

Article

Long-Term Production Performance and Stability of Alfalfa/Grass Mixtures in the Longdong Loess Plateau of China: Subjected to Various Species Combinations and Seeding Ratios

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Abstract

Stable productivity is the basis for efficient and sustainable use of perennial grasslands, holding both ecological and economic importance. Alfalfa-based mixtures have great potential to achieve this goal. There was limited information on the impact of species combination and seeding ratio on their long-term production performance and stability. We investigated forage yield, quality, and temporal stability over six years in alfalfa (*Medicago sativa*)/timothy (*Phleum pretense*) and alfalfa/smooth brome grass (*Bromus inermis*) mixtures at varying seeding ratios. Alfalfa/grass mixtures showed a yield advantage over grass monocultures with greater yield at higher alfalfa seeding proportions (50% or more). The mixtures showed advantages in crude protein and neutral detergent fiber. Crude protein content tended to increase with increasing alfalfa seeding proportion, while fiber contents barely changed. As stands grew older, forage yield increased and then declined and showed greater stability in mixtures compared with monocultures. The percentage of alfalfa yield tended to increase over the life of the stand. In contrast, forage quality varied over the life of the stand, with greater variability in mixtures than monocultures. Considering forage yield, quality, and stability across years, smooth brome grass would be more compatible with alfalfa in a mixture compared to timothy for the Longdong Loess Plateau of China and areas with similar climates.

Keywords: alfalfa/grass mixture; forage yield; forage quality; temporal stability; stand age



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1. Introduction

Cultivated grassland provides fundamental support for the development of animal husbandry [1] and holds irreplaceable ecological and economic importance [2,3]. In comparison to annual cropping, the cultivated perennial grassland offers diverse advantages such as reducing planting costs [4], extending the forage supply duration [5], reducing soil disturbance to stabilize C stocks [6], and decreasing soil erosion [7]. Intercropping of perennial forages (containing at least one perennial species, e.g., alfalfa (*Medicago sativa*)) has demonstrated significant benefits in improving yield and land use efficiency [8–10]. Stable and sustainable productivity are critical for farmers to reduce inputs and cope with climate change [11]. Therefore, the stability of high forage yield and quality of the perennial

grassland is a major concern in practice. However, research with cultivated perennial grasslands has paid less attention to variation in long-time production in temperate and dry regions (e.g., the Loess Plateau of China). Therefore, more prolonged investigations with various perennial grasslands are needed, especially to better understand factors supporting the stability of forage production over time.

Stability is vital for a perennial grassland, which is readily affected by multiple factors [12,13]. Sward stability can still be influenced by the living conditions such as climate or soil condition [14,15]. Seasonal and inter-annual climatic variation may lead to changes in forage yield and quality of mowed grasslands with multiple harvests per year [16,17]. Furthermore, soil fertility is closely related to the stability of agricultural systems [18]. Nutrient imbalance results in reduced yield, quality and thus compromises stability [19]. Therefore, management actions which affect living conditions will play important roles in controlling the stability of productivity. For example, fertilization and irrigation can change the temporal stability of community yield by changing plant growth conditions [20,21]. In forage mixtures, species combination and seeding ratio are the main determinants of stability. In mixtures, the effects of species combination and seeding ratio on production and stability are closely related to resource availability [22] and the interspecific relationship among forage species [12,23]. Therefore, altering species combination and seeding ratio would in turn affect production and even stability [24,25]. However, more long-term studies are still needed to uncover the temporal patterns of stability in response to various species combinations and seeding ratios.

Optimizing species combination and seeding ratio (the ratio of species seeding proportions) are fundamental strategies in precisely managing a perennial mixture. Legume species show advantages in establishing a successful cultivated grassland due to strong biological N₂ fixation (BNF), high yield and crude protein content, and strong adaptability. Legume/grass mixtures significantly increase yield stability compared to monocultures [12,23]. For example, mixtures of timothy (*Phleum pratense*) and red clover (*Trifolium pratense*) can sustain yields well for multiple years, and adoption of adaptive cultivars can further improve yield performance and enhance the persistence of stands [26]. Apart from species combination (i.e., the specific assembly of different forage species), species balance (i.e., species seeding proportion or seeding ratio) also strongly influences the temporal stability of grassland communities [27,28]. Adjusting species seeding proportion can change the dominance of species in the sward and the relationships among species [14], which in the long run leads to changes in community stability. For example, a high proportion of legume forage within legume/grass mixtures has shown to negatively impact forage yield stability [29]. However, how these two factors affect productivity stability and long-term persistence has received relatively little attention in perennial forage mixtures in the Loess Plateau.

Alfalfa is a widely grown perennial legume in China and is planted across 2300 thousand hectares [30]. Many studies on diverse alfalfa-based cropping systems have shown great advantages in yield and quality performance [31,32]. The strong BNF and deep root system make alfalfa a vital component in intercropping (including mixtures) and rotation systems. These systems can effectively improve resource utilization, avoid soil dryness, and alleviate nutrient limitation through the complementarity and synergy between species [33–35]. The stands of alfalfa monoculture may last from 2 to 10 years depending on the stability of pasture yield and soil water depletion [36,37]. In the Longdong Loess Plateau area, the optimal length of alfalfa phase in alfalfa–crop rotation systems should not be over 8 years [36,38] due to deep water depletion, which affects the stability of the systems. There is no research clearly reporting the optimal production duration of alfalfa/grass forage mixtures, although studies on alfalfa/grass mixtures have accumulated [33,39]. However, there were few studies on

assessing the stability of productivity of alfalfa mixtures for more than 5 years. Therefore, a better understanding of the variability and stability of productivity and sustainability of the mixture over time would allow for better recommendations on how to optimize alfalfa-based grassland production.

This study aimed to test the hypothesis that alfalfa/grass mixtures would result in more resilient forage productivity and nutritional quality compared with monocultures. The objectives of this study were to (1) assess the effects of species combination and seeding ratio on forage yield and quality of alfalfa/grass mixtures, and (2) assess the variation in productivity of the mixtures in successive years. To address these objectives, we conducted a field experiment testing combinations of alfalfa/grass mixtures at three seeding ratios over six production years.

