Nitrogen nutrition for cotton in a semi-arid environment

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Abstract

Nitrogen (N) is the most significant nutrient affecting crop growth and development for all types of crops, except legumes. The goal of this study was to optimize the N level for cotton grown in a semi-arid environment to enhance growth and development, determine N status, and increase seed cotton yield and biomass. Two independent field experiments each three years in duration were conducted, from 2007-2009 (Exp.-I) and 2018-2020 (Exp.-II). Experiments were laid out in a randomized complete block design with three replicates. The N treatments in Exp.-I were comprised of 0, 40, 80, 120, 160, 200, and 240 kg N/ha, while treatments in Exp.-II were comprised of 0, 70, 140, 210, and 280 kg N/ha. A wide range of data sets for cotton traits were recorded, including canopy height, leaf area index, the N status of the leaf and stem, seed cotton yield, and time series biomass data. The higher N rates 240 and 280 kg N/ha performed better for all these traits. However, the highest leaf N contents were recorded for 210 kg N/ha. Based on these results, it is suggested that under semi-arid conditions, slightly higher rates than optimum or recommended N rates could be applied as a strategy by cotton growers for a higher seed cotton yield. The findings of this study may also increase profitability in other cotton-growing areas that have similar weather conditions.

Keywords: Growth; Development; Component N status; *Gossypium hirsutum*

Introduction

The world's population is expected to rise to 8.3 billion in 2030 and to 9.3 billion in 2050, despite predictions from the UN that growth will dramatically slow down. Despite this slowdown in the population increase, there is a rising need for food, feed, fuel, and fiber (Ahmad *et al.*, 2021). Cotton (*Gossypium hirustum* L.) is commonly cultivated and then traded as fiber, yarn, fabric, or finished commodities (Ballester *et al*., 2021; Van der Sluijs, 2022; Ahmad *et al*., 2023). Customers prefer cotton fiber over synthetic fiber because it is a natural fiber that is soft to the touch, absorbs moisture well, and has a variety of uses (Thiry, 2011; Van der Sluijs and Johnson, 2011; Wakelyn *et al*., 2006; Ahmad *et al*., 2017, 2021). Because cotton is cultivated both as a fiber and as an oilseed crop, it has great social and economic importance. It is currently one of the top ten cash crops in the agricultural sector worldwide (de Oliveira Araujo *et al*., 2013; Khan *et al*., 2017; Tariq *et al*., 2017, 2018, 2020, 2021) and is a source of livelihood for 24.2 million people (International Cotton Advisory Committee, 2022). During 2021, it was grown on 32.6 million ha with a total production of 25 million tons of lint and 43 million tons of cotton seed for oil (International Cotton Advisory Committee, 2022). More than 70 countries grow cotton, with Asia contributing more than half of the total world production (Ahmad and Raza, 2014; Ahmad and Hasanuzzaman, 2020; Ahmad *et al*., 2023; Tariq *et al*., 2018, 2022).

Pakistan is a leading cotton-producing country, and cotton is Pakistan's most important cash crop (Ahmad *et al*., 2023; Tariq *et al*., 2018, 2022). Cotton produces both edible oil and yarn (Munir *et al*., 2020; Matloob *et al*., 2020; Rahman *et al*., 2020). Exports of lint and other value-added cotton goods account for 60% of Pakistan's overall foreign exchange profits, with cotton accounting for 0.7% of GDP and 2.9% of value-added agriculture (Govt. of Pakistan, 2024). Due to the cotton leaf curl virus and floods in 2021 and 2022, the cotton cropping area decreased to 1.94 million ha with a total production of 8.33 million bales. There are other factors responsible for this decrease, which include climate change, and improper crop nutrition (Tariq *et al*., 2018, 2022; Mubeen *et al*., 2021; Afzal *et al*., 2019; Ahmad *et al*., 2023; Rafi *et al*., 2015; Raphael *et al*., 2019; Rinehardt *et al*., 2004). Despite significant genetic improvement, global

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yield has been stagnant at around 750 kg lint/ha since 2010 (International Cotton Advisory Committee, 2022).

The most important nutrient for crop productivity is N, and the N fertilizer application recommendations are directly related to yield targets (Snider *et al*., 2021; Ahmad *et al*., 2023). The N availability affects cotton growth, development, physiological processes, and ultimately yields. Inadequate N leads to a series of deficiency symptoms that are generally recognized as N stress (Radin and Mauney, 1986; Snyder *et al*., 2009; Howard *et al*., 2001; Srivastava *et al*., 2018; Dhakal *et al*., 2019; Cochran *et al*., 2007; Chen *et al*., 2010). Low N exposure causes plants to grow more slowly, produce fewer fruiting sites, have lower leaf area, and mature earlier (Radin and Mauney, 1986; Reddy *et al*., 1997). The N deficiency also lead to shedding of fruit and thus fewer bolls and a decrease in yield (Bondada and Oosterhuis, 2001; Boquet and Breitenbeck, 2000).

Previous studies revealed that N application rates to cotton crops resulted in variable N accumulation in different plant parts (Hou *et al*., 2021), accumulation in the root structure (Chen *et al*., 2020a, b), and regulation of auxin (Krouk *et al*., 2010), abscisic acid, and salicylic acid (Chen *et al*., 2021). The role of N availability in the root-shoot relationship was elaborated by Chen *et al*. (2020a, b), showing that the growth of both the roots and the shoots was controlled by N. Genotype response to N supply is variable due to differences in traits such as root growth and architecture (Xu *et al*., 2012; Jiang *et al*., 2017). Conversely, increasing application rates of N can delay crop maturity but enhance vegetative growth and increase the retention of poor fruit at lower nodes of the main stem (Boquet and Breitenbeck, 2000; Hons *et al*., 2004). Because of these variations, measurements of crop growth and maturity should be included in assessments of cotton response to different N application rates. Measuring plant height or the number of main

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stem nodes over time, as well as using sigmoidal growth curves to determine a higher value for every parameter as well as the rate of growth for any given period in the season, are common methods for assessing plant growth response (Zhao *et al*., 2003). When N is applied to cotton before flowering, both the leaf area index (LAI) and blooming improve (Borowski, 2001). Higher N application rates promote vegetative growth, which delays crop maturity (Hons *et al*., 2004).

Experiments were conducted in the conventional cotton belt of southern Punjab, Pakistan, during 2007-2009 and 2018-2020 using a wide range of N application rates. The overall goal of this study was to determine the most efficient N levels for time series growth, N status, seed cotton yield, and time series biomass for cotton grown in a semi-arid environment.

Materials and methods

Experimental site and cotton cultivars

Cotton has been sown in research fields of the Central Cotton Research Institute (CCRI) since its establishment in 1970. Two field experiments were conducted at the experimental station of CCRI, Multan (30.19°N, 71.47°E, and 122 m above sea level), in southern Punjab, Pakistan for three years from 2007 to 2009 and for three years from 2018 to 2020. The soil is sandy clay loam in nature with fractions of sand, silt, and clay at 0.39, 0.44, and 0.17, respectively (Table 1). Composite soil samples from variable soil depths were drawn for various physical and chemical properties of the soil at the experimental site (Table 1). The data regarding soil properties showed that the soils were alkaline and low in fertility levels. The chemical properties of the study site were determined using the Bao (2000) methodology. The following techniques, i.e., potassium dichromate-volumetric, alkaline hydrolysis diffusion, Mo-Sb colorimetric, NH_4OAc

extraction were used for determining soil organic matter, alkali-hydrolyzed N, soil available P, and soil available K. The climate of the area is semi-arid with long, hot, and dry summers and short, very cold winters. Daily maximum and minimum temperature, rainfall, and solar radiation for the cotton growing seasons from 2007-2009 (Exp.-I) and 2018-2020 (Exp.-II) are presented in Fig. 1 and Fig. 2, respectively. The cotton cultivar MNH-886 was used for planting during both field experiments (Exp.-I and Exp.-II). This cultivar is commercially grown by farmers in the cotton belt of Pakistan.

