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Research article

Optimization of nitrogen and phosphorus fertilization for enhanced forage production and quality of *Festuca Krylovianacv.* Huanhu artificial grassland in alpine regions

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ABSTRACT

Artificial grasslands of *F. kryloviana* in the region surrounding Qinghai Lake have been observed to a decline in productivity following three years of establishment. Traditional fertilization practices, aimed at maintaining ecological balance, have predominantly focused on the application of phosphorus. However, it remains unclear whether phosphorus fertilizers offer a superior advantage over nitrogen fertilizers in sustaining productivity. Consequently, from 2017 to 2019, we conducted an experimental to assess the impact of nitrogen and phosphorus fertilization on forage yield and quality. We designed with four levels of phosphorus and two levels of nitrogen, resulting in eight distinct fertilizer combinations. Our experimental findings indicate that the degradation of artificial grasslands leads to a shift in the allocation pattern of aboveground biomass. There was a respective decrease of 68.2 % and 62.5 % in the biomass proportions of stems and ears, contrasted by a greater than 200 % increase in the biomass proportion of leaves. The application of nitrogen not only elevated the total aboveground biomass but also promoted a preferential allocation of biomass to stems and leaves, consequently enhancing the forage's crude protein content. Nitrogen fertilization significantly increased aboveground biomass, and crude protein content by 63.21 %, and 6 %, respectively. Phosphorus fertilization's impact varied annually but favored the distribution of biomass to stems and ears. The net photosynthetic rate improved by over 53.12 % with fertilizer application, although the differences among treatments were not statistically significant. The balanced application of nitrogen and phosphorus fertilizers significantly bolstered the aboveground biomass, ear biomass, stem biomass, leaf biomass, and crude protein content in varying years by 17.25 %–209.83 %, 34.7 %–438.9 %, 25.5 %–250.2 %, 18.4 %–133.3 %, and 10.21 %–25.62 %, respectively. Our analysis revealed that nitrogen-only fertilization exhibited the most optimal fertilizer use efficiency and economic returns. In conclusion, nitrogen fertilization is crucial for sustaining the productivity and quality of *F. kryloviana* artificial grasslands. The local practice of 75 kg ha^{-1} phosphorus fertilizer is detrimental to the maintenance of productivity in *F. kryloviana* artificial grasslands. This study offers valuable insights into the optimization of fertilization strategies for sustainable forage production within alpine regions.

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1. Introduction

Biomass partitioning is a pivotal part of the function-structure feedback mechanism [1]. For example, under nutrient-rich conditions, *Alhagi sparsifolia* distributes more biomass to the nutrition organs, especially to the leaves [2]. Conversely, under nutrient-poor conditions, plants allocate more resources to underground organs [3]. It is evident that by intervening in soil nutrient levels, one can alter the biomass allocation patterns of plants. Fertilization, as the most effective agricultural measure, controls the accumulation, translocation, and utilization efficiency of plant nutrients [4–6], thereby influencing the structural and functional aspects of plant organs. Leaves, as important nutritional organs of plants, are often used as a benchmark for forage productivity. The balance between high forage yield and excellent nutritional value is a central goal in forage cultivation [7,8]. Crude protein (CP) is a primary nutritional indicator for assessing forage quality, and its enhancement contributes to the improved yield and quality of livestock dairy products [9]. Conversely, a higher fiber content, such as acid detergent fiber (ADF) and neutral detergent fiber (NDF), diminishes forage quality [10]. Therefore, maximizing the allocation of plant resources to leaves is a crucial pathway for maintaining the balance between forage yield and quality.

Nitrogen and phosphorus fertilizers primarily enhance plant photosynthesis and nutrient absorption by the roots [4,11,12]. Insufficient nitrogen supply in the soil often leads to delayed plant growth, reduced leaf area, and decreased biomass [4,13]. Numerous studies have found that the coordinated application of nitrogen and phosphorus fertilizers can improve fertilizer utilization efficiency [9]. Alterations in the N to P relationships may not only alter plant photosynthate allocation to vegetative or reproductive organs, but also regulate the metabolic and growth rate of plant [14]. The differential nutrient requirements of various crops for nitrogen and phosphorus are well-documented [11,15]. Over-fertilization can reduce fertilizer utilization efficiency and even inhibit plant growth [16]. Reasonable application of fertilization volumes that match plant nutrient demands is an effective way to maintain a rational allocation of plant resources.

Unlike annual plants, perennial plants' underground root systems do not die off in the autumn and winter seasons and rapidly break dormancy to regrow during the growth season [17,18]. This characteristic reduces soil disturbance in cold regions, leading to a predominance of perennial plants in artificial grasslands in these areas. *Festuca kryloviana* Reverd is one of the most common companion grass species in the alpine grasslands around Qinghai Lake, contributing significantly to the local livestock forage supply and the ecological stability of the grasslands. Long-term observations have indicated a degradation in *F. kryloviana* artificial grasslands after the third year. However, it remains unclear whether the degradation of grasslands alters the allocation patterns of aboveground organs. Traditionally, the practice of maintaining ecological balance in grasslands has resulted in preferring the use of phosphorus fertilizer [19], neglecting the use of nitrogen fertilizer. The superiority of phosphorus over nitrogen fertilizer in sustaining the forage production performance of artificial grasslands is not yet established. Therefore, we conducted research on 3-year-old, 4-year-old, and 5-year-old *F. kryloviana* artificial grasslands to study the impact of adding nitrogen and phosphorus fertilizers on forage yield and quality. The objectives of our experiment were (1) to elucidate the aboveground biomass allocation patterns in degraded grasslands, and (2) to ascertain whether phosphorus fertilizer has an advantage over nitrogen fertilizer in maintaining the productivity of *F. kryloviana* artificial grasslands.

2. Materials and methods

2.1. Experimental site

The experiment was conducted at the National perennial herbage germplasm garden (100°52.848′ E, 36°59.36′ N, altitude 3156 m above sea level) in Xihai Town, Qinhai Province, China. A typically Alpine region where annual sunshine was 2980 h. The mean annual temperature was 0.5 °C that the lowest value (−27.3 °C) in January and the highest value (25 °C) in August. The annual average frost-free period was about 93 days and there was no absolutely frost-free period, that is, may occur at any time of the year. The annual total precipitation was 369.1 mm and focus on July, August and September. The annual evaporation was 1400 mm. The precipitation and temperature during the experiment period were showed in Fig. S1. The soil traits in the field with 8.43 pH, 32.48 g kg^{-1} organic matter, 1.56 g kg^{-1} total N content, 1.39 g kg^{-1} total P content, 88.8 mg kg^{-1} available nitrogen and 2.2 mg kg^{-1} available phosphorus in the 0–20 cm topsoil layer before experiment.

