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Short Communication

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

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ABSTRACT

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:

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

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 Soil pH = 7.57 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}	Apr. 10.41, May 31.75, Jun. 114.30, Jul. 58.17, Aug. 33.02.
Exp. 1	4–7	July 2012	2015	0, 45, 90, 135, 180, 225	18 June, 29 June, 11 July, 20 July		
Exp. 2	8–10	July 2013	2014	0, 30, 60, 90, 120, 150	24 June, 1 July, 8 July,	Soil type = Sandy loam Soil pH = 7.70 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}	Apr. 9.14, May 23.37, Jun. 73.66, Jul. 112.27, Aug. 16.51.
Exp. 2	11–14	July 2013	2015	0, 30, 60, 90, 120, 150	18 June, 29 June, 11 July, 20 July		