Experimental design, and management practices

A randomized complete block design (RCBD) with three replications was used for both experiments. The treatments in Exp.-I that were conducted from 2007 to 2009 were, i.e., 0, 40, 80, 120, 160, 200, and 240 kg N/ha, while the treatments in Exp.-II conducted from 2018 to 2020 were 0, 70, 140, 210, and 280 kg N/ha. The N levels (as per treatment in both experiments in the form of urea) and application methodology were consistent with other cotton-growing areas in the region and phosphorus and potassium were applied at the rate of 100 kg phosphorus, 90 kg potassium per hectare in the form of di-ammonium phosphate (DAP), and sulphate of potash, respectively). The total 10-12 irrigations were applied as per need of the crop based on soil, crop and environmental conditions. However, the source of water was tube well. Each experimental unit contained six cotton rows with a 75 cm row-to-row distance. Thinning was carried out at the two true-leaf stages to maintain the optimum plant population. Further field management practices, i.e., irrigation, weeding, and insect and disease treatments were uniform and carried out according to recommendations of the extension wing of the Punjab Agricultural Extension Department.

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Sampling and measurements

Seed cotton was picked manually from an area of 1 m^2 marked from the central rows of each experimental unit. The seed cotton yield of every picking/harvest was sun-dried and weighed to attain seed cotton yield. Eight times plants from an area of 1 m^2 from each plot were harvested manually at 15-day intervals during various vegetative and reproductive phases. Biomass was calculated from the sum of plant parts from an area of 1 m^2, which were separated into their parts, i.e., leaf, stem, and cotton seed. The plant material was packed in craft bags for drying at 105°C for 30 minutes, subsequently dried at 70ºC to obtain constant weight, and then weighed. The separated leaves from an area of 1 m^2 were used to calculate leaf area using a leaf area meter, and afterward, LAI was calculated as the ratio of leaf area to ground surface area.

Total plant nitrogen uptake

Leaf, stem, and cotton seed N were determined at different growth stages. A pair of scissors was used to separate plant parts (leaf, stem, and cotton seed) during both the vegetative and reproductive phases. These components were oven-dried at 75°C, and the oven-dried plant samples were milled and screened through a 0.5 mm sieve. The N concentration was estimated according to the micro-Kjeldahl technique (Bremner and Mulvaney, 1982) and presented on a dry weight basis. The N concentration of leaf, stem, and cotton seed was determined individually. For the calculation of N uptake, the N content (%) was multiplied by the biomass of respective plant components (leaf, stem, and cotton seed) using the following equation.

$$\text{N uptake (kg/ha)} = \text{Biomass x \% N} \qquad (1)$$

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Statistical analysis

Cotton crop parameters, i.e., leaf area index, biomass, seed cotton yield, leaf N, stem N, and cotton seed, N data sets were statistically analyzed using analysis of variance (ANOVA). To optimize the appropriate N level for cotton under a semi-arid environment, Statistics 8.1 software for RCBD with three replications was used. The main and interaction effects for N treatment means were compared for significance using the least significant difference (LSD) test at $p<0.01$.

Results (Experiments I and II)

Leaf area index

The leaf area index (LAI) in cotton increased considerably over time in all treatments in Exp.-I and Exp.-II. The diverse N regimes significantly affected LAI during both sets of field experiments conducted under semi-arid environmental conditions. For Exp.-I (2007-2009) and Exp.-II (2018-2020), LAI increased linearly for all N rates, and the cotton crop fully covered the ground area at 98 days after sowing (DAS) when it reached its peak value, and then decreased progressively (Fig. 3, Fig. 4, Fig. 5, and Fig. 6). For LAI, the highest values were recorded at 240 kg N/ha in Exp.-I and 280 kg N/ha in Exp.-II, while the lowest were recorded at 0 kg N/ha for both experiments (Table 2).

Seed cotton yield

The results presented in Table 2 for Exp.-I (2007-2009) and Exp.-II (2018-2020) showed that for both field experiments conducted under semi-arid environments, the seed cotton yield (SCY) was significantly affected by the diverse N regimes. The highest SCY was achieved at 240 kg

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N/ha in Exp.-I and 280 kg N/ha in Exp.-II and the lowest SCY was achieved at 0 kg N/ha for both experiments.

Lint yield

The results of the two field experiments conducted under semi-arid conditions depicted in Table 2 (Exp.-I; 2007-2009, and Exp.-II; 2018-2020) showed that LY was significantly affected by the diverse N rates. The highest LY was recorded at 240 kg N/ha in Exp.-I and 280 kg N/ha in Exp.-II, while the lowest LY was recorded at 0 kg N/ha for both field studies.

Above-ground biomass

The cotton crop biomass accumulation increased continuously over time for all treatments. The different N regimes significantly affected biomass accumulation for Exp.-I and Exp.-II (Table 2). In Exp.-I (2007-2009) and Exp.-II (2018-2020), biomass accumulation increased progressively for all N rates, and reached its peak values at 160 DAS (Fig. 3, Fig. 4, Fig. 5, and Fig. 7). The highest biomass accumulation values were recorded at the highest N rates 240 kg N/ha in Exp.-I and at 280 kg N/ha in Exp.-II, while the lowest were recorded at 0 kg N/ha for both experiments (Table 2).

Canopy height

The results revealed that for Exp.-II (2018-2020) the canopy height was significantly affected by the N treatments (Table 3). The highest canopy height was recorded at 280 kg N/ha and the lowest canopy height was recorded at 0 kg N/ha. On average across treatments and years, the canopy height ranged from 1.53 to 2.05 m during this study.

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Leaf nitrogen

The leaf-N was significantly affected by N treatments in Exp.-II (2018-2020). For leaf-N, a linearly increasing trend was observed at all N regimes, values peaked at 81 DAS, and afterward a decreasing trend was noticed (Fig. 8). The results revealed that the year-wise highest leaf-N values (65, 69, and 72 kg/ha; during 2018, 2019, 2020, respectively) were recorded at 210 kg N/ha, while the lowest (9.6, 11.5, and 11 kg/ha; during 2018, 2019, 2020, respectively; Table 3) were recorded at 0 kg N/ha.

Stem nitrogen

During Exp.-II (2018-2020), the stem-N was significantly affected by N regimes (Table 3). The stem-N was measured at a 15-day interval. The stem-N showed a linear increase during study, peaked at 81 DAS, and then a decreasing trend was noticed (Fig. 8). The data showed that year-wise the highest stem-N values (32.1, 32.7, and 32.4 kg/ha; during 2018, 2019, 2020, respectively) were recorded at 280 kg N/ha, while the lowest (4.6, 5.6, and 5.5 kg/ha; during 2018, 2019, 2020, respectively; Table 3) were recorded at 0 kg N/ha.

Cotton seed nitrogen

The results depicted in Table 3 reveal significant differences among N treatments for cotton seed-N. The cotton seed-N presented a sigmoid trend, and its highest values were recorded at 160 DAS (Fig. 9). The highest N rate 280 kg N/ha depicted the highest values (83.1, 101.1, and 100.5 kg/ha; during 2018, 2019, 2020, respectively), while the lowest N rate 0 kg N/ha recorded the lowest values 19, 22, and 24 kg/ha during 2018, 2019, 2020, respectively.

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Discussion

(a) Cotton growth (leaf area index and biomass)

In the present study, we evaluated growth time series LAI and biomass (Exp.-I: 2007-2009; and Exp.-II: 2018-2020) under a semi-arid environment. The study site was selected because it is the highest-yielding area, centrally located in the cotton region, and managed by the Federal Government of Pakistan. The highest values of time course LAI and biomass were reported at 240 kg N/ha in Exp.-I, while in Exp.-II the highest values of canopy height, time series LAI and biomass were reported at 280 kg N/ha. These optimum N rates in both studies were consistent with findings of large-scale N rate response studies conducted under semi-arid conditions by Ali *et al.* (2011) and Khan *et al.* (2014). The LAI and biomass accumulation were maximized at the highest applied N rate at the study site in the semi-arid environment in southern Punjab, Pakistan (Wajid *et al.*, 2010; Afzal *et al.*, 2019). However, Girma *et al.* (2007) and Rochester (2011) have documented 200 kg N/ha as the optimum rate. The higher amounts of N in the present study caused a considerable increase in time course growth and development during the entire cotton crop duration. The increased LAI and higher biomass were attained during the rapid leaf area development and biomass accumulation period (Liu *et al.*, 2022). This increase may also be due to higher N uptake and N accumulation by cotton crop components, i.e., leaf, stem, and cotton seed, which might have contributed to the higher LAI and biomass values for the higher N application of 240 or 280 kg/ha. The N being a vital component of cotton crop plant nutrition played a significant role in growth and development. The LAI and biomass were lowest at control or 0 kg/ha N application, and our results were consistent with Tariq *et al.* (2021), who also found that an improper N level reduces the leaf area development and biomass accumulation

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in cotton (Tariq *et al*., 2017, 2022). Control and lower N rates resulted in lower N availability rates for the crop required for fulfilling the cotton nutritional requirement, thus explaining why lower LAI and biomass were produced at these N rates (Tariq *et al*., 2018, 2022). The lower rates of N were not sufficient to meet the full N demands for cotton growth, which principally restricted leaf area development and biomass accumulation during cotton crop growth.

