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Morphological and physiological responses of *Calobota sericea* plants subjected to water limitation and subsequent rewatering

Francuois Müller^{1,2,3*} , Lincoln Raitt³, Lilburne Cyster³, Samson Chimphango⁴ , M Igshaan Samuels^{1,3} ,
Clement Cupido¹  and J Stephen Boatwright³ 

¹ Agricultural Research Council – Animal Production: Range and Forage Sciences, Roodeplaat, South Africa

² Agricultural Research Council – Animal Production: South African National Forage Genebank, Roodeplaat, South Africa

³ Department of Biodiversity and Conservation Biology, University of the Western Cape, Bellville, South Africa

⁴ Department of Biological Sciences, University of Cape Town, Cape Town, South Africa

*Correspondence: MullerF@arc.agric.za; francuoismuller@gmail.com

Calobota sericea is a native legume of South Africa, confined to the water-limited rangelands, and it has recently been prioritised for additional characterisation regarding its pasture potential. In this study, we examined the growth characteristics of *C. sericea* under glasshouse conditions where water limitation was implemented at different plant ages, and for different durations. Results indicate that preferential resource allocation to the roots, as well as reduced stomatal conductance and transpiration, were early responses to water limitation, irrespective of the age at which water limitation was imposed, or the duration of water limitation. Under water-limited conditions, increased production of protective pigments, such as carotenoids and anthocyanins, was also observed, which helped in recovery after rewatering. It was concluded that after rewatering, all negative impacts of water limitation on morphology and physiology of *C. sericea* plants were generally returned to well-watered levels. This suggests that *C. sericea* plants employ a wide range of phenotypic adjustments in response to water limitation, which makes the plants well adapted to areas with high rainfall variability.

Keywords: drought resistance, drought stress, photosynthetic rate, resource allocation, stomatal conductance

Supplementary material: available online at <https://doi.org/10.2989/10220119.2021.1954089>

Introduction

Large arid and semiarid regions around the world are generally characterised by high variability and unpredictability in rainfall, as well as rainfall that is insufficient for arable crop production under rain fed conditions (Abu-Zanat et al. 2004; Belkheiri and Mulas 2013). Within these drylands, plants are often exposed to a variety of environmental stresses, with drought stress or water limitation commonly regarded as the most significant factors, leading to substantial reductions in agricultural productivity (Lambers et al. 2008; Walter et al. 2011). Because of these stresses, most of the available lands are marginal to crop lands and are used primarily as grazing lands for extensive livestock production. Such rangelands have limited options to sustainably increase agricultural productivity, especially where irrigation is not an option (Palmer and Ainslie 2006; Belkheiri and Mulas 2013; Jordaan et al. 2013). These limitations to improving agricultural productivity within these water-limited rangelands are expected to worsen under the predicted future climate change conditions (IPCC 2007; Meissner et al. 2013).

Generally, for South Africa, it is predicted that the current unpredictability and variability in temporal and spatial rainfall distribution will likely increase in

the future. This will result in additional increases in marginal agro-ecological conditions, with increases in the duration and intensity of episodic drought events, resulting in further limitations to agricultural productivity (Kruger and Shongwe 2004; Benhin 2008; DEA 2013; Meissner et al. 2013). Therefore, in order to meet the future increase in the demand for livestock products in South Africa, the productivity of these water-limited rangelands will have to be improved. One of the ways to improve rangeland production is through better rangeland management, which includes the implementation of improved fodder flow programs that can adequately address the current dry season feed shortages occurring due to the seasonal deterioration of the natural veld (Müller et al. 2019a).

The current stock of commercial forage species suitable for these water-limited agro-ecological conditions in South Africa is limited (Dickenson et al. 2010; Truter et al. 2015). Recently, however, the Agricultural Research Council (ARC) of South Africa, along with various South African research institutions have started identifying and prioritising native South African legume species that can potentially be developed further as forages for use within these marginal areas especially where the current

commercially available forages are not suitable. These native species are naturally adapted to the marginal agro-ecological conditions and therefore have the potential to be effectively utilised in fodder flow programs that will allow for improved livestock production within these rangelands (Müller et al. 2017a; Trytsman et al. 2019; Chimphango et al. 2020). From these studies, *Calobota sericea* (Thunb.) Boatwr. and B-E van Wyk, a perennial legume species, which occurs within the semiarid rangelands of the Northern Cape and Western Cape provinces of South Africa (Boatwright et al. 2018), has been recognised as a species that should be evaluated for its agronomic potential within the semiarid rangelands of Namaqualand, and other areas experiencing similar bioclimatic and edaphic conditions (Samuels et al. 2016; Müller et al. 2017a, 2019a).

Recent work has indicated that *C. sericea* already forms an important part of the fodder flow plan of communal farmers within the Leliefontein communal rangelands of South Africa (Samuels et al. 2016). Therefore, if managed properly, this species has the potential to significantly reduce feed and nutrient shortages during the dry season within these rangelands (Müller et al. 2019a). However, apart from the requirements for seed germination and seedling establishment (Müller et al. 2017b, 2019b), very little is currently known about the ecophysiology of plant-water relations in *C. sericea*. This, in turn, limits our understanding of how well the species will respond to the predicted increased variability in rainfall and increased cycles of wet and dry conditions within these arid and semiarid agro-ecosystems.

In areas experiencing regular periods of water limitation, perennial plants, compared with annuals, have a greater diversity of physiological and/or biochemical responses, which allows them greater resistance to drought (Praba et al. 2009; Perez-Harguindeguy et al. 2013; Basu et al. 2016). Generally, the mechanism of drought resistance in perennial plants include drought avoidance, drought tolerance or a combination of these adaptive responses (Praba et al. 2009; Perez-Harguindeguy et al. 2013; Basu et al. 2016; Makonya et al. 2020). Drought avoidance is the ability of plants to maintain relatively higher water content, despite reduced soil moisture content (Perez-Harguindeguy et al. 2013; Basu et al. 2016). Plants that display drought avoidance mechanisms do so through adaptive responses, which either minimise the loss of water e.g. reduced stomatal conductance, transpiration rate or leaf area, or, through maintaining their ability to take up water e.g. increased root growth (Perez-Harguindeguy et al. 2013; Basu et al. 2016). Drought tolerance, on the other hand, is the ability of plants to endure low tissue water content through adaptive responses, such as the maintenance of cell turgor through osmotic adjustment (Basu et al. 2016). These responses to drought are crucial to the survival of plants until the stress has been relieved, but these responses usually negatively affects plant growth and production. Apart from drought tolerance and/or avoidance mechanisms, plants that are adapted to areas with episodic periods of drought should also have the ability to compensate for periods of stress when the stress is relieved. Therefore, how quickly and effectively a plant can

respond to, and recover from, water limitation is key to the survival of these plants. These kind of adaptive responses of plants to water limitation have been well documented for important agronomic crops, such as potato, sorghum and maize (Obidiegwu et al. 2015; Wang et al. 2017), with some plants, such as soybeans, able to compensate for growth upon rehydration (Dong et al. 2015). This recuperation after stress is often achieved through phenotypic plasticity. Phenotypic plasticity in plants can be defined as the capacity of a single genotype to generate alternative phenotypes in response to shifts in environmental conditions. It is a mechanism by which plants can respond quickly to changes in their environment (Bradshaw 1965, 2006; Nicotra et al. 2010; Arnold et al. 2019). This ability of a plant to shift developmental processes in response to the environment is key to the success of plants in natural and agro-ecosystems (Nicotra et al. 2010; Gray and Brady 2016). Determining whether *C. sericea* plants possess these recuperative abilities is therefore key to its implementation as a planted forage within semiarid and arid agro-ecological areas. This, in turn, can provide an indication of how quickly and effectively *C. sericea* plants can or will respond to, and recover from water limitation and give valuable information for future breeding and improvement initiatives.

The aim of the current study was to quantify the responses of *C. sericea* to different levels of water limitation, at different ages. This was done by quantifying the responses to water limitation, and subsequent rewatering at two, three and four months after establishment. We aimed to quantify: (1) plant growth and resource allocation, (2) water relations, and (3) gas exchange and photosynthetic pigment content in the leaves of *C. sericea* plants. We hypothesise that because of the natural distribution of *C. sericea* plants in water-limited areas, they would show a wide range of adaptive plasticity in morphological and/or physiological traits in response to water limitation, which would help *C. sericea* plants to cope in water-limited growing conditions.

