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Morpho-agronomical and physiological response of cotton seedlings to low nitrogen supply

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Abstract

Among the crop production factors, nitrogen is the most important limiting factor of crop production. The relationship between photosynthesis and nitrogen nutrition has been widely studied in different crops. However, in cotton leaf photosynthetic traits and nitrogen use efficiency are less clear at the seedling stage. It was found that low nitrogen supply significantly reduced cotton growth, photosynthesis, nitrogen content and ultimately the nitrogen use efficiency except root morphological traits. Correspondingly, all the parameters related to gaseous exchange were very sensitive to nitrogen deficiency and therefore reduced. However, the intercellular CO_2 concentration and nitrogen efficiency ratio were increased under low N supply. These results suggest the possibility of utilizing these traits as indicators for optimum nitrogen fertilization and development of nitrogen efficient genotypes. Further, this could lead to the development of sustainable agriculture for better crop productivity and profitability as well as environmental protection.

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Introduction

Nitrogen (N) availability is one of the most important factors limiting plant growth and productivity in both natural and agricultural environments. It is an essential input in agricultural production and is the main constituent of many macromolecules, secondary metabolites and signaling compounds, which are required for plant growth and development. N fertilizer applications have previously provided an important guarantee for increasing food, feed and fiber production and reducing the pressure of global population growth. However, sub-optimal N supply is a major constraint for crop production, causing up to 50% yield loss (Jones *et al.*, 2013; Iqbal *et al.*, 2015). For this reason, large amounts of N fertilizer are applied to improve plant growth and yield (Glass, 2003; Sarasketa *et al.*, 2014) with an expected three-fold increase in application rate in the future (Good *et al.*, 2004). Indeed, large production and consumption of N fertilizer (amount to 30% of worldwide levels) in China have made a significant contribution to Chinese agricultural development (Zhang *et al.*, 2013). However, excess N fertilizer applications for crop production exert adverse environmental impacts, resulting in higher N_2O emissions and the eutrophication of freshwater and marine ecosystems (Qiao *et al.*, 2012). Over application of nitrogen has been a common problem in China, resulting in low N use efficiency (NUE) and environmental pollution (Miao *et al.*, 2011).

Beside the over use, nitrogen deficiency has also negative impacts like reducing plant growth and development, photosynthesis, leaf area, and ultimately limits plant productivity (Chen *et al.*, 2016). For a sustainable crop production system, there is a requirement to properly manage nitrogen fertilizer input and increase nitrogen use efficiency. This may be achieved by understanding the relationship between N nutrition and the photosynthetic rate in the leaf (Mu *et al.*, 2016). Photosynthesis depends on many physiological and biochemical processes such as stomatal conductance, intercellular CO_2 concentration, photochemical capacity of PSII, and contents and activities of carbon fixation enzymes (Zhao *et al.*, 2005). Photosynthesis has a positive relationship with leaf nitrogen (Uribelarrea *et al.*, 2009). This is because about 70% of leaf nitrogen is located in the chloroplast (Ghannoum *et al.*, 2005). Nitrogen deficiency reduces the content of chlorophyll, Cyt f, coupling factor, N content of thylakoid in light reactions, as well as the electron transport chain (Mu *et al.*, 2016). The decreased photosynthesis in low nitrogen was mainly associated with lower stomatal conductance (Zhao *et al.*, 2005). Therefore, combination of photosynthesis and related parameters are very important to know the crop productivity and finally the nitrogen use efficiency (NUE). Clearly, NUE is a complex trait that must be encoded by many different genes and their environmental interactions, but it can be dissected into two components. Firstly, the ability of the plant to take up N from the soil termed “nitrogen uptake efficiency” and secondly the ability of the plant to transfer N to plant organs and yield, known as “nitrogen utilization efficiency” (Xu *et al.*, 2011). Several studies on model and crop species have highlighted the genetic variability and the complex regulatory mechanisms controlling NUE under low and high N supply (Krapp *et al.*, 2011). Given the importance of the topic, it is surprising that relatively few papers have compared measures of NUE for cotton growing in different environments. However, it can be improved by precision nitrogen management and nitrogen efficient genotypes. For both approaches, it is necessary to diagnose plant nitrogen nutrition status timely and precisely. The importance of optimizing N management together with the selection of efficient genotypes may decrease excess fertilizer applications (Good and Beatty, 2011) which will subsequently reduce nitrogen leaching and environmental damage (Good *et al.*, 2004; Sebilo *et al.*, 2013).

