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Seed nutrient is more stable than leaf in response to changing multiple resources in an alpine meadow

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Abstract

Background It has been long thought that nitrogen (N), phosphorus (P) concentrations and their ratios (N:P) in metabolically active or functional organs (i.e., leaves) are less responsive to environmental changes. Little attention, however, has been paid to the reproductive organs—seeds, while seeds may maintain their nutrients more stable for the evolutionary fitness of next generation.

Methods Here, we conducted a field experiment of N, P addition and drought in an alpine meadow, aiming to compare the difference of leaf and seed nutrients and stoichiometric ratios in response to these resource treatments and their interactions. Four dominant species were selected among grass and forb functional groups, including *Elymus nutans*, *Deschampsia caespitosa*, *Artemisia roxburghiana* and *Polygonum viviparum*.

Results Under natural conditions, leaf N and P concentrations were consistently lower than seed among species. However, leaf nutrients were much more sensitive than seed nutrients to N and P addition. Specifically, N or P addition accordingly increased leaf N or P concentration by 22.20–44.24% and 85.54–93.61%, while only enhanced seed N or P concentration by 5.15–17.20% and 15.17–32.72%, respectively. Leaf N or P concentration was significantly reduced by P or N addition, but seed nutrients remained unchanged. In contrast, drought did not change both organ nutrients. Similarly, nutrient addition and drought had synergistic interactions on leaf nutrients, but not on seed nutrients.

Conclusions This study highlights that seed nutrient concentrations could be more stable than metabolically active leaf organ when facing multidimensional resource changes. This complements the traditional view on the 'Stable Leaf Nutrient Hypothesis' with the involvement of reproductive organs. The less responsiveness of seed nutrients suggests the adaptive strategy to ensure the success of next generations and long-term plant demographic stability.

Keywords Alpine meadow, Leaf, Multiple resource change, Nitrogen concentration, N:P ratio, Phosphorus concentrations, Seed

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Introduction

Leaf and seed nutrient concentrations [i.e., nitrogen (N), phosphorus (P)] are essential for plant growth and reproduction (White and Veneklaas 2012). For example, leaf N, P concentrations and N:P ratio could influence plant photosynthesis (Reich et al. 1998; Wright et al. 2004; Wu et al. 2012). Seed nutrients and quality are critical to determine the evolutionary fitness of next generations (Ronnenberg et al. 2011; Tungate et al. 2002; Yang et al. 2021). Based on the different functions of these two organs, their nutrients may respond differently to changing soil resources (Gargallo-Garriga et al. 2014; Valladares et al. 2000). However, most research involved has focused on the response patterns and impacting factors of leaf nutrients along natural environmental gradients or in global change experiments (Han et al. 2005; Reich and Oleksyn 2004; Tian et al. 2018). Less attention has been paid to seeds—the reproductive organ, and it remains poorly understood how leaf versus seed nutrients and stoichiometry respond differentially to changing environments.

Soil N and P availability are critical for plant growth and reproduction. Leaf, as a metabolically active organ, requires enough nutrients and balanced stoichiometric ratio to sustain plant photosynthesis and growth (Kattge et al. 2009; Wright et al. 2004). This priority of nutrient investment in leaf may be at the expense of the nutrient pools from stem and root (Tang et al. 2018; Schreeg et al. 2014). Based on these, the proposed 'Stable Leaf Nutrient Hypothesis' states that leaf nutrients are less responsive than other organs under changing environments (Tang et al. 2018). However, this hypothesis focuses on nutrient response in vegetative organs, while ignores plant reproductive organ (i.e., seed). Generally, seed is closely related to the quality of plant next generation and evolutionary fitness, thus influencing long-term plant demographic stability (Luo et al. 2022). This most likely enables plants to adopt a conservative strategy in facing soil nutrient changes (Fernandez-Martinez et al. 2019), with seed nutrients more stable than vegetative organs, a hypothesis that has yet been tested.

Drought may also affect leaf and seed nutrients differently (Gao et al. 2011; Harpole et al. 2007). In dry regions, drought typically intensifies water limitation and reduces soil nutrient availability, resulting in decreased plant N and P uptake (Xiong et al. 2022). Though some previous studies reveal the negative effect of drought on leaf nutrients (Cramer et al. 2009; He and Dijkstra 2014; Sardans et al. 2012), less is known about seed nutrient responses. When faced with drought stress, plants normally invest resources in seed to avoid or escape from unfavorable environments. Thus, drought may have a minor effect on seed nutrients (Fernandez-Martinez et al. 2019; Tegeder and Masclaux-Daubresse 2018). In wet regions, however, drought causes less water limitation but reduces soil nutrient leaching (Knapp et al. 2008), which indirectly enhances leaf and seed nutrient concentrations (Olde Venterink et al. 2001; Stirling et al. 2020).

