agronomy

MDPI

Article

The Responses of Stem and Leaf Functional Traits of *Medicago sativa* and *Bromus inermis* to Different Mixed Planting Patterns

Fuhong Miao [1,2,†], Xiaoxu Yu [1,2,†], Xinkai Tang [1,2], Xindi Liu [1,2], Wei Tang [1,2], Yanhua Zhao [1,2], Chao Yang [1,2], Yufang Xu [1,2], Guofeng Yang [1,2] and Juan Sun [1,2,*]

[1] College of Grassland Science, Qingdao Agricultural University, Qingdao 266109, China; miaofh@qau.edu.cn (F.M.); tangxinki@163.com (X.T.); zhaoyanhua@qau.edu.cn (Y.Z.); yangchao@qau.edu.cn (C.Y.); xuyufang@qau.edu.cn (Y.X.); yanggf@qau.edu.cn (G.Y.)

[2] Key Laboratory of National Forestry and Grassland Administration on Grassland Resources and Ecology in the Yellow River Delta, Qingdao 266109, China

* Correspondence: sunjuan@qau.edu.cn; Tel.: +86-135-0642-0912

† These authors contributed equally to this work.

Abstract: This study investigated the differences in stem and leaf growth characteristics of *Medicago sativa* and *Bromus inermis* in the Jiaozhou region of China during 2019–2020 under three different planting modes of the two forages: monoculture, mixed species sowing in the same rows, and mixed species sowing in alternating rows. No special management of the experimental plots was carried out in this study to simulate as much as possible the growth of forages in their natural state. The stem and leaf characteristics influencing the dry matter weight were calculated using grey correlation. These characteristics included leaf length, leaf width, leaf thickness, leaf area, leaf fresh weight, stem length, stem diameter, stem fresh weight, stem–leaf ratio, fresh matter yield, dry matter yield, and protein yield of *M. sativa* and *B. inermis* under different sowing methods in different years. The results showed that the weight pattern of the characteristics affecting the yield of *M. sativa* and *B. inermis* production was leaf area > stem diameter > leaf length > stem length > leaf width > leaf thickness, leaf area > leaf length > stem length > leaf width > leaf thickness > stem diameter. Considering all the growth factors, the production capacity was ranked as mixed sowing in alternating rows > mixed sowing in same rows > monoculture. Thus, the suitable mode for *M. sativa*–*B. inermis* sowing was mixed sowing in alternating rows.

Keywords: *Medicago sativa*; *Bromus inermis*; sowing mode; stem–leaf characteristics

check for updates

Citation: Miao, F.; Yu, X.; Tang, X.; Liu, X.; Tang, W.; Zhao, Y.; Yang, C.; Xu, Y.; Yang, G.; Sun, J. The Responses of Stem and Leaf Functional Traits of *Medicago sativa* and *Bromus inermis* to Different Mixed Planting Patterns. *Agronomy* **2023**, *13*, 2733. https://doi.org/10.3390/agronomy13112733

Academic Editor: Aiming Qi

Received: 8 October 2023
Revised: 28 October 2023
Accepted: 28 October 2023
Published: 30 October 2023

1. Introduction

Selecting suitable legumes and grass forages to establish a high-yielding mixed grass system is an important way of solving the current grass–livestock imbalance since mixing perennial grass with legume forage improves the grass production and protein content per unit area [1–4]. It also increases the nitrogen nutrient and organic matter content in the soil [5–7]; improves soil fertility [8]; and reduces the application of industrial nitrogen fertilizer, production costs, and environmental pollution [9,10]. However, legumes are also known to contribute to the emission of N_2O, especially when their above-ground residues are incorporated into the soil [11,12]. This is why it is extremely important to find a suitable grass–legume combination that minimizes environmental impacts without compromising yield. Alfalfa (*Medicago sativa*) and awnless brome (*Bromus inermis*) are forage crops widely grown and utilized in agricultural and pastoral areas of China, with significant advantages, such as high protein and mowing resistance [13,14]. The mixed planting of alfalfa and awnless brome exhibit advantages in mixing legume and grass forage [15], such as occupying the different above-ground and below-ground spaces and maximizing the utilization of light resources and below-ground nutrients, thus significantly improving forage yield and quality [3]. Therefore, alfalfa and awnless brome can complement each other at several levels when planted together.

Previous studies mostly used forage nutritional quality and yield as important indicators to assess the forage mixing patterns [16,17], but this approach has some limitations. Since functional trait characteristics reflect species differences in resource competition, resistance to external pressure, and adaptation strategies, studies based on the functional traits of plant organs can better elucidate the interactions and coexistence mechanisms among species [18,19]. Therefore, this study analyzed the characteristics of stem and leaf functional traits of alfalfa–awnless brome in mixed planting to reveal the plasticity of these traits and their adaptive responses. We also analyzed the correlation between stem and leaf functional traits of legumes and grasses under mixed sowing, providing a theoretical basis for elucidating the mechanism of efficient production in legume–grass mixed sowing systems.

2. Materials and Methods

2.1. The Study Area

This study was conducted in Jiaozhou City, Shandong Province (36°26′22″ N, 120°04′43″ E), China. The study area has a warm temperate monsoon climate with a characteristic oceanic climate, an average annual temperature of 12.1 °C (Figure 1), an average annual precipitation of about 695.6 mm, an average annual air pressure of 1015.6 MPa, and an annual frost-free period of 205.5 d, the annual sunshine duration is 2573 h. And the soil type is sandy ginger black, the information of physical and chemical properties of soil at 0~30 cm is in Table 1.

Table 1. The physical and chemical properties of soil at 0~30 cm.

Index	pH	Total N ($g\cdot kg^{-1}$)	Available N ($mg\cdot kg^{-1}$)	Total P ($g\cdot kg^{-1}$)	Available P ($mg\cdot kg^{-1}$)	Available K ($mg\cdot kg^{-1}$)	Organic M ($g\cdot kg^{-1}$)	Organic C ($g\cdot kg^{-1}$)
Data	7.14	0.88	526.71	0.45	27.43	114.29	17.04	9.88

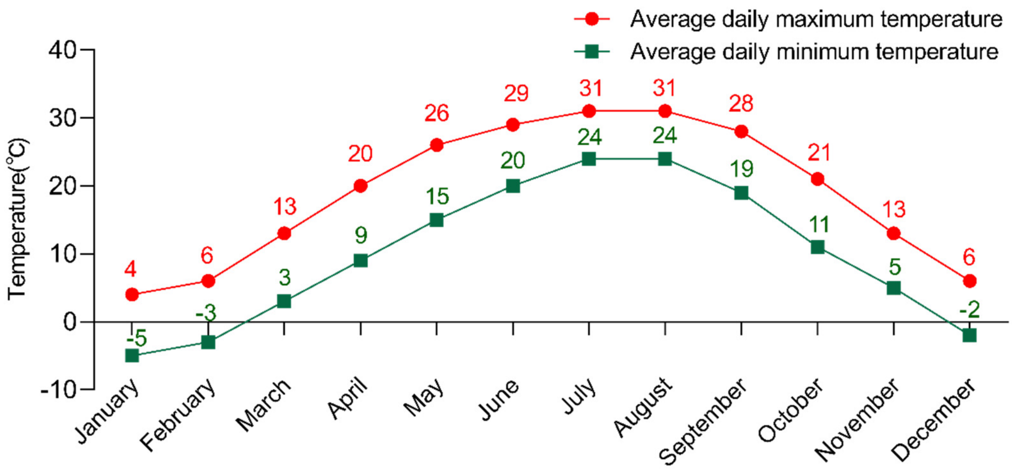

Figure 1. Annual average temperature of test field in Jiaozhou area. Data from China Meteorological Data Service Centre (http://data.cma.cn 8 February 2020).