2. Materials and Methods

2.1. Experimental Site Description

We carried out a field experiment during 2017–2023 at the National Field Scientific Observation and Research Station of Grassland Agro-ecosystems in Qingyang, Gansu Province, China (107°51' E, 35°40' N, 1297 m a.s.l.). It is a typical semi-arid rainfed agricultural area with continental monsoon climate. The region experiences a long-term average (from 1970 to 2023) annual temperature of 9.8 °C and an average annual rainfall of 544 mm. Mean monthly precipitation and temperature for the whole experiment (2017/9–2023/9) are shown in Figure 1. The prevailing type of soil in the area is classified as Heilu soil (Entisol of FAO classification). Prior to establishing the experimental plots, the soil basic properties in 0–90 cm layers were recorded and found to contain 3.71 kg m⁻² organic carbon, 0.44 kg m⁻² total nitrogen (N), and 0.86 kg m⁻² total phosphorus (P).

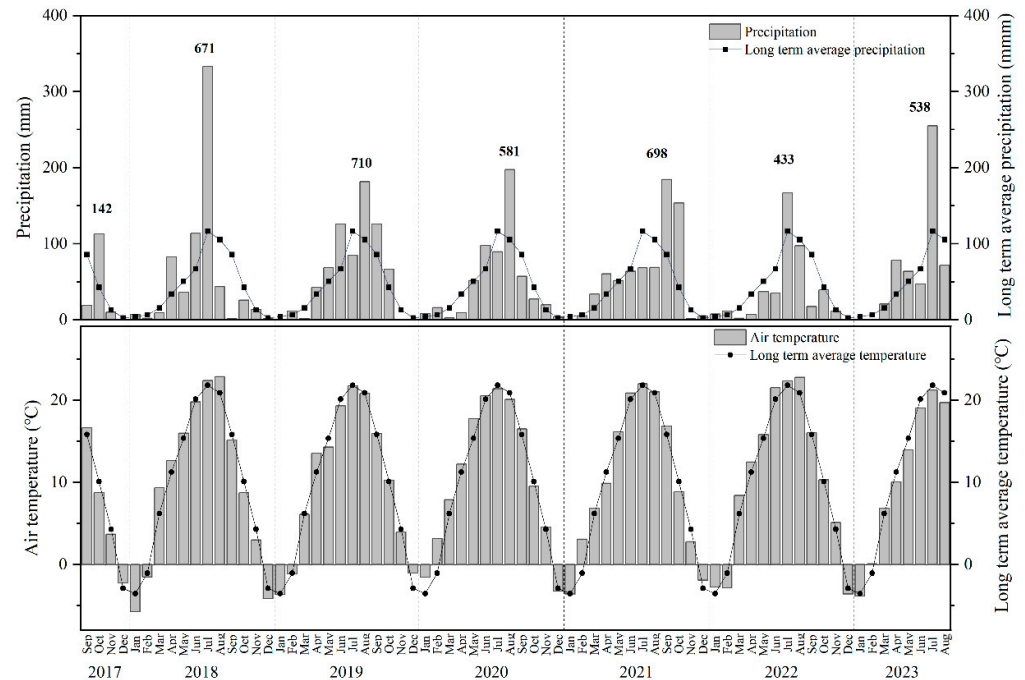


Figure 1. Changes in monthly precipitation and air temperature during 2017–2023, and the long-term (1970–2023) mean values at the study site. Data above the bars show annual precipitation in the experimental duration.

2.2. Experimental Design and Treatments

The field experiment was conducted in a completely randomized block design. One legume forage, alfalfa (*M. sativa* L. cv. Gannong No. 3, M), and two grasses, timothy

(P) and smooth brome grass (B), were sown in monoculture or legume/grass mixtures. The two species combinations included alfalfa/timothy mixture (MP) and alfalfa/smooth brome grass mixture (MB). Three seeding ratios were designed to create mixtures of 3:7 (alfalfa–grass, labeled as M3P7 and M3B7), 5:5 (labeled as M5P5 and M5B5), and 7:3 (labeled as M7P3 and M7B3). Alfalfa seeding proportion in the mixture would be 30%, 50%, and 70%. Seeding rates in monocultures were 15, 15 and 30 kg·ha^{−1} for alfalfa, timothy and smooth brome grass, respectively. Therefore, seeding rate of each species in a mixture was calculated by multiplying the seeding rate in the monoculture by designed seeding proportion in the mixture. In total, there were nine treatments (three monocultures and six mixtures) and three replicates for each treatment. All plots were established on 26 September 2017 (establishment year, considered as age 1) with seed sowing by hands. Sampling was conducted over six complete production years, from 2018 (age 2) to 2023 (age 7). The plot size was 3 m × 3 m and the interval between plots was 0.5 m. There were 10 lines of forage in each plot with a row width of 30 cm. The row width and the corresponding plant spacing are consistent with the local conventional cultivation practices for forage crops in the research area, and this setup is widely used in local forage production fields. Before sowing in 2017, base fertilizers were applied at 50 kg·N ha^{−1} (urea, containing 46% N) and 60 kg·P ha^{−1} (superphosphate, containing P₂O₅ 46%), respectively. No fertilizer was applied to all plots from 2018 to 2021. The plots of grass monocultures showed a clear decline in biomass yield. Therefore, from 2022, N and P fertilizers were added to all plots in equal amounts and same forms as the base fertilizers at the forage regreening stage each year. Note that the amount of fertilizer applied in this experiment was half of the conventional fertilization rate. This was to minimize the impact of additional fertilizer management on the BNF of alfalfa, as BNF is a key link in sustaining the productivity of this forage system. Conventional farmland management practices were employed, and no irrigation was applied in the experimental duration.

Smooth brome grass and timothy are the common grasses used in the experimental areas. These two grass species utilized in this study are unregistered local varieties and both show good compatibility with Gannong No. 3 for mixed cropping, exhibiting significant advantages in terms of forage yield and quality [40,41]. All three forages are commercial cultivars, and the seeds were bought from the market.