(b) In-Season N status

When the appropriate N rate is applied, efficient N uptake and utilization assure the formation of crop yields. Higher N application rates or availability improved the N supply capacity of the soil, which finally improves crop N uptake and utilization under semi-arid conditions (Wahab *et al*., 2022, 2024; Ahmad *et al*., 2018; Abbas *et al*., 2020, 2023). The study also determined that sampling of crop components in vegetative and reproductive growth phases could be a valuable indicator of in-season crop performance along with final productivity. In both experiments, higher N application rates of 240 kg/ha (Exp.-I) and 280 kg/ha (Exp.-II) in cotton significantly improved N supply capacity at all growth stages and phases compared with control or 0 kg N/ha. Our results showed that higher N uptake by cotton crop components, i.e., leaf, stem, and cotton seed, might have contributed to the greatest in-season N status at a higher N application. Rahman *et al*. (2018) and Ahmad *et al*. (2021) described that higher N fertilizer levels were obligatory to meet critical component (leaf, stem, and cotton seed) requirements and higher yields. Their findings corresponded closely with the results presented here where optimal N rates met critical a threshold for cotton growth in a semi-arid environment and hot climate (Rahman *et al*., 2020; Ahmad *et al*., 2017, 2023; Nouri *et al*., 2020; Ma *et al*., 2022). Liu *et al*. (2022) found that occurrences of higher N concentration in cotton parts, i.e., leaf, stem, and cotton seed resulted in

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higher growth and production. The control and lower rates of N application were insufficient to meet the requirements of the cotton crop through efficient uptake and utilization in our experiments, possibly due to lessor N fertilizer uptake and utilization and non-synchronization with cotton N demand during all growth stages and phases (Afzal *et al*., 2019; Tariq *et al*., 2020, 2021).

(c) Seed cotton yields

In this research, we also determined seed cotton yield in the two independent experiments (Exp.-I: 2007-2009; and Exp.-II: 2018-2020) under semi-arid conditions. The highest values of seed cotton yields were recorded at 240 kg N/ha in Exp.-I, while in Exp.-II at 280 kg N/ha. Seed cotton and final biomass yields gradually increased with increasing N application rates in both experiments (I and II) during all study years. The increased physiological traits and processes at appropriate N rates in cotton plants improved leaf area development and canopy cover that contributed towards higher seed cotton yield and final biomass production (Boquet and Breitenbeck, 2000; Yang *et al*., 2011, 2021; Ahmad *et al*., 2014; Ali *et al*., 2014). The higher seed cotton yield was attained by the cotton crop during the rapid reproductive phase (Liu *et al*., 2022). Overall, N nutrition determines the production and quality of cotton crops. Higher N application rates increased photosynthetic rates, in turn leading to a higher accumulation of photosynthates and metabolites and higher production and productivity. These two experiments demonstrated that 240 and 280 kg N/ha are optimum N levels and are adequate quantities to produce higher seed cotton yields under semi-arid environmental conditions. Gormus and El Sabagh (2016) found that overall improvement in crop performance and higher yield parameters was recorded with increasing N rates. Geng *et al*. (2015) found that cotton crop requires a

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continuous supply of N from the soil and that synchronizing N inputs with the needs of cotton is vital for yield formation. Geng *et al*. (2015) and Ghaffar *et al*. (2020) found that cotton crop needs not only a high supply of N but, more importantly, a greater proportion of N from the first bloom stage to the initial bloom opening. The results of this study revealed that better vegetative growth is a prerequisite for better reproductive growth in cotton (Fig. 3 - Fig. 9) under a semi-arid environment. Synchronization of timely optimum N application rates with optimum crop growth rates and application timing strongly influences time course crop productivity, as does partitioning of growth between vegetation and reproductive organs during vegetative and reproductive phases.

Way forward

Cotton as an indeterminate crop requires special N fertilizer management compared to determinate crops under semi-arid environmental conditions. Therefore, synchronization of timely optimum N application rates with crop N fertilizer requirements for optimum crop growth rates greatly influences the productivity of specific cultivars, especially for partitioning of photo-assimilates between vegetation and reproductive organs as it progresses simultaneously in cotton under a semi-arid environment. It is, therefore, recommended that under semi-arid conditions slightly higher doses of N could be safely used for obtaining higher seed cotton yields without having any negative impact on the environment through leaching or volatilization. The results of this study will be shared with farmers and extension workers of the region through outreach activities at the farmer fields. The N nutrition results for the semi-arid environment are also useful for policymakers and planner to help set priorities for the agricultural sector.

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Conclusions

The present study provided updated research results on cotton crop response to different N management regimes for semi-arid environmental conditions. The results showed that there were higher values for LAI, seed cotton yield, lint yield, biomass, and cotton plant components (leaf, stem, and cotton seed) N-status during the growing season for the higher N application rates. The rates used in these studies were similar to the agriculture department's recommendation of 250 kg ha^{-1} for this region.

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Ethical approval. Not applicable

Author contributions. GA and MT conceived and designed the study. ZF and AAW conducted data gathering. MA performed statistical analyses. SA wrote the article and CWJ and GH improved the final draft. All authors wrote the final draft before submission to journal.

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Table 1. Physical and chemical properties of study soil

Soil properties	Values
Physical analysis	
Sand (proportion)	0.39
Silt (proportion)	0.44
Clay (proportion)	0.17
Bulk density (g/cm^3)	1.35
Texture class	Sandy clay loam
Chemical analysis	
pH	8.11
EC (dS/m)	1.42
Organic matter (g/kg)	4.7
Total nitrogen (g/kg)	0.39
Available phosphorus (mg/kg)	6.92
Available potassium (mg/kg)	82.71

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Table 2. Effect of different N fertilizer levels on the maximum LAI, seed cotton yield, lint yield, and final biomass for Exp.-I and Exp.-II.

Exp.-I N levels (kg/ha)	**Maximum LAI (m^2/m^2)**			**Seed cotton yield (kg/ha)**			**Lint yield (kg/ha)**			**Final biomass (kg/ha)**		
	2007	**2008**	**2009**	**2007**	**2008**	**2009**	**2007**	**2008**	**2009**	**2007**	**2008**	**2009**
$N_0 = 0$	0.8 ± 0.05	0.9 ± 0.03	0.8 ± 0.02	904 ± 7.6	974 ± 6.5	783 ± 5.1	320 ± 2.8	345 ± 2.2	277 ± 3.2	2665 ± 6.0	2592 ± 4.1	1974 ± 3.9
$N_1 = 40$	1.6 ± 0.09	1.6 ± 0.07	1.4 ± 0.06	1284 ± 6.3	1765 ± 7.4	1074 ± 6.9	465 ± 5.0	638 ± 5.7	390 ± 5.4	3247 ± 5.2	4038 ± 6.0	2992 ± 4.3
$N_2 = 80$	2.1 ± 0.06	2.1 ± 0.05	1.9 ± 0.03	1374 ± 7.5	2138 ± 9.9	1392 ± 8.6	512 ± 6.8	794 ± 7.3	520 ± 8.1	4092 ± 5.9	5411 ± 6.9	3539 ± 6.1
$N_3 = 120$	2.5 ± 0.05	2.5 ± 0.09	2.2 ± 0.08	1838 ± 7.7	2883 ± 10.0	1665 ± 9.1	704 ± 7.8	1099 ± 8.9	638 ± 9.1	4949 ± 7.3	6929 ± 6.0	4266 ± 7.6
$N_4 = 160$	2.7 ± 0.02	2.7 ± 0.04	2.3 ± 0.06	2163 ± 6.4	3111 ± 9.3	1883 ± 9.9	847 ± 10.0	1215 ± 10.0	738 ± 10.3	5565 ± 9.9	7648 ± 9.0	4911 ± 9.5
$N_5 = 200$	2.7 ± 0.04	3.1 ± 0.03	2.6 ± 0.04	2211 ± 8.5	3465 ± 9.7	1929 ± 10.0	888 ± 9.9	1390 ± 10.5	775 ± 11.1	6211 ± 7.5	8512 ± 9.9	5356 ± 10.4
$N_6 = 240$	3.1 ± 0.05	3.2 ± 0.02	2.8 ± 0.05	2192 ± 6.8	3483 ± 8.9	1977 ± 9.8	880 ± 9.2	1395 ± 11.8	795 ± 15.1	6538 ± 9.9	8611 ± 11.6	5692 ± 12.8
LSD (5%)	0.19	0.12	0.15	65.0	77.0	56.0	32.0	47.0	35.0	277	311	247
Significance	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01
Exp.-II N levels	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**
$N_0 = 0$	1.1 ± 0.03	1.1 ± 0.04	1.1 ± 0.02	877 ± 3.1	992 ± 4.2	311 ± 2.3	351 ± 3.6	363 ± 3.2	347 ± 4.2	2577 ± 3.6	2556 ± 6.4	2263 ± 5.1

