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# Nitrogen, Phosphorus, and Potassium Resorption Responses of Alfalfa to Increasing Soil Water and P Availability in a Semi-Arid Environment

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**Abstract:** In semi-arid areas, alfalfa (*Medicago sativa* L.) is widely grown, but its growth is often restricted due to limited rainfall and soil nutrients, particularly phosphorus (P). Nutrient resorption is an effective strategy for dealing with nutrient shortages. Alleviation of these limited resources using film mulch and P fertilization—which are common practices in semi-arid areas—can affect the internal recycling of such nutrients. Little is known about such effects in alfalfa and the relationship between resorption efficiency and forage yield. We conducted a two-year field experiment in the semi-arid Loess Plateau of China using film mulch and P fertilization to investigate the response to long-term increasing soil water and P availability on leaf nitrogen (N), P, and potassium (K) concentrations and nutrient resorption characteristics in alfalfa. In green leaves, mulching significantly increased P concentration by an average of 5.5% but it had no significant effect on N concentration over two years, and it decreased K concentration by 16.1% in 2017. P fertilization significantly increased N concentrations to a greater degree in 2018 (8.1%) than 2017 (1.6%). P fertilization also significantly increased P concentrations by an average of 34.1% over two years. In contrast, P fertilization significantly decreased K concentration in the mulched treatment by an average of 17.3% in 2017 and 21.8% in 2018, but it had no effect in the no-mulch treatment. In senescent leaves, mulching significantly increased N concentration by an average of 3.9% and P concentration by an average of 16.7%, but it had no significant effect on K concentration over two years, while P fertilization significantly decreased N and K concentrations over two years by an average of 7.5%, and 32.8%, respectively. P fertilization significantly increased senesced P concentration by an average of 11.9% in 2017 and 17.5% in 2018; and year × mulching × P fertilization had a significant interaction on senesced leaf P concentration. For resorption efficiency, mulching decreased P resorption efficiency by an average of 3.0%, but it had no impact on N or K resorption efficiency, while P fertilization increased the N, P, and K resorption efficiencies in alfalfa by an average of 6.8%, 6.2%, and 76.4% over two years, respectively. Interactive effects of mulching and P fertilization were found on P and K resorption efficiencies over time. In addition, N and K resorption efficiencies were significantly higher in 2018 than in 2017. The application of P fertilizer without mulching resulted in positive correlations between forage yield and N, P, and K resorption efficiencies, but no correlations were observed under film mulch. That is, mulching changed the relationship between forage yield and N, P, and K resorption efficiencies in alfalfa, suggesting that N, P, and K resorption efficiencies may not be related to high yield. Our results provide new insights into the role of nutrient resorption in alfalfa in response to increasing soil water and P availability and the relationship between resorption efficiency and forage yield, which will help us to improve alfalfa yield in semi-arid regions.

**Keywords:** plant–soil interactions; nutrient cycling; nutrient addition; soil nutrient; perennial leguminous forage

## 1. Introduction

Nutrient resorption is an integral part of the recycling of internal nutrients in plants [1–3], where nutrients in senescing tissues are resorbed and either conserved or reused for further growth and development, which is important for growth, reproduction, and competitive ability [2]. Nutrient resorption before leaf death and abscission reduces the dependence of plants on the soil as a source of nutrients, especially in less fertile soils [4–6]. Nutrient resorption can be quantified as resorption efficiency (the proportion of a given nutrient resorbed from mature green leaves) and resorption proficiency (the concentration of a given nutrient in senescent tissue) [7]. Globally, the mean resorption efficiencies from senescent leaves are 62.1% for nitrogen (N), 64.9% for phosphorus (P), and 70.1% for potassium (K) [3]. In general, plants growing in nutrient-limited environments have higher resorption efficiencies than those growing in nutrient-rich environments [2,3,7]. Moreover, resorption efficiency decreases as the availability of nutrients in soil increases [2,6,8]. However, this relationship is far more complex, inconsistent, and varies between species, climates, plant age, and plant form or habit [2,9–11]. In addition, this relationship is affected by plant phenology, and the reproductive effort tends to increase nutrient resorption [2,12].

Forage legumes are widely grown in semi-arid areas, not only as a source of fodder but also to improve the ecological environment and soil quality [13,14]. The semi-arid Loess Plateau has a typical semi-arid monsoon climate. Alfalfa (*Medicago sativa* L.) is the dominant perennial N-fixing legume [15–17], and this region is the main production area of alfalfa in China (61% in 2015). However, forage yields of alfalfa are usually very low due to limited water and soil nutrient availability [15,17]. Most alfalfa in this region is rainfed due to the lack of water resources for irrigation. According to the National Alfalfa Industry Development Plan (2016–2020) issued by the Ministry of Agriculture of China, irrigated alfalfa yields will reach 12 t $ha^{-1}$, compared with 6.75 t $ha^{-1}$ for rainfed alfalfa, by 2020 on the semi-arid Loess Plateau. Limited access to water limits plant growth and productivity in semi-arid ecosystems [18,19]. Low soil moisture, due to limited precipitation and high evaporation, constrains soil microbial activity, litter decomposition, nutrient mineralization, and soil nutrient availability [20,21]. It is generally believed that N might not restrict alfalfa growth because it can fix N through biological nitrogen fixation [22]. In contrast, soil-available P declined as alfalfa plants grew, which was significantly positively correlated with alfalfa forage yield [23]. P restricts plant growth more than other elements due to its low mobility and bioavailability [24]. P mobility and bioavailability are often limited by a number of factors, including soil texture, precipitation, and surface adsorption [25]. On the semi-arid Loess Plateau, semi-arid climate and alkaline calcareous soil limit the mobility and bioavailability of P. Furthermore, as an N-fixing plant, the biological N fixation of alfalfa requires more P supply [17]. Therefore, improving soil moisture and P availability is vital for increasing alfalfa production.

Greater resorption is an effective strategy to counter nutrient deficiency [2]. Nutrient resorption in alfalfa would make it less reliant on soil nutrients, enhancing the adaptation of plants in fields. Furthermore, as a key determinant of litter quality, resorption can influence the recycling of soil nutrients in ecosystems due to its effect—positive or negative—on litter decomposition and nutrient release [3,26,27]. However, little definitive information is available on the effect of increasing soil nutrient availability on nutrient resorption efficiency in different environments and species [6,10,22,28,29]. Most studies have focused on woody plants and annual herbs, with few on perennial herbs, especially perennial legume herbs. The effect of changes in soil N availability on nutrient resorption has been studied extensively [5,18,20], but the relationship between plant nutrient resorption in N-fixing leguminous plants and P and/or water availability is unclear, particularly in semi-arid regions. In addition, the pattern of internal nutrient recycling in alfalfa may differ from that in other annual or perennial species. Large quantities of N, P, and K are removed by cutting alfalfa each year, and thus, they are lost to the system [17,30]. Alfalfa is harvested twice annually (average yield 3.9 t $ha^{-1}$ $year^{-1}$) in this area [13]; each ton of harvested dry matter removes about 30 kg of N, 2 kg of P, and 26 kg of K [31]. This removal may change the distribution of nutrients in the plant and their availability in soil, leading to changes in the status of each nutrient and its recycling, possibly influencing N, P, and K resorption. As an N-fixing plant, the N in alfalfa is acquired through biochemical fixation and soil. So, alfalfa has greater concentrations of N in leaves than plant without $N_2$-fixation capacity [22], which may affect the metabolism of other elements and nutrient resorption.

