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Lucerne Proportion Regulates Competitive Uptake for Nitrogen and Phosphorus in Lucerne and Grass Mixtures on the Loess Plateau of China

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Abstract: Mixtures of legume and grass are used worldwide to gain advantages in forage production and ecological maintenance. However, competition for nutrients by legumes in mixtures has not been fully explored. The aim was to determine how the forage proportion affected nutrient competition in legume and grass mixtures. Treatments included two species combinations and five sowing ratios. Competitive ratios (CR) of lucerne for nitrogen (N) and phosphorus (P) over two grasses were assessed to analyze how the lucerne proportion in mixtures affected the competition. Total N and P uptake were mostly lower under timothy-containing mixtures (MPs) than under smooth bromegrass-containing mixtures (MBs). Proportions of both N (N_M%) and P uptake (P_M%) of lucerne were higher under MPs than under MBs. Higher total N and P uptake were found under half-lucerne mixtures (M5P5 or M5B5) than under other grasslands. The N_M% and P_M% tended to be higher under half-lucerne mixtures, although they showed little difference among mixtures. Lucerne CR was greater under MPs than under MBs, and was greater than grass CR when lucerne was in lower proportion in the mixtures. There was little difference in soil N density among grasslands of the same cut, whereas soil P density was variable. Competitiveness of lucerne depends largely on the initial sowing ratio. High ratios of lucerne significantly reduce soil P density, leading to P limitation and reduced N and P uptake. On the Loess Plateau of China, mixing lucerne with smooth bromegrass is recommended to increase the uptake and harvest of N and P, specifically at the sowing ratio of 5:5.

Keywords: *Medicago sativa*; lucerne–grass mixture; forage proportion; nutrient uptake; competition ratio; soil nutrient density

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1. Introduction

Forage mixtures are fundamental in supporting the sustainable development of livestock husbandry and thus are increasingly applied worldwide [1]. Mixtures not only provide high-quality forages [1,2] but also help to improve soil fertility, consequently increasing the sustainability of agricultural production. Diverse forage crops in mixtures promote resource use through niche and facilitation effects, which can help mixtures to survive adverse environments [3,4] and maintain stable biomass [5]. However, the advantages of mixtures in production practice are controlled by competition among mixture components for various resources such as soil water and nutrients [6,7]. For example, in mixtures of legume and grass, legume species can help reduce the reliance on nitrogen (N) fertilizer application because of the high rates of biological N_2 fixation (BNF), which may also lead to changes in competition for N and other nutrients. Therefore, a better understanding of the competition for soil nutrients will help to optimize establishment and management of mixtures with legumes.

Competitiveness of forage is determined not only by genetic characteristics but also by environmental factors such as light, soil moisture, and nutrients [8,9]. Soil nutrients significantly affect forage competitiveness [10,11], with generally greater competitiveness under infertile conditions [12,13]. In general, legumes and grasses have different requirements for particular nutrients and different nutrient forms [14]. For example, legumes are primarily dependent on BNF as a source of N, and nutrients limiting legume growth are primarily P and potassium. Additionally, lucerne is more likely to absorb ammonium N (NH_4^+) than nitrate N (NO_{3-}) if it has to acquire N from the soil. By contrast, grasses primarily acquire N from soils, and the nutrient limiting growth is N. Competitiveness of forage crops for soil nutrients (especially N and P) is related to the cation exchange capacity of roots [15,16]. Roots release of H^+ can help activate P in soil and reduce competition for P among forages in mixtures [17]. Such results suggest that BNF may incidentally affect nutrient competition in legume-containing mixtures. Proportions of components in a mixture affect the total amount and availability of soil nutrients, partly by affecting organic matter that returns in roots and litter [18]. In addition, the mixture proportion affects the utilization of soil N by grasses, which changes the soil nutrition status and stimulates legume BNF [19]. It remains unclear how the forage proportion affects nutrient competition among forages in legume and grass mixtures.

The Loess Plateau is one of the main food-producing areas in China and has an important role in ecological maintenance. Shortages of water and poor soil fertility heavily restrict agriculture and livestock husbandry productivity in the area. Lucerne (*Medicago sativa* L.) is one of the most widely sown forages on the plateau, and mixtures are one efficient pattern in lucerne production [8,20]. Lucerne can fix N_2 and thus by reducing N fertilization, reduces leaching and volatilization of N [19]. Other forages in mixtures can then use available soil mineral N, and the reduction in soil N can feedback to facilitate further lucerne BNF. This response–feedback helps mixtures sustain good productivity. However, legume persistence in mixtures is often a challenge because of the competition with grasses [21] for nutrients. Therefore, optimized proportions of legume and grass would help to balance soil nutrients and alleviate competition to maintain legumes in mixtures.

This study tested the hypothesis that the proportion of lucerne would regulate the competition for nutrients between legume and grass in a mixture. The key objectives were the following: (1) to quantify N and P uptake in different mixtures; (2) to determine the effect of lucerne proportion on nutrient competitiveness; and (3) to correlate nutrient competitiveness with nutrient uptake and soil nutrient density.

2. Materials and Methods

2.1. Description of Experimental Site

The experiment was conducted at the Qingyang Loess Plateau Pastoral Agriculture Station (35°40′ N, 107°51′ E; 1298 m above sea level) of Lanzhou University. The station is in Shishe Township, Xifeng District, Qingyang City, Gansu Province, China. The area has a semiarid continental monsoon climate. Average annual precipitation is 543 mm (1980–2018), and 70% of the precipitation falls from July to September. During the experiment (September 2017 to August 2018), total precipitation was 655.8 mm, greatly exceeding the long-term average, whereas the annual pan evapotranspiration is 1504 mm. Annual sunshine duration is 2400 to 2600 h. Annual minimum and maximum temperatures are 4.5 °C and 14.0 °C, respectively. Before the experimental plots were established in September 2017, corn (*Zea mays* L.) was cultivated in the field. The soil is classified as a Heilu soil (Entisol of FAO classification), a silty loam with 70% silt, 23% clay, and 7% sand, representing the major cropping soil in the area. Soil physicochemical properties before seeding of lucerne and grasses are shown in Table 1. Note that these parameters were measured using the same methods as described in “Sampling and measurements”.

Table 1. Soil physicochemical properties before seeding of lucerne and grasses at the experimental site.