Cotton (*Gossypium* L.) known as the white gold is the backbone of textile natural fiber in the world, grown worldwide in more than 50 countries (Smith and Cothren, 1999). Among the production inputs, nitrogen has the most vital role in cotton. Nitrogen is an essential element for canopy area development and photosynthesis (Wadleigh, 1944). Providing the right N amount during the plant growth will provide healthy leaves with the photosynthetic capacity needed to support the growth of the reproductive components (Bondada and Oosterhuis, 2001). Therefore, like other crops, cotton also increase the root morphological traits as well as reduces their N assimilation activities in response to low nitrogen supply, so that to adapt to the low nitrogen condition with the allocation of N resources to the leaves, which enhance the photosynthetic rates (Li *et al.*, 2012). Although the relationship between nitrogen deficiency and photosynthesis has been widely studied in other crops, less is known about the response of the photosynthetic system and nitrogen use efficiency in cotton to low nitrogen supply. However, the response may be different depending on the genotypes and species, leading to variations in their growth characteristics, adaptability to the environment, and morphological traits. Cotton morphological and physiological responses to low nitrogen have not yet been investigated, and the use of these traits in breeding programs requires the preliminary characterization. Such information is important not only for improving photosynthesis and nitrogen use efficiency performance under low nitrogen supply but also for the development of potential tools for the diagnosis of plant nitrogen nutrition status. This could be used in both the selection of nitrogen-efficient genotypes and precision nitrogen fertilizer management. Therefore, our study aims at characterizing the performance of cotton morpho-physiological analysis along with nitrogen use efficiency at seedling stage in response to low nitrogen supply.

Materials and methods

The experiment was conducted in a growth chamber at Cotton Research Institute of Chinese Academy of Agricultural Sciences, Anyang Henan China. Seeds of the cotton cultivar "TM-1" were germinated in a mixture of sand and vermiculite for one week in germinator. After the full opening of two cotyledons, uniform and healthy seedlings were selected and transplanted into 7 L plastic containers in a growth chamber (16/8 h light/dark cycle, 28°C light/dark temperature regime, 60% relative humidity). At the first week after transplanting, seedlings were supplied with 1/2-strength, followed by full strength Hoagland solution (1mM KH_2PO_4, 2mM KCL, 2mM $MgSO_4$, 0.1mM EDTA·Fe·Na, 46.2uM H_3BO_3, 9.1uM $MnCl_2·4H_2O$, 0.8uM $ZnSO_4·7H_2O$, 0.3uM $CuSO_4$ ·5H2O, 1.0uM $(NH_4)_6Mo_7O_{24}.4H_2O$) containing $Ca(NO_3)_2$ at 0.25 and 5mM, representing low and high N levels, respectively. In low N treatment, a total concentration of 1mM·L^{-1} $CaCl_2$ was added to equalize calcium concentration between the treatments. The nutrient solution was refreshed every week and aerated using an electric pump. The experiment was carried out in a completely randomized block design with three replicates. After cultivation for four weeks, the agronomic and physiological characteristics of cotton seedlings were investigated.

Plant growth, dry matter and root development measurements

Plant growth was measured by using four uniform plants from each replication. Data on shoot length (cm) was recorded with the help of a ruler by selecting four plants randomly from each replication and then the average was worked out. Similarly, lengths and widths of each leaf of four randomly selected plants was measured, then mean single leaf area was calculated. After four weeks, the plants were harvested and divided into roots (plant part below the graft junction) and shoots (plant part above the graft junction), placed in paper bags, labeled, and placed in the oven at 105°C for 30 min and then at 80°C for 72h. The dry weight of root and shoot was measured with an electric balance. Part of the root system (approximately 2g) was excised from each plant and then scanned and analyzed by using WinRHIZO root analyzer system (WinRHIZO version 2007b, Regent Instruments Canada, Montreal, QC, Canada).

Gas-exchange measurements and SPAD value

The photosynthetic characteristics (i.e., photosynthetic assimilation rate (A), stomatal conductance (gs), intercellular CO_2 concentration (Ci) and transpiration rate (E) of the third fully expanded leaves of six selected plants were measured using a portable photosynthesis system (Li-Cor-6800; Li-Cor, Inc., Lincoln, NE, USA) from 9:00 to 11:00 a.m. in the growth chamber. The chlorophyll content was measured with a portable chlorophyll meter (SPAD 502 Meter, Minolta Corporation, Tokyo, Japan).

Determination of chlorophyll content

For chlorophyll determination, approximately 50mg fully expanded fresh leaves were incubated with 50ml of acetone and an anhydrous ethanol solution (1:1, v/v) under darkness at 25°C for 12h. Following centrifugation at 4000g, the absorbance was measured at 663, and 645nm using a UV-2401 spectrophotometer (Shimadzu Corp., Kyoto, Japan) to determine the concentrations of leaf chlorophyll a (Chl a), chlorophyll b (Chl b) and chlorophyll a+b (Chl a+b), respectively as determined by (Arnon, 1949). The pigment concentrations were calculated based on absorbance values as:

Chl a (mg g^{-1} FW) = (12.7 × $A663$ − 2.69 × $A645$) × $V/1000 \times W$

Chl b (mg g^{-1} FW) = (22.8 × $A645$ − 4.67 × $A663$) × $V/1000 \times W$

Chl a+b (mg g^{-1} FW) = (20.29 × $A645$ + 8.05 × $A663$) × $V/1000 \times W$

where A663 and A645 are the absorbance values at 663, and 645 nm, respectively, V is the volume of extraction solution (ml), and W is the fresh weight of a leaf sample.

