Evaluating soil evaporation and transpiration responses to alternate partial rootzone drying to minimise water losses

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Abstract

- *Purpose:* Partial rootzone drying (PRD) typically alternates the dry and irrigated parts of the rootzone, but how plant physiology and soil evaporation respond to this alternation are poorly understood.

- *Methods:* Dwarf tomatoes were grown in small split pots comprising two 250 cm^3 compartments and fully irrigated (WW: 100% ET_c) or subjected to three deficit irrigation treatments (75% ET_c): homogeneous rootzone drying (HRD; irrigation evenly distributed); fixed PRD (PRD-F, irrigation applied to one fixed compartment); alternated PRD (PRD-A: as PRD-F but alternating the irrigated compartment every three days). Stem diameter and evapotranspiration were monitored during alternation cycles. The day after alternating the irrigated side of the root system, whole-plant gas exchange and leaf water potential were measured following step increments of vapour pressure deficit.

-*Results*: Alternation did not affect stem diameter contractions or evapotranspiration, which were lower in HRD than in the two PRD treatments. However, soil evaporation was higher in HRD and PRD-A after alternation than in PRD-F. Following alternation, higher soil evaporation was counteracted by decreased transpiration compared with fixed PRD, despite similar overall soil water content. VPD increments did not change this pattern.

-*Conclusion:* Irrigation placement determined soil moisture distribution, which in turn affected soil evaporation and whole plant gas exchange. Optimising the frequency of PRD alternation to maximise water savings while ensuring productive water use needs to consider how soil moisture distribution affects both soil evaporation and plant water use.

Keywords: Irrigation efficiency, deficit irrigation, evapotranspiration, stem diameter variations, plant water status

Introduction

Water-saving irrigation techniques that decrease crop evapotranspiration (ET) can alleviate increasing pressure on water resources for agriculture. However, since crop yield is linearly related to ET and only saturates at high ET (Fereres and Soriano 2007), restricting transpiration (which generally accounts for the greatest proportion of ET) without decreasing yield is challenging. Nevertheless, deficit irrigation techniques that deliberately apply less irrigation volume than potential crop evapotranspiration (ET_c) while maintaining yields have been adopted. While most studies of deficit irrigation determine how little water can be applied without affecting yield, irrigation timing and placement within the rootzone can also improve crop water use efficiency at the same irrigation volumes (Dodd et al. 2015; Sebastian et al. 2016).

The partial rootzone drying (PRD) technique was developed to improve crop water use efficiency by changing the placement of deficit irrigation (During et al. 1996). Under PRD, a percentage of ET_c water volume is applied locally to one part (usually half) of the rootzone, which maintains leaf water status while decreasing stomatal conductance and shoot growth compared to evenly distributed irrigation at 100%ET_c (Kang et al. 1998; Dry and Loveys 1999). PRD increases xylem concentrations of the antitranspirant hormone abscisic acid (ABA) (Stoll et al. 2000; Liu et al. 2006; Pérez-Pérez et al. 2018), and changes other phytohormones (Kudoyarova et al. 2007; Pérez-Pérez et al. 2020) in many crops. ABA-mediated partial stomatal closure restricts transpiration while affecting photosynthesis less (Thompson et al. 2007), thereby increasing instantaneous leaf water use efficiency (the ratio of photosynthesis to transpiration). Sustaining this effect over the growing season allows PRD to improve crop water use efficiency (i.e. their yield per irrigation volume) compared to homogeneous application with the same irrigation volume (Dodd 2009).

However, instantaneous water use efficiency may only transiently increase during PRD because stomata re-open as the soil of the non-irrigated rootzone further dries (Dry et al. 2000; Stoll et al. 2000). This was attributed to reduced export of xylem-borne ABA from dry roots as their contribution to total sap flow declines with soil water content (Dodd et al. 2008a). This attenuation of PRD-induced signalling requires regular alternation of the irrigated side, to continue to restrict transpiration while maintaining shoot water status and growth. Other physiological measurements may help determine when PRD alternation should occur.

Despite much literature analysing the effects of PRD on different crops (Adu et al. 2018), the timing of PRD alternation is usually empirically based, causing variable effects on crop yield (Dodd et al. 2015). At the same irrigation volumes, alternation usually enhanced yield of field grown crops compared to fixed PRD, associated with higher stomatal conductance of pepper (Kang et al., 2001), but no differences in cotton plant water use (Du et al., 2006; Tang et al., 2005). These measurements were usually made in the middle of the alternation cycle, but few contradictory studies have evaluated the dynamics of physiological responses to alternation. Thus, alternation transiently decreased stomatal conductance of containerised tomato plants (receiving 50% less irrigation) associated with higher xylem ABA concentration (Dodd et al. 2006). Since sustained soil drying causes root ABA accumulation (Pérez-Pérez et al. 2020), re-watering likely substantially increased ABA export from those roots. However, when the wet side of the rootzone was maintained close to saturation, alternation did not decrease stomatal conductance or increase xylem ABA concentration in tomato (Pérez-Pérez and Dodd 2015; Pérez-Pérez et al. 2020). The intensity of the ABA signal, and hence magnitude of stomatal closure, depends not only on the soil moisture of the dry side of the rootzone but also of the wet side (Dodd et al. 2008b; Puértolas et al. 2016). Thus, xylem ABA increments and consequential partial stomatal closure following PRD alternation could be attributed to drying of the former wet side before full rehydration of the newly irrigated side.

Chemical long-distance signals interact with hydraulic signals to mediate shoot responses to water deficits (Tardieu 2016). Since PRD aimed to exploit long distance ABA signalling, potential changes in plant hydraulic conductance following PRD alternation have often been neglected. Re-watering previously dry roots can rapidly increase their hydraulic conductance (Kang et al. 2003) by enhancing aquaporin expression (McLean et al. 2011). Both hydraulic and chemical signalling models can simulate transpiration changes during PRD alternation (Huber et al. 2014), but the relative importance of each mechanism is still uncertain and may vary as evaporative demand changes during the day. Under low evaporative demand, progressive soil drying of half of the rootzone (with the remaining roots in wet soil) maintained leaf water potential, with root-sourced chemical signals triggering stomatal closure (Sobeih et al. 2004). However, under increasing evaporative demand, water supply from the wet rootzone might not meet greater transpirational requirements, thereby decreasing leaf water potential, causing turgor loss in guard cells and eliciting stomatal closure (Buckley 2019). Using soil-root water flow models to analyse the roles of chemical and hydraulic signals suggests that measuring plant transpiration and water potential at several evaporative demands can help discriminate the relative contribution of both mechanisms to stomatal control (Huber et al. 2015), thereby helping to predict the impact of PRD alternation under different environmental conditions.

Transpiration decreases xylem water potential causing water to flow from living vascular tissues into the xylem to partially sustain transpirational flow. Continuously monitoring the extent and velocity of stem diameter contractions, as applied in automated irrigation scheduling (Fernández and Cuevas 2010), can estimate changes in xylem tension (Klepper et al. 1971) between treatments during PRD alternation. Nevertheless, relatively few studies have measured these responses to PRD (e.g. Egea et al. 2011; Elsayed-Farag and Melgar 2015; Consoli et al. 2014).

Changing irrigation placement and timing can also affect soil evaporation rates. Since PRD usually waters only half the rootzone, it can reduce evaporation rates thereby increasing irrigation efficiency (defined as yield per irrigation volume unit), compared to homogeneous soil drying (Marsal et al. 2008). However, when irrigation is applied frequently enough, PRD alternation might increase soil evaporation from the whole rootzone. Evaporation increases with the wetted soil surface area (Leib et al., 2006), which increases dramatically immediately after alternating the irrigated side as surface soil moisture of both the newly and former irrigated sides is transiently high. Although some field studies have attempted to estimate whether PRD alters the evaporative component of ET (Leib et al. 2006; Marsal et al. 2008), dynamic changes in evaporation during PRD alternation cycles are poorly understood. While conventional rainfed cropping has emphasised the importance of minimising evaporative water losses while maximising transpiration to increase crop water use efficiency (Hatfield and Dold 2019), relatively few studies have determined how PRD affects crop evapotranspiration components (evaporation and transpiration) when wet and dry sides are alternated. Such information is essential to determine the potential water savings of the technique.

