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Physiological evaluations of recent drought-tolerant maize hybrids at varying management-imposed stress levels.

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ABSTRACT

Maize (*Zea mays* L.) improvement in drought-stress tolerance poses a great challenge as the global need for food, fiber, and fuel increases. Seed companies are developing and promoting drought-tolerant hybrids, but their physiological drought-tolerance mechanisms are not well understood. The research objective was to investigate the plant traits related to yield improvement for similar maturity hybrids classified as either drought-tolerant (non-transgenic) or conventional at varying plant density (PD) (two levels) and N rates (four levels) over two site-years in Northwestern Indiana. Physiological measurements included photosynthesis (*A*), transpiration *(E*), and leaf area index at multiple growth stages, as well as anthesis-silking interval, potential kernel number, grain yield (GY) and its components. Intensive heat and drought stress occurred in the 30d period prior to and during flowering in 2012, but not in 2011. Overall, similar maturity drought- and non-drought-tolerant hybrids did not markedly differ in GY or most other traits, and hybrid responses to varying PD and N rates were similar. In both seasons, GY was impacted most by N rates. *A* complex N rate effect on *A* and *E* was tightly related to water supply (i.e. higher N had positive impact under non-drought conditions). Hybrid differences in *A* and *E* were not significant at the leaf-scale, but one drought-tolerant hybrid had lower estimated cumulative *A* and *E* at the season-long canopy scale. Under the non-drought and specific-drought conditions in these single-location trials there was no indication that designated drought-tolerant hybrids were more tolerant to high crowding intensity and/or low N stresses.

Abbreviations: *A*, Photosynthesis; *E*, Transpiration; LAI, Leaf area index; VPD, vapor pressure deficit; KN, kernel number; KW, kernel weight; PKn, Potential kernel number; ASI, Anthesis-silking interval; BM, Biomass; HI, Harvest index; PD, Plant density; GY, Grain yield.

INTRODUCTION

Maize (*Zea mays* L.) is the world's largest grain crop in total production on a MT basis (FAOSTAT, 2010). Given the reliance on maize for food, fiber, and fuel, continued improvement in maize grain yields (GYs) is a substantial challenge. Maize, however, is highly sensitive to drought, specifically two weeks prior- and post-silking (Bänziger et al., 2000; Tollenaar and Lee, 2011). Drought is one of the most limiting factors constraining maize GYs in the United States (Campos et al., 2006). Compounding this problem is that greater drought frequency and intensity is being forecasted for major maize production areas (Rind et al., 1990; Campos et al., 2004; Markelz et al., 2011).

In response to these challenges, seed companies are employing diverse strategies for improving crop tolerance to drought stress. Non-transgenic drought-tolerant hybrids are already available commercially, and transgenic versions will be commercially available in 2013. Yield gains of 8.9% and 1.9% were claimed by a privately owned seed company under respective drought and non-drought conditions for the non-transgenic drought-tolerant versus conventional hybrids (Pioneer, 2013). These drought-tolerant hybrids have largely been evaluated in dryland production regions that experience regular drought and low to moderate grain yields (i.e. < 9.0 Mg ha^{-1}). Such hybrids, however, could prove beneficial for higher-yielding areas like the Eastern Corn Belt that experience periodic- or short-drought intervals if they remain competitive in non-drought conditions. Research efforts characterizing the physiological effects of drought and heat stresses (individually or combined) in maize has been increasingly documented (Jamieson et al., 1995; Cichino et al., 2010; Rattalino Edreira et al., 2011; Chen et al., 2012; Kebede et al., 2012). Nevertheless, due to the complexity inherent in combined drought and heat stresses, less is known concerning the effects of drought timing, duration, and intensity on the main crop physiological processes involved in GY formation.

Past research has investigated the physiological effects of water stress in maize (Denmead and Shaw; 1960; Acevedo et al., 1971; Sanchez, 1983; NeSmith and Ritchie, 1992a; Cakir, 2004; Markelz et al., 2011) as well as physiological traits that may confer increased drought tolerance (Bäzinger et al., 2000; Campos et al., 2004; 2006; Blum, 2009; Lopes et al., 2011). There are no known public and physiology-focused research publications that have investigated these recently released drought-tolerant hybrids. Physiological mechanisms suggested for increased tolerance in one group of new hybrids include reduced excess water use via improved stomatal control, and higher photosynthetic rates and increased stay green under drought stress conditions (Pioneer, 2013). Increased plant densities (PDs) have also been advocated for these newer hybrids (Grower System Trials, 2012), which may suggest differential behavior to the crowding intensity stress.

This study investigates physiological responses of both non-drought and commercially designated drought-tolerant hybrids of comparable maturity at varying PD and N rate levels with specific research objectives of: i) examining photosynthesis (*A*) and transpiration (*E*) patterns at multiple growth stages, ii) investigating and quantifying plant traits [leaf temperature, leaf vapor pressure deficit (VPD), leaf area index (LAI), anthesis-silking interval (ASI), potential kernel number (PKn), soil profile water changes , whole-plant biomass (BM), grain harvest index (HI), GY and its components (kernel number –KN- and weight – KW)] potentially associated with the drought tolerance property, and lastly, iii) improving scientific understanding of the complex interplay exerted by management factor levels and combinations on maize plant responses.

MATERIALS AND METHODS

Location and Experimental Design

The experiment was conducted over two growing seasons (2011-12) in Northwest Indiana at the Pinney Purdue Agricultural Center (PPAC) (41°26'49" N, 86°55'42" W). Non-irrigated field-experiments were established on Tracey sandy loam (coarse-loamy, mixed mesic Ultic Hapludalfs). In both seasons, the preceding crop was soybeans [*Glycine max* (L.) Merr.], and the tillage employed was fall chisel plow and spring secondary tillage.

A five replicate split-split plot design was utilized with hybrid as the main plot, plant density (PD) as the subplot, and N rate as the sub-subplot. In 2011, one hybrid designated as drought-tolerant (DuPont Pioneer AQUAmax^{TM} brand P1151 AM1, DuPont Pioneer Hi-Bred Intl., Inc., Johnston, IA) was compared with two similar 111 comparative relative maturity (CRM) hybrids with less drought tolerance (DuPont Pioneer brands P1162 XR and P1184 HR). Drought tolerance scores as determined by DuPont Pioneer on a 9 point scale (1= low, 9 = high) were 9, 8, and 7 for P1151, P1162, and P1184, respectively. In 2012, four hybrids were compared consisting of two pairings with different drought-tolerance: 111 (DuPont Pioneer AQUAmax^{TM} brand P1151 HR versus DuPont Pioneer brand P1162 HR, respectively) and 114 CRM hybrids (DuPont Pioneer AQUAmax^{TM} P1498 HR versus DuPont Pioneer 33D49 HR, respectively). Drought scores for P1498 and 33D49 were 9 and 7, respectively. For the results and discussion to follow: Hybrid 1 = AQUAmax^{TM} P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax^{TM} P1498, Hybrid 4 = 33D49, Hybrid 5 = P1184. The two PD levels were 79,000 (PD1) and 109,000 (PD2) for 2011, and 79,000 (PD1) and 104,000 (PD2) pl ha^{-1} for 2012. All plots received 26 kg N ha^{-1} (19-17-0) of starter N at planting, and side-dress UAN (28-0-0) treatments of either 0 (Nr1), 134 (Nr2), 202 (Nr3), or 269 (Nr4) kg N ha^{-1} band-applied around

V5 (Abendroth et al., 2011) in both years. Intensive measurements were performed in three replicates whose individual plots measured 4.6 meters wide (six 76 cm rows) by 27 meters long (18 m long for the remaining two replicates).

Maize development and growth stages were recorded in all plots both seasons (Table 1). For emergence (VE), silking (R1), and physiological maturity (R6), dates correspond to when 50% of plants reached each stage. Vegetative stages (V5, V10, and V15) were determined by the most recent fully expanded (collared) leaf and reproductive stage R3 when kernels were at milk stage. Soil fertility data analyzed by A&L Great Lakes Laboratories, Inc. was obtained from twenty 30-cm depth cores (2-cm diameter) (Table 1).

Photosynthesis (A) and Transpiration (E) at the Leaf Level

A Li-Cor 6400XT (LI-COR, Lincoln, NE) was used to determine rates of photosynthesis (*A*, CO_2 exchange rate) and transpiration (*E*), leaf temperature, and vapor pressure deficit (VPD); all parameters determined at the leaf-scale. Measurements were taken at R3, R4, and R5 stages in 2011 and at V10, V12, V15, R1, R3, R4, and R5 stages in 2012. Fewer sampling times during 2011 was due to unavailability of the Li-Cor 6400XT early in the season, as well as funding and labor restrictions. In 2012 at R1, Hybrids 1 and 2 were measured 7 days earlier than Hybrids 3 and 4 to correspond more closely with the mean 50% silking date. Measurements were performed in the center two rows on all hybrids and PDs but only at two N rates (Nr1 vs. Nr3), in three replications. Two plants were measured per plot and two measurements were taken at the midpoint on each leaf (opposite the mid-rib). During the vegetative phase the youngest collared leaf was selected, while the ear leaf was used during the reproductive period. An isolation technique was performed [Xia (2012), modified from Dwyer et al. (1995) and Earl and Tollenaar (1999)]. Representative plants were selected for measurements and neighboring plants within

0.50-0.75 m were removed to eliminate shading. Plants were allowed to acclimate to the full sunlight for 1 hour prior to measurements; however, in 2012 during a severe drought stress period, acclimation time was reduced to 20-30 minutes.