2.3. Sampling and Measurements

Plant sampling was carried out over six full production years from 2018 to 2023. The actual dates of each cut were shown in Table 1. All experimental plots were cut twice at the mid-flowering stage of alfalfa (the grasses were at the flowering stage) in 2018–2020 and three times at the early flowering stage of alfalfa (the grasses were at the heading stage) in 2021–2023. Alfalfa has clear, consistent flowering phenology in the Longdong Loess Plateau of China, making it a reliable benchmark to unify sampling across all plots. The growth stages of timothy and smooth brome grass were consistent between their monoculture and mixture plots. At the synchronous sampling time, all experimental plots shared the same climate conditions, which minimized phenological differences in forage between treatments. It was assumed that the biomass yield and forage quality under two cuts annually might be underestimated in comparison with those under three cuts annually. Three 0.5 m-long plant lines in each plot were randomly sampled at a stubble height of 5 cm using hand clippers. In the plots of mixtures, samples were separated into alfalfa and grass. Herbage samples were oven-dried at 105 °C for 30 min and then at 75 °C for 48 h to measure the dry weight before calculating dry matter yield (DMY). The DMY of the mixtures was calculated by summing the DMY of alfalfa and grass.

Table 1. Harvest time in the experimental duration.

Cut	Year (Stand Age)					
	2018 (2)	2019 (3)	2020 (4)	2021 (5)	2022 (6)	2023 (7)
1st	24 June	27 June	26 June	4 June	1 June	1 June
2nd	24 August	27 August	19 August	12 July	6 July	8 July
3rd	-	-	-	28 August	25 August	25 August

The dried sample was ground into a fine powder that could pass through a 1 mm sieve for further laboratory measurements. Total N (TN) concentration in the shoot was measured using the automatic Kjeldahl method with a Kjeldahl 8400 auto-analyzer (FOSS, Hilleroed, Denmark), and subsequently crude protein (CP) content was calculated by multiplying TN by a conversion factor of 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using filter bags, following the detergent fiber analysis method of Van Soest et al. [42]. Note that a higher temperature to dry the sample may lead to underestimation of forage fiber content. An older version of instruction has previously been referred to measure forage fiber content at the beginning of this study a few years ago. Thus, to maintain methodological consistency across experimental years, the drying protocol has been retained to drying at 75 °C. Although this approach may underestimate forage fiber content, the errors should remain consistent both across years and among treatments, exerting little effect on the change trends. Therefore, it is thought that this temperature for drying samples may not significantly alter the fundamental conclusion of the study.

2.4. Calculation

The annual DMY was calculated by summing DMY of all yearly cuts (Equation (1)). For the quality parameters (CP, NDF, and ADF), annual content was further calculated by computing weighted average content of the year (Equation (2)) [43].

$$\text{Annual DMY} = \sum_{i=1}^r \text{DMY}_i \quad (1)$$

$$\text{Annual content} = \sum_{i=1}^r \left(\text{Content}_i \times \frac{\text{DMY}_i}{\text{Annual DMY}} \right) \quad (2)$$

where i is the i^{th} cut, and r is the total number of cuts per year.

We used the coefficient of variation (CV, the ratio of standard deviation to mean) as an index of variation to evaluate the temporal stability of forage productivity from 2018 to 2023, with a lower value indicating lower variability and, thus, higher stability [43,44]. Inter-annual CV (CV_{inter}) was calculated to show the change in forage yield and quality across years (Equation (3)).

$$\text{CV}_{\text{inter}} = \left(\frac{\sum_{n=1}^y (A_n - \bar{A}_y)^2}{y - 1} \right)^{\frac{1}{2}} \times \frac{1}{\bar{A}_y} \quad (3)$$

where A_n is the annual DMY or the weighted average content of quality parameters in the n^{th} year. $\bar{A}_y$ is the mean of annual DMY or the mean of average content of quality parameter across years and y is the total number of years.

The Technique for Order Preference by Similarity to an Ideal Solution (TOPSIS) method based on the entropy weight coefficient was used to calculate the comprehensive score of

forage yield and quality [45]. The weights of evaluation indicators (DMY, CP, NDF, and ADF) were calculated using entropy, and the optimal value close to the ideal result was determined through TOPSIS. Thus, the feeding potential of forage was evaluated. The score and $CV_{\text{inter-DMY}}$ under each cropping system were presented in a two-dimensional scatter plot to evaluate the whole performance of the mixture.

2.5. Statistical Analysis

Data analysis was performed using SPSS 25.0. All data were assessed for normal distribution and homogeneity through relevant tests before analysis. Considering that the effect of stand age on forage was cumulative, a three-factor repeated-measures analysis of variance (ANOVA) within the general linear model framework was used to analyze the effect of species combination, seeding ratio, stand age, and their interactions on forage yield and quality, with species combination and seeding ratio as between-subjects factors and stand age as within-subjects factor. Two-way ANOVA was performed to assess the effects of species combination and seeding ratio on the inter-annual stability of forage yield and quality. When effect tests were significant, Duncan post hoc multiple comparison was used to analyze the differences among stand ages or seeding ratios at a significance level of $p < 0.05$.

3. Results

3.1. Forage Yield

Stand age (SA), seeding ratio (SR), and their interactions ($SA \times SR$) significantly affected annual dry matter yield (DMY), while species combination (SC) significantly affected annual DMY only under its interaction with SR and/or SA (Table 2). All alfalfa-containing swards showed greater annual DMY than grass monocultures (Table 3). For MPs (alfalfa/timothy mixtures), a long-term annual DMY was higher in M7P3, and there was no difference between the other two mixtures. M7P3 had a similar yield to alfalfa monoculture, while annual DMY in the other two mixtures was lower than that in alfalfa monoculture. For MBs (alfalfa/smooth brome grass mixtures) there were no difference in a long-term run annual DMY. M7B3 and M5B5 had similar yield as alfalfa monoculture, while annual DMY in M3B7 was lower than that in alfalfa monoculture. Overall DMY peaked in the third year (age 3, 2019) after establishment and was significantly higher than those in other years (stand ages) for all cropping treatments (Table 3). It declined afterwards but remained relatively stable, except for an increase at age 6 when a fertilizing treatment was applied. In sum, alfalfa monoculture or alfalfa/grass mixtures maintained high annual DMY. Both for MPs and MBs, annual DMY was higher when alfalfa was sown at a rate $\geq 50\%$ than at 30%. As stand age increased, annual forage DMY tended to increase and then decrease, finally remaining relatively stable.

