

A LARGER ROOT SYSTEM IN OAT (*AVENA NUDA* L.) IS COUPLED WITH ENHANCED BIOMASS ACCUMULATION AND HORMONAL ALTERATIONS UNDER LOW NITROGEN

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Abstract. Nitrogen (N), an essential macronutrient for crop growth and development, is well known for its economic losses and environmental hazards due to imbalanced application. Oat (*Avena nuda* L.), a food and fodder crop, is well adapted to marginal soils and requires minimum water and nutrients to produce biomass. A hydroponic experiment was conducted in China Agricultural University, Beijing, China to evaluate the morphological and physiological adaptations of oat to organic N (ON) and low N (LN) with respect to chemical N (CK as control). The results have shown that oat responded to different nitrogen supplies with response to LN having been the dominant one. Lowest SPAD value, net photosynthesis rate, transpiration rate, gas exchange and stomatal conductance showed that the oat was under LN stress. Highest level of root dry mass, as indicated by increased R/S ratio and C/N ratio, was at the cost of minimum nitrogen utility as depicted by increased nitrogen utilization efficiency in roots under LN. Accumulation of total soluble proteins and sugars elaborated the carbon allocation to nitrogen deprived roots. As a result of sufficient biomass provision in LN root, total root length increased which was coupled with increased concentrations of indole-3-acetic acid, gibberellic acid and zeatin riboside whereas decreased concentration of abscisic acid. Overall, oat is found to be a nitrogen use efficient crop which responded to LN through enhanced root system through biomass accumulation by the provision of nitrogen metabolites.

Keywords: *root architecture, photosynthesis, nitrogen utilization efficiency (NUE), nitrogen metabolites, hormonal accumulation*

Introduction

Nitrogen (N) is an important macronutrient required for the biosynthesis of chlorophyll, nucleic acids, amino acids, proteins and some organic acids. Its essentiality is obvious from its involvement in most of the biological and physiological processes such as photosynthesis, biomass production, hormonal accumulation, carbohydrate allocation, plant root architectural growth and development of reproductive organs of plants (Zhao et al., 2005; Miller and Cramer, 2005). To produce 1 kg biomass most of the non-legume plants absorb 20 to 50 g of N from the soil (Robertson and Vitousek, 2009). Nitrogen is present in soils in a patchy manner either as inorganic form (nitrate or ammonium) or organic form (amino acids, peptides and proteins). Plants uptake different nitrogen forms from the soil through roots with nitrate being the most preferential form (Yuan et al., 2007; Puangfoo-Lonhienne et al., 2008 and Wang et al., 2012). Being highly mobile element, nitrogen is subjected to losses once applied to the soil as inorganic form whereas nitrogen released by organic forms is slow which cannot meet the plant requirement.

Hence, farmers are compelled to use chemical nitrogen fertilizers which, more often than not, which damages soil physical and chemical health, and toxifies the environment. Approximately 10^{11} kg N per annum is applied in the soil globally with the aim to get highest crop production (Glass, 2003; Tilman et al., 2011; Liu et al., 2013; Chandna et al., 2011; Rawat et al., 2012). Hence, high levels of soil N are susceptible to losses (Zhu et al., 2002; Ju et al., 2004) which ultimately can reduce the N use efficiency (NUE) (Ata-Ul-Karim et al., 2014). On the other hand, deficiency of N reduces the biosynthesis of chlorophyll, affects plant organ development and finally results in reduced crop yield (Marschner, 1995). Plants have developed morphological and physiological alterations over evolution to cope with heterogeneous environmental conditions. Nitrogen is present in soil solution in mineral as well as organic form (Nasholm and Persson, 2001); thus, it depends on the adaptability of a plant to dwell under the conditions of its high or low availability (Glass, 2003; Garnett et al., 2009). The primary response of a plant to low external nitrogen availability is to modify its root system. Most plants expand their root system to capture N by exploring as much rhizosphere as possible (Palta, et al., 2011). Leguminous crops form root nodules to fix atmospheric N but non-leguminous crops like maize, rice produce longer roots (Wang et al., 2003; Chun et al., 2005a) whereas, foxtail millet produces shorter and thicker roots facilitating carbohydrate allocation perhaps through wider xylem vessels (Nadeem et al., 2018). Hence, the diversity is observed in the adaptive measures of different crops under different rhizosphere condition.

Naked oat (*Avena nuda* L.) and hulled oat (*A. sativa* L) are two types of oats cultivated in China. The fast growing nature of oat capacitates itself to produce ample quantity of fresh fodder within short period of time with high nutritional values. The high protein and β -glucose content along with the presence of hypo-glycemic fiber content in oat grain increase its value as a human food. Generally, rice, wheat, maize and other high yielding cereal crops generally occupy most of the agricultural land but crops like oat find very limited area and interest to be cultivated worldwide. Current study was aimed to characterize root morphological and physiological adaptations of oat in order to maximize its nitrogen utilization efficiency (NUE) in terms of biomass accumulation under different nitrogen supplies. The findings revealed oat as an extremely large root crop under low nitrogen (LN) condition with remarkable nitrogen use efficiency and biomass accumulation.

Materials and methods

The hydroponic experiment was carried out during April-July in a standard growth chamber with 28/22 °C alternate day night temperature, 14/10 h (day/night) photoperiod, 65% relative humidity and $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ illumination in China Agricultural University, Beijing, China. High quality seeds of oat (*Avena nuda* L.) variety Baiyan 2 were collected from Baicheng Academy of Agricultural Science, Jilin, China. Deionized H_2O was used to wash the seeds followed by sterilization with 10% H_2O_2 solution for 30 min and soaking in saturated CaSO_4 solution for 5 h with continuous aeration. Seeds were germinated on moist tray in the growth chamber. After emergence of approximately 2 cm primary roots, seedlings were wrapped with two layers of deionized water saturated filter paper, enfolded in cylinder shape and placed vertically in a growth holder containing distilled water to assure a continuous water supply to the seedlings. 12 uniform and consistent seedlings with two fully emerged leaves were selected and transplanted into well aerated 5-L vessels containing nutrient solution. The total nutrient solution (as

control or CK) consisted of 2 mM NH_4NO_3 , 0.75 mM K_2SO_4 , 0.25 mM KH_2PO_4 , 0.1 mM KCl, 0.2 mM Fe-EDTA, 0.65 mM MgSO_4 , 2 mM CaCl_2 , 1×10^{-3} mM ZnSO_4 , 1×10^{-3} mM MnSO_4 , 5×10^{-6} mM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 1×10^{-4} mM CuSO_4 , and 1×10^{-3} mM H_3BO_3 . 2 mM NH_4NO_3 was replaced by 4 mM glycine ($\text{C}_2\text{H}_5\text{NO}_2$) as a source of organic nitrogen (ON). Low nitrogen (LN) treatment contained 10% of 2mM NH_4NO_3 used in control (CK). Every treatment had six replicates. Each container had 12 plants. Oat seedlings were grown in nutrient solution for 21 days in total having 25% of CK solution for 3 days, 50% of CK solution for 4 days, 100% of CK solution for 7 days followed by the treatment application (CK, ON and LN) for 7 days. The pH of the nutrient solution was adjusted to 5.8-6 and the nutrient solution was changed every 2 days. The whole experiment was repeated three times.

Plant roots and shoots were harvested, frozen in liquid N and stored in -80°C for amino acids, soluble proteins, soluble sugars and hormone analysis. Root samples were, washed with deionized water 3 times, surfaced dried using a blotting paper prior to storage. For other physiological analysis, shoot and root samples were harvested and dried in an oven at 105°C for 30 min and then at 70°C until constant weight.

Gas exchange and SPAD values (Soil and plant analyzer developer) measurements

On the morning of 21st day, between 8.00 to 9.00 am, the 3rd fully expanded leaf of every plant of every replicate was measured in three points carefully with the help of a portable chlorophyll meter (SPAD-502, Minolta, Japan). Every pot had 12 seedlings. The three SPAD reads of every seedling were averaged to get 12 SPAD reads from every pot which served as technical replicates. On the same day a portable gas exchange system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) was used to measure photosynthetic rate (P_n), gas exchange (C_i), stomatal conductance (g_s) and transpiration rate (T_r). Measurements were taken on 6 cm^2 leaf area segment next to the mid-rib of the 4th leaf. Readings from 12 seedlings per pot served as 12 technical replicates and 6 pots of every treatment served as 6 biological replicates. Photosynthetic photon flux density provided by a red/blue LED light source (6400-02B, LI-COR Biosciences, Lincoln, NE, USA) was fixed to $600\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ and the concentration of ambient CO_2 was adjusted to $400\text{ }\mu\text{mol mol}^{-1}$ by CO_2 injection as reported by Geilfus et al. (2011).

Root length analysis

The root system was separated into crown and lateral roots. Each kind of roots was counted manually and crown roots were measured with a ruler. Roots were scanned using a scanner (Epson 1680, Indonesia). WinRHIZO software (version 5.0) (Regent Instruments Inc., Quebec City, QC, Canada) was used to analyze the scanned images to get total root length and average diameter following previously described methods (Peng et al., 2010). Lateral root length was obtained by subtracting crown root length from total root length. Lateral root density was defined as the number of lateral roots per unit length of crown root containing lateral roots. Each sample had three technical and six biological replicates.

N concentration analysis in the shoot and root

The dried plant samples were weighed and ground into fine powder. 0.3 g of ground sample was taken for digestion by $\text{H}_2\text{SO}_4\text{--H}_2\text{O}_2$ followed by total N analysis using a modified Kjeldahl digestion method (Baker and Thompson, 1992).