Soil Layer	SWC	TP	AP	TK	AK	TN	AN	NN	Bulk Density
(cm)	(%)	(mg g^{-1})	(mg Kg^{-1})	(mg g^{-1})	(mg Kg^{-1})	(mg g^{-1})	(mg Kg^{-1})	(mg Kg^{-1})	(g cm^{-3})
0–10	12.3	0.6	19.2	4.6	272.5	1.0	2.6	23.3	1.11
10–20	14.2	0.5	20.2	5.7	101.6	0.9	3.0	21.1	1.20
20–30	14.1	0.6	12.7	5.5	84.5	0.7	2.6	17.3	1.26
30–60	14.3	0.3	5.5	7.3	124.3	0.7	2.3	18.7	1.26
60–90	13.3	0.3	1.9	7.6	94.5	0.7	3.0	21.9	1.26

SWC: soil water content; TP: total phosphorus concentration; AP: available P concentration; TK: total potassium concentration; AK: available K concentration; TN: total nitrogen concentration; AN: ammonium N concentration; NN: nitrate N concentration.

2.2. Experimental Design

Treatments were arranged in a randomized complete block design with lucerne (*Medicago sativa*) and timothy (*Phleum pretense* L.) or smooth bromegrass (*Bromus inermis* L.). Mixtures were established with interlaced rows of lucerne and grass (alternating lucerne and grass). The treatments consisted of two species combinations (lucerne–timothy, MP; lucerne–smooth bromegrass, MB) and five sowing ratios of lucerne: grass (10:0, 7:3, 5:5, 3:7, 0:10), representing the different proportions of forages. There were three replicate plots for each treatment, and each plot (3 m × 4 m) contained 10 lines for all monocultures and mixtures (five lines lucerne and five lines grass). The row width was 30 cm.

Lucerne monoculture was sown at a rate of 13.5 g of seed/plot, and seeds were evenly distributed to the 10 lines. Timothy and smooth bromegrass monocultures were sown at 13.50 and 27.00 g of seed/plot, respectively. In mixtures, the proportion of a forage was calculated according to the actual seed weight in the monoculture. For example, in a 7:3 lucerne: timothy mixture, lucerne seed was applied at 9.45 g/plot, which was 70% of seed weight in the monoculture, and timothy seed was applied at 4.05 g/plot, which was 30% of seed weight in the monoculture (Table 2).

Table 2. Design to establish legume–grass mixtures and monocultures.

Species Combination	Sowing Ratio	Sowing Rate (g/plot)	Code
MP	Lucerne:timothy = 7:3	9.45 + 4.05	M7P3
	Lucerne:timothy = 5:5	6.75 + 6.75	M5P5
	Lucerne:timothy = 3:7	4.05 + 9.45	M3P7
MB	Lucerne:bromegrass = 7:3	9.45 + 8.10	M7B3
	Lucerne:bromegrass = 5:5	6.75 + 13.50	M5B5
	Lucerne:bromegrass = 3:7	4.05 + 18.90	M3B7
Monoculture	Lucerne 100%	13.50	M
	Timothy 100%	13.50	P
	Smooth bromegrass 100%	27.00	B

M: lucerne; P: timothy; B: smooth bromegrass.

According to local cultivation practice of lucerne production, sowing in autumn can help control weeds and establish the grassland. Seeds were sown on 26 September 2017. Before seeding, urea and superphosphate were applied as base fertilizers at 50 kg N ha^{-1} and 60 kg P ha^{-1}, respectively, which represented common practices in local farming systems. No additional fertilizers were applied in the following year. Experimental plots were completely rain fed, and manual weed control was performed as needed throughout the study. In order to ensure successful over-wintering of the grasslands in the establishment year, no cutting or harvesting was performed.

2.3. Sampling and Measurements

In 2018, forage stands were sampled on June 26 (first cut) and August 20 (second cut) when lucerne was at the early flowering stage. In each of the three replicates, two 50-cm sample segments for lucerne and/or grass (one for each) were cut with 5 cm stubble height, while for the monocultures, two 50-cm segments were sampled. Fresh biomass of samples was measured immediately in a nearby laboratory. Plant samples were oven-dried at 105 °C for 20 min and then at 75 °C for 3 d. Dry weights were obtained, and samples were ground into uniformly fine powder to pass through a 1.0-mm sieve. Samples were stored in plastic bags until measurements of the total N and P concentrations.

Soil samples were collected separately from the depths of 0–10, 10–20, 20–30, and 30–60 cm with a soil auger (40 mm diameter) when plants were sampled. In mixtures, three cores were collected from between-row and rows of the two forages and mixed thoroughly to obtain one soil sample. In monocultures, two soil cores were collected from each plot and mixed to obtain one sample. Soil samples were air-dried in the laboratory. Dried soil samples were passed through a 0.25-mm sieve in order to measure total N and P concentrations. Soil bulk density was measured immediately after seeding by the cutting ring method with five replicates.

Total N (TN) in plant and soil samples was digested with sulfuric acid and TN concentration was measured using a semimicro-Kjeldahl method with a Kjeldahl 8400 Auto-analyzer (Foss, Hilleroed, Denmark). Total P (TP) in plant and soil samples was digested with sulfuric acid, nitric acid and perchloric acid, and TP concentration was determined colorimetrically with a spectrophotometer (UV-2102 PCS, Shanghai Spectrum Instruments Co., Shanghai, China).

2.4. Calculations

Forage nutrient uptake (F_u, mg m^{-2}) was calculated as follows:

$$F_u = W_u \times C_u, \tag{1}$$

where W_u is the aboveground dry weight (g m^{-2}) of a forage and C_u is the aboveground nutrient concentration (mg g^{-1}). Therefore, total nutrient uptake of a mixture was calculated as lucerne nutrient uptake plus grass nutrient uptake of the aboveground part. Stoichiometric ratio of plant N and P was calculated as TN (mg g^{-1}):TP (mg g^{-1}). Proportions of lucerne N and P uptake of the totals were N_M% and P_M%, respectively, with those for grasses represented similarly.

Nutrient competitive ratio (CR) of lucerne was calculated as follows:

$$CR = \frac{(F_{umm}/F_{usm}) \times F_p}{(F_{ump}/F_{usp}) \times F_m}, \tag{2}$$

where F_{umm} and F_{usm} are lucerne nutrient uptake in mixtures and monoculture, respectively; F_{ump} and F_{usp} are timothy nutrient uptake in mixtures and monoculture, respectively; and F_m and F_p are the original sowing ratios of lucerne and timothy in mixtures, respectively. In MBs, the calculation method is consistent with MBs. The CRs for N and P (CR_N and CR_P) were then calculated. When $CR > 1.0$, the competitiveness of lucerne exceeds that of grass in a mixture, whereas when $CR < 1.0$, the competitiveness of grass exceeds that of legume in a mixture [22].

Soil nutrient density (SND, mg cm^{-2}) was calculated as follows:

$$\mathrm{SND} = \sum_{i=1}^{n} P_i \times C_i \times T_i, \tag{3}$$

where P_i is the soil bulk density (g cm^{-3}) in soil layer i; C_i is the nutrient concentration (mg g^{-1}) in soil layer i; i refers to the soil layer; and T_i is the thickness (cm) of soil layer i; n is the total number of soil layers [23]. Stoichiometric ratio of soil N and P ($SND_{N:P}$) was calculated as SND_N (mg cm^{-2}): SND_P (mg cm^{-2}). When the relationship between CR and SND was analyzed, soil nutrition status at the initial stage was defined as SND_i for cut 1, and the SND at the flowering stage in cut 1 was defined as SND_e. The SND at the flowering stage in cut 1 was defined as SND_i for cut 2, and the SND at the flowering stage in cut 2 was defined as SND_e for that cut.

