

## Article

# Effects of Main Land-Use Types on Plant and Microbial Diversity and Ecosystem Multifunctionality in Degraded Alpine Grasslands

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**Abstract:** Grassland resources occupy an important place in the national economy. However, grasslands in alpine regions of China are severely degraded, and the effects of land-use types on species composition, soil nutrients, and ecosystem multifunctionality of degraded alpine grasslands are less certain. To ascertain the effects of main land-use types (no-tillage reseeding and fertilization) on species diversity and ecosystem multifunctionality in alpine grasslands, we investigated the changes in these factors by subjecting specified areas. Using a standardized field survey, we measured the cover, richness, and evenness of plants. At each site, we measured microbial diversity and twelve soil variables critical for maintaining ecosystem multifunctionality in alpine grasslands. The results showed that: (1) the Margalef, Shannon–Wiener, and Simpson indices of plant community, and fungal diversity indices increased significantly in no-tillage reseeding and fertilization; (2) at the phyla level, the relative abundances of Basidiomycota, Olpidiomycota, and Proteobacteria increased significantly in no-tillage reseeding and fertilization, as well as, at the genus level, those of Coniochaeta, Solirubrobacter, Pseudonocardia, and Microvirga; (3) the soil physicochemical properties (except the C:N of soil) increased significantly in no-tillage reseeding and fertilization; (4) correlation analysis showed that species diversity was mainly correlated with soil nutrients in control check, while it was mainly correlated with soil physical properties in no-tillage reseeding and fertilization; (5) linear regression analysis showed significant positive relationships between Margalef, Shannon–Wiener, and Simpson indices of plant community and ecosystem multifunctionality. In addition, ecosystem multifunctionality was positively related to Pielou, Shannon–Wiener, and Simpson indices of the fungal community and it was positively related to Pielou and Shannon–Wiener indices of bacterial community. These observations indicated that no-tillage reseeding and fertilization of degraded alpine grasslands had the potential to improve ecosystem functions in many ways.

**Keywords:** species diversity; plant community; microbial community; ecosystem functions



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## 1. Introduction

The grasslands in China cover about 400 million ha or 40.7% of the national territory, a crucial ecological barrier for terrestrial ecosystems [1], which play a central role in maintaining national ecological security and income generation for farmers and herders. For instance, the most widely distributed and largest terrestrial ecosystem in Qinghai is

that of alpine grassland, a pivotal and irreplaceable element in local ecological protection. However, climate change, overgrazing, soil erosion, rodent damage, and other phenomena have resulted in various degrees of degradation in alpine grasslands [2]. Such damages have been manifested by changes in soil characteristics, loss of biological diversity, and simplification of ecosystem structure that has significantly affected ecosystem functions, the survival of pastoralists, and development of the livestock industry. Therefore, these reasonable improvement measures in alpine grasslands are key to improving the ecological environment of grasslands and promoting the rational use of grasslands. Measures for managing degraded grasslands in China are mainly reseeded, fertilizing, fencing, and controlling rodent pests. Artificial application of organic fertilizer and no-tillage reseeded are simple measures that require little investment and have a quick impact. They have had obvious effects on increasing species composition and vegetation cover and improving the yield and quality of crops [3,4]. Some studies indicated that organic fertilization could significantly affect the nutrient element contents in soil and improve crop yield and nutrition value in alpine grasslands [5–7]. Ma et al. [8] conducted organic fertilizer in alpine pastures, which improved the rhizosphere nutrition conditions of crops as well as the content of soil organic matter, total phosphorus, total potassium, available phosphorus, and available potassium. Jin et al. [9] found that the presence of organic fertilizer enhanced soil physical and chemical properties, as well as improving beneficial bacterial and fungal abundance and diversity. Furthermore, Liu et al. [10] indicated that organic fertilizers could effectively improve crop yield, soil fertility, and the condition of nitrogen limitation in arid and semi-arid areas. No-tillage reseeded could prevent soil erosion and improve the function of grasslands as a carbon sink, shorten the natural recovery process, improve grassland productivity and biodiversity, and restore the multifunctionality and stability of the grassland ecosystem [11–13]. In China, no-tillage reseeded was first carried out in the 1980s [14] and then widely applied to grassland types such as arid and semi-arid grassland, temperate grassland, desert grassland, lowland meadow, and mountain meadow, etc. Research on no-tillage reseeded has mainly focused on studying the effects on grassland productivity, plant diversity, and vegetation community characteristics. For example, Duan et al. [15] selected five native grasses in alpine meadows on the Qinghai–Tibet Plateau for no-tillage reseeded, which improved community stability and production; Wu et al. [16] selected native species in the desert grassland of Ningxia for no-tillage reseeded, which increased the aboveground biomass of the grassland community and improved community stability; Sun et al. [17] used no-tillage reseeded of *Leymus chinensis* (Trin.) Tzvel. and *Medicago falcata* L. in Hulunbuir grassland to maintain the species diversity and richness of grassland ecosystem at a high level and increase the importance of Fabaceae and Poaceae in degraded grassland. In Qinghai, the main land-use types of degraded alpine grasslands are the artificial application of organic fertilizer based on no-tillage reseeded [18]. Duan et al. [19] also showed that the combination of no-tillage reseeded and organic fertilizer increased plant diversity and soil nutrient content more significantly than the experimental treatments alone. However, most of the studies only investigated the effect of no-tillage reseeded and organic fertilizer as separate experimental treatments but did not combine the two, and the relationships among plant, microorganisms, and ecosystem multifunctionality in degraded grasslands under no-tillage reseeded and fertilization are not clear. We hypothesized that no-tillage reseeded and fertilization had positive effects on plant diversity, microbial diversity, and ecosystem functions and species diversity indices were positively correlated with ecosystem multifunctionality in no-tillage reseeded and fertilization. Therefore, in this study, we selected the typical degraded alpine grasslands in Gangcha County, Qinghai Province, and conducted no-tillage reseeded and fertilization to investigate (a) the changes in plant communities, microbial communities, and ecosystem functions, and (b) the relationships between species diversity and ecosystem multifunctionality.

## 2. Materials and Methods

### 2.1. Study Site

The study area was located in Jiermeng Township, Gangcha County, Haibei Tibetan Autonomous Prefecture, Qinghai Province (99°28'53" E, 37°18'27" N, about 3400 m above sea level), at the junction of three counties in Haibei Gangcha County, Haixi Tianjun County, and Hainan Gonghe County. The climate type is plateau continental climate, with a long cold period, and short cool temperate period, no obvious four seasons, windy, and a big temperature difference between day and night. The annual average temperature is  $-0.6\sim 5.7\text{ }^{\circ}\text{C}$ , the annual average precipitation is 324.5–522.3 mm, the annual evaporation is 1500.6–1847.8 mm and the annual sunshine hours are 3 037 h, cold in winter, warm and cool in summer and autumn, and there is no absolute frost-free period. The natural grassland type is mainly alpine grassland. The soil type was subalpine grassland soil, with a thickness of 25–30 cm. The dominant species were *Stipa purpurea* Griseb, *Carex alataensis* S. R. Zhang, *Elymus breviaristatus* (Keng) Keng f., *Sibbaldianthe bifurca* (L.) Kurtto and T. Erikss, and *Knorringia sibirica* (Laxm.) Tzvelev.

