

Article

Soil Nitrogen Prevails in Controlling Alpine Meadow Productivity Despite *Medicago ruthenica* Reseeding and Phosphorus Application

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Abstract

Under intensified global climate change and anthropogenic pressures, alpine ecosystems confront unprecedented stress. The degradation of alpine meadows has caused significant declines in productivity and in the abundance of high-quality forage species. This study aims to explore the effects of phosphorus (P) application and reseeded of *Medicago ruthenica* (L.) Trautv. on the biomass and quality of forage in degraded alpine meadows, and to identify the key soil factors influencing forage growth. Three reseeded rates (V_1 : low, V_2 : medium, V_3 : high) and three P levels (P_0 : none, P_1 : low, P_2 : high) were established in this experiment. The factors were arranged in a completely randomized design, resulting in nine distinct treatment combinations, that is V_1P_0 , V_1P_1 , V_1P_2 , V_2P_0 , V_2P_1 , V_2P_2 , V_3P_0 , V_3P_1 , and V_3P_2 . The results showed that the interaction between the reseeded and P addition exerts a significant effect on the biomass of *M. ruthenica*, forbs, and aboveground biomass ($p < 0.05$). Additionally, the interaction between the reseeded and P addition had a significant effect on crude protein content ($p < 0.05$). Phosphorus addition and the interaction between the reseeded and P addition had a significant effect on ether extract content ($p < 0.05$). However, it is only reseeded that can significantly influence the neutral detergent fiber content ($p < 0.05$). Grey correlation analysis revealed that the V_3P_2 treatment optimized both forage biomass and nutritional quality. Hierarchical partitioning further identified soil total nitrogen as the factor that contributed the most to forage biomass and quality following reseeded and phosphorus application.

Keywords: P addition; biomass and nutrient quality; soil chemical properties



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1. Introduction

The Tibetan Plateau, hosting the world's largest alpine meadow grasslands, serves as both a crucial natural gene pool for cold-adapted biodiversity and a vital ecological security barrier for both China and Asia [1]. However, alpine meadow degradation driven by climate change, overgrazing, and rodent activity, has triggered severe ecological impacts, such as reduced productivity, soil nutrient loss, and declining forage quality. These consequences are reflected in key degradation indicators: the loss of high-quality forage species, diminished biodiversity, and significant biomass reduction [1]. Forage is indispensable to livestock production systems, as it provides essential dietary roughage to support digestive function and nutrient utilization [2]. Thus, developing integrated solutions to mitigate

meadow degradation while enhancing forage biomass and nutritional quality has become a critical research priority in the region.

Previous studies have shown that reseeding and fertilization are the most commonly used methods to improve the forage quality and yield in degraded alpine meadows. Reseeding promotes the establishment of high-value forage species in plant communities, while fertilization enhances soil nutrient availability to support productivity [3]. Specifically, reseeding legumes into grass-dominated natural meadow ecosystems can significantly boost both forage biomass and quality [4]. For example, reseeding *Medicago falcata* L. in degraded grasslands increased aboveground net primary productivity by 28.3% [5], and reseeding the legume *Vicia villosa* Roth led to a 29.78% significant increase in crude protein content at the second-year harvest compared to controls [6].

Extensive evidence confirms that species selection is fundamental to reseeding effectiveness, with native species showing distinct advantages in ecological adaptation and population establishment [7]. For this reason, we selected the local native legume *Medicago ruthenica* as the reseeding species. *M. ruthenica* is an excellent forage with high nutritional value and biomass; it is cold-tolerant, drought-resistant, adaptable to poor soils, and trampling-resistant, making it well-suited to cold and arid regions [8]. As a native species, it is also well adapted to local climate and soil conditions, facilitating successful reseeding.

Notably, low soil P significantly limits legume growth and symbiotic nitrogen (N) fixation [9], and legumes have high P demands to optimize nodulation and nitrogen fixation efficiency [10]. To ensure the successful establishment of *M. ruthenica*, we therefore implemented targeted P supplementation. Phosphorus application after legume reseeding increases soil P content, which is critical for legume growth, development, and biological N fixation [11]. Beyond supporting legumes, P is a vital soil nutrient: its storage and supply capacity are essential for healthy plant growth, microbial survival, and soil ecosystem stability [12]. Additionally, P fertilization increases soil available P and improves grassland quality [13], and a meta-analysis of long-term field experiments in global natural and semi-natural herbaceous ecosystems showed that P addition increases aboveground biomass by an average of 33% [14].

Biomass and nutritional quality of forage serve as comprehensive biological indicators of soil fertility, integrating the synergistic effects of soil physicochemical properties, nutrient availability, and microbial activity [15]. Building on this, the study hypothesizes that the combined application of the reseeding and P addition will yield significantly greater improvements in forage biomass and nutritional quality than reseeding alone. Specifically, our objectives are to (1) identify the optimal treatment combination that maximizes forage productivity and quality; (2) determine the key soil factors influencing these forage parameters. Our research findings will offer practical guidance for the restoration of soil-forage systems in degraded alpine grasslands.

2. Materials and Methods

2.1. General Situation of the Study Site

The study site is located in the alpine meadow grasslands of Gannan (35°3'29", 102°22'30") on the northeastern edge of the Qinghai–Tibetan Plateau, with altitudes ranging from 1172 m to 4920 m. The region has a continental plateau climate. The mean annual air temperature is 2.6 °C, and the average annual precipitation is 516 mm, with most precipitation occurring during the growing season from April to September (Figure 1). The sampling site exhibits rich species diversity, with the dominant species being the grasses *Elymus nutans* Griseb. and *Poa annua* L. Common and taller weeds include *Ligularia sagitta* (Maxim.) Mattf., *Saussurea japonica* (Thunb.) DC. and *Picris hieracioides* L., *Gymnaconitum gymnanthum* (Maxim.), *Anemone rivularis* var. *flore-minore* Maxim. and *Delphinium grandiflorum* L., and

Elsholtzia densa Benth. Common low-growing forbs include *Geranium wilfordii* Maxim., *Lancea tibetica*, and *Thalictrum aquilegiifolium* var. *sibiricum* *Oxytropis kansuensis*.

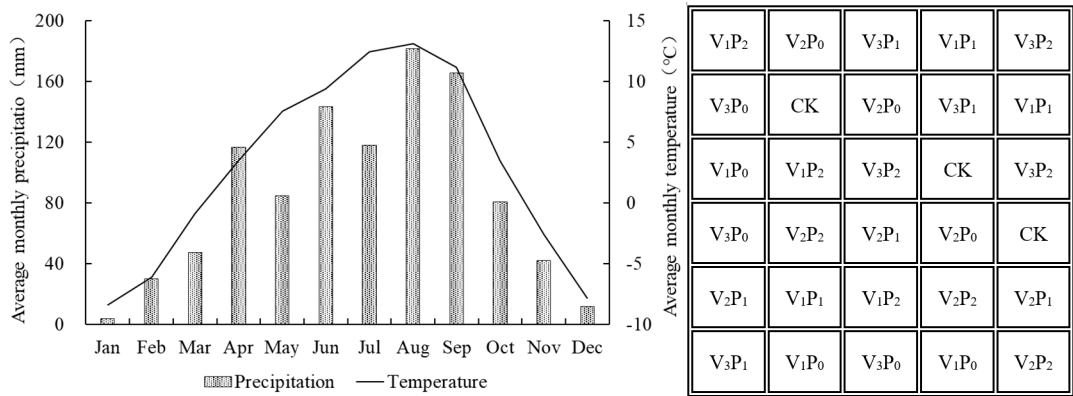


Figure 1. Meteorological data of the sampling plot and layout of the experiment.

