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Nitrogen fertilization improved water-use efficiency of winter wheat through increasing water use during vegetative rather than grain filling

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ABSTRACT

Water availability is a major constraint for wheat production in many semiarid regions of the world, including the Loess Plateau of China, and thus improving the water use efficiency (WUE) becomes a main research target. The impact of nitrogen (N) fertilizer on root growth, water use and WUE were examined for winter wheat grown in a semiarid farm on the Loess Plateau of China. A four-growing season field study (2011–2015) at the Changwu Agri-ecological Station, Changwu, Shaanxi, China with four rates of N fertilizer (0, 60, 120 and 180 kg N ha^{-1}) were conducted to determine soil water balance, root growth, yield and WUE in each growing season. Soil water content at final harvest in each growing season was lower and evapotranspiration (ETg) was higher under N supply than under non-N fertilization. N supply increased root growth and root length density (RLD) in deeper layers of the soil profile (80–140 cm), and improved water uptake, above-ground biomass and WUEb during vegetative growth stage. Grain yield under 60, 120 and 180 kg N ha^{-1}, increased by 12.8, 25.4 and 34.8%, respectively, with corresponding improvements in WUEg of 5.1, 13.8 and 29.3%, respectively, when compared to the non-N treatment. Our results demonstrated that N applications improved dryland winter wheat WUEg by increasing deep root growth and water use during vegetative.

1. Introduction

On the Loess Plateau of China, more than half of the precipitation is received during the fallow season from July to September (Wang et al., 2011). The precipitation storage efficiency (PSE), expressed as soil water accumulation divided by precipitation received during fallow periods, is approximately 35–40% (Shangguan et al., 2002). Therefore, maximizing water productivity is a major target in this region and will have a great impact at local and regional scales. Grain yield and water use efficiency (WUE) in wheat are primarily limited by the soil water deficit during the spring growth and through the grain-filling because of the high evaporation and the erratic distribution of rainfall. High wheat grain yields are often achieved by using N fertilizer and this practice has markedly increased in China to the point that a controversial environmental concern has been brought to grain production (Zhu and Chen, 2002). It is considered that high N fertilization will not be sufficient in the long-term maintenance of high grain yields and WUE because of the potential depletion of soil water under high N supply (Fan et al., 2005; Huang et al., 2003; Liu et al., 2013; Wang et al., 2011). The understanding of the mechanisms that control water use and WUE under N fertilization is critical for the efficient use of water in semiarid regions of the world, including the Loess Plateau of China.

One of the ways for efficient water use improvement by a crop is through increasing the depth of the root system, since in most dryland environments crops do not use all the water available in the soil profile due to restrictions to root growth (Turner, 2004). The growth and distribution of the crop root system is affected

Abbreviations: DM, dry matter; DMR, pre-anthesis DM remobilization; CDMR, the contribution of pre-anthesis DM remobilization to grain; RD, root diameter; RDW, root dry weight; RLD, root length density; RML, root mass per length; PSE, precipitation storage efficiency.

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by factors, such as cultivar, soil properties, timing and amount of irrigation and fertilization and tillage management (Huang et al., 2012; Outoukarte et al., 2010; Wang et al., 2014). For instance, fertilizer application stimulates deeper root growth in winter wheat, enabling access to more stored soil water and reducing the risk of water deficit (Read et al., 1982; Wang et al., 2011). A larger root system can also lead to increases in evapotranspiration (ET) through increases in the extraction of stored soil water (Cooper et al., 1987) and if the soil water is not sufficient, then highly fertilized crops may experience greater water deficit late in the season, limiting grain yield and WUE (Fan et al., 2005; Frederick and Camberato, 1995; Huang et al., 2003). Alternatively, if the crop has a poor developed root system, grain yield can be also limited because of insufficient capture of soil water by the roots (Johnson and Davis, 1980). For instance, a grain yield of 0.84 Mg ha^{-1} resulted from plots in which the soil water extraction was limited to a depth of 0.9 m in the soil profile, leaving 72 mm of available soil water in the 2.1 m of the soil. In contrast, a high yield of 2.3 Mg ha^{-1}, resulted when only 25 mm of available soil water was left in the 2.1 m profile because crops captured more water from deeper soil layers (Johnson and Davis, 1980). The role of roots system in acquiring N and water have increasingly gained focus in recent years (Fang et al., 2017; Palta and Watt, 2009; Palta et al., 2011; Wang et al., 2014), with valuable results for improving crop yields and the efficiency of water and N use.

On the Loess Plateau of China, limited and erratic rainfall is the primary water resource for rainfed wheat production, and no other water resources are available (Liu et al., 2013). Additionally, in most cases, the limited water resources are used inefficiently by crops; therefore, improving the efficient use of rainfall is a primary target in this region. An ideotype of root architecture seems to be important for improving the capture of water and nitrogen (Palta and Watt, 2009). However, the effects of N fertilization on the soil water balance, grain yield and WUE need to be assessed for wheat grown in a region with limited and erratic rainfall, before a root architecture is designed. The aims of this study were (i) to determine the effect of N application rate on the spatial distribution of the root system and its relationship with WUE and grain yield, and (ii) to evaluate the sustainability of obtaining high grain yield under high rates of N fertilizer. A field study was conducted during four consecutive growing seasons (2011–2015) and the N fertilizer rates of 0, 60, 120 and 180 kg N ha^{-1} were applied to a wheat crop in each growing season. It was hypothesized that (i) N application to winter wheat increase root length density (RLD) in deeper layers of the soil profile, increasing water use and grain yield, and (ii) high N application rates increasing grain yield and WUE adversely deplete the soil water and organic C.

2. Materials and methods

2.1. Experimental site

A four-growing season's field study was conducted at the Changwu Agri-ecological Station of the Loess Plateau (107°44.703′E, 35°12.787′N) in Changwu County, Shaanxi Province, China during 2011–2015 growing seasons. The site is located in a warm temperate zone with a continental monsoon climate with a long-term average rainfall of 570 mm (1984–2010) with more than half falling from July to September. The mean frost free period is 194 days and an annual water-surface evaporation demand of 1552 mm. The experimental site is 1220 m above sea level and has a slope of 0.07%. The soils are Cumuli-Ustic Isohumosols, according to the Chinese Soil Taxonomy (Gong, 1999), containing 37% clay, 59% silt, and 4% sand, the dominant clay mineral in this region is I/S mixed-layer mineral (approximately 69%). The pH measured in a suspension of soil in 0.01 M $CaCl_2$, is 8.4 in 0–20 cm soil layer. The field capacity is about 0.287 cm^3 $cm^{-3}$and the wilting point 0.098 cm^3 cm^{-3} (Chen et al., 2015a; Kang et al., 2000; Wang et al., 2013). The soil total N, organic matter content, available phosphorus, available potassium and bulk density at 0–20 cm depth were 0.93 g kg^{-1}, 14.1 g kg^{-1}, 16.3 mg kg^{-1}, 138.7 mg kg^{-1} and 1.3 g cm^{-3}, respectively.

2.2. Experimental design and crop management

The traditional practice in the region is to apply all N fertilizer before sowing as the base fertilizer. However, the late N amendment has been proposed as a strategy to achieve an improvement in grain yield in recent years because it stimulates photosynthate use in the growth processes and delays senescence of the photosynthetic apparatus (Fuertes-Mendizábal et al., 2010). So, we optimized this pattern using late N fertilizer application in this study. The four N treatments were as follows:

1. No N applied (N_0)
2. 60 kg N ha^{-1} (N_{60})
3. 120 kg N ha^{-1} (N_{120})
4. 180 kg N ha^{-1} (N_{180})

For treatments of N60, N120, N180, 36, 72 and 108 kg N ha^{-1}, urea was broadcast at planting as base fertilizer and an additional 24, 48 and 72 kg N ha^{-1} were broadcast at flag leaf visible stage (i.e. from April 20 to May 2) when there was some rainfall, respectively. Superphosphate was the P source and was broadcast at a rate of 120 kg P_2O_5 ha^{-1} at planting. The experimental plots were 12 m × 5 m (25 rows) and each N treatment was replicated four times for a total of 16 plots in each growing season. The plots with the different N application were arranged in a randomized complete block design and located in the same location for the four growing seasons. Winter wheat (cv. Changhan 58, a high-yielding cultivar) was sown at the rate of 150 kg ha^{-1} using a no-till disk drill with 20−cm row spacing. Wheat crops were sown in late September and harvest in late June of four consecutive growing seasons (2011–2015), all residual straw was removed after harvest for each growing season.

