Nitrogen fertilization affects maize grain yield through regulating nitrogen uptake, radiation and water use efficiency, photosynthesis and root distribution

7 Wennan Su [1,2,3], Shakeel Ahmad[2,3], Irshad Ahmad[2,3] , and Qingfang Han [1,2,3]*

9 1College of Agronomy, Northwest Agriculture and Forestry University

10 2Key Laboratory of Crop Physio-ecology and Tillage Science in North-western Loess Plateau, Ministry of Agriculture / College of Agronomy,

11 Northwest Agriculture and Forestry University

12 3Key Laboratory of Agricultural Soil and Water Engineering in Arid and Semi-arid Areas, Ministry of Education / Institute of Water Saving

13 Agriculture in Arid Areas of China, Northwest Agriculture and Forestry University

14 Corresponding Author:

15 Qingfang Han[1, 2, 3]

16 Yangling, Shaanxi, China

17 Email address: 1481860234@qq.com

Abstract

High external nitrogen (N) inputs can maximize maize yield but can cause a subsequent reduction in N use efficiency (NUE). Thus, it is necessary to identify the minimum effective N fertilizer input that does not affect maize grain yield (GY) and to investigate the photosynthetic and root system consequences of this optimal dose. We conducted a 4-year field experiment from 2014 to 2017 with four N application rates: 300 (N_{300}), 225 (N_{225}), 150 (N_{150}), and 0 kg ha^{-1} (N_0) in the Northwest of China. GY was assessed by measuring the photosynthetic capacity and root system (root volume, surface area, length density and distribution). Grain yield decreased by -3%, 7.7%, and 21.9% when the N application rates decreased by 25%, 50%, and 100% from 300 kg ha^{-1}. We found that yield reduction driven by N reduction was primarily due to decreased radiation use efficiency (RUE) and WUE instead of intercepted photosynthetically active radiation and evapotranspiration. In the N_{225} treatment, GY, WUE, and RUE were not significantly reduced, or in some cases, were greater than those of the N_{300} treatment. This pattern was also observed with relevant photosynthetic and root attributes (i.e., high net photosynthetic rate, stomatal conductance, and root weight, as well as deep root distribution). Our results suggest that application of N at 225 kg ha^{-1} can increased yield by improving the RUE, WUE, and NUE in semi-arid regions.

Introduction

In the past four decades, global maize production has greatly increased (FAO, 2018) mainly due to application of nitrogen (N) fertilizers. Worldwide, N fertilizer has widely been excessively applied to achieve higher grain yields (Cui et al., 2009; Meng et al., 2016; Liang et al., 2020). For example, the average dose of N fertilizer applied by the farmers was greater than 300 kg ha^{-1} (288±113kg ha^{-1}), which far exceeds the optimal N rates for maize demonstrated in field experiments (Zhang et al., 2015a; Chang et al., 2014; Yang et al., 2017). N fertilizer was applied in excess (350−600 kg ha^{-1} year^{-1}, Mueller et al., 2013) in an attempt to maximize yields in the North China Plain. However, excessive application of N fertilizer has negative effects on crops, greatly reduces N use efficiency (NUE), and causes significant nitrate leaching losses (more than 50% N to the environment) and contamination of groundwater (Erisman et al., 2013; Wang et al.,2014; McBratney and Field, 2015; Ahmad et al., 2018; Suchy et al., 2018; Wang et al., 2019). Reducing N input rates from this level to "moderate" levels in maize fields may improve NUE, maintain a fair level of maize grain yield (Zhao et al. 2010; Dai et al., 2015, Qiang et al., 2019), and display less negative environmental impacts. In order to implement reduced N input rates, it is necessary to assess the extent to which the N fertilizer application rate is consistent with crop N requirements to maximize resource utilization and maintain relatively high grain yields (Robertson and Vitousek, 2009; Zhang et al., 2015b).

Radiation interception and radiation use efficiency (RUE) form the basic framework for analyzing crop yield constraints. Variations in crop biomass due to abiotic factors may be attributed to intercepted photosynthetically active radiation (IPAR), RUE, or the combination of both IPAR and RUE (Fletcher et al., 2013). Reduced leaf area under low-N conditions is accompanied by a reduction in radiation interception (Massignam et al., 2012). Low-N

58   conditions primarily decrease the photosynthetic rate per unit area (Vos, Van Der Putten and
59   Birch, 2005), indicating that a low N effect on both leaf size and photosynthetic capacity may
60   affect the final grain yield. Understanding how maize production and resource use are affected
61   by varying N application rates will inform improvement of N fertilizer management to achieve
62   optimal grain yield and resource use efficiency. The appropriate amount of N fertilizer input can
63   improve utilization of precipitation during the crop season (Dai et al., 2015; Herrera et al., 2016;
64   Yang et al., 2017; Qiang et al., 2019). Previous studies of the effect of N fertigation on grain
65   yield and resource use efficiency primarily describe the effects of single resource utilization at
66   the leaf or plant level (Brown, Jamieson and Moot, 2012). A preceding study described the
67   relationship between maize water use efficiency (WUE) and NUE in pot conditions (Wang et al.,
68   2019). However, few comprehensive studies on the effects of N fertilizer on the utilization of
69   radiation, water, and N resources in field crops have been performed.
70   Enhancing photosynthesis is critical to promoting crop yield (Mu et al., 2017). In order to
71   understand the underlying mechanisms and differences in photosynthetic capacity, it is necessary
72   to determine the relevant photosynthetic parameters of ear leaves (Li et al., 2013; Zhang et al.,
73   2017; Lamptey et al., 2017). Root morphology and distribution also play key roles in the
74   acquisition of soil resources such as nutrients and water (Ristova and Busch, 2014; Lynch, 2013;
75   Yu et al., 2014). Mi et al. (2010) proposed the ideotype root architecture of "high yield and high
76   N efficiency" in maize, which provided references for root research. In field conditions, the
77   complexity of root sampling has limited efforts to understand the effects of N on roots to shoots.
78   Such research has been conducted under pot conditions (Wang et al., 2019); thus, it is not
79   possible to assess variation due to differences in light and temperature conditions as well as
80   nitrate N leaching, as occurs in field conditions. Limited knowledge about shoot and root traits
81   related to maize grain yield, NUE, RUE, and WUE under field conditions was investigated.
82   Therefore, exploring the response of photosynthetic parameters and root development to grain
83   yield reduction due to N reduction will provide an important reference for management of N
84   fertilizer inputs.
85   In the current study, we determined that N reduction results in maintenance of photosynthetic
86   activity and root development to maximize grain yield and radiation, water, and N use by maize
87   crops. The objectives of this study were to 1) investigate the effects of N reduction on water,
88   radiation, and N use efficiencies in maize crops, and 2) determine the effects of N reduction on
89   photosynthetic activity and root development in maize crops under semi-arid conditions. Our
90   findings provide key data to support enhanced maize production and resource use efficiency.

91 Materials and Methods

92 Field experiments

93   A 4-year field experiment was conducted on the same land at the agricultural experimental
94   station of Northwest A&F University in Yangling (34°20′N, 108°04′E, elevation, 455 m),
95   Shaanxi province, China in 2014, 2015, 2016, and 2017. The experimental site experienced an
96   annual average daylight of 2150 h, an annual average temperature of 12°C to 14°C, and an
97   average annual precipitation of 581 mm. The annual mean temperature and total precipitation of

98 the experimental area are shown in Fig. 1. The soil of the experimental site is classified as a dark
99 loess soil, and the former crop was winter wheat. Before sowing, soil chemical properties were
100 analyzed in the top 60 cm of soil for organic matter content, N, phosphorus (P), and potassium
101 (K) (Table 1).
102   The research work was carried out with a randomized complete block design with three
103 replications. The total sub plot size was 39 m^2 (7.8 m long and 5 m wide). The row-to-row
104 spacing was maintained at 60 cm and plant-to-plant spacing at 25 cm. The maize seeds were
105 planted manually, and in each hill, two seeds were sown at a depth of 5 cm in the middle of June
106 during each growing season. Plants were thinned manually for normal plant population densities
107 in the area of (67,500 pl. ha) at V3 (three-leaf stage) (Ritchie and Hanway, 1982). Plots were
108 kept free of weeds, insects, and diseases; and the weeding was controlled by hand and hoe during
109 each growing season. The N treatments applied in this study were: 1) no N (N_0, 100% reduction
110 from N_{300}); 2) 150 kg N ha^{-1} (N_{150}, 50% reduction from N_{300}); 3) 225 kg N ha^{-1} (N_{225}, 25%
111 reduction from N_{300}); and 4) 300 kg N ha^{-1} ($N_{300,}$ the traditional N dose applied by farmers in the
112 Loess Plateau of China). Fertilizer N was sourced from urea (46% N), evenly split in the
113 fractions of 1/2 at pre-sowing and side-banded deep (5 cm) into the soil on the sowing rows of
114 1/2 at the twelve-leaf stage. A total of 150 kg of phosphorus (calcium superphosphate, P_2O_5
115 16%) ha^{-1} and 150 kg of potassium (as potassium sulfate, K_2O 45%) ha^{-1} were applied 5 days
116 before sowing. Irrigation was applied at the twelve-leaf stage (75mm). The amount of irrigation
117 was controlled by a water meter (Zhejiang Ningbo Water Meter Co., Ltd.).