Measurement of nitrogen concentration, N accumulation, N efficiency ratio, NutE and NUpE

Total N concentration in plants was determined by the Kjeldahl method. The dried samples were ground into fine powder, and around 0.2g sample powder was weighed, digested with H_2SO_4-H_2O_2 and were then analyzed for N content according to (Li *et al.*, 2006). N values were used to estimate NUE based on different definitions as reported by (Abenavoli *et al.*, 2016). In particular, Total N Accumulation (TNA), calculated as the N concentration x total plant dry weight (NA= d. wt × NC) (mg N) (Lawlor, 2002); Nitrogen Efficiency Ratio (NER), calculated as the total plant dry weight divided by TNA (g TDW mg^{-1} N) (Gabelman and Gerloff, 1983); Nitrogen Utilization Efficiency (NUtE), calculated as the total plant dry weight divided by N concentration (g^2 TDW mg^{-1} N) (Siddiqi and Glass, 1981) and Nitrogen Uptake Efficiency (NUpE), calculated as TNA divided by root dry weight (mg N g^{-1} RDW) (Elliot and Laüchli, 1985), were determined.

Statistical analysis

A one-way ANOVAs were conducted to analyze the effects of nitrogen on cotton seedling using Statistix 10 software. Multiple comparisons were performed using the method of least significant difference (LSD) test. Graphs were generated using SigmaPlot software (SigmaPlot 13.0, United States). All the data results are expressed as mean ± standard error (SE) of three replications. *, **, and *** represent p ≤ 0.05, 0.01, and 0.001, respectively, and ns means not significant.

Results

Effect of low nitrogen on growth, dry matter and single leaf area

The growth and dry biomass of cotton seedlings were significantly affected by nitrogen supply (Table and Fig. 1). It was observed from the results that low nitrogen reduced shoot length by 24% at the end of the experiment. On the contrary, root dry matter was increased by 39% at low nitrogen treatment compared with the high nitrogen. Moreover, shoot dry matter (g plant^{-1}), total plant dry matter (g plant^{-1}) and single leaf area, in low nitrogen treated plants were reduced by 55%, 44% and 139%, respectively.

Table 1. Shoot length (cm), root dry weight (g), shoot dry weight (g), total plant dry weight (g) and single leaf area (cm^2) of cotton seedlings under low and high N conditions.

Low N	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)	Total plant dry weight (g)	Single leaf area (cm^2)
Range	14.2 ~ 17.1	0.51 ~ 0.54	1.22 ~ 1.49	1.7 ~ 2.0	58.24 ~ 70.84
Mean ± SD	15.97 ± 1.55	0.53 ± 0.01	1.38 ± 0.14	1.9 ± 0.08	63.61 ± 6.50
High N					
Range	20.2 ~ 22.1	0.23 ~ 0.39	2.99 ~ 3.23	3.33 ~ 3.47	92.01 ~ 118.65
Mean ± SD	21.1 ± 0.95	0.32 ± 0.08	3.09 ± 0.13	3.41 ± 0.04	105.25 ± 13.32
LSD	3.99	0.20	0.667	0.51	17.82
CV%	6.12	12.97	8.55	5.27	6.01
Significance	*	*	**	**	**

Note: Data are means ± SD (standard deviation). Whereas, ns stands for not significant (P > 0.05), * and ** significant at p < 0.05 and 0.01, respectively.

Effect of low nitrogen on root morphology and root to shoot ratio

As expected, low nitrogen availability significantly affected root to shoot ratio and root morphological traits (Table 2). The results indicated that root to shoot ratio was enhanced by low nitrogen supply as compared to high nitrogen. Similarly, root length (m), root projected area (cm^2), root surface area (cm^2), root diameter (mm) and root volume (cm^3) were significantly increased by 26%, 19%, 17%, 35% and 43% respectively under low nitrogen supply when compared with high nitrogen. This indicated that under low nitrogen condition, the roots had greater capacity to take up nitrogen and improve root growth than shoot growth for plant survival.

