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Short Communication

Development of a critical nitrogen dilution curve of Siberian wildrye for seed production

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A B S T R A C T

The critical nitrogen dilution curve (CNDC) is usually used as an efficient method to diagnose nitrogen (N) status of crop plants. However, there is no successfully developed CNDC for forage species seed production. The objectives of this study were to develop an appropriate CNDC in seed production and to manage nitrogen application accurately in seed fields of Siberian wildrye (*Elymus sibiricus* L.). Two experiments were carried out with N application treatments (0–225 kg N ha^{-1}) in two successive growing seasons (2014 and 2015) at Yuershan Farm in Hebei Province, China. Shoot biomass (t ha^{-1}), nitrogen concentration (percentage of dry matter), and seed yield (kg ha^{-1}) were measured to calculate critical N concentration, development and validation of the CDNC. The CNDC for Siberian wildrye seed production was developed with the equation $Nc = 3.00\,W^{-0.32}$ (determination coefficient 0.97), based on shoot biomass (between 0.9 and 7.1 t ha^{-1}) and its N concentration. According to the independent data set grouped by seed yield, the developed CNDC could adequately identify the situations of N-limiting seed yield and N non-limiting seed yield before and during anthesis stage, the optimum seed yield was reached at around NNI = 1. The CNDC developed in this study provides insight to improve N diagnosis and management in Siberian wildrye seed production under rain-fed conditions.

1. Introduction

Siberian wildrye (*Elymus sibiricus* L.) is a nitrogen (N) susceptible forage grass and N application can improve its seed yield at different stand ages (Mao et al., 2001; Zhang et al., 2001; Zhao et al., 2012). Consequently, N application is widely used in seed production and is one of the important factors affecting seed yield and quality. Insufficient N application results in reduced seed yield and reduced profits for growers. However, excessive N application does not produce a substantial increase in seed yield due to the principle of diminishing returns (Cassman et al., 2003) and results in increased costs. Moreover, excessive N fertilization exceeding plant requirements is a potential nitrate pollution source for surface and ground water (Mary, 1997). Optimum N application varies depending upon difference in plant density, soil fertility, and climate condition (Black and Reitz, 1969; Zhang et al., 2001; Gao et al., 2010). Therefore, an agronomic tool that could detect N deficiencies and excesses in crops or forage species should be investigated further.

The critical N dilution curve (CNDC) has the potential to diagnose the N status of crop plants. This curve is based on the concept of critical N concentration defined as the minimum N concentration required for maximum crop growth (Ulrich, 1952) and is derived from the set of critical N concentrations. The CNDC has been determined for a number of crop species, including rice (*Oryza sativa* L.; Ata-Ul-Karim et al., 2013), winter wheat (*Triticum aestivum* L.; Justes et al., 1994; Yue et al., 2012), winter rape (*Brassica napus* L.; Colnenne et al., 1998), corn (*Zea mays* L.; Ziadi et al., 2008) and spring wheat (*T. aestivum* L.; Ziadi et al., 2010). However, these critical N dilution curves reported by previous research were all different in the coefficient of the equation, which indicated interspecies dissimilarities (Justes et al., 1994). To manage N application precisely, every species should have its critical N dilution curve based on morphological and eco-physiological characteristics (Lemaire and Gastal, 1997).

Developing CNDC in forage crops is complicated due to difference in its usage. CNDCs for forage production have been developed in many forage species, such as annual ryegrass (*Lolium multiflorum* L.; Marino et al., 2004), tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh.; Lemaire and Denoix, 1987; Lemaire and Salette, 1984), alfalfa (*Medicago sativa* L.; Lemaire et al., 1985), and forage brassicas (Fletcher and Chakwizira, 2012). These developed CNDCs were based on management strategies for hay production, which are different in their coefficient of curves equation and have more than 0.66 in determination coefficient of their curves equation. However, in forage species developing the CNDC for seed production is more challenging than for hay

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production, and no suitable critical N dilution curve has been developed in grass for seed production (Gislum and Boelt, 2009). This is due to the fact that seed production in term of biomass represents only a very small fraction of the total above-ground biomass. There is no information on whether the minimum N concentration required for maximum crop growth could ensure the maximum seed production. Therefore, the CNDC developed in forage species for seed production should be further investigated.

Siberian wildrye is one of the most globally important perennial bunchgrass species and is usually used to build grasslands and recover degenerated rangeland due to its drought resistance and cold tolerance. The objective of this study was to develop a CNDC in Siberian wildrye for seed production, and to assess the reliability of this newly developed curve by validating it with other data and comparing this curve with existing critical N dilution curves for forage species. The projected results will provide a new strategy for N management in Siberian wildrye seed production in rain-fed conditions.