### 2.2. Experimental Design

Based on the classification criteria of degraded grasslands specified by Ma et al. [20], light to moderately degraded grassland with vegetation cover of 30–50%, slope less than  $25^{\circ}$ , thick soil layer, and obvious degradation characteristics were selected for the study. The field plots were set up for two treatments: no-tillage reseeding and fertilization and control check without any addition. Each treatment had three replicates (5 ha each), and a total of six plots. No-tillage reseeding methods were as follows: (1) no-tillage seeder and high-horsepower power machine were used for operation in late April 2021; (2) the seeding method was cross-seeding and the operation was carried out twice; (3) the seeds were selected from a mixture of *Elymus breviaristatus* (Keng) Keng f. and *Poa araratica* Trautv. in a ratio of 3:1, which were native grasses in Qinghai Province; (4) the total seeding amount was  $60\text{ kg}\cdot\text{ha}^{-1}$  and the seeding depth was 0.5–2 cm. Fertilizer type was organic fertilizer (organic matter  $\geq 45\%$ , total nutrients (N + P<sub>2</sub>O<sub>5</sub> + K<sub>2</sub>O)  $\geq 5\%$ , water  $\leq 30\%$ ):  $922.5\text{ kg}\cdot\text{ha}^{-1}$  [18].

### 2.3. Sampling and Computing Method

#### 2.3.1. Plant Diversity

In August 2021, four plant quadrats of  $0.5\text{ m} \times 0.5\text{ m}$  were randomly placed in each treatment for a survey, and the name, coverage, abundance, and height of plant species in the quadrats were recorded. Each index was investigated as follows: coverage was obtained by visual estimation to obtain total community coverage and species coverage; abundance was obtained by investigating the number of plants; natural height was measured by randomly selecting five plants of the same species (if there were less than 5 plants, all of them were measured); plant diversity was expressed using Margalef ( $H$ ), Shannon–Wiener ( $H'$ ), Simpson ( $D$ ), and Pielou evenness ( $P$ ) [21]:

$$H = \frac{S - 1}{\ln N} \quad (1)$$

$$H' = - \sum_{i=1}^S P_i \ln P_i \quad (2)$$

$$D = 1 - \sum_{i=1}^S P_i^2 \quad (3)$$

$$P = \frac{H'}{\ln S} \quad (4)$$

where  $S$  is the number of species,  $N$  is the total number of plant individuals in the quadrat, and  $P_i$  is the importance value of species  $i$ . The importance value of species = (relative coverage + relative height + relative abundance)/3.

### 2.3.2. Microbial Diversity

After mowing all plants in the quadrats, we used a soil auger (3 cm inner diameter) to collect 0–10 cm of soil by taking three drills along the diagonal. Three replicates were collected from each quadrat, mixed to form a composite soil sample, and stored at  $-80\text{ }^{\circ}\text{C}$  for DNA extraction. DNA was extracted using a DNA kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. The DNA was determined on the platform of Shanghai Paisano Biological Co. for high throughput sequencing, and the DNA concentration and quality were detected using 0.8% agarose gel electrophoresis. For the bacterial community, 338F/806R primer sets (338F: ACTCCTACGGGAGGCAGCA; 806R: GGACTACHVGGGTWTCTAAT) were used to amplify (triplicate reactions for each sample) the 16S V3–V4 region. For the fungal community, the ITS5f/ITS2 primer pair (ITS5F: GGAAGTAAAAGTCGTAACAAGG; ITS2R: GCTGCGTTCTTCATCGATGC) was selected to amplify the ITS\_V1 region of the rRNA gene. The amplification conditions were pre-denaturation at  $95\text{ }^{\circ}\text{C}$  for 5 min, denaturation at  $95\text{ }^{\circ}\text{C}$  for 15 s, and annealing at  $60\text{ }^{\circ}\text{C}$  for 30 s, for a total of 40 cycles [22]. The 16S rRNA amplicons and ITS amplicons were pooled separately and then sequenced with the Illumina MiSeq instrument. The sequence data were filtered, chimera checked and operational taxonomic unit (OTU) clustered, and taxonomy assignment was performed by QIIME (v1.9.0) pipeline. The sequence information of each treatment was categorized according to barcodes and clustered into OTUs for species classification, and the OTU similarity was set to 97%. Taxonomy was identified for each OTU using the Ribosomal Database Project Classifier trained on the Greengenes and UNiversal Intracellular Targeted Expression databases for bacterial and fungal sequences [23].

### 2.3.3. Selection of Ecosystem Functions

In recent studies on ecosystem multifunctionality, the selection of evaluation indicators mostly refers to Maestre et al. [24]. Since soil–vegetation was an interdependent and reciprocal feedback complex, and soil was the nutrient and seed bank of the ecosystem, soil indicators can largely reflect multiple functions of the ecosystem. The indicators related to soil fertility maintenance are OC, TN, TP, AN, AP, AMN, and NN; indicators related to water conservation are SWC and CP. The indicators related to nutrient transformation and cycling are pH, C:N, and BP [25]. These indicators are simple and easy to measure in production practices and relate to various aspects of soil water, fertilizer, gas, heat, and multiple ecosystem functions [26].

### 2.3.4. Measurement of Ecosystem Functions

We used a soil auger (5 cm inner diameter) to collect 0–10 cm of soil by taking three drills along the diagonal. Three replicates were collected from each quadrat, mixed to form a composite soil sample, stones and plant roots were removed, and then returned to the laboratory for determination of the soil's physicochemical properties. Soil organic carbon ( $\text{g}\cdot\text{kg}^{-1}$ , OC), total nitrogen ( $\text{g}\cdot\text{kg}^{-1}$ , TN), total phosphorus ( $\text{g}\cdot\text{kg}^{-1}$ , TP), available nitrogen ( $\text{mg}\cdot\text{kg}^{-1}$ , AN), available phosphorus ( $\text{mg}\cdot\text{kg}^{-1}$ , AP), ammonia nitrogen ( $\text{mg}\cdot\text{kg}^{-1}$ , AMN), nitric nitrogen ( $\text{mg}\cdot\text{kg}^{-1}$ , NN), soil water content (%), SWC), capillary porosity (%), CP), pH, C:N, and bulk porosity (%), BP) were measured using standard methods [27].

### 2.3.5. Assessing Ecosystem Multifunctionality

Before calculating the ecosystem multifunctionality, we standardized the ecosystem functions to be between 0 and 1 with min–max normalization and then used the average method to calculate the ecosystem multifunctionality [28,29]. The calculation formula is as follows:

$$F(x_i) = \frac{x_i - \min}{\max - \min} \quad (5)$$

where  $x_i$  represents the original value of ecosystem function  $i$ , max is the maximum value of ecosystem function  $i$ , and min is the minimum value of ecosystem function  $i$ .

$$EMF = \frac{\sum_{i=1}^n F(x_i)}{n} \quad (6)$$

where  $F(x_i)$  represents the standardized value of ecosystem function  $i$ ,  $n$  is the number of ecosystem functions.

### 2.3.6. Statistical Analysis

The Kolmogorov–Smirnov test and Shapiro–Wilk test were used to test whether the data conformed to the normal distribution, and for the data that obeyed the normal distribution, the independent samples T test was used to compare the differences between treatments, and for the data that did not obey the normal distribution, the nonparametric Mann–Whitney test was used, with the significance level set at  $p < 0.05$ . Correlation analysis was performed between plant and microbial diversity and ecosystem functions. We also analyzed the relationship between species diversity and ecosystem multifunctionality using linear regression. All analyses were performed in the software SPSS 25.0. The visualization of the data was performed using Origin 2021.

