

## Article

# Nitrate and Ammonium Nitrogen Addition Did Not Alter Nutrient Resorption of Dominant Plant in an Alpine Grassland

Lei Li <sup>1,2,3,4</sup> and Bo Liu <sup>5,\*</sup>

- <sup>1</sup> State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China; lilei@ms.xjb.ac.cn
  - <sup>2</sup> Cele National Station of Observation and Research for Desert-Grassland Ecosystem in Xinjiang, Cele 848300, China
  - <sup>3</sup> Xinjiang Key Laboratory of Desert Plant Roots Ecology and Vegetation Restoration, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China
  - <sup>4</sup> University of Chinese Academy of Sciences, Beijing 100049, China
  - <sup>5</sup> Shandong Provincial Key Laboratory of Soil Conservation and Environmental Protection, College of Resources and Environment, Linyi University, Linyi 276000, China
- \* Correspondence: liubo@lyu.edu.cn

**Abstract:** Worldwide increases in nitrogen deposition rates are influenced by human activities. Although the total amount of N deposition tends to be stable in our country, atmospheric N deposition of the reactive N forms ( $\text{NO}_4^+$ -N,  $\text{NH}_4^+$ ;  $\text{NO}_3^-$ -N,  $\text{NO}_3^-$ ) is remarkably different, and the ratios of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  change continuously. Nutrient resorption is a crucial driver of plant nutrient conservation strategies and litter quality. Therefore, the plant nutrient resorption pattern has remarkable ecological significance for nutrient cycling and the community structure of the ecosystem. However, previous studies have found that plants have different preferences in  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , and the response of nutrient resorption to different N forms remains unclear. In 2017 and 2018, we conducted field experiments simulating five  $\text{NO}_3^-$  and  $\text{NH}_4^+$  addition ratios to examine the responses of the nutrient resorption of the dominant species, *Stipa capillata*, in the alpine grassland of northwest China. Results showed that N addition treatments did not affect the nutrient resorption efficiencies. The results showed that N application had little effect on soil inorganic N composition, so there was no significant change in the nutrient resorption efficiencies. In addition, the nutrient resorption efficiencies were unaffected across the five different ratios of nitrate and  $\text{NH}_4^+$  addition, suggesting that nutrient resorption was not only controlled by the nutrient supply and nutrient form. Our results have important implications in understanding the significance of the frequency of N addition due to N loss through leaching and denitrification. The results also highlight that low intensity and high frequency N addition are required to investigate the response of plant nutrient resorption to the N supply in the future study.



**Citation:** Li, L.; Liu, B. Nitrate and Ammonium Nitrogen Addition Did Not Alter Nutrient Resorption of Dominant Plant in an Alpine Grassland. *Atmosphere* **2023**, *14*, 555. <https://doi.org/10.3390/atmos14030555>

Academic Editor: László Bencs

Received: 14 December 2022

Revised: 17 February 2023

Accepted: 9 March 2023

Published: 14 March 2023

**Keywords:** nitrogen addition; ammonium and  $\text{NO}_3^-$ ; nutrient resorption; nutrient concentration; alpine grassland



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Global atmospheric N deposition has dramatically increased by ten-fold, from 15 to 156 Tg N yr<sup>-1</sup> between 1860 and the early 1990s [1], due to the heavy use of fossil fuels and fertilizers by human activities over the past century [2,3], and it has complex effects on terrestrial ecosystems [4,5]. Simultaneously, with the increased development of agricultural production and industrial engineering, anthropogenic N deposition has increased significantly in China during the 30 years from 1980 to 2010 [6], generally increasing the availability of N in ecosystems. Since 2011, the temporal evolution of the total N deposition in our country has changed from a rapid increase to a stable level, but the relatively high level of N deposition still requires more attention [7]. Previous studies

have reported that plant community composition [8], plant residuals decomposition [9], nutrient resorption [10], and soil microbial community structure [11] were affected by N deposition [12]. Hence, the N deposition has led to a profound alteration of structure and function in ecosystems [3,5,6].

The atmospheric concentrations of reactive N forms (ammonium N,  $\text{NH}_4^+$ ; nitrate N,  $\text{NO}_3^-$ ) were remarkably different in the dry and wet depositions [13,14]. Although  $\text{NH}_4^+$  is the dominant form of N deposition, the rate of  $\text{NO}_3^-$  is increased, thereby decreasing the ratio of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  [5,14]. Therefore, these patterns of N deposition will lead to changes in the terrestrial ecosystem. The impact of N deposition on the terrestrial ecosystems depends on the direction of N inputs to the ecosystem, which rest with whether they enter the ecosystem in the form of reduction or oxidation [15]. In the gross, various plant species have preferences for the different forms of N utilization, and dominant species in the community give priority to the use of N with higher chemical forms in the soil [16]. Therefore, the input of different forms of N may lead to varied plant responses and nutrient utilization patterns in the community, and thus lead to different levels of N retention in the ecosystem. For instance, the above ground net primary productivity (ANPP) of forbs was considerably affected by N forms after five years of a different  $\text{NH}_4^+$ -N/ $\text{NO}_3^-$ -N ratio addition in a natural semi-arid grassland in northern China [16], and short-term  $^{15}\text{N}$  tracer experiments also indicated that plants preferred  $\text{NO}_3^-$  [17,18]. In addition, the input of different N forms may make P restriction more prominent in grassland ecosystems, and thus, gradually changing the nutrient restriction state from a N restriction to a P restriction or N and P co-restriction [19]. However, previous studies mainly focused on the effects of the amount of N addition on the ecosystem. In addition to affecting the nutrient cycling process of grassland ecosystems, changes in the N form ratio can also significantly alter the interspecific competition in grassland communities, resulting in changes in the community structure and a loss of diversity [10]. Thus, studying the effects of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  on plant nutrient resorption in alpine grasslands is critical to effectively predict how these ecosystems respond to N deposition.

Nutrient resorption is an important nutrient conservation strategy for plants to reduce their dependence on external nutrient substance supplies [20,21]. It is also a key process of the nutrient cycle because it determines the plant's residuals quality [21,22] and the plant's residuals decomposition [9], and then affects the nutrient inputs into the ecosystems [23]. Nutrient resorption was generally regulated by the nutrient status of the plant and soil [10,22,24], and the nutrient resorption capacity of plants also reflects the available nutrient status of soils, while the effect of nutrient enrichment by N deposition alters the nutrient resorption. Nitrogen addition improves P recovery efficiency by increasing the plant N content and reducing N [25], thus changing the balance between total N and P returned to soil, and ultimately affecting the plant and soil stoichiometric characteristics [26]. Although foliar nutrient resorption has been investigated from site to global scales [27–31], while the results were inconsistent. For instance, N addition had negative [32] and neutral [33] effects on the N resorption efficiency (NRE); those of P resorption efficiency (PRE) are even more dramatic [33–35]. These conflicting results limit our understanding of plant adaptation to environmental changes. A previous study on forest ecosystems reported that plant nutrient resorption efficiency was affected by N forms, but not by the N source [36]. However, a study on alpine grassland ecosystems is still unclear.