To better understand the dynamics of water losses from soil and crop during alternate partial rootzone drying, spatial and temporal variation in soil evaporation and plant physiological responses were investigated. Different irrigation treatments (homogeneous rootzone drying, and fixed and alternate PRD) were applied to split-root dwarf tomato (MicroTom) plants to measure whole plant gas exchange responses to changes in evaporative demand, both before and after PRD alternation. Unlike fast growing tall tomato genotypes, this variety dried the soil more slowly, allowing longer (three days) alternation cycles with substantial soil drying comparable to field studies. Diurnal stem contractions were readily monitored in this slow growing genotype, as these are overridden by fast diameter growth in tall tomato (Puértolas et al. 2020a). We hypothesised that

1. PRD alternation limits hydraulic conductance more than other deficit treatments, causing larger stem diameter contractions and restricting stomatal conductance (estimated from whole-plant gas exchange measurements) under increasing VPD.
2. PRD alternation transiently increases soil evaporation from the recently irrigated rootzone, causing greater evaporation from the whole rootzone than other deficit treatments.

Material and methods

Plant culture

Forty-eight seedlings of a dwarf tomato variety (*Solanum lycopersicum* cv. 'MicroTom') were raised in small (50 cm^3) pots filled with an organic loam (John Innes No.2, Westland Horticulture, Dungannon, UK) and grown for four weeks, so roots colonised the whole pot volume. By gently extracting and unpacking the soil plug, the root system was carefully divided and transplanted (ensuring the root system was equally distributed) into two square (8 x 8 cm) compartments of 250 cm^3 (7 cm in height) of a split-pot. Plants were grown for another two weeks, to ensure root colonisation of each compartment, in a ventilated greenhouse (day average temperature 25°C, night average temperature 18°C, average daytime air humidity 50%). During the photoperiod (14 hours), when natural light was below 200 µmol m^{-2} s^{-1}, high-pressure sodium lamps (Son-T, Phillips, Amsterdam, The Netherlands) provided 400 µmol m^{-2} s^{-1} at bench height. For six weeks before starting the treatments, pots were weighed daily (between 17:00-18:00) and irrigated to a pre-determined target weight near to pot capacity (to avoid transient rootzone anoxia). This weight was determined by irrigating pots at the end of the photoperiod until observing drainage, with pots weighed at the beginning of the next photoperiod (to guarantee all the gravitational water drained). Evaporative losses during the night period were not considered to ensure the irrigation target was slightly lower than actual pot capacity. Irrigation was distributed evenly over the entire soil surface, before randomly assigning plants to four irrigation treatments that were applied for 21 days:

Well-watered (WW): plants were watered as in the initial period, receiving an accumulated average of 3.02 L plant^{-1}, at 125 mL plant^{-1} day^{-1} (62.5 mL per compartment).

Fixed partial rootzone drying (PRD-F): 75% of the WW average irrigation volume was applied daily to only one compartment.

Alternate partial rootzone drying (PRD-A): Same irrigation as PRD-F, but swapping the irrigated compartment every three days, on days 4, 8, 12, 16 and 20 (Fig. 1).

Homogeneous soil drying (HRD): 75% of the WW average irrigation volume was distributed evenly between both compartments.

Frequency of irrigation (daily) and alternation (every three days) was determined from the rate of soil drying in preliminary measurements during the week before the experiment commenced. On average, WW plants lost 30% of the soil water volume in one day. Based on a substrate moisture release curve (see next section), daily irrigation was considered adequate for well-watered plants. After three days, water loss was 65% of the initial total water in the pot (estimated from soil volumetric water content measurements, see next section) representing moderate soil drying suitable for alternating the wet and dry sides. Although field-grown plants grown with unrestricted root systems have longer alternation frequencies, containerised tomato grown under greenhouse conditions usually swapped the wet and dry sides at intervals of less than 6 days (Kirda et al. 2004; Pazzagli et al. 2016; Yang et al. 2021). Thus, the alternation frequency was appropriate for the restricted volume of each compartment.

Water was slowly applied using a 60 mL plastic syringe to avoid drainage and to wet the whole surface of the compartment uniformly. No drainage was observed either upon alternation in PRD-A or after watering HRD.

Whole plant gas exchange responses to VPD (see gas exchange measurements section) were measured the day after the first, third and fifth alternation cycle, representing the entire treatment period. These time-consuming measurements (typically 2 hours per plant) allowed only one replicate of each treatment (four plants) each day. Since this would have limited replication (n=4), three groups of 12 plants (G1, G2, G3) were allocated, with each group starting the treatments on different days to ensure the first, third and fifth days of cycle for each group did not coincide on the same day. Also, since gas exchange measurements required leaf removal to normalise against leaf area, an additional set of 12 plants were kept intact until the end of the experiment to measure stem diameter variation. To avoid overlapping target alternation days, treatments started in G1 three days before G2 and six days before G3. Gas exchange was measured in one plant per irrigation treatment of each group the day after the first, third and fifth PRD-A alternation of the irrigated side (days 5, 13 and 21 after the start of the treatments; Fig. 1). This staggered application of irrigation treatments ensured that three replicates of each could be measured at the same time relative to when the treatments started. Whole-plant gas exchange measurements started two hours after the beginning of the photoperiod and finished before the evening irrigation. Treatments were distributed within each, day ensuring that the three replicates were measured at different times of the day to minimise time-related effects (diurnal changes and soil drying) on gas exchange.

Estimating the evaporative fraction of evapotranspiration.

Daily pot evapotranspiration on day n (ET) was measured as:

$ET = (PW_{n-1}+IV_{n-1})-PW_n$

Where PW_n is pot weight measured on that day (before irrigation), PW_{n-1} is pot weight measured on the previous day (before irrigation) and IV_{n-1} the irrigation volume applied after weighing the pot the day before (assuming a water density of 1 g L^{-1}).

Relative ET with respect to WW average (ET_{rel}) was calculated for each pot of the deficit irrigation treatments.

To estimate the evaporation component of ET, a separate experiment utilised fifteen single compartment pots without plants, each identical to a single compartment of the split-pots used in the main experiment. They were filled with the same substrate, and placed in the same environment, and distributed between three treatments to assess the effect of the different irrigation procedures applied to individual compartments.

WW: pots were watered to pot capacity every other day (as soil evaporative water losses over two days were similar to evapotranspirational losses from a single day in the main experiment).

WH: Water was withheld for nine days to replicate the irrigation procedure in the dry compartment of PRD-F, allowing soil to dry to the lowest level observed in that treatment.

RW: Pots were rewatered every two days but with 75% of the water applied to WW, to replicate HRD deficit irrigation and when a compartment was rewatered during PRD alternation.

Pots were weighed and soil volumetric water content ($\theta_{vsensor}$) measured with a soil moisture dielectric probe (ML2 Thetaprobe, Delta-T, Burwell, UK) immediately before each rewatering and θ_v calculated as described above. Evaporation (E) was calculated as the difference in weight between two consecutive days and the relative E (E_{rel}) compared to the average of WW was calculated for each day and pot of the WH and RW treatments. Since the θ_v vs E_{rel} relationship did not differ between both WH and RW treatments, a unique sigmoid function was fitted to the data to estimate E_{rel} from $\theta_{vsensor}$ measurements taken in plants instrumented with stem diameter sensors (see next section) before irrigation during the main experiment. E_{rel} was estimated separately for each compartment and the average E_{rel} was then calculated for the whole pot as the mean value of both E_{rel}.

Soil and plant water status

All pots without stem diameter sensors were weighed daily immediately before irrigation to estimate whole-pot soil volumetric water content. For the homogeneous irrigation treatments (WW and HRD), compartments were assumed to have similar water content. In the two PRD treatments, soil volumetric water content ($\theta_{vsensor}$) of each compartment was instantaneously measured with a Thetaprobe before and after each irrigation.