2.2. Experimental Material and Design

The reseeding material, *Medicago ruthenica* ‘Longzhong 1’ (Number: GS–CWV–2022–005), was developed through domestication and selection from wild populations native to Qinghai–Tibetan Plateau alpine meadows. Superphosphate ($P_2O_5 \geq 12\%$) was applied as the fertilizer. In 2023, we selected alpine meadows with fence protection, high grazing intensity, weak vegetation growth and toxic weeds, which were classified as moderately degraded according to the China’s National Standard GB19377-2003 “Parameters for degradation, sandification and salification of rangelands” as the experiment site [16]. As shown in Table 1, a completely randomized design was used in this experiment, with three levels of *M. ruthenica* reseeding: V_1 (low rate, 9 kg/hm²), V_2 (medium rate, 15 kg/hm²), and V_3 (high rate, 22.5 kg/hm²), as well as three levels of P application: P_0 (no addition), P_1 (low level, 75 kg/hm²), and P_2 (high level, 200 kg/hm²). This yielded nine unique combinations of reseeding and fertilization treatments, in addition to a control (CK) group that received neither reseeding nor fertilization. The plots were 10 m × 10 m in size, with a spacing of 2 m between them. Each treatment was replicated three times, making a total of 30 plots. The plots were treated at the end of May 2023. Reseeding and fertilization were performed manually. Prior to fertilization, the seeds were evenly hand-broadcast across plots and lightly incorporated into the topsoil (1–2 cm depth) using rakes to minimize surface exposure. Phosphorus fertilizer was applied on a rainy evening to enhance dissolution and soil incorporation.

Table 1. Each combination treatment and their abbreviations.

Treatment	Reseeding Rates (kg/hm ²)	P Levels (kg/hm ²)
CK	0	0
V ₁ P ₀	9 (Low rate)	0 (No P)
V ₁ P ₁	9 (Low rate)	75 (Low level)
V ₁ P ₂	9 (Low rate)	200 (High level)
V ₂ P ₀	15 (Medium rate)	0 (No P)
V ₂ P ₁	15 (Medium rate)	75 (Low level)
V ₁ P ₂	15 (Medium rate)	200 (High level)
V ₃ P ₀	22.5 (High rate)	0 (No P)
V ₃ P ₁	22.5 (High rate)	75 (Low level)
V ₃ P ₂	22.5 (High rate)	200 (High level)

2.3. Investigation of Plant Community Characteristics and Forage Sampling

Plant sampling was conducted during the peak plant growth period in mid-August 2024. The plant communities in the experimental area were surveyed, and the plants were identified to the species level. We randomly selected three 1 m × 1 m quadrats within each plot to measure the aboveground biomass of the plant communities. All plants within the selected quadrats were cut at ground level using scissors. The plants were categorized into three functional groups, that is legumes, grasses, and forbs. The legume group mainly refers to *M. ruthenica* after reseeding, as the primary legume before reseeding was a toxic weed (*Oxytropis kansuensis*) with no feeding value. The samples were placed into three separate envelopes, transported to the laboratory, and dried in an oven at 65 °C to determine the biomass of each functional group and the total aboveground biomass (AGB).

2.4. Methods for Forage Nutrition Analysis

To assess changes in forage nutrient quality under each treatment, the plants were ground to pass through a 2 mm sieve and their nutritional quality was subsequently measured. Crude protein (CP) content was determined using the Kjeldahl method [17]. Ether extract (EE) content was determined using the Soxtec™ 8000 extraction unit (Fuzhou, China) [17]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were determined using the Van Soest method [17].

2.5. Soil Sampling and Chemical Analysis

Soil samples were extracted from the 0–15 cm layer using a 5 cm diameter auger, which were then transported to the laboratory for analysis. To ensure uniformity in soil collection, we employed the diagonal sampling method, taking five subsamples per plot and mixing them evenly. The collected soil was taken to the laboratory, where impurities were removed, and the soil was air-dried subsequently. The soil nutrient content was measured after passing through a 1 mm sieve.

The contents of total N (TN), available N (AN), total P (TP), and available P (AP) were measured according to the method described by Bao [18]. Total N was quantified using the semi-micro Kjeldahl method. Total P was analyzed through digestion with H₂SO₄-HClO₄ and measured by the molybdenum–antimony colorimetric method. Soil organic matter (SOM) was measured using the K₂Cr₂O₇ oxidation method with external heating [18]. Available N was measured using the alkali diffusion method. Available P was measured by NaHCO₃ extraction–colorimetric method [18].

Soil microbial biomass carbon (MBC), microbial biomass N (MBN) and microbial biomass P (MBP) were determined by “Determination of soil microbial biomass—Fumigation-extraction method” in accordance with the China’s National Standard GB/T 39228-2020 [19]. For MBC and MBN determination, three fresh soil samples (10 g each) were placed at room temperature in a vacuum desiccator and fumigated with chloroform for 24 h. After fumigation, both fumigated and unfumigated soil samples were extracted with 0.5 mol/L K₂SO₄. The MBC and MBN were determined using the K₂Cr₂O₇ oxidation method with external heating and the semi-micro Kjeldahl method, respectively [19]. For MBP determination, the samples were fumigated with chloroform following the same procedure described above. Both fumigated and unfumigated soil samples were extracted with 0.5 mol/L NaHCO₃ and analyzed using the molybdenum-antimony colorimetric method [19].

2.6. Statistical Analyses

The Levene test and the Shapiro–Wilk normality test in R software (version 4.3.3; R Foundation for Statistical Computing, Vienna, Austria) confirmed that the data met the

assumptions of chi-square distribution and normality. The significance of the differences ($p < 0.05$) in biomass, nutrient quality, and soil chemical properties after the experimental treatment was analyzed using two-way ANOVA in SPSS 26.0. All bar charts were generated using Excel 2019. The effects of reseeding and phosphorus application on AGB and soil chemical properties were quantified and compared using the response ratio formula [20].

$$R = \ln\left(\frac{T}{C}\right)$$

T denotes the observed specific values of biomass or soil chemical properties for different treatment, and C represents the specific observed values of biomass or soil chemical properties within the control. Positive response ratios indicate an increase in the indicator under this treatment, while negative values suggest the opposite trend.

