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# Effects of nitrogen addition and drought on the relationship between nitrogen- and water-use efficiency in a temperate grassland

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## Abstract

**Background** Nitrogen- and water-use efficiency (NUE and WUE) reflect the capacity of plants to take up and utilize resources in the environments. Although N deposition and drought are known to affect plant growth and persistence, it remains elusive how plants adjust NUE, WUE and their relationship to adapt to the concurrent N deposition and drought under the context of global change. We conducted a field experiment in a temperate grassland in Inner Mongolia, in which N addition ( $10 \text{ g m}^{-2} \text{ yr}^{-1}$ ), drought (reduced 66% precipitation during growing season) and their combination were manipulated, and we explored the responses of both instantaneous ( $\text{NUE}_{\text{INST}}$  and  $\text{WUE}_{\text{INST}}$ ) and long-term ( $\text{NUE}_{\text{LT}}$  and  $\text{WUE}_{\text{LT}}$ ) WUE and NUE for six common plant species (*Leymus chinensis*, *Stipa baicalensis*, *Thermopsis lanceolata*, *Potentilla bifurca*, *Thalictrum squarrosus*, *Klasea centauroide*).

**Results** Across all the six species,  $\text{NUE}_{\text{INST}}$  decreased and  $\text{WUE}_{\text{INST}}$  increased with both N addition and drought;  $\text{NUE}_{\text{LT}}$  decreased with both N addition and drought, while  $\text{WUE}_{\text{LT}}$  decreased with N addition and increased with drought. A significant interactive effect between N addition and drought was detected, in that, drought weakened the negative effect of N addition on  $\text{WUE}_{\text{LT}}$  and  $\text{NUE}_{\text{LT}}$ , and N addition weakened the negative effect of drought on  $\text{NUE}_{\text{LT}}$ , but enhanced the positive effect of drought on  $\text{WUE}_{\text{LT}}$ . As a consequence, drought caused a trade-off between NUE and WUE, and N addition brought forth positive correlation between NUE and WUE. Meanwhile, the relationship between NUE and WUE varied among the species of different functional groups.

**Conclusions** Our study demonstrated that NUE, WUE and their relationship play an important role in determining their responses and adaptability to changes in soil nitrogen and water availability induced by N deposition and drought. Our results shed light on the antagonistic, synergetic and neutral effects between nitrogen deposition and drought on resource use efficiency, and further clarified the different adaptability among species of different functional groups, which will conduce to a comprehensive understanding on the role of NUE and WUE in plant persistence and coexistence in grassland ecosystems.

**Keywords** Functional group, Global change, Leaf carbon to nitrogen ratio, Nitrogen water coupling, Photosynthesis, Semi-arid grassland, Stable isotope

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## Introduction

Resource use efficiency (RUE: carbon assimilation per unit of resource) reflects the capacity of plants to take up and utilize resources in the environments, which is closely related to plants' surviving strategy, environmental adaptability and competitiveness (Liu et al. 2019; Hodapp et al. 2019). Plant growth is co-limited by nitrogen (N) and water availability in arid and semi-arid temperate grasslands (Lü et al. 2014, 2018), different species can exhibit different nitrogen use efficiency (NUE) and/or water use efficiency (WUE) to regulate interspecific resource competition, and therefore, NUE and WUE are expected to play primary roles in modulating plant persistence and coexistence in these ecosystems (Funk and Vitousek 2007; Luo et al. 2018a; Perez-Ramos et al. 2019; Godoy et al. 2020). For example, previous studies have found the positive correlation between species' abundances and WUE under water stress (Fowler 1986; Tsialtas et al. 2001; Gong et al. 2011). Tsialtas et al. (2001) found that in a water-limited upland grassland, *Poa pratensis*, a grass specie, had the highest abundance and had the highest WUE, while *Plantago lanceolata*, a non-leguminous forb specie, had both low abundance and low WUE, suggesting that higher WUE can render plants competitive superiority in water-limiting environment. Over the past few decades, researches on NUE and WUE have received increasing attention (Li et al. 2003; Chen et al. 2012; Yue et al. 2019; Habbib et al. 2020; Sun et al. 2020; Guo et al. 2022). Despite that, NUE and WUE together, rather than a single resource use efficiency, determine the population persistence, species interactions and biodiversity–productivity relationship in N and water co-limited grasslands. Moreover, NUE–WUE relationship is largely understudied (Sinclair and Rufty. 2012; Li et al. 2019; Luong et al. 2021) and remains elusive.

It is pervasively believed that plants cannot maximize both NUE and WUE, i.e., there is a trade-off between them (DeLucia and Schlesinger 1991; Gong et al. 2011). For example, Chen et al. (2005) and Gong et al. (2011) found that under nitrogen addition, *Leymus chinensis* would improve WUE (i.e., stable carbon isotope,  $\delta^{13}\text{C}$ ) at the cost of reducing NUE (i.e., carbon to nitrogen ratio, C/N), thereby achieving a balance between carbon gain in photosynthesis and water loss in transpiration. In this case, the trade-off between NUE and WUE may help plants to optimize the use of environmental nitrogen and water resources. However, as a result of variation in resource supply status or in resource uptake capability of species, WUE was thought to be unnecessary to always trade off with NUE (Wang et al. 2017a, b; Zhou et al. 2016). Instead, the change of WUE may also be independent or positively correlated with that of NUE. For example, high N supply could increase leaf nitrogen

content (LNC) and N/P, and strengthen phosphorus limitation which would decrease photosynthetic capacity, and consequently decoupled the trade-off between NUE and WUE (Huang et al. 2016). Therefore, exploring the NUE–WUE relationship in different functional groups can help to explain their response and adaptation to the environment, as well as to explain community dynamics.

Nitrogen deposition and drought frequency are increasing due to climate change and anthropogenic activities, and will undoubtedly affect plant NUE, WUE and their relationship through altering soil N and water availability, as well as plant physiological and ecological processes (Bobbink et al. 2010; Zhang et al. 2014). Herbaceous plants have a faster turnover rate, so grassland ecosystems are more responsive and sensitive to nitrogen deposition and drought (Bai et al. 2010; Heimann and Reichstein 2008; Fuchslueger et al. 2016). On one hand, the increased N deposition will reduce NUE by increasing leaf nitrogen content and promoting plant photosynthesis, and enhance WUE by increasing SLA and photosynthesis (Neff et al. 2002; Chen et al. 2004; Gong et al. 2011; Ning et al. 2016; Peng et al. 2016; Midolo et al. 2019; Diao et al. 2021). On the other hand, drought will reduce transpiration and SLA to improve WUE, and impede nutrient transport to decrease NUE (Swap et al. 2004; Zhang et al. 2017; Wellstein et al. 2017; Sanchowala et al. 2001; Yuan and Li 2007). In sum, with the increase of N deposition or drought, WUE will be enhanced, while NUE will be repressed, showing an apparent trade-off relationship. Furthermore, multiple global change factors might produce the neutral, antagonistic or synergistic interaction effects on plant NUE and WUE (Collins et al. 2017; Meng et al. 2021). Therefore, how plants adjust NUE, WUE and their relationship to adapt to the concurrent change of soil N and precipitation due to global changes remains controversial and thus needs further researches.

