



Maize water use efficiency and evapotranspiration response to N supply under contrasting soil water availability



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ABSTRACT

Water use efficiency (WUEg), the quotient between grain yield and crop evapotranspiration (ET), might be increased in maize crops (*Zea mays* L.) due to N supply. Most research has focused on understanding grain yield response to N supply; so there is little and contradictory information on the influence of N supply on WUEg in water limited environments and on ET response to N supply under contrasting water availability. The objectives of our research were to elucidate whether N supply affects WUEg in water limited environments; and to clarify the expected response to N supply of maize ET and its components under contrasting soil water availability. Maize crops were grown at Balcarce, Argentina during three seasons. Treatments included two water regimes (i.e. rain-fed and irrigated) and two rates of N (i.e. 120 kg N ha⁻¹ or non-fertilized). Measurements included (i) soil water content and intercepted photosynthetically active radiation (iPAR) during the whole crop season, and (ii) grain yield and shoot dry matter at physiological maturity. Crop ET was calculated by means of a water balance and soil evaporation was estimated by means of micro-lysimeters. Our results show that N supply did not influence WUEg in water limited environments; but N supply significantly increased ET (2–8%) under all water availability conditions. Maize seasonal ET increments were closely related to the improvement of seasonal iPAR in non-water limited environments, but not in water limited environments. In non-water limited environments, ET response to N supply was mediated by the concomitant effects of iPAR increments on increasing transpiration while reducing evaporation. In water limited environments, ET slightly increased in response to iPAR increments due to N supply. The low ET increment in water limited environments with frequent low superficial soil water content (i.e. ≤ 2 mm cm⁻¹) was probably not influenced by reductions in evaporation (E); but associated with stomata closure in response to water deficiencies. This is consistent with the fact that N supply did not promote improvements in radiation use efficiency for biomass production (RUEb) in these environments.

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1. Introduction

Water availability often limits grain yield of rain-fed crops; and 12–15% grain yield reductions due to water stress were estimated for maize grown in temperate regions (Edmeades et al., 2001; Eyhéabide et al., 1996). In addition, it is expected that dry periods will become more frequent due to climate change (Solomon et al., 2007). In this context, ways to increase crops water use

efficiency (i.e. grain yield per unit of evapotranspiration, WUEg) should be sought. Management practices like mulching (e.g. Zhang et al., 2014), row spacing reductions (Barbieri et al., 2012) and N supply (e.g. Viets, 1962; Ogola et al., 2002), among others, have the potential for increasing WUEg in maize and in other crops (Hatfield et al., 2001; French and Schultz, 1984).

It is well known that N supply increases grain yield and WUEg in N deficient soils with no water limitations (e.g. Olson et al., 1964; Eck, 1984; Kim et al., 2008; Al-Kaisi and Yin, 2003; Di Paolo and Rinaldi, 2008). In these environments, grain yield increments due to N supply were mainly related to shoot biomass increments, by means of (i) greater photosynthetically active radiation interception (iPAR; e.g. Wolfe et al., 1988; Bennett et al., 1989; Uhart and Andrade, 1995; Sinclair and Muchow, 1999; Boomsma et al.,

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2009); and (ii) greater radiation use efficiency (RUEb; e.g. Sinclair and Muchow, 1999; Boomsma et al., 2009). However, there is little information and conflicting results on the influence of N supply on the evapotranspiration (ET) component of WUEg. Some reports indicate that in non-water limited environments, N supply increased ET (Pandey et al., 2000; Ogola et al., 2002; Abbas et al., 2005; Adamtey et al., 2010; Barbieri et al., 2012) or there was not a clear trend in ET response to N supply (Reddy et al., 1980; Jones et al., 1986; Fernández et al., 1996; Kim et al., 2008; Teixeira et al., 2014).

In water limited environments, grain yield and WUEg response to N supply will be closely associated with the timing and intensity of the water and N deficiencies. Water deficiencies reduce shoot biomass production through reductions in iPAR (Jones et al., 1986; Wolfe et al., 1988) and/or RUEb (Stone et al., 2001). In addition, water deficiencies during the critical period for kernel set (i.e. 30 days bracketing silking) drastically reduce grain yield through reductions in harvest index (HI; Hall et al., 1981). These responses to water deficiencies might decrease N supply effects on biomass production and grain yield. In agreement, some reports have demonstrated that N supply does not increase grain yield in water limited environments (Eck, 1984; Kim et al., 2008; Di Paolo and Rinaldi, 2008), suggesting that N supply does not increase WUEg in these environments. By contrast, other papers indicate that N supply increased maize WUEg in dry environments (e.g. Ogola et al., 2002; Teixeira et al., 2014); or proposed that management practices (including N fertilization) that affect biomass production or the interception of radiation may increase WUEg under contrasting water availability (Hatfield et al., 2001). However, most of the studies dealing with maize grain yield response to N supply under different water regimes do not include crop ET measurements. The few reports accounting for maize ET in water limited environments indicated none (Ogola et al., 2002; Teixeira et al., 2014), 6% (Barbieri et al., 2012), or more than 60% crop ET increment (Jones et al., 1986; Pandey et al., 2000; Abbas et al., 2005) in response to N supply. Therefore, reports on the role of N supply on crop ET and on WUEg in water limited environments are scarce and not consistent, highlighting the need of a greater understanding of the N supply \times water regime interaction.

