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Intercropping of *Echinochloa frumentacea* with Leguminous Forages Improves Hay Yields, Arbuscular Mycorrhizal Fungi Diversity, and Soil Enzyme Activities in Saline–Alkali Soil

Yunlong Cheng [1], Xing Xu [1], Yang Zhang [2], Xudong Gu [1], Haojie Nie [3,4] and Lin Zhu [3,4,*]

[1] College of Forestry and Prataculture, Ningxia University, Yinchuan 750021, China
[2] Gansu Analysis and Research Center, Lanzhou 730000, China
[3] School of Ecology and Environment, Ningxia University, Yinchuan 750021, China
[4] Breeding Base for State Key Laboratory of Land Degradation and Ecological Restoration in Northwestern China, Yinchuan 750021, China
* Correspondence: zhulinscience@126.com

Abstract: Soil salinization is detrimental to crop growth, agricultural yields, and environmental protection. *Echinochloa frumentacea* (Roxb.) Link is a pioneer species for the alteration of saline–alkali lands. In this paper, we examined the effects of intercropping between *E. frumentacea* and leguminous forages on saline land improvement in the saline–alkali soil of the Hetao-Ningxia Plain, China. We found that intercropping increased the diversity and richness of the arbuscular mycorrhizal fungi (AMF) community in the rhizosphere soil of *E. frumentacea*. Glomus was the dominant genus in the saline–alkali soil of the Hetao-Ningxia Plain, where Glomeraceae, VTX00067, VTX000193, and VTX000165 were the dominant species. Intercropping improved the activities of soil urease, sucrase, alkaline phosphatase, and catalase. The hay yields of *E. frumentacea* were correlated positively with soil enzyme activities, Chao1 index, and ACE index, and negatively with total water-soluble salt content. Together, intercropping between *E. frumentacea* and leguminous forages enhances AMF diversity and soil enzyme activities, which provides an agricultural practice for improving sustainability of the agro-ecosystem in saline–alkali areas.

Keywords: intercropping; *Echinochloa frumentacea*; leguminous forages; arbuscular mycorrhizal fungi (AMF); enzyme activity; saline–alkali soil

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1. Introduction

In arid and semi-arid areas, because of the high evaporation of soil moisture, the dissolved salts in groundwater accumulate on the soil surface through capillary movement, resulting in so-called secondary salinization, which is harmful to farmland and leads to land degradation [1]. In addition, irrational irrigation and insufficient drainage systems further aggravate soil salinization in this area [2]. Globally, soil salinization has become one of the most important abiotic stresses limiting crop yield and agricultural sustainability [3]. In the past decades, salinity-induced soil degradation has increased in the world; about 75 countries in the world have soil affected by salinization, and salinity-induced soil represents about 15% of the arid and semi-arid regions of the world. Generally, saline–alkali land accounts for 20% of global irrigated land, and this value reaches 30% in arid and semi-arid countries due to unreasonable irrigation and insufficient drainage [4–6]. The Hetao-Ningxia Plain is located in the southeastern area of Huanghe Alluvial Plain where the developed irrigation system has been constructed. Approximately 50% of irrigated lands have been salinized in the northern area of Hetao-Ningxia Plain, which is attributed to the high groundwater table resulted from flooding, irrigation, and insufficient drainage systems [7,8].

Excessive salt in soil exerts a negative influence on the soil physical and chemical properties [9], as well as the activities of soil microorganisms and enzymes. According to

Singh et al. [10], the microbial communities (structure, function, and diversity) are affected significantly by soil salinity and alkalinity. Nevertheless, saline soil can be utilized following multiple ameliorative measures, of which biological improvement is an effective method. Biological improvement refers to planting salt-tolerant plant species that have a positive impact on soil physical and chemical properties [11]. *Echinochloa frumentacea* (Roxb.) Link is a gramineous forage with strong tolerance to salinity, a fast growth rate, high grass yield, and good palatability, which can be used to make hay or silage feed [12,13]. *E. frumentacea* is widely planted in the saline–alkali land of the Hetao-Ningxia Plain, China. Studies have shown that the cultivation of *E. frumentacea* reduces soil pH, total salt, and alkalinity, and increases organic matter, which is beneficial to the reclamation of saline–alkali land [14,15].

Arbuscular mycorrhizal fungi (AMF) are microorganisms that form symbionts with host plants. The fungal hyphae of AMF colonize plant root cells and form arbuscular mycorrhiza, which are branches and places for exchanging nutrients between plants and fungi. AMF assist their hosts to obtain nutrients and mineral elements needed for growth, and obtains carbon sources from their hosts in exchange, affecting the productivity and stability of plant communities [16,17]. Studies have shown that AMF are an important mediator of soil aggregation and an important factor for maintaining the stability of soil aggregates [18]. Glomalin-related soil proteins (GRSP) secreted by AMF and their mycelium play an important role in promoting soil improvement and fertilization, maintaining soil health and sustainable productivity [19]. AMF can alleviate the negative impacts of saline–alkali lands [20]. AMF can increase the salt tolerance of plants, regulating plant growth in many ways, and relieve salt stress injury in plants. AMF can improve nutrient use by host plants, especially the accumulation of N, Ca, Zn, Mg, P, Cu, and Fe and soluble proteins in plants, and maintain the K^+/Na^+ ratio, enhancing the salt tolerance of plants [21–23].

