1 Effect of irrigation regimes and nitrogen rates on water use
2 efficiency and nitrogen uptake in maize

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25 **Abstract**

26 The nutritional responses to drying and rewetting cycles of partial root-zone irrigation still
27 remains elusive. The effect of alternate partial root-zone irrigation (PRI) on water use efficiency and
28 nitrogen (N) accumulation compared with deficit irrigation (DI) and full irrigation (FI) were
29 investigated in maize (*Zea mays* L.) grown under three N-fertilization rates (1.5, 3.0, and 6.0 g N
30 pot⁻¹) and moderately and severely water-stressed levels (60 and 40% of soil water holding
31 capacity). The plants were grown in split-root pots and exposed to FI, DI and PRI treatments from
32 the fourth leaf to silking stage. Analysis across the N-fertilization treatments showed that both PRI
33 and DI significantly decreased plant water use as well as plant height, girth, leaf area and shoot
34 biomass, leading to similar WUE compared with the FI control. Carbon isotope composition ($\delta^{13}\text{C}$)
35 was highest in PRI plants indicating a fine-tuned long-term stomatal control over gas exchange.
36 Across the N-fertilization rates, FI plants accumulated significantly greater amount of N than deficit
37 irrigation treatments. PRI and DI plants had similar plant $\delta^{15}\text{N}$, indicating the similar soil N
38 mineralization. Plant dry biomass, which was linearly associated with plant N uptake, was similar
39 for PRI and DI plants. Both resulted in the equivalent amount of N accumulation in the shoots of
40 PRI and DI plants. It was noted that increased soil moisture level, e.g., from 40% to 60%, showed
41 the tendency of increasing N uptake for PRI plants relative to DI plants. Therefore, in order to
42 facilitate N uptake, the soil water availability in the wet soil compartment of PRI treatment should
43 remain at high water levels.

44 Keywords: Deficit irrigation; partial root-zone irrigation; water level; $\delta^{13}\text{C}$; $\delta^{15}\text{N}$

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46 **1. Introduction**

47 Water is the most limiting factor in reducing crop productivity particularly on the 40% of global

land under arid or semi-arid climatic conditions where irrigation is the only way to maintain a stable food production (Cattivelli et al., 2008). Increasing threats of freshwater shortage, reduction in arable land, and more frequent and severe drought due to climate change has stimulated research into water-saving irrigation strategies aiming at producing more ‘crops per drop’ (Morison et al., 2008; Dodd, 2009). Deficit irrigation (DI) and partial root-zone irrigation (PRI) are water-saving irrigation techniques being intensively studied in many regions of the world. DI is a method that irrigates the entire root zone with an amount of water less than the potential evapotranspiration and the minor stress that develops has minimal effects on the yield and thereby increases water use efficiency (WUE) (English and Raja, 1996). PRI is a further refinement of DI. The principle behind PRI is to alternately let one part of the root system be exposed to soil drying, while the other part is irrigated, in order to keep the leaves hydrated. It is widely believed that one of the underlying mechanisms for improved WUE under PRI is the induced abscisic acid (ABA) based root-to-shoot chemical signalling in the drying roots which causes partial closure of stomata and reduction of leaf expansion growth thereby curtailing the transpirational water loss (Davies et al., 2000; Liu et al., 2006; Dodd, 2007; Wang et al., 2010a).

When applying DI or PRI soil water dynamics are changed being more ‘static’ under DI than under PRI and this has influenced both plant physiological and soil water and nutrient mineralization processes (Wang et al., 2010a,b; Wang et al., 2012a,b,c). Until now, there has been considerable discussion in the literature regarding the regulation of chemical and hydraulic signals on stomata and plant growth under DI and PRI (reviewed by Dodd, 2009; Sadras, 2009; Jensen et al., 2010). A knowledge gap, however, still exists on the nutritional responses to drying and rewetting cycles under soil water dynamics by PRI (Dodd et al., 2015; Wang et al., 2016). We have observed that, with the same degree of water saving, PRI-treated tomatoes accumulated greater amount of N in shoots compared to DI plants, which partly contributes to the greater WUE in PRI