Materials and methods

Seed collection and pregermination treatments

Physiologically mature seeds of *C. sericea* were collected from naturally occurring populations in the semiarid rangelands of Leliefontein in Namaqualand, South Africa in November 2016. Within the Leliefontein communal area seeds were collected from a minimum of 75 plants per sampling location from the rangelands surrounding the Leliefontein, Tweerivier, Spoegrivier and Kharkhams villages, after which all seeds collected were pooled to form a single seed-lot for the Leliefontein area (Müller et al. 2019b). After collection, the seeds were removed from the seed pods by hand to reduce injury to the seeds, after which a portion of the seeds collected were mechanically scarified using an abrasive sand paper to remove the coat imposed seed dormancy (Müller et al. 2017b). Scarified seeds were pregerminated in 90 mm petri dishes on two layers of filter paper. Seeds were regarded as germinated when a radicle of ≥ 3 mm was visible. Seedlings were removed from the petri dishes and transplanted into pots.

Experimental design

A pot experiment, laid out in a complete randomised block design, was conducted under greenhouse conditions. Within the trial, three treatments (well-watered control, i.e. pots were watered to capacity once per week, water limited, i.e. water was withheld for specific durations, and rewatered after water limitation, i.e. watered to pot capacity) were implemented in plants of three ages, i.e. two, three and four months after establishment, with four water-limited periods (15, 30, 45 and 60 days) within each age (refer to Supplementary material Figure 1). Pots (15 cm diameter, 40 cm deep) were filled with soil collected from the locations where seeds were collected. No chemical amendments were made to the soil prior to planting. Three pregerminated (radicle ≥ 3 mm) seeds were planted at a depth of 1 cm in each pot. Thereafter the pots were separated into three trials based on when the different treatments would be implemented, i.e. two, three and four months after establishment. The seedlings were allowed to grow for one month before a half-strength stock nutrient solution (Plant Food- Starke Ayres) was applied to the pots to avoid nutrient deficiencies. At six weeks after sowing, the pots were thinned to two uniformly sized plants per pot within each trial, resulting in a total three pots and six plants per treatment. At each trial, i.e. age at which treatments were imposed, the day before the water limitation treatments were imposed, all pots were watered to saturated levels, and allowed to drain to pot capacity. This was to ensure that none of the plants were stressed before the water limitation treatments were imposed. Thereafter, watering was withheld for drought stressed plants, whereas well-watered pots were watered once a week. After each water limitation period, i.e. 15, 30, 45 and 60 days, drought stressed pots were rewatered to capacity once a week for 21 days before harvesting the recovered plants. At the end of the experiment, within each age category (two, three and four months after establishment), the plants in all treatments were harvested at the same day in order to compare the impacts of the treatments on plants of the same age. See the experimental design in the Supplementary material.

Measurements

The day before physiological measurements were made, all well-watered pots were watered and allowed to drain to field capacity. After 24 hours of watering, physiological measurements were conducted. Using one of the plants in each pot, the photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 (C_i) of the largest, fully expanded compound leaf were measured between 12h00 and 14h00, using a Li-Cor 6400 xt portable open gas system with a red/blue light source (LI-COR Biosciences, Lincoln, NE, USA). The reference CO_2 concentration was maintained at 400 ppm, flow rate was $400 \mu\text{mol s}^{-1}$, and the light in the chamber was set at $400 \text{ photons } \mu\text{mol s}^{-1}$. After measuring, the leaf was harvested and the leaf area immediately determined using a portable leaf area meter. The measurements obtained were thereafter adjusted for each leaf area measured. From the default measurements, photosynthetic water use efficiency (A/E) for each plant was calculated (Borba et al. 2017). Thereafter, all plant material was collected

and separated into roots, stems and leaves and the fresh mass (g) and root length (cm) determined. After fresh mass determination, the leaves were submerged in distilled H_2O for 24 hours at room temperature, after which they were reweighed to determine the turgor mass (g) (Turner 1981). All plant materials were thereafter oven dried at 60°C until a constant mass was achieved. The dried material was reweighed to determine the dry mass (g) of each plant component. Using this information, the relative leaf water content (RLWC) was calculated using Equation 1, and root water content (RWC) was calculated using Equation 2. Dry mass for shoots were calculated as the sum of leaf and stem dry mass.

$$\text{RLWC (\%)} = [(LFW - LDW) \div (LTW - LDW)] \times 100 \quad (1)$$

$$\text{RWC (\%)} = (RFW \div RDW) \times 100 \quad (2)$$

where: LFW = leaf fresh mass, LTW = leaf turgor mass, LDW = leaf dry mass, RFW = root fresh mass, RDW = root dry mass.

The other plant in the pot was used for pigment determination. The leaves of each plant were removed and cut into smaller pieces and mixed well, after which a 0.5 g sample (fresh mass) was mashed in a mortar and pestle with 80% acetone (v/v). The mixture was allowed to stand for 12 h, after which the extract was filtered through Whatman no. 1 filter paper. Absorbances of the filtrate using a UV-Vis spectrophotometer at 470, 537, 647 and 663 nm, respectively, were recorded and the concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll, anthocyanin (Kong et al. 2017) and carotenoids (Pompelli et al. 2013) were calculated, using Equations 3–7.

$$\text{Chlorophyll } a = 0.137 \times A_{663} - 0.000897 \times A_{537} - 0.003046 \times A_{647} \quad (3)$$

$$\text{Chlorophyll } b = 0.024 \times A_{647} - 0.004305 \times A_{537} - 0.005507 \times A_{663} \quad (4)$$

$$\text{Total chlorophyll} = \text{chlorophyll } a + \text{chlorophyll } b \quad (5)$$

$$\text{Anthocyanin} = 0.0817 \times A_{537} - 0.00697 \times A_{647} - 0.002228 \times A_{663} \quad (6)$$

$$\text{Carotenoids} = [(1\,000 \times A_{470}) - (2.13 \times \text{chlorophyll } a) - (97.64 \times \text{chlorophyll } b)] \div 209 \quad (7)$$

Statistical analyses

The Statistical Package for the Social Sciences Version 22 (SPSS Inc., Chicago, IL, USA) was used to test the data for normality using the Shapiro–Wilk test. When data deviated from normality, the data were log-transformed to achieve normality. A one-way ANOVA was done on all variables to determine whether significant differences ($p \leq 0.05$) existed between the different treatments, within each age category (two, three or four months after establishment). Where significant differences were observed, a Least Significant Difference (LSD) *post hoc* test was done to separate the means.

Results

Biomass production and resource allocation

Water limitation significantly influenced the growth and development of *C. sericea*. Shoot mass (Figure 1) decreased significantly in water-limited plants, irrespective of the duration of water limitation, or the age at which water limitation was imposed on the plants. With plants two and three months old, rewatering after water limitation generally resulted in the shoot mass recovering to, or better than well-watered levels. This was true in all incidences, except when rewatering occurred after 60 days at three months, where recovery was significantly higher than water-limited levels, but were still below well-watered levels (Figure

1). However, when water limitation was imposed on the plants at four months, although shoot mass increased from water-limited levels, recovery of shoot mass did not occur to well-watered levels. At 60 days of water limitation when water limitation was imposed on the plants at four months of age, no recovery after rewatering occurred.

Generally, root mass (Figure 2) and root length (Figure 3) in water-limited and rewatered plants was significantly heavier and longer than those of well-watered plants irrespective of the age at which water limitation was imposed on the plants, or the duration of water limitation. The exception to this was when water limitation was imposed on the plants for 60 days at four months. Here, both root mass (Figure 2) and root length (Figure 3) significantly

