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Original Research

Effect of Nitrogen Fertilizer and Seeding Rate on Yield of Alfalfa and Weeds

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Abstract

Applying nitrogen (N) to increase alfalfa (*Medicago sativa* L.) yield is a general practice in China that has caused serious weed infestation problems. Our objective was to determine the effect of N fertilizer and seeding rate on yields of alfalfa and weeds. The effects of four N fertilizer gradients (0, 50, 100, and 150 kg N hm^{-2}) and five alfalfa seeding rates (0, 5, 7.5, 10, and 15 kg hm^{-2}) were examined on dry matter of alfalfa and weeds using a three-year *in situ* controlled trial. The results showed:

- There were no significant differences among N fertilizer (N) for dry matter of alfalfa (DMA) during three years. Dry matter of grassy weeds (DMGW) and broadleaf weeds (DMBW) were significantly increased with the N fertilizer gradient in the seeding year.
- DMA was significantly increased with the seeding rates gradient in both 2012 and 2013. DMGW and DMBW were significantly decreased with the seeding rates gradient in all three years.
- Interactions between N fertilizer and seeding rates on DMA and DMBW in 2012 and DMGW and DMT in 2013 are significant ($p<0.05$). Although the study demonstrated that increasing seeding rate can suppress weed invasions, N fertilizer facilitated them.

Keywords: Nitrogen fertilizer, seeding rate, grassy weeds, broadleaf weeds, dry matter

Introduction

Weed infestation is a serious problem in an agroecosystem. It can decrease crop yield and degrade the environment. Under the background of global warming, weed management has become more complex and full of uncertainty. Though some studies show that CO_2 removal has been accomplished in a special urban city and park area, scientific observations show that the concentration of CO_2 is still increasing in the atmosphere [1-7]. Alfalfa (*Medicago sativa* L.) is one of the most important perennial forage legumes cultivated in the world because of its high nutritive quality and yield [8]. Weed invasion also is a serious problem for alfalfa cultivation, especially in seeding year and older stands [8-9]. Weed affects the stability of alfalfa stands because of competition with alfalfa for light, nutrients, and water resources, which result in decreased yields and quality [10-11]. Herbicide application is a widespread practice in many production systems for efficient weed control, but they have the potential to cause serious environmental pollution [12], particularly of ground and surface water [13-14]. In addition, some weeds become

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resistant to herbicides after a certain period of treatment, which leads to further increasing rates of herbicide. Therefore, it is important to develop a cultural method of weed control for alfalfa production that enhances the competitive ability of alfalfa and minimizes weed competition.

Fertilizer management is an important cultural method to control the dynamic of weed communities, which has been described by many authors [15-16]. Grassy weed is sensitive to nitrogen fertilizer. N fertilizer applications in spring will promote weed growth, which results in the need for more frequent herbicide applications or cultivation to control weeds [17]. As a general rule, Alfalfa can use biological nitrogen fixation to supply N nutrition and also improve soil fertility; the fertilizer nitrogen replacement value is about 143 kg N ha^{-1} [18]. In China, however, alfalfa is commonly grown on sandy and saline-alkali soils where the soil has poor nutrient and water-holding capacity. Applying N fertilizer to increase yield potential has been routine practice for many alfalfa growers [19]. Although applying more N helps achieve the desired yield [20-21], it also can increase the chances of invasive weed growth [22-23] and result in greater levels of environmental pollution [24].

High seeding rate, in a certain range, often results in high seedling density and total yield [25-26]. Hansen and Krueger found that alfalfa dry matter was increasing with the seeding rates from 4.5 to 18 kg hm^{-2} [27-28]. On the contrary, Bolger and Meyer found there is no difference in alfalfa yields from stands established at 9 to 22.4 kg hm^{-2} [29]. Further research showed that the threshold of seeding rate is about 17 kg hm^{-2}, and when seeding rate exceeds this it will no longer provide a long-term benefit to alfalfa stand establishment and total yield [30].

Many previous studies have identified N fertilizer rate and seeding rate impact weed infestation; much of the research on the effect of N application or seeding rate on weed diversity and community structure has been done in agricultural systems [9, 25]. The interaction of N fertilizer rate and seeding rate has rarely been reported. Therefore, the aim of this study was to determine the effect of N fertilizer application rate and seeding rate and their interaction on the yield of alfalfa and weeds.