Compared with the traditional monoculture, intercropping planting has positive impacts on the richness and diversity of AMF community, and contributes to the sustainable development of agricultural ecosystems [24]. According to Liu et al. [25] and Lithourgidis et al. [26], the intercropping system between gramineae and leguminous plants has several main advantages, such as higher total yield, better land use efficiency, and higher economic benefits, better utilization of sunlight, water, and nutrients, and helps to improve soil fertility. Soil nutrient cycle includes biochemical, chemical, and physicochemical reactions, which are catalyzed by soil enzymes. Since soil enzymes respond to changes faster than physicochemical indices, soil enzyme activities are often used as indicators of changes in microbial activity and soil fertility [27]. Therefore, it is necessary to study the diversity and community structure of the AMF community and soil enzyme activities in the intercropping system of gramineous and leguminous plants in saline–alkali soil.

We aim to examine the impact of intercropping between gramineous and leguminous forages on the diversity of the AMF community and soil enzyme activity in the rhizosphere soil of *E. frumentacea* grown in saline–alkali lands. We conducted field experiments that consisted of intercropped treatments between *E. frumentacea* and two leguminous grasses and monoculture control in the saline–alkali soil of the Hetao-Ningxia Plain, China. We analyzed AMF diversity and the soil enzyme activity in rhizosphere soil of *E. frumentacea*.

2. Materials and Methods

2.1. Experimental Materials

E. frumentacea cv. 'Haizi No.1' was bred by Lisheng Wan, working in Ningxia Grassland Station, Ningxia Hui Autonomous Region. Semi-wild soybean (*Glycine max. gracilis Skvortsov* cv. 'Dongsidou No.1') originated from the Dongying Academy of Agricultural Sciences, Shandong Province. Fodder soybean (*Glycine max* (L.) Merr. Cv. 'Mudanjiang MD') was bred by Guowen Cui who works in Northeast Agriculture University.

2.2. Background of the Experimental Site

The experimental site was located in Gaozhuang Township in Pingluo County of Ningxia Hui Autonomous Region, China (38°95′ N, 106°54′ E), which is located in the Hetao-Ningxia Plain, with an altitude of about 1057.8 m. It is a continental climatic region of temperate zone. The annual sunshine is from 2800 h to 3200 h with small rainfall, and high evaporation. The soil chemical properties were: 0–20 cm soil pH, 8.36; total salt, 4.87‰; organic matter, 13.41 g·kg^{-1}; total N, 0.71 g·kg^{-1}; available N, 43.03 mg·kg^{-1}; available P, 9.82 mg·kg^{-1}. The monthly precipitation and mean air temperature data from March to October for two years are shown in Figure 1.

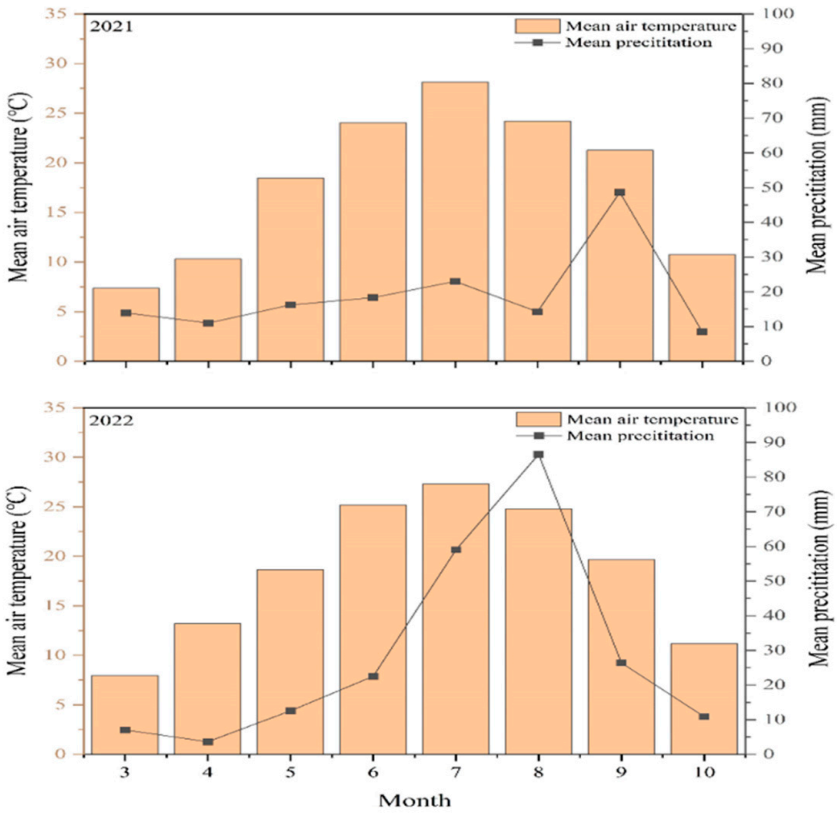

Figure 1. Monthly mean air temperature and precipitation in the experimental site from March to October in 2021 and 2022. Data from wheat A malt—agro-meteorological big data system V1.5.7b.