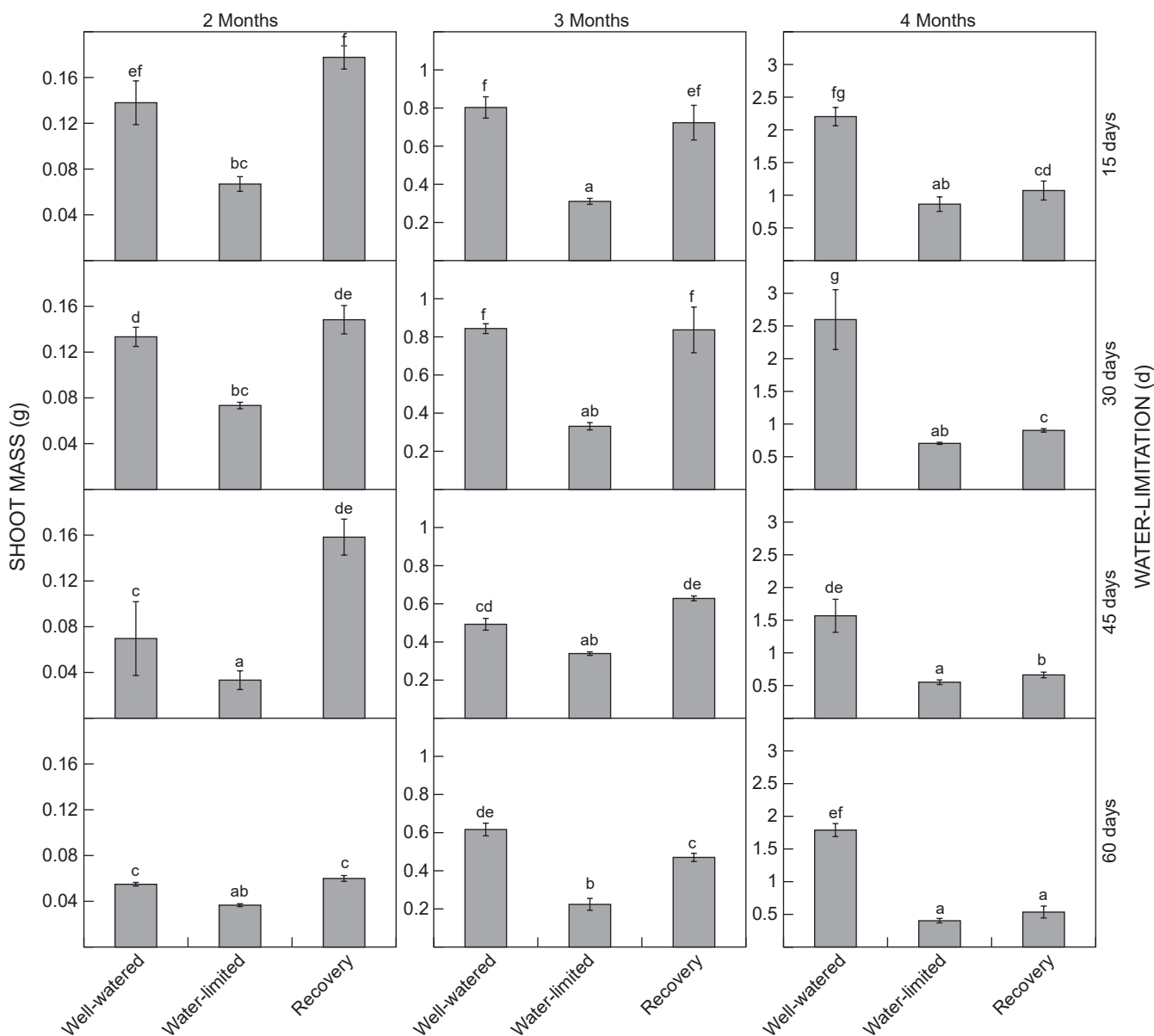


Figure 1: Shoot dry mass (g) of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

decreased from well-watered levels in both water-limited and rewatered plants, suggesting that at there was no recovery after rewatering.

When considering the root:shoot ratio mass ratio (Figure 4), significantly more resources were allocated to root production in water-limited plants, even after rewatering. The exception to this was when water limitation was imposed on the plants for 60 days at four months where water-limited and rewatered plants had a significantly lower root:shoot mass ratio than well-watered plants.

Plant water status

Relative leaf water content (Figure 5) decreased significantly from the well-watered levels in water-limited

plants, but after rewatering, generally increased back to well-watered levels, irrespective of the age at which water limitation was imposed on the plants, or the duration of water limitation. However, when water limitation was imposed on *C. sericea* plants at four months, for 60 days, even after rewatering, leaf water content did not recover from water-limited levels. Similarly, root water content (Figure 6) decreased significantly from well-watered levels in water limited plants, irrespective of the age at which water limitation was imposed on the plants, or the duration of water limitation. Generally, however, the longer water limitation was imposed on the plants, the lower the root water content. When these plants were rewatered, however, root water content significantly increased to and

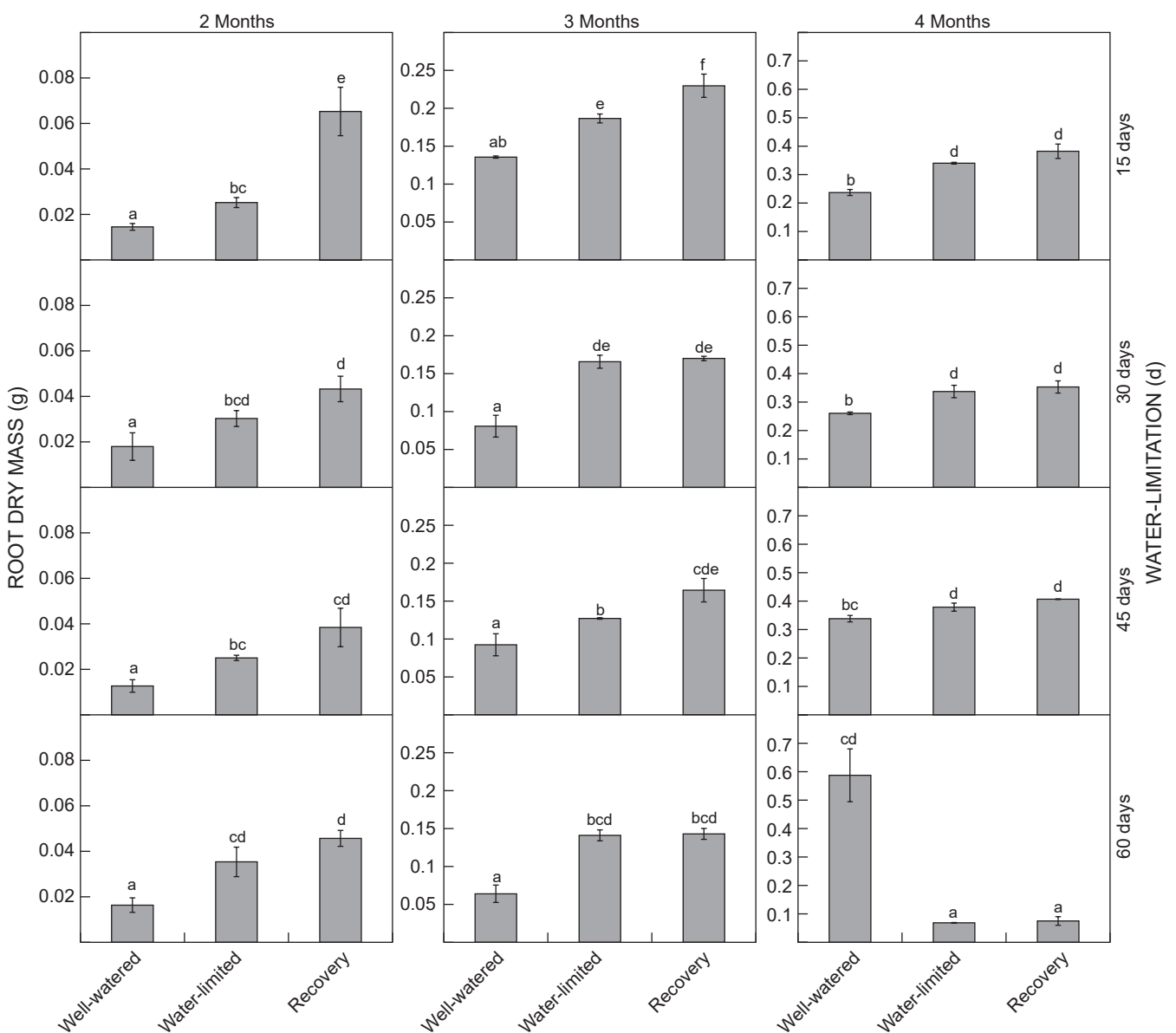


Figure 2: Root dry mass (g) of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

