

Interactive effects of CO₂ concentration elevation and nitrogen fertilization on water and nitrogen use efficiency of tomato grown under reduced irrigation regimes



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ABSTRACT

The interactive effects of CO₂ concentration elevation, N fertilization, and reduced irrigation regimes on water and nitrogen use efficiency (WUE and NUE) of tomato (*Solanum lycopersicum* L.) plants at both leaf and whole plant scales were investigated in a split-root pot experiment. The plants were grown in two separate climate-controlled greenhouse cells at atmospheric [CO₂] of 400 (a[CO₂]) and 800 (e[CO₂]) ppm, respectively. In each cell, plants were fertilized at either 1.5 or 3 g N per pot. The leaf physiological parameters, C and N content in stem, leaf and fruit were determined, and both WUE and NUE were evaluated. Plants harvested from 3 g N per pot associated with e[CO₂] environment possessed the greatest photosynthetic rate (P_n) and lowest stomatal conductance (g_s) and transpiration rate (T_r), resulting in the highest WUE at stomatal and leaf levels. Especially alternate partial root-zone irrigation (PRI) strategy coupled with e[CO₂] had the potential to synergistically reduce g_s and T_r while sustain P_n and leaf water status, and further improve tomato leaf WUE. e[CO₂] combined with sufficient N fertilization enhanced the biomass, C accumulation and N uptake of plants under reduced irrigation; yet the WUE and NUE at whole plant scale were affected solely by the N supply being greater in low N fertilizer. These findings provide useful knowledge on efficient irrigation and N management for adapting to the future water-limited and CO₂-enriched environment.

1. Introduction

Atmospheric carbon dioxide (CO₂) concentration has been constantly increasing and is predicted to reach nearly 800 ppm at the end of this century (IPCC, 2013). The elevated CO₂ concentration (e[CO₂]) in atmosphere could stimulate global warming, induce severe declining freshwater resources in agricultural regions around the world (Pazzagli et al., 2016). This has led to increased attention into the research of developing novel irrigation strategies for improved crop water use efficiency (WUE) (Wang et al., 2010a). On the other hand, e[CO₂] could decrease mineral contents, particularly nitrogen (N) in plants (Li et al., 2016), mostly due to dilution effect, and potentially exacerbate the prevalence of ‘hidden hunger’ (Loladze, 2002; Myers et al., 2014). Therefore, it is of great interest to understand the influences of reduced irrigation and limited N fertilizer on plant physiological response and nutrient status in e[CO₂] condition, which may provide further insights into the regulatory mechanisms of water and N availability as well as CO₂ environment effects on water and N use efficiency (WUE and NUE)

of plants.

It is widely accepted that alternate partial root-zone irrigation (PRI) and deficit irrigation (DI) are effective irrigation techniques, which have been extensively studied throughout the world (Davies et al., 2002; Du et al., 2015). DI is a method that irrigates the entire root zone with an amount of water less than the potential evapotranspiration and the mild stress that develops minimal effects on the yield (Dodd, 2009). PRI is a further refinement of DI, which has been demonstrated to maintain crop product quality and allows considerable water savings. The underlying mechanisms for the improved WUE from leaf to plant scale under PRI are to alternately allow one part of the root system to be irrigated to keep the leaves hydrated while the other part is exposed to soil drying, triggering stronger root-to-shoot abscisic acid (ABA) hormonal signaling, inducing partial closure of stomata and reduction in leaf expansion growth, thereby curtailing the transpirational water loss larger than photosynthesis (Davies et al., 2002; Kang and Zhang, 2004; Liu et al., 2006; Loveys et al., 2000; Wang et al., 2010a; Wei et al., 2016). In addition to enhancing crop WUE, recent studies have

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indicated that PRI could improve N nutrition as well as NUE as compared to DI with the same degree of water saving (Hu et al., 2009; Shahnazari et al., 2008; Wang et al., 2009, 2010a,b,c, 2012, 2013). The greater accumulated N nutrient accompanied with modified dry matter and N allocation of the plant might have increased leaf photosynthetic capacity, contributing to the higher WUE in the PRI plants (Wang et al., 2009, 2010a,b,c). The improved plant N nutrition of PRI treatment could be predominantly ascribed to an enhanced root N uptake capacity associated with an increased mineral N availability caused by the intensive drying and wetting cycles of the soil (Sun et al., 2013a; Wang et al., 2010a,c, 2012).

Previous evidence has demonstrated that $e[\text{CO}_2]$ could increase leaf photosynthetic rate (P_n) but decrease stomatal conductance (g_s) and transpiration rate (T_r) (Ainsworth and Long, 2005; Leakey et al., 2009) as well as modify the morphology of stomata (Haworth et al., 2016), hereby maintaining a better leaf water status (Tausz-Posch et al., 2015; Wullschlegel et al., 2002) and resulting in an improvement of WUE at leaf scale (da Silva et al., 2017; Li et al., 2017; Kang et al., 2002; Pazzagli et al., 2016; Yan et al., 2017). The higher photosynthesis and lower stomatal aperture are predominantly induced by stronger Rubisco activity and depolarization of guard cell membrane potential, respectively (Ainsworth and Rogers, 2007), probably leading to declined leaf transpiration and plant water consumption, therefore, further enhancing WUE of plant (da Silva et al., 2017; Kaminski et al., 2014; Pazzagli et al., 2016).

Despite improving WUE at both leaf and plant scale at $e[\text{CO}_2]$, there is generally a reduction in N concentration of plant, partly having an impact on the leaf photosynthetic and carbohydrate metabolic process (da Silva et al., 2017; Li et al., 2016; Sanz-Sáez et al., 2010), and such phenomenon has been proposed to attribute primarily to the dilution of N by increased biomass, altered root architecture and physiology, and changed requirement for N as protein cofactors or in other organic complexes as well as reduced root N uptake due to limited transpiration mass flow of plant resulting from partial stomatal closure (Taub and Wang, 2008; Loladze, 2002; McGrath and Lobell, 2013; Myers et al., 2014). Furthermore, NUE is commonly observed to enhance in plant harvested from $e[\text{CO}_2]$ environment resulted from the increased C and decreased N content in dry matter (Reddy et al., 2010). Nevertheless, to date no studies are available to elucidate the mechanism and response of combined effects of N fertilization and reduced irrigation regimes on plant WUE and NUE under $e[\text{CO}_2]$ environment.

In this study, tomato plants were grown in two atmospheric CO_2 concentrations (400 and 800 ppm) combined with two N fertilizer rates (1.5 and 3.0 g pot⁻¹) and subjected to three different irrigation regimes (FI, DI, and PRI) during flowering to fruiting stages. It was hypothesized that both $e[\text{CO}_2]$ and reduced irrigation would improve WUE from leaf to plant level simultaneously; moreover, $e[\text{CO}_2]$ would increase C and decrease N concentration, whilst PRI and greater N fertilization could improve plant nutrients uptake and may further enhance plant N nutrition and NUE at $e[\text{CO}_2]$. The objective was to investigate whether the interactive effects of $e[\text{CO}_2]$ and reduced irrigation could synergistically improve WUE at leaf and plant scales and NUE simultaneously of tomato under increased N fertilization in a future drier and CO_2 -enriched environment.