72 plants (Wang et al., 2010a). Using the ^{15}N isotope labeling technique, it was found that PRI induced
73 dry/wet cycles in the soil stimulated mineralization of organic N hence increasing the amount of
74 mineral N available for plant uptake (Wang et al., 2010b). The effect of increase in soil water
75 dynamics caused by PRI on plant N nutrition is shown in several additional studies; in maize, Kirda
76 et al. (2005) reported that PRI had the highest N-fertilizer recovery, compared with DI and FI, with
77 minimal mineral N residues left in the soil after harvest. Wang et al. (2012a) found significantly
78 higher N accumulation in maize leaves compared with DI and FI treatments. Likewise, Wang et al.
79 (2009) noted that in relation to DI, PRI increased N content in all organs of potato plants. Liu et al.
80 (2015) reported that PRI plants accumulated 23% and 34% more N than DI plants under the
81 treatments with and without P fertilization, respectively. However, Topcu et al. (2007) found no
82 significant differences in N accumulation and fertilizer recovery of tomato plants among FI, DI and
83 PRI treatments. In potatoes, Shahnazari et al. (2008) reported that N accumulation was similar
84 between PRI and DI treatment. Li et al. (2007) found similar shoot N accumulation in PRI
85 treatment compared to conventional irrigation in maize plants. Therefore, more studies are still
86 necessary to examine further whether PRI can improve crop N accumulation in order to fully
87 exploit the beneficial effect of the irrigation technique in crop production.

88 Carbon isotope composition ($\delta^{13}\text{C}$) in plant biomass provides a time-integrated measurement of
89 plant WUE under water stress (Farquhar and Richards, 1984). A greater $\delta^{13}\text{C}$ is always positively
90 associated with higher WUE, and this has been extensively used for many C_3 and C_4 plants
91 including maize (Dercon et al., 2006; Zhang et al., 2015). Plant ^{15}N has been widely used to
92 indicate the mineralization rate of soil organic N as soil microbes discriminate against ^{15}N during
93 mineralization and immobilization turnover process, leaving soil organic N enriched in ^{15}N (Kerley
94 and Jarvis, 1996). Therefore, plants grown in soils relying on more N derived from soil organic N
95 pool should be enriched in ^{15}N in the biomass than the plants obtaining N mainly from inorganic N

(Kohl et al., 1973). The objective of the present study is to investigate the effect of irrigation regimes and nitrogen rates on water use efficiency and nitrogen uptake in maize.

2. Materials and methods

2.1. Experimental setup

The pot experiment was conducted in April-June, 2013 under a rain shelter with natural light and temperature conditions at the experimental farm of Department of Agricultural Engineering, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, (06°41' N, 01°33' W). The climate in this area is humid tropical savannah with average maximum and minimum temperatures of 31°C and 23°C, respectively, during the experimental period. At the third leaf stage, maize plants (*Zea mays L.*) were transplanted into pots (28 cm in diameter at the top edge, 25 cm in diameter at the bottom, 32 cm in depth) filled with 19.0 kg of naturally air-dried soil per pot with a bulk density of 1.2 g soil cm⁻³. Before filling the pots, the soil was sieved passing through 1 cm mesh. The inside of all pots was evenly divided into two vertical compartments by a plastic separator which was glued to the walls of the pots such that water exchange between the two compartments was prevented. The bottoms were perforated with small holes which allow free drainage. The soil was classified as sandy loam, having a pH of 6.2, total N of 1.4 g kg⁻¹, total P of 0.7 g kg⁻¹, total K of 15.8 g kg⁻¹, soil organic matter content of 5.7% and soil water holding capacity of 19% (on the mass basis). The natural ¹⁵N-abundance of the soil N was 0.368 % (i.e., [¹⁵N:(¹⁴N + ¹⁵N)]%). Plants were placed at the middle of the pots so that their primary roots were fairly evenly distributed into the two compartments of pots.

2.2 . Irrigation and N-fertilization treatments

Treatments comprised two levels of soil moisture, three irrigation methods and three N-fertilization rates. The soil moisture regime was either 60% or 40% of soil water holding capacity (θ_{WHC}), representing moderately or severely water-stressed conditions. Soil water content regime

