



Research Article

Effects of nitrogen addition and mowing on nitrogen- and water-use efficiency of *Artemisia frigida* in a grassland restored from an abandoned croplandHuajie Diao^{1,2,3,†}, Paul Kardol^{4,†}, Kuanhu Dong^{1,3,*} and Changhui Wang^{1,2,3,*}

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Abstract

Aims Competition among plants in a community usually depends on their nitrogen (N)-use efficiency (NUE) and water-use efficiency (WUE) in arid and semi-arid regions. *Artemisia frigida* is an indicator species in heavily degraded grassland, however, how its NUE and WUE respond to N addition in different successional stages is still unclear, especially with mowing, a common management practice in semi-arid grasslands.

Methods Based on a long-term controlled experiment with N addition and mowing in an abandoned cropland from 2006 to 2013, we investigated the NUE and WUE of *A. frigida* in two patches (i.e. grass and herb patches) in 2013 which represented two potential successional stages from herb to grass communities. The coverage of *A. frigida* was higher (about 50%) in the herb patch than in the grass patch (about 10%). Stable isotopic C ($\delta^{13}\text{C}$) and N ($\delta^{15}\text{N}$) as well as C and N pools were measured in plants and soils. NUE was calculated as leaf C/N, and leaf $\delta^{13}\text{C}$ values were used as a proxy for WUE.

Important Findings N addition did not affect WUE of *A. frigida*, but significantly decreased NUE by 42.9% and 26.6% in grass and herb patches, respectively. The response of NUE to N addition was related to altering utilization of different N sources (NH_4^+ vs. NO_3^-) by *A. frigida* according to the changed relationship between leaf $\delta^{15}\text{N}$ /soil $\delta^{15}\text{N}$ and NUE. Mowing had no effect on NUE regardless of N addition, but significantly increased WUE by 2.3% for *A. frigida* without N addition in the grass patch. The addition of N reduced the positive effect of mowing on its WUE in grass patch. Our results suggested that decreased NUE and/or WUE of *A. frigida* under mowing and N addition could reduce its competition, and further accelerate restoration succession from the abandoned cropland to natural grassland in the semi-arid region.

Keywords mowing, nitrogen addition, nitrogen-use efficiency, semi-arid grassland, water-use efficiency

氮添加和刈割对内蒙古弃耕草地冷蒿氮和水分利用效率的影响

摘要: 在氮和水分限制的区域, 植物氮利用效率 (NUE) 和水分利用效率 (WUE) 决定了它们在群落中的竞争优势。冷蒿 (*Artemisia frigida*) 是半干旱草地重度退化的先锋物种, 在不同退化程度的草地中具有不

同的优势度，经常被认为是退化草地群落演替的指示物种。退化草地恢复过程中，氮添加和割草如何影响冷蒿的NUE和WUE尚不清晰。以内蒙古多伦县弃耕草地为研究对象，选取两个不同群落斑块（禾草和冷蒿为优势物种的斑块），经过长期（2006–2013）氮添加和刈割（对照、氮添加、刈割、氮添加+刈割）处理后，研究冷蒿的NUE（叶片碳氮比）和WUE（叶片碳同位素， $\delta^{13}\text{C}$ ）对氮添加、刈割及其交互作用的响应；结合植物和土壤的碳、氮同位素（ $\delta^{13}\text{C}$ 和 $\delta^{15}\text{N}$ ）及碳、氮库探究退化草地恢复过程中植物的资源利用策略及其机制。研究表明：（1）氮添加对冷蒿的WUE没有显著影响（ $P > 0.05$ ），但NUE在禾草和冷蒿斑块中分别显著降低了42.9%和26.6%（ $P < 0.05$ ）；（2）植物对不同氮源（ NH_4^+ 或 NO_3^- ）的利用会引起植物和土壤 $\delta^{15}\text{N}$ 的分馏，研究表明叶片和土壤的 $\delta^{15}\text{N}$ 与NUE呈现相反的变化趋势，因此冷蒿的NUE对氮添加的响应与不同氮源的利用有关；（3）刈割不影响冷蒿的NUE（ $P > 0.05$ ），但在禾草斑块，冷蒿的WUE在刈割处理下显著提高了2.3%（ $P < 0.05$ ）；（4）在禾草斑块，氮添加减缓了割草对冷蒿WUE的促进作用；（5）结构方程模型显示，土壤含水量直接或间接的调控着冷蒿的WUE和NUE。综上所述，在禾草斑块，氮添加+刈割处理维持较低的NUE和WUE，不利于冷蒿对资源的竞争，进一步降低其优势度，这也预示着氮添加+刈割处理会促进退化草地的恢复。

关键词：氮添加，刈割，氮利用效率，半干旱草地，水分利用效率

INTRODUCTION

Nitrogen (N) and water are two of the most important limiting resources for plant establishment and growth in arid and semi-arid grasslands (Bai *et al.* 2008). Thus, N-use efficiency (NUE) and water-use efficiency (WUE) of plants may determine their competition in the community (Field *et al.* 1983). Anthropogenic global N deposition has dramatically increased over the last decades (Galloway *et al.* 2008), which had important consequences for the functioning of terrestrial ecosystems (Kanakidou *et al.* 2016). Previous studies have shown that N addition decreased leaf C/N ratio, indicating lower NUE of studied species (Han *et al.* 2014; Hou *et al.* 2017), which was due to lower leaf N absorption efficiency from senescing leaves (Yuan and Chen 2015). Importantly, the uptake of different N sources (NO_3^- or NH_4^+) by plants varied with soil N availability (Högberg 1997), which would then affect NUE due to the different absorption and transformation processes between NO_3^- and NH_4^+ (Takushi and Hitoshi 2016). However, whether the effect of N addition on plant NUE is related to the utilization of different N sources is still largely unknown. Studies have shown that N addition could promote plant WUE (Chen *et al.* 2005; Gong *et al.* 2011) because of the relatively greater increase in photosynthetic rate compared with transpiration rate (Chen *et al.* 2005). Change in leaf functional traits also has been found to have a certain regulation effect on plant WUE (Gong *et al.* 2011). However, the effects of N addition on WUE are often strongly species specific (Chen *et al.* 2005). Furthermore, most of these studies were

conducted in natural grasslands. How N addition affects plant NUE and WUE in restored successional communities is still largely unknown, which may limit our understanding to predict the successional direction and magnitude for abandoned croplands.