2.3. Sampling and measurements

The dates of the developmental stages (phenostages) for anthesis and physiological maturity (flag leaves turned yellow; Hanft and Wych 1982) were recorded for each plot in each N treatment. Daily observations were made and the phenostage noted when 50% of the plants in each plot had achieved the particular stage. Phenostages were defined using the Zadoks' scale of cereal development (Zadoks et al., 1974). Comparisons between the two phenostages were made in days after sowing (DAS).

Rainfall was recorded daily at the experimental station. In addition, daily records of wet and dry bulb temperature, windspeed, and class-A pan evaporation were obtained from an automatic weather station located 500 m away from the experimental plots. Annual rainfall, fallow rainfall and growth-season rainfall were also calculated according to the periods of winter wheat growth in the region. The drought index (DI) for annual rainfall was used to assess variations and status in rainfall among the four growing seasons and calculated using the following equation (Fan et al., 2005; Guo et al., 2012):

$$DI = (Ar - M)/\sigma \quad (1)$$

Where Ar is the annual rainfall (the sum of fallow rainfall and seasonal rainfall), M is the average annual rainfall, and s is the standard deviation for annual rainfall. DI is used to distinguish among the wet

(DI > 0.35), normal (−0.35 ≤ DI ≤ 0.35), and dry (DI < −0.35) growing seasons (Guo et al., 2012). Similarly, the DIs for fallow rainfall and seasonal rainfall were also calculated to assess variations and status in rainfall among different growing seasons.

Soils from the surface to a depth of 200 cm were sampled before sowing, at start of elongation (GS30), anthesis (GS60), and after final harvest (GS92), respectively, for measurement of soil water content, soil water balance (ΔS), precipitation storage efficiency (PSE) and water use efficiency. Samples were collected at intervals of 10 cm within the first 100 cm depth and at 20 cm intervals from 101 cm to 200 cm. The fresh weight of the samples was determined immediately and the dry weight determined after drying in a forced-air oven at 105°C until a constant weight was achieved to calculate the soil moisture. The soil water storage (SWC), evapotranspiration (ET), soil water balance (ΔSWC), precipitation storage efficiency (PSE) were determined according to the following equations (Wang et al., 2011, 2013).

$$\mathrm{SWS} = (\mathrm{SD} \times \mathrm{R} \times \mathrm{Wm}) \times 100 \quad (2)$$

$$\Delta\mathrm{SWCf} = \mathrm{SWCph} - \mathrm{SWCs} \quad (3)$$

Where the SWS, SD, R and Wm are soil-water-storage (mm), soil depth (m), soil bulk density ($\mathrm{g\,cm^{-3}}$), and soil moisture at different depths (%), respectively. ΔSWCf is soil water storage during the fallow period (mm); SWCph is soil water content at previous harvest or the beginning of the fallow period (mm) and SWCs is the soil water content at wheat planting or the end of the fallow period (mm);

The precipitation storage efficiency (PSE) was calculated by:

$$\mathrm{PSE}\,(\%) = \frac{\Delta\mathrm{SWCf}}{\mathrm{Rf}} \quad (4)$$

Where Rf is the rainfall during the fallow period (mm).

The ETg (mm) was calculated by:

$$\mathrm{ETg} = \mathrm{SWCs} + \mathrm{Rg} - \mathrm{SWCh} \quad (5)$$

Where Rg is the seasonal rainfall (mm), SWCh is the soil water content at harvest (mm).

The change of soil-water storage (mm) was calculated by:

$$\Delta\mathrm{SWC} = \mathrm{P}' - \mathrm{ET}' \quad (6)$$

Where P′ is the rainfall occurring during the given period (mm); ET′ is the amount of evapotranspiration (mm), and DSWC is the change of soil-water storage within the soil profile (mm).

The soil water balance was measured for the fallow period (DSWCf), growing season (DSWCg) and the whole growing season from the beginning of the fallow period to the end of growing period (DSWCy), which represents the soil water recharge during the fallow period, the soil water depletion during growing season and annual soil water balance, respectively.

Three soil cores (diameter 4.5 cm, height 20 cm) were taken randomly in each plot before sowing in 2011, when the experiment was initiated, and at harvest in 2013–2015 growing seasons, respectively, for measurement of soil nutrient contents. Soil samples were air-dried, ground, and passed through a 2.0 mm sieve. Dried samples weighing 5 g each were added to 50 ml of 2 M KCl, shaken for 60 min, and analyzed with a FIAstar 5000 Analyzer (FOSS Tecator, Sweden) for nitrate nitrogen (NO3-N) and ammonium nitrogen (NH4-N). Available phosphorus (AP) was determined using the Olsen method (Olsen, 1954). Soil organic C concentration was determined by wet oxidation with $K_2Cr_2O_7$ and H_2SO_4 (Nelson and Sommers, 1996).

Roots measurements made only in the 2013–2014 and 2014–2015 growing seasons. In each plot, areas with uniform growth of wheat plants were selected, and wheat root samples were harvested with a root auger at the flowering stage. The radius and length of the drill bit of the root auger were 5 and 10 cm, respectively. The following sampling method was used. Two cores were collected per plot, one within the crop row and one in the midway between rows (Xue et al., 2003). Each soil layer was 20 cm in depth, to a total depth of 2 m. The mixtures of roots and soil were transferred into a 100-mesh nylon bag and then submerged in water for 30 min, and subsequently, samples were washed with tap water and the impurities were removed. The cleaned root samples were placed on the glass plate of a root system scanner for grey-scale scanning with an Epson Perfection V700 photo flatbed scanner (Seiko Epson Corp., Nagano, Japan). An analysis of files was conducted using root system analysis software (WinRHIZO 2008) to obtain root morphology parameters, such as root length density (RLD), root dry weight (RDW) and root mass per unit of root length (RML). Then, the root samples were dried at 85 °C to a constant dry weight to obtain root dry weight.

Root dry weight (RDW) per unit area refers to the root dry weight in unit area of soil. The RDW ($\mathrm{g\,m^{-2}}$) was calculated by:

$$\mathrm{RDW} = \frac{\mathrm{M}}{\mathrm{S}} \times 10^4 \quad (7)$$

Where M and S are the total root dry weight (g) within of 0–200 cm of the soil profile and the soil area ($\mathrm{cm^2}$), respectively.

The soil area ($\mathrm{cm^2}$) was calculated by:

$$\mathrm{S} = 3\Pi \mathrm{Pr}^2 \quad (8)$$

Where r is the radius of the drilling bit (r = 5 cm).

The RLD ($\mathrm{cm\,cm^{-3}}$) was calculated by:

$$\mathrm{RLD} = \frac{\mathrm{L}}{\mathrm{Sh}} \quad (9)$$

Where L and h are the root length (cm) of different soil layer and the sampling depth (h = 20 cm), respectively.

The RML ($\mathrm{mg\,m^{-1}}$) was calculated by:

$$\mathrm{RML} = \frac{\mathrm{RDW}}{\mathrm{L}'} \times 10^3 \quad (10)$$

Where L′ is the total root length within of 0–200 cm of the soil profile in unit area of soil ($\mathrm{m\,m^{-2}}$).

Aboveground dry matter (DM) accumulation was measured at the start of stem elongation (GS30) and at 50% flowering (GS60) by harvesting an area of $0.25\,\mathrm{m^2}$ per plot. At final harvest, aboveground DM was sampled in an area of $1\,\mathrm{m^2}$ with 5 replications per plot and that each replication was selected randomly. Sample collection and the determination of yield were carried on by manual operation, following previous studies (Chen et al., 2015a; Fang et al., 2010). Tiller number was recorded at 50% anthesis and final harvest and where appropriate, spikes were counted and dried in a fan-forced oven at 70 °C for at least 48 h, and then weighed, threshed by hand, and grain was redried and weighed. Grain number and weight was determined for each sample. Additional 50 mainstem spikes from each plot were sampled to determine kernel number per spike. Harvest index (HI) and water use efficiency (WUE) were calculated as:

$$\mathrm{HI} = \frac{\mathrm{GY}}{\mathrm{ET}} \quad (11)$$

$$\mathrm{WUEb} = \frac{\mathrm{BY}}{\mathrm{ET}} \quad (12)$$

$$\mathrm{WUEg} = \frac{\mathrm{GY}}{\mathrm{ET}} \quad (13)$$

Where HI, BY, GY, WUEb and WUEg, are harvest index, the biomass yield ($\mathrm{kg\,ha^{-1}}$), the grain yield ($\mathrm{kg\,ha^{-1}}$), water use efficiency for biomass yield ($\mathrm{kg{\cdot}mm^{-1}{\cdot}ha^{-1}}$), water use efficiency for grain yield ($\mathrm{kg{\cdot}mm^{-1}{\cdot}ha^{-1}}$), respectively.