118 ## Sampling and measurements

119 ## Intercepted Photosynthetically Active Radiation (IPAR)

120 During the V6–R3, IPAR was measured every 5–7 days (on clear and sunny days between 11:00
121 AM to 2:00 PM) using a SunScan Canopy Analyzer (Delta, UK). In each plot, three points were
122 chosen. When measuring, the line sensor was placed horizontally between the two ridges (5 cm
123 from the soil surface) and was used to collect three consecutive readings of transmitted
124 photosynthetically active radiation (Chen et al., 2016). Round-trip observations were used to
125 minimize error.

126 ## Soil Water Content

127 At sowing and maturity, soil water content from the 0–200 cm layer was determined using a
128 hand-held soil iron drill (Zhang et al., 2019). Between 0 and 20 cm, samples were collected
129 every 10 cm, and between 20 and 200 cm, samples were collected every 20 cm. Soil samples
130 were stored in a closed aluminum box and weighed before drying, oven-dried at 105°C for 24 h,
131 and weighed separately. The soil water content of each plot was calculated from the average of
132 three random soil core samples. The soil water content was calculated as the difference between
133 the fresh soil weight minus the dry soil weight divided by the dry soil weight.

134 ## Photosynthetic Parameters and Leaf Area Index (LAI)

135 In 2016 and 2017, 20 plants were marked prior to the eight-leaf stage (V8). At V8, the tasseling
136 stage (VT), milking stage (R3), and physiological maturity (R6), the three marked plants per plot
137 were selected to measure the net photosynthetic rate (Pn), intercellular CO_2 concentration (Ci),

138   and stomatal conductance (Gs) on the ear leaves (at VT, R3, and R6) or fully expanded leaves at
139   the top of a plant (at V8) using a photosynthesis analyzer system (LI-6400, LI-COR, Lincoln,
140   Nebraska, USA) on a clear sunny day between 9:00 AM to 11:00 AM (Zhang et al., 2017). The
141   same plants were used for measuring the green leaf area at V8, VT, R3, and R6. The green leaf
142   area index (LAI) was calculated as follows (Birch, Vos and Van Der Putten, 2003):
143   LAI=0.75×Leaf length ×maximum width ×number of plants within a unit area of land /area of
144   land

Root System

146   Three plants root were sampled using the soil profile method (Holanda et al., 1998) at the V8,
147   VT, R3, and R6 stages of maize. Each root system was excavated from an area of 0.15 m^2 (line
148   spacing 0.6 m× row spacing 0.25 m) soil around the center of the plant. Root sampling was
149   conducted at depth intervals of 0–30 cm (surface soil layer), 30–60 cm (middle soil layer), and
150   60–90 cm (deep soil layer) in each plot. Excavated roots were immersed overnight in a plastic
151   container filled with water and washed with tap water on a 0.25-mm screen until the roots were
152   free of soil. Roots were scanned using an HP Scanjet 8200 scanner, and each root image was
153   analyzed using a root analysis program (Regent Instruments Inc. WinRhizo Provision 5.0,
154   Canada) to obtain the root surface area (cm^2 plant^{-1}) and root length (mm). Root volume was
155   measured by the drainage method. Root length density was calculated as the average of three
156   plants' root lengths divided by the soil volume (Li et al., 2010). Root samples were dried for 48h
157   at 70°C in an oven to obtain the root dry weight per plant.

Biomass Yield, Shoot N Content, and Grain Yield

159   Four central rows were harvested randomly at 20 m^2 to measure the grain yield at harvest in each
160   plot. Ten ears were randomly selected from each plot and threshed separately to determine
161   moisture content and kernel number. Grain yield was estimated based on kernel weight and
162   water content and expressed as 14% (w/w) moisture content. Six plants were sampled at each
163   plot and were divided into leaves, stems, and grains. Before determining the N concentration, all
164   plant parts were dried at 70°C for 48 h and weighed. After weighing, the samples were ground
165   into powder using a willey-type mill (<1 mm mesh), weighed (0.3–0.4 g), and were mineralized
166   using H_2SO_4–H_2O_2; then, total N concentration was obtained by using an automatic Kjeldhal
167   microdistillation analyzer (FOSS, Sweden, Nelson and Sommers, 1973).

Statistical analysis

169   Daily intercepted solar radiation was calculated using the following equation (Liu et al., 2014):

170    $$LT = \frac{PAR_L}{PAR_T}$$ (1)

171   Where LT is the light transmission ratio, PAR_L is the intercepted photosynthetically active
172   radiation at the bottom of the canopy, and PAR_T is the intercepted photosynthetically active
173   radiation at the top of the canopy.

174   The measured intercepted photosynthetically active radiation value of the bottom layer was
175   analyzed by two-dimensional interpolation over 1 day to obtain the intercepted
176   photosynthetically active radiation of the entire canopy. Then, the intercepted photosynthetically
177   active radiation rate obtained by interpolation analysis is multiplied by the incident PAR
178   measured on the corresponding date by the meteorological observatory to determine the amount
179   of canopy IPAR.
180   Radiation use efficiency (RUE) was calculated using the following equation:

181   $RUE = \frac{Mh}{\sum Q} \times 10^{-7} \times 100\%$ (2)

182   Where $\sum Q$ (MJ m^{-2}) is the accumulated intercepted solar radiation, h (kJ kg^{-1}) is the heat energy
183   released of per kg of grain yield, and M is the grain yield (kg ha^{-1}).
184   Water use efficiency (WUE) was calculated as:

185   $WUE = \frac{GY}{ET}$ (3)

186   Where GY (kg ha^{-1}) is the grain yield, and ET (mm) is the evapotranspiration, as calculated as
187   by soil water balance equation (Huang et al., 2005).
188   The following equations were also used:

189   Internal N efficiency, $INE = \frac{GY}{SNC}$ (4)

190   Agronomic N use efficiency, $ANE = \frac{(GY_{Ni} - GY_{N0})}{Ni}$ (5)

191   Apparent N recovery efficiency, $REN\ (\%) = \frac{(SNC_{Ni} - SNC_{N0})}{Ni} \times 100$ (6)

192   N harvest index, $NHI\ (\%) = \frac{GNC}{SNC} \times 100$ (7)

193   where SNC (kg ha^{-1}) is the shoot N content calculated as biomass (kg ha^{-1}) multiplied by shoot
194   N concentration (kg kg^{-1}), i (N rates, kg ha^{-1}) is N application rates 150, 225, or 300, GY_{Ni} (kg
195   ha^{-1}) is grain yield in the N application plots, GY_{N0} (kg ha^{-1}) is grain yield in the no-N
196   application plots, and Ni is the N application rate. SNC_{Ni} (kg ha^{-1}) is the shoot N content in N
197   application plots, SNC_{N0} (kg ha^{-1}) is the shoot N content in the no-N application plots. GNC is
198   grain N content (kg ha^{-1}).
199   The experimental data were organized and processed using Microsoft and are presented
200   with standard error. SPSS18.0 (SPSS Institute Inc.) statistical analysis software was used for
201   variance analysis. The data was checked for normality (Kolmogorov–Smirnov test) and
202   homogeneity of variance (Bartlett–Box test). The effects of N rates, years, and their interactions
203   on the measured variables were tested using one- and two-way ANOVAs. To identify significant
204   treatments effects, multiple comparisons among different treatments were performed using
205   Duncan's multiple range test. Differences with $P < 0.05$ were considered statistically significant.

206 **Results**

207 **Grain yield, Biomass Yield, and Crop Resource Utilization**

208   Our results revealed that the year and N application rates showed significant effects on grain
209   yield (GY), biomass yield (BY), grain N content (GNC), agronomic N use efficiency (ANE),

