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Research Article

Effects of nitrogen application on seed yield, dry matter and nitrogen accumulation of Siberian wildrye (*Elymus sibiricus* L.)

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Abstract

Siberian wildrye (*Elymus sibiricus* L.) is a perennial and self-fertilizing forage grass that support animal husbandry and environmental sustenance in the world. In order to explore influence of nitrogen(N) application in Siberian wildrye seed production, the field experiments were conducted to evaluated the effect of different N treatments (of 0, 30, 60, 90 and 120 kg/hm²) on dry matter, N accumulation, transport and utilization, yield components and seed yield of Siberian wildrye DJ-01, which was generated and stored in our lab. The results showed that the 90 kg/hm² group significantly improved Fertile tillers m⁻², spikelets per fertile tiller, 1000-grain weight and seed yield. Furthermore, the seed yield of 90 kg/hm² group reached 899.3 kg/hm² and elevated 45.6% compared with 0(CK) group. Moreover, we found that Siberian wildrye exhibited more advantages in dry matter and N translocation. The increment of grain N depended on pre-anthesis dry matter translocation (Pre-DMT) and pre-anthesis N translocation (Pre-NT), but not post-anthesis dry matter accumulation (Post-DMA) and post-anthesis N uptake (Post-NU) to seed yield. With the increase of N, N partial productivity and N absorption efficiency gradually decreased. All data showed the 90 kg/hm² had the best effects on seed yield components, seed yield, dry matter accumulation (DMA), N accumulation and N efficiency. These results provide an ideal strategy to expand the plant area of Siberian wildrye.

Keywords: distribution and transport; dry matter accumulation; nitrogen level; seed yield; Siberian wildrye

Introduction

Siberian wildrye (*Elymus sibiricus* L.) is an important perennial tufted grass in the world. It is originated in the northern of the Eurasian continent and distributed from Sweden in Europe to Japan in East Asia, and even to Alaska and Canada in North America (De *et al.*, 2021). Siberian wildrye is one of the most widely distributed wildryes in the world and has lots of advantages, such as cold resistance and adaptability (Zhang *et*

al., 2022). These characters make it play an important role for the improvement of degraded grassland and the sustainable development of animal husbandry (Zhao et al., 2017). However, like many other grass species, the fluctuation of seed development and yield of Siberian wildrye may be affected by growth conditions, physiological processes of assimilation, production and distribution, and molecular genetic factors (Wang et al., 2018). These reasons limited Siberian wildrye to being planted in the world.

Forage production is gaining more and more attention in the tropics and the subtropics of both developed and developing countries (Boval and Dixon, 2012). N is the most important macronutrient for forage and food crop production. Dida *et al.* (2021) found appropriate rate of N fertilizer could get better agronomic performance (Faji Dida *et al.*, 2021). Besides, N management alleviate the damaging effects of adverse environment. Liu *et al.* (2019) found increasing N application could alleviate yield losses caused by high temperatures in super hybrid rice during the flowering stage (Liu *et al.*, 2019). The appropriate allocation of fertilizer is crucial to ensure the accurate use of N especially in the early stage of crop growth (Anas *et al.*, 2020). In Siberian wildrye, the suitable use of N may influence the relevant indicators of forage seed yield components and furtherly have the potential to improve seed yield.

The formation process of seed yield is actually the process of dry matter production, distribution and accumulation (Wang *et al.*, 2020). Studies have shown that the appropriate treatments of N fertilizer could improve the dry matter transport, accumulation, distribution and yield of wheat (Xu *et al.*, 2021; Zhou *et al.*, 2022). In the middle and late stage of rice growth, a certain amount of N fertilizer could promote the accumulation of dry matter in the aboveground part (Luo *et al.*, 2022; Xu *et al.*, 2021). In recent years, researches related to the effect of N fertilizer of Siberian wildrye mainly focus on the effects of N application to the constituent factors of seed yield and seed vigor of Siberian wildrye (Wang *et al.*, 2017). While there are few reports on the effects on dry matter accumulation and transport of Siberian wildrye.