RUEs can be categorized into instantaneous and long-term resource use efficiency. The former is measured at minute/second time scale, determined by plant physiological process and influenced by the immediate external environmental change (Field et al. 1983; Li et al. 2009a); the latter is a widely used indicator, assessed usually at growing-season time scale, determined by the long-term accumulation of morphological, physiological and metabolic processes in plants (Zhan et al. 2012). Therefore, we conducted a field manipulative experiment in a temperate grassland in Inner Mongolia, in which N addition, drought and their combination were implemented, with the purpose of investigating the responses of NUE, WUE and their relationship to the simulated global change factors. We hypothesized that (1) both nitrogen addition and drought could increase WUE but decrease NUE, and their interaction is synergistic; (2) NUE and WUE may

not only be a trade-off relationship, and it depends on the N and/or water availability and differs among species of different functional groups (grass, leguminous forb and non-leguminous forb).

## Materials and methods

### Study area

The study was carried out at Erguna Forest-Steppe Ecotone Research Station, Institute of Applied Ecology, Chinese Academy of Sciences (50.16° N, 119.39° E, 530 m a.s.l.), which is located in Hulunbier, Inner Mongolia (Additional file 1: Fig. S1a). The grassland in the region is characterized by a semiarid temperate continental climate. The vegetation type of the research site belongs to the meadow grassland, which is mainly composed of the perennial rhizomatous grass *L. chinensis* and bunch grass *Stipa baicalensis*. It is an important component of the grassland ecosystem in northern China. As it is located in the Forest-Steppe transition zone and the eastern Eurasian steppe region, it is more sensitive and responsive to climate change. The long-term (1970–2020) mean annual temperature and precipitation in this area were  $-2.4^{\circ}\text{C}$  and 353 mm, respectively, with most of the rainfall occurring from May to August. The soil type is chernozem and the pH of the top layer (0–10 cm) is 6.8 to 7.0. The contents of total nitrogen, total phosphorus and organic carbon are  $2.4\text{ g kg}^{-1}$ ,  $0.7\text{ g kg}^{-1}$  and  $25.0\text{ g kg}^{-1}$  (Lü et al. 2021), and the contents of bulk density, saturated moisture content, sand and clay + silt are  $1.13\text{ g cm}^{-3}$ , 47.21%, 49.43% and 50.56%, respectively (Ma et al. 2022).

### Experimental design

The experiment was set up in 2018 following randomized block design. In total, 24 plots were set up within a topographically flat area and were divided into six blocks, then four treatments including control (C), N addition (N), drought (D) and the combination of N addition and drought (ND) were randomly assigned to the four plots within each block (Additional file 1: Fig. S1b). Thus, each treatment had six replicates. Each experimental plot was  $6\text{ m} \times 6\text{ m}$  in size, and every two adjacent plots were separated by 2 m. Nitrogen addition was performed in mid-May by dissolving 1028.5 g of  $\text{NH}_4\text{NO}_3$  in 25 kg of pure water and applying it evenly to each N and ND plot with a sprayer, while in C and D plots, equal amount purified water was sprayed to eliminate the difference due to the unwanted water added to N and ND plots. According to the design method of Yahdjian and Sala (2002), the drought was simulated by intercepting 66% of rainfall amount from May to August with rainout shelters, which shield 2/3 vertical projected area of the shelter roof by evenly arrayed high light-transmittance polycarbonate strips (Additional file 1: Fig. S1c). Due to the high

visible light transmittance of polypropylene plastic plate, 90% photosynthetically active radiation is allowed to pass through, and its effect on solar effective radiation is negligible. The shelter roofs were supported by arch stainless-steel frames up to 2.5 m and 1 m above the ground at the highest point and lowest point, respectively, which make sure the air circulation was unhindered, with minimizing unnecessary greenhouse effect. To prevent the external subsurface water permeation into the drought plots, we hydrologically isolated the plots by trenching to a depth of 1 m and lining the trench with watertight aluminum plates and thick plastic around the plot.

### Measuring and monitoring

At the peak of the growing season in the third year (2020), since the experiment platform was established, one  $1 \times 1\text{ m}$  quadrat was set up in each plot and was divided into four sub-quadrats ( $0.5 \times 0.5\text{ m}$ ). All aboveground live plant materials were harvested and sorted into species in two diagonal sub-quadrats. The harvested plant materials of the same species from the two sub-quadrats were combined, then oven-dried and weighed to determine the species-level biomass.

We selected species based on the fact that each selected species should occur in the community in such frequency that can be found in all the experimental treatments and at least three repeated blocks for each treatment. In addition, main functional groups such as grass, leguminous forb and non-leguminous forb should be included, to find out whether and to what extent they respond differently to experimental treatments. These species included a perennial rhizomatous grass (*L. chinensis*), a perennial bunch grass (*S. baicalensis*), a leguminous forb (*Thermopsis lanceolata*, *T. lanceolata*), and three non-leguminous forbs (*Potentilla bifurca*, *P. bifurca*; *Thalictrum squarrosom*, *T. squarrosom*; *Klasea centauroide*, *K. centauroide*), whose cumulated relative biomass reached around 80% of total community biomass (Additional file 1: Table S1).

In mid-August 2020, the measurements of net photosynthetic rate ( $P_n$ ), transpiration rate ( $T_r$ ), stomatal conductance ( $G_s$ ) and intercellular carbon dioxide concentration ( $C_i$ ) were taken from 8:30 to 11:30 on cloudless and sunny days, using an LI-6400XT portable photosynthesis system (Li-COR Biosciences, Inc., Lincoln, Nebraska, USA). The Li-6400-02B red and blue artificial light source was put in the leaf chamber, with a  $\text{CO}_2$  concentration of  $400\text{ }\mu\text{mol mol}^{-1}$  (atmospheric  $\text{CO}_2$  concentration), a relative air humidity of 50–70%, a flow rate setting of  $500\text{ }\mu\text{mol s}^{-1}$  and a photosynthetically active radiation (PAR) gradient of  $1500\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$  (full light induction). The measurements were conducted on three fully expanded, healthy and mature leaves from 3 mature and disease-free individuals for each target

species per plot. According to the standard measurement method of Pérez-Harguindeguy et al. (2013), each plant individual in whole was cut from the ground surface, wrapped in wet filter papers and packed in a self-sealing bag, then put them in an incubator and bring back to the lab (Zhou et al. 2020). After saturated water absorption for 6–12 h, the target individual was removed from the self-sealing bag and its target leaf was separated, then the dry filter paper was used to wipe the surface moisture of the leaf, and Cano Scan LiDE 300 and ImageJ were used to estimate the photosynthetic leaf area and correct the gas exchange parameters. Afterward, the leaves were placed in the oven (75 °C) and then crushed using a ball mill (Retsch MM 400, Retsch, Haan, Germany). One part of the leaf sample was used to determine the leaf carbon (LCC, %) and nitrogen content (LNC, %) using an elemental analyzer (Vario Micro Cube, Langensfeld, Germany), and the other part was for measuring  $\delta^{13}\text{C}$  (‰), using stable isotope mass spectrometer (Finnigan MAT 253, Bremen, Germany) with a measurement accuracy of < 0.2‰.