2.2. Experimental design

The experimental material was the cultivar of *Festuca Krylovianacv.* Huanhu (from the Qinghai Academy of Animal Science and Veterinary Medicine). Our research was conducted over the period of 2017–2019. Cultivation commenced in May 2015 with a sowing density of 22.5 kg ha^{-1}, a sowing depth of 5 cm, and a row spacing of 30 cm. Given the perennial nature of the plant, the full growth cycle began in the third year (2017), as the plants were unable to complete the reproductive phase in the first year and only produced limited reproductive shoots in the second year. In alignment with local agricultural practices, the experienced phosphorus fertilization rate was established at 75 kg ha^{-1}. Our experimental design incorporated variations of ±15 kg ha^{-1} around this baseline to assess the impact on plant growth. Concurrently, to address the local neglect of nitrogen fertilization, we introduced nitrogen into our treatment regime. We designed four levels of phosphorus fertilizer (P_2O_5 15.5 %) at 0, 60, 75, and 90 kg ha^{-1}, denoted as P0, P60, P75, and P90, respectively, and two levels of nitrogen fertilizer (N 46 %) at 0 and 60 kg ha^{-1}, denoted as N0 and N60, respectively. This resulted in a total of eight fertilizer treatment combinations. The experimental layout was arranged in a randomized complete block (RCB) design, with each treatment comprising three replications. Each experimental plot was 5 m * 3 m in area. Fertilization was applied annually during the tillering stage following the plants' return to growth.

2.3. Sample collection and determination

2.3.1. Agronomic traits

The agronomic traits mainly recorded plant height, stem diameter (SD), and reproductive shoots (RS). At anthesis every year (when 60 % of the plants were in bloom), twenty reproductive shoots were randomly selected within each experimental plot to measure plant height and stem diameter. The absolute height of the reproductive shoots, as determined using a tape measure, was recorded as the plant height. The SD, representing the width of the stem at the nearest node to the ground, was recorded using a vernier caliper. Within a 1 m section of the plot designated for the assessment of plant biomass, the number of stem segments was manually counted in its entirety and documented as the number of RS.

2.3.2. Forage biomass and biomass allocation

At anthesis, three uniform 1-m segments within the experimental plots were harvested at ground level. All above-ground organs were collected and brought to the laboratory, where they were manually separated into stems, leaves, and ears. Each organ was subjected to a kill-green process at 105 °C for 30 min in a hot air oven, followed by adjustment of the oven temperature to 75 °C for drying until a constant weight was achieved. The dry biomass of each organ was then measured using an electronic balance, and the ratio of biomass for each organ was calculated. The biomass ratio of the ear, stem, and leaf organs was determined as follows:

Total aboveground biomass (TAB)= Biomass of ear + Biomass of stem + Biomass of leaf;

Ratio biomass of ear (ROE) = Biomass of ear/Total aboveground biomass;

Ratio biomass of stem (ROS) = Biomass of stem/Total aboveground biomass;

Ratio biomass of leaf (ROL) = Biomass of leaf/Total aboveground biomass.

The partial factor productivity and agronomic efficiency were calculated as follows:

Partial factor productivity (kg kg^{-1}) = Fertilization yield/Fertilization amount in the fertilization area;

Agronomic efficiency (kg kg^{-1}) = (Fertilization yield - unfertilized area yield)/Fertilization amount in the fertilization area.

2.3.3. Forage quality

After measuring the forage biomass, combined the plant samples (leaves, stems, and ears) of each plot, and then using a crusher ground into a fine powder and passed through a 2 mm mesh screen. For measurement of crude protein (CP), crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), and soluble total sugar (SS) content of samples. The CP, ADF, and NDF were measured as described by Ref. [20]. The CF and SS were measured as described by Ref. [21].

2.3.4. Gas exchange of leaves

Determination of flag leaves at anthesis, reference method [2]. The net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) were measured using a portable photosynthesis system (LI-6400, LI-COR Inc., USA) between 9:00 and 11:00 a.m. The relative humidity of the air, CO2 concentration, and photon flux density were maintained at 30–40 %, 400 μmol mol^{-1}, and 1000 μmol m^{-2} s^{-1} in all cases. The water-use efficiency (WUE) was calculated using the following equation: Pn/Tr.

2.3.5. Cultivation costs

In crop cultivation costs, the unit price of nitrogen fertilizer (N 46 %) is 2.1 ¥ kg^{-1}, the unit price of phosphorus fertilizer (P_2O_5 15.5 %) is 1.2 ¥ kg^{-1}, the mechanical cost is 600 ¥ ha^{-1}, and other expenses are 2160 ¥ ha^{-1}. The income comes from forage, and the purchase price of forage is 300 ¥ t^{-1}.

2.4. Statistical analysis

Analysis of variance (ANOVA) for the three-year data was performed in the SPSS 25.0 statistical software (SPSS Inc., Chicago, IL, USA). In this study, nitrogen, phosphorus and year were considered as fixed and main factors. The interaction effects, wherever found significant were also calculated and presented. Data processing and statistical analyses were performed using Microsoft Excel 2019 (Microsoft Corp., USA). The least significant difference Tukey's honestly significant difference was used to separate the mean values at the 5 % probability level. Origin 2023 (Origin Lab Corp., USA) was used for plotting, correlation Analysis and principal component analysis. Comprehensive evaluation of TOPSIS with EW used SPSSAU that a web-based data science algorithm platform system.

3. Results

3.1. Agronomic traits

The number of reproductive shoots, plant height, and total aboveground biomass were significantly influenced by nitrogen fertilizer, phosphorus fertilizer, and growth years, with plant height additionally affected by significant interactions among these factors ($P < 0.05$; Table 1). Stem diameter was not affected by phosphorus fertilizer, and the interaction of nitrogen and phosphorus fertilizers ($P < 0.01$). The reproductive shoots and total aboveground biomass decreased with the increase in growth years (Fig. 1A and D). Plant height and stem thickness increased with the extension of growth years, with increments exceeding 18.8 % and 35.7 %, respectively (Fig. 1B and C). Nitrogen application increased reproductive shoots and total aboveground biomass, with increases of 13.29 % and 63.21 % in 2018 and 2019, respectively. The impact of phosphorus fertilizer on reproductive shoots, plant height, stem diameter, and total aboveground biomass showed interannual variations. Overall, the combined application of nitrogen and phosphorus fertilizers favored the maintenance of larger total aboveground biomass. However, due to differences between years, the increase in total aboveground biomass in the N60P90 treatment compared to the unfertilized treatment ranged from 17.25 % to 209.83 %.

3.2. Biomass accumulation and distribution

The ear biomass and stem biomass decreased with the increase in growth years, and the corresponding organ biomass proportions exhibited similar trends (Tables 1 and 2). Our experiments revealed reductions of 88.8 % and 90.4 % in ear and stem biomass, respectively, while the ratio of biomass reductions for these organs were 62.5 % and 68.2 %. With the extension of growth years, there was a notable shift in resource allocation toward leaves, with ratio of biomass increasing by more than 200 %. Nitrogen fertilizer promoted the accumulation of stem and leaf biomass, with increments exceeding 28 % and 6 %, respectively. After nitrogen application, both ear biomass and its proportion decreased. Ear and stem biomass, as well as their proportions, showed an increasing trend with the application of phosphorus. The combined application of nitrogen and phosphorus significantly enhanced the aboveground organs biomass. In the N60P90 treatment, the increases in ear, stem, and leaf biomass were 34.7%–438.9 %, 25.5%–250.2 %, and 18.4%–133.3 %, respectively.