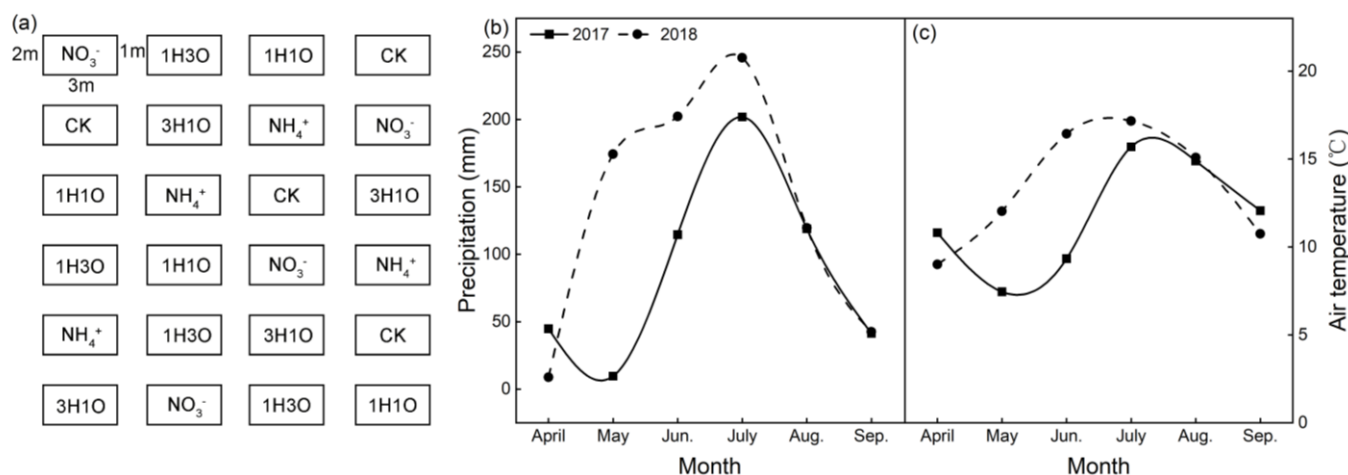
The alpine grassland of Kunlun Mountain is located on the north edge of the Tibetan Plateau and the south edge of the Taklimakan desert, is sensitive to global change factors, and experiences atmospheric N deposition [5]. Moreover, nutrient fertilization was widely used in the restoration and improvement of the degraded vegetation by overgrazing in grassland management [37]. However, nitrogenous fertilizers of N addition in field experiments differ with natural N deposition composition [14,22,35]. Hence, how does plant nutrient resorption respond to various nitrate and  $\text{NH}_4^+$  additions? The uncertainty in the answer is remarkably important for assessing the response of grasslands to climate change, in particular, for plants with difference preferences of N forms [17,38]. We conducted

field experiments with a gradient of nitrate and  $\text{NH}_4^+$  ratios to examine the intraspecific responses of the nutrient resorption of the dominant species, *S. capillata*, in the alpine grassland of northwest China. We hypothesized the following: (i) N addition decreases N resorption but increases P resorption. Thus, the N:P resorption ratios decrease because the plants have sufficient N and may suffer P limitation under the amendment of N. (ii) The variation of nitrate and  $\text{NH}_4^+$  addition would alter the nutrient resorption due to preference of plant N uptake.

## 2. Materials and Methods

### 2.1. Study Area and Experimental Setup

This study was conducted at an alpine grassland of Kunlun Mountain in 2017–2018, northwest of China. The area has an elevation of 3186 m with specific location at  $81^\circ 06' \text{ E}$ ,  $36^\circ 08' \text{ N}$ . The mean annual air temperature was  $3.7^\circ \text{ C}$ , ranging from  $-8.7^\circ \text{ C}$  in December to  $14.5^\circ \text{ C}$  in July. The mean total precipitation was 600 mm (2014–2018) with more than 80% in the growing season from May to October, and the precipitation patterns of experimental duration as shown in Figure 1. This study site area, of more than  $10,000 \text{ m}^2$ , was fenced since 2009 to prevent the grazing of large animals. The soil (0–20 cm) in the study area is sandy loam texture [39], and the mean bulk density is  $1.2 \text{ g cm}^{-3}$ . The soil pH is 8.0, and the total N content, soil organic matter content,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  content are  $0.70 \pm 0.07 \text{ g kg}^{-1}$ ,  $17.97 \pm 0.19 \text{ g kg}^{-1}$ ,  $26.09 \pm 0.06 \text{ mg kg}^{-1}$ , and  $18.19 \pm 0.23 \text{ mg kg}^{-1}$ , respectively. The dominant grass species are *Stipa capillata*, *Seriphidium rhodanthum*, *Astragalus polycladus*, and *Allium chrysanthum* Regel.



**Figure 1.** Experimental design (a), precipitation (b) and air temperature (c) in experimental duration between 2017 and 2018. CK, the control;  $\text{NH}_4^+$ , the total  $\text{NH}_4^+$ ; 3H1O, tripled  $\text{NH}_4^+:\text{NO}_3^-$ ; 1H1O,  $\text{NH}_4^+$  equal to  $\text{NO}_3^-$ ; 1H3O,  $\text{NH}_4^+:\text{tripled } \text{NO}_3^-$ ;  $\text{NO}_3^-$ , total  $\text{NO}_3^-$ .

In 2017 to 2018, five different ratios of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  N addition experiments (N fertilization  $16 \text{ g N m}^{-2} \text{ yr}^{-1}$ ) were simulated in  $2 \times 3 \text{ m}$  plots with six treatments and four replicates of each treatment. All plots were separated by at least 1 m, and each plot was randomly arranged (Figure 1). In late April, all of N (analytically pure) was fertilized and N was mixed with soil from the test plot and spread on the surface of the soil evenly. The nitrogen addition treatment is as follows: (1) the control (no fertilization, defined as CK), (2) the total  $\text{NH}_4^+$  (defined as  $\text{NH}_4^+$ ), (3) tripled  $\text{NH}_4^+:\text{NO}_3^-$  (defined as 3H1O), (4)  $\text{NH}_4^+$  equal to  $\text{NO}_3^-$  (defined as 1H1O), (5)  $\text{NH}_4^+:\text{tripled } \text{NO}_3^-$  (defined as 1H3O), and (6) total  $\text{NO}_3^-$  (defined as  $\text{NO}_3^-$ ). The  $\text{NH}_4^+$  of  $\text{NH}_4\text{Cl}$  was applied, as well as the  $\text{NO}_3^-$  of  $\text{KNO}_3$ .