$\text{NUE}_{\text{INST}}$  was estimated as the ratio of  $P_n$  to LNC, and  $\text{WUE}_{\text{INST}}$  was estimated as the ratio of  $P_n$  to  $T_r$  (Field et al. 1983; Zhan et al. 2012). Both of them take the time scale of minute/second as the measurement unit, which are susceptible to the direct impact of external environmental changes (Field et al. 1983; Li et al. 2009a).  $\text{NUE}_{\text{LT}}$  was characterized by the ratio of LCC to LNC (Chen et al. 2005; Diao et al. 2021), and  $\text{WUE}_{\text{LT}}$  was calculated as follows (Tian et al. 2021):

$$\delta^{13}\text{C}_p = \left( \frac{R_{\text{sam}}}{R_{\text{std}}} - 1 \right) \times 1000\text{‰}, \quad (1)$$

where  $R_{\text{sam}}$  is the relative abundance of  $^{13}\text{C}/^{12}\text{C}$  in plant sample;  $R_{\text{std}}$  is the relative abundance of  $^{13}\text{C}/^{12}\text{C}$  in the international standard substance VPDB (Vienna-Pee Dee Belemnite):

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_a - \delta^{13}\text{C}_p}{1 - \delta^{13}\text{C}_p/1000}, \quad (2)$$

$$\text{WUE}_{\text{LT}} = \frac{C_a(b - \Delta^{13}\text{C})}{1.6(b - a)}, \quad (3)$$

where  $\Delta^{13}\text{C}$  is the plant carbon isotope discrimination rate;  $\delta^{13}\text{C}_a$  is the carbon isotope ratio of atmospheric  $\text{CO}_2$  and is calculated as  $-9.04\text{‰}$ ;  $\delta^{13}\text{C}_p$  is the carbon isotope ratio of plant leaves;  $C_a$  is the atmospheric  $\text{CO}_2$  concentration and is calculated as  $387.91 \mu\text{mol mol}^{-1}$ ;  $a$  is the fractionation coefficient during  $\text{CO}_2$  diffusion with a value of  $4.4\text{‰}$ ;  $b$  is the fractionation coefficient during the carboxylation reaction with a value of  $27\text{‰}$ ; 1.6

is the ratio of stomatal conductivity to water vapor and  $\text{CO}_2$ .  $\text{NUE}_{\text{LT}}$  and  $\text{WUE}_{\text{LT}}$  are the widely used indicators, usually evaluated based on the growth season and determined by the long-term accumulation of plant form, physiology and metabolic process (Lu et al. 2007; Zhan et al. 2012).

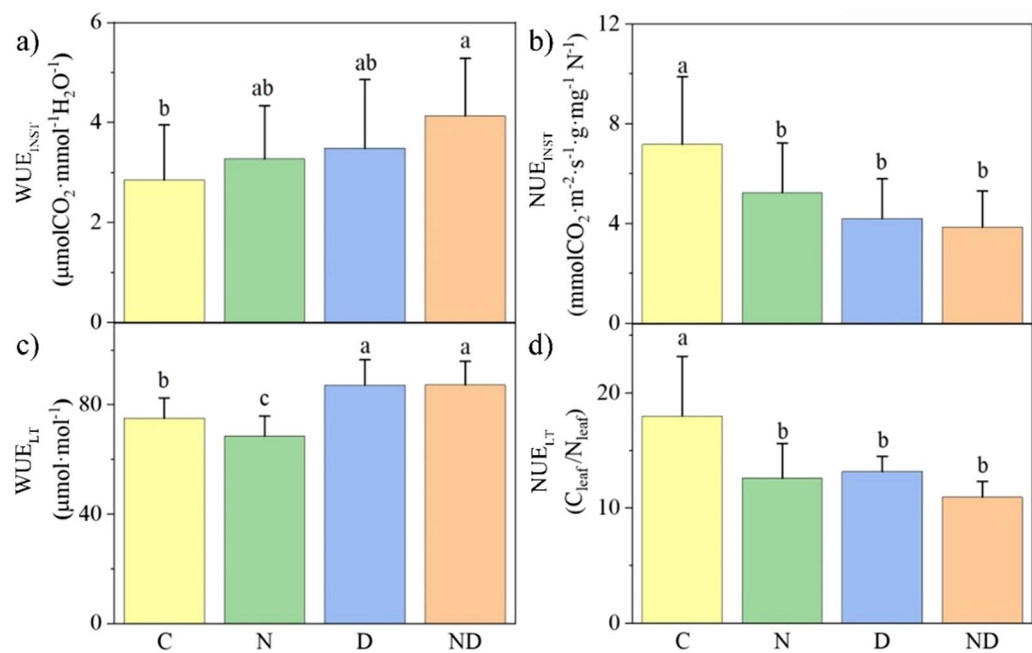
### Statistical analyses

Three-way ANOVAs were used to test the main effects of N addition, drought, species identity and their interaction on  $\text{NUE}_{\text{LT}}$ ,  $\text{WUE}_{\text{LT}}$ ,  $\text{NUE}_{\text{INST}}$  and  $\text{WUE}_{\text{INST}}$ , with N addition, drought and species as fixed factors and block as a random factor. For each species, two-way ANOVAs were performed to test the effect of N addition and drought on  $\text{NUE}_{\text{LT}}$ ,  $\text{WUE}_{\text{LT}}$ ,  $\text{NUE}_{\text{INST}}$  and  $\text{WUE}_{\text{INST}}$ , with N addition and drought as fixed factors and block as a random factor. When interactive effects of N addition and drought were significant, a post-hoc test of the binary treatments was performed. To quantify the magnitude and direction of WUE and NUE changes for each individual species in response to N addition and drought, we calculated the response ratio of RUE to N addition or drought treatment as follows:  $(\text{RUE}_{\text{N or D}} - \text{mean}(\text{RUE}_C))/\text{mean}(\text{RUE}_C)$  and  $(\text{RUE}_{\text{ND}} - \text{mean}(\text{RUE}_{\text{N or D}}))/\text{mean}(\text{RUE}_{\text{N or D}})$ , respectively, and the significant differences were tested by  $T$  test. Standardized major axis (SMA) was performed using the R package “smatr” to estimate the line of best fit between  $\text{NUE}_{\text{LT}}$  and  $\text{WUE}_{\text{LT}}$  or  $\text{NUE}_{\text{INST}}$  and  $\text{WUE}_{\text{INST}}$ , which is not restricted by the axes (Warton et al. 2011). Data were tested for normality using the Shapiro–Wilk test and for equality of variance using Levene’s test. The data were transformed if any of them did not satisfy the premises. All statistical analyses were performed in R 3.4.2 and Origin 2019.