2.3. Experimental Designings

Field experiments were carried out from April to August in 2021 and 2022. The crop in this field before this experiment started was alfalfa. Random block design was adopted with three treatments, including monoculture of *E. frumentacea*, intercropping between *E. frumentacea* and semi-wild soybean, and intercropping between *E. frumentacea* and fodder soybean. Each treatment was repeated three times. The area was 24 m^2 (4 m × 6 m) for every plot, surrounded by isolation belts with a width of 1 m.

The sowing rate of monocropped *E. frumentacea*, intercropped *E. frumentacea*, intercropped semi-wild soybeans, intercropped fodder soybeans was 15 kg·ha^{-1}, 9 kg·ha^{-1}, 19.5 kg·ha^{-1}, and 36 kg·ha^{-1}, respectively. The *E. frumentacea* tested crops were sown in line. As shown in Figure 2, the row space was 40 cm in monocropping treatment. For intercropping systems, and the proportion of intercropping rows was 2:2 for two species (i.e., two rows of *E. frumentacea* and two rows of legume intercropping). The same species, such as *E. frumentacea* or leguminous forages, were sowed with a row space of 30 cm, while a row interval of 50 cm was maintained between *E. frumentacea* and legumes. Apply compound fertilizer (N-P_2O_5-K_2O: 18-18-18) 450 kg·ha^{-1} during sowing, with topdressing once in June, applying compound fertilizer (N-P_2O_5-K_2O: 18-18-18) 150 kg·ha^{-1}, and urea

(N: 46.4%) 120 kg·ha^{-1}. During the experiment period, irrigated by the Yellow River water, the irrigation method is flood irrigation. Weeds in the plots were eliminated manually, spraying pesticides to control red spiders and aphids.

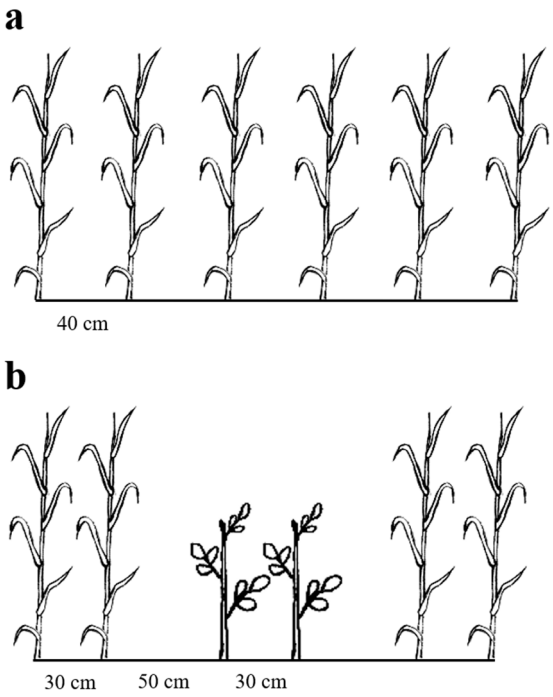

Figure 2. Diagram of planting system. (**a**) *E. frumentacea* monoculture; (**b**) *E. frumentacea* and legume grass intercropping.

2.4. Soil Sample Collection and Determination of Plant Yields

The sampling dates of rhizosphere soil of *E. frumentacea* in monoculture and intercropping treatments were 19 August 2021 and 20 August 2022. Six plants were dug randomly for each treatment. Loose soil was shaken off from the roots. The rhizosphere soil was removed from the roots with a sterilized soft bristle brush. After mixing evenly, the rhizosphere soil was put into a sterile sampling bag and brought back to the laboratory using sampling boxes containing ice. One-third of the soil sample was kept in sterilized centrifuge tubes and stored in an refrigerator at −80 °C for high-throughput sequencing analysis of the AMF community structure. The remaining soil samples were used for testing soil enzymes and to measure pH and total water-soluble salt content.

The measurement of fresh grass and hay yields of forages for the three treatments was determined with reference to Cheng et al. [15].

2.5. AMF Diversity Detection Method

DNA was extracted from soil using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany). DNA purity test method: NanoDrop2000, DNA concentration test method: NanoDrop2000, DNA integrity test method: Agarose gel electrophoresis. PCR amplification: The primers for the first round of amplification were AML1F (5′-ATCAACTTTCGATG GTAGGAGATAGA-3′) and AML2R (5′-GAACCCAACACTTGGTTCC-3′), and the primers for the second round of amplification were AMV4-5NF (5′-AAGCTCTTCGATGGTATTCG-3′) and AMDGR (5′-CCAACACTATCCACTTTGGTTCAT-3′). PCR official experimental used TransGen AP221-02: TransStart Fastpfu DNA Polymerase, 20 µL reaction system. Sequencing of amplicon libraries was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). The determination was completed by Majorbio Biopharm Technology Co., Ltd. (Shanghai, China).