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N_1 = 70	2.5 ± 0.04	2.5 ± 0.03	2.5 ± 0.05	1956 ± 5.8	2088 ± 5.4	707 ± 4.7	756 ± 6.9	774 ± 7.4	629 ± 6.1	5665 ± 7.4	5711 ± 6.3	5338 ± 5.5
N_2 = 140	3.6 ± 0.07	3.6 ± 0.05	3.5 ± 0.06	2839 ± 7.9	2865 ± 9.3	1055 ± 5.9	1065 ± 10.3	1077 ± 9.0	874 ± 8.8	8012 ± 8.0	7892 ± 9.7	7583 ± 7.0
N_3 = 210	4.5 ± 0.05	4.5 ± 0.06	4.4 ± 0.08	2999 ± 5.6	3611 ± 6.8	1144 ± 7.0	1375 ± 10.8	1368 ± 11.2	1029 ± 15.3	9983 ± 6.6	9965 ± 7.2	9029 ± 9.1
N_4 = 280	5.01± 0.05	5.1 ± 0.07	5.0 ± 0.09	3121 ± 10.3	3483 ± 9.2	1221 ± 8.1	1363 ± 8.7	1374 ± 10.1	1183 ± 9.9	11112 ± 8.1	11088 ± 12.0	10092 ± 10.1
LSD (5%)	0.16	0.13	0.15	93.0	89.0	38.0	56.0	65.0	311	280	309	311
Significance	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01

LAI = leaf area index; N = nitrogen; LSD = least significant difference; ** $P \leq 0.01$

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Table 3. Effect of different N levels on canopy height, leaf N, stem N, and cotton seed N for Exp.-II

N levels (kg/ha)	Canopy height (m)			Leaf N (kg/ha)			Stem N (kg/ha)			Cotton seed N (kg/ha)		
	2018	**2019**	**2020**	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**
$N_0 = 0$	1.5 ± 0.03	1.6 ± 0.04	1.5 ± 0.02	10 ± 4.0	12 ± 7.1	11 ± 5.7	5 ± 3.1	6 ± 2.1	6 ± 2.3	19 ± 6.7	22 ± 5.1	24 ± 6.0
$N_1 = 70$	1.6 ± 0.02	1.6 ± 0.03	1.6 ± 0.04	33 ± 6.1	39 ± 5.3	38 ± 6.2	11 ± 4.2	11± 3.5	11± 3.7	44 ± 5.6	48 ± 4.9	47 ± 3.9
$N_2 = 140$	1.7 ± 0.04	1.7 ± 0.03	1.7 ± 0.03	58 ± 3.0	59 ± 6.1	60 ± 5.0	18 ± 2.9	18 ± 2.1	18 ± 3.1	63 ± 4.5	70 ± 3.9	70 ± 4.1
$N_3 = 210$	1.8 ± 0.06	1.8 ± 0.05	1.8 ± 0.02	65 ± 2.0	69 ± 2.0	72 ± 3.1	23± 4.0	24 ± 3.1	24 ± 3.4	74 ± 6.3	89 ± 4.3	88 ± 5.2
$N_4 = 280$	2.0 ± 0.04	2.1 ± 0.02	2.0 ± 0.03	63 ± 1.15	65 ± 1.1	68 ± 2.0	32 ± 3.8	33 ± 4.6	32.38 ± 4.1	83 ± 5.0	101 ± 4.3	101 ± 6.1
LSD (5%)	0.014	0.013	0.021	18.11	19.29	17.65	1.08	0.87	0.96	2.54	2.61	2.19
Significance	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$

N = nitrogen; LSD = least significant difference; ** $P \leq 0.01$

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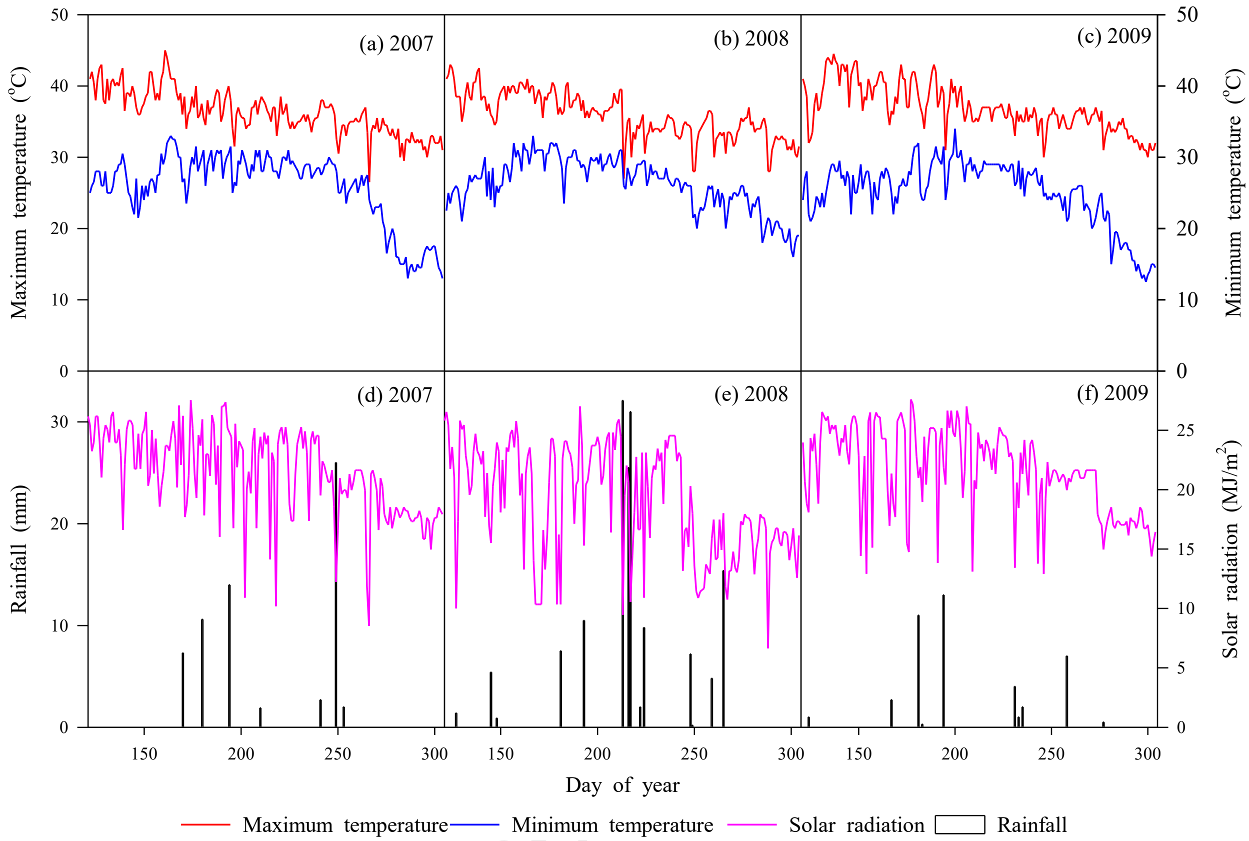

Figure 1. Maximum and minimum temperatures (a, b, and c), rainfall, and solar radiation (d, e, and f) for Exp.-I at the study site during 2007, 2008, and 2009.