## Results

### The effects of N addition, drought and their combination on $\text{WUE}_{\text{INST}}$ and $\text{NUE}_{\text{INST}}$

When averaged across six species, both N addition and drought, alone or in combination, increased  $\text{WUE}_{\text{INST}}$  and decreased  $\text{NUE}_{\text{INST}}$  (Fig. 1a, b).  $\text{WUE}_{\text{INST}}$  did not vary remarkably among species (Table 1) and was not significantly affected by N addition or drought (Table 2; Fig. 2a–c; Additional file 1: Fig. S2). By contrast,  $\text{NUE}_{\text{INST}}$  was significantly affected by drought, and such effect varied among species (Table 2; Fig. 2d–f). In particular, drought alone significantly reduced the  $\text{NUE}_{\text{INST}}$  of three species (*S. baicalensis*, *T. lanceolata* and *P. bifurca*) (Fig. 2d–f), and its combination with N addition only significantly reduced the  $\text{NUE}_{\text{INST}}$  of the bunch grass (*S. baicalensis*) (Fig. 2d). Furthermore, N addition and



**Fig. 1** Responses of averaged  $WUE_{INST}$  (a),  $NUE_{INST}$  (b),  $WUE_{LT}$  (c) and  $NUE_{LT}$  (d) to different treatments (C: control, N: N addition, D: drought, ND: combined drought and N addition). Data are shown as mean  $\pm$  SE. Different lowercase letters above any two bars indicate significant differences among the treatments at  $p < 0.05$

**Table 1** Results of three-way ANOVA to test the effects of N addition, drought and species identity on  $WUE_{INST}$ ,  $NUE_{INST}$ ,  $WUE_{LT}$  and  $NUE_{LT}$

	df	$WUE_{INST}$		$NUE_{INST}$		$WUE_{LT}$		$NUE_{LT}$	
		F	p value	F	p value	F	p value	F	p value
N addition (N)	1	3.81	0.055	8.61	<b>0.004</b>	17.23	<b>&lt; 0.001</b>	167.16	<b>&lt; 0.001</b>
Drought (D)	1	8.63	<b>0.004</b>	44.36	<b>&lt; 0.001</b>	247.64	<b>&lt; 0.001</b>	115.90	<b>&lt; 0.001</b>
Species (S)	5	1.39	0.267	13.09	<b>&lt; 0.001</b>	26.53	<b>&lt; 0.001</b>	54.61	<b>&lt; 0.001</b>
N $\times$ D	1	0.37	0.547	9.88	<b>0.002</b>	5.44	<b>0.022</b>	38.32	<b>&lt; 0.001</b>
N $\times$ S	5	0.21	0.959	2.11	0.074	0.89	0.490	2.77	<b>0.022</b>
D $\times$ S	5	0.49	0.781	2.09	0.076	3.73	<b>0.004</b>	25.53	<b>&lt; 0.001</b>
N $\times$ D $\times$ S	5	0.7	0.627	0.55	0.739	1.29	0.276	5.35	<b>&lt; 0.001</b>

Model: lmer (RUE ~ N addition  $\times$  Drought  $\times$  Species identity + 1 | Block)

df degrees of freedom

Significant p values (< 0.05) are bolded

drought had antagonistic effects on  $NUE_{INST}$  of two species (*L. chinensis* and *P. bifurca*) ( $P < 0.05$ , Additional file 1: Fig. S3a, c, d, f).

**The effects of N addition, drought and their combination on  $WUE_{LT}$  and  $NUE_{LT}$**

When averaged across the six species, N addition alone significantly decreased  $NUE_{LT}$  and  $WUE_{LT}$ , while drought and the combined treatment increased  $WUE_{LT}$  and decreased  $NUE_{LT}$  (Table 1; Fig. 1c, d). For the rhizomatous grass (*L. chinensis*), drought and the combined

treatment significantly increased  $WUE_{LT}$  and decreased  $NUE_{LT}$ , while N addition alone significantly decreased  $NUE_{LT}$  but had no effect on  $WUE_{LT}$  (Fig. 2g, j). For the bunch grass (*S. baicalensis*), N addition alone significantly decreased  $WUE_{LT}$  and  $NUE_{LT}$  at the same time, while drought and the combined treatment decreased  $NUE_{LT}$  but had no effect on  $WUE_{LT}$  (Fig. 2g, j). For all the four forb species,  $WUE_{LT}$  was increased by drought and its combined treatment, but did not respond to N addition alone (Fig. 2h, i);  $NUE_{LT}$  of two species (*P. bifurca* and *T. squarrosus*) was decreased by N addition, drought



**Table 2** Results of two-way ANOVA to test the effects of N addition (N) and drought (D) on  $WUE_{INST}$ ,  $NUE_{INST}$ ,  $WUE_{LT}$  and  $NUE_{LT}$  for the six studied species

Functional groups	Species	Factors	df	$WUE_{INST}$		$NUE_{INST}$		$WUE_{LT}$		$NUE_{LT}$	
				F	p	F	p	F	p	F	p
Grass	<i>L. chinensis</i>	N	1	1.13	0.307	4.12	0.063	0.22	0.645	38.48	<0.001
		D	1	3.95	0.068	2.9	0.113	51.94	<0.001	27.86	<0.001
		N×D	1	2.08	0.172	3.63	0.079	5.57	<b>0.032</b>	8.78	<b>0.010</b>
	<i>S. baicalensis</i>	N	1	0.59	0.367	3.20	0.103	8.05	<b>0.013</b>	115.54	<0.001
		D	1	2.37	0.145	47.28	<0.001	24.60	<0.001	197.66	<0.001
		N×D	1	0.03	0.856	0.99	0.342	15.85	<0.001	38.52	<0.001
Legumi-nous forb	<i>T. lanceolata</i>	N	1	1.45	0.250	19.85	<b>0.002</b>	3.54	0.083	7.84	<b>0.013</b>
		D	1	0.01	0.938	27.84	<0.001	47.52	<0.001	3.84	0.068
		N×D	1	0.41	0.533	13.53	<b>0.006</b>	0.53	0.526	0.73	0.406
Non-legumi-nous forb	<i>P. bifurca</i>	N	1	1.17	0.305	0.10	0.755	17.43	<0.001	24.89	<0.001
		D	1	1.82	0.207	9.43	<b>0.011</b>	83.49	<0.001	23.54	<0.001
		N×D	1	0.46	0.513	3.88	0.074	0.19	0.671	10.09	<b>0.006</b>
	<i>T. squarrosus</i>	N	1	0.01	0.911	5.19	<b>0.049</b>	1.61	0.229	9.58	<b>0.009</b>
		D	1	1.15	0.312	5.33	<b>0.046</b>	57.07	<0.001	12.95	<b>0.004</b>
		N×D	1	0.10	0.755	1.52	0.248	0.02	0.893	5.99	<b>0.031</b>
	<i>K. centauroide</i>	N	1	0.27	0.614	3.64	0.079	1.44	0.250	114.06	<0.001
		D	1	2.35	0.149	4.76	<b>0.048</b>	37.64	<0.001	1.61	0.221
		N×D	1	0.03	0.862	0.45	0.514	0.84	0.375	1.02	0.327

Model: lmer (RUE ~ N addition × Drought + 1 | Block)

df degrees of freedom

Significant p values (&lt;0.05) are bolded

and their combination, while that of *K. centauroide* was decreased under N addition and the combined treatment (Fig. 2l). Furthermore, drought weakened the effect of N addition on  $NUE_{LT}$  of four species (*L. chinensis*, *S. baicalensis*, *P. bifurca* and *T. squarrosus*) and  $WUE_{LT}$  of two species (*L. chinensis* and *S. baicalensis*) (Table 2; Figs. 3a, c, 4a), while N addition weakened the negative effect of drought on  $NUE_{LT}$  of four species (*L. chinensis*, *S. baicalensis*, *P. bifurca* and *T. squarrosus*) and enhanced the positive effect of drought on  $WUE_{LT}$  of two species (*L. chinensis* and *S. baicalensis*) (Table 2; Figs. 3d, f, 4d).