At the end of the experiment, soil moisture in both compartments of all plants was measured with the probes and the soil weighed after removing the roots. Soil was dried at 105˚C for

24 h to determine water mass in each compartment and calculate actual soil water content (θ_v) assuming a volume of 250 cm^3 for each compartment. A linear regression model between $\theta_{vsensor}$ and θ_v was fitted ($P<0.001$; $r^2=0.91$), and the equation ($\theta_V=2.06*\theta_{vsensor}$) used to transform measured $\theta_{vsensor}$ to actual θ_v. In plants that were not measured with the probes, whole pot volumetric and gravimetric water content was also calculated retrospectively in all treatments from daily pot weight measurements, considering dry soil, pot and plant fresh weight at harvest (plant fresh weight growth considered negligible). Only volumetric values are shown for consistency with probe measurements, but gravimetric values were used to estimate average soil water potential (Ψ_{soil}) from θ_g values using a previously determined moisture release curve (Puértolas et *al*. 2020b).

Before the last PRD alternation (around noon) in each group, half of the plants were sealed in a plastic bag and left in the dark for at least 24 hours to ensure soil and plant water potential equilibrated. Leaf water potential at null transpiration (Ψ_{E0}) was measured as a proxy of soil water potential averaged across the entire rootzone. The remaining plants in each treatment group were bagged the following morning (or after finishing gas exchange measurements in selected plants), once PRD was alternated the evening before. Leaf area was measured in all plants after Ψ_{E0} measurements.

Measuring stem diameter and gas exchange.

A linear variable displacement transducer (LVDT) (DF/2.5, Solartron Metrology, Bognor Regis, UK) was mounted on a metal alloy frame of low thermal expansivity (invar) and attached to the stem of four plants per irrigation treatment in G1. Each LVDT was connected to a multiplexer (AM416) + datalogger (CR1000) logging system (Campbell Scientific, Logan, UT, USA), which recorded stem diameter every 30 seconds. The stem swelled during the night and sharply contracted from the beginning of the photoperiod to around midday, as lamps were activated during that period (Fig. 2). After that, stems contracted at a lower rate and even temporarily swelled again when the greenhouse construction shaded the plants. Stem diameter consistently grew after irrigation and during the night period. To assess daily stem variations on the day of PRD alternation and the day after, maximum daily contraction (MDC) was calculated as the difference between the predawn maximum diameter and the minimum before watering, while afternoon contraction (AC) was the difference between the diameter at the end of the morning supplementary light period and the minimum before watering. Since the logging system failed to record during the first two alternation cycles in G1, the third and fourth cycles were analysed. To calculate water losses in the five WW plants with LVDT sensors, they were placed on portable balances (Scout Pro, Ohaus, Parsippany, NJ, USA) to continuously display pot weight. Weight at pot saturation was recorded right after installing the sensor and placing the pot on the balance.

Whole-plant gas exchange responses to step changes in air humidity were measured three times during the experiment in each group. For each group, one plant per treatment was measured the day after the first, third and fifth (last) alternation (Fig. 1). Initially, the whole pot and plant were placed in the custom-built whole-plant gas exchange system described previously (Jáuregui et al., 2018) with details summarised in S1:Appendix 1. The plant was acclimated for approximately 30 min to the light conditions of the chamber. Air relative humidity (RH) inside the chamber was initially set to ∼70% by passing dry air (RH=6 %; [CO_2] =420 ppm) through a humidifier compartment. Then the chamber was opened (for less than 30 s) to excise a fully expanded leaf to measure leaf water potential (Ψ_{leaf}), then determine leaf area (Model Li-3100, Li-Cor Inc., Lincoln, Nebraska, USA). After closing the chamber again, gas exchange was allowed to stabilise again (typically 10 min), and then RH inside the system was reduced to 50% by introducing a mixture of dry and humidified air to the chamber. After the following RH level was achieved (typically 3 min), plant gas exchange was allowed to stabilise (typically 20-30 min). Each plant was exposed to four sequentially decreasing humidity levels achieved by increasing the ratio of dry to humid air (70%, 50%, 35%, 6%), approximately corresponding to vapour pressure deficit (VPD) values of 1, 1.5, 2 and 3 kPa (measurements taken at ambient temperature of ~25˚C). VPD levels were controlled accurately, with a maximum standard error of 0.15 kPa for each treatment and date combination. At the lowest VPD level, Ψ_{leaf} and area were again measured.

Stability of Ψ_{soil} was assumed during the gas exchange measurements, as the total volume of water transpired was less than 10 g in well-watered plants (with θ_V=0.60 cm^3cm^{-3}) and even less in drier plants (typically around 5 g for plants with θ_v=0.20 cm^3cm^{-3}). Thus θ_v decreased by 0.02 and 0.01 cm^3cm^{-3} respectively, corresponding to Ψ_{soil} decreasing from -0.010 to -0.012 and from -0.70 to -0.78 MPa, respectively. Such changes were considered negligible in the context of the treatments imposed.

Net photosynthesis (A_n), transpiration rate (T), water use efficiency (*WUE*), and canopy conductance (G_c) were calculated from the difference in [CO_2] and [H_2O] of the air entering and exiting the chamber and the air flow as described in S1:Appendix 2.

Statistical analyses

Whole pot θ_v, ET_{rel} and estimated E_{rel} were analysed by repeated measures ANOVA. Since WW values of θ_v greatly exceeded the deficit irrigation treatments ($P<0.001$), a second analysis included only the three deficit irrigation treatments. Treatment differences were determined by a Tukey test ($P<0.05$). Maximum stem diameter contraction (MDC) and afternoon contraction (AC) were analysed by a mixed linear model, with irrigation treatment and time with respect to alternation (the day of, and after, alternation) as fixed factors and cycle (third or fourth cycle) and plant as random factors. Treatment differences were assessed by pairwise comparisons using the Bonferroni adjustment. The response of T, G_c, A_n, *WUE*, and Ψ_{leaf} to VPD on the day after alternation was analysed by a mixed model, with cycle, VPD step and treatment as fixed factors (VPD step as repeated factor), and plant as a random factor. Treatment and time with respect to alternation effects on Ψ_{E0} were assessed by two-way ANOVA and differences between treatments with a post-hoc test (Tukey, $P<0.05$). All these analyses were performed with SPSS 24 (IBM, Armonk, NY, USA). The significance of the relationship between θ_v and G_c measured at the second VPD level (1.5 kPa) was determined by linear regression using Sigmaplot 12.0 (Systat Software Inc, San Jose, CA, USA).

Results

Estimating the evaporative fraction of evapotranspiration

Soil volumetric water content (θ_v) was sigmoidally related to evaporation relative to WW plants (E_{rel}). Relative evaporation (E_{rel}) of pots with θ_v >0.4 was close to 1, and similar to WW pots (Fig. 3). Predicted E_{rel} fluctuated around 0.7 in PRD-A and HRD treatments, and continued to decrease in PRD-F reaching 0.5 at the end of the experiment. Then, E_{rel} of the wet and dry compartments were~0.9 and ~0.1 respectively (Fig. 4).

In PRD-A, the alternation of irrigated sides dictated fluctuations in predicted E_{rel}, which increased the day after each alternation but progressively decreased before the next alternation (Fig. 5a). Predicted E_{rel} was higher in HRD and PRD-A than in PRD-F. In all treatments, it started to decrease 3 to 6 days after applying treatments (two first PRD-A cycles).

Actual relative evapotranspiration (ET_{rel}) was lowest in HRD, with intermediate, similar values for the two PRD treatments (Table 1). Following homogenous root drying, ET_{rel} of HRD plants initially remained close to 1 then dropped sharply 3 days after withholding water, but increased again the day after. ET_{rel} progressively decreased in HRD, but remained consistently below the PRD treatments at 0.6 (Fig. 5b). Evapotranspiration of the PRD treatments progressively decreased during the first week, then stabilised until the end of the experiment at 0.75. Overall, PRD alternation did not affect ET, although it transiently increased the day after the third alternation.