2.2. Experimental Design

The perennial leguminous forage alfalfa variety WL525HQ and graminaceous awnless brome variety HARANO were obtained from Beijing Zhengdao Seed Industry Co., Ltd., Beijing, China, for this study. The two forages were planted on 22 March 2019, in a mixed legume–grass seeding system, and the forage samples were collected at the early flowering stage of alfalfa on 4 June, 10 July, 14 August, and 21 September 2019 and on 3 June, 7 July, and 11 August 2020. A 1 m^2 sampling area was set up in each plot, and all plants within the sampling area were harvested manually after mowing at a 5 cm stable height. The fourth sampling in the second year (2020) was affected by an epidemic, and the alfalfa was already at the pod stage at the time of collection; thus, only seven crops were harvested across the two years. The awnless brome was not sampled after the first two sampling points to prevent thinning because the growth of the awnless brome was reduced after the first two sampling points each year. The alfalfa and awnless brome were sown individually as controls, while the sowing combination involved alfalfa–awnless brome peer mixing and planting in different rows. There were four treatments: alfalfa single sowing, awnless brome single sowing, alfalfa–awnless brome mixed sowing in alternating rows, and alfalfa–awnless brome mixed sowing in the same rows, all of which were sown in strips.

The experimental plots were designed in a randomized block design with three replications of each treatment, with a total of 12 plots. Each plot was 12 m^2 (3 m $\times$ 4 m), and all plots were manually furrowed with a row spacing of 20 cm and a sowing depth of 3–4 cm. Based on the seed germination and thousand seed weight of awnless brome and alfalfa, the sowing rate was 37.5 kg/ha and 18.75 kg/ha for awnless brome and alfalfa, respectively, in order to ensure that all the mixed treatments were sown at a plant ratio of 1:1.

2.3. Sample Collection

2.3.1. Sample Collection

Fresh forage samples were sampled on 4 June 2019, for the first crop and on 10 July, for the second crop, when alfalfa was in the early flowering stage. Five sample points were selected in each plot for the W-shaped sampling [20,21]. Two plants in good growth conditions were selected from each sample point by category, mowed and placed in ice boxes (YMJ-A from Hangzhou Lvbo Instrument Co., Ltd., Hangzhou, China) to be transported to the laboratory for storage at 4 °C. After sampling, three 1 m $\times$ 1 m plots were randomly selected in each plot and mowed with 4–5 cm of stubble height, with three rows of awnless brome and two rows of alfalfa in the mixed pattern. The yield of each forage was measured after sorting the mixed forages by species. After that, 500 g of mixed samples were randomly taken from each plot according to the quartering method and transported to the laboratory for further analysis. All plots were mown as a whole after sample collection.

2.3.2. Determination Indices and Methods

Measurement of the fresh samples: Leaf length, leaf width, leaf thickness, and leaf area were measured using a leaf area meter (YMJ-A from Hangzhou Lvbo Instrument Co., Ltd., Hangzhou, China), while the stem diameter was measured using a diameter meter (MA-YH from Nanjing Ming'ao Instrument Co., Ltd., Nanjing, China), and stem length was measured using a steel ruler (MA-YH from Nanjing Ming'ao Instrument Co., Ltd., Nanjing, China). Leaf and stem fresh weights were weighed on an electronic scale (YMJ-A from Hangzhou Lvbo Instrument Co., Ltd., Hangzhou, China).

Dry matter yield measurement: The forage was weighed in bags after mowing and oven-dried at 105 °C for 1 h and then 85 °C to constant weight to calculate the dry matter yield.

Determination of the crude protein yield: The forage samples were dried and crushed with an ultra-micro crusher, after which the total nitrogen content of the pasture was determined with an automatic Kjeldahl nitrogen tester. The crude protein yield of the mixed sown forage was calculated as forage dry matter yield (kg/ha) $\times$ forage protein content (%).

2.4. Data Analysis

2.4.1. Principal Component and Gray Correlation Analyses

Principal component and gray correlation analyses were used to analyze the dry matter yield. Since dry matter yield is a more accurate indicator of grass production capacity [22], the dry matter yield of all treated alfalfa and awnless brome materials was selected as the reference column, which was recorded as {X_0(k) (k = 1, 2, 3, 4, . . ., n). Each index was used as the evaluation index in the comparison column, i.e., the set of observed values of the participating indices, and the indices were recorded as {(k)} (i = 1, 2, 3, 4, . . ., m); k = (1, 2, 3, 4, . . ., n). The traits used for the analysis included leaf length, leaf width, leaf area, leaf thickness, stem length, stem diameter, crude protein yield, and dry matter yield. The mowed stubble was denoted as X, and the traits were denoted as k; thus, the values of mowed stubble X at trait k formed the comparison series, X_i. X_0 was used to construct the reference series. The correlation coefficients were calculated according to the formula (k), while the equal-weighted correlations were calculated using the formula r_i. ρ is the resolution coefficient, ρ ∈ [0, 1], where 0.5 represents a moderate positive correlation. Here, we utilized the value 0.5.

Correlation coefficients:

$$\varepsilon_i(k) = \frac{\mathbf{min_i min_k |X_0k - X_ik| + \rho min_i min_i |X_0k - X_ik|}}{\mathbf{|X_0k - X_ik| + \rho max_i max_i |X_0k - X_ik|}},$$

where $|X_0(k) - X_i(k)|$ is the absolute difference, denoted as Δi(k):

$$\Delta i\mathbf{k} = |\mathbf{X_0k - X_ik}|$$

Equal-weighted correlations:

$$\mathbf{r_i} = \frac{1}{\mathbf{n}} \sum_{\mathbf{k=0}}^{\mathbf{n}} \varepsilon_i(\mathbf{k})$$

where n is the number of samples. Weighting factor $\omega_1 = \frac{r_i}{\sum r_i}$.

According to the principal of correlation analysis, the larger the weighted coefficient, the greater the influence of the trait on the evaluation index, and the smaller the weighted coefficient, the smaller the influence on the evaluation index.

2.4.2. ANOVA and LSD Methods

Microsoft Excel 2019 (Redmond, WA, USA) was used for preliminary organization of the data, and one-way analysis of variance (ANOVA) and the least significant difference (LSD) method were used to compare and test the significance of differences among the indicators using SPSS 26.0 (BM, Inc, Armonk, NY, USA) statistical software, and Sigma Plot 12.5 (Systat Software, San lose, CA, USA) was used to generate the graphs.

3. Results

3.1. Effects of Cutting Times and Sowing Patterns on Stem and Leaf Characteristics and Production Characteristics

According to the variance analysis of stem and leaf traits and nutritional quality indices of herbage in the alfalfa and awnless brome seeding model, it was found that cutting stubble times and seeding mode had significant effects on leaf length, leaf width, leaf thickness, leaf area, stem length, stem diameter, leaf fresh weight, protein yield, dry matter yield, and fresh matter yield ($p < 0.05$). Leaf length, leaf width, leaf thickness, leaf area, stem length, stem diameter, protein yield, and dry matter yield were all affected by mowing and seeding pattern (Table 2).

Table 2. Effects of cutting times and sowing patterns on stem and leaf characteristics and production characteristics.

Index	Probability of Significance		
	Cutting Times	**Mixture Model**	**Cutting Times × Mixture Model**
Leaf length	<0.001	<0.001	<0.001
Leaf width	<0.001	<0.001	<0.001
Leaf thickness	<0.001	<0.001	<0.001
Leaf area	<0.001	0.024	<0.001
Stem length	<0.001	<0.001	0.036
Stem diameter	<0.001	<0.001	<0.001
Leaf fresh weight	0.011	0.127	0.392
Stem fresh weight	0.053	0.111	0.386
Amount of protein	<0.001	<0.001	0.012
Dry matter mass	0.001	<0.001	0.021
Fresh matter mass	0.011	<0.001	0.142

3.2. Effects of Different Sowing Patterns and Stubble on Stem and Leaf Traits of Alfalfa and Awnless Brome

3.2.1. Effect of Different Sowing Patterns and Stubble on Leaf Traits of Alfalfa and Awnless Brome

During the first crop sampling in the first year, the leaf length, leaf width, and leaf thickness of alfalfa under the alfalfa–awnless brome peer mixture were significantly higher than those under the hetero-row mixture and monoculture treatments, with the values of 17.89 mm, 9.28 mm, and 0.19 mm, respectively. Leaf area showed significant differences ($p < 0.05$) under all three sowing patterns, with the ranking of heterocomplex > peer mix > monoculture, thus indicating that the monoculture sowing pattern had the lowest leaf area (Table 3). The leaf length of the first sampled awnless brome (147.38 mm) was significantly lower under the heterogeneous row mix treatment than under the monoculture treatment. Similarly, the leaf width of the first sampled awnless brome (5.43 mm) was significantly lower under the peer mix treatment than the monoculture treatment.