Crop ET response to N supply will be the result of N effects on its components, crop transpiration (T) and soil evaporation (E). Soil water availability, iPAR and RUEb affect transpiration (Jones et al., 1986; Matthews et al., 1988; Earl and Davis, 2003); whereas the degree of soil cover by the crop and superficial soil water content are the primary factors influencing E rates from soil beneath crop canopies (Ritchie and Burnett, 1971; Ritchie, 1972; Villalobos and Fereres, 1990; Allen et al., 1998). In environments with low soil water content that limits evaporation (Ritchie, 1972; Villalobos and Fereres, 1990), ET response to N supply will mostly depend on transpiration response to N supply; whereas in non-water limited environments, ET response to N supply will be mediated by both, an increased transpiration and a reduced evaporation.

The objectives of this work were (i) to elucidate whether N supply affects WUEg in water limited environments, and (ii) to clarify the expected response to N supply of maize ET and its components under contrasting soil water availability.

2. Materials and methods

2.1. Site and crop management

Maize crops were grown at Balcarce, Argentina (37°45' S, 58°18' W; elevation 130 m), during 2011–2012 (Season 1), 2012–2013 (Season 2) and 2013–2014 (Season 3). The soil was a complex of a fine mixed Typic Argiudoll and a fine thermic

Petrocalcic Paleudoll (petrocalcic horizon at 140 cm), with a loam texture at the surface layer (0–25-cm depth), loam to clay-loam at sub-surface layers (25–110-cm depth) and sandy-loam below 110-cm depth (C-horizon) with 5.4% topsoil organic matter. Maximum water holding capacity (3.7 mm cm^{-1}) was determined according to Cassel and Nielsen (1986) and the permanent wilting point (2 mm cm^{-1}) was determined according to Richards and Weaver (1943). Experiments were conducted under conventional tillage. Crops were fertilized with 30 kg P ha^{-1} before sowing. Weeds and insects were effectively controlled. The location of the plots and treatments during Seasons 2 and 3 corresponded with those during Season 1; and initial mean soil N-NO_3^- in non-N fertilized plots were 101 ± 8.6 (Season 1), 99 ± 7.8 (Season 2) and $94 \pm 7.3 \text{ kg ha}^{-1}$ (Season 3) for the top 60 cm at sowing. Local studies indicated that grain yield responses to N supply are expected at these soil N-NO_3^- contents at sowing (Echeverría and Sainz Rozas, 2001) and thresholds of 177 kg ha^{-1} of N-NO_3^- (0–60 cm) to attain maximum grain yields were reported for no-till maize in the same location (Pagani et al., 2008).

Cumulative photosynthetically active radiation and mean air temperature during the growing seasons were close to the mean value for a series of 30 years (Table 1). Water input from rain accumulated from emergence to physiological maturity was 387 mm in season 1, 588 mm in Season 2, and 364 mm in Season 3; and water input distribution along the growing season differed among years (Table 1 and Fig. 1). In addition, reference ET accumulated from emergence to physiological maturity was 631 mm in Seasons 1 and 3 and 614 mm in Season 2. Consequently, rain-fed treatments of Seasons 1 and 3 were considered water limited environments; and irrigated and rain-fed treatments of Season 2 and irrigated treatments of Seasons 1 and 3 were considered non-water limited environments.

2.2. Plant material and experimental design

Maize hybrid DK 747 MGRR was sown on 25 Oct, Seasons 1 and 2 and on 18 Oct, Season 3. Treatments included two water regimes (rain-fed and irrigated) and two rates of N supply (0 and 120 kg N ha^{-1}). Maize plant density was 8 plants m^{-2} ; plots were over-sown and thinned to the desired plant densities at V3 (Ritchie and Hanway, 1982). The treatments were arranged in a split plot design with three replications; irrigation treatments were assigned to the main plots and fertilizer treatments were assigned to the sub plots. Sub plots comprised five rows 12 m long. Drip irrigation was performed to maintain soil water availability above 60% of soil available water during the growing cycle. Although irrigation was applied, it was not effective at maintaining soil water content above this value during Seasons 1 and 3. Fig. 1 indicates moments and amounts of irrigation for the different treatments. Fertilized treatments received 120 kg N ha^{-1} at V6 in Seasons 1 and 2 and at sowing in Season 3; N was applied broadcast in the three seasons.