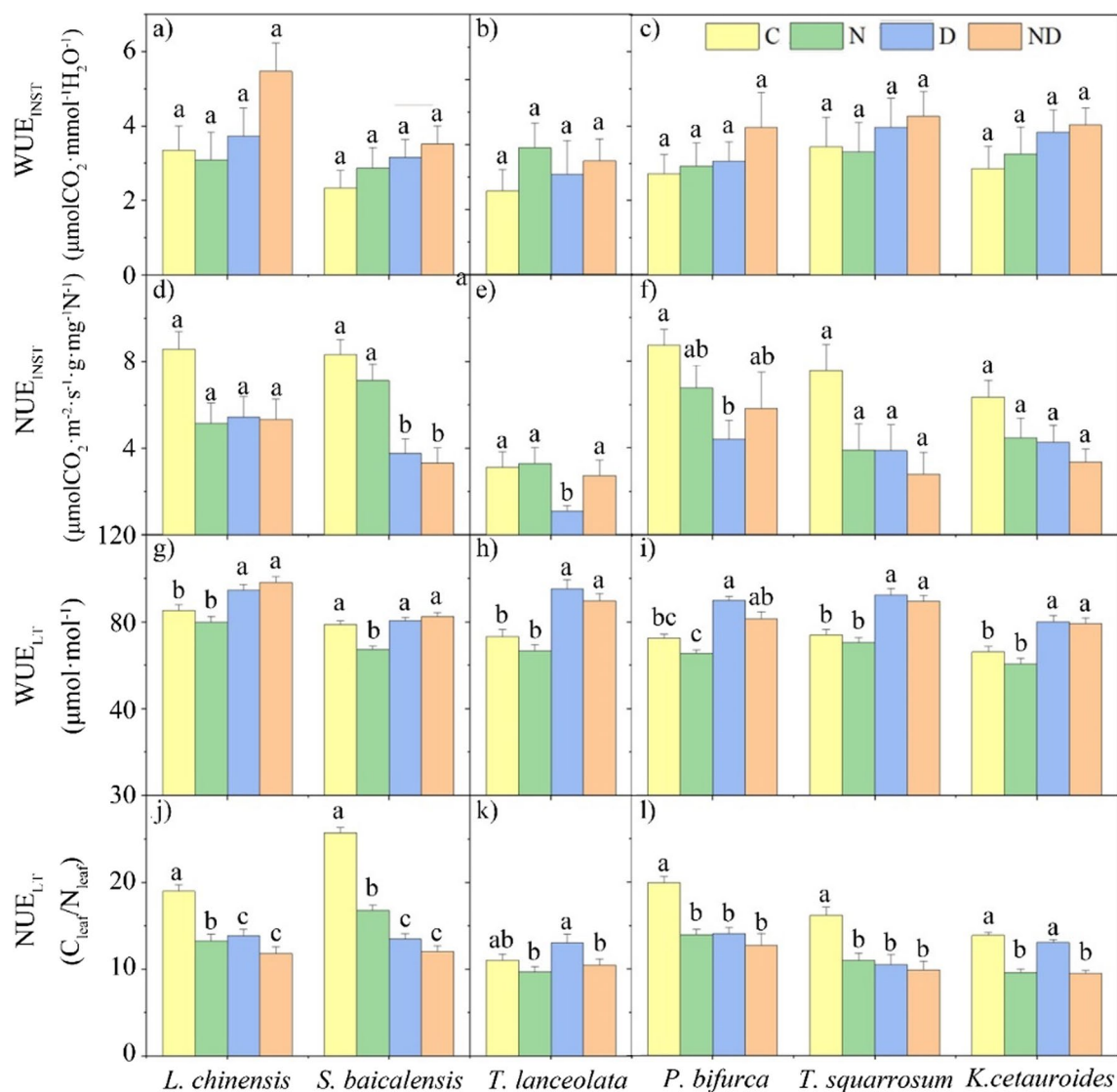
### Relationship between NUE and WUE

Under natural condition, there was a significant trade-off between  $NUE_{LT}$  and  $WUE_{LT}$  for one non-leguminous forb (*K. centauroide*) ( $R^2 = 0.79$ ;  $P = 0.01$ ), while no significant relationship was found for any other species (Additional file 1: Fig. S4). The correlations between WUE and NUE under N addition and drought were significantly different among functional groups. Drought did not engender significant correlation between  $NUE_{INST}$  and  $WUE_{INST}$  for any studied species, whether with or without N addition (Fig. 5a, b), but caused a trade-off between the  $NUE_{LT}$  and  $WUE_{LT}$  in three non-leguminous forb species (*P. bifurca*, *T. squarrosus* and

*K. centauroide*) without N addition and in the only bunch grass (*S. baicalensis*) under N addition (Fig. 5e, f). Nitrogen addition brought forth the positive correlation between  $NUE_{INST}$  and  $WUE_{INST}$  for the leguminous forb (*T. lanceolata*) and between  $NUE_{LT}$  and  $WUE_{LT}$  for two grass species (*L. chinensis* and *S. baicalensis*) without drought (Fig. 5c, g), while between  $NUE_{INST}$  and  $WUE_{INST}$  for two non-leguminous forb species (*T. squarrosus* and *K. centauroide*) and between  $NUE_{LT}$  and  $WUE_{LT}$  for one non-leguminous forb (*P. bifurca*) with drought (Fig. 5d, h).

### Discussion

We found high plasticity of plant NUE and WUE in response to N addition and drought. More importantly, the interactions of the two factors for NUE and WUE were antagonistic, synergetic or neutral, that is, one factor might weaken, exacerbate or keep the impacts of the other on RUE. Although mounting evidence shows a negative correlation between NUE and WUE, our results demonstrated that there was no universal trade-off between NUE and WUE, and this relationship could be positive, negative or neutral, varying among the species



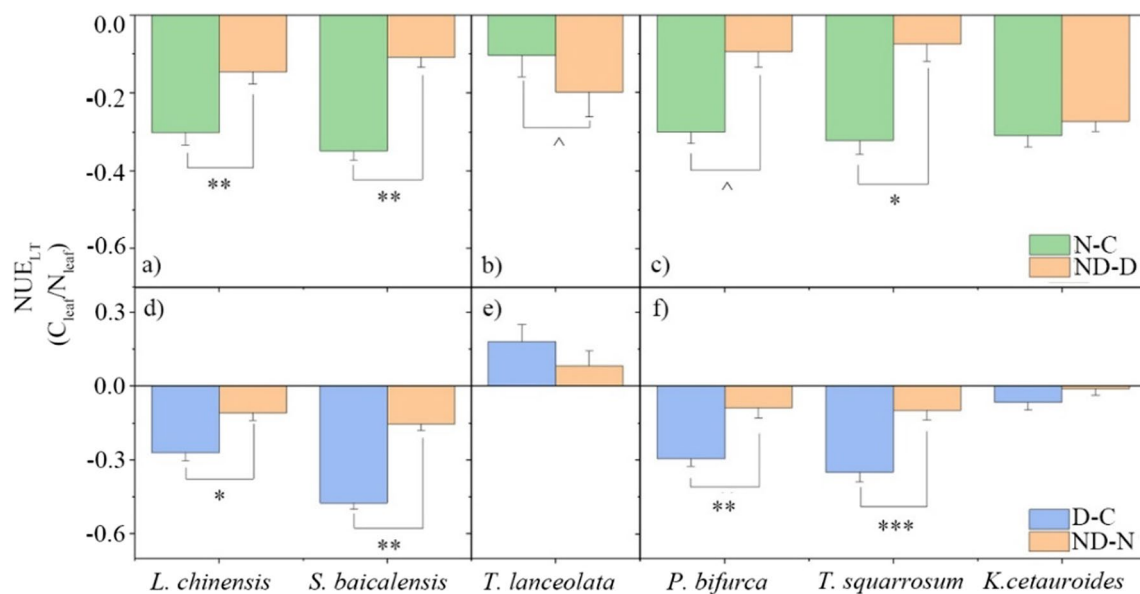
**Fig. 2**  $WUE_{INST}$  (a–c),  $NUE_{INST}$  (d–f),  $WUE_{LT}$  (g–i) and  $NUE_{LT}$  (j–l) of the six studied species under different treatments (C: control, N: N addition, D: drought, ND: combined drought and N addition). Data are shown as mean  $\pm$  SE. Among them, **a, d, g** and **j** show the changes in grasses, **b, e, h** and **k** show the changes in the leguminous forb, **c, f, i** and **l** show the changes in non-leguminous forbs. Different lowercase letters above any two bars indicate significant differences among the treatments at  $p < 0.05$