Soil N, P and water are often coupled and changing simultaneously, likely leading to complex interactions on leaf and seed nutrients (Brookshire and Weaver 2015). For example, N and P enrichment generally have a synergistic interaction on plant nutrients (Elser et al. 2007; Peng et al. 2019). Drought may reduce the effects of soil nutrient availability on plant nutrient uptake via restricting the solubility and mobility of soil ions in dry grasslands (Zhou et al. 2020). In wet grasslands, however, drought mainly reduces soil nutrient leaching, which may indirectly enhance the impacts of nutrient enrichment on plant nutrient uptake (Knapp et al. 2008). In the face of these complex interactions, seeds may prefer a conservative nutrient strategy to ensure the quality of offspring (Nadeem et al. 2011). As a result, seed nutrients may be less responsive to the interactions of multiple soil resource than leaf nutrients.

The Qinghai-Tibet Plateau is known as the third pole of the world, characterized by high altitude and low temperature. In this region, alpine grasslands are greatly limited by soil N and P, due to the slow nutrient cycle by temperature limitation (Elser et al. 2007; Zhang et al. 2018; Zhou et al. 2021). Moreover, greater climate warming rates in this area cause more frequent drought events recently (IPCC 2013). These resource changes may have complex interactions on alpine plant nutrients and growth (Lower and Orians 2003). Thus, we conducted an experiment of drought, N and P addition in a wet alpine meadow, aiming to compare the difference between leaf and seed nutrient responses. Specifically, we hypothesize that: (i) N or P addition increases leaf nutrients more than seed nutrients; (ii) drought tends to enhance plant nutrients, but with a less extent in seed; (iii) multiple resource changes have synergistic interactions on leaf nutrients, but weaker interactions on seed nutrients.

Materials and methods

Experiment site

This study was conducted in an alpine meadow located within the Sichuan Zoige Alpine Wetland Ecosystem National Observation and Research Station. The study site is at the eastern Qinghai-Tibet Plateau (32° 48′ N, 102° 33′ E, 3500 m a.s.l.), which belongs to the Hongyuan County, Sichuan Province of China. The long-term (1961–2017) mean annual precipitation (MAP) and mean annual temperature (MAT) are 750 mm and 1.5 °C, respectively. The dominant species are *Elymus nutans* and *Deschampsia caespitosa* (grasses), *Carex*

enervis (sedge) and *Artemisia roxburghiana* and *Polygonum viviparum* (forbs).

Experimental design

This experiment was established in 2017. Following the randomized block design, seven treatments with four replications were included to test our hypotheses, such as control treatment (CK), N addition (+N), P addition (+P), drought (D), N+P, N+D and N+P+D. In total, there were 28 plots (4 m × 4 m) with the distance between plots being 2 m. Consistent with our joined Nutrient Network (Borer et al. 2014), we also applied the same level (10 g m^{-2} yr^{-1}) of N and P addition in early May of each year since 2018. The N and P fertilizers were slow-released urea (CH_4N_2O with a pure N content of 46.6%) and double superphosphate ($Ca(H_2PO_4)_2{\cdot}H_2O$ with a P_2O_5 content of 12%), respectively. In accordance with the Drought Network (Smith 2015), our drought treatment was achieved by removing 50% of ambient precipitation, using passive rainout shelters (6-m length × 6-m width × 3-m height).

Plant sampling and measurement

In the August of 2020, we randomly collected mature green leaves and seeds of four species, including two dominant grass species (*Elymus nutans* and *Deschampsia caespitosa*), and two dominant forb species (*Artemisia roxburghiana* and *Polygonum viviparum*). We chose these species mainly based on the following reasons: species dominance in terms of relative biomass; covering different functional groups (grass and forb); and enough seed production used for the measurement of nutrient concentration. In the lab, all samples were oven-dried at 65 °C for 48 h. Subsequently, we ground dry samples using a ball mill (MM400 ball mill, Retsch, Germany) for chemical determination. The N and P concentrations in plant samples were measured by an elemental analyzer (Vario macrocube elemental analyzer, Elementar, Germany) and inductively coupled plasma-optical emission spectrometry (iCAP 6300 ICP-OES Spectrometer, Thermo Scientific, USA) after microwave digestion by HNO_3, respectively.