Table 3. The dynamics of the leaf traits of alfalfa.

Cut	Seeding Method	Leaf Length (mm)	Leaf Width (mm)	Leaf Thickness (mm)	Leaf Area (cm^2)
First cut	P	17.89 ± 4.09 Aa	9.28 ± 2.62 Aa	0.19 ± 0.04 Aa	1.16 ± 0.60 Aa
	H	16.88 ± 3.77 Ab	8.34 ± 2.42 Ab	0.18 ± 0.05 Ab	1.17 ± 0.64 Aa
	M	17.14 ± 3.74 Ab	8.02 ± 2.32 Ab	0.16 ± 0.05 Ab	0.95 ± 0.48 Ab
Second cut	P	14.72 ± 3.73 Bb	6.79 ± 2.25 Bb	0.12 ± 0.03 Ba	0.61 ± 0.39 Bab
	H	16.65 ± 5.66 Aa	8.20 ± 3.49 Aa	0.13 ± 0.03 Ba	1.02 ± 0.91 Aa
	M	16.03 ± 3.73 Bab	8.34 ± 2.68 Aa	0.12 ± 0.03 Cb	0.89 ± 0.41 Ab

Peer mix (P), heterocomplex (H), monoculture (M). Capital letters indicate significant differences in the same planting pattern at different stubble times at the 0.05 level ($p < 0.05$). Lowercase letters indicate significant differences in the different planting patterns at the same stubble times at the 0.05 level ($p < 0.05$).

The leaf length, leaf width, leaf thickness, and leaf area of the second sampled alfalfa were significantly lower than the first sampled crop under the alfalfa–awnless peer mixture treatment. The leaf width (6.79 mm) and leaf area (0.61 cm^2) of alfalfa were significantly lower under the peer row mix treatment than under the heterogeneous mixture and the monoculture treatments (Table 3). The leaf length of awnless brome (16.65 mm) under the heterogeneous row mixture treatment was significantly higher than that of the peer mixture treatment. Moreover, the leaf thickness under the monoculture treatment was significantly lower than that of the other two treatments. There was no significant difference in the fresh weight of leaves among the three treatments. However, the leaf length of the second sampled awnless brome was significantly higher compared to the first sampled crop under the hetero-row mixture and monoculture treatments, with values of 186.62 mm and 169.88 mm, respectively. The leaf width of the second sampled awnless brome (5.21 mm) was significantly lower compared to the first sampled crop under the monoculture treatment, and the leaf area of the monoculture treatment (6.95 cm^2) was significantly lower than that of the hetero-row mixture treatment.

For the first crop sampling in the second year, the leaf length of alfalfa under the alfalfa–awnless brome hetero-row mixture was significantly higher than that under the peer mixture and monoculture treatments (Table 4), with a value of 20.15 mm. The leaf thickness under alfalfa–awnless brome peer and hetero-row mixture treatments was significantly lower than that under the other treatments, with values of 0.14 mm and 0.15 mm, respectively. Moreover, the stem length was significantly higher (78.68 cm) in the alfalfa–awnless brome mixed treatment than in the monoculture treatment, while stem diameter was significantly higher (3.21 mm) in the alfalfa–awnless brome allopathic mixed treatment than in the monoculture treatment.

The leaf length of the second sampled alfalfa crop was significantly higher in alfalfa–awnless brome peer (24.87 mm) and hetero-row mixtures (24.67 mm) than in the other treatments. The leaf width under alfalfa–awnless brome peer treatment (9.47 mm) was significantly higher than that under monoculture treatments. Similarly, the leaf area under alfalfa–awnless brome peer and hetero-row mixture treatments was significantly higher than that under the monoculture, with a value of 1.77 cm^2, and there were no significant differences in stem length and diameter between the treatments ($p > 0.05$).

Table 4. The second-year Alfalfa stem and leaf trait dynamics under different seeding methods.

Cut	Seeding Method	Leaf Length (mm)	Leaf Width (mm)	Leaf Thickness (mm)	Leaf Area (cm^2)
First cut	P	18.83 ± 4.04 Bab	9.10 ± 1.83 Abc	0.14 ± 0.04 Ab	1.39 ± 0.66 Babc
	H	20.15 ± 2.60 Ba	8.75 ± 1.94 Abc	0.15 ± 0.03 Bb	1.21 ± 0.38 Bbc
	M	17.57 ± 3.43 Bb	8.30 ± 2.71 Ac	0.18 ± 0.05 Aa	1.07 ± 0.44 Bc
Second cut	P	24.87 ± 4.07 Aa	9.47 ± 2.69 Aa	0.18 ± 0.04 Ab	1.77 ± 0.67 Aa
	H	24.67 ± 3.94 Aa	9.03 ± 1.85 Aab	0.20 ± 0.03 Aa	1.69 ± 0.45 Aa
	M	21.67 ± 4.05 Ac	8.43 ± 1.92 Aab	0.19 ± 0.04 Aab	1.33 ± 0.43 Abc

Peer mix (P), heterocomplex (H), monoculture (M). Capital letters indicate significant differences in the same planting pattern at different stubble times at the 0.05 level ($p < 0.05$). Lowercase letters indicate significant differences in the different planting patterns at the same stubble times at the 0.05 level ($p < 0.05$).

3.2.2. Effects of Different Sowing Patterns and Stubble on Stem Traits of Alfalfa and Awnless Brome

For the first crop sampling in the first year, alfalfa stem length was the highest under the alfalfa–awnless brome monoculture treatment at 36.32 cm. The stem length of awnless brome was significantly lower in the single sowing treatment (5.30 cm) than in the other treatments ($p < 0.05$); however, there were no significant differences in stem diameter and stem fresh weight among the three treatments. For the second crop sampled in the same year, alfalfa stem length and diameter were higher under the alfalfa–awnless brome peer mixture than under the other two treatments, and the highest alfalfa stem fresh weight was 15.52 g under the heterogeneous row mixture treatment. The stem diameter of awnless brome significantly differed from its stem length and stem fresh weight, with the most significant differences occurring under the heterogeneous row mixture. However, there was a non-significant difference in stem length and stem fresh weight among the three sowing pattern treatments ($p > 0.05$).

For the first crop sampled in the second year, the stem diameter (3.21 mm) and stem fresh weight (1.40 g) of alfalfa were higher under the alfalfa–awnless brome hetero-row mix treatment than under the other treatments. The stem length under the peer-row mix treatment was the longest (78.68 cm), and the stem length and diameter under the monoculture treatment significantly differed from those of the other treatments ($p < 0.05$).

The alfalfa stem diameter of the second crop sampled was significantly higher (with an increase of 0.49 mm) compared to the first crop sampled in the same year under the alfalfa–awnless brome monoculture treatment. Similarly, the stem length of the second sampled alfalfa was significantly higher than that of the first sampled crop under the heterogeneous mixed and monoculture treatments by 77.21 cm and 70.29 cm, respectively. The stem fresh weight of the second sampled awnless brome was significantly lower under all treatments compared to the first sampled crop, considering that awnless brome growth was affected by climate. However, there were no significant differences in the stem length, stem diameter, and stem fresh weight among the three treatments (Table 5).

Table 5. The dynamics of the stem traits of alfalfa and awnless brome.