### 2.2. Field Sampling and Measurements

In August, at the plant peak biomass period, from 2017 to 2018, a subplot ( $1 \text{ m} \times 1 \text{ m}$ ) was randomly selected in each plot, and then the *S. capillata* plant samples were collected.

At the same time, four soil samples (0–10 cm) were randomly collected using a 2 cm diameter soil auger from each plot, thoroughly mixed, and combined as a single composite sample. Foliar samples were oven-dried at 75 °C for 48 h. After the samples were completely dried, they were cooled naturally and then ground, screened, bagged, labeled, and finally analyzed chemically. In late October, at the time when the plant was entirely senesced, the senescent foliar of *S. capillata* were also collected, dried, and ground. C and N concentrations in plant tissues were analyzed with a CN elemental analyzer (Eurovector, Milan, Italy). Phosphorus concentration was determined with persulfate oxidation, followed by colorimetric analysis. All soil samples were sieved by a 2 mm mesh to remove the plant roots and other impurities. A fresh soil sample of 20 g was weighed, 100 mL of 1 mol/L KCl solution was added to it, and the bottle was capped tightly, before being put on an oscillator for 30 min. After the filtration extraction, a flow injection automatic analyzer (FIAstar 5000, Foss Tecator, Denmark) was used to determine the content of inorganic N in the fresh soil. Soil available P concentrations were extracted by NaHCO<sub>3</sub>, 150 rpm, shaken for 30 min, filtered by filter paper, and analyzed using the ammonium molybdate method. The soil inorganic N and available P concentrations were based on the dry soil weight, which was measured by drying the soil at 105 °C for 48 h [40].

### 2.3. Calculation of Nutrient Resorption

N and P resorption efficiency (NuRE) was calculated by the following equations:

$$\text{NuRE} = (1 - \text{Nutrient}_{\text{senesced}} / \text{Nutrient}_{\text{green}}) \times 100\% \quad (1)$$

where  $\text{Nutrient}_{\text{senesced}}$  and  $\text{Nutrient}_{\text{green}}$  are senesced and green leaf nutrient concentrations (N and P), respectively, and were expressed on a dry weight mass basis [19].

A two-way ANOVA was performed to examine the year and the nutrient form effects, as well as their interaction effects on the soil available contents, leaf nutrient concentration, and resorption. A one-way ANOVA and independent sample t-test were performed to examine the differences in the soil available contents, leaf nutrient concentration, and resorption related to years and treatments. Prior to the analysis, a normality test was performed using the Shapiro–Wilk criteria. A significance level of  $\alpha = 0.05$  was used in the statistical analyses. All statistical analyses were performed in the SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

### 3. Results

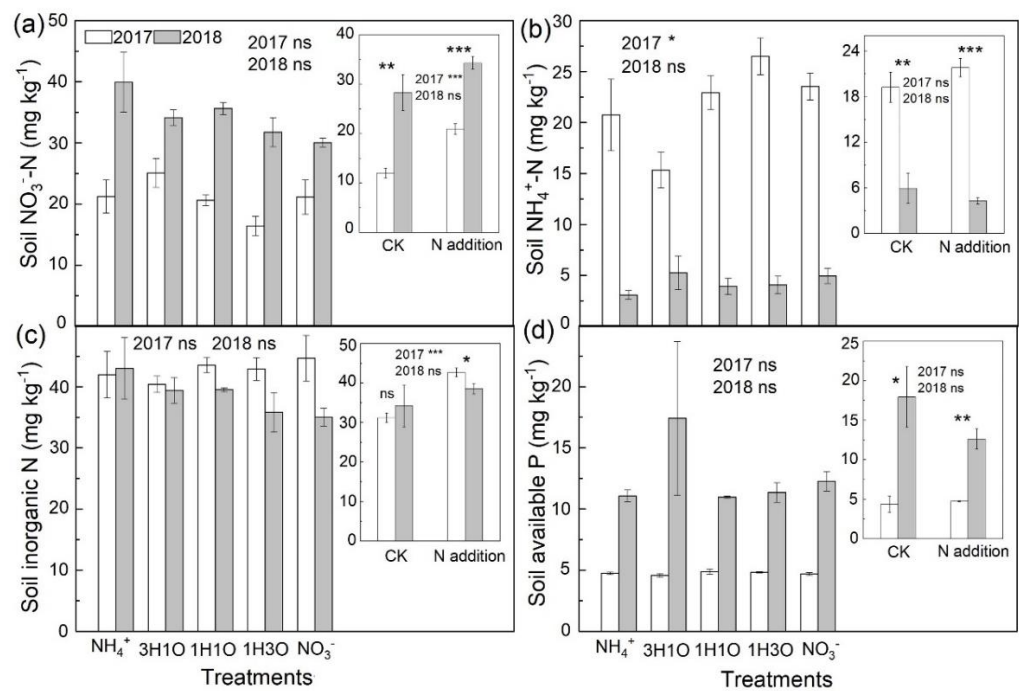
Various N form fertilization increased the soil NO<sub>3</sub><sup>−</sup>-N and inorganic N contents, but did not affect the soil NH<sub>4</sub><sup>+</sup>-N and available P contents compared with the control (Table 1, Figure 2). Soil NO<sub>3</sub><sup>−</sup>-N contents increased in 2017, but no changes were observed in 2018, as well as inorganic N. In addition, soil NO<sub>3</sub><sup>−</sup>-N and inorganic N contents showed a decreasing trend in 2018 under different proportions of N fertilizer treatments. In 2017 and 2018, no significant differences were found among various N form fertilization treatments, except for the soil NH<sub>4</sub><sup>+</sup>-N contents in 2017 ( $p < 0.05$ ). Different N fertilization had significant effects on the soil NO<sub>3</sub><sup>−</sup>-N, while the overall effect on the soil nutrient content was small, and the available P contents of 2018 were higher than those of 2017. However, the soil NH<sub>4</sub><sup>+</sup>-N contents were lower than in 2017.

