

Temporal and spatial patterns of soil water extraction and drought resistance among genotypes of a perennial C₄ grass

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Abstract. The objective of this study was to investigate patterns of soil water extraction and drought resistance among genotypes of bermudagrass (*Cynodon* spp.) a perennial C₄ grass. Four wild Australian ecotypes (1–1, 25a1, 40–1, and 81–1) and four cultivars (CT2, Grand Prix, Legend, and Wintergreen) were examined in field experiments with rainfall excluded to monitor soil water extraction at 30–190 cm depths. In the study we defined drought resistance as the ability to maintain green canopy cover under drought. The most drought resistant genotypes (40–1 and 25a1) maintained more green cover (55–85% vs 5–10%) during water deficit and extracted more soil water (120–160 mm vs 77–107 mm) than drought sensitive genotypes, especially at depths from 50 to 110 cm, though all genotypes extracted water to 190 cm. The maintenance of green cover and higher soil water extraction were associated with higher stomatal conductance, photosynthetic rate and relative water content. For all genotypes, the pattern of water use as a percentage of total water use was similar across depth and time. We propose the observed genetic variation was related to different root characteristics (root length density, hydraulic conductivity, root activity) although shoot sensitivity to drying soil cannot be ruled out.

Additional keywords: drought tolerance, green couch grass, turfgrass, water use.

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Introduction

Plant production depends on the magnitude of the available water, which is a balance between the water supply from the root system and demand from the atmosphere (Robertson *et al.* 1993a). The supply of water provided by the root system in a drying soil profile of an annual crop has been described by a framework defined by Monteith (1986) and is a combination of models for the downward movement of the root system at a constant velocity, and a function reflecting the decrease in available soil water content once the root system reaches a given layer. Based on this framework, water extraction pattern can be analysed to estimate plant maximum extractable water, water extraction rate and extraction front velocity at each measured depth of soil profile (Robertson *et al.* 1993a). Recently, an empirical model was also developed to predict water uptake without measuring root length density (Dardanelli *et al.* 2004); however, these techniques require prior information and have limited value when used for the first time on a species. The water extraction pattern may be related to drought resistance in annual crops. Generally, drought resistance is associated with greater soil water extraction at depth. For example, the critical zone that explained differences between genotypes was 30–90 cm in wheat (*Triticum aestivum* L.) (Reynolds *et al.* 2007; Gutierrez *et al.* 2010), 45–70 cm for rice (*Oryza sativa* L.) genotypes (Kato *et al.* 2007) and 40–280 cm for peanut (*Arachis hypogaea* L.) (Collino *et al.* 2000). Conversely, for grain sorghum (*Sorghum bicolor* (L.) Moench) (Robertson

et al. 1993a) and barley (*Hordeum vulgare* L.) (Thomas *et al.* 1995) there were no differences for water extraction, water extraction rate and extraction front velocity measured at 50–130 cm depth of soil profile between drought resistant and drought susceptible genotypes growing under conditions of continuous soil drying. This may be because the studied genotypes were able to employ other mechanisms to adapt to drought rather than extracting more water from deep parts of the soil profile (Turner 1996).

Water extraction patterns and drought resistance mechanisms of perennial grasses may be different from that of annual crop species. First, perennial grasses may establish over more than one season after which the root systems would be well developed before drought stress. In addition, perennial species have possibly evolved another strategy of drought adaptation which is not maintenance of green leaf area but rather the ability to survive and recover rapidly after rewatering (Munns *et al.* 2010).

Generally, plants exhibit two types of drought resistance: dehydration avoidance and dehydration tolerance (Chaves *et al.* 2003). Dehydration avoidance describes a plant's capacity to survive a dry period by maintaining a high plant water status (Levitt 1980). In the turfgrass industry, visual appearance is a key component of turf quality, therefore, the ability to maintain green cover under drought conditions has always been regarded as the main criterion evaluated for drought resistance (Beard 1973; Fry and Huang 2004) including our

previous studies (Kearns *et al.* 2009; Zhou *et al.* 2009, 2013b). Extracting more water from deeper zones of the soil profile is one of the most important dehydration avoidance mechanisms turfgrasses can employ under water deficits (Fry and Huang 2004). For example, for experiments maintained for 2–3 years after grasses were established tall fescue (*Festuca arundinacea* Schreb.) cultivars had less leaf firing and wilting during drought and were characterised by greater water extraction at 90 cm depth, when compared with other species e.g. Kentucky bluegrass (*Poa pratensis* L.), bermudagrass (*Cynodon* spp.), buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) and zoysiagrass (*Zoysia japonica* Steud.) (Qian *et al.* 1997; Ervin and Koski 1998). Similarly, Bonos and Murphy (1999) studied Kentucky bluegrass genotypes at 3 and 4 years after establishment and found that the Kentucky bluegrass genotypes with higher turf quality and cooler canopies in summer drought periods were able to extract more water at 15–30 cm depth in the field. However, the measurements of soil water content in previous turfgrass research were neither deep nor consistent during the whole drought period, therefore, no analysis of water extraction pattern in turfgrass species has been reported under the field conditions.

As a C₄ grass, bermudagrasses are widely used around the world for both turf and pasture. Intra-specific variation for drought resistance of bermudagrass has been evaluated in the conditions of both shallow profile e.g. lysimeters (Kearns *et al.* 2009; Zhou *et al.* 2009, 2013b) and deep profile e.g. field conditions using automated rainout shelters (Steinke *et al.* 2011; Zhou *et al.* 2013a). However, there are few studies that have focussed on drought resistance mechanisms related to water extraction. For example, Hays *et al.* (1991) found that when drought stress started at 45 days after planting, turf quality of 10 bermudagrass genotypes during drought was correlated with root biomass at depths of 30–60, 60–90, 90–120, and 120–150 cm ($r=0.72-0.86$), suggesting greater water extraction at those depths. However, this experiment was a pot trial conducted in a glasshouse with a short growth period and water extraction was not monitored.

The objectives of this paper were to quantify temporal and spatial patterns of soil water extraction among bermudagrass genotypes under drought conditions and determine their contribution to drought resistance.

Materials and methods

The experimental site was located at Redlands Research Station (27°32'S, 153°15'E) near Brisbane in southern Queensland, Australia. The soils of this site were typical kandosols as

described by Isbell (2002). Eight genotypes of bermudagrasses (*Cynodon* spp.) were planted in two sets of plots grown side by side and each arranged as a randomised complete block design (RCBD) with three replications (see Fig. S1, available as Supplementary Material to this paper). The eight genotypes included four cultivars Legend, Wintergreen, Grand Prix and CT2 and four Australian ecotypes 1–1, 25a1, 40–1 and 81–1 collected from Queensland, Australia. On 1 December 2008, each 1.5 × 1 m plot was established by planting 8 plugs of each genotype (Fig. 1). For each grass, eight plugs were planted evenly across the plot. Canopy closure occurred by stoloniferous growth from each plug. Roots grew from each node that was in contact with the soil surface. During establishment the plots were irrigated weekly with 40 mm of water, fertilised with 30 kg N ha⁻¹ as urea every month and maintained at 30 mm height. By August 2009 when all the grass plots had established, the herbicide Basta (Glufosinate-ammonium; Bayer CropScience, Monheim am Rhein, Germany) (7 mL L⁻¹) was sprayed weekly along the border of each plot to avoid contamination among genotypes. On 15 April 2010, a steel rainout igloo covered with clear plastic was installed over the entire experimental area, but irrigation continued for another month to all the plots. There were four experiments conducted in these two fields as shown in Fig. 1. There were three replications in each experiment with the plots arranged as a RCBD.

Experiments 1 and 2

On 11 May 2010, experiments 1 and 2 conducted in adjacent fields were started (Fig. 1). For experiment 1, irrigation was maintained with 40 mm water per week and a water deficit was imposed on experiment 2 by stopping irrigation. A clear plastic barrier was placed between the experiments to prevent any spray drift.