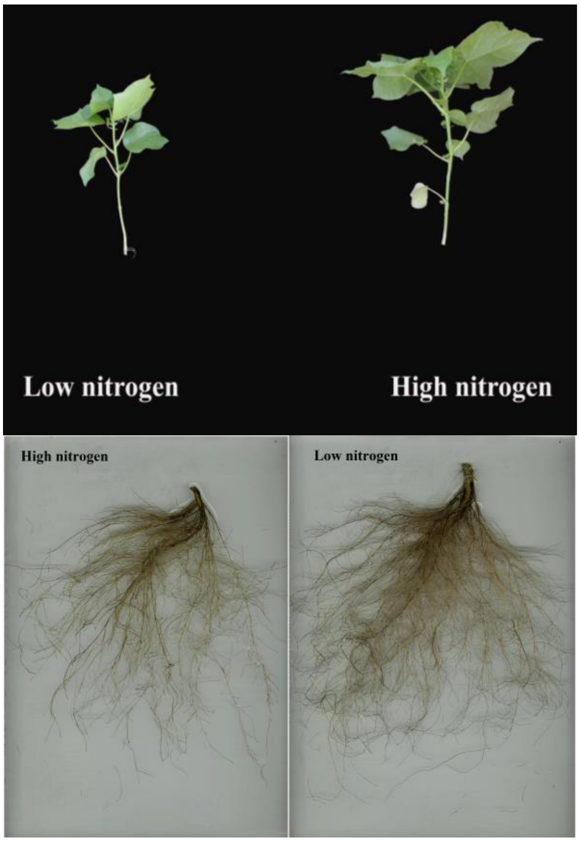

Fig. 1. Root and shoot phenotypes of cotton seedlings in response to low and high levels of nitrogen.

Effect of low nitrogen on chlorophyll content and SPAD value

Chlorophyll content and SPAD value of cotton seedlings were significantly affected by nitrogen application (Table 3). The chlorophyll a, chlorophyll b, and chlorophyll a+b concentration was lower (7%, 9% and 7% respectively) in low nitrogen treated plants compared with high nitrogen plants. Similarly, SPAD value was also significantly reduced by 10% under low nitrogen compared with high nitrogen treatment.

Table 2. Root shoot ratio, root length (m), projected root area (cm^2), root surface area (cm^2), root diameter (mm) and root volume (cm^3) of cotton seedlings under low and high N conditions.

Low N	Root shoot ratio	Root length (m)	Projected root area (cm^2)	Root surface area (cm^2)	Root diameter (mm)	Root volume (cm^3)
Range	0.35 ~ 0.43	18.84 ~ 22.20	138.7 ~ 169.3	480 ~ 532	0.88 ~ 0.93	8.93 ~ 9.83
Mean ± SD	0.39 ± 0.05	20.48 ± 1.68	153.8 ± 15.3	498 ± 29	0.91 ± 0.03	9.46 ± 0.47
High N						
Range	0.08 ~ 0.13	14.39 ~ 19.24	120.9 ~ 137.9	417 ~ 433	0.64 ~ 0.75	6.10 ~ 7.32
Mean ± SD	0.11 ± 0.03	16.20 ± 2.65	129.5 ± 8.5	427 ± 8	0.68 ± 0.06	6.63 ± 0.63
LSD	0.1511	3.87	16.93	61.88	0.2166	2.72
CV%	19.62	6.02	5.3	8.3	8.02	9.65
Significance	**	*	*	*	*	*

Note: Data are means ± SD (standard deviation). Whereas, ns stands for not significant ($P > 0.05$), * and ** significant at $p < 0.05$ and 0.01, respectively.

Table 3. Chlorophyll a (mg g^{-1}), chlorophyll b (mg g^{-1}), chlorophyll a+b (mg g^{-1}) and SPAD value of cotton seedlings under low and high N conditions.

Low N	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	SPAD value
Range	7.73 ~ 7.99	2.52 ~ 2.61	10.31 ~ 10.66	29.30 ~ 33.50
Mean ± SD	7.86 ± 0.13	2.56 ± 0.04	10.48 ± 0.17	31.57 ± 2.12
High N				
Range	8.30 ~ 8.53	2.75 ~ 2.85	11.12 ~ 11.44	33.60 ~ 36.40
Mean ± SD	8.42 ± 0.11	2.80 ± 0.05	11.28 ± 0.16	35.17 ± 1.43
LSD	0.37	0.014	0.38	1.7389
CV%	7.1	8.1	5.3	4.8
Significance	***	***	***	**

Note: Data are means ± SD (standard deviation). Whereas, ns stands for not significant ($P > 0.05$), * and *** significant at $p < 0.05$, and 0.001, respectively.

Effect of low nitrogen on photosynthetic characteristics and water use efficiency

As expected, low nitrogen significantly reduced photosynthetic characteristics and water use efficiency except stomatal conductance (Table 4). Application of low nitrogen significantly reduced the photosynthetic rate by 40% as compared to high N. Transpiration rate is lower (33%) in low nitrogen treatment compared with high nitrogen treatment. In contrast, the intercellular CO_2 concentration in low nitrogen treatment was 22% high than high nitrogen treatment. Water use efficiency was reduced by 11% under low nitrogen treatment, while it was high in the high nitrogen.