2. Materials and methods

2.1. Field experiments

Two field experiments were conducted at the Grassland Research Station at China Agricultural University located at the Yuershan farm, Hebei Province, China (41°44′ N, 116°8′ E, elevation 1455 m) from 2012 to 2015. Both experiments for N application rates (0–225 kg N ha^{-1}) were arranged in a completely randomized block design with four replications. For experiment 1, plot size was 6 m × 6 m and application rates were from 0 to 225 kg N ha^{-1} with an interval of 45 kg N ha^{-1}. For experiment 2, plot size was 5 m × 4 m and application rates were from 0 to 150 kg N ha^{-1} with an interval of 30 kg N ha^{-1} (Table 1). The field experiments were tilled with a chisel plow and a disk harrow before establishment. The seeding rate of each experiment was 33 kg ha^{-1} (96.8% purity, 71% germination), and the row spacing was 0.3 m. At sowing, 60 kg phosphorus (P_2O_5) ha^{-1} was applied as calcium superphosphate. Urea was used as the N source in both experiments and was applied at the initiation of tillering stage. After N application, plots were irrigated (60 mm) to minimize volatilization, after which no further supplemental irrigation was provided. Weeds were controlled by hand removal.

2.2. Plant sampling and tissue N determination

Plant samples were cut at ground level with three repetitions from each plot. Each cut was a 0.3 m row segment. The sampling period (Table 1) varied but the main sampling occurred from active tilling to the anthesis stage. Each sampling time in each experiment was considered its own dataset. Shoot biomass (t ha^{-1}) was determined after oven-drying each sample at 80 °C for 24 h. The samples were subsequently ground to powder to pass through the sieve (1 mm) in a mill. After that, total N concentration in shoot biomass was determined by the micro-Kjeldahl method (Bremner and Mulvancy, 1982). When seed moisture content was down to 40%–45% (Mao et al., 2003), three 1 m row segments were harvested carefully by hand in each plot. Samples were threshed and cleaned when the moisture content was approximately 10%. Then, seed yields (kg ha^{-1}) were calculated after the samples were weighed.

2.3. Determination of critical N concentration

To determine the critical N concentration (Nc), the datasets (1–10) were used to determine the Nc according to the method described by Justes et al. (1994). In each dataset, the shoot biomass (t ha^{-1}) under different N rates and the corresponding N concentration were compared by the analysis of variance (ANOVA). The datasets, where it could be divided into the N limiting group and the N non-limiting group, were used to determine the Nc. The N limiting group was defined by an increasing in N application rate having significant effects on shoot biomass. The N non-limiting group was defined by an increasing in N application rate having no significant effect on the shoot biomass but having a positive effect on N concentration. Then, two linear regression models were developed on shoot biomass and N concentration based on the two groups. The critical point was the intersection of the vertical regression line and an oblique regression line. These data points were used to fit the most commonly used power function as shown in the following equation:

$$Nc = a\,W^{-b} \tag{1}$$

where Nc is the critical N concentration at one sampling, W is the corresponding shoot biomass at the same sampling, and a and b are unknown parameters to be estimated.

2.4. Validity of the established CNDC

The developed CNDC was validated qualitatively using independent datasets (11–14) from Exp.2 conducted in 2015 by two ways. One way was that the datasets were divided into N limiting growth and N non-limiting growth group according to the method of determining Nc. Then, the two groups' data points were plotted together with the CNDC to test whether the established CNDC was reliable in discriminating between them. The other method was to test whether the CNDC could show if N limited the seed yield according to the method described by Gislum and Boelt (2009). In each dataset, the effects of N application on seed yields were compared by ANOVA. If the effect of N application was

Table 1
Basic information about field experiments.

Experiment	Dataset	Sowing date	Harvest year	N application rate (kg ha^{-1})	Sampling date	Soil characteristics	Mean monthly precipitation (mm)
Exp. 1	1–3	July 2012	2014	0, 45, 90, 135, 180, 225	24 June, 1 July, 8 July,	Soil type = Sandy loam	Apr. 10.41, May 31.75,
Exp. 1	4–7	July 2012	2015	0, 45, 90, 135, 180, 225	18 June, 29 June, 11 July, 20 July	Soil pH = 7.57	Jun. 114.30, Jul. 58.17,
						OM = 12.82 g kg^{-1} Available N = 46.48 mg kg^{-1} Available P = 1.33 mg kg^{-1} Total K = 22.99 g kg^{-1}	Aug. 33.02.
Exp. 2	8–10	July 2013	2014	0, 30, 60, 90, 120, 150	24 June, 1 July, 8 July,	Soil type = Sandy loam	Apr. 9.14, May 23.37,
Exp. 2	11–14	July 2013	2015	0, 30, 60, 90, 120, 150	18 June, 29 June, 11 July, 20 July	Soil pH = 7.70	Jun. 73.66, Jul. 112.27, Aug. 16.51.
						OM = 30.55 g kg^{-1} Available N = 96.42 mg kg^{-1} Available P = 3.80 mg kg^{-1} Total K = 22.78 g kg^{-1}	