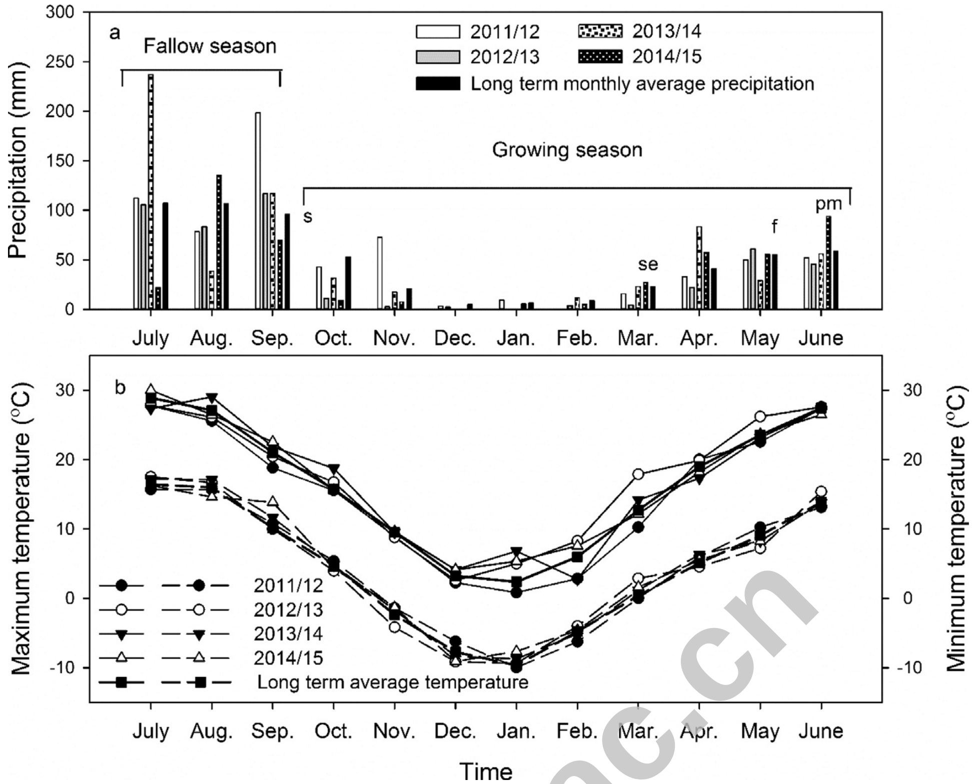

Fig. 1. Monthly rainfall (a) and maximum and minimum temperature (b) for each of the 4 years of the study. The letters indicate sowing (s), beginning of stem extension (se), flowering (f), and physiological maturity (pm).

Apparent DM remobilization and remobilization efficiency were calculated following (Arduini et al., 2006; Masoni et al., 2007; Ercoli et al., 2008).

1.) DM remobilization (DMR) = DM at anthesis – DM of leaves + culms + chaff at final harvest;
2.) Contribution of DM remobilization to grain (CDMR) = (DMR/grain yield at maturity) × 100.

2.4. Statistical analysis

Analysis of variance (ANOVA) was conducted using the SAS software (SAS Institute, Inc., Cary, North Carolina). All data were statistically analyzed as a completely randomized design with four replications using analysis of variance (ANOVA). The effects of the N rate, growing seasons and their interactions on the measured variables were tested using one- and two-way ANOVAs. When F-values were significant, the least significant difference (LSD) test was used to compare means. The significant differences between the means were estimated at 95% confidence level. Drawing figures were conducted by using Sigmaplot software (Jandel Scientific, Corte Maders, CA).

3. Results

3.1. Weather conditions and crop phenology

There was a large variation in rainfall among the four growing seasons (Fig. 1a), with more than half of the annual rainfall received during the summer fallow period (July to September). Compared with the long-term average of 569 mm (1984–2010), the annual rainfall in 2014–2015 was similar to the long-term average, but lower than that in 2011–2012 (667 mm) and 2013–2014 (644 mm), and higher than that in 2012–2013 (456 mm). The fallow rainfall and seasonal rainfall were not parallel to the annual rainfall pattern. Compared with the yearly average of the fallow rainfall (305 mm), fallow rainfall in 2012–2013 was in similarity, but the rest growing seasons were wetter. The seasonal rainfall in 2013–2015 was similar to the yearly average, but it was wetter in 2011–2012 (289 mm) and drier in 2012–2013 (169 mm). The annual rainfall in the dry growing season (2012–2013) was 113 mm less than that of the long-term average (1984–2010), whereas for others growing seasons, the annual rainfall was 75–98 mm more than the long-term average. The rainfall deficit in 2012–2013 (116 mm) coincided with the wintering and jointing stages (Table 1). Minimum temperatures occurred at wintering stage each growing seasons and were not significant difference between growing seasons (Fig. 1b). Severe frost damage at stem elongation (GS30) and ear emergence (GS55) and rare hail damage during grain filling (GS65) adversely influenced the growth of wheat in the 2014–2015 growing season. During the growth season, maximum temperatures occurred at physiological maturity in each growing season (Fig. 1b).

Time to flowering and physiological maturity was affected by the N supply rates and growing season of the study (Table 2). Time to flowering of both N_{120} and N_{180} treatments was slightly delayed when compared with non-N application treatment (Table 2). Time to physiological maturity was delayed when N supply was increased from N_0 to N_{180} by 4 days in 2012–2015 seasons and by 6 days in 2011–2012 ($P < 0.05$); (Table 2). The physiological maturity was delayed by 8 days in 2011–2012 ($P < 0.05$), by 9 days in 2013–2014 ($P < 0.05$) and by 1 days in 2014–2015 ($P > 0.05$), respectively, when compared with 2012–2013 (Table 2). The duration of grain filling was delayed when N supply was increased from N_0 to N_{180} by 4 days in 2011–2012 ($P < 0.05$), by 2 days in 2012–2013 and 2014–2015 ($P < 0.05$), and by 1 days in 2013–2014 ($P > 0.05$). The duration of grain filling was delayed by 5–6 days

Table 1
Annual rainfall, fallow season rainfall and seasonal rainfall for the experimental years at the Changwu Agri-ecological Station.

Growing seasons	Annual rainfall (mm)	Drought index (DI) for annual rainfall	Condition	Fallow rainfall (mm)	DI for fallow rainfall	Condition	Seasonal rainfall (mm)	DI for seasonal rainfall	Condition
2011–2012	667	0.81	wet	378	0.68	wet	289	0.50	wet
2012–2013	456	−0.93	dry	287	−0.17	normal	169	−1.89	dry
2013–2014	644	0.62	wet	392	0.81	wet	252	−0.24	normal
2014–2015	607	0.31	normal	346	0.38	wet	261	−0.06	normal
Mean (1984–2010)	569	–	–	305	–	–	264	–	–

Years are classified as 'Dry', 'Normal' and 'Wet' based on the drought index (DI) values for annual rainfall, fallow season rainfall and seasonal rainfall, respectively, i.e.: DI <−0.35, −0.35 ≤ DI ≤ 0.35, and DI>0.35, respectively.

Table 2
Time (days) from sowing to 50% flowering, and physiological maturity for the experimental years.

Growing seasons	N rates kg ha^{-1}	Flowering 50% (days)	Physiological Maturity (days)	The duration of grain filling (days)
2011–2012	N_0	230a	271b	41b
	N_{60}	230a	272b	42ab
	N_{120}	231a	275ab	44ab
	N_{180}	232a	277a	45a
2012–2013	N_0	228a	264b	36a
	N_{60}	228a	265ab	37a
	N_{120}	229a	266ab	37a
	N_{180}	230a	268a	38a
2013–2014	N_0	231a	273b	42a
	N_{60}	232a	274ab	42a
	N_{120}	233a	276ab	43a
	N_{180}	234a	277a	43a
2014–2015	N_0	224a	265a	41a
	N_{60}	224a	266a	42a
	N_{120}	225a	267a	42a
	N_{180}	226a	268a	43a
Average	N_0	236a	277b	41b
	N_{60}	236a	278ab	42ab
	N_{120}	237a	280ab	43ab
	N_{180}	238a	281a	44a

Different lowercase letters represent significant differences between treatments for the same growing season ($P \leq 0.05$).

in 2011–2012, 2013–2014 and 2014–2015 when compared with 2012–2013 ($P < 0.05$); (Table 2).