## 3. Results

### 3.1. Effects of Main Land-Use Types on Plant Diversity, Microbial Diversity, and Soil Microbial Community Structure

Plant diversity, microbial diversity, and soil microbial community structure varied widely between treatments. Compared with control check, the Margalef, Shannon–Wiener, and Simpson indices of plant community increased significantly in no-tillage reseeding and fertilization ( $p < 0.05$ ). However, there were no significant differences in the Pielou index of plant community between treatments ( $p > 0.05$ , Table 1). The results showed that the coverage of soil microbial community ranged from 99.06 to 99.98%, indicating that the sequencing results represent the true picture of the microorganisms in samples. The fungal diversity indices in no-tillage reseeding and fertilization were significantly higher than those in control check ( $p < 0.05$ ), but the changes in bacterial diversity indices in different treatments were not significant ( $p > 0.05$ , Table 2). In no-tillage reseeding and fertilization, the Margalef index of plant community showed a positive correlation with the Shannon–Wiener index of the bacterial community ( $p < 0.05$ ). In the control check, the Margalef, Shannon–Wiener, and Simpson indices of the plant community were positively correlated with the Pielou, Shannon–Wiener, and Simpson indices of the microbial community ( $p < 0.05$ ) (Table 3).

**Table 1.** Changes in plant diversity under main land-use types (mean  $\pm$  SD).

Treatment	Index			
	Margalef	Shannon–Wiener	Simpson	Pielou
NF	1.34 $\pm$ 0.17 <sup>a</sup>	1.81 $\pm$ 0.18 <sup>a</sup>	0.80 $\pm$ 0.06 <sup>a</sup>	0.88 $\pm$ 0.05 <sup>a</sup>
CK	0.67 $\pm$ 0.22 <sup>b</sup>	1.16 $\pm$ 0.25 <sup>b</sup>	0.65 $\pm$ 0.10 <sup>b</sup>	0.88 $\pm$ 0.07 <sup>a</sup>

Notes: NF: no-tillage reseeding and fertilization. CK: control check. Different lowercase letters within the same column represent significant differences between different treatments ( $p < 0.05$ ).

Most of fungal sequences belonged to the phyla Ascomycota, Basidiomycota, and Mortierellomycota. At the fungal phyla level, the relative abundance of Ascomycota decreased significantly in no-tillage reseeding and fertilization ( $p < 0.05$ ). In addition, the abundance of Kickxellomycota and Zoopagomycota presented in CK and did not present in no-tillage reseeding and fertilization (Figure 1A). At the genus level, the dominant fungi included Archaeorhizomyces, Mortierella, Acremonium, Didymella, and Pseudogymnoascus. An abundance of Gibberella, Alternaria, Coniochaeta, Ilyonectria, Cephalotrichum, Didymella, and Tricharina presented in the control check, whereas Dermoloma presented

in no-tillage reseeding and fertilization (Figure 1B). Moreover, the relative abundance of Coniochaeta increased significantly in no-tillage reseeding and fertilization ( $p < 0.05$ ). Most of bacterial sequences belonged to the phyla Actinobacteria, Proteobacteria, and Acidobacteria. The relative abundance of Proteobacteria increased significantly in no-tillage reseeding and fertilization ( $p < 0.05$ ). Moreover, the relative abundance of Chloroflexi increased significantly in control check (Figure 1C). At the genus level, the dominant bacteria differed significantly in treatments. For example, the relative abundance of Solirubrobacter, Pseudonocardia, and Microvirga increased significantly, whereas KD4-96 decreased significantly in no-tillage reseeding and fertilization ( $p < 0.05$ ) (Figure 1D).

**Table 2.** Changes in microbial diversity under main land-use types (mean  $\pm$  SD).

Category	Treatment	Index				
		Chao1	Pielou	Shannon–Wiener	Simpson	Coverage/%
Fungi	NF	460.22 $\pm$ 29.56 <sup>a</sup>	0.69 $\pm$ 0.03 <sup>a</sup>	6.08 $\pm$ 0.18 <sup>a</sup>	0.95 $\pm$ 0.01 <sup>a</sup>	99.98
	CK	351.14 $\pm$ 60.73 <sup>b</sup>	0.64 $\pm$ 0.07 <sup>b</sup>	5.33 $\pm$ 0.51 <sup>b</sup>	0.93 $\pm$ 0.02 <sup>b</sup>	99.97
Bacteria	NF	4299.04 $\pm$ 431.27 <sup>a</sup>	0.90 $\pm$ 0.01 <sup>a</sup>	10.76 $\pm$ 0.04 <sup>a</sup>	1.00 $\pm$ 0.00 <sup>a</sup>	99.18
	CK	3986.51 $\pm$ 550.43 <sup>a</sup>	0.89 $\pm$ 0.01 <sup>a</sup>	10.61 $\pm$ 0.25 <sup>a</sup>	0.99 $\pm$ 0.00 <sup>a</sup>	99.06

Note: different lowercase letters within the same column represent significant differences between NF and CK ( $p < 0.05$ ).

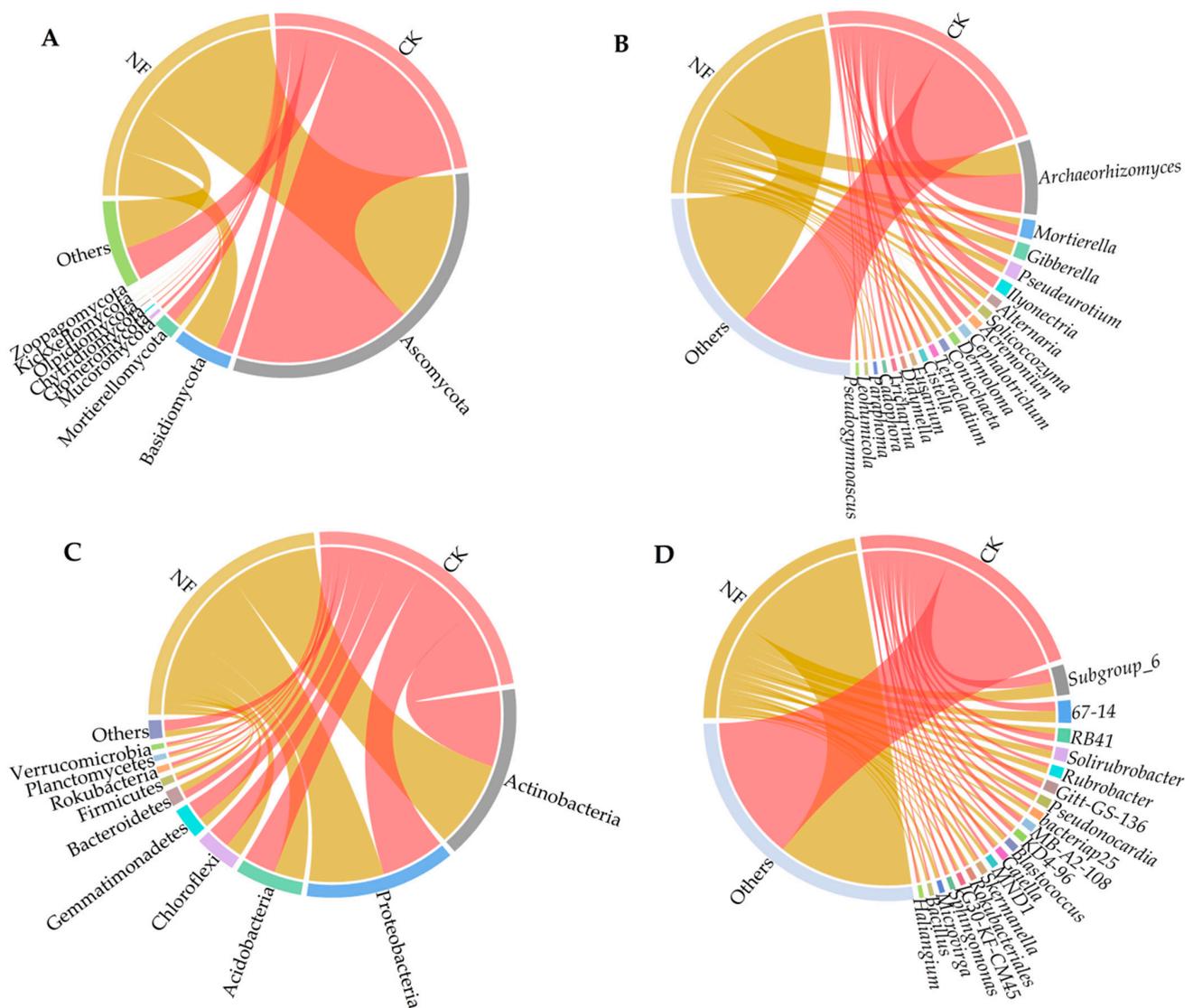
**Table 3.** Correlation analysis of plant diversity with microbial diversity under main land-use types.