Analysis of mineral concentration

For the analysis of mineral contents of the plant using inductively coupled plasma-mass spectrometry (ICP-MS; Agilent Technologies 7700 Series, Böblingen, Germany), the dried (60 °C) plant tissues were ground into fine powder. 100 mg of fine powder was digested with 10 mL of 69% HNO₃ (ROTIPURAN Supra for ICP, 69%) in an 1800 W microwave oven (MARS 6 Xpress; CEM, Matthews, MC, USA) at 100 °C for 2 min, at 120 °C for 1 min and at 180 °C for 20 min followed by 20 min of cooling. Samples were diluted to 100 mL using sterilized deionized water (18.2 MΩ cm conductivity) and stored at 4 °C till analysis. Similar procedure also followed for blank digestions. A certified reference material NCSDC 73350 from the Beijing vegetable research institute (Beijing, China) was used for standardized analytical technique. Internal macro- and micronutrient standards and reference material samples were tested as internal control.

Hormone extraction and quantification by enzyme linked immunosorbent assay (ELISA)

Approximately 0.5 g fresh plant sample was ground into powder and homogenized by using 2 mL of 80% methanol (containing 40 mg l⁻¹ butylated hydroxytoluene as an antioxidant), incubated 48 h at 4 °C, and then centrifuged at 1900 × g for 15 min at 4 °C. The supernatant was passed through C18 Sep-Pak cartridges (Waters Corp.), and the hormone fraction was eluted with 10 mL of 100% (v/v) methanol and then 10 mL ether. The elute was N₂-dried at 20 °C. The N₂ dried extracts were dissolved in 2.0 mL phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween-20 and 0.1% (w/v) gelatin (pH 7.5) to analyze the concentration of free indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA) and zeatin riboside (ZR) by enzyme linked immunosorbent assay (ELISA) following a well-established protocol (Weiler et al., 1981).

Extraction of metabolite and analyses

The soluble proteins were extracted and analyzed by using a standard kit (Coomassie Protein assay reagent; Bio-Rad, Hercules, CA, USA) with bovine serum albumin as a reference. Chlorophyll was extracted from 0.2 g fresh leaf disks using 25 mL mixture of alcohol and acetone (1:1 v/v). The absorbance of the extract was measured at 663, 645, and 470 nm using a UV-VIS spectrophotometer (UV-2600, Shimadzu, Japan) to estimate chlorophyll a, chlorophyll b and total carotenoids according to the previously described methods Wellburn and Lichtenthaler (1984). The concentration of total free amino acids was measured according to the Rosen ninhydrin colorimetric method using leucine as a standard (Rosen, 1957). The concentration of soluble sugars was determined using a commercially available kit (Boehringer Mannheim, Germany). Individual amino acids profile was analyzed using Waters e2695 HPLC (Waters Corp, Milford, MA, USA) by incubating 100 mg of fresh tissue powder with 1.5 mL of the 5% (w/v) Trichloroacetic acid (TCA) solution for 1 h at room temperature. ~50 mg plant tissues were loaded into an Elementar vario Macro CN (Elementar Technologies, Hanau, Germany) analyzer for the C: N ratio analysis.

Statistical analysis

Statistical analysis was performed using a windows software package (IBM SPSS Statistics 22). Means of different treatments were compared using the least significant

difference at a 0.05 or 0.01 level of probability. Correlation analysis was performed using Pearson correlation function of SPSS. Graphical rendering was carried out using sigma plot 13.0 windows software. All the data were presented as means $\pm$ standard errors (SE) of six replicates.

Results

Root and shoot morphology of oat and biomass accumulation

Oat seedlings were subjected to control (CK), organic nitrogen (ON) and low nitrogen (LN) treatments for one week. At harvest, the seedlings showed obvious symptoms of various nitrogen regimes having green, mild green and light green (yellowish) shoot color in CK, ON and LN respectively (*Fig. 1A, B*).

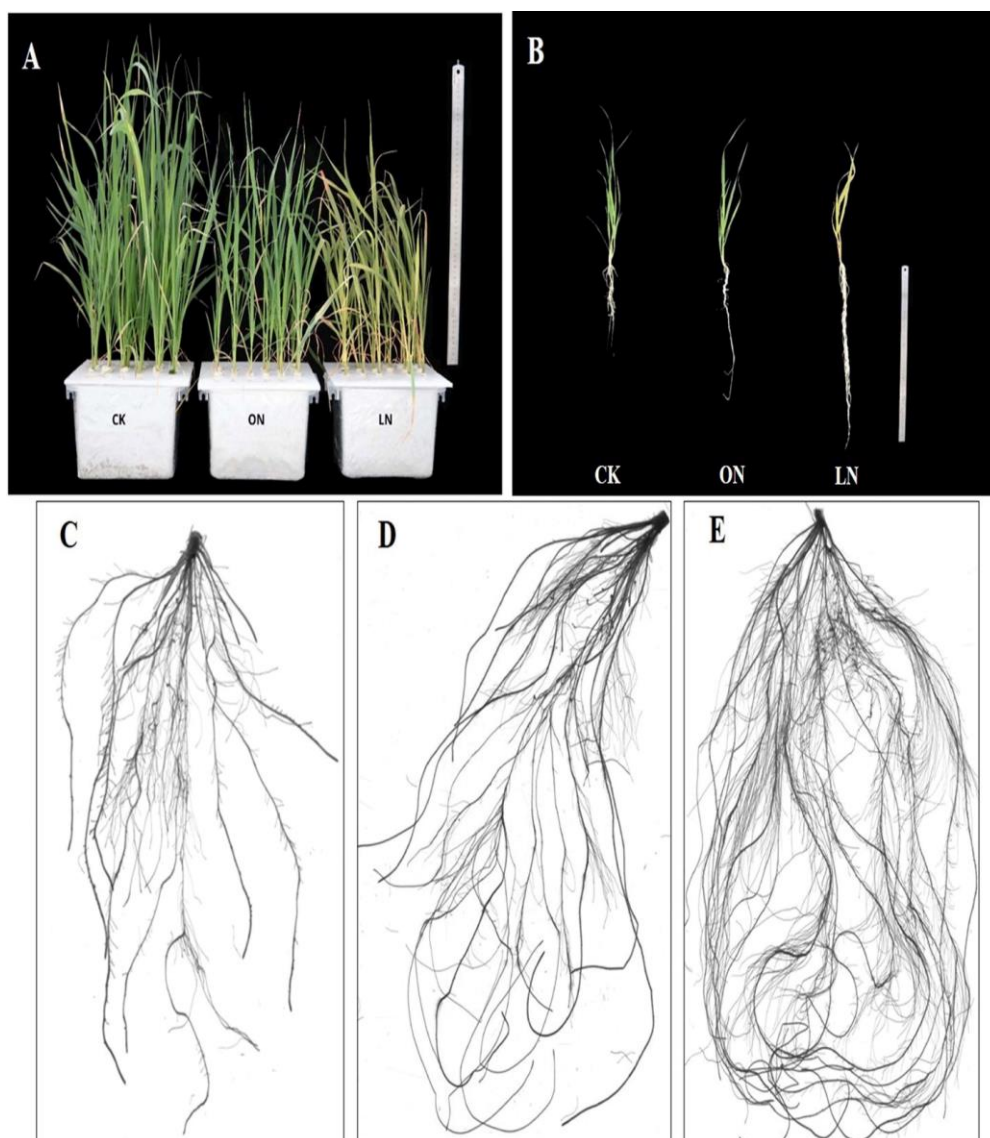


Figure 1. Shoot and root responses of oat to different form and level of N. Plants grown under CK, ON and LN condition in hydroponic pots (A), plants with the intact root and shoot (B), The whole scan picture of root under Ck (C), ON (D) and LN (E). Treatment CK = control, ON = organic N, LN = low N

During the harvesting time, each seedling under LN condition revealed the deficiency symptoms of N. Limitation of N generally hinders oat plant growth. In current study, shoot length, leaf area and shoot dry weight decreased whereas root dry weight and root to shoot ratio increased in ON and LN treatments (Fig. 2A-E). Reduction in plant height was observed in the ON and LN treatment which was almost 14% and 28%, respectively, in comparison with CK (Table A1 in the Appendix). Compared to CK and ON treatment 45.77% and 35.77% less shoot dry weight and 82.61% and 31.25% higher root DW was produced under LN condition, respectively, (Table A2). Interestingly, root to shoot ratio resulted in 241.27% and 109.76% increment in LN treatment with respect to CK and ON respectively. Similar was the case in ON treatment where 62.7% increase in root to shoot ratio was observed as compared to CK (Table A2). Talking about root architectural traits (Fig. 1C-E), LN treatment increased total root length (112.3% and 64.65%), crown root length (116.27% and 75.91%), lateral root length (111.57% and 63.46%), crown root number (96.67% and 40.47%), and lateral root number (34.1% and 16.39%) and density (128.21% and 67.92%) with respect to CK and ON whereas, ON treatment only increased lateral root number (15.22%) and length (29.43%) and total root length (28.78%) significantly as compared to CK (Table 1; Table A3 and A4). Average diameter of roots decreased in LN significantly (Table 1).