Soil samples were collected randomly at 0–10 cm depth in each plot and then sieved through a 2-mm sieve. Then, we divided the samples into two parts. One part was used to measure soil-available N with an automatic continuous flow analyzer (AA3, SEAL Analytical GmbH, Germany). The other part was air-dried to measure soil-available P by $NaHCO_3$ maceration-molybdenum antimony spectrophotometer (Murphy and Riley 1962).

Statistical analysis

Before analysis, we calculated the individual treatment effects on leaf and seed nutrient and their ratio as relative changes: (Treatment − CK)/CK × 100%. The interactions of treatments were calculated as follows:

$$\text{Interactive effects} = \left(T_{\text{mul}} - \sum_{i=1}^{n} T_i\right) \Big/ \sum_{i=1}^{n} T_i \times 100\%,$$

where T_i and T_{mul} represent plant nutrient concentrations in the ith individual and combined treatment, respectively. n is the count of interacting factors. For example, when T_{mul} represents a three-factor treatment of N, P addition and drought, the sum of T_i is the expected additive effects among these individual treatments.

First, we conducted three-way ANOVA to examine the main effects and interactions of N addition, P addition and drought treatment on leaf and seed nutrient concentrations and their ratios. Secondly, independent t-test was used to test whether the changes in plant nutrients caused by individual or combined treatments differ from zero. Thirdly, linear regression was employed to analyze the relationships of plant nutrient concentrations and their ratios with soil nutrient availability across all the treatments. Specifically, we used a standard major axis (SMA) test to compare the difference between the slopes of leaf and seed nutrient with soil nutrient availability, by using the "smart" package in R software (Warton et al. 2006). All statistical analyses were performed by the SPSS 21.0, OriginPro 2021, and R 4.0.2., and the figures were made by the OriginPro 2021.

Results

Different responses of leaf and seed nutrient to individual treatments

Under ambient condition, leaf N and P concentrations were significantly lower than seed among four species (Fig. 1a, b). However, leaf nutrients were greater stimulated than seed by N or P addition (Fig. 2). Specifically, N addition significantly enhanced leaf N by 37.99%, 44.24%, 28.35% and 22.20% for *E. nutans*, *D. caespitosa*, *A. roxburghiana* and *P. viviparum*, respectively. However, seed N concentration was only stimulated by 14.39%, 14.68%, 5.15% and 17.20% for *E. nutans*, *D. caespitosa*, *A. roxburghiana* and *P. viviparum*, respectively (Fig. 2a–d). Similarly, P addition, respectively, enhanced leaf P concentration by 85.54%, 87.63%, 89.12% and 93.61%, while increased seed P 15.17%, 23.78%, 32.72%, and 26.23% for *E. nutans*, *D. caespitosa*, *A. roxburghiana* and *P. viviparum*, respectively (Fig. 2e–h). Accordingly, we found that N:P ratio of these species increased with N addition but

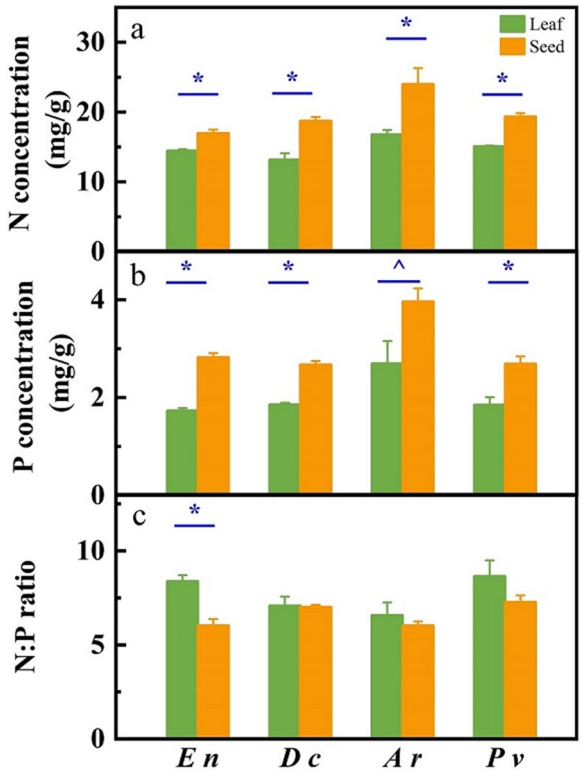

Fig. 1 N and P concentrations and N:P ratio in leaf versus seed of four alpine dominant species in ambient condition. *En*, *Dc*, *Ar*, *Pv* represent *Elymus nutans*, *Deschampsia caespitosa*, *Artemisia roxburghiana*, and *Polygonum viviparum*, respectively. * and ^, respectively, indicate a significant difference at $P < 0.05$ and $P < 0.1$ between leaf and seed