Mowing is one of the most widely used management strategies in grassland around the world, especially in semi-arid areas (Han *et al.* 2014). Mowing would inhibit photosynthesis as a result of removing aboveground biomass and then reduce NUE through increase in leaf N or decrease in leaf C/N (Han *et al.* 2014; Yang *et al.* 2020). However, Lü *et al.* (2012) reported that mowing did not affect leaf C/N and plant NUE in a semi-arid grassland of northern China. This discrepancy may due to the different species and habitats involved in different studies. Mowing also alters soil microclimate (e.g. increased soil temperature [ST] and decreased surface soil moisture [SM]; Wan *et al.* 2002; Wang *et al.* 2011), leading to the changes in soil microbial activity and litter decomposition (Keeler *et al.* 2009; Li *et al.* 2019). The decrease in soil water content induced by mowing could accelerate water limitation and then improve the WUE of plants. Yuan and Li (2007) found that SM conditions regulated N utilization by plants. So, we hypothesized that mowing could alter the effect of N addition on NUE indirectly by affecting WUE due to changes in SM.

The natural abundance of stable isotope ratios ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) integrates across several biogeochemical processes in ecosystem carbon and N cycling (Bai and Houlton 2009; Högberg 1997; Robinson 2001). The stable isotope ratios of soils and plants could provide

valuable information on the utilization strategy of resources for a reaction or a biogeochemical process, because a light isotope is usually more abundant in the product, and a heavy isotope is usually enriched in the substrate (Craine *et al.* 2015; Högberg 1997). Thus, the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ abundances in plant leaves and soils can give insights into the utilization of different N sources by plants. Leaf $\delta^{13}\text{C}$ value is related to leaf stomatal conductance, and therefore can be used as a proxy for plant WUE (Farquhar *et al.* 1982; Peri *et al.* 2012).

The temperate steppe in Inner Mongolia, northern China, represents a typical arid and semi-arid vegetation type of the Eurasian continent. During the past half century, most of the grassland in this area has suffered from heavy human disturbance, such as overgrazing and cropping pressure, resulting in severe land degradation. With the development of the 'Conversion from Cropland into Grassland Program' implemented by China's central government, some abandoned croplands are undergoing restoration succession to natural grasslands through different successional processes, and thus developed different vegetation patches. As an indicator species of heavily degraded grassland, *Artemisia frigida* usually occupies high coverage in the community in the early restoration stage and then its coverage decreases with progressive restoration succession (Li *et al.* 2002, 2011). In different vegetation patches, *A. frigida* had different resource utilization efficiencies (i.e. NUE and WUE), which regulated its competition and coverage in community. However, little is known about the effect of N deposition and mowing on the WUE and NUE of *A. frigida*, which limits us to understand the underlying mechanisms of restoration succession. We hypothesized that (i) N addition reduces its NUE through improved WUE due to accelerated N turnover; (ii) mowing increases its WUE because of increased water stress, while mowing decreases its NUE due to the trade-off between NUE and WUE; (iii) the interactions between N addition and mowing on the NUE and WUE of *A. frigida* are different with vegetation patches.

MATERIALS AND METHODS

Study area

This study was conducted in a semi-arid grassland located in Duolun County (116°40' E, 42°27' N, 1324 m above sea level), Inner Mongolia, northern China. The long-term (1953–2007) mean annual temperature and precipitation in this area were 2.1 °C and 382.2 mm, with more than 80% of the rainfall occurring during the

growing season (from June to September) (Wang *et al.* 2011). The area of experimental site is an abandoned cropland without any further human management since 1995, and then undergoing natural restoration succession to grassland. Different vegetation patches were developed during the process of restoration succession due to the differences in nutrients or water condition. Two types of vegetation patches (grass and herb patches) were chosen to represent two restoration stages: early restoration stage for the herb patch and middle restoration stage for the grass patch. *A. frigida* is a dominant species in herb patch (>50% of total coverage in the community), while its coverage is less than 10% in the grass patch which are dominated by the grass *Pennisetum centrasiaticum*. The soil in this area is chestnut soil (FAO classification) according to the Food and Agriculture Organization classification. The proportions of sand, silt and clay in the 20 cm top soil are 63%, 20% and 17%, respectively. Aboveground and belowground biomass are twice higher in grass patch than in herb patch. More detailed information about the study area can be found in the study of Wang *et al.* (2011).

Experimental design

Twenty-four plots (4 m × 4 m) were established in 2005 with two kind of patches (i.e. grass and herb patches), making a total of 48 plots in our experiment. The distance between the two vegetation patches was approximately 18 m, and any two plots in each patch were separated by a 2 m buffer zone. In each patch, four different treatments were organized in a randomized block design with six replicates of each treatment. Four treatments in each block were as follows: (i) control (CK, no N addition and no mowing), (ii) N addition (N), (iii) mowing (M) and (iv) a combination of mowing and N addition (N + M). The ambient atmospheric N deposition in this area was estimated at 1–2 g N m⁻² year⁻¹. The detailed experimental design has been reported previously (Wang *et al.* 2011). The N addition treatment received NH₄NO₃ at a rate of 10 g N m⁻² year⁻¹ on 1 day in early July before precipitation. Mowing was conducted every year in mid-August leaving 3 cm of aboveground biomass. Nitrogen addition and mowing treatments were conducted every year from 2006 to 2013.

Field sampling and chemical analysis

At the beginning of August 2013, the peak growing period for *A. frigida*, healthy and fully expanded green leaves of *A. frigida* were collected from each plot. The leaves were oven-dried at 105 °C for 0.5 h,