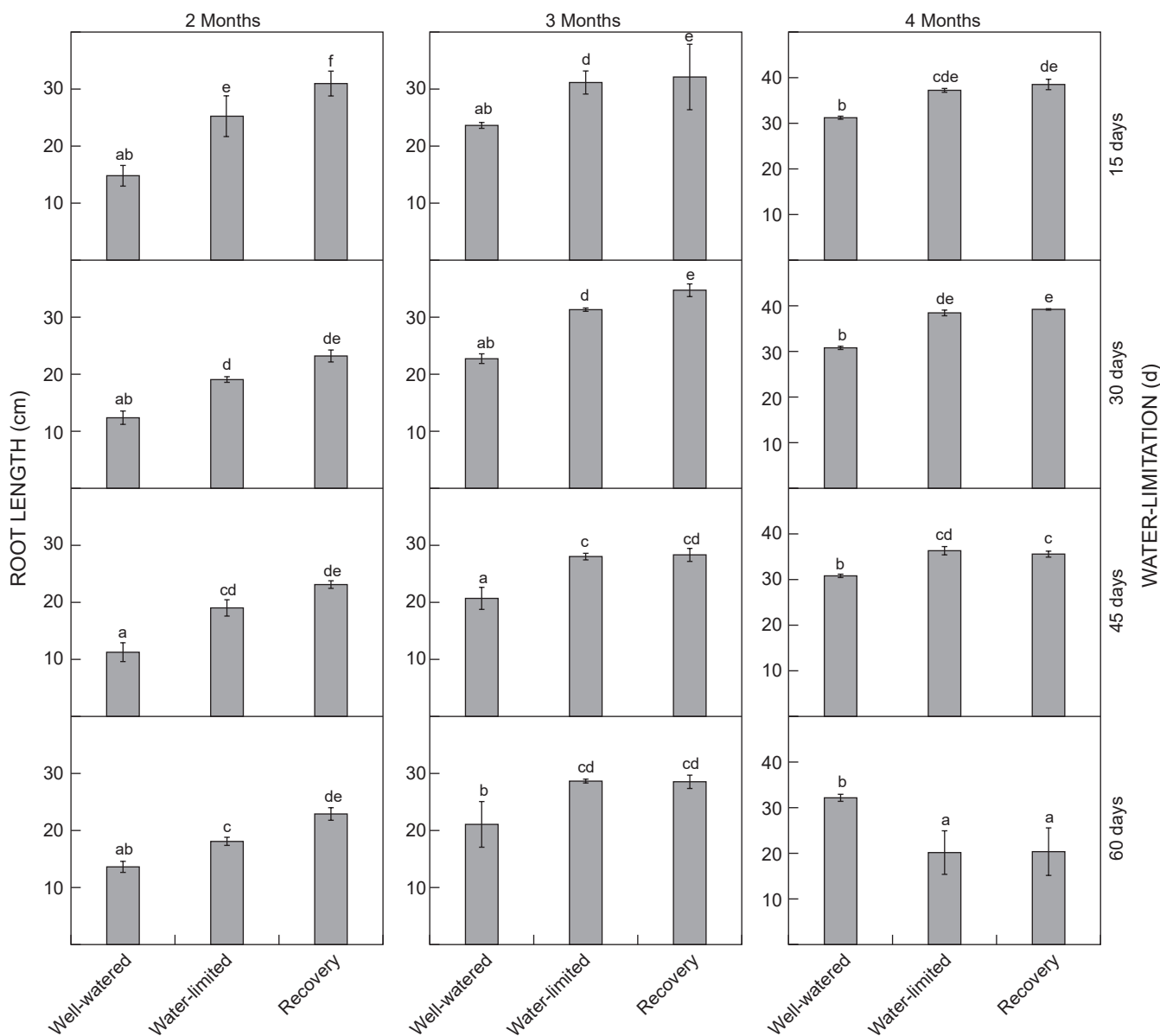


Figure 3: Root length (cm) of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

sometime above well-watered levels. The exceptions to these were at 60 days of water limitation, where even though the root water content increased from water-limited levels, it did not increase back to well-watered levels.

Gas exchange

Stomatal conductance (Figure 7), C_i (Figure 8) and E (Figure 9) decreased significantly from well-watered levels in water-limited plants, irrespective of the age at which water limitation was imposed on the plants and the duration of water limitation. The decreased g_s and C_i concentrations also resulted in reduced A (Figure 10) in water-limited plants. When water limitation was imposed on the plants for 15 and 30 days at two months after

establishment, however, no significant differences in A was observed between the well-watered, water-limited and rewatered plants. At 45 and 60 days of water limitation, and when water limitation was imposed on the plants three and four months after establishment, irrespective of the duration of water limitation, A decreased significantly from well-watered levels in water-limited plants. After rewatering, however, g_s , C_i , E and A increased significantly from water-limited levels. This was true for all water-limited periods at all ages at which water limitation was imposed on the plants, except when water limitation was imposed for 60 days at four months.

With the decrease in E , because of the closure of stomata in water-limited plants, Photosynthetic Water Use Efficiency

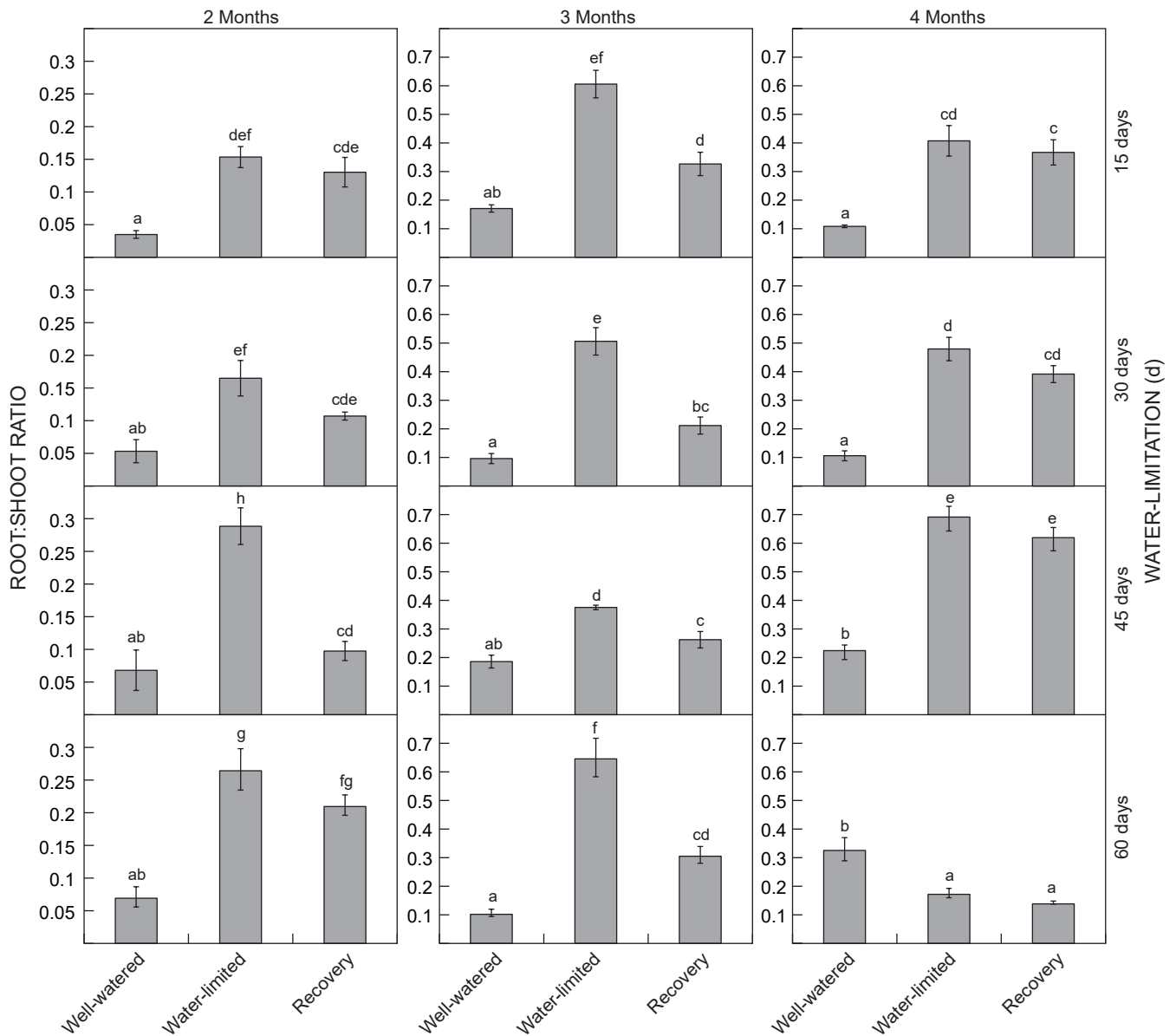


Figure 4: Root:shoot mass ratio of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

(PWUE; Supplementary material Figure 2) increased significantly in water stressed plants, irrespective of the age at which water limitation was imposed on the plants, or the duration of water limitation. The exception to this was when water limitation was imposed for 60 days at four months. Rewatering after 15 and 30 days of water limitation, at two months, resulted in a significant decrease in PWUE from water-limited levels back to well-watered levels. Rewatering after 45 and 60 days of water limitation, however, resulted in PWUE not differing between water-limited and rewatered plants. Similarly, rewatering after 15 days of water limitation at three months after establishment and after 15 and 30 days of water limitation at four months after establishment resulted in a significant decrease in PWUE

from water-limited levels. However, when rewatering occurred only after 30, 45 and 60 days of water limitation in three-month-old plants, and 45 and 60 days of water limitation in four-month-old plants, PWUE of the rewatered plants did not differ from water-limited levels.