decreased with P addition, with greater leaf responsive than seed (Fig. 2i–l).

Furthermore, N addition significantly reduced leaf P concentration among four species, but did not decrease seed P concentration (Fig. 2e–h). Similarly, leaf N concentration of *A. roxburghiana* was decreased by P addition, but unaffected for seed N concentration (Fig. 2a–d).

Drought had no effect on leaf and seed nutrients and N:P ratio, except for leaf P concentration in *P. viviparum* (Fig. 2). Furthermore, we found no relationship of leaf and seed nutrients with soil water (Additional file 1: Fig. S1).

Different responses of leaf and seed nutrients to the interactions of multiple resources treatments

N and P addition had no interaction on leaf and seed N concentration (Fig. 3a–h). However, these two treatments showed a synergistic effect on leaf P concentration, not for seed P concentration (Fig. 3a–h). Accordingly, N and P addition exerted an antagonistic interaction on leaf N:P ratio (Fig. 3i–l). For the combination of N addition and drought, there was an antagonistic effect on seed N and P concentrations in *E. nutans*, and on seed P concentration in *P. viviparum* (Fig. 3a, e, h). Differently, leaf N concentration was synergistically stimulated by combined N addition and drought (Fig. 3a–h).

For the higher-order interactions, drought, N and P addition synergistically increased leaf N and P concentrations (except for *A. roxburghiana*) (Fig. 3a–h). Based on a greater increase of leaf P than leaf N concentration, leaf N:P ratio was antagonistically reduced by this combined treatment (Fig. 3i–l). However, less interaction was found on seed nutrients and their ratios under this three-factor treatment (Fig. 3).

Relationships of leaf versus seed nutrients with soil nutrient availability

Our results showed that N and P addition significantly increased soil N and P availability, respectively. Moreover, leaf and seed nutrients and their ratios showed positive and linear relationships with soil-available nutrients and their ratios, respectively (Fig. 4). However, the sensitivity (slopes) of leaf nutrients was significantly larger than seed nutrients in response to soil nutrient availability (Fig. 4).

Discussion

By comparing the difference in leaf versus seed nutrient responses to experimental multiple resource changes (N, P addition and drought), we found that seed nutrients were more stable than leaf nutrients in response to changing environments. The combinations of two or three soil resource factors had synergistic interactions on leaf nutrients, but not on seed nutrients. To our best knowledge, this study is the first to directly compare the difference of leaf versus seed nutrient responses to manipulated multiple resources. These new findings complement the 'Stable Leaf Nutrient Hypothesis' with the involvement of reproductive organ response, beyond vegetative organ nutrient responses to changing environments (Tang et al. 2018). Overall, the more stable nutrient response of seed than leaf suggests the conservative reproductive strategy for evolutionary fitness (Tungate et al. 2002; Nadeem et al. 2011), highlighting the importance of including plant reproductive organ in future stoichiometric research.

Effects of individual treatments on leaf and seed nutrients

In line with our first hypothesis, we found that seed nutrients were more stable than leaf in response to N or P addition. Moreover, the linear slopes of leaf nutrients against soil nutrient were larger than seed nutrients, again supporting this result. However, our result