2.5. Statistical Analyses

Three-way ANOVAs were used to analyze effects of cut, species combination, and sowing ratio of mixtures and their interactions on total nutrient uptake, proportionate nutrient uptake of lucerne, nutrient competition ratio of lucerne, and soil N, P, and N:P ratio in GENSTAT (VSN International, Hemel Hempstead, UK). Differences in total nutrient uptake, proportionate nutrient uptake of lucerne, and soil N, P, and N:P ratio among different mixtures were examined using one-way ANOVA. Differences in nutrient uptake between lucerne and grass in the same mixture were analyzed using a two-sample t-test. Differences in nutrient uptake, N and P CR of lucerne, and N:P ratio of forages between two cuts were also analyzed using a two-sample *t*-test. Correlations were analyzed using GENSTAT.

3. Results

3.1. Aboveground Total N Uptake of Lucerne and Grass, and Proportion of Lucerne N Uptake

Species combination, sowing ratio, cut, and their interactions significantly affected the total N uptake of the mixtures (Table 3). In cut 1, there was no significant difference in total N uptake among grass-containing grasslands (7.5–11.3 g m^{-2}), all of which was lower than that under lucerne monoculture (15.5 g m^{-2}; Table 4). Total N uptake under half-lucerne mixtures (M5P5 or M5B5) was higher than that under other grasslands, except the lucerne monoculture, but the differences were not significant. There were also no significant differences between the combinations of lucerne with timothy (MPs) and smooth bromegrass (MBs). In cut 2, variability in total N uptake increased among different grasslands, ranging from 3.3 to 19.3 g m^{-2}. Half-lucerne mixtures had the highest total N uptake, and mixtures had significantly higher total N uptake than that of the monocultures (except M7P3 and M3P7). Total N uptake of timothy-containing grasslands was lower than that of smooth bromegrass-containing grasslands, except M5P5. Smooth bromegrass-containing mixtures had higher total N uptake than that under lucerne monoculture.

Table 3. Three-way ANOVAs on effects of species combination (SC), sowing ratio (SR), and cut (C) on aboveground total N and total P uptake of lucerne and grasses and proportions of lucerne N ($N_{M\%}$) and P ($P_{M\%}$) uptake in aboveground total N and P uptake.

Source of Variation	Total N Uptake		$N_{M\%}$		Total P Uptake		$P_{M\%}$	
	F	P	F	P	F	P	F	P
SC	26.64	<0.01	257.09	<0.01	14.20	<0.01	75.93	<0.01
SR	31.74	<0.01	3.49	0.05	14.61	<0.01	4.55	0.02
SC × SR	8.90	<0.01	0.20	0.82	3.39	0.05	2.13	0.14
C	187.59	<0.01	9.08	0.01	34.52	<0.01	0.01	0.94
C × SC	31.64	<0.01	19.86	<0.01	8.42	0.01	17.06	<0.01
C × SR	3.92	0.03	2.55	0.10	4.37	0.02	1.08	0.36
C × SC × SR	5.15	0.01	3.00	0.07	0.18	0.84	4.43	0.02

Table 4. Aboveground total N and P uptake of lucerne and grass in different grasslands.

Grasslands	Total N Uptake (g m^{-2})		Total P Uptake (g m^{-2})	
	Cut 1	Cut 2	Cut 1	Cut 2
P	7.7 ± 1.08b *	3.3 ± 1.01d	0.74 ± 0.08	0.39 ± 0.07f
M3P7	9.8 ± 0.55b *	13.4 ± 0.7b	0.78 ± 0.12	0.82 ± 0.07de
M5P5	11.1 ± 0.18b *	17.9 ± 1.96a	0.74 ± 0.16 *	1.25 ± 0.14b
M7P3	8.1 ± 1.03b	8.4 ± 1.20c	0.57 ± 0.08	0.60 ± 0.02ef
M	15.5 ± 3.37a	12.0 ± 0.74b	0.83 ± 0.22	0.91 ± 0.09cd
M7B3	8.9 ± 0.51b *	17.4 ± 1.95a	0.78 ± 0.33	1.17 ± 0.05b
M5B5	11.3 ± 2.53b	19.3 ± 1.61a	0.93 ± 0.48	1.72 ± 0.09a
M3B7	8.2 ± 0.82b **	17.2 ± 0.31a	0.55 ± 0.09 *	1.07 ± 0.14bcd
B	7.5 ± 0.91b	10.3 ± 1.51bc	0.48 ± 0.05	1.11 ± 0.16bc

Values are the mean ± SD (n = 3). Different lowercase letters in the same column indicate significant differences ($p < 0.05$) among mixtures of the same cut. Asterisks (*) show significant differences between two cuts at $p < 0.05$.

Species combination and cut had significant effects on $N_{M\%}$, and their interaction effect was also significant ($p < 0.01$; Table 3). In cut 1, $N_{M\%}$, which exceeded 70% under

MPs, was higher than under MBs (Figure 1a). There was no significant difference in $N_{M\%}$ among MPs or MBs, but M5B5 tended to have the highest $N_{M\%}$ of the smooth bromegrass treatments. In cut 2, $N_{M\%}$, which exceeded 70% under MPs, was also significantly higher than under MBs There was no significant difference in $N_{M\%}$ among MPs or MBs, but M5P5 tended to have the highest $N_{M\%}$ of the timothy grass treatments.