Table 4. Photosynthetic assimilation (Pn; µmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mol m^{-2} s^{-1}), intercellular CO_2 (Ci; µmol CO_2 mol^{-1} air), stomatal conductance (gs; mmol H_2O m^{-2} s^{-1}) and water use efficiency (WUE; µmol CO_2/mmol H_2O) of cotton seedlings under low and high N conditions.

Low N	Pn	E	Ci	gs	WUE
Range	4.66 ~ 6.12	3.45 ~ 4.01	250 ~ 275	0.23 ~ 0.27	1.16 ~ 1.77
Mean ± SD	5.61 ± 0.82	3.82 ± 0.32	266 ± 14	0.25 ± 0.02	1.49 ± 0.31
High N					
Range	9.06 ~ 9.63	4.74 ~ 6.74	213 ~ 228	0.25 ~ 0.30	1.39 ~ 1.91
Mean ± SD	9.36 ± 0.29	5.72 ± 1.00	218 ± 9	0.27 ± 0.03	1.67 ± 0.26
LSD	2.25	1.85	28.69	0.27 ± 0.03	0.11
CV%	8.55	11.06	7.3	12.22	6.2
Significance	**	*	**	ns	*

Note: Data are means ± SD (standard deviation). Whereas, ns stands for not significant ($P > 0.05$), * and ** significant at $p < 0.05$ and 0.01, respectively.

Effect of low nitrogen on root and shoot nitrogen content and accumulation

Root and shoot nitrogen content and shoot nitrogen accumulation of cotton seedlings were significantly affected by nitrogen application (Table 5). The root and shoot nitrogen content was significantly reduced by 34% and 42% under low nitrogen treatment compared with high nitrogen treatment. Similarly, shoot nitrogen accumulation of cotton seedlings was reduced by 74% in the low nitrogen as compared to high nitrogen application. However, nitrogen application had no significant effect on root nitrogen accumulation.

Table 5. Root N content (mg g^{-1}), shoot N content (mg g^{-1}), root N accumulation and shoot N accumulation of cotton seedlings under low and high N conditions.

Low N	Root N content (mg g^{-1})	Shoot N content (mg g^{-1})	Root N accumulation	Shoot N accumulation
Range	16.80 ~ 24.50	24.50 ~ 27.60	8.88 ~ 12.59	33.54 ~ 36.41
Mean ± SD	20.77 ± 3.86	25.75 ± 1.63	10.92 ± 1.09	35.31 ± 0.89
High N				
Range	28.50 ~ 35.40	38.70 ~ 51.30	6.68 ~ 13.70	117.70 ~ 155.12
Mean ± SD	31.63 ± 3.49	44.40 ± 6.39	10.34 ± 2.03	134.67 ± 10.94
LSD	9.97	17.72	ns	4.61
CV%	10.84	14.39	17.23	15.43
Significance	*	*	ns	*

Note: Data are means ± SD (standard deviation). Whereas, ns stands for not significant ($P > 0.05$), * significant at $p < 0.05$.

Effect of low nitrogen on total nitrogen content and nitrogen use efficiency

Total nitrogen content, N accumulation, N efficiency ratio, and N uptake efficiency of cotton seedlings was significantly affected by nitrogen application except N utilization efficiency (Table 6). Applying different common definitions, NUE in cotton seedlings supplied with low and high nitrogen treatments was calculated (Table 4). The total nitrogen content of cotton seedlings was significantly reduced by 39% under low nitrogen treatment as compared to high nitrogen. Total nitrogen accumulation under low nitrogen treatment drastically reduced by 68% than that of high nitrogen. In contrast, nitrogen efficiency ratio increased by 76% under low nitrogen as compared to high nitrogen. Low nitrogen treatment had no significant effect on nitrogen utilization efficiency, while nitrogen uptake efficiency was reduced by 81% in comparison to high nitrogen treatment.

Table 6. Total N content (mg g^{-1}), total N accumulation (mg N), nitrogen efficiency ratio (g DW mg^{-1} N), N utilization efficiency (g^2 DW mg^{-1} N) and N uptake efficiency (mg N g^{-1} RDW) of cotton seedlings under low and high N conditions.