During the growth of Siberian wildrye, the demand of N fertilizer is rapidly growing (LI *et al.*, 2011). However, the relationship between seed yield and N application is contradictory in Siberian wildrye. It may depend on the agronomic management, such as timing of sowing, amount and method of pesticide application, the use of irrigation and the proportion of N (Dai *et al.*, 2017; Zahoor *et al.*, 2019). Therefore, it is important to declare the relationship between seed yield and N by seed yield components in different N application.

Here, we found that N treatments increased seed yields of Siberian wildrye by fertile tillers m^{-2} , spikelets per fertile tiller, seed number per plant and 1000-grain weight seed yield. Furtherly, the DMA, distribution and transport were significantly regulated by N treatments. Moreover, these effects promoted the N accumulation and transport and improved the N use efficiency. In the process of N treatments, we proved the 90 kg/hm² was an ideal treatment concentration to seed yields of Siberian wildrye, whose seed yields was 899.3 kg/hm². In summary, our study demonstrated the appropriate N was beneficial to growth and provided an ideal agronomic optimization strategy to seed yield of Siberian wildrye.

Materials and Methods

Experimental sites and varieties

The experiments were conducted in the forage and lawn experiment station of Sanping of Xinjiang Agricultural University (Xinjiang, China). It is located in the southern edge of Junggar basin. The annual precipitation is approximately 228.8 mm and the average annual evaporation is 2647 mm. The mean annual temperature is $7.2\,^{\circ}$ C. The terrain slopes gently and the 0-10 cm soil layer of the test site contains organic matter 21.50 g/kg, alkali hydrolysable N 27.00 mg/kg, available phosphorus 9.22 mg/kg, available potassium 238.94 mg/kg and pH 8.39, respectively.

Experimental design

Treatments were arranged in a randomized complete block design with three replicates. The experimental unit was 3×5 m. The field trial was established on October 2017 with seeds of 'DJ-01' Siberian wildrye, which was generated, named and stored in our lab. Seeds plots were 30 cm row spacing and sowing rate was 100 grains/m (seed purity 95% and seed germination rate 85%). N treatments were 0 (CK), 30, 60, 90 and 120 kg/hm² with urea applied at the tillering stage of Siberian wildrye (April 21, 2019). No additional N was applied in subsequent years. Weeds were controlled by hand removal. Other field management methods such as irrigation (the average irrigation amount was about 750 m³/hm² per time; the average irrigation time was 5 during the whole growth period) and weeding were the same as those of the local field.

Determination items and methods

Seed yield and its components

Yield components were measured before seed harvest except for seed weight. Fertile tillers m^{-2} were measured in three randomly selected five 50 cm sample sections in each community samples at anthesis. Thirty fertile tillers and thirty spikelets were selected randomly to determine the number of spikelets per fertile tiller, florets per spikelet at anthesis, and seeds per spikelet at the milk stage. 1000-grain weight was determined by select 200 seeds weight of three subsamples multiply by 5 from the harvested seeds obtained after air drying and cleaning.

Seed yield data were collected in 2019. At the later stage of wax ripening, cutting the reproductive branches, threshing, cleaning and weighing after natural drying, and calculating the seed yield of each plot.

Dry matter and total nitrogen of plants

At the elongation stage, flowering stage and ripening stage, sampled plants were divided into the vegetative growth (leaf + stem + leaf sheath) and reproductive stage (rachis + glume + grain). The plant parts were dried to constant weight and ground into a powder which was 105 °C for 30 minutes, baking them in an oven at 80 °C for 24 hours, weighing them and determining the DMA of Siberian wildrye. After grinding with a high-speed pulverizer, the total N content of the plant was measured with an element analyzer (Euro EA 3000 type). Plants were harvested by selecting 10 plants with consistent growth and good development in each plot at each sampling time (three replicates). The dry matter (DM) values were used to calculate and analyze dry matter production and distribution, and the total N values were used to calculate and analyze total N uptake, translocation and relevant indexes of N use efficiency (Seepaul *et al.*, 2019; Wu *et al.*, 2018).