In order to minimize environmental influence, measurements were taken on cloudless days and artificial light was used. Settings for the Li-Cor 6400XT were: i) photosynthetic photon flux density (PPFD) set at 1500 µmol photons m^{-2} s^{-1}; ii) sample chamber CO_2 held constant by the CO_2 mixer at 400 µmol CO_2 mol^{-1} air; and iii) flow set at 400 µmol air s^{-1}.

Leaf Area Index (LAI)

Leaf area index (LAI) was estimated by using the Li-Cor 2200 Plant Canopy Analyzer (LI-COR, Lincoln, NE) in both years. Three measurements were taken at ground level in the center rows, one at the ¼ row position, one at the ½ row position, and one at the ¾ row position. Individual plot LAI values were means of these three measurements.

Grain Yield and Components

Potential kernel number (PKn) was determined in 2012 to analyze whether drought conditions resulted in significant treatment differences. A procedure similar to one utilized by Ciampitti (2012) was followed in which 5 consecutive ears were harvested at R1 from all treatments in three replications and PKn were counted using magnification. At maturity, GY and its components were determined in both seasons. Actual kernel number per plant (KN) and kernel weight (KW, adjusted to 155 g kg^{-1}) were determined from the average of 20 consecutive ears from the center rows at R6 in each treatment for three replications. Biomass (BM) and grain harvest index (HI) were calculated from 10 plants harvested from center rows of each treatment for three replications. Grain HI was calculated as the grain weight to the total BM ratio at maturity. Plot GYs (adjusted to 155 g kg^{-1}) were calculated after harvesting (plot combine) the central two rows.

Soil Water Balance

Soil moisture meters (Field Connect, John Deere, Moline, IL) were inserted to a depth of 1.0 m in just four plots (equipment/funding constraints) within close proximity to minimize topographical and soil variation. Hybrids 1 and 2 were selected at PD1 and at Nr1 and Nr3 to coincide with *A* measurements. Meters were installed at V5 stage, calibrated by John Deere for soil type, and volumetric soil moisture was recorded every thirty minutes at five depths (10, 20, 30, 50, 100 cm). These soil moisture values were interpolated across depths, and integrated daily within growth stage intervals at each soil depth range in order to calculate cumulative changes in soil water balance for the entire maize growing season.

Statistical Analysis

The analysis of variance (ANOVA) was executed using SAS PROC MIXED (SAS Institute, 2004) and differences among treatment means were also analyzed (Table 2). For photosynthetic parameters, the VPD factor was found to be an influential and significant covariable at V12 and V15 stages in 2012. Models evaluated were fit using GraphPad Prism 5 software (Motulsky and Christopoulos, 2003).

RESULTS AND DISCUSSION

Phenology and Growing Seasons

Soil parameters were similar across years while timing of phenological development varied slightly due to different planting dates and climate conditions (Table 1; Fig. 1A, B). The 2011 growing season experienced near normal temperatures and precipitation (historical trend can be reviewed at Ciampitti and Vyn, 2011) with 209 mm of rainfall occurring during the critical months of June and July. Above-normal temperatures and record drought conditions with only 61 mm rain from June 1 to mid-July in 2012 resulted in severe plant stress being most evident during the V12-R1 interval (Table 1).

Overall Photosynthesis and Transpiration Patterns

Treatment effects of all reported parameters can be viewed in Table 2 while photosynthetic (*A*, CO_2 exchange rate) and transpiration (*E*) rates are presented in Tables 3 and 4, respectively. Measurements of *A* at leaf- and canopy-scales have been shown to decline steadily during the growing season in field-grown maize (Wolfe et al., 1988; Kang et al., 2000; Kim et al., 2006; Leakey et al., 2006; Echarte et al, 2008; Markelz et al., 2011; Xia, 2012). In this study, both years generally followed this growing-season pattern (following the senescence process).

In 2012, heat and drought stresses substantially reduced *A* and *E* during the late vegetative-phase (V12, V15 and R1) (Fig. 1C; Tables 3 and 4). Declining *A* at reduced leaf water potentials in maize is well documented at the leaf-scale (Lawlor and Fock, 1978; Ackerson 1983, Schussler and Westgate, 1991b; Zinselmeier et al., 1995; Kang et al., 2000; Cruz de Carvalho et al., 2011; Markelz et al., 2011) and has also been noted at the canopy-level (Suyker et al., 2004). Drought effects in 2012 were ameliorated somewhat by precipitation events during late R1 that elevated the *A* and *E* rates for the remaining grain-fill period (Fig. 1C; Tables 3 and 4).

Heat and drought intensity reduced CER and then BM as evidenced by the lower LAI in 2012 as compared with 2011 season (Fig. 1D, Table 5). In addition, LAI readings were also affected by leaf rolling; thus, LAI reflected more “effective” than actual plant leaf area.

Reduction in LAI impacted not only the incident photosynthetically active radiation (PAR) captured but also the C utilization efficiency (Xianshi et al., 1998; Stone et al., 2001). The 2012 drought conditions occurred when maize is most sensitive to water stress not only at flowering but also during the late-vegetative-phase (as documented by Denmead and Shaw, 1960; Claasen and Shaw, 1970a, b; Chapuis et al., 2012). In addition, similar reductions in leaf area and/or BM due to drought stress were documented by Hall et al. (1981), Sinclair et al. (1990), and Otegui et al. (1995), and in GY (and its components) by Shaw (1977), Westgate and Boyer (1986), Grant et al. (1989), and NeSmith and Ritchie (1992a,b), among several others.

Growth stage evaluation: Photosynthesis (*A*) and Transpiration (*E*) per unit leaf area

In 2011, *A* was found to be primarily affected by N rate at each measured stage (Table 2 and 3). Averaged across growth stages, *A* increased by ~6 micromoles CO_2 m^{-2} s^{-1} when moving from low to high N rate. In 2012, N rate significantly affected *A* only at R4 and R5 stages (Table 2 and 3). The lack of N rate effect earlier in the season might be associated with the interaction between N and water supply: low water status prior to silking caused a delay in the positive impact of N rate on maize leaf *A* until later in the season (when water was available). The only hybrid difference between drought-tolerant and non-drought tolerant hybrids occurred at R5 with Hybrid 3 > Hybrid 4 (Δ 7 units; Table 3). Apparent hybrid variation at V12 and V15 was mainly dependent on vapor pressure deficit (VPD) and leaf temperatures associated with sampling time (with similar trend for *E*). Higher leaf temperatures and VPD due to later sampling time within a day (11 a.m.) during combined stress conditions (drought + heat) resulted in reduced *A* (Supplementary Fig. 1). Similar results of reduced *A* under elevated temperatures (above 38 ^{o}C) have been documented by Crafts-Brandner and Salvucci (2002), with a comparable leaf temperature critical threshold value (~37-38 ^{o}C).

Similar N rate and PD effects on *E* were observed in 2011 (Table 2). Nitrogen rate exerted a positive and significant influence on *E* by ~1.6 units at R3 and R4, and by 0.3 units at R5, while the impact of PD was minor (Table 4). In 2012, the higher N rate significantly increased *E* during the late reproductive period by ~0.8 units (Table 4). A hybrid by PD interaction was observed at R4 with Hybrid 4 showing the negative effect of higher PD on *E*.

In summary, across both years, the N rate was the main factor that positively influenced *A* and *E*, especially at later reproductive stages. A previous maize study in Indiana also showed similar N rate effects (Xia, 2012). In two studies comparing varied N rates on older and newer maize hybrids, *A* increased with higher N rates, with a more significant difference later in the season (Ding et al., 2005; Echarte et al., 2008). Both *A* and *E* were often similar across hybrids at each individual stage. A similar lack of hybrid differences in *A* for lines differing in drought tolerance was also documented in a study by Bunce (2010). However, Bunce (2010; 2011) observed significant differences in *E* for maize inbreds and hybrids. Genetic variation at the inbred-level was recently documented by Benešová et al. (2012) with major improvements in *A* for some inbreds under drought conditions but with lesser variation in *E*. In this study, hybrid differences in *E* were not significant (Table 4).

Drought and Non-Drought Periods (2012 Season)

Two periods related to differences in water supply and observed plant stress were arbitrarily separated and individually analyzed for *A* and *E* during the 2012 season (Fig. 2). For the R1 component of the drought stress period (V12, V15, and R1), the hybrid effect was artificial and due to sampling time (Tables 2, 3, and 4). For combined V12 and V15 stages, VPD significantly contributed to variation in *A* and *E* (Supplementary Fig. 1), and was employed as a covariable for the statistical analysis. A minor negative effect (P = 0.056) of increasing N rate on *A* and *E* was observed. Across hybrids and PD, as N rate increased, *A* decreased by ~1 unit while *E* decreased by ~0.1 unit. For maize, a negative N effect on *A* when no irrigation was applied versus a well-watered (irrigated) scenario can be observed from Wolfe et al. (1988). In summary, environmental conditions exerted a much greater influence on *A* and *E* than treatment factors evaluated during the drought period.