210 apparent N recovery efficiency (REN), and N harvest index (NHI) (Table 2). The interaction
211 between N application rate and year had no significant effect on the above parameters. We
212 observed no significant differences in GY, BY, and GNC between N_{225} and N_{300} in all growing
213 seasons. Compared with N_{300}, the GY of N_{150} and N_0 decreased ranging from 4.7% to 13.6% and
214 19.7% to 22.8%, respectively, while the grain yield of treatment N_{225} increased ranging from
215 1.5% to 3.7% averaged of four years. Treatment N_{300} increased biomass yield ranging from 28%
216 to 34%, compared with N_0, while N_{225} increased biomass yield ranging from 27% to 32% in the
217 four years. The GY difference among years may be due to the rainfall amount and seasonal
218 distribution. Rainfall was 390 mm, 284 mm, 311 mm, and 371 mm in 2014, 2015, 2016, and
219 2017, respectively (Fig. 1). Among the experimental years, GY was higher in 2016 and 2017
220 compared to 2014 and 2015. 2017 was a more suitable year for maize growth. Although the
221 rainfall in 2016 was reduced, the distribution was relatively uniform throughout the growth
222 period. The early rainfall ensured the regularity and vegetative growth of maize seedlings. The
223 lower GY in 2015 can be explained by the reduced rainfall. In 2014, the greater rainfall was
224 mainly due to the large amount of rainfall occurring 70 days after sowing. Continuous rainfall
225 from the silking to the flowering stage affected maize pollination, and severe stalk rot disease
226 occurred during the grain filling stage, which caused pro-senescence (Fig. 1), causing lower GY.
227 The optimal rate of N application increased the AEN, REN, and NHI, except for the AEN of N_{150}
228 during the 2016 growing season. The NHI was highest for the N_{225} and N_{150} (2014, 2015, and
229 2017) or N_{225} (2016) treatments.
230   Throughout the crop growth cycle, differences in ET among N treatments were significant
231 ($P<0.05$, Table 3). During growing seasons 2015, 2016, and 2017, we observed that the rate of N
232 application showed no significant effect on IPAR. The IPAR of N_0 was less than that of N_{300} in
233 the 2014 growing season. SNC was increased by increasing the N application rate. For N_{300},
234 SNC increased ranging from 73% to 92% compared with N_0, and N_{225} application increased
235 SNC ranging from 68% to 81% compared with the N_0 treatment in the four years. IEN was
236 increased with a low rate N application. Application of N at 225 kg ha^{-1} increased IEN ranging
237 from 8.5% to 12.3% compared with N_{300}, while N_{150} decreased IEN ranging from 9.7% to 20.7%
238 compared with N_{300} in the four years. N application at the N_{300} and N_{225} rates were similar, while
239 N_{300} increased RUE ranging from 19% to 26%, whereas N_{225} increased RUE ranging from 23%
240 to 29% compared with N_0 in the four years. Reduced N application was associated with reduced
241 WUE. Our results showed that N_{225} increased grain WUE ranging from 25% to 26% compared to
242 N_0 in the four years. WUE was also significantly increased in the N_{300} treatment but to a lesser
243 extent (19%–22%). RUE and NUE exhibited non-linear responses to the N application rate,
244 indicating that the maximum grain yield can occur with N_{150} or N_{225} treatments. The differential
245 grain yield between N_{225} and N_{300} was relatively small.

Photosynthetic parameters and LAI

247 N application rates and year had significant effects on Pn, Gs, and Ci, but the interaction between
248 N application rates and year did not display significant correlated (Fig. 2). In both 2016 and
249 2017, the N_0 and N_{150} treatments resulted in lower values of Pn and Gs when compared with N_{225}

250   and N_{300}; however, the effect of N_{225} was relatively greater than that of the N_{300} treatment at R6.

251   This finding indicates that N fertilizer inputs can increase Gs and improve the photosynthetic

252   capacity of maize crops. Conversely, the Pn and Gs of the N_{300} treatment were reduced compared

253   with N_{225}. Pn of N_{150}, N_{225}, and N_{300} increased ranging from 16% to 81% across growth stages

254   compared with N_0 averaged of two years. N application rates significantly affected 1-Ci/Ca ($P <$

255   0.05). The 1-Ci/Ca of the N_0 treatment was significantly lower than that of N_{300} in all

256   measurements (Fig. 2).

257   Overall, the leaf area index (LAI) was unaffected ($P = 0.55$) by N application rates, with an

258   average of 2.6 and 3.7 at the V8 and VT stages (Fig. 2). N application rates significantly affected

259   LAI at the R3 and R6 stages. Compared with N_{300}, the LAI of N_{225}, N_{150} and N_0 decreased by

260   1%, 6%, 11%, respectively, and 1%, 7%, and 13%, respectively, at the R3 and R6 stages.

261 **Root System**

262   The root dry weight reached a maximum value with the N_{225} treatment. Root dry weight of N_{225}

263   increased ranging from 35% to 67% compared with N_0, while N_{300} increased the root dry weight

264   ranging from 29% to 53% compared with N_0 average of two years (Table 4). We observed clear

265   differences in the vertical distributions of roots as well as root morphology between N

266   application rates, including root volume, root surface area, and root length density, which varied

267   with N application rates and years (Table 5). After the VT stage, the root system distributed in

268   the surface layer (0–30 cm) accounted for greater than 88 % of the root weight for all treatments.

269   At the V8 stage, the root ratio was proportional to the N application rate and increased

270   significantly in the middle layer (30–60 cm). In the N_0 treatment, almost all roots were

271   concentrated in the surface soil layer. The N_0 treatment exhibited significant increases in the

272   deep-layer (60–90cm) root ratio at the VT stage. The root ratio in the deep soil layer decreased

273   with increasing N application after the VT stage. At the R3 and R6 stages, the surface root ratio

274   increased with increasing N application rate. For the N_0 treatment, the deep-layer root ratio

275   decreased after the VT stage. Similar to the dry root weight, root morphology indexes (root

276   volume, surface area, and length density) increased initially and then decreased with increasing

277   N application rate. These indexes reached their maximum values under the N_{225} treatment. At the

278   V8 stage, root morphology indexes were in the order of $N_{225} > N_{300} > N_{150} > N_0$ in the surface

279   soil layer, which differed from the middle soil layer ($N_{300} > N_{225} > N_{150} > N_0$). Larger roots

280   appeared in deep soil layers at the VT stage when compared to the V8 stage, and the maximum

281   value (root volume, surface area, and length density) for the N_0 treatment was observed in the

282   middle or deep soil layers. At the R3 and R6 stages, trends in root morphology indexes varied

283   between soil layers; in the surface layer, the indexes followed the order $N_{225} > N_{300} > N_{150} > N_0$;

284   in the middle layer, $N_{225} > N_{150} > N_{300} > N_0$; and in the deep layer, $N_{225} > N_{150} > N_0 > N_{300}$.

285 **Discussion**

286   An optimal rate of N application is expected to produce a balance between crop demand and N

287   supply and ensure maximum crop production while conserving resources and protecting against

288   environmental damage (Ciampitti and Vyn, 2011; Peng, Li and Fritschi, 2014). Our results

289   showed that during the 2017 growing season, even when photosynthesis was significantly

290   affected by a 50% reduction in N application (N_{150}), grain yield was not significantly decreased.
291   In 2016, photosynthetic parameters decreased further for the N_{150} and N_0 treatments, and the
292   grain yield also decreased significantly. Over 4 years, we observed no significant reduction in
293   grain yield for the N_{225} treatment (Table 2), which even displayed a grain yield higher than that
294   of the N_{300} treatment, although the difference was not significant. Our research indicated that the
295   N dose could be reduced by at least 25% without compromising grain production. Our results are
296   similar to those of Li et al. (2007) and Lamptey et al. (2017), who reported an optimal N
297   fertilization range for summer maize of 200 to 300 kg N ha^{-1}. Under the N_0 and N_{150} treatments,
298   dry matter accumulation was limited, and the difference between N_{300} and N_{225} treatments was
299   not significant. The reduction in crop yield induced by N reduction can be explained by various
300   factors, as described below.
301   Our experiments also addressed the mechanism by which maize yield is affected by N
302   application rates. We found that for most years, IPAR was not affected by N fertilizer application
303   rates, a finding similar to that described in previous studies (Vos, Van Der Putten and Birch,
304   2005; Massignam et al., 2012). N application rate significantly affected RUE ($P<0.05$). For N_0
305   and N_{150}, the RUE was significantly less than that of N_{300}. However, the RUE of the N_{225}-treated
306   crop was greater than that of the N_{300}-treated crop. In this case, lower grain yield driven by lower
307   N treatment corresponded to lower RUE. These results indicate that under such production
308   conditions, N application rate mainly affects grain yield by affecting RUE rather than IPAR.
309   Vos, Van Der Putten, and Birch (2005) also found that maize tends to sacrifice specific leaf N
310   and RUE while maintaining leaf area [small changes to leaf area index (LAI, Fig. 2) in
311   comparison with the large decrease in Pn (Fig. 2) at low N application rates]. Therefore, in the
312   condition of high IPAR, improving the RUE may represent a valid mechanism to achieve high
313   maize grain yield.
314   The actual ET involves two components: crop transpiration and soil evaporation. N
315   application can increase ET during the reproductive period due to high leaf transpiration under
316   high N conditions (Lamptey et al., 2017; Rudnick et al., 2017). The relatively low sensitivity of
317   IPAR to N supply in maize may also be consistent with the low sensitivity of soil evaporation to
318   N supply. In this case, the lower grain yields associated with the N_0 treatments corresponded to
319   lower ET and WUE values. In addition, WUE of the N_{300}-treated crop was lower than that of the
320   N_{225}-treated crop. This is due to a higher sensitivity to soil water by the plant at higher N
321   application rates. An increase in N application rates is usually accompanied by a decrease in
322   NUE (Ju et al., 2015). Significant effects of N were also observed on N use efficiency (NUE
323   including AEN, REN, NHI, and INE) in our study. Conversely, reducing the N application rate
324   can create a balance between crop demand and N supply (Lamptey et al., 2017). The desired N
325   concentration of the plant, under the N_0 treatment, has a high INE value, indicating that the plant
326   N concentration or yield are low and that the amount of N in the plant absorbed from the soil is
327   small. N accumulation increased with increasing N application, but N accumulation in the grain
328   was not significantly different between N_{225} and N_{300} treatments, and the value of NHI at N_{225}

329   was greater than that at N_{300}, which indicated that increased N in the plant did not transfer to the

330   grain, resulting in excessive N absorption and residual N in the vegetative organs.