2. Materials and methods

2.1. Experimental setup

The experiment was conducted in a climate controlled greenhouse at the experimental farm of the Faculty of Science, University of Copenhagen, Taastrup, Denmark. The tomato seeds (*Solanum lycopersicum* L., cv. Elin) were sown on 26th Sept. 2016. The seedlings were transplanted into 1.5 L pots at the fourth leaf stage. From sowing, half of the plants (24) were grown in a greenhouse cell with ambient CO_2 concentration of 400 ppm ($a[\text{CO}_2]$), and another half were grown in a

cell with elevated CO_2 concentration of 800 ppm ($e[\text{CO}_2]$). In both greenhouse cells, the $[\text{CO}_2]$ was sustained or achieved by emission of pure CO_2 from a bottled tank, released in one point and distributed evenly in the cells through internal ventilation. The $[\text{CO}_2]$ in the glasshouse cells was monitored every 6 s by a CO_2 Transmitter Series GMT220 (Vaisala Group, Helsinki, Finland). The climatic conditions in the two glasshouse cells were set at: $23/16 \pm 2^\circ\text{C}$ day/night air temperature, 60% relative humidity, 16 h photoperiod, and $> 500 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic active radiation (PAR) supplied by sunlight plus LED lamps.

Five weeks after sowing, tomato seedlings were transplanted into 10 L pots (17 cm diameter and 50 cm depth) in the greenhouse, filled with 14.5 kg of air-dried soil. The pots were divided vertically into two equal-sized compartments with plastic sheets such that the water exchange between the two compartments was prevented. The soil used was classified as sandy loam, with a pH of 6.7, total C 10.3 g kg^{-1} , total N 1.0 g kg^{-1} , $\text{NH}_4^+ - \text{N}$ 0.1 mg kg^{-1} , $\text{NO}_3^- - \text{N}$ 5.3 mg kg^{-1} . The soil was sieved through 5 mm mesh before filling the pots. The soil had a volumetric soil water content (% vol.) of 18.0% and 5.0% at pot water holding capacity and permanent wilting point, respectively. 0.7 g P and 0.88 g K fertilizers were applied as KH_2PO_4 into the soil of each pot. In each $[\text{CO}_2]$ greenhouse cell, 1.5 g N was applied as NH_4NO_3 per plant into half of the pots, and 3.0 g N per plant was applied into the other half.

2.2. Treatments

The experiment was conducted in two CO_2 concentration greenhouse cells, one with ambient CO_2 concentration ($a[\text{CO}_2]$, 400 ppm) and the other with elevated CO_2 concentration ($e[\text{CO}_2]$, 800 ppm). Two N fertilizer rates in each cell, that is N1 (1.5 g N pot^{-1}) and N2 (3.0 g N pot^{-1}).

The tomato plants were well-watered in the first three weeks after transplanting. Thereafter, the plants were subjected to three irrigation treatments: (1) full irrigation (FI) where both soil compartments were watered daily at 15:00 h to water content of 18% to compensate the full evapotranspiration water loss during the previous day; (2) alternative partial root-zone irrigation (PRI), where half of the root system was watered to 70% of the FI irrigation volume while the other half was allowed to dry to ca. 6%, then the irrigation was shifted between the two soil compartments; and (3) deficit irrigation (DI) where the same amount of water for PRI was evenly irrigated to the two soil compartments. The experiment was a complete randomized design with four replicates in each treatment. The water used for the irrigation was tap water with negligible concentrations of nutrients. The irrigation treatments lasted 40 days and each soil compartment of the PRI plants had experienced five drying/wetting cycles. The average soil water content in the pot was monitored by a time domain reflectometer (TDR, TRASE; Soil Moisture Equipment Corp., Santa Barbara, USA) with probes (35 m in length) installed in the middle of each soil compartment. The changes of soil water content in the pots were presented elsewhere (Wei et al., unpublished).

2.3. Measurements

On days 1, 4, 8, 12, 16, 24, 28 and 40 after initiation of the irrigation treatment with four replicates in each treatment during each measurement, leaf gas exchange rates, including photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r) were determined on upper canopy fully expanded leaves around 10:00 h with a portable photosynthetic system (LiCor-6400XT, LI-Cor, NE, USA). Measurements were performed on one leaf per plant at 20°C chamber temperature and $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density, and at a $[\text{CO}_2]$ of 400 ppm for $a[\text{CO}_2]$ and 800 ppm for $e[\text{CO}_2]$ treatment, respectively. Intrinsic water use efficiency (WUE_i , $\mu\text{mol mol}^{-1}$) was calculated as the ratio of P_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$) to g_s ($\text{mol m}^{-2} \text{s}^{-1}$) and instantaneous water use

efficiency (WUE_n , mmol mol^{-1}) was calculated as the ratio of P_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$) to T_r ($\text{mmol m}^{-2} \text{s}^{-1}$). In order to examine the overall effects of various treatments on leaf gas exchange rates, here only the average values of the eight measurements obtained at different dates are presented.

On days 1, 8, 16, 24 and 40 after initiation of the irrigation treatment with four replicates in each treatment during each measurement, midday leaf water potential (Ψ_l) was measured on the same leaves used for gas exchange rates measurement with a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA). After measuring Ψ_l , the leaf was immediately packed in aluminum foil and frozen in liquid nitrogen and stored at -80°C for later determination of osmotic potential (Ψ_π). Ψ_π was determined after thawing the leaf sample at 20°C by using a psychrometer (C-52 sample chambers, Wescor Inc., Logan, UT, USA) connected to a microvoltmeter (HR-33T, Wescor, Logan, UT, USA). Turgor pressure (Ψ_p) was then calculated as the subtraction of Ψ_l to Ψ_π . The average values of the five measurements obtained at different dates are presented.

After the irrigation treatments, all plant materials were harvested. Plant samples were divided into stem, leaf and fruit, and dry matter was determined after oven drying at 70°C to constant weight. The dry samples were thoroughly grounded to a fine powder and analysed for C and N concentration using a CHNS/O Elemental Analyser (Flash 2000, Thermo Fisher Scientific, Cambridge, UK). Total above ground dry matter (TDM) was calculated as the sum of stem, leaf and fruit dry matter. The plant total C accumulation (TC) and N uptake (TN) was calculated as the sum of the multiplication of C and N concentration with dry matter in stem, leaf and fruit, respectively. The above measurements were done for four samples of each treatment.

Plant harvest index (HI) was calculated as the ratio of plant fruit dry matter to the shoot dry matter. Plant water use (PWU) during the experimental period was calculated based on the amount of irrigation and changes in the soil water content. Plant WUE (WUE_p) was calculated as the ratio between TDM and PWU during the treatment period. Plant N use efficiency (NUE) was calculated as the ratio of total plant C to N.

2.4. Statistical analyses

Three-way ANOVA was performed for the independent variables: CO_2 concentration ($[\text{CO}_2]$), N fertilizer (N), and irrigation regime (IRRI); as well as for their interactions. Data were analyzed with SPSS version 18.0 (IBM Electronics).