During the non-drought period (V10, R3, R4, R5 stages), Hybrid 1 had significantly ($P < 0.05$) lower *A* than comparable maturity Hybrid 2 (Δ5 units) (Fig. 2B). Nitrogen rate showed a positive significant ($P < 0.05$) influence on *A* causing it to increase by 2 units as N increased. Similar to the individual stage analysis, N rate was also the main factor influencing *E* ($P < 0.05$), with *E* increasing by 0.4 units as N increased. The positive N rate effect on *A* and *E* when water is non-limiting has also been observed in other studies (Ding et al., 2005; Echarte et al., 2008; Xia, 2012). At the leaf-level, water and N supply interactions were investigated by Wolfe et al. (1988) with 50% reduction in *A* capacity under N deficiency. This positive relationship between N rate and *A* and *E* during the non-drought period, in contrast to the inverse relationship observed during the drought period, is likely due to interaction with water supply (as documented by Wolfe et al., 1988).

Potential (PKn) and Actual Kernel Number (KN), and Relative Kernel Failure (RKF)

The main factors influencing PKn were the single effects of hybrid and N rate. For hybrid differences ($P = 0.0001$): Hybrid 4 (885) > Hybrid 3 (833), while Hybrid 1 (791) = Hybrid 2 (745 kernels ear^{-1}) (Fig. 3A, B). The single factor, N rate, significantly ($P < 0.01$) affected PKn, with the lowest N rate different from two higher N levels: Nr1 (790) < Nr3 (821) = Nr4 (835 kernels ear^{-1}). In summary, PKn was more influenced by hybrid (and CRM) than by PD and Nr factors. The minor PD and Nr effect on PKn was also supported by Lemcoff and

Loomis (1986) and Ciampitti (2012) who found neither management variables exerted a main impact over this parameter. Even the hybrid variation in PKn in this study presents relatively minor differences; all hybrids fall within a normal range of PKn that has not changed over time in non-prolific hybrids according to information summarized by Ciampitti (2012).

Drought conditions led to drastically reduced final per-plant KN in 2012 (Fig. 3A, B). Hybrid 4 had significantly ($P < 0.01$) greater KN than other hybrids [Hybrid 4 (325) > Hybrid 2 (246) = Hybrid 1 (208) = Hybrid 3 (196 kernels ear^{-1})] (Fig. 3A, B). Plant density was also significant ($P < 0.05$) as a single factor and, as anticipated, PD1 > PD2 (264 vs. 224 kernels ear^{-1}). Similar reductions in KN under water stress were documented by several investigations (Hall et al., 1981; Grant et al., 1989; Sinclair et al., 1990; NeSmith and Ritchie, 1992a, b; Otegui et al., 1995; among others).

At the plant-scale, relative kernel failure (RKF) was calculated as the proportion of PKn that failed to remain established by R6 (either non-fertilized or aborted). The RKF was extremely high and experienced a similar pattern as documented for the KN (Fig. 3A, B). Plant density was significant ($P < 0.01$) as higher RKF occurred at PD2 as compared with PD1 (73% > 68%). Hybrid order rankings for RFK were: Hybrid 3 (76%) > Hybrid 1 (74%) > Hybrid 2 (67%) > Hybrid 4 (63%).

Severe drought and heat stress occurred 3 weeks preceding flowering and continued into the flowering period in 2012. High temperatures, limited water during flower set, and supra-optimum PD are stress factors that clearly influence final KN and the RKF (Schussler and Westgate, 1991a, b; Lemcoff and Loomis, 1994; Schussler and Westgate, 1995; Zinselmeier et al., 1995; Otegui et al., 1997; Andrade et al., 2002; Cichino et al., 2010; Rattalino Edreira et al., 2011). Pollination failure as opposed to kernel abortion was observed as the major cause of high RKF (visual observations) in the 2012 growing season.

Two physiological measurements that assist understanding of mechanisms underlying the witnessed pollination failure and hybrid differences in RKF are: i) limited water availability during the flowering period (measured by soil water balance 0-125 cm using Field Connect sensors), and ii) varied anthesis-silking intervals (ASI). For the ASI, the major factor influencing the ASI was hybrid (Supplementary Table 1). Both Hybrids 1 and 2 (CRM 111) began flowering around the same period in July during high temperatures and water stress (Fig. 3C). Hybrid 2, however, had the lowest ASI and lower RKF, while Hybrid 1 had the greatest ASI asynchrony and high RKF. For water supply, although replications with the soil moisture meters were not possible, it is still noteworthy that Hybrid 2 had greater changes in the cumulative soil water balance deeper in the profile at the high N rate (202N) around the drought period (Fig. 4; Supplementary Table 2). This presumably suggests different root architecture (water conservation strategies) between hybrids with Hybrid 2 being deeper rooting (accessing more water) than Hybrid 1 during the intense water and heat stress period. Greater water access and related lower ASI are, therefore, possible factors that influenced lower RKF in Hybrid 2 (58% at PD1 and 202N) compared with Hybrid 1 (80% at PD1 and 202N) (Fig 3B). Hybrids 3 and 4 had similar ASI, but the latter showed more delayed flowering onset than the former. This delay allowed Hybrid 4 to experience more precipitation events during its flowering period, which benefited relative final KN.

Water deficit was shown to negatively impact ovary establishment (Schussler and Westgate, 1995; Zinselmeier et al., 1995) even when ASI asynchrony is eliminated by hand pollination of delayed extruding silks with fresh pollen (Otegui et al., 1995; Zinselmeier et al., 1995). Therefore, high RKF was most likely due to severe water stress for developing ovules compounded by asynchrony in the ASI [more related to the female organ: lower silk elongation rate (Herrero and Johnson, 1981); reduced silk receptivity (Basetti and Westgate, 1993). Even with successful fertilization, embryo development can abort under low water potential (Westgate and Boyer, 1986)] with resulting hybrid variation in RKF due to slight advantages in water access.

Grain Yield and its Components

In 2011, KN was only significantly affected by N rate (Table 2). In 2012, however, N rate did not significantly affect KN, likely due to more limiting drought conditions around the flowering time. In 2011, KN for the highest N rate was two-fold greater than for the low N supply (4200 vs. 2100 kernels m^{-2}, respectively) while in 2012 the difference was much smaller (2300 vs. 2000 kernels m^{-2}, respectively) (Table 6). The most significant factor influencing KN in 2012 was hybrid (via water availability) with Hybrid 4 (3100) > Hybrid 2 (2190) = Hybrid 1 (1840) = Hybrid 3 (1820 kernels m^{-2}). A minor hybrid x PD interaction in 2012 also occurred because KN increased substantially at PD2 with Hybrid 4 but not in other hybrids. Genotypic variation in seed number as drought intensity changes was also documented by Chapuis et al. (2012), Moradi et al. (2012), and Shahin Abad et al. (2013).

In 2011, KW was solely affected by N rate (Nr4 -336- > Nr1 -280- mg g^{-1}) (Tables 2 and 6). In 2012, however, all single effects of hybrid, PD, and N rate, as well as the hybrid by PD interaction, were significant. Hybrid 4 KW was more negatively affected by higher PD compared with other hybrids since it was the only hybrid with increased KN at higher PD. Mean KW (averaged for hybrids and N rates) was higher at low versus high PD (346 vs. 334 mg g^{-1}). Not surprisingly, mean hybrid effects on KW were in the reverse order compared with KN:

Hybrid 3 (352) > Hybrid 1 (347) > Hybrid 2 (337) > Hybrid 4 (323 mg g^{-1}). Late season precipitation in 2012 permitted adequate source supply for the limited KN retained following the drought stress period. Poor N supply (Nr1) reduced KW compared to when N was applied (Nr2-3-4) (321 vs. 346 mg g^{-1}). While N rate was not significant for KN, the alleviation of drought stress early in the grain-fill period (Fig. 1) lead to a positive N rate influence on KW (Lemcoff and Loomis, 1986; 1994; Melchiori and Caviglia, 2008; Ciampitti and Vyn 2011; Ciampitti, 2012).

In 2011, the GY response (155 g kg^{-1} moisture, determined from plot-combine harvest) indicated single treatment effects for all three variables (Table 2). The PD (1 > 2) and N rate (4 > 3 > 2 > 1) effects were as expected when water was non-limiting (Table 6). In this “normal” growing season non-drought-tolerant Hybrid 2 out yielded drought-tolerant Hybrid 1 (11.7 vs. 10.9 Mg ha^{-1}), and Hybrid 1 suffered a greater yield decline (0.8 Mg ha^{-1}) at the supra-optimal PD than Hybrid 2 (0.4 Mg ha^{-1}). In 2012, yield levels were severely reduced by the extreme drought and heat stresses (high RKF). Hybrid by PD interaction significantly affected GY (Table 2) with both Hybrids 1 and 2 negatively impacted as the crowding intensity increased (Δ yield ~2.4 Mg ha^{-1}). While hybrids differing in drought tolerance may have been expected to differ significantly in GYs with drought occurring, this was not the case. The unique intensity and timing of the 2012 drought, combined with damaging heat stress, are likely explanations. In another study comparing varied drought-tolerant hybrids and controlled water stress treatments, hybrid differences were observed with water stress occurring 1 week prior to flowering, but not when stress was imposed 3 weeks prior to flowering (Bruce et. al, 2002).