2.3. Measurements

Soil water content was measured with a neutron probe (Troxler Electronic Lab., Troxler 4300, NC, USA) in each experimental unit from 12 (Seasons 1 and 2) or 13 days (Season 3) after emergence until physiological maturity. The method combined gravimetric measurements between 0 and 10 cm depth and the use of the neutron probe in 10 cm increments between 10 and 40 cm depth and in 20 cm increments from 40 to 140 cm depth. Total soil water content in each experimental unit was determined as the sum of the water content in all layers. One access tube per experimental unit was placed midway between the two harvest rows and soil water was measured approximately every 7–15 days.

Table 1
Mean photosynthetically active radiation (PAR), mean air temperature, cumulative rainfall, irrigation and reference evapotranspiration (ET_0) every month during the 2011–2012 (S1), 2012–2013 (S2) and 2013–2014 (S3) growing seasons and their corresponding mean (for PAR, mean air temperature and ET_0) or median (for rainfall) of a 30 years of data (H) at Balcarce, Argentina.

	PAR ($MJ m^{-2} d^{-1}$)				Mean air temperature ($^{\circ}C$)				Rainfall (mm)				Irrigation (mm)			ET_0 (mm)			
	S1	S2	S3	H	S1	S2	S3	H	S1	S2	S3	H	S1	S2	S3	S1	S2	S3	H
October	8	7	8	8	13	15	14	13	41	51	103	91				91	96	93	90
November	10	11	9	9	19	18	17	16	151	100	127	63				134	137	125	116
December	12	11	11	10	20	20	22	19	36	239	27	100	72	21	96	152	165	169	145
January	12	10	11	10	23	21	22	20	58	152	111	103	163	27	91	176	143	179	151
February	10	11	9	9	22	21	20	20	105	33	73	71	115	36		135	133	108	117
March	8	8	7	7	18	16	19	18	67	114	153	75				99	90	81	95

Daily values for reference evapotranspiration (ET_0), precipitation and global radiation were provided by the EEA INTA Balcarce meteorological station situated at less than 1 km from the field experiment.

Soil evaporation was measured during Season 2 (at 31, 40, 45, 54, 62, 68, 81, 95, 103, 117 and 129 days from emergence) and Season 3 (at 36, 49, 69, 76, 91, 99, 105, 120 days from emergence) using micro-lysimeters. The micro-lysimeters consisted of a plastic pipe (inside diameter 0.1 m, 0.15 m long with a wire mesh at the bottom) filled with non-disturbed soil samples taken from the inter-row. The micro-lysimeters were weighed and placed in the same spot from where the soil samples were taken. Micro-lysimeters were weighed again after 48 h. Previous evaporation measurements indicated that soil within the micro-lysimeter was representative of real evaporation for up to 48 h (Valenzuela, 2010). This time period is in agreement with Boast and Robertson (1982) and Allen (1990). Water loss from those micro-lysimeters did not account for root activity (Villalobos and Fereres, 1990).

Grain yield was determined at physiological maturity by collecting ears of consecutive plants in 10 m rows per plot. Shoot dry matter and grain yield were determined in samples of 10 plants for harvest index (HI) estimation. In all cases, the samples were taken from the third and fourth rows of each subplot, leaving borders of at least 1 m. Shoots and grain were oven-dried (forced air at $60^{\circ}C$) to constant weight and weighed.

Physiological maturity date was taken to occur when grain moisture reached 32%. Grain moisture at maturity was determined from kernel weight and kernel moisture determinations every 5–7 days during the grain-filling period, by collecting five kernels in the central part of the ear row in five consecutive plants in the second row per subplot, in all the treatments during Season 3 (not shown). The value of grain moisture at maturity obtained in this work is in the range of values reported in the literature (Carter and Ponleit, 1973). Grain moisture was measured non-destructively every 3–5

days with a calibrated ear moisture-meter (Delmhorst Instrument Co., ECT-1, Towaco, NJ, USA) during the three seasons; in the central part of ears of 5 consecutive plants in the second row per subplot at the end of the grain-filling period.

2.4. Calculations and statistical analysis

Crop evapotranspiration (ET) was calculated as precipitation plus irrigation minus the change in soil water storage between two observation dates and minus drainage. Water drainage was estimated as the difference between maximum water holding capacity and measured soil water content. Crop ET calculations assumed precipitation was effective, the application efficiency of supplemental irrigations was 100%, and runoff was zero. These assumptions were based on that no slopes were evident in the experiment layout, and the land was well drained.

Soil water content in each experimental unit was expressed either in mm or as a percentage of soil available water (AW), which was calculated as:

$$AW(\%) = \frac{SW - PWP}{SAW} * 100 \quad (1)$$

where, SW is soil water content (i.e. the sum of the water content in all layers for each date of measurement expressed in millimeters), PWP (mm) is permanent wilting point, and SAW is soil available water (i.e. the difference between maximum water holding capacity and permanent wilting point expressed in millimeters).

