agronomy

Article

Integrating Native Plant Mixtures and Arbuscular Mycorrhizal Fungi Inoculation Increases the Productivity of Degraded Grassland

Jiechao Chang [1], Kang Li [1], Jiayao Xie [1], Yanxia Zhang [1], Sitong Wang [1], Haiyan Ren [1,*] and Manqiang Liu [2]

[1] College of Agro-Grassland Science, Nanjing Agricultural University, Nanjing 210095, China
[2] Soil Ecology Lab, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China
* Correspondence: hren@njau.edu.cn

Abstract: Intense human activities break the grassland–livestock balance and accelerate grassland degradation. We evaluated the use of native dominant species combined with arbuscular mycorrhizal fungi (AMF) in order to recover grassland and restrain grassland degradation. We conducted a full factorial greenhouse experiment to evaluate the interaction effects of native species of distinct traits grass *Lolium perenne* (L) and legume *Trifolium repens* (T) with arbuscular mycorrhizal fungi (AMF) inoculation on grass productivity and soil properties across non-degraded, lightly degraded, and severely degraded soils. The grass–legume mixture was manipulated with five ratios (T:L = 1:0, T:L = 1:1, T:L = 3:1, T:L = 1:3, T:L = 0:1). The results showed that *L. perenne* significantly increased grassland productivity at different grass–legume ratios, regardless of AMF presence or absence. AMF inoculation increased plant N and P content uptake and improved the productivity of degraded grasslands, especially in severely degraded grasslands. The NO_3^--N and available P concentrations increased in soil when the legume component increased from T:L = 0:1 (grass monoculture) to T:L = 1:0 (legume monoculture). This may be because the presence of *Lolium perenne* (L) can promote nitrogen fixation in legumes. Structural equation modeling indicated that grass–legume mixtures directly affected plant biomass, whereas AMF affected plant biomass via providing plant nutrients. A soil quality index based on minimum datasets indicated a significant positive effect of artificial grassland establishment on soil quality. We conclude that planting T:L = 0:1 and T:L = 1:3 combined with AMF inoculation can be used to recover degraded grassland production, and planting T:L = 1:1 and T:L = 1:3 plus AMF inoculation can be applied for grassland nutrient accumulation and stability maintenance.

Keywords: arbuscular mycorrhizal fungi; grassland restoration; grass–legume mixture; planting ratio; plant–soil interaction

Citation: Chang, J.; Li, K.; Xie, J.; Zhang, Y.; Wang, S.; Ren, H.; Liu, M. Integrating Native Plant Mixtures and Arbuscular Mycorrhizal Fungi Inoculation Increases the Productivity of Degraded Grassland. *Agronomy* **2023**, *13*, 7. https://doi.org/10.3390/agronomy13010007

Academic Editors: Kesi Liu and Xinqing Shao

Received: 1 November 2022
Revised: 5 December 2022
Accepted: 14 December 2022
Published: 20 December 2022

1. Introduction

Grassland degradation is an ecological process of reverse evolution caused by a series of human and natural events [1] At present, one-third of grasslands in China are degraded. Nanshan Pasture in Hunan Province is an important pastoral grassland area in southern China. However, due to excessive grazing and climate factors, most of the grassland area in Nanshan has seriously degraded, accompanied with plant biomass and species diversity loss, soil nutrients loss, and soil pH increase [2]. In response to these environmental disasters, various interventions have been implemented, such as fencing pastures, rodent and pest control, fertilization, and farming; however, these strategies require long recovery periods and have unstable effects [3].

The establishment of artificial grassland offers a sound approach for the restoration of degraded grasslands and to slow down the pace of grassland degradation [4,5]. Studies have shown that the establishment of artificial or semi-artificial grasslands by reseeding had

a positive effect on productivity and soil structure in degraded grasslands. In addition, the soil microbial community plays a vital role in nutrient cycling, soil structure maintenance, and ecosystem productivity [6]. As the interactions between plant and microorganism are altered in degraded grassland ecosystems, the regulation of soil microorganisms is expected to improve the effect of sowing native grass species for the effective restoration of degraded grassland ecosystems.

Arbuscular mycorrhizal fungi (AMF), as substantial components of microbial communities in soils, form mutualistic associations with the roots of most terrestrial plants, helping them access soil nutrients (especially phosphorus), while accumulating plant carbohydrates [7,8]. Mycorrhizal symbionts can improve the absorption of soil mineral elements by the host plant, increase plant biomass, and improve soil quality and soil aggregate stability, thus affecting the stability of the plant–soil system. The role of AMF is most pronounced in vegetation restoration in degraded areas [9]. Rosales et al. (1997) illustrated that AMF should be considered for the restoration of degraded areas by reintroducing them or increasing their inoculum [10]. Therefore, the use of mycorrhizal biotechnology to restore degraded grassland ecosystems has been a main focus in research regarding the restoration of severely degraded grasslands [11]. However, the effects of AMF inoculation on plant species composition and soil–plant synergism in degraded grasslands remains unclear.

Grass–legume mixtures, which are widely used in mixed pastures and agricultural intercropping systems, can enhance community productivity and stability [12–14]. As two different functional groups, their characteristics of physiology and nutrient utilization benefit each other. The establishment of *Lolium perenne* and *Trifolium repens* mixture grassland has been widely used to reconstruct the grassland ecosystem in southern China [15]. The reseeding of *T. repens* and *L. perenne* in degraded grasslands is known to increase soil nitrogen availability, improve biomass yield, and maintain biodiversity [16]. However, the addition of AMF in leguminous and non-leguminous mixed seeding systems reduces nitrogen loss and improves nitrogen utilization efficiency [17]. Previous studies mostly focused on the complementary effects of grass–legume mixtures, and less research is related to the effects of exogenous beneficial microorganisms on vegetation restoration in mixed grass–legume planting grassland, thus restricting the recovery of degraded grasslands.

Interactions between above-ground and below-ground communities are vital in ecosystem functions, including improving ecosystem stability, maintaining biodiversity, and enhancing soil nutrient stability [18]. In this study, we introduced AMF combined with locally dominant grass species to regulate soil–plant interactions to accelerate grassland restoration. The objectives of this study were to investigate whether the addition of AMF was effective in restoring degraded grassland and to study the effects of the combination of two dominant species at different composition ratios and AMF inoculation on plant biomass, nutrient accumulation, and soil nutrients with the aim to aid the appropriate management schemes for the protection and restoration of degraded grasslands.

2. Materials and Methods

2.1. Study Area

Nanshan Pasture in Hunan province (Figure 1) is located in the southwest of Chengbu Miao Autonomous County in Shaoyang City, Hunan Province, China. It was established in March 1956. The soil of Nanshan Pasture is mainly mountain meadow soil and mountain yellow brown soil, and the pH is neutral to slightly acidic. Natural vegetation mainly includes *Arundinella yunnanensis Keng*, *Cathaya argyrophylla Chun et Kuang*, *Emmenopterys henryi Oliv*, and *Holcus lanatus*. The dominant grass species at this site are *Trifolium repens* (T) and *Lolium perenne* (L). The mean annual temperature is around 11 °C, and the average annual rainfall is approximately 1900 mm.

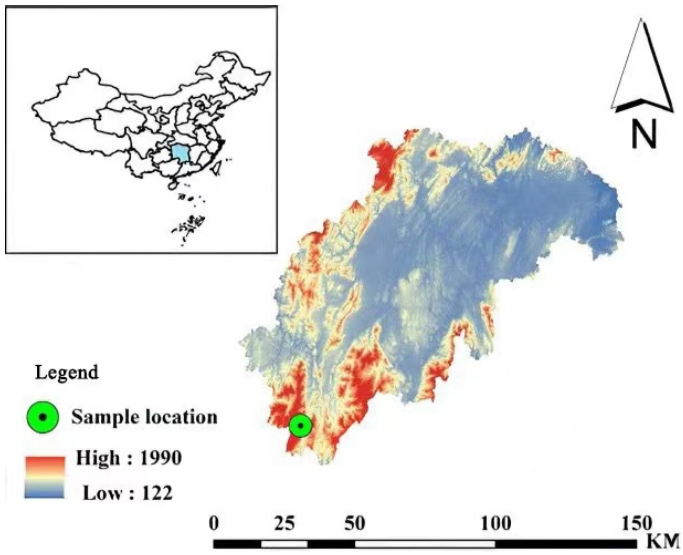

Figure 1. Soil sampling site at Nanshan Pasture, Hunan Province. The inset shows the location of Hunan Province in China. Data Center for Resource and Environmental Sciences, Chinese Academy of Sciences, 2021.

2.2. Soil and Inoculum Preparation

Based on the evaluation criteria of grassland degradation (BG19377-2003), non-degraded (NDG), lightly degraded (LDG), and severely degraded (SDG) grasslands were selected for sampling. Soil samples were collected on 30 July 2021. At each plot, we collected 60 kg of soil (5–20 cm deep) using shovels, and between samples, the shovels were sterilized with a 70% ethanol solution. The soil samples were transported to the laboratory, mixed, and passed through a 0.5 cm sieve to remove rocks and plant debris. The homogenized soil was divided into two equal parts. One part was stored at 4 °C. The other part was heat-sterilized (autoclaving at 121 °C for 60 min) and stored in a cool place for later use. A major disadvantage of sterilization is that the concentration of soil nutrients increases due to decomposition of the killed soil organisms [19]. Autoclaving increases the soil organic carbon (SOC) content. In general, the side effects of sterilization can be largely avoided with the addition of small amounts of living and sterilized soil to the sterilized background soil [20,21]. To minimize the effect of autoclaving on soil, we mixed the fresh soil or sterilized soil with each degradation degree and sterilized mixed soil (soil + sand) with the same degradation degrees at a ratio of 1:10, which were non-sterilized and sterilized soils.

