



# Alternate partial root-zone drying irrigation improves nitrogen nutrition in maize (*Zea mays* L.) leaves

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## ABSTRACT

The effects of alternate partial root-zone drying (PRD) irrigation as compared with conventional deficit irrigation (DI) and full irrigation (FI) on leaf nitrogen (N) accumulation were investigated in maize (*Zea mays* L.) grown under three N-fertilization rates (1.5, 3.0, and 6.0 g N pot<sup>-1</sup>). The plants were grown in split-root pots in a glasshouse and were exposed to FI, PRD, and DI treatments from the seventh leaf to tasselling/silking stage. The results showed that neither irrigation nor N-fertilization treatments influenced shoot biomass and plant leaf area; while PRD plants had the highest root biomass and root to shoot ratio compared to DI and FI plants. Increase of N rate significantly increased leaf N accumulation; across the N-fertilization rates, PRD and FI plants accumulated significantly greater amount of N in leaves than did DI plants. Leaf  $\delta^{15}\text{N}$  decreased significantly with increasing N-fertilization rate, and was significantly higher in PRD and FI than in DI plants. Water use efficiency (WUE) was the highest in PRD, followed by DI and the lowest in FI; while N-fertilization rate had no effect on WUE. It was concluded that an enlarged root system and an enhanced soil N availability under PRD might have contributed to the greater N accumulation in maize leaves.

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## 1. Introduction

Agriculture represents 70% freshwater withdrawals globally, and this percentage rises above 90% in many arid countries (WRI, 2005). However, irrigation water has become less available in many regions due to global climate change and an increased competition for water by industry, domestic purposes, and the environment (Jensen et al., 2010). To confront this challenge, there is an urgent need to develop water-saving irrigation techniques in order to maximize crop WUE.

Deficit irrigation (DI) and partial root-zone drying (PRD) are water-saving irrigation strategies (Kang and Zhang, 2004). DI is a method that irrigates the entire root zone with an amount of water less than the potential evapotranspiration, and the mild stress has minimal effects on the yield (English and Raja, 1996). PRD is a further development of DI; it involves irrigating only part of the root zone leaving the other part to dry to a predetermined level before the next irrigation (Kang and Zhang, 2004). By alternately wetting

and drying part of the root zone, PRD allows the induction of the abscisic acid (ABA)-based root-to-shoot chemical signalling to regulate growth and water use thereby increasing WUE (Liu et al., 2005). Across several crop species including maize (*Zea mays* L.), PRD has shown a great potential in saving water and increasing crop WUE (Kirda et al., 2005; Liu et al., 2006). Accumulated evidence has also demonstrated that, given a same degree of water saving, PRD is superior to DI in terms of yield maintenance and increase of WUE (Dodd, 2009; Wang et al., 2010a). It is generally believed that the significant improvement of crop WUE under PRD is attributed mainly to a lowered stomatal conductance and a restricted leaf expansion growth induced by the root-to-shoot ABA signalling (Liu et al., 2006). In addition, the drying and wetting cycles in the soil imposed by the PRD treatment may influence soil bio-physicochemical processes and solutes' transportation hence affecting the soil nutrient availability and crop nutrient uptake (Shahnazari et al., 2008). Skinner et al. (1999) observed that alternate furrow irrigation successfully increased crop N uptake and reduced the potential of  $\text{NO}_3^-$  leaching. Likewise Kirda et al. (2005) reported that PRD improved N fertilizer recovery in maize resulting in lower mineral N left in the soil as compared with the full irrigation (FI) and DI treatments. Consistent with these findings, an enhanced N uptake under PRD was also observed by Li et al. (2007)

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and Hu et al. (2009) in pot-grown maize. However, until now the mechanisms underlying the improvement of crop N uptake under PRD remain largely illusive.