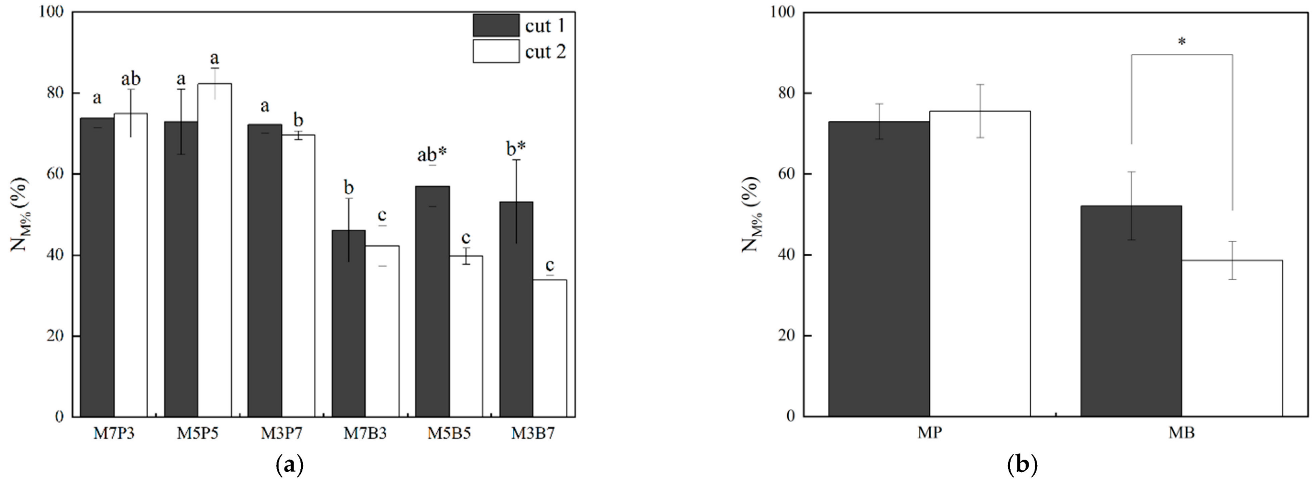

Figure 1. The proportion of lucerne N uptake in total grassland (**a**), ($N_{M\%}$) in different cuts of mixtures, and (**b**) cut-level $N_{M\%}$ under different species combinations. Values are the means ± SD (n = 3 in a and n = 9 in b). Different lowercase letters indicate significant differences ($p < 0.05$) among mixtures of the same cut. Asterisks (*) show significant differences between two cuts of the same mixture at $p < 0.05$.

Cut-level $N_{M\%}$ exceeded 70% under MPs in the two cuts, but there was no significant difference between cut 1 and cut 2 (Figure 1b). Under MBs, cut-level NM% was lower than 60% and was significantly higher ($p < 0.05$) in cut 1 than in cut 2.

3.2. Aboveground Total P Uptake of Lucerne and Grass, and Proportion of Lucerne P Uptake

Species combination, sowing ratio, and cut had significant effects on total P uptake of the mixtures ($p < 0.01$; Table 3). Interactions of the three factors on total P uptake were also significant ($p < 0.05$), except the three-way interaction (Table 3). In cut 1, there was no significant difference in total P uptake among different grasslands (0.48–0.93 g m^{-2}; Table 4), and the highest P uptake was under M5B5. In cut 2, total P uptake was highly variable among different grasslands, ranging from 0.39 to 1.72 g m^{-2}. Total P uptake was highest under half-lucerne mixtures and was higher under MBs than under MPs.

Species combination, sowing ratio, and the interaction between combination and cut significantly affected $P_{M\%}$ ($p < 0.05$; Table 3). In cut 1, $P_{M\%}$ approached 50% under MPs and M5B5, with the highest $P_{M\%}$ in M5B5 (Figure 2a). There was no significant difference among mixtures, except for a significantly lower uptake in M7B3. In cut 2, $P_{M\%}$ tended to exceed 60% under MPs, whereas it was lower than 30% under MBs (Figure 2a). There was no significant difference in $P_{M\%}$ among MPs or MBs.

Cut-level $P_{M\%}$ in cut 1 under MPs was significantly lower ($p < 0.05$) than that in cut 2, although both exceeded 50% (Figure 2b). By contrast, cut-level $P_{M\%}$ in cut 1 under MBs tended to be higher than that in cut 2. Cut-level $P_{M\%}$ in both cuts under MBs was below 40%.

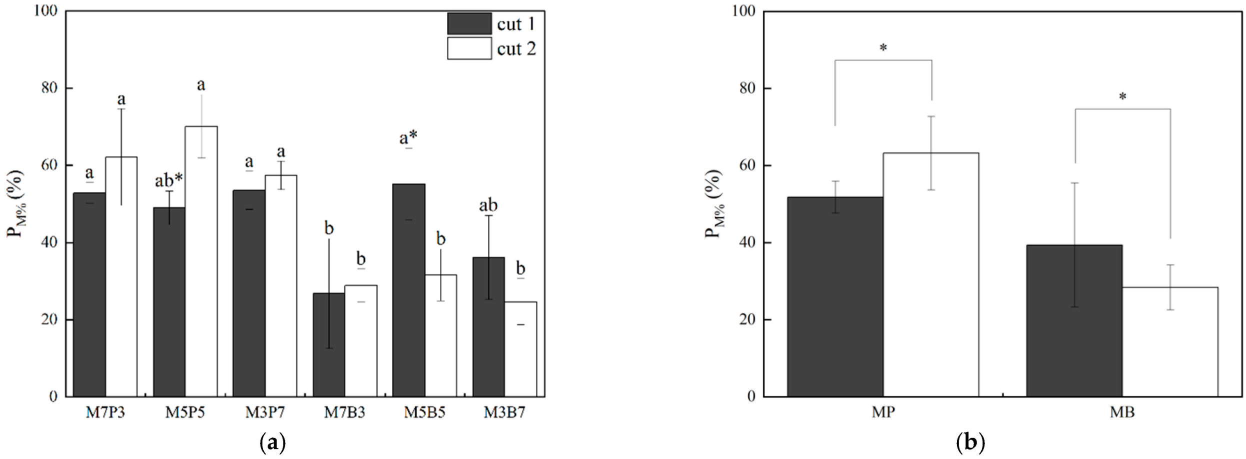

Figure 2. The proportion of lucerne P uptake in total grassland (**a**), ($P_{M\%}$) in different cuts of mixtures and (**b**) cut-level $P_{M\%}$ under different species combinations. Values are the means ± SD (n = 3 in a and n = 9 in b). Different lowercase letters indicate significant differences ($p < 0.05$) among mixtures of the same cut. Asterisks (*) show significant differences between two cuts of the same mixture at $p < 0.05$.

3.3. Lucerne N Competitive Ratio (CR_N) and P Competitive Ratio (CR_P) in Mixtures

Species combination, sowing ratio, cut, and their interactions significantly affected ($p < 0.05$) the CR_N of lucerne, and species combination, sowing ratio, and their interaction significantly affected the CR_P ($p < 0.01$; Table 5). The CR_N was significantly greater ($p < 0.05$) than 1.0 under M3P7 in the two cuts (Table 6). Under the same combinations (MPs or MBs), the CR_N tended to increase with the decreasing proportion of lucerne in the mixtures. The CR_P was significantly greater ($p < 0.05$) than 1.0 only under M3P7 in cut 1, and the maximum CR_P (1.36) was also under M3P7 in cut 2. Under the same combinations (MPs or MBs), the CR_P tended to increase with decreasing proportion of lucerne in the mixtures. The minimum values of CR_N and CR_P were under M7B3 in the two cuts.

Table 5. Three-way ANOVAs on effects of species combination (SC), sowing ratio (SR), and cut (C) on competitive ratios for N (CR_N) and P (CR_P) of lucerne.