Species	Cut	Seeding Model	Stem Length (cm)	Stem Diameter (mm)	Stem Weight (g)
Medicago sativa First year	First cut	P	31.61 ± 19.73 Aa	2.53 ± 0.72 Aa	13.13 ± 4.82 Aa
		H	30.04 ± 15.87 Aa	2.00 ± 0.76 Ba	10.97 ± 2.07 Ab
		M	36.32 ± 20.41 Aa	2.49 ± 0.82 Aa	12.28 ± 5.14 Aa
	Second cut	P	38.91 ± 18.00 Aa	2.08 ± 0.64 Ab	12.18 ± 6.21 Aa
		H	35.92 ± 14.02 Aa	2.05 ± 0.62 Aa	15.52 ± 3.62 Aa
		M	34.14 ± 9.21 Aa	1.77 ± 0.58 Ab	8.93 ± 1.53 Aa
Medicago sativa Second year	First cut	P	78.68 + 13.10 Aa	3.00 + 0.61 Aa	0.62 + 0.12 Aa
		D	71.34 + 8.46 Aa	3.21 + 0.52 Aa	1.40 + 0.80 Aa
		M	58.86 + 7.50 Ab	2.64 + 0.43 Ab	0.71 + 0.02 Aa
	Second cut	P	73.36 + 12.67 Aa	3.06 + 0.53 Aa	0.34 + 0.11 Aa
		H	77.21 + 12.55 Aa	3.24 + 0.56 Aa	0.37 + 0.04 Aa
		M	70.29 + 7.77 Aa	3.13 + 0.42 Aa	0.34 + 0.03 Aa
Bromus inermis First year	First cut	P	6.86 ± 1.78 Aa	1.89 ± 1.94 Aa	8.59 ± 0.67 Aa
		H	6.45 ± 2.28 Aa	1.81 ± 0.57 Aa	8.07 ± 1.55 Aa
		M	5.30 ± 1.65 Bb	1.92 ± 0.62 Aa	9.15 ± 1.01 Aa
	Second cut	P	6.76 ± 2.97 Aa	1.40 ± 0.30 Abb	7.13 ± 0.56 Aa
		H	7.12 ± 2.91 Aa	1.35 ± 0.27 Bb	7.10 ± 0.26 Aa
		M	6.43 ± 2.67 Aa	1.56 ± 0.42 Ab	7.78 ± 1.07 Aa
Bromus inermis Second year	First cut	P	53.16 + 16.00 Aa	2.84 + 0.48 Aa	0.56 + 0.18 Aa
		H	62.60 + 12.79 Aa	2.96 + 0.39 Aa	0.71 + 0.33 Aa
		M	45.82 + 12.29 Aa	2.87 + 0.34 Aa	0.46 + 0.07 Aa
	Second cut	P	11.23 + 4.87 Aa	1.93 + 0.52 Aa	0.23 + 0.04 Aa
		H	12.95 + 5.52 Aa	1.47 + 0.34 Aa	0.29 + 0.04 Aa
		M	8.20 + 3.57 Ab	1.89 + 0.73 aA	0.25 + 0.05 Aa

Peer mix (P), heterocomplex (H), monoculture (M). Capital letters indicate significant differences in the same planting pattern at different stubble times at the 0.05 level ($p < 0.05$). Lowercase letters indicate significant differences in the different planting patterns at the same stubble times at the 0.05 level ($p < 0.05$).

3.2.3. Effect of Different Sowing Patterns and Stubble on the Stem-to-Leaf Ratio of Alfalfa and Awnless Brome

The stem-to-leaf mass ratio indicates the distribution of plant material in the above-ground tissues [23,24], and a smaller stem-to-leaf ratio indicates a more favorable distribution of nutrients to the leaves, better palatability, and higher protein content [25,26]. Furthermore, the average leaf aspect ratio and stem length/diameter ratio indicate the geometric fineness of leaves and stems, and larger values indicate that the leaf and stem growth tend to be more vertical, while smaller values indicate that leaves and stems tend to grow laterally.

In the first year, the alfalfa stem length/diameter ratio of the first crop to be sampled was significantly higher under the alfalfa–awnless brome heterogeneous mixture than under the peer mix treatment, indicating that its stem growth tended to be more vertical. The leaf aspect ratio (27.32), stem length-to-diameter ratio (2.93), and stem fresh weight ratio (0.62) of awnless brome under the first monoculture treatment were the smallest among all treatments, indicating that awnless brome had shorter and wider leaves, thicker and shorter stems, and relatively higher leaf biomass and smaller stem biomass under the monoculture treatment (Table 6).

The alfalfa leaf aspect ratio (2.24) of the second crop sampled under the alfalfa–awnless brome peer mixture treatment was the highest of all treatments, indicating a greater tendency for vertical leaf growth. There was a significant decrease in the leaf aspect ratio of the second crop under the alfalfa monoculture treatment and a significant increase in stem length-to-diameter ratio in the peer mix versus monoculture treatment. A significant decrease also existed in the stem-to-leaf fresh weight ratio in the monoculture treatment. The stem length-to-diameter ratio of the second crop of awnless brome under the monoculture treatment was the smallest among the treatments, and the leaf length-to-width ratio was significantly increased in the monoculture treatment. All three sowing patterns of awnless brome had significantly higher stem length-to-diameter ratios in the second crop, indicating that the stems tended to grow vertically (Table 6).

Table 6. The dynamics of the stem–leaf ratio of alfalfa and awnless brome.

Species	Cut	Seeding Model	Leaf Aspect Ratio	Stem L/D Ratio	Stem/Leaf Weight Ratio
Medicago sativa	First cut	P	2.24 ± 4.46 aA	11.51 ± 5.71 bB	1.39 ± 0.19 aA
		H	2.09 ± 0.41 aA	16.71 ± 11.52 aA	1.33 ± 0.13 aA
		M	2.21 ± 0.45 aA	13.75 ± 5.71 bAB	1.47 ± 0.36 aA
	Second cut	P	2.24 ± 0.42 aA	18.84 ± 5.16 aA	1.35 ± 0.42 aA
		H	2.10 ± 0.35 aB	17.94 ± 5.59 aA	1.45 ± 0.01 aA
		M	2.00 ± 0.40 bB	19.91 ± 4.01 aA	1.13 ± 0.15 bA
Bromus inermis	First cut	P	32.52 ± 12.61 aA	4.13 ± 1.12 bA	0.65 ± 0.05 aAB
		H	32.03 ± 13.77 aA	3.71 ± 1.13 bB	0.73 ± 0.10 aA
		M	27.32 ± 13.44 bB	2.93 ± 1.14 bC	0.62 ± 0.05 aB
	Second cut	P	34.28 ± 14.10 aA	4.80 ± 1.71 aAB	0.93 ± 0.37 aA
		H	35.51 ± 13.50 aA	5.42 ± 2.39 aA	0.74 ± 0.05 aA
		M	39.46 ± 26.13 aA	4.17 ± 1.49 aB	0.70 ± 0.03 aA

Peer mix (P), heterocomplex (H), monoculture (M). Capital letters indicate significant differences in the same planting pattern at different stubble times at the 0.05 level ($p < 0.05$). Lowercase letters indicate significant differences in the different planting patterns at the same stubble times at the 0.05 level ($p < 0.05$).

3.3. Effects of Different Sowing Patterns and Stubble on Yield Traits of Alfalfa and Awnless Brome

In the first year, the crude protein, dry matter, and fresh matter yield of the first sampled alfalfa were significantly higher in the alfalfa–awnless brome hetero-row mix, with the values of 0.81 t/ha, 4.51 t/ha, and 14.72 t/ha, respectively, than those of the other two sowing patterns. Similarly, the crude protein yield (1.19 t/ha) and dry matter yield (5.57 t/ha) of the second sampled alfalfa were significantly higher in the alfalfa–awnless brome hetero-row mix than in the single sowing. There was no significant difference in fresh matter yield between the three sowing patterns (Table 7).