3.2. Effect of N rate on PSE, soil water profile and water balance

Precipitation storage efficiency (PSE) varied among N rates and growing seasons (Fig. 2) and was lower under N_0 than under the other N rates. On average, the N_{60}, N_{120} and N_{180} rates, significantly increased the PSE by 11.9%, 18.2% and 19.2%, respectively, when compared to N_0 treatment. Soil water recharge during the fallow period (ΔSWCf) was affected by N fertilization. Application of N-fertilizer resulted in greater water replenishment during the fallow period in all growing seasons (Table 3). Over the 4 years, the N_{60}, N_{120} and N_{180} rates increased soil water replenishment during the fallow period by 11 ($P > 0.05$), 24 and 25 mm ($P < 0.05$), respectively, when compared to non-N treatment (Table 3). On the contrary, N fertilization reduced evapotranspiration during the fallow period (ETf) when compared N_0 treatment (Table 3). The soil water storage at planting (SWCs) was not significantly different among the N treatments (Table 3).

At physiological maturity (GS92), the soil water storage was lower under all N rates than under the treatment without N (Table 3). Under the N_{60}, N_{120} and N_{180} rates, the soil water storage at harvest decreased by 16 ($P > 0.05$), 31 and 36 mm ($P < 0.05$), respectively, in 2011–2012 growing season; 41, 55 and 56 mm, respectively, in 2012–2013 growing season ($P < 0.05$); 33, 44 and 48 mm, respectively, in 2013–2014 growing season ($P < 0.05$); and 49, 74 and 89 mm, respectively, in 2014–2015 growing season ($P < 0.05$) when compared to non-N treatment (Table 3). Over the 4 years, the N_{60}, N_{120} and N_{180} rates increased water uptake by 11.6 ($P > 0.05$), 22.3 and 24.8 mm ($P < 0.05$), respectively, in the 0–80 cm layers of the soil profile; 15.9, 20.1 and 22.9 mm, respectively ($P < 0.05$), in the 80–140 cm soil layers and 7.5, 8.6 and 8.3 mm, respectively ($P > 0.05$), in the 140–200 cm soil layers when compared to the non-N treatments (data not shown). The interaction N rate × soil depth was statistical significant for soil water content ($P < 0.05$; data not shown). Thus, on average, the soil water storage at harvest decreased by 35, 51 and 56 mm under the N_{60}, N_{120} and N_{180} rates when compared to non-N treatment respectively ($P < 0.05$); (Table 3). The soil water recharge during the growing period (ΔSWCg) always increased with fertilizer N application (Table 3). Over the 4 years, the N_{60}, N_{120} and N_{180} rates increased significantly the ΔSWCg by 30, 55 and 48 mm when compared to non-N treatment, respectively (Table 3). The distribution of SWCg among different growing seasons was consistent with the seasonal rainfall (Table 3).

The annual soil water balance (ΔSWCy) varied among different years and N treatments (Table 3). The distribution of ΔSWCy among different growing seasons was not consistent with the annual precipitation in 2011–2013 growing seasons (Table 3), and soil water was in deficit in all treatments except for N_0 in 2012–2013 growing season, whereas in 2013–2015 growing seasons, soil water was in surplus for all treatments (Table 3). Application of N-fertilizer resulted in depletion of soil water, over the 4 growing seasons of the study, ΔSWCy was 10, −2, −8 and −11 mm under N_0, N_{60}, N_{120} and N_{180}, respectively (Table 3).

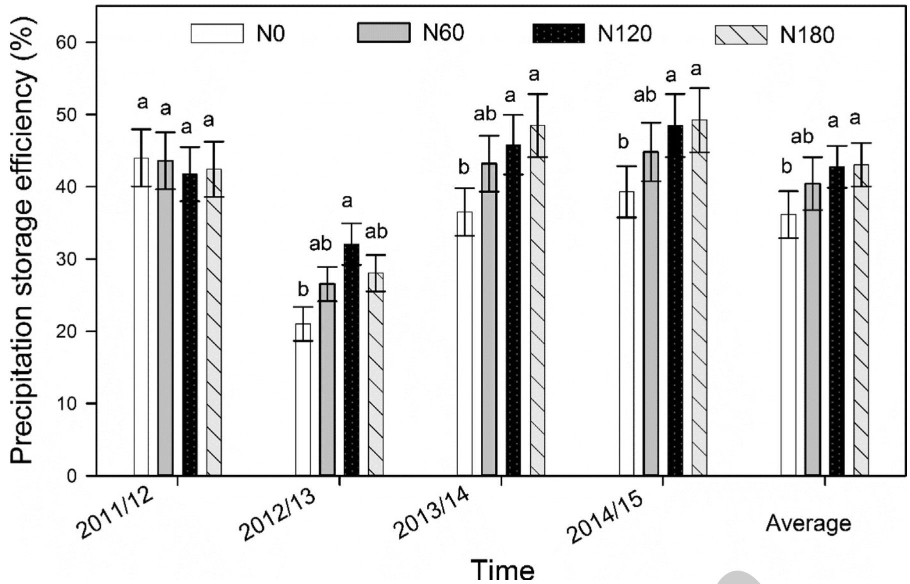

Fig. 2. Effects of 4 rates of N application (N_0, N_{60}, N_{120}, N_{180}) on precipitation storage efficiency (PSE) during the summer fallow. Data points are means ± standard deviations. Different lowercase letters represent significant differences between treatments for the same growing season ($P \le 0.05$).

Table 3
Components of the soil water balance in the 0–2 m under 4 rates of N application.

Growing seasons	N rates	SWCph	SWCs	SWCh	Fallow period		Growing period		ΔSWCy (mm)
	kg ha^{-1}	(mm)	(mm)	(mm)	ΔSWCf (mm)	ETf (mm)	ΔSWCg (mm)	ETg (mm)	
2011–2012	N_0	421	601a	382a	180a	198a	−219b	508b	−39a
	N_{60}	421	602a	366ab	181a	197a	−236b	525b	−55b
	N_{120}	421	600a	351ab	179a	199a	−249ab	538ab	−70c
	N_{180}	421	606a	346b	185a	193a	−260a	549a	−75c
2012–2013	N_0	382	481a	393a	99b	187ab	−88b	258b	11a
	N_{60}	366	451a	352b	85c	201a	−99b	268ab	−13c
	N_{120}	351	460a	338b	109a	177b	−105ab	275a	−13c
	N_{180}	346	449a	337b	104a	183b	−112a	282a	−9b
2013–2014	N_0	393	588a	423a	195c	197a	−165c	417c	30c
	N_{60}	352	570a	390b	218b	174b	−180b	432bc	38b
	N_{120}	338	583a	379b	245a	147c	−223a	475a	41a
	N_{180}	337	582a	375b	245a	147c	−207ab	459ab	38b
2014–2015	N_0	423	577a	462a	154b	238a	−115c	376c	39a
	N_{60}	390	578a	413b	188ab	204b	−165b	426b	23b
	N_{120}	379	570a	388c	191a	201b	−186ab	447ab	9c
	N_{180}	375	568a	378c	193a	199b	−190a	451a	3d
Average	N_0	–	562a	415a	157b	205a	−142c	385c	10a
	N_{60}	–	550a	380b	168ab	194ab	−172b	412b	−2b
	N_{120}	–	553a	364c	181a	182b	−197a	439a	−8c
	N_{180}	–	551a	359c	182a	181b	−190ab	433ab	−11d

SWCph is soil water content at previous harvest; SWCh is soil water content at harvest; SWCs is soil water content at sowing; ΔSWCf is the change in soil water content during the fallow period; ΔSWCg is the change in soil water content during the growing season; ΔSWCy is the change in soil water content during the whole year; ETf is the evapotranspiration during the fallow period; ETg is the evapotranspiration during the growing season. Different lowercase letters represent significant differences between treatments for the same growing season ($P \le 0.05$).

3.3. Effect of N rate on evapotranspiration

The evapotranspiration from sowing to the beginning of stem elongation (GS30) (ET1) was not significantly different among the N application rates in the 4 growing seasons of the study (Table 4). The evapotranspiration from the beginning of stem elongation (GS30) to flowering (GS60) (ET2), increased with the increase of N application rates (Table 4). From flowering to physiological maturity (GS92) evapotranspiration (ET3) also increased with the rate of N application (Table 4). Compared with N_0, the N_{60}, N_{120} and N_{180} rates increased ET2 by 2.3 ($P > 0.05$), 11.5 and 22.1 mm ($P < 0.05$), respectively, and ET3 by 19.9, 49.3 and 28.2 mm ($P < 0.05$), respectively. Soil water consumption varied with the growing season and, in 2011–2012 growing season (extremely wet growing season), was higher than that in other growing seasons because of higher precipitation in the summer fallow period and growing season ($P < 0.05$); (Table 4). In 2013–2014 growing season (wet growing season) and 2014–2015 growing season (normal growing season), ET2 was higher than in 2011–2013 growing seasons because of greater precipitation during the stem elongation and grain-filling stages ($P < 0.05$). Evapotranspiration during the growing season (ETg) was high in 2011–2012 growing season due to a wet growing season and low in 2012–2013 growing season as a result of a dry growing season (Table 4). For the 4 growing seasons of the study, the ETg increased significantly by 27, 54 and 48 mm for N_{60}, N_{120} and N_{180}, respectively, when compared with N_0 (Table 4).