Soils were inoculated with AMF strains purchased from the College of Resources and Environment Sciences, Nanjing Agricultural University, Nanjing, China. The five AMF strains included *Acaulospora scrobiculata Trappe*, *Glomus mosseas*, *Glomus caledonium*, *Glomus versiforme*, and *Glomus intraradices*. AMF spores contained in soil inoculation were propagated in autoclaved (121 °C for 120 min for one cycle) substrate (sand/soil, 1:2) with maize for two successive propagation cycles (three months for each cycle). The inoculum consisted of a mixture of the five strains at a dry weight ratio of 1:1:1:1:1.

2.3. Greenhouse Experiment

Seed was used of the ryegrass (*Lolium perenne*) Bond varieties, and of the legume (*Trifolium repens*) Sulky varieties. Mixtures are henceforth referred to by the first letters of their names. We carried out a full factorial greenhouse experiment, in which soil degradation degree (NDG, LDG, and SDG), planting ratio (T1L0, T3L1, T1L1, T0L1, and T1L3), and soil treatment (non-sterilized, sterilized, and sterilized + AMF) were the three factors (Table 1). The plant density in each pot remained unchanged, with 16 plants per pot and three replicates. One of five *T. repens* to *L. perenne* ratios (0:1, 1:3, 3:1, 1:0, and 1:1) was

randomly assigned to each pot. All seeds were surface-disinfected (2 min in a 1% chloride solution), rinsed, and germinated on Petri dishes.

Table 1. A complete factorial design of 3 levels of soil degradation degree × 3 levels of soil biological treatment × 5 levels of plant mixtures.

Soil Degradation Degree	Soil Biological Treatment	Planting Ratios
Non-degraded/lightly/severely degraded	Sterilized soil	*Lolium perenne* monoculture *Trifolium repens* monoculture *L. perenne*:*T. repens* = 3:1 *L. perenne*:*T. repens* = 1:3 *L. perenne*:*T. repens* = 1:1
	Sterilized + AMF	*L. perenne* monoculture *T. repens* monoculture *L. perenne*:*T. repens* = 3:1 *L. perenne*:*T. repens* = 1:3 *L. perenne*:*T. repens* = 1:1
	Non-sterilized soil	*L. perenne* monoculture *T. repens* monoculture *L. perenne*:*T. repens* = 3:1 *L. perenne*:*T. repens* = 1:3 *L. perenne*:*T. repens* = 1:1

At the planting stage, the experiment included 135 pots (3 soil degradation degrees × 5 planting ratios × 3 soil treatments × 3 replicates). Plants were grown in plastic pots containing 500 g of steam-sterilized coarse sand covered with 2000 g of sterilized soil of different degradation levels and 200 g of fresh soil or 2200 g of sterilized soil mixture. For the AMF inoculation treatment, we added 250 g of AMF inoculum per pot, whereas for the non-inoculation treatment, 250 g of sterilized AMF inoculum was added per pot. Seedlings that died during the first week were immediately replaced, and pots were positioned randomly in greenhouses with 70% relative humidity. The plants were exposed to 16 h of light at 21 °C (day) and 8 h of darkness at 16 °C (night). The pots were watered every other day, and initial soil moisture (17% dry weight of soil) was measured twice a week.

2.4. Harvesting and Measurements

After 18 weeks, shoots were harvested, oven dried at 65 °C for 72 h to a constant weight, and weighed. The soil in the pots was collected through a 2 mm sieve and dried in a ventilated place to test the soil properties. Nitrate (NO_3^--N) was detected using a colorimetric method (220 nm minus 275 nm) after extraction with 2 mol/L of KCl [22], and ammonium (NH_4^+-N) was analyzed using a colorimetric method (625 nm) after indophenol blue-KCl extraction [23]. The available phosphorus (mg/g) was measured using the Olsen method [23].

2.5. Data Analysis and Statistical Analysis

A general linear model (GLM) was used to determine the effect of soil degradation degree, soil treatment, planting ratio, and their interactions on above-ground biomass, above-ground N and P contents, soil NH_4^+-N, soil NO_3^--N, and soil available P (Table 2). One-way ANOVA was used to evaluate the significant differences among treatments. All of the mentioned parameters were Box-Cox-transformed to ensure normality and homogeneity using a Shapiro–Wilk test. Duncan's multiple comparison was used to test the difference between the mean values of each treatment. Linear regression analysis was used to analyze the relationship between plant biomass and soil nutrients and plant N and P content. R 3.6.0 was used for statistical analysis (p-values of less than 0.05 were considered significant). The location map of the studied area was created by using ArcMap of ArcGIS software. SigmaPlot 12.5 was used to create the figures.

Table 2. Analysis of variance of the effects of soil degradation degree (S), planting ratio (P), and soil biological treatment (B) on plant and soil indicators.

Treatment	DF	Biomass	NH_4^+-N	NO_3^--N	Available P	Shoot N	Shoot P	Plant N/P
Soil degradation (S)	2	901.0 ***	73.25 ***	110.13 ***	498.13 *	426.19 *	909.14 *	108.26 ***
Planting ratio (P)	4	278.20 ***	5.39 ***	24.892 ***	1.97	82.96 *	90.02 *	4.70 *
Biological treatment (B)	2	10.99 ***	7.29 ***	19.648 *	0.05	5.83 *	10.19 *	13.99 ***
S × P	8	3.92 ***	2.80 ***	8.116 *	2.59 *	5.84 *	5.57 *	4.92 ***
S × B	4	15.24 ***	0.86	3.016 *	0.94	10.73 *	12.02 *	5.28 *
B × P	8	3.73 ***	1.42	1.188	1.83	2.67	1.53	1.41
S × P × B	16	2.48 ***	1.26	1.651	1.36	3.19	2.78 *	2.03 *

Note: * indicates significant difference ($p < 0.05$); *** indicates highly significant difference ($p < 0.01$).

A soil quality Index (SQI) was calculated using the minimum dataset (MDS) indicators that best represented soil functions. Using PCA, PCs with eigenvalues > 1 that explained >5% of the total variation were assumed to represent soil quality for MDS (Table 3). We then used the following sigmoidal curve to normalize and score the MDS indicators [24].

$$NL - SF(Y) = \frac{\mathrm{a}}{\left(1 + \left(\frac{\mathrm{x}}{x_0}\right)b\right)}$$

where *NL—SF* (*Y*) is the non-linear score of each indicator ranging from 0 to 1, a is the maximum value (a = 1), x is the value of the selected indicator, x_0 is the average value of each indicator, and b is the slope of the equation and was set to –2.5 for "more is better" functions and 2.5 for "less is better" functions.

Table 3. Principal component analysis of soil quality indicators.

Variable	PC 1	PC 2	PC 3
Available P	0.949	−0.092	−0.057
SOC	0.883	0.035	−0.127
Shoot P	0.855	0.025	0.159
Biomass	0.0747	−0.252	−0.449
Shoot N	−0.072	0.831	0.4
pH	−0.084	−0.587	0.599
NO_3^--N	0.613	−0.031	0.67
NH_4^+-N	0.466	0.543	−0.168
Eigenvalues	3.576	1.405	1.197
Cumulative contribution (%)	44.696	62.264	77.222

The final step for the soil quality assessment combined the selected indicators into an overall SQI using the following weighted additive equation [25].:

$$\mathrm{SQI} = \sum_{i=0}^{n} \mathrm{WiSi}$$

where W is the weighting factor for the soil property that equals the explanation of each PC divided by the total percentage of variation and S is a non-linear (NL− SQI) score. The SQI value is considered to represent overall soil quality, with higher values indicating better soil quality.

Structural equation models (SEMs) were used to analyze potential pathways which estimate AMF addition and planting ratio exert effects and indirect relationships between soil nutrients and plant shoot nutrient content on plant biomass. Soil nutrients (organic C, available N, and available P) were decreased separately through principal component analysis (PCA). The first principal component (PC1) in each group was used for the subsequent SEM analysis. The PCA results showed that in non-degraded grasslands, PC1 explained 73% and 80% of the total variance in soil nutrients and plant nutrients,

respectively. In lightly degraded grasslands, PC1 explained 66% and 70% of the total variance of soil nutrients and plant nutrients, respectively, while in severely degraded grasslands, PC1 explained 77% and 66% of the total variance of soil nutrients and plant nutrients, respectively (Table 4).

Table 4. Partial correlation coefficients of principal components analysis (PCA) of variables in categories of plant nutrients and soil nutrients in soil degradation degree.