Calculation Equation of the Relevant Indicators

The relevant indicators were calculated according to the following equation (Moradi *et al.*, 2022; Prey *et al.*, 2019).

Pre-anthesis DM translocation (Pre-DMT, g plant⁻¹) = Total aboveground DM at anthesis – DM of vegetative parts (leaves and stems) at maturity.

Pre-anthesis DM translocation efficiency (Pre-DMTE, %) = Pre-DMT/Total above ground DM at anthesis $\times 100$.

Post-anthesis DM accumulation (Post-DMA, g plant $^{-1}$) = Total aboveground DM at maturity - Total aboveground DM at anthesis.

Ratio of post-anthesis DM accumulation to total DM accumulation (Post-DMR, %) = Post-DMA/Total DM accumulation×100.

Contribution of pre-anthesis translocation or post-anthesis accumulation of DM to grain yield (DMC, %) = Pre-DMT or Post-DMA/Grain weight at maturity×100

Pre-anthesis N translocation (Pre-NT, mg plant⁻¹) = Total aboveground N at anthesis – N of vegetative parts at maturity.

Pre-anthesis N translocation efciency (Pre-NTE, %) = Pre-NT/Total aboveground N at anthesis×100.

Post-anthesis N uptake (Post-NU, mg $plant^{-1}$) = Total aboveground N at maturity – Total aboveground N at anthesis.

Ratio of post-anthesis N uptake to total N accumulation (Post-NR, %) = Post-NU/Total N accumulation $\times 100$.

Contribution of pre-anthesis translocation or post-anthesis uptake of N to grain yield (NC, %) = Pre-NT or Post-NU/Grain N at maturity×100.

Protein content (g / kg) = nitrogen content of the sample \times 6.25

Protein yield (g / kg) = protein content × Dry matter mass

Partial productivity of nitrogen fertilizer (kg/kg) = grain yield/ nitrogen application

Nitrogen harvest index (%) = grain nitrogen uptake / plant nitrogen uptake

Nitrogen absorption efficiency (%) = plant nitrogen accumulation / nitrogen application × 100%

Nitrogen use efficiency (%) = grain yield / plant nitrogen accumulation \times 100%

Data processing and analysis

The experiments were repeated two times independently, and each data point was the mean of three replicates at least. All the statistical analyses were performed using SPSS 25.0 software. All data have been represented in mean \pm SD (in case of the continuous variable) and expressed as number and percentages (in case of categorical variables). Analysis of variance was used for comparison of continuous data. Differences among the treatments were determined based on Duncan multiple comparison and independent sample t-test. All experimental data were processed by GraphPad Prism (version 6.01) software that was used to generate plot histograms. P < 0.05 was considered statistically significant in all analysis. Means followed by different letters within the same column are significantly different at P < 0.05. And means followed by the same letters are not significantly different at P < 0.05.

Results

Seed yield components and seed yield

Our results showed that fertile tillers m^{-2} increased at first and then descend with the increase of N concentration (Figure 1A). And the 90 kg/hm² group got the highest value and was significantly higher than that in CK (P < 0.05). However, we found the spikelets per fertile tiller of each groups maintained the similar number (Figure 1B), The 90 kg/hm² was significantly higher than that in CK (P < 0.05). Next, we evaluated the number of florets per spikelet and found the same trends as that in spikelets per fertile tiller (Figure 1C). The number of seeds per plant was unacted on the N and each group represented same level (Figure 1D). At last, we calculated the 1000-grain weight of each group, that 90 kg/hm² was also the best group (Figure 1E). And all data showed the trends that increased at first and then descend with the N concentration increasing (P < 0.05).

Moreover, we found that the seed yield showed a level that was from high to low with the N increasing (Figure 1F). The seed yield of each N application treatment increased by 154.0 kg/hm², 134.4 kg/hm², 410.7 kg/hm² and 235.0 kg/hm² respectively compared with that in CK (P < 0.05).