The percentage of alfalfa DMY in the total exhibited considerable variation across years, tending to rise from age 2 to age 7 (Figure 2). As the stand grew older, the variation in alfalfa DMY percentage gradually diminished among different seeding ratios for both MPs and MBs. Change in alfalfa DMY percentage with seeding ratio differed between species combinations and among stand ages. For MPs, alfalfa DMY percentage tended to be greater in M7P3 than other ratios, while for MBs, no clear trend was observed. At age 2, alfalfa DMY percentage was less than 50% in M3P7, M5B5, and M3B7 and at age 3, alfalfa DMY percentage was less than 50% in M5P5 and M3B7. However, alfalfa DMY percentage was greater than 50% and even exceeded 70% at ages 5–7. Moreover, alfalfa DMY percentage tended to be higher in MBs than MPs at the same seeding ratio.

Table 2. Effects (*F* value with significance) of stand age (SA), species combination (SC), seeding ratio (SR), and their interactions on forage yield and quality.

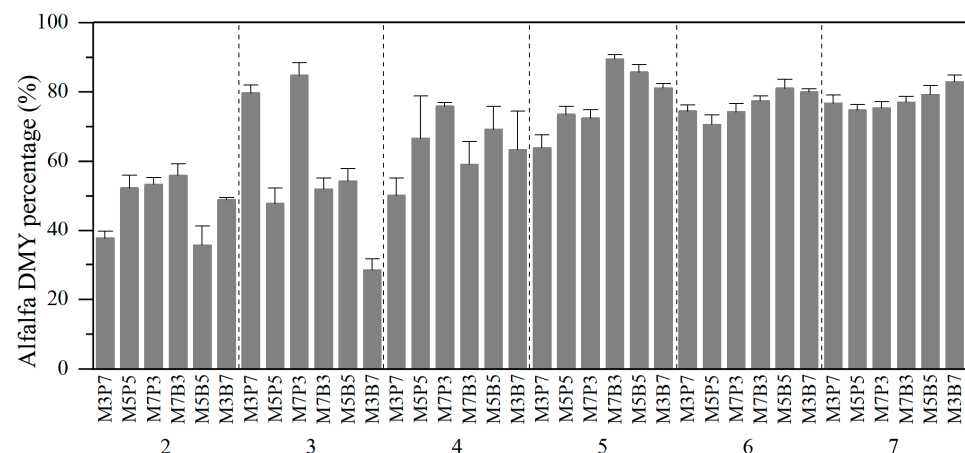
Effector	DMY (t ha ⁻¹)	CP (%)	NDF (%)	ADF (%)
Stand age (SA)	261.363 ***	547.679 ***	372.233 ***	189.28 ***
Species combination (SC)	2.436 NS	216.923 ***	2.312 NS	0.256 NS
Seeding ratio (SR)	462.084 ***	181.157 ***	60.893 ***	8.885 ***
SA × SC	4.284 **	45.851 ***	2.031 NS	0.974 NS
SA × SR	21.246 ***	161.493 ***	18.062 ***	5.443 ***
SC × SR	5.265 **	95.222 ***	1.992 NS	6.164 **
SA × SC × SR	4.635 ***	12.813 ***	2.199 **	3.153 ***

DMY, dry matter yield; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber. **, and *** show significant effects at the levels of 0.01 and 0.001, respectively. NS means no significance.

Table 3. Annual dry matter yield (DMY, t ha⁻¹) in different cropping treatments at different stand ages.

Cropping Treatment	Stand Age						Average
	2	3	4	5	6	7	
P	8.0 ± 1.1 Ad	7.7 ± 1.2 Af	5.1 ± 0.4 Bc	3.3 ± 0.3 Cd	3.5 ± 0.4 Cd	3.0 ± 0.2 Cf	5.1 ± 2.2 f
M3P7	13.2 ± 1.1 Cab	20.2 ± 2.7 Ad	11.7 ± 4.4 Cb	15.4 ± 0.8 BCbc	18.1 ± 1.2 ABb	14.2 ± 0.6 BCcd	15.5 ± 3.5 e
M5P5	11.7 ± 0.8 Bc	21.0 ± 2.3 Ad	12.2 ± 4.0 Bb	15.2 ± 0.4 Bbc	19.0 ± 0.9 Ab	15.1 ± 0.5 Bbc	15.7 ± 3.8 de
M7P3	14.8 ± 0.5 Ca	28.8 ± 1.0 Ab	18.5 ± 2.6 Ba	14.2 ± 0.6 Cc	18.4 ± 0.4 Bb	14.1 ± 0.5 Cd	18.1 ± 5.3 a
M	9.8 ± 1.5 Dc	33.9 ± 2.2 Aa	11.8 ± 1.6 Db	15.8 ± 1.2 Cab	20.6 ± 0.6 Ba	14.4 ± 0.1 Ccd	17.7 ± 8.3 ab
M7B3	14.6 ± 1.2 Ca	23.8 ± 2.3 Acd	11.2 ± 1.6 Db	16.8 ± 0.9 BCa	18.1 ± 1.1 Bb	15.8 ± 0.4 BCb	16.7 ± 4.1 bcd
M5B5	13.3 ± 0.7 Bab	26.1 ± 2.4 Abc	15.0 ± 3.6 Bab	15.3 ± 0.7 Bbc	16.5 ± 0.1 Bc	15.9 ± 0.9 Bb	17.0 ± 4.6 bc
M3B7	13.6 ± 0.6 Ca	23.1 ± 3.1 Acd	12.8 ± 1.1 Cb	13.9 ± 1.1 Cc	18.2 ± 1.4 Bb	17.1 ± 0.7 Ba	16.4 ± 3.8 cde
B	6.6 ± 0.6 Bd	14.9 ± 1.0 Ae	4.8 ± 0.7 Cc	3.2 ± 0.1 Dd	1.8 ± 0.1 Ee	4.2 ± 0.2 CDe	5.9 ± 4.5 f
Average for MPs (n = 15)	11.6 ± 3.2 D	24.4 ± 6.6 A	11.1 ± 3.9 D	13.0 ± 5.2 C	15.0 ± 7.0 B	13.5 ± 4.9 C	
Average for MBs (n = 15)	11.5 ± 2.7 C	22.3 ± 9.4 A	11.9 ± 5.1 C	12.8 ± 4.9 C	15.9 ± 6.5 B	12.2 ± 4.8 C	