Variables	Component 1		
	Non-Degraded	Lightly Degraded	Severely Degraded
Soil nutrients			
AN (mg kg^{-1})	0.753	0.651	0.657
AP (mg kg^{-1})	0.752	0.822	0.775
SOC (mg kg^{-1})	0.388	−0.617	0.724
Cumulative (%)	72.75%	66.42%	77.36%
Plant nutrients			
Shoot N (mg g^{-1})	0.893	0.772	0.751
Shoot P (mg g^{-1})	0.893	−0.772	−0.751
Cumulative (%)	79.82%	69.60%	66.41%

3. Results

3.1. Changes in Plant Community Productivity under Different Treatments

All three treatments, i.e., the soil treatment (non-sterilized, sterilized, and sterilized + AMF), soil degradation degree (NDG, LDG, and SDG), and planting ratio (T1L0, T3L1, T1L1, T0L1, and T1L3), had significant effects on above-ground biomass, and there were significant interactions among them (Table 2). The highest plant biomass of 10.45 g DM/pot was observed under T1L3 in NDG non-sterilized soil, followed by 9.07 g DM/pot under L1T0 in NDG non-sterilized soil inoculated with AMF (Figure 2). Compared to the non-sterilized and sterilized treatments, AMF treatment of NDG soil induced a decrease in above-ground biomass (Figure 2). Compared with the non-sterilized and sterilized treatments, AMF inoculation significantly increased the above-ground biomass in both LDG and SDG soils, especially in communities with a high proportion of *L. perenne* (Figure 2). The aboveground biomass under different treatments was in the following order: T1L3 in NGD non-sterilized soil > T0L1 in NDG soil with AMF > T1L1 in NDG non-sterilized soil > T1L3 in NDG sterilized soil > T0L1 in NDG sterilized soil > T1L1 in NDG sterilized soil > T0L1 in LDG soil with AMF > T0L1 in LDG sterilized soil > T1L1 in LDG soil with AMF, and these above-ground biomass levels were significantly higher than those under the other treatments (Figure 2).

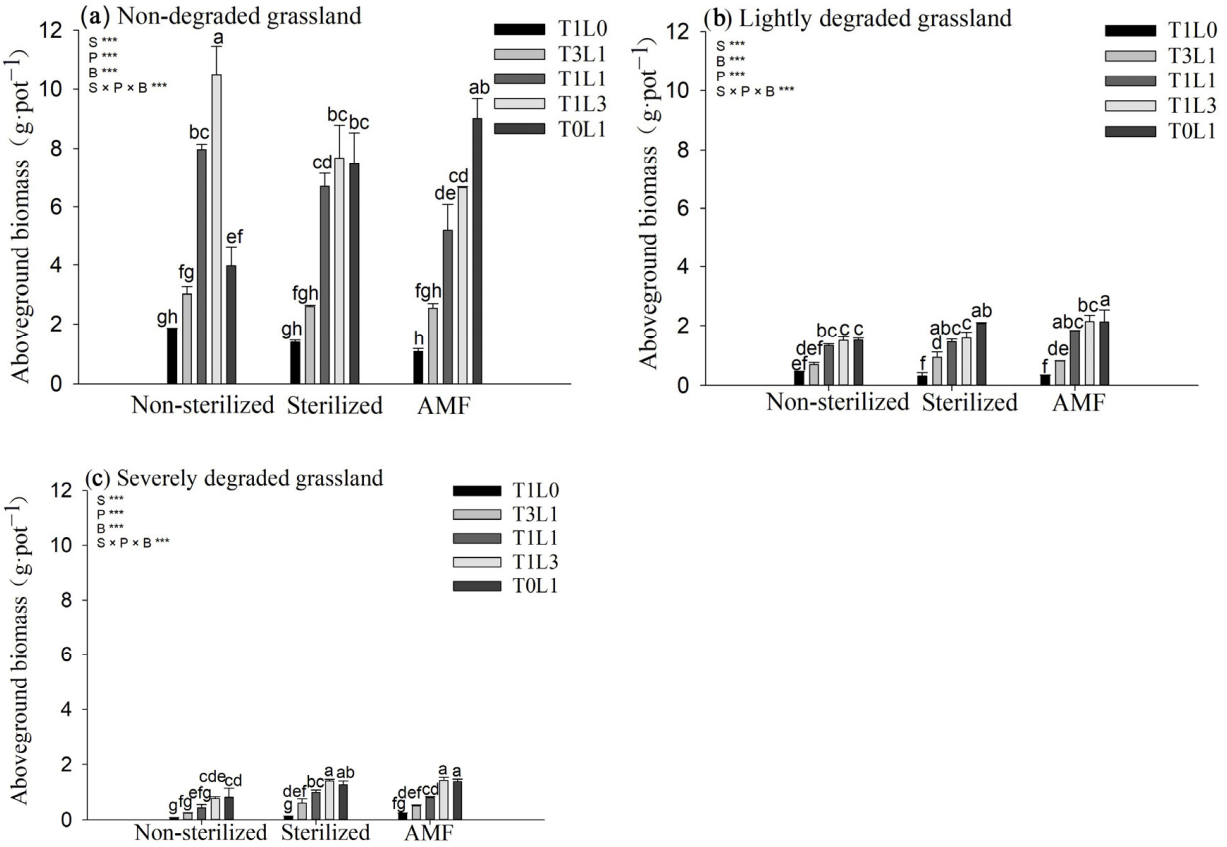

Figure 2. Effects of different treatments on plant community productivity. (**a**) Effects of soil type (S), planting ratio (P) , biological treatment (B) and their interactions (S × P × B) on aboveground biomass in non-degraded grassland soil (**b**) Effects of soil type (S), planting ratio (P) , biological treatment (B) and their interactions (S × P × B) on aboveground biomass in lightly degraded grassland soil. (**c**) Effects of soil type (S), planting ratio (P) , biological treatment (B) and their interactions (S × P × B) on aboveground biomass in severely degraded grassland soil. Different letters indicate the significant differences among treatments ($p < 0.05$). *** $p < 0.001$ indicate the significance level of each treatment. Note: soil degradation degree (S); planting ratio (P); soil biological treatment (B). T1L0, T3L1, T1L1, T1L3, T0L1 mean planting ratio of *T. repens* and *L. perenne*: 1:0, 3:1, 1:1, 1:3, 0:1, respectively.

3.2. Changes in Aboveground N and P Contents in Plants under Different Treatments

Arbuscular mycorrhizal fungi inoculation improved the plant nutrient contents (Figures 3b and 4b,c). In LDG and SDG soils, AMF inoculation increased the plant shoot N and P contents. However, AMF inoculation did not improve the plant shoot N and P contents in NDG soil (Figure 3b). The plant shoot N content was the highest under T1L0 in NDG soil. AMF inoculation increased the plant shoot P content in plant communities with a high proportion of *T. repens* in LDG and SDG soils (Figure 4). The plant N/P ratio in NDG soil was significantly lower than that in LDG and SDG soils (Figure 5). Overall, for NDG soil, the average plant N:P ratio was 3.66 ± 0.34 (mean ± standard error); for LDG soil, it was 18.36 ± 1.25; and for SDG soil, it was 15.42 ± 0.91.

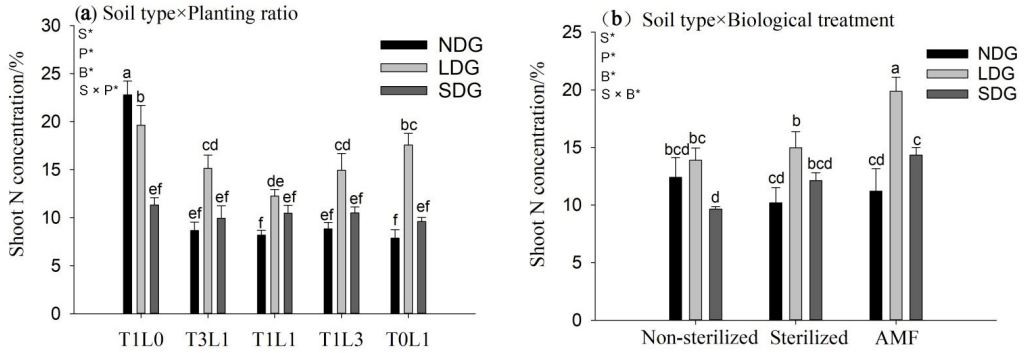

Figure 3. Effects of different treatments on shoot N content. (**a**) Effects of soil type (S), planting ratio (P) and their interactions (S × P) on shoot N concentration. (**b**) Effects of soil type (S), biological treatment (B) and their interactions (S × B) on shoot N concentration. Different letters indicate the significant differences among treatments ($p < 0.05$). * $p < 0.05$, $p < 0.1$ indicate the significance level of each treatment. Note: soil degradation degree (S); planting ratio (P); soil biological treatment (B); NDG: non-degraded grassland; LDG: lightly degraded grassland; SDG: severely degraded grassland. T1L0, T3L1, T1L1, T1L3, T0L1 mean planting ratio of *T. repens* and *L. perenne*: 1:0, 3:1, 1:1, 1:3, 0:1, respectively.