significant, the seed yields were divided into the N limiting seed yield and N non-limiting seed yield groups. The N limiting seed yield group is defined as a group for which supplemental N application leads to a significant increase in seed yield. The N non-limiting seed yield group is defined as a group for which the seed yield is already at its highest and which supplemental N application could not significantly increase. The corresponding plant N concentration and shoot biomass of the two groups were plotted together with the established CNDC to show whether the data from the two groups were placed above or below the CNDC.

2.5. Calculation of nitrogen nutrition index

The developed CNDC was also validated quantitatively using independent datasets (11–14) from Exp. 2 conducted in 2015 by a relative seed yield response curve to N nutrition index. The N nutrition index (NNI) was determined by dividing the actual N concentration of shoot biomass (Na) by Nc according to previous reports by Lemaire et al. (1989), as Eq. (2).

$$\mathrm{NNI} = \frac{\mathrm{Na}}{\mathrm{Nc}} \tag{2}$$

If NNI = 1, N nutrition at that N treatment is considered optimal, while NNI > 1 indicates excess N and NNI < 1 indicates N deficiency.

The relative seed yield (RSY) was calculated with Eq. (3).

$$\mathrm{RSY} = \frac{\mathrm{Ya}}{\mathrm{Yc}} \tag{3}$$

where Ya is the actual seed yield and Yc is the lower seed yield in the Non-limiting seed yield group.

2.6. Data analysis

The ANOVA were performed using SAS v.8.0 software (Cary, 2001) and means used to assess difference between different treatments were compared by Duncan's new multiple rage test with a significance level of 0.05. The parameters of the power function were conducted using the standard curve fitting tool in the program Sigmaplot 12.0 (Systat Software, San Jose, California, USA).

3. Result

3.1. Determination of a CNDC for Siberian wildrye seed production

There were 9 datasets that fulfilled the statistical criteria for the Nc calculation from 10 datasets. Nc points, which were determined by the intercept between the oblique and vertical lines in each dataset, presented a gradually declining trend (Fig. 1). Nc decreased from a maximum of 3.1% to a minimum of 1.73% while the corresponding shoot biomass increased from a minimum of 0.9 t ha^{-1} to a maximum of 7.1 t ha^{-1}. The declining trends of Nc points were fitted with the most commonly used power function with the equation Nc = 3.0 W$^{-0.32}$, with a determination coefficient (R^2) of 0.97. The model accounted for 97% of the total variance (Fig. 2).

3.2. Validation of the CNDC

The CNDC could effectively discriminate between N limiting and N non-limiting situations within the range for which it was established. All data points from N limiting group were located under the curve, while those from N non-limiting group were close to and above the curve (Fig. 3). When the CNDC was validated with datasets grouped basing on seed yield, all data points from N limiting seed yield treatments were under the curve, whereas those from N non-limiting seed yield treatments regularly located on both sides of the CNDC. Data points of N non-limiting seed yield sampled on June 18 and 29 were

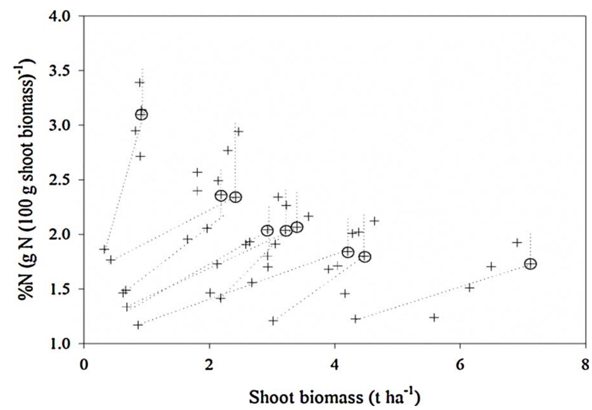

Fig. 1. Shoot N concentration (%N) vs. shoot biomass for datasets (+). Nc is defined as the intersection data between the vertical and oblique dotted lines for each dataset indicated with a circle.