In the second year, the crude protein (1.82 t/ha) and dry matter (10.25 t/ha) yields of the first sampled alfalfa crop in the second year were significantly higher in the alfalfa–awnless brome hetero-row mix than in the other sowing methods. Similarly, the crude protein (2.99 t/ha) and dry matter (14.81 t/ha) yields of the second sampled alfalfa crop were significantly higher under alfalfa–awnless brome peer-row mix than under the other sowing methods, but there was no significant difference in fresh matter yield among the treatments ($p > 0.05$). The crude protein, dry matter, and fresh matter yields of the first sampled awnless brome under the alfalfa–awnless brome mix were the lowest among the three sowing patterns, with the values of 0.12 t/ha, 0.52 t/ha, and 1.06 t/ha, respectively. The second sampled awnless brome under the monoculture pattern had the best performance, with the crude protein, dry matter, and fresh matter yields of 1.2 t/ha, 4.92 t/ha, and

11.90 t/ha, respectively. Furthermore, there was no significant variation between stubbles for alfalfa and awnless brome in the same sowing patterns (Table 7).

Table 7. The dynamics of the yield of alfalfa and awnless brome.

Species	Cut	Speeding Model	Crude Protein Yield (t/ha)	Dry Matter Yield (t/ha)	Fresh Forage Yield (t/ha)
Medicago sativa First Year	First cut	P	0.34 ± 0.09 aB	2.05 ± 0.52 aB	7.13 ± 1.73 aB
		D	0.81 ± 0.25 aA	4.51 ± 1.39 aA	14.72 ± 4.28 aA
		M	0.41 ± 0.09 aB	2.55 ± 0.55 aB	8.49 ± 1.69 aB
	Second cut	P	1.05 ± 0.67 aAB	5.17 ± 3.31 aAB	13.48 ± 8.17 aA
		D	1.19 ± 0.37 aA	5.57 ± 1.75 aA	17.67 ± 5.35 aA
		M	0.63 ± 0.26 aB	2.98 ± 1.24 aB	9.40 ± 4.38 aA
Medicago sativa Second Year	First cut	P	1.32 ± 0.21 Bbc	8.09 ± 0.86 Bb	26.33 ± 3.33 Ab
		D	1.82 ± 0.46 Aab	10.25 ± 1.14 Ab	44.92 ± 7.25 Aa
		M	0.97 ± 0.18 Ac	5.75 ± 0.62 Ac	21.87 ± 1.31 Ab
	Second cut	P	2.99 ± 0.42 Aa	14.81 ± 2.05 Aa	18.07 ± 2.50 Bb
		D	1.37 ± 0.16 Abc	6.48 ± 0.49 Bbc	21.13 ± 0.88 Bb
		M	1.05 ± 0.28 Ac	5.23 ± 1.44 Ac	12.97 ± 4.38 Bb
Bromus inermis First Year	First cut	P	0.12 ± 0.097 aB	0.52 ± 0.42 aB	1.06 ± 0.58 aB
		D	0.34 ± 0.11 aA	1.42 ± 0.48 aA	4.35 ± 1.76 aA
		M	0.28 ± 0.08 aA	1.25 ± 0.36 aA	3.88 ± 1.67 aA
	Second cut	P	0.13 ± 0.036 aB	0.57 ± 0.16 aB	1.75 ± 0.64 aB
		D	0.40 ± 0.14 aB	1.44 ± 0.51 aB	4.48 ± 1.21 aB
		M	1.20 ± 0.96 aA	4.92 ± 3.93 aA	11.90 ± 6.09 aA
Bromus inermis Second Year	irst cut	P	0.29 ± 0.10 b	2.83 ± 0.52 b	8.73 ± 1.57 b
		D	0.46 ± 0.22 a	2.94 ± 1.46 b	11.89 ± 5.48 b
		M	0.86 ± 0.43 a	4.95 ± 1.92 ab	16.70 ± 7.17 ab
	Second cut	P	0.14 ± 0.04 b	1.03 ± 0.32 b	2.77 ± 0.50 a
		D	0.46 ± 0.24 a	3.14 ± 1.70 a	9.28 ± 5.72 a
		M	0.18 ± 0.09 ab	1.05 ± 0.49 b	3.15 ± 1.48 a

Peer mix (P), heterocomplex (H), monoculture (M). Capital letters indicate significant differences in the same planting pattern at different stubble times at the 0.05 level ($p < 0.05$). Lowercase letters indicate significant differences in the different planting patterns at the same stubble times at the 0.05 level ($p < 0.05$).

3.4. Correlation Coefficients of Alfalfa and Awnless Brome Stem and Leaf Traits with Their Dry Matter Yields under Different Treatments

The equal-weight correlation degree was derived using forage dry matter yield as the reference column; however, the equal-weight correlation degree can only evaluate the significance of different varieties under the condition that the traits are equally important. The data of correlation coefficients among different cuts and treatments of alfalfa is in Table 8. The data of correlation coefficients among different cuts and treatments of awnless brome is in Table 9.

Practically, the importance of different traits in alfalfa is different, and their weights are expressed according to the magnitude of the correlation degree. The values obtained were $\omega1 = 0.1674$, $\omega2 = 0.1609$, $\omega3 = 0.1742$, $\omega4 = 0.1582$, $\omega5 = 0.1654$, and $\omega6 = 0.1739$. A higher value means that the trait contributes more to the dry matter yield. Therefore, the order of weight of each trait in the evaluation index was leaf area > stem diameter > leaf length > stem length > leaf width > leaf thickness (Figure 2). The weighted index of each trait in awnless brome was $\omega1 = 0.1703$, $\omega2 = 0.1668$, $\omega3 = 0.1760$, $\omega4 = 0.1613$, $\omega5 = 0.1672$, and $\omega6 = 0.1584$, with the order of evaluation index being leaf area > leaf length > stem length > leaf width > leaf thickness > stem diameter (Figure 3).

Table 8. Correlation coefficients among different cuts and treatments of alfalfa.

Cut–Treatment	Leaf Length	Leaf Width	Leaf Area	Leaf Thickness	Stem Length	Stem Diameter
First cut of P	0.45	0.42	0.37	0.38	0.55	0.40
Second cut of P	0.81	0.72	0.68	0.54	0.81	0.96
First cut of H	0.63	0.63	0.89	0.67	0.50	0.55
Second cut of H	0.41	0.41	0.36	0.46	0.43	0.39
First cut of M	0.57	0.62	0.55	0.59	0.56	0.49
Second cut of M	0.79	0.71	0.96	0.83	0.76	1.01

Peer mix (P), heterocomplex (H), monoculture (M).

Table 9. Correlation coefficients among different cuts and treatments of awnless brome.

Cut–Treatment	Leaf Length	Leaf Width	Leaf Area	Leaf Thickness	Stem Length	Stem Diameter
First cut of P	0.51	0.51	0.51	0.57	0.48	0.45
Second cut of P	0.49	0.51	0.51	0.55	0.50	0.59
First cut of H	0.85	0.88	0.90	0.77	0.87	0.99
Second cut of H	1.00	0.90	0.97	0.77	1.00	0.68
First cut of M	0.88	0.86	0.99	0.86	0.81	0.75
Second cut of M	0.37	0.35	0.35	0.37	0.37	0.36

Peer mix (P), heterocomplex (H), monoculture (M).