Source of Variation	CR_N		CR_P	
	F	P	F	P
SC	55.55	<0.01	34.96	<0.01
SR	70.50	<0.01	55.53	<0.01
SC × SR	6.22	0.007	8.31	0.002
C	16.77	<0.01	2.73	0.112
C × SC	8.05	0.009	4.25	0.050
C × SR	9.43	0.001	3.28	0.055
C × SC × SR	3.56	0.044	7.31	0.003

Cut-level CR_N values of lucerne under MPs were greater than those under MBs (Figure 3a). Under MPs, cut-level CR_N in cut 1 was significantly greater ($p < 0.05$) than that in cut 2, whereas in under MBs, there was no significant difference between the two cuts. Cut-level CR_P values of lucerne under MPs tended to be higher than those under MBs (Figure 3b). There was no significant difference in CR_P between the two cuts under MPs or MBs.

Table 6. Competition ratios for N (CR_N) and P (CR_P) of lucerne under different mixtures.

Mixture	CR_N		CR_P	
	Cut 1	Cut 2	Cut 1	Cut 2
M7P3	0.60 ± 0.08bc *S	0.37 ± 0.10c *	0.43 ± 0.05bc *S	0.31 ± 0.07cd *
M5P5	1.46 ± 0.63b	1.32 ± 0.36c	0.87 ± 0.16b	1.09 ± 0.45ab
M3P7	3.02 ± 0.30a *S	1.46 ± 0.07c *	2.43 ± 0.46a *S	1.36 ± 0.20a
M7B3	0.18 ± 0.06c *	0.27 ± 0.06b *	0.09 ± 0.03c *	0.22 ± 0.04d *
M5B5	0.65 ± 0.14bc *	0.56 ± 0.05ab *	0.75 ± 0.29bc	0.58 ± 0.19bcd
M3B7	1.36 ± 0.53b	1.02 ± 0.05a	0.80 ± 0.34bc	0.96 ± 0.32abc

Values are the mean ± SD (n = 3). Asterisks (*) show the ratio is significantly higher or lower ($p < 0.05$) than 1.0. Different lowercase letters in the same column indicate significant differences ($p < 0.05$) among mixtures. The letter "S" indicates a significant difference between two cuts at $p < 0.05$.

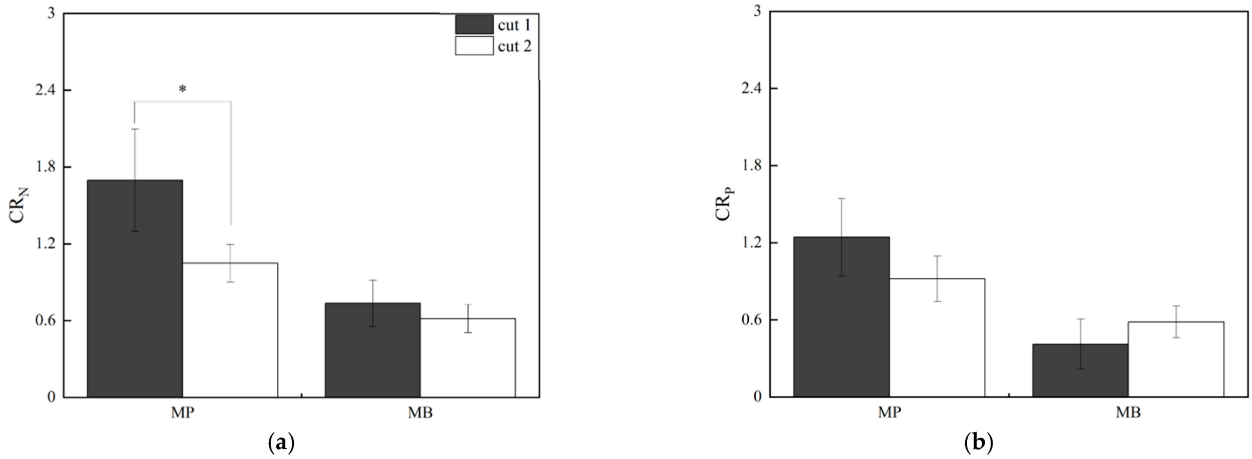

Figure 3. Cut-level competition ratios for (**a**) N (CR_N) and (**b**) P (CR_P) of lucerne under different species combinations. Values are the means ± SD (n = 9). The asterisk (*) indicates a significant difference between the two cuts of the MP mixtures at $p < 0.05$.

3.4. Soil N and P Density and Their Ratio

Interactions between cut and species combination and between sowing ratio and combination significantly affected SND_N ($p < 0.05$; Table 7). There were no significant differences in SND_N between cut 1 and cut 2 (Figure 4a). There were also no significant differences in SND_N among grasslands of the same cut, except between M5P5 and timothy monoculture and M3B7 in cut 1. In cut 2, timothy monoculture, M7P3 and lucerne monoculture had significantly lower SND_N than smooth bromegrass monoculture and all the smooth bromegrass mixtures.

Table 7. Three-way ANOVAs on effects of species combination (SC), sowing ratio (SR), and cut (C) on soil N density (SND_N), soil P density (SND_P), and their stoichiometric ratio ($SND_{N:P}$).

Source of Variation	SND_N		SND_P		$SND_{N:P}$	
	F	P	F	P	F	P
SC	2.65	0.08	26.86	<0.01	11.20	<0.01
SR	2.40	0.11	37.96	<0.01	21.72	<0.01
SC × SR	5.47	0.01	7.74	<0.01	1.30	0.29
C	3.40	0.07	629.12	<0.01	274.11	<0.01
C × SC	9.09	<0.01	18.30	<0.01	17.31	<0.01
C × SR	1.93	0.16	59.97	<0.01	21.56	<0.01
C × SC × SR	0.01	0.99	5.71	0.01	0.07	0.93