Table 4
Evapotranspiration (ET) between sowing and the beginning of stem elongation (GS30), between GS30 and flowering (GS60) and between GS60 and physiological maturity (GS92) under 4 rates of N application.

Growing seasons	N rates	ET (mm)		
	kg ha^{-1}	(ET1) Sowing-GS30	(ET2) GS30-GS60	(ET3) GS60-GS92
2011–2012	N_0	252.9a	132.6c	122.1c
	N_{60}	246.8a	122.4c	155.7b
	N_{120}	232.3a	180.5b	176.1a
	N_{180}	241.2a	201.8a	149.8b
2012–2013	N_0	129.6b	86.6a	41.9a
	N_{60}	138.6a	84.7a	45.3a
	N_{120}	138.0b	85.2a	51.4a
	N_{180}	144.4ab	83.7a	54.1a
2013–2014	N_0	164.9a	176.9c	75.4b
	N_{60}	154.0a	210.6b	67.8b
	N_{120}	140.9a	212.0ab	122.2a
	N_{180}	154.9a	221.7a	82.3b
2014–2015	N_0	101.6b	173.4b	101.2c
	N_{60}	115.6ab	178.2b	132.1b
	N_{120}	126.9a	162.4b	157.4a
	N_{180}	112.8ab	202.5a	135.9ab
Average	N_0	163.9a	142.1b	78.5c
	N_{60}	168.8a	144.4b	98.4b
	N_{120}	157.6a	153.6ab	127.8a
	N_{180}	162.0a	164.2a	106.7b

Different lowercase letters represent significant differences between treatments for the same growing season ($P \le 0.05$).

Table 5
Effects of 4 rates of N application (N_0, N_{60}, N_{120}, N_{180}) on root length density (RLD) at anthesis in the upper 200 cm of the soil profile for each treatment in 2013–2014 and 2014–2015 growing seasons.

Growing seasons	Sampling location	N rates	Soil depth (cm)				
		kg ha^{-1}	0–20	20–40	40–80	80–140	140–200
2013–2014	RLD within the row	N_0	1.876b	0.916b	0.275c	0.211b	0.216a
		N_{60}	2.168ab	1.017a	0.314b	0.246a	0.220a
		N_{120}	2.486a	0.988ab	0.329ab	0.261a	0.225a
		N_{180}	2.394a	0.979ab	0.350a	0.271a	0.219a
	RLD between the rows	N_0	0.654d	0.382b	0.215c	0.118d	0.117a
		N_{60}	0.798c	0.564a	0.256b	0.132c	0.119a
		N_{120}	1.035b	0.597a	0.312a	0.230b	0.121a
		N_{180}	1.283a	0.566a	0.328a	0.288a	0.123a
2014–2015	RLD within the row	N_0	1.353d	0.940b	0.380c	0.228b	0.243a
		N_{60}	1.873c	0.873b	0.616ab	0.259b	0.235a
		N_{120}	2.100b	1.956a	0.605b	0.343a	0.254a
		N_{180}	2.679a	2.046a	0.685a	0.332a	0.256a
	RLD between the rows	N_0	0.971d	0.812b	0.480c	0.220c	0.206a
		N_{60}	1.432c	1.285a	0.647b	0.394b	0.209a
		N_{120}	1.687b	1.389a	0.803a	0.386a	0.208a
		N_{180}	2.014a	1.344a	0.790a	0.379a	0.211a
Average		N_0	1.347c	0.818b	0.334c	0.203c	0.207a
		N_{60}	1.719b	0.938b	0.461b	0.256b	0.206a
		N_{120}	1.982b	1.312a	0.497ab	0.304a	0.215a
		N_{180}	2.241a	1.327a	0.531a	0.312a	0.214a

Different lowercase letters represent significant differences between treatments at the same sampling location for the same growing season ($P \le 0.05$).

3.4. Effect of N rate on root growth

The root dry weight (RDW), average root diameter (RD) and root length density (RLD) were affected by N application (Fig. 3; Table 5). N_{60}, N_{120} and N_{180} treatments increased RDW by 22.0, 23.6 and 25.1%, respectively ($P < 0.05$), in 2013–2014 growing season and 10.9 ($P > 0.05$), 17.5 and 16.1% ($P < 0.05$), respectively, in 2013–2014 growing season when compared to N_0 (Fig. 3a). Application of N-fertilizer reduced RD in all growing seasons when compared to the non-N treatment ($P < 0.05$); (Fig. 3b). However, RDW and RD were not affected under N fertilization above 60 kg N ha^{-1} (Fig. 3). Compared to the non-N treatment, N_{60}, N_{120} and N_{180} treatments increased RLD in the 0–140 cm layers of the soil profile between the rows in each growing season of the study ($P < 0.05$); (Table 5). However, RLD within-row under 120 and 180 kg N ha^{-1} application was significantly increased in the 0–140 cm layers of the soil profile in both 2013–2014 and 2014–2015 growing seasons when compared N_0 (Table 5), but RLD within-row under 60 kg N ha^{-1} application was different only in 20–140 cm soil layers in 2013–2014 growing season and in 0–20 and 40–80 cm soil layers in 2014–2015 growing season, respectively, when compared N_0 (Table 5). RLD between the rows was higher in 2014–2015 growing season than that in 2013–2014 growing season ($P < 0.05$); (Table 5). The rate of N application affected the root distribution down the soil profile, the total root length in the 80–140 cm layers of the soil profile accounted for 15.0, 16.1, 17.2 and 16.8% of the total root length,

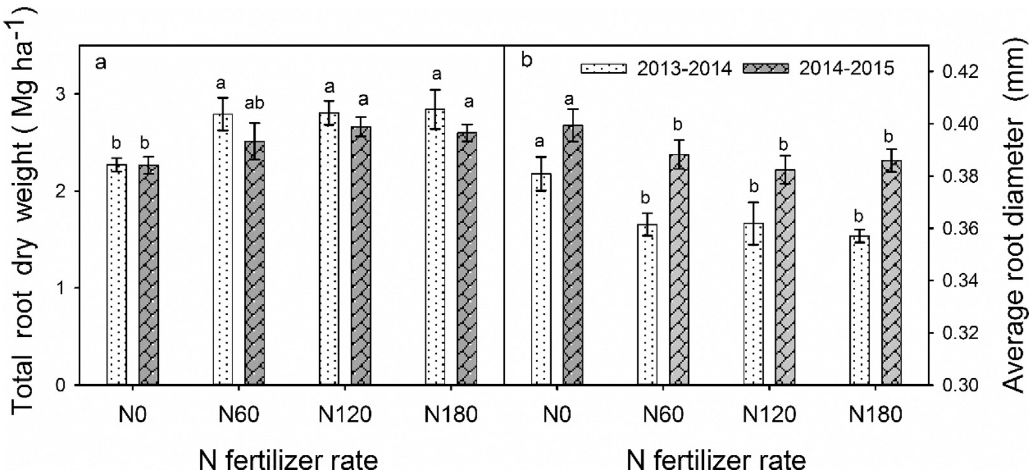

Fig. 3. Effects of 4 rates of N application (N_0, N_{60}, N_{120}, N_{180}) on root dry weight (a) and average root diameter (b). Data points are means ± standard deviations, different lowercase letters represent significant differences between treatments for the same growing season ($P \leq 0.05$).