Material and Methods

Site Description

A field experiment was conducted from 2012 to 2014 at the Huanghua Forage Experimental Station (38.49° N, 117.58° E, 2 m a.s.l.) in Hebei Province of northern China. The annual mean maximum and minimum air temperature at the site were 20.7 and 9.1ºC, respectively, and the annual mean temperature was 12.5ºC. Annual precipitation was 600-650 mm, less than 70 percent of which falls from June to October. The soil type in the experimental field is salinized fluvo-aquic soil. The soil texture is 12.3% sand, 66.9% silt, and 20.8% clay. The organic matter content of 0-20 cm topsoil was 1.87% and available nitrogen was 76.8 mg kg^{-1}. The average bulk density of the soil to the depth of 0-5 m was 1.32 g cm^{-3}. The crop for the two previous years before the study was corn (*Zea mays* L.). There are six species of grassy weeds and seven species of broadleaf weeds in the experimental site, which belong to eight families (Table 1). The nomenclature used is according to the International Plant Names Index (IPNI 2017) [31].

Table 1. Species recorded at the experimental site, including their families, life cycle, and weed type.

Species	Family	Life cycle	Weed type
Echinochloa crus-galli L.	Poaceae	Annual	Grassy weed
Digitaria sanguinalis L.	Poaceae	Annual	Grassy weed
Eleusine indica L.	Poaceae	Annual	Grassy weed
Setaria viridis L.	Poaceae	Annual	Grassy weed
Phragmites australis (Cav.) Trin	Poaceae	Perennial	Grassy weed
Eriochloa villosa (Thunb.) Kunth	Poaceae	Annual	Grassy weed
Chenopodium album L.	Chenopodiaceae	Annual	Broadleaf weed
Amaranthus retroflexus L.	Amaranthaceae	Annual	Broadleaf weed
Portulaca oleracea L.	Portulacaceae	Annual	Broadleaf weed
Persicaria lapathifolia L.	Polygonaceae	Annual	Broadleaf weed
Xanthium strumarium L.	Asteraceae	Annual	Broadleaf weed
Solanum nigrum L.	Solanaceae	Annual	Broadleaf weed
Acalypha australis L.	Euphorbiaceae	Annual	Broadleaf weed

Experimental Design

The treatments consisted of five alfalfa seeding rates (0, 5, 7.5, 10, and 15 kg hm^{-2} designated S1, S2, S3, S4, and S5, respectively) and four levels of N fertilizer (0, 50, 100, and 150 kg N hm^{-2}, designated N0, N50, N100, and N150, respectively). The factorial combinations of the treatments were arranged in a randomized complete block design and replicated three times. The plot size with 50 cm spacing between plots was 4 × 5 m (20 m^2) consisting of 19 rows, with 20 cm spacing between rows.

Urea (46% N) was used as the source of N, and alfalfa seeds were sown by hand on 25 April 2012 in rows with spacing of 20 cm and seeding depth of 1-1.5 cm. The green-up (spring regrowth) dates in the following years (2013 and 2014) were 13 April and 15 April, respectively. Prior to alfalfa green-up in the spring of each subsequent year, N was broadcast into each plot at the same rate as in the seeding year. The alfalfa cultivar (Sanditi) was obtained from Barenbrug (Tianjin) International Co., Ltd.

Data Collection

Precipitation and air temperature during 2012-14 were recorded by Huanghua Ranch meteorology station located 10 km from the experimental site.

Alfalfa harvests in the seeding year began in early July and were repeated three times each year, followed by a 35- to 40-d harvest interval that corresponded with an approximate 10% alfalfa bloom. Harvests in each subsequent year began in mid-May and then followed a 35- to 40-d harvest schedule. Prior to harvesting, forage composition was determined in the field by hand harvesting a 1 m^2 sample per plot. Total herbage was separated into alfalfa and broadleaf and grassy weeds. At each cut, three subsamples of 200-1,000 g fresh forage (including alfalfa, grass, and broadleaf weeds) were taken from each plot, oven-dried at 65ºC for 24 h to a constant weight, and weighed to obtain dry matter yield.

