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Original Article

Estimation of Nitrogen Uptake and Utilization Efficiency in Cotton by the Fertilizer-Response Model

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Abstract

Due to the indeterminate growth habit of cotton crops, a better understanding of N status at the rational fertilizer regime is important to promote lint yield. The fertilizer-response model was employed to evaluate N status by analyzing data of shoot dry mass, N content and N concentration at different growing stages. A field study was conducted on drip-irrigated cotton plants with N fertilizer addition in total amounts of 0 (N0), 120 (N1), 240 (N2), 360 (N3) and 480 (N4) kg ha^{-1} in Xinjiang, China in 2016. Thirty percent of total fertilizers were applied at planting and the rest 70% were applied over six applications. The N fertilizer treatment at the accumulative rate of 70 kg ha^{-1} was enough to induce the N status of steady state accumulation 60 days after germination. Since 90 days the treatments that delivered the N amount between 120 and 240 kg ha^{-1} was deficient for cotton demand, higher rates from 360 and 480 kg ha^{-1} induced inherent N reserve and resulted in the highest level of yield. With regard to the practical meaning, the N fertilizer dose of 360 kg ha^{-1} can be used for cotton growth. The N fertilizer dose of 120 kg ha^{-1} can be recommended when the yield of 5,840 kg ha^{-1} lint can meet the goal of cotton culture.

Keywords: luxury uptake; optimum dose; nutrient limit; nutrient loading

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most widely fiber crops across a broad range of climates with varied soils and cultivating regimes (Wang *et al*., 2011; Shah *et al*., 2016). Cotton is cultivated in 76 countries in occupation of 32 million hectares of crop lands (Saranga *et al*., 2001). In the past six years, China was the leader of cotton-production all over the world (Khan *et al*., 2017a). The Xinjiang Uyghur Autonomous Region is the largest and most dominant production base of cotton in China (Yao *et al*., 2017). During the years between 2011 and 2014, the cotton yield was 3.41 × 10^{6} t from a total planting area of 1.758 × 10^{6} ha in Xinjiang, accounting for 38% of area and 56% of yield at the national scale of China, respectively (Bai *et al*., 2017). The high input into production cropping systems and new varieties have encouraged overuse of fertilizers due to increasing consumer demand in Xinjiang. Nitrogen (N) is the major necessary nutrient element limiting cotton growth and development. As a result of production achievement, the consumption of synthetic N fertilizer has grown exponentially in recent decades (Macdonald *et al*., 2017). Due to the indeterminate growth habit of cotton crops, a better understanding of cotton growth and development under N supply at the rational fertilizer regime is important in the continuing efforts of growers to produce lint and seed yield more efficiently and profitably.

Nitrogen is required in larger amounts than other nutrients by cotton plants (Hou *et al*., 2007, 2009; Chen *et al*., 2010). Synchronization of N uptake for demands along with supply is crucial for generating the optimum N use efficiency in cotton (Khan *et al*., 2017b). The N demand by cotton is strongly related to yield potential, which was in turn associated with N supply (Macdonald *et al*., 2017; Zhang *et al*., 2017). Either N deficiency or excess of N supply can cause a stress for cotton by influencing growth and yield through disrupting several growth processes (Gerik *et al*., 1998; Lokhande and Reddy, 2015). Compared to cotton plants with optimum N application, those under deficient-N condition showed stunted growth and insufficient biomass accumulation with declines in leaf area, number of fruiting braches, and lint yield (Jaynes *et al*., 2001; Lokhande and Reddy, 2015). At the early growing stage, N limitation reduces leaf expansion with decreased

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cell division and cell expansion (Chapin 1980; Tang *et al.*, 2012). During the reproductive growing stage of cotton, N deficiency can suppress vegetative growth through stimulating inherent N translocation to reproductive organs, but the fruiting ability and boll formation were also impacted at the same time (Gerik *et al.*, 1998; Lokhande and Reddy, 2015). At the fiber development stage, limited N supply would reduce fiber strength and quality (Read *et al.*, 2006). In contrast, excessive N supply may prolong the vegetative growth to elongate the reproductive growth in cotton plants, driving them to face a larger risk of early-autumn frost (Ayissa and Kebede, 2011; Marschner, 2013; An *et al.*, 2019). The excessive application of N would also result in problems of N leaching (Macdonald *et al.*, 2016), runoff (Mchugh *et al.*, 2008) and atmospheric losses (Macdonald *et al.*, 2015). Current studies about the evaluation on N status in response to exogenous supply were scattered by cultivars, locations and fertilizer doses. These results are still insufficient to predict the cotton N status under a given application dose.