The GY levels attained are partially explained by lower per-plant BM (dry basis) at maturity in 2012 than in 2011 (Table 6). Biomass responses in both years demonstrated significant PD and N rate effects (Table 2) with patterns similar to those reported by Ciampitti and Vyn (2011). Mean biomass (averaged over years, hybrids and N rates) for PD1 was greater than PD2 (76 vs. 42 g pl^{-1}). The mean ranking of per-plant BM (averaged over hybrids and PD levels) improved consistently with N rates in both years (Table 6). On the other hand, significant PD and N rate effects on grain harvest index (HI) were only documented in 2011 (Table 2). Grain HI was higher for PD1 than PD2 (0.56 vs. 0.54 units), and HI was lower with Nr1 than with the three higher N rates (0.46 vs. 0.58 units; Table 6). In 2012, HI differences were strongest among hybrids [mean ranking: Hybrid 4 (0.48) > Hybrid 2 (0.42) > Hybrid 1 (0.35) = Hybrid 3 (0.35)], although there were significant, but minor PD influences on HI within each hybrid (Tables 2 and 6).

To investigate the genotype by environment interaction, a stability index was calculated for Hybrids 1 and 2 in both 2011 and 2012 (Supplementary Fig. 2). Yield variations for both hybrids (across PD and N rates) were primarily related to the growing season. For the 2011 season (normal environment), both drought and non-drought tolerant hybrids were highly responsive to the N supply at both PDs evaluated. In 2012, the drought stress constrained hybrid GY responses to PD and N rate levels (with very flat trends across all N rates) (Supplementary Fig. 2). Only negligible changes in hybrid GY ranking were documented in 2012.

In summary, PD and N rate effects were apparent in 2011 for GY, BM, and grain HI parameters. Biomass differences between minimum and maximum PD and N rate levels (43% for PD, and 92% for N rate, respectively) were greater than changes in HI (4% for PD, and 28% for N rate, respectively) and were, therefore, more influential on final GY at maturity. In 2012, PD and N rate treatment effects occurred for GY and BM, but not for HI. Under the 2012 drought (combined with heat) stress, grain HI order was identical to that of final KN (Hybrid 4 >

2 > 1 > 3) at maturity. Hybrid differences, however, were not seen for GY suggesting that drought-tolerant Hybrids 1 and 3 conferred no advantage in final GY compared to non-drought-tolerant Hybrids 2 and 4 given the specific duration, intensity, and timing of drought stress experienced at this location.

Cumulative Photosynthesis (*A*) and Transpiration (*E*)

Our earlier discussions about *A* and *E* responses to treatments focused at the leaf-scale. Mean leaf *A* (over the season) has been strongly correlated with maize BM and GY at maturity (Peng et al., 1991). Understanding whole plant processes (i.e. canopy-level) over the entire growing season can provide more insight to variables like BM or GY than the study of a portion of a leaf. While others have investigated canopy level *A* or *E* correlations with BM for different crops (Hufstetler et al., 2007; Di Paolo and Rinaldi, 2008; Balota et al., 2010) a novel approach was used to "scale-up" measurements of *A* and *E* from leaf to canopy-level cumulatively over the growing season (Fig. 5). Using this cumulative approach over 2012 season, *A* (adjusted by mean LAI and PD parameters) correlates well to plant mass ($r^2 = 0.51$) and the slope (0.84) is close to a 1:1 ratio (Fig. 5A). Hybrid, PD, and N rate variables did not modify the slope observed between BM and *A* processes. While the association is reasonable, cumulative *A* appears to underestimate actual plant mass. This may be due to measurements not beginning until the V10 stage which constrained the period evaluated for cumulative *A*. A second possible explanation may be that plants were more photosynthetically active prior to measurements being recorded (i.e. earlier in the morning) during the drought-stress period. The resulting integration over the growing season would therefore underestimate total plant BM. In addition, it should be acknowledged that different photosynthetic rates are to be expected in the canopy profile, so it may be incorrect to assume that the leaf being measured represents the mean photosynthetic value among leaves within the canopy. Notwithstanding these constraints, the "scaling up" method used to calculate cumulative *A* as a prediction of plant mass helps to demonstrate the relevance of the methodology used in acquiring leaf-level *A*.

Cumulative *E* was calculated in a similar manner as cumulative *A* and was correlated with BM (Fig. 5B). Cumulative *E* was less correlated to plant mass than cumulative *A* (r^2 = 0.47) but treatment dispersion patterns were more evident. While hybrid differences in *E* were insignificant on individual measurement dates, hybrid differences in *E* (as also noted by Bunce, 2010, 2011), were more evident when cumulative *E* was considered. Drought-tolerant Hybrid 1 (blue colored points) generally appeared to have the lowest cumulative *E* ranging from 50-75 kg pl^{-1}. Water conservation seems to be a strategy employed by Hybrid 1, suggesting drought avoidance rather than drought-tolerance. This water conservation tendency is supported by the trends of lower observed transpiration (Table 4, Fig. 2C, D, Fig. 5B), lower observed LAI (Table 5) and less water uptake as suggested by the smaller change during V10-R1 stage in cumulative soil water balance in deeper soil layers (Fig. 4). Cumulative *E* in drought-tolerant Hybrid 3 ranged from 75 to 110 kg pl^{-1}, while non-drought-tolerant Hybrids 2 and 4 at the lowest PD fell between 115 to 140 kg pl^{-1}. It is interesting that Hybrids 2 and 4 appeared more PD dependent in terms of cumulative *E*, while Hybrids 1 and 3 were less-density dependent. This suggests greater stability of *E* at varying PD levels for the designated drought-tolerant Hybrids 1 and 3. Hybrids 2 and 4, on the other hand, displayed higher *E* at the lower PD than Hybrids 1 and 3, but were unable to sustain those rates as PD increased. Close associations between biomass production and water consumption are anticipated given that ET depends on the water supply factor. For sorghum, whole-plant BM was more strongly correlated with the transpiration efficiency (TE, dry mass to water use ratio) than with the water transpired during the entire season (Xin et al., 2009). The present research suggests that genetic diversity in physiological drought responses is more apparent in the association between BM and cumulative *E* (TE slope) than in specific leaf *E* measurements.

CONCLUSIONS

This research was conducted under natural climatic conditions, and the fairly normal weather year of 2011 was followed by severe drought stress in 2012. For both seasons, hybrid differences in GY, *A* and *E* parameters were minimal across site-years and management treatment factors. The N rate, rather than hybrid or PD, was the primary factor governing leaf *A* and *E* responses at late reproductive stages, and when analyzed during drought and non-drought periods. In addition, higher N rates had a positive impact on *A* and *E* under adequate moisture, but a minor negative effect during drought conditions. Following canopy-level “scaling up” for cumulative *A* and *E*, hybrid differences were more apparent.

In summary, all hybrids whether labeled as more drought-tolerant or less so, responded similarly (for GY and parameters measured) to PD and N treatment factors in both drought and non-drought environments. Both GY and KN consistently declined as the crowding intensity increased; while KW and plant BM at maturity were positively impacted as the N supply increased. Conclusions regarding the lack of superiority of drought-tolerant hybrids during the drought year are pertinent only to the specific environmental conditions encountered in the particular location tested.

Future research should, where possible, focus on evaluating hybrids and management intensities in environments were the three main components of the drought process: timing, duration and intensity can be controlled. As increasing numbers of drought-tolerant hybrids are being developed, continuing efforts on improving drought tolerance might benefit by focusing on: i) maintaining elevated rates of *A* under low leaf water potential, ii) minimizing ASI asynchrony under stress, iii) reducing RKF to maximize KN, and iv) exploring alternative physiological mechanisms associated with drought tolerance (e.g. stomatal closure, root architecture, shoot:root partitioning, among others).

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FIGURE CAPTIONS

Fig. 1. Weather conditions (maximum and minimum air temperature and mean precipitation) for 2011 and 2012 maize growing seasons at Pinney-Purdue Agricultural Center (PPAC) in North-Western Indiana (panels A, B) paired with season measurements of photosynthesis [*A*] and transpiration [*E*] (panel C), and LAI (panel D), all measurements averaged across plant density and N rates in both years, for 2 hybrids in 2011 and four hybrids in 2012.

Fig. 2. Photosynthetic (A, B) and transpiration (C, D) rates for the drought (A, C) and non-drought periods (B, D) for all four hybrids (Hybrid 1 = AQUAmax^{TM} P1151, Hybrid 2 = P1162, Hybrid 3 =AQUAmax^{TM} P1498, Hybrid 4 = 33D49) at only two N rates (Nr1 = 0 and Nr3 = 202 kg N ha^{-1}) across plant densities for the 2012 growing season at PPAC experimental site. For the drought period the *A* rates for each treatment combination were averaged across V12, V15 and R1, while for the non-drought period the mean value is obtained from the average of V10, R3, R4 and R5. Error bars represent the standard error of treatment means over time across three replications. Similar procedure was followed for the calculations of *E* rates.