Data Analysis

We examined the effects of nitrogen, and seedings were analyzed by N fertilizer (N), seeding rates (S), and N fertilizer × seeding rates interaction as sources of variance using the GLM model of SPSS software (version 19.0, SPSS Inc., Chicago, Illinois, USA, 2004). Means were separated using the least significant difference (LSD) test. Results from the statistical analyses of the data were graphed using Microsoft Excel software.

Results

Precipitation and Air Temperature

Precipitation and air temperatures varied somewhat over the three years of the study (Fig. 1). Average air

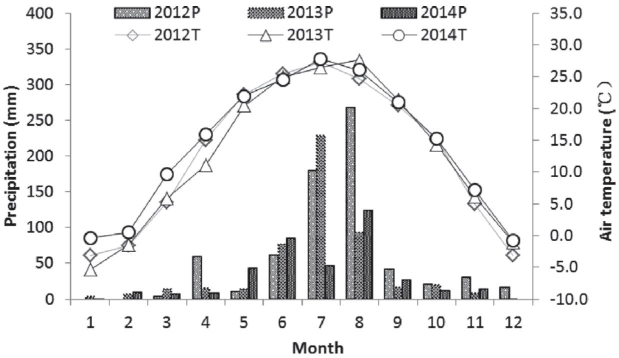

Fig. 1. Precipitation distribution and air temperature in the experimental field, 2012-14.

temperatures were 12.7ºC, 12.5ºC, and 14.1ºC in 2012, 2013, and 2014, respectively. Annual precipitation was 691 mm in 2012, and deceased to 497 mm and 375 mm in 2013 and 2014, respectively.

Effect of N fertilizer on DMA, DMGW, DMBW, and DMT

There were no significant differences among N fertilizer (N) for DMA during three years (Fig. 2a). DMGW was 2.6 and 2.3 Mg hm^{-2} in N3 and N4 in 2012, respectively, and both are greater than that of N1 and N2 (1.3 and 1.7 Mg hm^{-2}; $p<0.05$), and significantly increased with the N fertilizer gradient. In 2013 DMGW was 2.1 Mg hm^{-2} in N3 – greater than that of the other three treatments. There were no significant differences among N fertilizer (N) for DMGW in 2014 (Fig. 2b). IN 2012 DMBW was 1.6 Mg hm^{-2}, greater than that of N3 and N2 (1.2 and 1.0 Mg hm^{-2}; $p<0.05$), and then DMBW in N3 was greater than that of N1 0.8 Mg hm^{-2}, significantly increased with the N fertilizer gradient. There were no significant differences among N fertilizer (N) for DMBW in both 2013 and 2014 (Fig. 2c). DMT was 6.3 Mg hm^{-2} in 2012, greater than

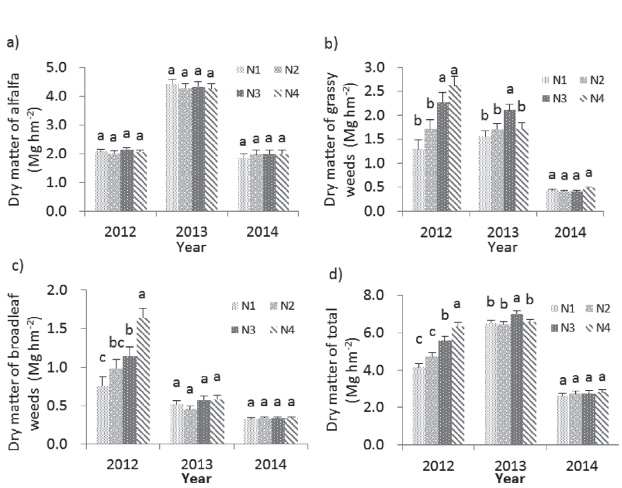

Fig. 2. Effect of N fertilizer on dry matter of alfalfa, grassy weeds, broadleaf weeds, and total.

that of N3 (5.6 Mg hm^{-2}; p<0.05), and then greater than that of N2 and N1 (4.7 and 4.1 Mg hm^{-2}; p<0.05), significantly increasing with the N fertilizer gradient. In 2013 DMT was 7.0 Mg hm^{-2} in N3, greater than that of the other three treatments. There were no significant differences among N fertilizer (N) for DMT in 2014 (Fig. 2d).