Treatment	Index	C(F)	P(F)	H'(F)	D(F)	C(B)	P(B)	H'(B)	D(B)
NF	Margalef	−0.33	0.43	0.43	0.40	−0.28	0.43	0.633 *	0.31
	Shannon–Wiener	−0.13	0.27	0.30	0.18	−0.08	0.24	0.45	−0.03
	Simpson	−0.03	0.18	0.21	0.05	0.02	0.13	0.33	−0.16
	Pielou	−0.16	0.13	0.12	0.11	−0.17	0.20	0.23	0.10
CK	Margalef	−0.43	0.68 *	0.62 *	0.72 **	0.79 **	0.81 **	0.81 **	0.76 **
	Shannon–Wiener	−0.51	0.78 **	0.71 **	0.84 **	0.85 **	0.80 **	0.85 **	0.72 **
	Simpson	−0.54	0.74 **	0.65 *	0.81 **	0.72 **	0.67 *	0.72 **	0.58 *
	Pielou	0.05	0.32	0.37	0.27	0.14	0.08	0.13	0.15

Notes: \*  $p < 0.05$ , \*\*  $p < 0.01$ . C(F), P(F), H'(F), D(F), C(B), P(B), H'(B), and D(B): Chao1 index of fungal community, Pielou index of fungal community, Shannon–Wiener index of fungal community, Simpson index of fungal community, Chao1 index of the bacterial community, Pielou index of the bacterial community, Shannon–Wiener index of the bacterial community, Simpson index of the bacterial community, respectively.

### 3.2. Effects of Main Land-Use Types on Ecosystem Functions and Multifunctionality

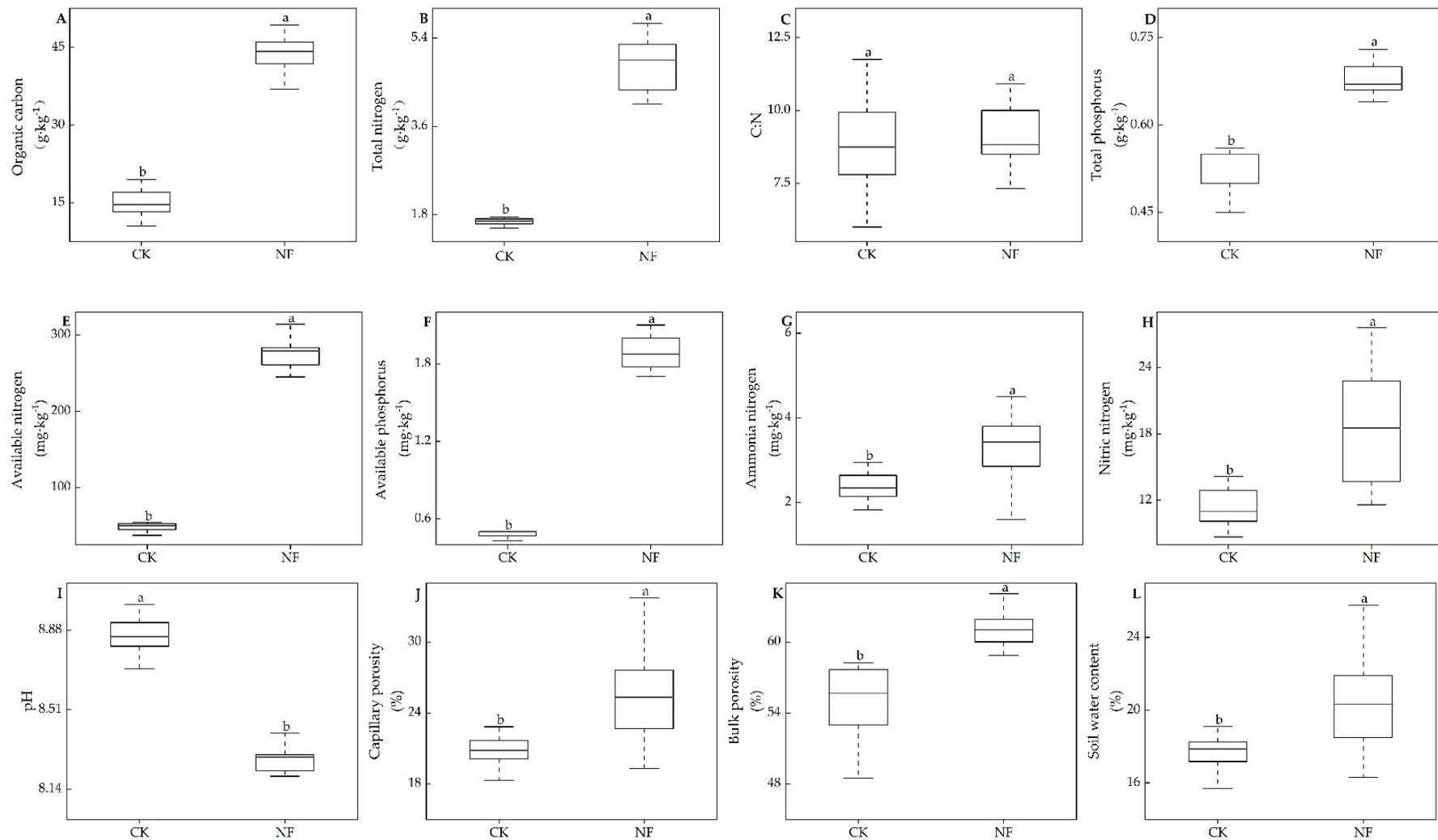
Twelve ecosystem functions were examined in this study. The no-tillage reseeding and fertilization had significant positive effects on ecosystem functions related to soil fertility maintenance, i.e., OC, TN, TP, AN, AP, AMN, and NN. In no-tillage reseeding and fertilization, the indices of soil fertility maintenance increased by 82.25%, 73.79%, 66.01%, 65.15%, 39.21%, 28.96%, and 22.58%, respectively ( $p < 0.05$ ) (Figure 2A,B,D–H). Compared with the control check, the indices of water conservation increased in no-tillage reseeding and fertilization, i.e., CP and SWC increased by 22.70% and 15.44%, respectively ( $p < 0.05$ ) (Figure 2J,L). The pH and BP related to nutrient transformation and cycling decreased by 6.67% and increased by 11.03%, respectively ( $p < 0.05$ ) (Figure 2I,K). In addition, the differences between treatments in the three main functions of soil fertility maintenance, water conservation, and nutrient transformation and cycling were not significant. Meanwhile, ecosystem multifunctionality showed a nonsignificant response in no-tillage reseeding and fertilization ( $p > 0.05$ ) (Figure 3A–D).