331   The plasticity of root morphology allows it to respond to soil mineral nutrients (Peng, Li and

332   Li, 2012; Yu et al., 2014). We found that root dry weight reached a maximum with the N_{225}

333   treatment, which suggested that the relationship between N input and the root system is not linear

334   and positive; N input may even have a negative impact on root growth and development. In the

335   present study, application of N fertilizer promoted growth of roots in the 0–60 cm soil layer and

336   increased the proportion of roots in this layer, indicating that N application improved growth of

337   the upper layer roots. In the late growth stage (after VT), the N_0 treatment exhibited a negative

338   effect on the root dry weight and proportion in the 30–60 cm and 60–90 cm soil layers,

339   indicating that N deficiency would be detrimental during the accelerated aging of deep layer

340   roots. Not only are root morphology and nutrient absorption closely related, but the spatial

341   distribution of roots is also closely connected to crop growth and productivity (Mi et al., 2010;

342   Lynch, 2013). In both years, the root system exhibited the optimal distribution under the N_{225}

343   treatment, with a higher root length density in the observed soil layer, resulting in larger and

344   deeper infiltration scales. Slower root senescence in the N_{225} treatment is also a major contributor

345   to N rate–induced increases in grain yield. Studies have shown that the relative stability of the

346   deep root environment is beneficial in promoting the buffer capacity of the root system in

347   adverse soil environments and achieving high grain yield and resource use efficiency (Chen et

348   al., 2010; Mi et al., 2010; Wasson et al., 2012; Saengwilai et al., 2014). The results of our study

349   during the 2016 and 2017 growing seasons showed that excessive N (N_{300}) application

350   negatively affects early deep root growth compared with N_{225}. High external N input also

351   appeared to generate an overall inhibitory effect on later root growth. There are many reasons for

352   the observed reduction in crop yield. Slight reductions in crop yield induced by excessive N

353   application may be due to negative impacts on root growth during the early growth stage or may

354   be caused by differing mechanisms of aging leading to N loss and relative N deficiency during

355   the reproductive stage. N deficiency induces root thinning and increases longitudinal expansion

356   by promoting root growth in the deep soil, while high N inhibits vertical expansion of roots

357   (Trachsel et al., 2013; Mu et al., 2015). This study explained the effect of excessive N and N

358   deficiency on yield from the perspective of root morphology and growth.

359   The role of N in grain formation is mainly explained by photosynthesis, and N reduction

360   usually negatively impacts photosynthetic performance in maize (Massignam et al., 2012;

361   Olszewski et al., 2014). Although the N_{150} treatment significantly reduced Pn in the 2017

362   growing season, the grain yield for the N_{150} treatment was not significantly reduced, indicating

363   that the plant's transient photosynthetic parameters were more sensitive than the dry mass factors

364   in responding to changes in N application rates. Our results illustrate the physiological basis for

365   utilizing the 225 kg ha^{-1} N rate to improve the stress resistance of summer maize plants in the

366   Loess Plateau. For the N_{300} treatment, decreased Pn was due to lower Gs, which may be due to

367   N-associated increased sensitivity of plant to soil water and results in lower WUE than the N_{225}

368   treatment at the R6 stages. In the low-N treatment, maize plants showed reduced leaf rolling

369   under soil water stress compared with the high N treatment and thus obtained higher WUE
370   (Wang et al., 2019).
371   At different measurement dates, the maximum value of Gs was not always associated with the
372   N_{300} treatment (Fig. 2). In general, plants' water requirements are expected be greater under high
373   N conditions. Thus, high-N fertilizer plots are more likely to be water-deficient if the soil
374   moisture is inadequate, which aggravates the plant's stomatal limitations (1-Ci/Ca) and resulting
375   in reduction of Gs and Pn. Similar findings have been described in wheat (Zhang et al., 2017).
376   Previous studies also pointed out that under soil water-stress, ABA (as the signal carrier)
377   transmitted to the shoot, reducing the Gs and increasing stomatal limitations in the initial drought
378   (Larcher, 2003; Li and Xu, 2014; Yan et al., 2017). The inadequate soil moisture of the N_{300}
379   treatment is not serious enough to affect non-stomatal factors restricting photosynthetic carbon
380   assimilation. The Pn of the N_{225} treatment was similar to that of the N_{300} treatment during the VT
381   stage, but it was less than that of the N_{225} treatment in R6. Although the N_{225} treatment showed a
382   higher photosynthetic rate in the R6 than the N_{300} treatment, the ratio did not result in a
383   significantly higher yield, which may be caused by the small contribution of high photosynthetic
384   capacity to a grain yield (Acciaresi et al., 2014). In addition, the photosynthetic capacity may
385   also be related to the differences in N nutrition characteristics among different maize genotypes
386   (Chen et al., 2014; Li et al., 2015). Thus, while the application rate of 225 kg N ha^{-1} was may
387   still be high, the current N rate was relatively effective in improving resource use efficiencies.
388   Under production conditions, large amounts of N input are often used as an "insurance" against
389   higher yields to ensure further increases in maize production (Huang et al., 2007; Chen et al.,
390   2012). However, this behavior results in a significant reduction in NUE (Chen et al., 2010) and a
391   slight reduction of RUE and WUE. Previously, Chen et al. (2016) and Mu et al. (2018) reported
392   that N is used primarily for cell morphogenesis, that N in leaves is N redundant, and excess N is
393   mainly stored in soluble protein and light-harvesting pigment-protein complexes. Therefore, in
394   our study, the grain yield of N_{225} treatment did not display significantly different results
395   compared to the N_{300} treatment, and the root system and photosynthetic capacity showed certain
396   advantages of N_{225} treatment. From these findings, we can conclude that it is achievable to
397   improve resource use efficiencies while ensuring grain yield. Actually, maize genotypes and soil
398   moisture also affect GY and physiological characteristics (Ciampitti et al., 2013). In the current
399   experiment, these factors are not taken into consideration. In future studies, we will focus on the
400   effects of genotype with the aim of maximizing GY and resource use efficiency.

401 ## Conclusions

402 Decreased grain yield due to N reduction was driven by reduced radiation utilization efficiency
403 and water use efficiency; the impact of radiation interception and total water evapotranspiration
404 were relatively small. An application rate of 225 kg N ha^{-1} could be used as a reference for
405 optimal N application in the Loess Plateau of China. This N application rate optimized the eco-
406 physiological responses of the plant, a finding which was confirmed by measuring
407 photosynthetic activity and the root system. This response to optimizing N input resulted in
408 higher grain yield, RUE, WUE, and NUE. Reducing N application rates has strong recoverability

409   in maize production and can maximize the capture and utilization of resources, increasing the
410   maize grain yield.

411

412   **References**

413   Acciaresi HA, Tambussi EA, Antonietta M, Zuluaga MS, Andrade FH, Guiamét JJ. 2014.
414   Carbon assimilation, leaf area dynamics, and grain yield in contemporary earlier- and later-
415   senescing maize hybrids. European Journal of Agronomy |59:29–38 DOI
416   10.1016/j.eja.2014.05007.

417   Ahmad I, Kamran M, Ali S, Bilegjargal B, Cai T, Ahmad S, Meng XP, Su WN, Liu TN, Han
418   QF. 2018. Uniconazole application strategies to improve lignin biosynthesis, lodging
419   resistance and production of maize in semiarid regions. Field Crops Research 222:66–77
420   DOI 10.1016/j.fcr.2018.03.015.

421   Brown HE, Jamieson PD, Moot DJ. 2012. Predicting the transpiration of Lucerne. European
422   Journal of Agronomy 43:9–17 DOI 10.1016/j.eja.2012.03.012.

423   Birch CJ, Vos J, Van Der Putten PEL. 2003. Plant development and leaf area production in
424   contrasting cultivars of maize grown in a cool temperate environment in the field. European
425   Journal Agronomy 19:173–188 DOI 10.1016/S1161-0301(02)00034-5.

426   Chen YL, Wu DL, Mu XH, Xiao CX, Chen FJ, Yuan LX, Mi GH. 2016. Vertical distribution of
427   photosynthetic nitrogen use efficiency and its response to nitrogen in field-grown maize.
428   Crop Science 56(1):397–407 DOI 10.2135/cropsci2015.03.0170.

429   Chang YL, Liu JM, Li YH, Sun BH, Zhang SL, Yang XY. 2014. Investigation and evaluation of
430   nutrient input under winter wheat and summer maize rotation system in Guanzhong Plain of
431   Shaanxi Province. Journal Northwest A &F University 42:51–61 (in Chinese).

432   Chen GP, Gao JL, Zhao M, Dong ST, Li SK, Yang QF, Liu YH, Wang LC, Xue JQ, Liu JG, Li
433   CH, Wang YH, Wang YD, Song HX, Zhao JR. 2012. Distribution, yield structure, and key
434   cultural techniques of maize super-high yield plots in recent years. Acta Agronomica Sinica
435   38(1):80–85 DOI 10.3724/SP.J.1006.2012.00080.