Values are presented as mean values ± standard deviation (*n* = 3). Different lowercase letters represent significant differences among cropping treatments and different uppercase letters represent significant differences among stand ages. MPs, alfalfa/timothy mixtures; MBs, alfalfa/smooth brome grass mixtures.

**Figure 2.** Percentage of alfalfa DMY in the totals of different mixtures at different ages. Error bars are standard deviation.

3.2. Forage Quality

SA, SC, SR, and their interactions significantly affected annual crude protein (CP) content (Table 2). Annual CP content was higher in all alfalfa-containing swards than grass monocultures (Figure 3a). For MPs, M7P3 had a higher annual CP content and there was no difference between the other two mixtures. All MPs had lower annual CP content than alfalfa monoculture at ages 2–4. For MBs, annual CP content was lower in M3B7 and there was no difference between the other two mixtures (M7B3 the highest). All MBs had similar annual CP content as alfalfa monoculture. MPs and timothy monoculture had lower annual CP content than MBs and smooth brome grass monoculture. Annual CP content

was highest at age 7 and was significantly higher than those at ages 2–5, while there was no difference between age 2 and 3, and age 6 and 7 (Figure 3a). In total, an alfalfa seeding proportion over 50% in MBs resulted in an obvious mixing advantage of annual CP content, while there was no such advantage in MPs, showing species differences. As stand age increased, annual CP content increased.

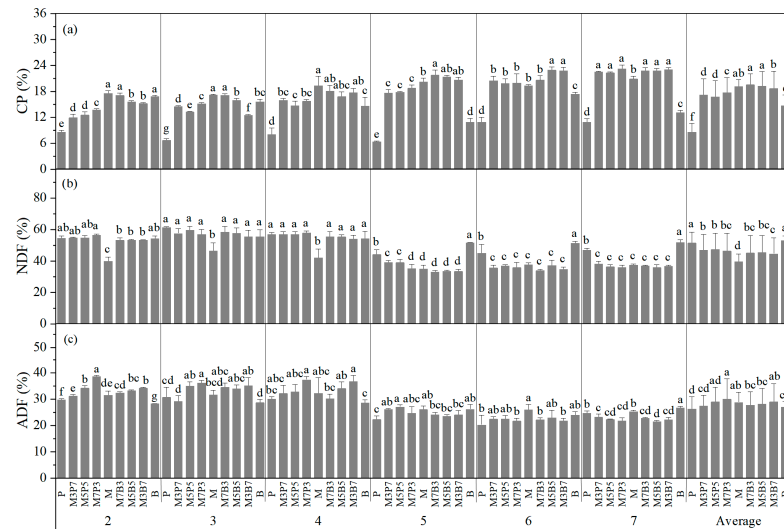


Figure 3. Forage crude protein (CP, (a)), neutral detergent fiber (NDF, (b)), and acid detergent fiber (ADF, (c)) content in different cropping treatments at different stand ages. Error bars are standard deviation. Different lowercase letters indicate significant ($p < 0.05$) differences among cropping treatments.

SA, SR, SA $\times$ SR, and SA $\times$ SC $\times$ SR significantly affected annual neutral detergent fiber (NDF) content (Table 2). Annual NDF content was the lowest in alfalfa monoculture and the highest in grass monocultures, which was significantly different from those in the mixtures (Figure 3b). For MPs or MBs, there was no difference among the three mixtures. There was no difference in annual NDF content between MPs and MBs except at age 5 when annual NDF contents in M3P7 and M5P5 were significantly higher than those in MBs. Annual NDF content peaked at age 3 and decreased afterwards, and it was significantly higher at ages 2–4 than ages 5–7 (Figure 3b). In conclusion, alfalfa inclusion could reduce annual NDF content. With the increase in stand age, annual NDF content tended to increase and then decrease, finally remaining relatively stable.

SA, SA $\times$ SR, SC $\times$ SR, and SA $\times$ SC $\times$ SR significantly affected annual acid detergent fiber (ADF) content (Table 2). Annual ADF content in all alfalfa-containing swards tended to be higher than those in grass monocultures (Figure 3c). There was no difference in annual ADF content among alfalfa-containing swards, except that in MPs, the annual ADF content in M3P7 was significantly lower than those in other alfalfa-containing swards. As stand age increased, annual ADF content decreased, and it was significantly higher at ages 2–4 than ages 5–7 (Figure 3c). Conclusively, there was little mixing advantage of annual ADF content. As stand age increased, annual ADF content tended to decrease and finally remained relatively stable.

3.3. Inter-Annual Stability of Forage Yield and Quality

DMY-based coefficient of variation across years ($CV_{\text{inter-DMY}}$) was significantly affected by SC, SR, and SC $\times$ SR (Table 4). In the mixtures, $CV_{\text{inter-DMY}}$ was significantly lower than those in the monocultures (Figure 4a). $CV_{\text{inter-DMY}}$ in smooth brome grass monoculture was higher than those in timothy and alfalfa monocultures, and the latter two had no difference. There was no difference among all mixtures. Conclusively, mixing increased the stability of

DMY across years compared to monocultures. The stability of timothy monoculture was greater than that of smooth brome grass monoculture, while mixing with alfalfa alleviated the difference.