Effect of Seeding Rates on DMA, DMGW, DMBW, and DMT

DMA was significantly increased with the seeding rates gradient in both 2012 and 2013. In 2012 DMA was 4.0 Mg hm^{-2} in S5 and greater than that of S4 (2.7 Mg hm^{-2}; p<0.05), and then DMA in S4 was greater than that of S3 (2.1 Mg hm^{-2}; p<0.05), and then greater than that of S2 (1.5 Mg hm^{-2}; p<0.05). There is no DMA for S1 treatment. In 2013 DMA was 6.3 Mg hm^{-2} in S5, greater than that of S4 and S3 (5.6 and 5.2 Mg hm^{-2}; p<0.05), and then greater than that of S2 (4.4 Mg hm^{-2}; p<0.05). There were no significant differences among seeding rates (S) for DMA in 2014 (Fig. 3a).

DMGW was significantly decreased with the seeding rates gradient in all three years. In 2012 DMGW was 3.4 Mg hm^{-2} in S1, greater than that of S2 (2.6 Mg hm^{-2}; p<0.05), and DMGW in S2 was greater than that of S3 and S4 (1.8 and 1.3 Mg hm^{-2}; p<0.05), respectively. DMGW in S3 was greater than that of S5 (0.8 Mg hm^{-2}; p<0.05). In 2013 DMGW was 4.0 Mg hm^{-2} in S1, greater than that of S2 and S3 (1.8 and 1.4 Mg hm^{-2}; p<0.05), and DMGW in S2 was greater than that of S4 (1.1 Mg hm^{-2}; p<0.05). DMGW in both S3 and S4 was greater than that of S5 (0.5 Mg hm^{-2}; p<0.05). In 2014 DMGW was 0.7 Mg hm^{-2} in S1, greater than that of S2 (0.5 Mg hm^{-2}; p<0.05), and DMGW in S2 was greater than that of S3 (0.4 Mg hm^{-2}; p<0.05). DMGW in S3 was greater than that of S4 and S5 (0.3 and 0.2 Mg hm^{-2}; p<0.05) (Fig. 3b).

DMBW was significantly decreased with the seeding rates gradient, too. In 2012 DMBW was 1.6 and 1.7 Mg hm^{-2} in S1 and S2, respectively, greater than that of S3 and S4 (1.1 and 0.8 Mg hm^{-2}; p<0.05), and then DMBW in S3 was greater than that of S5 (0.5 Mg hm^{-2}; p<0.05). In 2013 DMBW was 0.9 Mg hm^{-2} in S1, greater than that of S2 and S3 (0.7 and 0.5 Mg hm^{-2}; p<0.05), and then DMBW in S2 and S3 was greater than that of S4 and S5 (0.3 and 0.2 Mg hm^{-2}; p<0.05). In 2014 DMBW was 0.4 Mg hm^{-2} in S1, greater than that of S4 and S5 (0.3 and 0.3 Mg hm^{-2}; p<0.05) (Fig. 3c).

In 2012 DMT was 5.8 Mg hm^{-2} in S2, greater than that of S1, S3, and S4 (5.0, 4.8, and 4.8 Mg hm^{-2}; p<0.05), respectively. There were no significant differences among seeding rates among others. In both 2013 and 2014, DMT of planting alfalfa treatments (S2, S3, S4 and S5) was 6.8, 7.1, 7.1, and 7.2 Mg hm^{-2}, greater than that of S1 (5.0 Mg hm^{-2}, p < 0.05). There were no significant differences among planting alfalfa treatments (Fig. 3d).

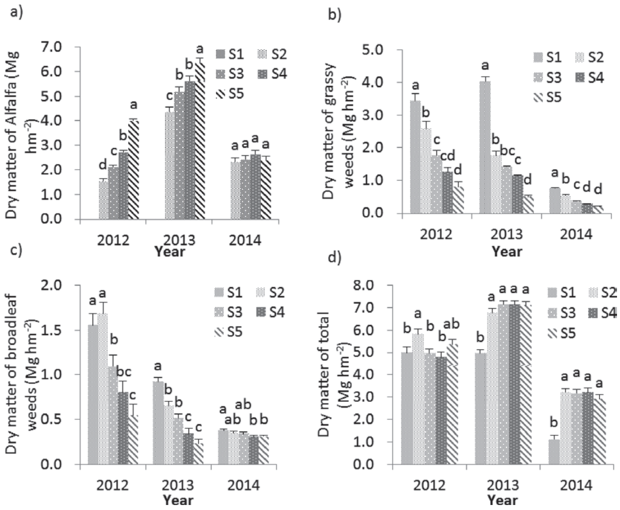

Fig. 3. Effect of seeding rates on dry matter of alfalfa, grassy weeds, broadleaf weeds, and total.