Plastic film mulch is a common practice in semi-arid areas because mulching slows evaporation and increases soil moisture, thereby making soil nutrients more easily available to plants [32]. Another common practice is P fertilization, which can change the forms of soil P and increase the amount of available P [33]. Both practices improve soil nutrient availability, particularly P, which is crucial for growth and forage yield in alfalfa [15–17]. While many studies have investigated the effect of changing soil water and P availability on the growth and yield of alfalfa, the effects on N, P, and K resorption have received little attention. Changes in nutrient availability will affect the absorption and utilization of different nutrients by alfalfa and, thus, its yield. In addition, soil nutrient deficiency can affect alfalfa growth and yield. For example, P deficiency caused leaf senescence in alfalfa, which decreased its growth and yield [34]. Nutrient resorption can alleviate nutrient deficiency, making the plant less reliant on soil nutrients [2]. However, the relationship between forage yield and nutrient resorption has not been examined.

Here, we investigated the nutrient resorption characteristics of alfalfa in a semi-arid environment in response to long-term mulching and P fertilization and the relationship between nutrient resorption and forage yield. The experiment tested the following four hypotheses: (1) mulching decreases N, P, and K resorption efficiencies, thereby increasing their availability in soil by increasing soil moisture content; (2) P fertilization increases N and K resorption efficiency and decreases P resorption efficiency due to the relatively greater demand for N and K and lesser demand for P; (3) yearly harvesting (cutting) of forage and continued mulching and P application increases plant resorption of N, P, and K over time; and (4) resorption efficiency is positively correlated with forage yield due to efficient nutrient utilization.

## 2. Materials and Methods

### *2.1. Site Description*

The experiment was conducted in 2017 and 2018 on the Loess Plateau at the Semi-Arid Ecosystem Research Station of Lanzhou University. The site is in Zhonglianchuan village (36°02′ N, 104°25′ E, 2400 m above sea level) in Yuzhong county in Gansu province, which is part of the northern mountainous region of northwestern China. The climate is medium temperate and semi-arid with a mean annual air temperature of 6.5 °C. The mean monthly maximum of 19.0 °C is reached in July, and the minimum of −8.0 °C is reached in January. The mean annual precipitation is 320 mm, of which about 60% is received during the main part of the growing season between June and September, and the average annual free-water evaporation is about 1300 mm [35]. Groundwater is not available for plant growth and not a feasible source of irrigation, and crops are grown entirely under rainfed conditions [35]. The soil is classified locally as Heima soil—Calcic Kastanozem (Siltic)—with the following properties: maximum water-holding capacity (field capacity), 0.196 g $g^{-1}$; permanent wilting coefficient, 0.047 g $g^{-1}$; sand, 12.3%; silt, 66.9%; clay, 20.8%; pH, 8.8; organic carbon, 5.28 g $kg^{-1}$; total N, 0.34 g $kg^{-1}$; and total P, 0.66 g $kg^{-1}$ [17]. During the alfalfa growing season (April–June), the mean air temperature was 10.8 °C in 2017 and 11.8 °C in 2018; the corresponding values for precipitation were 130.8 mm and 138.6 mm.

### 2.2. Experimental Design and Field Management

The experiment, involving a combination of mulching with plastic film and the application of P fertilizer, was initiated in 2011 and continues to date. More detailed information on the experimental design is in an earlier study [17]. Briefly, the experiment comprised a split-plot with randomized blocks and two main treatments—ridge–furrow planting with plastic film as the mulch (M1) and flat planting without mulch (M0)—as the main plots and four levels of P in the form of calcium superphosphate—P0 (0 kg $ha^{-1}$), P1 (9.73 kg $ha^{-1}$), P2 (19.3 kg $ha^{-1}$), and P3 (28.9 kg $ha^{-1}$)—as the split plots. The eight treatments were distinguished as follows: M0P0, M0P1, M0P2, M0P3, M1P0, M1P1, M1P2, and M1P3. The ridges (30 cm wide and 15 cm high) acted as rainwater-harvesting structures, and alfalfa was grown within the furrows, which were V-shaped in the cross-section. The ridges were formed and covered with plastic film (0.008 mm thick sheets of polyethylene) on 29 June 2011, and a local alfalfa cultivar, 'Longzhong', was sown at 15 kg $ha^{-1}$ the same day. Each year after 2011, when the plants turned green, the plastic film of the previous year was removed, and the designated dose of P was applied. The ridges were mulched by hand with new plastic film, and the adjacent plastic film was brought together in the furrow in between. Each such joint was retained in its place by heaping some soil on top. Each plot was 5 m long and 1.5 m wide, and each treatment was replicated three times. The crop was harvested twice each year (in mid-July and mid-October from 2011 to 2016 and at the end of June and August from 2017 to 2018).

### 2.3. Sampling and Measurements

Leaf samples were randomly collected from each plot at the end of June in 2017 and 2018 (early flowering stage). The samples consisted of mature leaves (green and fully expanded trifoliate leaves from the upper one-third of the shoot) and senescent leaves (yellow leaves still clinging to the plant, but easily detached from it with a gentle shake) collected from about 40 plants, which were immediately taken to the laboratory after collection. The leaves were oven-dried at 65 °C for 48 h, ground, and mixed evenly for elemental analysis. For each sample, a 0.2 g subsample was digested with $H_2SO_4$–$H_2O_2$ [36] to determine N, P, and K concentrations in leaf tissue using the following methods [37]: Kjeldahl method for N, molybdenum-antimony anti-spectrophotometry for P, and flame photometry for K.

The nutrient resorption efficiency (NuRE) for each nutrient was calculated as follows:

$$\text{NuRE (\%)} = ((\text{Nu}_{\text{gre}} - \text{Nu}_{\text{sen}})/\text{Nu}_{\text{gre}}) \times 100\% \quad (1)$$

where $\text{Nu}_{\text{gre}}$ and $\text{Nu}_{\text{sen}}$ are the nutrient (N, P, or K) concentrations (in g per kg leaf tissue) in mature green leaves and senescent leaves, respectively.

Six soil cores were collected from each plot, three from the ridges and three from the furrows, chosen at random, using a soil auger 20 cm long and 8 cm in diameter. The samples—collected each year when alfalfa returned to green (the end of April)—were mixed to form a composite sample. Root fragments and other plant debris were removed from the soil, which was then passed through a 2 mm sieve. Each soil sample was divided into two parts: one was stored at 4 °C for determining soil inorganic N ($NO^{3-}$–N + $NH^{4+}$–N), and the other was air-dried for determining soil-available P and soil-available K. Soil inorganic N was measured using a flow injection autoanalyzer (Skalar, Breda, the Netherlands) after extraction with 0.5 mol $L^{-1}$ $K_2SO_4$ [38]. Available soil P was estimated by extraction with 0.5 mol $L^{-1}$ $NaHCO_3$ (pH = 8.5) and determined using the Olsen-P method [37]. Available soil K was determined using a digital flame photometer (M410, Sherwood Scientific Ltd., Cambridge, UK) after extraction with 1.0 mol $L^{-1}$ $CH_3COONH_4$ [37].