120 was controlled by weighing the pots and irrigating the plants during the experimental period.
121 Irrigation methods include (1) DI in which soil compartments were irrigated to 60 or 40% of θ_{WHC}
122 in each irrigation event; (2) PRI in which one soil compartment was watered with exactly the same
123 amount as the DI treatment, while the other was allowed to dry for about 8 days, then the irrigation
124 was shifted to the other soil compartment; (3) FI as a well-watered control treatment in which both
125 of the soil compartments were watered to 80% of θ_{WHC} in each irrigation event. Three N-
126 fertilization rates included low N (N1, 1.5 g N pot⁻¹), medium N (N2, 3.0 g N pot⁻¹) and high N (N3,
127 6.0 g pot⁻¹). The experimental plan yielded 15 treatments (i.e. $2 \times 2 \times 3 + 3 \times 1$), with four
128 replicates in each treatment. At the commencement of the experiment 1.5 g N pot⁻¹, 0.65 g P pot⁻¹,
129 and 1.25 g K pot⁻¹ was applied in the form of commercial granulated NPK-fertilizer into the surface
130 layer of pots. Additional urea-N was applied for the medium and high N treatments with irrigation
131 water just before the initiation of irrigation treatments. The maize plants were well-watered in the
132 first 10 days after transplanting, and then the water treatments were initiated. Water was applied
133 every second (0-24 days after irrigation treatment (DAT)) or every day (25-51 DAT). The
134 experiment lasted seven weeks, during which each soil compartment of PRI-treated plants
135 experienced six drying/rewetting cycles.

136 2.3. Sampling, measurements and analysis

137 The plants were harvested on 19th June at 51 DAT. Plant height, stem girth, leaf length and
138 width were measured by a flexible measuring tape. Leaf length was defined as the distance from the
139 tip of the leaf to the branching point of the main vein. Leaf width was measured as the widest
140 region across the lamina perpendicular to the length. Leaf area was estimated by multiplying leaf
141 length, leaf maximum width and a constant (0.73) (Stewart and Dwyer, 1999). Leaves, stems,
142 reproductive organs (including tassels and young ears) were harvested separately. Dry biomass was
143 determined after oven drying at 70°C to constant weight.

144 After grinding in a ball mill, the plant samples were analysed for total N, ^{15}N , total C, and ^{13}C
145 content using the Dumas dry combustion method in a system consisting of an ANCA-SL Elemental
146 Analyser coupled to a 20–20 MassSpectrometer (Sercon Instruments, Crewe, UK). Natural $\delta^{15}\text{N}$ in
147 plant biomass was calculated as:

$$148 \quad \delta^{15}\text{N} = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$$

149 where R_{sample} and R_{standard} are the $^{15}\text{N}:(^{14}\text{N} + ^{15}\text{N})$ ratios of the plant sample and the standard,
150 respectively. The carbon isotope composition of plant samples was calculated as:

$$151 \quad \delta^{13}\text{C} = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$$

152 where R_{sample} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ of the PDB (Pee Dee
153 Belemnite) standard. $\delta^{13}\text{C}$ was used as an indicator of the integrated long-term WUE. Crop water
154 consumption (evapotranspiration, ET) was calculated from the pot water balance as:

$$155 \quad \text{ET} = I - \theta_{\text{H}}$$

156 where I is the total amount of irrigation water (liters) during plant growth, and θ_{H} is the amount of
157 soil water stored in the pots at the harvesting date (litres). There was no leakage in this experiment
158 and the soil was air-dried at the beginning of the experiment. At the whole plant level, WUE was
159 calculated as the ratio between the plant above-ground dry biomass to the crop water consumption
160 during the entire experimental period.

161 *2.4. Statistical analyses*

162 Two-way analysis of variance (ANOVA) was performed using the linear model-univariate
163 procedure from the SPSS software. ANOVAs were done on the effects of irrigation treatments and
164 N-fertilization rates as well as their interactions. All the treatment means and their interactions were
165 compared for any significant differences using Duncan's multiple range test at a significant level of
166 $P < 0.05$. Regression analysis was used to determine the relationships between the measured
167 parameters.

168 3. Results

169 3.1. Plant growth

170 Both irrigation treatments and N-fertilization rates had significant effect on plant growth (Table
171 1). When analyzed across N-fertilization treatments, plant height, girth and leaf area in FI treatment
172 were significantly higher than other irrigation treatments. For the water-stressed treatments, PRI60
173 had the highest plant height, girth and leaf area, followed by DI60, and the lowest in PRI40 and
174 DI40 treatments. Analysis across the irrigation regimes showed that N1 and N2 plants had
175 significantly higher plant height, girth and leaf area than did N3 plants.