Daily soil evaporation (E) was calculated as:

$$E(\text{mm day}^{-1}) = \frac{\Delta W 150 \text{ mm}}{Vs \rho_w 2 \text{ d}} \quad (2)$$

where ΔW is the difference between weight of the micro-lysimeter at the beginning and at the end of the period, Vs is soil volume, 150 mm is the length of the tube, ρ_w is water density (1.0 g cm^{-3}) and 2 days.

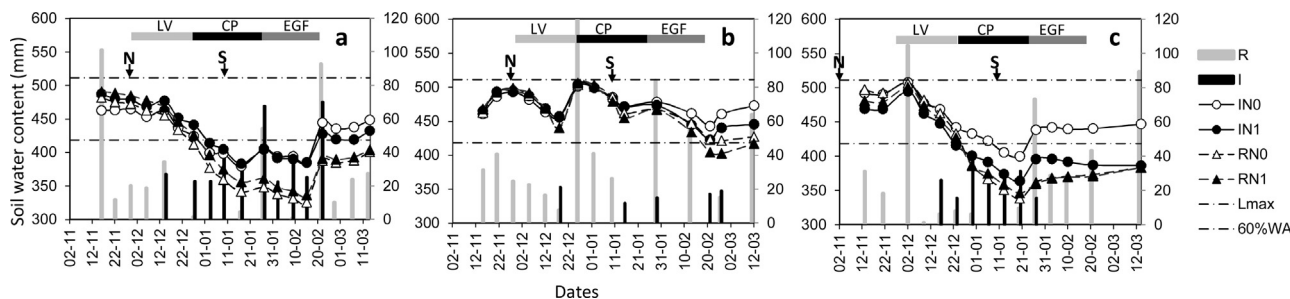


Fig. 1. Soil water content (mm) to a depth of 140 cm as a function of dates (November 2 to March 22), for crops grown under two nitrogen supplies (N0, non N fertilized and N1, fertilized) and two water regimes (I, irrigated and R, rain-fed); for Season 1 (a), Season 2 (b) and Season 3 (c). Circles and Triangles correspond to irrigated and rain-fed treatments, respectively; and white and solid symbols correspond to non N fertilized and N fertilized treatments, respectively. Upper and bottom lines indicate maximum soil water holding capacity and 60% of soil available water, respectively. Vertical gray and black bars indicate accumulated precipitation and irrigation, respectively between two water content measurements. Horizontal bars indicate phenological stages: LV, late vegetative; CP, critical period for kernel set; EGF, early grain-filling. The LGF lasted 11–17 days, with longer durations corresponding to N fertilized treatments. Emergence dates were November 2 (Seasons 1 and 2) and October 31 (Season 3). Vertical arrows labeled “N” and “S” indicate timings of N fertilizer addition and silking date, respectively.

Mean daily ET and mean E were averaged for five periods during the growing season (i) early vegetative (EV), (ii) late vegetative (LV), (iii) critical period for kernel set (CP), (iv) early grain-filling period (EGF) and (v) late grain-filling period (LGF; Fig. 1). Each growing period elapsed approximately 26–31 days; except the late grain-filling period that ranged from 11 to 17 days depending on the physiological maturity date. Longer cycle duration corresponded to N supplied treatments.

Percent photosynthetically active radiation interception (%iPAR) was calculated as $[1 - (I_t/I_0)] \times 100$, where I_t is the incident light at ground level or just below the lower level of green leaves and I_0 is the incident light at the top of the canopy. The values of I_t and I_0 were obtained with a line quantum sensor (Cavadevices. BAR-RAD 100. Buenos Aires. Argentina); measurements were done in clear sky days around noon, from 14 days after emergence until physiological maturity, following the method of Gallo and Daughtry (1986). Global radiation was transformed to PAR by multiplying by 0.5. Absolute iPAR for each day of measurement was estimated as daily incident PAR \times %iPAR; and values were interpolated and accumulated between measurements dates until physiological maturity to estimate accumulated iPAR during the season (i.e. seasonal iPAR).

Water use efficiency for grain production (WUE_g) was estimated by dividing grain yield by the accumulated seasonal crop evapotranspiration. Radiation use efficiency for biomass production (RUEb) was estimated by dividing shoot biomass by the accumulated seasonal iPAR.

Analysis of variance, using the proc mixed procedure (SAS v9) was used to test the effect of water regime, nitrogen supply and their interaction on grain yield, its determinants (i.e. HI and shoot biomass), crop ET, E , accumulated iPAR, RUEb and WUE_g . Class values were block, water (W) and nitrogen supply (N). The model statement was parameter = W|N. When interactions were significant, means were separated by test of contrasts.