Interaction of N fertilizer and Seeding Rates on Dry Matter of DMA, DMGW, DMBW, and DMT

Interactions between N fertilizer and seeding rates on DMA in 2012, DMGW in 2013, DMBW in 2012, and DMT in 2013 are significant (P<0.05). In N4 treatment of 2012, DMA was 1.0 Mg hm^{-2} in S2 and less than that of N1 to N3 treatments (p<0.05), but increased to 4.4 Mg hm^{-2} in S5, greater than that of N1 and N2 (3.7 and 3.4 Mg hm^{-2}, p<0.05) (Fig. 4a). DMBW was 3.1 and 1.7 Mg hm^{-2} in S2 and S3 – greater than that of others. However, there is no significant difference when in S4 and S5 conditions (Fig. 4c). In N4 treatments of 2013, DMGW was 4.8 Mg hm^{-2} in S1, greater than that of N1 to N3 (p<0.05), but decreased to 1.2 and 0.6 Mg hm^{-2} in S3 and S4, respectively – less than that of N2 and N3

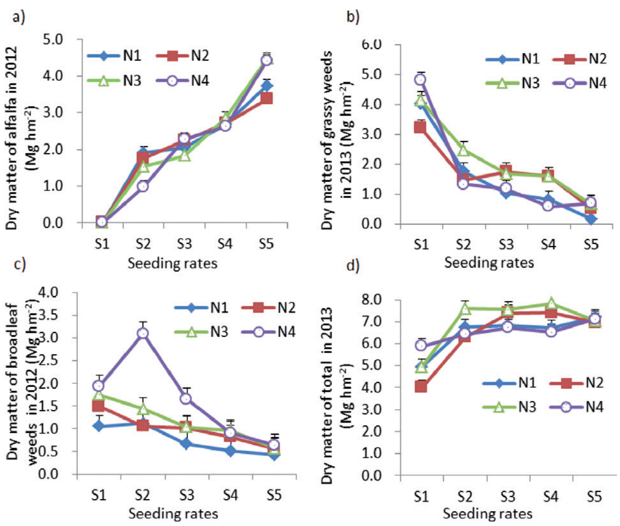

Fig. 4. Interaction plot for dry matter versus N fertilizer and seeding rates.

($p<0.05$) (Fig. 4b). DMT was 5.9 Mg hm^{-2} in S1, greater than that of N1 to N3 ($p<0.05$). However, when in S3 and S4 conditions, N4 treatments was less than that of N2 and N3 (Fig. 4d).

Discussion

Weed seed germination is triggered by various factors, including soil temperature, soil moisture, rainfall, light, and soil available N [9, 32]. In the alfalfa seedling year, broadleaf weeds are often an early competitor with alfalfa because they emerge earlier than grassy weeds and their growth peak is early and long. Alfalfa growth was slow from germination to the fourth week, while some broadleaf weeds such as *Chenopodium album* L., *Amaranthus retroflexus* L., and *Portulaca oleracea* L., germinated in soil and grew rapidly under appropriate moisture and nutrient conditions. In contrast, the peak growth period of grassy weeds was later than that of broadleaf weeds; they emerged and grew rapidly with the arrival of the rainy season.