The grey correlation analysis (GRA) method was used to identify the optimal treatment for forage nutritional quality and biomass. The optimal values of the eight indicators were set as the ideal reference sequence (X_0), and the performance of these indicators were treated as the comparison sequence X_i ($i = 1, 2, 3, \dots, 8$), from which the grey correlation degree was calculated [21]. The analysis steps are as follows: suppose the reference number column is X_0 , the comparison number column is X_i , and $i = 1, 2, 3, \dots, 8$, and $X_0 = \{X_0(1), X_0(2), X_0(3), \dots, X_0(8)\}$, $X_i = \{X_0(1), X_0(2), X_0(3), \dots, X_0(8)\}$, then $\zeta_i(k)$ is called the correlation coefficient between X_0 and X_i at the K point, and the calculation formula is as follows:

$$\zeta_i(k) = \frac{\frac{\min_i \min_k |x_0(k) - x_i(k)| + \rho \frac{\max_i \max_k |x_0(k) - x_i(k)|}{\frac{\max_i \max_k |x_0(k) - x_i(k)|}}{|x_0(k) - x_i(k)| + \rho \frac{\max_i \max_k |x_0(k) - x_i(k)|}{\frac{\max_i \max_k |x_0(k) - x_i(k)|}}}}$$

$$\text{Equal-weight correlation degree: } r_i = \frac{1}{n} \sum_{k=1}^n \zeta(k)$$

$$\text{Weighted correlation degree: } r_i = \frac{1}{n} \sum_{k=1}^n \zeta(k) w_i$$

In the formula, $x_0(k) - x_i(k)$ represents the absolute difference between the X_0 sequence and the X_i sequence at point k , denoted as $\Delta_i(k)$, where $\frac{\min_i \min_k |x_0(k) - x_i(k)|}{\frac{\max_i \max_k |x_0(k) - x_i(k)|}$ is the second-order minimum difference, $\frac{\max_i \max_k |x_0(k) - x_i(k)|}{\frac{\max_i \max_k |x_0(k) - x_i(k)|}$ is the second-order maximum difference, and ρ is the resolution coefficient, which is generally taken as 0.5. w_i represents the weighting coefficient.

To identify the key soil factors influencing forage biomass and quality, hierarchical partitioning analysis was performed using the “rdacca.hp” package in R software (version 4.3.3; R Foundation for Statistical Computing, Vienna, Austria), calculating the contribution rate and significance of each soil factor [22]. Our goal was to identify the key factors affecting the nutritional quality of forage. Therefore, we first treated biomass and quality as response variables, performed redundancy analysis using soil factors as explanatory variables, and then conducted hierarchical partitioning.

3. Results

3.1. The Effects on Biomass

3.1.1. The Effects of Reseeding and P Addition on the Biomass of Different Functional Groups

Table 2 shows the effects on biomass of different functional groups in the alpine meadow after the reseeding and P addition. The reseeding $\times$ P addition interaction had a significant effect on the biomass of *M. ruthenica* and forbs ($p < 0.05$). Reseeding and the reseeding $\times$ P addition interaction had a significant impact on aboveground biomass ($p < 0.05$). Figure 2 shows that the biomass of *M. ruthenica* in the V_1P_1 treatment was

significantly lower than in the other treatments ($p < 0.05$). The V_3P_2 treatment increased grass biomass by 55.3%. AGB was highest under V_3P_2 treatment and significantly increased by 74.48% ($p < 0.05$).

Table 2. Analysis of variance of reseeding, phosphorus application, and their interaction on biomass.

Factor	Significance			
	Legume (<i>M. ruthenica</i>)	Grasses	Forbs	Aboveground Biomass
V	ns	ns	ns	*
P	ns	ns	ns	ns
V $\times$ P	***	ns	*	*

Note: V: reseeding; P: P addition; V $\times$ P: the interaction between reseeding and P addition. Results of ANOVA models are indicated with asterisks (* $0.01 < p < 0.05$; *** $p < 0.001$).

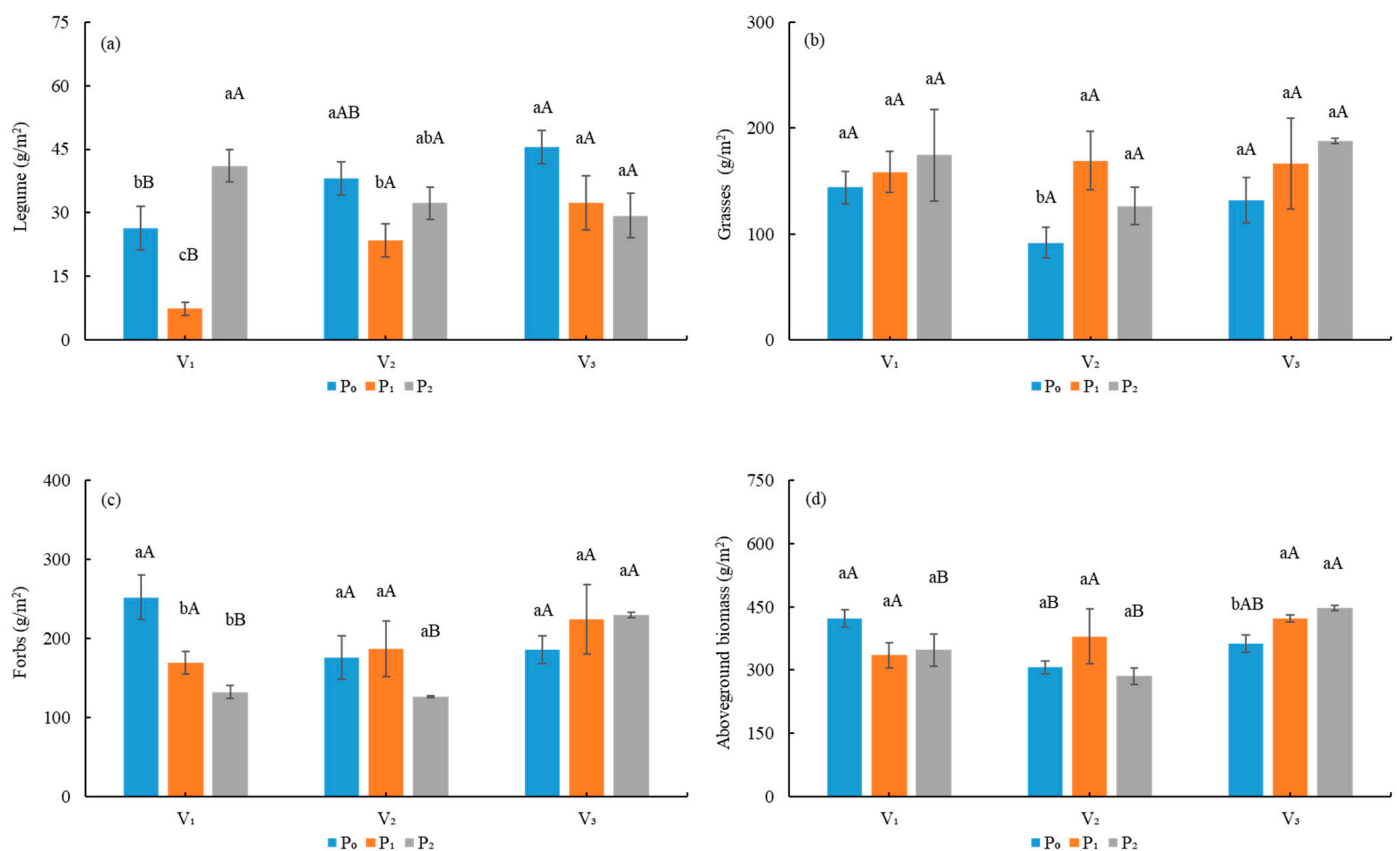


Figure 2. The effects of reseeding and P addition on the biomass of different functional groups. Note: Lowercase letters indicate significant differences among phosphorus levels within the same reseeding level. Uppercase letters indicate significant differences among reseeding levels within the same phosphorus level. (a) Biomass of legume (*M. ruthenica*); (b) Biomass of grasses; (c) Biomass of forbs; (d) Aboveground Biomass.

3.1.2. The Response Ratios of Biomass After Reseeding and Fertilization Under Each Treatment

The response ratio more clearly illustrated the impact of various treatments on the biomass of different functional groups. As shown in Figure 3, except V_2P_0 , reseeding and fertilization treatments had a significant positive impact on the biomass of grasses ($p < 0.05$). Different treatments exhibited both positive and negative effects on forbs biomass. Among all treatments, both V_1P_0 and V_3P_2 had a significant positive effect on forbs biomass ($p < 0.05$). In addition, V_1P_2 and V_2P_2 had significant negative effects on forbs biomass.