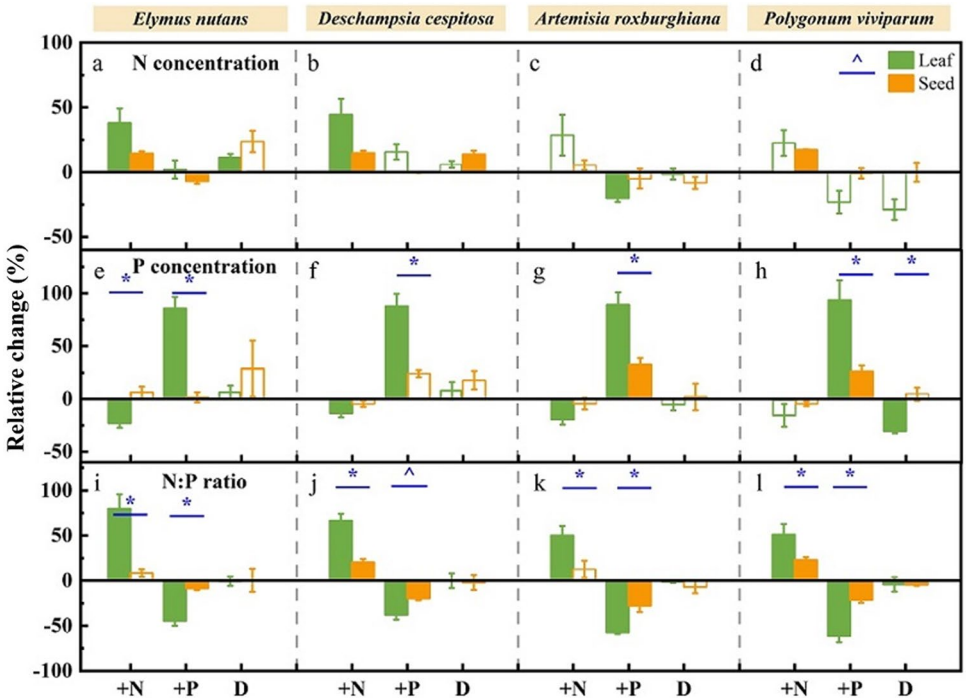

Fig. 2 Relative changes of N, P concentrations and N:P ratio in leaf versus seed of four species (*Elymus nutans*, *Deschampsia caespitosa*, *Artemisia roxburghiana*, and *Polygonum viviparum*) caused by individual treatments. Solid columns indicate a significant response to treatment. Hollow columns indicate no significant response. * and ^, respectively, indicate a significant difference at $P < 0.05$ and $P < 0.1$ between leaf and seed

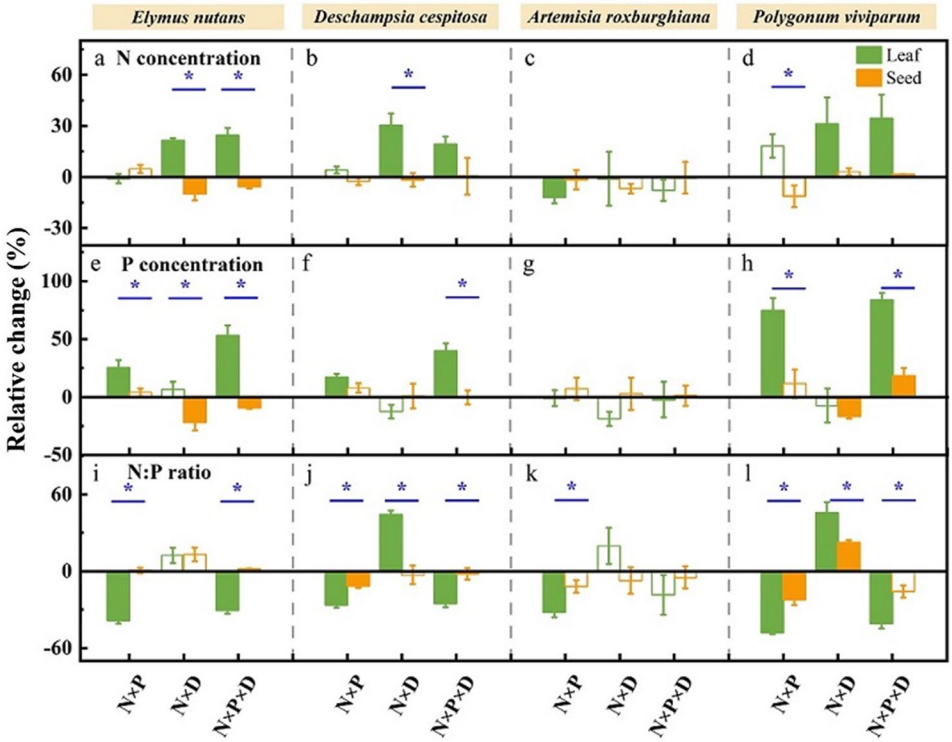

Fig. 3 Relative changes of N, P concentrations and N:P ratio in leaf versus seed of four species (*Elymus nutans*, *Deschampsia caespitosa*, *Artemisia roxburghiana*, and *Polygonum viviparum*) by treatment interactions. Solid columns indicate a significant response to treatment interactions. Hollow columns indicate no significant response. * and ^, respectively, indicate a significant difference at $P < 0.05$ and $P < 0.1$ between leaf and seed