Effect of N fertilizer on Yield of Alfalfa and Weeds

Many studies have found that the application of N fertilizer did not increase alfalfa yield or quality, instead of reducing alfalfa yield [33-34]. This is because biologically fixed N is sufficient to meet the N needs of legumes if the symbiosis is functioning properly. In most agricultural soils, soil available N is sufficient for early growth of legumes prior to the establishment of an effective N_2 fixation system. The application of N fertilizer improved the growth of invasive grass weeds that respond to N availability [22, 24]. However, some studies have shown that alfalfa needs supplemental N when it is grown on extremely low N soils (e.g., saline-alkali and sandy soils with nitrate levels below 15 mg kg^{-1} or organic matter below 15 g kg^{-1}), or under conditions that limit the N_2 fixation symbiosis (e.g., low temperature) [35]. In our experimental site, the organic matter and available nitrogen of topsoil (0-20cm) is 1.87% and 76.8 mg kg^{-1} both of which are higher than the extremely low N level. This is consistent with the results of our study, where DMA did not benefit from the application of fertilizer N (Fig. 2a). However, DMGW and DMBW increased significantly with increasing N application in the seeding years (Figs. 2b-c). It also demonstrated that the part of increasing DMT is coming from the DMGW and DMBW in 2012 (Fig. 2d). So, N fertilizer was unfavorable for alfalfa, since it facilitated weeds.

Effect of Seeding Rate on Yield of Alfalfa and Weeds

In this study, with increasing seeding rate, the DMA increased significantly in 2012 and 2013 ($P<0.05$), but was not significantly different in 2014 (Fig. 3a). This is consistent with the results of Jefferson and Cutforth, who reported that alfalfa yield increased with increasing seeding rate within the range of 6-18 kg hm^{-2} [36]. Alfalfa yield in this study did not differ among different seeding rates in the third year, one reason being that annual precipitation is 375 mm in 2014 (Fig. 1), drought limited growth potential, and cutting increased the number of alfalfa branches and therefore increased alfalfa yield for all seeding rates [29]. The yield increased with increased seeding density within a certain range, but outside of this range increasing density resulted in reduced yield caused by the number of branches per individual plant and the decline in individual plant biomass [30, 37].

Low seeding rate resulted in a large number of invasive weeds, and ultimately affected alfalfa yield and quality; this was confirmed by the results of our study that DMY of grass and broadleaf weeds decreased significantly with increased seeding rate (Fig. 3). Alfalfa had higher and stronger stems at low seeding rates, resulting in higher lignification. In contrast, alfalfa had small and thin stems at high seeding rates, resulting in low lignification and better quality; the bottom of alfalfa communities with high seeding rate had bad ventilation and light, resulting in faster senescence for alfalfa leaves at the bottom, which to some extent explains the adverse effects on forage quality [38]. So, S4 and S5 treatments (10-15kg hm^{-2}) are the favorable seeding rate for alfalfa planting.

Interaction of N fertilizer and Seeding Rates on Yield of Alfalfa and Weeds

There is an interacted effect between N fertilizer and seeding rates on DMA and DMBW in 2012 and DMGW and DMT in 2013. In a seeding year, the effect of N fertilizer on DMA increased with the seeding rates gradient. In S2 conditions, DMA of the N4 treatment was 1.0 Mg hm^{-2} in S2, less than that of N1 to N3 treatments ($p<0.05$), but increased to 4.4 Mg hm^{-2} in S5, greater than that of N1 and N2 ($p<0.05$) (Fig. 4a). This illustrates that alfalfa can benefit from N fertilizer when there is enough community density, but not for the consequent years since there is enough nitrogen supply by symbiotic N fixation.

Broadleaf weeds are strong competitors in the seeding year. The effect of N fertilizer on DMBW decreased with seeding rates gradient. In S2 and S3 conditions, DMBW of N4 treatment was greater than that of other N treatments. However, there is no significant difference in S4 and S5 conditions (Fig. 4c). In 2013 the dry matter of grassy weeds of N4 treatment was significantly decreased with seeding rate gradients. DMGW of N4 in S1 conditions was greater than that of N1 to N3 ($p<0.05$), and less than that of N2 and N3 ($p<0.05$) in S3 and S4 conditions. The dry matter of total yield of N4 treatment was significantly different between seeding (S2 to S5) and no seeding (S1) treatments. DMT of N4 in S1 condition was greater than that of N1 to N3 ($p<0.05$), and less than that of N2 and N3 ($p<0.05$) in S3 and S4 conditions.

Conclusions

N fertilizer will facilitate weed invasion and cannot increase alfalfa yield. The favorable seeding rate for alfalfa planting is 10-15 kg hm^{-2}, which can suppress weed invasion and keep a higher yield. Alfalfa can benefit from N fertilizer in higher seeding rates in seeding years, but not for the consequent years since there is enough nitrogen supplied by symbiotic N fixation.

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