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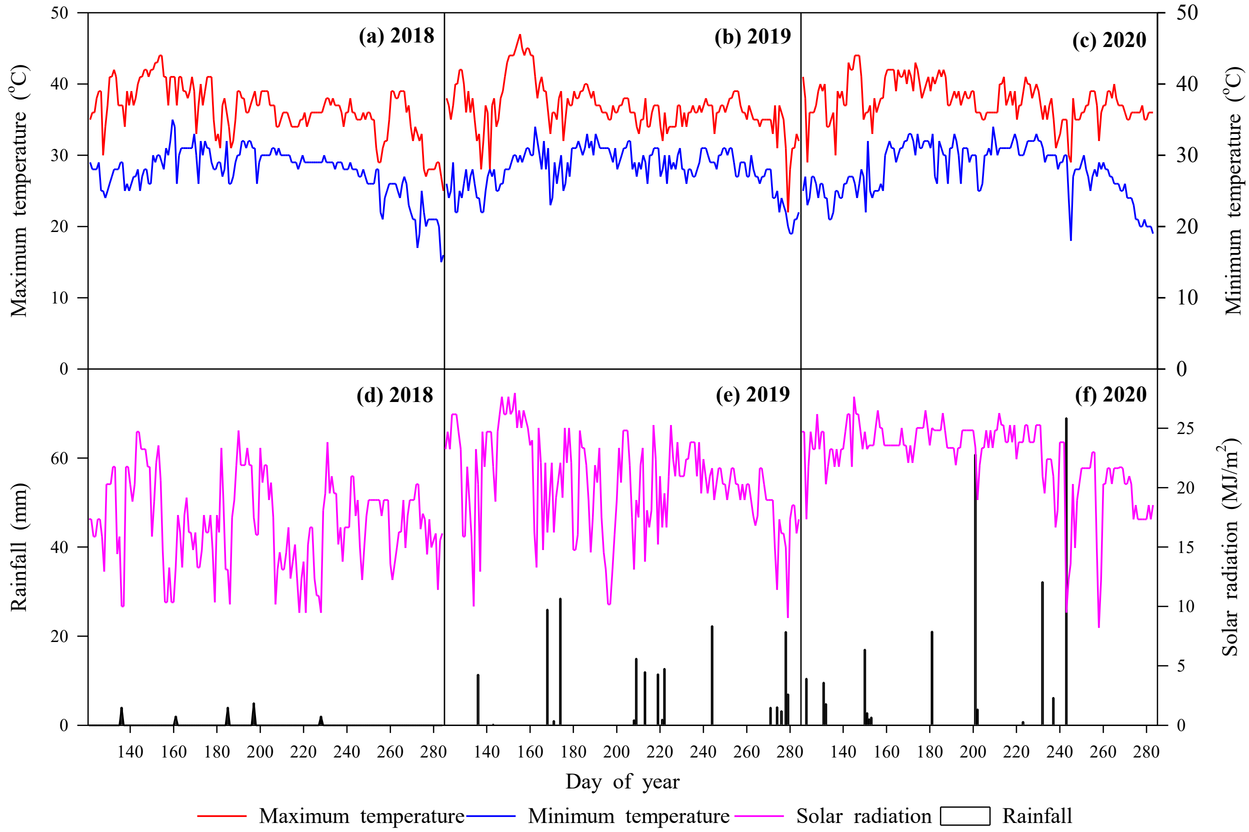

Figure 2. Maximum and minimum temperatures (a, b, and c), solar radiation, and rainfall (d, e, and f) for Exp.-II during 2018 (a and d), 2019 (b and e), and 2020 (c and f) growing seasons.

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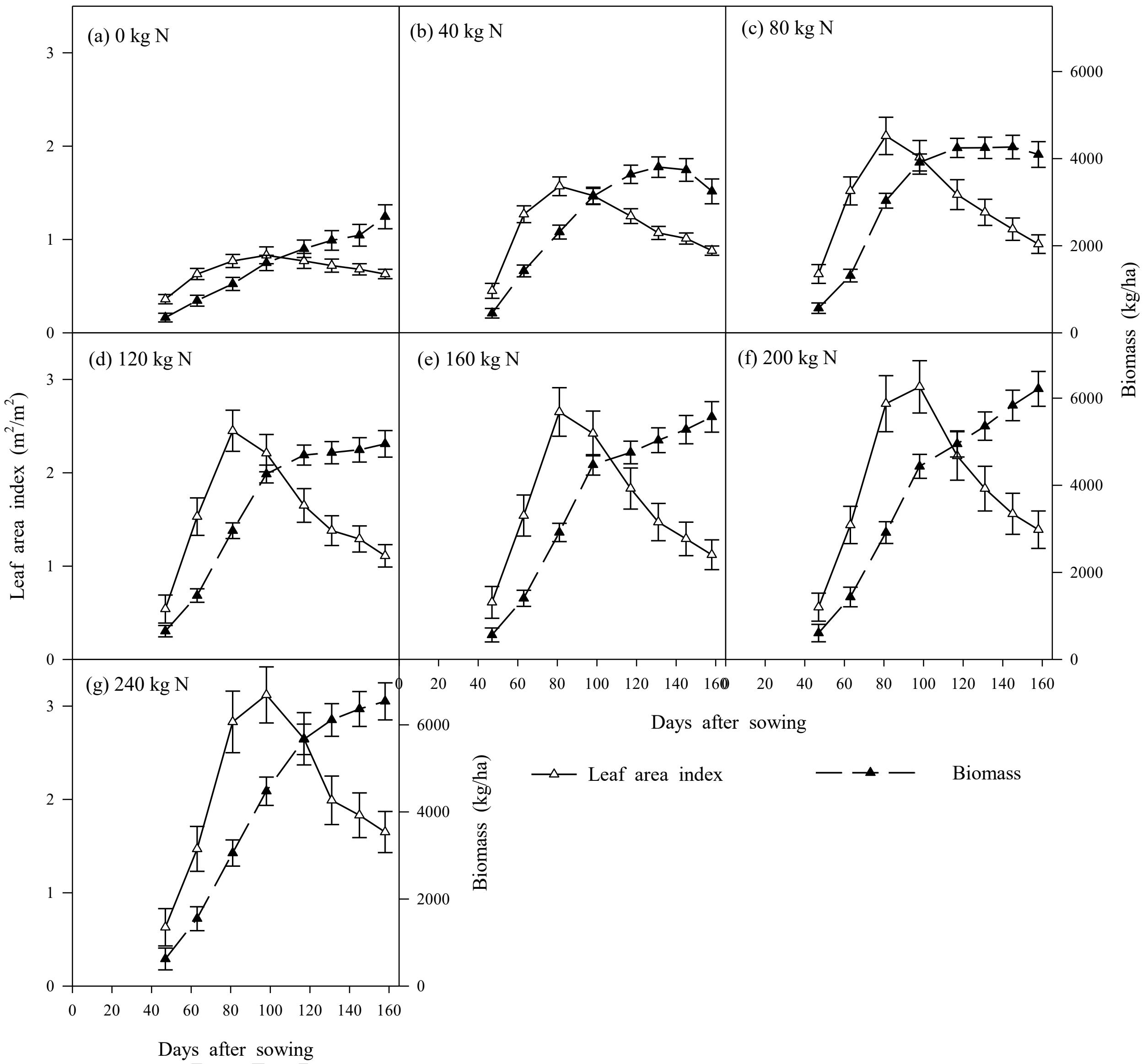

Figure 3. Effect of different nitrogen fertilizer application rates on the leaf area index and total biomass of cotton for Exp.-I at the study site during 2007 at 0 kg N/ha (a), 40 kg N/ha (b), 80 kg N/ha (c), 120 kg N/ha (d), 160 kg N/ha (e) 200 kg N/ha (f), and 240 kg N/ha (g). Bars represent standard errors.