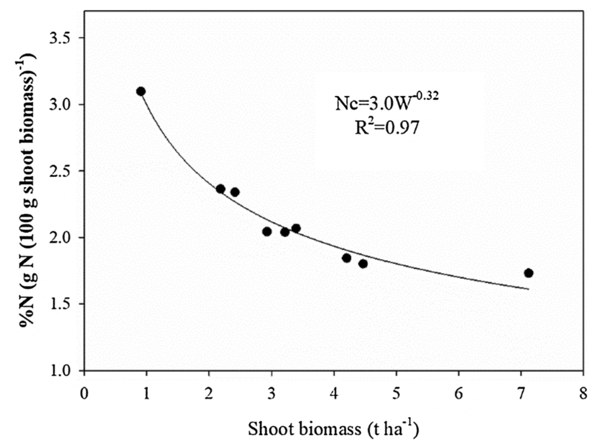

Fig. 2. Nc points and CNDC obtained by non-linear fitting under different N application rates in experiments conducted during 2014 and 2015.

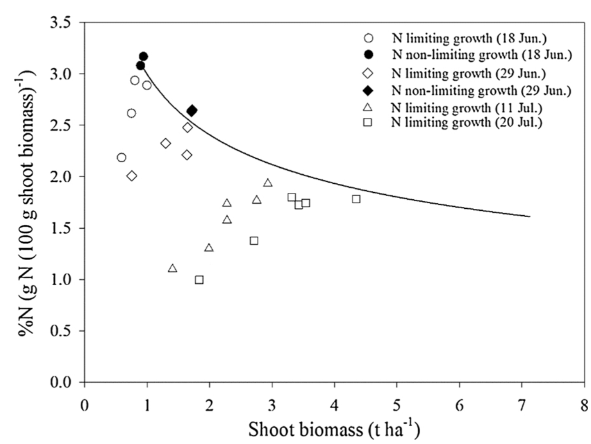

Fig. 3. Validation of the CNDC using independent datasets (from Exp. 2 conducted in 2015) grouped by growth.

close to and above the curve, while those sampled on July 11 and 20 were close to and below the curve (Fig. 4).

3.3. Relationship between NNI and RSY

NNI was plotted against RSY at different sampling times in Exp. 2 in 2015 to quantify the relationship between NNI and RSY (Fig. 5). Both NNI and RSY increased with N fertilization level. Additionally, there was a linear relationship between NNI and RSY in four sampling time.

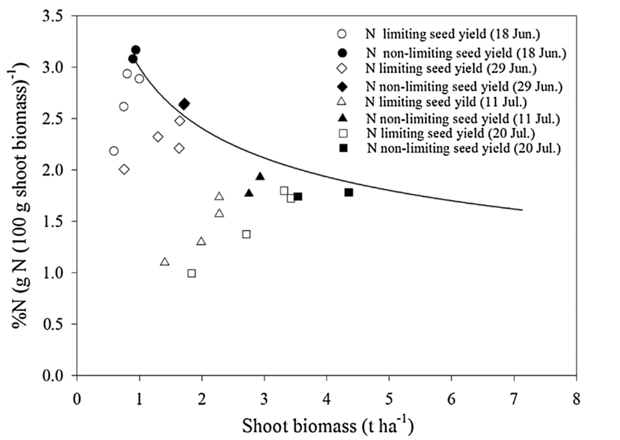

Fig. 4. Validation of the CNDC using independent datasets (from Exp.2 conducted in 2015) grouped by seed yield.

At the heading (June 18; Fig. 5, A) and anthesis stages (June 29; Fig. 5, B), RSY increased linearly with NNI and reached 1 at around NNI = 1.0. However, at milk and dough stage, NNI could not reach 1.0. RSY was reached 1 at around NNI = 0.8.