In general, the N application treatment promoted the N and P contents of the green and senesced leaf compared to the control treatment, but the effects of the different proportions of N fertilizer on the N and P contents of green and senesced leaf was inconsistent. The green leaf N concentrations (Ng) and senesced leaf N concentrations (Ns) of 2017 increased after N fertilization ( $p < 0.05$ ). However, no significant changes were found in 2018 ( $p > 0.05$ ) (Figure 3). On the contrary, N fertilization reduced the senesced leaf P concentrations (Ps) in 2018. In 2017 and 2018, no significant differences in leaf nutrient contents among various N form fertilization treatments were observed. Different treatment years significantly affected Ng, Ns, and green leaf P concentrations (Pg), but did not affect Ps.

**Table 1.** Results of the two-way ANOVA for nutrient concentrations and nutrient resorption efficiencies as dependent on years (Y), treatments (T), and their interactions.

Factors	df	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N	IN	AP	Ng	Pg	N:Pg	Ns	Ps	NPs	NRE	PRE	NRE:PRE
T	5	5.1 ***	2.1	2.7 *	1.0	9.4 ***	2.5	3.8 **	2.4	1.6	9.6 ***	0.7	1.2	0.2
Y	1	97.4 ***	284.5 ***	3.0	49.2 ***	1.5	23.3 ***	13.4 **	20.3 ***	0.1	58.6 ***	15.9 ***	8.1 **	69.9 ***
T×Y	10	1.4	3.3	1.3	1.3	1.5	1.4	2.2	0.7	1.0	1.4	0.5	1.0	1.1

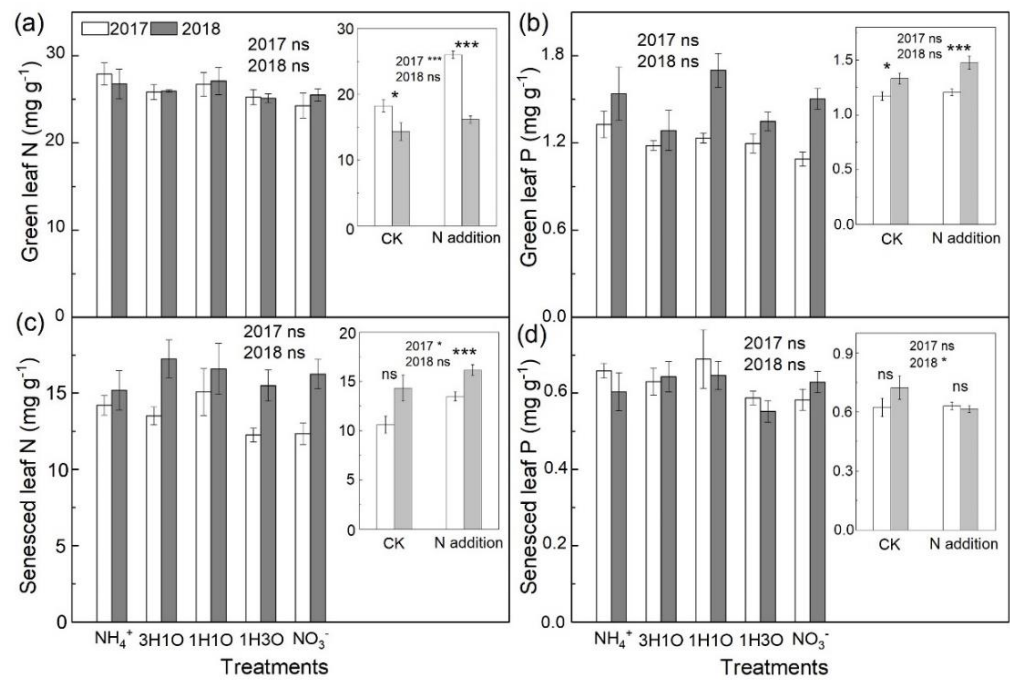
The F-values with the level of significance presented. NO<sub>3</sub><sup>-</sup>-N represents soil nitrate N, NH<sub>4</sub><sup>+</sup>-N represents soil ammonium N, IN represents soil inorganic N, AP represents soil available P, Ng and Pg represent N and P concentrations in green leaves, respectively; Ns and Ps represent N and P concentration in senesced leaves, respectively; NRE and PRE represent N resorption efficiency and P resorption efficiency, respectively. \* *p* < 0.05; \*\* *p* < 0.01; \*\*\* *p* < 0.001.



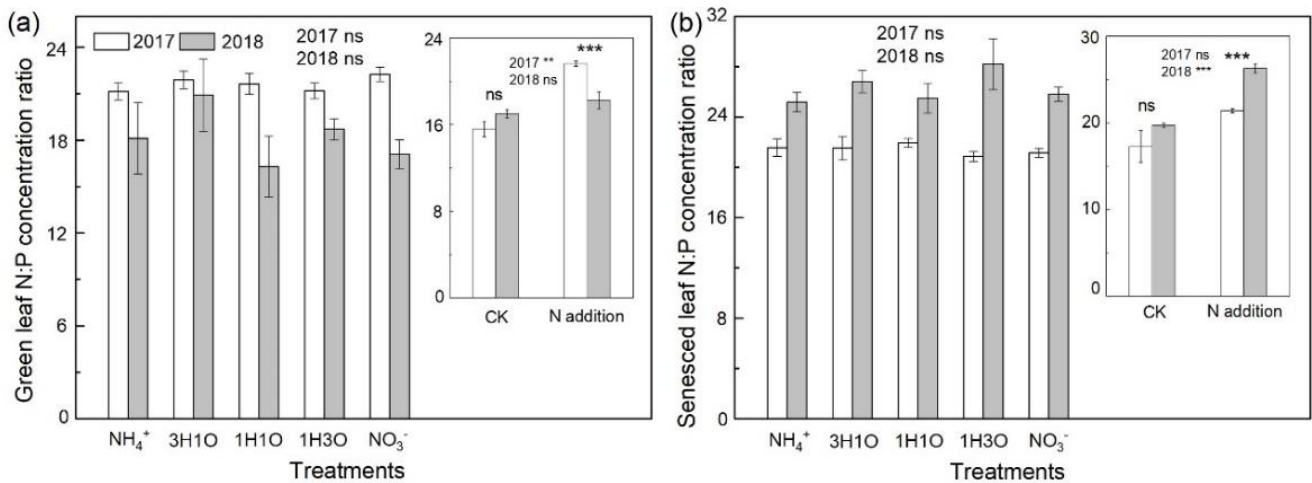
**Figure 2.** Response of soil NO<sub>3</sub><sup>-</sup>-N (a), NH<sub>4</sub><sup>+</sup>-N (b), inorganic N (c) and available P (d) to various N form fertilizations. The asterisks and ns above the white and gray bar graphs represent the significance between 2017 and 2018. The asterisks and ns following the number of years inside the little figure represent the significance in control (*n* = 4) and N addition (average, *n* = 20). The asterisks and ns following the number of years outside the figure represent the significance in N addition groups. \* *p* < 0.05; \*\* *p* < 0.01; \*\*\* *p* < 0.001; ns, not significant.