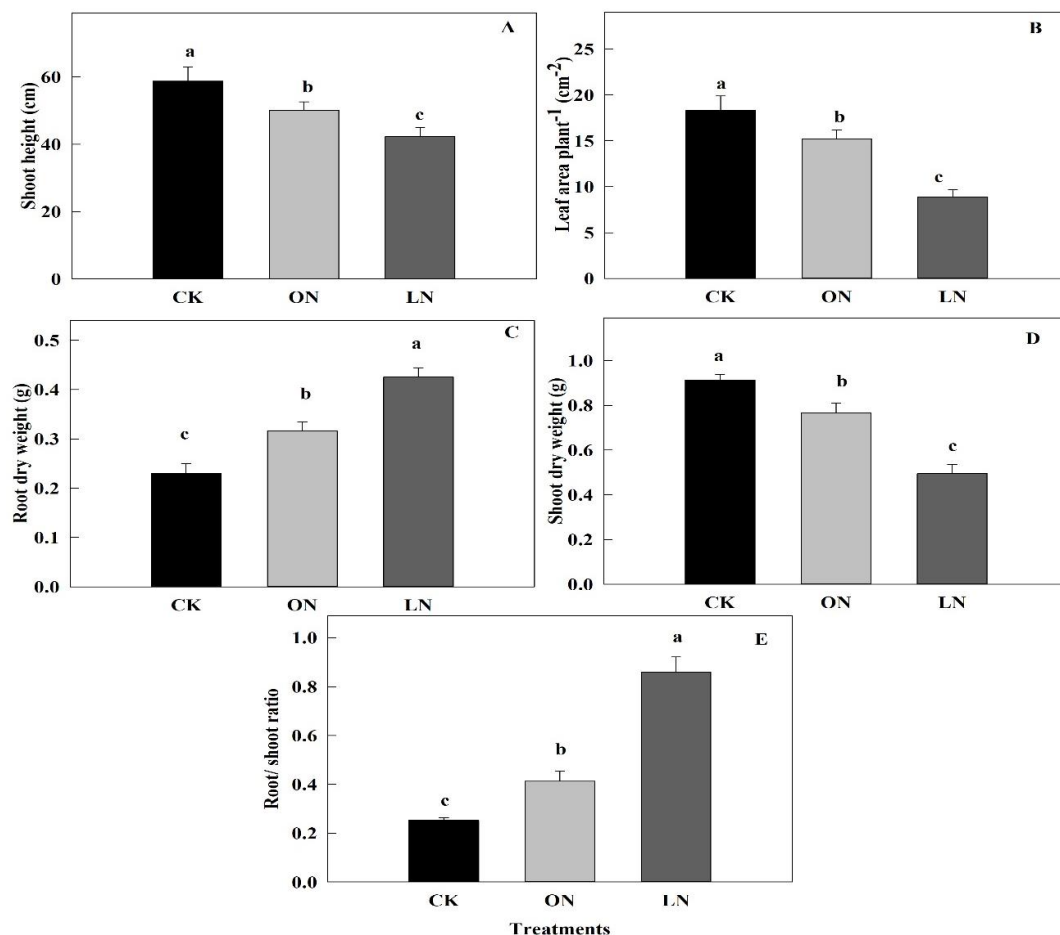


Figure 2. The oat plant responses to different form and level of N. Shoot height (A), leaf area plant⁻¹(B), root dry weight (C), shoot dry weight (D) and root/shoot ratio (E). Error bars represented standard error of six biological replicates, different letters indicate significant differences ($P < 0.05$). Treatment CK = control, ON = organic N, LN = low N

Table 1. Root morphological characteristics under CK, ON and LN condition during harvesting

Treatment	Crown root number	Lateral root number	Lateral root density	Crown root length (cm)	Lateral root length (cm)	Total root length (cm)	Average diameter (cm)
CK	7.5±0.87b	1736.17±15.2c	7.8±0.98b	143.34±10.56b	1288.16±82.2c	1431.5±97.36c	0.073±0.0039a
ON	10.5±1.08b	2000.4±123.3b	10.6±1.76b	176.23±13.98b	1667.22±102.1b	1843.45±131.23b	0.078±0.27a
LN	14.75±2.22a	2328.17±155.8a	17.8±2.01a	310±17.76a	2725.3±178.3a	3035.33±198.3a	0.052±0.45b

In each column lower case lettering is used to show the significant differences between different types of treatments at $P < 0.05$ level. Values show Standard errors (SE) $\pm$ mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N

Attributes of photosynthesis and nitrogen accumulation

We found a continuous and significant decrease in SPAD value, Chlorophyll a, b and total carotenoids from CK to LN (Fig. 3A-D; Table A5). Moreover, net photosynthesis (P_n), intercellular carbon dioxide (C_i), transpiration rate (Tr) and stomatal conductance (g_s) followed the same pattern across all three treatments (Fig. 4A-D). Shoot and root nitrogen concentrations were decreased in the order of CK>ON>LN (Fig. 5A, B; Table A6), and vice versa for whereas for carbon to nitrogen (C/N) ratio and nitrogen utilization efficiency (cumulative biomass per unit of nitrogen; g DW g⁻¹ N in the plant; NUtE) in both shoot and root (Fig. 5C-F).

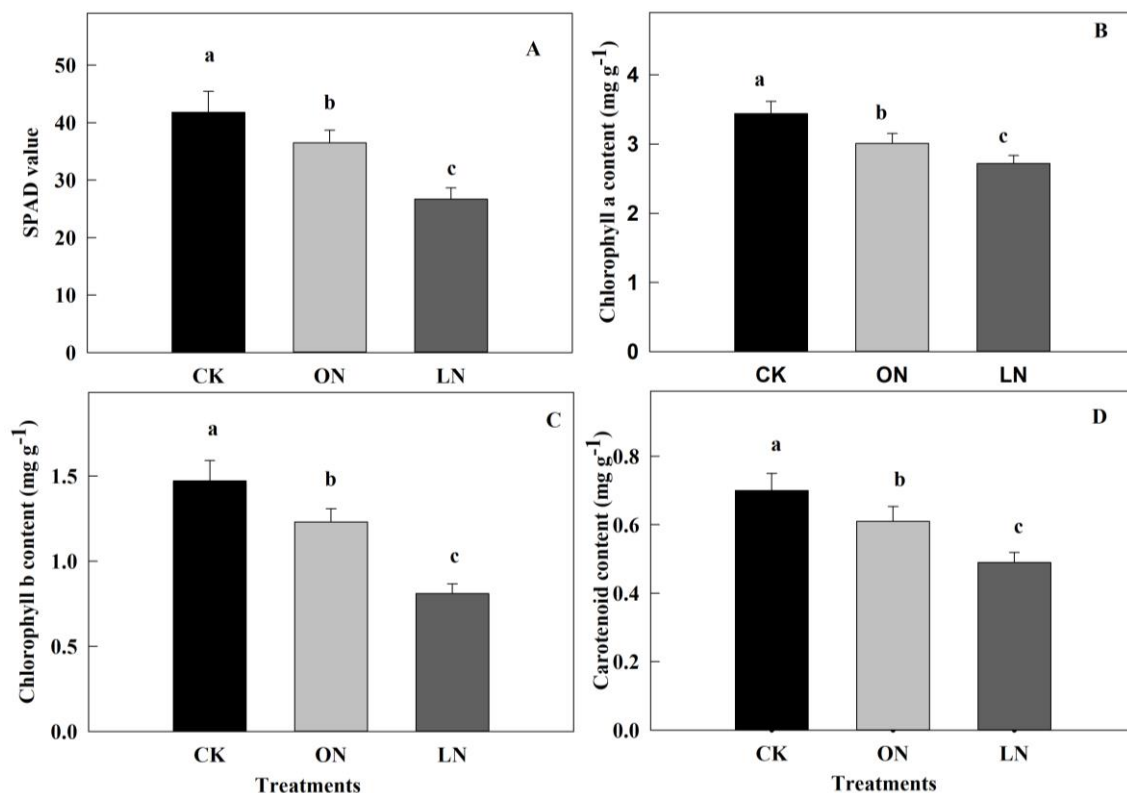


Figure 3. Comparison in chlorophyll contents of oat leaf grown under different sources and doses of N. SPAD value (A), chlorophyll a content (B), chlorophyll b content (C), carotenoid content (D). Error bars represented standard error of six biological replicates, different letters indicate significant differences ($P < 0.05$). Treatment CK = control, ON = organic N, LN = low N

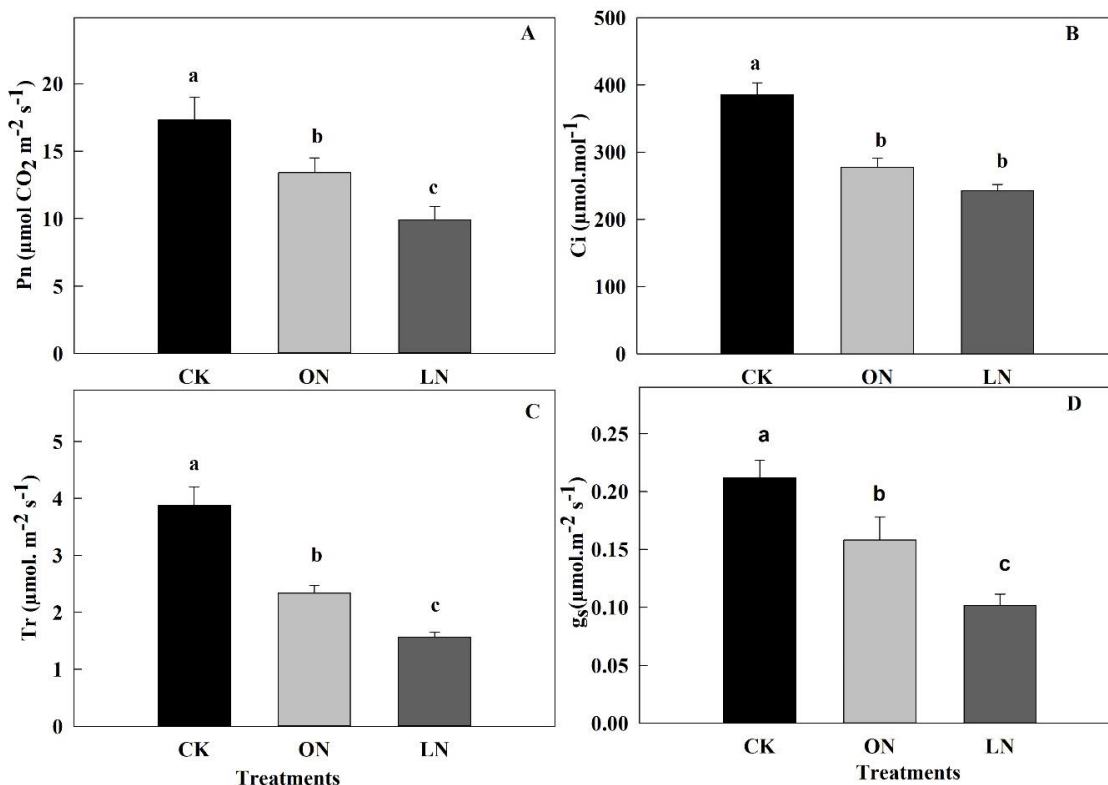


Figure 4. Trend of photosynthetic parameters in oat leaf under different treatment. Net photosynthetic rate Pn (A), inter cellular CO₂, Ci (B), transpiration rate, Tr (C), stomatal conductance, g_s (D) in response to N treatments. Error bars represented standard error of six biological replicates, different letters indicate significant differences ($P < 0.05$). Treatment CK = control, ON = organic N, LN = low N