3.3. Photosynthetic parameters of leaves

Following fertilization, the net photosynthetic rate of the leaves increased by more than 53.12 % compared to the unfertilized, but the differences among various fertilization treatments were not statistically significant (Fig. 2A). Transpiration rates were enhanced under treatments of phosphorus or nitrogen, but with a trend of initially increasing and then decreasing with the amount of applied phosphorus. When combined application nitrogen and phosphorus, the transpiration rate of leaves decreased with the increasing phosphorus application, with a reduction ranging from 27 % to 47 % (Fig. 2B and C). Combining the data from three years of experiments, co-application of nitrogen and phosphorus, without affecting stomatal conductance, improved water use efficiency of leaves by enhancing photosynthetic rates and reducing transpiration rates (Fig. 2A, B, 2C and 2D).

3.4. Nutritional quality of forage

Crude fiber and crude protein were significantly influenced by nitrogen, phosphorus, and the growth years ($P < 0.01$, Table 3). The interactions among these factors predominantly affected neutral detergent fiber, crude protein, and soluble sugar content ($P < 0.01$). Compared to 2017, the levels of crude fiber, soluble sugar, acid detergent fiber, and crude protein in 2018 and 2019 increased by 50.6 %, 60.6 %, 3.5 %, and 2.94 %, respectively (Table 4). Nitrogen fertilization led to an increase in crude protein by about 6 %. The three-year data revealed diverse trends in nutritional indicators. The highest soluble sugar content was observed in only phosphorus treatment, while the maximum crude protein occurred in the N60P90 treatment, with values 10.21–25.62 % higher than the unfertilized.

3.5. Correlation and principal component analysis

We conducted correlation analyses among agronomic traits, organ biomass, nutritional quality, and photosynthetic characteristics. The results revealed a highly significant correlation between crude protein content and crude fiber, leaf biomass, ratio biomass of organs, and leaf water use efficiency (Fig. 3A, B and 3C). Crude fiber content exhibited significant correlations with acid detergent fiber, soluble sugar, organ biomass and ratio biomass of organs. Ear biomass, stem biomass, and their proportions were negatively correlated with various nutritional indicators. It is evident that the organ allocation pattern significantly influences forage quality.

Table 1

Analysis of covariance (ANCOVA) of agronomic traits among nitrogen fertilizer and phosphorus fertilizer in the period 2017–2019.

Factor	Reproductive shoots	Plant height	Stem diameter	Total aboveground biomass	Ear	Stem	Leaf
N	159.016**	145.988**	8.949**	490.785**	23.55**	15.919**	723.527**
P	30.984**	8.751**	2.626ns	19.149**	12.606**	38.257**	2.451ns
Y	766.932**	246.698**	80.288**	285.788**	599.526**	574.732**	132.677**
N*P	0.463ns	15.127**	2.218ns	1.086ns	0.855ns	7.487**	1.838ns
N*Y	236.778**	39.19**	25.844**	48.125**	101.361**	232.605**	60.01**
P*Y	19.792**	9.983**	2.652*	1.559ns	6.568**	13.395**	9.103**
N*P*Y	0.725ns	13.269**	6.642**	3.125*	2.012ns	8.164**	15.056**

N, P and Y refer to nitrogen fertilizer, phosphorus fertilizer and growth years. The numbers in the table represent the *F* value of ANCOVA.**refer to $P < 0.01$,*refer to $P < 0.05$, ns refer to $P > 0.05$, respectively.

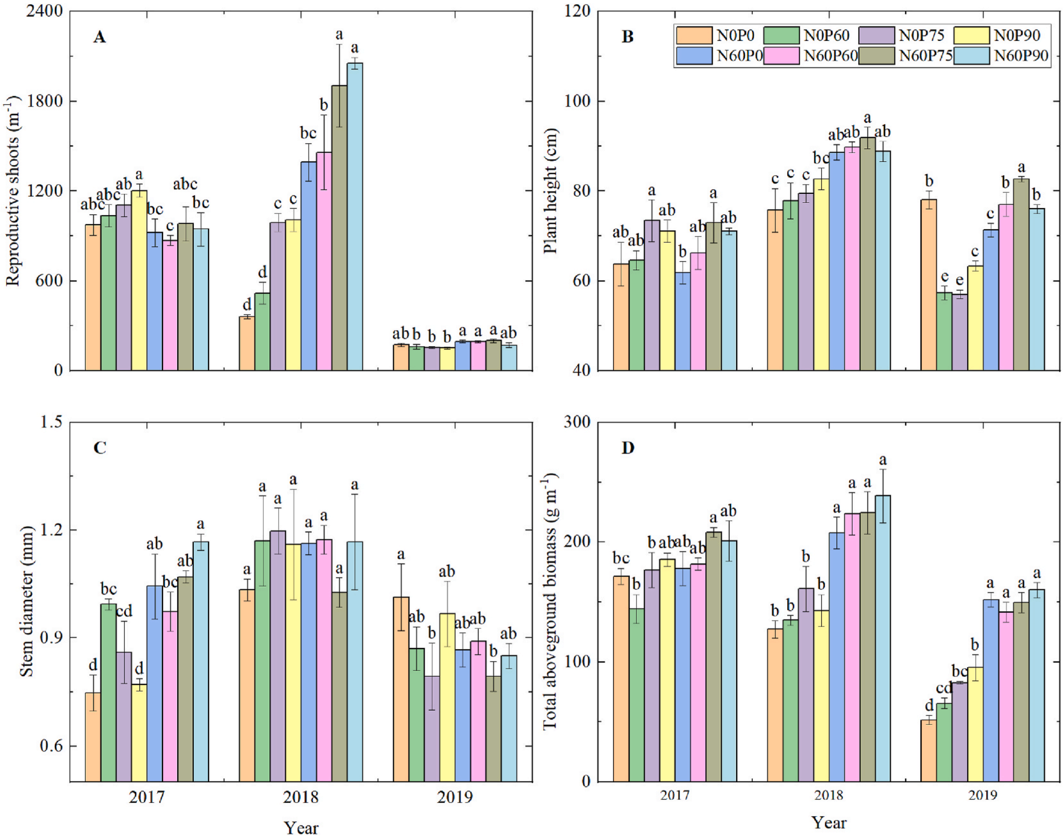

Fig. 1. Effect of nitrogen and phosphorus fertilization on the agronomic characteristics of *F. Kryloviana* in 2017–2019, with reproductive shoots (A), plant height (B), stem diameter (C) and total aboveground biomass (D). Different letters in the figure indicate significant differences between treatments in the same year.