N fertilization significantly affected the green and senesced leaf N:P ratios (Figure 4). Although there was no significant difference in N:P ratio between 2017 and 2018, the N:P ratio of green leaf in 2017 was higher than that in 2018 under N application (*p* < 0.01), while N:P ratio of senesced leaf in 2018 was significantly higher (*p* < 0.001). In addition, the different treatment years significantly affected the N addition treatments; no significant changes were observed in the control group.

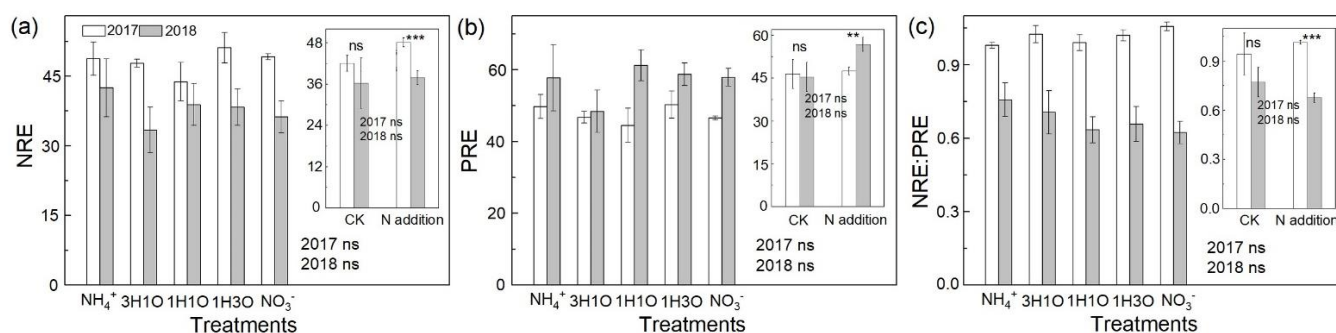
Although there were no significant changes in the nutrient resorption efficiencies between N fertilization and control treatments, both the N and P resorption efficiencies showed an upward trend (Figure 5). In 2017 and 2018, no significant differences in the nutrient resorption efficiencies were observed among various N form fertilization treatments, but different treatment years significantly affected N addition treatments, and no changes in control were found. In addition, NRE:PRE changed from an increase in 2017 to a decrease under N application, and showed an opposite trend under different N application proportions in 2017 and 2018.



**Figure 3.** Response of leaf nutrient concentrations (including green leaf N (a) and P (b), senesced leaf N (c) and P (d)) to various N fertilization forms. The asterisks and ns above the white and gray bar graphs represent the significance between 2017 and 2018. The asterisks and ns following the number of years inside the little figure represent the significance in control ( $n = 4$ ) and N addition (average,  $n = 20$ ). The asterisks and ns following the number of years outside the figure represent the significance in N addition groups. \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ ; ns, not significant.



**Figure 4.** Response of ratios of N:P in green (a) and senesced leaf (b) to various N form fertilizations. The asterisks and ns above the white and gray bar graphs represent the significance between 2017 and 2018. The asterisks and ns following the number of years inside the little figure represent the significance in control ( $n = 4$ ) and N addition (average,  $n = 20$ ). The asterisks and ns following the number of years outside the figure represent the significance in N addition groups. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; ns, not significant.



**Figure 5.** Response of leaf nutrient resorption efficiencies (including NRE (a), PRE (b), NRE:PRE (c)) to various N form fertilizations. The asterisks and ns above the white and gray bar graphs represent the significance between 2017 and 2018. The asterisks and ns following the number of years inside the little figure represent the significance in control ( $n = 4$ ) and N addition (average,  $n = 20$ ). The asterisks and ns following the number of years outside the figure represent the significance between N addition groups. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; ns, not significant.

#### 4. Discussion

In this study, we set up a field experiment to research the nutrient resorption of *Stipa capillata* by changing the addition ratio of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . Our results showed that the N application increased the soil  $\text{NO}_3^-$ -N content on the whole, but had no significant effect on soil  $\text{NH}_4^+$ -N and available P contents, and there was no obvious change in the plant leaf nutrient content under the five N fertilizer addition ratios. Contrary to our hypothesis, our results showed that the nutrient resorption efficiencies of ambient conditions in the studied alpine grassland have no significant changes compared with N addition treatments, as well as across the five different ratios of nitrate and  $\text{NH}_4^+$  addition. However, N resorption decreased and P resorption increased; thus, reduction in the N:P resorption ratios was found in 2017, compared with 2018 (Figure 5). Since plant growth was previously limited only by N, the absorption of N by plants increased during the initial N addition, which promoted plant growth. At the same time, plant non-restrictive nutrient P was consumed, and plants tended to absorb more P and improve the resorption efficiency of P to maintain the dynamic balance of the N/P ratio. Although N addition increased soil inorganic N content, it may also increase soil leaching and the  $\text{N}_2\text{O}$  emission rate without changing the total N content [41]. Therefore, the change of the N addition ratio may have little effect on soil nutrients, and thus have no obvious change on plant nutrient uptake. Previous studies found that plants have different preferences in nitrate and  $\text{NH}_4^+$  [17,18], while our results clearly showed that the fertilization of different N forms had no effects on *S. capillata* nutrient resorption efficiencies in an alpine grassland.

Soil available P contents in 2018 were significantly more than two times higher than those in 2017 (Figure 2), and it is possible that the higher inorganic N content may stimulate the activity of phosphatase [42], enabling plants and microorganisms to allocate excess N to phosphatase enzymes, thus relieving P restrictions [43]. Moreover, our results showed that the soil  $\text{NO}_3^-$ -N contents of 2017 were significantly lower than those of 2018, whereas  $\text{NH}_4^+$ -N showed contrasting results with that of  $\text{NO}_3^-$ -N (Figure 2). However, N fertilization did not increase the soil available N contents in 2018, and they were conflicting with other reports that the available N contents were positive with N addition rates [10,22]. These novel results might be attributed to higher soil available P in 2018 which relieved the P limitation of nitrifying bacteria and stimulated their activity and enhance nitrification. Hence,  $\text{NO}_3^-$ -N increased and  $\text{NH}_4^+$ -N pools decreased, leading to N loss by greenhouse gas emission [44].