Fig. 3. A.B. Actual kernel number (full bars), determined at maturity, and potential kernel number – PKn – ("spikelets", dashed bars), measured at silking, for the different maize hybrids [Hybrid 1 = AQUAmaxTM P1151 (blue bar), Hybrid 2 = P1162 (red), Hybrid 3 = AQUAmaxTM P1498 (green) and Hybrid 4 = 33D49 (yellow)] at each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha-1) and N rate levels (0N = 0, and 202N = 202 kg N sidedress ha-1) for one site (PPAC) and season (2012). Numbers inside each bar denote the reproductive relative kernel failure (RKF, ratio between the actual to PKn. C. Anthesis-silking interval (ASI), anthesis and silking duration (expressed as 10-90% range), for each hybrid across plant density and N rates (as related to the maximum temperature and precipitation evolutions during the flowering time).

Fig. 4. Apparent water consumption (mm) in soil profile (from 0 to 125 cm soil depth) for the different maize hybrids [Hybrid 1 = AQUAmax^{TM} P1151, and Hybrid 2 = P1162] at only two N rate levels (0N = 0, and 202N = 202 kg N sidedress ha^{-1}) and at a single plant density level (PD1 = 79,000 pl ha^{-1}) for one site (PPAC) and season (2012). Water sensors were installed at V5 growth stage, calibrated by John Deere for soil type, and volumetric soil moisture was recorded every thirty minutes at five depths (10, 20, 30, 50, 100 cm). Values were interpolated across depths, and integrated across growth stages in order to calculate cumulative changes in the soil water balance for the entire maize growing season.

Fig. 5. Association between the final plant mass on a dry basis (determined at physiological maturity) versus the cumulative photosynthesis [panel A, expressed in grams per plant, and calculated as the cumulative area under the *A* evolution curve (Fig. 1) from V10 till R5 period – values taken from Table 3) during the entire maize season adjusted by the mean leaf area index value (from Table 5), and corrected by the plant density values] and the cumulative transpiration [panel B, expressed in kg per plant, following the same procedure as detailed for the cumulative photosynthesis calculation, cumulative transpiration values were taken from Table 4 (from V10 till R5 period)] for four maize hybrids [Hybrid 1 =

AQUAmax^{TM} P1151 (blue color), Hybrid 2 = P1162 (red color), Hybrid 3 = AQUAmax^{TM} P1498 (green color) and Hybrid 4 = 33D49 (yellow color)] at each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha^{-1}) and N rate level (0N = 0, and 180N = 180 kg N sidedress ha^{-1}) for 2012. Each data point represents the replication value at individual plot-level. Subfigures within panels A and B show the residual distribution for each association.

Supplementary Fig. 1. Photosynthetic rates (A) at the most fully developed (collared) leaf versus leaf temperature (A), and vapor pressure deficit – VPD – (B) at V15 growth stage (drought plus heat stresses) for all four hybrids (Hybrid 1 = AQUAmax^{TM} P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax^{TM} P1498, hybrid 4 = 33D49) across plant densities and N rate levels for the 2012 season at PPAC site. Each individual point corresponds to plot-basis values (mean from four individual numbers obtained from two plants per plot). In panel A, the subfigure represents the association between leaf and air temperature in parallel when the *A* values were recorded. All points were obtained on two consecutive days, but Hybrids 1 and 2 were sampled 1 hour later than Hybrids 3 and 4.

Supplementary Fig. 2. Grain yield (Mg ha^{-1}, 155 g kg^{-1} moisture) stability of two hybrids (Hybrid 1 = AQUAmax^{TM} P1151 and Hybrid 2 = P1162) at each plant density (PD1 = 79,000; and PD2 = 104,000 pl ha^{-1}) and N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 180, and Nr4=269 kg N sidedress ha^{-1}) over the 2011 and 2012 growing seasons at the PPAC experimental site.

Table 1. Soil analysis values at the beginning of the maize growing season for non-fertilized plots [inorganic nitrogen (NO_3^-–N/NH_4^+-N), organic matter content (OM), soil pH, potassium content (K) and phosphorous Bray-P 1 (P)] in the uppermost 0.3 m of the soil profile, and phenological data [dates of planting, emergence, and harvest date, five (V5), ten (V10), and fifteen (V15) leaves stages, silking (R1), milk stage (R3), and physiological maturity (R6)], expressed in growing degree days after emergence (thermal time-TT-$^{\circ}$C d^{-1}), for each growing season (2011 and 2012), averaged across hybrids. For the soil analysis information, the value within parenthesis refers to its standard deviation.

Soil parameters/Sites	Growing Seasons	
	2011	2012
	PPAC	PPAC
NO_3^-/NH_4^+-N (mg kg^{-1})	N/A	23.3 (5.7)/1.8 (0.7)
OM content (g 100 g^{-1})	1.5 (0.2)	1.6 (0.3)
pH units	6.4 (0.1)	6.1 (0.2)
K (mg K kg^{-1})	104 (8.4)	103 (13.8)
P (mg P kg^{-1})	45 (9.3)	43 (9.4)
Phenological data		
Planting date	May 10	April 24
Emergence date (VE)	May 21 (0 $^{\circ}$C d^{-1})	May 7 (0 $^{\circ}$C d^{-1})
V5 stage	June 12 (251°C d^{-1})	May 29 (230°C d^{-1})
V10 stage	July 3 (497 $^{\circ}$C d^{-1})	June 21 (506°C d^{-1})
V15 stage	July 14 (654 $^{\circ}$C d^{-1})	July 5 (729°C d^{-1})
Silking date (R1)	July 24 (829°C d^{-1})	July 14 (891°C d^{-1})
Milk Stage (R3)	August 8 (1054 $^{\circ}$C d^{-1})	July 28 (1154°C d^{-1})
Physiological maturity (R6)	October 11 (1554 $^{\circ}$C d^{-1})	September 13 (1714°C d^{-1})
Harvest	October 31 (1554 $^{\circ}$C d^{-1})	October 18 (1823°C d^{-1})

Table 2. Statistical analysis for leaf photosynthesis (A) (micromol CO_2 m^{-2} s^{-1}), leaf transpiration (E) (mmol H_2O m^{-2} s^{-1}), leaf area index (LAI) (m^2 m^{-2}), grain yield (GY) (Mg ha^{-1}), kernel number (KN) (kernel m^{-2}), kernel weight (KW) (mg $kernel^{-1}$), grain harvest index (HI), and biomass (BM) (g $plant^{-1}$) for the 2011 and 2012 growing seasons at PPAC experimental site.

2011								
Variable	A (R_3/R_4/R_5)	E (R_3/R_4/R_5)	LAI (R_1/R_4)	GY	KN	KW	HI	BM
Hyb	ns/ns/ns	ns/ns/ns	*/*	*	ns	ns	ns	ns
PD	**/ns/*	*/ns/*	***/**	***	ns	ns	**	***
Nr	***/***/**	***/**/**	***/***	***	***	***	***	***
Hyb x PD	ns/ns/ns	ns/ns/ns	ns/ns	ns	ns	ns	ns	ns
Hyb x Nr	ns/ns/ns	ns/ns/ns	ns/ns	ns	ns	ns	ns	ns
PD x Nr	ns/ns/ns	ns/ns/ns	ns/*	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns/ns/ns	ns/ns/ns	ns/ns	ns	ns	ns	ns	ns
2012								
Variable	Photosynthesis (A) ‡ (V_{10}/V_{12}/V_{15}/R_1/R_3/R_4/R_5)	Transpiration (E) ‡ (V_{10}/V_{12}/V_{15}/R_1/R_3/R_4/R_5)	LAI (V_{10}/V_{12}/V_{15}/R_1/R_3/R_4)	GY	KN	KW	HI	BM
Hyb	ns/ns/ns/*/*/ns/*	ns/ns/ns/**/ns/ns/ns Ŧ	ns/ns/ns/ns/ns/ns	ns	**	*	**	ns
PD	ns/ns/ns/ns/ns/ns/ns	ns/ns/ns/ns/ns/ns/ns	***/ns/**/**/***/ns	***	ns	*	ns	***
Nr	ns/ns/ns/ns/ns/***/*	ns/ns/ns/ns/ns/***/*	ns/ns/ns/ns/ns/ns	**	ns	**	ns	*
Hyb x PD	ns/ns/ns/ns/ns/ns/ns	ns/ns/ns/ns/ns/**/ns	ns/ns/ns/ns/ns/ns	**	**	**	*	ns
Hyb x Nr	ns/ns/ns/ns/ns/ns/ns	ns/ns/ns/ns/ns/ns/ns	*/ ns/ns/ns/ns/ns	ns	ns	ns	ns	ns
PD x Nr	ns/ns/ns/ns/ns/ns/ns	ns/ns/ns/ns/ns/ns/ns	ns/ns/ns/ns/ns/ns	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns/ns/ns/ns/ns/ns/ns	ns/ns/ns/ns/ns/ns/ns	ns/ns/ns/*/ns/ns	ns	ns	ns	ns	ns
VPD †	–/***/***/ns/–/–/–	–/***/***/ns/–/–/–	–/–/–/–/–/–	–	–	–	–	–

† VPD was used as a covariate for statistical analysis at V12 and V15 growth stages. ‡

‡ Hybrid difference at R1 was due to rain event which elevated measurements performed later on 114 relative maturity Hybrids 3 and 4.