3. Results

3.1. Leaf gas exchange rates

Leaf photosynthetic rate (P_n) was significantly affected by $[\text{CO}_2]$ and N as well as $[\text{CO}_2] \times \text{N}$ (Fig. 1a; Table 1). Irrespective of IRRI treatment, plants grown at $e[\text{CO}_2]$ had notably 30.40 and 40.15% greater P_n than those grown at $a[\text{CO}_2]$ under N1 and N2 treatment, respectively. Both stomatal conductance (g_s) and transpiration rate (T_r) were significantly affected by $[\text{CO}_2]$, N and IRRI (Fig. 1b and c; Table 1). g_s and T_r was lower in $e[\text{CO}_2]$, N1 and reduced irrigation (DI and PRI) plants (decreased by 27.39, 17.70 and 22.19% for g_s and 22.77, 13.31 and 21.16% for T_r) as compared with $a[\text{CO}_2]$, N2 and FI plants, respectively. Both intrinsic water use efficiency (WUE_i) and instantaneous water use efficiency (WUE_n) were remarkably affected by $[\text{CO}_2]$, N and IRRI as well as $[\text{CO}_2] \times \text{IRRI}$ (Fig. 1d and e; Table 1). WUE_i and WUE_n was separately greater in $e[\text{CO}_2]$, N2 and reduced irrigation (DI and PRI) plants (increased by 101.44, 13.34 and 41.74% for WUE_i and 84.85, 23.22 and 31.16% for WUE_n) as compared with $a[\text{CO}_2]$, N1 and FI plants, respectively. Reduced irrigation and $e[\text{CO}_2]$ could synergistically improve both WUE_i and WUE_n across N treatment.

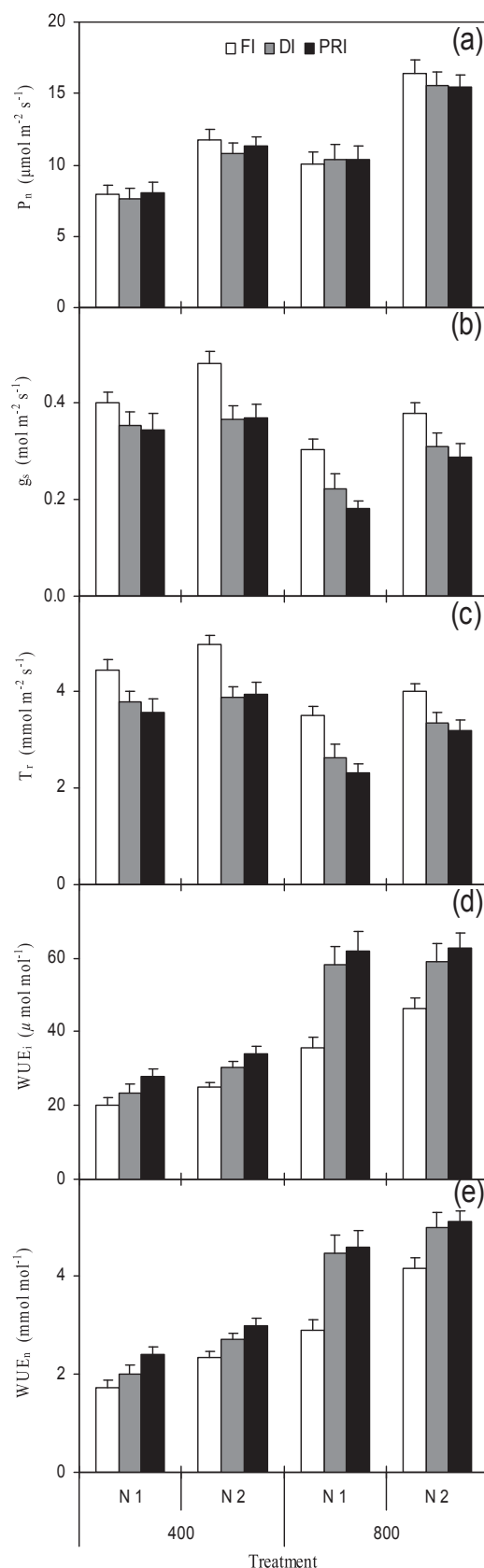


Fig. 1. Leaf photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r), intrinsic water use efficiency (WUE_i) and instantaneous water use efficiency (WUE_n) of tomato plants as affected by the atmospheric $[\text{CO}_2]$ (400 and 800 ppm), N fertilizer rates (N1 and N2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; and alternative partial root-zone irrigation, PRI). Error bars indicate standard error of the mean ($n = 32$). Statistical comparisons among the treatments are presented in Table 1.

Table 1

Out put of three-way ANOVA for leaf photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r), intrinsic water use efficiency (WUE_i), instantaneous water use efficiency (WUE_n), leaf water potential (Ψ_l), osmotic potential (Ψ_π) and turgor pressure (Ψ_p) of tomato plants as affected by the atmospheric $[CO_2]$ (400 and 800 ppm), N fertilizer rates (N1 and N2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; and alternative partial root-zone irrigation, PRI). The data is presented in Figs. 1 and 2.

Factors	P_n	g_s	T_r	WUE_i	WUE_n	Ψ_l	Ψ_π	Ψ_p
$[CO_2]$	***	***	***	***	***	**	ns	*
N	***	***	***	***	***	ns	ns	ns
IRRI	ns	***	***	***	***	**	ns	ns
$[CO_2] \times N$	*	ns	ns	ns	ns	**	ns	ns
$[CO_2] \times$ IRRI	ns	ns	ns	**	*	ns	ns	ns
N \times IRRI	ns	ns	ns	ns	ns	ns	ns	ns
$[CO_2] \times N \times$ IRRI	ns	ns	ns	ns	ns	ns	ns	ns

The table reported the significance results of the three-way ANOVA on photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r), intrinsic water use efficiency (WUE_i), instantaneous water use efficiency (WUE_n), leaf water potential (Ψ_l), osmotic potential (Ψ_π) and turgor pressure (Ψ_p) of tomato plants as affected by the CO_2 environment ($[CO_2]$), nitrogen fertilizer rates (N) and irrigation regimes (IRRI) and their interactions.

*, ** and *** indicate significance levels at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; ns denotes no significance.

3.2. Leaf water potential, osmotic potential and turgor pressure

Leaf water potential (Ψ_l) was significantly affected by $[CO_2]$, IRRI and $[CO_2] \times N$ (Fig. 2a; Table 1). Plants grown at $e[CO_2]$ had a 8.71% increase in Ψ_l than those grown at $a[CO_2]$ under N1. Ψ_l was 4.43% lower in the reduced irrigation plants in relation to the FI plants. Osmotic potential (Ψ_π) was unaffected by any of the $[CO_2]$, N and IRRI treatments (Fig. 2b; Table 1). Turgor pressure (Ψ_p) was only remarkably affected by $[CO_2]$ (Fig. 2c; Table 1). Regardless of N and IRRI treatments, $e[CO_2]$ plants possessed 19.09% higher Ψ_p than $a[CO_2]$ plants.

3.3. Stem, leaf, fruit and total dry matter

Stem dry matter (SDM) was solely affected by $[CO_2]$ (Fig. 3a; Table 2). Across N and IRRI treatments, plants grown at $e[CO_2]$ had 8.02% greater SDM than those grown at $a[CO_2]$. Leaf dry matter (LDM) was significantly affected by $[CO_2]$, N and IRRI as well as $N \times$ IRRI (Fig. 3b; Table 2). LDM was 6.33, 20.96 and 8.23% higher in $e[CO_2]$, N2 and FI plants as compared with $a[CO_2]$, N1 and reduced irrigation (DI and PRI) plants, respectively. Fruit dry matter (FDM) was notably affected by $[CO_2]$, N and $N \times$ IRRI (Fig. 3c; Table 2). $e[CO_2]$ and N2 plants had 13.39 and 52.91% greater FDM than $a[CO_2]$ and N1 plants, respectively. Plant total above ground dry matter (TDM) was remarkably affected by $[CO_2]$, N and IRRI as well as $N \times$ IRRI (Fig. 6a; Table 2). TDM was 9.56, 30.37 and 9.05% greater in $e[CO_2]$, N2 and FI plants as compared with $a[CO_2]$, N1 and reduced irrigation plants, respectively.