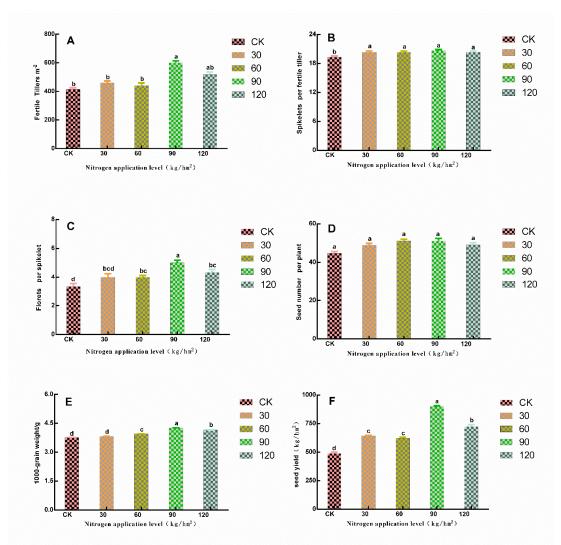


Figure 1. Effects of different nitrogen application levels on seed yield components of Siberian wildrye (*Elymus sibiricus* L.)

Notes: P<0.05 was considered statistically significantly in all analysis. Means followed by different letters within the same column are significantly different at P<0.05. And means followed by the same letters are not significantly different at P<0.05 The same as below.

Dry matter accumulation, distribution and transport

With the increase of N application, the DMA in each growth period of Siberian wildrye increased first and then decreased. And the DMA differed in different growth period (Figure 2A). We found that 60 kg/hm^2 , 90 kg/hm^2 and 120 kg/hm^2 treatments was significantly higher than that in CK in elongation stage (P < 0.05). But, the flowering stage of 60 kg/hm^2 and 120 kg/hm^2 increased more in DMA (P < 0.05). The trend of ripening stage and flowering stage was about the same, and there was a significant difference with it in control group.

Next, we furtherly found that Pre-DMT and Post-DMA of 90 kg/hm² group showed a significant change compared with that in CK group, The Pre-DMT was significantly higher than it in CK, and the Post-DMA was significantly lower than that in CK (Figure 2B, D). The Pre-DMTE and Post-DMR of 60 kg/hm² and 90 kg/hm² was significant difference with that in control group (Figure 2C, E), The Pre-DMTE was

significantly higher than that in CK, and the Post-DMR was significantly lower than it in CK. Moreover, we found that Pre-DMC increased first and then decreased with the increase of N treatments (Figure 2F). And 60 kg/hm² was the best group compared with other groups (increased by 44.97% (CK), 36.07% (30 kg/hm²), 6.96% (90 kg/hm²) and 15.69% (120 kg/hm²), respectively). However, Post-DMC showed a converse result that gradually decreased with the increase of N treatments. The level of 60 kg/ hm² was obviously lower than that in other groups.

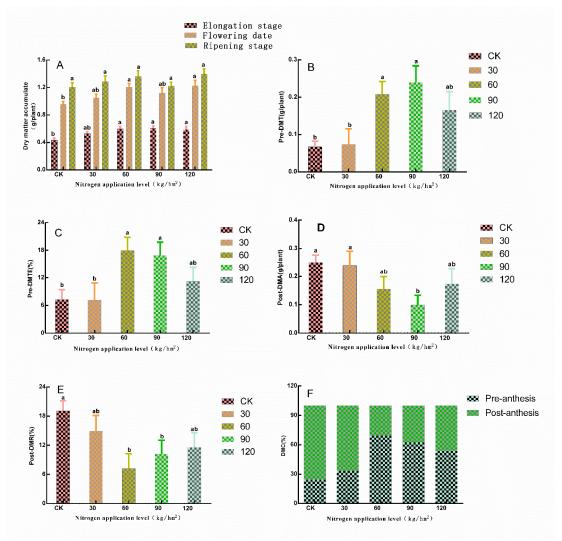


Figure 2. Characteristics of dry matter accumulation and translocation subjected to levels in nitrogen application of Siberian wildrye (*Elymus sibiricus* L.)

(A)Dry matter accumulation. (B) Pre-DMT, pre-anthesis dry matter translocation. (C) Pre-DMTE, pre-anthesis dry matter translocation efficiency. (D) Post-DMA, post-anthesis dry matter accumulation. (E) Post-DMR, ratio of Post DMA to total dry matter accumulation. (F) DMC, contribution of Pre-DMT or Post-DMA to grain of *Elymus sibiricus* L., respectively.