Wang et al. (2009) proposed that at least two factors might have contributed to the improved crop N uptake under PRD treatment; namely an enlarged root system for N uptake and an increased N availability in the soil. Earlier studies have observed that root growth particularly lateral roots can be stimulated by PRD in maize (Kang et al., 1998) and tomato (Mingo et al., 2004), which may increase the root surface area facilitating water and nutrient uptake. It is known that N in the soil is prevalent in the organic form, and the nature of PRD is frequent wetting and drying of the soil profile in the root zone. Birch (1958) demonstrated that rewetting the drying soil could stimulate mineralization of soil organic N and increase the mineral N available to the plants. These phenomena have been confirmed by numerous experimental studies and have been known collectively as the 'Birch effect' (e.g., Jarvis et al., 2007 where a detailed explanation of the 'Birch effect' was given). The microbial stress and substrate supply mechanisms have been postulated to elucidate the increased mineralization rate of soil organic N during the dry/wet cycles (Xiang et al., 2008; Butterly et al., 2009). Using  $^{15}\text{N}$  isotope labeling technique, our recent findings on tomatoes have explicitly indicated that PRD irrigation could increase mineralization rate of maize residuals in the soil (Wang et al., 2010b). Additionally, earlier studies have also demonstrated that the natural stable isotope abundance of N (i.e.,  $\delta^{15}\text{N}$ ) in the plant biomass can indicate the relative contribution of different N sources in the soil to the N accumulated in the plant (Högberg, 1997), and thus could be used as an indirect indicator for assessing the mineralization rate of soil organic N. It has been well established that the microbes in the soil discriminate against  $^{15}\text{N}$  during decomposition, mineralization (Nadelhoffer et al., 1996; Handley and Raven, 1992) and denitrification (Piccolo et al., 1996), leaving the soil organic N enriched in  $^{15}\text{N}$  (Kerley and Jarvis, 1996). Therefore, plants grown in soils relying on much more N derived from soil organic N pool should be enriched in  $^{15}\text{N}$  in the biomass than the plants obtaining N mainly from inorganic mineral N (Kohl et al., 1973). Accordingly, if PRD enhances soil organic N mineralization rate,  $\delta^{15}\text{N}$  of PRD-treated plants would be greater than those of the DI plants since they might have taken up more  $^{15}\text{N}$  derived from decomposition of soil organic N. Oppositely, increase of inorganic N-fertilization rate might cause decrease in plant  $\delta^{15}\text{N}$  due to a greater amount of mineral  $^{14}\text{N}$  in the soil which is easily available for plants uptake.

The objective of this study was to examine whether PRD improves N nutrition and facilitates the mineralization of soil organic N in maize plants. To test this, N accumulation and  $\delta^{15}\text{N}$  in maize leaves under different irrigation regimes and N-fertilization rates were determined. In addition, root biomass and root to shoot ratio were determined to investigate if an enlarged root system may also contribute to plant N accumulation under the PRD treatment.

## 2. Materials and methods

### 2.1. Experimental setup

The experiment was conducted from April 1st to June 24th, 2010 in a climate controlled greenhouse at the experimental farm of the Faculty of Life Sciences, University of Copenhagen, Taastrup, Denmark. At the fifth leaf stage, maize (*Z. mays* L.) seedlings were transplanted into 19.6l pots (25 cm diameter and 40 cm deep). The pots were evenly divided into two vertical compartments by plastic sheets; such that water exchange between the two compartments was prevented. The pots were filled with 20.2 kg of naturally