*Hyb = hybrid, PD = plant density, Nr = N rate; ns = not significant, * = P<0.05; ** = P<0.01; *** = P<0.0001.*

Table 3. Leaf photosynthesis (*A*) (micromol CO_2 m^{-2} s^{-1}) using Licor 6400XT (LI-COR, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmax^{TM} P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax^{TM} P1498, Hybrid 4 = 33D49, Hybrid 5 = P1184) at both densities (PD1 = 79,000 pl ha^{-1}, PD2 = 104,000 – 109,000 pl ha^{-1}) and at two N rates (Nr1 = 0 kg N ha^{-1}, Nr3 = 202 kg N ha^{-1}) for two growing seasons (2011 and 2012) at PPAC experimental site. Each value represents three replications (total 4 points per plot) from field isolated, light acclimated plants on the youngest fully expanded leaf at vegetative stages and the ear leaf at reproductive stages.

2011 *A*			
	R3	R4	R5
Hybrid			
Hyb 1	25	23	10
Hyb 2	25	22	9
Hyb 5	27	22	12
PD			
PD1	27 a	23	11 a
PD2	25 b	21	10 b
Nr			
Nr1	23 b	18 b	9 b
Nr3	29 a	27 a	12 a

2012 *A*							
	V10	V12 [†]	V15 [†]	R1 [‡]	R3	R4	R5
Hybrid							
Hyb 1	36	10	20	12 b	29 ab	26	17 ab
Hyb 2	42	12	22	14 ab	33 a	31	21 ab
Hyb 3	41	13	16	22 ab	29 ab	29	22 a
Hyb 4	41	11	18	23 a	28 b	30	15 b
PD							
PD1	40	12	19	17	30	29	18
PD2	40	11	19	17	30	29	19
Nr							
Nr1	41	12	19	18	29	27 b	17 b
Nr3	39	11	19	17	31	31 a	20 a

[†] *VPD was used as a covariate for statistical analysis at V12 and V15 growth stages.*

[‡] *Hybrid difference at R1 was due to rain event which elevated measurements performed later on 114 relative maturity Hybrids 3 and 4.*

Hyb= Hybrid; PD= Plant density; Nr= N rate; ns = not significant.

Table 4. Leaf transpiration (*E*) (mmol H_2O m^{-2} s^{-1}) obtained using Licor 6400XT (LI-COR, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmax^{TM} P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax^{TM} P1498, Hybrid 4 = 33D49, Hybrid 5 = P1184) at both densities (PD1 = 79,000 pl ha^{-1}, PD2 = 104,000 – 109,000 pl ha^{-1}) and at only two N rates (Nr1 = 0 kg N ha^{-1}, Nr3 = 202 kg N ha^{-1}) for two growing seasons (2011 and 2012) at PPAC experimental site. Each value represents three replications (total 4 points per plot) from field isolated, light acclimated plants on the youngest fully expanded leaf at vegetative stages and the ear leaf at reproductive stages.

2011 *E*

	R3	R4	R5
Hybrid			
Hyb 1	5.3	6.0	1.2
Hyb 2	5.5	5.6	1.4
Hyb 5	6.4	5.4	1.5
PD			
PD1	6.0 a	5.9	1.5 a
PD2	5.5 b	5.4	1.3 b
Nr			
Nr1	4.9 b	4.8 b	1.2 b
Nr3	6.5 a	6.5 a	1.5 a

2012 *E*

	V10	V12 †	V15 †	R1 ‡	R3	R4	R5
Hybrid							
Hyb 1	4.5	2.1	3.9	1.6 b	5.0	4.5	3.1
Hyb 2	5.9	2.4	4.9	1.5 b	5.9	5.6	4.1
Hyb 3	5.9	2.3	2.9	3.2 a	4.9	5.5	4.2
Hyb 4	6.1	2.1	3.4	3.5 a	4.8	5.9	2.9
PD							
PD1	5.6	2.2	3.8	2.5	5.2	5.5	3.3
PD2	5.6	2.2	3.8	2.4	5.2	5.2	3.8
Nr							
Nr1	5.7	2.3	3.8	2.4	5.0	4.9 b	3.2 b
Nr3	5.5	2.1	3.7	2.4	5.3	5.9 a	3.9 a

† *VPD was used as a covariate for statistical analysis at V12 and V15 growth stages.*

‡ *Hybrid difference at R1 was due to rain event which elevated measurements performed later on 114 relative maturity Hybrids 3 and 4.*

Hyb= Hybrid; PD= Plant density; Nr= N rate.

Table 5. Leaf area index (LAI) (m^2 m^{-2}) obtained using Licor 2200 (LI-COR, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmax$^{\text{TM}}$ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax$^{\text{TM}}$ P1498, Hybrid 4 = 33D49, Hybrid 5 = P1184) at both densities (PD1 = 79,000 pl ha^{-1}, PD2 = 104,000 – 109,000 pl ha^{-1}) and at all N rates (Nr1 = 0 kg N ha^{-1}, Nr2 = 134 kg N ha^{-1}, Nr3 = 202 kg N ha^{-1}, Nr4 = 269 kg N ha^{-1}) at various growth stages throughout the season. Mean values represent the average of 3 replications of 3 measurements per plot.

2011 LAI

	R1	R4
Hybrid		
Hyb 1	3.6 b	3.4 b
Hyb 2	4.1 a	3.9 a
Hyb 5	4.0 ab	3.6 ab
PD		
PD1	3.5 b	3.5 b
PD2	4.3 a	3.8 a
Nr		
Nr1	2.8 b	2.4 c
Nr2	4.0 a	3.8 b
Nr3	4.3 a	4.1 a
Nr4	4.4 a	4.1 a

2012 LAI

	V10	V12	V15	R1	R3	R4
Hybrid						
Hyb 1	2.0	2.0	2.5	2.3	1.9	1.8
Hyb 2	2.2	2.0	2.7	2.6	2.2	1.9
Hyb 3	2.0	1.9	2.4	2.5	2.1	1.9
Hyb 4	1.7	1.8	2.3	2.6	2.3	2.3
PD						
PD1	1.9 b	1.8	2.4 b	2.4 b	2.0 b	1.9
PD2	2.1 a	2.0	2.6 a	2.6 a	2.2 a	2.0
Nr						
Nr1	2.0	1.9	2.4	2.5	2.0	1.8
Nr2	2.0	–	2.5	2.5	2.2	1.9
Nr3	2.1	2.0	2.5	2.6	2.2	2.1
Nr4	2.0	–	2.5	2.5	2.1	2.0

Hyb= Hybrid; PD= Plant density; Nr= N rate.

Table 6. Grain yield (155 g kg^{-1} moisture) from combine harvest, kernel number (KN), kernel weight (KW), grain harvest index (HI), and total plant biomass (BM) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax^{TM} P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax^{TM} P1498, Hybrid 4 = 33D49, and Hybrid 5 = P1184) grown at two plant densities (PD1=79,000, PD2=104,000-109,000 pl ha^{-1}) and four N rates (Nr1 = 0, Nr2 = 134, Nr 3 = 202, and Nr 4 = 269 kg ha^{-1}) in 2011 and 2012 at PPAC experimental site.

	Grain yield (Mg ha^{-1})					**Kernel number (KN)** (kernel m^{-2})					**Kernel weight (KW)** (mg kernel^{-1})					**Harvest index (HI)**					**Biomass (BM)** (g. plant^{-1})			
	2011 2012					2011 2012					2011 2012					2011 2012					2011 2012			
Hybrid 1	Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4
PD1	5.9 7.2	12.1 8.0	13.1 7.9	14.1 9.0		1819 1874	3829 1761	3798 1390	4325 2379		305 359	313 372	347 351	343 322		0.48 0.44	0.59 0.39	0.59 0.30	0.58 0.44		149 188	271 186	282 176	301 204
PD2	5.0 5.4	11.6 5.4	12.2 6.0	13.1 5.5		1425 2121	4213 2010	3826 1459	4051 1747		295 336	293 342	328 339	327 356		0.42 0.42	0.58 0.32	0.59 0.24	0.60 0.32		90 146	192 147	202 152	194 155
Hybrid 2	Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4
PD1	6.2 6.9	12.7 8.2	14.0 8.3	14.6 8.9		2387 1881	3767 2732	3840 2556	4040 2615		276 310	308 329	309 346	328 337		0.48 0.37	0.58 0.50	0.60 0.41	0.59 0.43		150 168	261 209	260 206	279 214
PD2	5.8 4.4	12.1 7.1	13.3 5.3	13.3 6.1		2383 1751	4304 2271	4076 1830	4417 1870		296 330	323 345	317 351	313 349		0.47 0.41	0.55 0.44	0.58 0.42	0.59 0.40		114 134	182 142	197 162	231 156
Hybrid 3	Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4
PD1	na 7.1	na 7.3	na 7.7	na 8.2		na 2014	na 2182	na 1295	na 1428		na 339	na 376	na 361	na 351		na 0.45	na 0.35	na 0.28	na 0.25		na 183	na 200	na 189	na 187
PD2	na 5.9	na 6.1	na 7.8	na 6.9		na 1752	na 1849	na 2055	na 1976		na 328	na 359	na 353	na 353		na 0.35	na 0.37	na 0.38	na 0.39		na 140	na 147	na 185	na 170
Hybrid 4	Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4
PD1	na 8.3	na 8.6	na 9.2	na 9.2		na 2614	na 2333	na 2456	na 2949		na 319	na 359	na 359	na 348		na 0.49	na 0.43	na 0.41	na 0.47		na 205	na 226	na 227	na 223
PD2	na 7.1	na 9.8	na 8.5	na 8.0		NA 3296	NA 3865	NA 3491	NA 3804		NA 244	NA 329	NA 320	NA 305		NA 0.50	NA 0.54	NA 0.54	NA 0.51		NA 133	NA 195	NA 180	NA 183
Hybrid 5	Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4		Nr1	Nr2	Nr3	Nr4
PD1	6.3 na	12.6 na	13.0 na	13.8 na		2648 na	3951 na	4063 na	3880 na		266 na	304 na	338 na	377 na		0.45 na	0.59 na	0.60 na	0.60 na		181 na	281 na	311 na	314 na
PD2	5.4 na	11.3 na	12.4 na	12.3 na		2063 na	4393 na	4307 na	4700 na		246 na	278 na	310 na	327 na		0.43 na	0.54 na	0.58 na	0.60 na		112 na	193 na	221 na	199 na