### 2.4. Statistical Analysis

Microsoft Excel 2019 (Microsoft, Redmond, WA, USA) was used for data processing. Statistical analysis of variance was carried out using GenStat ver. 18.1 (VSN International, Hemel Hempstead, UK), and graphs were plotted using Origin Pro 2015 (Origin Lab, Northampton, Massachusetts, MA, USA). The effects of year, mulching, and P fertilization on all the response variables were analyzed by linear mixed models in the randomized blocks of the split-plot design. Differences within a year were examined using ANOVA in randomized blocks of the split-plot design. Regression analyses were conducted to determine the relationships between soil nutrient concentrations and leaf nutrient concentrations and nutrient resorption efficiencies. All reported values are the means of three replicates.

## 3. Results

### 3.1. Soil-Available Nutrients

(1) Mulching and the interaction of mulching and year significantly affected soil inorganic N concentration (Table 1): mulching increased soil inorganic N concentrations by 105% in 2017 but had no significant effect on it in 2018 (Figure 1). P fertilization, year, and other interactions did not affect soil inorganic N concentration significantly (Table 1). (2) P fertilization significantly increased soil-available P concentration in both years—the highest concentrations were recorded at the highest dose (Table 1; Figure 1), while mulching and other factors did not affect soil available P concentrations significantly (Table 1). (3) Year, mulching, P fertilization, and the interaction of mulching and P fertilization had significant effects, but other interactions had no effects on soil available K concentration (Table 1). Soil available K concentrations decreased with P fertilization level in mulching treatments, but that was not the case in the no-mulch treatment (Figure 1). In addition, mulching significantly increased soil water content by 24% in 2017 and 28% in 2018 compared with with no mulch, while P fertilization had no effect on soil water content (data not shown).

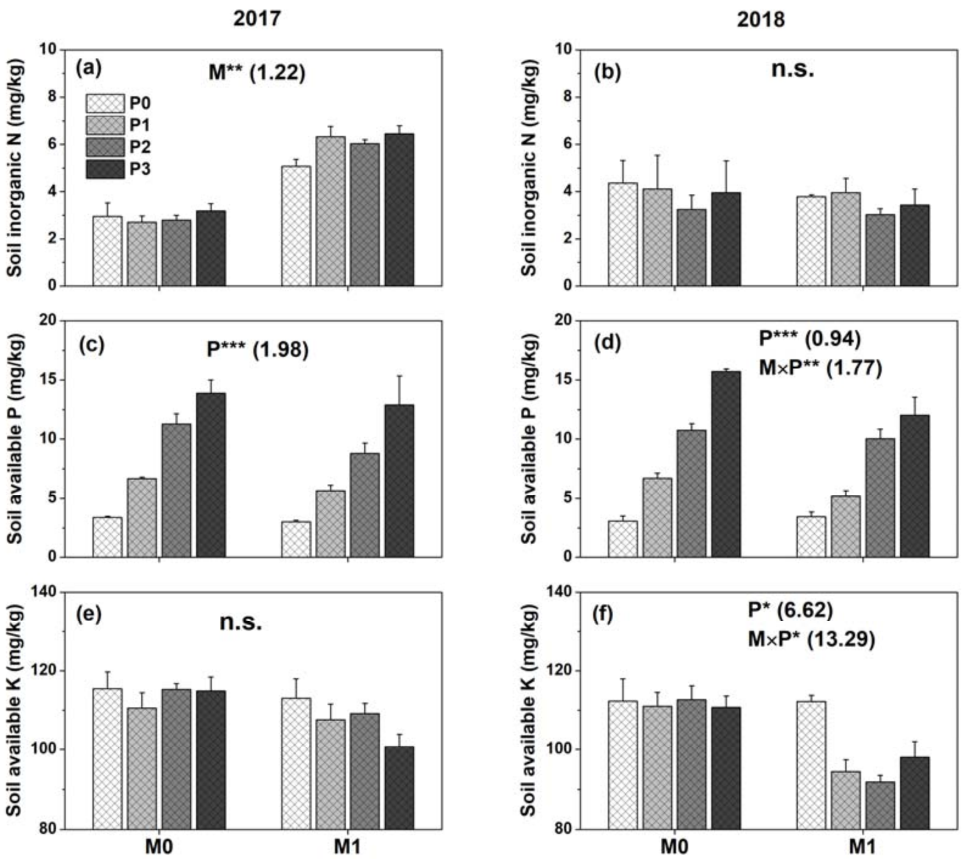


**Figure 1.** Effect of film mulch (M) and P fertilization (P) on (**a**,**b**) inorganic N, (**c**,**d**) available P, and (**e**,**f**) available K concentrations in soils in 2017 and 2018. M0P0, M0P1, M0P2, M0P3, M1P0, M1P1, M1P2, and M1P3 represent no mulch (M0) and mulch (M1) with four levels of P (P0, P1, P2, and P3), respectively. ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$. Numbers in parentheses are the least significance differences (LSD) among treatments ($p = 0.05$). Error bars show one standard error of the mean.

**Table 1.** Results of linear mixed models analysis of variance on the effects of film mulch (M), P fertilization (P), and cropping year (Y), and their interactions on forage yield, inorganic nitrogen (IN), available phosphorus (AP), and available potassium (AK) in soil, nutrient concentrations in mature and senescent leaves, and nutrient resorption efficiency.

| | Yield | IN | AP | AK | $[N]_{gre}$ | $[P]_{gre}$ | $[K]_{gre}$ | $[N]_{sen}$ | $[P]_{sen}$ | $[K]_{sen}$ | NRE | PRE | KRE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Y | 122 *** | 5.2 | 0.1 | 7.9 * | 3.1 | 0.0 | 1.5 | 9.5 * | 0.7 | 7.7 * | 8.4 * | 0.9 | 9.1 * |
| M | 2.3 | 10.2 * | 4.7 | 18.0 * | 0.0 | 16.3 * | 6.6 | 9.3 * | 42.6 ** | 0.6 | 3.6 | 22.0 ** | 0.0 |
| P | 7.7 *** | 0.6 | 166 *** | 4.4 * | 10.9 *** | 97.0 *** | 11.4 *** | 13.1 *** | 22.8 *** | 20.3 *** | 24.0 *** | 55.3 *** | 15.2 *** |
| Y*M | 1.2 | 16.6 * | 0.0 | 1.8 | 0.3 | 0.1 | 8.0 * | 0.0 | 4.7 | 3.6 | 0.2 | 3.7 | 0.8 |
| Y*P | 0.5 | 0.9 | 0.2 | 1.2 | 5.7 ** | 0.8 | 0.0 | 0.2 | 0.2 | 0.7 | 1.7 | 2.4 | 1.2 |
| M*P | 5.9 ** | 0.4 | 1.9 | 3.1 * | 1.2 | 0.6 | 3.6 * | 0.6 | 0.2 | 1.3 | 0.8 | 0.6 | 1.4 |
| Y*M*P | 0.5 | 0.2 | 1.8 | 2.0 | 0.9 | 1.3 | 0.8 | 0.6 | 4.1 * | 2.2 | 0.8 | 6.8 ** | 5.3 ** |

**Note.** F-ratios are presented, together with their level of significance. ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$. $[N]_{gre}$, $[N]_{sen}$, $[P]_{gre}$, $[P]_{sen}$, $[K]_{gre}$, $[K]_{sen}$, NRE, PRE, and KRE are nitrogen, phosphorus, and potassium concentrations in green and senescent leaves and resorption efficiencies for nitrogen, phosphorus, and potassium, respectively.