Low N	Total N content	Total N accumulation	Nitrogen efficiency ratio	NUtE	NUpE
Range	44.40 ~ 49.00	42.42 ~ 49.00	0.0408 ~ 0.0416	0.039 ~ 0.043	80.26 ~ 95.34
Mean ± SD	46.52 ± 2.32	46.23 ± 3.41	0.041 ± 0.0004	0.041 ± 0.002	87.84 ± 7.54
High N					
Range	71.70 ~ 82.30	131.4 ~ 165.8	0.0203 ~ 0.0261	0.041 ~ 0.046	393.6 ~ 588.4
Mean ± SD	76.03 ± 5.56	145.01 ± 18.27	0.023 ± 0.0029	0.044 ± 0.003	470 ± 125
LSD	8.18	41.17	0.0143	ns	319
CV%	6.8	12.25	5.51	5.21	32.5
Significance	**	**	**	ns	*

Note: Data are means ± SD (standard deviation). Whereas, ns stands for not significant ($P > 0.05$), * and ** significant at $p < 0.05$ and 0.01, respectively.

Correlations among morphological and physiological traits

Correlations among morphophysiological traits of cotton seedlings are shown in Table 7. Out of total correlations, 35 were positive, 17 negative and 15 were not significant. The lower number of negative and high number of positive correlations suggests that cotton seedlings had strong coordination among different morphophysiological traits, and therefore continue to grow under low nitrogen supply giving preferences for some specific traits that are different in different N conditions. Most of the traits within the same group (morphological–morphological and physiological–physiological) were correlated with each other the percentage of positive correlations among morphological traits were only 50%, while that of physiological traits the positive correlations were double than the negative. This results suggested that under low nitrogen supply, plants mostly rely on physiological growth and efficiency of N metabolism. There was a non-significant correlation between leaf area and shoot nitrogen content in any of the N nutrition conditions. The most likely reason is that most of the variation for leaf area was constrained by a huge genetic effect as well as nutrition, whereas shoot nitrogen was affected by nutrition. This result suggested that most of the variation of morphological traits were caused by genetic and nutrition effects together, while in the case of physiological traits the main source of variation was nutrition alone as described previously by (Ikram *et al.*, 2011).

Table 7. Pearson's correlations among morphophysiological traits and N efficiency under low and high N conditions.

	SL	RD	SD	LA	PS	SN	RN	TN	TNA	NER	NUpE	NUtE
SL	1											
RD	-0.87*	1										
SD	0.94**	-0.94**	1									
LA	0.79*	-0.80*	0.92**	1								
PS	0.84*	-0.83*	0.94**	0.93**	1							
SN	0.90*	-0.86*	0.90*	0.73^{ns}	0.90*	1						
RN	0.88*	-0.69^{ns}	0.88*	0.87*	0.80*	0.72^{ns}	1					
TN	0.96**	-0.85*	0.96**	0.84*	0.93**	0.96**	0.89*	1				
TNA	0.96**	-0.89*	0.96**	0.84*	0.94**	0.98**	0.85*	1.00**	1			
NER	-0.95**	0.91**	-0.97**	-0.84^{ns}	-0.94**	-0.98**	-0.83*	-0.99**	-1.00**	1		
NUpE	0.91*	-0.99**	0.95**	0.79*	0.87*	0.92**	0.71^{ns}	0.90*	0.93**	-0.95**	1	
NUtE	0.57^{ns}	-0.62^{ns}	0.71^{ns}	0.86*	0.63^{ns}	0.35^{ns}	0.70^{ns}	0.52^{ns}	0.51^{ns}	-0.52^{ns}	0.57^{ns}	1

Note: Data are means ± SD (standard deviation). Whereas, ns stands for not significant ($P > 0.05$), * and ** significant at $p < 0.05$ and 0.01, respectively.

Among different traits, the nitrogen efficiency ratio was negatively correlated with all the traits except root dry weight, suggesting that under low nitrogen condition the plants tend to improve the root system for more nitrogen absorption and thus the nitrogen efficiency ratio improved. Other than nitrogen efficiency ratio, root dry weight had a negative correlation with all other morphophysiological traits in this experiment. Under low nitrogen, roots growth was enhanced, while that of the shoot were reduced, which are supported by their highly negative correlation in our experiment. Among the nitrogen use efficiency components, nitrogen utilization efficiency had no significant correlation with most of the morphophysiological traits except leaf area. As the leaf is the ultimate organ for nitrogen utilization that's why both are positively correlated.

Discussion

Nitrogen is an essential mineral nutrient required for plant growth and development. The excessive application of N fertilizers has led to increases in crop production but causing at the same time environmental pollution (Ding *et al.*, 2015). Indeed, crop plants are only able to acquire 30-40% of all the N fertilizer applied (Raun and Johnson, 1999), while the remaining N is immobilized in organic matter or adsorbed to the soil matrix, and/or lost by nitrate leaching, denitrification from the soil and loss of ammonia to the atmosphere, causing deleterious environmental effects (Glass, 2003). Hence, understanding how crops respond at physiological, morphological and molecular levels to different N rates is important for breeding new cultivars with high nitrogen use efficiency (NUE) and minimizing the agriculture environmental impacts. Plants enhance N uptake to maintain normal growth under low N conditions (Hakeem *et al.*, 2011). Most plants adapt to their environment by changing their growth, morphology and physiology (Sakakibara *et al.*, 2006). Relative biomass or dry weight is often used as an indicator of plant tolerance to low nutrition stress (Hermans *et al.*, 2006).