4. Discussion

The shoot biomass is an important factor in determining the application of the CNDC. In the present study, the developed CNDC was suitable for shoot biomass ranging from 0.9 and 7.1 t ha^{-1}, which was different from that of other forages and crops presented in Table 2. In addition to difference in plant growth characteristics between species, this may be due to the sampling period and planting density in the experiments. Plant growth is a process of dry matter accumulation, and the shoot biomass increases with plant development: thus, in the crop development stage, the longer the sampling period interval, the greater the range of suitable shoot biomass. Studies on perennial ryegrass and red fescue (Gislum and Boelt, 2009) showed that the developed CNDC was suitable for a shoot biomass between 2.3 and 13.8 t ha^{-1} for sampling from the elongation to the maturity stages, while when sampling from the elongation to the heading stage, the established CNDC was suitable for a shoot biomass between 2.3–6 t ha^{-1}. Similar results found in annual crops, including studies on the developing CNDC of maize (Plenet and Lemaire, 2000) showed that the shoot biomass ranged from 1 to 11.7 t ha^{-1} for sampling from emergence to the silking stage, while when sampling from emergence to maturity, the shoot biomass ranged from 1 to 22 t ha^{-1}. This indicated that the maximum shoot biomass increased with the later sampling stage. However, when the sampling period was the same, the maximum shoot biomass of the CNDC developed in annual ryegrass was lower than the annual crops of maize, winter wheat, and rice (Table 2). Planting density may also influence the shoot biomass range of the developed CNDC. Wang et al. (2012) developed a CNDC in cotton at the 7.5×10^4, 9.8×10^4, and 12×10^4 plants ha^{-1} planting density: the corresponding shoot biomass range was 0.12–7.07, 0.15–8.97, and 1.55–9.52 t ha^{-1}, respectively. This indicated that the shoot biomass range increased with increasing planting density (range of routine crop density). Similar responses have been shown in brassica crops. The shoot biomass range of the CNDC developed in forage brassicas (Fletcher and Chakwizira, 2012) was from 3.4 to 27 t ha^{-1}, while for the CNDC developed in oilseed rape (Colnenne et al., 1998), the shoot biomass was from 0.88 to 6.3 t ha^{-1}. The row spacing of forage brassicas was 0.15 m, which was much narrower than oilseed rape row spacing, which was set at 0.4 m. In forage crops, row spacing of forage seed fields was usually wider than 0.3 m, whereas forage fields usually used broadcast sowing or row spacing narrower than 0.3 m. This resulted in different shoot biomass per unit area in same forage species intended for different usages, thus leading to different shoot biomass ranges and CNDCs.

Parameters of CNDC are major factors in determining its curve trend and specificity. Parameter *a* represents the N concentration in the shoot

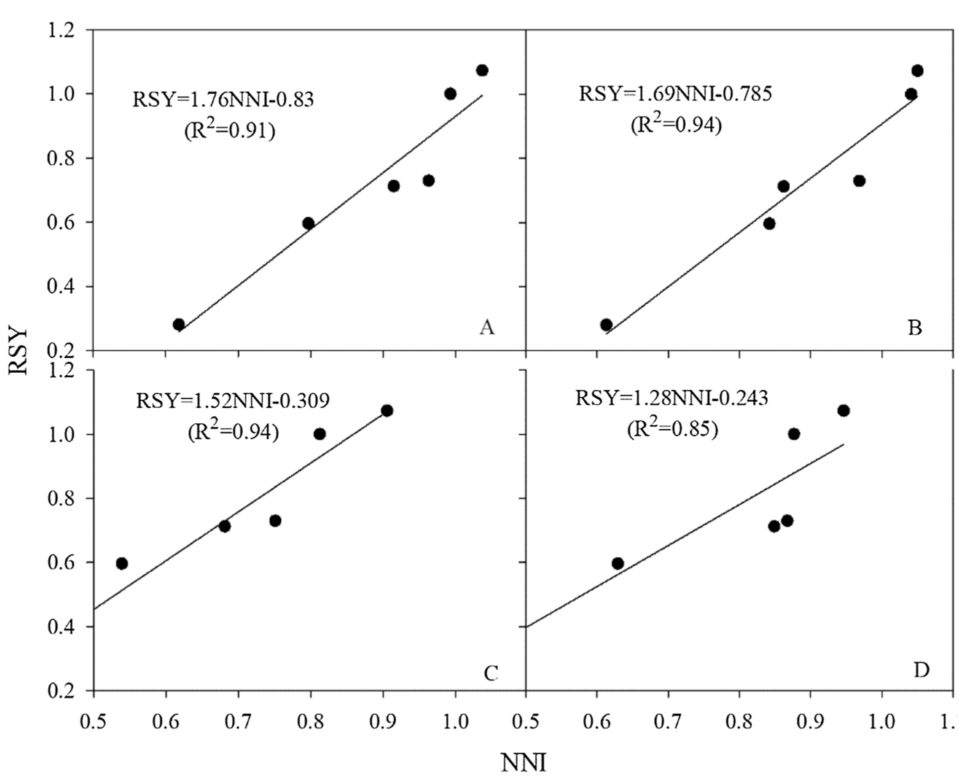

Fig. 5. Changes of N nutrition index (NNI) in different sampling times with relative seed yield (RSY) for Siberian wildrye conducted in Exp. 2 in 2015. (A, June 18; B, June 29; C, July 11; D, July 20).