Green cover (GC) defined as the percentage of leaves that remained green, was measured using the digital image analysis method modified from Zhou *et al.* (2013b). An image of the grass in each plot was taken by a digital camera (500D, Canon, Tokyo, Japan); subsequently, the percentage of green leaves in the images was estimated based on the criteria that Hue ranged from 40 to 120 and Saturation ranged from 0 to 100 using SigmaScan Pro (ver.5.0.0, SPSS Inc., Chicago, IL, USA) software. Colour charts (Royal Horticultural Society, London, UK) were used to adjust different light intensities that may have existed when photos were taken.

GC of both experiments was collected every 2 weeks. In experiment 2 (the drought experiment), the relationship between

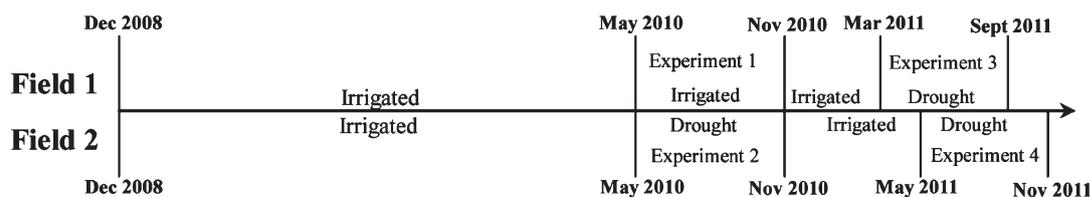


Fig. 1. Flow chart describing time period and irrigation/drought treatments of four bermudagrass (*Cynodon* spp.) experiments conducted in two adjacent fields located at Redlands Research Station near Brisbane in southern Queensland, Australia from 2008 to 2011. (See Fig S1, available as Supplementary Material to this paper, for actual field plan).

GC and days after water withheld (DAWW) of every plot fitted very well to a sigmoid model (Zhou *et al.* 2013):

$$GC = \frac{k}{1 + e^{a+bt}}, \quad (1)$$

where GC was green cover in one plot; t was DAWW, a and b were parameters of the curve and k was the maximum GC i.e. 100% at 0 DAWW in this experiment. Using the model, GC_{50} for each experimental unit was calculated as the time at which the rate of decrease in GC was a maximum (Tipton 1984) and given by:

$$GC_{50} = -\frac{a}{b}. \quad (2)$$

Soil water was measured by neutron moisture meter (NMM) (503 DR, CPN International Inc., Martinez, CA, USA). Aluminium access tubes of 50 mm diameter and 2 m long were installed into the middle of each plot in both fields. A linear regression between the NMM reading and soil volumetric water content was determined. Soil water content (SWC) was measured at 40, 60, 80, 100, 120, 140, 160 and 180 cm depth of soil profile. Therefore, based on the change of SWC from 0 DAWW and because each NMM reading applies to a volume of soil with 10 cm radius, the actual amount of extracted water (EW_a) by genotypes could be calculated at 30–50, 50–70, 70–90, 90–110, 110–130, 130–150, 150–170 and 170–190 cm depth of soil profile. The NMM measurements started at 0 DAWW with an interval of 14 days until the end of the experiment. Soil water extraction from the upper 30 cm of profile was not measured in the present experiments, partly because it was not safe to use NMM in this zone and partly because traditional gravimetric methods would have damaged the grass swards confounding water extraction measurements. However, with well established root systems in perennial grasses, water extraction to wilting point could be assumed in the top 30 cm of soil.

As reported by Robertson *et al.* (1993b), a sigmoid model was used to describe the relationship between SWC and DAWW for each 20 cm layer of soil for each experimental unit (Fig. S2).

$$\theta = \theta_1 + \frac{k}{1 + e^{a+bt}}, \quad (3)$$

where θ was SWC (mm), t was DAWW (day) and a , b , k and θ_1 were the parameters of the curve. Therefore, θ_1 represents the lower limit of SWC for each genotype in each layer of the soil profile (Fig. S2).

Maximum extractable water (EW_{max}) was defined as a genotypic specific estimate of the maximum amount of water the grasses could extract from each layer of the soil profile. Therefore, EW_{max} (mm) was calculated as:

$$EW_{max} = \theta_0 - \theta_1, \quad (4)$$

where θ_0 (mm) was the SWC at 0 DAWW (Fig. S2). Maximum water extraction rate (Q_{max}) was calculated based on the method from work by Lilley and Fukai (1994).

Canopy net photosynthetic rate (Pn) was measured for all grasses of both experiments 1 and 2 at 127 DAWW when it was a clear sunny day. An LI-6400 portable gas exchange system

(Li-Cor Inc., Lincoln, NE, USA) with a modified canopy chamber was used to determine Pn. The acrylic canopy chamber of volume 30.5 cm² area \times 13 cm height was firmly placed over each grass to make a seal for canopy gas exchange measurements. Flow rate to the sample cell was set to 700 $\mu\text{mol s}^{-1}$ and the reference CO₂ was adjusted to 600 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ using the CO₂ mixer. The area covered by the chamber was 30.5 cm².

Leaf relative water content (RWC) of grasses was also determined in both experiments also at 127 DAWW. Six fully expanded young leaves were collected in each plot and pooled to determine leaf RWC. Leaf RWC was determined according to the method of Turner (1981).

Canopy temperature differential (CTD) was calculated as the canopy temperature minus air temperature. CTD in both experiments 1 and 2 was measured eight times after the experiments commenced during a time period between 11am and 1pm when the weather was clear and sunny without wind. An infrared thermometer (Model AG-42, Telatemp Co., Fullerton, CA, USA) and a weather station were used to measure canopy temperature and air temperature, respectively. The infrared thermometer was held vertically at 5 cm above the canopy of the plot. Canopy temperature of each plot was the average of three measurements.

All the measurements in experiments 1 and 2 ceased at 196 days (23 November 2010) after the commencement date when the average GC of the grasses in the drought experiment reached ~20%.

Experiments 3 and 4

After experiments 1 and 2 were completed (23 November 2011), both fields were irrigated with 40 mm weekly and fertilised with 30 kg N ha⁻¹ of urea every month and were used for experiments 3 and 4 (Fig. 1).

For experiment 3, irrigation ceased on 10 March 2011 and the dry period began (Fig. 1). For experiment 4, irrigation ceased on 27 May 2011 when the grasses used in experiment 2 had recovered to 100% green cover (except genotype 1–1). It should be noted that experiment 4 only included seven genotypes because genotype 1–1 which was the most drought susceptible in experiment 1 did not recover.

GC, SWC and soil water extraction were determined every 14 days until 196 DAWW (22 September 2011) in experiment 3 and 154 DAWW (28 October 2011) in experiment 4, and GC_{50} , EW_{max} and Q_{max} of experiments 3 and 4 were also calculated using the same method as described above for experiments 1 and 2. There were differences between experiments for SWC at the start of the drought treatment. These differences affect the estimated maximum extraction. CTD was also measured on clear sunny days using the method described above, seven and five times for experiments 3 and 4, respectively. In addition, daily vapour pressure deficit (VPD) during the whole period of each experiment was calculated using the method described by Allen *et al.* (1998). The average daily VPD of experiments 2, 3 and 4 was 0.95, 1.12 and 1.14 KPa respectively.

Statistical analyses

Each experiment was analysed individually. Nonlinear regressions using a sigmoid model were produced with

SigmaPlot (ver. 11.0, Systat Software Inc., San Jose, CA, USA). For characteristics e.g. GC, SWC at each depth of soil profile and CTD measured in different time periods for the same experimental unit, repeated-measures was applied using mixed models of SAS (SAS Institute Inc., Cary, NC, USA) (Wang and Goonewardene 2004). Other variables e.g. GC_{50} , EW_{max} , Q_{max} , Pn and RWC were analysed as RCBD using the general linear model (GLM) option in Minitab (Minitab Inc., State College, PA, USA).

Results

Green cover (GC) and GC_{50}

The patterns of decline in green cover (GC) and ranking of genotypes were consistent across the drought experiments 2, 3 and 4 (Fig. 2). GC of all the genotypes was high at the beginning of the drought treatment and then reduced sharply after an initial lag phase. This lag phase was variable among genotypes and longer for drought resistant 40-1 and 25a1 and shortest for drought susceptible 1-1 in experiments 2 and 3. For example, at 112 days after water withheld (DAWW), 40-1 and 25a1 still had 55–85% GC, significantly higher than CT2 (15% in experiment 4) or 1-1 (5–10% in experiments 2 and 3) whereas the other genotypes were intermediate.