3. Results

3.1. Soil water content

Soil water content dynamics was influenced by water regimes and N supply; and differed among seasons (Fig. 1). Soil water content was, in general, lower than 60% of available water during the critical period for kernel set (i.e. 30 days around silking) and from silking until physiological maturity during Seasons 1 and 3; whereas soil water content was above 60% of available water during most of Season 2, except at the end of the grain-filling period for the N fertilized treatment in rain-fed condition. Minimum soil AW values averaged across N supply treatments were lowest for the rain-fed treatments of Season 1 (23% AW) and of Season 3 (27% AW); intermediate for the irrigated treatments of Season 1 (43% AW) and Season 3 (44% AW); and highest for the rain-fed (58% AW) and irrigated (66% AW) treatments of Season 2.

3.2. Grain yield, evapotranspiration and water use efficiency

Grain yield and seasonal crop ET were closely and positively related across seasons and water regimes; and N supply effects on grain yield were only evident in non-water limited environments (i.e. irrigated treatments of Seasons 1 and 3 and irrigated and rain-fed treatments of Season 2; Fig. 2). In these environments grain yield increments ranged from 17 to 46% ($P < 0.05$; Fig. 2). Grain yield response to N supply increased across the three seasons, a response possibly associated with the soil N depletion with time (i.e. from 101 kg ha⁻¹ in Season 1, to 94 kg ha⁻¹ in Season 3 of N-NO₃⁻ in the top 60 cm of soil). In water limited environments, N supply did not

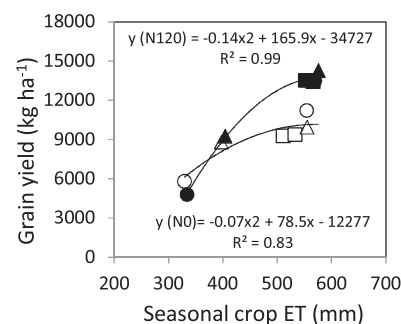


Fig. 2. Mean grain yield (kg ha⁻¹) as a function of mean seasonal crop evapotranspiration (ET, mm) for two N supply treatments (0N, white symbols; 120 kg ha⁻¹ N, solid symbols) across water regimes and three seasons (Season 1, circles; Season 2, squares; Season 3, triangles). Standard errors for grain yield were 460, 280 and 465 kg ha⁻¹ for Seasons 1, 2 and 3, respectively. Standard errors for seasonal crop ET were 7.2, 8.9 and 5.6 mm for Seasons 1, 2 and 3, respectively.

increase grain yield (i.e. rain-fed treatment of the dry Seasons 1 and 3; Fig. 2).

Grain yield increments due to N supply in non-water limited environments were in accordance with significant shoot biomass and HI increments during Seasons 2 and 3 ($P < 0.05$) and with a clear trend of biomass and HI increments during Season 1 (Table 2). The lack of yield response to N supply for the water limited environments was in agreement with the lack of biomass or HI response to N supply (Table 2). Grain yield was associated with shoot biomass across water regimes, N supply and seasons ($R^2 = 0.96$, $P < 0.05$; data not shown).

Crop ET response to N supply did not interact with water regime in any of the three seasons (Table 2). Crop ET significantly increased due to N supply in Seasons 2 and 3 ($P < 0.05$, Table 2). Mean ET increments due to N supply were 6% under no water limitations (i.e. irrigated treatment of Season 3 and rain-fed and irrigated treatments of Season 2) and 2% in a water limited environment (i.e. rain-fed treatment of Season 3; Table 2). The same trend was detectable during Season 1, but ET increments were lower (i.e. 3% for the irrigated and 1% for the rain-fed treatment) and not significant (Table 2). Larger accumulated ET values in response to N supply were evident during the grain filling period (data not shown); and were attributed to (i) greater daily ET rates during the early grain filling period in Seasons 2 and 3 ($P < 0.05$). As such, daily ET rates of N fertilized treatments were 5.4, 5.2, 4.7 and 3.7 mm d⁻¹, for irrigated and rain-fed treatments of Seasons 2 and 3, respectively; and ET rates of non-N fertilized treatments were 4.9, 4.5, 4.2 and 3.5 mm d⁻¹, for irrigated and rain-fed treatments of Seasons 2 and 3, respectively; and to (ii) longer cycle duration (Seasons 1–3) and greater ET rates (Season 3) during the late grain filling period. Mean length cycle duration across treatments and seasons was 126 days; N supply increased, on average, 3 days the length cycle duration. The increment was only significant during Season 2 ($P < 0.05$; N supply \times water regime interaction not significant).

Larger increments in grain yield than in crop ET in response to N supply resulted in significant increments (30%; $P < 0.05$) of WUE_g in non-water limited environments; whereas N supply did not affect WUE_g in water limited environments ($P > 0.05$; Table 2).