3.4. Stem, leaf, fruit C concentration and total C accumulation

Stem C concentration (SCC) was significantly affected by N and IRRI (Fig. 4a; Table 2). Plants grown at N1 and FI had 1.73 and 1.73% lower SCC than those grown at N2 and reduced irrigation, respectively. Leaf C concentration (LCC) was affected by N, IRRI and $[CO_2] \times$ IRRI as well as $[CO_2] \times N \times$ IRRI (Fig. 4b; Table 2). N2 + reduced irrigation plants had 2.96 and 2.65% higher LCC than other treatments in $a[CO_2]$ and $e[CO_2]$ environment, respectively. Fruit C concentration (FCC) was unaffected by any of the $[CO_2]$, N and IRRI treatments (Fig. 4c; Table 2). Total C accumulation (TC) was significantly affected by $[CO_2]$, N and IRRI as well as $N \times$ IRRI (Fig. 6b; Table 2). Plants grown under $e[CO_2]$, N2 and FI treatment had 9.29, 33.21 and 7.97% greater TC than those grown under $a[CO_2]$, N1 and reduced irrigation treatment, respectively.

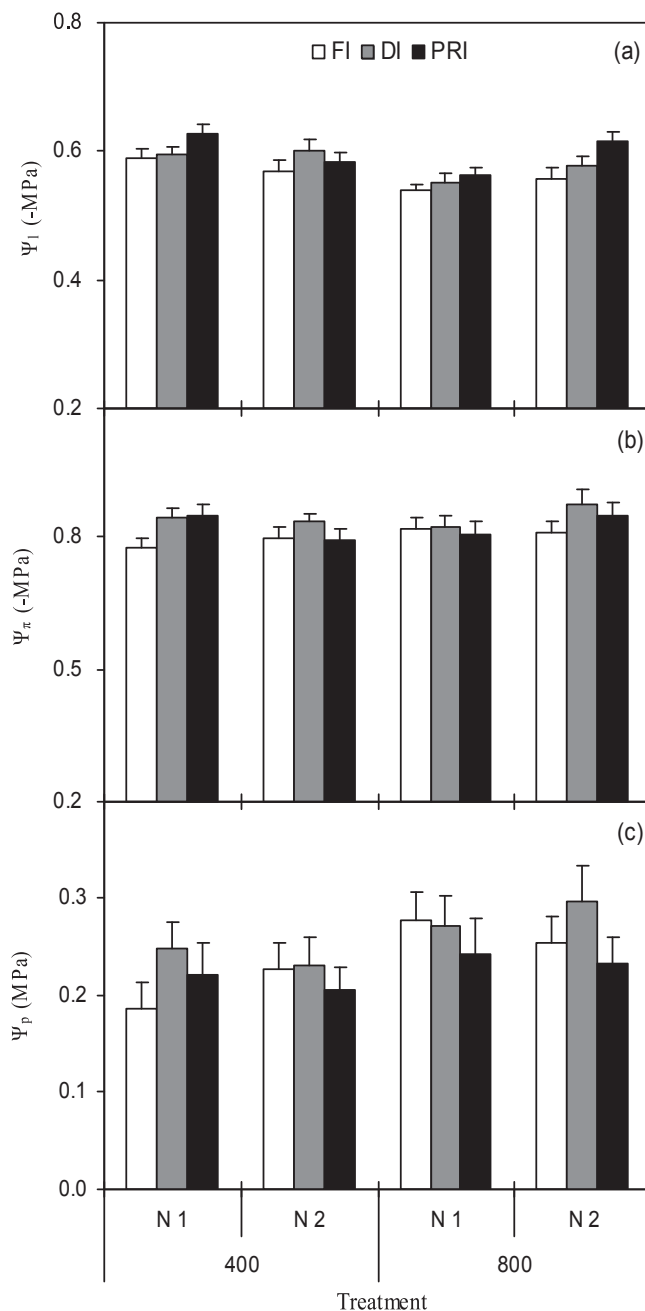


Fig. 2. Leaf water potential (Ψ_l), osmotic potential (Ψ_π) and turgor pressure (Ψ_p) of tomato plants as affected by the atmospheric $[CO_2]$ (400 and 800 ppm), N fertilizer rates (N1 and N2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; and alternative partial root-zone irrigation, PRI). Error bars indicate standard error of the mean ($n = 20$). Statistical comparisons among the treatments are presented in Table 1.

3.5. Stem, leaf, fruit N concentration and total N uptake

Stem N concentration (SNC) was only significantly affected by N treatment (Fig. 5a; Table 2). Across $[CO_2]$ and IRRI treatments, plants grown at N2 had 53.55% greater SNC than those grown at N1. Leaf N concentration (LNC) was affected by $[CO_2]$, N and IRRI as well as $N \times$ IRRI (Fig. 5b; Table 2). LNC was 6.68, 45.96 and 14.55% higher in $a[CO_2]$, N2 and reduced irrigation (DI and PRI) plants as compared with $e[CO_2]$, N1 and FI plants, respectively. Fruit N concentration (FNC) was remarkably affected by N, $[CO_2] \times$ N and $N \times$ IRRI (Fig. 5c; Table 2). N2 plants possessed 19.62% greater FNC than N1 plants. Total N uptake (TN) was solely affected by N treatment (Fig. 6c; Table 2). Across $[CO_2]$ and IRRI treatments, plants grown at N1 had 44.03%