of different functional groups and depending on habitats with different soil nitrogen and/or water availability.

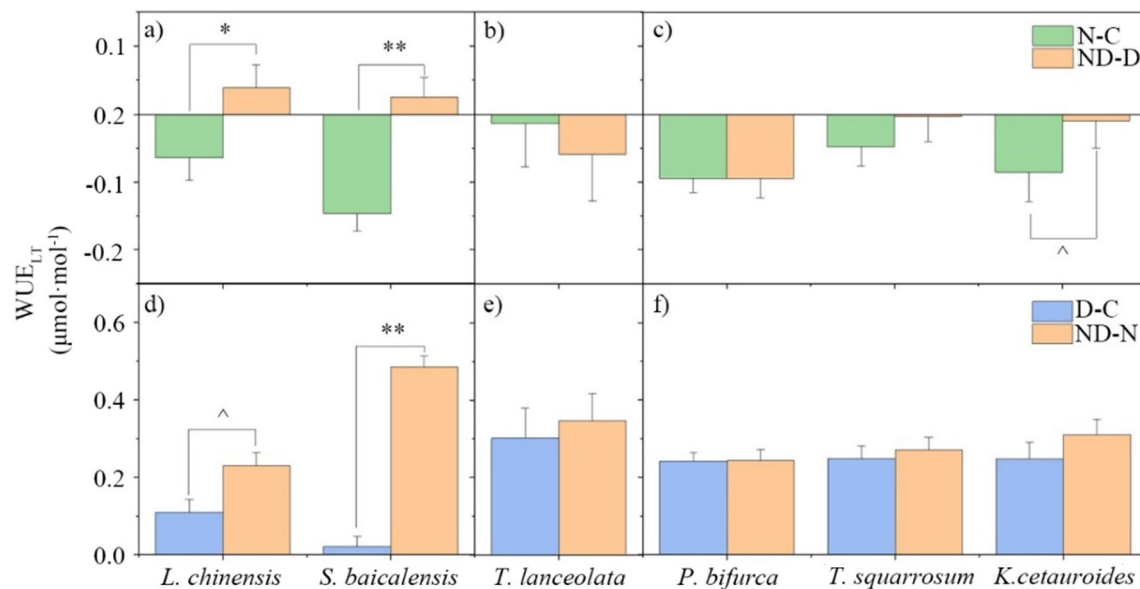
#### Consistent effects of N addition and drought on NUE but not on WUE

Previous studies have shown that drought will increase WUE (i.e.,  $\delta^{13}\text{C}$ ) (Swap et al. 2004; Mariotte et al. 2013) and decrease NUE (i.e.,  $C/N$ ) (Sun et al. 2020; Luong et al. 2021; Guo et al. 2022) of grassland plants, such as *L. chinensis* (Yue et al. 2019), and the process is mainly through the reduction of stomatal conductance and transpiration rate while hindering nutrient transport (Swap et al. 2004;

Wang et al. 2010). Similar results were found in our study, with drought significantly reducing soil water availability,  $NUE_{INST}$  and  $NUE_{LT}$  decreased and  $WUE_{LT}$  increased in most species, but the response mechanism may be attributed to the increase of LNC and the decrease of natural height. By contrast, although  $NUE_L$  was significantly reduced by N addition,  $WUE_L$  was not significantly increased but remained unchanged or decreased in N addition plots, which was consistent with previous researches (Chen et al. 2005; Diao et al. 2021). Global synthetic analyses showed that N addition can increase plant carbon uptake by stimulating leaf production and/or leaf



**Fig. 3** Changes of  $NUE_{LT}$  (a–f) of the six studied species under N addition and drought. Data are shown as mean  $\pm$  SE. Among them, a and d show the changes in grasses, b and e show the changes in the leguminous forb, c and f show the changes in non-leguminous forbs. “N–C” and “ND–D” represent the relative changes of RUE caused by N addition without and with drought, respectively; “D–C” and “ND–N” represent the relative changes of RUE caused by drought without and with N addition, respectively.  $^{\wedge}0.05 < p < 0.1$ ,  $*0.01 < p < 0.05$ ,  $**0.001 < p < 0.01$ ,  $***p < 0.001$