Hyb = Hybrid; PD = Plant density; Nr = N rate; na = not available,

Supplementary Table 1. Anthesis (anthers suspended) and silking (silk extrusion ≥ 1cm) information averaged across N rates for all hybrids (Hybrid 1 = AQUAmax^{TM} P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax^{TM} P1498, Hybrid 4 = 33D49) and plant densities (PD1 = 79,000 pl. ha^{-1}, PD2 = 104,000 pl. ha^{-1}) for the 2012 growing season at PPAC experimental site. Values were averaged across 3 replications of 20 marked plants surveyed daily from the start of flowering.

Hybrid	**Density**	**Anthesis Duration** (Days from 10%-90%)	**Silk Duration** (Days from 10%-90%)	**Anthesis Silking Interval (ASI)** (Days from 50% anthesis to 50% silking)	**Date of 50% Anthesis** (July 2012)	**Date of 50% Silk** (July 2012)
1	PD1	4.3	13.2	5.1 (a)	6	11
	PD2	5.1	13.8	6.1 (a)	6	13
	mean	4.7	13.5	5.6	6	12
2	PD1	4.2	8.9	1.9 (a)	7	9
	PD2	5.4	9.4	5.0 (ab)	8	13
	mean	4.8	9.2	3.5	8	11
3	PD1	5.9	8.0	4.5 (ab)	10	14
	PD2	5.9	8.1	4.3 (ab)	11	15
	mean	5.9	8.0	4.4	10	15
4	PD1	6.3	6.8	5.8 (a)	11	17
	PD2	6.3	6.1	5.3 (a)	13	19
	mean	6.3	6.5	5.6	12	18
ANOVA [ASI] (Hyb - Hyb*PD) SE [ASI] (0.49 - 0.68)						

Supplementary Table 2. Cumulative soil water changes (mm) in soil profile (from 0 to 125 cm soil depth) for the different maize hybrids [Hybrid 1 = AQUAmax^{TM} P1151, and Hybrid 2 = P1162] at only two N rate levels (0N = 0, and 202N = 202 kg N sidedress ha^{-1}) and at a single plant density level (PD1 = 79,000 pl ha^{-1}) for one site (PPAC) and season (2012). Water sensors were installed at the V5 growth stage, calibrated by John Deere for soil type, and volumetric soil moisture was recorded every thirty minutes at five depths (10, 20, 30, 50, and 100 cm). Values were interpolated across depths, and integrated across growth stages in order to calculate cumulative changes in the soil water balance for the entire maize growing season.

Depth (cm)	**Hybrid 1, 0N** V_{10-12} / V_{12-15}/ V_{15}-R_1/ R_{1-3}/ R_{3-4}/ R_{4-5}	**Hybrid 1, 202N** V_{10-12} / V_{12-15}/ V_{15}-R_1/ R_{1-3}/ R_{3-4}/ R_{4-5}	**Hybrid 2, 0N** V_{10-12} / V_{12-15}/ V_{15}-R_1/ R_{1-3}/ R_{3-4}/ R_{4-5}	**Hybrid 2, 202N** V_{10-12} / V_{12-15}/ V_{15}-R_1/ R_{1-3}/ R_{3-4}/ R_{4-5}
0-10	-37.1/-4.3/0.9/-1.5/18.8/-3.8/-5.2	-32.4/-5.4/1.1/-1.6/14.5/-3.4/-6.3	-34.7/-5.4/0.9/-1.7/19.5/-1.6/-7.4	-27.5/-5.8/1.6/-3.5/20.4/-5.2/-8.2
10-20	-15.8/-3.7/0.4/-1.4/7.9/-3.5/2.4	-6.7/-4.7/0.3/-1.4/4.6/-2.0/1.6	-13.6/-5.1/0.0/-1.9/10.1/-4.4/1.6	-8.1/-4.9/0.2/-2.3/10.3/-5.4/0.4
20-30	-2.1/-5.9/-0.3/-1.7/8.6/-3.8/3.9	0.0/-6.7/-0.9/-2.2/6.6/-3.3/3.5	-6.1/-5.9/-1.1/-2.8/11.4/-5.5/5.1	-2.2/-5.3/-0.9/-3.3/9.7/-4.9/2.7
30-50	0.2/-5.7/-1.7/-2.4/6.3/-3.3/0.4	-2.1/-3.2/-1.9/-2.3/-0.2/-0.7/0.9	-10.2/-11.0/-3.3/-6.5/9.2/-5.9/1.8	-7.9/-6.2/-1.4/-3.6/5.9/-3.0/0.2
50-100	-4.2/-5.2/-4.0/-8.8/-10.6/-1.8/-0.9	-4.3/-3.7/-3.6/-4.8/-9.6/-2.8/-0.8	-3.9/-5.4/-4.2/-9.2/-10.7/-1.4/-1.4	0.0/-11.0/-15.0/-11.6/-5.9/-3.5/-2.2

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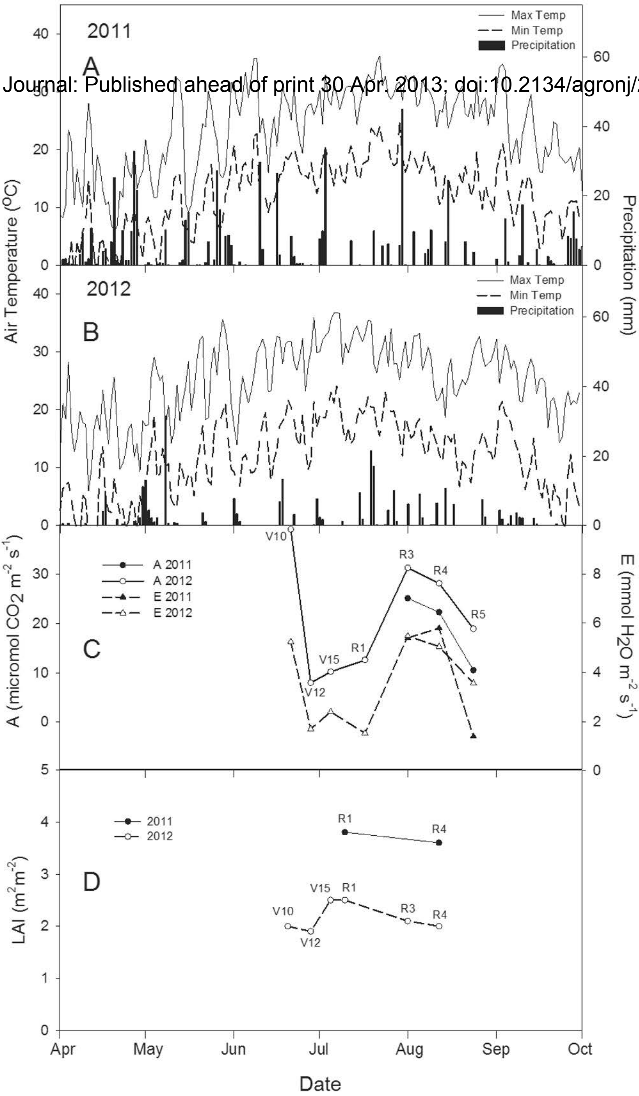