Table 4. Effects (*F* value with significance) of species combination (SC), seeding ratio (SR), and their interactions on inter-annual coefficients of variation (CV_{inter-}) of forage yield and quality.

Effector	CV_{inter-}			
	DMY	CP	NDF	ADF
Species combination (SC)	8.413 **	24.259 ***	2.533 NS	1.508 NS
Seeding ratio (SR)	45.732 ***	40.332 ***	54.012 ***	11.592 ***
SC $\times$ SR	9.996 ***	4.389 *	6.266 **	7.991 **

DMY, dry matter yield; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber. *, **, and *** show significant effects at the levels of 0.05, 0.01, and 0.001, respectively. NS means no significance.

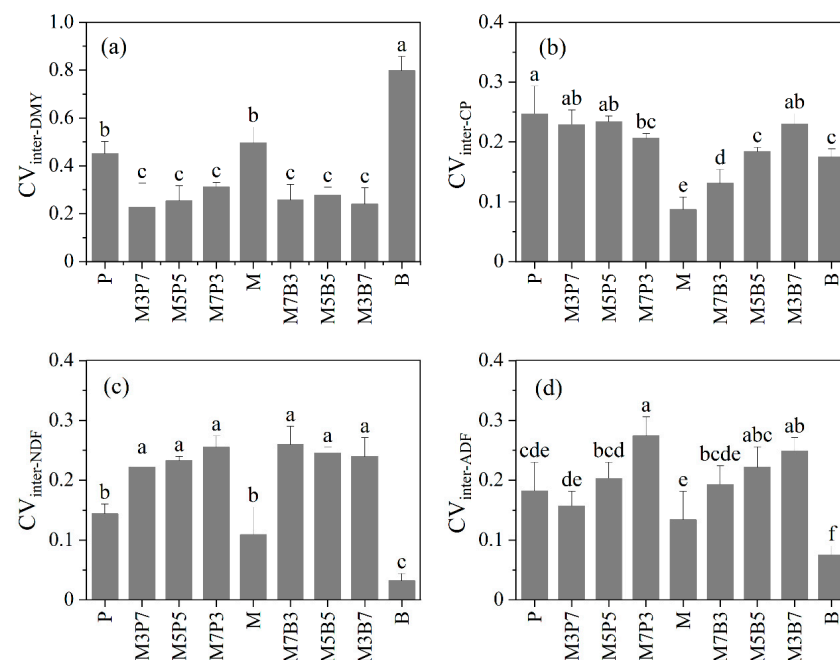


Figure 4. Inter-annual coefficient of variation in forage yield and quality in different cropping treatments. (a): DMY-based, $CV_{inter-DMY}$; (b): CP-based, $CV_{inter-CP}$; (c): NDF-based, $CV_{inter-NDF}$; (d): ADF-based, $CV_{inter-ADF}$. Error bars are standard deviation. Different lowercase letters indicate significant ($p < 0.05$) differences between cropping treatments.

CP-based coefficient of variation across years ($CV_{inter-CP}$) was significantly affected by SC, SR, and SC $\times$ SR (Table 4). $CV_{inter-CP}$ in alfalfa monoculture was lower than those in other swards, while it tended to be the highest in timothy monoculture (Figure 4b). For MPs, M7P3 had lower $CV_{inter-CP}$ than the other mixtures, while for MBs, increasing alfalfa seeding proportion led to a decrease in $CV_{inter-CP}$. To summarize, inter-annual stability of CP in alfalfa monoculture was higher than that in the grass monoculture and all mixtures, and the higher the seeding proportion of alfalfa in the mixtures, the greater the stability.

NDF-based coefficient of variation across years ($CV_{inter-NDF}$) was significantly affected by SR and SC $\times$ SR (Table 4). In the mixtures, $CV_{inter-NDF}$ was higher than those in the monoculture (Figure 4c). $CV_{inter-NDF}$ was lower than those in alfalfa and timothy monocultures, and there was no difference between the latter two. There was no difference between mixtures. Above all, inter-annual stability of NDF in monocultures was higher than those in all mixtures, and there was no significant difference among seeding ratios.

ADF-based coefficient of variation across years ($CV_{inter-ADF}$) was significantly affected by SR and SC $\times$ SR (Table 4). There was no significant difference between $CV_{inter-ADF}$ in

alfalfa and timothy monocultures, while both were higher than that in smooth brome grass monoculture (Figure 4d). $CV_{\text{inter-ADF}}$ in alfalfa monoculture was significantly lower than that in the mixtures where the alfalfa seeding proportion was over 50% for MPs or below 50% for MBs. $CV_{\text{inter-ADF}}$ in timothy monoculture was lower than that in M7P3 and showed no difference from that in the other MPs. $CV_{\text{inter-ADF}}$ in smooth brome grass monoculture was significantly lower than those in all MBs. As alfalfa seeding proportion increased, $CV_{\text{inter-ADF}}$ in MPs tended to increase, while in MBs, it tended to decrease. $CV_{\text{inter-ADF}}$ in MPs was lower than that in MBs at 30% alfalfa, equaled at 50% alfalfa, and higher at 70% alfalfa. In short, inter-annual stability of ADF showed little mixing advantage, and the effect of seeding ratio on ADF stability was influenced by the mixing component.

3.4. Comprehensive Assessment

There were higher comprehensive scores in all alfalfa-containing swards than grass monocultures, and alfalfa monoculture had the highest score (Table 5). For MPs, the scores decreased and then increased with alfalfa seeding proportion, peaking at 70% alfalfa (M7P3). For MBs, there was little variation among seeding ratios. MBs performed better than MPs. As stand age increased, the score tended to increase, except that it was higher at age 3 than age 4.

Table 5. Comprehensive score for forage yield and quality in different cropping treatments at different stand ages.