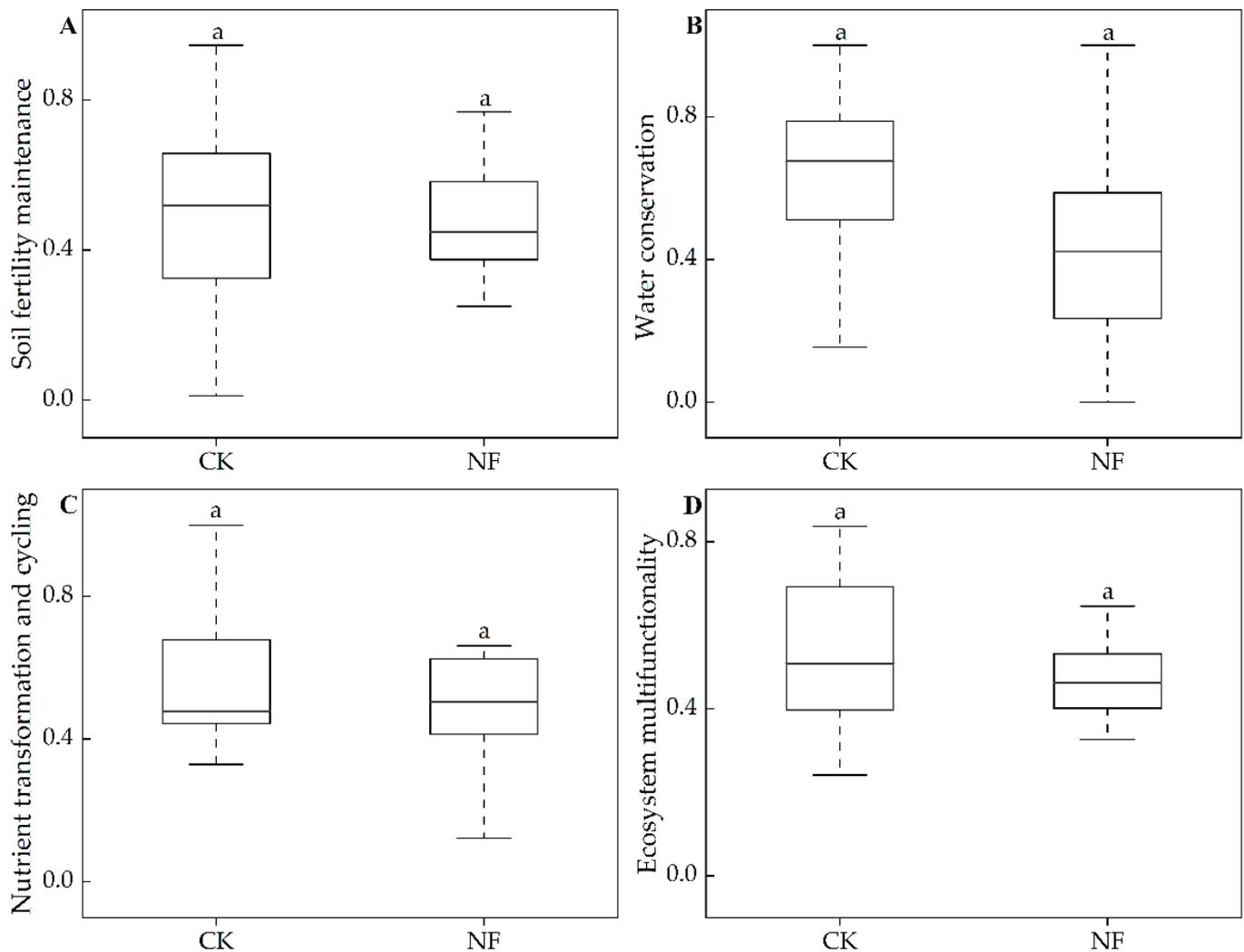
**Figure 1.** The distribution of abundant taxa with relative abundances > 1% in at least one sample. (A), Soil fungal community at phylum level; (B), Soil fungal community at genus level; (C), soil bacterial community at phylum level; (D), soil bacterial community at the genus level. NF: no-tillage reseeding and fertilization. CK: control check.

### 3.3. Correlation Analysis of Plant and Microbial Diversity and Ecosystem Functions

We also analyzed the relationships between plant and microbial diversity indices and ecosystem functions. The Pearson correlation heatmap showed significant positive relationships between plant and microbial diversity indices and organic carbon, ammonia nitrogen, and nitric nitrogen in control check (Figure 4A,C). The Simpson and Pielou indices of the bacterial community were positively related to pH, and the Chao1 index of the bacterial community was positively related to CP in the control check (Figure 4C). In addition, Shannon–Wiener, Margalef, and Pielou indices of plant community were positively related to CP and SWC in no-tillage reseeding and fertilization. The Shannon–Wiener and Margalef indices of plant community were negatively related to pH in no-tillage reseeding and fertilization (Figure 4B). The microbial diversity indices were positively related to OC in no-tillage reseeding and fertilization. Meanwhile, Shannon–Wiener and Simpson indices of the bacterial community were negatively related to BP in no-tillage reseeding and fertilization (Figure 4D).



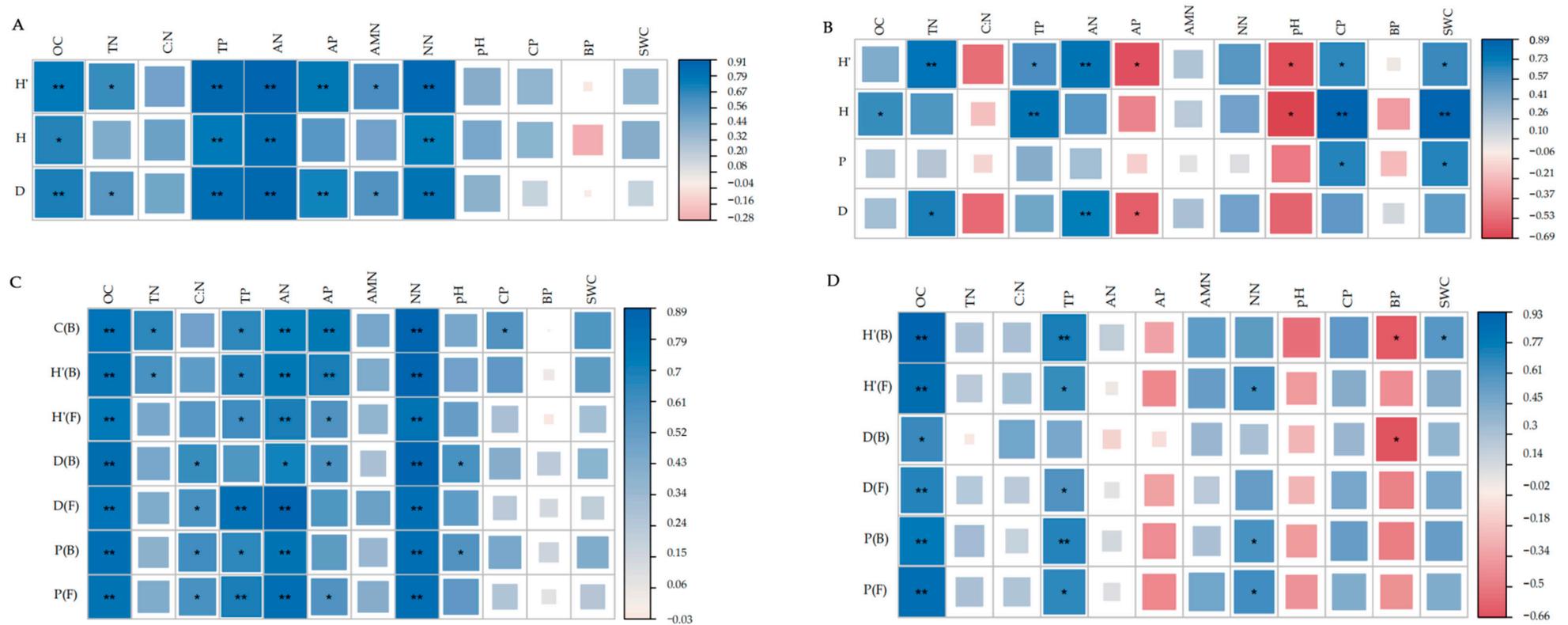
**Figure 2.** Effects of main land-use types on single ecosystem functions. (A), organic carbon; (B), total nitrogen; (C), C:N; (D), total phosphorus; (E) available nitrogen; (F), available phosphorus; (G), ammonia nitrogen; (H), nitric nitrogen; (I), pH; (J), capillary porosity; (K) bulk porosity; (L), soil water content. a/b: Different lowercase letters represent significant differences between NF and CK ( $p < 0.05$ ).