Table 2
The related values found in the literature about the regression Nc = aW^{-b}, where Nc is the critical shoot N (in g N (100 g shoot biomass)$^{-1}$) and W is the shoot biomass dry weight (in t ha^{-1}), a and b are the coefficients of the regression, sample date is the plant development stage to build the regression, and biomass range is the application range of the regression.

Species	a	b	R^2	Sample date	Biomass range (t ha^{-1})	References
Siberian wildrye	3.0	0.32	0.97	elongation to dough stage	0.91–7.13	This article
Annual ryegrass	4.07	0.38	0.69	Tillering to anthesis stage	0.5–7	Marino et al. (2004)
Perennial ryegrass and	6.36	0.71	–	elongation to maturity	2.3–13.8	Gislum and Boelt (2009)
Red fescue	–	0.60		elongation to heading stage	2.3–6	
Tall fescue	4.8	0.32	–	–	–	Lemaire and Denoix (1987)
Alfalfa	4.6–5.5	0.29–0.36	0.98	–	–	Lemaire et al. (1985)
Forage brassicas	5.53	0.47	0.78	three-leaf to anthesis stage	3.4–27	Fletcher and Chakwizira (2012)
Winter oilseed rape	4.48	0.25	–	three-leaf to anthesis stage	0.88–6.3	Colnenne et al. (1998)
Sunflower	4.53	0.42	–	Four-leaf to anthesis stage	0.75–8.60	Debaeke et al. (2012)
Rice	3.53	0.28	0.803	Tillering to anthesis stage	1.55–12.73	Ata-Ul-Karim et al. (2013)
Winter wheat	4.15	0.38	0.87	Tillering to anthesis	1–10	Yue et al. (2012)
Maize	3.41	0.373	0.99	Emergence to maturity	1–22	Plenet and Lemaire (2000)
	3.39	0.367	0.99	Emergence to silking stage	1–11.7	
Cotton						Wang et al. (2012)
(7.5 × 10^4 plants hm^{-2})	4.09	0.277	0.96	Final singling to boll	0.12–7.07	
(9.8 × 10^4 plants hm^{-2})	4.19	0.248	0.91	opening stage	0.15–8.97	
(12 × 10^4 plants hm^{-2})	4.38	0.252	0.90		1.55–9.52	

biomass when W = 1 t ha^{-1}. For Siberian wildrye in this study, parameter a (3.0) was lower than other species presented in Table 2, and was close to rice (3.53; Ata-Ul-Karim et al., 2013) and maize (3.4; Plenet and Lemaire, 2000). Except the physiological properties among species, this may be due to the different biomass per unit area caused by row spacing in different experiments. As plant growth and development progressed, shoot biomass increased while N concentration decreased. The shoot biomass of crops grown in narrow row spacing reaching 1 t ha^{-1} was earlier than wide row spacing in the development stage, resulting in higher N concentration in narrow row spacing (Cruz and Lemaire, 1986). Thus, the narrow row spacing leading to a higher parameter a (Wang et al., 2012). In the present study, row spacing was 30 cm. The row spacing of other forage species presented in Table 2, which was used to produce hay, was less than 30 cm. Therefore, the value of a in our study is lower. The row spacing of wheat and rice was usually approximately 20 cm, thus the value of a is higher than the current study. It was also possible that this experiment, which was conducted under rain-fed condition, the water condition in the soil influenced N absorbtion and allocation. Errecart et al. (2014) showed that the Nc of tall fescue growing without water deficiency was higher than fescue growing under water-deficient conditions. Parameter b describes the declining slope in Nc with shoot biomass (Flenet et al., 2006). Parameter b of Siberian wildrye in the current study was in the middle of the range of reported values in Table 2, similar to values of rice (Ata-Ul-Karim et al., 2013), and same with values of tall fescue (Lemaire and Denoix, 1987). This suggested that the declining slope in Nc with shoot biomass increasing in Siberian wildrye was consistent with tall fescue and rice. However, parameter b of perennial ryegrass and red fescue and annual ryegrass was higher than the values of Siberian wildrye, suggesting Nc of perennial ryegrass and red fescue and annual ryegrass decreased quickly with increasing shoot biomass and indicated differences in the N accumulation process with Siberian wildrye. This may be due to the difference in biological characteristics and sampling periods among species (Gislum and Boelt, 2009).