The N status of agricultural crops is often assessed by analyzing the actual N concentration of above-ground dry matter and the critical N concentration. The 'critical N concentration' is defined as the minimum demand of a crop plant for necessary N concentration to achieve the highest growth rates with a given biomass accumulation (Chakwizira *et al.*, 2016). Therefore, the N concentration dilution curve (NCDC) was further developed to evaluate N status for dry mass production in a wide range of crops, Thereafter, NCDC of cotton was figured out by Xue *et al.* (2007). Due to the exorbitant standard of data selection and over-scattered data regression across studies, results calculated by the NCDC model were limited for the use of theoretical prediction of N requirement of cotton in reproductive growing stage. The diagnosis and recommendation integrated system (DRIS) approach was also employed to monitor the nutrient status of cotton plants. Their conclusions, however, were mainly drawn by inter-element ratios which were insufficient in relation with growth and yield data (Singh *et al.*, 2012).

With the aim to characterize nutrient status in tree seedlings, Timmer (1991) proposed an integrated model with analysis on the relationships among dry mass, nutrient content, and nutrient concentration (Fig. 1). This model was developed from the "steady-state nutrient status" concept and characterized nutrient status of seedlings into those of deficiency, luxury consumption and toxicity with the increase of nutrient supply. These statuses were classified by two critical nutrient-supply doses, named sufficient and optimum points. Any nutrient supply between these two doses would cause the increase of nutrient content and concentration without significant changes of dry mass. Although this model was pre-conditioned and developed by the nutrient loading technique (Timmer, 1996; Salifu and Timmer, 2003; Salifu and Jacobs, 2006), its usage can be extended to study the response of nutrient status to doses of nutrient supply through several other fertilizer regimes (Wei *et al.*, 2012, 2013, 2014; Wang *et al.*, 2016; Li *et al.*, 2017, 2018). Current knowledge about the evaluation of nutrient status by fertilizer-response model was mainly from studies on vegetative growing stage of tree seedlings, quite little is known about its usage in field crop research at the reproductive stage. The N status in cotton plants has rarely been evaluated by the analysis through this model.

In this study, a field study on cotton was conducted in Xinjiang. Dry mass, N content and N concentration were measured in aerial part of cotton plants with N-fertilizer supply at five doses up to 480 kg N ha^{-1} at different days after germination. It was hypothesized that: (1) cotton plants would show significant responses of symptoms as deficiency, luxury consumption and toxicity to the increase of fertilizer N doses, and (2) the status induced by the optimum N supply dose would also result in highest yield and N use efficiency.

Materials and Methods

Site description

A field experiment was conducted at the Cotton Crop Study Center (44°39'15" N, 86°07'36" E), Liuhudi Village, Manasi County, Xinjiang, China in 2016. The soil was a loam which contained organic matter of 19.13 g kg^{-1}, hydrolyzed N of 50.80 mg kg^{-1}, available phosphorus (P) of 19.8 mg kg^{-1}, available potassium (K) of 160.1 mg kg^{-1}, and bulk density of 1.43 g cm^{-3} at the depth of 0-20 cm. Average temperature, available cumulative temperature over 10 °C, and frost-free time is 5 °C, 3,500-4,000 °C, and 180 d, respectively.

Experimental design and fertilizer treatment

The study consisted of five N fertilizer treatments at application rates of 0, 120, 240, 360, and 480 kg ha^{-1} which were labeled as the treatments of N0, N1, N2, N3, and N4, respectively. The experiment was arranged as a randomized complete block design with three replicate blocks. Each block consisted of five plots receiving five fertilizer treatments. Each plot had an area of 25 m^2 (2.5 m × 10 m) with a central planting area of 20.5 m^2 (2.05 m × 10 m) and two buffer-line areas of 4.5 m^2 (0.225 m × 10 m × 2). The two buffer-lines were placed along the two east-west directing lines of the northern and southern boundaries. The central planting area was mulched with drip irrigation for six planting-rows of cotton plants spaced 0.66 m apart and 0.1 m within-row. Thirty percent of the total fertilizer-N was applied at seeding with the mixture of urea (N-P_2O_5-K_2O, 46.4-0-0), 150 kg ha^{-1} of calcium superphosphate (N-P_2O_5-K_2O, 0-16-0), and 150 kg ha^{-1} of potassium sulphate (N-P_2O_5-K_2O, 0-0-50). The rest of 70% of N fertilizers were delivered using urea to cotton plants through drip irrigation over seven applications. Specific schedule of fertilizer applications is shown in Table 1.