3.6. Comprehensive evaluation of nitrogen and phosphorus fertilizer

Using the TOPSIS with EW, the EW of the twenty forage quality indicators in the comprehensive evaluation were calculated to reflect the different role of each indicator in the evaluation (Table S1). The comprehensive relative quality scores and ranks of the treatment were obtained by using the TOPSIS with EW (Fig. 4A, B, 4C and Table S2). The two-dimensional scatter distribution diagrams of relative quality score and total aboveground biomass for the treatment were drawn a visualized overall assessment result showed that the combination of nitrogen and phosphorus fertilizers contributed to the forage performance of *F. Kryloviana* (Fig. 5 A and 5B and 5C). Through analysis of fertilizer use efficiency, we observed that fertilizer partial factor productivity and fertilizer agronomic efficiency were significantly higher in the N60P0 treatment compared to other treatments, with increases of more than 55.98 % and 81.25 %, respectively (Fig. 6A and B). Additionally, the limited enhancement of aboveground biomass by phosphorus fertilizer resulted in a decrease in fertilizer partial factor productivity and agronomic efficiency in the treatment with combined nitrogen and phosphorus fertilization. Considering the economic perspective, the net income under the N60P0 treatment was four times higher than that under the N0P60 treatment at the same fertilization level (Fig. 6C). Consequently, our findings suggest that nitrogen fertilizer is more advantageous than phosphorus fertilizer in fertilization practices for artificial grassland of *F. Kryloviana*.

4. Discussion

4.1. Degradation of F. Kryloviana artificial grassland accompanies changes in organ biomass allocation

The reduction in aboveground biomass is a primary characteristic of declining productivity in artificial grasslands [22]. Our experiment found that not only does the extension of growth years decrease the total aboveground biomass of *F. Kryloviana*, but it also alters the allocation pattern of aboveground organ biomass (Fig. 1D and Table 2). Specifically, the biomass and proportion of ear and stem organs decrease with extended growth years, while the leaf organs show the opposite trend (Tables 1 and 2). Growth and reproduction are among the most fundamental activities in plants [23]. For perennial plants, the ear and stem organs represent the plant's reproductive potential, and the reduction in biomass allocation to these organs reflects a decreased investment of resources in reproduction. By reducing the investment in reproductive organs, plants allocate more resources to vegetative organs, promoting

Table 2
The application of nitrogen and phosphorus fertilizers affected the accumulation and distribution of aboveground organ biomass during 2017–2019.

Year	Nitrogen	Phosphorus	Biomass (g m^{-1})			Ratio of biomass (%)		
			Ear	Stem	Leaf	Ear	Stem	Leaf
2017	N0	P0	42.40 ± 3.60ab	86.70 ± 5.00bc	42.30 ± 1.80c	24.71a	50.56bc	24.73b
		P60	34.85 ± 2.45bc	66.40 ± 6.10cd	42.95 ± 3.55c	24.19a	46.03c	29.78b
		P75	42.12 ± 4.60ab	100.22 ± 16.28ab	34.37 ± 8.07c	23.83a	56.48ab	19.68bc
		P90	46.65 ± 0.75a	113.75 ± 4.25a	24.90 ± 0.70c	25.19a	61.38a	13.43c
	N60	P0	34.23 ± 3.74bc	60.57 ± 6.60d	83.00 ± 15.32b	19.29b	34.2d	46.52a
		P60	32.05 ± 4.65c	51.35 ± 7.85d	98.20 ± 7.40ab	17.61b	28.21d	54.18a
		P75	38.70 ± 2.20abc	69.55 ± 4.25cd	100.05 ± 1.95ab	18.6b	33.37d	48.03a
		P90	35.57 ± 2.97bc	59.23 ± 5.39d	106.17 ± 8.91a	17.71b	29.46d	52.83a
2018	N0	P0	6.18 ± 0.54c	17.29 ± 1.35d	103.69 ± 5.77abc	4.85d	13.59d	81.56a
		P60	11.23 ± 1.43c	32.07 ± 4.29cd	91.41 ± 2.67bc	8.36cd	23.75c	67.89b
		P75	15.44 ± 4.59bc	40.60 ± 7.78b	104.96 ± 6.55abc	9.46bc	25.07c	65.48bc
		P90	15.88 ± 1.63bc	43.62 ± 2.19b	83.44 ± 9.64c	11.1abc	30.6b	58.3cd
	N60	P0	26.99 ± 6.83 ab	70.50 ± 9.66a	110.04 ± 4.12ab	12.91 ab	33.87ab	53.22de
		P60	28.34 ± 2.10a	72.74 ± 0.80a	122.58 ± 14.75a	12.67 ab	32.64ab	54.69de
		P75	31.13 ± 7.41a	77.68 ± 3.11a	115.74 ± 7.71a	13.75a	34.67 ab	51.58de
		P90	33.31 ± 3.86a	86.40 ± 12.83a	118.73 ± 5.70a	13.95a	36.11a	49.94e
2019	N0	P0	4.73 ± 0.58bc	8.31 ± 1.08c	38.64 ± 2.12e	9.12a	16.04bc	74.84cd
		P60	6.32 ± 1.23ab	12.56 ± 1.91abc	46.65 ± 1.30de	9.58a	19.1ab	71.32d
		P75	7.70 ± 0.44a	18.20 ± 1.85a	56.83 ± 3.34d	9.31a	22.02a	68.67d
		P90	4.93 ± 1.87bc	13.20 ± 3.90abc	77.15 ± 7.93c	5.11b	13.78cd	81.11bc
	N60	P0	2.56 ± 0.44c	10.64 ± 0.17bc	138.70 ± 5.38a	1.68c	7.01e	91.31a
		P60	7.04 ± 0.74ab	16.75 ± 0.99 ab	117.74 ± 6.96b	4.97b	11.84cde	83.18b
		P75	5.80 ± 0.31ab	14.25 ± 3.78abc	129.47 ± 4.20 ab	3.88b	9.45de	86.67ab
		P90	6.37 ± 0.43ab	18.41 ± 2.04a	135.33 ± 4.53a	3.98b	11.47cde	84.55b

Different letters in the table indicate significant differences among treatments in the same year.

vegetative growth. The architecture of a plant is a crucial determinant of resource acquisition and influences plant growth and biomass partitioning [24]. Leaves, as the primary vegetative organs, provide energy and carbon sources for plant growth through photosynthesis [25]. Studies have shown that a reduction in aboveground biomass decreases the total nutrient demand of plants, prompting a shift in plant competition from underground to aboveground organs [26]. The main method is by increasing leaf area, leaf biomass, and plant height to enhance the capture of light energy [25,27]. Our experiment results, which show an increase in leaf biomass, leaf biomass proportion, and plant height despite a decrease in aboveground biomass, can be considered a result of the plant's internal structural adjustment (Fig. 1B and Table 2) [28].