### *3.2. Nutrient Concentrations in Mature Green Leaves*

(1) P fertilization and the interaction of P fertilization and year significantly affected N concentrations (Table 1): N concentrations in green leaves with P fertilization increased more in 2018 (8.1%) than 2017 (1.6%) (Figure 2). Meanwhile mulching, year, and other interactions did not affect N concentrations significantly (Table 1). (2) Both mulching and P fertilization increased P concentrations significantly (Table 1; Figure 2). (3) P fertilization, Y × M, and M × P interaction significantly affected K concentration (Table 1). Mulching decreased K concentration by 16.1% in 2017 ($p = 0.06$) but had no significant effect on it in 2018 ($p > 0.1$; Figure 2). P fertilization significantly decreased K concentrations in the mulching treatment by an average of 17.3% in 2017 and of 21.8% in 2018, but that was not significant in no mulch over two years (Table 1; Figure 2).

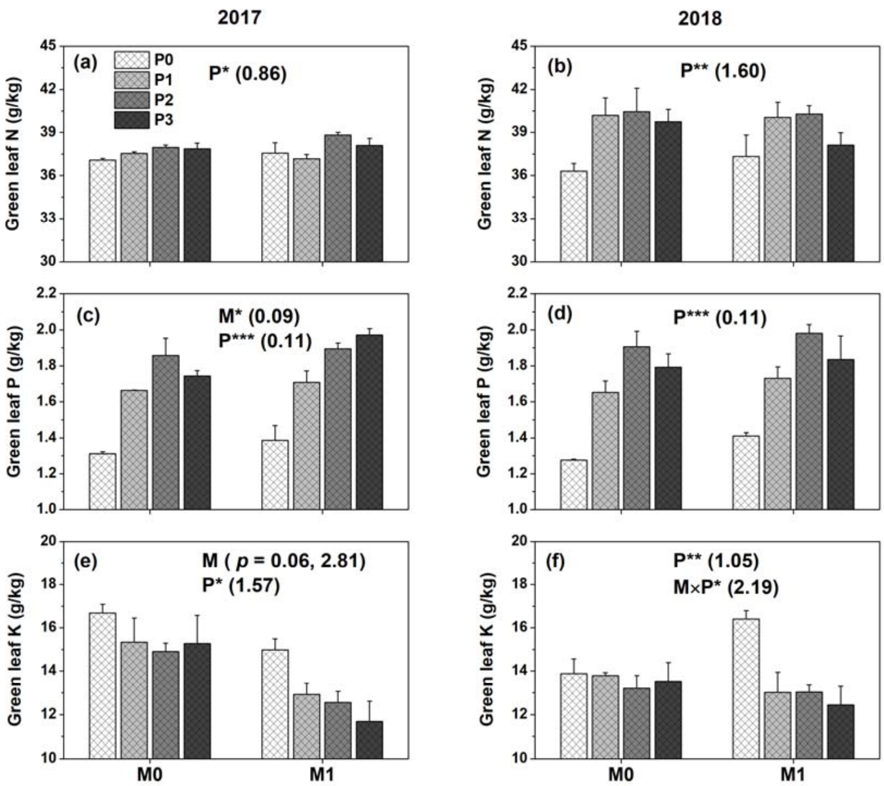


**Figure 2.** Effect of film mulch (M) and P fertilization (P) on concentrations in green leaves of (**a**,**b**) N, (**c**,**d**) P, and (**e**,**f**) K in 2017 and 2018. M0P0, M0P1, M0P2, M0P3, M1P0, M1P1, M1P2, and M1P3 represent no mulch (M0) and mulch (M1) with four levels of P (P0, P1, P2, and P3), respectively. ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$. Numbers in parentheses are LSD values among treatments ($p = 0.05$). Error bars show one standard error of the mean.

## 3.3. Resorption of Nutrients

### 3.3.1. Nutrient Concentrations in Senescent Leaves

(1) Year, mulching, and P fertilization had significant effects, but their interactions had no effects on N concentrations (Table 1). Mulching increased senesced leaf N concentration by an average of 3.9%, but P fertilization significantly decreased N concentrations by an average of 7.5% over two years. The N concentration was significantly higher in 2017 than in 2018 (Table 1; Figure 3). (2) Significant effects of mulching, P fertilization, and Y × M × P interaction on P concentration were found (Table 1). Mulching increased P concentrations by an average of 16.7% over two years and P fertilization increased senesced P concentrations by an average of 11.9% in 2017 and 17.5% in 2018 (Figure 3). (3) Year and P fertilization significantly affected K concentrations, while mulching and other factors had no effects (Table 1). P fertilization significantly decreased K concentrations by 30.2% in 2017 and 36.6% in 2018. The K concentration was significantly higher in 2017 than in 2018 (Table 1; Figure 3).

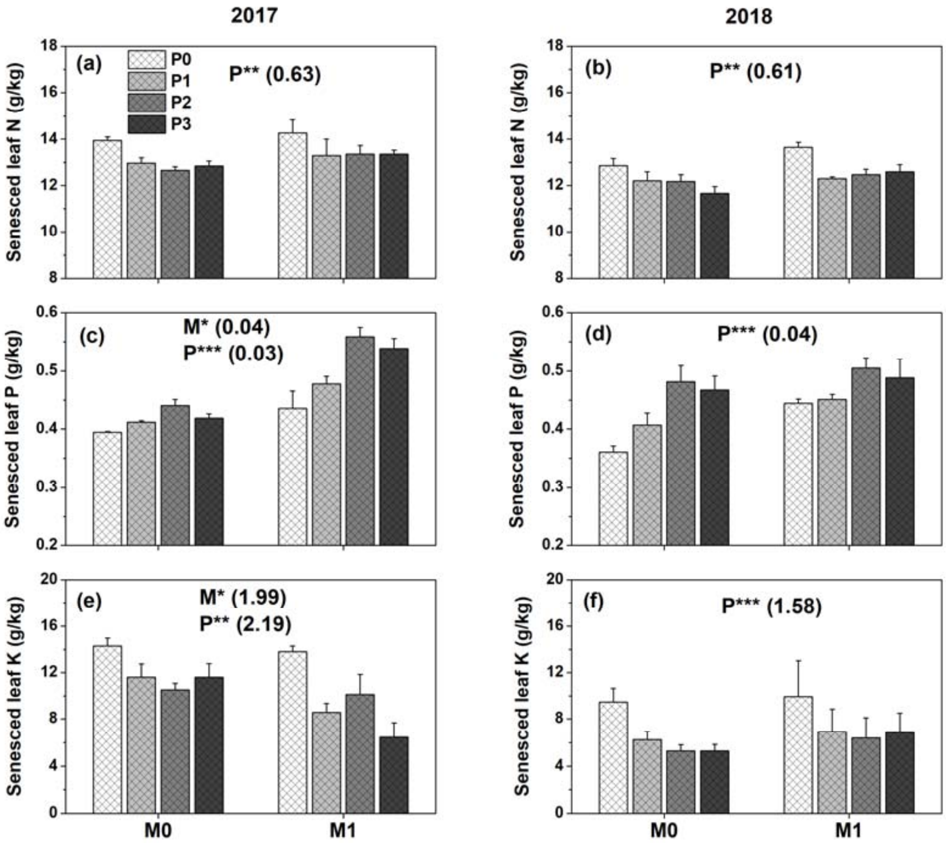


**Figure 3.** Effect of film mulch (M) and P fertilization (P) on concentrations in senescent leaves of (**a**,**b**) N, (**c**,**d**) P, and (**e**,**f**) K in 2017 and 2018. M0P0, M0P1, M0P2, M0P3, M1P0, M1P1, M1P2, and M1P3 represent no mulch (M0) and mulch (M1) with four levels of P (P0, P1, P2, and P3), respectively. ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$. Numbers in parentheses are LSD values among treatments ($p = 0.05$). Error bars show one standard error of the mean.