Sampling and determination

Both dry mass and N status were assessed five days after the second fertilization with drip irrigation. At each sampling, a plastic pipe in diameter of 5 cm was placed along the planting line in the plot. Three cotton plants were randomly selected and cut at the ground line with pruning shears along the pipe length. Pre-harvest sampled aboveground plant parts were bagged separately by plot and transported to the laboratory, where samples were dried to a constant weight at 55 °C in a forced-air drying oven. At the last sampling 145 days after germination, field plants were randomly selected along the pipe length and the fresh weight of lint and seed was recorded as the yield. Dried samples were ground with a cyclone sample mill to pass through a 1 mm screen. Dry mass N concentration was determined by the Dumas combustion technique using a LECO CNS-200 analyser (LECO Corporation, St Joseph, MI, USA). Total N content was calculated as the product of dry mass and the concentration.

Data analysis

To evaluate nutrient use efficiency and yield production ability, four variables were proposed in this study. N utilization index (*NUI*) was modified from Hawkins (2007) to illustrate total dry mass in the shoot part supported by N assimilation:

$$NUI = DM_{shoot} / \%N_{shoot}$$

where DM_{shoot} is dry mass accumulation (kg ha^{-1}) in the shoot part and $\%N_{shoot}$ is percentage of N content per dry mass (%). Agronomic NUE (*aNUE*) is the increase in seed cotton yield divided by per unit of fertilizer N applied (Novoa and Loomi, 1981):

$$aNUE = (Y_f - Y_0 / F_{appl}$$

where Y_f and Y_0 indicate the cotton yield (kg ha^{-1}) in response to applied fertilizer N (F_{appl}) in kilograms per hectare. Internal NUE (*iNUE*) is the increase in cotton yield per unit increase in N uptake (Witt *et al.*, 1999):

$$iNUE = Y_{l\,\mathrm{int}} / TNU_f$$

where Y_{lint} is the lint yield and TNU_f is the total N uptake (kg ha^{-1}) by cotton plants from the fertilized plots. Yield per unit N uptake (*YUNU*) was modified from Ciampitti and Vyn (2011):

$$YUNU = Y_{l\,\mathrm{int}} / \%N_{shoot}$$

Repeated one-way ANOVA was conducted on parameters at each sampling date but results were graphed by dynamic trend lines across sampling dates. When significant effect was indicated (a=0.05) results were arranged according to means difference at the significance given by Turkey test. To facilitate integrated analysis of dry mass and N data, results in each fertilizer treatment were further standardized to range between 0 and 100 with the N0 treatment as 100. The gross N status of synergic dry mass, N content and N concentration in a certain fertilizer treatment was analyzed and diagnosed according to Fig. 1. The nutrient status of seedlings under a given nutrient dose according to this model was suggested to be interpreted by the vector nomograph with standardized data of dry mass, content and concentration (Timmer and Miller, 1991; Timmer, 1996). Specific N status was evaluated by the nomograph proposed by Salifu and Timmer (2003) and Salifu and Jacobs (2006).

Table 1. The dynamic of experiment implements

Date	Implement	Fertilizer percent	Days
4-11	Sowing	30%	
4-23	Germination		1
6-17	Fertilization	7%	49
6-23	Fertilization	7%	55
6-28	Sampling		60
7-1	Fertilization	14%	65
7-6	Sapling		68
7-10	Fertilization	14%	72
7-15	Sampling		77
7-22	Fertilization	14%	84
7-28	Sampling		90
8-5	Fertilization	7%	98
8-12	Sampling		105
8-20	Fertilization	7%	113
9-3	Sampling		125
9-23	Boll yield		145

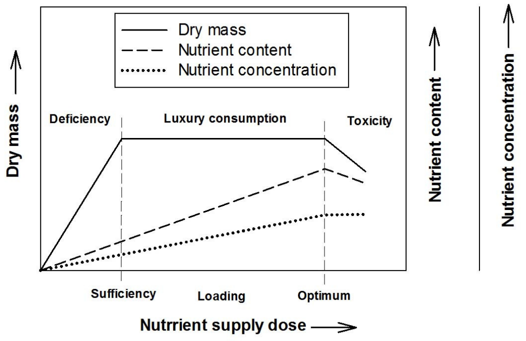

Fig. 1. The integrated model of conceptual relationships among changes of dry mass, nutrient content and nutrient concentration in response to exogenous N supply at increasing doses

Results

The dynamic of dry mass

At the 60 days after germination, no difference of shoot dry mass was observed among N fertilizer treatments (Fig. 2a). Shoot dry mass was greater in the N3 and N4 treatments than in the N0 treatment since the 68 days after germination. Shoot dry mass in the N2 treatment was greater than that in the N0 treatment at 68 ($P<0.0001$), 90 ($P=0.0027$), and 105 ($P=0.0006$) days after germination. At 77 and 125 days after germination, shoot dry mass in the N2 treatment was not different from either that in the N0 treatment or that in the N1 treatment. At 77 days after germination, the averaged shoot dry mass in the N3 and N4 treatments was greater by 144% than that in the N0 treatment ($P=0.0164$); at the 125 days after germination, the averaged shoot dry mass in the N3 and N4 treatments was greater by 211% than that in the N0 treatment ($P=0.0342$).