Free amino acids, total soluble proteins, total soluble sugars, amino acid profile and mineral status of oat

Low nitrogen treatment reduced total free amino acids (-26.78% and -18.97%) in shoot as well as root (-36.03% and -25.76%) with respect to CK and ON however, no significance was found between CK and ON (Fig. 6A, B; Table A7). Total soluble proteins and sugars in shoot of oat decreased significantly (CK>ON>LN) and vice versa in roots without any significance between CK and ON (Fig. 6C-F). Total soluble protein synthesis in shoot was found to be the least under LN treatment having decrements of -73.42% and -48.78% from CK and ON respectively, however, in roots they were increased up to 195% and 99.71% from CK and ON respectively under LN treatment (Table A7). Similarly, total soluble sugars in shoot of oat seedling were found to be minimal (-57.73% and -33.87%) whereas highest in roots (159.68% and 80.9%) under LN treatment with respect to CK and ON (Table A7). In current study amino acids profile of shoot and root of oat seedlings under CK, ON and LN treatments was generated and found that 18 amino acids (Glu, ser, Gln, Ala, β-Ala, Val, Asp, Asn, Lys, Ile, Gly, Tyr, Trp, His, Leu, Orn, Cit, Tau) in shoot and 15 amino acids (Glu, Ser, Gln, Arg, Ala, β-Ala, Asp, Lys, Gly, Tyr, Leu, Orn, Cit, Tau) in root were decreased in LN treatment as compared to CK; however, Thr increased in both shoot and root of LN seedlings (Table 2). Among these amino acids Ala, β-Ala and Lys are the amino acids

which co-decreased significantly in shoot and root following the order CK>ON>LN (Table 2) As far as mineral accumulation in shoot and root of oat seedlings is concerned, P, Mg, Na, Cu, B, Mn and Zn concentration increased while that of K and S decreased in LN root and shoot as compared to CK and ON (Table 3).

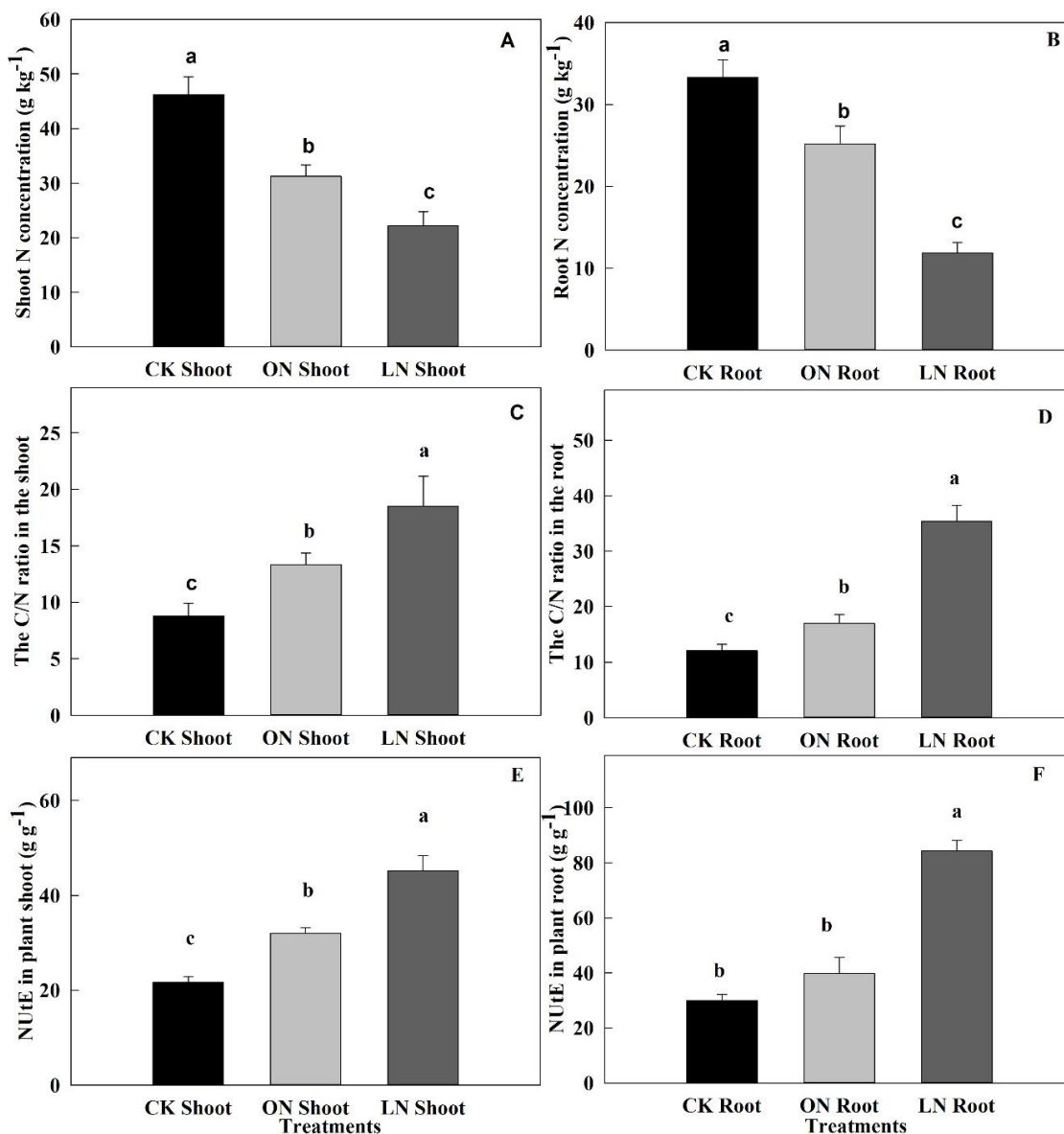


Figure 5. N accumulation in oat plant parts under different treatment. Shoot N concentration (A), root N concentration (B), C/N ratio in the shoot (C), C/N ratio in the root (D), NUtE in the shoot (E), and NUtE in the root (F) in response to LN. Error bars represented standard error of six biological replicates, different letters indicate significant differences ($P < 0.05$). Treatment CK = control, ON = organic N, LN = low N

Alterations in hormone accumulation

Plant growth and development is regulated by plant hormones (Beveridge et al., 1997; Marsch-Martinez and de Folter, 2016). In this study, the concentrations of indole-

3-acetic acid (IAA/auxin), gibberellic acid (GA3) and zeatin-riboside (ZR; a kind of cytokinins) decreased in shoots of ON as well as LN treated oat seedlings (Fig. 7A, C, G). In contrast to shoot, the concentration of aforementioned hormones reversed and increased in roots of LN treated seedlings as compared to CK and ON (Fig. 7B, D, H). On the other hand, abscisic acid concentration increased in both ON and LN treated shoots as compared to CK (CK < ON < LN) with LN treated shoots depicting the highest number than the rest and vice versa in roots (Fig. 7E, F).

Discussion

Nitrogen is an important macronutrient for plant growth and development. As an imperative component of chlorophyll, it is required for proper functioning of photosynthesis apparatus whereas being a building block of peptides, it is required for protein biosynthesis (Hirel et al., 2007; Wang et al., 2012). Owing to the heterogeneous availability of nitrogen in soil, the adaptation of plants to uptake nitrogen from its immediate vicinity becomes vital (Wang et al., 2007), hence different plants respond to external N availability differentially through physiological, morphological and molecular adaptations. Legumes form nodules to fix atmospheric nitrogen whereas non-legumes like maize, rice and foxtail millet may alter their root architecture in order to explore the nitrogen patches in soil (Postgate, 1998; Wang et al., 2003; Chun et al., 2005a; Nadeem et al., 2018). Long-term nitrogen deficiency hinders plant root and shoot growth (Chun et al., 2005b; Guo et al., 2005; Goron et al., 2015). It is well established that rice and wheat crops produced longer root system under nitrogen deprivation in order to explore as much soil surface as possible to reach nitrogen rich patches (Cai et al., 2012; Guo et al., 2014). Oat can be cultivated in marginal lands with little nutrient and/or water requirements. The morphological and physiological adaptation of oat to chemical nitrogen (CK), organic nitrogen (ON) and low nitrogen (LN) remains unknown to date. In this study, oat produced the longest root system, shortest shoot and smallest leaf area under LN as compared to CK and ON whereas it produced the longer root system, shorter shoot and smaller leaf area under ON with respect to CK (Figs. 1A-E and 2A, B) as a primary response to various nitrogen regimes.