### 3.3.2. Nutrient Resorption Efficiency

(1) Year and P fertilization significantly affected N resorption efficiency (NRE), while mulching and other interactions had no impact. NRE in P fertilization treatments was significantly higher than that in P0 treatment under each mulching treatment (Figure 4). NRE was significantly higher in 2018 than in 2017. (2) Significantly effects of mulching, P fertilization, and Y × M × P interaction were found for P resorption efficiency (PRE). PRE in mulching treatment was lower by an average of 3.0% than in the no-mulch treatment, while P fertilization increased PRE by an average of 6.2% over two years

(Table 1; Figure 4). (3) Year, P fertilization, and Y × M × P interaction had significant effects on K resorption efficiency (KRE). P fertilization significantly increased KRE by an average of 76.4% over two years, and KRE was significantly higher in 2018 than in 2017 (Table 1; Figure 4).

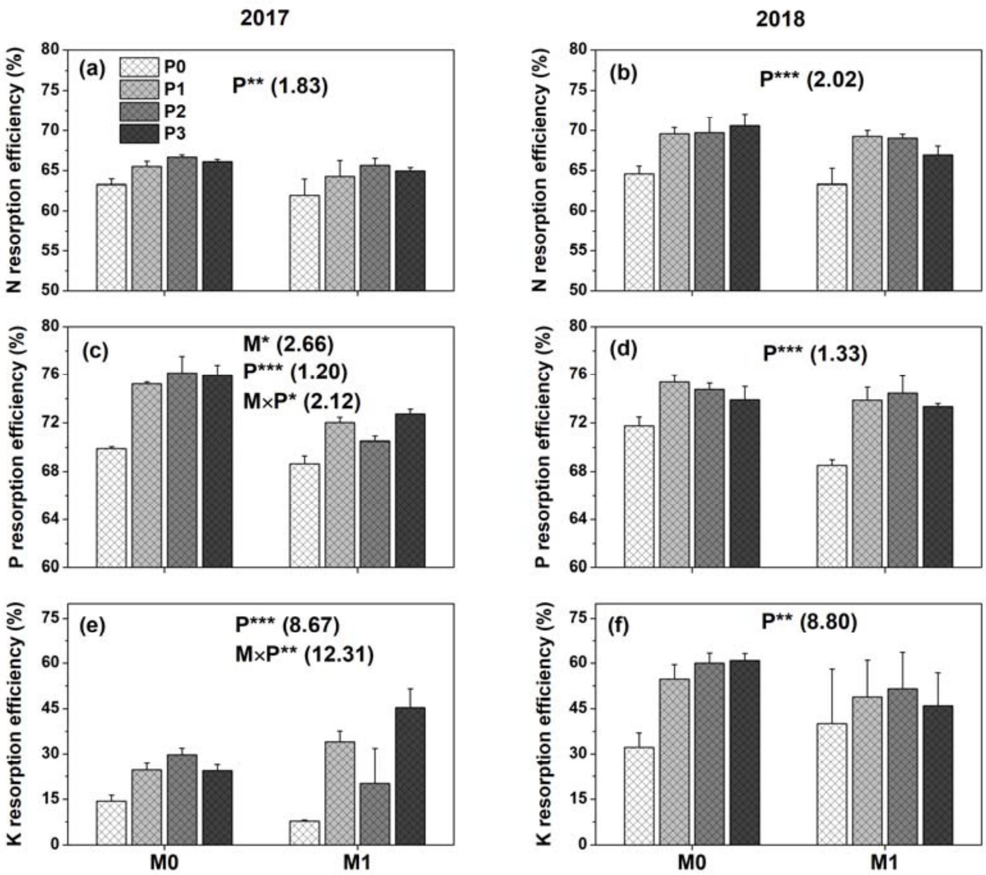


**Figure 4.** Effect of film mulch (M) and P fertilization (P) on resorption efficiencies for (**a**,**b**) N, (**c**,**d**) P, and (**e**,**f**) K in 2017 and 2018. M0P0, M0P1, M0P2, M0P3, M1P0, M1P1, M1P2, and M1P3 represent no mulch (M0) and mulch (M1) with four levels of P (P0, P1, P2, and P3), respectively. ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$. Numbers in parentheses are LSD values among treatments ($p = 0.05$). Error bars show one standard error of the mean.

A second-degree polynomial model was well-fitted to NRE ($y = -0.013\ x^2 + 0.50x + 63.16$, $R^2 = 0.33$, $p < 0.001$), PRE ($y = -0.012\ x^2 + 0.47x + 69.94$, $R^2 = 0.44$, $p < 0.001$), and KRE ($y = -0.035\ x^2 + 1.65x + 24.64$, $R^2 = 0.12$, $p < 0.05$) with P level as the independent variable.

### *3.4. Correlation between Resorption and Nutrient Concentration in Soil and Leaves*

The correlation analysis showed that N and P concentrations in green leaves increased with increasing levels of soil-available P, but P concentrations in green leaves decreased with increasing levels of soil-available K (Table 2). The K concentrations in green leaves were negatively correlated with soil-available P but positively correlated with soil-available K (Table 2). N concentrations in senescent leaves decreased with decreasing levels of soil-available P (Table 2). P concentrations in senescent leaves were positively correlated with soil inorganic N and available P but negatively correlated with soil-available K (Table 2). K concentrations in senescent leaves increased with increasing levels of soil-available K but decreased with increasing levels of soil-available P (Table 2).

In green leaves, N concentration was positively correlated with P concentration but negatively correlated with K concentration (Table 2), and P concentration was negatively correlated with K concentration. In senescent leaves, (1) N concentration was negatively correlated with N and P concentrations in green leaves, but it was positively correlated with K concentration in green leaves (Table 2); (2) P concentration was positively correlated with N and P concentrations in green leaves, but it was negatively correlated with K concentration in green leaves (Table 2), and (3) K concentration was positively correlated with K concentration in green leaves and N concentration in senescent leaves, but it was negatively correlated with N and P concentrations in green leaves and P concentration in senescent leaves (Table 2).