A suitable CNDC in grass species used for seed production is difficult to develop, as the characteristic of grass species is breeding for forage use. No Nc dilution curve existed in grass species for seed production before Gislum and Boelt (2009). They developed a CNDC based on perennial ryegrass and red fescue for seed production, but failed in validating it with data grouped by seed yield. The CNDC developed in this study could distinguish the N limiting growth group and the N non-limiting growth group very well (Fig. 3). It could also distinguish N limiting seed yield group and N non-limiting seed yield group before and at the anthesis stage, but failed in distinguishing the two groups after the anthesis stage. The data of N non-limiting seed yield was diagnosed as N limited by the CNDC after anthesis (Fig. 4). This indicated that the growth and N demand of Siberian wildrye began to diverge between plants intended for seed production and for herbage production after anthesis. The N demand of seed production was lower than that of herbage production. Excess N increased growth of new vegetative tillers, biomass dry matter accumulation, and N concentration, but did not influence seed production (Warringa and Kreuzer, 1996). The relationship of RSY and NNI also indicated that the RSY reached 1 when NNI was approximately 0.85 at milk and dough stage (Fig. 5). Based on the findings above, the application of the developed CNDC to assess plant N status should occur at or before anthesis stage. Moreover, N application before and during anthesis could be utilized more effectively (Gislum and Boelt, 1998).

5. Conclusions

The Nc decreased with increasing shoot biomass. Based on above-ground biomass N concentration, a unique CNDC in Siberian wildrye intended for seed production was developed and described by the equation Nc = 3.0 W$^{-0.32}$ with the determination coefficient of 0.97, when shoot biomass was between 0.9 and 7.1 t ha^{-1} under rain-fed conditions. The developed CNDC could effectively distinguish the N status of Siberian wildrye for seed production before and at anthesis. We recommend application of the CNDC to diagnose Siberian wildrye N status for seed production before and at the anthesis stage. Additionally, the relationship between RSY and NNI indicated optimum seed yield was reached at around NNI = 1. The CNDC developed in the current study provides an insight into plant N nutrition and can serve as a guide to improve N diagnosis and management in Siberian wildrye under rain-fed conditions.

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References

Ata-Ul-Karim, S.T., Yao, X., Liu, X., Cao, W., Zhu, Y., 2013. Development of critical nitrogen dilution curve of Japonica rice in Yangtze river reaches. Field Crops Res. 149, 149–158.
Black, A., Reitz, L., 1969. Row spacing and fertilization influences on forage and seed yields of intermediate wheatgrass Russian wildrye, and green needlegrass on dryland. Agron. J. 61, 801–805.
Bremner, J.M., Mulvancy, C.S., et al., 1982. Nitrogen-total. In: Page, A.L. (Ed.), Methods of Soil Analysis, Part 2. American Society of Agronomy, Madison, WI, pp. 595–624.

Cassman, K.G., Dobermann, A., Walters, D.T., Yang, H., 2003. Meeting cereal demand while protecting natural resources and improving environmental quality. Annu. Rev. Environ. Resour. 28, 315–358.

Colnenne, C., Meynard, J.M., Reau, R., Justes, E., Merrien, A., 1998. Determination of a critical N dilution curve for winter oilseed rape. Ann. Bot. 81, 311–317.

Cruz, P., Lemaire, G., 1986. Analyse des relations de compétition dans une association de luzerne (Medicago sativa L.) et de dactyle (Dactylis glomerata L.). II − Effets sur la nutrition azotée des deux espèces. Agronomie 6, 735–742.

Debaeke, P., Oosterom, E.J., Justes, E., Champolivier, L., Merrien, A., Aguirrezabal, L.A.N., González-Dugo, V., Massignam, A.M., Montemurro, F., 2012. A species-specific critical nitrogen dilution curve for sunflower (*Helianthus annuus* L.). Field Crops Res. 136, 76–84.

Errecart, P.M., Agnusdei, M.G., Lattanzi, F.A., Marino, M.A., Berone, G., 2014. Critical nitrogen concentration declines with soil water availability in tall fescue. Crop Sci. 54, 318–330.

Flenet, F., Guerif, M., Boiffin, J., Dorvillez, D., Champolivier, L., 2006. The critical N dilution curve for linseed (*Linum usitatissimum* L.) is different from other C3 species. Eur. J. Agron. 24, 367–373.

Fletcher, A.L., Chakwizira, E., 2012. Developing a critical nitrogen dilution curve for forage brassicas. Grass Forage Sci. 67, 13–23.

Gao, P., Li, C., Chen, B., Wang, Y., Miao, L., Zhang, Y., 2010. Effects of nitrogen fertilizer on seed yield, seed constitutive factors and seed vigor of *Elymus sibiricus* L. J. Northeast Normal Univ. (Nat. Sci. Ed.). 2, 126–131.

Gislum, R., Boelt, B., 1998. Timing of spring nitrogen application in amenity-types of *Lolium perenne* L. grown for seed. J. Appl. Seed Prod. 16, 67–70.