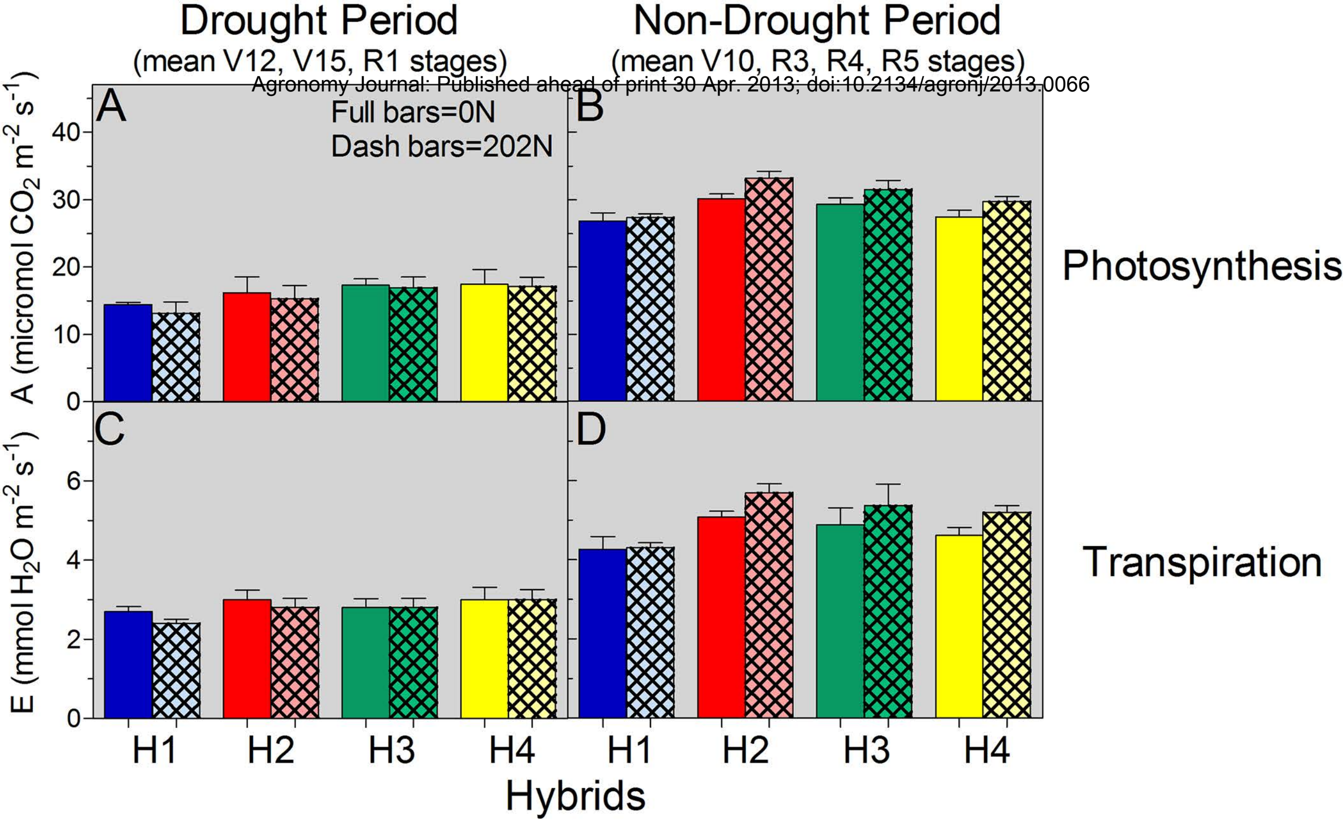
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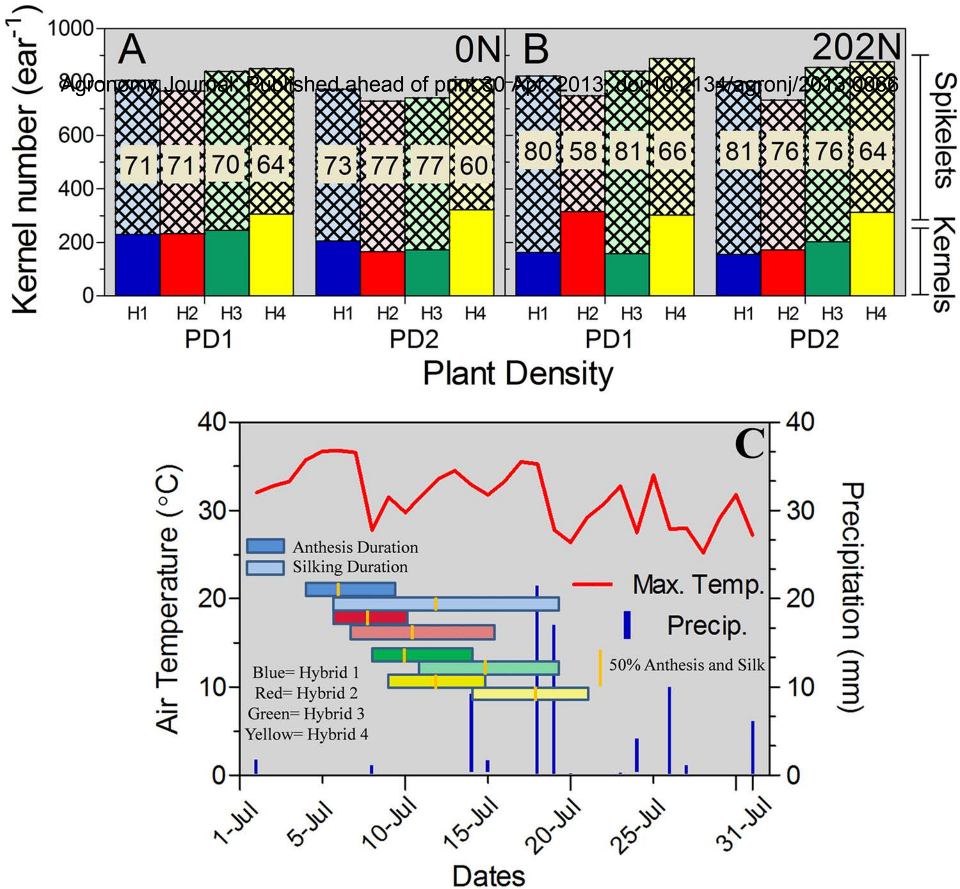
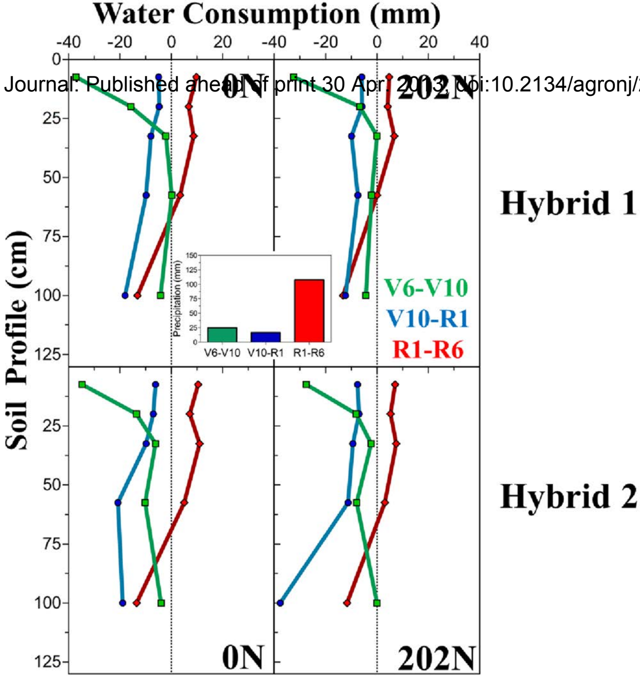
Non-Drought Period
(mean V10, R3, R4, R5 stages)
A
B
C
D
Full bars=0N
Dash bars=202N
A (micromol CO_2 m^{-2} s^{-1})
E (mmol H_2O m^{-2} s^{-1})
0
10
20
30
40
0
2
4
6
Photosynthesis
Transpiration
H1
H2
H3
H4
H1
H2
H3
H4
Hybrids


Kernel number (ear^{-1})
1000
800
600
400
200
0
A
0N
71
71
70
64
73
77
77
60
B
202N
80
58
81
66
81
76
76
64
Spikelets
Kernels
H1
H2
H3
H4
PD1
PD2
Plant Density
C
Air Temperature (°C)
Precipitation (mm)
40
30
20
10
0
Anthesis Duration
Silking Duration
Max. Temp.
Precip.
50% Anthesis and Silk
Blue= Hybrid 1
Red= Hybrid 2
Green= Hybrid 3
Yellow= Hybrid 4
1-Jul
5-Jul
10-Jul
15-Jul
20-Jul
25-Jul
31-Jul
Dates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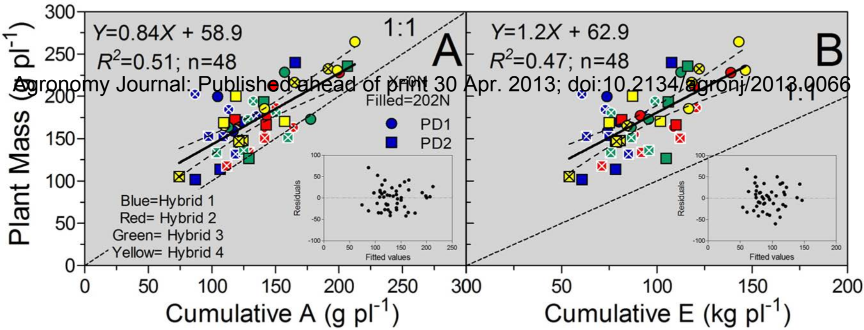
-20
0
20
-40
-20
0
20
40
Soil Profile (cm)
0
25
50
75
100
125
0N
202N
Hybrid 1
Hybrid 2
V6-V10
V10-R1
R1-R6
Precipitation (mm)
150
125
100
75
50
25
0
V6-V10
V10-R1
R1-R6
0N
202N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R^2=0.51; n=48
1:1
A
X=0N
Filled=202N
PD1
PD2
Blue=Hybrid 1
Red= Hybrid 2
Green= Hybrid 3
Yellow= Hybrid 4
Residuals
Fitted values
Plant Mass (g pl^{-1})
Cumulative A (g pl^{-1})
Y=1.2X + 62.9
R^2=0.47; n=48
B
1:1
Cumulative E (kg pl^{-1})
0
50
100
150
200
250
300