Photosynthetic pigments

When water limitation was imposed on the plants two months after establishment (Table 1), the chlorophyll content in water-limited plants was generally, but not always lower than in well-watered plants. After rewatering, chlorophyll (chlorophyll *a*, chlorophyll *b* and total chlorophyll) content returned to well-watered levels. Anthocyanin and carotenoid pigment content in these

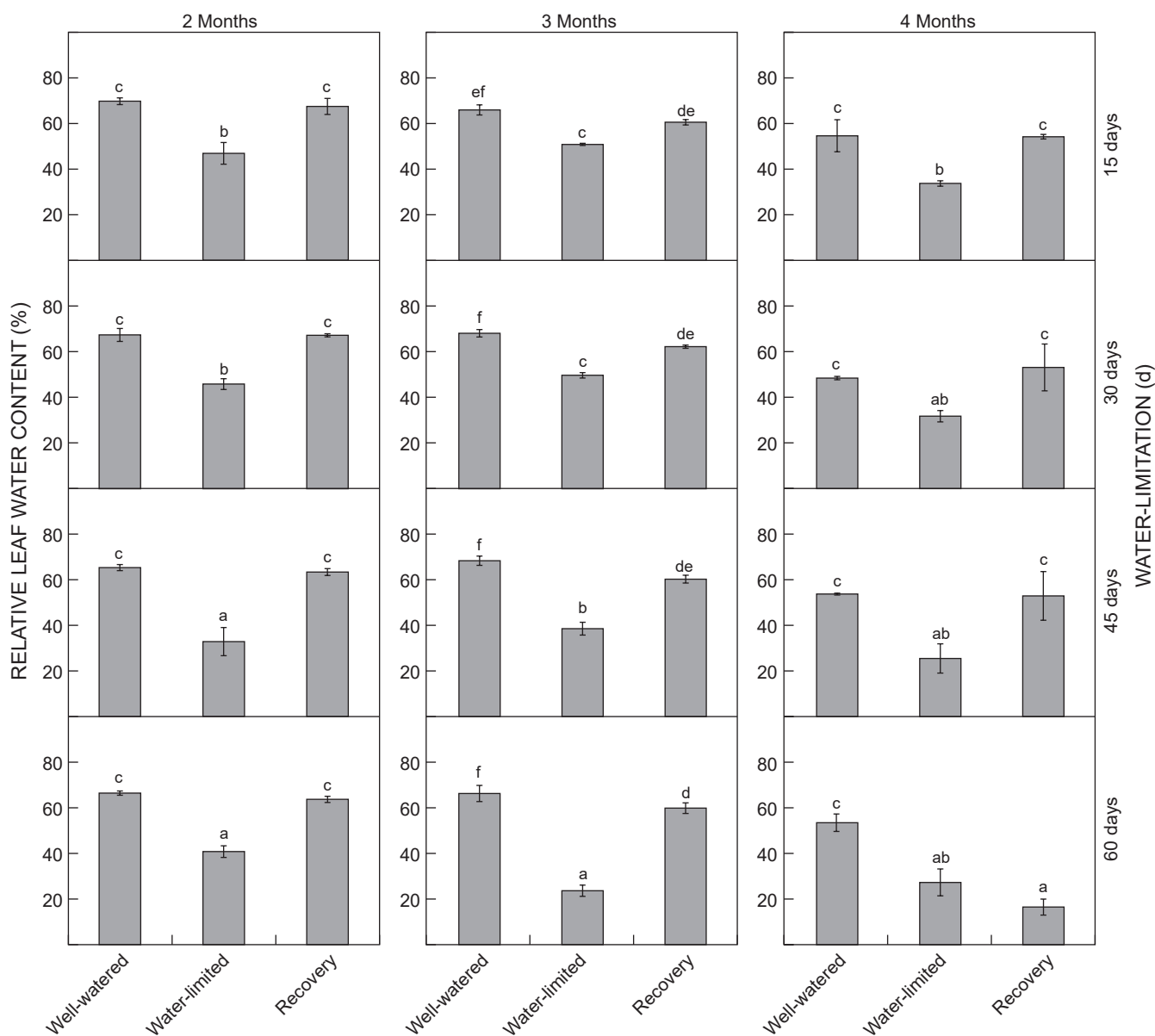


Figure 5: Relative leaf water content (%) of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

plants, however, increased significantly in water-limited plants, irrespective of the duration of water limitation.

When water limitation was imposed on the plants three months after establishment (Table 2). Chlorophyll content (chlorophyll *a* and total chlorophyll) was generally significantly lower in water-limited plants, but after rewatering, chlorophyll content in the plants recovered to well-watered levels. Chlorophyll *b* content, however, did not differ between well-watered, water-limited and rewatered plants. Anthocyanin and carotenoid pigment content within these plants increased significantly from well-watered levels in water-limited plants. After rewatering, anthocyanin and carotenoid pigment content decreased with anthocyanin levels generally, but not always decreasing to

well-watered levels, whereas carotenoid pigment content, although lower, was still significantly higher than for the well-watered levels.

When water limitation was implemented on the plants four months after establishment (Table 3), total chlorophyll content decreased significantly from well-watered levels in water-limited plants, irrespective of the duration of water limitation. After rewatering, the chlorophyll content increased significantly from water-limited levels. The exception was plants that were subjected to 60 days of water limitation, where no increases occurred. Anthocyanin and carotenoid pigment content in these plants was significantly higher in water-limited plants, as well as rewatered plants, irrespective of the duration of water limitation.

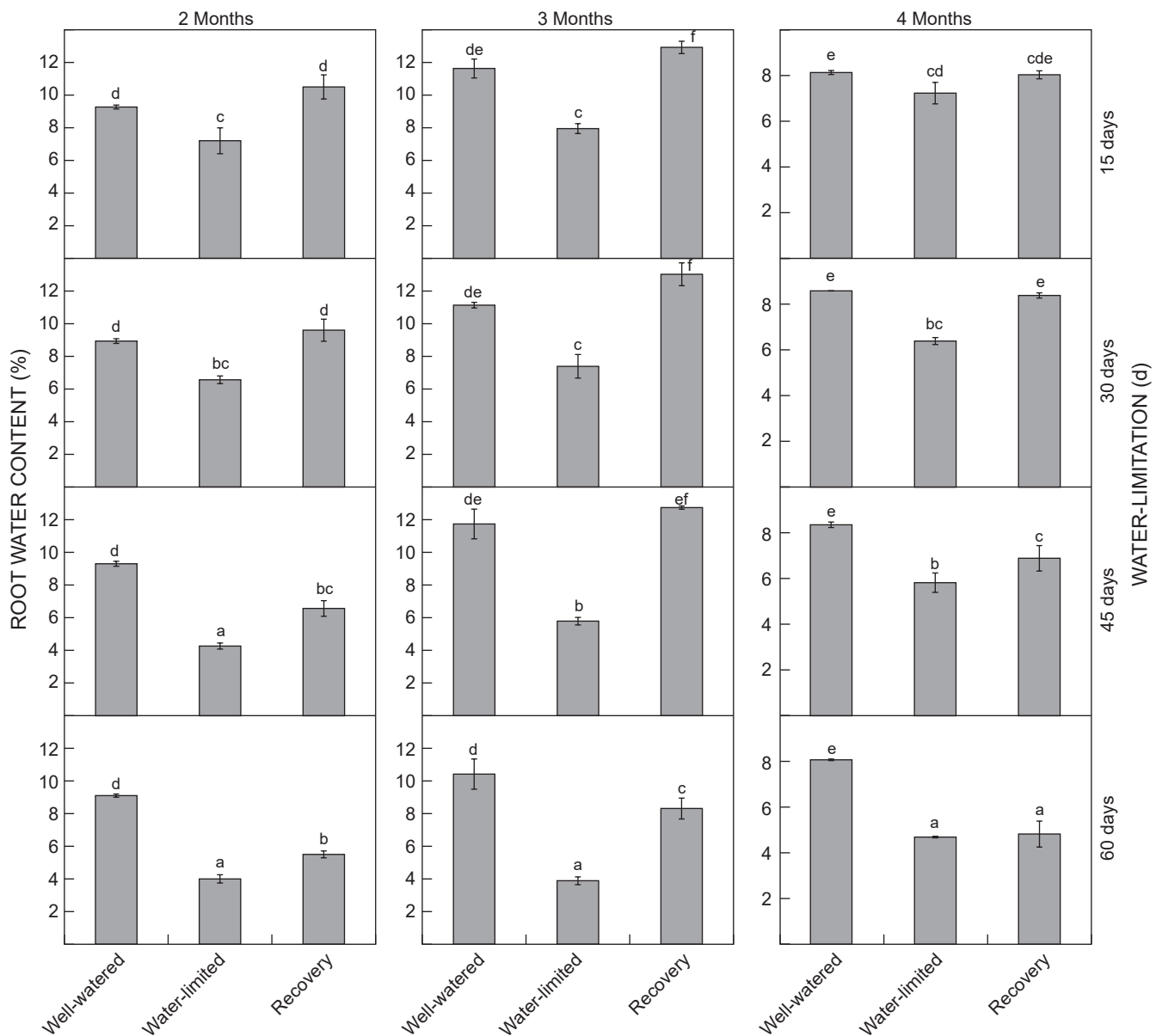


Figure 6: Root water content (%) of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