Characteristics of nitrogen accumulation and transport

We found that the N accumulation of Siberian wildrye, which reached the highest at ripening stage, has an increasing trend with the increase of N treatment in different growth period (Figure 3A). Moreover, the N accumulation of elongation stage also increased in same trends (90 kg/hm^2 and 120 kg/hm^2 reached a

significant level (P<0.05)). According to the elongation stage, the flowering stage got the similar results that the treatments of 60 kg/hm², 90 kg/hm² and 120 kg/hm² reached significant levels (P<0.05). Then, we also evaluated the N accumulation of ripening stage and found an increasing trend that 90 kg/hm² group got the highest level.

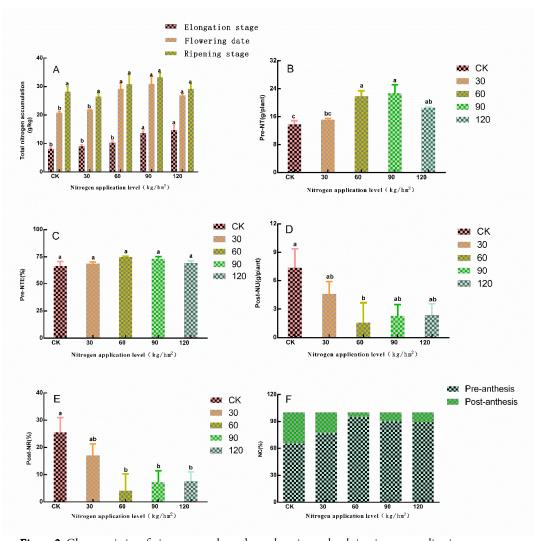


Figure 3. Characteristics of nitrogen uptake and translocation to levels in nitrogen application (A) Total nitrogen translocation. (B) Pre-NT, pre-anthesis nitrogen translocation. (C) Pre-NTE, pre-anthesis nitrogen translocation efficiency. (D) Post-NU, post-anthesis nitrogen uptake. (E) Post-NR, ratio of Post-NU to total nitrogen accumulation. (F) NC, contribution of Pre-NT or Post-NU to grain of Siberian wildrye (*Elymus sibiricus* L.) respectively.

Next, we evaluated the N transport in Siberian wildrye. And the Pre-NT and Pre-NTE showed the trends that increased firstly and then decreased (Figure 3B, C). The Pre-NT of 90 kg/hm² group was significantly higher than that in CK group. 90 kg/hm² group was significantly higher than that in control group. Then, we detected the Post-NU and Post-NR (Figure 4D, E). The Post-NU of 60 kg/hm² group was significantly lower than that in CK group. At last, we explored the relations with NC (Figure 3F). The contribution of Pre-NC increased firstly and then decreased. The 60 kg/hm² group increased by 29.07% compared with that in CK.

Nitrogen use efficiency

Protein yield increased first and then decreased with the increase of N application level, and reached the highest at 60 kg/hm² N application level (Figure 4A). Then with the increase of N treatments, N partial productivity and N absorption efficiency decreased significantly (P < 0.05) and the N use efficiency increased firstly and then gradually decreased, which the highest level was in 90 kg/hm² group (27.32%) and increased 9.88% than that in CK group (Figure 4B, C, D). And the N harvest index was not obvious with the increase of N application (Figure 4E).

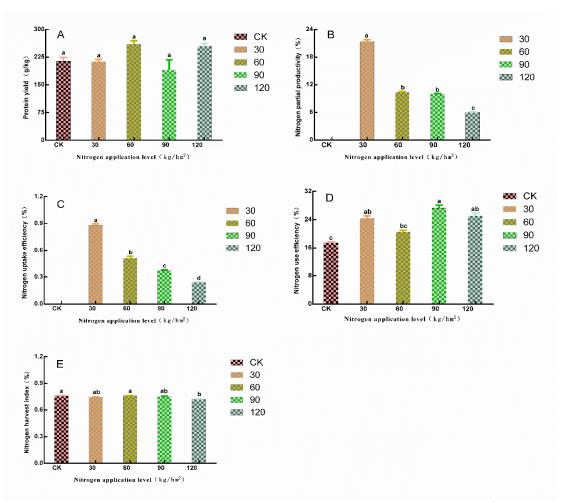


Figure 4. Effects of different nitrogen application levels on nitrogen efficiency of Siberian wildrye (*Elymus sibiricus* L.)