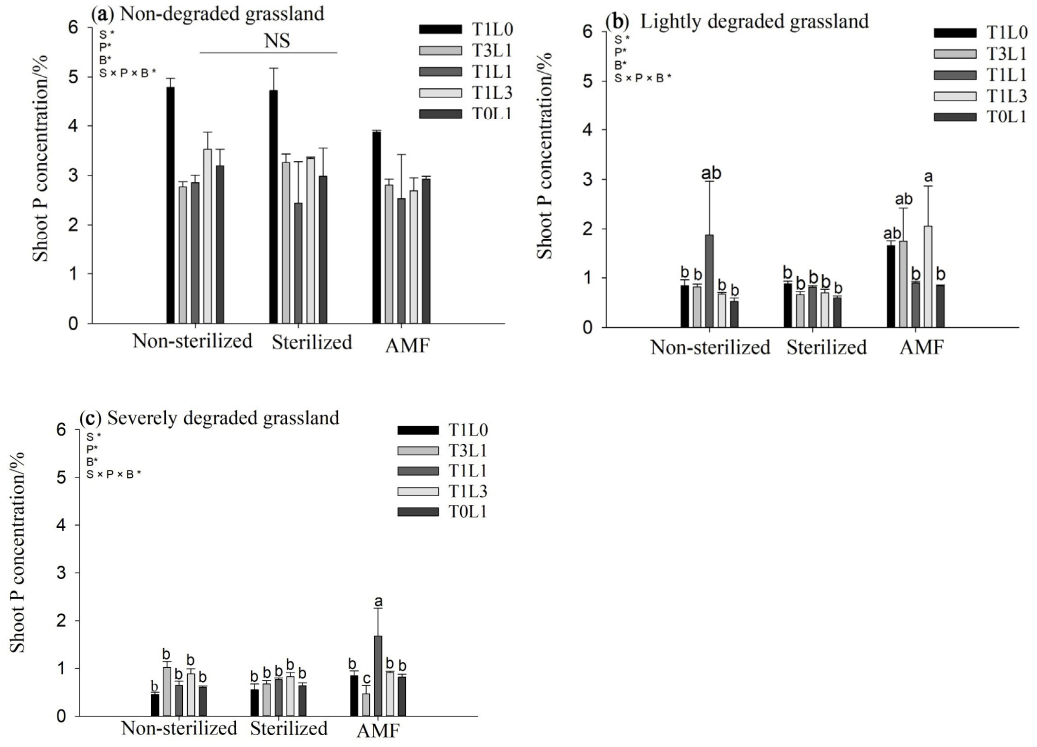

Figure 4. Effects of different treatments on shoot P content. (**a**) Effects of soil type (S), planting ratio (P) , biological treatment (B) and their interactions (S × P × B) on shoot P concentration in non-degraded grassland soil (**b**) Effects of soil type (S), planting ratio (P) , biological treatment (B) and their interactions (S × P × B) on shoot P concentration in lightly degraded grassland soil. (**c**) Effects of soil type (S), planting ratio (P) , biological treatment (B) and their interactions (S × P × B) on shoot P concentration in severely degraded grassland soil. Different letters indicate the significant differences among treatments ($p < 0.05$). * $p < 0.05$, $p < 0.1$ indicate the significance level of each treatment. Note: soil degradation degree (S); planting ratio (P); soil biological treatment (B). T1L0, T3L1, T1L1, T1L3, T0L1 mean planting ratio of *T. repens* and *L. perenne*: 1:0, 3:1, 1:1, 1:3, 0:1, respectively.

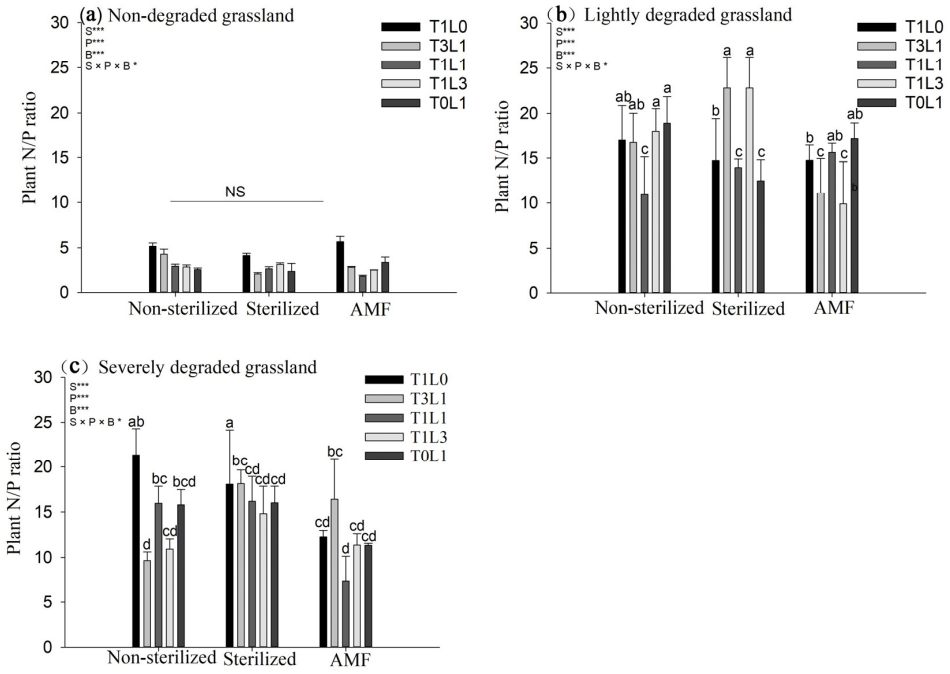

Figure 5. Effects of different treatments on plant N/P ratio. (a) Effects of soil type (S), planting ratio (P) , biological treatment (B) and their interactions (S × P × B) on plant N/P ratio in non-degraded grassland soil (b) Effects of soil type (S), planting ratio (P) , biological treatment (B) and their interactions (S × P × B) on plant N/P ratio in lightly degraded grassland soil. (c) Effects of soil type (S), planting ratio (P) , biological treatment (B) and their interactions (S × P × B) on plant N/P ratio in severely degraded grassland soil. Different letters indicate the significant differences among treatments ($p < 0.05$). *** $p < 0.001$, * $p < 0.05$, $p < 0.1$ indicate the significance level of each treatment. Note: soil degradation degree (S); planting ratio (P); soil biological treatment (B). T1L0, T3L1, T1L1, T1L3, T0L1 mean planting ratio of *T. repens* and *L. perenne*: 1:0, 3:1, 1:1, 1:3, 0:1, respectively.

3.3. Changes in Soil Nutrients under Different Treatments and SQI Values

The soil quality indexes after treatments are shown in Figure 6. The PCA based on soil physicochemical parameters showed that the eigenvalues of the first two PCs were >1, and PC1 explained 71.4% of the total variance (Table 3). Further, 77.22% of the total variance was explained by three PCs (Table 3). PC-1 was dominated by AP, SOC, and NO_3^--N, which accounted for 44.69% of the total variance. PC-2 was characterized by shoot N, pH, and NH_4^+-N, which accounted for 17.56% of the total variance. These variables were not correlated. Biomass was selected from PC-3 (explaining 14.95% of the variance).

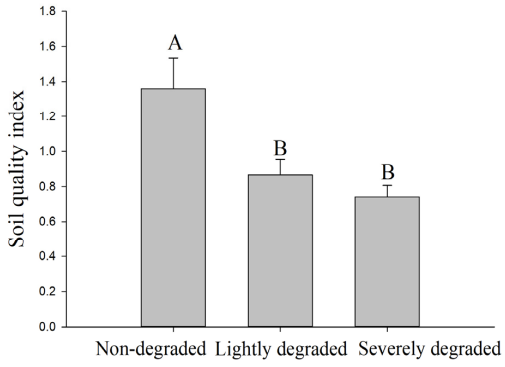

Figure 6. Soil quality index after vegetation restoration and AMF inoculation. Different letters indicate the significant differences among treatments ($p < 0.05$).

The soil treatment, planting ratio, and soil degradation degree significantly affected the soil nutrient contents (NH_4^+-N and NO_3^--N (Table 2). The soil degradation degree also significantly affected the soil's available P content (Table 2). The NH_4^+-N content was higher under T1L0, T0L1, and T1L1 in LDG soil (Figure 7a). The NO_3^--N and NH_4^+-N content were increased when the legume component increased (Figure 8a). The available nitrogen content in the three soil types decreased by 65%, 56.9%, and 16.8% after the treatments. There were also significant differences in the available P content under the different treatments. The available P content was higher in the communities with large proportions of legumes (Figure 9). The available P content in the three soil types decreased by 48%, 24%, and 13%. Soil pH generally increased by 77%. The SOC content significantly reduced by 66%, 40%, and 29%, and it reduced most in non-degraded soil. The soil treatment also significantly affected the soil NH_4^+-N and NO_3^--N contents. Compared with the sterilized and AMF treatments, the non-sterilized treatment reduced the soil NH_4^+-N content (Figure 7b). In NDG and LDG soils, AMF inoculation reduced the soil NO_3^--N content compared to the sterilized and non-sterilized treatments (Figure 8b).