Soil and plant water status

Before irrigation, whole pot θ_v of WW plants remained at 0.5 $cm^3\ cm^{-3}$ (Ψ_{soil}= -0.05 MPa) throughout the experiment. In the three deficit irrigation treatments (which were statistically similar, P=0.55), it steadily decreased from that value to around 0.23 $cm^3\ cm^{-3}$ (Ψ_{soil}= -0.88 MPa) during the first ten days and remained stable thereafter (Fig. 5c). In PRD-A, θ_v before irrigation generally dropped to 0.2 cm^{3}cm^{-3} in the dry side, recovered to 0.4 cm^{3}cm^{-3} the day after irrigation, and gradually increased to 0.5 cm^{3}cm^{-3} before the next change of irrigation side, as daily irrigation replenished pot capacity (Fig. 6a). Before irrigation, θ_v of the wet side of PRD-F was stable at 0.5 cm^{3}cm^{-3} throughout the experiment. Withholding water from the dry side rapidly decreased θ_v to 0.3 cm^{3}cm^{-3} in the first three days, then slowly decreased θ_v to 0.12 cm^{3}cm^{-3} in the following 11 days before it stabilised (Fig. 6b). The 3 deficit treatments receiving the same irrigation volumes varied in their spatial distribution of soil moisture.

Pot average θ_V of WW plants was always higher than in the three deficit irrigation treatments (0.57 vs 0.40 cm^{3}cm^{-3}). However, θ_v of the wettest side was significantly higher in WW and PRD-F (0.62 and 0.58 cm^{3}cm^{-3}) than in PRD-A and HRD (0.42 cm^{3}cm^{-3} for both treatments). Water potential at null transpiration (Ψ_{E0}) was significantly ($P<0.001$) higher in WW plants than the three statistically similar deficit irrigation treatments (Table 1). Neither alternation nor its interaction with treatment ($P=0.52$ and 0.50 respectively) affected Ψ_{E0}. Thus, the two groups of PRD plants had similar access to soil water both before and after alternation in PRD-A.

Stem diameter and gas exchange measurements

Maximum daily contraction (MDC) was consistently around 50-100 µm with θ_v>0.35 cm^{3}cm^3, and increased as average θ_v decreased below that threshold (Fig. 7a, b). MDC did not statistically differ ($P=0.25$) between treatments, with MDC of PRD-A plants remaining low (generally below 100 µm) and not changing with alternation. Afternoon contraction (AC) followed a similar trend as MDC (Fig. 7c, d), but the treatments significantly differed ($p=0.006$), with AC significantly higher in HRD than WW, while PRD treatments had intermediate values (not significantly different from HRD or WW; Table 1). Thus, stem diameter measurements in the afternoon during the period of supplementary lighting could distinguish the treatments.

When measured after alternation, leaf gas exchange (T, G_c and A_n) was similar between WW and PRD-F plants and between PRD-A and HRD plants. These three variables were significantly higher (P=0.006, 0.005, 0.04 respectively) in WW and PRD-F than in PRD-A and HRD treatments (Fig. 8). However, irrigation treatment did not affect Ψ_{leaf} and instantaneous water use efficiency (P=0.13 and P=0.69 respectively, data not shown).

Canopy conductance and Ψ_{leaf} decreased with VPD, while T and WUE increased (P<0.001 for G_c, Ψ_{leaf}, T, and WUE, Fig. 8). None of these variables (A_n, G_c, Ψ_{leaf} and WUE) interacted with treatment (P>0.35) although T increased with VPD in WW and PRD-F plants more than in PRD-F and HRD plants (P=0.02 for the Treatment x VPD interaction, Fig. 8a). Net photosynthesis increased from the first to the second VPD step change, and then decreased below the initial value from the third to the fourth VPD level (Fig. 8c).

For the deficit irrigation treatments, whole-pot average θ_v and G_c measured at the second VPD level (1.5 kPa, maximum difference between treatments as in Fig. 8b) were not related, as PRD-F (with higher G_c) had similar θ_v to the other two treatments (Fig. 9a). However, G_c significantly increased with θ_v of the wettest compartment, with both higher in PRD-F (Fig. 9b). Thus, fixed PRD maintained similar whole plant gas exchange to WW plants, while PRD alternation and homogenous soil drying decreased it. Greater stomatal closure caused by PRD alternation, and comparing HRD plants to fixed PRD, limited any VPD-induced increase in transpiration.

Discussion

Developing a miniaturised model system of PRD allowed precise whole plant measurements of photosynthesis and transpiration at controlled VPDs (Jauregui et al. 2018), while continuously measuring stem diameter as a proxy of shoot water status. Furthermore, this system readily distinguished the impacts of different irrigation treatments on both evaporative and transpiration components of ET. Variable impacts of PRD alternation on these components may account for variation in crop yield and water use efficiency (Dodd 2009; Adu et al. 2018), but most PRD studies have emphasised leaf-level physiological or whole plant agronomic responses without reconciling these effects across scales. Although the components of evapotranspiration were not directly dissected, the differences in both transpiration (measured in the whole-plant gas exchange chamber) and evaporation (measured in a dedicated experiment) suggest that PRD alternation restricts plant water use but enhances evaporative losses. These contrasting effects might explain the lack of net water savings of alternate compared with fixed application of PRD in our model system (Table 1, Fig. 10). Since fixed PRD maintained photosynthesis relative to well-watered plants and better suppressed soil evaporation alternating the wet and dry sides of PRD plants may be unnecessary to maximise water savings. Further field experiments should confirm that these effects are not restricted to the miniaturised crop system used here. Nevertheless, since PRD-F is less complex to operate than alternate PRD, this could facilitate greater farmer adoption. Irrespective, minimising soil evaporative losses in irrigated agriculture (e.g. through mulching) seems essential if gains in leaf-level water use efficiency (stimulated by our enhanced understanding of plant responses to heterogeneous soil drying) are to bring maximal benefits to water scarce cropping systems.

The presence of wet soil layers in otherwise dry rootzones (PRD) maintains transpiration compared with uniformly dry soil of the same water content (HRD). This was attributed to the maintenance of high root water potential and hence lower ABA accumulation of roots growing in heterogeneously rather than homogenously dry soil (Puértolas et al. 2017, 2020b), due to hydraulic redistribution from wet to dry roots (Bauerle et al. 2008). Both PRD-F and PRD-A treatments (with different soil moisture distributions but always a wetted part of the root system) had similar leaf water potential at null transpiration (Table 1) and during gas exchange measurements, and consequently stem diameter contractions (Fig. 7). Thus, increased root-sourced chemical signalling after PRD alternation might decrease gas exchange. Under PRD, most water uptake occurs in the irrigated rootzone, where root-sourced signals are presumably low (Puértolas et al. 2015), but alternation rapidly decreases water uptake from this side while increasing it from the formerly dry side (Pérez-Pérez and Dodd 2015). Together with increasing soil drying in the formerly wet side, this allows both sides of the root system to transiently export root-sourced signals. Although Ψ_{E0} is lower in PRD-F than WW plants, their similar gas exchange suggests soil moisture distribution is a key variable in stomatal regulation under mild soil water deficits.

Moreover, alternating wet and dry sides during partial rootzone drying irrigation transiently homogenised soil moisture distribution compared to fixed PRD, as soil moisture of the previously dry side only partially recovered to field capacity (Fig. 6a). Thus, soil water content in both sides of PRD-A was lower than 0.5 cm^3cm^{-3} while the wet side of PRD-F was above that threshold, allowing higher stomatal conductance of PRD-F than PRD-A plants (Fig. 8b). Thus, continuously irrigating to part of the rootzone while withholding irrigation from the other. could maintain whole plant gas exchange and biomass accumulation. Treatment differences in whole-plant transpiration occurred after PRD alternation (higher in WW and PRD-F than HRD and PRD-A - Fig. 8), but all deficit irrigation treatments had lower ET than WW plants, especially the HRD treatment that was lower than both PRD treatments (Fig. 5b). The effect of irrigation placement on evaporation (E) also partly explained variation in ET. Irrigating the whole pot surface in HRD was estimated to increase E compared to irrigating only half of the pot, which might have attenuated treatment differences in ET caused by the low T of HRD than PRD-F. Interestingly, alternation in PRD-A transiently increased E (Fig. 5a) and maintained it higher than PRD-F. This might explain the similar ET of both treatments, even though PRD-F had higher T than PRD-A after alternation (Fig. 10). While the presence of a wet part within the rootzone with heterogeneous soil drying (PRD) enhanced plant transpiration, evaporation from the non-irrigated side of the rootzone was restricted.