Table 2. Amino acid concentrations ($\mu\text{mol g}^{-1}$ FW) in shoot and root of oat grown hydroponically low N, organic N and Control (high chemical N) condition

Amino acid	Shoot			Root		
	CK	ON	LN	CK	ON	LN
Glutamic acidGlu	2.72 $\pm$ 0.80a	1.39 $\pm$ 0.20b	1.27 $\pm$ 0.11b	0.76 $\pm$ 0.24a	0.54 $\pm$ 0.13b	0.29 $\pm$ 0.15c
Serine (Ser)	1.42 $\pm$ 0.04a	1.25 $\pm$ 0.25b	1.09 $\pm$ 0.17c	1.02 $\pm$ 0.24a	0.91 $\pm$ 0.16a	0.75 $\pm$ 0.15b
Glutamine (Gln)	1.12 $\pm$ 0.80a	0.78 $\pm$ 0.27b	0.63 $\pm$ 0.25b	3.61 $\pm$ 0.97a	1.85 $\pm$ 0.18b	1.68 $\pm$ 0.64b
Arginine (Arg)	0.06 $\pm$ 0.01a	0.06 $\pm$ 0.01a	0.07 $\pm$ 0.01a	0.04 $\pm$ 0.01c	0.07 $\pm$ 0.02b	0.13 $\pm$ 0.06a
Alanine (Ala)	1.70 $\pm$ 0.04a	1.67 $\pm$ 0.66ab	1.17 $\pm$ 0.14c	1.25 $\pm$ 0.81a	0.62 $\pm$ 0.13b	0.45 $\pm$ 0.03c
β Alanine (β -Ala)	0.62 $\pm$ 0.02a	0.51 $\pm$ 0.01b	0.40 $\pm$ 0.03c	0.33 $\pm$ 0.03a	0.17 $\pm$ 0.01b	0.09 $\pm$ 0.01c
Valine (Val)	0.27 $\pm$ 0.01a	0.18 $\pm$ 0.04b	0.18 $\pm$ 0.00b	0.69 $\pm$ 0.26a	0.47 $\pm$ 0.20b	0.61 $\pm$ 0.18a
Aspartic acid (Asp)	2.88 $\pm$ 0.28a	1.98 $\pm$ 0.28b	1.49 $\pm$ 0.13c	0.43 $\pm$ 0.06a	0.36 $\pm$ 0.13ab	0.21 $\pm$ 0.01b
Asparagine (Asn)	1.55 $\pm$ 0.66a	1.53 $\pm$ 0.75a	1.15 $\pm$ 0.68b	1.18 $\pm$ 0.70a	1.18 $\pm$ 0.34a	0.74 $\pm$ 0.43b
Lysine (Lys)	0.09 $\pm$ 0.00a	0.07 $\pm$ 0.01ab	0.03 $\pm$ 0.00c	0.06 $\pm$ 0.01b	0.09 $\pm$ 0.02a	0.03 $\pm$ 0.01c
Isoleucine (Ile)	0.07 $\pm$ 0.00a	0.05 $\pm$ 0.00b	0.023 $\pm$ 0.01c	0.45 $\pm$ 0.16a	0.43 $\pm$ 0.24a	0.32 $\pm$ 0.20b
Methionine (Met)	0.03 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.00	0.1 $\pm$ 0.07a	0.03 $\pm$ 0.04c	0.07 $\pm$ 0.02b
Threonine (Thr)	0.51 $\pm$ 0.10c	0.71 $\pm$ 0.16b	1.49 $\pm$ 0.01a	0.70 $\pm$ 0.36c	0.95 $\pm$ 0.45b	1.14 $\pm$ 0.36a
Glycine (Gly)	0.10 $\pm$ 0.01b	0.19 $\pm$ 0.05a	0.08 $\pm$ 0.02b	0.23 $\pm$ 0.06b	0.48 $\pm$ 0.05a	0.18 $\pm$ 0.02b

Tyrosine (Tyr)	0.04 ± 0.01a	0.01 ± 0.01b	0.02 ± 0.00b	0.16 ± 0.04a	0.12 ± 0.06b	0.09 ± 0.04b
Tryptophan (Trp)	0.11 ± 0.03a	0.06 ± 0.02b	0.04 ± 0.00b	0.17 ± 0.09a	0.15 ± 0.08b	0.14 ± 0.02b
Phenylalanine (Phe)	0.07 ± 0.00a	0.03 ± 0.01b	0.02 ± 0.01b	0.04 ± 0.02a	0.04 ± 0.02a	0.03 ± 0.00a
Histidine (His)	0.20 ± 0.03a	0.10 ± 0.02b	0.09 ± 0.01b	0.27 ± 0.12a	0.17 ± 0.09b	0.15 ± 0.03b
Leucine (Leu)	0.04 ± 0.01a	0.02 ± 0.001ab	0.01 ± 0.01b	0.61 ± 0.35a	0.45 ± 0.26b	0.06 ± 0.21c
Ornithine (Orn)	0.14 ± 0.01a	0.09 ± 0.02b	0.06 ± 0.00b	0.12 ± 0.01a	0.10 ± 0.02a	0.04 ± 0.00b
Citrulline (Cit)	0.12 ± 0.01a	0.11 ± 0.01a	0.07 ± 0.00b	0.13 ± 0.01a	0.09 ± 0.01b	0.06 ± 0.00b
Taurine (Tau)	0.02 ± 0.00a	0.01 ± 0.00b	0.009 ± 0.00b	0.04 ± 0.01a	0.02 ± 0.001ab	0.01 ± 0.00b

Tissue samples were taken just prior to harvesting. In each column lower case letters indicated the significant differences between different types of treatments at $P < 0.05$ level. Values show Standard errors (SE) ± mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N

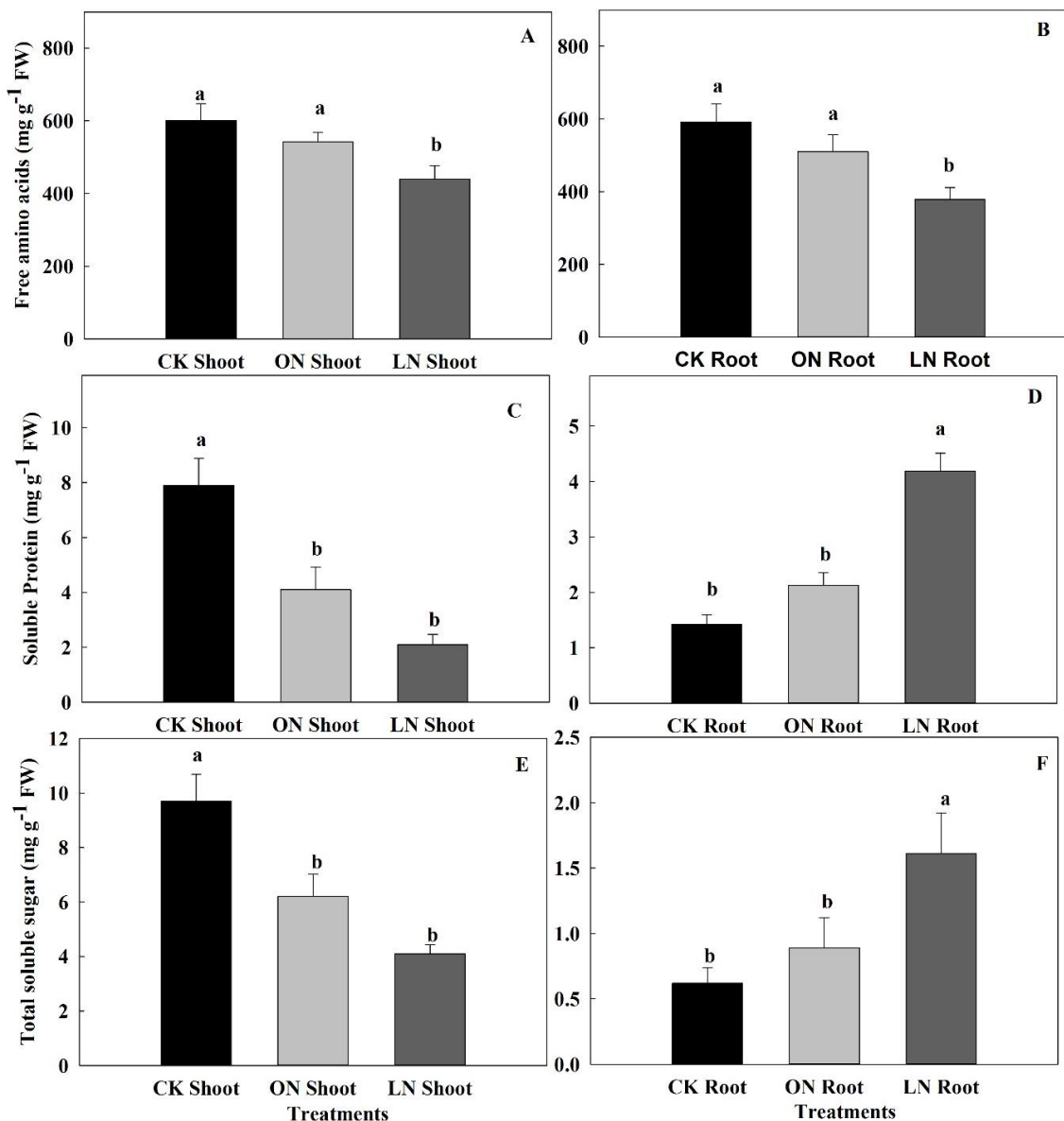


Figure 6. Concentrations of N metabolites in shoot and root of oat plant under different N treatment, free amino acids (A, B), soluble proteins (C, D), and soluble sugars (E, F). Error bars represented standard error of six biological replicates, different letters indicate significant differences ($P < 0.05$). Treatment CK = control, ON = organic N, LN = low N