Dynamics of N concentration and N content

In contrast to dry mass, shoot N concentration declined with time (Fig. 2b). The N0 treatment resulted in lower shoot N concentration than the N2, N3 and N4 treatments by 17-25% ($P=0.0001$), 10-13 % ($P=0.0071$), 9-11% ($P=0.0034$), 14-21% ($P=0.0012$), and 25-29 % ($P=0.0002$) at 60, 77, 90, 105 and 125 days after germination, respectively. At 60 and 125 days after germination, the N3 and N4 treatment resulted in higher N concentration than the N1 treatment. At 68 days after germination, N fertilizer treatment had no effect on shoot N concentration.

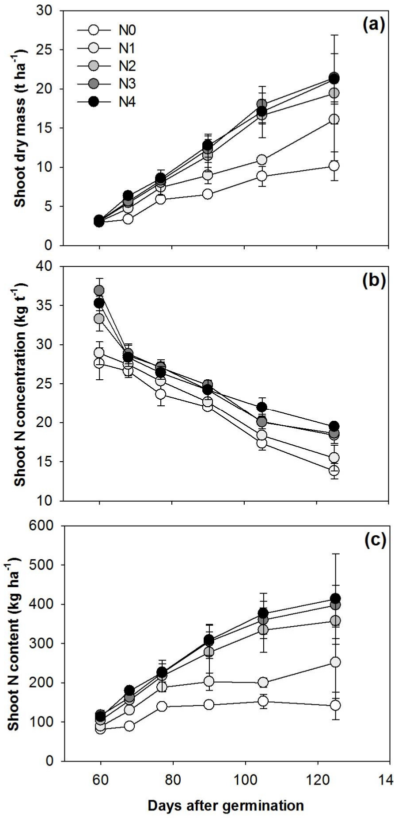

Fig. 2. The dynamic changes of dry mass (a), nitrogen (N) concentration (b) and N content (c) in cotton plants with N fertilizer addition in total amounts of 0 (N0), 120 (N1), 240 (N2), 360 (N3) and 480 (N4) kg ha^{-1} in Xinjiang, China in 2016. Cotton plants were sampled at 60, 68, 77, 90, 105 and 125 days after germination. Error bars indicate standard errors of the average of three replicates

Shoot N content increased with time but the increasing trend levelled off toward 90 days after germination (Fig. 2c). Compared to the N0 treatment, the N2, N3 and N4 treatments resulted in greater N content by 29-44 % ($P=0.0009$), 76-103 % ($P<0.0001$), 57-63% ($P=0.0043$), 94-116 % ($P=0.0013$), 119-147 % ($P=0.0002$), 153-193% ($P=0.0077$) at 60, 68, 77, 90, 105 and 125 days after germination, respectively. The N1 treatment did not result in greater N content than the N0 treatment at most sampling days except 68 days after germination when shoot N content was greater in the N1 treatment by 47% than that in the N0 treatment.

Evaluation of the dynamic N status

At 60 days after germination, shoot dry mass increased with N dose but results were not statistically different among treatments (Fig. 3a). Instead, both N concentration and N content increased with N doses from 0 (N0) to 158.4 kg ha^{-1} (N3) (Table 1) then kept constant even under more N addition (N4). These changes together suggested the N status of luxury consumption (Fig. 4a) where N addition probably had acted as nutrient loading (Fig. 1).

At 68 days after germination, shoot dry mass started to increase with N supply doses (Fig. 3b). However, N concentration remained statistically unchanged resulting N content increased with dry mass. At this sampling day, accumulative N supply was in the range between 69.6 and 278.4 kg ha^{-1}. These results together suggest the N supply was sufficient (Fig. 4b) because N content accumulated in a steady state without the increase of N concentration as well.

At 77 days after germination, N status was differed by two N supply dose ranges (Fig. 3c). In the first range, shoot dry mass increased with N supply doses from 0 (N0) to 172.8 kg ha^{-1} (N2) (Table 1). Meanwhile, both N concentration and N content increased with N supply doses within this range, together suggesting the N status of deficiency (Fig. 4c). In the second N supply dose range from 172.8 kg ha^{-1} (N2) to 345.6 kg ha^{-1} (N4), although N concentration showed a tiny decline both dry mass and N content showed apparent increasing trends (Fig. 3c). These results suggested the N status of luxury consumption with stable N accumulation (Fig. 4c).