dried soil with a bulk density of  $1.14\text{ g cm}^{-3}$ . The soil was classified as sandy loam, having a pH of 6.7, total C  $12.9\text{ g kg}^{-1}$ , total N  $1.4\text{ g kg}^{-1}$ ,  $\text{NH}_4^+-\text{N}$   $0.7\text{ mg kg}^{-1}$ ,  $\text{NO}_3^--\text{N}$   $19.1\text{ mg kg}^{-1}$ . The natural  $^{15}\text{N}$ -abundance of the soil N was 0.369 at.% (i.e.,  $[^{15}\text{N}:(^{14}\text{N} + ^{15}\text{N})]\%$ ). The soil was sieved passing through a 2 mm mesh and had a volumetric soil water content (vol.%) of 30.0% and 5.0% at pot water holding capacity and permanent wilting point, respectively. The average soil water content in the soil was monitored by a time domain reflectometer (TDR, TRASE, Soil Moisture Equipment Corp., CA, USA) with probes (33 cm in length) installed in the middle of each soil compartment. The climate conditions in the greenhouse were set at:  $26 \pm 2^\circ\text{C}$  and  $20 \pm 2^\circ\text{C}$  day and night air temperature respectively, 15 h photoperiod and  $>500\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$  photosynthetic active radiation (PAR) supplied by sunlight plus metal-halide lamps.

### 2.2. N-fertilization and irrigation treatments

Three N-fertilization rates, viz., low N ( $\text{N1}$ ,  $1.5\text{ g N pot}^{-1}$ ), medium N ( $\text{N2}$ ,  $3.0\text{ g N pot}^{-1}$ ) and high N ( $\text{N3}$ ,  $6.0\text{ g N pot}^{-1}$ ), were included in the experiment. The N fertilizer supplied as  $\text{NH}_4\text{NO}_3$  was mixed thoroughly with the soil before filling the pots. In addition, P and K were also applied as  $\text{KH}_2\text{PO}_4$  ( $7.6\text{ g pot}^{-1}$ ) and  $\text{K}_2\text{SO}_4$  ( $2.6\text{ g pot}^{-1}$ ) into the soil to meet the nutrient requirement for plant growth. The maize plants were well-watered in the first 10 days after transplanting. Thereafter, the plants were exposed to three irrigation regimens: (1) FI in which both soil compartments were watered daily at 18:00 h to a soil water content of 28% to compensate the full evapotranspiration water loss during the previous day; (2) PRD in which one soil compartment was watered daily to a soil water content of 28% while the other was allowed to dry to about 10 days, then the irrigation was shifted to the other compartment; and (3) DI in which the same amount of water used for the PRD plants was irrigated evenly to the whole pot. The experiment was a complete factorial design comprising nine treatments and each treatment had four replicates. Plant water use (i.e., sum of the daily evapotranspiration from the pots) during the treatment period was computed based on the amount of irrigation and soil moisture depletion in the pots. The plants were irrigated manually with tap water which contains negligible concentrations of nutrients. The irrigation treatments lasted for 8 weeks, during which each soil compartment of the PRD plants had experienced six drying/wetting cycles.

### 2.3. Measurements and calculations

The plants were harvested on 24th June after 51 days of irrigation treatments. Leaf area (LA) was measured using an Area Meter (LI-3100, LI. Cor. Inc. Lincoln, NE, USA). Biomass of leaves, stem, reproductive organs (including tassel and young ears) and roots, was determined after drying in an oven at  $70^\circ\text{C}$  for 72 h. Crop water use efficiency (WUE) was calculated as the ratio between the increments of plant dry biomass (shoot + root) to the plant water use during the treatment period.

Dry samples of leaves were ground to a fine powder for  $^{15}\text{N}$  and total-N concentration analysis using the Dumas dry combustion method in a system consisting of an ANCA-SL Elemental Analyser coupled to a 20-20 Tracer Mass Spectrometer (Europa Scientific Ltd., Creve, UK). Natural  $\delta^{15}\text{N}$  in leaf dry biomass was calculated as:

$$\delta^{15}\text{N} = 1000 \left[ \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right]$$

$R_{\text{sample}}$  and  $R_{\text{standard}}$  are the  $^{15}\text{N}:(^{14}\text{N} + ^{15}\text{N})$  ratios of the leaf sample and the standard, respectively, where  $R_{\text{standard}} = 0.3663\text{ at.}\% ^{15}\text{N}$ .

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