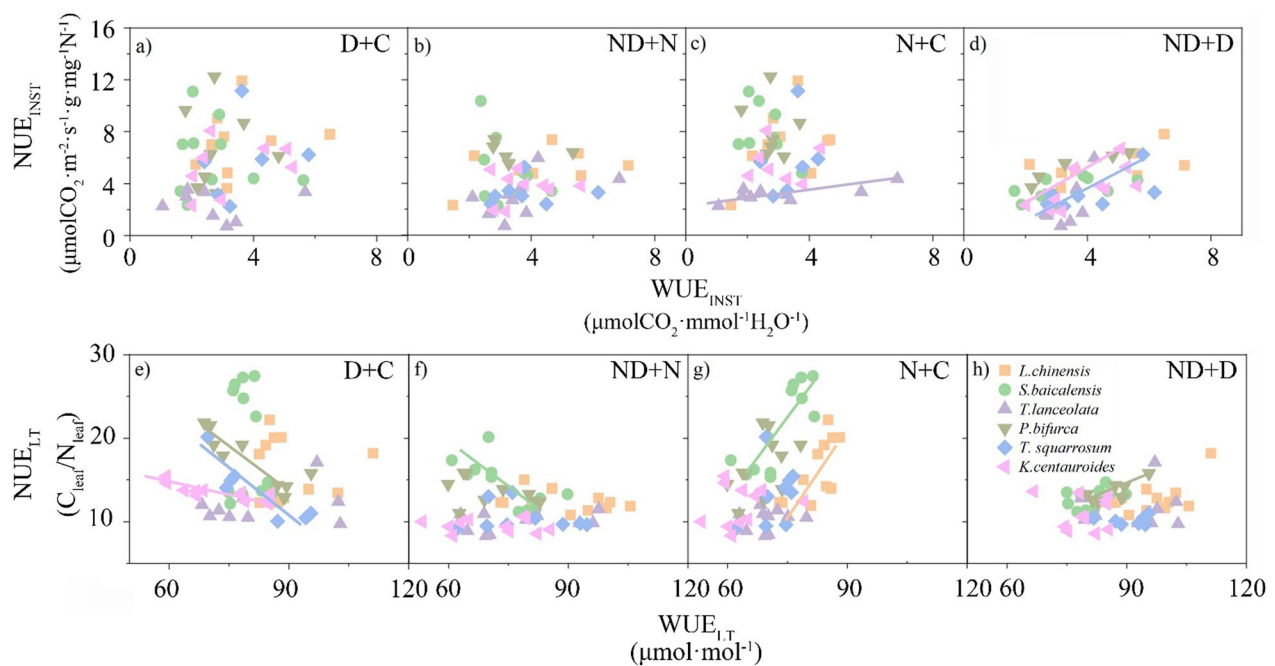


**Fig. 4** Changes of  $WUE_{LT}$  (a–f) of the six studied species under N addition and drought. Data are shown as mean  $\pm$  SE. Among them, a and d show the changes in grasses, b and e show the changes the leguminous forb, c and f show the changes in non-leguminous forbs. “N–C” and “ND–D” represent the relative changes of RUE caused by N addition without and with drought, respectively; “D–C” and “ND–N” represent the relative changes of RUE caused by drought without and with N addition, respectively.  $^{\wedge}0.05 < p < 0.1$ ,  $*0.01 < p < 0.05$ ,  $**0.001 < p < 0.01$ ,  $***p < 0.001$

photosynthetic capacity, so there is a strong promoting effect of N addition on  $WUE_{LT}$  (Liang et al. 2020). However, it is worth noting that  $WUE_{LT}$  was not significantly increased but remained unchanged or decreased under N addition, and it might be attributed to unchanged gas

exchange parameters during our experimental period (Additional file 1: Fig. S5a–l). Meanwhile, the downward trend of  $WUE_{LT}$  could be explained by vegetation height. The natural height of *L. chinensis* is significantly higher than other plants (Additional file 1: Fig. S5p), which will





**Fig. 5** Relationship between  $WUE_{INST}$  and  $NUE_{INST}$  (a–d), and between  $WUE_{LT}$  and  $NUE_{LT}$  (e–h) of the six studied species. Each panel is based on the pooled-together data from two experimental treatments as indicated in its upper right corner, to enlarge the span of data points along the WUE and NUE gradients. Across the panels, the effects of N addition (b and f relative to a and e, respectively) and drought (d and h relative to c and g, respectively) on WUE–NUE relationship can be seen

hinder the evapotranspiration of water in soil surface and leaves, keeping the soil moisture and compensating the plants' water demand as biomass increases with N addition. Furthermore, higher vegetation canopy will reduce the PAR and increase the  $C_i/C_a$  (the ratio of intercellular to atmospheric  $CO_2$  partial pressure) of the under-canopy plants, thus decreased WUE (i.e., a lower  $\delta^{13}C$  value; Chen et al. 2004).

#### Antagonism and synergy between N addition and drought

Nitrogen addition and drought are not independent, but rather interact with each other to influence plant physiological and ecological processes (Gong et al. 2011; Meng et al. 2021). However, a synthetic analysis showed that the effects of multiple global change factors on ecosystem processes are generally additive (Yue et al. 2017). Although the impacts of N addition and drought on  $NUE_{LT}$  were in the same direction in our study, their combination did not produce greater effect compared with either of them alone. Thus, our results provide support for the view that the interactive effect of N addition and drought was antagonistic for  $NUE_{LT}$ . For example, drought could reduce the negative effect of N addition on  $NUE_{LT}$  in all selected species except the leguminous forb, *T. lanceolata*. Although N addition alleviated soil nutrient limitation and decreased  $NUE_{LT}$ , it might not when combining with drought, because low soil water

availability induced by drought suppressed nutrient diffusion in soils, nutrient uptake by roots and nutrient transport in plant tissues (Luo et al. 2018b). Similarly, even drought could suppress nutrient transport and decrease NUE (Swap et al. 2004), this negative effect might be alleviated by N addition. For example, drought significantly decreased the  $NUE_{LT}$  of *S. baicalensis* with or without N addition, with the effect size being lower in the presence of N addition than otherwise.

Besides, N addition strengthened the positive effect of drought on  $WUE_{LT}$  of two grass species, suggestive of the synergistic effect between the two global change factors. In our study, the two grass species had higher  $WUE_{LT}$  than forb species, and, therefore, were better resistant to drought without N addition. However, N addition could increase the water requirement of plants due to the increasing LNC and/or increasing specific leaf area (Jin et al. 2022). In this case, the  $WUE_{LT}$  of grasses under the combination of drought and N addition increased much more than that under drought alone, indicating that N addition increased the sensitivity of grass  $WUE_{LT}$  to drought, which was inconsistent with Shovon et al. (2019). Given *L. chinensis* and *S. baicalensis* are the dominant species in the temperate grassland community, this synergistic effect between N addition and drought on the two species may scale up to the community level according to the dominance/mass ratio hypothesis, which

means that N addition could exacerbate drought stress, further improve species' tolerance, and enable communities, even ecosystems, to have higher WUE.

#### **WUE–NUE relationship varied across functional groups and depended on environmental context**

Numerous studies have shown that plants could alter resource utilization strategies in response to the changes of limited resources, which means that the reduction of one type of limited resource was assumed to stimulate plants to increase the use efficiency for this resource at the expense of that of another type of less limited resource (Field et al. 1983; Patterson et al. 1997). Therefore, in grassland ecosystems co-limited by nutrient and water, plants were thought to adopt a common trade-off strategy under N addition or drought (Yao et al. 2011; Chen et al. 2005). In line with previous studies, we found a strong negative correlation between  $NUE_{LT}$  and  $WUE_{LT}$  under drought. However, this trade-off varied between different functional groups, depending on whether nitrogen is added or not. It was observed in the three non-leguminous forbs without N addition and in the only bunch grass with N addition in our study. This might suggest that the species of different functional groups adopt different resource use strategies to adapt to the changes in nitrogen and water availability, partly because the extent of water restriction induced by drought varied across species of different functional groups. Compared to grasses, forbs were more sensitive to precipitation change (Ma et al. 2022; Hoover et al. 2014), and thus drought-induced intense water restriction forced forb species to increase  $WUE_{LT}$  at the cost of  $NUE_{LT}$ , leading to a trade-off. Instead, in N-enriched condition, drought did not engender the trade-off in forb species, because greater soil nutrient availability might offset the drought-induced negative effect on  $NUE_{LT}$ , while for grass species, the increase of biomass and LNC due to greater soil nutrient availability could increase their water requirements under drought, and thus would increase  $WUE_{LT}$  at the expense of  $NUE_{LT}$ .

In addition, we also observed a strong positive correlation between  $NUE_{LT}$  and  $WUE_{LT}$  under N addition, which is out of our expectation. This positive correlation might be attributed to the simultaneous decrease in  $NUE_{LT}$  and  $WUE_{LT}$  under N addition, which may suggest that plant growth in N-enriched conditions was less limited by soil nitrogen and water than by other resources, such as phosphorus or light. In fact, in this study area, N addition can indeed significantly increase LNC and reduce leaf phosphorus content (LPC), thus increasing N/P. Moreover, drought with N addition can further improve the limiting effect of phosphorus on plant

growth (Zhou et al. 2020; Liang et al. 2022). Furthermore, several studies have reported the positive correlation between LPC with WUE and photosynthetic capacity, and some have shown that many P-limited plants have lower NUE (Singh et al. 2000; Graciano et al. 2005; Talbi Zribi et al. 2011; Crous et al. 2017).