Discussion

In this study, the morphological responses of *C. sericea* plants to water limitation generally corresponded to what is found in the literature, in that there was a greater inhibition of shoot growth and a preferential allocation of resources to root growth (Pang et al. 2011; Foster et al. 2012; Poorter et al. 2012; Lotter et al. 2014; Eziz et al. 2017). This finding corresponds to the optimum partitioning theory (Bloom et al. 1985; Mao et al. 2012; Gargallo-Garriga et al. 2014; Eziz et al. 2017) in that *C. sericea* plants under water-limited conditions allocated more resources towards the roots, which are tasked with capturing the limited resource. After rewatering, leaf water content and biomass

production generally recovered. Interestingly, even though shoot growth recovered after rewatering, the proportion of biomass allocated to the roots of these rewatered plants was still significantly higher than that of the well-watered plants. Rapid recovery in shoot biomass after rewatering can partially be explained by the increased uptake of the now available water resources, by a better-developed and deeper root system. This, in turn, is partially responsible for rapid refilling of embolised xylem vessels, allowing for improved water movement through the plant (Holbrook et al. 2001; Lambers et al. 2008; Foster et al. 2015). The development of deeper and better-developed root systems by plants, such as beans (Beebe et al. 2013; Fenta et al. 2014; Polania et al. 2017), under water-limited conditions

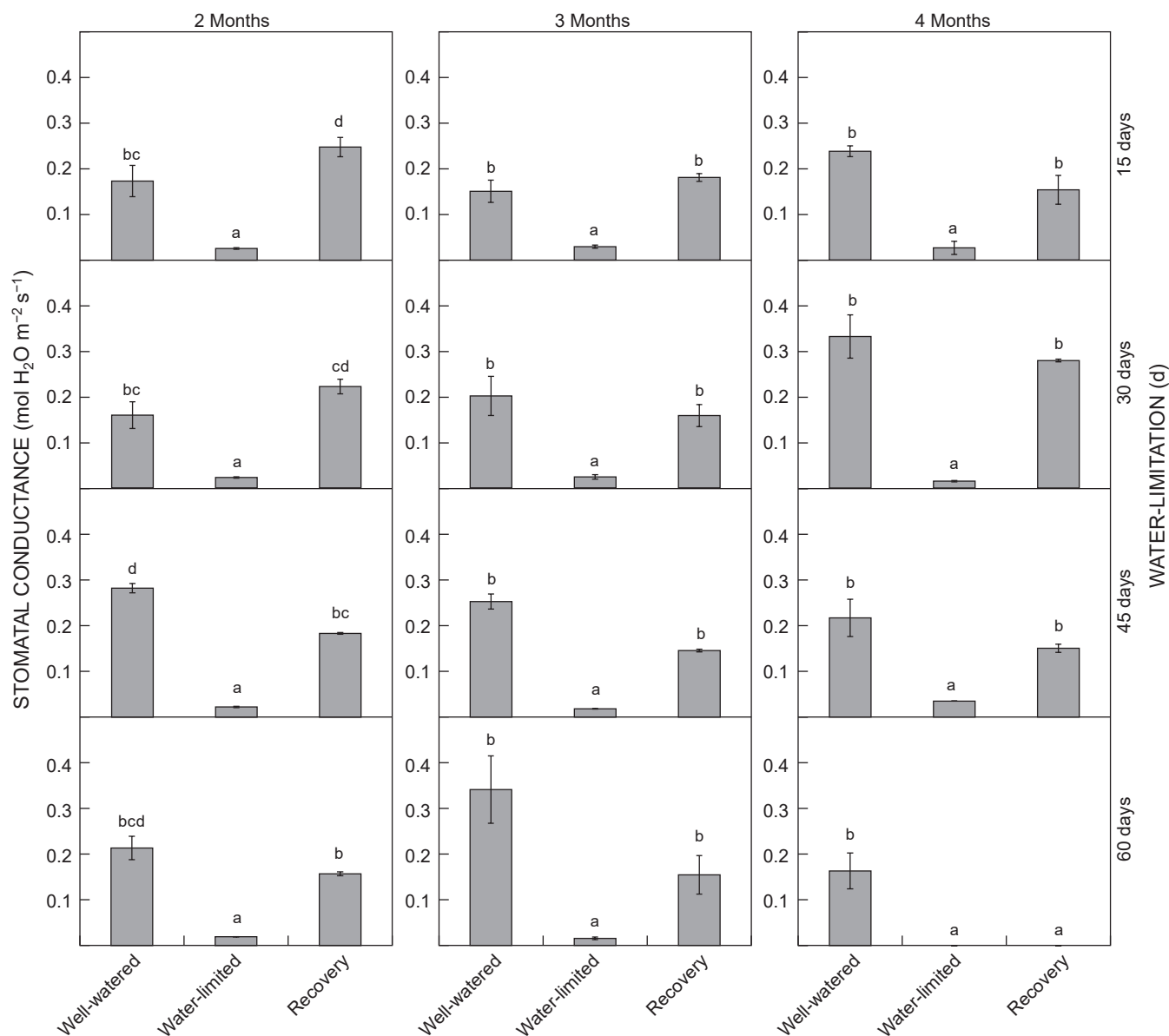


Figure 7: Stomatal conductance of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

has been shown to improve drought tolerance (Comas et al. 2013; Polle et al. 2019). The importance of root morphology in drought tolerance in plants has resulted in it becoming one of the targeted traits for plant breeders for improving water harvesting from deeper water resources from the soil (Polle et al. 2019). Therefore, the improved root traits developed by *C. sericea* plants under water limitation may also result in better adaptation of these plants to subsequent water-limited conditions, which is expected in arid and semiarid areas, where low and variable rainfall is the major limiting factor for production (DEA 2013).

Results from this study also indicated that *C. sericea* plants subjected to water limitation used multiple physiological adaptive responses to optimise the plant

performance under the marginal conditions. Rapid stomatal closure, even under moderate durations of water limitation in *C. sericea* plants could be one of the first adaptive responses to minimise dehydration, resulting in reduced g_s and E . Along with these, the reduced water availability and subsequent closure of the stomata resulted in decreased A and photosynthetic pigments, and therefore also carbon assimilation. This, in turn, could explain the significant reduction in biomass production under the water-limited conditions. The results generally correspond to the literature, because many researchers believe that the first reaction of most plants to water limitation is the closure of their stomata to prevent the loss of water through transpiration (Casson and Hetherington 2010; Anjum et