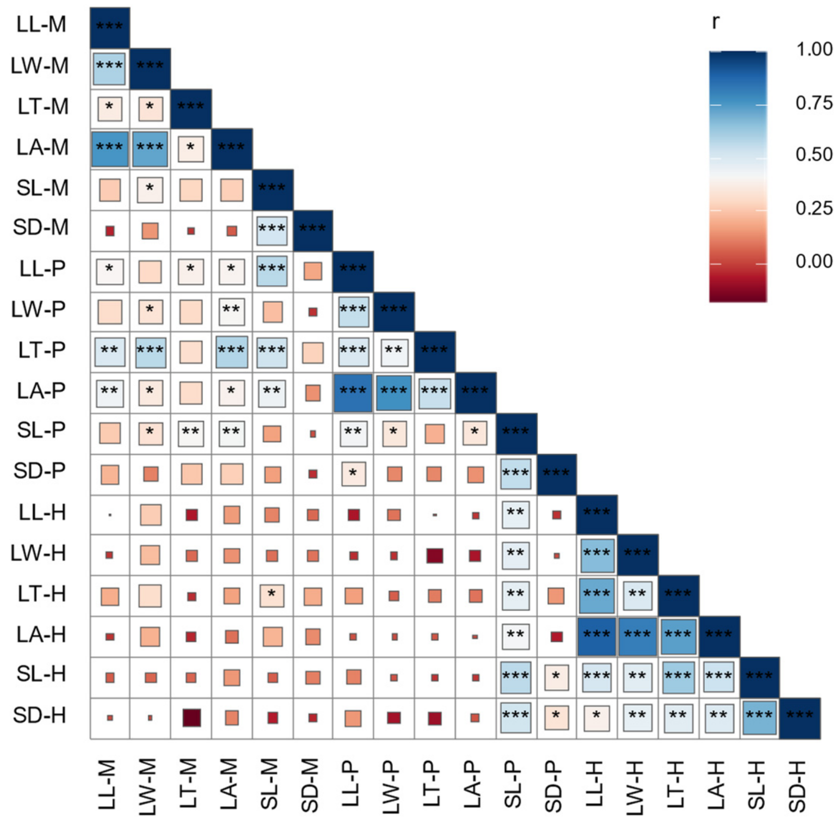

Figure 2. Correlation of *Medicago sativa* stem and leaf indicators under different sowing methods. LL is leaf length. LW is leaf width. LT is leaf thickness. LA is leaf area. SL is stem length. SD is stem diameter. Peer mix (P), heterocomplex (H), monoculture (M). Significance levels are as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

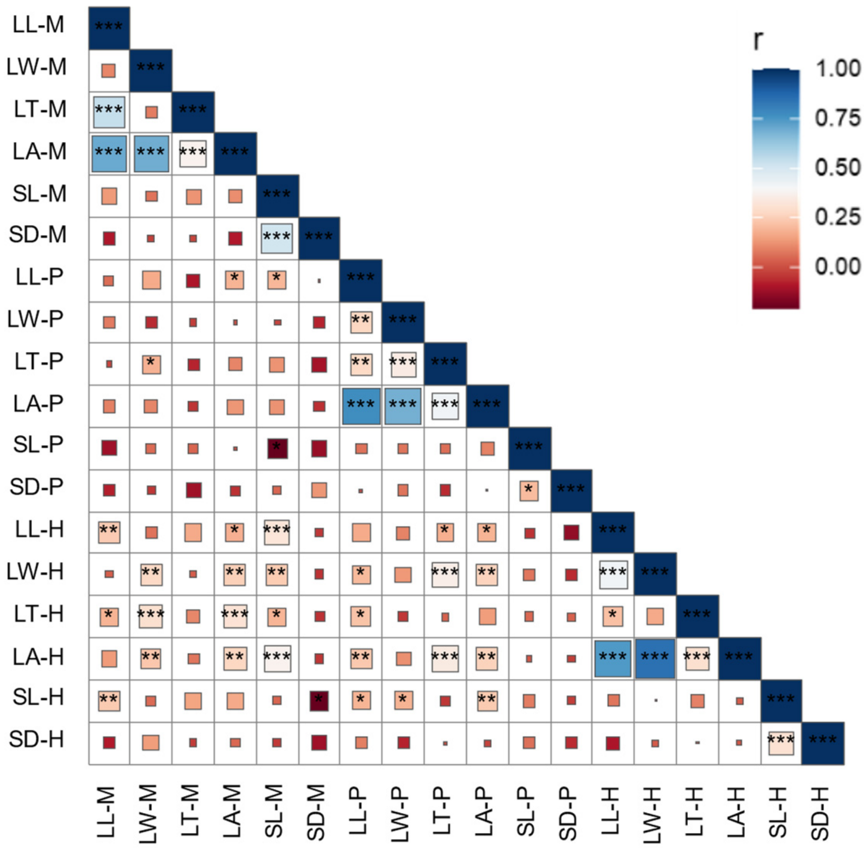

Figure 3. Correlation of *Bromus inermis* stem and leaf indicators under different sowing methods. LL is leaf length. LW is leaf width. LT is leaf thickness. LA is leaf area. SL is stem length. SD is stem diameter. Peer mix (P), heterocomplex (H), monoculture (M). Significance levels are as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

4. Discussion

4.1. Effect of Different Sowing Patterns on Forage Stem and Leaf Traits

Spatial distribution changes of plant populations (in the same row and alternate rows) can directly affect their access to light energy, interspecific competition above and below ground, and yield potential [27]. Stem and leaf traits are intrinsic physiological and external morphological measures of adaptive responses developed by plants in response to environmental changes. The traits are closely related to plant biomass, plant access to and utilization efficiency of resources [28], and the survival strategies adopted by plants to obtain maximum material harvest [29]. In this study, we found that alfalfa stem and leaf traits of the first sampled crop in the first year were significantly higher under the alfalfa–awnless brome mixed treatment than under the hetero-row mixed and monoculture treatments. However, the stem and leaf traits of the second sampled alfalfa crop were significantly lower than those of the first sampled crop in the monoculture and alfalfa–awnless brome hetero-row mixed treatments. This phenomenon may be because awnless brome has poor regeneration and growth rate compared to alfalfa after mowing, and awnless brome is a low plant, while alfalfa is a high plant in the mixed population [30]. After mowing, awnless brome was subjected to stronger shading by alfalfa under the same row mix treatment compared to the different row mix treatment, inhibiting the growth and development of awnless brome, eventually resulting in poor development of awnless brome stems and leaves. The leaf width and diameter of awnless brome in the first year were significantly lower under the alfalfa–awnless brome treatment compared to the other treatments. In addition, alfalfa stem and leaf traits of the second sampled crop in the second year were significantly improved under the alfalfa–awnless brome hetero-row mix treatment than under the peer mix and monoculture treatments. Moreover, the stem and leaf traits were significantly higher in the second sample alfalfa crop compared to the first sampled crop, indicating that the growth of awnless brome under the hetero-row mix treatment was promoted with increasing planting years and crop frequency, but suppressed under the other two sowing methods. This could be because mowing stimulates tillering of grasses and branching of leguminous plants, and the more adequate exposure of awnless brome to light after mowing under the inter-row mix treatment reduces the interspecific competition and promotes the compensatory growth of awnless brome [31,32].

4.2. Effects of Different Sowing Patterns on the Quantitative Traits of Forage Grasses

Forage yield is among the most important indicators for forage evaluation. Different plant species differ significantly due to their interspecific competitiveness, which affects the population size and the yield of mixed planted grass [33]. DU Jun-ying [30] showed that alfalfa yield was significantly higher under alfalfa–awnless brome mixed sowing treatment than under the monoculture system. In this study, the protein, dry matter, and fresh matter yields of the first and second sampled alfalfa crops under the alfalfa–awnless brome peer mixed sowing pattern were not significantly different from those under single sowing. However, the yield of alfalfa crops under the alfalfa–awnless brome heterogeneous mix was significantly higher than that of the peer mix and monoculture. This was consistent with the results of Yuqiang Tian [34], which showed that the heterogeneous row mix treatment significantly increased the relative density of grass forage compared to the peer mix treatment, significantly increasing the yield.

The protein, dry matter, and fresh matter yields of the first and second sampled awnless brome crops were significantly higher under the monoculture treatment than under the peer and heterogeneous row mix treatments. There was no significant difference in all yields of awnless brome under the peer and heterogeneous row mix treatments. The difference between the awnless brome yield reported in our study and the results reported by Wang Bin [35] may be because awnless brome was mowed at the same time as alfalfa (at the early flowering stage of alfalfa) when it was in the gestation stage. The effect of light intensity and wind speed within the grass population during the mowing period ranked as monosowed awnless brome > peer mixed and inter-row mixed > monosowed alfalfa. For the temperature, the pattern was monosowed awnless brome > monosowed alfalfa > peer mix, inter-row mix. The microclimatic conditions of awnless brome under monoculture were better than those under the other treatments. Moreover, the mixed sowing changed the biomass accumulation pattern of awnless brome [36], eventually reducing awnless brome yield in the mixed sowing system.