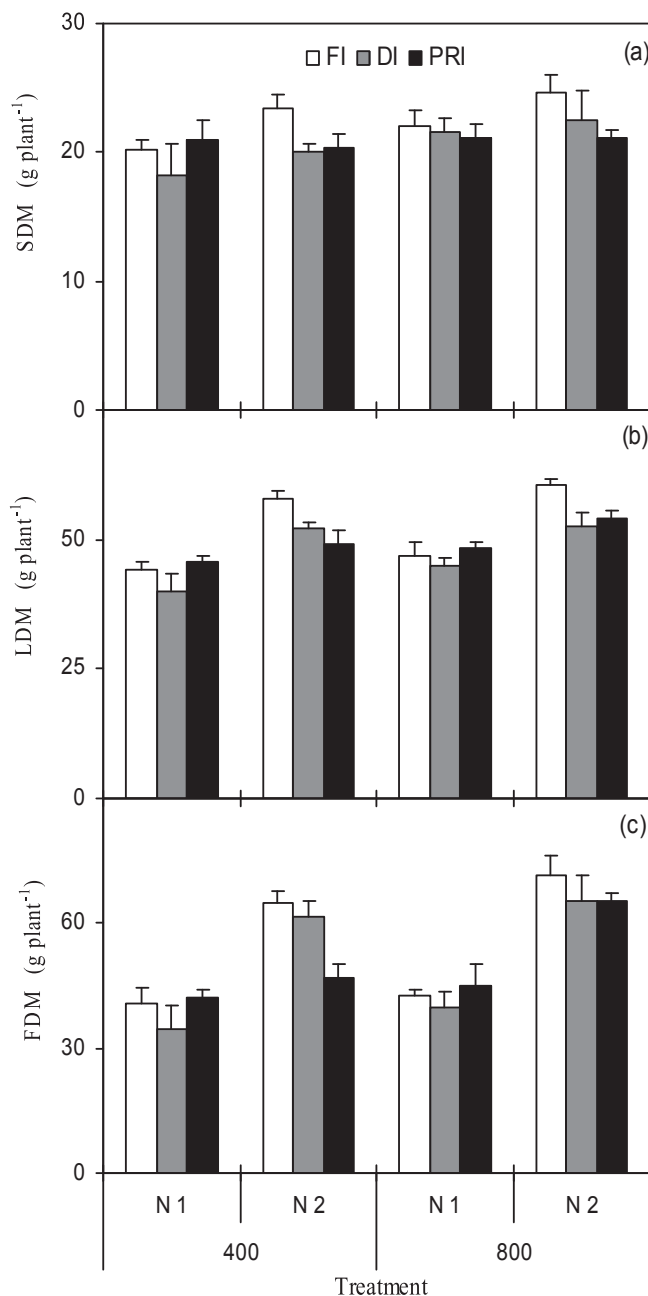


Fig. 3. Stem dry matter (SDM), leaf dry matter (LDM) and fruit dry matter (FDM) of tomato plants as affected by the atmospheric $[\text{CO}_2]$ (400 and 800 ppm), N fertilizer rates (N1 and N2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; and alternative partial root-zone irrigation, PRI). Error bars indicate standard error of the mean ($n = 4$). Statistical comparisons among the treatments are presented in Table 2.

lower TN than those grown at N2 fertilization.

3.6. Harvest index, plant water use, plant WUE and N use efficiency

Plant harvest index (HI) was only significantly affected by N treatment (Fig. 7a; Table 2). Across $[\text{CO}_2]$ and IRRI treatments, plants grown at N2 had 31.16% higher HI than those grown at N1. Plant water use (PWU) was remarkably affected by N and IRRI (Fig. 7b; Table 2). Regardless of $[\text{CO}_2]$ environment, N1 and reduced irrigation plants had 36.52 and 30.00% lower PWU than N2 and FI plants, respectively. Plant WUE (WUE_p) was only significantly affected by N treatment (Fig. 7c; Table 2). Across $[\text{CO}_2]$ and IRRI treatments, plants grown at N1 had 34.13% higher WUE_p than those grown at N2. Reduced irrigation

showed a 15.29% increased trend in WUE_p as compared with FI across $[\text{CO}_2]$ and N treatments, though the increase was not statistically significant. N use efficiency (NUE) was notably affected by N and $\text{N} \times \text{IRRI}$ (Fig. 7d; Table 2). Plants grown at N1 had 34.06% higher NUE than those grown at N2 treatment. The $e[\text{CO}_2]$ plants showed a 4.77% enhanced trend in NUE in relation to $a[\text{CO}_2]$ plants, though the improvement was not statistically significant.

4. Discussion

It is widely believed that reduced irrigation improves leaf WUE by equivalent or slight decreased photosynthetic rate (P_n) but significant declined stomatal conductance (g_s) along with transpiration rate (T_r) at leaf scale as compared with FI (Kang and Zhang, 2004). Moreover, PRI plants normally bring about greater leaf WUE than DI plants due to the alternately stimulate the higher ABA signaling of drying soil transported up to the leaf inducing stomatal closure and reducing transpiration rate, while sustaining plant-water relation and photosynthesis (Davies et al., 2002; Loveys et al., 2000; Wang et al., 2010a). In the present study, this was clearly illustrated in Fig. 1a–e; Table 1, where reduced irrigation, especially PRI strategy could enhance both leaf intrinsic and instantaneous WUE (WUE_i and WUE_n) as a result of maintained P_n coupled with lowered g_s and T_r in tomato leaf throughout the treatment period, which was in good agreement with the earlier studies (Du et al., 2006; Liu et al., 2006; Wei et al., 2016). Additionally, low N supply could limit leaf photosynthetic capacity and have an impact on the control of stomatal closure and plant growth (Bouranis et al., 2014). Here, it was found that lower N (N1) led to significantly decreased P_n larger than g_s and T_r , thereby resulting in reduced leaf WUE when compared to higher N (N2) applied (Fig. 1a–e; Table 1).

It is well known that an enhancement in leaf P_n , accompanied with reduction in g_s and T_r are generally achieved by plants grown at $e[\text{CO}_2]$ (da Silva et al., 2017; Kang et al., 2002; Pazzagli et al., 2016). The higher photosynthesis is driven by the acceleration of carboxylation and inhibition of oxygenation reaction through the modulation of Rubisco activity at the chloroplast (Ainsworth and Long, 2005), while the reduction in stomatal aperture is mainly attributed to the increase in intercellular CO_2 concentration (C_i) and depolarization in the guard cells (Ainsworth and Rogers, 2007), leading to declined leaf transpiration. Consistent with this, here a remarkable increased leaf WUE was found in plants grown under $e[\text{CO}_2]$ (Fig. 1a–e; Table 1). Accordingly, the results in this study explicitly demonstrated that in spite of the disparate mechanism of different treatment factor on modulation of leaf WUE, the reduced irrigation, particularly PRI combined with greater N fertilizer had the ability to synergistically improve WUE at leaf scale under $e[\text{CO}_2]$ environment.

It has been reported that $e[\text{CO}_2]$ plants would conserve water and improve plant water relation resulted from decreased leaf g_s (Wullschlegel et al., 2002), whereas, reduced irrigation commonly induced lower leaf water potential (Ψ_1) as compared to full irrigation (Dodd et al., 2006; Sun et al., 2013b). In good consensus with this, in the current study, plants grown in $e[\text{CO}_2]$ together with FI normally had enhanced Ψ_1 (less negative) than those grown in $a[\text{CO}_2]$ with reduced irrigation (Fig. 2a; Table 1). Here, there was similar osmotic potential (Ψ_π) among all treatments (Fig. 2b; Table 1), which may imply an equivalent solutes accumulation in the leaf cells. Furthermore, irrespective of irrigation and N treatments, a greater leaf turgor pressure (Ψ_p) was noticed in the $e[\text{CO}_2]$ -treated plants (Fig. 2c; Table 1), in line with previous findings (Yan et al., 2017).

Dry matter of stem (SDM), leaf (LDM) and fruit (FDM) as well as total above ground (TDM) was higher at $e[\text{CO}_2]$ than $a[\text{CO}_2]$ (Figs. Figure 3a–c and Figure 6a; Table 2), and FI plants commonly possessed greater LDM and TDM than the plants with reduced irrigation strategy (Figs. Figure 3b, Figure 6a; Table 2). Meanwhile, plants harvested from N1 had lower LDM, FDM, TDM and harvest index (HI) than those from N2 (Figs. Figure 3b, c, Figure 6a, and Figure 7a; Table 2). Consistently,

Table 2

Out put of three-way ANOVA for stem dry matter (SDM), leaf dry matter (LDM), fruit dry matter (FDM), stem C concentration (SCC), leaf C concentration (LCC), fruit C concentration (FCC), stem N concentration (SNC), leaf N concentration (LNC), fruit N concentration (FNC), total plant dry matter (TDM), total plant C accumulation (TC), total plant N uptake (TN), harvest index (HI), plant water use (PWU), plant WUE in dry matter (WUE_p) and plant N use efficiency (NUE) of tomato plants as affected by the atmospheric $[CO_2]$ (400 and 800 ppm), N fertilizer rates (N1 and N2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; and alternative partial root-zone irrigation, PRI). The data is presented in Figs. 3–7.