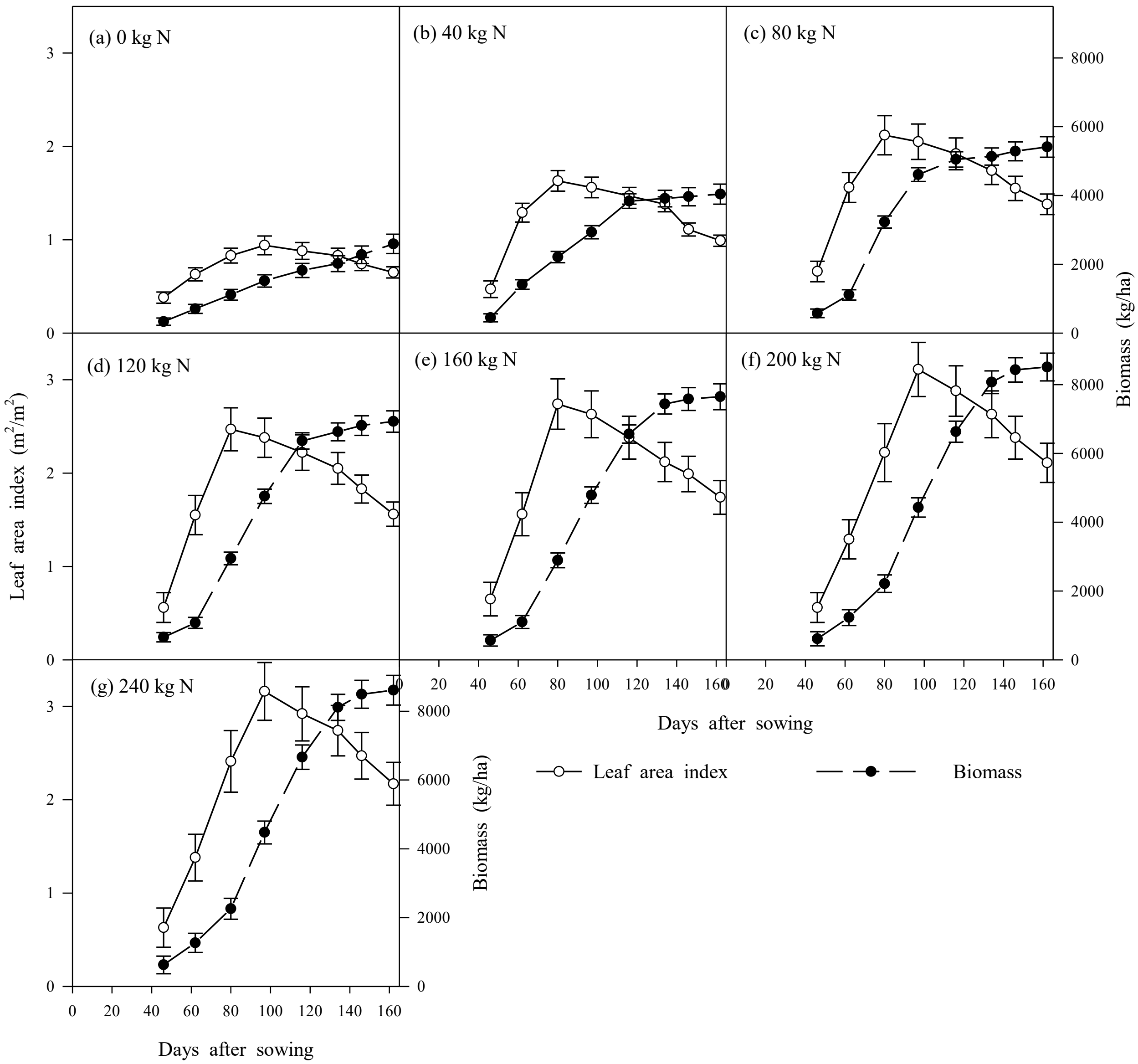

Figure 4. Effect of different nitrogen fertilizer application rates on the leaf area index and total biomass of cotton for Exp.-I at the study site during 2008 at 0 kg N/ha (a), 40 kg N/ha (b), 80 kg N/ha (c), 120 kg N/ha (d), 160 kg N/ha (e) 200 kg N/ha (f), and 240 kg N/ha (g). Bars represent standard errors.

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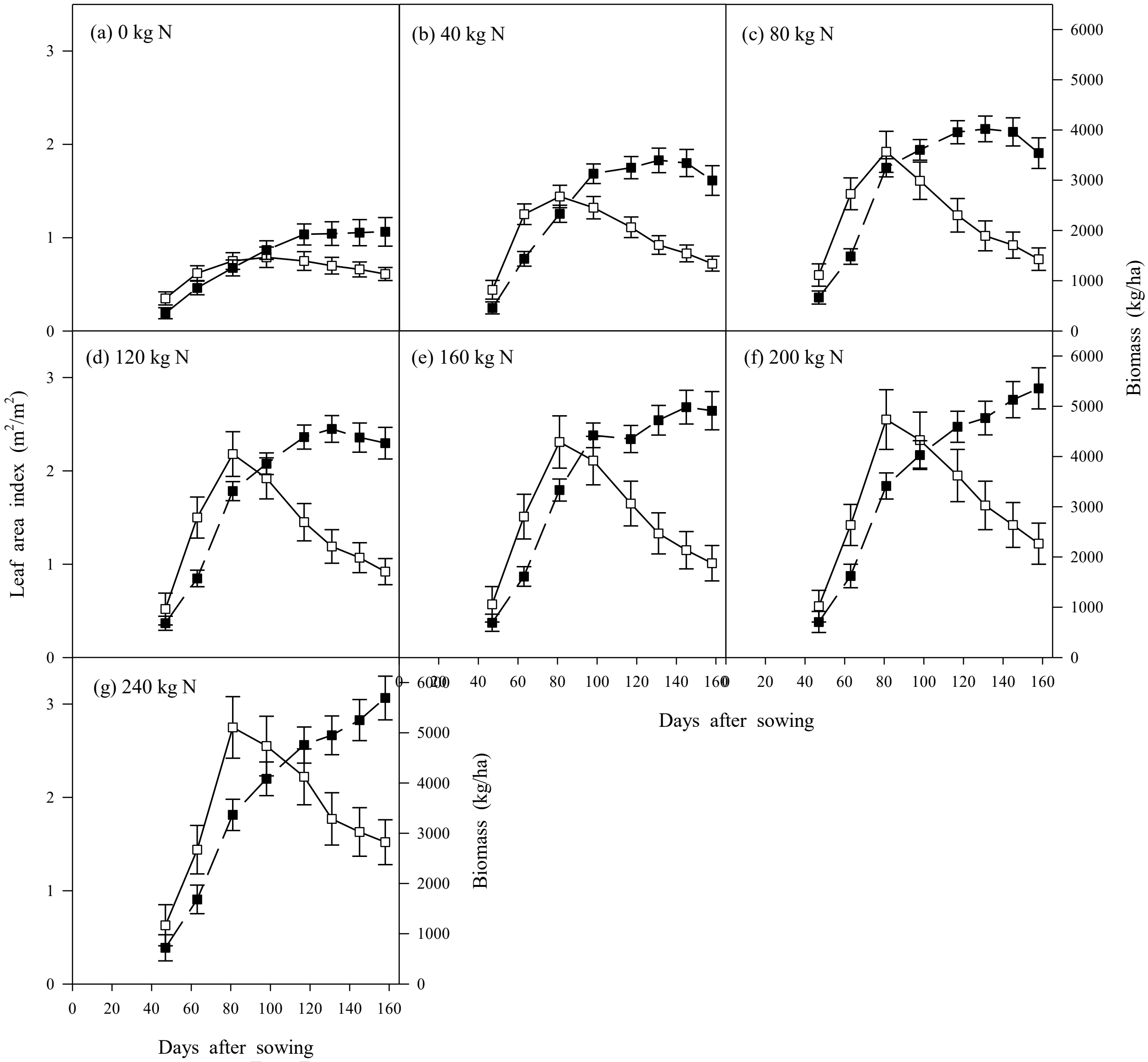

Figure 5. Effect of different nitrogen fertilizer application rates on the leaf area index and total biomass of cotton for Exp.-I at the study site during 2009 at 0 kg N/ha (a), 40 kg N/ha (b), 80 kg N/ha (c), 120 kg N/ha (d), 160 kg N/ha (e) 200 kg N/ha (f), and 240 kg N/ha (g). Bars represent standard errors.