4.2. Nitrogen fertilizer promotes biomass allocation to leaves

Plant plasticity for the adjustment of various organ phenotypic traits to adapt to the environment [29]. Research has shown that *Alhagi sparsifolia* allocates more biomass to resource-acquiring organs, especially leaves and fine roots, under nutrient-rich conditions [2]. Our experiment found that nitrogen fertilizer has an advantage over phosphorus fertilizer in promoting the development of leaf organs. Nitrogen application primarily increases the biomass and proportion of stems and leaves, while phosphorus fertilizer mainly increases the biomass and proportion of ears and stems (Table 2). Increasing the allocation of plant resources to leaves is beneficial for enhancing forage quality. Enhancing the photosynthetic fixation of light energy is a prerequisite for improving forage quality. There are two main ways to enhance plant photosynthetic capacity: increasing photosynthetic efficiency and increasing the number of photosynthetic organs. Nitrogen fertilizer stimulates leaf nitrogen content and photosynthetic protein enzyme activity, enhancing photosynthesis and carbon assimilation. Phosphorus fertilizer improves plant phosphorus status and photosynthetic rate, leading to enhanced non-structural carbohydrate transfer within the plant [30]. Crude protein content is a major indicator of forage quality, and higher levels of acid detergent fiber and neutral detergent fiber reduce forage quality (Fig. 4A, B, and 4C) [9,10]. Nitrogen application reduces ADF and increases CP in switchgrass [31]. However, excessive nitrogen fertilizer can increase plant cell wall components and fiber content, thereby reducing CP content [7,32]. Our experimental results indicate that compared to phosphorus fertilizer, nitrogen fertilizer is more advantageous in increasing forage CP (Table 4). There is a strong correlation between CP and leaf water use efficiency, with the highest values occurring in the treatment with combined nitrogen and phosphorus fertilization (Figs. 2D–3A and 3B, 3C and Table 4). Additionally, when nitrogen and phosphorus fertilizers are co-applied, they maintain stomatal conductance, enhance photosynthetic rate, reduce transpiration rate, and then enhance high-efficiency water use at the leaf level (Fig. 2A, B, 2C, 2D). This is particularly important for plants in alpine regions where precipitation is far less than evaporation (Fig. S1) [33].

4.3. Only phosphorus fertilization is unfavorable for the maintenance of grassland productivity

A global-scale study has shown that, in grassland ecosystems, the phenotypic traits of aboveground biomass and plant height in Poaceae plants are more sensitive to nitrogen than phosphorus [34]. In our experiment, the promotion effect of nitrogen fertilizer on

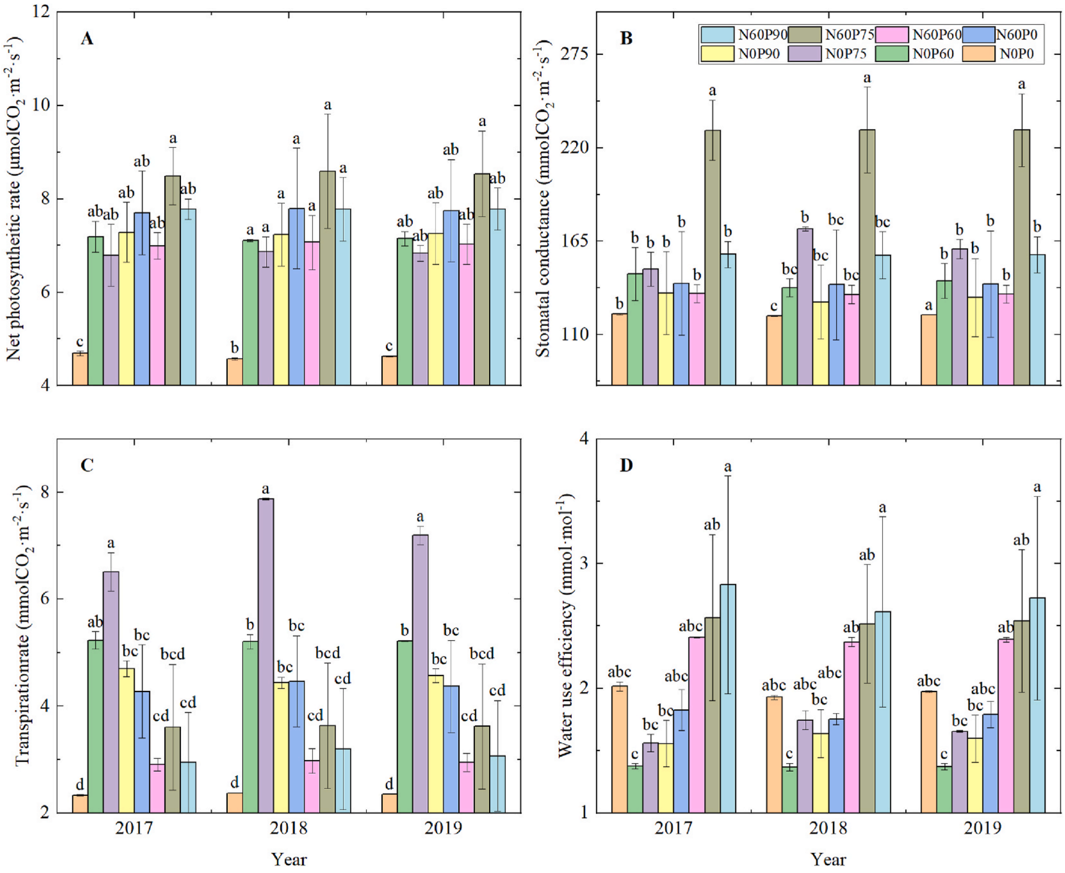

Fig. 2. The photosynthetic parameters of leaves with nitrogen fertilizer and phosphorus fertilizer in 2017–2019, with net photosynthetic rate (A), stomatal conductance (B), transpiration rate (C) and water use efficiency (D). Different letters in the figure indicate significant differences between treatments in the same year.

Table 3
Analysis of covariance (ANCOVA) of forage quality among nitrogen fertilizer and phosphorus fertilizer in the period 2017–2019.

Factor	CF	ADF	NDF	CP	SS
N	8.704**	1.646ns	0.081ns	72.659**	64.434**
P	3.321*	0.194ns	3.247*	9.302**	2.441ns
Y	1853.054**	25.831**	0.178ns	25.011**	43.883**
N*P	1.447ns	0.435ns	4.105*	3.07*	3.612*
N*Y	0.335ns	2.257ns	1.527ns	1.022ns	26.445**
P*Y	1.368ns	0.273ns	1.562ns	0.545ns	1.16ns
N*P*Y	0.613ns	0.306ns	1.727ns	1.115ns	1.67ns

aboveground biomass, plant height, and CP was significantly higher than that of phosphorus fertilizer under the same fertilization level (Fig. 1B and D and Table 4). The lower values of aboveground biomass and fertilizer agronomic efficiency in the sole phosphorus application treatment indicate that phosphorus fertilizer, compared to nitrogen fertilizer, is less effective in enhancing these parameters (Fig. 1A, D and Fig. 6B). Phosphorus fertilizer, compared to nitrogen fertilizer, is less prone to leaching and can be fixed in the soil to maintain long-term fertility [35,36]. Plants exhibit a tolerance to phosphorus nutrition, and under low phosphorus conditions, the activity of phosphatase enzymes in the rhizosphere is high, accelerating the mineralization of soil organic phosphorus and thus improving soil fertility [37–39]. Phosphorus accumulation and translocation and the efficiency of p utilization were mainly determined by the genotype in relation to environmental condition of growth [15,16]. Phosphorus fertilizer has been shown to enhance plant biomass more significantly under water-stressed conditions in a maize-grass pea intercropping systemin [39]. However, a study on the perennial legume (*Medicago sativa* L.) found that the synergistic effect of phosphorus fertilizer and water varied with the age of the plants. Phosphorus fertilizer promoted the consumption of soil water in two-year-old alfalfa but did not enhance soil water consumption in five-year-old plants [40]. Our experiment, conducted in years with low rainfall in 2018 and 2019 (Fig. S1), showed that regardless of whether the amount of phosphorus fertilizer was increased or decreased based on the local customary application,

Table 4
The forage quality with nitrogen fertilizer and phosphorus fertilizer in 2017–2019.