3.3. Intercepted photosynthetically active radiation and radiation use efficiency

Seasonal iPAR increased in response to N supply in all water regimes (7% mean increment, $P < 0.05$; Table 2); and %iPAR differences between N supply treatments began around silking in the three seasons (not shown). Radiation use efficiency for shoot biomass production, however, was increased due to N supply only

Table 2
Shoot biomass, harvest index (HI), seasonal evapotranspiration (ET), water use efficiency for grain production (WUEg), seasonal intercepted photosynthetically active radiation (iPAR) and radiation use efficiency for shoot biomass (RUEb) for maize grown under two contrasting N supply (0 and 120) and two contrasting water regimes (irrigated and rain-fed) for Seasons 1–3. Significance of factor effects within each season are also shown.

Season	Water regime	N supply	Biomass (kg ha ⁻¹)	HI	ET (mm)	WUEg (kg mm ⁻¹)	iPAR (MJ m ⁻²)	RUEb (g MJ ⁻¹)	
1	Irrigated	120	27,899	0.48	569	23.7*	939*	3.0	
		0	25,859	0.44	554	20.2	894	2.9	
	Rain-fed	120	14,265	0.34	334	14.5	855*	1.7	
		0	14,535	0.40	329	17.6	803	1.8	
	ANOVA								
	Water (W)			<0.0001	0.005	<0.0001	0.003	0.001	<0.001
Nitrogen (N)			ns	ns	ns	ns	0.014	ns	
W × N			ns	ns	ns	0.047	ns	ns	
2	Irrigated	120	26,018**	0.52**	566**	23.7**	964**	2.7**	
		0	19,902	0.47	533	17.6	895	2.2	
	Rain-fed	120	26,310**	0.51**	552**	24.5**	1002**	2.6**	
		0	20,067	0.46	511	18.1	913	2.2	
	ANOVA								
	Water (W)			ns	ns	ns	ns	ns	ns
Nitrogen (N)			<0.001	<0.001	0.003	<0.001	<0.001	<0.001	
W × N			ns	ns	ns	ns	ns	ns	
3	Irrigated	120	27,855**	0.51**	576**	23.6**	928**	3.0**	
		0	21,004	0.47	555	17.9	875	2.4	
	Rain-fed	120	19,374	0.48	403*	22.9	878**	2.2	
		0	18,547	0.48	397	22.1	788	2.4	
	ANOVA								
	Water (W)			0.001	0.008	<0.001	0.044	0.009	0.009
Nitrogen (N)			0.002	0.043	0.049	0.002	0.008	ns	
W × N			0.008	0.043	ns	0.005	ns	0.013	

ns stands for non-significant effect.

* Significant differences between N treatments within water regime at $P < 0.1$.

** Significant differences between N treatments within water regime at $P < 0.05$.

in non-water limited conditions of Seasons 2 and 3; and RUEb increments averaged 22% ($P < 0.05$; Table 2). The response of RUEb to N supply presented the same trend in the irrigation treatment of Season 1, but RUEb increment was lower (3%) and not significant (Table 2).

In general, the onset of differences in ET corresponded to the onset of significant differences in iPAR (data not shown); and seasonal crop ET increments were closely related to iPAR increments in response to N supply in non-water limited environments (Fig. 3; $P < 0.05$). As such, seasonal iPAR increments ranging from 5 to 10% were associated with seasonal ET increments ranging from 3 to 8%. In contrast, in water limited environments, although iPAR increments due to N supply were evident and ranged from 7 to 11%, seasonal ET increments were lower than 2% (Fig. 3).

3.4. Evaporation

Water content in the 0–10 cm soil layer during the reproductive period ranged from 12 to 32 mm across water regimes, N

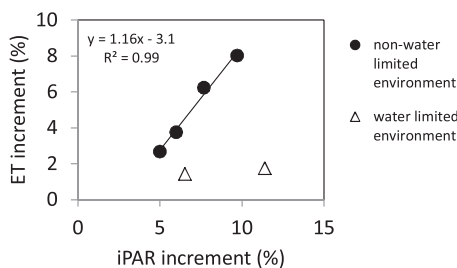


Fig. 3. Mean seasonal crop ET and mean seasonal iPAR increments due to N supply in a non-water limited environment (circles) and in a water limited environment (triangles), for maize grown under two contrasting N supply (0 and 120 kg N ha⁻¹) across three seasons. Standard errors for seasonal crop ET increments were 2.7, 1.1 and 1.3% for Seasons 1, 2 and 3, respectively. Standard errors for seasonal iPAR increments were 3.0, 2.6 and 2.6% for Seasons 1, 2 and 3, respectively.