Factors	SDM	LDM	FDM	SCC	LCC	FCC	SNC	LNC	FNC	TDM	TC	TN	HI	PWU	WUE_p	NUE
$[CO_2]$	*	*	**	ns	ns	ns	ns	*	ns	***	**	ns	ns	ns	ns	ns
N	ns	***	***	**	***	ns	***	***	***	***	***	***	***	***	*	***
IRRI	ns	**	ns	*	**	ns	ns	**	ns	**	*	ns	ns	*	ns	ns
$[CO_2] \times N$	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
$[CO_2] \times IRRI$	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$N \times IRRI$	ns	**	*	ns	ns	ns	ns	*	*	**	**	ns	ns	ns	ns	*
$[CO_2] \times N \times IRRI$	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

The table reported the significance results of the three-way ANOVA on plant stem dry matter (SDM), leaf dry matter (LDM), fruit dry matter (FDM), stem C concentration (SCC), leaf C concentration (LCC), fruit C concentration (FCC), stem N concentration (SNC), leaf N concentration (LNC), fruit N concentration (FNC), total above ground dry matter (TDM), plant total C accumulation (TC), plant total N uptake (TN), harvest index (HI), plant water use (PWU), plant WUE in dry matter (WUE_p) and N use efficiency (NUE) of tomato plants as affected by the CO_2 environment ($[CO_2]$), nitrogen fertilizer rates (N) and irrigation regimes (IRRI) and their interactions.

*, ** and *** indicate significance levels at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; ns denotes no significance.

as mentioned previously, either $e[CO_2]$ and/or associated with high N supply could improve leaf photosynthetic capacity, thereby motivating translocation of photosynthates into the fruit, and further enhancing TDM of plants (Bénard et al., 2009; Li et al., 2017; Moretti et al., 2010). Moreover, low N fertilization would have a strong influence on the magnitude of primary and secondary metabolites due to the source-sink balance hypothesis (Bénard et al., 2009), hence decreasing the allocation to fruit and reduce the HI of plant. The results indicated increased N fertilization could enhance the allocation of biomass into fruit.

The $e[CO_2]$ environment has been suggested to result in lower plant water use (PWU) induced by the decline in leaf g_s (Leakey et al., 2009). Nonetheless, decreased g_s could increase leaf temperature and conversely enhance the rates of leaf transpiration (Reddy et al., 2010). On the other hand, greater LDM of $e[CO_2]$ plants most probably coincided with larger leaf area, thus promoting the transpiration of whole plant along with PWU (Fleisher et al., 2008; Li et al., 2017). In addition, the increased root system of plant at $e[CO_2]$ resulted from the improved fraction of assimilates allocated to roots enabled to exploit more water from surrounding soil ameliorating the water stress (Wullschlegel et al., 2002). Thereby, plants grown at $e[CO_2]$ might totally offset the effect of lowered g_s and ultimately bring about an increase in transpiration at plant level. In the present study, PWU was equivalent between $e[CO_2]$ and $a[CO_2]$ treatment (Fig. 7b; Table 2), which could be interpreted by the aforementioned findings. Moreover, the significant lower PWU in N1 as compared with N2 plant (Fig. 7b; Table 2) would be ascribed to the smaller leaf area of the plants.

In this study, plants grown under N1 had remarkably higher plant WUE (WUE_p) than those grown under N2 treatment (Fig. 7c; Table 2). Whilst, accumulated evidence has shown that reduced irrigation, particularly PRI strategy has a predominant advantage on improving WUE at plant level related to FI or DI treatment due to the larger decrease in water consumption than plant production (da Silva et al., 2017; Kaminski et al., 2014; Pazzagli et al., 2016; Wang et al., 2010a). Similarly, here we found that irrespective of $[CO_2]$ and N treatments, reduced irrigation presented an increased WUE_p trend as compared to FI although the increase was not statistically significant (Fig. 7c; Table 2). As the same PWU was found in plants grown under $e[CO_2]$ and $a[CO_2]$ environment, there was no notable enhancement in WUE_p of $e[CO_2]$ plants.

It is well documented that N status plays an important role in modulating C metabolism of plants as N is an essential ingredient for all of the enzymes involving in carbohydrate transport, metabolism and utilization in the plants (Nunes-Nesi et al., 2010). The relative lower plant matter and maintained photosynthesis in the reduced irrigation

would account mainly for the greater C fixation and concentration in the plant (Wang et al., 2010b). This was clearly illustrated in Fig. 4a–c; Table 2, where C concentration of stem and leaf (SCC and LCC) was higher under N2 fertilization together with reduced irrigation, despite having no effect on fruit C concentration. Most interestingly, across irrigation strategy, plants grown under $e[CO_2]$ and N2 treatment generally had greater C accumulation of fruit and total dry matter (TC), especially under N2 fertilization (Figs. Figure 3c, Figure 4c and Figure 6b; Table 2) as a result of obvious increased dry matter in N2 treatment under $e[CO_2]$ environment, indicating that $e[CO_2]$ along with sufficient N applied could be beneficial for C partitioning to fruit and attenuate the detrimental effect of water deficit on C accumulation in tomato plants.

As mentioned above, adequate N supply could predominantly improve plant development and N nutrition in plants (Bénard et al., 2009). Here, the greater N concentration of stem, leaf and fruit (SNC, LNC and FNC) as well as plant total N uptake (TN) were found in N2 than N1 plants (Figs. Figure 5a–c and Figure 6c; Table 2). While, reduced irrigation, especially PRI generally possessed higher LNC, FNC and TN than FI treatment (Figs. Figure 5a–c and Figure 6c; Table 2). It has been suggested that PRI induced spatially and temporally heterogeneous distribution of soil moisture could stimulate root growth, improve nutrients availability in the soil and facilitate uptake of water and nutrients from the wetting soils (Wang et al., 2012). Moreover, a decreased leaf g_s and T_r in PRI plants might reduce the hydrostatic tension in xylem and enhance xylem connection to the fruit, hence allowing more water and nutrients movement into the fruit (Davies et al., 2000; Sun et al., 2013b). Besides, $e[CO_2]$ environment had notably lower LNC and no influence on SNC, FNC and TNU (Figs. Figure 5a–c and Figure 6c; Table 2). It is well known that there is generally a reduction in N concentration of plants grown under $e[CO_2]$, which is mainly ascribed to the dilution of N in the greater biomass (Li et al., 2016; Loladze, 2002; McGrath and Lobell, 2013; Myers et al., 2014). Nevertheless, the increased dry matter and identical water use at $e[CO_2]$ as compared to $a[CO_2]$ condition would enhance N requirement from the root system and contribute to the no reduction in TN of $e[CO_2]$ plants. Therefore, the reduced irrigation, especially PRI associated with sufficient N supply had the capacity to enhance plant N status simultaneously under $e[CO_2]$ environment.