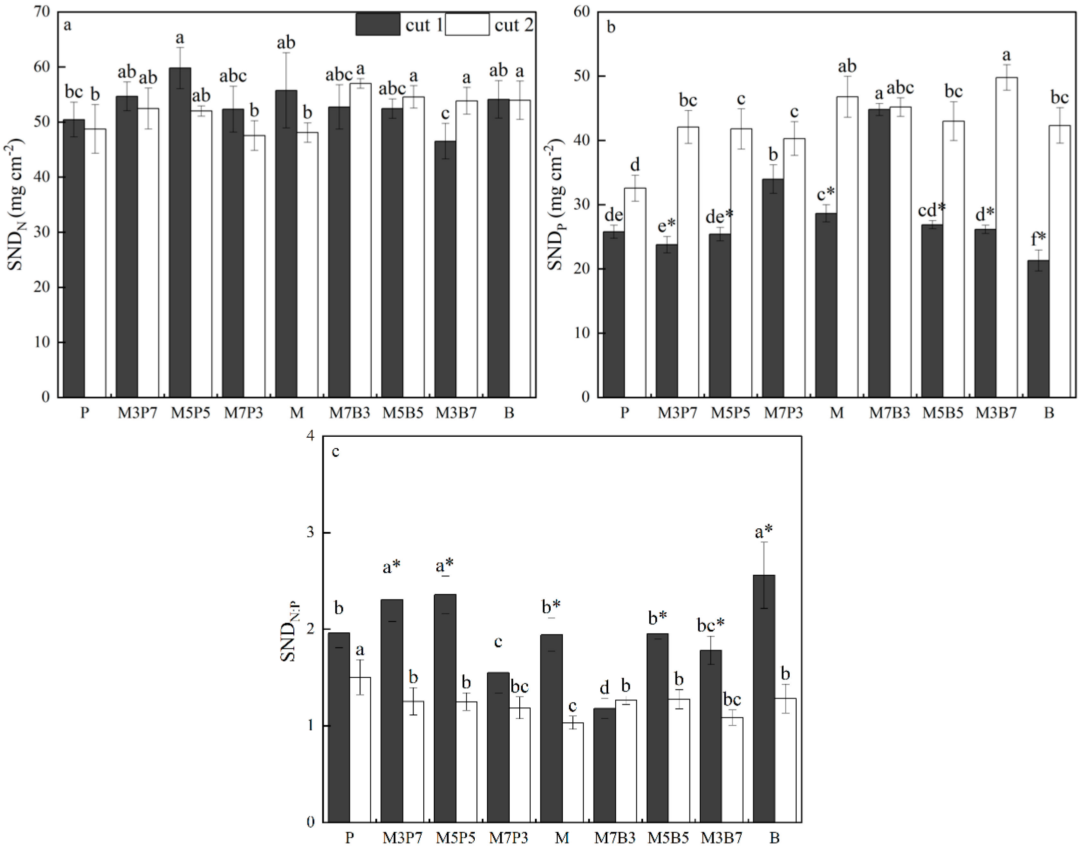

Figure 4. Soil (**a**) N density (SND_N), (**b**) P density (SND), and (**c**) N:P density ratio ($SND_{N:P}$) under different grasslands. Values are the means ± SD (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$) among grasslands of the same cut. Asterisks (*) show significant differences between two cuts of the same grassland at $p < 0.05$.

Species combination, sowing ratio, cut, and their interactions significantly affected SND_P ($p < 0.01$; Table 7). The SND_P in cut 1 was significantly lower than that in cut 2 except under 7:3 ratios and timothy monoculture (Figure 4b). In cut 1, the SND_P under M7B3 was significantly higher than that under other grasslands. In cut 2, the SND_P under M3B7 was higher than that under other grasslands, although there were no significant differences among lucerne monoculture, M7B3, and M3B7.

Species combination, sowing ratio, cut, and their interactions (except SC × SR and C × SC × SR) significantly affected $SND_{N:P}$ ($p < 0.01$; Table 7). The $SND_{N:P}$ in cut 1 was significantly higher than that in cut 2 except under 7:3 ratio and timothy monoculture (Figure 4c). In cut 1, the $SND_{N:P}$ under M5P5, M3P7, and smooth bromegrass monoculture was significantly higher than that under other grasslands. In cut 2, the $SND_{N:P}$ under timothy monoculture was significantly higher than that under other grasslands, and the lowest value was under lucerne monoculture. The minimum $SND_{N:P}$ appeared under the 7:3 ratios of both MPs and MBs in cut 1, and the $SND_{N:P}$ tended to increase with decreasing proportion of lucerne in cut 2.

Cut-level SND_N was significantly higher ($p < 0.05$) at the initial stage (beginning of green returning) than that at the flowering stages in both cuts (Figure 5a). Cut-level SND_N was significantly higher ($p < 0.05$) at the flowering stage in cut 1 than that in cut 2 under MPs, whereas the opposite result was observed under MBs. Cut-level SND_P increased significantly ($p < 0.05$) from the initial stage to the flowering stage in cut 2 (Figure 5b). Cut-level $SND_{N:P}$ at the initial stage was significantly higher ($p < 0.05$) than that at the flowering stages in cuts 1 and 2, and it was significantly lower ($p < 0.05$) in cut 2 than in cut 1 (Figure 5c).