However, since this study was conducted under controlled environmental conditions, there are some limitations. First, unlike the continuous natural nitrogen deposition at a low rate, our nitrogen addition was implemented once during a year with a much higher rate. Second, both abiotic factors (e.g., soil water, soil nutrient and soil texture) and biotic factors (e.g., plant functional trait and other resource use efficiency except nitrogen and water) that may influence plant NUE and WUE are not taken into account for the sake of simplicity and feasibility of the study (Lambers et al. 2008; Li et al. 2009b; Belnap 2011; Pettigrew 2008; Chen 2014; Sardans and Peñuelas 2015; He et al. 2021). Despite that, our findings will contribute to a comprehensive understanding about the variations of NUE–WUE relationship across plant functional groups depending on environmental contexts. Variations of plant NUE, WUE and their relationship in response to global changes might underlie the changes of community composition and ecosystem functionality of grasslands. As found in our study, the NUE, WUE and their relationship of component species or functional groups in the communities showed adaptive changes under the global changes, which would result in the shift of their competitive superiority, and ultimately alter the community composition if the global change scenario last long enough. For instance, drought-tolerant species perform much better than drought-sensitive species in response to drought, and nitrophilous species perform better than non-nitrophilous species under N addition. Both the changes of NUE, WUE and their relationship, and the resulted changes of community composition, will undoubtedly impact the ecosystem functionality. Hence, our future work should link plant NUE vs. WUE changes with their competitive ability or fitness, and further with the modulation of community structure and ecosystem functionality, adopting more realistic experimental treatments with more critical factors taken into account and finer functional groups categorized.

#### **Conclusion**

Our research suggested that NUE and WUE and their relationship play an important role in determining their responses and adaptability to N deposition and drought. Drought-induced stronger water limitation promoted plants to increase WUE at the expense of NUE, showing an apparent trade-off relationship, while plant growth in N-enriched conditions might be less limited by soil

nitrogen and water than by other resources, and therefore, NUE and WUE simultaneously decreased under N addition. Meanwhile, our results shed light on the antagonistic or synergetic effect between N addition and experimental drought by demonstrating that drought weakened the negative effect of N addition on WUE and NUE, and N addition weakened the negative effect of drought on NUE while enhanced the positive effect of drought on WUE. Moreover, we further clarified the different responses and adaptability among species of different functional groups (grasses, the leguminous forb and non-leguminous forbs) to N addition and drought. These findings may have implications for grassland management particularly in terms of fertilizer application, e.g., by nitrogen fertilizing at appropriate rate according to drought severity to balance and optimize the NUE and WUE to gain maximum benefit with special respect to ecosystem productivity.

#### Abbreviations

RUE	Resource use efficiency
NUE	Nitrogen use efficiency
WUE	Water use efficiency
NUE <sub>INST</sub> and WUE <sub>INST</sub>	Instantaneous water and nitrogen use efficiency
NUE <sub>LT</sub> and WUE <sub>LT</sub>	Long-term water and nitrogen use efficiency
C	Control
N	Nitrogen addition
D	Drought
ND	The combination of N addition and drought
P <sub>n</sub>	Net photosynthetic rate
Tr	Transpiration rate
G <sub>s</sub>	Stomatal conductance
C <sub>i</sub>	Intercellular carbon dioxide concentration
PAR	Photosynthetically active radiation
LCC and LNC	The leaf carbon and nitrogen content

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13717-023-00448-7>.

**Additional file 1: Figure S1.** The geographical location of the research site (a), the randomized block design of the study (b) and the picture of rainout shelters used in the manipulative drought experiment (c). In Fig. S1a, the rectangular frame in the map of China is the territory of Hulunbuir City, Inner Mongolia, and the solid circle in the map of Hulunbuir City shows the location of the research site. Fig. S1b shows the six blocks of the experimental platform, each containing eight different treatments (C: control, N: N addition, D: drought, S: snowmelt delay, ND: combination of drought and N addition, NS: combination of N addition and snowmelt delay, DS: combination of drought and snowmelt delay, NDS: combination of drought, N addition and snowmelt delay). **Figure S2.** The relative change of WUE<sub>INST</sub> (a–f) of the six studied species induced by N addition and drought. Data are shown as mean ± SE. Among them, panel a and d show the changes in grasses, panel b and e show the changes in the leguminous forb, panel c and f show the changes in non-leguminous forbs. “N–C” and “ND–D” represent the relative changes caused by N addition without and with drought, respectively; “D–C” and “ND–N” represent the relative changes caused by drought without and with N addition, respectively. <sup>^</sup>0.05 < *p* < 0.1, \*0.01 < *p* < 0.05, \*\*0.001 < *p* < 0.01, \*\*\**p* < 0.001. **Figure S3.** The relative change of NUE<sub>INST</sub> (a–f) of the six studied species induced by N addition and drought. Data are shown as mean ± SE.

Among them, panel a and d show the changes in grasses, panel b and e show the changes in the leguminous forb, panel c and f show the changes in non-leguminous forbs. “N–C” and “ND–D” represent the relative changes caused by N addition without and with drought, respectively; “D–C” and “ND–N” represent the relative changes caused by drought without and with N addition, respectively. <sup>^</sup>0.05 < *p* < 0.1, \*0.01 < *p* < 0.05, \*\*0.001 < *p* < 0.01, \*\*\**p* < 0.001. **Figure S4.** Relationships between NUE<sub>INST</sub> and WUE<sub>INST</sub> (a), and between NUE<sub>LT</sub> and WUE<sub>LT</sub> (b) for the six studied species under natural conditions. **Figure S5.** Mean net photosynthetic rate (*P<sub>n</sub>*, a–c), stomatal conductance (*G<sub>s</sub>*, d–f), transpiration rate (*T<sub>r</sub>*, g–i), intercellular carbon dioxide concentration (*C<sub>i</sub>*, j–l), leaf nitrogen content (LNC, m–o) and plant height (p–r) of the six studied species under different treatments (C: control, N: N addition, D: drought, ND: combined drought and N addition). Data are shown as mean ± SE. Among them, panels a, d, g, j, m and p show the changes in grasses, panels b, e, h, k, n and q show the changes in the leguminous forb, panels c, f, i, l, o and r show the changes in non-leguminous forbs. Different lowercase letters above any two bars indicate significant differences among the treatments at *p* < 0.05. **Table S1.** Information of the six studied species common to experimental plots.

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#### Author contributions

HJX carried out the field measurement and sampling, indoor survey, data analysis and manuscript drafting. MW and WZW conceived the experimental design and helped with manuscript revision. All authors have read and approved the final manuscript.

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#### Availability of data and materials

If paper is accepted, all data and code used will be archived in Figshare.

#### Declarations

##### Ethics approval and consent to participate

Not applicable.

##### Consent for publication

Not applicable.

##### Competing interests

The authors declare that they have no competing interests.

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