**Table 2.** Correlation between nutrient concentrations in mature and senescent leaves and soil-available nutrients.

| | $[N]_{gre}$ | $[P]_{gre}$ | $[K]_{gre}$ | $[N]_{sen}$ | $[P]_{sen}$ | $[K]_{sen}$ | IN | AP | AK |
|---|---|---|---|---|---|---|---|---|---|
| $[N]_{gre}$ | - | 0.59 *** | –0.32 * | –0.48 *** | 0.38 ** | –0.54 *** | –0.12 | 0.38 ** | –0.26 |
| $[P]_{gre}$ | - | - | –0.55 *** | –0.40 ** | 0.75 *** | –0.51 *** | 0.09 | 0.73 *** | –0.36 * |
| $[K]_{gre}$ | - | - | - | 0.38 ** | –0.49 *** | 0.74 *** | –0.28 | –0.32 * | 0.51 *** |
| $[N]_{sen}$ | - | - | - | - | –0.06 | 0.62 *** | 0.14 | –0.47 *** | 0.27 |
| $[P]_{sen}$ | - | - | - | - | - | –0.31 * | 0.43 ** | 0.40 ** | –0.34 * |
| $[K]_{sen}$ | - | - | - | - | - | - | –0.02 | –0.43 ** | 0.43 ** |

**Note.** ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$. $[N]_{gre}$, $[N]_{sen}$, $[P]_{gre}$, $[P]_{sen}$, $[K]_{gre}$, $[K]_{sen}$, NRE, PRE, and KRE are nitrogen, phosphorus, and potassium concentrations in green and senescent leaves and resorption efficiencies for nitrogen, phosphorus, and potassium, respectively. Inorganic nitrogen (IN), available phosphorus (AP), and available potassium (AK).

NRE decreased with increasing soil-available K concentration but increased with increasing soil-available P concentration (Table 3). PRE increased with increasing soil-available P concentration but decreased with increasing soil inorganic N concentration (Table 3). KRE was positively correlated with soil-available P concentration but negatively correlated with soil-available K concentration (Table 3). The resorption efficiencies of N, P, and K increased with soil-available P concentration (Table 3).

**Table 3.** Correlation between nutrient concentrations in mature and senescent leaves and soil-available nutrients.

| | NRE | PRE | KRE | $[N]_{gre}$ | $[P]_{gre}$ | $[K]_{gre}$ | $[N]_{sen}$ | $[P]_{sen}$ | $[K]_{sen}$ | IN | AP | AK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NRE | – | 0.57 *** | 0.68 *** | 0.79 *** | 0.56 *** | –0.42 ** | –0.91 *** | 0.23 | –0.68 *** | –0.15 | 0.51 *** | –0.31 * |
| PRE | – | – | 0.42 ** | 0.40 ** | 0.56 *** | –0.24 | –0.55 *** | –0.13 | –0.40 ** | –0.38 ** | 0.59 *** | –0.12 |
| KRE | – | – | – | 0.55 *** | 0.43 ** | –0.52 *** | –0.61 *** | 0.18 | –0.95 *** | –0.10 | 0.41 ** | –0.33 * |

**Note.** ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$. $[N]_{gre}$, $[N]_{sen}$, $[P]_{gre}$, $[P]_{sen}$, $[K]_{gre}$, $[K]_{sen}$, NRE, PRE, and KRE are nitrogen, phosphorus, and potassium concentrations in green and senescent leaves and resorption efficiency for nitrogen, phosphorus, and potassium, respectively. Inorganic nitrogen (IN), available phosphorus (AP), and available potassium (AK).

The resorption efficiencies of N, P, and K were consistently and positively correlated with N and P concentrations in green leaves but negatively correlated with K concentration in green leaves and N and K concentrations in senescent leaves (except for PRE versus $[K]_{gre}$) (Table 3). The efficiency of any one of the three major nutrients (N, P, and K) was also significantly positively correlated with that of the other two nutrients (Table 3).

### *3.5. Correlation between Nutrient Resorption and Forage Yield*

P fertilization and the interaction between mulching and P fertilization significantly affected alfalfa forage yield: P fertilization significantly increased forage yield in the no-mulch treatment, but it had no effect in the mulched treatment ((Table 1; Figure 5). Alfalfa forage yield was significantly higher in 2017 than 2018 (Figure 5). Alfalfa forage yield was positively correlated with NRE, PRE, and KRE in the no-mulch treatment, but there were no correlations with NRE, PRE, and KRE in the mulched treatment (Figure 6).

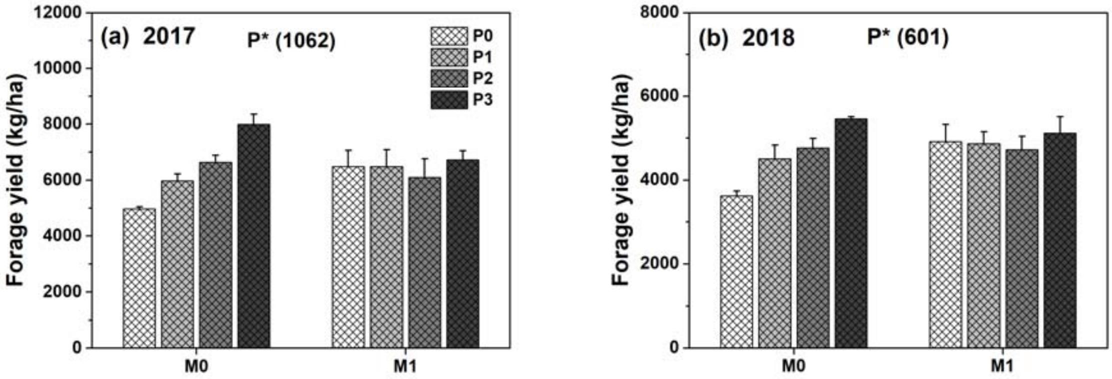


**Figure 5.** Effect of film mulch (M) and P fertilization (P) on alfalfa forage yield in (**a**) 2017 and (**b**) 2018. M0P0, M0P1, M0P2, M0P3, M1P0, M1P1, M1P2, and M1P3 represent no mulch (M0) and mulch (M1) with four levels of P (P0, P1, P2, and P3), respectively. *, $p \leq 0.05$. Numbers in parentheses are LSD values among treatments ($p$ = 0.05). Error bars show one standard error of the mean.