For each plot, a sigmoid model was fit to the change of GC during drought stress and the correlation coefficient (r) was higher than 90% in each case. Therefore, GC_{50} was considered an effective parameter to describe genetic variation of green cover reduction with each drought treatment. With respect to GC_{50} the genotypes fell into four general groups. Genotypes, 40-1 and 25a1 reached GC_{50} much later than all other genotypes in every drought experiment (Fig. 2; Table 1). For example, GC_{50} of 40-1 and 25a1 was over 160, 140 and 100 days for the three drought experiments, respectively, ~1–2 times longer than 1-1, which had the lowest value (Table 1). The GC_{50} of commercial cultivars Legend and Grand Prix were in the second highest group and were not significantly different from each other. Wintergreen, 81-1 and CT2 grouped and had the third highest GC_{50} , ~30–70 days significantly shorter than 40-1 and 25a1. In order to present the genotypic variation in each figure clearly, we show only genotypes 40-1, 25a1, Legend, CT2 and 1-1 in figures, although we did measure all the genotypes in each experiment except for 1-1 in experiment 4 because it died.

Physiological traits

Under well-watered conditions of experiment 1, canopy temperature differential (CTD) of all grasses was 0–3 degrees below ambient across the entire experiment (Fig. 3a) and genotypic variation was quite small and variable depending on the measurement time. The patterns of CTD across drought experiments were similar with a linear increase in CTD as the drought period lengthened (Fig. 3b–d) and again the genotypic variation of CTD and the ranking of genotypes were similar across these experiments. Genotypes 40-1 and 25a1 always had significantly lower CTD than others, especially in severe dry conditions. For example, the CTD of 40-1 and 25a1 measured in the late stage of experiments (at 171, 175 and 140 DAWW of experiments 2, 3 and 4 respectively) was ~5–7°C lower than CT2 and 1-1.

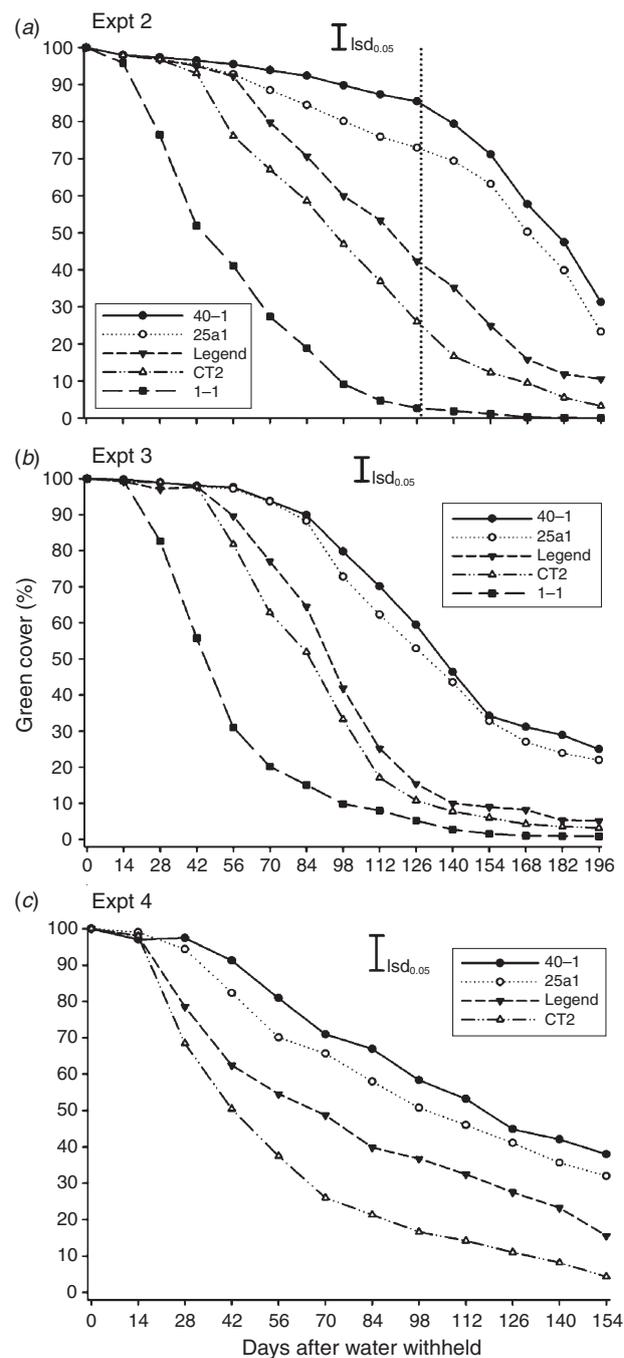


Fig. 2. Green cover of five bermudagrass (*Cynodon* spp.) genotypes in (a) experiments 2 and (b) 3; and four genotypes in (c) experiment 4 under a rainout igloo at Redlands Research Station near Brisbane in southern Queensland, Australia from 2008 to 2011. The error bar indicated least significant difference (l.s.d.) ($P=0.05$) of each measurement based on the analysis of all the eight genotypes (seven genotypes in experiment 4). The vertical dotted line indicates the time when photosynthetic rate and relative water content were measured (Table 1).

Photosynthetic rate (Pn) and relative water content (RWC) of 40-1 and 25a1 in the middle stages (127 DAWW) of the drought experiments were the highest, nearly three times and twice as high

Table 1. Bermudagrass (*Cynodon* spp.) traits measured in four field experiments conducted at Redlands Research Station near Brisbane in southern Queensland, Australia from 2008 to 2011

Experiment 1 was under irrigated conditions and experiments 2–4 under drought. Traits included GC₅₀ indicating the days required for each experimental unit to decline to 50% of the maximum green cover, photosynthetic rate (Pn) and relative water content (RWC) at 127 days after experiments started, and maximum extractable water (EW_{\max}) at 30–190 cm depth of soil profile. Within each column, means followed by the different letters are significantly different based on least significant difference (l.s.d.) ($P=0.05$): Expt, experiment

Genotype	GC ₅₀ (days)			Pn at 127 days after experiment started ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)			RWC at 127 days after experiment started (%)			EW_{\max} at 30–190 cm of soil profile (mm)		
	Expt 2	Expt 3	Expt 4	Expt 1	Expt2	Expt 2/ Expt 1(%)	Expt1	Expt2	Expt 2/ Expt 1(%)	Expt 2	Expt 3	Expt 4
40–1	181a	143a	116a	61.0a	51.0a	83.7	96.5a	93.7a	97.1	165.3a	145.1a	121.2a
25a1	166b	140a	107a	62.3a	48.4b	77.7	99.7a	87.3a	87.6	154.4a	144.3a	116.8a
Legend	128c	106b	87b	62.5a	42.4c	67.8	97.7a	66.8b	68.4	135.8bc	116.7b	98.5b
Grand Prix	121cd	103b	92b	60.1a	41.3c	68.7	98.8a	66.5b	67.3	143.5b	106.7b	101.8b
Wintergreen	108de	107b	75c	60.3a	30.6d	50.8	97.0a	44.6d	46.0	127.7c	118.6b	81.5c
81–1	94e	105b	86b	57.6a	34.6d	60.1	98.4a	54.2c	55.1	130.1bc	115.3b	98.4b
CT2	101e	101b	66c	57.5a	30.5d	53.1	99.1a	41.4d	41.7	124.0c	107.5b	84.6c
1–1	56f	64c	– ^A	60.2a	18.8e	31.2	98.0a	42.1d	42.9	107.6d	77.9c	– ^A

^ANo data collected in respective experiment.

as 1–1, respectively (Table 1), although in the irrigated experiment, the genotypic variation of Pn and RWC was not significant.

Soil water content

Generally, for all the genotypes except 1–1 there were similar patterns of soil water content change. For all genotypes and experiments, SWC at each layer declined with no or very short lag period when the drought treatment commenced and significantly fit a sigmoidal model with $r > 95\%$ (Fig. 4d–l). For genotype 1–1 SWC decreased after a long lag period. For example, at 70–90 cm depth of experiment 2, the lag period of 1–1 reached ~56 days (Fig. 4g), but for other genotypes the lag period was found only in the deep profile of experiment 2 (Fig. 4j). There was no lag period of water extraction when the entire soil profile (30–190 cm) was considered (Fig. 4a–c).