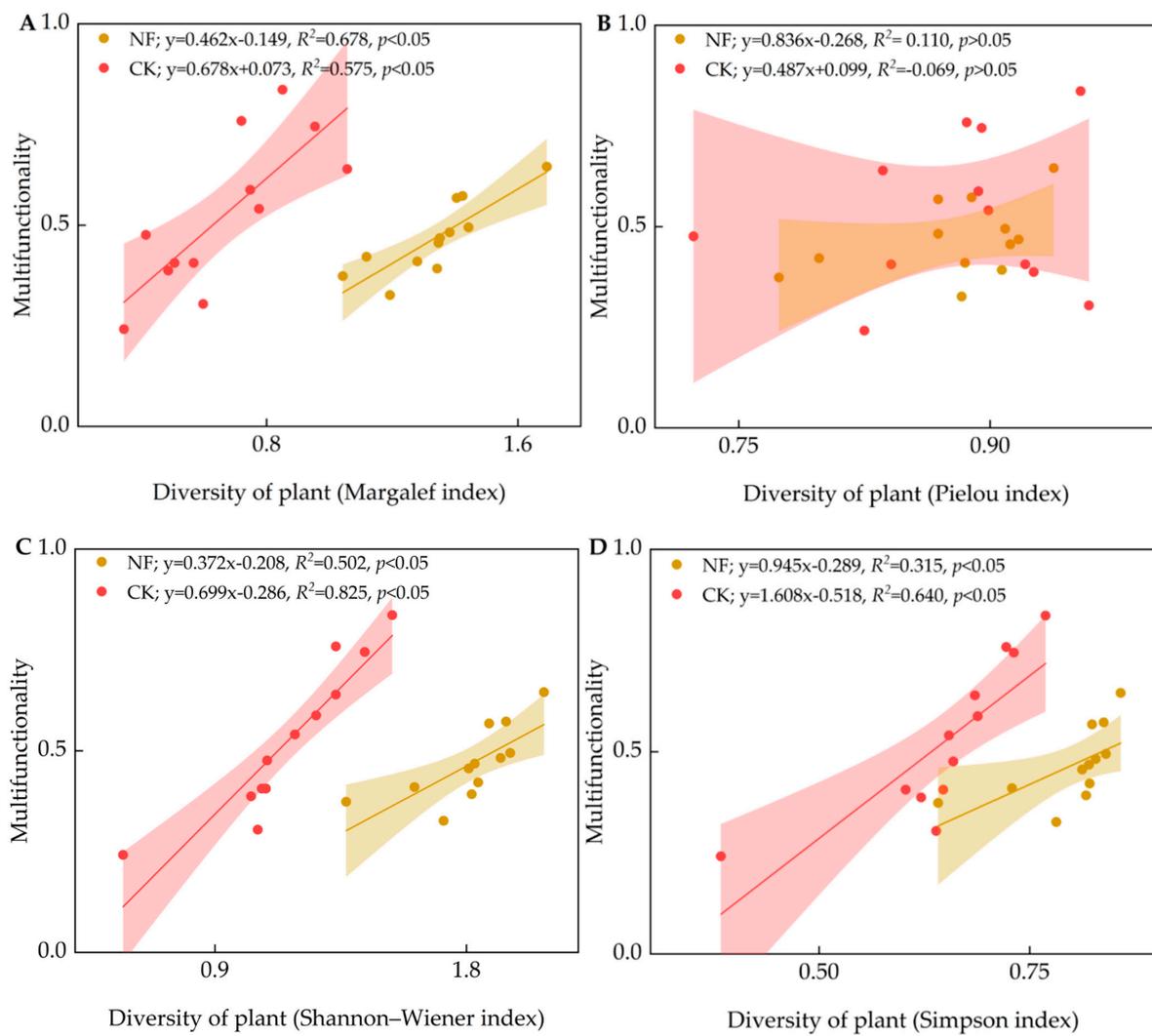
**Figure 3.** Effects of main land-use types on ecosystem functions. (A), soil fertility maintenance; (B), water conservation; (C), nutrient transformation and cycling; (D), ecosystem multifunctionality. a: Same lowercase letters represent nonsignificant differences between NF and CK ( $p > 0.05$ ).

### 3.4. Relationship of Plant and Microbial Diversity with Ecosystem Multifunctionality

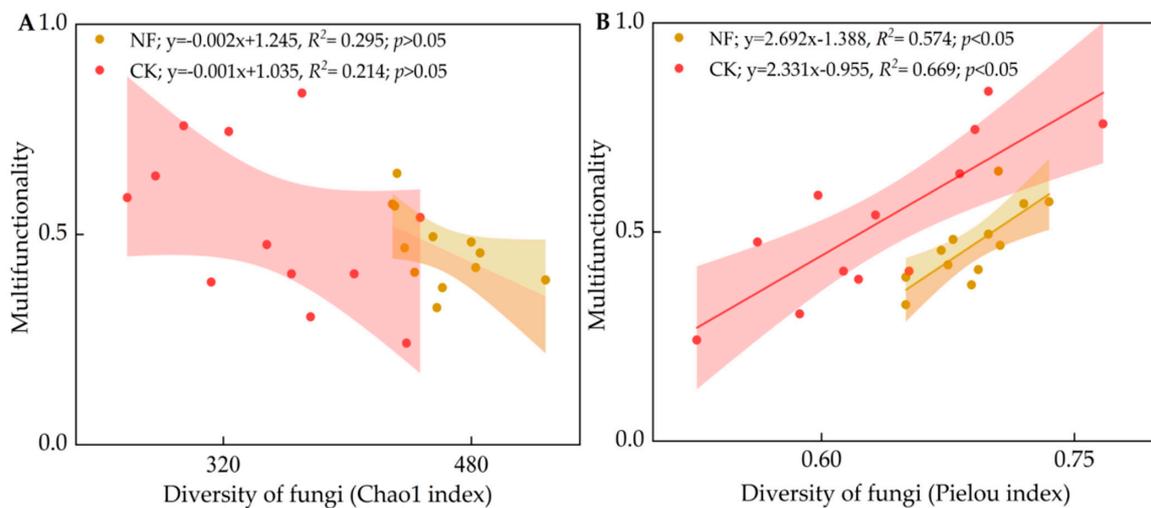
Possible relationships between plant diversity, microbial diversity, and ecosystem multifunctionality were further uncovered based on linear regression analysis (Figures 5 and 6). The linear regression analysis showed significant positive relationships between Margalef, Shannon–Wiener, and Simpson indices of plant community and ecosystem multifunctionality ( $p < 0.05$ ) (Figure 5A,C,D). Overall, ecosystem multifunctionality varied with plant diversity. When evaluating the relationship between microbial diversity and ecosystem multifunctionality, we observed ecosystem multifunctionality was positively related to Pielou, Shannon–Wiener, and Simpson indices of the fungal community ( $p < 0.05$ ) (Figure 6B–D). In addition, ecosystem multifunctionality was positively related to Pielou and Shannon–Wiener indices of bacterial community ( $p < 0.05$ ). The Chao1 and Simpson indices of the bacterial community had significant positive relationships with ecosystem multifunctionality in CK ( $p < 0.05$ ).