treatments and phenological stages (CP, EGF, LGF; Fig. 4a). Normalized E (i.e. E/ET_0) increased with increasing soil moisture ($P < 0.05$; Fig. 4a). Normalized E variability was larger at soil water content higher than 2 mm cm⁻¹; and residuals of the fitted equation between normalized ET and soil water content (i.e. observed–estimated) were significantly explained by %iPAR ($P < 0.05$; Fig. 4b). Frequency of occurrence of soil water content (0–10 cm) ≤ 2 mm cm⁻¹ for a total of 9 observations during the reproductive period, was 0 and 0.1 for the irrigated and rain-fed treatments of Season 2, and 0.4 and 0.6 for the irrigated and rain-fed treatments of Season 3, respectively. Significant daily evaporation rate reductions occurred during the critical period (i.e. mean 18% E reduction; $P \leq 0.07$) and the early grain-filling period (i.e. mean 24% E reduction; $P < 0.05$) in Season 2; and during the late grain-filling period (i.e. mean 30% E reduction; $P < 0.05$) in Season 3. These E reductions were in accordance with high superficial soil water contents and %iPAR increments in response to N

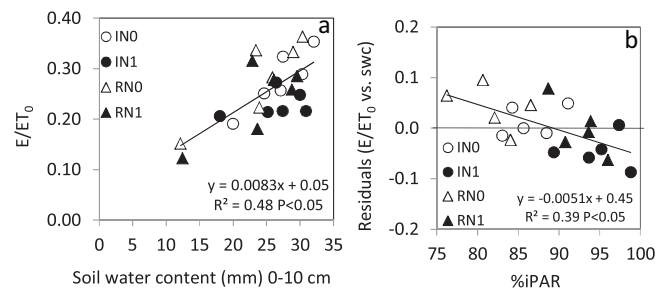


Fig. 4. (a) Daily soil evaporation normalized by reference daily evapotranspiration (E/ET_0) as a function of soil water content (mm) on the top 10 cm; (b) residuals of equation fitted to the E/ET_0 –soil water content relationship as a function of %iPAR for maize grown under two contrasting N supply (0 and 120) and two contrasting water regimes (irrigated and rain-fed) during Seasons 2 and 3. Soil water content and %iPAR represent the mean values for each phenological stage during the reproductive period (CP, EGF, LGF) for each year and treatment. Residuals corresponding to soil water content ≤ 20 mm are not shown in (b).

supply. Thus, mean superficial water content values were 3 and 2.4 mm cm⁻¹, and %iPAR increased from 87% to 90% and from 85 to 96% during the critical period and the early grain-filling periods in Season 2, respectively. During the late grain-filling period in Season 3, mean superficial water content was 3.1 mm cm⁻¹ and %iPAR increased from 80 to 96% in response to N supply.

4. Discussion

The distribution and quantity of precipitations along with irrigation treatments provided contrasting patterns of soil water content over the seasons. Severe soil drying during the critical period for kernel occurred during Seasons 1 and 3, and a late moderate soil drying during Season 2 (Fig. 1). Grain yield was closely associated with seasonal ET (Fig. 2); and results of this study were in accordance with the boundary functions for WUE derived from Grassini et al. (2009) for non N-deficient maize crops.

Water use efficiency for grain production was increased due to N supply in non-water limited environments only (i.e. irrigated treatments in Seasons 1 and 3 and irrigated and rain-fed treatments in Season 2), and increments ranged from 17 to 35% (Table 2). Other reports have also shown WUEg increments at high or moderate soil water availability (Olson et al., 1964; Kim et al., 2008; Al-Kaisi and Yin, 2003; Di Paolo and Rinaldi, 2008). Greater WUEg in crops well supplied with N was the result of larger grain yield increments (38% mean grain yield increment) than of ET increments (5% mean ET increment) for environments with non-water limitations (Fig. 2; Table 2). The ecophysiological processes underlying shoot biomass and grain yield response to N supply were well documented in the literature (e.g. Uhart and Andrade, 1995; Boomsma et al., 2009). Briefly, shoot biomass response to N supply was associated with an increased leaf area (Bennett et al., 1989), an extended green leaf area duration (Wolfe et al., 1988; Echarte et al., 2008), a greater RUE (Sinclair and Muchow, 1999) and greater leaf photosynthesis (Echarte et al., 2008). These effects are in accordance with the N effects on crop physiological variables found in this work; which were mainly evident during the reproductive periods (data not shown). Nitrogen supply also increased HI in non-water limited environments; however, N effects on biomass production were greater (26%) than those in HI (10%). Harvest index increments due to N supply have also been reported in the literature, and were associated with plant growth rate increments during the critical period for kernel set (Uhart and Andrade, 1995).