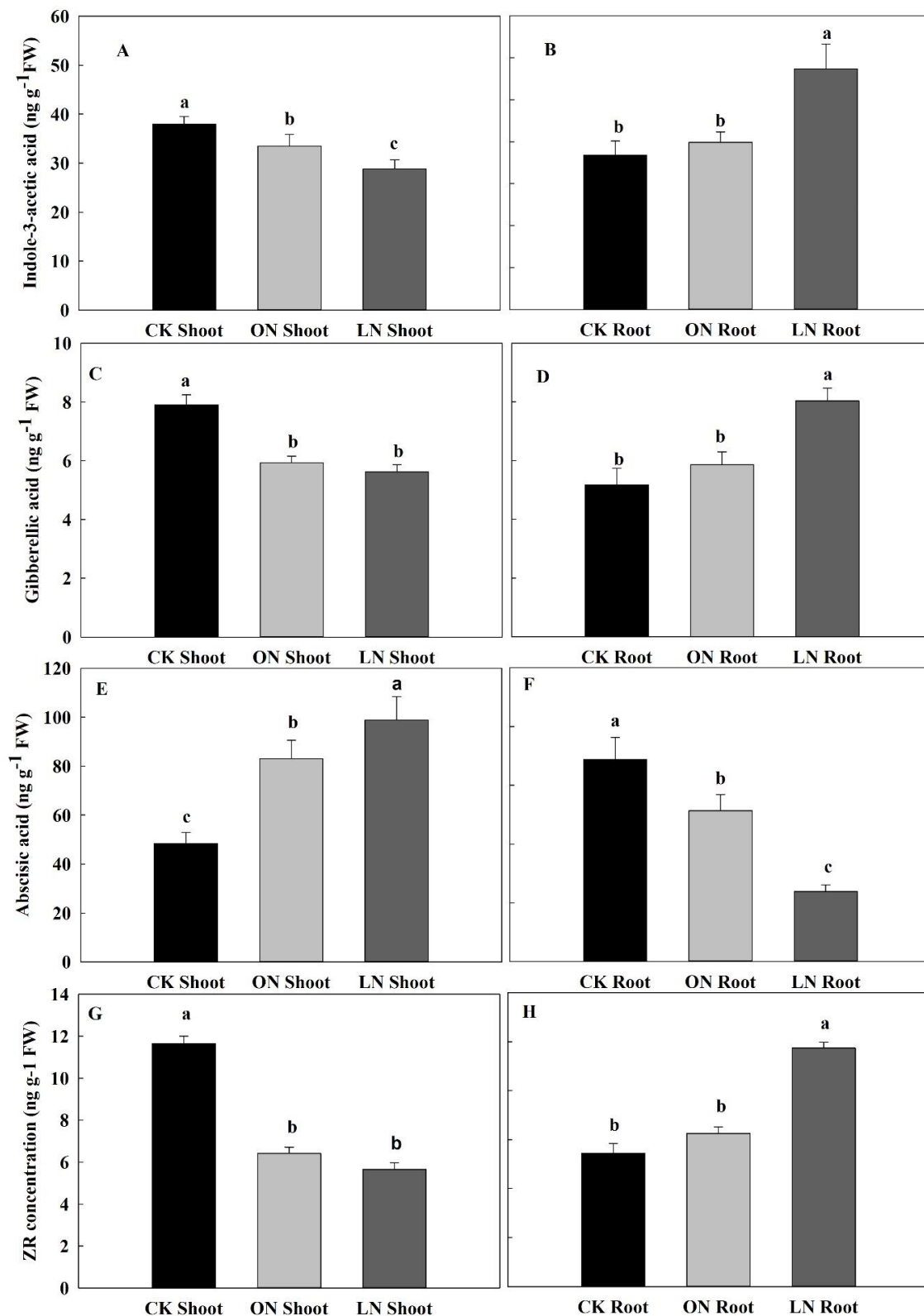


Figure 7. Effects of N deficiency on hormone accumulation in the oat shoot and root. (A, B) IAA. (C, D). GA. (E, F). ABA, (G, H). ZR. Error bars represented standard error of six biological replicates, different letters indicate significant differences ($P < 0.05$). Treatment CK = control, ON = organic N, LN = low N

Table 3. Minerals concentration in Shoot and root after harvesting of oat under different treatment

	Treatment	P	K	Ca	Mg	Fe	Na	Cu	Zn	Mn	S	B
		(mg kg ⁻¹)										
Shoot	CK	6750c	31900a	2463.3c	1345c	115.83c	131.5c	3.88c	12.82c	10.85c	2698.33a	3.85c
	ON	8016.67b	29583.3b	5300a	1865b	229.5a	330.5b	5.73b	15.27b	18.53b	2451.66b	5.48b
	LN	9133.33a	25600c	5116.67b	1983.3a	170.5b	687.5a	6.25a	20.3a	39.17a	2263.33c	6.91a
Root	CK	5676.54b	17733.15a	2457.52c	1432.9c	256.57c	1508.51c	4.7c	39.66b	14.1c	3915.43a	3.26c
	ON	5838.3ab	12400b	5075a	2592.5b	1105a	3000b	6.65b	42.7b	46.75b	2997.5b	3.91b
	LN	6500a	10625b	2750b	5766.67a	438.83b	5877.5a	13.775a	60a	56.63a	1938.33c	5.84a

In each column lower case lettering is used to show the significant differences between different types of treatments at $P < 0.05$ level. CK = 100% chemical N, ON = 100% organic N and LN = Low N

Large root system, hormonal variations and subsequent biomass accumulation in oat

Nitrogen starvation produced larger root system in maize (Han et al., 2015). Roots are the means by which plants uptake nutrients and these are the organs directly sensing their availability. In current study, oat seedlings exposed to LN treatment produced largest root system in terms of crown root number and length, lateral root number, length and density, and total root length. In ON treated seedlings, lateral root number, length and total root length increased as compared to CK only (Table 1). The enhancement of total root system of oat under low nitrogen and lateral root system under ON could be due to increased carbon allocation from shoot towards the root as shown by subsequently decreased shoot dry weight and increased root dry weight and root to shoot ratio under ON and LN treatments (Fig. 2C-E). Consistent with the previous studies (Han et al., 2015; Nadeem et al., 2018) the specific root length (SRL) of oat was found to be 6227 cm g⁻¹, 5760 cm g⁻¹, and 7145 cm g⁻¹ under CK, ON and LN treatments respectively. Extremely large SRL of oat under LN indicated its adaptation and primary response strategy to explore nitrogen under nitrogen limitation conditions. On one hand the increased total root length under LN was due to increased crown root number and length, and lateral root number and length whereas on the other hand it could also be due to decreased average root diameter (Table 1) which illustrated the longitudinal growth of roots. It is well established now that if only NO₃⁻ used as a source of N (no NH₄⁺) LN caused reduced total root length including crown and lateral root length and number in maize (Wang et al., 2005; Tian et al., 2008; Gao et al., 2015). Hence the differential response of oat to ON treatment in terms of root system architecture remained consistent with previous findings and it could be the innate response of oat where different plant species respond to different nitrogen sources differentially. A significant positive correlation was revealed between concentration of P, Mg, Cu, Mn, Zn, B and Na in root tissue with root morphological traits (Table A8). These attributes possibly influenced the ion balance within oat root leading to alterations in root morphology which needs to be further investigated in future. The most crucial role played by hormones is to regulate the speed of growth and development of the individual parts of the plant to adapt the surrounding environment (Wolters and Jürgens, 2009). Auxin is the most essential hormone which modulates different physiological processes of the plants, i.e. cell division, elongation and differentiation of different parts of plant (Reed, 2001) including root growth and development regulation (Jiang and Feldman, 2003). Gibberellic acids have crucial role

of plant organ differentiation and development, regulation of the tall phenotypic character of plants (Yamaguchi, 2008), shoot internode extension, enlargement of leaf surface area and enhancement of apical dominance. Cytokinins help to regulate the mobilization of carbohydrate and N signaling (Ashikari et al., 2005; Sakakibara, 2006). On the other hand, the special cytokinin “zeatin-riboside” helps in the bio-synthesis and maintenance of auxin (Jones et al., 2010). IAA, GA3 and ZR decreased in ON and LN shoot whereas increased in respective roots in this study (*Fig. 7A-D, G-H*). This finding is in accordance to shorter shoot and larger root system observed in ON and LN treated oat seedlings (*Fig. 2A; Table 1*). ABA stimulates plant capacity to cell division or intermediates adaptive responses to the stress environment as well as N starvation (Chin and Beevers, 1970; Santner et al., 2009). This study found increased ABA concentration in LN shoot followed by ON and CK shoot whereas, in total contrast to shoot, the concentration of ABA totally reversed in roots of oat seedlings having least concentration under LN followed by ON and CK (*Fig. 7E, F*). This finding illustrated that oat roots were not under stress in LN condition and can act as the basis to develop plant varieties which can adapt to stress conditions efficiently.

Nitrogen concentration, mineral accumulation and photosynthesis

Chlorophyll (Chl) concentration in plant leaves affects photosynthesis by regulating photosynthetic capacity and ultimately plant growth (Li et al., 2018). The most important source of energy required for plant growth is photosynthesis (Baker, 2008). A photosynthetic reaction is generally split into three steps: primary reaction, electron transport and photophosphorylation, and carbon assimilation. Chl a and Chl b are the most essential pigments to complete primary reaction. Chl a can absorb red to orange light whereas Chl b absorbs blue to purple light. SPAD value, Chl a, Chl b and total carotenoids decreased under ON and LN treatment in current study (*Fig. 3A-D*) which resulted in subsequent decreases in net photosynthesis (Pn), intercellular carbon dioxide (Ci), transpiration rate (Tr) and stomatal conductance (gs) (*Fig. 4A-D*). The reason to this could be the decreased N concentration in shoot of oat under ON and LN treatment (*Fig. 5A*) which could have hindered chlorophyll biosynthesis and disturbed the functioning of photosynthesis apparatus. These findings are consistent with previous study in sorghum (Muchow and Sinclair, 1994) and corn (Zhao et al., 2003; Long et al., 2004). Another possibility could have been the gradually reduced leaf area due to less N accumulation under ON and LN (*Fig. 1B*) which provided less surface area for light absorption and hence resulted in malfunctioned photosynthetic bio-machinery similar to maize (Zhao et al., 2003). Another reason which could have led to decreased photosynthesis was the reduction of potassium (K) concentration and increment in sodium (Na) concentration in LN shoot (*Table 3*). Lower the ratio of K/Na, lower will be the opening of stomata (Willmer et al., 1983) which could have reduced stomatal conductance in current study gradually from CK to LN conditions.