then at 65 °C for 48 h to a constant weight. Then the leaf samples were ground and passed through an 80-mesh sieve to measure leaf total carbon (C) and N content, as well as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. At the same time, three soil cores (0–10 cm depth) were collected at the center of each plot using a soil auger 3 cm in diameter. The soil samples were mixed and bulked into one composite sample per plot, and sieved through a 2 mm mesh to remove large roots. A thermometer and TDR-300 probe were used to measure ST and SM at 10 cm soil depth once a week during the growing season from May to September. A pH meter was used to determine soil pH with a 1:5 (weight:volume) ratio of soil to water and shaking for 30 min. Ammonia (NH_4^+) and nitrate (NO_3^-) in 10 g soil samples were extracted with 50 mL of 0.5 mol L⁻¹ K_2SO_4 solution and then analyzed using an Auto Analyzer (Continuous-Flow Analysis-CFA, SEAL, Germany). Total C and N in leaves and soils were measured using an elemental analyzer (Euro EA 3000, EuroVector, Milan, Italy). $\delta^{15}\text{N}$ (isotope ratio of $^{15}\text{N}/^{14}\text{N}$) and $\delta^{13}\text{C}$ (isotope ratio of $^{13}\text{C}/^{12}\text{C}$) in both soils and leaves were determined using an isotope ratio mass spectrometer (IRMS, Delta Plus XP, Thermo Finnigan, Berlin, Germany) interfaced with an elemental analyzer (Euro EA 3000, EuroVector, Milan, Italy). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were expressed as: $\delta^{15}\text{N} (\%) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, $\delta^{13}\text{C} (\%) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R_{sample} is the ratios of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ in the sample and R_{standard} is the ratios of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ in the standard (Wu *et al.* 2018). The standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were Pee Dee Belemnite and atmospheric N_2 , respectively. High values of $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ indicate the enrichment of ^{13}C or ^{15}N , while low values of $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ indicate the depletion of ^{13}C or ^{15}N . In our study, plant WUE was defined as leaf $\delta^{13}\text{C}$ values according to the leaf $\delta^{13}\text{C}$ values reflected by the C_i/C_a ratio, which is an important physiological index concerning stomatal behavior and WUE (Peri *et al.* 2012; Tanaka-Oda *et al.* 2010). Plant NUE was calculated by the ratio of the leaf C/N, which indicates the plant carbon gain of per unit of N (Chen *et al.* 2005; Livingston *et al.* 1999).

Statistical analysis

Three-way analysis of variance (ANOVA) was used to determine the main and interactive effects of N addition, mowing and vegetation type on soil pH, soil and plant C, soil and plant N and the ratios of soil and plant C/N. The effects of N addition and mowing on NUE and WUE and other variables (soil $\delta^{15}\text{N}$, soil N, leaf $\delta^{15}\text{N}$, leaf N) were tested using a two-way

ANOVA test for each patch. Multiple comparisons were performed to test the differences between treatments (CK, N, M, N + M) using Tukey's HSD test. Linear or nonlinear regression analyses were selected according to equation power to examine the relationships of WUE, NUE, ST, SM, leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$, NH_4^+ and NO_3^- . The above analyses were performed using SPSS version 25 (SPSS Inc., Chicago, IL, USA). To elucidate the internal regulating mechanisms of the variation of NUE and WUE under N addition and mowing, structural equation modeling (SEM) was conducted to analyze the direct and indirect effects of N addition and mowing on the NUE (leaf C/N) and WUE (leaf $\delta^{13}\text{C}$) of *A. frigida*. Soil $\delta^{15}\text{N}$, soil $\delta^{13}\text{C}$, soil C, SM, leaf $\delta^{15}\text{N}$ and leaf N were involved in the model. The SEM was implemented using Amos version 21.0 (Amos Development Corporation, Chicago, IL, USA).

RESULTS

Soil microenvironment and C and N pools

N addition and mowing and their interaction had no significant effects on ST or SM ($P > 0.05$, Table 1). Mowing significantly decreased SM by 1.4% only in the grass patch ($P < 0.05$), while N addition alone or combined N addition with mowing had no significant effect on it ($P > 0.05$). There was a significant difference in ST and SM between the two patches for all treatments and plots ($P < 0.05$, Table 1). In the control plot of each patch, SM in the grass patch was significantly higher by 3.6% than in the herb patch ($P < 0.001$), while no significant differences in ST were found ($P > 0.05$).

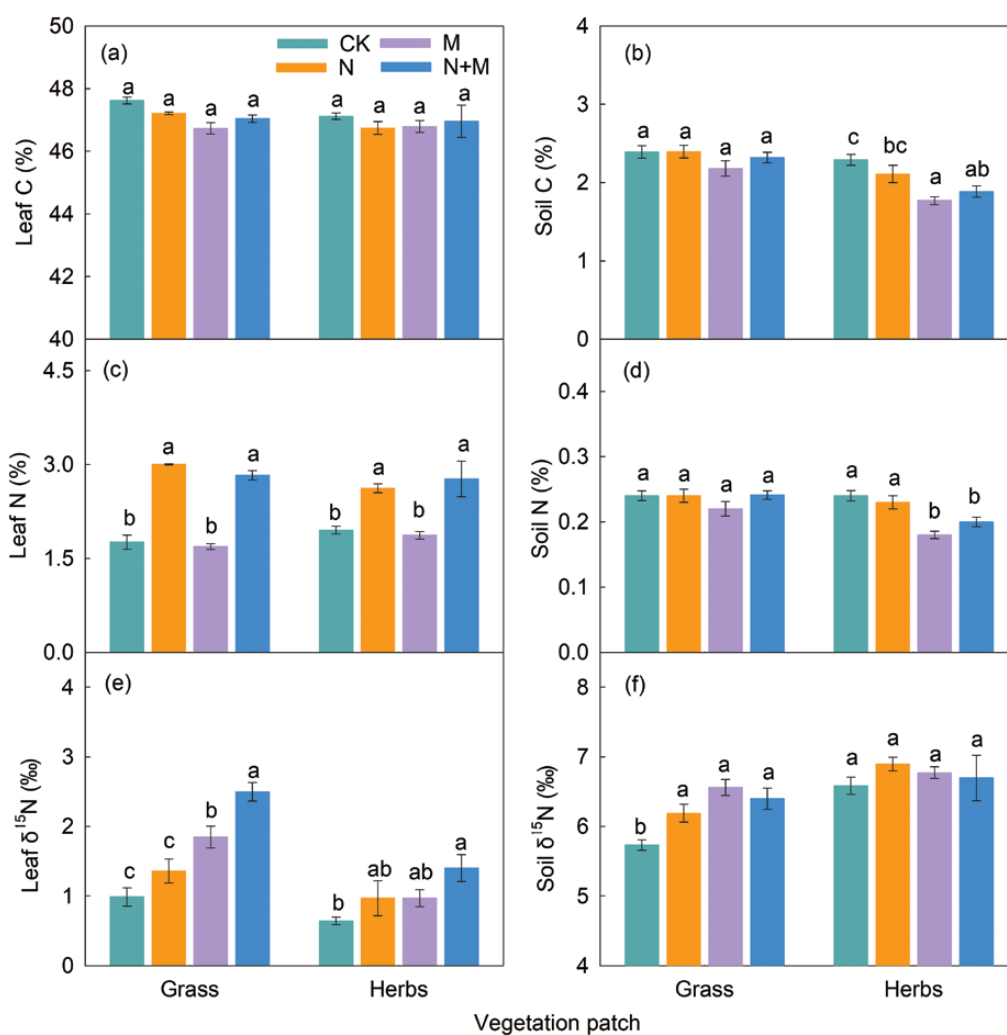
N addition had no effect on soil C or N content ($P > 0.05$, Table 1). Mowing had significant effect on soil total C and N content ($P < 0.01$, Table 1), but had no significant effect on soil C/N ratio in either patch ($P > 0.05$, Table 1). Mowing significantly decreased soil C and N in the herb patch ($P < 0.001$, Fig. 1b and d). The interactive effects of N addition and mowing significantly decreased soil C and soil N in the herb patch ($P < 0.001$, Fig. 1b and d). No significant differences in soil C and N contents in control plots were found between the two patches ($P > 0.05$, Fig. 2b and e). Soil pH values in the grass patch (7.2 ± 0.15) were significantly lower than in the herb patch (7.4 ± 0.09) ($P < 0.01$). N addition significantly decreased soil pH by 0.7 and 0.6 units in grass and herb patches, respectively ($P < 0.001$, Table 1).