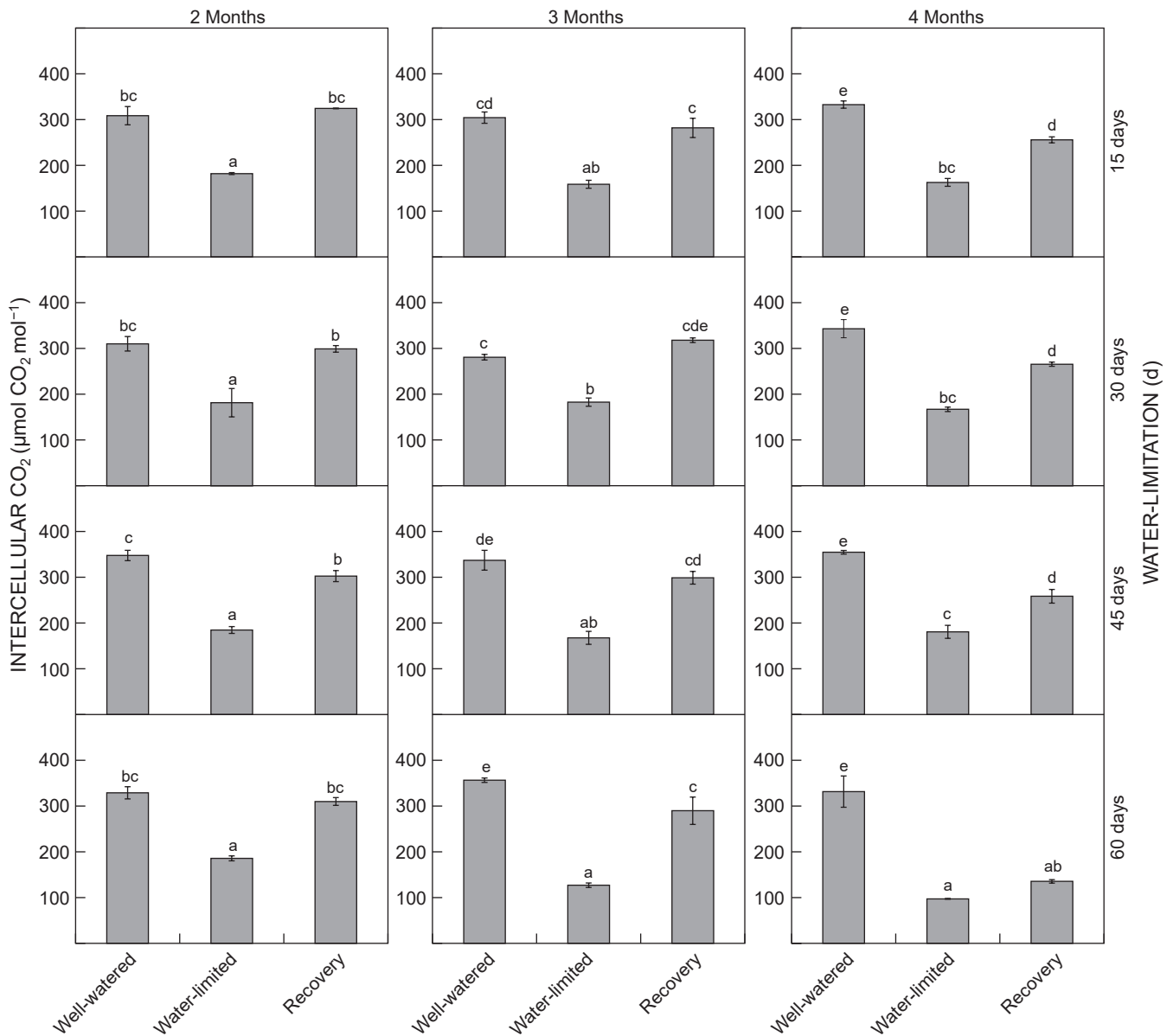


Figure 8: Intercellular CO₂ concentrations of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

al. 2011; Torres-Ruiz et al. 2013; Osakabe et al. 2014; Nemeskéri et al. 2015). Similarly, Mutava et al. (2015) revealed that under drought stress, reduced g_s in soybean was responsible for reduced A . The rapid closure of the stomata in this study also resulted in a reduced E , which generally resulted in an increased PWUE in water-limited plants. Similar results were reported by Kobata et al. (1996) and Tolk and Howell (2003) who reported that reduced g_s in rice, and reduced evapotranspiration in sorghum were associated with higher water use efficiency (Blum 2005).

Chlorophyll content in *C. sericea* plants that were subjected to water limitation generally decreased from well-watered levels. A reduction in g_s has been shown to disrupt photosynthetic pigments, because of the damage

that occurs to the chloroplasts caused by reactive oxygen species (ROS) produced under drought stress (Anjum et al. 2011). This in turn further reduces gas exchange and eventually leads to a reduction in plant growth and productivity (Anjum et al. 2011; Osakabe et al. 2014; Mutava et al. 2015; Pirasteh-Anosheh et al. 2016). The decrease in chlorophyll content is a commonly observed phenomenon under water-limited conditions (Bijanazadeh and Emam 2010; Din et al. 2011). Similar findings were reported for other legume species, such as mung bean (Batra et al. 2014), soybean (Makbul et al. 2011; Basal et al. 2020), chickpea (Mafakheri et al. 2010) and pea (Iturbe-Ormaetxe et al. 1998). Results from the current study also indicated that under water-limited conditions,

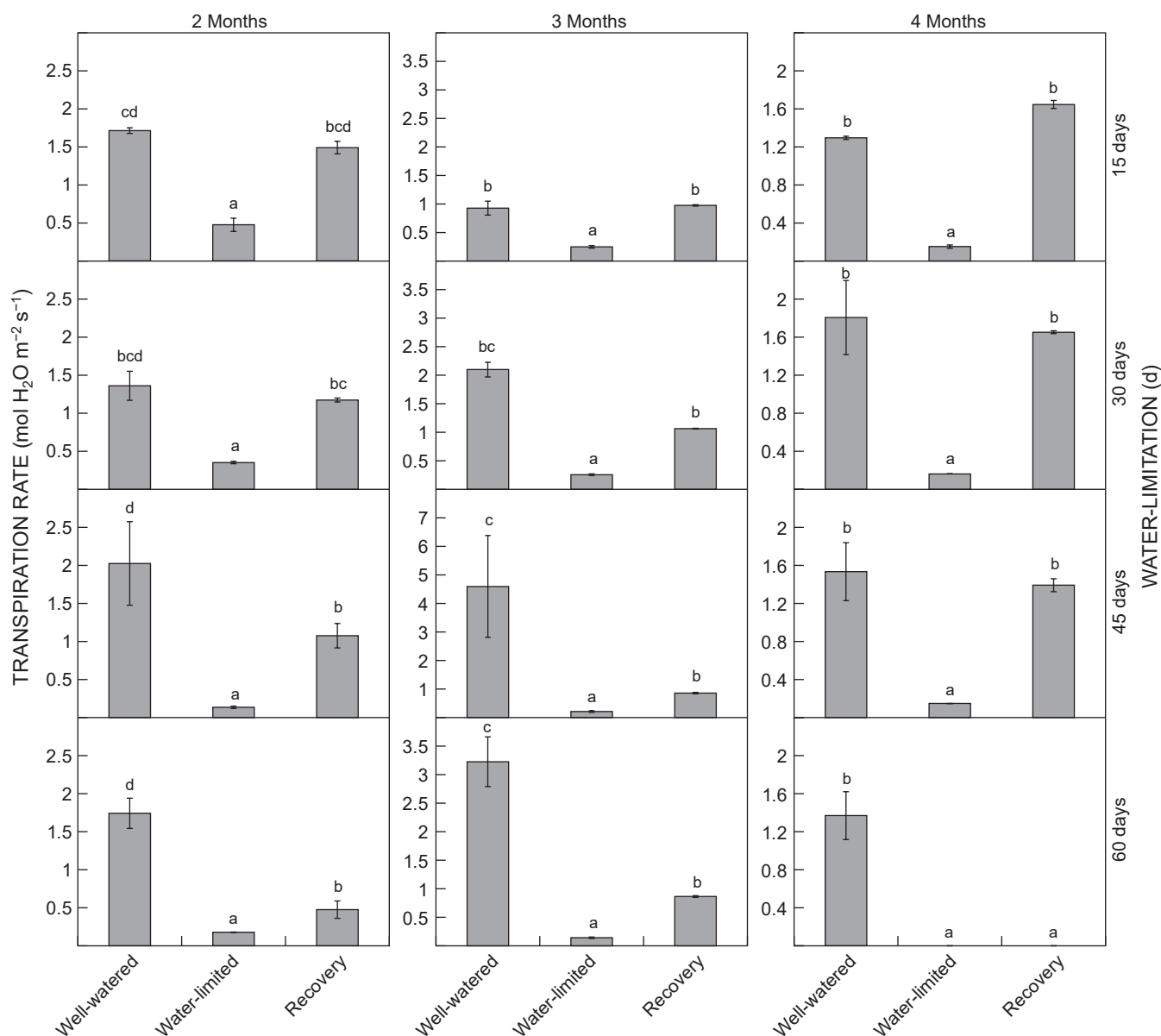


Figure 9: Transpiration rate of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were

there was an increased production of protective pigments (carotenoid and anthocyanin). One of the ways that plants have evolved to protect themselves against ROS-induced damage under water-limited conditions, is the synthesis of protective pigments, such as carotenoids and anthocyanin (Efeoğlu et al. 2009; Batra et al. 2014; Basal et al. 2020). These pigments are believed to have contributed to the avoidance of severe damage to the photosynthetic machinery of the *C. sericea* plants during the water-limited conditions, and allowed for a faster recovery of the photosynthetic activity after rewatering (Hörttensteiner 2009; Frosi et al. 2017).

Rewatering of the *C. sericea* plants in this study generally resulted in recovery of all the photosynthetic parameters

evaluated and a return to well-watered levels. After rewatering, E and g_s returned to normal levels resulting in increased A . These recoveries to the photosynthetic machinery of *C. sericea* plants, along with the improved uptake of water through the deeper and better-developed root system, generally enabled shoot biomass to rapidly recover after the stress was removed. It is well known, however, that the extent of recovery after water limitation can be limited by the intensity and duration of the preceding drought, before rewatering, a phenomenon called 'predrought limitation' (Flexas et al. 2009; Wang et al. 2017). This was evident when water limitation was imposed on the plants for 60 days, four months after establishment. Here, recovery after rewatering did not occur.