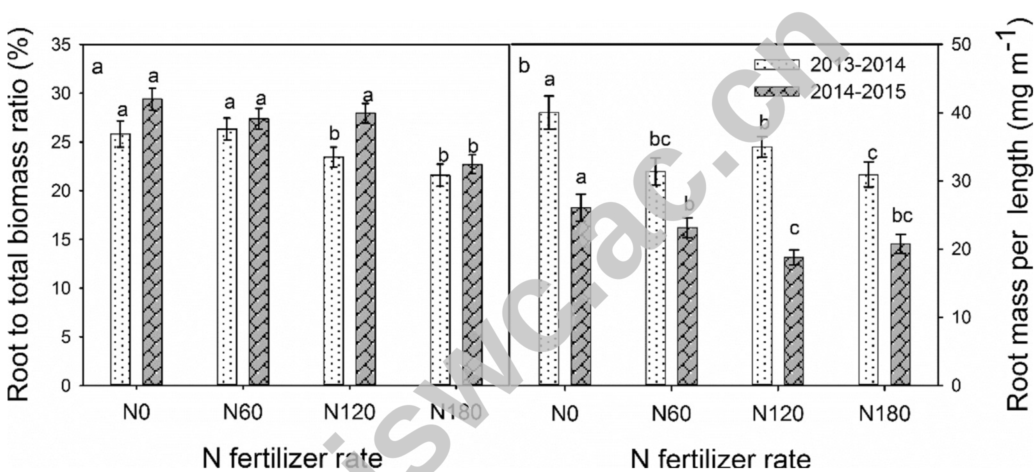

Fig. 4. Effects of 4 rates of N application (N_0, N_{60}, N_{120}, N_{180}) on the proportion of total biomass allocated to roots by anthesis (a) and root mass per unit of root length (b). Data points are means ± standard deviations, different lowercase letters represent significant differences between treatments for the same growing season ($P \leq 0.05$).

within of 0–200 cm of the soil profile, under N_0, N_{60}, N_{120} and N_{180}, respectively (data not shown). Similarly, the total root length in the 140–200 cm layers of the soil profile accounted for 10.1, 8.3, 7.7 and 7.5% of the total root length, within 2 m of the soil profile for N_0, N_{60}, N_{120} and N_{180}, respectively (data not shown). The interaction N application × soil depth was statistical significant for RLD ($P < 0.05$; data not shown).

Regardless root samples collected between rows or within a row, compared to the non-N treatment (N_0), on average, RLD in the 0–80 cm layers in the three N supplied treatments significantly increased by 20.8, 33.9 and 38.8%, respectively, by 20.7, 33.2 and 34.9% in the 80–140 cm layers, respectively, but there was no significant difference in RLD in the 140–200 cm soil layers among all N treatments (Table 5). RLD and RD varied with the growing season of the study (Table 5; Fig. 3a). In 2014–2015, the average wet growing season, RLD and RD were higher than in 2013–2014, a wetter growing season ($P < 0.05$). Although the RDW increased as the N rate increased (Fig. 3a), the changes in the proportion of root-to-total biomass and root mass per unit of root length (RML) were not consistent with the increase in RDW. Increases in the N application rate reduced the proportion of root-to-total biomass (allocation of total biomass to the root system) and RML in each growing season (Fig. 4a, b). The proportion of root-to-total biomass and RML varied with precipitation. In 2013–2014 growing season, the proportion of root-to-total biomass was lower than in 2014–2015 growing season (Fig. 4a). RML was lower in 2014–2015 growing season than in 2013–2014 growing season ($P < 0.05$); (Fig. 4b). Regardless of the season, the N_{60}, N_{120} and N_{180} rates reduced the RML by 16.4, 20.2 and 21.5%, respectively ($P < 0.05$); (Fig. 4b).

3.5. Effect of N application on biomass and remobilization of stored DM

Biomass accumulation varied with the growing season of the study. In 2011–2012 growing season, an extremely wet growing season, biomass accumulation increased with the increase in the N rate (Table 6). In 2012–2013 growing season, an extremely dry growing season, and in 2013–2014 growing season, an average wet growing season, biomass accumulation was not affected under N applications above 120 kg N ha^{-1} (Table 6). The increased N-fertilizer application markedly increased biomass accumulation (Table 6). The interaction between N rate and growing season for biomass accumulation was significant only at flowering (data not shown). For the 4 growing seasons, biomass accumulation at flowering (GS60) under N applications was increased by 19.3, 39.6 and 53.1%, respectively ($P < 0.05$); (Table 6). The changes in biomass accumulation were not corresponding with changes in evapotranspiration (Table 6). For instance, the evapotranspiration from July to March was approximately 57% of the total evapotranspiration ($ET_f + ET_g$), but biomass accumulation from sowing to the onset of

Table 6
Effect of 4 rates of N application on biomass accumulation at stem elongation (GS30), at flowering (GS60) and at physiological maturity (GS92), water-use efficiency for biomass yield at vegetative growth stage (WUEb′) and at grain-filling stage (WUEb″), pre-anthesis DM remobilization (DMR) and the contribution of pre-anthesis DM remobilization to grain (CDMR).

Growing seasons	N rates	Biomass ($g\,m^{-2}$)			DMR	CDMR	WUEb′	WUEb″
	$kg\,ha^{-1}$	GS30	GS60	GS92	$g\,m^{-2}$	%	$kg\,mm^{-1}\,ha^{-1}$	
2011–2012	N_0	203c	653d	1065c	211c	33.86a	16.94c	33.74ab
	N_{60}	278b	832c	1299c	231b	32.08a	22.54b	29.99bc
	N_{120}	303b	995b	1491b	278a	35.92a	24.10ab	28.17c
	N_{180}	378a	1182a	1737a	272a	32.88a	26.68a	37.05a
2012–2013	N_0	160d	503c	712c	114b	35.27c	23.27c	49.88a
	N_{60}	216c	619b	846b	106b	36.77bc	27.72b	50.11a
	N_{120}	271b	756a	961a	147a	41.70ab	33.87a	39.88b
	N_{180}	305a	770a	974a	164a	44.57a	33.76a	37.71b
2013–2014	N_0	197c	827c	1159b	171c	33.96b	24.20b	44.03b
	N_{60}	256b	901b	1245b	207b	39.77a	24.71b	50.74a
	N_{120}	308a	1062a	1534a	210b	36.45ab	30.09a	38.63b
	N_{180}	284ab	1135a	1562a	236a	35.62ab	30.14a	51.88a
2014–2015	N_0	147c	544c	599c	206bc	78.94a	19.78b	5.43d
	N_{60}	179b	666b	765b	227b	69.66a	22.67ab	7.49c
	N_{120}	218a	686b	889b	174c	46.11b	23.71a	12.90b
	N_{180}	217a	784a	1015a	315a	48.26b	24.87a	17.00a
Average	N_0	177c	632d	959b	175c	45.51a	20.65c	41.66a
	N_{60}	232b	754c	1055b	193bc	43.57ab	24.37b	30.59bc
	N_{120}	275a	882b	1219a	212b	37.55c	28.34a	26.37c
	N_{180}	296a	993a	1322a	247a	40.33b	30.44a	30.83b

Different lowercase letters represent significant differences between treatments for the same growing season ($P \leq 0.05$).

stem elongation was only 21% of the total biomass at final harvest (Table 6).

The remobilization of pre-anthesis stored DM (DMR) increased with increased the rate of N application (Table 6). DMR under N_{60}, N_{120} and N_{180} was greater than under N0 by 13.0, 9.9 and 26.7%, respectively ($P < 0.05$). The DMR was affected by seasonal rainfall. In 2011–2012 growing season (extremely wet growing season), the DMR were greater than in the other growing seasons ($P < 0.05$). The contribution of pre-anthesis stored DM to the grain (CDMR) was significantly affected by the N application rate, with the exception in 2011–2012 growing season (Table 6). Application of N-fertilizer increased CDMR in 2012–2014 growing seasons, whereas in 2014–2015 growing season, the CDMR reduced with N rate increased because of the hail damage at grain filling stage (Table 6). Pre-anthesis stored DM remobilization contributed up to approximately 37–45% of the final grain yield for the four growing seasons (Table 6).

3.6. *Effect of N application rates on yield, yield components and WUE*

Thousand-grain weight (TGW) was slightly reduced in N treatments regardless N rates applied than the non-N treatment ($P > 0.05$), but application of N did not affect HI ($P > 0.05$); (Table 7). The increase of grain yield owing the N application was mainly from the increase of ear number per m^2 and grain number per m^2 (Table 7). On average for the four-growing season, the ear number per m^2 under N_{60}, N_{120}, and N_{180} increased significantly by 16.4, 29.2 and 46.2%, respectively, whereas the grain number increased by 9.4 ($P > 0.05$), 32.6 and 41.3% ($P < 0.05$), respectively (Table 7). The grain yield significantly increased with increased fertilizer application in all four growing seasons (Table 7), particularly under N_{120} and N_{180}. Over the 4 growing seasons, N_{60}, N_{120} and N_{180} increased the yield of wheat by 12.8, 25.4 and 34.8%, respectively ($P < 0.05$). The increases in yield varied among growing seasons (Table 7). In 2012–2013 growing season (drier growing season), the yield of wheat increased by 3.0, 8.9 ($P > 0.05$) and 14.0% ($P < 0.05$) in treatments N_{60}, N_{120}, and N_{180}, respectively; however, in 2011–2012 and 2013–2014 growing seasons (wet growing seasons), the yields were higher by 12.2, 24.3 and 32.8%, and increased by 13.6, 27.7 and 31.9%, respectively ($P < 0.05$), compared with N_0 (Table 7). In 2014–2015 growing season (severe frost and rare hail damage), yields were higher than that for N_0 by 25.0, 44.3 and 70.9% for treatments N_{60}, N_{120}, and N_{180}, respectively ($P < 0.05$); (Table 7).