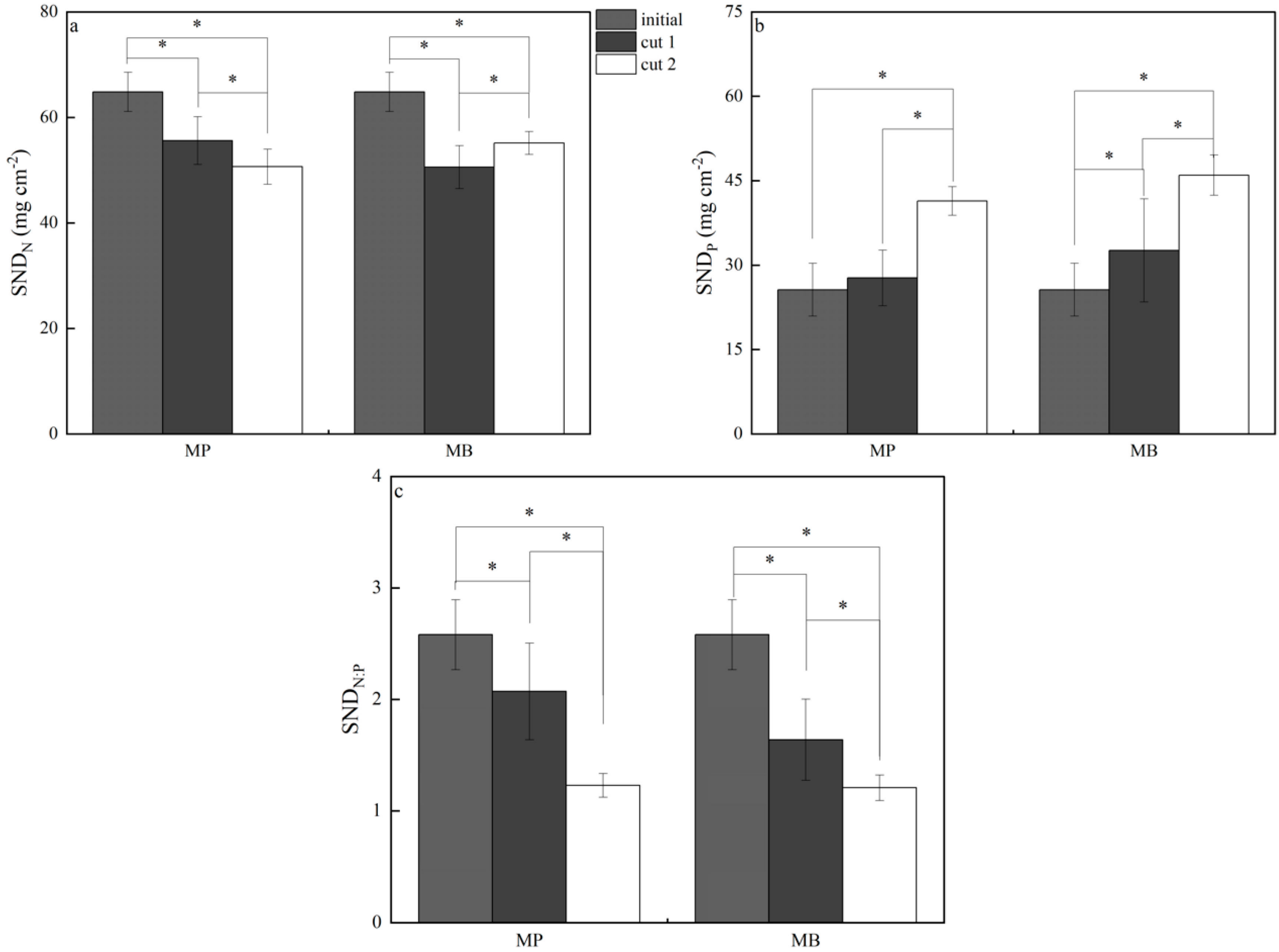

Figure 5. Cut-level soil (**a**) N density (SND_N), (**b**) P density (SND_P), and (**c**) N:P density ratio ($SND_{N:P}$) under different species combinations. Values are the means ± SD (n = 9). Soil nutrition status was measured at the initial stage and the flowering stage of cuts 1 and 2. Asterisks (*) show significant differences between the initial, cut 1, and cut 2 values of the same grassland at $p < 0.05$.

3.5. Correlations of Competitiveness for N and P of Lucerne with N and P Uptake and Soil N and P Density

Lucerne CR_N was positively correlated ($r = 0.94$ and 0.91, respectively) with CR_P at the two cuts, and there were few correlations of CR with other parameters (Figure 6). In cut 1, lucerne CR_N and CR_P were significantly positively correlated ($r = 0.56$ and 0.49, respectively) with lucerne $N_{M\%}$, and CR_P was significantly positively correlated ($r = 0.51$) with $P_{M\%}$ (Figure 6a). There were significant negative correlations between CR and SND_{Pe} ($r = -0.66$ for CR_N and -0.65 for CR_P) and significant positive correlations between CR and $SND_{N:Pe}$ ($r = 0.64$ for CR_N and 0.63 for CR_P). In cut 2, there were significant negative correlations between CR and SND_{Pi} ($r = -0.78$ for CR_N and -0.75 for CR_P) and significant positive correlations between CR and $SND_{N:Pi}$ ($r = 0.82$ for for CR_N and 0.79 for CR_P; Figure 6b).

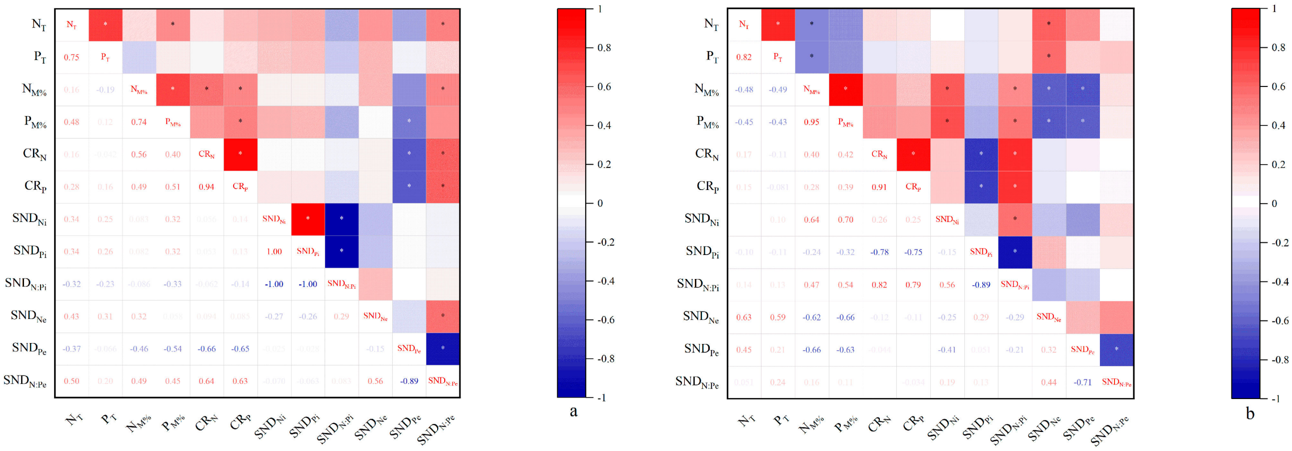

Figure 6. Correlations between nutrient competitiveness, nutrient uptake, and soil nutrition status at (**a**) cut 1 and (**b**) cut 2 $N_{M\%}$ and $P_{M\%}$: proportion of lucerne N and P uptake, respectively; N_T and P_T: total N and P uptake, respectively, in the mixtures; SND_N: soil N density; SND_P: soil P density; $SND_{N:P}$: soil N:P density ratio; i: at the initial stage; e: at the flowering stage. Asterisks (*) show a significant correlation at $p < 0.05$.

4. Discussion

4.1. Effect of Species Combination and Lucerne Proportion on Competitive Ratios for N and P of Lucerne

Among plant species, there are significant differences in characteristics such as individual size and root distribution, which lead to heterogeneous demands by plants for resources [24]. Such demands are measures of the plant niche and definitively shape the competition for resources among species. Generally, the greater the similarity between niches is, the stronger the competition among plants [11,25]. However, the competitiveness of a plant species may change when in mixtures with different species. In this study, under mixtures with the same proportion of lucerne, nutrient competitiveness of lucerne was greater under MPs (with timothy grass) than under MBs (with smooth bromegrass). This result suggested that in competition with lucerne, timothy expressed weaker competitiveness for soil nutrients than that of smooth bromegrass. The result is consistent with those found under mixtures of sainfoin (*Onobrychis viciifolia* Scop.) and different grasses, including timothy and smooth bromegrass [26]. On the one hand, vertical growth (height) of smooth bromegrass is not significantly affected under mixtures, whereas it is seriously limited for timothy [26]. Taller forage can obtain sufficient light energy and thereby increase the accumulation of nutrients and its competitiveness [27]. Branching and tillering can also be beneficial and increase nutrient sink capacity, helping to strengthen nutrient competitiveness. On the other hand, CO_2 assimilation and assimilate transportation are weak under a weak light environment, resulting in much more allocation of photosynthates to aboveground parts than to roots [28]. Hence, root morphology is affected, and length, surface area, and volume decrease [17]. Such a decrease could then affect the competitive ability of a grass such as timothy to obtain soil nutrients and water.