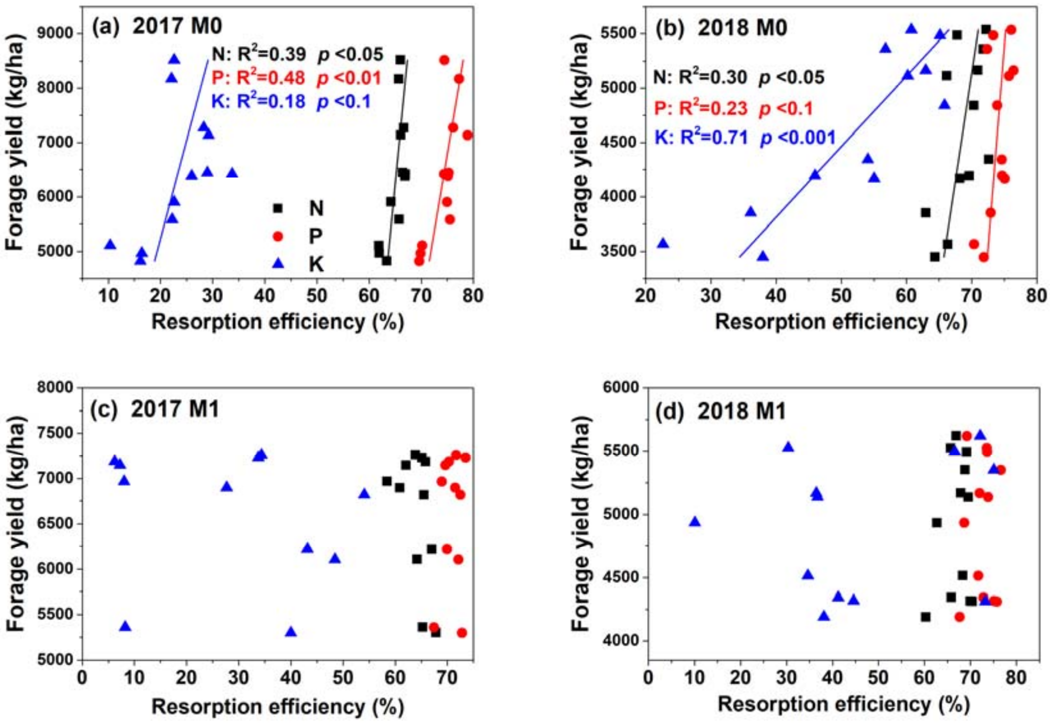


**Figure 6.** Relationships between N, P, and K resorption efficiencies and alfalfa forage yield in the (**a**,**b**) no-mulch treatment (M0) and (**c**,**d**) mulched treatment (M1) in (**a**,**c**) 2017 and (**b**,**d**) 2018.

## 4. Discussion

Alfalfa, a typical perennial forage legume, loses large amounts of N, P, and K at each harvest (when fresh shoots are cut for forage). In this study, the amount of cumulative forage harvested in the past six years was 25 t ha$^{-1}$ in M0P0, 35 t ha$^{-1}$ in M0P1, 43 t ha$^{-1}$ in M0P2, 45 t ha$^{-1}$ in M0P3, 52 t ha$^{-1}$ in M1P0, 66 t ha$^{-1}$ in M1P1, 64 t ha$^{-1}$ in M1P2, and 58 t ha$^{-1}$ in M1P3. Over the same period, about 553, 792, 976, 1063, 1047, 1421, 1447, and 1380 kg ha$^{-1}$ of N, 36.6, 57.6, 76.7, 85.1, 79.4, 115, 120, and 121 kg ha$^{-1}$ of P, and 451, 600, 756, 762, 856, 1025, 1061, and 891 kg ha$^{-1}$ of K were cumulatively removed from the soil in M0P0, M0P1, M0P2, M0P3, M1P0, M1P1, M1P2, and M1P3, respectively.

The M0P0 treatment (no mulch or P fertilizer) had significantly lower amounts of soil inorganic N and available P (Figure 1) than the practices (NY/T2700 2015) recommended by the Ministry of Agriculture, People's Republic of China [39]. Commonly, soil nutrient levels are very low in semi-arid regions, limiting plant growth. Mulching significantly increased soil inorganic N in 2017, whereas P fertilization increased soil-available P in the mulched/no-mulch treatments but decreased soil-available K in the mulched treatments. Changes in the levels of soil-available N, P, and K brought about by mulching and P fertilization probably affect the concentrations of those nutrients in leaves and, in turn, the strategies adopted by the plant to conserve nutrients.

### *4.1. Effect of Mulching and P Fertilization on Nutrient Concentrations in Green Leaves*

Mulching has other benefits, including increased soil temperature and moisture, increased mineralization of N, reduced volatilization of ammonia, and increased soil inorganic N concentration [40]; however, mulching had no effect on N content in green leaves (Table 1; Figure 2), which may be due to the low level of inorganic N in soil (2.7–6.4 mg $kg^{-1}$). Mulching can also enhance P uptake [41] and caused green leaves to have significantly higher P contents than the no-mulch treatment. However, mulching increased alfalfa yields over the past six years, depleting large amounts of K from the soil (cumulative removal 958 kg $ha^{-1}$, or 49% more than the no-mulch treatment), resulting in lower K concentrations in green leaves in 2017.

P fertilization did not affect the soil inorganic N concentration but significantly increased N concentration in green leaves in both years, especially in 2018. This may be the result of P-promoted absorption and assimilation of soil N in alfalfa [24]. P can also increase the N content of N-fixing plants indirectly by improving their ability to fix atmospheric N [27,42]. It is no surprise that P fertilization significantly increased soil-available P concentration, thereby increasing leaf P content, as reported by others [11,14,28,43]. However, P fertilization significantly decreased K concentration in green leaves in the mulched treatment in both years, which was probably because P fertilization decreased the level of soil-available K as alfalfa increased its aboveground biomass, removing K from the soil year after year for six years [17]. Furthermore, the aboveground biomass in the mulched treatment did not significantly differ between years, thereby lowering the amount of K in leaves per unit weight. In addition, some studies have shown that the nutrient concentrations in leaves are related to the availability of those nutrients in soil [2,11,22], and the present study is no exception (except for N).

### *4.2. Effect of Mulching and P Fertilization on Nutrient Concentrations in Senescent Leaves*

Complete and incomplete nutrient resorption can be evaluated by measuring nutrient concentrations in senescent leaves [7]; this approach has been widely used in many studies [11,21,22,26]. Usually, N and P concentrations less than 7 g $kg^{-1}$ and 0.5 g $kg^{-1}$, respectively, in senescent leaves of deciduous species indicate complete resorption; those greater than 10 g $kg^{-1}$ and 0.8 g $kg^{-1}$, respectively, indicate incomplete resorption [7]. In the present study, N concentrations in senescent leaves were greater than 10 g $kg^{-1}$ (11.3–15.4 g $kg^{-1}$), and P concentrations were less or slightly higher than 0.5 g $kg^{-1}$ (0.34–0.58 g $kg^{-1}$). This threshold points to the incomplete resorption of N and complete resorption or intermediate resorption of P by alfalfa. Mean N (12.9 g $kg^{-1}$) and P (0.45 g $kg^{-1}$) concentrations in senescent leaves were less than those recorded in global N-fixing species (15.5 g $kg^{-1}$ for N and 0.74 g $kg^{-1}$ for P), whereas the mean K concentration (9.0 g $kg^{-1}$) was higher than the global average (5.1 g $kg^{-1}$) [44]. Average resorption efficiencies for N and P showed the opposite pattern, being 66.3% and 72.9%, respectively, and higher than the global average values for N-fixing species (49.9% for N and 60% for P [44]). Compared to the global average K resorption efficiency for N-fixing species of 48.6% [44], that of alfalfa was lower in 2017 (25.0%) and higher in 2018 (49.3%), which suggests that relatively more K was resorbed to make up for the limited stocks of available K in soil over time. Overall, alfalfa showed the higher resorption of the three major nutrients, indicating that more N, P, and K were remobilized and used by alfalfa in this study and the alfalfa have the high risk of growth being limited by N, P, and K, compared to other N-fixing legume species.

In senescent leaves, the high N and P concentrations in the mulched treatment, relative to the no-mulch treatment, may have been due to more significant root activity in the mulched plots leading to a greater assimilation of N and P. As reported in earlier studies, P application increases the P content of senescent leaves [6,11,43]. Compared with non-fertilized alfalfa, alfalfa supplied with P through fertilizers has a greater capacity to transfer N and K to maintain nutrient stoichiometric balance in green leaves [45,46], while P fertilization significantly lowered the concentration of N and K in senescent leaves. In addition, senescent leaves are an important part of nutrient recycling in the plant–soil system, and leaf nutrient contents affect the amount of nutrients returned to the soil. Senescent leaves eventually become litter, which returns nutrients to soil [4,26,43]. In the present study, mulching increased the return of N and P, whereas P fertilization increased the return of P and decreased the return of N and K. Thus, mulching led to positive plant–soil feedback for N and P, whereas P fertilization without mulching led to positive plant–soil feedback for P but negative feedback for N and K. This difference warrants further attention to the input of N and K, especially in perennials.