Sequencing data processing: Cut the Barcode sequence and PCR amplification primer sequence from the sequenced sequence, and then use FLASH (v1.2.11) to splice the reads

of each sample to obtain raw tags. After strict filtering, quality control, and removal of chimeric sequences, obtain the final effective tags. Use USEARCH11 software to cluster all effective tags with 97% consistency for OTUs (Operational Taxonomic Units), and use maarjam081/AM database to annotate OTUs representative sequences for species to obtain taxonomic information.

2.6. Determination of Soil Enzyme, pH and Total Salts

The activities of soil urease, sucrase, alkaline phosphatase, and catalase were determined with soil enzyme kits (Comin, Suzhou, China). Enzyme activities were defined as follows: one unit of soil urease activity was defined as the production of 1 μg NH_3-N per gram of soil per day at 37 °C; a unit of soil sucrase activity was defined as the production of 1 mg reducing sugar per gram of soil per day; a unit of soil alkaline phosphatase activity was defined as releasing 1 μmol paranitrophenol per gram of soil per day; a unit of soil catalase activity was defined as the degradation of 1 μmol H_2O_2 per gram soil per day. The pH meter was used to measure soil pH. The total water-soluble salt content (TS) was obtained by measuring the electrical conductivity of 1:5 (soil/water) suspension with a conductivity meter (Multiparameter SevenCompact™, Mettler Toledo, Shanghai, China) [15].

2.7. Statistical Analysis

The Shannon diversity index (Shannon), Simpson diversity index (Simpson), and richness index (Chao1, ACE) of AMF in rhizosphere soil of *E. frumentacea* in different treatments was calculated by Mothur (version v.1.30.2) software. PCoA statistical analysis and mapping of species community column chart (bar chart) were finished using R language (version 3.3.1). Correlation analysis and mapping was finished by Origin 2021, Excel 2019 and SPSS 23 were used for statistical variance analysis of the diversity of the AMF community and soil enzyme activities between different treatments.

3. Results

3.1. Hay Yields per Unit Area of E. frumentacea under Different Intercropping Treatments

During the 2-year experiments, treatments of intercropping with different leguminous forages increased the hay yields per unit area of *E. frumentacea* ($p < 0.05$) (Figure 3). For the same sowing area, compared with EE, the hay yields per unit area of ES and EF increased by 39.66% and 38.55% in 2021, and by 45.57% and 44.51% in 2022, respectively.

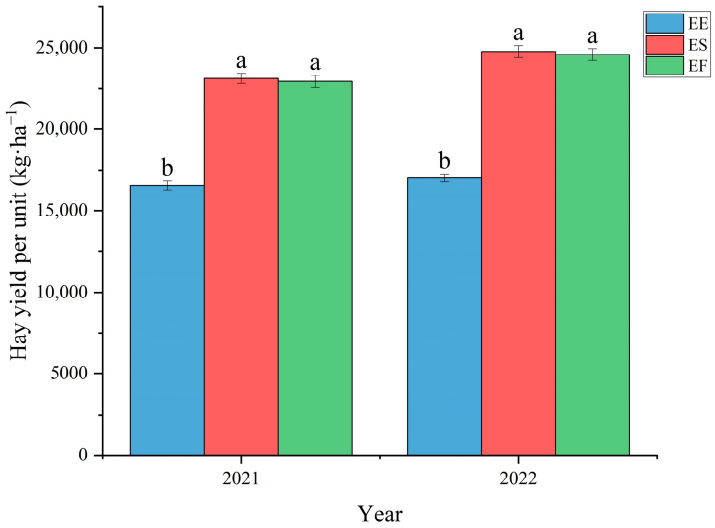

Figure 3. Hay yields of *E. frumentacea* under different treatments. EE, monocropped *E. frumentacea*; ES, *E. frumentacea* in intercropping system with semi-wild soybeans; EF, *E. frumentacea* in intercropping system with fodder soybeans. Different letters above the columns in the same year indicate significant differences among different treatments by Duncan's test ($p < 0.05$).

3.2. Venn Diagram of AMF OTU Distributions in Different Treatments

As shown in Figure 4, the sequencing results showed that there were 38 OTU types shared with the soils of the 3 different treatments. *E. frumentacea* intercropped with fodder soybeans (EF) had the highest number of OTUs, at 64 types, followed by *E. frumentacea* intercropped with semi-wild soybeans (ES), with 58 types. The total number of OTUs in rhizosphere soil for monocropped *E. frumentacea* (EE) was the lowest, at 45 types.