4.3. Effects of Different Stem and Leaf Traits on Forage Yield Traits

Stem and leaf traits are important indicators of plant growth, and as the main photosynthesis organs, the leaves play a critical role in light energy acquisition, water and nutrient uptake, and organic matter synthesis [37,38]. The stem length and diameter of forage grasses reflect the growth and development of forage grasses and grass productivity [39], indicating their important role as indicators of the agronomic traits of forage grasses. The gray correlation analysis and the dry matter yield of forage stem and leaf traits showed leaf area, stem diameter, and leaf length were the top three stem and leaf traits that contributed more to the dry matter yield of alfalfa [40]. The slower-growing species in the mixed sowing system were affected by the shade effect because shade strongly affects the development of forages by changing their biomass allocation strategy and reducing the growth height of the forage [41–43]. The stem length of awnless brome contributed more to its dry matter yield than stem diameter, suggesting that adequate light resources increase the dry matter yield of awnless brome.

5. Conclusions

Mixing alfalfa with awnless brome in different rows can improve the stem and leaf functional traits, dry matter yield, and crude protein yield of both forages. The inter-row mixing significantly improved the stem and leaf functional traits of alfalfa but limited the growth and development of awnless brome in the late growth stage. The weighting order of the evaluation indices was dry matter yield > protein yield > leaf area > leaf length > leaf width > stem diameter > leaf thickness > stem length for alfalfa and dry matter yield > protein yield > leaf area > leaf length > leaf width > stem length > leaf thickness > stem diameter for awnless brome. In summary, heterogeneous row mixing is more suitable than the other treatments considered for alfalfa and awnless brome mixed planting.

Author Contributions: Conceptualization, X.Y. and F.M.; methodology, F.M.; software, X.T.; validation, X.L.; formal analysis, X.Y.; investigation, W.T.; resources, C.Y.; data curation, G.Y.; writing—original draft preparation, Y.Z.; writing—review and editing, J.S.; visualization, X.Y.; supervision, Y.X.; project administration, J.S.; funding acquisition, J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the China Agriculture Research System (CARS-34), the Natural Science Foundation of Shandong Province (ZR2020QC189), the National Key Research and Development Program of China (No. 2022YFD1300802), the Doctoral Scientific Research Startup of Qingdao Agricultural University (6631116024), the Shandong Forage Research System (SDAIT-23-01), and the First-Class Grassland Science Discipline Program of Shandong Province (1619002), the "Youth Innovation Team Plan" of Universities in Shandong Province (2022KJ166).

Data Availability Statement: Not applicable.

Acknowledgments: The authors are thankful for the support of the foundation and thank the teachers and students for their help and guidance.

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

References

1. Liu, X.; Tahir, M.; Li, C.; Chen, C.; Xin, Y.; Zhang, G.; Cheng, M.; Yan, Y. Mixture of Alfalfa, Orchardgrass, and Tall Fescue Produces Greater Biomass Yield in Southwest China. *Agronomy* **2022**, *12*, 2425. [CrossRef]
2. Zhu, L.; Li, X.; He, J.; Zhou, X.; Wang, F.; Zhao, Y.; Liang, X.; Nan, X.; Li, Y.; Qin, K.; et al. Development of *Lycium barbarum*–Forage Intercropping Patterns. *Agronomy* **2023**, *13*, 1365. [CrossRef]
3. Zheng, E.; Zhu, Y.; Qin, M.; Chen, P.; Liu, M.; Qi, Z. Effects of Organic Fertilizer Replacement Nitrogen Fertilizer on Nitrogen Utilization and Growth of Mung Bean: Evidence from 15N-Tracing Technology. *Agronomy* **2023**, *13*, 235. [CrossRef]
4. Suter, M.; Huguenin-Elie, O.; Lüscher, A. Multispecies for multifunctions: Combining four complementary species enhances multifunctionality of sown grassland. *Sci. Rep.* **2021**, *11*, 3835. [CrossRef]
5. Li, Y.; Moinet, G.Y.K.; Clough, T.J.; Whitehead, D. Organic matter contributions to nitrous oxide emissions following nitrate addition are not proportional to substrate-induced soil carbon priming. *Sci. Total Environ.* **2022**, *851*, 158274. [CrossRef]
6. Fan, F.; Yang, Q.; Li, Z.; Wei, D.; Cui, X.; Liang, Y. Impacts of organic and inorganic fertilizers on nitrification in a cold climate soil are linked to the bacterial ammonia oxidizer community. *Microb. Ecol.* **2011**, *62*, 982–990. [CrossRef]
7. Sun, R.; Guo, X.; Wang, D.; Chu, H. Effects of long-term application of chemical and organic fertilizers on the abundance of microbial communities involved in the nitrogen cycle. *Appl. Soil Ecol.* **2015**, *95*, 171–178. [CrossRef]
8. Ghani, M.U.; Kamran, M.; Ahmad, I.; Arshad, A.; Zhang, C.; Zhu, W.; Lou, S.; Hou, F. Alfalfa-grass mixtures reduce greenhouse gas emissions and net global warming potential while maintaining yield advantages over monocultures. *Sci. Total Environ.* **2022**, *849*, 157765. [CrossRef]
9. Meza, K.; Vanek, S.J.; Sueldo, Y.; Olivera, E.; Ccanto, R.; Scurrah, M.; Fonte, S.J. Grass—Legume Mixtures Show Potential to Increase Above- and Belowground Biomass Production for Andean Forage-Based Fallows. *Agronomy* **2022**, *12*, 142. [CrossRef]
10. Finn, J.A.; Kirwan, L.; Connolly, J.; Sebastià, M.T.; Helgadottir, A.; Baadshaug, O.H.; Bélanger, G.; Black, A.; Brophy, C.; Collins, R.P.; et al. Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: A 3-year continental-scale field experiment. *J. Appl. Ecol.* **2013**, *50*, 365–375. [CrossRef]
11. Jensen, E.S.; Peoples, M.B.; Boddey, R.M.; Gresshoff, P.M.; Hauggaard-Nielsen, H.; Alves, B.J.R.; Morrison, M.J.J.A.f.S.D. Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. *Agron. Sustain. Dev.* **2012**, *32*, 329–364. [CrossRef]
12. Pappa, V.A.; Rees, R.M.; Walker, R.L.; Baddeley, J.A.; Watson, C.A. Nitrous oxide emissions and nitrate leaching in an arable rotation resulting from the presence of an intercrop. *Agric. Ecosyst. Environ.* **2011**, *141*, 153–161. [CrossRef]
13. Kumar, S. Biotechnological advancements in alfalfa improvement. *J. Appl. Genet.* **2011**, *52*, 111–124. [CrossRef]
14. Singer, S.D.; Hannoufa, A.; Acharya, S. Molecular improvement of alfalfa for enhanced productivity and adaptability in a changing environment. *Plant Cell Environ.* **2018**, *41*, 1955–1971. [CrossRef] [PubMed]