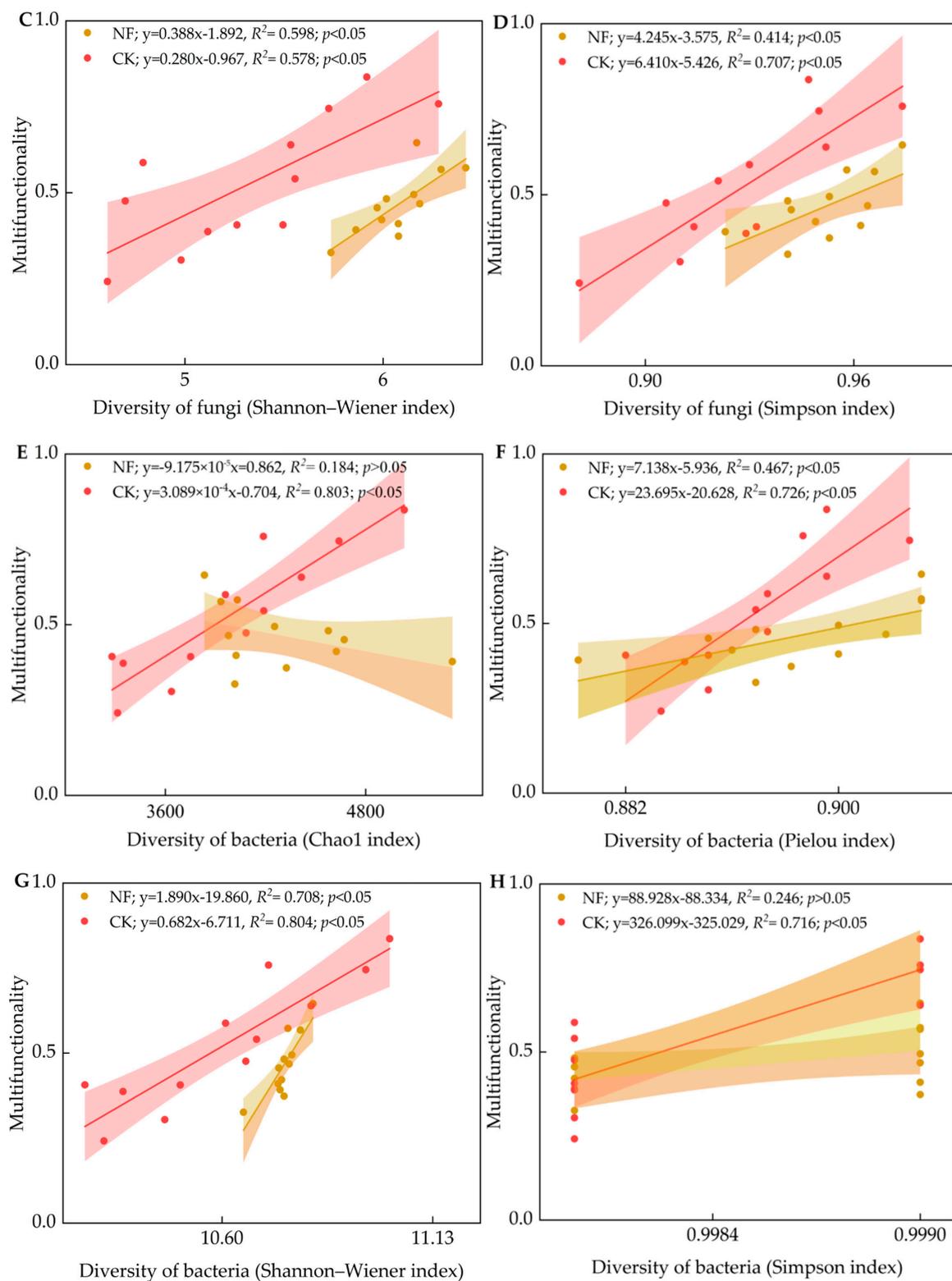
**Figure 4.** Correlation analysis between species diversity indices and single ecosystem functions in NF and CK. \*  $p < 0.05$ , \*\*  $p < 0.01$ . (A), correlation analysis between plant diversity indices and single ecosystem functions in CK; (B), correlation analysis between plant diversity indices and single ecosystem functions in NF; (C), correlation analysis between bacterial diversity indices and single ecosystem functions in CK; (D), correlation analysis between bacterial diversity indices and single ecosystem functions in NF. H, H, D, and P: Margalef index of the plant community, Shannon–Wiener index of the plant community, Simpson index of the plant community, and Pielou index of the plant community. C(F), P(F), H'(F), D(F), C(B), P(B), H'(B), and D(B): the Chao1 index of fungal community, Pielou index of fungal community, Shannon–Wiener index of fungal community, Simpson index of fungal community, Chao1 index of the bacterial community, Pielou index of the bacterial community, Shannon–Wiener index of the bacterial community, Simpson index of the bacterial community. OC, TN, C:N, TP, AN, AP, AMN, NN, pH, CP, BP, and SWC: organic carbon, total nitrogen, C:N, total phosphorus, available nitrogen, available phosphorus, ammonia nitrogen, nitric nitrogen, pH, capillary porosity, bulk porosity, and soil water content.



**Figure 5.** The relationship between plant diversity and ecosystem multifunctionality. Yellow and red colored dots indicate NF and CK, respectively. The translucent areas indicate 95% confidence intervals. (A), Margalef index of plant diversity; (B), Pielou index of plant diversity; (C), Shannon–Wiener index of plant diversity; (D), Simpson index of plant diversity.



**Figure 6.** Cont.



**Figure 6.** The relationship between microbial diversity and ecosystem multifunctionality. Yellow and red colored dots indicate NF and CK, respectively. The translucent areas indicate 95% confidence intervals. (A), Chao1 index of fungal diversity; (B), Pielou index of fungal diversity; (C), Shannon–Wiener index of fungal diversity; (D), Simpson index of fungal diversity; (E), Chao1 index of bacteria diversity; (F), Pielou index of bacteria diversity; (G), Shannon–Wiener index of bacteria diversity; (H), Simpson index of bacteria diversity.

## 4. Discussion

### 4.1. Response of Plant Diversity, Microbial Diversity to No-Tillage Reseeding and Fertilization

How plant and microbial community diversity changes during no-tillage reseeded and fertilization is critical to maintaining sustainable ecosystem development. Our results showed that plant diversity increased significantly in no-tillage reseeded and fertilization. Consistent with other studies [30,31], the increase in Margalef index was mainly because no-tillage reseeded added new grass species, broke the ecological niche of original species, and changed the spatial pattern of plant community, increasing the number of species and making the community composition relatively complex. The Shannon–Wiener and Simpson indices of the plant community increased in no-tillage reseeded and fertilization, which could be explained by the reseeded plants. In detail, the reseeded plants were more adaptable and grew well because they were native grasses in Qinghai Province [32]. In addition, Chen et al. [33] showed that fertilizer application can increase the soil nutrient content in grassland, thus promoting the rhizosphere environment and improving the survival rate and diversity of crops [34].

Microbes are the main decomposers of organic matter and play an important role in the ecosystem nutrient cycle. Previous studies have reported that bacteria are the main component of the soil microbial community in artificial grassland [35,36], which was consistent with our results. The fungal diversity indices were higher in no-tillage reseeded and fertilization than in the control check. This could be because soil microbial community structure was affected by restoration of alpine grasslands, and the fungal community was more sensitive to grassland succession than the bacterial community [37]. Moreover, plant diversity had significant positive correlations with most of the microbial diversity indices in our study. The growth of plants might promote the accumulation of litter and sufficient nutrients in the soil, which provides more nutrient resources to soil microbes [38,39].