176 3.2. Plant dry biomass, plant water use, WUE and $\delta^{13}\text{C}$

177 Significant differences in plant water use, plant dry biomass, WUE and $\delta^{13}\text{C}$ were found across
178 both irrigation and N-fertilization treatments except WUE across N treatments (Table 2). Analysis
179 across N-fertilization rates showed that plant water use and dry biomass were similar for DI and
180 PRI plants at 60% and 40% soil moisture levels, and were significantly less than those of FI plants.
181 Thus, WUE was similar among irrigation treatments ($P = 0.110$). Significant positive linear
182 relationship between WUE and $\delta^{13}\text{C}$ in the shoot biomass was observed for DI and PRI treatment
183 (Fig. 1); however, it is notable that FI treatment did not follow the same relationship pattern. Across
184 the irrigation treatments, N1 plants had significantly higher water use, dry biomass, WUE and $\delta^{13}\text{C}$
185 than other N treatments, intermediate in N2 treatment, and the lowest in N3 treatment.

186 3.3. Plant N accumulation and $\delta^{15}\text{N}$

187 Irrigation and N-fertilization treatments had significant effect on N accumulation and $\delta^{15}\text{N}$ in
188 plant biomass (Table 3). Across the N-fertilization treatments, FI plants accumulated significantly
189 more N than other irrigation treatments. For the water deficit treatments, PRI and DI had similar N
190 accumulation; however, PRI60 increased N accumulation by 8.3% compared with DI60. Across the
191 irrigation treatments, N1 and N2 plants accumulated 7.0% and 14.1% more N than did N3 plants.

192 When analyzed across the irrigation treatments, $\delta^{15}\text{N}$ in plant biomass increased significantly with
193 increasing N-fertilization rates, whereas analysis across the N-fertilization rates showed that DI and
194 PRI treatments had similar $\delta^{15}\text{N}$ in plant biomass, which was significantly higher than FI treatment.

195 **4. Discussion**

196 A large body of evidence has demonstrated that PRI induces partial closure of stomata and
197 reduction of leaf expansion growth thereby improving water use efficiency (Stoll et al., 2000; Liu et
198 al., 2006; Sadras, 2009; Dodd, 2009; Wang et al., 2010a; Wang et al., 2012b). In the present study,
199 the water stress caused by PRI and DI treatments significantly decreased plant leaf area (Table 1).
200 The amount of water applied was greatly reduced in DI and PRI treatments, resulting in decreased
201 plant water consumption by 33%-50% compared with FI plants. Simultaneously, the plant dry
202 biomass was decreased significantly by water stress, leading to similar WUE for all the irrigation
203 treatments (Table 2). Nonetheless, PRI plants increased WUE by 12.4% and 7.3%, respectively, at
204 the two moisture levels, compared to FI plants. In DI, the WUE remained below that of PRI plants
205 and was increased only by 4.7% and 5.2%, respectively, compared to FI plants. Across the irrigation
206 treatments, when excessive N was supplied in the high N treatment, the plant growth and dry
207 biomass was significantly reduced, leading to the lower WUE (Tables 1 and 2).

208 Earlier studies have shown that both PRI and DI can induce the ABA-based root-to-shoot
209 chemical signals regulating stomatal conductance and leaf expansion growth thereby curtailing
210 plant water consumption (Dodd, 2007; Wang et al., 2010a). However, accumulated evidence
211 indicates that, at a similar degree of soil water deficit, PRI plants possess greater xylem ABA
212 concentration relative to DI plants and hence leading to a better control of plant gas exchange
213 thereby causing further improvement to WUE (Dodd, 2007; 2009; Wang et al., 2010a). In the
214 current study, compared to both FI and DI treatments, WUE was the highest for PRI plants, though
215 the differences were not statistically significant (Table 1). Moreover, it was found that $\delta^{13}\text{C}$ in the

216 shoot biomass was significantly higher in PRI plants than in DI plants (Table 2) and WUE was
217 linearly positively correlated with $\delta^{13}\text{C}$ for PRI and DI treatments (Fig. 1) (Wang et al., 2010a; Sun
218 et al., 2013). This demonstrated further that fine-tuned long-term stomatal control over gas
219 exchange in PRI plants have contributed to the increased WUE (Wang et al., 2010a).