The morphological and physiological changes during plant adaptation to low N include a reduction in growth and photosynthesis, transfer of N from old to new leaves, and accumulation of photoprotective anthocyanin pigments (Li *et al*., 2013). A wide range of alterations in morphophysiological traits have been extensively studied in plants like rice, maize, oil rape under different N conditions (Ikram *et al*., 2011; Kessel *et al*., 2012; Abdel-Ghani *et al*., 2013). However less is known about the morphological and physiological variations in cotton seedlings at low nitrogen supply.

In our study morphological traits like shoot length, shoot dry weight and leaf area of cotton seedlings were significantly reduced by 24%, 55% and 40% respectively, under low nitrogen supply. However, root morphological traits are strongly affected by the N availability. In addition, the N supply leads to different effects on root growth; N deficiency induced longer roots, greater surface area and greater biomass. In our results, it was observed that root dry weight, root length (m), root projected area (cm^2), root surface area (cm^2), root diameter (mm) and root volume (cm^3) were significantly increased by 39%, 26%, 19%, 17%, 35% and 43% respectively under low nitrogen supply when compared with high nitrogen. The results suggested that plants tend to increase root under low nitrogen supply for maximum nitrogen absorption and limited shoot growth. The increase in root system under low nitrogen treatment might be due to more photosynthates partitioning to roots to form a large root system for better nitrogen absorption (Eghball and Maranville, 1993). The reduction in shoot growth and development has also been observed in previous studies (North *et al*., 2009; Barraclough *et al*., 2010). Plants grown in different N environments affect the normal growth, which limits the overall productivity of the plants (Kant *et al*., 2010). The decrease in the overall plant growth and productivity is due to N deficiency, as it is a fundamental constituent of different leaf cell components, especially those associated with the photosynthetic system, including carboxylating enzymes and membranous proteins (Mattson Jr, 1980; Pandey *et al*., 2000).

Therefore plants tends to adapt and cope with low N environment and obtain high yield and production (Kant *et al*., 2010). This adaptation of plants to low N comprised of composite morphological, physiological and developmental responses (Yang *et al*., 2011). Plants sense external N availability and respond accordingly via hierarchical morphological, physiological, and molecular adaptations, although long-term low N eventually inhibits both shoot and root growth (Goron *et al*., 2015). Unlike low nitrogen, high nitrogen treatment significantly increased shoot growth, leaf area and SPAD value of cotton seedlings. The increase in leaf area under high N supply might be due to the enhanced protein synthesis and consequently higher vegetative growth, which resulted in increased photosynthetic surface and stimulated further growth (ELTELIB, 2004).

Plant productivity depends on the plant and the metabolic expenditure (Cooke *et al*., 2003). Photosynthesis is the primary limiting factor of plant productivity. N is a constituent of the photosynthetic machinery, and N-containing compounds play an essential role in CO_2 fixation (Xu *et al*., 2012). Increased photosynthesis with less input of land, water, nutrients, etc., is essential to sustainably meet global food and bioenergy demands (Evans, 2013). New models have been proposed to increase the efficiency of light capture, light energy conversion, and carbon capture and conversion, possibly by rapidly developing genetic engineering technologies (Ort *et al*., 2015). Plants are often sensitive to low N condition (Illman *et al*., 2000). Photosynthesis has a close relationship with leaf nitrogen. In our study, leaf area was reduced by 24% in low nitrogen treatment. The gas exchange parameters like transpiration rate, stomatal conductance and water use efficiency was also reduced by 33%, 8% and 11% respectively, while intercellular CO_2 concentration was enhanced by 22% under low nitrogen condition. The photosynthetic rate in low nitrogen plants was 40% lower than in high nitrogen plants. Thus, the decrease in photosynthesis is the main reason for the decreasing biomass. Like cotton, photosynthesis and biomass were significantly reduced in Arabidopsis, rice, maize, wheat and other plants under low nitrogen supply (Beatty and Good, 2018; Makino, 2011; Vidal *et al*., 2015).

The mechanisms determining photosynthetic performance are generally evaluated in terms of stomatal and non-stomatal factors like photosynthetic enzymes and chlorophyll content (Markelz *et al.*, 2011). The reduction in overall photosynthetic efficiency of the low N treated cotton seedlings were accompanied by enhanced intercellular CO_2 concentration, which indicates that the decline in photosynthesis is due to inhibition in carboxylation efficiency rather than stomatal limitations. Similar findings were reported in sunflower and rice (Huang *et al.*, 2004).