### 4.2. Response of Soil Microbial Community Composition to No-Tillage Reseeding and Fertilization

Fungal communities were overwhelmingly dominated by Ascomycota and Basidiomycota. Ascomycota and Basidiomycota were the predominant fungal phylum, playing an important role in the lignin decomposition process and nutrient cycles [40]. The finding was consistent with Rong et al. [41]. The relative abundance of Ascomycota increased in the control check. This observation could be explained by the Ascomycota's physiological ability to break down the biochemical structure of plant litter in the degraded alpine steppes [42]. Moreover, Kickxellomycota and Zoopagomycota only existed in control check, which might be due to the soil available nutrients and water content changed in no-tillage reseeded and fertilization, resulting in a change in the soil pH; changes in these soil indices might reduce the importance of certain fungal community in the nutrient uptake process of the plant, resulting in plant response to fungal changes in community composition [43,44]. The change in Proteobacteria may be because the bacteria involved in the nitrogen cycle were increased categories would vary depending on the habitat, and their relative abundance might also have a relationship with the surrounding environmental conditions [45]. However, the denitrification dominated by Chloroflexi likely caused the loss of nitrogen nutrients in the soil and accelerated grassland degradation; therefore, Chloroflexi was more abundant in degraded grassland [46]. Meanwhile, the relative abundance of Solirubrobacter, Pseudonocardia, Microvirga, and KD4-96 in Actinobacteria varied greatly between different treatments. These dominant bacteria are Gram-positive bacteria that have good adaptability to soil properties and vegetation changes in no-tillage reseeded and fertilization.

### 4.3. No-Tillage Reseeding and Fertilization Impacts on Ecosystem Functions and Multifunctionality

Soil, as the basis for a plant's survival, affects the composition of the plant community and the structure and function of the ecosystem [47]. Our study showed that the application of organic fertilizer was beneficial to increasing the OC content and promoting

plant growth, which was consistent with the results of other studies [19,48]. Meanwhile, the dense vegetation growth and high plant diversity developed in the alpine grassland after no-tillage reseeding could reduce the rate of soil erosion caused by wind and water while increasing the sequestration of carbon [49]. Wang et al. [50] found that fertilization increased the content of plant roots and root exudates and increased the TN content in soil, which is consistent with our findings. The study areas of control check were degraded, and the above-ground vegetation coverage and biomass were decreased, resulting in the protective effect on soil particles also diminishing. Consequently, the impact of wind erosion gradually rose, which led to a reduction in the TP content [51]. Our results indicated moderate jamming facilitated the compensatory growth of plants, which accelerated the mineralization rate of carbon, nitrogen, and phosphorus in the soil, thus increasing AP and AN. This implication agrees with the conclusions made by Shao et al. [52]. The indices for the SWC and CP were increased in no-tillage reseeding and fertilization, which were consistent with the data obtained by Ji et al. [53] because no-tillage reseeding disturbed the surface of the soil, resulting in reduced soil compactness and enhanced capillary porosity. Huo et al. [54] found, in the study of alkali-saline in Jingyuan County, Gansu Province, that adding organic fertilizer could significantly improve soil physical properties, reduced soil bulk density, and increased soil total porosity. At the same time, plant diversity rose after reseeding, reducing the area of exposed soil as well as causing the loss of surface soil water from evaporation. Moreover, pH and BP, which are related to nutrient transformation and cycling, differed between treatments. Notably, pH decreased during no-tillage reseeding, which is consistent with the results recorded by Li et al. [55] regarding the influence of global conservation tillage on pH. Hong et al. [56] also found that the soil pH treated with organic fertilizer showed a downward trend. BP in no-tillage reseeding and fertilization areas was higher, which was beneficial to the growth of plant roots. This study's findings showed there were no differences in soil fertility, water content, nutrient conversion, and ecosystem multifunctionality. The calculation method used here may weaken the effect of no-tillage reseeding and fertilization on soil single functions, resulting in nonsignificant changes to the ecosystem [57].

#### *4.4. The Roles of Plant and Microbial Diversity in Mediating Ecosystem Functions and Multifunctionality*

Some surveys have posited that ecosystem functions expand with increasing species diversity [58]. From our study, it was concluded the ecosystem functions of soil water, fertilizer, gas, and heat had different correlations with species diversity depending on treatment. There was a strong correlation between plant diversity in the control check and functional indices related to soil fertility, but not with those related to either water conservation or nutrient transformation and cycling. This might be because plant communities with higher species diversity tend to also have higher productivity and increase the input of rhizosphere microbial carbon sources [59]. By contrast, plant diversity in no-tillage reseeding and fertilization areas was positively correlated with pH, CP, and SWC. This meant plant diversity in degraded alpine grasslands was easily restricted by soil water and nutrient functional indices, resulting in limited plant growth and development, species settlement, and colonization [24,60]. Published work has observed that not only is microbial diversity related to community productivity and stability, but also to soil functions [61,62]. Our analysis led to the conclusion that microbial diversity is closely interrelated with the physical and chemical properties of soil, which was consistent with conclusions made in other studies [63–65]. Experimental data showed soil functions played a role in affecting the microbial community related to soil fertility, which agrees with the work of Luo et al. [66], who reported microbial participation in multiple ecosystem functions when influenced by global changes.

Except for the direct effect on ecosystem functions, no-tillage reseeding and fertilization also mediate ecosystem multifunctionality by affecting plant and microbial diversity. In this study, the plant diversity indices were positively correlated with ecosystem multifunc-

tionality in no-tillage reseeding and fertilization. It was necessary to pay attention to plant diversity because the positive correlation between this property and multifunctionality was also confirmed in temperate grassland, microbial, and aquatic communities [67]. Ecosystem multifunctionality of the alpine grassland ecosystem was predominantly affected by the abundance and uniformity of the plant community after reseeding and fertilization. This might have been caused by the community with more species possessing different ecological niches when using limited resources, and the uniform distribution of species could make the complementary use of resources more reasonable, thus enhancing ecosystem multifunctionality [61]. However, when microbial diversity indices were considered, the Shannon–Wiener and Pielou indices of the microbial community were positively correlated with ecosystem multifunctionality, which was consistent with other studies [28,68]. This could be attributed to the fact that richer microbial diversity promoted the decomposition rate of waste material, increased soil organic matter content and microbial activity, and accelerated organic decomposition and nutrient accumulation [69]. The Pielou index is often neglected, but in our study, the Pielou index was considered to be critical for maintaining ecosystem multifunctionality, and microbes contributed to the improvement of soil physicochemical properties through the rational use of spatial distribution patterns and changing the distribution of resources. Meanwhile, the Simpson index of fungal community and Chao1 index of bacterial community were both positively correlated with ecosystem multifunctionality in the control treatment. This relationship implies the number of dominant species in the fungal and bacterial communities in alpine degraded grasslands is a key limiting factor affecting ecosystem functions and stability.

## 5. Conclusions

No-tillage reseeding and fertilization significantly improved the diversity of plant species and fungal communities, as well as the ecosystem functions of degraded alpine grasslands. These effects indicate a positive contribution to the soil environment and grassland plant community of such regions, which can arise from a reasonable improvement from reseeding and fertilizer. Although environmental factors did not directly affect ecosystem multifunctionality, they could do so indirectly by influencing species diversity indices. In the field of ecosystem management, the results of this study can provide a reference for biodiversity conservation and measures to improve ecosystem multifunctionality by regulating biodiversity in degraded alpine grasslands in Qinghai province.

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