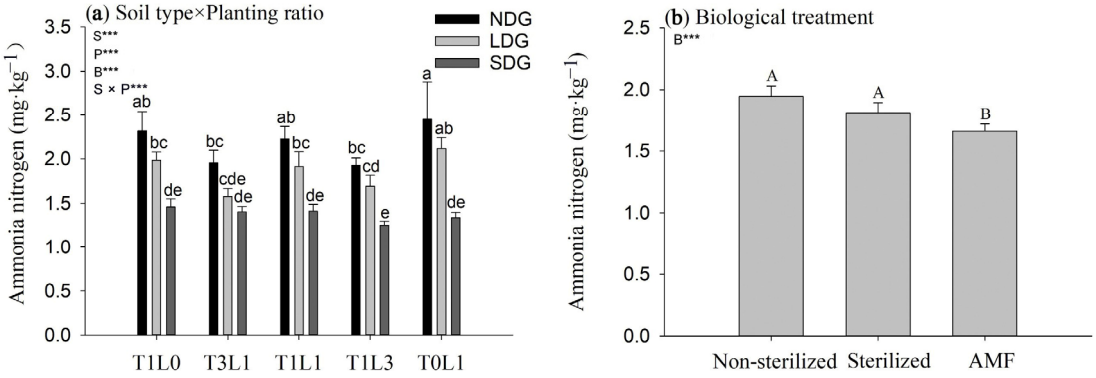

Figure 7. Effects of different treatments on soil NH_4^+-N content. (**a**) Effects of soil type (S), planting ratio (P) and their interactions (S × P) on soil ammonia nitrogen concentration. (**b**) Effects of biological treatment (B) on soil ammonia nitrogen concentration. Different capital and lowercase letters indicate significant differences between treatments. *** $p < 0.001$ indicate the significance level of each treatment. Note: soil degradation degree (S); planting ratio (P); soil biological treatment (B); NDG: non-degraded grassland; LDG: lightly degraded grassland; SDG: severely degraded grassland. T1L0, T3L1, T1L1, T1L3, T0L1 mean planting ratio of *T. repens* and *L. perenne*: 1:0, 3:1, 1:1, 1:3, 0:1, respectively.

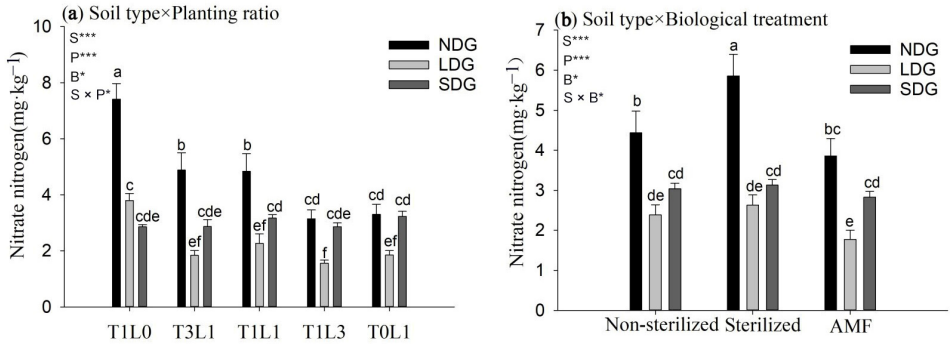

Figure 8. Effects of different treatments on soil NO_3^--N content. (**a**) Effects of soil type (S), planting ratio (P) and their interactions (S × P) on soil nitrate nitrogen concentration. (**b**) Effects of biological treatment (B) on soil nitrate nitrogen concentration. Different letters indicate the significant differences among treatments ($p < 0.05$). *** $p < 0.001$, * $p < 0.05$, $p < 0.1$ indicate the significance level of each treatment. Note: soil degradation degree (S); planting ratio (P); soil biological treatment (B); NDG: non-degraded grassland; LDG: lightly degraded grassland; SDG: severely degraded grassland. T1L0, T3L1, T1L1, T1L3, T0L1 mean planting ratio of *T. repens* and *L. perenne*: 1:0, 3:1, 1:1, 1:3, 0:1, respectively.

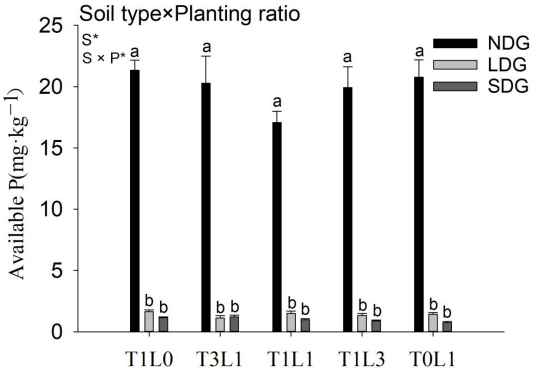

Figure 9. Effects of different treatments on soil available phosphorus content. Effects of soil type (S), planting ratio (P) and their interactions (S × P) on soil available P concentration. Different letters indicate the significant differences among treatments ($p < 0.05$). * $p < 0.05$, $p < 0.1$ indicate the significance level of each treatment. Note: soil degradation degree (S); planting ratio (P); soil biological treatment (B); NDG: non-degraded grassland; LDG: lightly degraded grassland; SDG: severely degraded grassland. T1L0, T3L1, T1L1, T1L3, T0L1 mean planting ratio of *T. repens* and *L. perenne*: 1:0, 3:1, 1:1, 1:3, 0:1, respectively.

3.4. Relationships between Plant Parameters and Soil Properties

The regression analysis showed that the community above-ground biomass increased with increasing NH_4^+-N and available P in the soil (Figure 10). The above-ground N content showed positive correlations with soil NH_4^+-N and NO_3^--N contents, but not with available P content (Figure 11). The above-ground P content showed positive correlations with the NH_4^+-N, NO_3^--N, and available P content in the soil (Figure 12).

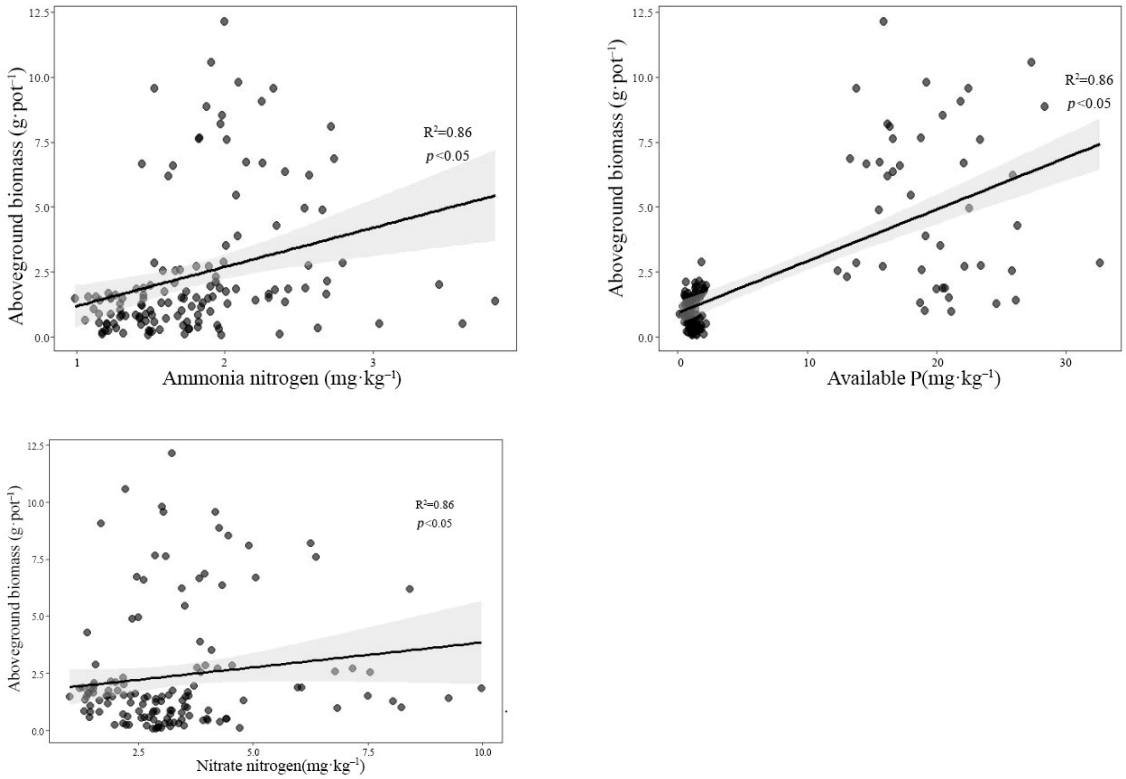

Figure 10. Relationships between available P, NH_4^+-N, and NO__-N contents in soil and plant community productivity.