At the beginning, there was no significant difference of total SWC (30–190 cm) in the soil profiles of different genotypes. As the drought period increased, the variation in SWC in the whole measured profile (30–190 cm) under each genotype became larger and significant (Fig. 4a–c). These differences were driven by large variation for SWC at 70–90 cm depth (Fig. 4g–i). The variation for SWC was smaller at both shallow e.g. 30–50 cm (except experiment 3) (Fig. 4d–f) and deep profiles e.g. 150–170 cm (Fig. 4j–l). Thus, 40–1 and 25a1 had lower SWC by ~9–16, 14–24 and 26–32 mm than Legend, CT2 and 1–1 at 42 DAWW, respectively, whereas at 140 DAWW this difference increased to 15–23, 31–36 and 38–58 mm for Legend, CT2 and 1–1, respectively (Fig. 4a–c). For the other genotypes not presented in Fig. 4, their SWC was in between the highest e.g. 1–1 or CT2 and the lowest e.g. 40–1 or 25a1.

Soil water extraction

For some genotypes, a fraction of extractable soil water remained in the soil profile because the experiments were terminated before every grass had reached 0% green cover. This fraction was estimated as the difference between EW_{\max} estimated from

the sigmoidal equation and EW_a at the end of the experiment. Therefore, we define EW_{fr} as this difference in percentage terms.

$$EW_{\text{fr}}(\%) = \frac{EW_{\max} - EW_a}{EW_{\max}} \times 100. \quad (5)$$

EW_{fr} was small across the three droughted experiments. For example, it was ~1–6% for the layers at 30–110 cm depth, and increased to ~6–10% for the layers at 120–190 cm depth. In addition, EW_{fr} was significantly and highly correlated with the GC measured when experiments terminated (Fig. S2) and genotypes 40–1 and 25a1 with higher GC when the experiments terminated also had higher EW_{fr} (Fig. S2). Therefore, small EW_{fr} and high correlation between EW_{fr} and GC at experimental end indicated a small magnitude of error for the estimation of the lower limit of SWC and calculation of EW_{\max} from the sigmoidal model.

Genotypes 40–1 and 25a1 had the highest EW_{\max} values with ~120–160 mm, ~18–32, 30–53 and 50–91% significantly higher than Legend, CT2 and 1–1, respectively (Table 1). Based on the whole soil profile, on average, genotypes in experiment 2 had higher values of EW_{\max} than those in experiments 3 and 4: values from experiment 3 being higher than in experiment 4 (Table 1). Part of the reason for the difference in EW_{\max} was caused by the difference in SWC at the start of the drought treatment. Generally, EW_a of genotypes 40–1 and 25a1 at the end of the experiments was significantly higher than other genotypes at every depth of soil profile except some deep layers e.g. 140 cm of experiment 3, and variation for EW_a was larger between 50–110 cm than at other depths (Fig. 5a, c, e). For example, EW_a of 40–1 and 25a1 at 70–90 cm depth was 15.6–24.9 mm, which was ~2.9–5.4, 5.4–7.4 and 8.7–15.6 mm higher than Legend, CT2 and 1–1, respectively. At 150–170 cm, EW_a of 40–1 and 25a1 was 8.3–10.9 mm, but about only 0.05–1.7, 2.1–3.2 and 1.1–10.9 mm higher than Legend, CT2 and 1–1, respectively. For the other of eight genotypes not presented in Fig. 5, their EW_a was intermediate between the highest e.g. 40–1 or 25a1 and the lowest e.g. 1–1 or CT2. In addition, all the genotypes left more water in the profile with depth as indicated by the increase in difference between EW_a

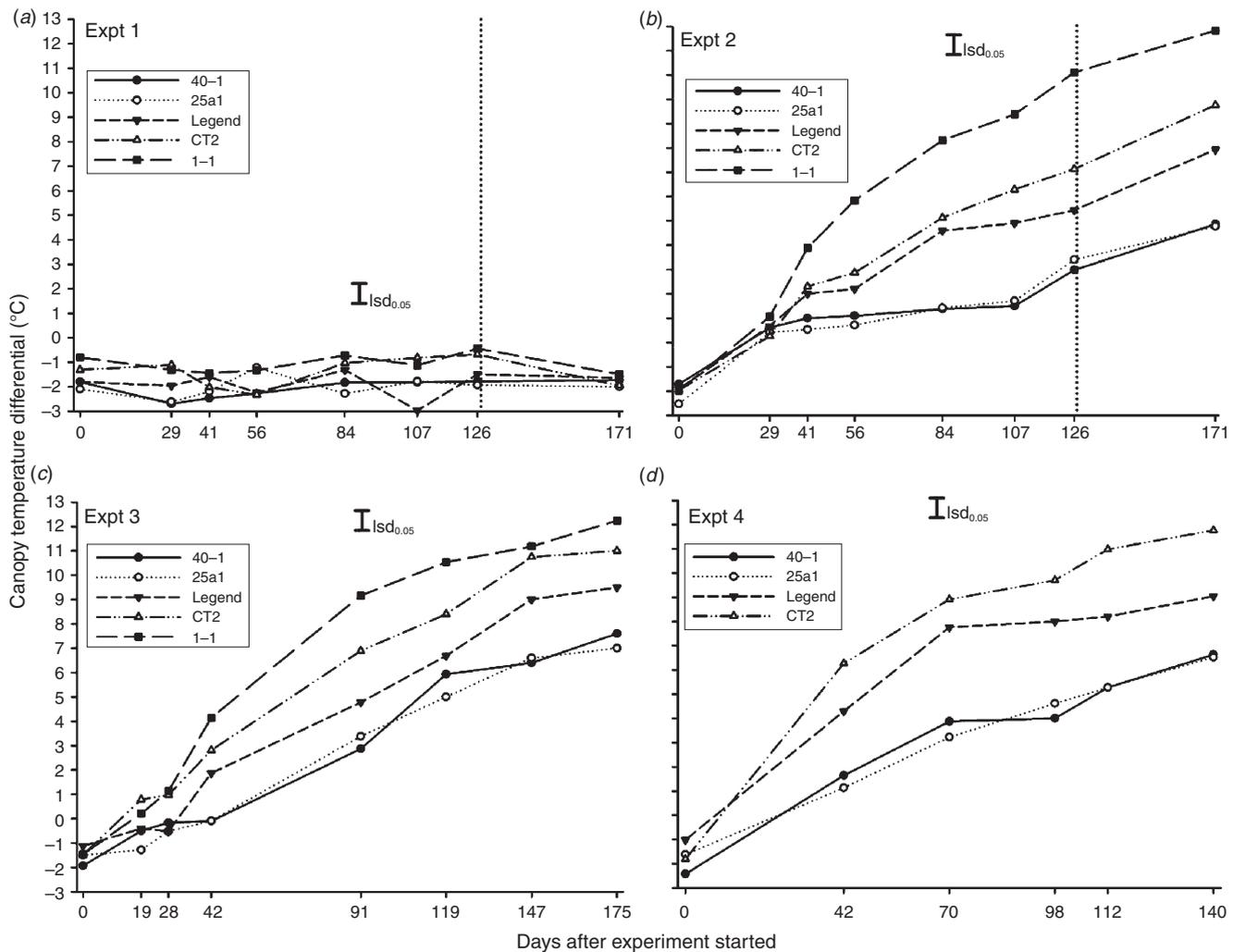


Fig. 3. Canopy temperature differential (CTD) of five bermudagrass (*Cynodon* spp.) genotypes in (a) experiments 1, (b) 2 and (c) 3; and four genotypes in (d) experiment 4. Experiment 1 was irrigated, whereas experiments 2–4 were droughted. The experiments were conducted at Redlands Research Station near Brisbane in southern Queensland, Australia from 2008 to 2011. The error bar indicated least significant difference (l.s.d.) ($P=0.05$) at each measurement based on the analysis of all the eight genotypes (seven genotypes in experiment 4). The vertical dotted line indicates the time when photosynthetic rate and relative water content were measured (Table 1).

and total available water (defined as SWC at field capacity minus SWC at wilting point) with depth (Fig. 5a, c, e).