Although the three deficit treatments had similar overall soil moisture (Fig. 5c), the more homogeneous soil moisture distribution across the entire rootzone in HRD and PRD-A plants maintained higher overall evaporation than PRD-F. Similar results occurred when comparing PRD with sustained deficit irrigation (DI), and between different dripper distributions under DI (Marsal et al. 2008). The temporal dynamics of E in PRD-A supports a positive relationship between soil moisture homogeneity and evaporation. Alternation tended to equalise soil moisture in both compartments (Fig. 6a), which transiently (for one day) increased E_{rel} (Fig. 5a). Between alternation events, as θ_v increasingly differed between compartments, predicted E_{rel} decreased since soil water content was non-linearly related to evaporation (Fig. 3, Wythers et al. 1999). Thus, soil evaporation in each side of treatments with homogeneously moderately dry rootzone (HRD and PRD-A) is only slightly lower than the wet side of PRD-F, but much higher than in the dry side (Fig. 4), thereby resulting in higher overall evaporation than in PRD-F (Fig. 5a). Thus, both irrigation placement and timing affected not only plant water use but also soil evaporation, which determines irrigation efficiency.

Although canopy shading of the soil surface may modify the relationship between θ_v and E by decreasing E, this relationship should still be proportional to the wetted soil volume. Thus, techniques that lower soil evaporation (such as sub-surface irrigation or soil cover) could maximise water savings during deficit irrigation (Abdelraouf and Ragab 2018; Gil et al. 2018; Iqbal et al. 2021). However, applying PRD-F when the irrigated side is covered to minimise soil evaporation might be the most effective technique, as it does not reduce G_c and A_n either transiently (PRD-A) or consistently (HRD) (Fig. 8). In some production systems, covering the soil of only the irrigated side of the rootzone could save time and money while maintaining high irrigation efficiency. However, in field experiments comparing fixed and alternate application of PRD, the latter consistently outyielded the former (Dodd et al., 2015 and references therein). Daily irrigation of our experiment was unable to maintain optimal water content of the wet side of PRD plants. Since soil moisture levels of the irrigated side explain physiological responses to PRD (Dodd et al., 2008a), more homogeneous water uptake between sides of PRD-A plants (the dry side does not dry as much as in fixed PRD) might explain some favourable results for alternate PRD (Kang et al., 2001). However, it also suggests that effective PRD application should consider an adequate frequency of irrigation.

Irrigation regime determined overall whole-plant gas exchange, but not its response to evaporative demand. Soil moisture content or distribution did not seem to affect stomatal sensitivity to VPD (Fig. 8). Hydraulic models indicate that stomatal conductance is controlled only by changes in leaf water potential, which in turn, depends on both soil water potential and transpiration rate (Buckley 2019). As stomatal aperture also controls transpiration rate, this configures a feedback model that predicts greater differences in G_c between plants with contrasting soil water potential at low VPD levels, which would decrease as VPD increases (Sperry et al. 2017). This occurs because limited water loss at low VPD levels, and differences in G_c, reflect differences in soil water potential. As the VPD increases, plant transpiration in drier soil will be more limited than in wetter soil, inducing proportionally greater stomatal closure. In our experiment, this difference in G_c was similar across the whole VPD range tested (1-3kPa, Fig. 8b), reinforcing that observed differences in gas exchange in response to the moderate water deficits elicited by these irrigation treatments are not associated to hydraulic constraints. Thus root-sourced signals regulate differences in G_c at low VPD, with increasing VPD enhancing transpiration and decreasing leaf water potential equally across all the treatments, reducing G_C similarly.

Conclusions

Hypothesis 1, that alternation would impose hydraulic limitations that increase stem diameter contractions and reduce G_c and A_n, was not sustained. Instead, alternation transiently decreased G_c and A_n coincident with the homogenisation of rootzone soil moisture, caused by the disappearance of the wet (close to field capacity) region within the rootzone. Decreasing soil moisture heterogeneity transiently increases fluxes of root-sourced chemical signals to the shoot to elicit stomata closure (Dodd et al. 2008b; Puértolas et al. 2016). However, homogenising soil moisture across the rootzone also transiently increased soil evaporation, confirming Hypothesis 2, thereby offsetting the decreased G_c and resulting in similar crop water losses (Fig. 10). While partial rootzone drying already minimises soil evaporation by irrigating only half the rootzone, further water savings could be achieved by reducing evaporative losses from irrigated areas (by covering them or using sub-surface irrigation) when applying PRD. Since applying PRD-F and covering only the irrigated half of the rootzone could minimise the cost of soil cover, further field trials are needed to empirically assess these concepts at different irrigation frequencies, and the consequences for irrigation efficiency.

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Statements and declarations

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Figure Legends

Figure 1. Experimental schedule. For each group of plants, the timing of side alternation in alternated PRD (black drops) and gas exchange measurements (asterisks) is shown for the 21 days of treatment application

Figure 2. Example of stem diameter variation from a HRD plant between 19:00 and the same hour on the following day. Stem diameter is shown as variation with respect to the value at 19:00 on the first day (marked with a horizontal dashed line). The shaded and stripped areas denote the nocturnal and afternoon period respectively. Double-headed arrows show the maximum daily contraction (MDC), and the afternoon contraction (AC) while a single-headed arrow indicates when the plant was irrigated.

Figure 3. Relationship between compartment soil volumetric water content (θ_v) and soil evaporation relative to the average of WW compartments for pots from which water was withheld (WH) or rewatered (RW). The sigmoid function fitted for the whole dataset (no statistical differences between WH and RW) is depicted, together with its equation and the correlation coefficient.

Figure 4. Daily estimated evaporation (E) relative to WW plants (n=4; mean ± s.e.) from each side of the pot (side A, black circles, solid lines; Side B, hollow circles, dashed lines) in PRD-Alternated (A), PRD-Fixed (B) and HRD (C) treatments. The dashed vertical lines denote when sides were alternated in PRD-A.

Figure 5. Daily estimated evaporation (E, A) and evapotranspiration (ET,B) relative to WW plants, and whole pot average soil volumetric water content (θ_v, C) (n=8 for A, B, n=4 for C; mean ± s.e.). The dashed vertical lines denote when sides were alternated in PRD-A (last alternation on day 20 not shown as half of the plants were harvested before irrigation). WW: hollow triangles, HRD: solid triangles, PRD-F: hollow circles, PRD-A: solid circles.

Figure 6. Soil volumetric water content (n=5, error bars not shown for clarity) estimated from soil moisture probe readings in each compartment taken immediately before and after each irrigation in the two partial rootzone drying treatments (alternated (A) and fixed (B)). Side X (solid line) is the initially irrigated side (dry side after alternation in alternated PRD), while Side Y (dotted line) is the initially dry side. The dashed vertical lines denote when sides were alternated in PRD-A.

Figure 7. Relationship between soil volumetric water content averaged across both compartments and both maximum daily contraction (A, B) and afternoon contraction (C, D) on the day of (A, C) and the day after (B, D) alternation for the three deficit irrigation treatments. HRD: solid triangles, PRD-F: hollow circles, PRD-A: solid circles. For significant regressions, P-values and regression coefficient for the nonlinear (quadratic) regression fitted for each relationship are shown (P=0.10 for regression in panel D, not shown)

Figure 8. Transpiration rate (A), canopy conductance (B) and net photosynthesis rate (C) for the four irrigation treatments (WW: hollow triangles, HRD: solid triangles, PRD-F: hollow circles, PRD-A: solid circles) at four incremental VPD steps (1=1 kPa, 2=1.5 kPa, 3=2.5 kPa, 4=3kPa; s.e<0.15 kPa). Data points represents the average of nine plants (three plants and three measurement dates; after alternation at the end of the first, third and fifth PRD-A cycles).