### *4.3. Effect of Mulching and P Fertilization on Nutrient Resorption Efficiencies*

In this study, mulching lowered the resorption efficiency for P due to enhanced P uptake, but it had no effect on that for N or K. This was somewhat different from what was expected from Hypothesis (1). High doses of P reduced N and K in senescent leaves, resulting in higher NRE and KRE in alfalfa. Despite being inconsistent with the findings of several earlier studies [6,43] and Hypothesis (2), the present study found that higher doses of P increased P the resorption efficiency. A few other studies report similar findings [11,12,29,46], namely that the resorption of P also increased with increasing amounts of soil P. This result may be due to the following: (1) The requirements for P increased with the amount of P applied despite more P in the soil following fertilization. There may be P restrictions, even if P is applied. Such speculation is supported by the N: P ratios (>20) in green leaves following P fertilization (mean 21.6 mg kg$^{-1}$), indicating that alfalfa might be limited by P according to the criteria by [45]. The NRE to PRE ratios (mean 0.91) in the P fertilization treatments were less than 1, which is another indication of P limitation [47]. (2) There may be a physiological limit of root of alfalfa to take up P from the soil owing to old age (7–8 years old) or due to the soil hydrothermal conditions, prompting the plant to increase its dependence on the resorption of P. (3) Nutrient resorption is not determined by soil nutrients alone, but also by reproductive effort [12]. Alfalfa supplied with P may increase seed yield [48], resulting in higher reproductive demands, which increase nutrient resorption in response to phenological demand. (4) Plant growth relies on the absolute quantities of a given nutrient that are available, as well as the balance between that nutrient and other nutrients [45,46]: if P fertilization significantly increased the resorption efficiencies for N and K, but not P, it may disturb the coordinated resorption of nutrients. This is supported by the NRE, PRE, and KRE, which were significantly positively related to each other.

With aboveground productivity stimulated by yearly mulching and P fertilization over the past six years [17], given the nutrient-poor site, the supply of N and K may not have matched the demand. The plants are likely to have altered their strategy for conserving and using nutrients over time [28,30,49]. To maximize N and K-use efficiencies and meet the demand for N and K, more conservative strategies of using N and K were observed, which were manifest in the decreased concentrations of N and K in senescent leaves and increased resorption efficiencies for the two nutrients. Consistent with the third hypothesis, NRE and KRE increased over time, pointing to a greater dependence on the internal recycling of N and K, especially K. The other reason for the increase in NRE over time is the decline in N-fixing ability with age, particularly at this semi-arid site [30,50]. Therefore, the plants probably relied more on N from soil—the levels of which have also been declining over time. Biological nitrogen fixation will affect the status of N and other elements in leaves. However, this study did not study the relationship between biological N fixation and nutrient resorption, which remains to be explored. To prevent N and K from limiting alfalfa growth in semi-arid environments over time, we suggest that N and K fertilizers be supplied in adequate quantities to ensure the sustainable management of land.

However, for P, no significant change was observed between its concentration in mature green leaves and its resorption efficiency in two years. This result is inconsistent with our third hypothesis, which is probably because P fertilizer is slow-acting and can release available P continuously for absorption by plants. However, P resorption efficiency in M0P0 increased over time, which is a finding that is consistent with that of Wang et al. [30] and with our third hypothesis. Overall, the adaptive fertilizer management strategies may be important for older alfalfa stands. The internal recycling of nutrients in perennial forage legumes, such as alfalfa, differs from that in other species, and may be due to the frequent cutting of shoots each year and high demand for P.

### *4.4. Relationship between Nutrient Resorption Efficiency and Forage Yield*

Generally, nutrient resorption is an effective strategy for dealing with nutrient shortages and improving nutrient-use efficiencies in nutrient-stressed environments. Higher nutrient resorption in alfalfa would reduce the reliance on soil nutrients, ensuring a relatively sufficient supply of nutrients to green leaves, to benefit photosynthesis and increase productivity [1,51]. Consequently, alfalfa forage yield would be positively correlated with NRE, PRE, and KRE. However, in this study, we found that these relationships were only present in the no-mulch treatment. Mulching changed the relationship between the N, P, and K resorption efficiencies and alfalfa forage yield, indicating that the internal recycling of nutrients in plants is not necessarily related to high yield. The reasons for this need further investigation.

### *4.5. Relationship between Resorption and Nutrient Concentrations in Soil and Leaves*

Previous studies have shown inconsistent relationships between nutrient resorption and leaf nutrient concentrations [1,3,30,52]. In present study, NRE, PRE, and KRE were positively correlated with green leaf N and P, but negatively correlated with senesced leaf N and K. This suggests that N and P concentrations in green leaves, as well as N and K concentrations in senescent leaves, are reliable indicators of the extent of nutrient resorption in perennial N-fixing forage legumes. However, more research is needed to ascertain whether these indicators work equally well under different levels of nutrients or different doses of N and K. In addition, there was strong correlations between soil-available P concentration and nutrient resorption efficiency, and soil-available P concentration and nutrient concentrations in mature and senescent leaves, indicating that soil P may be a good indicator of nutrient resorption in perennial forage legumes.

In general, nutrient resorption efficiency has been negatively correlated with nutrient concentrations in the corresponding green leaves [53] because nutrient concentrations in green leaves reflect soil nutrient availability to some extent [1,11,12,22]. In this study, K showed this relationship, while N and P did not. This may because of the following. (1) As an N-fixing plant, alfalfa is less dependent on the availability of soil N because N is acquired through biochemical fixation [22]. Such plants have higher N concentrations in senescent leaves and lower N resorption efficiency than plants that cannot fix atmospheric N [8,44]. This is consistent with the N concentrations in both green and senescent leaves and the lack of association between N resorption efficiency and inorganic soil nitrogen. (2) Alfalfa need more P for $N_2$ fixation, reproductivity, and maintain a stoichiometric balance. (3) Nutrient resorption efficiencies are not controlled by a single nutrient, but rather by multiple nutrients [29], as evidenced by the relationships between resorption efficiency and leaf nutrient concentration in the corresponding nutrient and other nutrients. For example, N resorption efficiency was significantly correlated with leaf N concentration as well as leaf P and K concentrations and soil P and K availability. These results suggest that the nutrient resorption processes in alfalfa are more complex and need further investigation.

## 5. Conclusions

This is the first study to report that plastic film mulching and P fertilization alter nutrient resorption in alfalfa. Mulching decreased the resorption efficiency for P and did not change that for N and K, and P fertilization significantly increased the resorption efficiency for N, P, and K. Our findings clarified the understanding of nutrient resorption by alfalfa in response to changes in the availability of water and P in semi-arid regions, revealing how alfalfa adapts to changes in the availability of water and P and suggesting that alfalfa adaptation can be enhanced in fields by managing fertilizer and water. This study did not establish solid links between nutrient resorption and forage yield. Thus, further studies under different nutrient conditions are needed to understand the relationship between internal nutrient cycling and yield to provide a reference for high yield and management of grasslands.

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