436   Chen XP, Zhang FS, Cui ZL, Li F, Li JL. 2010. Optimizing soil nitrogen supply in the root zone
437   to improve maize management. Soil Science Society of America Journal 74:1367–1373
438   DOI 10.2136/sssaj2009.0227.

439   Chen QW, Mu XH, Chen FJ, Yuan LX, Mi GH. 2016. Dynamic change of mineral nutrient
440   content in different plant organs during the grain filling stage in maize grown under
441   contrasting nitrogen supply. European Journal of Agronomy 80:137–153 DOI
442   10.1016/j.eja.2016.08.002.

443   Ciampitti IA, Vyn TJ. 2011. A comprehensive study of plant density consequences on nitrogen
444   uptake dynamics of maize plants from vegetative to reproductive stages. Field Crops
445   Research 121(1):2–18 DOI 10.1016/j.fcr.2010.10.009.

446   Ciampitti IA, Murrell ST, Camberato JJ, Tuinstra M, Xia Y, Friedemann P, Vyn TJ. 2013.
447   Physiological dynamics ofmaize nitrogen uptake and partitioning in response to plant

448   density and nitrogen stress factors: II. Reproductive phase. Crop Science 53:2588–2602
449   DOI 10.2135/cropsci2013.01.0041.
450 Cui ZL, Zhang FS, Mi GH, Chen FJ, Li F, Chen XP, Li JL, Shi LF. 2009. Interaction between
451   genotypic difference and nitrogen management strategy in determining nitrogen use
452   efficiency of summer maize. Plant Soil 317(1-2):267–276 DOI 10.1007/s11104-008-9807-
453   x.
454 Dai J, Wang ZH, Li FC, He G, Wang S, Li Q, Cao HB, Luo LC, Zan YL, Meng XY, Zhang
455   WW, Wang RH, Malhi SS. 2015. Optimizing nitrogen input by balancing winter wheat
456   yield and residual nitrate-N in soil in a long-term dryland field experiment in the Loess
457   Plateau of China. Field Crops Research 181:32–41 DOI 10.1016/j.fcr.2015.06.014.
458 Erisman JW, Galloway JN, Seitzinger S, Bleeker A, Dise NB, Petrescu AMR, Leach AM, de
459   Vries W. 2013. Consequences of human modification of the global nitrogen cycle.
460   Philosophical transactions of the Royal Society of London. Series B, Biological sciences
461   368(1621): 20130116 DOI 10.1098/rstb.2013.0116.
462 FAO. FAOSTAT-Agriculture Database. 2018. Available online: http://faostat.fao.org/site/
463 Fletcher AL, Johnstone PR, Chakwizira E, Brown HE. 2013. Radiation capture and radiation use
464   efficiency in response to N supply for crop species with contrasting canopies. Field Crops
465   Research 150(20):126–134. DOI 10.1016/j.fcr.2013.06.014.
466 Herrera JM, Rubio G, Häner LL, Delgado JA, Lucho-Constantino CA, Islas-Valdez S, Pellet D.
467   2016. Emerging and established technologies to increase nitrogen use efficiency of cereals.
468   Agronomy 6(2):25 DOI 10.3390/agronomy6020025.
469 Holanda FSR, Mengel DB, Paula MB, Carvaho JG, Bertoni JC. 1998. Influence of crop rotations
470   and tillage systems on phosphorus and potassium stratification and root distribution in the
471   soil profile. Communications in Soil Science and Plant Analysis 29(15-16): 2383–2394 DOI
472   10.1080/00103629809370118.
473 Huang YL, Chen LD, Fu BJ, Huang ZL, Gong J. 2005. The wheat yields and water-use
474   efficiency in the loess plateau: straw mulch and irrigation effects. Agricultural Water
475   Management 72(3):209–222 DOI 10.1016/j.agwat.2004.09.012.
476 Huang ZX, Wang YJ, Wang KJ, Li DH, Zhao M, Liu JG, Dong ST, Wang HJ, Wang JH, Yang
477   JS. 2007. Photosynthetic characteristics during grain filling stage of summer maize hybrids
478   with high yield potential of 15,000 kg ha−1. Scientia Agricultura Sinica 40(9):1898–1906
479   (in Chinese).
480 Ju CX, Buresh RJ, Wang ZQ, Zhang H, Liu LJ, Yang JC, Zhang JH. 2015. Root and shoot traits
481   for rice varieties with higher grain yield and higher nitrogen use efficiency at lower nitrogen
482   rates application. Field Crops Research 175:47–55 DOI 10.1016/j.fcr.2015.02.007.
483 Lamptey S, Li LL, Xie JH, Zhang RZ, Yeboah S, Antille DL. 2017. Photosynthetic response of
484   maize to nitrogen fertilization in the semiarid western loess plateau of China. Crop Science
485   57(5):2739–2752 DOI 10.2135/cropsci2016.12.1021.

486 Larcher W. 2003. Physiological plant ecology ecophysiology and stress: Physiology of
487   functional groups. 4th ed. Springer Verlag, Berlin, Heidelberg pp513 DOI 10.1007/978-3-
488   662-05214-3.
489 Li DD, Tian MY, Cai J, Jiang D, Cao WX, Dai TB. 2013. Effects of low nitrogen supply on
490   relationships between photosynthesis and nitrogen status at different leaf position in wheat
491   seedlings. Plant Growth Regulation 70:257–263 DOI 10.1007/s10725-013-9797-4.
492 Li Q, Luo YH, Yu DH, Kong FL, Yang SM, Yuan JC. 2015. Effects of low nitrogen stress on
493   photosynthetic characteristics and chlorophyll fluorescence parameters of maize cultivars
494   tolerant to low nitrogen stress at the seedling stage. Journal of Plant Nutrition and Fertilizer
495   21(5):1132–1141 DOI 10.11674/zwyf.2015.0505.
496 Li QQ, Dong BD, Qiao YZ, Liu MY, Zhang JW. 2010. Root growth, available soil water, and
497   water-use efficiency of winter wheat under different irrigation regimes applied at different
498   growth stages in North China. Agricultural Water Management 97(10):1676–1682 DOI
499   10.1016/j.agwat.2010.05.025.
500 Li X, Xu K. 2014. Effects of exogenous hormones on leaf photosynthesis of *Panax ginseng*.
501   Photosynthetica 52:152–156 DOI 10.1007/s11099-014-0005-1.
502 Li XX, Hu CS, Delgado JA, Zhang YM, Ouyang ZY. 2007. Increased nitrogen use efficiencies
503   as a key mitigation alternative to reduce nitrate leaching in north China plain. Agricultural
504   Water Management 89(12):137–147 DOI 10.1016/j.agwat.2006.12.012.
505 Liang HY, Shen PF, Kong XZ, Liao YC, Liu Y, Wen XX. 2020. Optimal nitrogen practice in
506   winter wheat-summer maize rotation affecting the fates of 15 N-labeled fertilizer.
507   Agronomy 10:521 DOI 10.3390/agronomy10040521.
508 Liu CA, Zhou LM, Jia JJ, Wang LJ, Si JT, Li X, Pan CC, Siddique KHM, Li FM. 2014. Maize
509   yield and water balance is affected by nitrogen application in a film-mulching ridge–furrow
510   system in a semiarid region of China. European Journal Agronomy 52:103–111 DOI
511   10.1016/j.eja.2013.10.001.
512 Lynch JP. 2013. Steep, cheap and deep: an ideotype to optimize water and N acquisition by
513   maize root systems. Annal of Botany 112:347–357 DOI 10.1093/aob/mcs293.
514 Massignam AM, Chapman SC, Hammer GL, Fukai S. 2012. Effects of nitrogen supply on
515   canopy development of maize and sunflower. Crop Pasture Science 62:1045–1055.
516 McBratney A, Field D. 2015. Securing our soil. Soil Science and Plant Nutrition 61:587–591
517   DOI 10.1080/00380768.2015.1071060.
518 Meng QF, Yue SC, Hou P, Cui ZL, Chen XP. 2016. Improving yield and nitrogen use efficiency
519   simultaneously for maize and wheat in China: a review. Pedosphere 26(2):137–147 DOI
520   10.1016/S1002-0160(15)60030-3.
521 Mi GH, Chen FJ, Wu QP, Lai NW, Yuan LX, Zhang FS. 2010. Ideotype root architecture for
522   efficient nitrogen acquisition by maize in intensive cropping systems. Science China-Life
523   Sciences 53(12):1369–1373 DOI 10.1007/s11427-010-4097-y.