Except the V_2P_0 treatment, all other treatments had significant positive effects on AGB ($p < 0.05$).

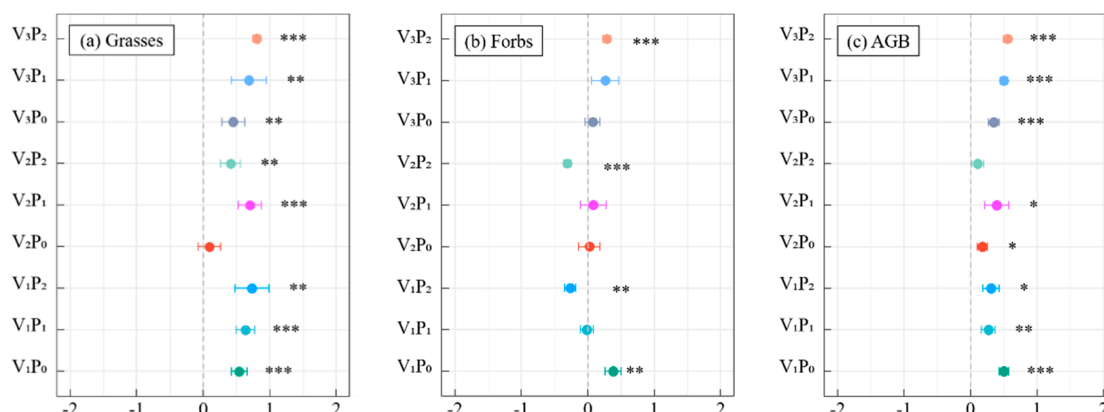


Figure 3. The response ratios of biomass to reseeding and fertilization under each treatment. Note: Asterisks indicate significant differences from zero. * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$.

3.2. The Effects on Forage Nutritional Quality

3.2.1. The Effects of Reseeding and P Addition on Nutritional Quality

Table 3 demonstrates that reseeding and the reseeding $\times$ P addition interaction had a significant effect on CP content in forage ($p < 0.05$). P addition and the reseeding $\times$ P addition interaction had a significant effect on EE content in forage ($p < 0.05$). Reseeding had a significant effect on NDF content in forage ($p < 0.05$). Figure 4 shows that the V_3P_1 treatment resulted in a maximal CP content increase of 41.68% ($p < 0.05$). EE levels reached their maximum (65.5%) under V_3P_2 treatment ($p < 0.05$).

Table 3. Analysis of variance of reseeding, phosphorus application, and their interactions on forage quality.

Factor	Significance			
	Crude Protein	Ether Extract	Neutral Detergent Fiber	Acid Detergent Fiber
V	***	ns	*	ns
P	ns	**	ns	ns
V $\times$ P	***	**	ns	ns

Note: V: reseeding; P: P addition; V $\times$ P: the interactions between reseeding and P addition. Results of ANOVA models are indicated with asterisks (* $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$).

3.2.2. The Response Ratios of Forage Nutritional Quality Under Each Treatment

Figure 5 shows that all treatments except V_1P_0 and V_1P_1 had a significant positive impact on the CP content of forage ($p < 0.05$). Every treatment except V_1P_1 had a significant positive impact on the EE content of forage ($p < 0.05$). All treatments had a significant negative impact on the NDF content of forage ($p < 0.05$). These results indicated that reseeding and phosphorus application synergistically enhance forage nutritional quality.

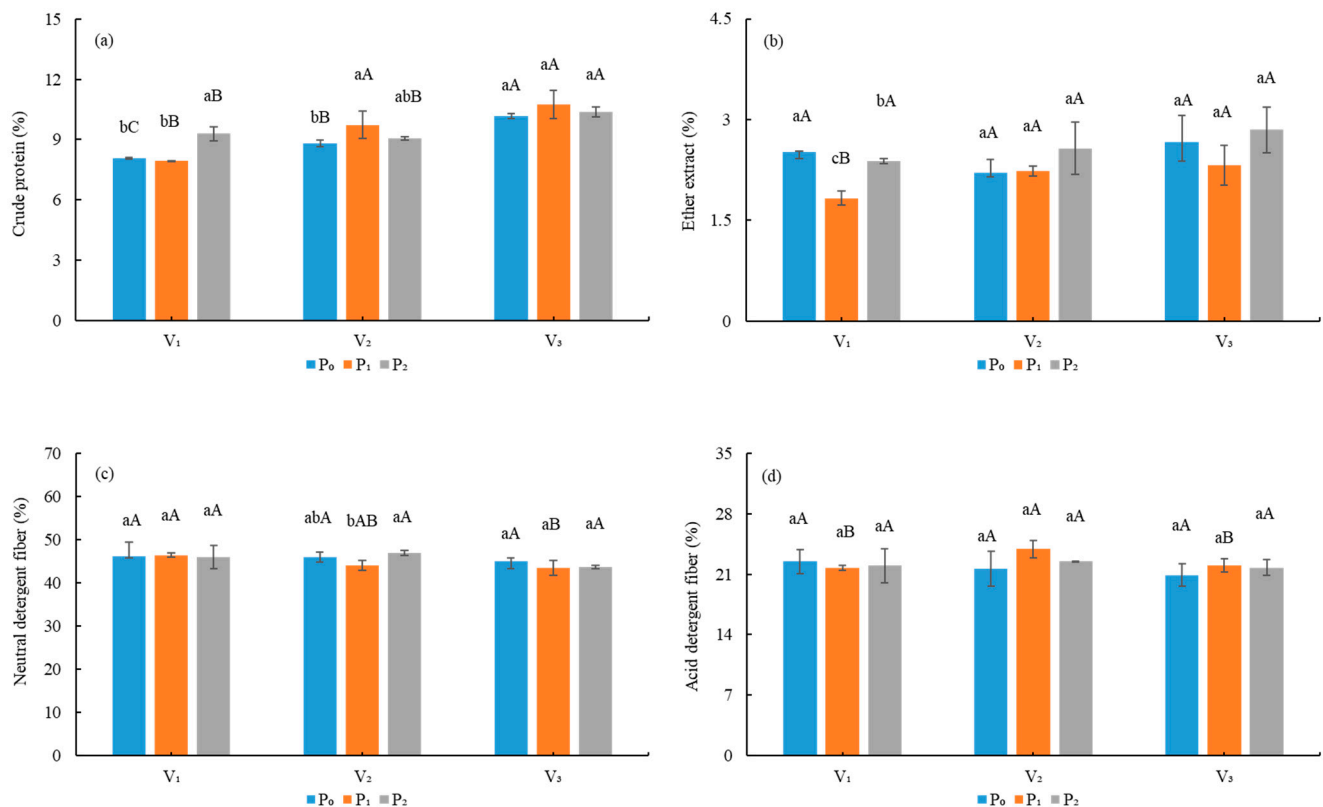


Figure 4. The effects of reseeding and P addition on forage nutritional quality. Note: Lowercase letters indicate significant differences among phosphorus levels within the same reseeding level. Uppercase letters indicate significant differences among reseeding levels within the same phosphorus level. (a) Crude Protein content of forage; (b) Ether Extract content of forage; (c) Neutral Detergent Fiber content of forage; (d) Acid Detergent Fiber content of forage.