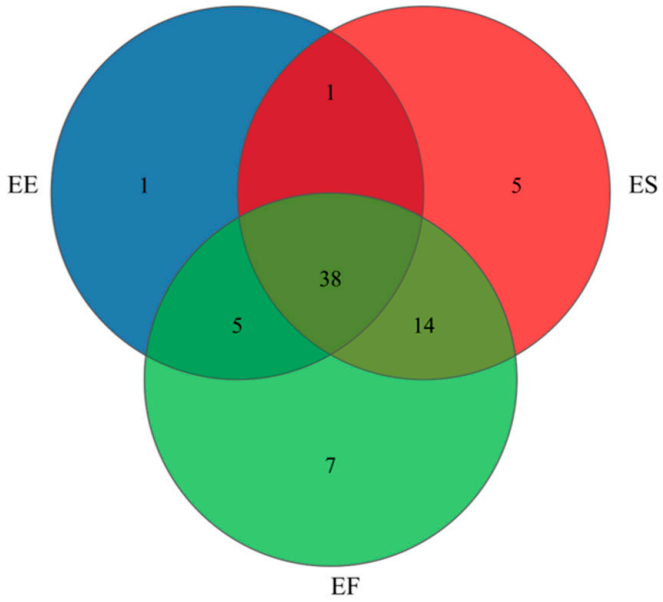

Figure 4. Venn diagram of AMF OTU distribution in rhizosphere soil of *E. frumentacea* under different plantings. EE, monocropped *E. frumentacea*; ES, *E. frumentacea* in intercropping system with semi-wild soybeans; EF, *E. frumentacea* in intercropping system with fodder soybeans.

3.3. Diversity of AMF in Rhizosphere Soil of E. frumentacea in Different Treatments

AMF in the rhizosphere soil of *E. frumentacea* displayed higher Shannon, Chao1, and ACE indices in intercropping treatments than those in monoculture treatment ($p < 0.05$), while a lower Simpson index of the AMF community was found in the rhizosphere soil of *E. frumentacea*. Compared with EE, the Shannon index of ES and EF increased by 33.89% and 28.16%, respectively. The Chao 1 index of ES and EF increased by 74.03% and 68.83%, respectively, and the ACE index of ES and EF increased by 84.43% and 70.4%, respectively (Table 1).

Table 1. AMF diversity in rhizosphere soil of *E. frumentacea* in the three treatments.

Treatment	Diversity Index		Community Richness Index	
	Shannon	Simpson	Chao1	ACE
EE	2.091 ± 0.31 b	0.185 ± 0.05 a	25.667 ± 8.74 b	25.655 ± 8.72 b
ES	2.799 ± 0.19 a	0.093 ± 0.02 a	44.667 ± 4.96 a	47.315 ± 3.47 a
EF	2.679 ± 0.07 a	0.129 ± 0.03 a	43.333 ± 3.06 a	43.716 ± 3.06 a

Note: EE, monocropped *E. frumentacea*; ES, *E. frumentacea* in intercropping system with semi-wild soybeans; EF, *E. frumentacea* in intercropping system with fodder soybeans. Different letters in the same column indicate statistically significant differences based on Duncan's test ($p < 0.05$).

3.4. Community Compositions of AMF in Rhizosphere Soil of E. frumentacea under Different Treatments

According to the results of high-throughput sequencing and species annotation, AMF communities in rhizosphere soil of *E. frumentacea* in monoculture and intercropping treatments were classified into 3 families, 3 genera, and 20 species. At the species level, unclassified_g__Glomus_f__Glomeraceae exhibited the highest relative abundance among all treatments (21.31–58.12%). Other dominant species were Glomus-mosseae-VTX00067

(15.43–28.54%), Glomus-group-B-Glomus-lamellosu-VTX00193 (5.519–17.43%), Glomus-sp.-VTX00165 (0.9803–21.9%), Glomus-MO-G23-VTX00222 (3.375–4.439%). Compared with EE, the AMF community in the rhizosphere soil of ES displayed higher abundance of unclassified_g__Glomus_f__Glomeraceae and s__Glomus-Wirsel-OTU16-VTX00156, and a lower abundance of Glomus-sp.-VTX00165 and Glomus-group-B-Glomus-lamellosu-VTX00193. In contrast, the AMF community in the rhizosphere soil of EF was found to have a higher abundance of Glomus-Group-B-Glomus-GlBb1.2-vtx00055 and a lower abundance of Glomus-sp.-VTX00165 than those of EE (Figure 5).