524   Mu XH, Chen QW, Chen FJ, Yuan LX, Mi GH. 2017. A RNA-Seq analysis of the response of
525   photosynthetic system to low nitrogen supply in maize leaf. International Journal of
526   Molecular Sciences 18(12):2624 DOI 10.3390/ijms18122624.
527   Mu XH, Chen FJ, Wu QP, Chen QW, Wang JF, Yuan LX, Mi GH. 2015. Genetic improvement
528   of root growth increases maize yield via enhanced post-silking nitrogen uptake. European
529   Journal Agronomy 63:55–61 DOI 10.1016/j.eja.2014.11.009.
530   Mu XH, Chen QW, Chen FJ, Yuan LX, Mi GH. 2018. Dynamic remobilization of leaf nitrogen
531   components in relation to photosynthetic rate during grain filling in maize. Plant Physiology
532   and Biochemistry 129:27–34 DOI 10.1016/j.plaphy.2018.05.020.
533   Nelson DW, Sommers LE. 1973. Determination of total nitrogen in plant material. Agronomy
534   Journal 65(1):109 DOI 10.2134/jeq1972.00472425000100040020x.
535   Olszewski J, Makowska M, Pszczółkowska A, Okorski A, Bieniaszewski T. 2014. The effect of
536   nitrogen fertilization on flag leaf and ear photosynthesis and grain yield of spring wheat.
537   Plant Soil and Environment 60(12):531–536 DOI 10.17221/880/2013-PSE.
538   Peng YF, Li C, Fritschi FB. 2014. Diurnal dynamics of maize leaf photosynthesis and
539   carbohydrate concentrations in response to differential N availability. Environmental and
540   Experimental Botany 99:18–27 DOI 10.1016/j.envexpbot.2013.10.013.
541   Peng YF, Li XX, Li CJ. 2012. Temporal and spatial profiling of root growth revealed novel
542   response of maize roots under various nitrogen supplies in the field. PLoS ONE
543   7(5):e37726 DOI 10.1371/journal.pone.0037726.
544   Qiang SC, Zhang Y, Fan JL, Zhang FC, Xiang YZ, Yan SC, Wu Y. 2019. Maize yield, rainwater
545   and nitrogen use efficiency as affected by maize genotypes and nitrogen rates on the Loess
546   Plateau of China. Agricultural Water Management 213:996–1003 DOI
547   10.1016/j.agwat.2018.12.021.
548   Ristova D, Busch W. 2014. Natural variation of root traits: from development to nutrient uptake.
549   Plant Physiology 166(2):518–527 DOI 10.1104/pp.114.244749.
550   Ritchie SW, Hanway JJ. 1982. How a corn plant develops. Spec. Rep. 48. Rev. Iowa State
551   University, Coop. Ext. Serv., Ames IA.
552   Robertson GP, Vitousek PM. 2009. Nitrogen in agriculture: balancing the cost of an essential
553   resource. Annual Review of Environment and Resources 34(1):97–125 DOI
554   10.1146/annurev.environ.032108.105046.
555   Rudnick DR, Irmak S, Djaman K, Sharma V. 2017. Impact of irrigation and nitrogen fertilizer
556   rate on soil water trends and maize evapotranspiration during the vegetative and
557   reproductive periods. Agriculture Water Management 191:77-84 DOI
558   10.1016/j.agwat.2017.06.007.
559   Saengwilai P, Nord EA, Chimungu JG, Brown KM, Lynch JP. 2014. Root cortical aerenchyma
560   enhances nitrogen acquisition from low-nitrogen soils in maize. Plant Physiology
561   166(2):726–735 DOI 10.1104/pp.114.241711.
562   Suchy M, Wassenaar LI, Graham G, Zebarth B. 2018. High-frequency NO3- isotope (delta N-15,
563   delta O-18) patterns in groundwater recharge reveal that short-term changes in land use and

564   precipitation influence nitrate contamination trends. Hydrology and Earth System Science
565   22:4267–4279 DOI 10.5194/hess-22-4267-2018.

566 Trachsel S, Kaeppler SM, Browm KM, Lynch JP. 2013. Maize root growth angles become
567   steeper under low N conditions. Field Crops Research 140:18–31 DOI
568   10.1016/j.fcr.2012.09.010.

569 Vos J, Van Der Putten PEL, Birch CJ. 2005. Effect of nitrogen supply on leaf appearance leaf
570   growth leaf nitrogen economy and photosynthetic capacity in maize (Zea mays L). Field
571   Crops Research 93(1):64–73 DOI 10.1016/j.fcr.2004.09.013.

572 Wang GL, Ye YL, Chen XP, Cui ZL. 2014. Determining the optimal nitrogen rate for summer
573   maize in China by integrating agronomic, economic, and environmental aspects.
574   Biogeosciences 11:3031–3041 DOI 10.5194/bg-11-3031-2014.

575 Wang SQ, Wei SC, Liang HY, Zheng WB, Li XX, Hu CS, Currell MJ, Zhou F, Min LL. 2019.
576   Nitrogen stock and leaching rates in a thick vadose zone below areas of long- term nitrogen
577   fertilizer application in the North China Plain: A future groundwater quality threat. Journal
578   of Hydrology 576:28–40 DOI 10.1016/j.jhydrol.2019.06.012.

579 Wang Y, Zhang XY, Chen J, Chen AJ, Wang LY, Guo XY, Niu YL, Liu SR, Mi GH, Gao Q.
580   2019. Reducing basal nitrogen rate to improve maize seedling growth, water and nitrogen
581   use efficiencies under drought stress by optimizing root morphology and distribution.
582   Agriculture Water Management 212:328–337 DOI 10.1016/j.agwat.2018.09.010.

583 Wasson AP, Richards RA, Chatrath R, Misra SC, Prasad SVV, Rebetzke GJ, Kirkegaard JA.
584   2012. Traits and selection strategies to improve root systems and water uptake in water-
585   limited wheat crops. Journal of Experimental Botany 63(9):3485–3498 DOI
586   10.1093/jxb/ers111.

587 Yan H, Wu LF, Filardo F, Yang XX, Zhao XM, Fu DH. 2017. Chemical and hydraulic signals
588   regulate stomatal behavior and photosynthetic activity in maize during progressive drought.
589   Acta Physiologiae Plantarum 39:125 DOI 10.1007/s11738-017-2418-5.

590 Yang XL, Lu YL, Ding Y, Yin XF, Raza S, Tong YA. 2017. Optimising nitrogen fertilisation: A
591   key to improving nitrogen-use efficiency and minimising nitrate leaching losses in an
592   intensive wheat/maize rotation (2008–2014). Field Crops Research 206:1–10 DOI
593   10.1016/j.fcr.2017.02.016.

594 Yu P, White PJ, Hochholdinger, F, Li C. 2014. Phenotypic plasticity of the maize root system in
595   response to heterogeneous nitrogen availability. Planta 240:667–678 DOI 10.1007/s00425-
596   014-2150-y.

597 Zhang SL, Gao PC, Tong YA, Norse D, Lu YL, Powlson D. 2015a. Overcoming nitrogen
598   fertilizer over-use through technical and advisory approaches: a case study from Shaanxi
599   province, northwest China. Agriculture Ecosystems &Environment 209:89–99 DOI
600   10.1016/j.agee.2015.03.002.

601 Zhang X, Davidson EA, Mauzerall DL, Searchinger TD, Dumas P, Shen Y. 2015b. Managing
602   nitrogen for sustainable development. Nature 528(7580):51–59 DOI 10.1038/nature15743.

603   Zhang XD, Yang LC, Xue XK, Kamran M, Ahmad I, Dong ZY, Liu TN, Jia ZK, Zhang P, Han
604   QF. 2019. Plastic film mulching stimulates soil wet-dry alternation and stomatal behavior to
605   improve maize yield and resource use efficiency in a semi-arid region. Field Crops
606   Research 233:101-113 DOI 10.1016/j.fcr.2019.01.002.

607   Zhang YQ, Wang JD, Gong SH, Xu D, Sui J. 2017. Nitrogen fertigation effect on
608   photosynthesis, grain yield and water use efficiency of winter wheat. Agriculture Water
609   Management 179:277–287 DOI 10.1016/j.agwat.2016.08.007.

610   Zhao BQ, Li XY, Li XP, Shi XJ, Huang SM, Wang BR, Zhu P, Yang XY, Liu H, Chen Y,
611   Poulton PR, Powlson DS, Todd AD, Payne RW. 2010. Long-term fertilizer experiment
612   network in China: crop yields and soil nutrient trends. Agronomy Journal 102(1):216–230
613   DOI 10.2134/agronj2009.0182.

Figure 1

Daily mean temperature and precipitation during the maize growing seasons in 2014 (a), 2015(b), 2016(c) and 2017 (d) at the experimental site.