Relationships between Nitrogen and seed yield traits

N directly and significantly influenced Pre-DMT and Flowering date DMA (P<0.01). Then, these alterations significantly increased N use efficiency and 1000-grain weight (P<0.01). Eventually, N indirectly and significantly increased seed yield (P<0.01). Besides, we also observed Pre-DMT could indirectly and significantly increased 1000-grain weight and seed yield.

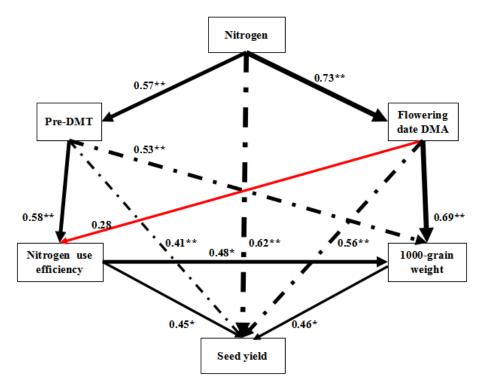


Figure 5. Structural equation modeling analysis for direct and indirect effects of nitrogen on the seed yield via pathways of yield components. Black lines indicate a positive correlation, whereas red lines indicate a negative correlation. Solid lines indicate direct relationships and dashed lines indicate indirect relationships. The width of the line is proportional to the strength of the path coefficient. * And * * represent significant differences at the 0.05 and 0.01 levels, respectively. This hypothetical model is consistent with our data, $\chi 2 = 3.631$, P = 0.135, GFI = 0.993, CFI = 0.991

Discussion

As an important grass species worldwide, Siberian wildrye has been recently researched from different perspectives (Xie *et al.*, 2020; Xie *et al.*, 2017; Zhang *et al.*, 2016). The seed yield of grass species is greatly dependent on inflorescence morphological traits, starting with spikelets per inflorescence and seeds per spikelet, to kernel size, and then to awns (Dreccer *et al.*, 2019; Severova *et al.*, 2022). Besides, Seed yield in grasses is also known to be associated with a number of seed yield components, such as by fertile tillers m⁻², spikelets per fertile tiller, seed number per plant and 1000-grain weight seed yield (Bhutto *et al.*, 2016; Vafa *et al.*, 2021; Ye *et al.*, 2019). These traits are important and complex to realize high seed yields. Analysing the processes influencing seed yield components contribute to improve the understanding of seed yield (Jing *et al.*, 2021). N plays an important role in growth and development of crops. Some studies have showed that N application significantly increased fertile tillers of *Lolium multiflorum*, seeds number per spikelet and seed number per unit area (Svečnjak *et al.*, 2022). A large number of studies have proved that appropriate N fertilizer treatment is beneficial to improve grass seed yield (Canto *et al.*, 2020). Here, we proposed that 90 kg/hm² was an ideal concentration for seed yields of Siberian wildrye, and the seed yields was 899.3 kg/hm². And our study demonstrated the appropriate N was beneficial to the growth of Siberian wildrye and provided an ideal strategy to seed yield.