Y	N	P	CF (%)	ADF (%)	NDF (%)	CP (%)	SS (%)
2017	N0	P0	19.17 ± 3.02a	44.03 ± 3.66a	77.80 ± 3.80ab	6.26 ± 0.49b	1.73 ± 0.15a
		P60	20.37 ± 2.02a	44.70 ± 0.66a	74.17 ± 3.22ab	6.72 ± 0.35ab	1.23 ± 0.15a
		P75	18.63 ± 2.45a	43.25 ± 2.85a	73.10 ± 5.40ab	6.74 ± 0.37 ab	1.20 ± 0.00a
		P90	20.35 ± 0.45a	44.75 ± 0.95a	77.60 ± 0.20ab	6.61 ± 0.16ab	1.20 ± 0.10a
	N60	P0	20.50 ± 0.66a	42.50 ± 5.07a	69.70 ± 4.17b	6.63 ± 0.50ab	1.37 ± 0.15a
		P60	20.17 ± 0.15a	42.53 ± 0.59a	73.80 ± 0.36ab	7.14 ± 0.44ab	1.60 ± 0.10a
		P75	20.57 ± 0.15a	42.70 ± 0.10a	74.27 ± 0.15ab	7.35 ± 0.46ab	1.30 ± 0.20a
		P90	20.23 ± 0.42a	41.80 ± 2.04a	78.80 ± 0.80a	7.62 ± 0.35a	1.47 ± 0.47a
2018	N0	P0	41.27 ± 1.50a	46.37 ± 1.24a	74.43 ± 2.34a	6.48 ± 0.24b	3.13 ± 0.93ab
		P60	40.37 ± 2.03a	47.40 ± 1.57a	72.73 ± 1.80a	6.65 ± 0.40b	4.27 ± 0.35a
		P75	41.37 ± 0.9a	48.57 ± 2.92a	76.2 ± 3.69a	6.91 ± 0.26ab	2.90 ± 0.79ab
		P90	40.50 ± 1.78a	46.27 ± 0.45a	73.03 ± 1.91a	6.64 ± 0.08b	2.90 ± 0.82ab
	N60	P0	43.83 ± 0.67a	48.47 ± 2.20a	75.53 ± 0.58a	6.83 ± 0.66ab	1.50 ± 0.10b
		P60	41.20 ± 0.76a	47.23 ± 2.15a	74.70 ± 1.45a	7.55 ± 0.48ab	1.63 ± 0.35b
		P75	40.77 ± 1.33a	48.17 ± 2.1a	74.1 ± 1.82a	7.84 ± 0.62ab	1.60 ± 0.66b
		P90	41.07 ± 0.23a	47.43 ± 1.01a	75.60 ± 3.82a	8.14 ± 0.73a	1.97 ± 0.29b
2019	N0	P0	41.77 ± 1.68b	45.20 ± 1.76a	76.12 ± 2.36a	7.25 ± 0.10c	2.43 ± 0.45ab
		P60	40.37 ± 1.85b	46.05 ± 1.08a	73.45 ± 2.43a	7.16 ± 0.18c	2.75 ± 0.25a
		P75	40.43 ± 1.78b	45.8 ± 0.56a	74.4 ± 0.96a	7.27 ± 0.18c	2.42 ± 0.08ab
		P90	40.50 ± 1.73b	45.51 ± 0.70a	75.32 ± 0.98a	7.37 ± 0.30c	2.05 ± 0.38abc
	N60	P0	44.33 ± 0.21a	45.48 ± 3.25a	72.62 ± 1.98a	7.47 ± 0.16bc	1.43 ± 0.13c
		P60	41.20 ± 0.35b	44.88 ± 1.06a	74.25 ± 0.85a	8.43 ± 0.22a	1.62 ± 0.20bc
		P75	41.38 ± 0.49b	44.77 ± 0.76a	75.77 ± 1.51a	8.21 ± 0.15a	1.7 ± 0.26bc
		P90	41.57 ± 1.10b	44.62 ± 0.68a	77.20 ± 2.22a	7.99 ± 0.13 ab	1.72 ± 0.38bc

Different letters in the table indicate significant differences among treatments in the same year. N, P and Y refer to nitrogen fertilizer, phosphorus fertilizer and growth years. CF, ADF, NDF, CP and SS refer to crude fiber, acid detergent fiber, neutral detergent fiber, crude protein and soluble total sugar, respectively.

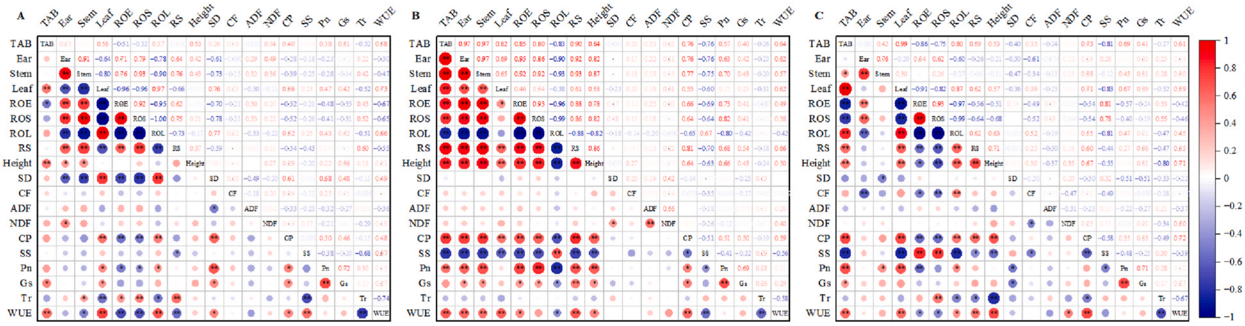

Fig. 3. Correlation analysis between agronomic traits and quality of each year. 2017 (A), 2018 (B) and 2019 (C). In the figure, TAB, Ear, Stem, Leaf, RS, SD, CF, ADF, NDF, CP, SS, ROE, ROS, ROL, Pn, Gs, Tr, WUE refer to total aboveground biomass, biomass of ear, biomass of stem, biomass of leaf, reproductive shoots, stem diameter, crude fiber, acid detergent fiber, neutral detergent fiber, crude protein, soluble total sugar, ratio biomass of ear, ratio biomass of stem, ratio biomass of leaf, net photosynthetic rate, stomatal conductance, transpiration rate and water use efficiency, respectively.