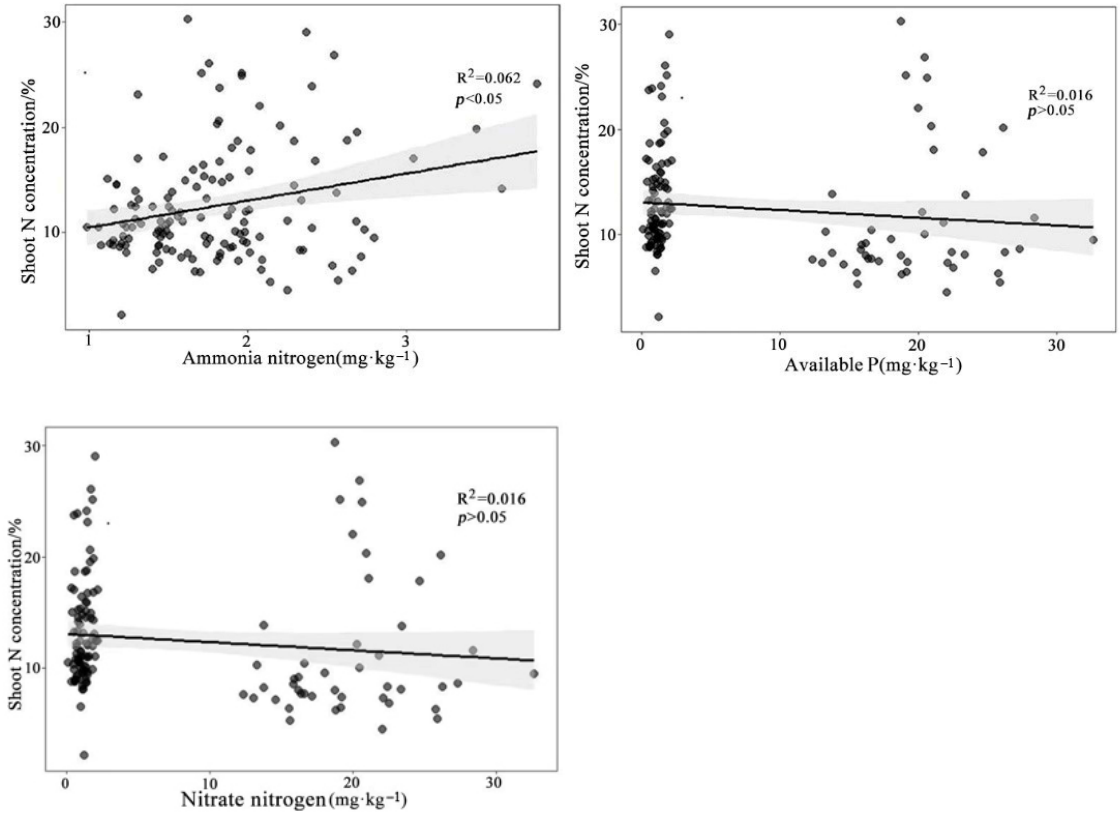

Figure 11. Relationships between available P, NH_4^+-N, and NO_-N contents in soil and plant N.

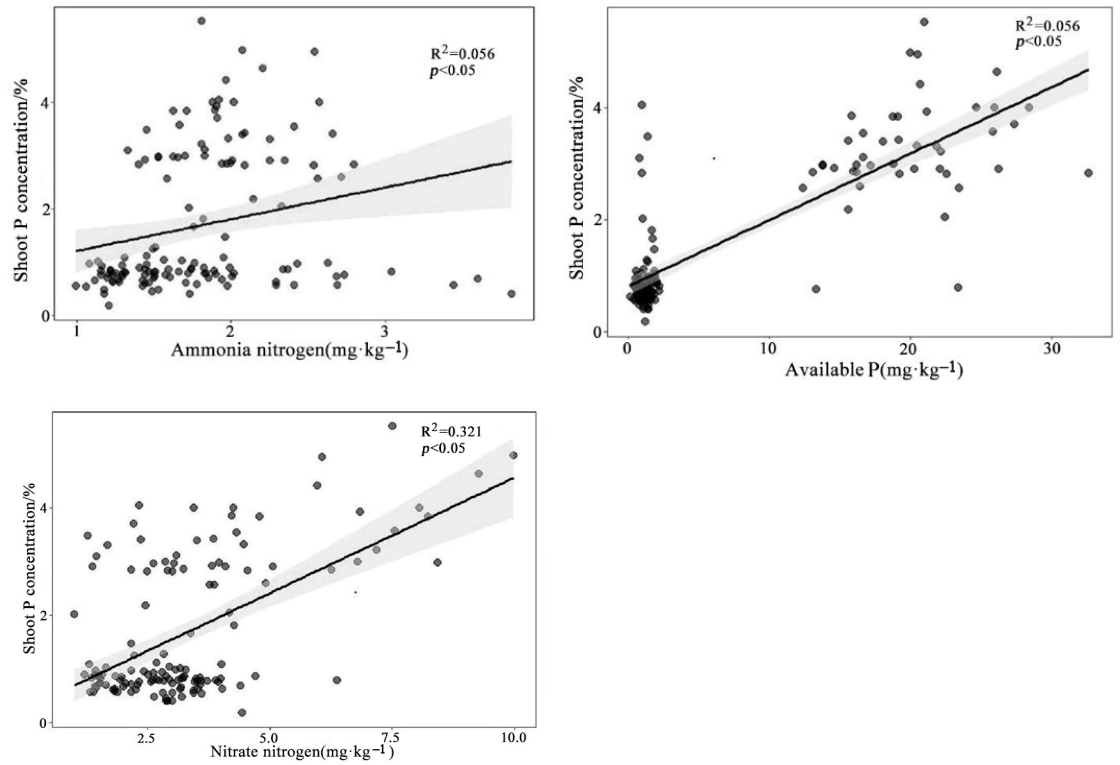

Figure 12. Relationships between available P, NH_4^+-N, and NO_3^--N contents in soil and plant P.

3.5. Pathways Determining Biomass

Two SEMs revealed that the planting ratio and AMF inoculation altered the plant biomass via different pathways in soils with different degrees of degradation, according to significant standardized path coefficients. Under different degrees of degradation, the planting ratio was the main pathway determining biomass, and it indirectly influenced the plant biomass through effects on shoot N and P contents. In LDG soil, AMF influenced the plant biomass through effects on soil nutrient contents. In SDG soil, AMF affected the plant biomass directly and indirectly by the affecting shoot N and P contents (Figure 13).

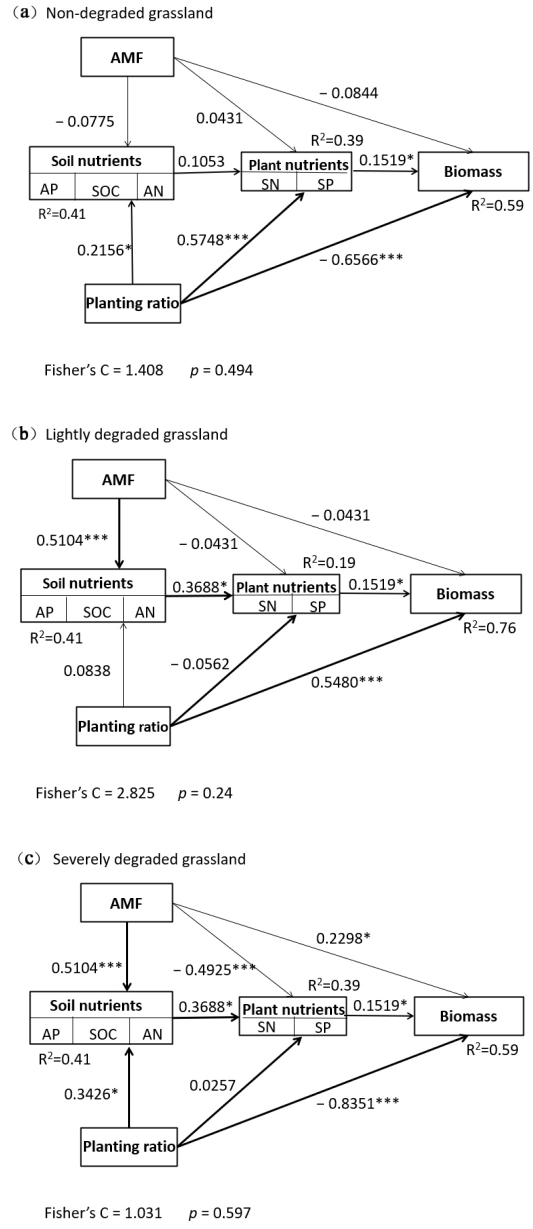

Figure 13. Structural equation models showing the effects of AMF addition and planting ratio on plant shoot biomass through soil nutrients and plant shoot nitrogen and phosphorus contents. (**a**) Non-degraded grassland. (**b**) Lightly degraded grassland. (**c**) Severely degraded grassland. Square boxes denote variables included in the models. Soil nutrient variables include soil NH_4^+-N, NO_3^--N, available P, and soil organic carbon. Soil nutrients are synthetic variables derived from the first axis of principal component analyses. The thickness of arrows is proportional to the standardized path coefficient. *** $p < 0.001$, * $p < 0.05$, $p < 0.1$ indicate the significance level of each effect. R^2 values represent the proportion of variance explained for each endogenous variable.

4. Discussion

Above-ground plant communities were affected by AMF inoculation, planting ratio, and grassland degradation state, as well as their interactions. The soil treatment and planting ratio regulated the plant shoot nutrient content and soil nutrients under different state of soil degradation. AMF inoculation increased the N and P contents in plant shoots, especially in nutrient-deficient environments, and enhanced the absorption of mineral elements by plants. One possible hypothesis is the strong nitrogen fixation ability of *T. repens*; communities with a large proportion of *T. repens* in the sowing mixture showed increased soil N accumulation.