Figure 9. Relationship between canopy conductance (G_c) measured at the second VPD level (1.5 kPa) and soil volumetric water content (θ_v) averaged across the whole pot (A) and of the wettest compartment (B) for the three deficit irrigation treatments. HRD: solid triangles, PRD-F: hollow

circles, PRD-A: solid circles. For B panel, P-value and regression coefficient for the linear regression fitted is shown ($P=0.18$ for the regression in panel A, not shown)

Figure 10. Schematic representation of the relative contribution of the components of irrigation efficiency (Net photosynthesis: A_n, Transpiration (T), Evaporation (E), Evapotranspiration (ET)) as affected by soil moisture (θ) distribution. Relative size of arrows and letters are proportional to the observed size of effect. For alternated PRD, effects observed after alternation. Sub-indices A and B for E represent the evaporation from either side.

Table 1. Average ± s.e. of accumulated evapotranspiration (ET) at the end of the experiment (n=8), afternoon stem contraction (AC) (n=4), and leaf water potential at null plant transpiration (Ψ_{E0}) (n=12) for the four irrigation treatments. AC and Ψ_{E0} are averaged across the four and two measurement dates, respectively, as no time significant effect was observed. For each variable, different letters within a row denote statistical differences between treatments.

	WW	PRD-F	PRD-A	HRD
ET (Kg H_20)	2.40±0.05 a	1.98±0.01 b	1.95±0.01 b	1.57±0.18 c
AC (µm)	6.7±2.8 b	51.5±19.6 ab	33.2±10.9 ab	125.9±20.4 a
Ψ_{E0} (MPa)	-0.16±0.01 b	-0.26±0.02 a	-0.26±0.02 a	-0.32±0.03 a

Figure 1.

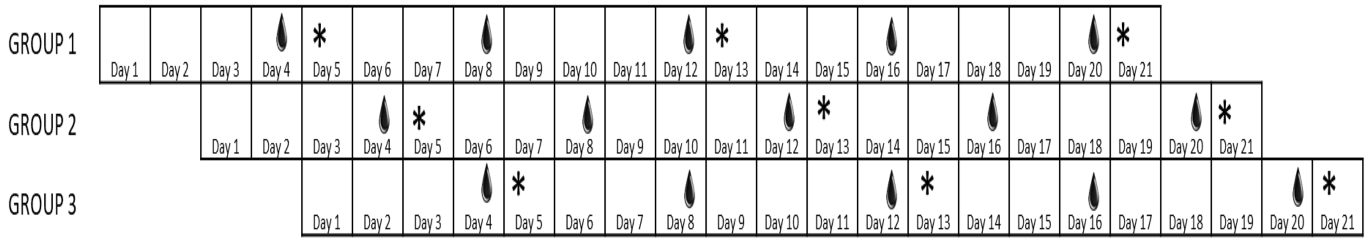

Figure 2

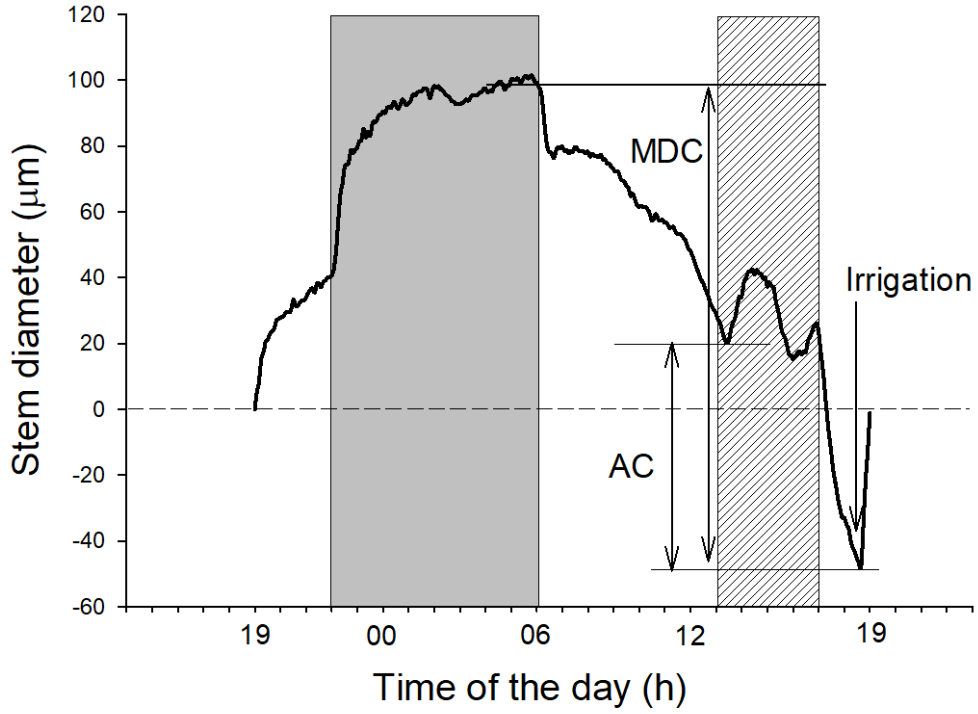

Figure 3

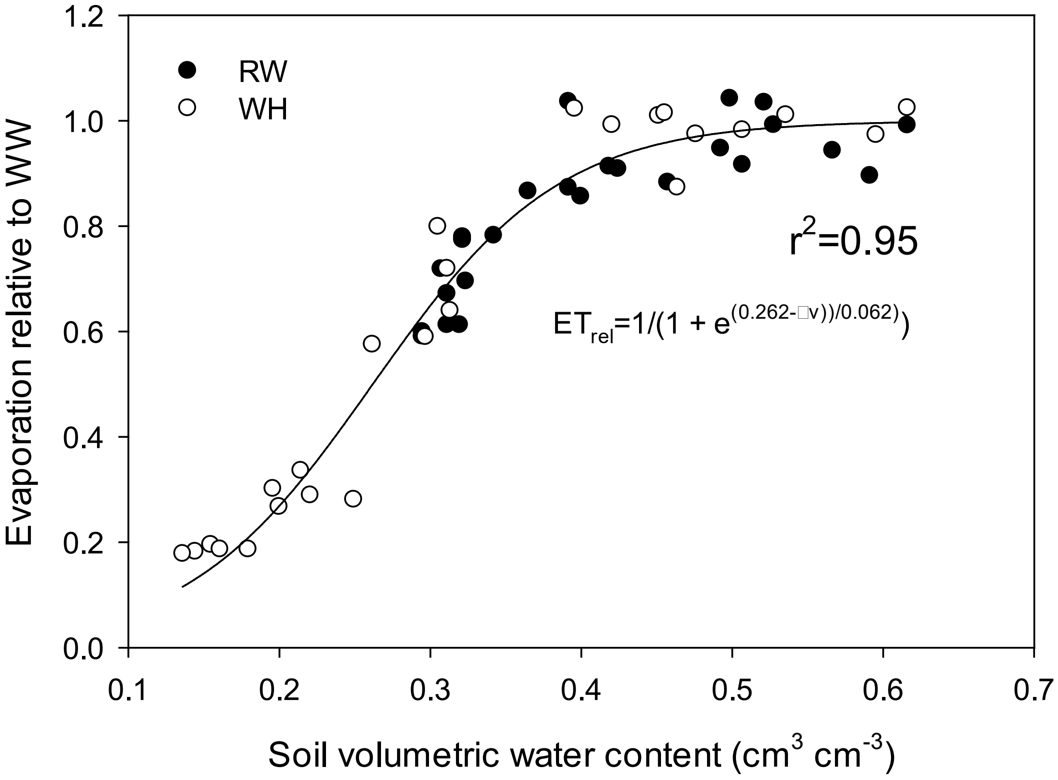
Evaporation relative to WW
1.2
1.0
0.8
0.6
0.4
0.2
0.0
RW
WH
r^2=0.95
$ET_{rel}=1/(1 + e^{(0.262-\square v))/0.062)})$
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Soil volumetric water content (cm^3 cm^{-3})