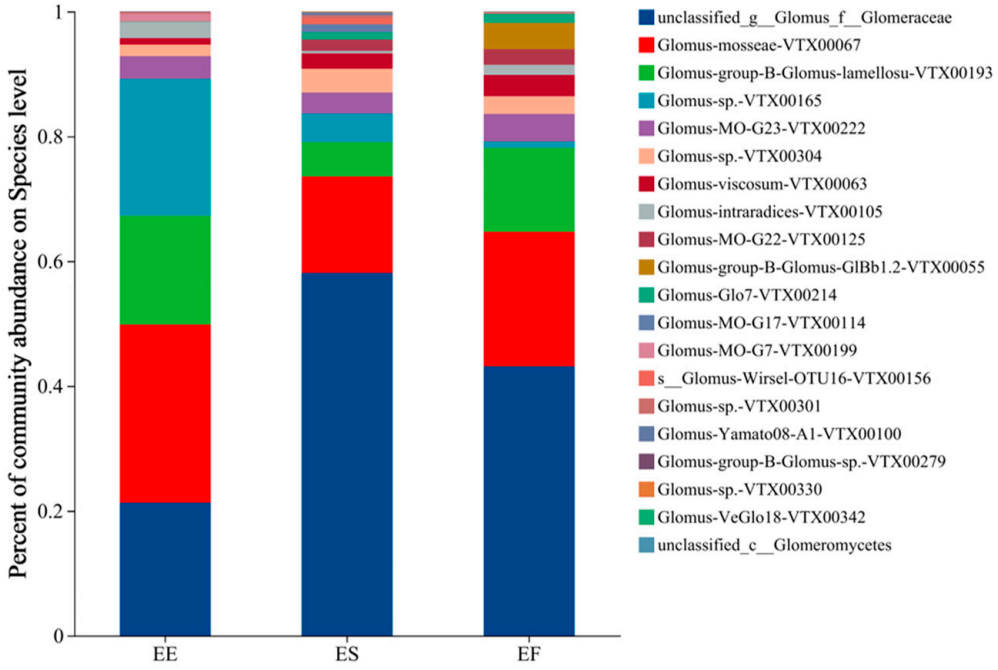

Figure 5. AMF species-level classification of *E. frumentacea* rhizosphere soil in three treatments. EE, monocropped *E. frumentacea*; ES, *E. frumentacea* in intercropping system with semi-wild soybeans; EF, *E. frumentacea* in intercropping system with fodder soybeans.

3.5. Beta Diversity Analysis of AMF in Rhizosphere Soil of E. frumentacea in Different Treatments

As shown in Figure 6, principal component 1 (PC1) could explain 33.77% of the variance of all variables, while principal component 2 (PC2) could explain 28.01% of the variance of all variables. The first two principal components could explain 61.78% of the total variance. AMF communities of all experimental treatments could be clearly divided into three clusters. The AMF flora in the *E. frumentacea* soil of the monoculture has a negative correlation with PC1, and the AMF flora in the intercropping soil has a positive correlation with PC1. The planting patterns of monoculture and intercropping have great influence on the AMF communities in the rhizosphere soil of *E. frumentacea*.

3.6. Analysis of Soil Enzyme Activities, pH, Total Water-Soluble Salt under Different Treatments

As shown in Table 2, in 2021, compared with EE, the activities of urease, sucrase, alkaline phosphatase, and catalase in ES rhizosphere soil, and the activities of sucrase and catalase in EF rhizosphere soil increased significantly ($p < 0.05$). In 2022, the activities of urease, sucrase, alkaline phosphatase, and catalase in ES rhizosphere soil, and the activities of urease, sucrase, alkaline phosphatase, and catalase in EF rhizosphere soil increased significantly ($p < 0.05$). Total water-soluble salt contents were lower in ES and EF than those in EE in the two consecutive years ($p < 0.05$).

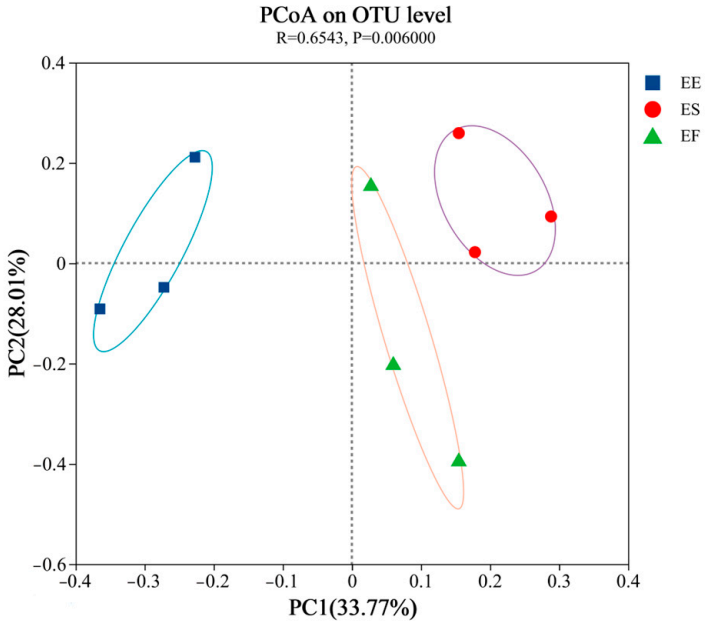

Figure 6. Principal component analysis of AMF beta diversity in the rhizosphere soil of *E. frumentacea* in three treatments. EE, monocropped *E. frumentacea*; ES, *E. frumentacea* in intercropping system with semi-wild soybeans; EF, *E. frumentacea* in intercropping system with fodder soybeans.

Table 2. Soil enzyme activities in the rhizosphere soil of *E. frumentacea* under different planting patterns.