15. Singh, A.K.; Singh, J.B.; Singh, R.; Kantwa, S.R.; Jha, P.K.; Ahamad, S.; Singh, A.; Ghosh, A.; Prasad, M.; Singh, S.; et al. Understanding Soil Carbon and Phosphorus Dynamics under Grass-Legume Intercropping in a Semi-Arid Region. *Agronomy* **2023**, *13*, 1692. [CrossRef]
16. Salama, H.S.A.; Nawar, A.I.; Khalil, H.E. Intercropping Pattern and N Fertilizer Schedule Affect the Performance of Additively Intercropped Maize and Forage Cowpea in the Mediterranean Region. *Agronomy* **2022**, *12*, 107. [CrossRef]
17. Tahir, M.; Wei, X.; Liu, H.; Li, J.; Zhou, J.; Kang, B.; Jiang, D.; Yan, Y. Mixed legume-grass seeding and nitrogen fertilizer input enhance forage yield and nutritional quality by improving the soil enzyme activities in Sichuan, China. *Front. Plant Sci.* **2023**, *14*, 1176150. [CrossRef]
18. Pan, J.; Miao, Q.; Zhang, W.; Wang, H.; Wang, W.; Xue, Y.; Qi, S.; Cui, Z. Increasing Soil Organic Carbon for Higher Wheat Yield and Nitrogen Productivity. *Agronomy* **2023**, *13*, 198. [CrossRef]
19. Pampana, S.; Arduini, I.; Andreuccetti, V.; Mariotti, M. Fine-Tuning N Fertilization for Forage and Grain Production of Barley–Field Bean Intercropping in Mediterranean Environments. *Agronomy* **2022**, *12*, 418. [CrossRef]
20. Hau, F.C.; Campbell, C.L.; Beute, M.K. Inoculum distribution and sampling methods for Cylindrocladium crotalariae in a peanut field. *Plant Dis.* **1982**, *66*, 568–571. [CrossRef]
21. Lin, C.S.; Poushinsky, G.; Mauer, M. An Examination of Five Sampling Methods under Random and Clustered Disease Distributions Using Simulation. *Can. J. Plant Sci.* **1979**, *59*, 121–130. [CrossRef]
22. Miller, C.M.F.; Fadel, J.G.; Heguy, J.M.; Karle, B.M.; Price, P.L.; Meyer, D. Optimizing accuracy of protocols for measuring dry matter and nutrient yield of forage crops. *Sci. Total Environ.* **2018**, *624*, 180–188. [CrossRef] [PubMed]
23. Liu, J.; Lu, S.; Liu, C.; Hou, D. Nutrient reallocation between stem and leaf drives grazed grassland degradation in inner Mongolia, China. *BMC Plant Biol.* **2022**, *22*, 505. [CrossRef] [PubMed]
24. Poorter, H.; Niklas, K.J.; Reich, P.B.; Oleksyn, J.; Poot, P.; Mommer, L. Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytol.* **2012**, *193*, 30–50. [CrossRef] [PubMed]
25. Hahn, P.G.; Maron, J.L. A Framework for Predicting Intraspecific Variation in Plant Defense. *Trends Ecol. Evol.* **2016**, *31*, 646–656. [CrossRef]
26. Li, X.-L.; Liu, Z.-Y.; Ren, W.-B.; Ding, Y.; Ji, L.; Guo, F.-H.; Hou, X.-Y. Linking nutrient strategies with plant size along a grazing gradient: Evidence from *Leymus chinensis* in a natural pasture. *J. Integr. Agric.* **2016**, *15*, 1132–1144. [CrossRef]
27. Faligowska, A.; Kalembasa, S.; Kalembasa, D.; Panasiewicz, K.; Szymańska, G.; Ratajczak, K.; Skrzypczak, G. The Nitrogen Fixation and Yielding of Pea in Different Soil Tillage Systems. *Agronomy* **2022**, *12*, 352. [CrossRef]
28. Emam, M.A.; Abd El-Mageed, A.M.; Niedbała, G.; Sabrey, S.A.; Fouad, A.S.; Kapiel, T.; Piekutowska, M.; Mahmoud, S.A. Genetic Characterization and Agronomic Evaluation of Drought Tolerance in Ten Egyptian Wheat (*Triticum aestivum L.*) Cultivars. *Agronomy* **2022**, *12*, 1217. [CrossRef]
29. Dong, S.; Wang, G.; Li, X.; Kang, Y. A Trade-Off between the Growing Performance and Sowing Density of Sunflower (*Helianthus annuus L.*) under Fertigation in an Arid Saline Area. *Agronomy* **2023**, *13*, 179. [CrossRef]
30. Chen, S.; Yang, P.; Zhang, Y.; Dong, W.; Hu, C.; Oenema, O. Responses of Cereal Yields and Soil Carbon Sequestration to Four Long-Term Tillage Practices in the North China Plain. *Agronomy* **2022**, *12*, 176. [CrossRef]
31. Chen, K.; Ma, T.; Ding, J.; Yu, S.E.; Dai, Y.; He, P.; Ma, T. Effects of Straw Return with Nitrogen Fertilizer Reduction on Rice (*Oryza sativa L.*) Morphology, Photosynthetic Capacity, Yield and Water–Nitrogen Use Efficiency Traits under Different Water Regimes. *Agronomy* **2022**, *13*, 133. [CrossRef]
32. Chen, G.; Fan, W.; Yin, W.; Fan, Z.; Wan, S.; Zhai, Y.; Zhang, X. Soil Aggregates Are Governed by Spacing Configurations in Alfalfa-Jujube Tree Intercropping Systems. *Agronomy* **2023**, *13*, 264. [CrossRef]
33. Centurión, N.; Mariscal-Sancho, I.; Navas, M.; Gabriel, J.L.; Ulcuango, K.; Ibáñez, M.Á.; Moliner, A.; Hontoria, C. Increasing Legume Input through Interseeding Cover Crops: Soil and Crop Response as Affected by Tillage System. *Agronomy* **2023**, *13*, 1388. [CrossRef]
34. Tian, Y.; Sun, W.; Song, M.; Zhao, Y.; Wen, S.; Cui, Y.; Li, X.; Xu, X. Effects of grass-legume mixture on plant production and inorganic nitrogen acquisition. *Rhizosphere* **2021**, *20*, 100447. [CrossRef]
35. Wang, B.; Yang, Y.-J.; Dong, X.; Li, M.-Y.; Shen, X.-T.; Cao, L.-J.; Lan, J. Effects of Reseeding Grass on Production Performance and Forage Quality of Degraded Alfalfa Grassland. *Acta Agrestia Sin.* **2021**, *29*, 618–624. [CrossRef]
36. Zhang, H.-H.; Shi, S.-L.; Wu, B.; Li, Z.-L.; Li, X.-L. A study of yield interactions in mixed sowings of alfalfa and three perennial grasses. *Acta Prataculturae Sin.* **2022**, *31*, 159–170. [CrossRef]
37. Wright, I.J.; Reich, P.B.; Westoby, M.; Ackerly, D.D.; Baruch, Z.; Bongers, F.; Cavender-Bares, J.; Chapin, T.; Cornelissen, J.H.; Diemer, M.; et al. The worldwide leaf economics spectrum. *Nature* **2004**, *428*, 821–827. [CrossRef]
38. Firn, J.; McGree, J.M.; Harvey, E.; Flores-Moreno, H.; Schütz, M.; Buckley, Y.M.; Borer, E.T.; Seabloom, E.W.; La Pierre, K.J.; MacDougall, A.M.; et al. Leaf nutrients, not specific leaf area, are consistent indicators of elevated nutrient inputs. *Nat. Ecol. Evol.* **2019**, *3*, 400–406. [CrossRef]
39. Mitchell, R.B.; Redfearn, D.; Moore, K.J. Growth and Development. *Forages* **2020**, *2*, 127–148.
40. Putnam, D.H. Factors Influencing Yield and Quality in Alfalfa. In *The Alfalfa Genome*; Yu, L.-X., Kole, C., Eds.; Springer International Publishing: Cham, Switzerland, 2021; pp. 13–27.
41. Moreno, L.S.B.; Boote, K.J.; Sollenberger, L.E.; Dubeux, J.C.B.; Kohmann, M.M.; Pequeno, D.N.L. Shade and nitrogen fertilization affect forage accumulation and nutritive value of C4 grasses differing in growth habit. *Crop. Sci.* **2022**, *62*, 512–523. [CrossRef]

42. Angadi, S.V.; Umesh, M.R.; Begna, S.; Gowda, P. Light interception, agronomic performance, and nutritive quality of annual forage legumes as affected by shade. *Field Crop. Res.* **2022**, *275*, 108358. [CrossRef]
43. Norton, B.; Wilson, J.; Shelton, H.; Hill, K. *The Effect of Shade on Forage Quality*; ACIAR: Canberra, Australia, 1991.