3.3. The Effects on Soil Chemical Properties

3.3.1. The Effects of Reseeding and P Addition on Soil Chemical Properties

Table 4 shows the different effects of reseeding, P addition, and the reseeding $\times$ P addition interaction on soil chemical properties. Reseeding, P addition, and their interaction significantly influenced soil AN ($p < 0.05$). P addition and the reseeding $\times$ P addition had a significant effect on soil AP. Reseeding and the reseeding $\times$ P addition interaction had significant effect on soil MBC ($p < 0.05$). Reseeding had a significant effect on soil MBN ($p < 0.05$). As shown in Figure 6, the highest AN content was observed under V₃P₂, with a 26.16% increase ($p < 0.05$). Significant increases in soil AP content were observed under V₁P₁, V₁P₂, V₂P₁, V₃P₁ and V₃P₂ treatments, ranging from 39.02% to 104.86% ($p < 0.05$). Soil MBC content peaked under V₁P₁ treatment, with a significant 64.79% enhancement ($p < 0.05$). The V₃P₂ treatment significantly enhanced soil MBN content, showing the highest levels among all treatments with a 43.78% increase ($p < 0.05$).

3.3.2. The Response Ratios of Soil Chemical Properties Under Each Treatment

Figure 7 demonstrated that treatments V₂P₀, V₂P₂, V₃P₁, and V₃P₂ had significant positive effects on soil TN ($p < 0.05$), while V₁P₀ and V₃P₁ had significant positive effects on soil TP ($p < 0.05$). All treatments except V₁P₀ and V₂P₀ positively affected soil AN, while phosphorus application significantly enhanced AP ($p < 0.05$). SOM responded positively to all treatments except V₃P₁ ($p < 0.05$).

Table 4. Analysis of variance of reseeding, phosphorus application, and their interactions soil chemical properties.

Factor	Significance							
	TN	TP	AN	AP	SOM	MBC	MBN	MBP
V	ns	ns	*	ns	ns	***	**	ns
P	ns	ns	**	**	ns	ns	ns	ns
V × P	ns	ns	***	***	ns	*	ns	ns

Note: V: reseeding; P: P addition; V × P: the interactions between reseeding and P addition. Results of ANOVA models are indicated with asterisks (* $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$). TN: total nitrogen; TP: total phosphorus; AN: available nitrogen; AP: available phosphorus; SOM: Soil organic matter; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; MBP: microbial biomass phosphorus.

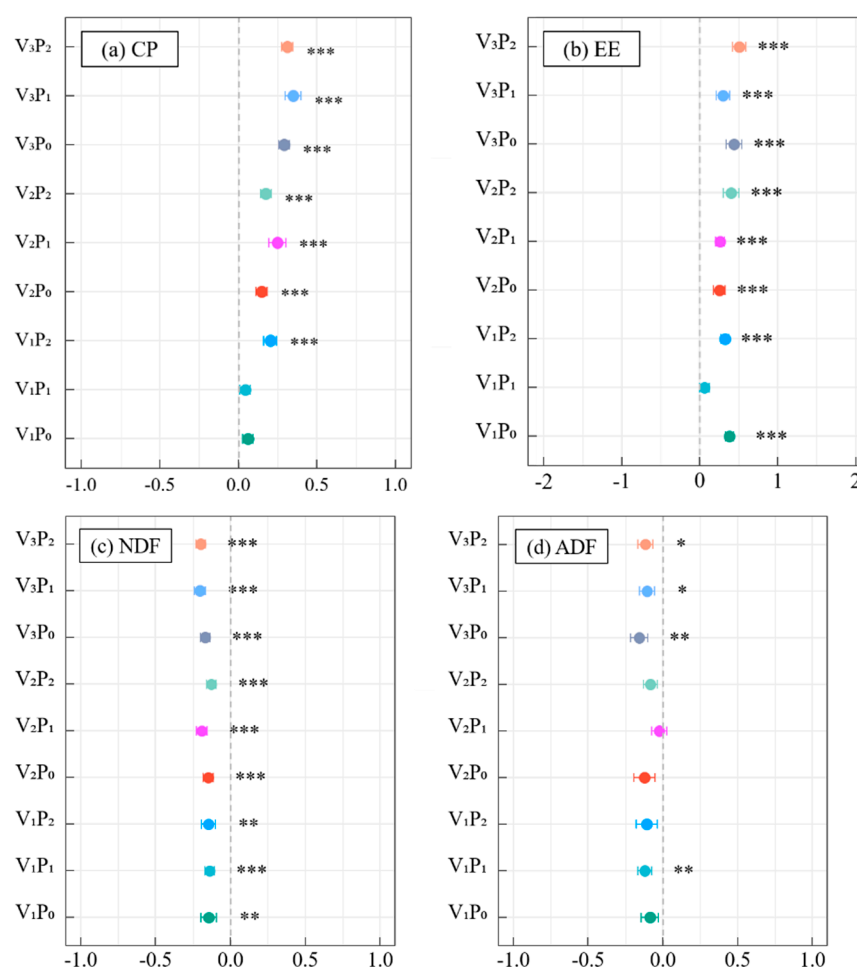


Figure 5. The response ratios of forage nutritional quality to reseeding and fertilization under each treatment. Note: Asterisks indicate significant differences from zero. * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$. (a) Crude Protein content of forage; (b) Ether Extract content of forage; (c) Neutral Detergent Fiber content of forage; (d) Acid Detergent Fiber content of forage.

Figure 7 illustrated that differential responses of soil microbial biomass (MBC, MBN, MBP) to reseeding and phosphorus application treatments. Low reseeding with phosphorus (V₁P₁ and V₁P₂) and all high reseeding treatments (V₃P₀, V₃P₁ and V₃P₂) ($p < 0.05$) had a significant positive impact on soil MBC, while high phosphorus with reseeding (V₂P₂) and high reseeding with phosphorus (V₃P₁ and V₃P₂) had a significant positive impact on soil MBN ($p < 0.05$). MBP showed the broadest treatment response among measured parameters, with all treatments except V₁P₀ having a significant positive impact on MBP levels ($p < 0.05$).