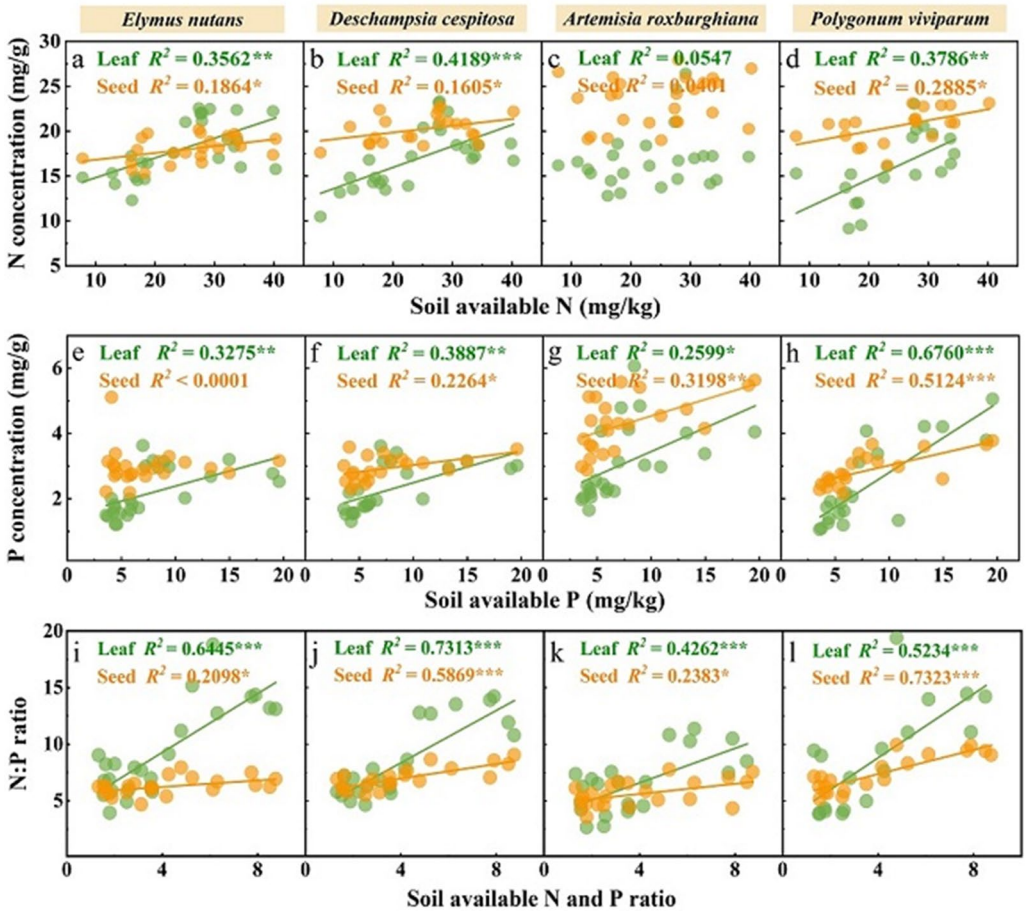

Fig. 4 Relationships of leaf versus seed N concentration with soil-available N in four species (**a**–**d**); those of leaf versus seed P concentration with soil-available P (**e**–**h**); and those of leaf versus seed N:P ratio with soil-available N:P (**i**–**l**). ***, ** and *, respectively, indicate significance levels at $P < 0.001$, $P < 0.01$, and $P < 0.05$

differs from the 'Stable Leaf Nutrient Hypothesis' stating that leaf nutrients are less responsive among vegetative organs to changing environments. This is based on the fact that leaf is the actively metabolic organ that sustains photosynthesis (Tang et al. 2018; Schreeg et al. 2014), which requires the priority of nutrient investment in leaf to ensure the basic provision of energy and material (Luo et al. 2021; Tang et al. 2018; Xiong et al. 2022). However, seed nutrients are closely related to the quality of next generations and evolutionary fitness (Sinclair and Park 1993; Fernandez-Martinez et al. 2019). Thus, these suggest that plants develop a more conservative strategy of seed than leaf nutrient to maintain long-term plant fitness.