In general, crop N requirement is mainly determined by dry-matter production. The dry-matter production of different crop species is influenced by the crop growth stage and photosynthetic variations (Raza et al., 2020). The DMA is a complex process, under a strong influence of the environment, cultivar and their interaction (Bhattacharya, 2021; Lammerts et al., 2017; Pszczółkowski et al., 2021). Characterizing the ecophysiological factors responsible for DMA is essential for understanding the high-yielding conditions for Siberian wildrye. It has been reported that each 1.45-kilogram increase in total DMA, the yield increases by 1 kilogram. And these data varied by specific field environments (Gaspar et al., 2017). Likewise, our results indicated that appropriate N treatments could improve the DMA, Pre-DMT and Pre-DMTE of Siberian wildrye. These results proved that N application could promote the distribution of dry matter to improve seed yield. Furtherly, our data suggested that fertilization could significantly promote the accumulation, distribution and translocation of dry matter and furtherly affect the seed formation, but the dry matter weight would decrease when the appropriate amount of fertilizer was exceeded. Some studies showed that suitable N application increased the dry matter weight of wheat and promoted the transfer of Pre-DMC from plant vegetative organs to grains, but excessive N application inhibited the transport of Pre-DMT, pre anthesis dry matter from leaves (Du et al., 2022), stems and sheaths to grains, which was consistent with our results.

It has been proved that proper N fertilizer promote the accumulation and transport of N in wheat, and furtherly improve the N use efficiency (Ranta *et al.*, 2021). The increase in DMA is largely a consequence of increased N treatments to some extent. Previous studies showed that N treatments could increase dry matter accumulation and transport efficiency, which was the primary method to obtain high seed yield (Yu *et al.*, 2021). Huang *et al.* (2022) proved that the improvement of N use efficiency was a consequence of enhancing N and dry matter translocation. Besides, Ma and Herath (2016) found aNUE and N-utilisation efficiency decreased with increasing N fertiliser rates, but NupE varied among environments with increasing preplant and side-dressed N application (Ma and Herath, 2016). Our data showed the advantage of Siberian wildrye N accumulation mainly lying in the Pre-NT at maturity date under different N application treatments. With the increase of N application rate, the Pre-NC gradually increased, which indicated that increasing yield should pay attention to the Pre-NT that is conducive to improving the transport of nutrients to grain before flowering and furtherly getting higher seed yield.

Previous studies have revealed that N treatments increase dry matter accumulation at anthesis (Liu *et al.*, 2020,). However, the same N treatments also directly affect 1000-grain weight (Wang *et al.*, 2020). In this study, our results showed that the effect on 1000-grain weight of N treatments was mainly mediated by the accumulation of dry matter at flowering stage, which contributed to the increase in 1000-grain weight and subsequently increased seed yield. Moreover, it was also found that N could increase the amount of Pre-DMT and promote N use efficiency, which in turn increased seed yield. The Post-DMA is essential for seed yield improvement (Wu *et al.*, 2018). In the present study, we found that the Pre-DMT was a major influence factor determining the seed yield of Siberian wildrye. While the Post-DMA had a minor effect on seed yield of Siberian wildrye. Interestingly, these traits were not consistent with those in annual crops. As far as the great majority of perennial crops, there is a tendency that lots of nutrients are transferred to be subsurface nutrients during the post growth period, and then stored in the roots, which enable the crop plants to survive in winter and enhance the ability of plants regeneration in the next year (Nkebiwe *et al.*, 2016). As a perennial forage, Siberian wildrye may also have the same tendency that nutrients are transferred to the root system during the late period of growth, which reduce the impact of Post-DMA to seed yield.

Conclusions

N application can promote the amount of dry matter and N accumulation of Siberian wildrye, and furtherly improve the Pre-DMT, Pre-DMTE, Pre-DMC, Pre-NC, eventually enhance the N use efficiency and

boost seed yield by increasing the number of reproductive branches, spikelets and 1000-grain weight. Besides, we ensure the 90 kg/hm 2 N fertilization treatment is an ideal application for the best seed yield and N use efficiency of Siberian wildrye. These results provide an innovation insight of popularizing Siberian wildrye to be planted in the world.

Authors' Contributions

R.W. and F.T. performed the entire experiment, analyzed all results, and drafted the manuscript. S.Z. designed the entire experiment and was in charge of manuscript revisions. W.X. and Y.W. interpreted the results and prepared the manuscript. B.Z., Y.W., S.Z. and Y.Z. provided resources. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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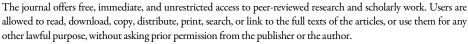
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