Figure 4

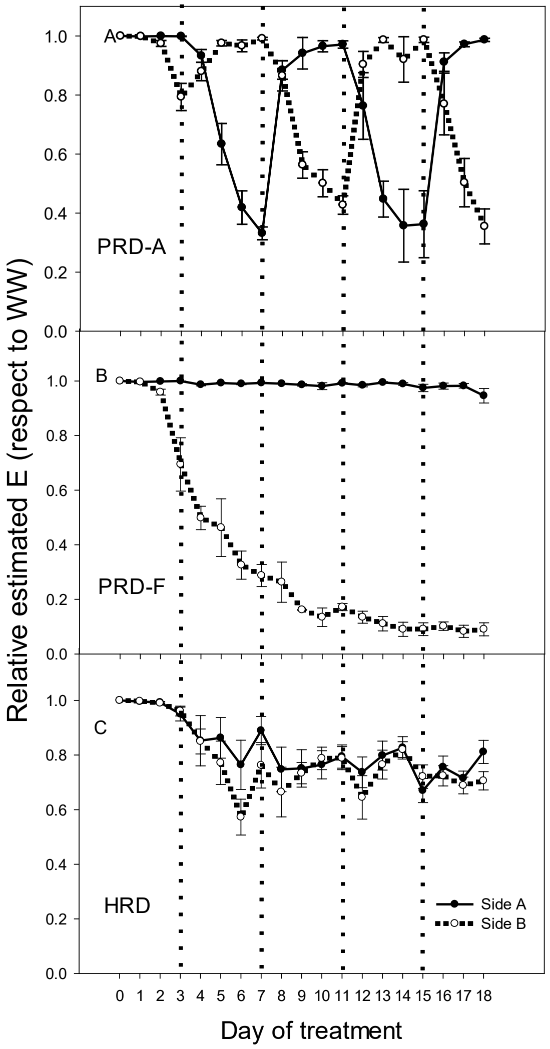
A
PRD-A
B
PRD-F
C
HRD
Side A
Side B
Relative estimated E (respect to WW)
Day of treatment
0.0
0.2
0.4
0.6
0.8
1.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure 5

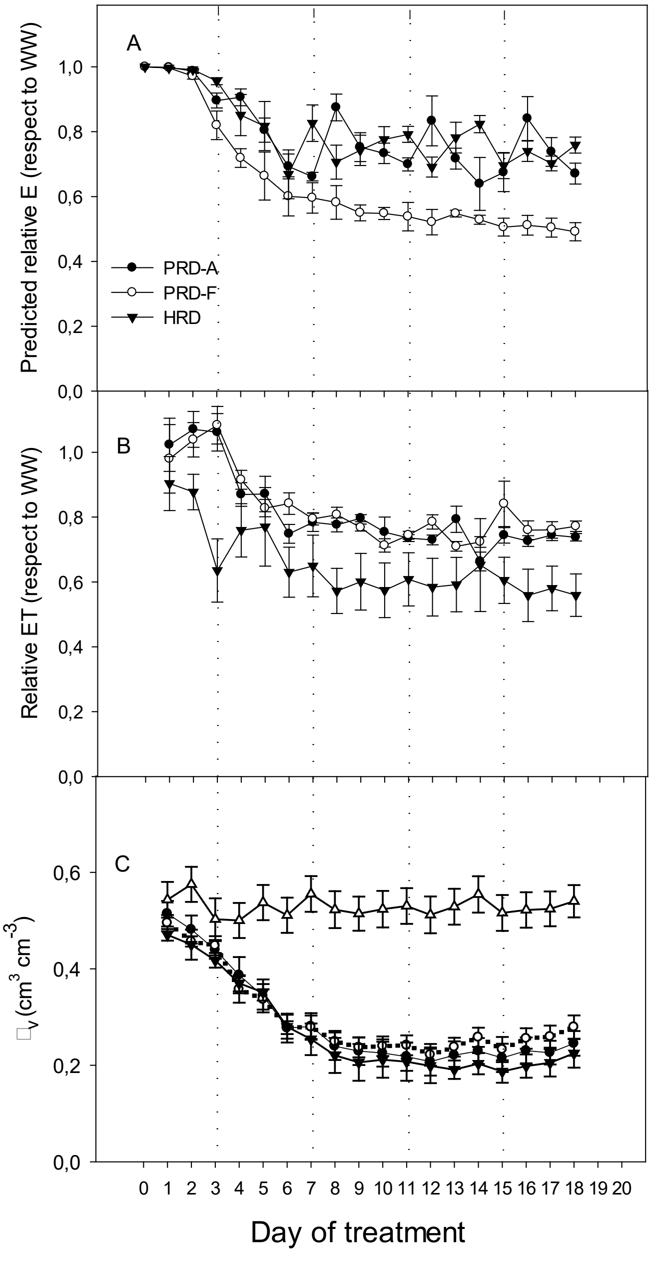
A
B
C
Predicted relative E (respect to WW)
Relative ET (respect to WW)
□V(cm3 cm-3)
0,0
0,2
0,4
0,6
0,8
1,0
PRD-A
PRD-F
HRD
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Day of treatment

Figure 6

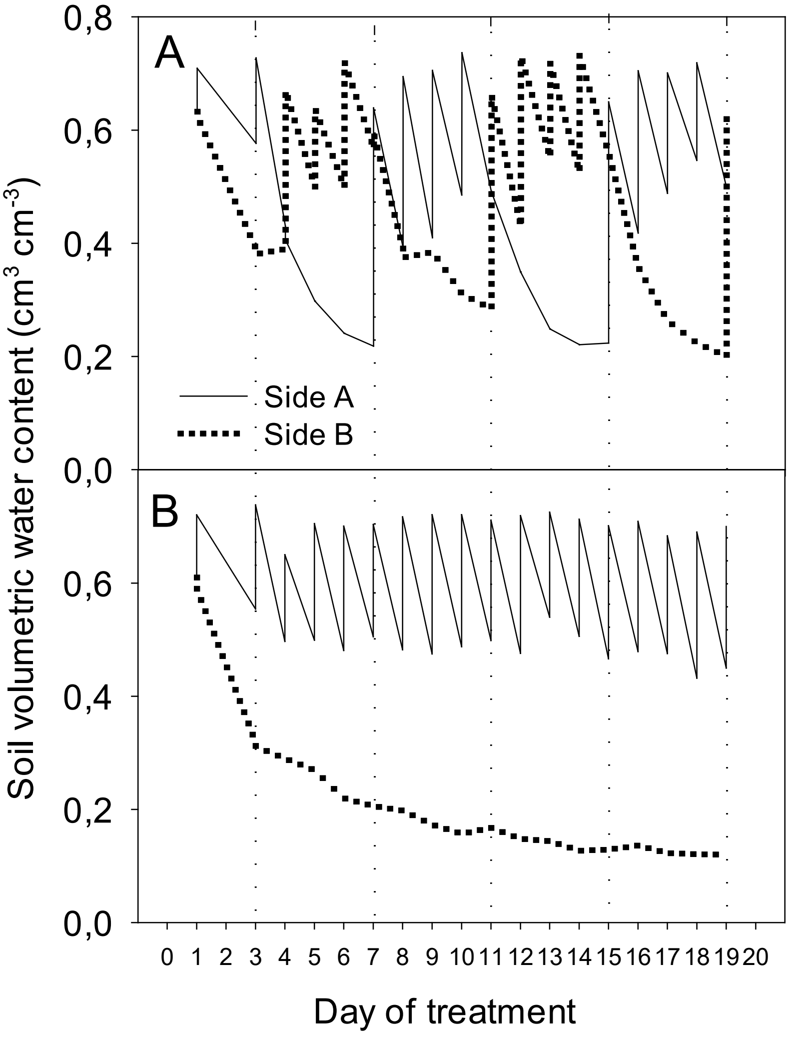
Soil volumetric water content (cm^3 cm^{-3})
0,8
0,6
0,4
0,2
0,0
A
B
Side A
Side B
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Day of treatment