Furthermore, we found the reduction in leaf P concentration by N addition, but not in seed P. The decreased leaf P is consistent with previous studies, which show the negative effects of N addition on leaf P concentration in N-limited ecosystems (You et al. 2018). This phenomenon might be explained by the increased plant biomass diluting leaf P concentration and intensified plant P limitation under N enrichment (Gusewell 2002; Kemp et al. 1994; Sardans et al. 2016; Liu et al. 2021). However, no reduction in seed P concentration could facilitate the quality of next generations, despite intensified P limitation under N enrichment (Gutterman 2000).

Different with our second hypothesis, we found no effect of drought on leaf and seed nutrients. This likely suggests a weak soil nutrient leaching, despite high precipitation in our studied grassland (750 mm). It may be because our experiment site has a unique climate of combined low temperature (3500 m a.s.l.) and high precipitation. Low temperature tends to suppress microbial activities and soil nutrient mineralization, further reducing soil nutrient availability (Li et al. 2017). Moreover, high precipitation in the studied area has long washed soil nutrient away from topsoil, finally leading to strong soil nutrient limitation (Song et al. 2023). This likely causes the intense soil nutrient competition between

plant roots and microbes (Sun and Wang 2016). For example, this alpine grassland has a greater root biomass allocation than many other types of grassland (Yang et al. 2010). These together result in weak soil nutrient leaching in this alpine grassland.

Interactions of multiple soil resources on leaf versus seed nutrients

We revealed the synergistic interactions of multiple soil resources on leaf nutrient concentrations, but not on seed nutrient concentrations. This result supports our third hypothesis, which is partly supported by a meta-analysis showing that the combined N and P addition increases leaf P more than single N or P addition (Jiang et al. 2019). Moreover, despite the weak soil nutrient leaching in the natural condition of our studied alpine grassland, the N leaching became much stronger under N enrichment with increasing soil water. Thus, drought could significantly reduce soil nutrient leaching, indirectly enhancing the effects of N and P addition on plant nutrients. The likely mechanism for the synergistic interactions of N, P addition and drought is that this combined treatment further lessens the co-limitation of plant nutrient uptake (Xu et al. 2015; Zhang et al. 2020). However, seed nutrients had less interactions than leaf nutrients under multiple soil resource changes, which suggests that seed tends to develop a conservative strategy of reducing its nutrient variations and further ensure plant evolutionary fitness (Tungate et al. 2002; Nadeem et al. 2011). Therefore, all these raise our attention to considering the role of reproductive organ nutrients in affecting ecosystem nutrient and stoichiometric coupling, especially in the context of multiple resource changes.

Conclusions

As opposed to previous studies focusing on plant vegetative organ nutrients, we compared the difference in seed versus leaf nutrient responses using an experiment of drought, N and P addition in an alpine meadow. Our results demonstrated that seed nutrient concentrations were more stable than leaf in response to individual soil resource changes. Moreover, seed nutrients were less responsive than leaf to the interactions of multiple soil resources. These new findings suggest that plants tend to develop a more conservative strategy of seed than leaf nutrients for evolutionary fitness no matter nutrient-rich or nutrient-poor condition. Overall, this study provides new insights into the role of seed nutrients in regulating ecosystem nutrient and stoichiometric coupling relationships in an evolutionary view. These findings also have important implications for our understanding of future grassland community regeneration in face of multidimensional resource changes.

Abbreviations

N	Nitrogen
P	Phosphorus
MAP	Mean annual precipitation
MAT	Mean annual temperature
CK	Control
+N	Nitrogen addition
+P	Phosphorus addition
D	Drought
N × P	Nitrogen and phosphorus interaction
N × D	Nitrogen and drought interaction
N × P × D	Nitrogen, phosphorus and drought interaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-023-00454-9.

Additional file 1: Figure S1. Relationships of leaf versus seed N concentration with soil moisture in four species (a–d); those of leaf versus seed P concentration with soil moisture (e–h); and those of leaf versus seed N:P ratio with soil moisture (i–l). ^Indicate significance levels at $P < 0.1$.

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Author contributions

DT and SN conceived the study. JL, DT and QZ conducted the experiment and wrote the manuscript draft. All authors contributed to the revision of manuscript and approved the final version of manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed and approved the manuscript for publication in *Ecological Processes*.

Competing interests

The authors declare no competing financial interests.

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