Nitrogen metabolism, carbon accumulation and re-allocation

In the process of inorganic N assimilation amides and amino acids are the first stable products (Yang et al., 2015) and act as the building blocks for proteins. The changes in several amino acids concentration or total free amino acids accumulation regulates many processes in plants including the N metabolism process and storage (Miller and Cramer, 2005; Cañas et al., 2009). The free amino acids concentration in the plant shoot

and root tissues has been reported to fluctuate significantly with different N levels and sources. A higher N dose can boost the concentration of total free amino-acid in leaves of many plant species (Yang et al., 2015). The total free amino acids concentration in wheat root was dramatically decreased due removal of the N from the nutrient solution (Rodgers and Barneix, 1989) but drastically increased upon resupply of NH_4^+ or NO_3^- the N-deficient plants (Causin and Barneix, 1993). Free amino acids concentration in shoot and root of oat seedlings in current study decreased in ON and LN treatments (Fig. 6A, B) which is consistent with related low nitrogen concentrations in shoot and root under ON and LN (Fig. 5A, B) resulting in lesser biosynthesis of amino acids. Reduced free amino acids concentration resulted in reduced soluble proteins biosynthesis in shoot under ON and LN treatments respectively (Fig. 6C). Similar trend was observed for total soluble sugars concentration in shoot under ON and LN respectively (Fig. 6E). In contrast to shoot, total soluble sugars and total soluble proteins concentrations increased in root of oat seedling under both ON and LN treatment (Fig. 6D, F) probably due to the co-transport of carbon and amino acids to nitrogen deficient root. This phenomenon resulted in increased carbon and nitrogen metabolites accumulation in nitrogen deficient root leading to a larger root system (Fig. 1B, D, E) to cope nitrogen limitation. There occurred a positive correlation of total soluble proteins and total soluble sugars with shoot and root dry weight (Fig. 8B, C, E, F) whereas the correlation of free amino acids with shoot dry weight was positive but that of with root dry weight remained negative (Fig. 8A, D) which again emphasized our argument of possible co-transport of carbon and N-metabolites to N-deficient root as an adaptive response to cope low nitrogen.

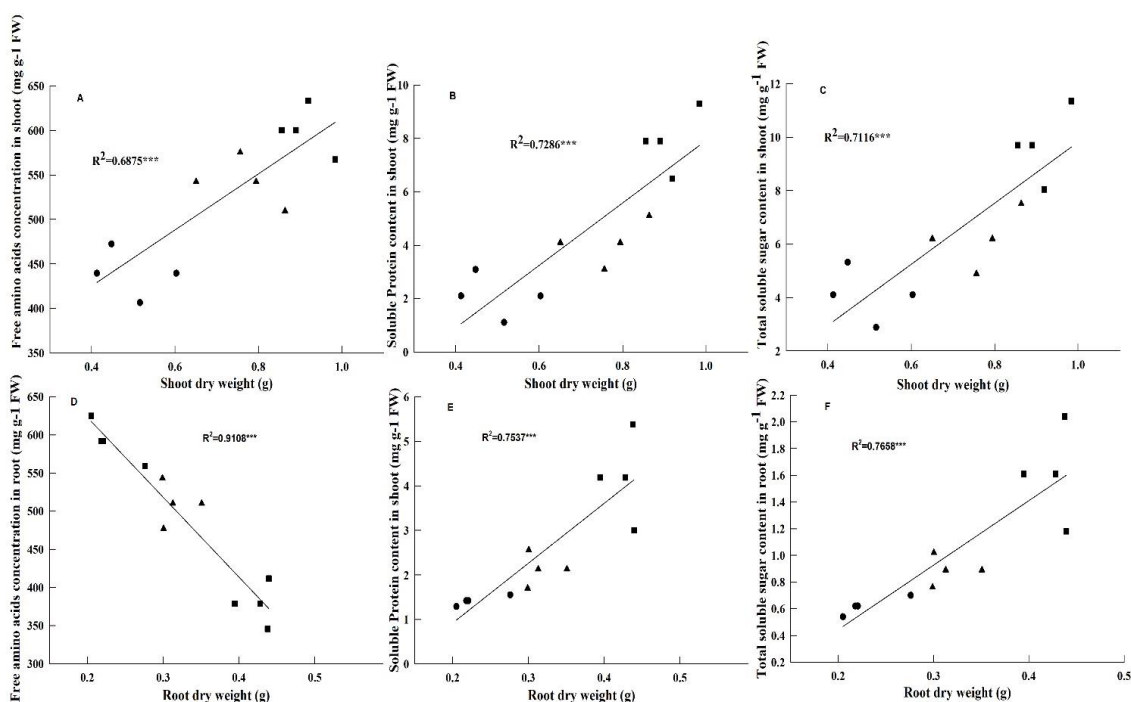


Figure 8. Relation between N metabolites and root and shoot dry weight of oat. Free amino acids and shoot dry weight (A), SOLUBLE protein and shoot dry weight (B), soluble sugar and shoot dry weight (C), free amino acids and root dry weight (D), soluble protein and root dry weight (E) and soluble sugar and root dry weight (F). Values were obtained from four replicates

Furthermore, free amino acids to N concentration ratio, total soluble proteins to N concentration ratio and total soluble sugars to N concentration ratio significantly increased in LN roots (*Table 4*) which further elucidated the shoot to root transport of carbon and nitrogen metabolites in order to let oat roots acclimatize to N-deprivation. In the mechanism of ammonium assimilation, the first product is glutamine and glutamate is its precursor whereas asparagine, aspartate and serine generally form the dominant pool of the amino acids in the leaves of many plants (Yang et al., 2015). Sometimes other amino acids may also become dominant depending on the plant species and N concentration of the surrounding environment (Xu et al., 2012; Pratelli and Pilot, 2014). Particularly, Gln and Asn are the most crucial mediators for synthesis and integration of different amino acids and also storage proteins (Miller and Cramer, 2005; Seebauer et al., 2004). Lower N concentration in shoot and root might have contributed to decreased biosynthesis of Gln (*Table 2*). Similar was the case with Asn in both shoot and root which could have altered protein synthesis negatively as revealed by decreased soluble proteins concentration in the LN shoot (*Table 2, Fig. 6C*). Altogether, the amino acid profile depicted a complicated regulation of N-metabolism which needs to be further dissected in future studies. shoot due to Carbon to nitrogen ratio was the highest in shoot as well as root under LN followed by ON treatment (*Fig. 5C, D*). Similar was the trend of nitrogen utilization efficiency (NUE) (*Fig. 5E, F*). These results further suggested the higher accumulation of carbon in shoot and root and subsequent re-allocation within the oat tissues as the N availability shifted from CK to LN conditions.

Table 4. Nitrogen metabolites/ N concentration ratio in oat shoot and root after harvesting

	Treatment	Free amino acids/N concentration ratio	Soluble protein/N concentration ratio	Soluble sugar/N concentration ratio
Shoot	CK	12.99 ± 0.5c	0.17 ± 0.01a	0.21 ± 0.01a
	ON	17.37 ± 0.84b	0.13 ± 0.01ab	0.2 ± 0.01a
	LN	19.88 ± 1.83a	0.096 ± 0.01b	0.19 ± 0.02a
Root	CK	17.76 ± 0.75b	0.043 ± 0.001b	0.019 ± 0.001b
	ON	20.52 ± 1.61b	0.085 ± 0.001b	0.04 ± 0.002b
	LN	31.95 ± 1.4 a	0.35 ± 0.04a	0.14 ± 0.01a

In each column lower case lettering is used to show the significant differences between different types of treatments at $P < 0.05$ level. Values show Standard errors (SE) ± mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N

Conclusion

Nitrogen, a vital macronutrient for crop growth and development, is often subjected to losses through various means and causes environmental hazards through its excessive deposition in the ecosystem. Hence, nitrogen use efficient crops can minimize the use of chemical nitrogen and thus reverse the environmental deteriorations. In this study, Oat (a cereal crop) was studied for its adaptation to different nitrogen regimes (CK, ON, LN). Root dry weight, R/S ratio, C/N ratio and NUE increased under LN treatment which indicated the allocation of carbon to continuously growing root as suggested by increased crown root number and length, lateral root number and length, and total root length under low nitrogen. Highest levels of total soluble proteins and total soluble sugars were in total contrast to lowest N concentration in roots under LN which further emphasized the carbon provision of roots at the cost of minimum photosynthesis.

Furthermore, highest levels of IAA, GA3 and ZR in Low nitrogen roots was perhaps the innate adaptive strategy of oat allowing the roots to expand under low nitrogen stress as shown by highest level of abscisic acid. Altogether, these findings suggested that oat is an extremely nitrogen use efficient crop which can be well adapted to N-poor soils.

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Author contributions. TAK, FN, ZZ, and YH designed the experiment. TAK, FN, YG, and YY, performed the research. TAK and FN analyzed the data and wrote the manuscript. XW, ZZ and YH revised the manuscript. All authors approved the final manuscript.