N addition significantly elevated the green and senesced leaf N, consistent with the results of previous studies. For instance, the leaf N of two dominant plant species (*Leymus chinensis* and *Stipa grandis*), perennial rhizomatous and bunchgrass, positively increased with the N addition rate ranging from 0–64  $\text{g N m}^{-2} \text{ yr}^{-1}$  [22]. However, leaf P

of senesced decreased, not affecting those of green. Although other studies have reported the positive [22] and neutral effects [33,35] of N application on leaf N, our results suggested that N application improves P demand and limitation, thereby resulting in a conservative P use [17,24,45]. Plants could alter their nutrient resorption to reduce the dependence on soil available nutrient [20,21], and it is a theoretical possibility that the N application increases the available N by reducing NRE, while PRE increases in order to maintain stoichiometric stability due to high P requirements. Contrary to our initial hypothesis, NRE and PRE showed no response to N addition, consistent with a global meta-analysis that more than half of cases of nutrient resorption (NRE, 63%,  $n = 60$  and PRE, 57%,  $n = 37$ ) showed no response to the increased nutrient availability [20]. This finding suggested that nutrient supply has unclear nutritional controls on nutrient resorption efficiency. Our previous study indicated that N addition reduced the NRE, and the values of NRE were higher than in this study [10]. We speculate that these findings may relate to higher leaf nutrient concentrations [27], reflecting that nutrient resorption was not only controlled by nutrient supply [20].

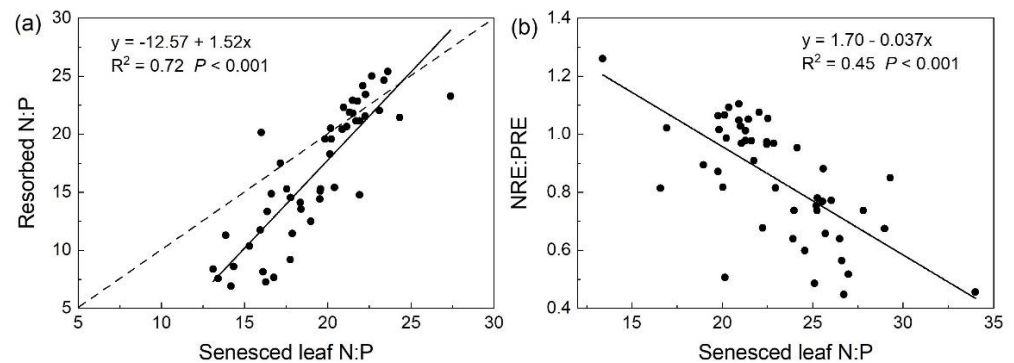
Generally, plants absorb N from the soil in the form of nitrate and ammonium, and the preference of plants for absorption is nitrate [18]. These unexpected results indicate that the variations of the N form had no effects on the plant leaf concentrations and nutrient resorption efficiencies (Figure 5). Previous studies demonstrated the occurrence of simultaneous nitrification and denitrification after the addition of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  fertilizers, and most N rapidly disappeared by the emission of gas N. For example, after applying N, approximately 25% to 75% of the added  $\text{NO}_3^-$ -N and nearly 50% of the  $\text{NH}_4^+$ -N disappeared within 7 days [46]. Therefore, the simultaneous nitrification and denitrification treatment kept the composition of soil inorganic N unchanged regardless of the form of initial N fertilization, and thus had no effect on plant nutrient uptake. Our results highlight that the study of plant nutrient resorption response to the N source should increase the frequency of N addition to maintain the fertilizing effect in the future given that various N sources were tested in the previous field experiments. Significant differences were found between the two years of treatment; NRE decreased and PRE increased, resulting in decreased NRE:PRE ratios in the second year compared with the first year (Figure 5). Precipitation was the main determinant of the influence of the  $\text{NH}_4^+/\text{NO}_3^-$  ratios on plant growth [16]; moreover, precipitation patterns showed a remarkable difference between the experimental duration. These partial results are in line with our first hypothesis which suggested that plants decreased the demand of N and increased the P intraspecific cycling, indicating an interannual effect of nutrient addition on plant nutrient resorption by precipitation.

We observed stoichiometry control, nutrient limitation control, and nutrient concentration control for leaf nutrient resorption [27]. Our results showed that resorbed N:P was positively correlated with senesced leaf N:P, suggesting that the plant nutrient resorption pattern was regulated by stoichiometry control. On the contrary, NRE:PRE was negatively correlated with senesced leaf N:P (Figure 6), indicating that plants adopt a nutrient limitation control strategy. Moreover, in theory, resorbed N:P would be positively correlated with senesced leaf N:P, following a 1:1 line. Our results showed that most data fall below the 1:1 line, indicating that more P was resorbed, fitting well into the N addition and leading to a P-limitation of the alpine grassland. In summary, these results were consistent with a study from a secondary forest ecosystem, indicating that stoichiometric and nutrient limitation controls were coexistent [27].

In conclusion, the results from this study showed that the simultaneous nitrification and denitrification, after the addition of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  fertilizers, kept the composition of soil inorganic N unchanged, so the leaf concentration and nutrient resorption efficiencies of plants in the studied alpine grassland were not affected, indicating that nutrient resorption was not only controlled by the nutrient supply. Moreover, our results showed that across the five different ratios of nitrate and  $\text{NH}_4^+$  addition, the nutrient resorption efficiencies were unaffected. Furthermore, our results have important implications for the potential



risks of a one-time N application due to N loss by nitrification–denitrification, and a high frequency N addition is required to avoid the potential risks from the response of plant nutrient resorption to the N supply.



**Figure 6.** Relationship between senesced leaf N:P ratios and resorbed N:P ratios (a) and NRE:PRE ratios (b). The dash line represents the 1:1 line.

**Author Contributions:** Conceptualization, Methodology, Funding acquisition, L.L. and B.L.; Writing-Original draft preparation, Software, Data curation, Writing-review and editing, L.L.; Supervision, Visualization, Project administration, B.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by [Natural Science Foundation of Xinjiang Uygur Autonomous Region] grant number [2022D01E100], [Shandong Province College Youth Innovation Technology Support Program] grant number [2020KJE009], [Linyi Key Research and Development Plan] grant number [2021029], [Shandong Provincial Natural Science Foundation] grant number [ZR2020MC040], [Youth Innovation Promotion Association of the Chinese Academy of Sciences] grant number [2020434].