Gislum, R., Boelt, B., 2009. Validity of accessible critical nitrogen dilution curves in perennial ryegrass for seed production. Field Crops Res. 111, 152–156.

Justes, E., Mary, B., Meynard, J.M., Machet, J.M., Thelier-Huche, L., 1994. Determination of a critical N dilution curve for winter wheat crops. Ann. Bot. 74, 397–407.

Lemaire, G., Denoix, A., 1987. Croissance estivale en matière sèche de peuplements de fétuque élevée (Festuca arundinacea Schreb.) et de dactyle (Dactylis glomerata L.) dans l'Ouest de la France. I. Etude en conditions de nutrition azotée et d'alimentation hydrique non-limitantes. Agronomie 7, 373–380.

Lemaire, G., Gastal, F., 1997. N uptake and distribution in plant canopies. In: Lemaire, G. (Ed.), Diagnosis of the Nitrogen Status in Crops. Springer-Verlag, Heidelberg, pp. 3–43.

Lemaire, G., Salette, J., 1984. Relation entre dynamique de croissance et dynamique de prèlévement d'azote pour un peuplement de graminées fourragères. I. Etude de l'effet du milieu. Agronomie 4, 423–430.

Lemaire, G., Cruz, P., Gosse, G., Chatier, M., 1985. Etude des relations entre la dynamique de préevement d'azote et la dynamique de croissance en matiére séche d'un peuplement de luzerne (*Medicago sativa* L.). Agronomie 5, 685–692.

Lemaire, G., Gastal, F., Salette, J., 1989. Analysis of the effect of N nutrition on dry matter yield of a sward by reference to potential yield and optimum N content. In: Proceedings of the 16th International Grassland Congress. Nice, France. pp. 179–180.

Mao, P., Han, J., Wang, Y., Liu, G., 2001. Effects of fertilization on seed yield and seed quality of *Elymus sibiricus*. Pratac. Sci. 18, 7–12.

Mao, P., Han, J., Wu, X., 2003. Effects of harvest time on seed yield of Siberian wildrye. Acta Agrestia Sin. 11, 33–37.

Marino, M.A., Mazzanti, A., Assuero, S.G., Gastal, F., Echeverria, H.E., Andrade, F., 2004. Nitrogen dilution curves and nitrogen use efficiency during winter-spring growth of annual ryegrass. Agron. J. 96, 601–607.

Mary, B., 1997. N hazards to crops and environment. In: Lemaire, G. (Ed.), Diagnosis on the Nitrogen Status in Crops. Springer-Verlag, Heidelberg, pp. 229–236.

Plenet, D., Lemaire, G., 2000. Relationships between dynamics of nitrogen uptake and dry matter accumulation in maize crops. Determination of critical N concentration. Plant Soil 216, 65–82.

Ulrich, A., 1952. Physiological bases for assessing the nutritional requirements of plants. Annu. Rev. Plant Physio. Plant Mol. Biol. 3, 207–228.

Wang, Z., Jin, L., Zhao, W., Wu, X., Zhao, Z., Wang, Y., Zhou, Z., 2012. Preliminary development and application of a critical nitrogen concentration dilution model for cotton grown in northeast China. Cotton Sci. 24, 427–435.

Warringa, J., Kreuzer, A., 1996. The effect of new tiller growth on carbohydrates: nitrogen and seed yield per ear in *Lolium perenne* L. Ann. Bot. 78, 749–757.

Yue, S., Meng, Q., Li, F., Cui, Z., Zhang, F., Chen, X., Zhao, R., 2012. Critical nitrogen dilution curve for optimizing nitrogen management of winter wheat production in the North China plain. Agron. J. 104, 523–529.

Zhang, J., Li, Q., Li, X., 2001. Studies on the effects of nitrogen and phosphate on *Elymus sibiricus* grown without irrigation. Grassland China 23, 38–41.

Zhao, L., Wang, M., Mao, P., A, N., 2012. Selected nitrogen and phosphorus fertilizer applications affect seed yield, seed yield components and roots of *Elymus sibiricus*. Acta Agrestia Sin. 4, 662–668.

Ziadi, N., Brassard, M., Bélanger, G., Cambouris, A.N., Tremblay, N., Nolin, M.C., Claessens, A., Parent, L., 2008. Critical nitrogen curve and nitrogen nutrition index for corn in eastern Canada. Agron. J. 100, 271–276.

Ziadi, N., Bélanger, G., Claessens, A., Lefebvre, L., Cambouris, A.N., Tremblay, N., Nolin, M.C., Parent, L.É., 2010. Determination of a critical nitrogen dilution curve for spring wheat. Agron. J. 102, 241–250.