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APPENDIX

Table A1. Percentage changes in the shoot height and leaf area of oat

Treatment	Shoot length (cm)	Percentage change (%)			Leaf area plant ⁻¹ (cm ²)	Percentage change (%)		
		CK to ON	CK to LN	ON to LN		CK to ON	CK to LN	ON to LN
CK	58.75 ± 4.11a				18.3 ± 1.6a			
ON	50 ± 2.58 b	-14.89	-28.09	-15.5	15.2 ± 0.97b	-16.94	-51.37	-41.45
LN	42.25 ± 2.63c				8.9 ± 0.81c			

Different letters after the values within the same column indicated significant differences ($P < 0.05$). Values show standard errors (SE) ± mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N. Percentage change CK to ON = [(value under ON – Value under CK)/Value under CK] × 100, CK to LN = [(value under LN – Value under CK)/Value under CK] × 100, ON to LN = [(value under LN – Value under ON)/Value under ON] × 100

Table A2. Percentage changes in the shoot dry weight, root dry weight and root shoot ratio

Treatment	Shoot dry weight (g)	Percentage change (%)			Root dry weight (g)	Percentage change (%)			Root/shoot ratio	Percentage change (%)		
		CK to ON	CK to LN	ON to LN		ON to CK	LN to CK	LN to ON		CK to ON	CK to LN	ON to LN
CK	0.91 ± 0.03a				0.23 ± 0.02c				0.252 ± 0.01c			
ON	0.77 ± 0.04b	15.38	-45.77	-35.75	0.32 ± 0.02b	39.13	82.61	31.25	0.41 ± 0.04b	62.7	241.27 (3.41 fold more)	109.76 (2.1 fold more)
LN	0.49 ± 0.04c				0.42 ± 0.02a				0.86 ± 0.06a			

Different letters after the values within the same column indicated significant differences ($P < 0.05$). Values show standard errors (SE) ± mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N. Percentage change CK to ON = [(value under ON – Value under CK)/Value under CK] × 100, CK to LN = [(value under LN – Value under CK)/Value under CK] × 100, ON to LN = [(value under LN – Value under ON)/Value under ON] × 100

Table A3. Percentage changes in the N concentration C/N ratio, N utilization efficiency in the shoot and root of oat

	Treatment	N concentration (g kg ⁻¹)	Percentage change (%)			C/N ratio	Percentage change (%)			NUE	Percentage change (%)		
			CK to ON	CK to LN	ON to LN		CK to ON	CK to LN	ON to LN		CK to ON	CK to LN	ON to LN
Shoot	CK	46.2 ± 3.23a				8.76 ± 1.12c				21.65 ± 1.19c			
	ON	31.23 ± 2.09b	-32.4	-52.01	-29.01	13.32 ± 1.04b	52.05	111.42	39.04	32.02 ± 1.16b	47.9	108.36	40.88
	LN	22.17 ± 2.64c				18.52 ± 2.64a				45.11 ± 3.19a			
Root	CK	33.33 ± 2.12a				12.08 ± 1.12c				30 ± 2.19c			
	ON	25.2 ± 2.19b	-24.39	-64.39	-52.9	16.97 ± 1.57b	40.48	193.23	108.78	39.68 ± 5.97b	32.27	180.9	112.37
	LN	11.87 ± 1.32c				35.43 ± 2.78a				84.27 ± 3.82a			

Different letters after the values within the same column indicated significant differences ($P < 0.05$). Values show standard errors (SE) ± mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N. Percentage change CK to ON = [(value under ON – Value under CK)/Value under CK] × 100, CK to LN = [(value under LN – Value under CK)/Value under CK] × 100, ON to LN = [(value under LN – Value under ON)/Value under ON] × 100

Table A4. Percentage changes in the crown root number, lateral root number and lateral root density under different treatment

Treatment	Crown root number	Percentage change (%)			Lateral root number	Percentage change (%)			Lateral root density	Percentage change (%)		
		CK to ON	CK to LN	ON to LN		ON to CK	LN to CK	LN to ON		CK to ON	CK to LN	ON to LN
CK	7.5 ± 0.87b				1736.17 ± 145.2c				7.8 ± 0.98b			
ON	10.5 ± 1.08b	40	96.67	40.47	2000.4 ± 123.3b	15.22	34.1	16.39	10.6 ± 1.76b	35.9	128.21	67.92
LN	14.75 ± 2.2a				2328.17 ± 155.8a				17.8 ± 2.01a			

Different letters after the values within the same column indicated significant differences ($P < 0.05$). Values show standard errors (SE) ± mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N. Percentage change CK to ON = [(value under ON – Value under CK)/Value under CK] × 100, CK to LN = [(value under LN – Value under CK)/Value under CK] × 100, ON to LN = [(value under LN – Value under ON)/Value under ON] × 100

Table A5. Percentage changes in the crown root length, lateral root length and total root length under different treatment

Crown root length (cm)	Percentage change (%)			Lateral root length (cm)	Percentage change (%)			Total root length (cm)	Percentage change (%)		
	CK to ON	CK to LN	ON to LN		ON to CK	LN to CK	LN to ON		CK to ON	CK to LN	ON to LN
143.34 ± 10.56b				1288.16 ± 82.2c				1431.5 ± 97.36c			
176.23 ± 13.98b	22.95	116.27	75.91	1667.22 ± 102.1b	29.43	111.57	63.46	1843.45 ± 131.23b	28.78	112.3	64.65
310 ± 17.76a				2725.3 ± 178.3a				3035.33 ± 198.3a			

Different letters after the values within the same column indicated significant differences ($P < 0.05$). Values show standard errors (SE) ± mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N. Percentage change CK to ON = [(value under ON – Value under CK)/Value under CK] × 100, CK to LN = [(value under LN – Value under CK)/Value under CK] × 100, ON to LN = [(value under LN – Value under ON)/Value under ON] × 100

Table A6. Percentage changes in the chlorophyll a, chlorophyll b and carotenoid in oat leaf under different treatment

Treatment	Chlorophyll a content/mg g ⁻¹ FW	Percentage change (%)			Chlorophyll b content/mg g ⁻¹ FW	Percentage change (%)			Carotenoid content/mg g ⁻¹ FW	Percentage change (%)		
		CK to ON	CK to LN	ON to LN		ON to CK	LN to CK	LN to ON		CK to ON	CK to LN	ON to LN
CK	3.44 ± 0.09a				1.47 ± 0.12a				0.7 ± 0.02a			
ON	3.01 ± 0.07b	-12.5	-20.93	-9.63	1.23 ± 0.08b	-16.36	-44.9	-34.15	0.61 ± 0.02b	-12.86	-30	-19.67
LN	2.72 ± 0.05c				0.81 ± 0.06c				0.49 ± 0.01c			

Different letters after the values within the same column indicated significant differences ($P < 0.05$). Values show standard errors (SE) ± mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N. Percentage change CK to ON = [(value under ON – Value under CK)/Value under CK] × 100, CK to LN = [(value under LN – Value under CK)/Value under CK] × 100, ON to LN = [(value under LN – Value under ON)/Value under ON] × 100

Table A7. Percentage changes in the concentration of free amino acids, total soluble protein and soluble sugars in the shoot and root of oat

	Treatment	Free amino acids (mg g ⁻¹ FW)	Percentage change (%)			Total soluble protein (mg g ⁻¹ FW)	Percentage change (%)			Total soluble sugar (mg g ⁻¹ FW)	Percentage change (%)		
			CK to ON	CK to LN	ON to LN		CK to ON	CK to LN	ON to LN		CK to ON	CK to LN	ON to LN
Shoot	CK	600.29 ± 47.4a				7.9 ± 0.99a				9.7 ± 0.98a			
	ON	542.46 ± 26.3a	-9.6	-26.78	-18.97	4.1 ± 0.82b	-48.1	-73.42	-48.78	6.2 ± 0.83b	-36.08	-57.73	-33.87
	LN	439.56 ± 37.3b				2.1 ± 0.38b				4.1 ± 0.33b			
Root	CK	592.04 ± 49.25a				1.42 ± 0.18b				0.62 ± 0.12b			
	ON	510.14 ± 45.96a	-13.83	-36.03	-25.76	2.13 ± 0.2b	50	195	99.71	0.89 ± 0.23b	43.54	159.68	80.9
	LN	378.71 ± 32.66b				4.19 ± 0.32a				1.61 ± 0.31a			

Different letters after the values within the same column indicated significant differences ($P < 0.05$). Values show Standard errors (SE) ± mean of four replicates. CK = 100% chemical N, ON = 100% organic N and LN = Low N. Percentage change CK to ON = [(value under ON – Value under CK)/Value under CK] * 100, CK to LN = [(value under LN – Value under CK)/Value under CK] * 100, ON to LN = [(value under LN – Value under ON)/Value under ON] * 100

Table A8. Correlation coefficient between root morphological traits and nutrient concentration in oat plant root

Nutrient	Crown root number	Seminal root number	Seminal root density	Crown root length	Lateral root length	Total root length	Average root diameter
P	0.656*	0.54	0.619*	0.579*	0.662*	0.651*	0.56
K	-0.713**	-0.785**	-0.718**	-0.731**	-0.749**	-0.759**	-0.853**
Ca	-0.12	0.05	-0.15	-0.22	-0.16	-0.17	0.001
Mg	0.907**	0.877**	0.902**	0.956**	0.967**	0.977**	0.963**
Fe	-0.017	0.14	-0.04	-0.13	-0.06	-0.07	0.10
Cu	0.925**	0.868**	0.922**	0.958**	0.976**	0.983**	0.953**
Mn	0.764**	0.803**	0.759**	0.776**	0.804**	0.814**	0.905**
Zn	0.808**	0.706*	0.819**	0.918**	0.886**	0.904**	0.897**
B	0.925**	0.877**	0.916**	0.938**	0.969**	0.973**	0.941**
S	-0.817**	-0.773**	-0.822**	0.886**	-0.891**	-0.907**	-0.942**
Na	0.878**	0.899**	0.866**	0.927**	0.941**	0.954**	0.954**

Tissue samples were taken just prior to harvesting. Significant decreases or increases were indicated by ‘*’ or ‘**’. * $P < 0.05$; ** $P < 0.01$