The N use efficiency (NUE) has been used as a long-term indicator on availability of N utilization for C acquisition in plants (Wang et al., 2010a). Grown in lower N condition would exacerbate the shortage of leaf N relative to C in plant (Stitt and Krapp, 1999). In accordance with this, the present study observed that NUE was greater in N1 than N2

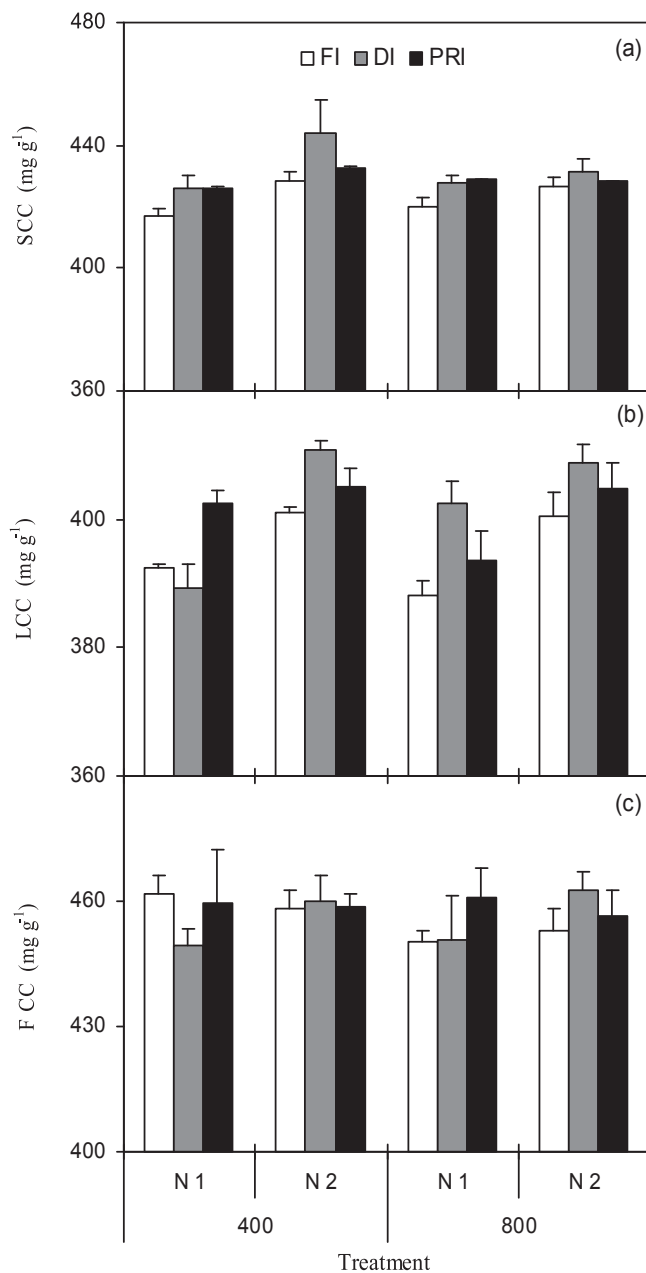


Fig. 4. Stem C concentration (SCC), leaf C concentration (LCC) and fruit C concentration (FCC) of tomato plants as affected by the atmospheric [CO₂] (400 and 800 ppm), N fertilizer rates (N1 and N2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; and alternative partial root-zone irrigation, PRI). Error bars indicate standard error of the mean (n = 4). Statistical comparisons among the treatments are presented in Table 2.

fertilizer (Fig. 7d; Table 2). Additionally, there was normally an improvement in NUE under *e*[CO₂] environment (Reddy et al., 2010), however, such response could be affected by diverse plant species, growing and nutrients circumstance (Gifford et al., 2000). Here, regardless of N and irrigation treatment, the *e*[CO₂] revealed an increasing tendency in NUE as compared with *a*[CO₂] plants, despite the enhancement was not statistically significant (Fig. 7d; Table 2), which was mostly resulted from the more improvement in uptake of C than N in the plants accompanied with the similar N uptake from *a*[CO₂] to *e*[CO₂] environment.

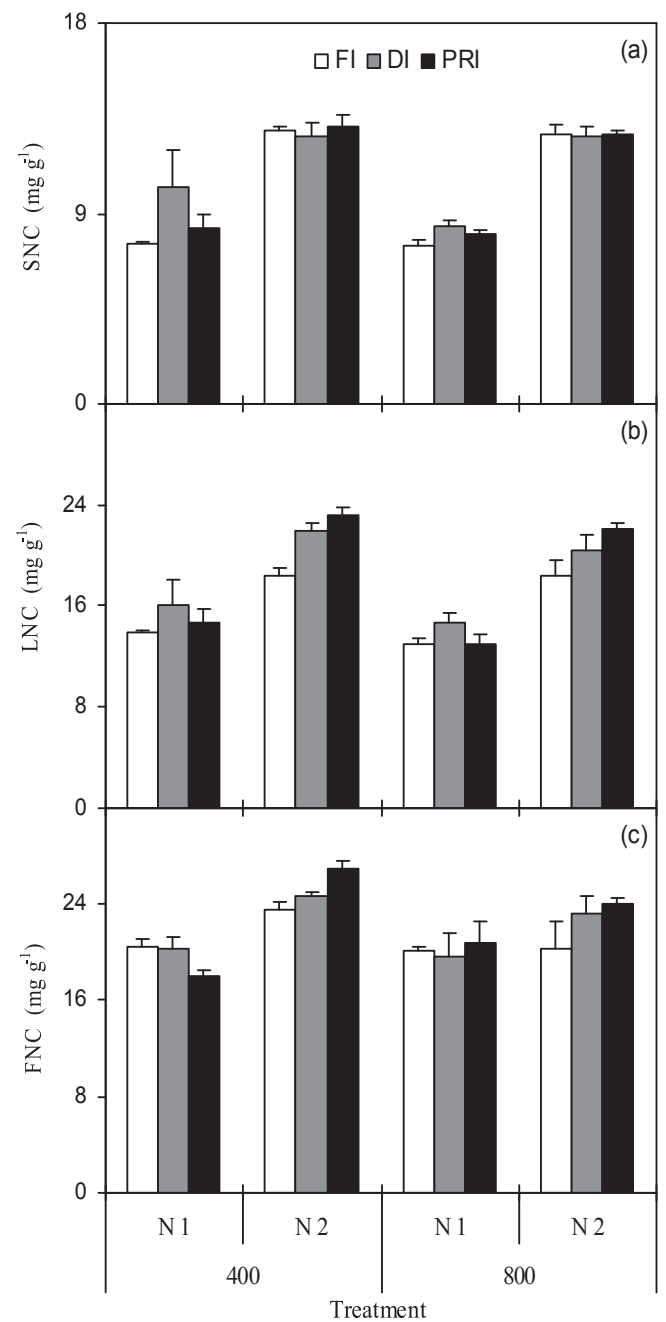


Fig. 5. Stem N concentration (SNC), leaf N concentration (LNC) and fruit N concentration (FNC) of tomato plants as affected by the atmospheric [CO₂] (400 and 800 ppm), N fertilizer rates (N1 and N2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; and alternative partial root-zone irrigation, PRI). Error bars indicate standard error of the mean (n = 4). Statistical comparisons among the treatments are presented in Table 2.