Figure 7

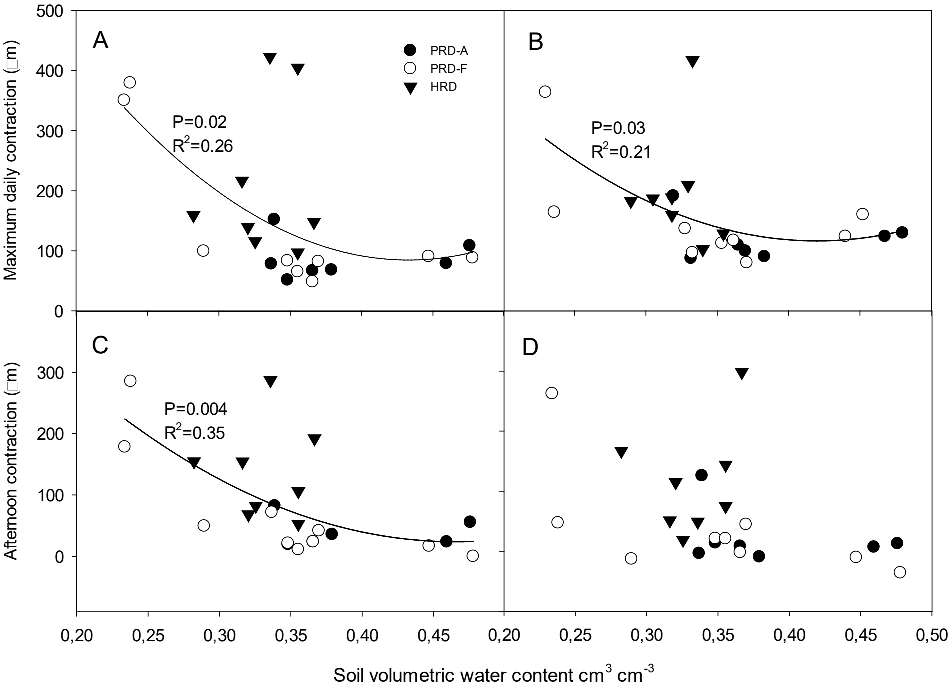
A
B
C
D
PRD-A
PRD-F
HRD
P=0.02
R^2=0.26
P=0.03
R^2=0.21
P=0.004
R^2=0.35
Maximum daily contraction (µm)
Afternoon contraction (µm)
500
400
300
200
100
0
300
200
100
0
0,20
0,25
0,30
0,35
0,40
0,45
0,20
0,25
0,30
0,35
0,40
0,45
0,50
Soil volumetric water content $cm^3\ cm^{-3}$

Figure 8

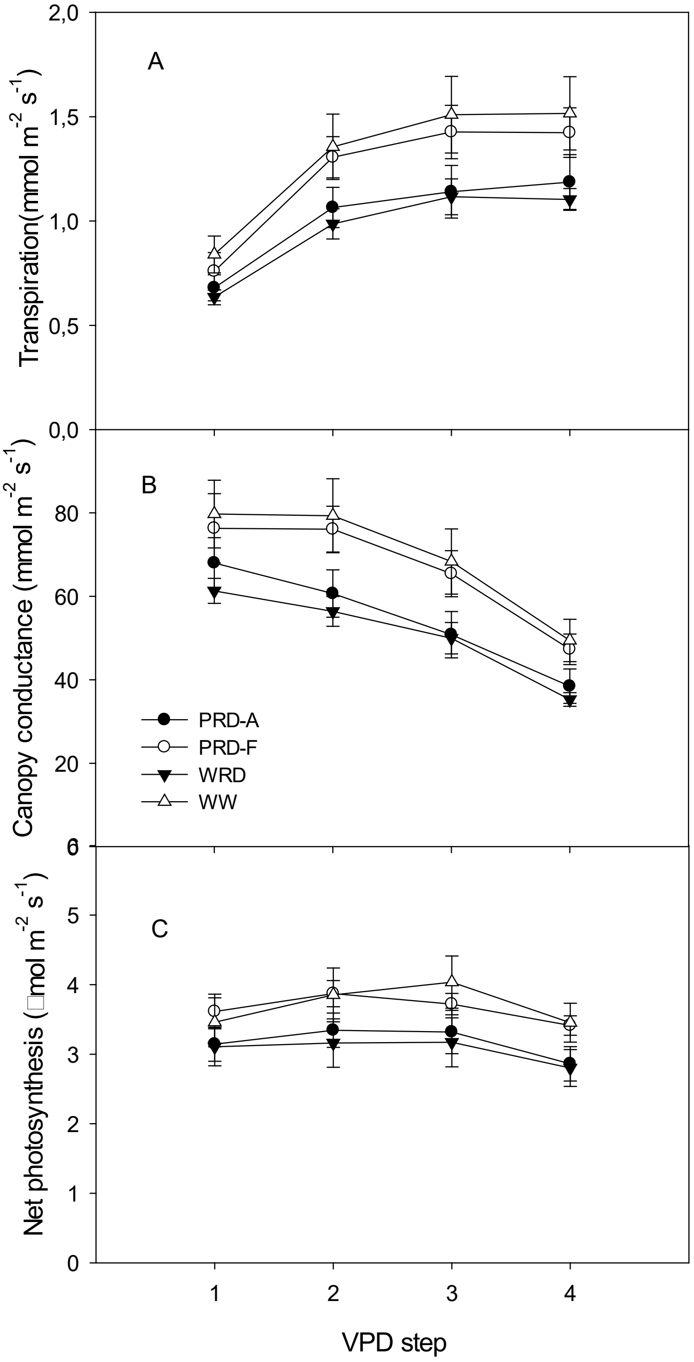
A
Transpiration(mmol m^-2 s^-1)
2,0
1,5
1,0
0,5
0,0
B
Canopy conductance (mmol m^-2 s^-1)
80
60
40
20
0
PRD-A
PRD-F
WRD
WW
C
Net photosynthesis (□mol m^-2 s^-1)
6
5
4
3
2
1
0
1
2
3
4
VPD step

Figure 9

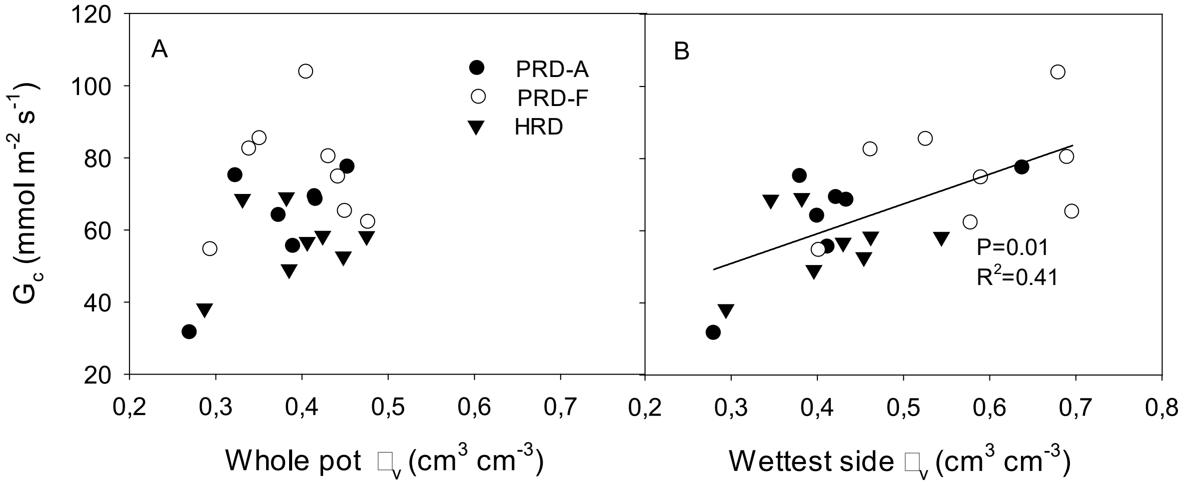

Figure 10