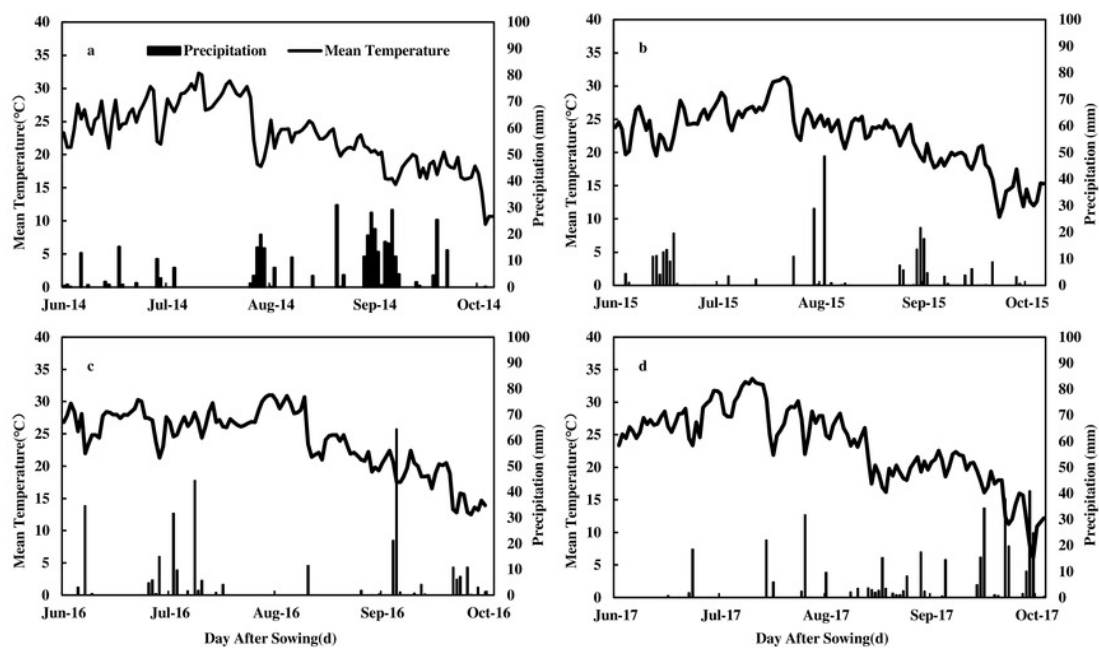
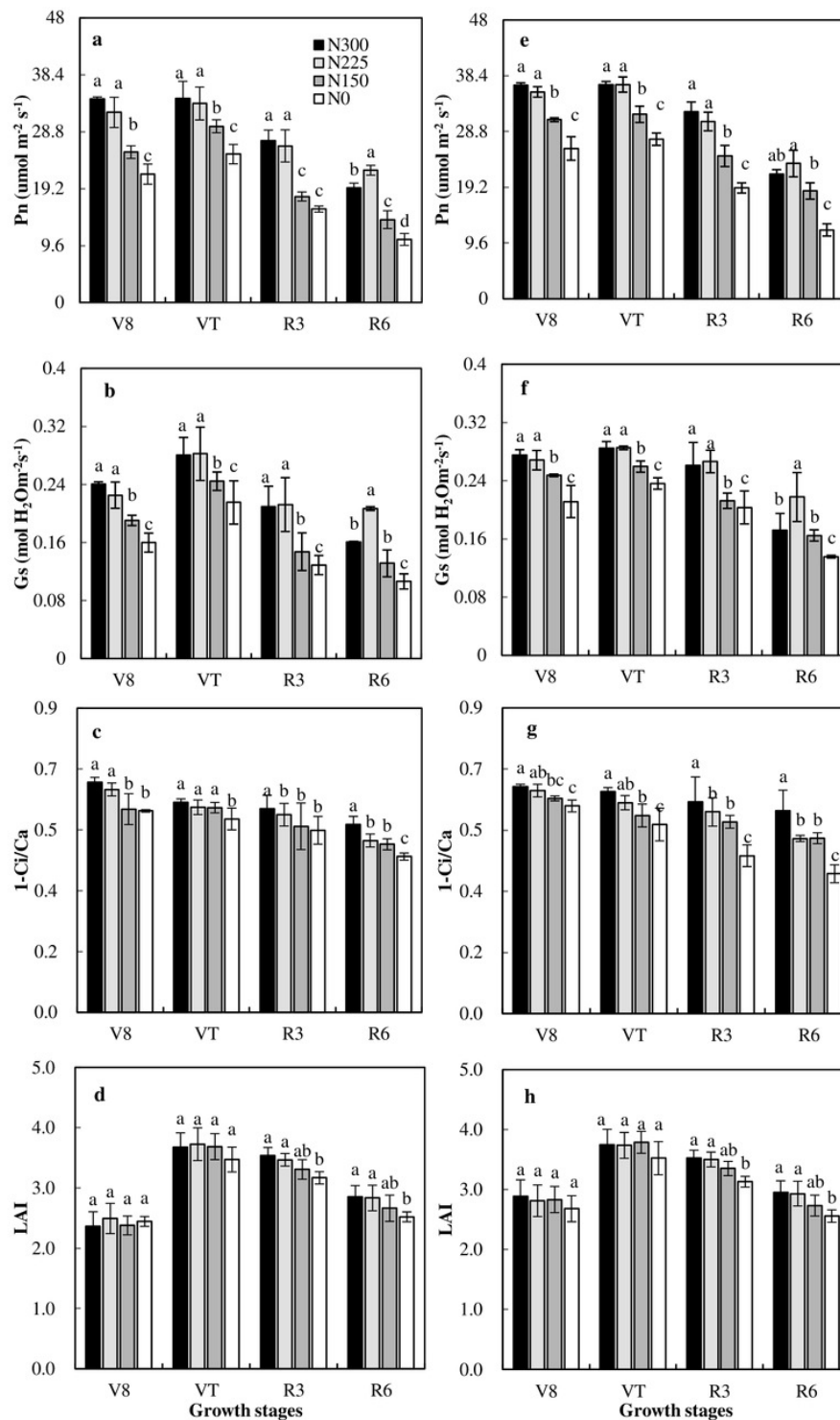



Figure 2

Effects of nitrogen application rates on Pn, Gs, 1-Ci/Ca and leaf area index (LAI) in 2016 (a-c) and 2017 (d-f) growing seasons

N_{300}, N_{225}, N_{150} and N_0 represent application of nitrogen at a rate of 300, 225, 150 and 0 kg ha^{-1}. Vertical bars represent the (means ± SD) (n = 3).

Soil chemical properties of the top 0-60 cm layers in the experimental fields

Layer (cm)	Organic matter (g kg^{-1})	Total nitrogen (g kg^{-1})	Alkaline nitrogen (mg kg^{-1})	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})
0-20	15.43	1.203	67.38	12.19	161.17
20-40	14.02	0.987	44.89	9.03	117.21
40-60	13.46	1.069	45.98	6.96	102.67

Effects of nitrogen application rates on grain yield (GY), biomass yield (BY), grain nitrogen content (GNC), N harvest index (NHI) and agronomic N use efficiency (AEN), apparent N recovery efficiency (RNE)

Means followed by different lowercase letters indicate significantly different ($p < 0.05$) within the same column and the same year. N_{300}, N_{225}, N_{150} and N_0 represent application of nitrogen at a rate of 300, 225, 150 and 0 kg ha^{-1}. * Significant at the 0.05 probability level. ** Significant at the 0.01 probability level. ns, non-significant

Year	Nitrogen rates	GY (t ha^{-1})	BY (kg ha^{-1})	GNC (kg ha^{-1})	NHI	AEN (kg kg^{-1})	REN (kg kg^{-1})
2014	N$_{300}$	10.2a	18.1a	127a	59.4b	7.6b	38.4b
	N$_{225}$	10.5a	17.9a	128a	64.5a	11.5a	44.5a
	N$_{150}$	9.4a	16.4b	105b	64.3a	9.9ab	43.1ab
	N$_{0}$	7.9b	13.6c	61c	62.0ab	-	-
2015	N$_{300}$	10.7a	18.6a	134a	63.1b	8.1b	35.3b
	N$_{225}$	11.1a	18.6a	133a	67.3a	12.5a	41.0a
	N$_{150}$	10.2a	17.3b	111b	66.1a	12.8a	41.3a
	N$_{0}$	8.3b	14.4c	68c	64.7b	-	-
2016	N$_{300}$	11.3a	20.0a	142a	60.5b	8.5b	38.7b
	N$_{225}$	11.4a	19.8a	134a	63.6a	12.1a	41.6ab
	N$_{150}$	9.7b	18.4b	113b	59.0b	6.9b	48.6a
	N$_{0}$	8.7c	15.6c	70c	59.1b	-	-
2017	N$_{300}$	11.0a	20.0a	137a	61.4b	7.2b	37.2b
	N$_{225}$	11.4a	20.0a	138a	65.1a	11.5a	45.0a
	N$_{150}$	10.5a	18.5b	116b	63.2ab	11.1a	47.7a
	N$_{0}$	8.8b	15.6c	70c	62.7b	-	-
Source of variation							
	Nitrogen rates (N)	**	**	**	**	**	**
	Year(Y)	**	**	**	**	**	**
	N x Y	ns	ns	ns	ns	ns	ns

Effect of nitrogen application rates on evapotranspiration (ET), the accumulated intercepted solar radiation (IPAR), shoot nitrogen content (SNC), internal N use efficiency (INE), radiation use efficiencies (RUE), and water use efficiency (WUE)

Means followed by different lowercase letters within each column indicate significantly different ($p < 0.05$). N_{300}, N_{225}, N_{150} and N_0 represent application of nitrogen at a rate of 300, 225, 150 and 0 kg ha^{-1}. ET, total evapotranspiration (mm); IPAR, the accumulated intercepted solar radiation (MJ m^{-2}), SNC, shoot nitrogen content (kg ha^{-1}), IEN, internal N use efficiency (kg kg^{-1}); RUE, radiation use efficiency; WUE, water use efficiency. * Significant at the 0.05 probability level. ** Significant at the 0.01 probability level. ns, non-significant.