5. Conclusions

Conclusively, *e*[CO₂] coupled with higher N supply had the capacity to improve P_n and reduce g_s and T_r, resulting in the greatest WUE at leaf level. Particularly plants grown under PRI strategy associated with *e*[CO₂] environment simultaneously possessed the lower g_s and T_r without decreased leaf water status and P_n, contributing to the enhanced WUE of tomato leaf. Plants grown in *e*[CO₂] combined with greater N fertilizer could increase the plant biomass, C accumulation and N uptake under reduced irrigation regimes. Disregarding [CO₂]

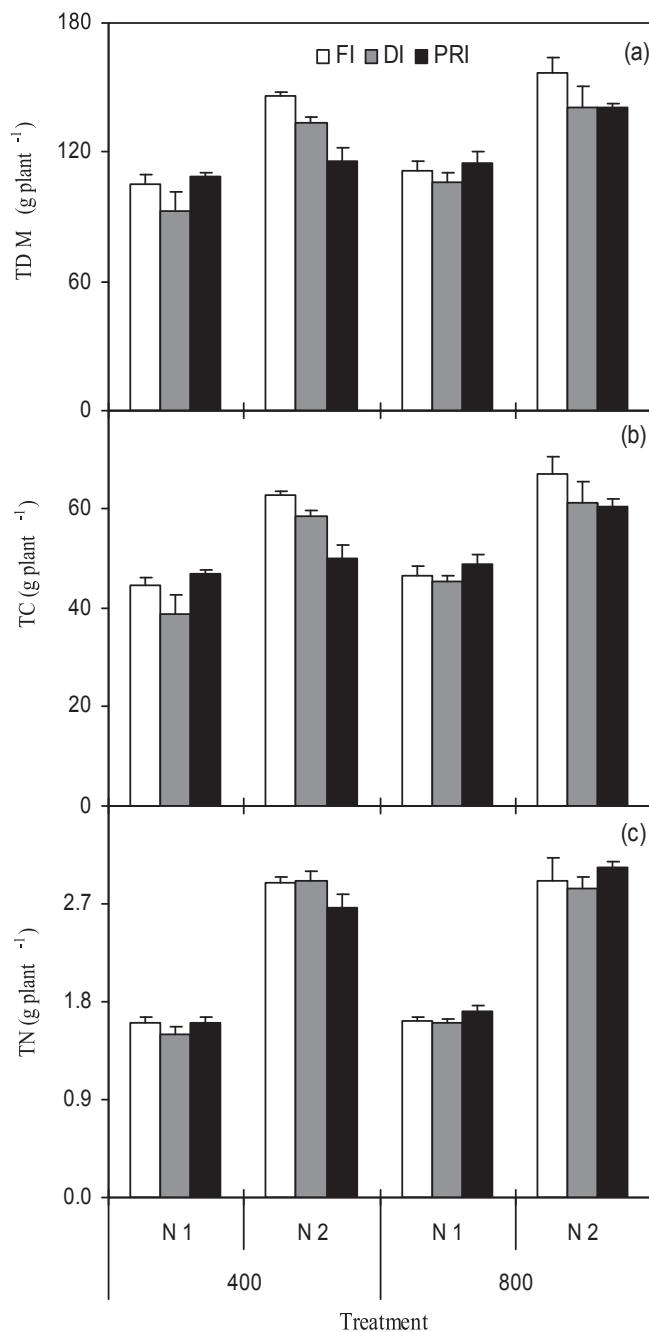


Fig. 6. Total plant dry matter (TDM), total plant C accumulation (TC) and total plant N uptake (TN) of tomato plants as affected by the atmospheric [CO₂] (400 and 800 ppm), N fertilizer rates (N1 and N2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; and alternative partial root-zone irrigation, PRI). Error bars indicate standard error of the mean (n = 4). Statistical comparisons among the treatments are presented in Table 2.

environment, both WUE and NUE at plant scale were only higher in low N fertilization. These findings will be considerably conducive to guide fertilization and irrigation strategies to utilize water and N more efficiently in a future drier and CO₂-enriched environment.

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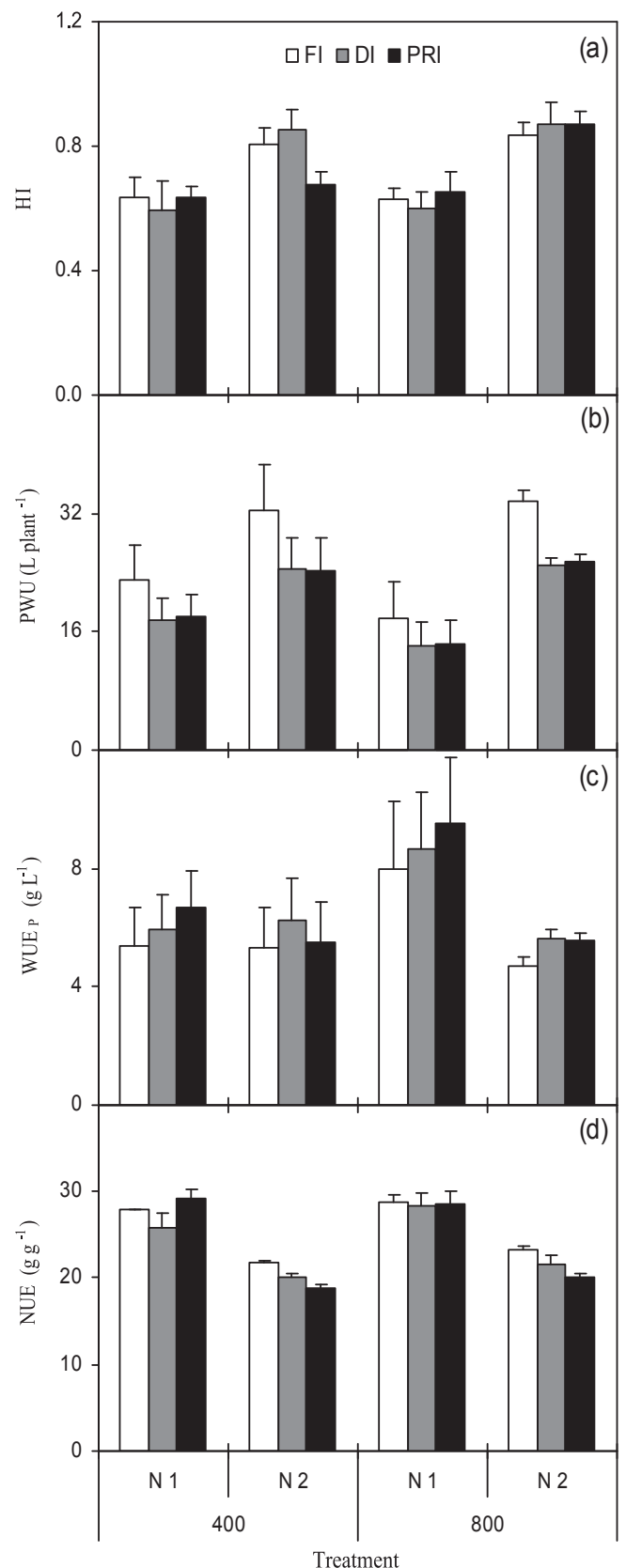


Fig. 7. Harvest index (HI), plant water use (PWU), plant WUE in dry matter (WUE_p) and plant N use efficiency (NUE) of tomato plants as affected by the atmospheric [CO₂] (400 and 800 ppm), N fertilizer rates (N1 and N2) and irrigation regimes (full irrigation, FI; deficit irrigation, DI; and alternative partial root-zone irrigation, PRI). Error bars indicate standard error of the mean (n = 4). Statistical comparisons among the treatments are presented in Table 2.

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