4.1. Effects on Degraded Grassland Plant Communities

Soil microorganisms play an important role in plant growth [26]. Beneficial microorganisms, as ecosystem mediators, can be used to increase crop yields and modify microorganism communities to restore degraded grasslands [27]. Our findings revealed that plants grown in non-sterilized soil performed better than those grown in sterilized soil and sterilized soil inoculated with AMF in NDG soil. This difference could be attributable to the higher beneficial microbial diversity in non-sterilized soil and the antagonistic effects of dominant beneficial microorganisms against pathogens in the microbial community, which contributes to a better growth of the plants [28]. We detected no significant differences in plants growth between LDG non-sterilized and sterilized soils, attributing to the negative effects of soil-borne pathogens accumulated in the rhizosphere of the plant communities and the positive effects of AMF and other beneficial microorganisms [29]. Appropriate soil nutrients promoted the positive feedback effect of AMF on the plant community, whereas nutrient enrichment suppressed the beneficial effects of AMF on plant performance, negatively affecting the positive feedback of AMF on the plant community [30]. AMF have a more positive effect on plant growth in nutrient-poor soil [31]. In line with this, our study showed the AMF inoculation promoted plant community productivity more significantly in LDG and SDG soil than in NDG soil.

N and P are the main limiting nutrients for plant growth in terrestrial ecosystems [32]. The symbiotic relationships between plants and AM fungi can significantly promote N and P uptake and thus improve plant biomass [33] In degraded soil, AMF inoculation was found to promote the plant shoot N and P contents. AMF inoculation contributed to an increase in the above-ground biomass of *L. perenne*. There was no significant increase in the above-ground biomass of *T. repens*, which was partly attributed to the higher individual biomass of *L. perenne*, which reduced the competitive ability of *T. repens*. In addition, this differential growth response could be attributable to the fact that *T. repens* is not strongly dependent on AM fungi. *L. perenne* forms strong symbiotic relationships with AMF, which enables *L. perenne* to obtain and efficiently utilize mineral nutrients, thereby promoting growth.

The vegetation N:P ratios served as indicators of nutrient limitation, and it has been suggested that an N/P ratio <14 generally indicates N limitation, whereas a ratio >16 indicates P limitation, and some transitional states may exist when N/P ratios are between 14 and 16 [34]. In the present study, given that in the NDG soil in the study area, the mean N/P ratio was lower than 16 under all treatments, we established the NDG soil to be N-limited. However, the LDG and SDG soils were P-limited soils. In future efforts for the restoration of degraded grassland, inoculation with beneficial rhizosphere microorganisms combined with a higher proportion of legumes in the planting community or adding P fertilizer can contribute to the alleviation of N and P limitations, thereby accelerate the restoration of degraded grasslands.

4.2. Effects on Soil Properties of Degraded Grassland

Artificial grassland establishment increases soil's nutrient content by recruiting native vegetation, such as by sowing legumes [35]. Nitrogen is a main limiting factor for grassland productivity [36]. It has been widely reported that introducing legumes into grasslands leads to higher soil N availability [37,38]. Although NO_3^--N is highly mobile and can

leach or denitrify, our study demonstrated that grass–legume mixtures at an appropriate proportion significantly increased the soil N content, regardless of the soil treatment. In addition, the soil'sNO_3^--N concentration decreased with the increase in the grass component. This may have been due to the large amount of NO_3^- absorbed by grass to maintain the soil mineral N at a low level and then to reduce the inhibition of legume N fixation. We concluded that the relative abundance of legumes had a great impact on the soil's N status in this grassland ecosystem. Furthermore, when the proportion of legumes increased in the mixed plant community, the soil's N mineralization and fixation increased, and the soil's N consumption decreased. The available phosphorus content in soil did not change obviously, possibly because soil's nutrient recovery requires more time. There is a clear need for further research to examine the effects of vegetation restoration on soil nutrients for longer periods of time if this approach is to be adopted to restore degraded grasslands.

Arbuscular mycorrhizal fungi (AMF) play a crucial role in promoting nutrient acquisition [39]. They can enhance a host plant's uptake of soil nutrients and transfer large amounts of inorganic N to the host plant. AM fungi can obtain substantial amounts of N from decomposing organic materials and directly utilize N from organic compounds [40], and then finally promote the efficiency of plant uptake and the utilization of soil N, thus reducing the residual N in the soil [41]. In this study, regardless of the planting ratio, the soil's NH_4^+-N and NO_3^--N contents decreased after AMF inoculation. This was because of the activation of soil nutrients via AMF inoculation, which improved soil nutrient availability and nutrient uptake, and thus reduced the soil's N content [42]. However, regardless of AMF addition, plants have a weak absorbability of available P, which may be determined by the nutritional requirements of plants themselves.

Plant–soil interaction is the most important feedback component in grassland restoration [43]. Previous research has indicated that soil nitrogen pools were strongly correlated with biomass [44]. In the present study, a regression analysis showed that above-ground biomass and plants' N and P contents were positively related with the soil's NH_4^+-N, NO_3^--N, and available P contents. Artificial vegetation establishment may increase the C and N storage by altering the quantity and quality of litter returned to the soil and further promote plant community recovery [45]. This research result was also reflected in the soil quality index. The soil quality index values showed that after vegetation restoration and AMF addition, there was little difference in the soil quality index between LDG and SDG. From a restoration perspective, the combination of AMF and grass–legume mixtures may enhance the uptake and utilization of soil nutrients and could be an effective strategy for degraded grassland restoration.

5. Conclusions

The restoration of vegetation and soil fertility reclamation is important for the recovery of degraded ecosystems. Grass–clover mixtures of *Lolium* (grass) and *Trifolium* (legume) on degraded grassland obviously improves soil quality. AMF increases plant community productivity by enhancing nutrient uptake, especially of P and N. SEM indicated that the planting ratio directly affects plant biomass, whereas AMF indirectly influences plant biomass via providing plant nutrients. Increasing the proportion of *L. perenne* can further improve plant community productivity. *T. repens* has a limited effect on community biomass but has a positive effect on forage quality and soil in mixed planting communities. We suggest that planting T:L = 0:1 and T:L = 1:3 combined with AMF inoculation can be used to recover degraded grassland production, and planting T1L1 and L3T1 with AMF inoculation can be applied for grassland nutrient accumulation and diversity conservation.

Author Contributions: H.R. designed the experiment; H.R. and J.C. Chang 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 XUEKEN2022020.