N addition only significantly increased soil $\delta^{15}\text{N}$ by 7.9% in the grass patch ($P < 0.05$, Fig. 1f),

Table 1: Results (*F* values) of three-way ANOVA to test the effects of vegetation patch (P), mowing (M), N addition (N) and their interactions on soil C, soil N, soil C/N, soil $\delta^{15}\text{N}$, soil $\delta^{13}\text{C}$, soil pH, SM and ST

Source of variation	Soil N (%)	Soil C (%)	Soil C/N	Soil $\delta^{15}\text{N}$ (‰)	Soil $\delta^{13}\text{C}$ (‰)	ST (°C)	SM (v/v)	pH
Patch (P)	13.1**	31.7***	54.7***	39.6***	170***	12.7**	100***	14.2**
Mowing (M)	17.1**	22.2***	0.01	9.61**	0.06	0.54	0.18	0.25
N addition (N)	0.97	0.10	10.9**	2.63	0.62	0.05	0.07	126***
P × M	7.98**	4.28*	20.4**	10.2**	0.80	0.02	2.53	0.16
P × N	0.35	0.97	0.74	0.03	0.98	0.01	0.87	0.17
M × N	3.15	3.79	0.16	9.30**	2.38	0.09	5.56	0.27
P × M × N	0.52	0.50	0.01	0.48	0.49	0.01	1.15	2.84

*, ** and *** indicate statistically significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

**Figure 1:** The effects of N addition and mowing on (a) leaf C, (b) soil C, (c) leaf N, (d) soil N, (e) leaf $\delta^{15}\text{N}$ and soil (f) $\delta^{15}\text{N}$ in Grass and Herbs patches. Data are shown as means \pm SE. Lowercase letters above bars indicate significant difference among treatments ($P < 0.05$). CK: control; N: nitrogen addition; M: mowing; N+M: nitrogen addition plus mowing.

while it had no effect on leaf $\delta^{15}\text{N}$ in either the grass or herb patch ($P > 0.05$, Fig. 1e). Mowing significantly increased leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ in the

grass patch ($P < 0.01$, Fig. 1e and f), but had no effect on them in the herb patch ($P > 0.05$, Fig. 1e and f). The highest leaf $\delta^{15}\text{N}$ occurred in grass and

herb patches under both N addition and mowing treatments (Fig. 1e).

Response of WUE (leaf $\delta^{13}\text{C}$) of *A. frigida*

WUE of *A. frigida* ranged from -30.39% to -28.33% with a mean value of -29.23% in experimental plots. There were significant differences in WUE of *A. frigida* between grass and herb patches, with a higher value in the herb patch (-28.95%) and a lower value in the grass patch (-29.56%) regardless of treatment ($P < 0.001$, Table 2, Fig. 2d).

N addition had no effects on WUE of *A. frigida* regardless of mowing in both the grass and herb patches ($P > 0.05$, Fig. 3a), while mowing significantly increased WUE of *A. frigida* by 2.28% compared with no mowing in the grass patch ($P < 0.05$, Fig. 3a). Significant interactive effects were found between N addition and mowing on WUE of *A. frigida* in the grass patch ($P < 0.05$, Table 2, Fig. 3a). N addition reduced the positive effect of mowing on WUE in the grass patch (Fig. 3a). WUE of *A. frigida* had a significant negative relationship with SM ($P < 0.001$, Fig. 3b), while there was no relationship between WUE of *A. frigida* and ST ($P > 0.05$, Fig. 3c). In addition, no significant relationship was found between WUE and leaf N or soil N of *A. frigida* ($P > 0.05$).

Response of NUE (leaf C/N) of *A. frigida*

NUE of *A. frigida* ranged from 15.1 to 32.7 with a mean value of 21.6 in the grass and herb patches for all

treatments. There were no significant differences in NUE of *A. frigida* between the two patches in the control plots ($P > 0.05$, Fig. 2a). N addition significantly decreased NUE of *A. frigida* by 42.9% and 26.6% regardless of mowing in the grass and herb patches, respectively ($P < 0.001$, Fig. 4a). However, mowing had no effect on NUE of *A. frigida* regardless of N addition in both grass and herb patches ($P > 0.05$, Fig. 4a, Table 2). There were no interactive effects on NUE of *A. frigida* between N addition and mowing ($P > 0.05$, Table 2). The relationship between leaf $\delta^{15}\text{N}$ /soil $\delta^{15}\text{N}$ and NUE of *A. frigida* showed an inverse trend both with and without N addition (Fig. 4b). Leaf $\delta^{15}\text{N}$ increased with NUE under no N addition, but decreased with NUE under N addition (Fig. 4b). There was a significant relationship between NUE and $\Delta\delta^{15}\text{N}$ ($\Delta\delta^{15}\text{N} = \text{soil } \delta^{15}\text{N} - \text{leaf } \delta^{15}\text{N}$) (Fig. 4c). NUE showed a significant positive relationship with NO_3^- , while no relationship between NUE and NH_4^+ was found.