In experiments 3 and 4, similar to the genetic variation of EW_a , genotypes 40–1 and 25a1 also had highest maximum water extraction rate (Q_{max}) at every layer of 50–110 cm (Fig. 5d, f), $\sim 10 \times 10^{-2}$ – 15×10^{-2} mm day $^{-1}$ higher than the lowest one 1–1 and CT2 in experiments 3 and 4 respectively (Fig. 5d, f). In contrast, in experiment 2, Q_{max} of 1–1 became the highest at 50–70 and 70–90 cm depth, whereas there was no significant difference among other genotypes (Fig. 5b).

Assuming that the total available soil water at 0–30 cm depth was extracted, the evapotranspiration rate which was equal to the total amount of water extracted from 0–190 cm depth in the whole period (196 days in experiments 2 and 3, and 154 days in experiment 4) was 1.27, 1.17 and 0.91 mm day $^{-1}$ for experiments 2, 3 and 4 respectively.

Spatial and temporal pattern of water extraction

Despite genetic variation for EW_{max} and EW_a , the amount of water extracted at each depth as a percentage of total water extracted from the whole profile was consistent across three contrasting genotypes (Fig. 6). In the early stage of drought e.g. first 6 weeks, the percentage of water extracted decreased with each layer of the profile (Fig. 6). Genotype 1–1 extracted more than 50% of its water at 30–50 cm depth (Fig. 6g), whereas 40–1 and Legend had extracted 20–30% at the same depth (Fig. 6a, d). In the middle stage of drought (middle 6 weeks), 40–1 and Legend kept similar water extraction pattern to the early stage whereas 1–1 had a lower percentage at 30–50 cm depth, $\sim 12\%$, and then decreased gradually from 16% at 50–70 cm to $\sim 4\%$ at 160–190 cm depth (Fig. 6h). In the late stage of drought (final 6 weeks), for all the eight genotypes percentage of water extracted increased slightly starting from the shallow (50–70 cm) to deep profile (Fig. 6c, f, i).

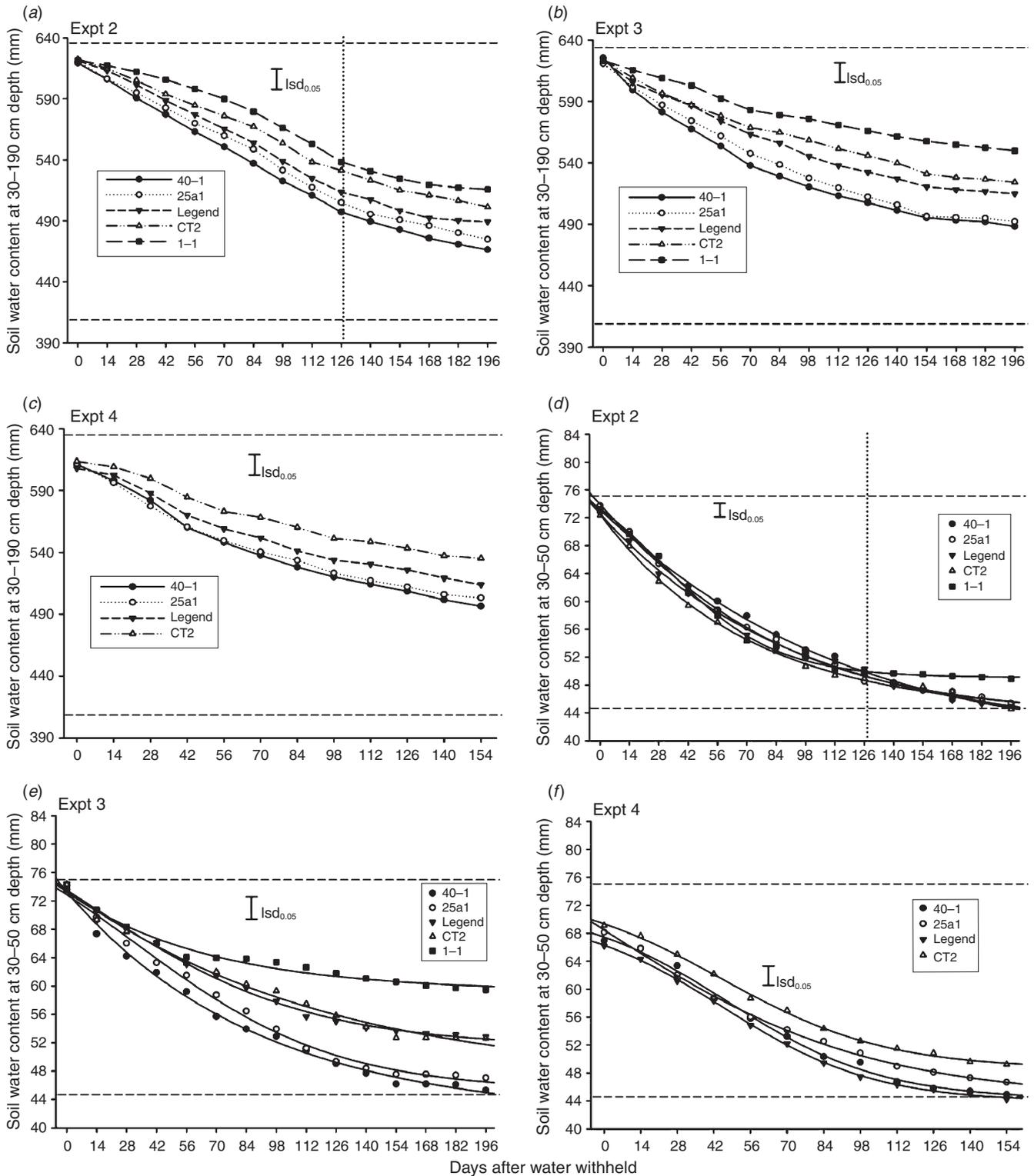


Fig. 4. Change in soil water content with time of bermudagrass (*Cynodon* spp.) genotypes in experiments 2–4; (a–c) whole soil profile (30–190 cm depth), (d–f) 30–50 cm depth, (g–i) 90–110 cm depth; and (j–l) 150–170 cm depth. All experiments were conducted under drought conditions at Redlands Research Station near Brisbane in southern Queensland, Australia from 2008 to 2011. The error bar indicated least significant difference (l.s.d.) ($P=0.05$) at each depth based on the analysis of eight genotypes (seven genotypes in experiment 4). Upper and lower dashed line indicated field capacity and wilting point respectively. (d–l) Curves represent the regression lines after fitting a sigmoidal model describing the relationship between soil water content and days after water withheld. The vertical dotted line indicates the time when photosynthetic rate and relative water content were measured (Table 1).