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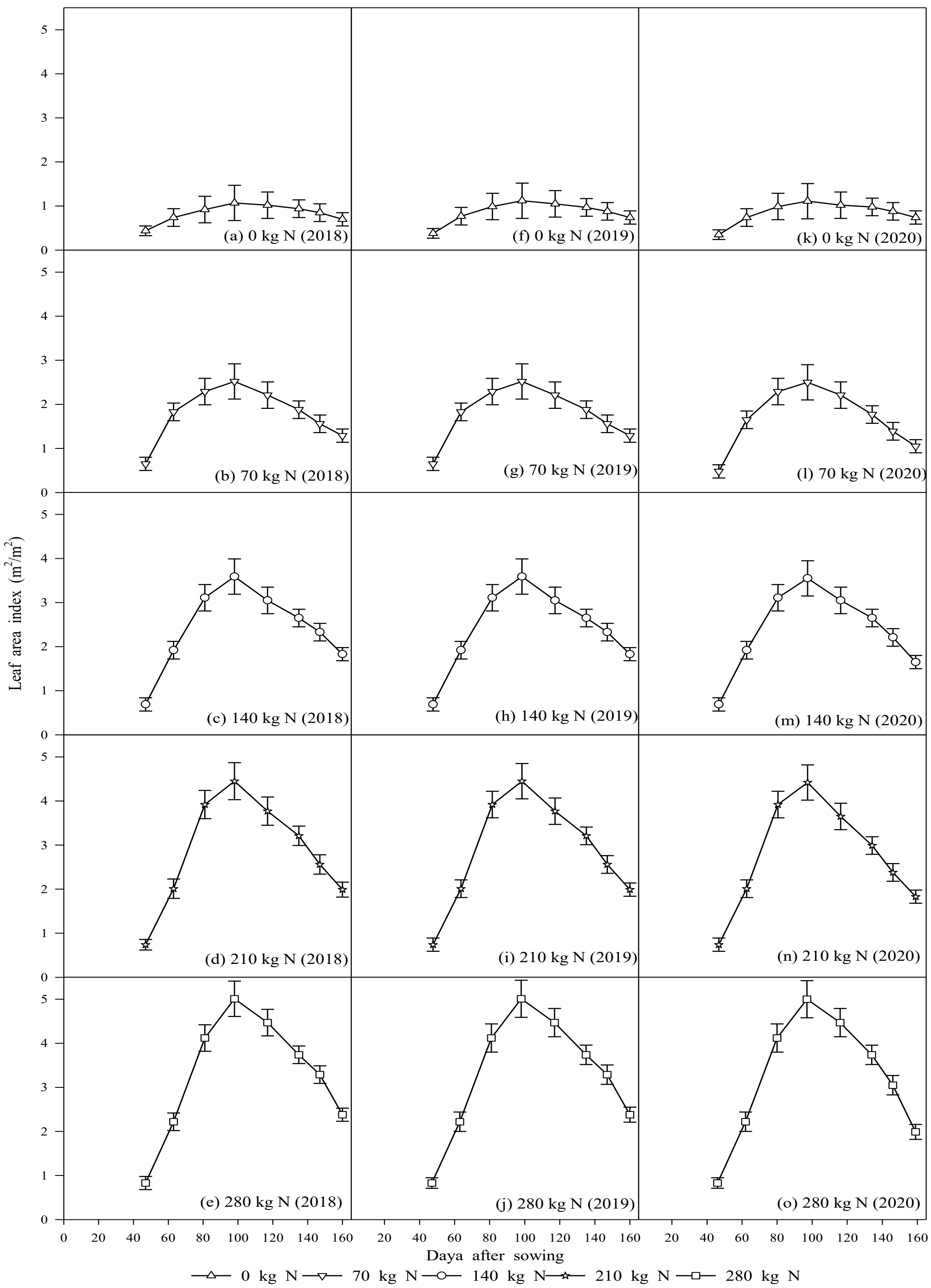

Figure 6. Effect of different nitrogen fertilizer application rates on the leaf area index of cotton for Exp.-II during the 2018 (a-e), 2019 (f-j), and 2020 (k-o) growing seasons at 0 kg N/ha (a, f,

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and k), 70 kg N/ha (b, g, and l), 140 kg N/ha (c, h, and m), 210 kg N/ha (d, i, and n), and 280 kg N/ha (e, j, and o). Bars represent standard errors.

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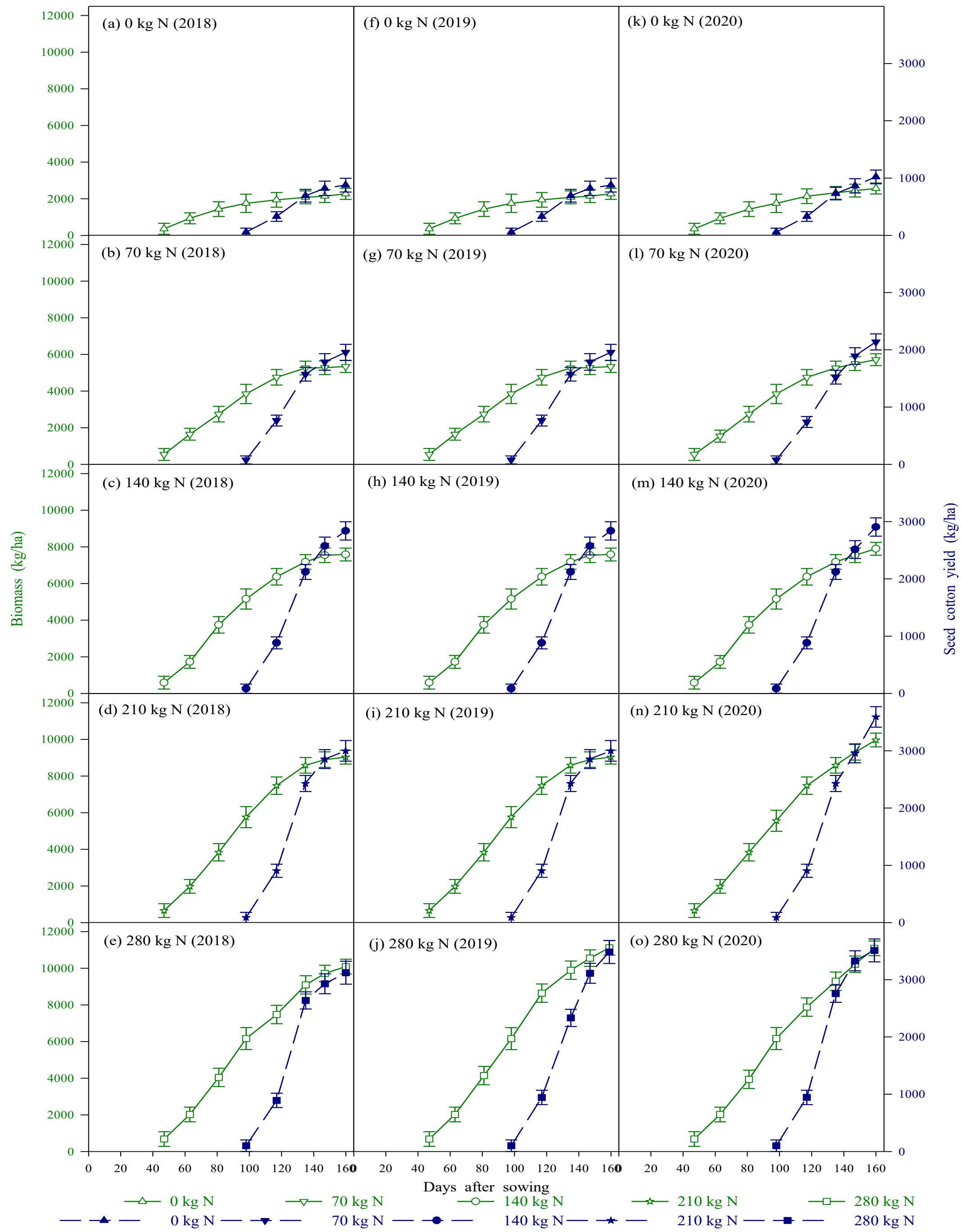

Figure 7. Effect of different nitrogen fertilizer application rates on total biomass (left-side) and seed cotton yield (right-side) for Exp.-II during 2018 (a-e), 2019 (f-j), and 2020 (k-o) cotton growing seasons at 0 kg N/ha (a, f, and k), 70 kg N/ha (b, g, and l), 140 kg N/ha (c, h, and m),

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210 kg N/ha (d, i, and n), and 280 kg N/ha (e, j, and o) at study site. Bars represent standard errors.