Photosynthesis is dependent upon several physiological and biochemical processes like stomatal conductance, transpiration and chlorophyll content (Ziadi *et al.*, 2008). As nitrogen is a constituent of chlorophyll, photosynthetic enzymes (included Rubisco, PEPc and PPDK), and thylakoid membranes. These cellular features are located in chloroplast and about three-quarters of total nitrogen is found in chloroplast (Ghannoum *et al.*, 2005; Poorter and Evans, 1998). In our study, chlorophyll a, b and a+b was reduced by 7%, 9% and 7% respectively under low nitrogen compared to high nitrogen, indicating the pivotal role of N in Chlorophyll synthesis (Evans and Terashima, 1988; Bondada and Syvertsen, 2003; Ghannoum *et al.*, 2005). Previous studies of other plant species have shown that chlorophyll content as well as the photosynthetic rate was significantly reduced under low N conditions (Markelz *et al.*, 2011; Pinto *et al.*, 2014). About 70-80% of cell N lies within the chloroplast (Makino and Osmond, 1991), which is a prerequisite for every step of photosynthetic process including formation of the light-harvesting complexes of the antenna (Bungard *et al.*, 1997). As reduced chlorophyll has been detected under low N conditions, it is reasonable to infer that the negative effects of low N on photosynthesis may be due to the depression of photosynthesis enzymatic activities or chlorophyll content. Another restriction may result from the down-regulation of nitrogen metabolism as low N conditions can reduce the activities of NR and GS in rice (Duan *et al.*, 2007).

In the current study, root, shoot and total nitrogen content were significantly reduced by 34%, 42% and 39% respectively by low nitrogen treatment compared with high nitrogen treatment. Similarly, total nitrogen accumulation and uptake efficiency were reduced by 68% and 81% under low nitrogen treatment. However, nitrogen utilization efficiency was not significant, while the nitrogen efficiency ratio was significantly increased by 76% under low nitrogen than high nitrogen (Fig. 4). This reduction may be due to the contribution of the different photosynthetic components mentioned above (Uribelarrea *et al.*, 2009; Mu *et al.*, 2016). In Arabidopsis, it was found that most of the nitrate taken up by root transporters is reduced in roots or shoots and then assimilated to synthesize amino acids mainly in shoots thus under shoot contain more nitrogen under high N and vice versa (Masclaux-Daubresse *et al.*, 2010). The reduction in the nitrogen content might be due to a decrease in nitrogen absorption and remobilization to cope with the need for N to sustain plant growth. The decrease in the shoot and root nitrogen content is one of the adaptations of metabolism to low N availability (Lemaître *et al.*, 2008; Wang and Tsay, 2011).

Nitrogen use efficiency is an essential characteristic of agricultural cops, which is evaluated extensively during genotype selection mostly under low nitrogen treatment (Xu *et al.*, 2012). It has been reported that the increase in total available N with more biomass production under low N enhances NUE (Raun and Johnson, 1999). However, under high nitrogen supply, NUE will decline due to inconsistent increase between N absorption and biomass production. Therefore under such circumstances, plants cannot assimilate enough nitrogen, leading more nitrogen losses (Dawson *et al.*, 2008). Several scientists have worked on increasing NUE in different crops (Anbessa *et al.*, 2009; Sylvester-Bradley and Kindred, 2009). As a function of multiple interacting genetic and environmental factors, NUE is a complex trait, which can be divided into two key plant physiological components, NUpE and NutE (Xu *et al.*, 2012). In our study, nitrogen utilization efficiency ratio was not significant under low nitrogen supply, suggested that the utilization efficiency of cotton seedlings is the same for low and high nitrogen supply. However, nitrogen uptake efficiency was reduced by low nitrogen supply, which suggested that cotton seedlings cannot absorb the low available nitrogen efficiently.

Conclusions

In conclusion, our results show that low nitrogen supply results in the increase in root morphological traits and reduction in shoot growth, photosynthetic traits including chlorophyll content and nitrogen use efficiency. These findings suggest the potential to utilize photosynthetic as well as chlorophyll parameters and nitrogen use efficiency as indicators of plant nitrogen nutrition status. In addition, this could be used to develop new tools to make precise nitrogen fertilizer recommendations and select nitrogen-efficient genotypes. Further, these results provide the basis for the molecular investigation and exploitation of the genetic resources to develop high yielding cotton genotypes under reduced N fertilization, which would surely be another boost in sustainable agriculture for the betterment of mankind and environmental protection.

Author contributions

MS, AI and DQ designed the experiment and wrote the manuscript. AI, and WZ conducted the experiment. HPG helped in nitrogen analysis. NJ helped in data collection and plant materials. WXR, ZHH, NCP analyzed the data.

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Conflicts of interest

All the authors declare no conflicts of interest.

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