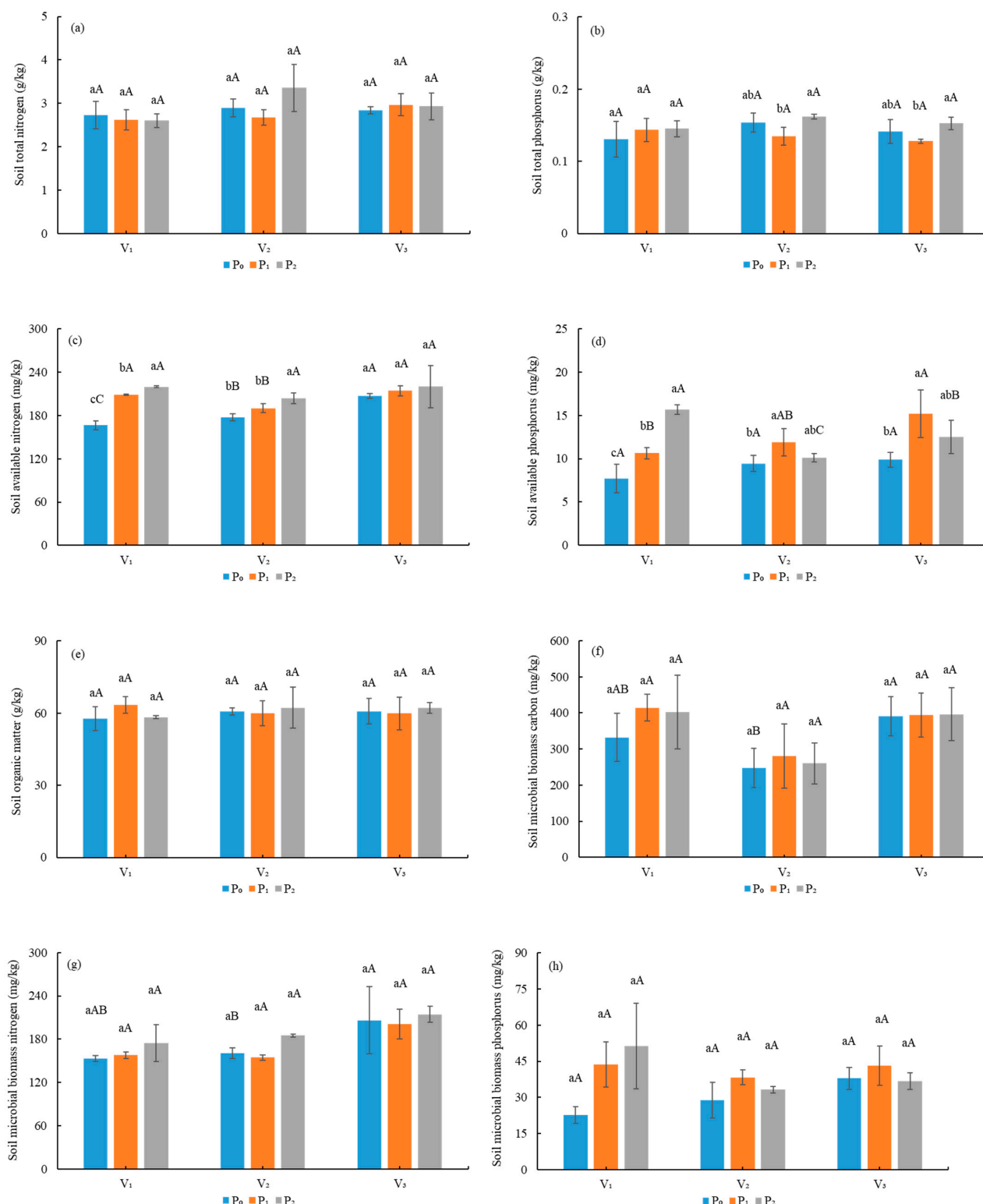


Figure 6. The effects of reseeding and P addition on soil chemical properties. Note: Lowercase letters indicate significant differences among phosphorus levels within the same reseeding level. Uppercase letters indicate significant differences among reseeding levels within the same phosphorus level. (a) Soil total nitrogen content; (b) Soil total phosphorus content; (c) Soil available nitrogen content; (d) Soil available phosphorus content; (e) Soil organic matter content; (f) Soil microbial biomass carbon content; (g) Soil microbial biomass nitrogen content (h) Soil microbial biomass phosphorus content.

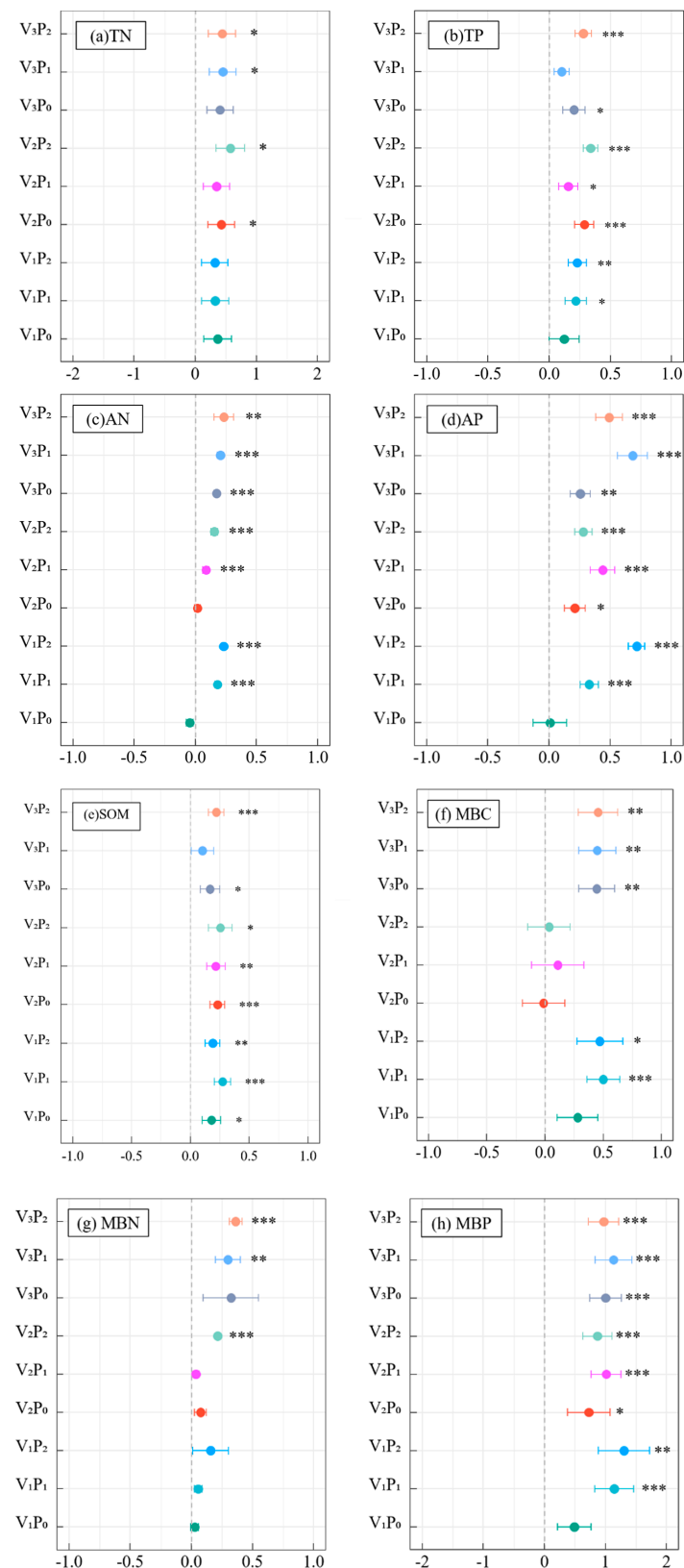


Figure 7. The response ratios of soil chemical properties after reseeding and phosphorus application. Note: Asterisks indicate significant differences from zero. * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$. (a) Soil total nitrogen content; (b) Soil total phosphorus content; (c) Soil available nitrogen content; (d) Soil available phosphorus content; (e) Soil organic matter content; (f) Soil microbial biomass carbon content; (g) Soil microbial biomass nitrogen content (h) Soil microbial biomass phosphorus content.

3.4. Comprehensive Grey Relational Analysis

We performed GRA on eight forage performance indicators to rank treatment efficacy, calculating grey relational grades for integrated assessment. Table 5 presented the grey correlation degree ranking. All treatments exhibited higher grey relational values compared to the CK. The top three performing treatments were V₃P₂, V₃P₁, and V₃P₀, indicating superior forage biomass and quality. Among these, the combination of a high reseeding rate with high phosphorus fertilization (V₃P₂) achieved the optimal performance in both forage biomass and quality.