The N status at 90 days after germination was similar with that at prior sampling day (Fig. 3d). All shoot parameters of dry mass, N content and N concentration increased with N supply doses from 0 (N0) to 206.4 kg ha^{-1} (N2), suggesting the N status of deficiency (Fig. 4d). Subsequently, N content showed stable accumulation and suggested the N status of luxury consumption between N doses of 206.4 kg ha^{-1} (N2) and 412.8 kg ha^{-1} (N4).

The N status at 105 days after germination also showed two-phase statuses (Fig. 3e). When N supply dose increased from 0 (N0) to 223.2 kg ha^{-1} (N2), the cotton plants showed the N status of deficiency; when N supply dose increased from 223.2 kg ha^{-1} (N2) to 446.4 kg ha^{-1} (N4) (Fig. 4e).

At 125 days after germination, the N supply doses for the N status of deficiency ranged from 0 (N0) to 360 kg ha^{-1} (N3), and the N supply doses for the N status of luxury consumption ranged from 360 kg ha^{-1} (N3) to 480 kg ha^{-1} (N4) (Fig. 3f; Fig. 4f).

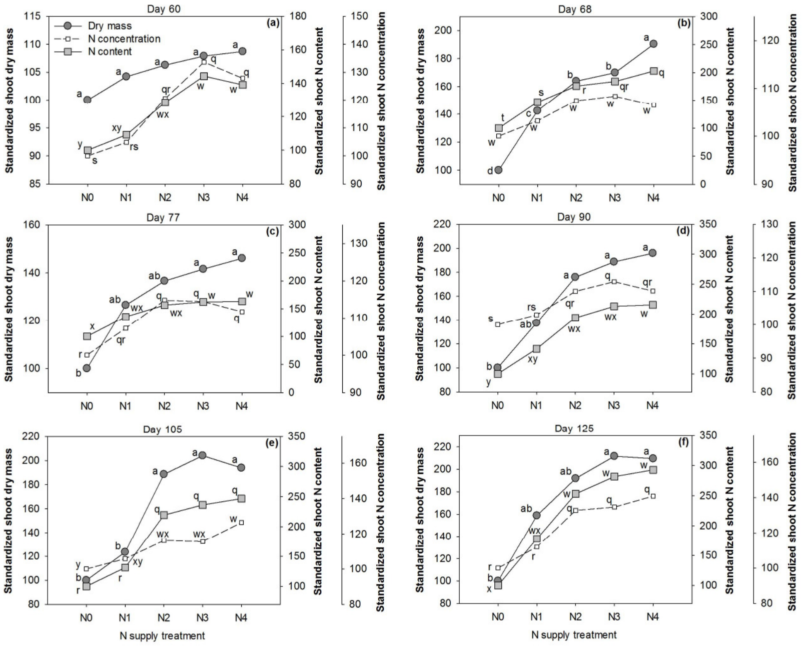

Fig. 3. The dynamic changes of dry mass, nitrogen (N) concentration and N content in cotton plants with N fertilizer addition in total amounts of 0 (N0), 120 (N1), 240 (N2), 360 (N3) and 480 (N4) kg ha^{-1} in Xinjiang, China in 2016. Cotton plants were sampled at 60 (a), 68 (b), 77 (c), 90 (d), 105 (e) and 125 (f) days after germination. All data are transformed to be standardized values between 0 and 100 with results in the N0 treatment to be the reference of 100. Different letters indicate significant difference at 0.05 level