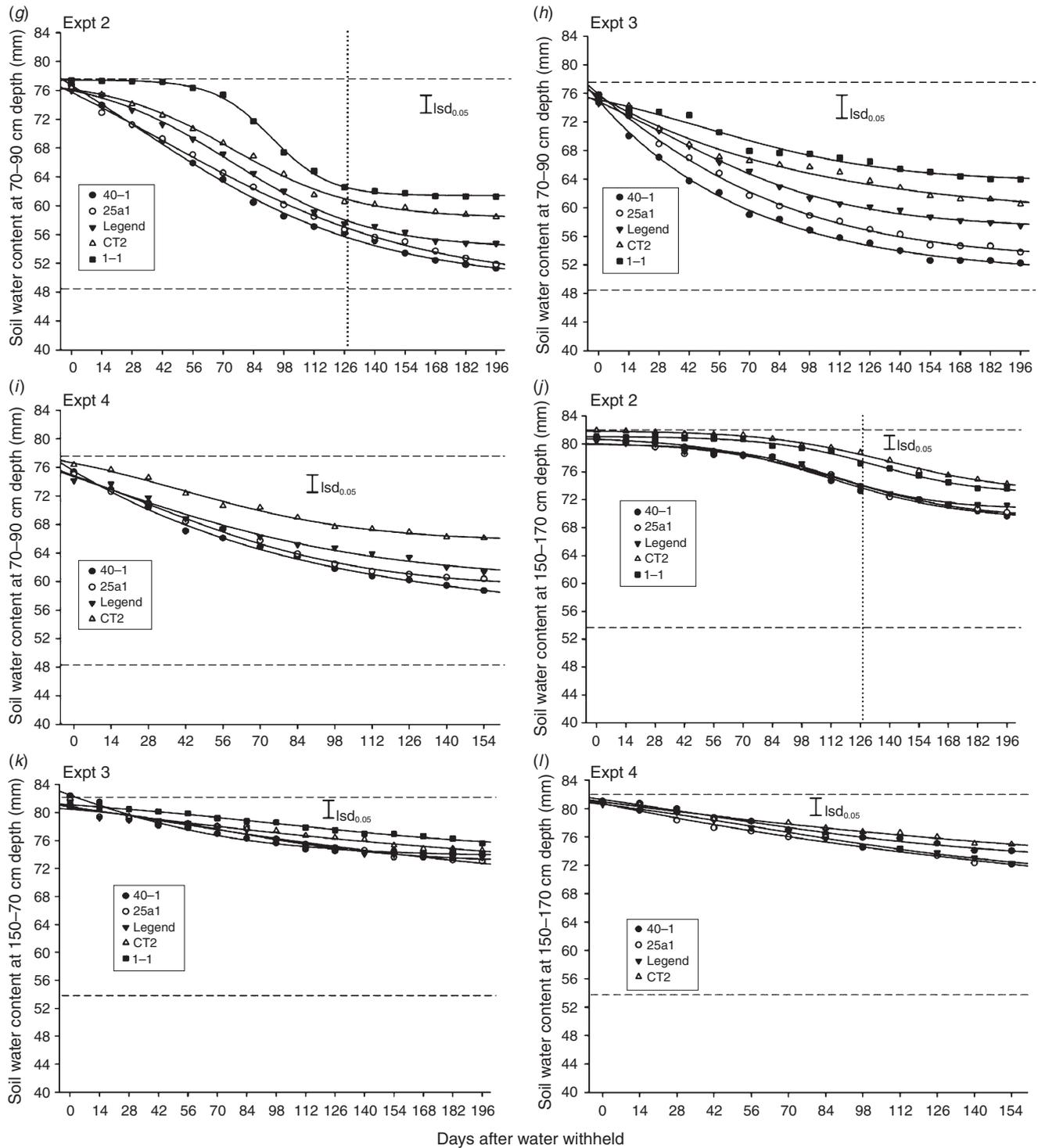


Fig. 4. (continued)

Relationships between measured attributes

At 0 DAWW, all the genotypes had 100% GC but as soil water extraction increased, GC declined after an initial lag phase (Fig. 7). The reduction of GC for genotypes 40-1 and 25a1 was at the point where more soil water was extracted than other

genotypes. For example, when GC reached 50%, soil water extraction of 40-1 and 25a1 was estimated as 141, 116 and 94 mm for three drought experiments, ~39–42, 57–75 and 95–124 mm higher than Legend, CT2 and 1-1, respectively. Whereas when GC reached 90%, soil water extraction of 40-1

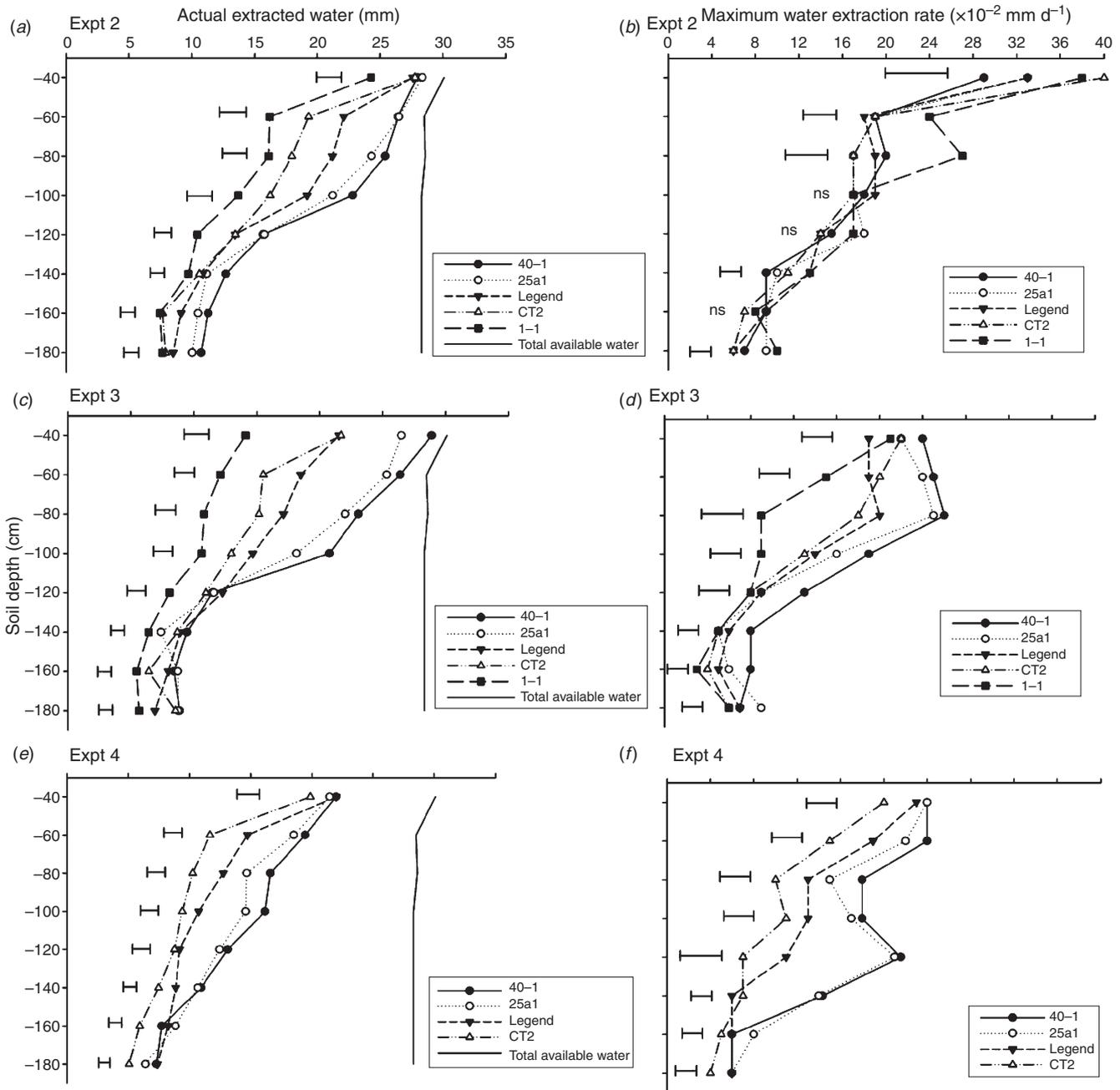
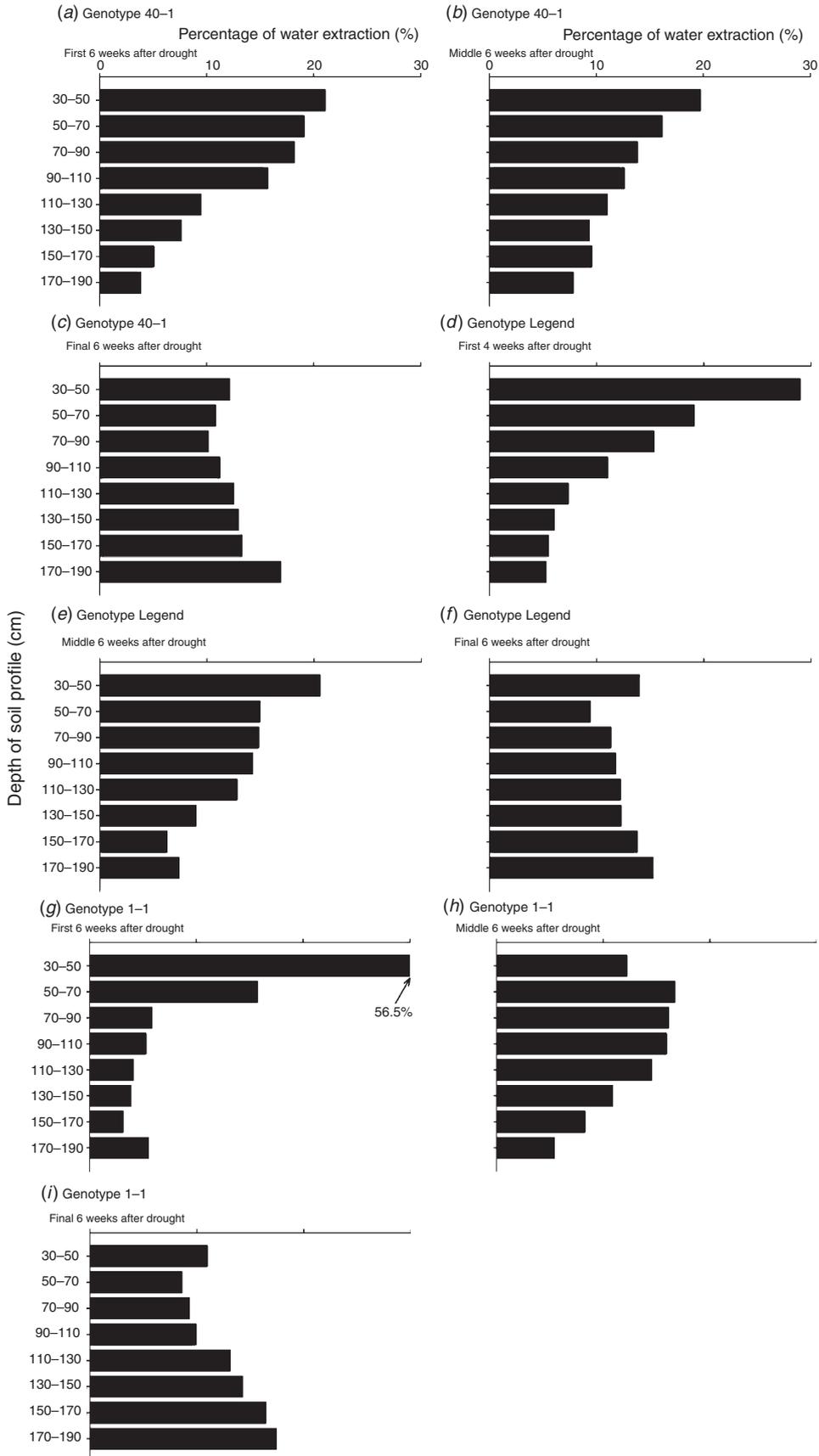