Direct and indirect effects on WUE and NUE of *A. frigida*

A weak positive correlation was found between WUE and NUE of *A. frigida* ($R^2 = 0.07$, $P = 0.064$). The result of SEM model showed that 47% of the variation in WUE of *A. frigida* was explained by soil $\delta^{13}\text{C}$, soil $\delta^{15}\text{N}$, SM, soil C, soil NO_3^- and leaf $\delta^{15}\text{N}$ in this degraded grassland, and was mainly controlled directly or indirectly by SM (Fig. 5). About 85% of variation in NUE of *A. frigida* was explained by soil $\delta^{13}\text{C}$, soil $\delta^{15}\text{N}$, SM, soil C, soil NO_3^- , leaf

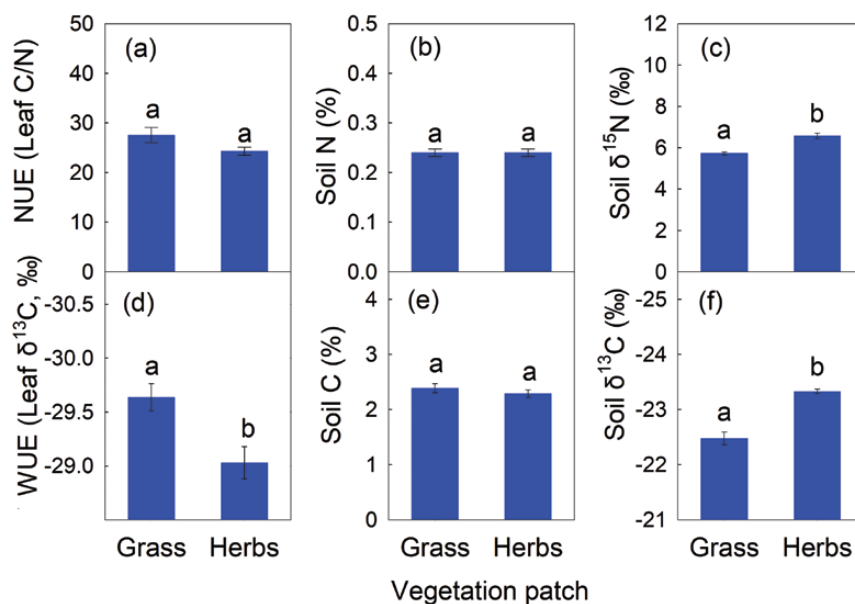


Figure 2: The effects of vegetation patches (Grass and Herbs, data in control plots) on NUE (a, leaf C/N), (b) soil N, (c) soil $\delta^{15}\text{N}$, WUE (d, leaf $\delta^{13}\text{C}$), (e) soil C and (f) soil $\delta^{13}\text{C}$. Data are shown as means \pm SE. Lowercase letters above bars indicate significant difference between vegetation patches ($P < 0.05$).

Table 2: Results (*F* values) of three-way ANOVA to test the effects of vegetation patch (P), mowing (M), N addition (N) and their interaction on leaf N, leaf C, leaf C/N, leaf $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of *A. frigida*

Source of variation	Leaf N (%)	Leaf C (%)	Leaf C/N	Leaf $\delta^{15}\text{N}$ (‰)	Leaf $\delta^{13}\text{C}$ (‰)
Patch (P)	0.07	5.38*	2.26	41.6***	42.9***
Mowing (M)	0.56	7.32*	0.23	42.9***	2.43
N addition (N)	334***	0.50	283***	17.9**	4.45*
P × M	2.12	4.82*	0.26	8.59**	4.80*
P × N	13.9**	0.07	14.3**	0.40	12.3**
M × N	0.28	8.67**	0.12	0.80	4.92*
P × M × N	2.17	0.16	1.13	0.17	1.56*

*, ** and *** indicate statistically significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

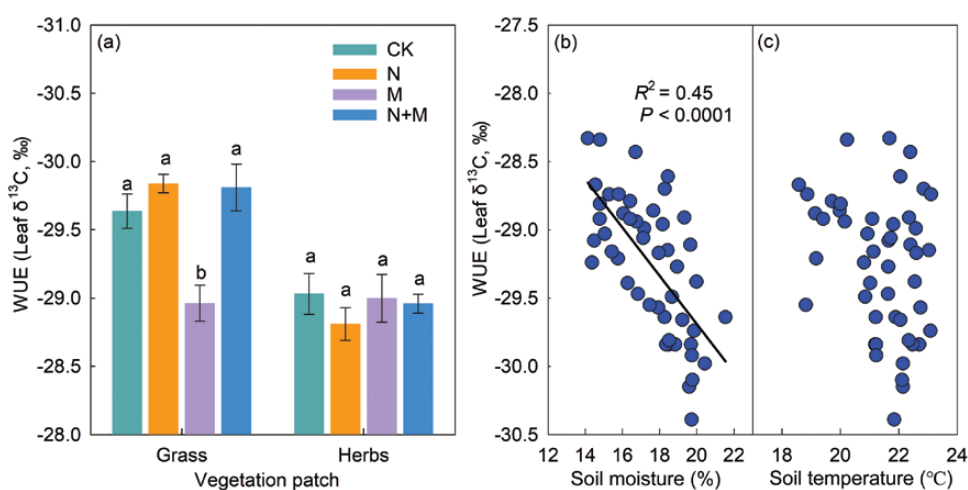


Figure 3: The effects of N addition and mowing on WUE (leaf $\delta^{13}\text{C}$) of *A. frigida* in Grass and Herbs patches (a), and the relationship between WUE and (b) soil moisture/(c) soil temperature. Data are shown as means \pm SE. Lowercase letters above bars indicate significant difference among treatments ($P < 0.05$). R^2 denotes the linear regression coefficient. CK: control; N: nitrogen addition; M: mowing; N+M: nitrogen addition plus mowing.

$\delta^{15}\text{N}$ and WUE, which was mainly directly affected by N addition (Fig. 5). Results of the SEM model showed that SM played an important role in regulating WUE and further indirectly affected the NUE of *A. frigida* (Fig. 5).

DISCUSSION

Effects of N addition and mowing on WUE

WUE is an important trait for plants adapted to arid and semi-arid environments (Chen *et al.* 2005; Gong *et al.* 2011). We found that N addition had no effect on the WUE of *A. frigida* regardless of mowing in grass and herb patches, while mowing significantly increased WUE of *A. frigida* in the grass patch. Moreover, interactive effects between N addition and mowing on WUE of *A. frigida* were found, and the addition of N reduced the positive effect of mowing on WUE in grass patch.