Cropping Treatment	Stand Age						Average
	2	3	4	5	6	7	
P	0.256	0.244	0.220	0.405	0.295	0.198	0.270
M3P7	0.324	0.433	0.276	0.566	0.756	0.745	0.517
M5P5	0.276	0.289	0.269	0.524	0.750	0.813	0.487
M7P3	0.297	0.386	0.316	0.653	0.768	0.805	0.537
M	0.770	0.741	0.622	0.615	0.684	0.643	0.679
M7B3	0.417	0.374	0.326	0.759	0.777	0.806	0.576
M5B5	0.373	0.392	0.324	0.776	0.720	0.875	0.577
M3B7	0.370	0.393	0.315	0.707	0.801	0.866	0.575
B	0.345	0.483	0.312	0.179	0.220	0.130	0.278
Average for MPs ($n = 15$)	0.385	0.419	0.341	0.553	0.650	0.641	0.498
Average for MBs ($n = 15$)	0.455	0.477	0.380	0.607	0.640	0.664	0.537

Values are presented as mean values ($n = 3$). MPs, alfalfa/timothy mixtures; MBs, alfalfa/smooth brome grass mixtures.

Figure 5 showed the evaluation of the whole performance of mixtures and monocultures based on comprehensive score and inter-annual stability of DMY (i.e., inverse of $CV_{\text{inter-DMY}}$). Alfalfa-containing swards demonstrated greater yield and quality (i.e., higher scores), while mixtures exhibited stronger stability compared to monocultures. Neither grass monocultures outperformed the other swards in terms of comprehensive score. M7P3 achieved higher score than two other MPs; however, stability was superior in M3P7. MBs shared a similar score, while stability ranked as M3B7 > M7B3 > M5B5. In sum, alfalfa/grass mixtures performed well, and 30% alfalfa were the best among all seeding ratios.

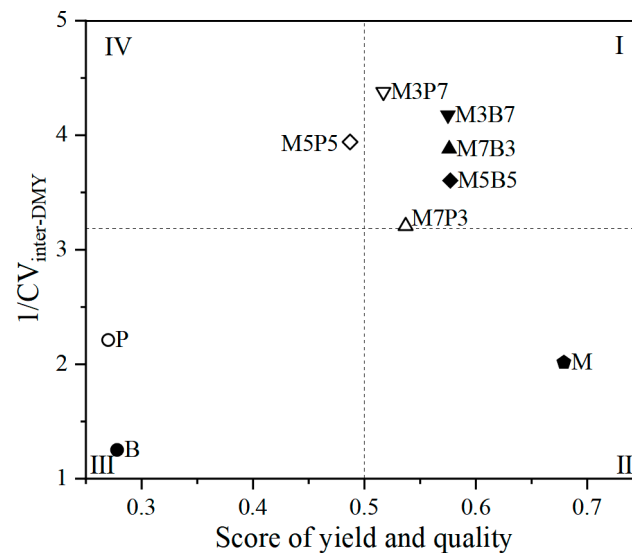


Figure 5. The evaluation of performance of various cropping systems based on a comprehensive score of forage dry matter yield, quality parameters, and stability ($1/CV_{inter-DMY}$), using TOPSIS with entropy weight. The score for each cropping system is the average of the scores for the six production years. The dashed lines represent the average of comprehensive score and average reciprocal of $CV_{inter-DMY}$. Cropping treatments in quadrant I indicate that they are above average in stability, yield, and quality; in quadrant II, they are below average in stability and above average in yield and quality; in quadrant III it indicates that they are below average in stability, yield, and quality; in quadrant IV it indicates that they are above average in stability, while yield and quality were below average.

4. Discussion

4.1. Effects of Species Combination and Seeding Ratio on Forage Yield and Quality

The mixtures generally exhibited higher productivity as reported in other studies [46,47]. However, in this study, only M7P3 (alfalfa/timothy mixture) showed a long-term yield advantage compared to alfalfa monoculture, and no such mixing advantage was measured in other MPs and all MBs. When compared to grass monocultures, the mixtures showed obvious mixing advantage in forage yield. This suggested that mixing advantage would depend on species combination and/or seeding ratio. Different types of grasses have completely different production traits [48]. For alfalfa mixtures, accompanying species, i.e., timothy or smooth brome grass, generally produce less biomass per unit area in the Longdong Loess Plateau [49], and therefore, would likely reduce the overall forage yield of the field compared to alfalfa monoculture. Previous studies have shown that alfalfa is suppressed by rhizomatous grass like smooth brome grass [50,51]. However, alfalfa seeding proportion (70%) of M7P3 was very close to that of sole alfalfa cropping (100%), resulting in high biomass production and yield advantage over alfalfa monoculture. This may be partly attributed to a positive interaction between the species in mixture. The interaction between alfalfa and grass species should include an improved soil nutrient status [52], especially N availability. More N can be released through strong BNF of alfalfa in the mixture with a higher alfalfa seeding proportion, i.e., 70% compared to that in mixtures with lower alfalfa seeding proportions. The released N can be taken up by the companion grass to promote the growth [53], and the depletion of soil N by grass can actually promote BNF, finally promoting the biomass production of both crops. Therefore, in this study, an obvious mixing advantage in yield was observed compared to alfalfa or grass monoculture at ages 2, 4, and 7, suggesting that interactions between two species in the mixture change with stand age.

Appropriate seeding ratios can ensure that the competition between various forages is balanced, thus promising higher yields [39,54]. It is widely recognized that forage mixtures with excessive legume proportions often exhibit reduced biomass due to intensified inter-

specific competition [50]. This aligns with Sanderson et al. [27], who found that 40–50% of the legume component in legume/grass mixtures resulted in higher biomass yields. These suggested that forage yield in mixtures should be mainly influenced by the seeding proportion of dominant species [55], i.e., legume crops [27,56]. The optimal legume proportion varies accordingly with regions and management practices. In this study, seeding ratio had a significant effect on DMY, and the highest yields were achieved when alfalfa seeding proportion was $\geq 50\%$, i.e., M7P3 and M5B5. The results partly confirmed that an increased seeding proportion of legumes in the mixture is positively correlated with yield enhancement. This reflects the competitive advantage of alfalfa/grass mixtures in the Longdong Loess Plateau region under specific management practices.