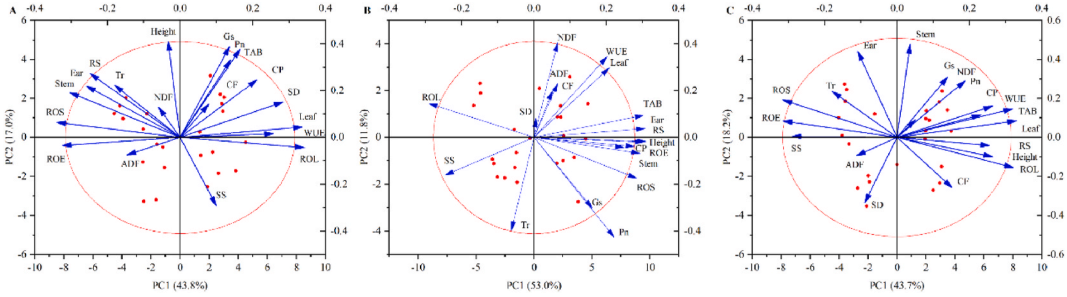

Fig. 4. The interrelationship patterns among forage quality between agronomic traits indicators based on the PCA diagrams during 2017–2019. 2017 (A), 2018 (B) and 2019 (C).

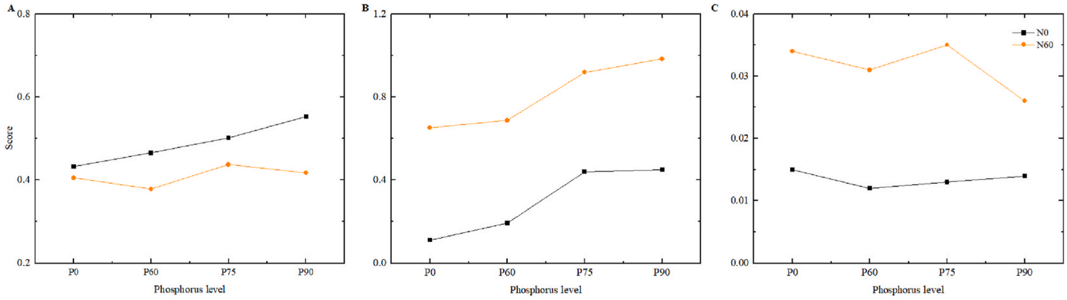

Fig. 5. The evaluation of forage production and quality based on TOPSIS with EW in the period 2017–2019. 2017 (A), 2018 (B) and 2019 (C).

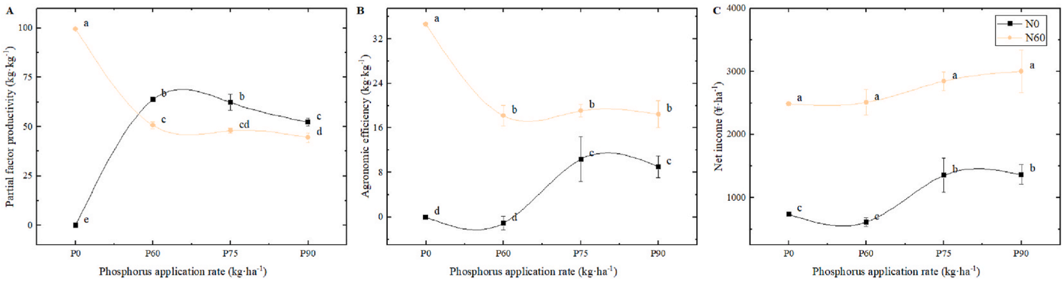

Fig. 6. Fertilizer use efficiency and net income under different fertilization treatments during 2017–2019. 2017 (A), 2018 (B) and 2019 (C).

the aboveground biomass and leaf-level water use efficiency were low. In contrast, the addition of nitrogen fertilizer led to opposite effects (Figs. 1D and 2D). This further suggests that the long-term application of phosphorus fertilizer is not conducive to maintaining the productivity of *F. Kryloviana* grasslands.

4.4. Future fertilization management

A comprehensive evaluation of agronomic traits, biomass allocation, photosynthetic parameters, and nutritional quality indicates that the local common practice of applying only 75 kg ha^{-1} of phosphorus fertilizer is disadvantages for forage production. In contrast, the combined application of nitrogen and phosphorus fertilizers shows advantages (Fig. 5A, B and 5C). However, considering the fertilizer use efficiency and economic benefits (Fig. 6A, B and 6C), we recommend a focus on nitrogen management in artificial grasslands of *F. Kryloviana*. Due to the susceptibility of nitrogen to runoff and leaching [36], resulting in lower actual fertilizer use efficiency. Future experiments will consider effective nitrogen fertilization methods to promote efficient management of *F. Kryloviana* grasslands.

5. Conclusions

Our experiment demonstrated that the interactive effects between nitrogen and phosphorus fertilizers, by altering the biomass allocation patterns of aboveground organs in *F. kryloviana*, had an advantage in sustaining both forage yield and nutritional quality. The traditional fertilization practice, which was predominantly reliant on phosphorus, was found to be detrimental to the stable maintenance of productivity in *F. kryloviana* artificial grasslands. Given the higher forage productivity, fertilizer utilization efficiency, and economic value observed after nitrogen application, we recommend an emphasis on nitrogen fertilization in future artificial grassland management practices.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Zhenghai Shi: Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Guolin Liang:** Writing – review & editing, Methodology. **Wenhui Liu:** Project administration, Funding acquisition, Conceptualization. **Sida Li:** Writing – original draft, Visualization. **Yan Qin:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wenhui Liu reports financial support was provided by Qinghai innovation platform construction project. Wenhui Liu reports financial support was provided by China Agriculture Research System. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35116.

References

[1] W. Zhang, et al., An aboveground biomass partitioning coefficient model for rapeseed (Brassica napus L.), Field Crops Res. 259 (2020).

[2] Z. Zhang, et al., Nitrogen application mitigates drought-induced metabolic changes in Alhagi sparsifolia seedlings by regulating nutrient and biomass allocation patterns, Plant Physiol. Biochem. 155 (2020) 828–841.

[3] V. Kumar, C.R. Babu, Phenotypic responses of some functional traits in four native perennial grass species grown on fly ash dump and native soil, Front. Plant Sci. 13 (2022) 805568.

[4] A. Liu, et al., One-off basal application of nitrogen fertilizer increases the biological yield but not the economic yield of cotton in moderate fertility soil, Field Crops Res. 288 (2022).

[5] J. Zhang, et al., PRDI can maintain aboveground biomass and increase economic benefits in alfalfa through regulating N:P ratios in roots and leaves, Field Crops Res. 253 (2020).