Our results showed that N addition increased leaf N content, but there was no relationship between WUE and leaf N content of *A. frigida*, which was consistent with previous studies (Li *et al.* 2015; Wang *et al.* 2016). In their study, WUE was mainly affected by environmental changes rather than by plant nutrient content. We found significant negative correlation between WUE and SM, while N addition had no effect on SM of the top soil (0–10 cm) during our experimental period, which led to no significant changes in WUE. However, this is inconsistent with other studies that N addition could increase plant WUE (Brueck *et al.* 2010; Chen *et al.* 2005; Gong *et al.* 2011). N deposition could enhance plant photosynthetic C gain of terrestrial plants (Liang *et al.* 2020). Chen *et al.* (2005) showed that there was a greater increase in photosynthetic rate than transpiration rate under N addition, which led to a lower water cost

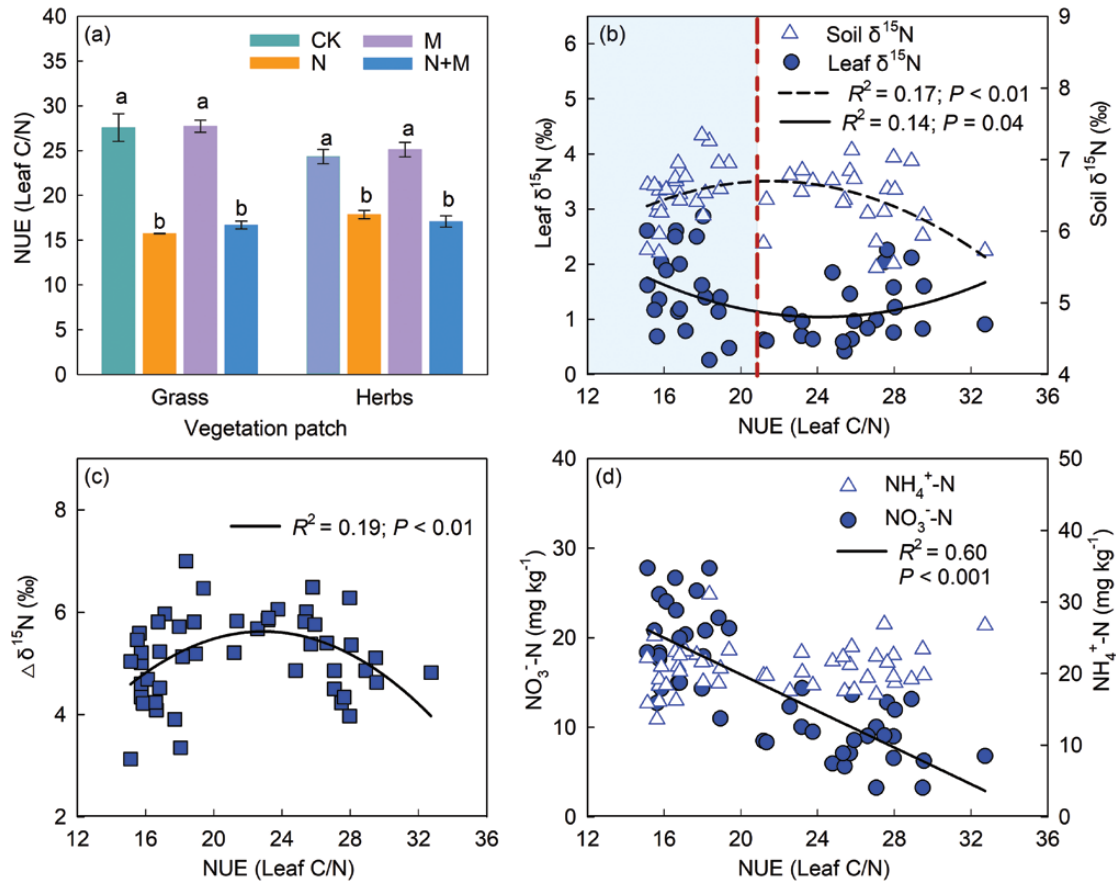


Figure 4: The effects of N addition and mowing on NUE (leaf C/N) of *A. frigida* in Grass and Herbs patches (a), and the relationship between NUE and $\delta^{15}\text{N}$ in leaf and soil (b). The relationship between NUE and $\Delta\delta^{15}\text{N}$ (soil $\delta^{15}\text{N}$ - leaf $\delta^{15}\text{N}$, c). The relationship between NUE and soil inorganic N ($\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$, d). The shaded or unshaded portion in the scatter plot (b) represents with or without N addition. Data are shown as means \pm SE. Lowercase letters above bars indicate significant difference among treatments ($P < 0.05$). R^2 denotes the quadratic regression coefficient. CK: control; N: nitrogen addition; M: mowing; N+M: nitrogen addition plus mowing.

accompanied by higher carbon sequestration through higher photosynthetic rate, thus presenting a higher WUE. In addition, increase in WUE under N addition occurred via altering plant functional traits (Gong *et al.* 2011; Liang *et al.* 2020), and showed that higher WUE under N addition was related to lower leaf N content per area (N_{area}), which was the main determinant of WUE of dominant species in the semi-arid grassland (Gong *et al.* 2011). The effect of N addition on WUE was species dependent. Chen *et al.* (2005) found that N supply had no significant effects on WUE of *Leymus chinensis*, but significantly increased WUE of *Stipa grandis*, which was mainly due to the different responses of photosynthetic rate and stomatal conductance to N addition. Gong *et al.* (2011) reported that N addition slightly increased WUE of *L. chinensis* and *Agropyron cristatum*, but had no significant effect on WUE of *S. grandis* and *A. frigida*. These studies were consistent with our results and indicate that *A. frigida* could adapt to changes in soil N conditions.