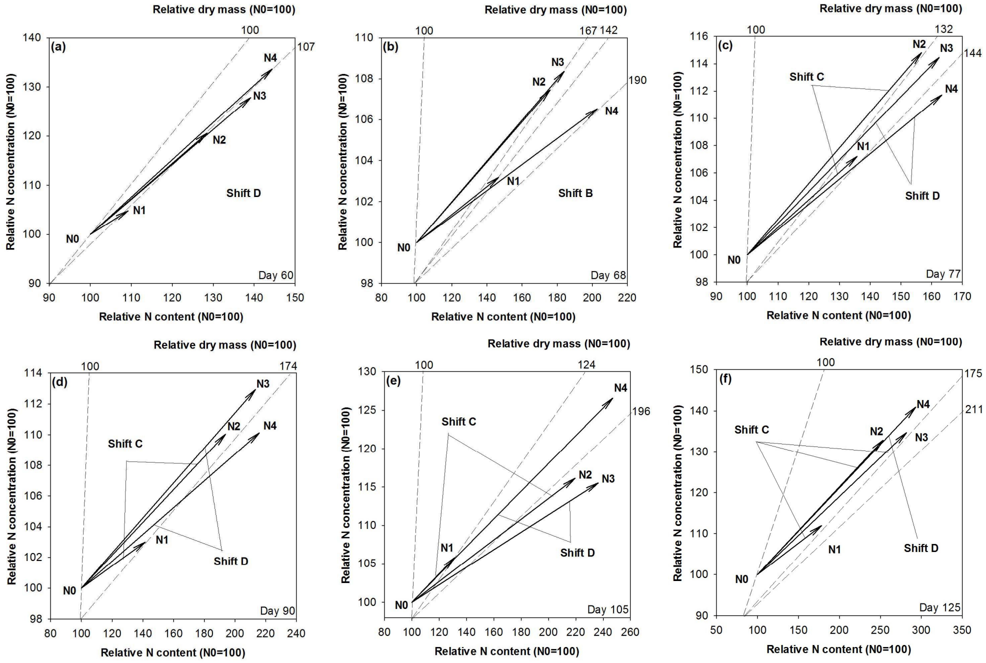

Fig. 4. Vector nomogram of N status of dry mass, nitrogen (N) content and N concentration in fertilized cotton plants compared to those in the controlled plots without N addition. Numbers at the end of dashed lines quantify relative dry mass. The type of nutritional response induced by enrichment is characterized by shift (or vector) direction and magnitude described by Salifu and Timmer (2003). Shift B, sufficiency at the steady-state status; shift C, deficiency at the limiting status; shift D, constant accumulation at the luxury consumption stage

Verification of the model

The preceding results showed that since the 90 d after germination the N2 treatment (240 kg N ha^{-1}) resulted in the critical change of shoot biomass until the 125 d after germination when any N addition over the rate of N3 treatment (360 kg N ha^{-1}) did not cause any significant biomass increment. Therefore, the time course of 90 d after germination appeared to be the critical time of growth management and the N application dose between the 240 and 360 kg N ha^{-1} appeared to be the optimum one for cotton plants. Therefore, another experiment was conducted on the cotton cultivar of 'Xinlu Early 45' with N application at the rate of 300 kg N ha^{-1} in 2017 and sampled at the same days after germination in 2016 (Fig. 5). Both biomass and N content increased with time until 90 d after germination (Fig. 5a). Thereafter, more N application did not cause any significant change of both biomass and N content. However, N concentration always declined throughout the second experiment. Vector analysis represented that before 90 d after germination the increase of biomass was faster than that of N concentration which generally resulted in the N dilution (Fig. 5b). Since the 90 d on, N addition began to cause antergic N excess.

Yield and N use efficiency

At harvest time, N fertilizer treatment increased lint yield compared to the N0 treatment and the N3 treatment resulted in the highest yield among N fertilizer treatments (Table 2). Likewise, the N3 treatment resulted in the highest N utilization index (*NUI*) among all treatments as well in spite results among the N2, N3 and N4 treatments were not statistically different. In contrast, N fertilizer treatment tended to decrease N use efficiencies (Table 2). Compare to the N1 treatment, *aNUE* in the N3 and N4 treatments declined by 37% and 65%, respectively. Compared to the N0 treatment, *iNUE* and *YUNU* generally declined by 54-60% and 10-18% in the averaged value of N2, N3 and N4 treatments, respectively.

Discussion

The public attitude to the symptom of N dilution between field crops and tree seedlings are different. N dilution is defined by the same meaning of N concentration decline during plant growth for both crop and forest plants. For field crops N dilution is widely taken as a natural symptom and the pre-condition to promote vegetative growth under the condition that N loading was not of the special concern (Xue *et al*., 2007; Chakwizira *et al*., 2016). To feed field crops with sufficient N will neither limit plant growth by N deficiency nor cause the problem of excessive N supply in toxicity performance and environmental contamination (Macdonald *et al*., 2015, 2016; Shah *et al*., 2017). A great effort has been made to detect the NCDC by evaluating the minimum concentration of N necessary at maximum shoot biomass in a wide variety of crop-plants including cotton (Xue *et al*., 2007). The NCDC method may be more useful to evaluate crops wherein yield is mainly derived from the dry matter accumulation than those from reproduction (Chakzizira *et al*., 2016). When the target