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

1. Peng, F.; Xue, X.; You, Q.; Huang, C.; Dong, S.; Liao, J.; Duan, H.; Tsunekawa, A.; Wang, T. Changes of soil properties regulate the soil organic carbon loss with grassland degradation on the Qinghai-Tibet Plateau. *Ecol. Indic.* **2018**, *93*, 572–580. [CrossRef]
2. Fayiah, M.; Dong, S.; Khomera, S.W.; Ur Rehman, S.A.; Yang, M.; Xiao, J. Status and challenges of Qinghai–Tibet Plateau's grasslands: An analysis of causes, mitigation measures, and way forward. *Sustainability* **2020**, *12*, 1099. [CrossRef]
3. Panakhyd, G.; Kotyash, U.; Yarmoluk, M.; Mizernyk, D.; Mashchak, Y. Improvement effect on the productivity of degraded grasslands. *Ştiinţa Agric.* **2014**, *2*, 3–8.
4. Carbon and nitrogen mineralization in soils under agro-pastoral systems in subtropical central Brazil. *Soil Sci. Plant Nutr.* **2002**, *48*, 179–184. [CrossRef]
5. Chen, K.; Zhou, H.; Lu, B.; Wu, Y.; Wang, J.; Zhao, Z.; Li, Y.; Wang, M.; Zhang, Y.; Chen, W.; et al. Single-Species Artificial Grasslands Decrease Soil Multifunctionality in a Temperate Steppe on the Qinghai–Tibet Plateau. *Agronomy* **2021**, *11*, 2092. [CrossRef]
6. Fisher, J.P.; Estop-Aragonés, C.; Thierry, A.; Charman, D.J.; Wolfe, S.A.; Hartley, I.P.; Murton, J.P.; Williams, M.; Phoenix, G.K. The influence of vegetation and soil characteristics on active-layer thickness of permafrost soils in boreal forest. *Glob. Change Biol.* **2016**, *22*, 3127–3140. [CrossRef]
7. Gonzalez-Chavez, C.; Harris, P.J.; Dodd, J.; Meharg, A.A. Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on Holcus lanatus. *New Phytol.* **2002**, *155*, 163–171. [CrossRef]
8. Bao, X.; Zou, J.; Zhang, B.; Wu, L.; Yang, T.; Huang, Q. Arbuscular Mycorrhizal Fungi and Microbes Interaction in Rice Mycorrhizosphere. *Agronomy* **2022**, *12*, 1277. [CrossRef]
9. Tian, H.; Gai, J.P.; Zhang, J.L.; Christie, P.; Li, X.L. Arbuscular mycorrhizal fungi in degraded typical steppe of Inner Mongolia. *Land Degrad. Dev.* **2009**, *20*, 41–54. [CrossRef]
10. Rosales, J.; Cuenca, G.; Ramírez, N.; De Andrade, Z. Native colonizing species and degraded land restoration in La Gran Sabana, Venezuela. *Restor. Ecol.* **1997**, *5*, 147–155. [CrossRef]
11. Coutinho, E.S.; Barbosa, M.; Beiroz, W.; Mescolotti, D.L.; Bonfim, J.A.; Berbara, R.L.L.; Fernandes, G.W. Soil constraints for arbuscular mycorrhizal fungi spore community in degraded sites of rupestrian grassland: Implications for restoration. *Soil Biol.* **2019**, *90*, 51–57. [CrossRef]
12. Hebeisen, T.; LÜSCHER, A.; Zanetti, S.; Fischer, B.; Hartwig, U.; Frehner, M.; Hendrey, G.; Blum, H.; NÖSBERGER, J.O.S.E.F. Growth response of Trifolium repens L. and Lolium perenne L. as monocultures and bi-species mixture to free air CO_2 enrichment and management. *Glob. Chang. Biol.* **1997**, *3*, 149–160. [CrossRef]
13. Li, B.; Li, Y.Y.; Wu, H.M.; Zhang, F.F.; Li, C.J.; Li, X.X.; Lambers, H.; Li, L. Root exudates drive interspecific facilitation by enhancing nodulation and N2 fixation. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 6496–6501. [CrossRef] [PubMed]
14. Issah, G.; Schoenau, J.J.; Lardner, H.A.; Knight, J.D. Nitrogen fixation and resource partitioning in alfalfa (*Medicago sativa* L.), cicer milkvetch (*Astragalus cicer* L.) and sainfoin (Onobrychis viciifolia Scop.) using 15N enrichment under controlled environment conditions. *Agronomy* **2020**, *10*, 1438. [CrossRef]
15. Crème, A.; Rumpel, C.; Gastal, F.; de la Luz Mora Gil, M.; Chabbi, A. Effects of grasses and a legume grown in monoculture or mixture on soil organic matter and phosphorus forms. *Plant Soil* **2016**, *402*, 117–128. [CrossRef]
16. Ledgard, S.F.; Steele, K.W. Biological nitrogen fixation in mixed legume/grass pastures. *Plant Soil* **1992**, *141*, 137–153. [CrossRef]
17. Verzeaux, J.; Hirel, B.; Dubois, F.; Lea, P.J.; Tétu, T. Agricultural practices to improve nitrogen use efficiency through the use of arbuscular mycorrhizae: Basic and agronomic aspects. *Plant Sci.* **2017**, *264*, 48–56. [CrossRef]
18. He, J.S.; Dong, S.; Shang, Z.; Sundqvist, M.K.; Wu, G.; Yang, Y. Above-belowground interactions in alpine ecosystems on the roof of the world. *Plant Soil* **2021**, *458*, 1–6. [CrossRef]
19. Berns, A.E.; Philipp, H.; Narres, H.D.; Burauel, P.; Vereecken, H.; Tappe, W. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. *Eur. J. Soil Sci.* **2008**, *59*, 540–550. [CrossRef]
20. De Long, J.R.; Kardol, P.; Sundqvist, M.K.; Veen, G.F.; Wardle, D.A. Plant growth response to direct and indirect temperature effects varies by vegetation type and elevation in a subarctic tundra. *Oikos* **2015**, *124*, 772–783. [CrossRef]
21. Gundale, M.J.; Kardol, P.; Nilsson, M.C.; Nilsson, U.; Lucas, R.W.; Wardle, D.A. Interactions with soil biota shift from negative to positive when a tree species is moved outside its native range. *New Phytol.* **2014**, *202*, 415–421. [CrossRef]
22. Zhang, S.; Huang, J.; Wang, Y.; Shen, Q.; Mu, L.; Liu, Z. Spatiotemporal heterogeneity of soil available nitrogen during crop growth stages on mollisol slopes of Northeast China. *Land Degrad. Dev.* **2017**, *28*, 856–869. [CrossRef]

23. BAO S, D. Soil and agricultural chemistry analysis. *Agric. Publ.* **2000**, 355–356.
24. Andrews, S.S.; Karlen, D.L.; Mitchell, J.P. A comparison of soil quality indexing methods for vegetable production systems in Northern California. *Agric. Ecosyst. Environ.* **2002**, *90*, 25–45. [CrossRef]
25. Andrews, S.S.; Mitchell, J.P.; Mancinelli, R.; Karlen, D.L.; Hartz, T.K.; Horwath, W.R.; Pettygrove, S.R.; Scow, K.M.; Munk, D.S. On-farm assessment of soil quality in California's Central Valley. *Agron. J.* **2002**, *94*, 12–23.
26. Hayat, R.; Ali, S.; Amara, U.; Khalid, R.; Ahmed, I. Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann. Microbiol.* **2010**, *60*, 579–598. [CrossRef]
27. Coban, O.; De Deyn, G.B.; van der Ploeg, M. Soil microbiota as game-changers in restoration of degraded lands. *Science* **2018**, *375*, abe0725. [CrossRef] [PubMed]
28. Zhou, X.; Zhang, J.; Pan, D.; Ge, X.; Jin, X.; Chen, S.; Wu, F. p-Coumaric can alter the composition of cucumber rhizosphere microbial communities and induce negative plant-microbial interactions. *Biol. Fertil. Soils.* **2018**, *54*, 363–372. [CrossRef]
29. Wang, G.; Koziol, L.; Foster, B.L.; Bever, J.D. Microbial mediators of plant community response to long-term N and P fertilization: Evidence of a role of plant responsiveness to mycorrhizal fungi. *Glob. Chang. Biol.* **2022**, *28*, 2721–2735. [CrossRef]
30. Lin, G.; McCormack, M.L.; Guo, D. Arbuscular mycorrhizal fungal effects on plant competition and community structure. *J. Ecol.* **2015**, *103*, 1224–1232. [CrossRef]
31. Liang, J.F.; An, J.; Gao, J.Q.; Zhang, X.Y.; Yu, F.H. Effects of arbuscular mycorrhizal fungi and soil nutrient addition on the growth of Phragmites australis under different drying-rewetting cycles. *PLoS ONE* **2018**, *13*, e0191999. [CrossRef]
32. Ren, Z.; Niu, D.; Ma, P.; Wang, Y.; Fu, H.; Elser, J.J. Cascading influences of grassland degradation on nutrient limitation in a high mountain lake and its inflow streams. *Ecology* **2019**, *100*, e02755. [CrossRef] [PubMed]
33. Song, Z.; Bi, Y.; Zhang, J.; Gong, Y.; Yang, H. Arbuscular mycorrhizal fungi promote the growth of plants in the mining associated clay. *Sci. Rep.* **2020**, *10*, 1–9. [CrossRef] [PubMed]
34. van de Weg, M.J.; Meir, P.; Grace, J.; Atkin, O.K. Altitudinal variation in leaf mass per unit area, leaf tissue density and foliar nitrogen and phosphorus content along an Amazon-Andes gradient in Peru. *Plant Ecol. Divers.* **2009**, *2*, 243–254. [CrossRef]
35. Conant, R.T.; Paustian, K.; Elliott, E.T. Grassland management and conversion into grassland: Effects on soil carbon. *Ecol. Appl.* **2001**, *11*, 343–355. [CrossRef]
36. Liu, Y.; Men, M.; Peng, Z.; Houx, J.H., III; Peng, Y. Nitrogen availability determines ecosystem productivity in response to climate warming. *Ecology* **2022**, *103*, e3823. [CrossRef]
37. Mortenson, M.C.; Ingram, L.J. Carbon sequestration in rangelands interseeded with yellow-flowering alfalfa (*Medicago sativa* ssp. falcata). *Environ. Manag.* **2004**, *33*, S475–S481. [CrossRef]
38. Rao, S.C.; Northup, B.K.; Phillips, W.A.; Mayeux, H.S. Interseeding novel cool-season annual legumes to improve bermudagrass paddocks. *Crop. Sci.* **2007**, *47*, 168–173. [CrossRef]
39. Ramasamy, K.; Joe, M.M.; Kim, K.Y.; Lee, S.M.; Shagol, C.; Rangasamy, A.; Chung, J.B.; Sa, T.M. Synergistic effects of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria for sustainable agricultural production. *Korean J. Soil Sci. Fertil.* **2011**, *44*, 637–649. [CrossRef]
40. Hodge, A.; Fitter, A.H. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13754–13759. [CrossRef]
41. Xu, H.; Shao, H.; Lu, Y. Arbuscular mycorrhiza fungi and related soil microbial activity drive carbon mineralization in the maize rhizosphere. *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109476. [CrossRef] [PubMed]
42. Chen, S.; Zhao, H.; Zou, C.; Li, Y.; Chen, Y.; Wang, Z.; Jiang, Y.; Liu, A.; Zhao, P.; Wang, M. Combined inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. *Front. Microbiol.* **2017**, *8*, 2516. [CrossRef] [PubMed]
43. Wang, C.T.; Wang, G.X.; Liu, W.; Wang, Y.; Hu, L.; Ma, L. Effects of establishing an artificial grassland on vegetation characteristics and soil quality in a degraded meadow. *Isr. J. Ecol. Evol.* **2013**, *59*, 141–153. [CrossRef]
44. Zak, D.R.; Grigal, D.F.; Gleeson, S.; Tilman, D. Carbon and nitrogen cycling during old-field succession: Constraints on plant and microbial biomass. *Biogeochemistry* **1990**, *11*, 111–129. [CrossRef]
45. Sun, D.S.; Wesche, K.; Chen, D.D.; Zhang, S.H.; Wu, G.L.; Du, G.Z.; Comerford, N.B. Grazing depresses soil carbon storage through changing plant biomass and composition in a Tibetan alpine meadow. *Plant Soil Environ.* **2011**, *57*, 271–278. [CrossRef]