Under N_{60}, N_{120}, and N_{180} rates, WUE for biomass production during vegetative growth (WUEb′) was higher than in N_0 by 33.0, 42.3 and 57.5%, respectively, in 2011–2012 growing season ($P < 0.05$); 19.1, 45.6 and 45.1%, respectively, in 2012–2013 growing season ($P < 0.05$); 2.1 ($P > 0.05$), 24.4 and 24.6% ($P < 0.05$), respectively, in 2013–2014 season; and 14.6, 19.9 and 25.7%, respectively, in 2014–2015 growing season ($P < 0.05$); (Table 6). Over the 4 growing seasons, treatments N_{60}, N_{120} and N_{180} increased significantly WUEb′ by 16.6, 37.2 and 47.4%, respectively, compared with that in N_0 (Table 6). However, increased fertilizer application greatly decreased WUE for biomass production during the grain-filling (WUEb″) compared with that in N_0; on average, treatments N_{60}, N_{120} and N_{180} decreased WUEb″ by 26.6, 36.8 and 26.1%, respectively ($P < 0.05$); (Table 6).

WUE for grain yield (WUEg) varied with the N application rate and growing season (Table 7). In 2012–2013 growing season, WUEg was not significantly different among N application rates, but in the other three growing seasons, WUEg increased with increasing N application (Table 7). WUEg did not increase when applications of N were less than 60 $kg\,ha^{-1}$ ($P > 0.05$). WUEg increased over the 4 growing seasons under N_{120}, and N_{180} rates by 14.4 and 29.1% compared with N_0, respectively ($P < 0.05$); (Table 7).

3.7. *Effect of N application rates on soil nutrient contents*

120 and 180 $kg\,N\,ha^{-1}$ application significantly increased soil mineral N in 0–200 cm layers of the soil profile in each growing season of the study when compared to the non-N treatment (Table 8). Whereas 60 $kg\,N\,ha^{-1}$ application only significantly increased soil mineral N in 0–200 cm layers of the soil profile in 2013–2014 growing season and in 20–140 cm soil layers in 2014–2015 growing season, respectively (Table 8). Over the 4 years,

Table 7
Effect of 4 rates of N application on grain yield, yield components and water-use efficiency for grain yield (WUEg).

Growing seasons	N rates kg ha^{-1}	Ear no. m^{-2}	Grain no. $\times 10^4$ m^{-2}	TGW g	HI	Yield kg ha^{-1}	WUEg kg mm^{-1} ha^{-1}
2011–2012	N_0	439c	1.17c	53.13a	0.50a	6225c	12.26b
	N_{60}	454bc	1.32c	52.69a	0.50a	6984c	13.31ab
	N_{120}	504b	1.56b	49.66b	0.51a	7739ab	13.14ab
	N_{180}	625a	1.77a	46.65c	0.52a	8265a	13.94a
2012–2013	N_0	295c	0.88ab	39.95a	0.34a	3229b	12.51a
	N_{60}	342b	0.81b	39.75a	0.33a	3327ab	12.40a
	N_{120}	359b	0.89ab	39.71a	0.34a	3516ab	12.78a
	N_{180}	461a	0.95a	38.78a	0.35a	3680a	13.04a
2013–2014	N_0	460b	1.16b	43.20a	0.43b	5027c	12.05b
	N_{60}	582a	1.28b	44.45a	0.46a	5711b	13.21ab
	N_{120}	645a	1.78a	36.00b	0.43b	6417a	13.51ab
	N_{180}	642a	1.65a	40.20ab	0.42b	6632a	14.45a
2014–2015	N_0	316c	0.66d	39.57a	0.44a	2611d	6.94c
	N_{60}	377b	0.82c	39.71a	0.43a	3263c	7.67bc
	N_{120}	441a	0.91b	41.09a	0.43a	3767b	8.43b
	N_{180}	475a	1.11a	40.26a	0.44a	4461a	9.89a
Average	N_0	377c	9694b	43.96a	0.43a	4273c	11.29b
	N_{60}	439b	10609b	44.15a	0.43a	4821b	12.02b
	N_{120}	487b	12856a	41.62a	0.43a	5360a	12.35ab
	N_{180}	551a	13695a	41.47a	0.43a	5760a	13.24a

TGW is the thousand-grain weight; HI is the harvest index. Different lowercase letters represent significant differences between treatments for the same growing season ($P \le 0.05$).

Table 8
Effects of 4 rates of N application (N_0, N_{60}, N_{120}, N_{180}) on the soil mineral N (mg kg^{-1}), available P (mg kg^{-1}) and organic C (g kg^{-1}) in the upper 200 of the soil profile at sowing in 2011–2012 growing season and at harvest in 2013–2015 growing seasons.

Growing seasons	Soil nutrient	N rates	Soil depth (cm)				
		(kg ha^{-1})	0–20	20–40	40–80	80–140	140–200
2011–2012	Mineral N		19.38	18.9	24.19	27.49	23.63
	Available P		16.75	9.04	4.16	3.72	3.07
	Organic C		8.14	7.86	7.07	6.8	6.35
2013–2014	Mineral N	N_0	14.89d*	13.50d*	13.69d*	16.16c*	16.01c*
		N_{60}	18.57c	17.05c	17.82c*	19.24b*	19.64b*
		N_{120}	28.76b*	30.26b*	26.26b	26.03a	26.21a
		N_{180}	34.78a*	35.26a*	34.00a*	29.21a	28.39a*
	Available P	N_0	19.95a*	8.41a	4.72a	3.94a	3.42a
		N_{60}	17.67b	8.66a	4.40a	3.77a	3.22a
		N_{120}	16.77b	8.20a	4.56a	3.72a	3.21a
		N_{180}	16.48b	8.45a	4.54a	3.75a	3.12a
	Organic C	N_0	8.61a	7.92a	7.15a	6.80a	6.55a
		N_{60}	8.09ab	7.90a	7.26a	6.56a	6.45a
		N_{120}	7.85ab	7.75a	7.23a	6.53a	6.52a
		N_{180}	7.74b	7.25a	7.05a	6.55a	6.33a
2014–2015	Mineral N	N_0	15.62c*	13.27d*	13.89d*	13.27d*	14.17b*
		N_{60}	17.66c	16.26c	16.86c*	16.83c*	18.49b*
		N_{120}	24.02b*	26.85b*	27.16b	29.17b	26.25a
		N_{180}	29.34a*	31.40a*	38.50a*	37.81a*	30.15a*
	Available P	N_0	21.47a*	8.18a	4.13a	3.79a	3.36a
		N_{60}	17.75b	8.37a	4.17a	3.92a	3.45a
		N_{120}	18.86ab	8.41a	4.38a	3.94a	3.35a
		N_{180}	18.92ab	8.38a	3.99a	3.72a	3.20a
	Organic C	N_0	8.73a*	7.60a	7.01a	6.36a	6.33a
		N_{60}	8.01ab	7.43a	6.86a	6.41a	6.30a
		N_{120}	7.78b	7.23a	6.82a	6.79a	6.26a
		N_{180}	7.54b*	7.26a	6.83a	6.42a	6.30a

Different lowercase letters represent significant differences between treatments for the same soil layer each growing season ($P \le 0.05$).
* Indicated significant differences in soil nutrient contents between sowing in 2011 and harvest in 2014 and 2015 for the same soil layer ($P \le 0.05$).