Table 5. The ranking of the grey correlation analysis between biomass and quality of forage.

Treatment	CP	EE	NDF	ADF	Grasses Biomass	Forbs Biomass	Legume Biomass	AGB	Score	Rank
V ₁ P ₀	0.67	0.81	0.89	0.87	0.68	1.00	0.54	0.90	0.80	4
V ₁ P ₁	0.66	0.58	0.88	0.93	0.76	0.60	0.37	0.67	0.70	9
V ₁ P ₂	0.79	0.75	0.90	0.91	0.88	0.51	0.84	0.69	0.79	5
V ₂ P ₀	0.73	0.69	0.90	0.93	0.49	0.62	0.76	0.61	0.73	7
V ₂ P ₁	0.84	0.70	0.98	0.78	0.83	0.66	0.51	0.77	0.77	6
V ₁ P ₂	0.76	0.84	0.86	0.87	0.61	0.50	0.63	0.58	0.72	8
V ₃ P ₀	0.90	0.89	0.93	1.00	0.63	0.65	1.00	0.73	0.85	3
V ₃ P ₁	1.00	0.73	1.00	0.90	0.82	0.82	0.63	0.90	0.86	2
V ₃ P ₂	0.93	1.00	0.99	0.92	1.00	0.85	0.58	1.00	0.92	1
CK	0.63	0.56	0.69	0.74	0.47	0.61	0.33	0.54	0.58	10
Correlation degree	0.79	0.76	0.90	0.89	0.72	0.68	0.62	0.74	6.09	
Weight	0.13	0.12	0.15	0.15	0.12	0.11	0.10	0.12	1.00	

Note: CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fiber; ADF: acid detergent fiber; AGB: aboveground biomass.

3.5. Hierarchical Partitioning Analysis

To explore the key soil factors driving forage biomass and quality under different re-seeding and P application, we conducted hierarchical segmentation based on redundancy analysis (RDA). RDA analysis was performed using soil nutrient properties as the explanatory variables and forage nutrient quality and biomass as the response variables (Figure 8). The first two RDA axes collectively explained 79.91% of the total variation in forage nutrient quality and biomass, indicating that soil nutrient availability in alpine meadows is a dominant driver of forage productivity. TN, TP, AN, AP, and MBC all exhibited significant effects on forage biomass and quality ($p < 0.05$), with TN emerging as the most influential factor.

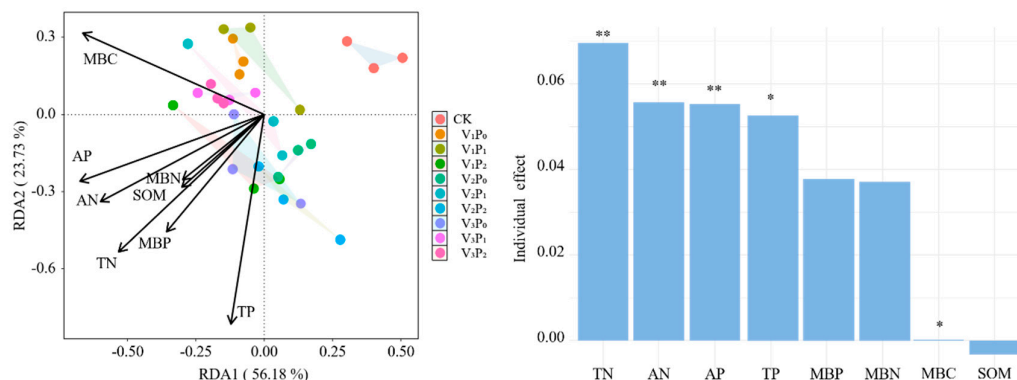


Figure 8. Hierarchical partitioning analysis based on RDA. Note: * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$. TN, total nitrogen; TP, total phosphorus; AN, available nitrogen; AP, available phosphorus; SOM, Soil organic matter; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus.

4. Discussion

4.1. Reseeding Coupled with Phosphorus Application Boosts Forage Biomass and Nutritional Value

Our findings demonstrated that P fertilization combined with reseeded effectively enhances alpine meadow productivity, corroborating previous reports by Wang et al. [23] regarding soil nutrient improvement and forage quality enhancement. As evidenced by our experimental data (Table 2), reseeded and the reseeded $\times$ P addition interaction enhanced *M. ruthenica* establishment and biomass production. The higher reseeded rate and higher P addition levels directly increased the per-unit-area plant density of *M. ruthenica*, effectively suppressing competing forbs through physical occupation of niche space. Additionally, the elevated population density may have conferred competitive advantages to *M. ruthenica* through pre-emptive resource acquisition, consistent with findings by Liu et al. [24] on density-dependent competition between legumes and forbs. Phosphorus management emerges as a critical determinant of *M. ruthenica* productivity under low reseeded rate, while the low-rate treatment (V_1P_0) yielded fewer plants per unit area; it maintained a substantial biomass output, demonstrating that initial reseeded rate alone does not limit production capacity. This phenomenon may be attributed to two key factors, namely enhanced individual plant adaptability under low-density conditions, and reduced interspecific competition from forbs in the absence of exogenous nutrient inputs, as supported by Xia et al. [25]. These findings suggested that *M. ruthenica* exhibits remarkable ecological plasticity, achieving competitive biomass yields through compensatory growth mechanisms rather than density-dependent effects. The lowest biomass yield of *M. ruthenica* was observed under the V_1P_1 treatment. This phenomenon may be explained by P fertilization stimulating competitive growth of companion forbs, which subsequently reduced light availability, intensified nutrient competition, and ultimately depressed *M. ruthenica* productivity. This phenomenon is consistent with findings reported by Su et al. [12] in similar grassland systems. Under the low reseeded rate with high P supplement (V_1P_2), the abundant phosphorus availability not only supports the growth of other companion species but also significantly enhances *M. ruthenica* productivity. This suggests that at lower reseeded densities, P serves as a key limiting factor for *M. ruthenica* performance, and its supplementation can overcome interspecific competition pressures to boost target species biomass.

The effects of the reseeded $\times$ P addition interaction on forbs biomass were significant, as both significant positive and negative responses were observed (Table 2). This variation likely arises from the superior competitive ability of grasses in nutrient acquisition and light interception, enabling them to rapidly dominate after reseeded or fertilization, which in turn suppresses the growth of forbs. Under combined fertilization and reseeded treatments, the accelerated growth of grasses can lead to early canopy closure, significantly reducing photosynthetically active radiation availability for forb species. This light limitation consequently suppresses forb biomass accumulation [26]. This competitive exclusion mechanism is particularly pronounced in high-fertility conditions where grasses typically exhibit superior light interception efficiency [27]. Almost all the treatments had a significant positive effect on the grasses (Figure 3). This positive response may reflect distinct root character. Most grasses have relatively shallow root systems, allowing them to rapidly utilize soil surface-applied nutrients resources [28]. In contrast, deep-rooted forbs experience a reduced competitive advantage in nutrient-rich environments [29,30]. As Figure 3 demonstrates, the V_3P_2 treatment generated synergistic effects: it had a significant positive effect on the biomass across all functional groups. Notably, the inclusion of *M. ruthenica* (a N-fixing legume) contributed substantially to this enhancement through its rhizobial symbiosis (*Sino rhizobium meliloti*), which converts atmospheric N_2 into plant-available ammonium [9]. The N fixed by *M. ruthenica* enhanced the community productivity through

root exudates, litter decomposition, and mycorrhizal networks [9]. This multi-pathway N transfer alleviates N limitation and promoting their growth. Critically, P exerts synergistic effects, being vital for energy metabolism, nucleic acid synthesis, and biological N fixation [31]. The reseeding $\times$ P addition interaction directly addressed plants phosphorus requirements, notably enhancing the nitrogen fixation efficiency of *M. ruthenica*. Furthermore, P-sensitive forbs exhibited enhanced tiller production and photosynthetic efficiency under elevated phosphorus availability [32]. This ultimately leads to an increase in biomass.