220 In addition to improving crop WUE, studies have shown that PRI can improve plant N nutrition,
221 contributing to higher WUE in PRI plants (Wang et al., 2010a,b). In maize, Wang et al. (2012a)
222 found that across three N-fertilization rates, N accumulation in the leaves of PRI plants increased
223 significantly by 4.8% compared to DI plants. Similarly, in potato, Wang et al. (2009) observed a
224 20% increase in N accumulation in PRI compared to DI plants. Liu et al. (2015) noted that PRI
225 plants increased plant N uptake by 23-34% compared with DI plants. In the present study, PRI
226 plants accumulated 8.3% more N than did DI plants, however, no significant difference was found
227 between these two treatments. Similar N uptake under PRI and DI treatments was also found by
228 Kirda et al. (2005) and Shahnazari et al. (2008).

229 It is known that plant N uptake is regulated by both soil N bioavailability and plant growth
230 (Gastal and Lemaire 2002; Lemaire et al. 2007; Wang et al., 2010b, 2013). Earlier studies have
231 shown that the drying and rewetting cycles under PRI stimulate soil organic N mineralization
232 thereby increasing mineral N available to plants (Wang et al., 2010b). In the present study, we have
233 measured plant $\delta^{15}\text{N}$ to reflect the mineralization rate of soil organic N (Högberg, 1997). It was
234 found that across the N-fertilization rates, plant $\delta^{15}\text{N}$ in both DI and PRI treatments was
235 significantly higher than that of FI treatment (Table 3) indicating a greater contribution of N derived
236 from soil organic N pool to the N uptake in these treatments. In a previous study, PRI has been
237 shown to have similar $\delta^{15}\text{N}$ in maize leaves as in the FI treatment, and was significantly higher than
238 the DI treatment (Wang et al., 2012a). The present study has found that DI and PRI plants had
239 similar plant $\delta^{15}\text{N}$, indicating the similar soil organic N mineralization and thereby the similar soil N

240 bioavailability. Consequently, the N uptake was equivalent for PRI and DI treatments. In
241 accordance with this, Sun et al (2013) also found similar plant $\delta^{15}\text{N}$ in PRI and DI plants. For DI
242 soil, the upper soil layers also experience frequent drying and rewetting processes, which might
243 have also enhanced soil N mineralization similar to the PRI soil, leading to the similar plant $\delta^{15}\text{N}$ in
244 these two treatments.

245 By analyzing the variation of $\delta^{13}\text{C}$ in the soil as a proxy of decomposition rate of soil organic
246 compounds, our recent study has even found a significantly greater amount of incorporated maize
247 residue was mineralized under DI than under PRI treatment (Wang et al., 2013). However, DI also
248 simultaneously resulted in significantly more mineralized N from maize residue or soil organic N
249 immobilized due to low soil water content and less water dynamics compared with PRI treatment,
250 resulting in less bioavailable N for plant uptake (Wang et al., 2013). Additionally, in PRI, the drying
251 and rewetting cycles caused flush of soil nutrients may be a transit (Denef et al., 2001a, b; Mikha et
252 al., 2005), and after several drying and rewetting cycles this pulse of nutrients could disappear with
253 the progress of irrigation treatments, leading to similar soil bioavailable N compared to DI
254 treatment. Most interestingly, it is noteworthy that across the irrigation treatments, plant $\delta^{15}\text{N}$
255 increased significantly with increasing N fertilization rate. This might be associated with the form
256 of N applied in the medium and high N treatments, where urea fertilizer was used, and urea must be
257 firstly immobilized and then mineralized before it can be taken up by plants. Under this
258 circumstance, soil N contribution became more important for plant N uptake in these two
259 treatments, resulting in significantly higher plant $\delta^{15}\text{N}$ in the medium and high N treatments. Plant
260 N uptake is not only affected by soil bioavailable N but also by plant growth. In the current study,
261 the significant linear correlation was observed between plant N accumulation and dry biomass (Fig.
262 2), indicating that plant N accumulation was intimately associated with dry biomass accumulation.
263 The similar dry biomass in PRI and DI treatments created equivalent plant N demand, and thereby

the similar N uptake in the two deficit irrigation treatments (Table 3). It was noted that increased soil moisture level, e.g., from 40% to 60%, showed the tendency of increasing N uptake for PRI plants compared to DI plants. This indicates that sustaining favourable soil water content in the wet zone of PRI treatment is critical for improving N uptake for PRI strategy, as good soil water conditions in the wet zone of the PRI root system could facilitate soil N mass flow/diffusion and a better contact of water with root system to increase water and nutrient uptake. More studies are still needed to quantify the uptake of nutrients from the respective wet and dry soil zones in the soil profile and their contribution to N uptake in deficit irrigation treatments.