[6] Z. Liu, et al., Timing and splitting of nitrogen fertilizer supply to increase crop yield and efficiency of nitrogen utilization in a wheat–peanut relay intercropping system in China, The Crop Journal 7 (1) (2019) 101–112.

[7] M. Kamran, et al., Irrigation and nitrogen fertilization influence on alfalfa yield, nutritive value, and resource use efficiency in an arid environment, Field Crops Res. 284 (2022).

[8] J. Hakl, et al., Impact of long-term manure and mineral fertilization on yield and nutritive value of lucerne (Medicago sativa) in relation to changes in canopy structure, Eur. J. Agron. 123 (2021) 126219.

[9] Q. Zhang, et al., Optimizing the nutritional quality and phosphorus use efficiency of alfalfa under drip irrigation with nitrogen and phosphorus fertilization, Agron. J. 112 (4) (2020) 3129–3139.

[10] X. Mao, et al., Application of molybdenum fertilizer enhanced quality and production of alfalfa in northern China under non-irrigated conditions, J. Plant Nutr. 41 (8) (2018) 1009–1019.

[11] B.H. Andrianary, et al., Phosphorus application affects lowland rice yields by changing phenological development and cold stress degrees in the central highlands of Madagascar, Field Crops Res. 271 (2021).

[12] G.S. Demirer, et al., Phosphate deprivation-induced changes in tomato are mediated by an interaction between brassinosteroid signaling and zinc, New Phytologist 239 (4) (2023) 1368–1383.

[13] A. Hernandez-Cruz, et al., Nitrate reductase activity, biomass, yield, and quality in cotton in response to nitrogen fertilization, Phyton, International Journal of Experimental Botany 84 (2) (2015) 454–460.

[14] J. Zhang, et al., Long-term N and P additions alter the scaling of plant nitrogen to phosphorus in a Tibetan alpine meadow, Sci. Total Environ. 625 (2018) 440–448.

[15] C. Dordas, Dry matter, nitrogen and phosphorus accumulation, partitioning and remobilization as affected by N and P fertilization and source–sink relations, Eur. J. Agron. 30 (2) (2009) 129–139.

[16] D.K. Papakosta, Phosphorus accumulation and translocation in wheat as affected by cultivar and nitrogen fertilization, J. Agron. Crop Sci. 173 (3-4) (1994) 260–270.

[17] S. Munné-Bosch, Do perennials really senesce? Trends Plant Sci. 13 (5) (2008) 216–220.

[18] S. Wahl, P. Ryser, Root tissue structure is linked to ecological strategies of grasses, New Phytol. 148 (3) (2000) 459–471.

[19] H. Cui, et al., Phosphorus addition regulates the responses of soil multifunctionality to nitrogen over-fertilization in a temperate grassland, Plant Soil (2020) 1–15.

[20] A.D. Iwaasa, et al., Beef cattle grazing behaviour differs among diploid and tetraploid crested wheatgrasses (Agropyron cristatumandA. desertorum), Can. J. Plant Sci. 94 (5) (2014) 851–855.

[21] A.K. Hegazy, et al., Chemical ingredients and antioxidant activities of underutilized wild fruits, Heliyon 5 (6) (2019) e01874.

[22] G. Zhang, et al., Spatio-temporal variation in grassland degradation and its main drivers, based on biomass: case study in the Altay Prefecture, China, Global ecology and conservation 20 (2019) e00723.

[23] X.-F. Xie, et al., Biomass allocation of stoloniferous and rhizomatous plant in response to resource availability: a phylogenetic meta-analysis, Front. Plant Sci. 7 (2016) 186064.

[24] J. Gu, et al., Quantifying differences in plant architectural development between hybrid potato (Solanum tuberosum) plants grown from two types of propagules, Ann. Bot. 133 (2) (2024) 365–378.

[25] X. Liang, et al., Global response patterns of plant photosynthesis to nitrogen addition: a meta-analysis, Global Change Biol. 26 (6) (2020) 3585–3600.

[26] U. Niinemets, Key plant structural and allocation traits depend on relative age in the perennial herb Pimpinella saxifraga, Ann. Bot. 96 (2) (2005) 323–330.

[27] A.W. Larkum, et al., Selection, breeding and engineering of microalgae for bioenergy and biofuel production, Trends Biotechnol. 30 (4) (2012) 198–205.

[28] R. Pierik, et al., Architecture and plasticity: optimizing plant performance in dynamic environments, Plant Physiol 187 (3) (2021) 1029–1032.

[29] A. Atlan, et al., Phenotypic plasticity in reproductive traits of the perennial shrub Ulex europaeus in response to shading: a multi-year monitoring of cultivated clones, PLoS One 10 (9) (2015) e0137500.

[30] W. Zhang, et al., Soil phosphorus availability alters the correlations between root phosphorus-uptake rates and net photosynthesis of dominant C3 and C4 species in a typical temperate grassland of Northern China, New Phytol. 240 (1) (2023) 157–172.

[31] M.K. Kering, et al., Effect of nitrogen fertilizer rate and harvest season on forage yield, quality, and macronutrient concentrations in midland Bermuda grass, Commun. Soil Sci. Plant Anal. 42 (16) (2011) 1958–1971.

[32] M. Islam, et al., Effects of irrigation and rates and timing of nitrogen fertilizer on dry matter yield, proportions of plant fractions of maize and nutritive value and in vitro gas production characteristics of whole crop maize silage, Anim. Feed Sci. Technol. 172 (3–4) (2012) 125–135.

[33] Z. Zhang, et al., The response of lake area and vegetation cover variations to climate change over the Qinghai-Tibetan Plateau during the past 30 years, Sci. Total Environ. 635 (2018) 443–451.

[34] W. Li, et al., Nitrogen effects on grassland biomass production and biodiversity are stronger than those of phosphorus, Environ. Pollut. 309 (2022) 119720.

[35] O. Urrutia, et al., Theoretical chemical characterization of phosphate-metal–humic complexes and relationships with their effects on both phosphorus soil fixation and phosphorus availability for plants, J. Sci. Food Agric. 93 (2) (2013) 293–303.

[36] R.L. Whetton, et al., Communicating nitrogen loss mechanisms for improving nitrogen use efficiency management, focused on global wheat, Nitrogen 3 (2) (2022) 213–246.

[37] S.-G. Zhu, et al., Soil water and phosphorus availability determines plant-plant facilitation in maize-grass pea intercropping system, Plant Soil 482 (1–2) (2022) 451–467.

[38] S.-G. Zhu, et al., Soil phosphorus availability and utilization are mediated by plant facilitation via rhizosphere interactions in an intercropping system, Eur. J. Agron. 142 (2023).

[39] S.-G. Zhu, et al., Plant facilitation shifts along with soil moisture and phosphorus gradients via rhizosphere interaction in the maize-grass pea intercropping system, Ecol. Indicat. 139 (2022).

[40] J.W. Fan, et al., Forage yield, soil water depletion, shoot nitrogen and phosphorus uptake and concentration, of young and old stands of alfalfa in response to nitrogen and phosphorus fertilisation in a semiarid environment, Field Crops Res. 198 (2016) 247–257.