In this study, nutrient competitiveness of lucerne (CR_N and CR_P) under 3:7 treatments (M3P7 and M3B7) were mostly greater than 1.0 (except CR_P under M3B7 in the two cuts), whereas most of the CRs under 7:3 and 5:5 treatments were significantly lower than 1.0 in the two cuts. These results indicated that the competitiveness of lucerne for soil nutrients was stronger than grasses when lucerne was in lower proportion in the mixtures, and vice versa. Generally, the competitiveness of grass is stronger than that of lucerne under mixtures [29]. The greater competitiveness of grass is partly because grasses (such as timothy and smooth bromegrass) are relatively high competitive-grade plants with relatively large leaf area and high leaf position [30]. Additionally, more N in grass is allocated to the root system, leading to increased proportions of fine roots and expansion of root space, which increase competitiveness [31]. However, under legume–grass mixtures, competitiveness of grass and legume depends largely on their initial sowing ratio, which affects legume BNF and intraspecific competition. With a decreasing number of lucerne plants, gross BNF may decrease, but BNF of individual lucerne plants should increase, which can increase nutrient competitiveness [32]. Additionally, with a high number of grass individuals, intraspecific competition intensifies, which alleviates the competitiveness of grasses [19].

4.2. Effects of Species Combination and Lucerne Proportion on N and P Uptake

Generally, nutrient uptake is attributed primarily to nutrient concentration and biomass accumulation, which are easily influenced by genetic background and growth characteristics in diverse environments [8]. In this study, total N and P uptake were mostly lower under timothy-containing grasslands than under smooth bromegrass-containing grasslands, whereas both $N_{M\%}$ and $P_{M\%}$ were higher under MPs than under MBs. The more highly restricted growth and lower production of timothy in the mixtures might explain the lower nutrient uptake, regardless of the less changeable nutrient concentration. This result is also consistent with the weaker competitiveness for N and P of timothy than smooth bromegrass in competition with lucerne. There is a coordinated change in nutrient uptake with photosynthate accumulation [33]. Improved nutrient acquisition increases support for growth and biomass production [14], resulting in higher nutrient accumulation. In this study, lucerne CR was significantly positively correlated with $N_{M\%}$ and $P_{M\%}$ in cut 1. Lucerne grew well because of the advantage in N acquisition, which incidentally promoted P acquisition, and in mixtures, it performed better with timothy, because timothy growth was more restricted than that of smooth bromegrass. The differences resulted in higher proportion of lucerne nutrient uptake because of relatively greater biomass yield than that of grass.

Notably, there was generally higher total N and P uptake under half-lucerne mixtures (M5P5 or M5B5) than in other grasslands. The explanation for the greater uptake is unclear, but the sowing ratio (lucerne proportion) may help construct a relatively stable community with less competition for nutrients and thus balanced growth. This possibility is partly supported by higher biomass yield of individual species. Therefore, $N_{M\%}$ and $P_{M\%}$ changed little among mixtures of different sowing ratios but tended to be higher under half-lucerne mixtures. For example, M5B5 tended to have the highest $N_{M\%}$ and $P_{M\%}$ in cut 1, and M5P5 tended to have the highest values in cut 2.

4.3. Effects of Species Combination and Lucerne Proportion on Soil N and P Density and Their Ratio

In this study, there were few differences in SND_N among grasslands of the same cut, whereas SND_P was more variable. This result suggested that the N source was likely adequate and not the main factor limiting growth of a lucerne-containing grassland in this area [18,34]. The N source may be sufficient primarily because lucerne can contribute 65% of the total N uptake through BNF [35], and as a result, a large amount of fixed N is released into soils, helping to stabilize the soil N source. By contrast, P deficiency was likely a constant condition in this area, which is easily influenced by the crops used [18,36]. In both cuts, there were significant correlations of lucerne CR with soil P density, but not with soil N density, providing indirect evidence that soil P availability is influenced by the competitive use of various forages in grasslands. In addition, the slow release of plant available P also explains why P availability usually limits growth [34,36]. Thus, P availability is more sensitive to changes in plant species.

Lucerne CR and soil P density (SND_{Pe} and $SND_{N:Pe}$) were significantly correlated at the flowering stage of cut 1, whereas lucerne CR values were primarily affected by initial soil P density (SND_{Pi} and $SND_{N:Pi}$) at cut 2. In spring, when forage begins to green again, mineral P accumulated in rhizomes and stored in roots during winter supports the regrowth in cut 1. Thus, there is little competition between forages for P, as well as N, resulting in the absence of correlation between CR and initial nutrition status. However, rapid consumption and slow replenishment during growth reduces the leftover P, and therefore, CR was strongly negatively correlated with P density at the flowering stage and positively correlated with the corresponding N:P density ratio. Afterward, P availability and plant available P largely determine growth because they are the main limiting factors, resulting in the strong negative correlations between SND_{Pi} and CR and positive correlations between initial soil N:P density ratio and CR. In previous research, high levels of soil P significantly improve lucerne competition for soil P and N [37]. This improvement occurs because an increase in P availability can improve the branching ability of lucerne and promote requirements for nutrients. However, why CR and soil nutrition status were not significantly correlated at the flowering stage remains unclear. There are many factors that affect SND_e, including litter decomposition, SND_i, CR, and leaching. Additional efforts are needed to clarify which are the key effectors.

5. Conclusions

Total N and P uptake were mostly lower under timothy-containing grasslands than under smooth bromegrass-containing grasslands, and both $N_{M\%}$ and $P_{M\%}$ were higher under MPs (with timothy) than under MBs (with smooth bromegrass). There was generally higher total N and P uptake under half-lucerne mixtures (M5P5 or M5B5) than under other grasslands. Although there was little difference in $N_{M\%}$ and $P_{M\%}$ among mixtures of different sowing ratios, they also tended to be higher under half-lucerne mixtures. Lucerne CR was greater under MPs than under MBs and was greater than that of grasses when lucerne was in lower proportion in the mixtures. Lucerne CR was significantly positively correlated with $N_{M\%}$ and $P_{M\%}$ only in cut 1. There were few differences in SND_N among grasslands of the same cut; whereas SND_P was more variable. Lucerne CR was correlated with soil P density but not soil N density. The competitiveness of lucerne depends largely on the initial sowing ratio. High ratios of lucerne significantly reduce soil P density, leading to P limitation and reduced N and P uptake. In the rain-fed agricultural area on the Loess Plateau of China, mixing lucerne with smooth bromegrass is recommended to increase the uptake and harvest of N and P, specifically at the sowing ratio of 5:5 (a half-lucerne grassland).

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