In conclusion, water deficit significantly reduced both plant water use and dry biomass, leading to the similar WUE as well as significantly lower plant N uptake compared to well-watered treatment. The similar soil N mineralization and thereby soil N bioavailability and plant dry biomass resulted in the equivalent amount of N accumulation for PRI and DI plants. There was a tendency of increased plant N accumulation by increasing soil moisture level in PRI treatment compared to DI treatment. In order to facilitate N uptake, the soil water availability in the wet soil compartment of PRI treatment should remain at high water levels.

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384 **Table 1**

385 Summary of analysis of variance on the effect of of irrigation treatment and N fertilization rate and
 386 their interaction on plant height, stem girth, and leaf area of maize plants. Different letters within a
 387 column for each experimental factor indicate significant differences at $P < 0.05$ by Duncan's
 388 multiple range tests.

Factor	Plant height (m)	Stem girth (mm)	Leaf area (cm ² plant ⁻¹)
Irrigation treatment			
FI	2.3a	6.5a	5848a
DI60	2.0b	5.4bc	4502bc
PRI60	2.2ab	5.7b	4706b
DI40	1.7c	5.2cd	3971c
PRI40	1.8c	4.9d	3865c
<i>P</i> -value	< 0.001	< 0.001	< 0.001
N rate			
N1	2.1a	6.1a	5133a
N2	2.0a	5.6b	4843a
N3	1.8b	5.0c	3798b
<i>P</i> -value	< 0.001	< 0.001	< 0.001
Irrigation × N rate			
<i>P</i> -value	0.033	0.596	0.319

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Table 2
 Summary of analysis of variance on the effect of experimental factors and their interaction on plant water use, plant dry biomass, water use efficiency (WUE), and $\delta^{13}\text{C}$ of maize plants. Different letters within a column for each experimental factor indicate significant differences at $P < 0.05$ by Duncan's multiple range tests.

Factor	Plant water use (L plant ⁻¹)	Plant dry biomass (g plant ⁻¹)	WUE (g L ⁻¹)	$\delta^{13}\text{C}$ (‰)
Irrigation treatment				
FI	32.1a	138.8a	4.3a	-12.66a
DI60	19.3b	89.3b	4.5a	-13.37c
PRI60	19.3b	93.0b	4.8a	-13.11b
DI40	15.0c	68.1c	4.5a	-13.58d
PRI40	15.2c	69.6c	4.6a	-13.36c
<i>P</i> -value	< 0.001	< 0.001	0.110	< 0.001
N rate				
N1	24.5a	117.7a	4.8a	-12.99a
N2	20.3b	92.5b	4.6a	-13.19b
N3	16.0c	66.5c	4.2b	-13.43c
<i>P</i> -value	< 0.001	< 0.001	0.001	< 0.001
Irrigation $\times$ N rate				
<i>P</i> -value	0.667	0.648	0.236	0.109

Table 3

Summary of analysis of variance on the effect of irrigation treatment and N-fertilization rate and their interaction on N accumulation (g plant^{-1}) and $\delta^{15}\text{N}$ in plant biomass (‰) of maize plants. Different letters within a column for each experimental factor indicate significant differences at $P < 0.05$ by Duncan's multiple range tests.

Factor	N accumulation (g plant^{-1})	$\delta^{15}\text{N}$ in plant biomass (‰)
Irrigation treatment		
FI	1.78a	4.29b
DI60	1.33bc	7.66a
PRI60	1.44b	7.02a
DI40	1.14c	7.56a
PRI40	1.16c	7.00a
<i>P</i> -value	< 0.001	< 0.001
N rate		
N1	1.37ab	2.17c
N2	1.46a	6.19b
N3	1.28b	11.44a
<i>P</i> -value	0.094	< 0.001
Irrigation $\times$ N rate		
<i>P</i> -value	0.710	0.015

415 **Figure legend:**

416 **Fig. 1.** Relationship between WUE and carbon isotope composition ($\delta^{13}\text{C}$) in the shoot biomass of
417 maize plants after seven weeks for FI, DI and PRI treatments. The regression line is for the data for
418 the DI and PRI treatments. *** represent significance of the regression line at $P < 0.001$.

419 **Fig. 2.** Correlation between plant dry biomass and N accumulation of maize plants after seven
420 weeks for FI, DI and PRI treatments. *** represent significance of the regression line at $P < 0.001$.

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Fig. 2