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** No new data were created or analyzed in this study. Data sharing is not applicable to this article.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Galloway, J.N.; Dentener, F.J.; Capone, D.G.; Boyer, E.W.; Howarth, R.W.; Seitzinger, S.P.; Asner, G.P.; Cleveland, C.C.; Green, P.A.; Holland, E.A.; et al. Nitrogen Cycles: Past, Present, and Future. *Biogeochemistry* **2004**, *70*, 153–226. [[CrossRef](#)]
- Davidson, E.A. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nat. Geosci.* **2009**, *2*, 659–662. [[CrossRef](#)]
- Gruber, N.; Galloway, J.N. An Earth-system perspective of the global nitrogen cycle. *Nature* **2008**, *451*, 293–296. [[CrossRef](#)] [[PubMed](#)]
- Li, W.; Jin, C.; Guan, D.; Wang, Q.; Wang, A.; Yuan, F.; Wu, J. The effects of simulated nitrogen deposition on plant root traits: A meta-analysis. *Soil Biol. Biochem.* **2015**, *82*, 112–118. [[CrossRef](#)]
- Liu, X.J.; Zhang, Y.; Han, W.X.; Tang, A.H.; Shen, J.L.; Cui, Z.L.; Vitousek, P.; Erisman, J.W.; Goulding, K.; Christie, P.; et al. Enhanced nitrogen deposition over China. *Nature* **2013**, *494*, 459–462. [[CrossRef](#)]
- Liu, X.; Duan, L.; Mo, J.; Du, E.; Shen, J.; Lu, X.; Zhang, Y.; Zhou, X.; He, C.; Zhang, F. Nitrogen deposition and its ecological impact in China: An overview. *Environ. Pollut.* **2011**, *159*, 2251–2264. [[CrossRef](#)]
- Lu, X.; Mo, J.; Zhang, W.; Mao, Q.; Liu, R.; Wang, C.; Zheng, M.; Wang, S.; Taiki, M.; Mao, J.; et al. Effects of simulated atmospheric nitrogen deposition on forest ecosystems in China: An overview. *J. Trop. Subtrop. Bot.* **2019**, *27*, 500–522.
- Zhao, Y.A.; Yang, B.; Li, M.X.; Xiao, R.Q.; Rao, K.Y.; Wang, J.Q.; Zhang, T.; Guo, J.X. Community composition, structure and productivity in response to nitrogen and phosphorus additions in a temperate meadow. *Sci. Total Environ.* **2019**, *654*, 863–871. [[CrossRef](#)]
- Zhang, W.D.; Chao, L.; Yang, Q.P.; Wang, Q.K.; Fang, Y.T.; Wang, S.L. Litter quality mediated nitrogen effect on plant litter decomposition regardless of soil fauna presence. *Ecology* **2016**, *97*, 2834–2843. [[CrossRef](#)]
- Li, L.; Gao, X.; Li, X.; Lin, L.; Zeng, F.; Gui, D.; Lu, Y. Nitrogen (N) and phosphorus (P) resorption of two dominant alpine perennial grass species in response to contrasting N and P availability. *Environ. Exp. Bot.* **2016**, *127*, 37–44. [[CrossRef](#)]