The nutritional quality of meadow forage is not only crucial for the healthy growth and development of livestock, but also impacts local animal husbandry productivity [33]. Forage quality assessment relies on three key biochemical parameters, CP, NDF, and ADF. Crude Protein content represents the most reliable indicator of forage nutritional value [33]. In contrast, ADF and NDF serve as critical predictors of forage palatability and intake potential [34]. The lower the fiber content, the better the palatability, which benefits livestock feeding and digestion [35]. As demonstrated in Table 3, the reseeding $\times$ P addition interaction had a significant effect on the forage quality, while the V_3P_1 and V_3P_2 treatments significantly enhanced the CP and EE contents in forage by 41.68% and 65.51%, respectively. These results highlight the reseeding $\times$ P addition interaction; especially, the application of the high P and high reseeding rate of *M. ruthenica* significantly improved the nutritional quality of forage in degraded alpine meadows. As a nitrogen-fixing legume, *M. ruthenica* exhibits optimal growth performance under high-density reseeding and P supplementation (V_3P_2). The biological N fixation by *M. ruthenica* not only increases the CP content but also enhances N availability for companion grasses through rhizosphere processes, thereby enhancing N utilization efficiency across the entire forage system [4]. Phosphorus application significantly enhanced N uptake and assimilation in forage species, as P plays vital roles in both energy metabolism and enzymatic activation within N assimilation pathways [32]. The combined application of P fertilization and legume reseeding synergistically enhanced the forage nutritional value [36]. As presented in Figure 4, reseeding had a significant effect on the NDF content in forage. However, the effect on the ADF content in forage was not significant. Moreover, all the treatments had a significant negative effect on it (Figure 5), indicating that reseeding in alpine meadows can significantly reduce the content of NDF in forage. This reduction may be attributed to their compositional differences. While both contain cellulose and lignin, only NDF includes the more labile hemicellulose fraction. The introduction of legumes combined with enhanced soil fertility promoted tiller development and increased the proportion of juvenile vegetative tissues, consequently reducing the NDF content through decreased lignification [37]. These results demonstrated that the combined application of P fertilization and legume reseeding effectively restores degraded alpine meadows and forage quality.

4.2. Reseeding and Phosphorus Application Significantly Enhances Soil Nutrient Availability in Alpine Meadows

Soil nutrients provide the essential material foundation for plant growth and development, playing a decisive role in the structure and function of plant communities. Reseeding and P addition are common restorative measures for degraded alpine meadows, significantly influencing soil nutrient content [38]. In this study, the P addition and the reseeding $\times$ P addition interaction had significant impacts on soil AN and AP (Table 4). However, the effects on the TN and TP content in soil are not significant (Figure 6). This could be because the available nutrients, as the immediate N and P source for plants, exhibit faster responses to management practices, whereas changes in TN and TP require longer-term and more intensive interventions to become notable [39]. Although reseeding, the P addition, and the reseeding $\times$ P addition interaction had no significant effects on

soil TN, TP, SOM, and MBP (Table 4), each treatment still had a significantly positive effect on soil SOM and MBP (Figure 7). This phenomenon can be explained by the fact that initial soil nutrient stocks were low, and the reseeding rate and P addition were insufficient to trigger nutrient accumulation [40]. The introduction of *M. ruthenica* may promote soil nutrient depletion through its substantial nitrogen and P uptake during the initial stage establishment, limiting its influence on soil nutrient levels [41]. There is another possible reason for increasing the AN and AP content, namely that P fertilization directly increased the concentrations of soil AP, promoting *M. ruthenica* growth. Through root nodule activity, *M. ruthenica* boosted biological nitrogen fixation, converting inert soil N into plant-available forms [42]. Additionally, as observed in the V₃P₁ and V₃P₂ treatments (Figure 7), the combined effect of the high reseeding rate and P application was essential for improving MBC, MBN, and MBP. Soil microbial biomass is a fundamental driver of the SOM transformation and nutrients cycling, serving as both a key reservoir of nutrients and a primary source of bioavailable elements for plant uptake [43]. The dynamics of soil microbial biomass are governed by microbial metabolic activity and the stoichiometric balance of C:N:P of soil. Reseeding combined with P fertilization can mitigate N and P limitations, promote SOM accumulation, and subsequently stimulate microbial biomass assimilation, leading to increased soil microbial biomass content [39].

These combinations ensured an optimal supply of nutrient content to meet both plant growth and microbial needs, facilitating soil nutrient cycling and activity of soil microorganisms. This study demonstrated that integrated reseeding and P application can synergistically enhance nutrient cycling, soil health, and ecosystem recovery in degraded soils.

4.3. Soil Total Nitrogen Contributes the Most to the Biomass and Nutrient Quality of Forage

Nitrogen, as a fundamental element for plant growth and development, has been widely recognized for its importance. It is not only the basic raw material that constitutes key nutrients such as proteins in forage grasses, directly determining the nutritional value of forage grasses, but also can indirectly affect the accumulation of other nutrients in forage grasses by participating in photosynthesis [12]. Previous study had shown that fertilization can significantly increase the TN content, which in turn enhances both the biomass and nutritional quality of forage [44]. A similar pattern was observed in this study: soil TN, AN, AP, and TP regulated the changes in plant biomass and nutrient quality in alpine meadows, among which TN contributes the most, followed by AN (Figure 8). The results of this study echoed the previous understanding that N is the primary limiting factor for regulating productivity of forage in grassland ecosystems [33,45]. Compared with other nutrients such as P, soil N levels can more directly and comprehensively influence the nutritional characteristics of forage grasses. It has a significant impact on the production performance and nutritional quality of forage [46]. Therefore, based on the results of this study and previous research, it can be clearly stated that N plays an irreplaceable and crucial role in the formation of the nutritional quality of forage and is the core for enhancing the nutritional value of forage.

5. Conclusions

Reseeding and P addition can significantly enhance both the nutritional quality and biomass of forage in degraded alpine meadows. The V₃P₂ treatment (high reseeding rate + high P) produced the highest forage biomass and the best nutritional quality, demonstrating the synergistic benefits of combined intervention. Under low reseeding condition (V₁), high P addition (P₂) compensated for lower seed input, still boosting the biomass of *M. ruthenica*, highlighting the reseeding and P addition interaction for its growth process.

N was the most critical element governing both the forage quality and biomass based on our data, indicating that N remains the primary limiting nutrient in alpine meadows. The combined practice of the reseeding and P application enhanced N cycling through biological N fixation, microbial mineralization, and mitigating inherent N deficiencies. Long-term meadow restoration should focus on sustaining soil N through integrated plant–microbe–phosphorus management. While the study successfully identified optimal treatments for improving alpine meadow productivity and determined key limiting factors, these findings remain preliminary as they are based on data from a single growing season. To establish robust conclusions, long-term monitoring is required to verify treatment effect and account for interannual variability.

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