Year	Treatment	Soil Enzyme Activities				pH	Total Water-Soluble Salt ($g \cdot kg^{-1}$)
		Urease ($\mu g \cdot g^{-1} \cdot d^{-1}$)	Sucrase ($\mu mol \cdot g^{-1} \cdot d^{-1}$)	Alkaline Phosphatase ($\mu mol \cdot g^{-1} \cdot d^{-1}$)	Catalase ($mg \cdot g^{-1} \cdot d^{-1}$)		
2021	EE	283.87 ± 7.68 b	10.60 ± 0.54 c	4.32 ± 0.25 b	17.46 ± 0.67 b	8.24 ± 0.03 a	1.74 ± 0.03 a
	ES	301.85 ± 9.59 a	13.92 ± 0.67 a	4.78 ± 0.06 a	20.29 ± 0.69 a	8.23 ± 0.02 a	1.57 ± 0.05 b
	EF	296.02 ± 4.87 ab	12.79 ± 0.31 b	4.53 ± 0.27 ab	20.18 ± 0.57 a	8.21 ± 0.02 a	1.56 ± 0.05 b
2022	EE	302.71 ± 8.41 b	12.91 ± 0.69 b	4.66 ± 0.45 b	19.24 ± 0.60 b	8.19 ± 0.02 a	1.66 ± 0.02 a
	ES	331.28 ± 2.67 a	15.64 ± 0.55 a	6.38 ± 0.31 a	21.58 ± 0.92 a	8.16 ± 0.02 a	1.50 ± 0.04 b
	EF	324.28 ± 5.34 a	15.36 ± 0.38 a	6.48 ± 0.50 a	21.21 ± 0.84 a	8.17 ± 0.02 a	1.47 ± 0.04 b

Note: EE, monocropped *E. frumentacea*; ES, *E. frumentacea* in intercropping system with semi-wild soybeans; EF, *E. frumentacea* in intercropping system with fodder soybeans. Different letters in the same column in the same year indicate statistically significant differences based on Duncan's test ($p < 0.05$).

3.7. Correlation Analyses among Hay Yields, pH, Total Water-Soluble Salt Content, AMF Diversity, and Soil Enzyme Activities in Rhizosphere Soil of E. frumentacea

As shown in Figure 7, the hay yields per unit area of *E. frumentacea* were positively correlated with urease, alkaline phosphatase, catalase, Chao1 index, and ACE index. Total water-soluble salt content was negatively correlated with Shannon, Chao1, and ACE indices, and positively correlated with Simpson index, and showed a trend of negative correlation with hay yields. The content of total salts was negatively correlated with urease, sucrase, and alkaline phosphatase. Urease was positively correlated with the Shannon, Chao1, and ACE indices, and negatively correlated with the Simpson index. Sucrase was found to be positively correlated with the Shannon and ACE indices and negatively correlated with the Simpson index. Positive correlation was recorded between alkaline phosphatase and Chao1 index. Catalase was positively related to the Shannon, Chao1, and ACE indices.

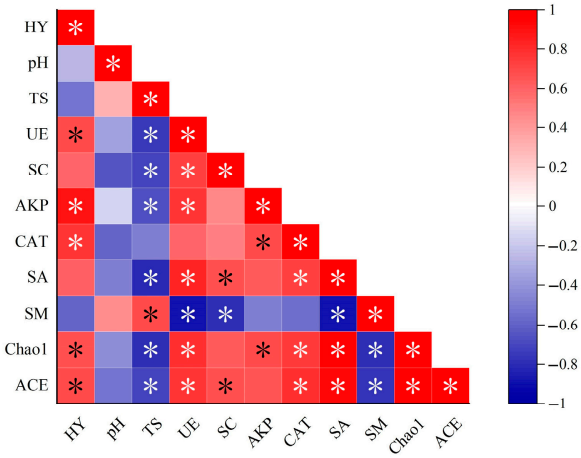

Figure 7. Correlation analysis of rhizosphere soil pH, total salts, soil enzyme activities, and AMF diversity under different planting patterns. HY: hay yields per unit area; TS: total salts; UE: urease; SC: sucrase; AKP: alkaline phosphatase; CAT: catalase; SA: Shannon; SM: Simpson. * indicates significance at the 0.05 probability level.

4. Discussion

4.1. Intercropping Improves AMF Diversity in Rhizosphere Soil of E. frumentacea

Studies have shown that monoculture reduces the richness of AMF due to the extremely low diversity of plant hosts, which is unfavorable to the community composition of AMF, leading to a decline in yield [28]. On the contrary, the increased plant diversity in the intercropping system increases AMF colonization rate and spore number, resulting in a high richness of AMF [29], which counteracts the negative impact of agricultural intensive monoculture on AMF and provides a potential means to improve the function and sustainability of agricultural ecosystems [30,31]. It has been proved that leguminous species in an intercropping system increases the spore concentration of AMF in soil [32]. The presence of leguminous plants increases rhizosphere nitrogen through different pathways, and AMF can promote the direct transfer of nitrogen from leguminous plants to non-leguminous plants through a mycelial network, which can promote the growth of non-leguminous plants [33]. In this study, interplant treatments improved the diversity of AMF in the rhizosphere soil of *E. frumentacea*. Meanwhile, the richness of AMF in the intercropping system was about two times higher than that in single cultivation. We also found a positive association between the hay yields of *E. frumentacea* and diversity of AMF. Our findings are in agreement with the report mentioned above, in that the introduction of leguminous forages into the monoculture system could improve AMF community composition favorable for agricultural production. Intercropping is an effective way to promote the recovery of AMF diversity and richness after long-term single cultivation by providing more diverse plant hosts.