Mowing significantly decreased SM in the grass patch after long-term treatment, which increased WUE of *A. frigida* during our experimental period. However, mowing treatment did not alter the WUE of *A. frigida* in the herb patch due to lower biomass and coverage compared with the grass patch. Mowing obviously increased surface soil exposure and evaporation only in the grass patch due to the higher biomass and coverage, thus leading to the significant change in SM in the grass patch. In addition, the change in SM induced by mowing would cause change in leaf traits, which would affect WUE. Gong *et al.* (2011) found that water addition had a significant effect on leaf N content per area (N_{area}), which regulated WUE. The significant difference in WUE of *A. frigida* in grass and herb patches in our study also resulted from the difference in SM. Studies have shown that plants tend to have higher WUE when aridity increases due to closing their stomata (Swap *et al.* 2004). The SEM model also showed that the WUE of *A. frigida*

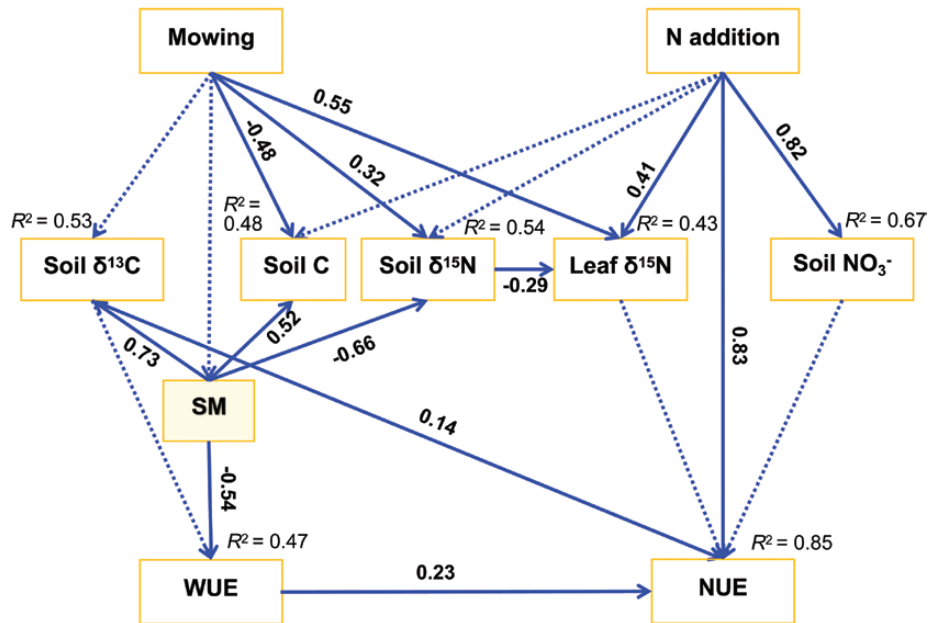


Figure 5: Structural equation model (SEM) of the effects of N addition and mowing on the NUE (leaf C/N) and WUE (leaf $\delta^{13}\text{C}$, ‰) of *A. frigida*; Soil $\delta^{15}\text{N}$, soil $\delta^{13}\text{C}$, soil C, SM, soil NO_3^- , and leaf $\delta^{15}\text{N}$ were also in the model; Results of SEM fitting: $\chi^2 = 27.63$, $P = 0.276$, $df = 24$, $n = 48$ (Note: high P -values indicate good model fit to data). Solid arrows indicate significant effects ($P < 0.1$), and dashed arrows represent the directions and effects that were not significant ($P > 0.1$). Values associated with arrows represent standardized path coefficients. The R^2 values associated with response variables indicate the percentage of variation explained by the explanatory variables.

was mainly regulated by SM. Our results suggested that mowing would increase the competitiveness of *A. frigida* in grass patch by increasing its WUE.

There was an interactive effect between N addition and mowing on WUE of *A. frigida* in the grass patch. We found that mowing increased WUE while N addition had no effect on WUE of *A. frigida* in the grass patch, but WUE did not significantly change under mowing and N addition treatment (Fig. 3a). This suggested that the addition of N mediated the positive effect of mowing on WUE in the grass patch. No significant response of SM was found under N addition and mowing treatments compared with the control. Our results indicated that N addition and mowing could promote nutrients absorption and conversion by maintaining WUE of *A. frigida*, which should be an adaptation strategy of *A. frigida* for optimizing growth under limited resources (water and N).

Effects of N addition and mowing on NUE

NUE is another important adaptation strategy for plants in infertile environments (Bridgham *et al.* 1995). We found that N addition significantly decreased NUE of *A. frigida* in grass and herb patches, while no significant differences were found under the mowing treatments. No interactive effects between N

addition and mowing were found, and mowing did not change the negative effect of N addition on NUE of *A. frigida*.

N addition significantly decreased NUE of *A. frigida* in both patches, which was consistent with our hypothesis and previous research (Han *et al.* 2014; Yuan and Chen 2015), and which was mainly due to the significant increase in leaf N content. N addition would be beneficial to plant photosynthesis, accelerate nutrient turnover, thus presenting a lower NUE. Mowing had no effect on NUE of *A. frigida* in our study due to unchanged leaf N content, which is consistent with the study of Lü *et al.* (2012), but inconsistent with Han *et al.* (2014). This could be due to species-specific N-use strategies of plants in response to mowing. Han *et al.* (2014) reported that mowing decreased NUE of *Achnatherum sibiricum*, *A. cristatum*, *Koeleria cristata*, *S. grandis* and *Carex korshinskii*, but had no effect on *L. chinensis* or *Cleistogenes squarrosa*. However, Lü *et al.* (2012) found that the NUE of *L. chinensis* and *S. grandis* were unchanged under mowing. Thus, the response of NUE to mowing is also related to plant habitat.

The significant response of NUE to N addition in our study related to the utilization of different N resources according to variation in $\delta^{15}\text{N}$ in plants and soils. $\delta^{15}\text{N}$ has been used as an indicator of