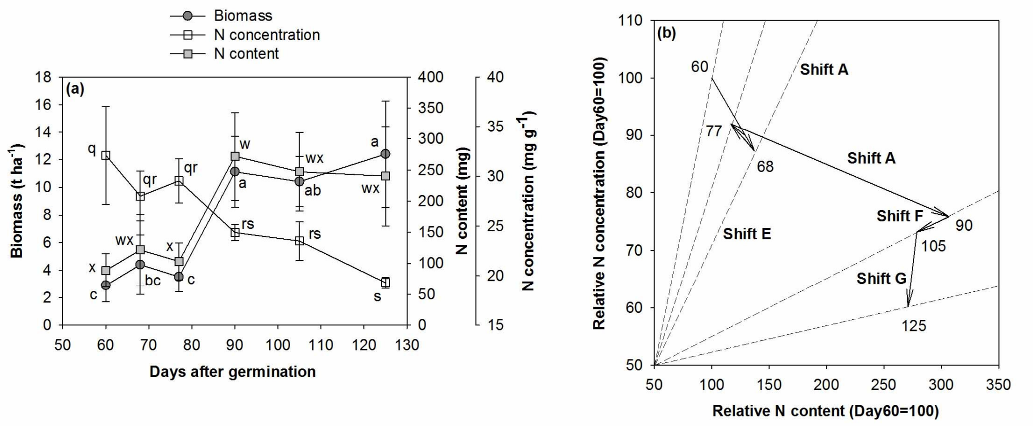

Fig. 5. Verification of the model using dry mass, nitrogen (N) content and N concentration in fertilized the cotton cultivar of 'Xinluzao 45' raised 60, 68, 77, 90, 105 and 125 days after germination (a) and the changes of relative N status in these days (b)

Table 2. Yield and N utilization indices [mean and (SE)] of cotton crops in response to N supply treatments at application rates of 0 (N0), 120 (N1), 240 (N2), 360 (N3), 480 (N4) kg N ha^{-1}

N supply treatment	Yield (t ha^{-1})		*NUI*		*aNUE*		*iNUE*		*YUNU*	
N0	5.22d	(0.09)	51.15c	(8.82)	-	-	38.78a	(9.93)	37.92a	(2.64)
N1	5.84c	(0.12)	59.4bc	(1.61)	5.18a	(0.45)	26.27ab	(11.5)	38.12a	(4.39)
N2	6.27ab	(0.11)	82.89ab	(15.88)	4.4ab	(0.29)	17.77b	(2.31)	34.28b	(2.07)
N3	6.40a	(0.11)	90.05a	(12.65)	3.28bc	(0.2)	16.3b	(2.26)	34.4b	(0.98)
N4	6.08bc	(0.10)	77.88abc	(4.34)	1.8c	(1.03)	15.62b	(4.47)	31.26b	(0.37)
Pr>F	<0.0001		0.0058		0.0008		0.0182		0.0494	

crop plant had the critical N concentration its dry mass during vegetative growth can be maximized but the yield during reproductive growth can be underestimated with low N input (Zhao *et al*., 2014). However, N dilution in tree seedlings during culture may impact growth or even results in mortality after transplant because most tree seedlings are cultured to plant in field (Timmer, 1996). Studies on tree seedlings showed that to counter nutrient dilution through N loading is the potential aim to improve transplant performance (Wei *et al*., 2013, 2014; Wang *et al*., 2016).

Because N content is the product of dry mass and N concentration, increased N content with time resulted from the increase of dry mass in spite of the tiny decline of N concentration. According to Jia *et al*. (2014), the Richards function best fit the nonlinear growth pattern of dry mass accumulation of cotton plants with time, wherein the key parameter was the product of thermal effectiveness and PAR in Xinjiang. The thermal and PAR conditions in Xinjiang promoted dry mass accumulation in cotton crops and subsequently benefited N content increase. Among the four N fertilizer treatments in our study, the N1 treatment increased N content to the least extent with time since 77 days after germination. In addition, both N concentration and N content in rates between the N1 and N0 treatments were not statistically different throughout the experiment. These results suggest that N fertilizer at the rate of 120 kg ha^{-1} was insufficient to maintain necessary N uptake for utilization. However, the N fertilizer rate of 92 kg ha^{-1} was already taken as the higher and better dose than the rate of 69 kg ha^{-1} (Ayissa and Kebede, 2011). N demand and initial soil N supply may both impact the evaluation on response to additional N dose; hence it is necessary to evaluate N status by the integrated model according to a range of N.

Generally, with the increase of dry matter accumulation N demand for growth by cotton plants would be enlarged with time .This resulted in the probably change of N status generating the dynamic response. This conclusion was the same with our results that the N status of steady-state uptake at the initial stage but it then changed to the deficiency state in the near lint production. Our results for cotton plants were agreed with by those about winter barley (Zhao, 2014) wherein the N-limited growth pattern also changed with time. Imo and Timmer (1997) specified the change of N status in mesquite (*Prosopis chilensis*) seedlings with time and found that N status can be elevated and promoted by N fertilizer regimes even at the same N addition doses. Current studies on N status in cotton plants mainly concerned the response to N fertilizer dose (Khan *et al*., 2017; Shah *et al*., 2017). Quite less information is known about fertilization regime effect on cotton plants compared to that on tree seedlings.