Studies have shown that Glomus is the dominant genus of AMF in saline–alkali soil [34,35], because it has greater environmental adaptability and is more tolerant to environmental changes due to its unique reproductive characteristics and high spore rate [36,37]. Our results confirmed these reports, in which we found that Glomeraceae, VTX00067, VTX000193, and VTX000165 were the most widely distributed species in the saline–alkali soil of our experimental site, indicating that these four species may have a higher level of adaptability to the saline–alkali environment.

4.2. Intercropping Improves Soil Enzyme Activities in the Rhizosphere of E. frumentacea

A multiple cropping system increases the total carbon input and the diversity of compounds (namely glucose, cellulose, and protein) in the soil [38]. In addition, intercropping can increase soil enzyme activity [39]. Organic nitrogen is the main state for most nitrogen (>95%) in the soil, and organic nitrogen can only be absorbed by plants

after it is transformed into inorganic nitrogen under the action of microorganisms and soil enzyme [40]. Urease can promote ammoniation and urea hydrolysis of nitrogen in plant rhizosphere soil, and promote the transformation of soil organic nitrogen [41]. Sucrase and urease are the most important enzymes for carbon and nitrogen transformation in the soil [42]. Dai et al. [43] showed that peanut and Atractylodes lancea intercropping significantly improved the activities of sucrase and urease. Alkaline phosphatase can promote the mineralization of soil organic phosphorus, which is positively correlated with the available phosphorus content in the soil [44]. Intercropping can effectively increase the available phosphorus content in crop rhizosphere [45]. Tiemann et al. [46], also reported that when the cropping system was diversified, enzyme activities related to nitrogen and phosphorus acquisition were higher.

Our results showed that there was a positive correlation between the hay yields of *E. frumentacea* and the soil enzymes, indicating that soil enzyme activities could have a beneficial effect on the yields of *E. frumentacea*. Intercropping improved urease activity in the rhizosphere soil of *E. frumentacea* compared with monoculture, indicating that intercropping may improve the nitrogen supply of *E. frumentacea* through urease. Intercropping with leguminous grasses could significantly improve the activities of sucrase and urease in the rhizosphere soil of *E. frumentacea*, indicating that intercropping is beneficial to soil carbon and nitrogen cycles and improves soil nutrient supply. Intercropping promoted the increase in alkaline phosphatase activity in the rhizosphere soil of *E. frumentacea*, indicating that intercropping played a positive role in promoting the mineralization of organic phosphorus in the rhizosphere soil of *E. frumentacea* and increased the utilization of available phosphorus. The activity of catalase in soil is mainly affected by the number of soil microorganisms [47]; in this study, increased activity of catalase in the rhizosphere of *E. frumentacea* in the intercropping system compared with monoculture were observed. A positive correlation between the diversity and richness of AMF and soil enzyme activities has been observed in our study. Previous studies also showed that AMF improved the soil quality by increasing the activity of soil enzyme such as urease and sucrase [48]. Together, intercropping with leguminous grasses enhances the soil enzyme activities, which is beneficial to the transformation of soil nutrients and ultimately the yields of *E. frumentacea*.

4.3. Soil Salts Affect Hay Yields, AMF Diversity, and Soil Enzyme Activities

Studies have shown that soil salinity affects the species distribution and sporulation of AMF, as well as the formation of mycorrhiza and colonization of hyphae [49]. The results of this study showed that the community diversity of AMF was negatively correlated with the content of total salts, suggesting that salt stress affects the community structure and root colonization of AMF. Intercropping of *E. frumentacea* and leguminous grass reduces soil salt content [15], which is beneficial to the composition and diversity of the AMF community. Xie et al. [50] reported that there is negative association of soil enzyme activity with the concentrations of Na^+ and Cl^- ions. Our results showed that the activities of soil enzymes were negatively correlated with the content of total salts, which was consistent with the previous report [51]. Intercropping with leguminous grass promoted the growth of *E. frumentacea*, reduced the content of total salts, increased the amount of AMF and root exudates and microbial activities, and finally improved the hay yield performance of *E. frumentacea*.

5. Conclusions

Intercropping between *E. frumentacea* and leguminous forages in saline–alkali soil arising from irrational irrigation and insufficient drainage significantly improved hay yields of *E. frumentacea*, and enhanced the diversity and richness of the AMF community and soil enzyme activities in *E. frumentacea* rhizosphere soil. There were positive correlations of the hay yields per unit area of *E. frumentacea* with the diversity indices of AMF and soil enzyme activities. Soil enzyme activities correlated with the Shannon, Chao1, and ACE indices, and were negatively related to Simpson index of AMF. It can be concluded that

intercropping is key to improving the diversity and richness of the AMF community and the soil enzyme activity, and ultimately improve the yields of *E. frumentacea*. Intercropping between *E. frumentacea* and leguminous forages is beneficial to the sustainable development of agricultural ecosystem in saline–alkali soil arising from irrational irrigation and inadequate drainage in irrigated regions in the world.

Author Contributions: Y.C. undertook the intercropping experiments, analyzed the data, wrote the manuscript. X.X. supervised the study and revised the manuscript. Y.Z. and X.G. performed the experiments and collected data. H.N. performed review and editing. L.Z. organized and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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