In mixtures, forage quality is related to species characteristics [8,23], and the dominant species is the main driver of forage quality [57]. Not surprisingly, alfalfa greatly affected CP content in this study, and there was more CP accumulation with more alfalfa in mixtures. Adjusting species seeding proportion in the mixtures is a commonly used agronomic measure to achieve an improved forage quality [32,58]. In both MPs and MBs, CP content tended to increase with the increase in seeding proportion of alfalfa, and this was consistent with previous findings [50]. For NDF and ADF contents, however, there was no significant difference among seeding ratios. The composition of mixture is more critical in influencing the nutritional quality than legume/grass seeding ratio [31]. This may be because variables related to crude fiber and digestibility are influenced by both temperature and precipitation [59]. In addition, Burity et al. [60] found that rhizomatous grasses, such as smooth brome grass, had greater total N yield due to more effective N root transfer compared to bunchgrasses like timothy. Therefore, MBs had higher CP content than MPs in this study, also indicating that alfalfa/smooth brome grass mixtures may be more suitable for this region than alfalfa/timothy mixtures.

4.2. Effects of Species Combination and Seeding Ratio on the Stability of Forage Yield and Quality

It is generally known that the production of a perennial cultivated grassland exhibits a trend of initially increasing and then decreasing, mainly due to the regular law of crop growth. In this study, annual DMY increased and then declined (across six production years), as expected, but remained relatively stable during older ages. This tendency may be attributed mainly to alterations in forage growth characteristics as the stand grows older [61] and the inter-annual climate fluctuates, resulting in subsequent changes in productivity [62]. The annual DMY was higher at age 3. This may be primarily because the annual precipitation was the highest in this year, exceeding the long-term average precipitation by 30%. Additionally, it could also be attributed to the grassland being in its prime functional period. In this study, the inter-annual stability of DMY was significantly higher in MPs and MBs compared to all monocultures. The yield of grass monocultures declined by ca 50% of the maximum yield after age 5, suggesting monocultures had been in an irreversible state of degradation. In contrast, in all mixtures, annual DMY declined by ca 30% or less, which confirms the positive role of crop mixing in sustaining productivity and stability [63,64]. However, the yield decline after peak productivity can vary considerably depending on intrinsic properties of component species, environmental variability, and the spatial and temporal scales [65–67]. In this study, in comparison to the yields at stand age 6, annual DMY were only 3.5–12.3% lower in MBs at age 7, but 21.0–23.5% lower in MPs. It suggested that the MBs better maintained yield during maturity and inter-annual stability. Smooth brome grass has the potential to become sod-bound, which can ultimately result in a decrease in yields [68]. Nevertheless, sod formation can be effectively mitigated after its mixing with alfalfa. Timothy is less drought-resistant [69], so should have been

more affected by precipitation variation. In addition, alfalfa competition for nutrients was greater under MPs than MBs [70], indicating MPs helped to maintain the yield.

In addition to instability caused by crop characteristics (lifetime, adaptability, etc.), management practices can also affect the stability of grassland communities, such as fertilizer application [65,71]. In this study, no fertilizer was applied at ages 2–5, potentially causing limitations in soil nutrients, especially for grass monocultures, which led to obvious decline in yield at ages 4 and 5. In this case, the mixing advantage was significant. Conversely, following a minor application of supplementary fertilizers at stand age 6, annual DMY increased by 30.4% in alfalfa monoculture and by 19.2% in all mixtures compared to those at age 5, and there was no mixing advantage in this year. However, at age 7, annual DMY decreased in all swards except smooth brome grass monoculture, and the mixing advantage appeared again. These results suggested that the mixing advantage in yield would be alleviated by fertilizer application, and importantly in an aged legume/grass mixture. However, the effect of fertilizer application on productivity recovery requires further exploration, and more precise controls over the amount and timing of fertilizer application is needed.

In this study, forage quality varied considerably between production years, and CP content was greater at ages 5–7 than ages 2–4, while NDF and ADF contents were lower. These may be due to several reasons including changes in mixture composition, annual precipitation, harvesting time [72,73], and management, e.g., fertilization. Three cuts were sampled annually since age 5, which supposedly has led to higher CP content and lower NDF and ADF contents than ages 2–4. Fertilizer application can stimulate growth and nutrient accumulation, thus leading to higher CP content and lower ADF content at age 6 than age 5 when applying fertilization at age 6 to recover the productivity. These were in accordance with that the comprehensive scores of forage yield and quality were higher in the last three production years compared to ages 2–4. Notably, forage quality of the mixtures changed more than that of monocultures, suggesting that mixtures did not improve the stability of forage quality. This may be partly because forage quality is more sensitive to the external environment [74]. Annual precipitation in this area is highly variable, ranging from 433 mm to 710 mm during the experiment. Considering that precipitation can be correlated with forage quality [59], variable precipitation may have contributed to the lower stability measured in mixtures.

5. Conclusions

Over the six years of this study, alfalfa/grass mixtures showed a yield advantage over grass monocultures with greater yield at higher alfalfa seeding proportions (50% or more). The mixtures showed advantages in CP and NDF, but not in ADF over grass monocultures. CP content tended to increase with increasing alfalfa seeding proportion, while NDF and ADF contents barely changed with seeding ratio. As the stand grew older, forage DMY increased and then declined to finally stabilize with more yield stability in mixtures than monocultures. The percentage of alfalfa DMY tended to increase over the life of the stand. In contrast, forage quality changed considerably across the production years with greater variability in mixtures than monocultures. Alfalfa/timothy mixture performed better in the stability of forage yield and quality with lower alfalfa seeding proportions. Considering both forage yield and quality, and the stability across years, smooth brome grass is considered to be more compatible with alfalfa in a mixed cropping compared to timothy for the Longdong Loess Plateau of China and areas with similar climate conditions.

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