11. Widdig, M.; Heintz-Buschart, A.; Schleuss, P.M.; Guhr, A.; Borer, E.T.; Seabloom, E.W.; Spohn, M. Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. *Soil Biol. Biochem.* **2020**, *151*, 12. [[CrossRef](#)]
12. Vitousek, P.M.; Aber, J.D.; Howarth, R.W.; Likens, G.E.; Matson, P.A.; Schindler, D.W.; Schlesinger, W.H.; Tilman, D. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol. Appl.* **1997**, *7*, 737–750. [[CrossRef](#)]
13. Li, K.H.; Song, W.; Liu, X.J.; Shen, J.L.; Luo, X.S.; Sui, X.Q.; Liu, B.; Hu, Y.K.; Christie, P.; Tian, C.Y. Atmospheric reactive nitrogen concentrations at ten sites with contrasting land use in an arid region of central Asia. *Biogeosciences* **2012**, *9*, 6627–6649. [[CrossRef](#)]
14. Yu, G.; Jia, Y.; He, N.; Zhu, J.; Chen, Z.; Wang, Q.; Piao, S.; Liu, X.; He, H.; Guo, X.; et al. Stabilization of atmospheric nitrogen deposition in China over the past decade. *Nat. Geosci.* **2019**, *12*, 424–429. [[CrossRef](#)]
15. Gurmesa, G.A.; Wang, A.; Li, S.; Peng, S.; de Vries, W.; Gundersen, P.; Ciaia, P.; Phillips, O.; Hobbie, E.A.; Zhu, W.; et al. Retention of deposited ammonium and nitrate and its impact on the global forest carbon sink. *Nat. Commun.* **2022**, *13*, 880. [[CrossRef](#)]
16. Ren, Z.R.; Zhang, Y.Q.; Zhang, Y.H. Nitrogen deposition magnifies the positive response of plant community production to precipitation: Ammonium to nitrate ratio matters. *Environ. Pollut.* **2021**, *276*, 116659. [[CrossRef](#)]
17. Liu, J.; Peng, B.; Xia, Z.; Sun, J.; Gao, D.; Dai, W.; Jiang, P.; Bai, E. Different fates of deposited  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in a temperate forest in northeast China: A  $^{15}\text{N}$  tracer study. *Glob. Change Biol.* **2017**, *23*, 2441–2449. [[CrossRef](#)]
18. Liu, Q.; Qiao, N.; Xu, X.; Xin, X.; Han, J.Y.; Tian, Y.; Ouyang, H.; Kuzyakov, Y. Nitrogen acquisition by plants and microorganisms in a temperate grassland. *Sci. Rep.* **2016**, *6*, 22642. [[CrossRef](#)]
19. Peñuelas, J.; Poulter, B.; Sardans, J.; Ciaia, P.; Van Der Velde, M.; Bopp, L.; Boucher, O.; Godderis, Y.; Hinsinger, P.; Llusia, J.; et al. Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat. Commun.* **2013**, *4*, 2934. [[CrossRef](#)]
20. Aerts, R. Nutrient resorption from senescing leaves of perennials: Are there general patterns? *J. Ecol.* **1996**, *84*, 597–608. [[CrossRef](#)]
21. Killingbeck, K.T. Nutrients in senesced leaves: Keys to the search for potential resorption and resorption proficiency. *Ecology* **1996**, *77*, 1716–1727. [[CrossRef](#)]
22. Lü, X.T.; Reed, S.; Yu, Q.; He, N.P.; Wang, Z.W.; Han, X.G. Convergent responses of nitrogen and phosphorus resorption to nitrogen inputs in a semiarid grassland. *Glob. Change Biol.* **2013**, *19*, 2775–2784. [[CrossRef](#)] [[PubMed](#)]
23. Zhang, J.F.; Li, J.; Fan, Y.X.; Mo, Q.F.; Li, Y.W.; Li, Y.X.; Li, Z.A.; Wang, F.M. Effect of nitrogen and phosphorus addition on litter decomposition and nutrients release in a tropical forest. *Plant Soil* **2020**, *454*, 139–153. [[CrossRef](#)]
24. Li, L.; Li, X.; Liu, B.; Lei, J.; Yue, Z.; Li, C. Imbalanced stoichiometric patterns in foliar nutrient resorption response to N and P addition in grazing alpine grassland. *Acta Oecol.* **2020**, *102*, 103505. [[CrossRef](#)]
25. Kobe, R.K.; Lepczyk, C.A.; Iyer, M. Resorption efficiency decreases with increasing green leaf nutrients in a global data set. *Ecology* **2005**, *86*, 2780–2792. [[CrossRef](#)]
26. Ye, X.; Li, H.; Zhang, L.; Chai, R.; Tu, R.; Gao, H. Amendment damages the function of continuous flooding in decreasing Cd and Pb uptake by rice in acid paddy soil. *Ecotox. Environ. Safe.* **2018**, *147*, 708–714. [[CrossRef](#)]
27. Chen, H.; Reed, S.C.; Lü, X.; Xiao, K.; Wang, K.; Li, D. Coexistence of multiple leaf nutrient resorption strategies in a single ecosystem. *Sci. Total Environ.* **2021**, *772*, 144951. [[CrossRef](#)]
28. He, M.S.; Yan, Z.B.; Cui, X.Q.; Gong, Y.M.; Li, K.H.; Han, W.X. Scaling the leaf nutrient resorption efficiency: Nitrogen vs. phosphorus in global plants. *Sci. Total Environ.* **2020**, *729*, 8. [[CrossRef](#)]
29. Tong, R.; Zhou, B.; Jiang, L.; Ge, X.; Cao, Y. Spatial patterns of leaf carbon, nitrogen, and phosphorus stoichiometry and nutrient resorption in Chinese fir across subtropical China. *Catena* **2021**, *201*, 105221. [[CrossRef](#)]
30. Vergutz, L.; Manzoni, S.; Porporato, A.; Novais, R.F.; Jackson, R.B. Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecol. Monogr.* **2012**, *82*, 205–220. [[CrossRef](#)]
31. Yuan, Z.Y.; Chen, H.Y.H. Global-scale patterns of nutrient resorption associated with latitude, temperature and precipitation. *Glob. Ecol. Biogeogr.* **2009**, *18*, 11–18. [[CrossRef](#)]
32. Shi, B.; Ling, X.; Cui, H.; Song, W.; Gao, Y.; Sun, W. Response of nutrient resorption of *Leymus chinensis* to nitrogen and phosphorus addition in a meadow steppe of northeast China. *Plant Biol.* **2020**, *22*, 1123–1132. [[CrossRef](#)] [[PubMed](#)]
33. Huang, G.; Su, Y.G.; Mu, X.H.; Li, Y. Foliar nutrient resorption responses of three life-form plants to water and nitrogen additions in a temperate desert. *Plant Soil* **2018**, *424*, 479–489. [[CrossRef](#)]
34. Lü, X.T.; Hou, S.L.; Reed, S.; Yin, J.X.; Hu, Y.Y.; Wei, H.W.; Zhang, Z.W.; Yang, G.J.; Liu, Z.Y.; Han, X.G. Nitrogen Enrichment Reduces Nitrogen and Phosphorus Resorption through Changes to Species Resorption and Plant Community Composition. *Ecosystems* **2020**, *24*, 602–612. [[CrossRef](#)]
35. Wang, M.; Murphy, M.T.; Moore, T.R. Nutrient resorption of two evergreen shrubs in response to long-term fertilization in a bog. *Oecologia* **2014**, *174*, 365–377. [[CrossRef](#)]
36. Choi, W.J.; Chang, S.X.; Hao, X.Y. Soil retention, tree uptake, and tree resorption of  $^{15}\text{NH}_4\text{NO}_3$  and  $\text{NH}_4^{15}\text{NO}_3$  applied to trembling and hybrid aspens at planting. *Can. J. For. Res.* **2005**, *35*, 823–831. [[CrossRef](#)]
37. Conant, R.T.; Paustian, K.; Elliott, E.T. Grassland management and conversion into grassland: Effects on soil carbon. *Ecol. Appl.* **2001**, *11*, 343–355. [[CrossRef](#)]
38. Li, S.; Gurmesa, G.A.; Zhu, W.; Gundersen, P.; Zhang, S.; Xi, D.; Huang, S.; Wang, A.; Zhu, F.; Jiang, Y.; et al. Fate of atmospherically deposited  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in two temperate forests in China: Temporal pattern and redistribution. *Ecol. Appl.* **2019**, *29*, e01920. [[CrossRef](#)]

39. Ma, Z.; Gao, X.; Tenuta, M.; Kuang, W.; Gui, D.; Zeng, F. Urea fertigation sources affect nitrous oxide emission from a drip-fertigated cotton field in northwestern China. *Agric. Ecosyst. Environ.* **2018**, *265*, 22–30. [[CrossRef](#)]
40. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*; USDA Circular 939; United States Department of Agriculture: Washington, DC, USA, 1954.
41. Fang, H.J.; Cheng, S.L.; Yu, G.R.; Yang, X.M.; Xu, M.J.; Wang, Y.S.; Li, L.S.; Dang, X.S.; Wang, L.; Li, Y.N. Nitrogen deposition impacts on the amount and stability of soil organic matter in an alpine meadow ecosystem depend on the form and rate of applied nitrogen. *Eur. J. Soil Sci.* **2014**, *65*, 510–519. [[CrossRef](#)]
42. Ratliff, T.J.; Fisk, M.C. Phosphatase activity is related to N availability but not P availability across hardwood forests in the northeastern United States. *Soil Biol. Biochem.* **2016**, *94*, 61–69. [[CrossRef](#)]
43. Marklein, A.R.; Houlton, B.Z. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol.* **2012**, *193*, 696–704. [[CrossRef](#)]
44. DeForest, J.L.; Otuya, R.K. Soil nitrification increases with elevated phosphorus or soil pH in an acidic mixed mesophytic deciduous forest. *Soil Biol. Biochem.* **2020**, *142*, 107716. [[CrossRef](#)]
45. Deng, Q.; Hui, D.; Dennis, S.; Reddy, K.C. Responses of terrestrial ecosystem phosphorus cycling to nitrogen addition: A meta-analysis. *Glob. Ecol. Biogeogr.* **2017**, *26*, 713–728. [[CrossRef](#)]
46. Abbasi, M.K.; Adams, W.A. Gaseous N emission during simultaneous nitrification–denitrification associated with mineral N fertilization to a grassland soil under field conditions. *Soil Biol. Biochem.* **2000**, *32*, 1251–1259. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.