Fig. 5. Actual extracted water (EW_a) at the end of the experiment and maximum water extraction rate at each measured depth of soil profile of five bermudagrass (*Cynodon* spp.) genotypes in (a, b) experiments 2 and (c, d) 3; and four genotypes in (e, f) experiment 4 under continuous drought at Redlands Research Station near Brisbane in southern Queensland, Australia from 2008 to 2011. Total available water defined as soil water content at field capacity minus wilting point is also shown. The NMM moisture probe was placed at 20 cm intervals between 40–180 cm, therefore, the shallowest and deepest part of the profile measured was 30 and 190 cm respectively. The error bar indicated least significant difference (l.s.d.) ($P=0.05$) at each depth based on the analysis of all the eight genotypes (seven genotypes in experiment 4).

and 25a1 was estimated as 78, 87 and 47 mm for three drought experiments, ~32–38, 40–48 and 73–76 mm higher than Legend, CT2 and 1–1, respectively.

Based on all the genotypes ($n=7$ or 8), GC_{50} was significantly correlated with EW_a of the whole profile (30–190 cm)

($r=0.94–0.97$) as well as the depths of 30–50 cm ($r=0.81–0.98$), 50–70 cm ($r=0.95–0.98$), 70–90 cm ($r=0.94–0.97$) and 90–110 cm ($r=0.97–0.98$), EW_{max} at 30–190 cm ($r=0.97–0.99$), Pn ($r=0.83$) and RWC ($r=0.85$) in all drought experiments.



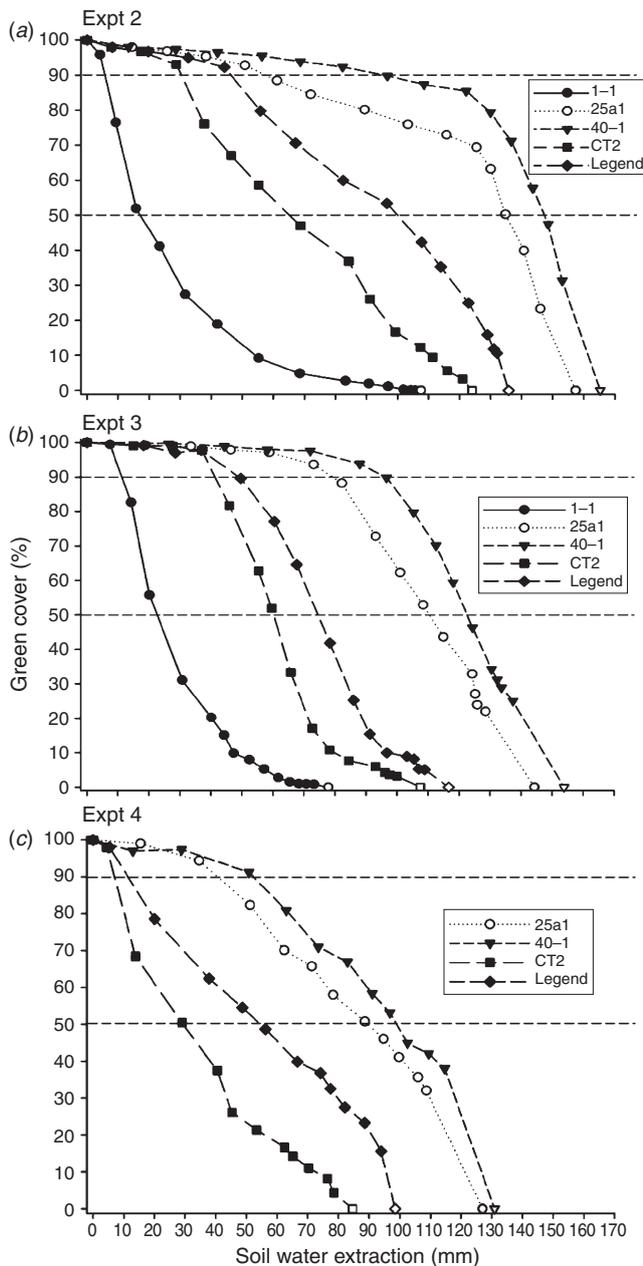


Fig. 7. Change in green cover with soil water content measured at the same time for five bermudagrass (*Cynodon* spp.) genotypes in (a) experiments 2 and (b) 3; and four genotypes in (c) experiment 4 under continuous drought at Redlands Research Station near Brisbane in southern Queensland, Australia from 2008 to 2011. For each genotype, the last point (open circle) indicated 0% green cover and maximum extractable water estimated from the model (see 'Materials and methods'). The dashed lines indicate green cover at 50 and 90%.

Discussion

Analysis of water extraction pattern

To our knowledge this is the first report that details soil water extraction of a C_4 perennial grass to depths of 190 cm of soil profile throughout a drought period of almost 200 days, although a previous study measured soil water extraction of US grassland species to 2 m depth under natural rainfall (Nippert and Knapp 2007). Our results show that water was extracted from depths of 190 cm for all the bermudagrass genotypes. The extraction from the bottom 20 cm was small, less than 7% of 30–190 cm depth in all genotypes. Nippert *et al.* (2012) reported that perennial C_4 grass species in mesic tallgrass prairie of North America at soil layers deeper than 1 m, had significant decreases in root biomass, total root length, and theoretical hydraulic conductivity and, therefore, limited significance for water uptake. At 30–50 cm depth, for two of the three experiments in our study, the grasses were able to extract the majority of total available soil water with little variation between genotypes and we suggest that similar patterns of extraction would have occurred at 0–30 cm. For turfgrasses, soil evaporation from the soil surface, higher root-density, -viability and -hydraulic conductivity can result in almost complete extraction of the available soil water in shallow profiles (Huang and Fry 1999). However, it was difficult to estimate water extraction at 0–30 cm for experiment 3 because water extraction in the upper layers of the profile (e.g. 30–50 cm) was much lower than the total available water and there were large differences between genotypes. This pattern of soil water extraction in the upper layers of the soil profile was not observed in experiment 3 and are discussed below.