Year	Nitrogen rate	ET (mm)	IPAR (MJ m^{-2})	SNC (kg ha^{-1})	IEN (kg kg^{-1})	RUE	WUE
2014	N$_{300}$	350a	974a	214a	47.8c	1.96a	29.1a
	N$_{225}$	346a	960a	198a	53.1b	2.05a	30.4a
	N$_{150}$	343a	989a	163b	57.6b	1.77b	27.4b
	N$_0$	333b	906b	98c	81.6a	1.63c	23.8c
2015	N$_{300}$	336a	1181a	212a	50.5d	1.69a	31.8a
	N$_{225}$	337a	1179a	198a	55.9c	1.75a	32.9a
	N$_{150}$	317ab	1131a	168b	60.8b	1.68a	32.1a
	N$_0$	303b	1138a	106c	78.1a	1.35b	27.2b
2016	N$_{300}$	414a	1141a	234a	48.1c	1.84a	27.2a
	N$_{225}$	416a	1124a	212a	54.0b	1.89a	27.5a
	N$_{150}$	399ab	1120a	191b	51.0b	1.62b	24.4b
	N$_0$	396b	1102a	118c	74.2a	1.47c	22.0c
2017	N$_{300}$	420a	1134a	223a	49.5d	1.82a	26.3ab
	N$_{225}$	417a	1121a	213a	53.7c	1.91a	27.5a
	N$_{150}$	409ab	1122a	183b	57.5b	1.75b	25.8b
	N$_0$	392b	1100a	112c	79.4a	1.50c	22.6c
Source of variation							
	Nitrogen (N)	*	*	**	**	**	**
	Year(Y)	**	**	**	*	*	**
	N x Y	ns	ns	ns	ns	ns	ns

Effect of nitrogen application rates on root dry matter and root ratio

Means followed by different lowercase letters within each column indicate significantly different ($p < 0.05$). N_{300}, N_{225}, N_{150} and N_0 represent application of nitrogen at a rate of 300, 225, 150 and 0 kg ha^{-1}.

Year	Soil layer	Nitrogen rate	Root dry matter (g plant^{-1})				Root ratio %			
			V8	VT	R3	R6	V8	VT	R3	R6
2016	0-30	N_{300}	2.81a	19.89b	19.09ab	16.45b	0.99	0.93	0.93	0.93
		N_{225}	3.05a	20.78a	20.43a	17.34a	0.99	0.92	0.92	0.91
		N_{150}	1.53b	20.18b	17.50b	14.41b	0.99	0.94	0.92	0.9
		N_{0}	0.97c	15.59c	13.19a	10.37c	1	0.89	0.91	0.9
	30-60	N_{300}	0.03a	1.52b	1.22b	1.23bc	0.01	0.07	0.06	0.07
		N_{225}	0.03a	1.65a	1.45a	1.45a	0.01	0.07	0.07	0.08
		N_{150}	0.01b	1.30c	1.26b	1.26b	0.01	0.06	0.07	0.08
		N_{0}	0.00b	1.49b	1.22b	0.99c	0	0.08	0.08	0.09
	60-90	N_{300}		0.03b	0.13b	0.08c	0	0	0.01	0
		N_{225}		0.05b	0.32a	0.34a	0	0	0.01	0.02
		N_{150}		0.07b	0.26a	0.26b	0	0	0.01	0.02
		N_{0}		0.52a	0.12b	0.12c	0	0.03	0.01	0.01
2017	0-30	N_{300}	3.06b	22.09a	19.90ab	17.33b	0.96	0.93	0.93	0.92
		N_{225}	4.67a	22.99a	21.90a	18.80a	0.97	0.93	0.92	0.91
		N_{150}	2.03c	21.99a	19.42b	15.81b	0.98	0.94	0.91	0.9
		N_{0}	1.36c	15.32b	14.20c	11.11c	1	0.88	0.9	0.89
	30-60	N_{300}	0.12a	1.52b	1.32c	1.24c	0.04	0.06	0.06	0.07
		N_{225}	0.14a	1.65a	1.61a	1.53a	0.03	0.07	0.07	0.07
		N_{150}	0.03b	1.30c	1.48b	1.42b	0.02	0.06	0.07	0.08
		N_{0}	0.01b	1.49b	1.36c	1.19c	0	0.09	0.09	0.1
	60-90	N_{300}		0.03b	0.21c	0.19b	0	0	0.01	0.01
		N_{225}		0.05b	0.40a	0.41a	0	0	0.02	0.02
		N_{150}		0.07b	0.34b	0.33b	0	0	0.02	0.02
	60-90	N_{0}		0.65a	0.21c	0.12b	0	0.04	0.01	0.01

Effect of nitrogen application rates on root volume, root surface and root length density

Means followed by different lowercase letters within each column indicate significantly different ($p < 0.05$). N_{300}, N_{225}, N_{150} and N_0 represent application of nitrogen at a rate of 300, 225, 150 and 0 kg ha^{-1}.

Year	Soil layer	Nitrogen rate	Root volume (cm³ plant⁻¹)				Root surface (cm² plant⁻¹)				Root length density (mm cm⁻³)			
			V8	VT	R3	R6	V8	VT	R3	R6	V8	VT	R3	R6
2016	0-30	N_{300}	31.2a	80.7ab	80.7a	68.6b	306.19a	536.5b	695.8b	569.3b	0.86a	1.24b	0.82b	1.52b
		N_{225}	37.7a	91.2a	89.9a	76.6a	339.78a	611.6a	744.9a	639.5a	0.98a	1.54a	2.30a	1.78a
		N_{150}	17.3b	86.8b	64.0b	52.4c	167.56b	652.4a	563.5c	472.6c	0.50b	1.23b	1.54c	1.11c
		N_0	10.7c	54.0c	31.7c	26.9d	132.53c	405.1a	281.8d	228.5d	0.40b	0.90c	0.70d	0.53d
	30-60	N_{300}	1.9a	6.7b	6.2a	2.9b	10.53a	101.4b	73.9b	51.1b	0.05a	0.34bc	0.48a	0.18b
		N_{225}	0.8b	9.0a	6.7a	5.5a	8.94a	117.4a	138.0a	100.2a	0.03a	0.39b	0.54a	0.4a
		N_{150}	0.1c	4.2c	3.3b	2.6b	0.97b	88.5b	90.8b	66.5b	0.003b	0.31c	0.44b	0.35a
		N_0	0.06c	7.1b	2.9b	1.9c	0.17c	112.5a	14.7c	36.5c	0.000b	0.46a	0.22c	0.13b
	60-90	N_{300}		1.1b	2.2b	0.9c		11.1c	44.1c	26.2c		0.01c	0.19c	0.07b
		N_{225}		3.2a	5.2a	3.9a		37.4b	118.0a	87.9a		0.17b	0.39a	0.24a
		N_{150}		1.5b	3.1b	2.6b		26.1b	91.0b	72.1b		0.07c	0.25b	0.21a
		N_0		4.6a	2.0b	1.0c		118.0a	47.9c	31.3c		0.35a	0.12c	0.09b
2017	0-30	N_{300}	36.8a	92.3b	85.6b	71.6b	428.83b	624.4b	726.8a	611.8b	1.08b	1.37b	1.77b	1.29b
		N_{225}	42.9a	112.9a	103.8a	94.3a	520.53a	683.4a	779.8a	690.1a	1.12a	1.74a	3.05a	2.67a
		N_{150}	21.2b	100.4a	73.9b	63.5b	248.31c	690.1a	609.6b	487.3c	0.56c	1.43b	1.92b	1.54b
		N_0	11.8c	58.4c	38.4c	32.6c	166.37d	560.2c	345.4c	286.7d	0.52d	1.00c	0.97c	0.58c
	30-60	N_{300}	2.7a	8.3b	7.2b	3.3b	11.37a	115.7a	100.0b	64.2c	0.05a	0.49b	0.57a	0.33b
		N_{225}	1.2b	11.0a	8.4a	6.6a	14.15a	133.0b	168.5a	146.1a	0.06a	0.53a	0.60a	0.52a
		N_{150}	0.4c	5.5c	4.4bc	3.4b	1.91b	110.4a	110.2b	96.0b	0.02b	0.44b	0.53b	0.48a
		N_0	0.3c	8.5b	3.8c	2.6b	0.34c	145.5b	29.0c	54.9c	0.03b	0.58a	0.33c	0.25c
	60-90	N_{300}		1.4b	3.2b	1.1c		29.3d	76.2b	49.1c		0.09c	0.29c	0.10bc
		N_{225}		4.8a	6.3a	4.3a		84.3b	145.7a	126.3a		0.30b	0.47a	0.31a
		N_{150}		2.3b	3.7b	3.1b		46.2c	101.1b	87.9b		0.13c	0.36b	0.30b
		N_0		5.5a	3.0b	2.0c		145.1a	60.2c	36.3c		0.44a	0.20c	0.10c