Water use efficiency for grain production was not affected by N supply in water limited environments (i.e. rain-fed treatments of Seasons 1 and 3; Table 2, Fig. 2). In these environments, grain yield was not influenced by N supply (Fig. 2; Table 2) whereas seasonal ET slightly increased due to N supply (significant in 1 of 2 years; Table 2). Other reports also indicate that N level had no effect on either shoot biomass or grain yield under severe water stress (Eck, 1984; Bennett et al., 1989; Di Paolo and Rinaldi, 2008). The lack of a significant yield response to N supply in the water limited environments of this work was associated with no effects of N supply on RUEb and on HI (Table 2); however, N supply increased seasonal iPAR in water limited environments (Table 2). In contrast, Ogola et al. (2002) and Teixeira et al. (2014) reported WUEg increments due to N supply in dry environments; associated probably with a positive trend of RUEb in response to N supply (Teixeira et al., 2014). Also, limitations to N mineralization and/or to nutrient transport to the root surface in dry soils might have influenced in part the lack of grain yield response to N supply (Buljovic and Engels, 2001). Hatfield et al. (2001) proposed that WUEg variation within a season can be attributed to any soil management practice that affects biomass production or the interception of radiation

for plant growth. Our results confirm that contention and demonstrated further that N supply increased both, WUEg and water use, in a non-water limited environment. Moreover, N supply did not increase WUEg in a water limited environment that did not promote biomass response to N addition (Table 2). The lack of biomass response to N, however, was attributed mainly to no RUEb response to N supply (Table 2).

Seasonal crop ET was significantly increased by 5% due to N supply during Seasons 2 and 3, and a similar trend was evident in Season 1 (Table 2). The lack of a significant effect of N supply on ET during the first season of this work might be related to a lower soil N deficiency compared with the other seasons. The low but significant ET increment is in close agreement with a previous work of our group dealing with row spacing and N supply in maize (Barbieri et al., 2012). Lenka et al. (2009) and Ogola et al. (2002) also showed significant ET increments due to N supply at low and at high soil water availability. Evapotranspiration increments due to N supply were attributed mainly to consistently greater accumulated ET during the grain-filling period (not shown); associated probably with greater sustained leaf photosynthesis under high-N conditions (Echarte et al., 2008). As such, crop ET increments were attributed to both, longer cycle duration and daily ET rates increments due to N supply. Greater daily ET rates during the early grain-filling period might have been the result of a greater soil water extraction capacity by the roots (Lenka et al., 2009) and/or a greater transpiration capacity by the canopy (i.e. greater leaf area index and/or greater leaf stomata conductance).

Soil water evaporation increased at increasing superficial soil water content (0–10 cm; Fig. 4a) in agreement with previous reports (e.g. Ritchie, 1972; Villalobos and Fereres, 1990; Allen et al., 1998). It was evident a threshold of superficial soil water content ≤ 2 mm cm⁻¹ below which *E* rates were entirely influenced by soil moisture; whereas *E* variability above that threshold was explained in part (40%) by %iPAR (Fig. 4b). A similar threshold of soil water content for the first 15 cm was established by Villalobos and Fereres (1990) for soils beneath different crop canopies. Therefore, results of this study indicate that in water limited environments (i.e. frequent superficial soil water content ≤ 2 mm cm⁻¹; Fig. 4a), the slight ET increment in response to N supply (Table 2) was likely attributed to a small increment in transpiration that was not partially offset by a reduced evaporation. In contrast, in non-water limited environments, crop ET increments in response to N supply were mediated by both, an increased transpiration and a reduced *E*. However, the contribution of *E* reductions to ET response to N supply varied with soil water content (Fig. 4a).

Maize seasonal ET increments in response to N supply were closely associated with seasonal iPAR increments in non-water limited environments (Fig. 3). In water limited environments, lower ET increments were evident in response to iPAR increments due to N supply; indicating further limitations possibly due to stomatal closure in response to water deficiencies (Bennett et al., 1989). In agreement, N supply did not increase RUEb in these water limited environments (Table 2). The close association between seasonal ET and iPAR responses to N supply (Fig. 3) provides a possible reason for the discrepancies among published work accounting for ET response to N supply (Reddy et al., 1980; Jones et al., 1986; Fernández et al., 1996; Pandey et al., 2000; Ogola et al., 2002; Abbas et al., 2005; Kim et al., 2008; Adamtey et al., 2010; Barbieri et al., 2012; Teixeira et al., 2014). As such, low ET response to N supply can be expected in environments with no water limitations and low iPAR response to N supply (e.g. Teixeira et al., 2014); while large ET response might be expected in non-water limited environments with large iPAR improvements due to N supply. In water limited environments, low ET increments due to N supply can be expected.

5. Conclusions

Our results clearly demonstrate that N supply did not increase WUEg in our water limited environments; and that N supply significantly increases seasonal ET under all water conditions. In addition, our results indicate a close association between seasonal ET and iPAR in response to N supply in non-water limited environments, but not in water limited environments. In non-water limited environments, ET response to N supply was mediated by the concomitant effects of iPAR increments on increasing transpiration while reducing evaporation. In water limited environments, N supply slightly increased seasonal ET. The low ET response to N supply in these environments was probably not influenced by evaporation reductions; but may have been associated with stomata closure in response to water deficiency. In agreement with this, N supply did not promote RUEb improvements in water limited environments. Our results demonstrated for the first time the close relationship between seasonal crop ET and iPAR in response to N supply in non-water limited environments; thus, elucidating uncertainties regarding expected crop ET responses to N supply.

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