120 and 180 kg N ha^{-1} application gradually increased soil mineral N in 0–200 cm soil layers compared to that in 2011, but 0 and 60 kg N ha^{-1} application slowly reduced soil mineral N compared to that in 2011. The N rate $\times$ soil depth interaction was significant for soil mineral N ($P < 0.05$; data not shown). Compared to the non-N treatment, application of N-fertilizer reduced soil available P content at 0–20 cm soil layer in each growing season, but there was no change in 20–200 cm layers of the soil profile (Table 8). The content of soil organic C at the 0–20 cm layer of the soil profile was lower under fertilizer-N applications than under non-applications of N. The soil organic C content in the 20–200 cm layers of the soil profile was not significantly different among the N application rates. With the increase in the rate of N application, soil organic C was reduced from 8.6 to 7.7 g kg^{-1} at the 0–20 cm soil layer

in 2013–2014 growing season and from 8.7 to 7.6 g kg^{-1} at the 0–20 cm soil layer in 2014–2015 growing season. The soil organic C content at the 0–20 cm soil layer under N_{180} and N_{120} was not significantly different with that in N_{60} (Table 8). Over the 4 years, non-applications of N-fertilizer treatment increased soil organic C content by 0.59 g kg^{-1} at 0–20 cm soil layer, but N_{60}, N_{120} and N_{180} treatments reduced soil organic C content by 0.13, 0.36 and 0.60 g kg^{-1} at 0–20 cm soil layer, respectively (Table 8).

4. Discussion

4.1. N applications affected root growth and water uptake

Application of N significantly enhanced root growth and increased RLD in deeper soil layers in wheat, one hand, mainly because of an increase in soil mineral N in deeper soil layers, one the other hand, likely because of a decrease in root mass per unit of root length and average root diameter (Liao et al., 2004, 2006; Russell, 1978). The high precipitation at the jointing stage in the 2013–2014 growing season, increased RDW and reduced RLD and RD compared to low rainfall growing season of 2014–2015, indicating that soil water available at the jointing stage increased RDW rather than root length. Similar responses have been reported for spring wheat in Canada (Read et al., 1982) and grasslands in Patagonia (Schulze et al., 1996). In contrast, low rainfall at the jointing stage increased root branching and lateral root extension, agreeing with findings in maize (Anderson, 1998). Application of N-fertilizer enhanced RLD in deeper layers of the soil profile (80–140 cm), which could be beneficial in environments prone to end-of-season drought or terminal drought (Palta and Watt, 2009; Palta et al., 2011) because roots at deeper layers of the soil profile are able to extract the deep available water (Kirkegaard et al., 2007; Manschadi et al., 2006). The activity of these roots was evidenced through the N applications of more soil water used by plants from deep layers than those in the non-N supply treatments.

4.2. N applications influenced biomass allocation

Application of N-fertilizer reduced the proportion of the total biomass allocated to the root system, as it has been shown in the studies of Ercoli et al. (2008) and Kamiji et al. (2014), presumably because the decreased RD and RML. A low RD and RML as a result of the N-fertilizer application would enhance RLD without more increasing root biomass. Thus, the water uptake and aboveground biomass significantly increased with the increase in the N rate. Accordingly, the proportion of the total biomass allocated to the root system was reduced by N-fertilizer application compared to N_0. In addition, a lower partitioning of assimilates to roots has been associated with a higher grain yield and WUE (Fang et al., 2010; Hu et al., 2015; Ma et al., 2010; Reynolds et al., 1994).

4.3. Effect of nitrogen rates on yield and WUE

The high biomass accumulation before anthesis under high N supply, improved grain yield, mainly because of the significant increase in ear number and grain number per m^2 (Ercoli et al., 2010; Estrada-Campuzano et al., 2012; Ferrante et al., 2012; Peltonen-Sainio et al., 2007; Sinclair and Jamieson, 2006). Application of N fertilizer in the season with high precipitation (2011–2012 and 2013–2015) prolonged the duration of grain filling likely because of a delay in senescence of the photosynthetic apparatus, which improved grain yield (Altenbach et al., 2003; Fuertes-Mendizábal et al., 2010). However, the duration of grain filling as short in the season with low precipitation (2012–2013), mainly because drought stress and high air temperature during grain filling paced plant senescence (Altenbach et al., 2003; Wiegand and Cuellar, 1981).

N applications had lower WUE for biomass yield during grain-filling, mainly because the greater biomass accumulation creates excessive transpiration and water loss from the crop canopy, which in turn causes severe water deficits during later critical crop development stage (Fan et al., 2005; Frederick and Camberato, 1995; Huang et al., 2003; Wang et al., 2011), ultimately reducing WUEb during grain-filling. However, N applications increased WUEb during vegetative growth stage, mainly because the large increase in biomass accumulation before anthesis reduced soil evaporation through increasing canopy cover and enhanced crop transpiration (Asseng et al., 2001; Kang et al., 2002; Turner, 2004; Zhang et al., 1998, 1999).

In cereals such as rice, maize and wheat, it is often accepted that a high HI under water-limited conditions is essential in obtaining a high yield and WUE (Kang et al., 2002; Perry and d'Antuono, 1989; Siddique et al., 1989). However, our results indicated that the improved yield and WUEg as a result of N fertilization arise mainly from a greater biomass accumulation rather than from a high HI. This is likely because HI in wheat is now approaching a plateau and further increases in grain yield will require an increase in biomass accumulation (Parry et al., 2010). Therefore, increased N fertilization did not increase the contribution of pre-anthesis stored dry matter to the grain, although the DMR increased as the N rate increased. These results of this study highlight that the application of N increased dryland winter wheat WUEg through the more efficient utilization of the available soil water during vegetative growth stage.

4.4. Effect of N application rates on PSE and soil water balance

In this study, the PSE fluctuation was dependent on the summer rainfall and the average PSE less than 43% is common for the region, in which a conventional wheat-summer fallow system varies from 12 to 36% in different years (Jin et al., 2007). Rainfall during the fallow period of the study was abundant, but a high evaporation loss from the soil surface failed to store enough water for the spring growth and during grain filling. Therefore, the high evaporation during the fallow period and through the winter, severely limited the yield and WUE. The PSE and ΔSWCf increased with an increase in N fertilizer rate, presumably as a result of a high soil reservoir available to store precipitation during the fallow period. It is likely that this high soil reservoir resulted from the high soil water uptake by the succeeding wheat (Wang et al., 2011).

Since ETg during the winter wheat growing season on the Loess Plateau of China, is much higher than the rainfall (Chen et al., 2015b; Huang et al., 2003; Jin et al., 2007; Zhang et al., 2011). The high levels of N applications (i.e. N_{120} and N_{180}) increased water uptake and resulted in severe soil water depletion during the growing season ($\Delta SWC_y < 0$), mainly because the large increase in RLD under N_{120} and N_{180} treatments significantly increased water uptake in deeper layers of the soil profile. This suggests that wheat grown under increased N fertilization depleted more soil water to supply high demand and to generate high yield. It is likely that in the long term, high N fertilizer supply might not be sufficient to maintain high yields (Fan et al., 2005; Huang et al., 2003; Liu et al., 2013). The high rates of N supply, N_{120} and N_{180}, reduced soil organic C content at 0–20 cm, likely because the slow straw rotting failed to increase the organic matter of soil due to the drying soil environments in the Loess Plateau (Sun et al., 2013). In addition, application of N could significantly increase soil respiration (Bowden et al., 2004; Burton et al., 2002; Pregitzer et al., 2000), leading to decrease in soil C content (Cao and Woodward, 1998; Ding et al., 2007). However, Wang et al. (2013) found that increased N fertilization can be sufficient to maintain high yields and WUEg due to increasing soil organic matter and water stored compared to no N fertilization. This discrepancy may be associated to differences in planting densities, since some studies have been conducted under low planting density (80 kg ha^{-1}) and with yield variation from 1.2 to 4.2 Mg ha^{-1} (Wang et al., 2013). Under these conditions, depletion of soil water and loss of soil organic C could not be clearly observed. Our results suggest that when grain yield was higher than 4.2 Mg ha^{-1}, increases in N fertilization resulted in adverse effects that included the depletion of soil water and loss of soil organic C, which approves our second hypothesis. However, we suggest that N fertilization can improve high yields and WUEg by facilitating the more efficient use of soil water.

5. Conclusions

The increase in root branching and RLD in deeper layers of the soil profile with the increases of N applications improved water uptake efficiency despite the reduction in root-shoot ratio. Aboveground biomass accumulation at anthesis was positively associated with the N application rates, water-use efficiency during vegetative growth stage and grain yield, respectively. However, the increases in the PSE and root biomass did not prevent the depletion of soil water and loss of soil organic C that resulted from increased fertilizer application. N fertilization improved dryland winter wheat yield and WUEg by increasing deep root growth and water use during vegetative other than grain filling. It is notable that N fertilization may result in depletion of soil water and loss of soil organic C, and thus in the long term, winter wheat monoculture with increased N fertilization might not be sufficient to maintain high yields and WUEg.

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