the N transformation process (Craine *et al.* 2015; Robinson 2001; Thompson *et al.* 2005). In our study, NUE (leaf C/N) and leaf $\delta^{15}\text{N}$ of *A. frigida* showed a pattern of first negative and then positive correlation, but an opposite trend between NUE and soil $\delta^{15}\text{N}$ was observed (Fig. 4b). Our results indicated that the absorption of different N sources (^{15}N or ^{14}N) was altered with the change in NUE related to the mechanism of isotope fractionation (Högberg 1997). Previous studies have shown that the $\delta^{15}\text{N}$ in plant or the difference of $\delta^{15}\text{N}$ between plant and soil could be used to indicate plant uptake of different N sources according to the different ^{15}N abundances of nitrate (NO_3^-) and ammonium (NH_4^+) (Falkengren-Grerup *et al.* 2004; Kahmen *et al.* 2008; Miller and Bowman 2002). In general, NO_3^- is often ^{15}N -depleted compared with NH_4^+ (Högberg 1997). Falkengren-Grerup *et al.* (2004) found that plant $\delta^{15}\text{N}$ value increased with a decrease in the ratio of $\text{NO}_3^-:\text{NH}_4^+$ due to the higher absorption in NH_4^+ . We deduced that *A. frigida* preferentially absorbed NH_4^+ rather than NO_3^- with increasing NUE according to the increased leaf $\delta^{15}\text{N}$ and decreased soil $\delta^{15}\text{N}$ under no N addition (Fig. 4b). This may be due to the root system preferentially selecting NH_4^+ with low assimilation energy consumption and also contributing to N conversion into plants which in turn promotes growth when N is limited (Bloom *et al.* 1992). While *A. frigida* mainly absorbed NO_3^- with increased NUE according to the decreased leaf $\delta^{15}\text{N}$ while it increased soil $\delta^{15}\text{N}$ under N addition (Fig. 4b). These two different trends (at lower and higher ranges of NUE) were related to N fertilization. Therefore, the effect of N addition on NUE of *A. frigida* was related to the absorption of different N sources to adapt to different N status (Högberg 1997). We also found a significant relationship between NUE and $\Delta \delta^{15}\text{N}$, which further indicated that the changes in NUE were related to the absorption of different N sources according to isotope fractionation. A significant negative relationship between NUE and soil NO_3^- was found, while there was no relationship between NUE and NH_4^+ . This can be explained as follows: with increasing NUE (slight N limitation), *A. frigida* preferentially absorbed NO_3^- , and further reduced soil NO_3^- . With a further increase in NUE (severe N limitation), *A. frigida* preferentially absorbed NH_4^+ , which indirectly increased soil denitrification due to the decrease in soil NH_4^+ , thus leading to lower soil NO_3^- under higher NUE. In this process, NH_4^+ did not change significantly, which was due to the continuous and stable process of organic matter decomposition. Our results showed that the

changes in soil NO_3^- induced by N addition regulates N utilization.

Soil $\delta^{15}\text{N}$ can comprehensively reflect soil N dynamics, which allows us to further study the mechanisms of changes in plant NUE under N addition. Our former study showed that N addition accelerated soil N transformation (Wang *et al.* 2015). Soil $\delta^{15}\text{N}$ value would decrease due to the dilution effect of N fertilizer, as the $\delta^{15}\text{N}$ value in fertilizer was lower than that in soil and plants (Amundson *et al.* 2003). However, N addition increased soil $\delta^{15}\text{N}$ in our study, which was caused by ^{15}N -depleted components leaving the soil (Liu *et al.* 2017; Stevenson *et al.* 2010), such as the volatilization of NH_3 and N_2O (Frank *et al.* 2004; Högberg 1997; Liu and Greaver 2009). N uptake by plants also led to the enrichment of soil $\delta^{15}\text{N}$ according to the mechanism of isotope fractionation (Högberg 1997). Therefore, the enrichment of soil $\delta^{15}\text{N}$ in the grass patch in our results indicated an increase in plant absorption, consistent with Lü *et al.* (2014), which showed that N addition enhanced plant N uptake. Kahmen *et al.* (2008) showed that soil $\delta^{15}\text{N}$ was significantly positively related to soil net mineralization rate. N addition could promote the transformation and absorption of N and accelerate the turnover of N between plants and soil, which indirectly reduces NUE to maximize plant growth. In our study, mowing also increased soil $\delta^{15}\text{N}$, while did not affect NUE. This was mainly caused by a decrease in the dilution effect for ^{15}N -depleted leaf litter returning to the soil, as plants had relatively depleted $\delta^{15}\text{N}$ compared with soil according to the fractionation mechanism (Högberg 1997).

The relationship between NUE and WUE

No trade-off between long term NUE and WUE of *A. frigida* was found in our study (Fig. 5), which did not support our hypothesis and was not consistent with previous studies (Field *et al.* 1983). Different species have different NUE and WUE. Chen *et al.* (2005) reported that a trade-off between WUE and NUE existed in *L. chinensis*, but not in *S. grandis*. However, Gong *et al.* (2011) showed that trade-offs between WUE and NUE existed in four kinds of C_3 species, including *A. frigida*, which may be related to plant habitat. In our study, the weak positive correlation between NUE and WUE of *A. frigida* occurred only in the grass patch, where *A. frigida* biomass was lower and there was more competition for survival, which showed a higher WUE and NUE to maximize competing resources. Mowing had no effect on NUE, but increased WUE of *A. frigida* in the grass patch due to changes in soil water

content, which indirectly maintained N utilization, indicating that mowing would contribute to the growth and competition of *A. frigida* in the grass patch. Our results showed that mowing would improve the competition of *A. frigida* by increasing its WUE in middle restoration stage (such as the grass patch in our study). N addition decreased NUE of *A. frigida*, while it had no effect on its WUE with or without mowing treatment, which maintained lower NUE and WUE at the same time. This showed that lower NUE and/or WUE of *A. frigida* under mowing and N addition could reduce its competition, and further accelerate restoration succession from the abandoned cropland to natural grassland. Vegetation patch had no effect on NUE of *A. frigida*, although there was a higher WUE in the herb patch due to the lower SM content and the negative correlation between WUE and soil water content, which indicated that *A. frigida* could regulate its physiology to adapt to different nutrient and water habitats (Wright *et al.* 2001). Therefore, SM plays an important role in regulating WUE and further affects NUE, which then affects restoration succession from the abandoned cropland to natural grassland in the semi-arid region.

CONCLUSIONS

N addition had no effect on WUE of *A. frigida*, but decreased its NUE, which was related to the difference in utilization of N resources (NO_3^- and NH_4^+). Mowing had no effect on the NUE of *A. frigida* regardless of N addition, while it improved WUE without N addition in the grass patch by decreasing SM. Mowing did not alter the effect of N addition on NUE, while the addition of N mediated the positive effect of mowing on WUE in the grass patch due to the unchanged SM in the N addition plus mowing treatment. There was no trade-off between the NUE and WUE of *A. frigida*, suggesting that *A. frigida* could regulate its NUE and WUE to adapt to changes in resources and environment. We highlighted that *A. frigida* could alter the absorption of N source to adapt to differences in soil N status, and N addition plus mowing treatment (maintained lower NUE and WUE in the grass patch) could accelerate restoration succession from the abandoned cropland to natural grassland. This experiment presented a general pattern of WUE and NUE of *A. frigida* based on the indicative role of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, which provided a scientific basis for studying ecosystem nutrient utilization and community succession under N enrichment and mowing.

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