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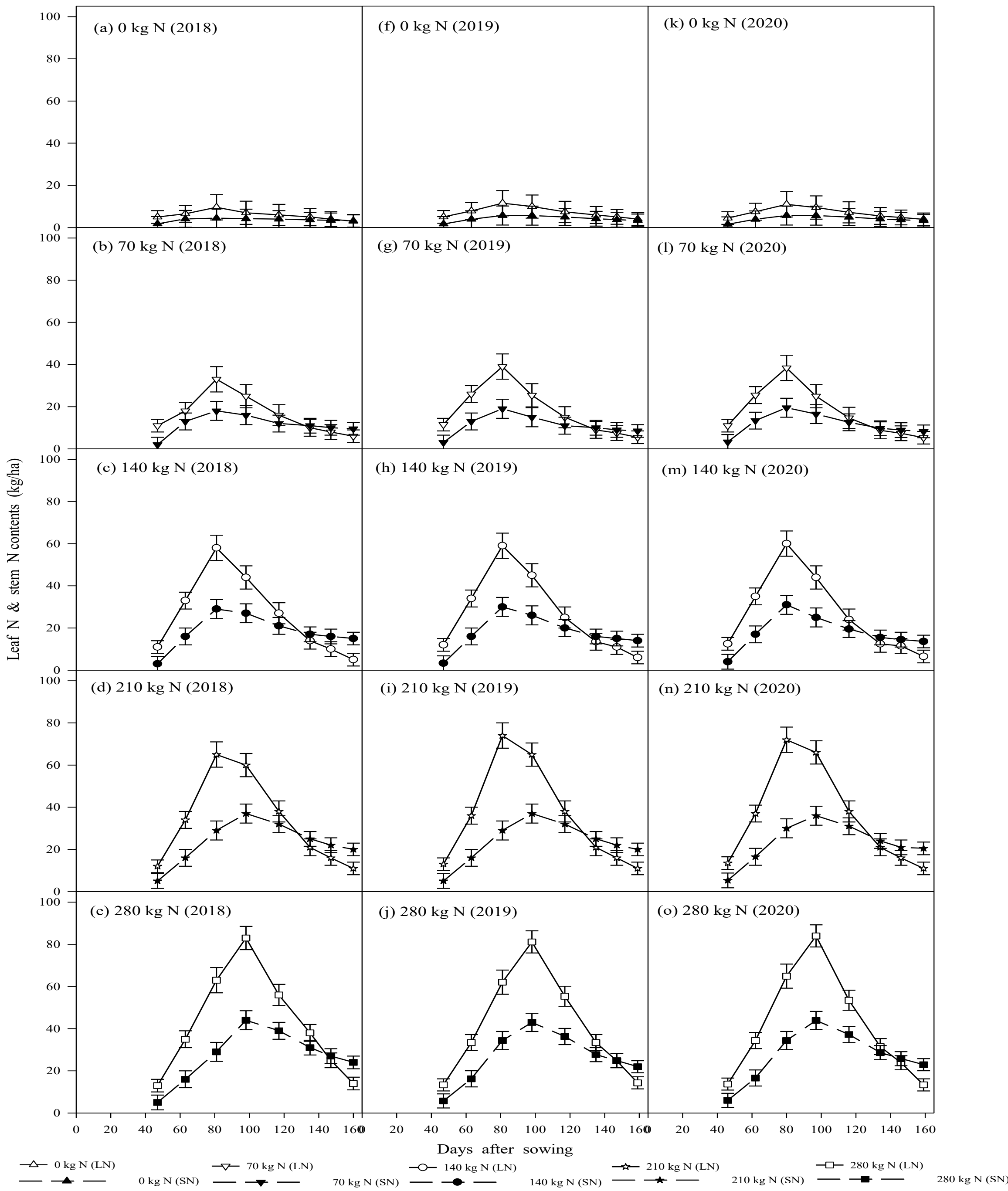

Figure 8. Effect of different nitrogen fertilizer application rates on the leaf-N and stem-N for Exp.-II during 2018 (a-e), 2019 (f-j), and 2020 (k-o) cotton growing seasons at 0 kg N/ha (a, f, and k), 70 kg N/ha (b, g, and l), 140 kg N/ha (c, h, and m), 210 kg N/ha (d, i, and n), and 280 kg N/ha (e, j, and o) at study site. Bars represent standard errors.

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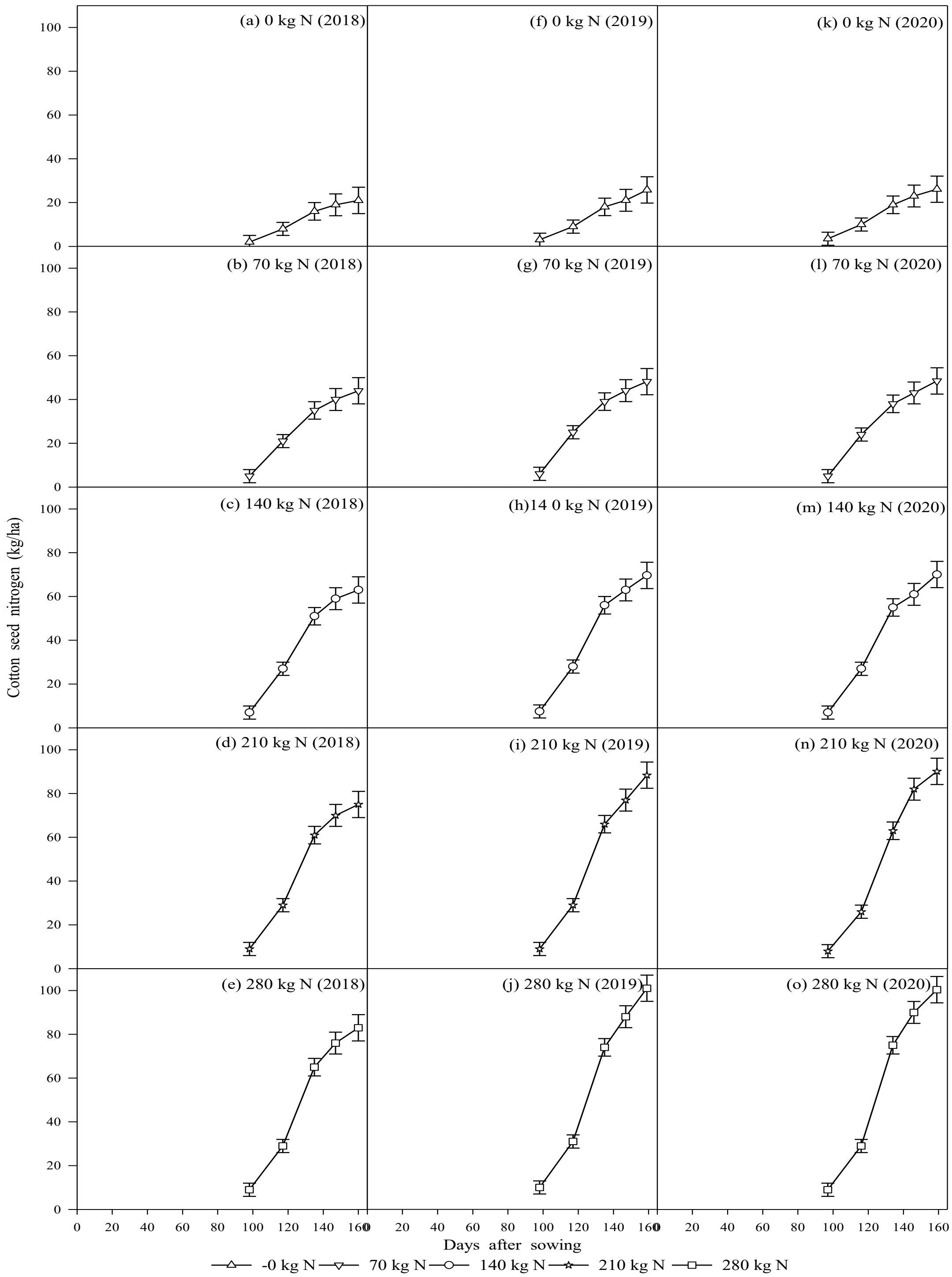

Figure 9. Effect of different nitrogen fertilizer application rates on cotton seed nitrogen for Exp.-II during 2018 (a-e), 2019 (f-j), and 2020 (k-o) cotton growing seasons at 0 kg N/ha (a, f,

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and k), 70 kg N/ha (b, g, and l), 140 kg N/ha (c, h, and m), 210 kg N/ha (d, i, and n), and 280 kg N/ha (e, j, and o) at study site. Bars represent standard errors.

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