With respect to temporal and spatial patterns of water extraction, despite large genetic variation of extractable water (EW_{max} and EW_a), the amount of extracted water at each depth as a percentage of water extracted in the whole soil profile was not different among genotypes, except genotype 1–1 (Fig. 6). In the early stage of drought, more water was extracted in the upper layers of the soil profile and less water was extracted with depth, whereas in the late stage the trend was reversed with more water extracted in the deeper layers. Similar spatial patterns of water extraction have not been reported in perennial crops. Evidence from annual crops is variable, for example, similar patterns to the present study were observed in C_3 crop species rice (Lilley and Fukai 1994), chickpea (*Cicer arietinum*) and barley (Thomas *et al.* 1995), but different patterns were observed between sunflower and sorghum, because sunflower was able to extract water at depths lower than 2 m whereas sorghum could not (Stone *et al.* 2002).

Higher maximum water extraction rate was associated with more water extraction, especially at depths of 50–110 cm. Similarly, more water extraction was correlated with higher extraction rate in rice (Lilley and Fukai 1994) and in peanut genotypes (Collino *et al.* 2000), whereas this relationship was

Fig. 6. Percentage of bermudagrass (*Cynodon* spp.) genotype extracted water at each depth to the whole soil profile (30–190 cm) during the first 6 weeks, middle 6 weeks and final 6 weeks after water was withheld for genotypes 40–1 (a–c), Legend (d–f) and 1–1 (g–i). Data presented for the three genotypes was the average of experiments 2, 3 and 4. All three experiments were conducted under continuous drought at Redlands Research Station near Brisbane in southern Queensland, Australia from 2008 to 2011. Note for Fig. 6g the percentage of water extraction at 30–50 cm was 56.5%.

not found in sunflower (Dardanelli *et al.* 1997). In the study of sorghum, Robertson *et al.* (1993a) showed that extracted water was fairly stable across experiments whereas water extraction rate varied markedly. They concluded that water extraction rate was not a conservative parameter for describing water extraction for a particular combination of species, soil type and environment. An exception to this relationship was noted in the present study, where in one experiment (experiment 2) genotype 1–1 had lower soil water extraction but higher water extraction rate probably due to its smaller effective duration of water extraction.

Mechanisms of water extraction

We are unable to be explicit about the mechanisms that underlie the variation in soil water extraction observed among the genotypes of this study. Our data does not allow for differentiation between the role of shoot–root–traits, or combinations of these. Water is extracted through a soil–plant–atmosphere continuum which is largely controlled by the resistances along the path from both shoot and root (Blum 2011). One of the key components of shoot control of water uptake is stomatal sensitivity. In addition, any reduction in the size of the green canopy (green leaves) could decrease transpiration and water extraction. The studies of C₄ turfgrasses including bermudagrass had reported that water uptake was significantly and positively correlated with root length density (Carrow 1996), root viability (Huang *et al.* 1997) and root hydraulic conductivity (Llobet *et al.* 2012). The fact that early in the drought period of the present study the most drought susceptible grass 1–1 showed a rapid decline in GC (Fig. 2a) despite the roots having access to adequate soil water suggested extreme shoot sensitivity to dry soil near or at the soil surface led to rapid leaf firing and reduced water uptake. However, we cannot reject the counter argument that the reduced water uptake was due to extreme sensitivity of the root system to the drying profile near or at the soil surface. Nevertheless, both shoot and root characteristics should be considered when describing the factors responsible for water uptake. Clearly, more research is needed in C₄ perennial grasses to elucidate the mechanisms associated with soil water extraction during exposure to drought.

Drought resistance and its relationship to water extraction

Using GC₅₀ as the criteria to select for drought resistance (Karcher *et al.* 2008; Steinke *et al.* 2011; Zhou *et al.* 2013b), the genotypic ranking of drought resistance from best to worst based on this study was 40–1 and 25a1 > Legend and Grand prix > 81–1, Wintergreen and CT2 > 1–1. Drought resistant grasses were also characterised by higher stomatal conductance, as suggested by lower canopy temperature, higher photosynthetic rate and higher water status during drought. Therefore, the maintenance of physiological functions led to higher visual turf quality.

Our study indicates that the drought resistant grasses were able to maintain GC longer and resistance was associated with the ability to extract more water from the soil profile especially at depths of 50–110 cm. Based on this significant correlation ($r > 0.90$), higher water extraction was possibly one of the mechanisms for drought resistance. Greater soil water extraction contributing to drought resistance has been

observed previously for annual crops wheat (Reynolds *et al.* 2007; Gutierrez *et al.* 2010), rice (Gowda *et al.* 2011), sorghum (Vadez *et al.* 2011) and peanut (Collino *et al.* 2000). However, perennial grasses may have other strategies to adapt to drought. Drought resistance evaluated in the present research referred to the ability to keep canopy green during a long period of drought. In evolutionary terms, species adapted to drought have a high level of physiological plasticity allowing exploitation of resources (Baker 1974; Williams and Black 1994). For example, perennial grass species can reduce canopy transpiration and water uptake quickly during dry-down to have more stored water in soil to survive and recover rapidly after rewatering (Munns *et al.* 2010). Therefore in our study, one might expect those grasses that lost green cover earliest may have recovered quicker after rewatering. We were unable to test this hypothesis in the present study, because genotypes reduced their GC at different rates during drought, therefore, it was not possible to use a re-watering treatment that could be applied on the same day. Genotype 1–1 lost most green leaves at ~140 DAWW of experiment 2 and didn't recover after rewatering at 196 DAWW when the remaining genotypes had green leaves. This showed that 1–1's rapid reduction of GC conferred no survival advantage under the drought and rewatering conditions of this study and cast doubt on this strategy as a drought survival mechanism.

Genotype and environment interaction

Genotypic variation for drought resistance and water extraction was consistent across three drought experiments in this study despite the existence of different environmental/management conditions for the periods after establishment for each experiment. The grasses in all experiments had at least 1.5 years growth after planting, which meant they had an established root system and dense canopy when drought stress commenced. This was similar to our previous findings that showed drought resistance of 47 bermudagrass genotypes was consistent despite the canopy age at the time of drought treatment (Zhou *et al.* 2013a).

Different green cover and water extraction between experiments was probably due to different growth conditions and soil water status. For example, the grasses in experiment 3 had lower GC₅₀ and lower EW_{max} than in experiment 2 probably because experiment 3 grew under reduced light conditions (~80% full sun) of the igloo for a longer period before drought treatment was imposed. Bermudagrasses are generally more sensitive to shading, compared with other C₄ and C₃ turfgrasses (Beard 1973). Wet and shaded conditions can result in lower total non-structural carbohydrates in roots of bermudagrass (Baldwin *et al.* 2009), subsequently, smaller water extraction and shorter GC₅₀ may be expected. In addition, experiment 4 had the lowest averages for GC₅₀ and EW_{max} , first because plots in experiment 4 were recovered after the drought period of experiment 2. Therefore, the drought of the previous experiment may have caused long-term impairment of shoot and root function although the canopy had reached 100% GC. Second, the soil water content at the commencement of the drought treatment was lower in experiment 4 compared with other experiments despite our best efforts to water the soil profile to field capacity.

Conclusions

The amount of extracted water at each depth as a percentage of water extracted in the whole soil profile was not different among genotypes. The extraction from the bottom 20 cm of the measured soil profile was small. The drought resistant grasses were able to maintain GC longer and resistance was associated with the ability to extract more water from the soil profile especially at depths of 50–110 cm. However, we are unable to be explicit about the mechanisms that underlie the variation in soil water extraction observed among the genotypes of this study. Our data could not differentiate the role of shoot–root-traits, or combinations of these and further research is necessary to understand these mechanisms.

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