The initial soil for cotton planting in our study contents available N of 50.80 mg kg^{-1}, which was lower than that in farmland at Xinjiang (1.1 g kg^{-1}) (Zhang *et al*., 2017) and the experimental site in Wuhan (81.7 mg kg^{-1}) (Khan *et al*., 2017b). These results demonstrate that initial soil N content only contributed to a limited effect on N availability for planted cotton, generating an appropriate condition for cotton to uptake added N. With regard that the N-response curve in our study covered the range from N deficiency to N loading, initial soil N unlikely modified the general response of N uptake to exogenous N applications. Hence, our experiment was acceptable to be popularized as a model for cotton planting in other regions.

In our study, stem dry matter only accumulate up to 15-40% to the annual amount at the initial stage of vegetative growth. Therefore, the demand of stem for nitrogen absorption was very limited at the start. At this stage, N addition at the low rate of 50 kg ha^{-1} already induced cotton plants into the N status of luxury consumption up to the rate of 210 kg ha^{-1}. To the economic meaning, the N fertilizer dose of ~50 kg ha^{-1} (the N1 treatment) was recommended at this stage because it can already result in the greatest dry mass. However, to the physiological meaning, the N fertilizer dose may be recommended to be ~160 kg ha^{-1} (the N3 treatment) because it resulted in the highest N concentration without any toxic symptom.

At the second stage at 68 days after germination, the increase of N fertilization rates from 70 to 280 kg ha^{-1} increased dry mass without any effect on N concentration. This suggested that exogenous N addition at this stage was mainly used for utilization of biomass production. According to Khan *et al*. (2017b) N utilization at this stage should be driven by the translocation to reproductive organs because it was the time of first bloom of cotton plants in Xinjiang. In the same study area near ours, Khan *et al*. (2017b) applied all N fertilizers at this time when Zhang *et al*. (2017) did not conduct any N fertilization. According to our results, N fertilization at this stage is necessary. However, Khan *et al*. (2017b) chose to feed cotton plants with N fertilizers at the rate of only 180 kg ha^{-1}, which was not sufficient to support bloom.

Cotton N status showed similar trends in response to N fertilizer treatments from 77 days after germination (Figs. 3 and 4, c-f). The N amount from fertilizers in the N1 and N2 treatments was not sufficient for cotton demand but that in the N3 and N4 treatments was sufficient enough to induce luxury consumption due to unchanged dry mass but synchronized increases of N content and concentration. Accordingly, the N3 treatment (360 kg N ha^{-1}) resulted in the highest yield and N utilization efficiency. Therefore, it is the first time by our results to reveal that the N status of luxury consumption can also mean the highest yield in cotton. The term of “luxury consumption” was put forward by Timmer’s group on tree seedlings (Timmer and Miller, 1991; Timmer, 1996; Imo and Timmer, 1997; Salifu and Timmer, 2003) to describe the response to exponential fertilization. This status was established by the label of N reserve without utilization. In our study, the N3 and N4 treatments fed cotton plants with proper dose of N at each stage and induced the N reserve which facilitated abundant N utilization for growth and reproduction at the late growing stages (Khan *et al*., 2017b; Zhang *et al*., 2017). In the verified experiment, the dose of 300 kg N ha^{-1} failed to compensate the N dilution. Therefore, the rate of 360 kg N ha^{-1} should be the most close to the critical value of N fertilization. We did not employ any nutrient loading technique; hence our N fertilizer effect resulted from the dose but not the fertilization regime, which concurred with results by Wei *et al*. (2013). With regard to the practical meaning, the N fertilizer dose of 360 kg ha^{-1} in this treatment was recommended for cotton planting in Xinjiang. However, due to higher N utilization efficiencies at lower rates of N fertilizer doses, the N fertilizer dose of 120 kg ha^{-1} can be recommended when a lower yield of 5840 kg lint ha^{-1} can meet the goal of cotton culture.

Conclusion

Our study employed the fertilizer-response model involving with data of dry mass, N content and N concentration in shoot part of cotton plants to evaluate their N status in Xinjiang, China. We found that N status changed with time throughout the experiment season. At the early stage of 60 days after germination, N fertilizer treatment at the accumulative rate of as low as about 70 kg ha^{-1} was enough to induce the N status of steady state accumulation. Since 90 days after germination, the treatments delivering the total N fertilizer amount between 120 and 240 kg ha^{-1} was deficient for cotton utilization, but that between 360 and 480 kg ha^{-1} was sufficient to induce inherent N reserve and resulted in the highest level of yield. Our study supplied a new sight to evaluate N status in cotton plants, and our results can be meaningful for the practical culture of cotton plants. We recommend the N fertilizer regime at the rate of 360 kg ha^{-1} over six applications for cotton culture to obtain the greatest lint yield.

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Conflict of Interest

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

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