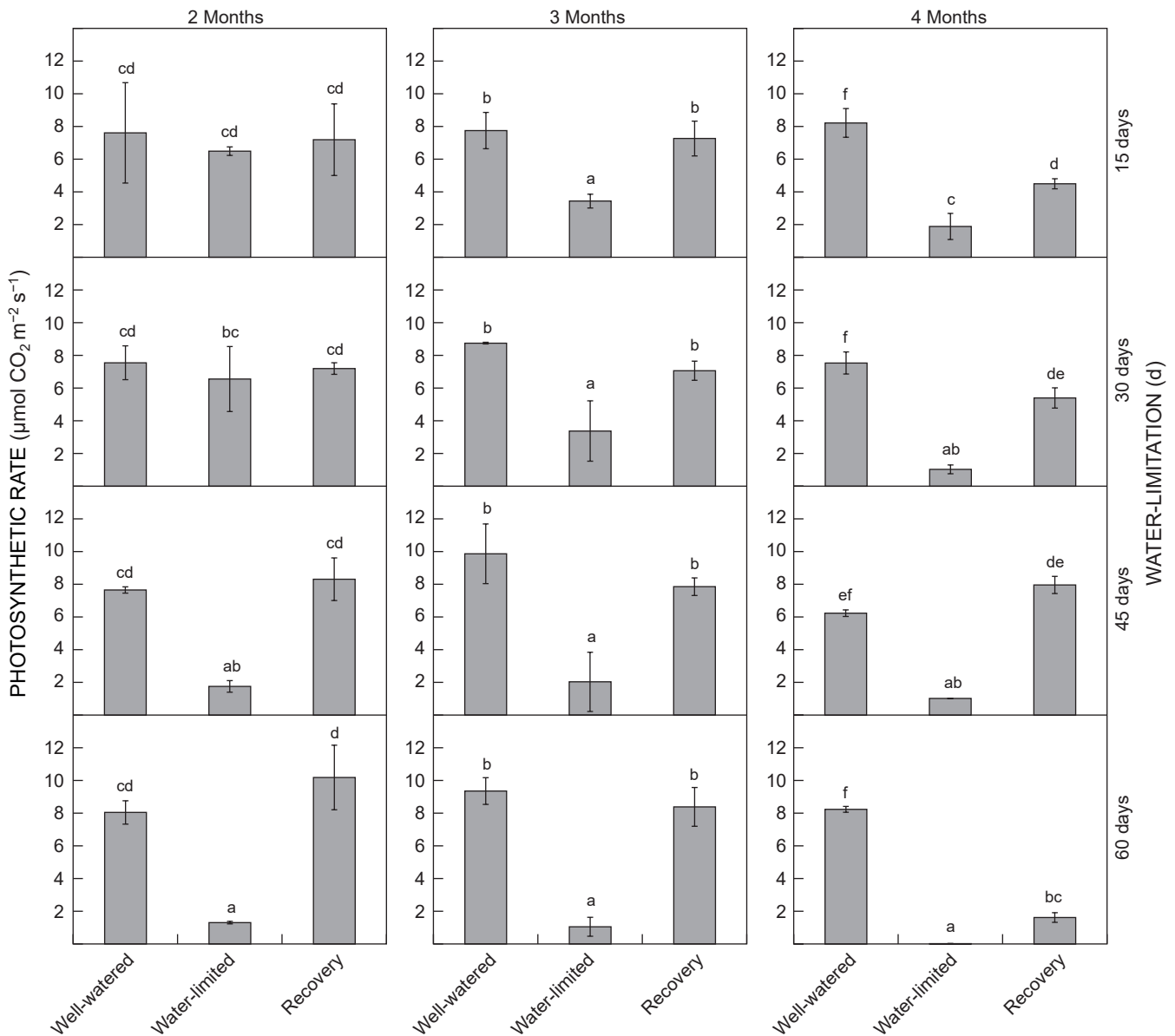


Figure 10: Photosynthetic rate of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

Conclusion

In the current study, we examined the effects of reduced water availability and subsequent rewatering on the morphological and physiological traits of *C. sericea*. We hypothesised that because of the natural distribution of *C. sericea* in water-limited areas, it would display a wide range of adaptive plasticity in morphological and/or physiological traits in response to water limitation, which would help *C. sericea* plants to cope in water-limited growing conditions. Results from the current study did indicate that *C. sericea* displayed a wide range of adaptive responses to water limitation, including increased allocation of resources to root growth,

closure of stomata resulting in reduced loss of water through transpiration, and the development of protective pigments to aid in a faster recovery of the photosynthetic machinery after rewatering. Although *C. sericea* plants were significantly negatively influenced by water limitation, rapid responses in both morphology and physiology allowed for rapid recovery once the stress had been removed. The extent to which the plants were able to cope with water limitation, and whether or not they were able to recover after the stress was removed was, however, primarily dependent on the intensity/duration of the stress and not necessarily on the age at which the stress was imposed on the plant.

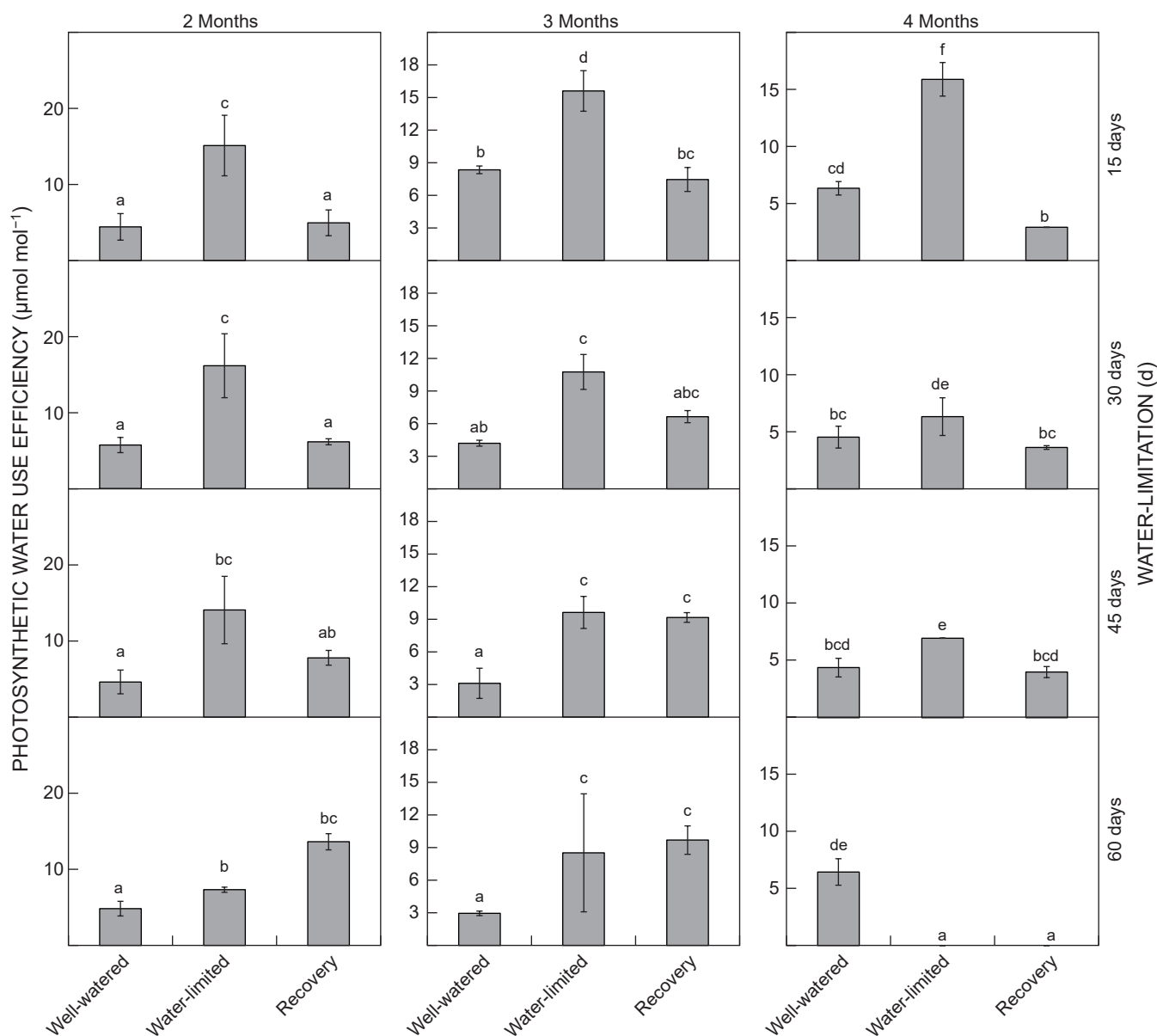


Figure 11: Photosynthetic water use efficiency of *Calobota sericea* plants at different ages under different durations of water limitation and subsequent recovery after rewatering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age

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ORCIDiDs

Francois Müller: <https://orcid.org/0000-0002-7211-6163>
 Samson BM Chimphango: <https://orcid.org/0000-0002-9755-790X>
 M Igshaan Samuels: <https://orcid.org/0000-0002-5594-2623>
 Clement Cupido: <https://orcid.org/0000-0002-0908-0049>
 Stephen Boatwright: <https://orcid.org/0000-0003-1250-4874>

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Table 1: Photosynthetic pigment content in two months old *Calobota sericea* plant leaves subjected to different durations of water limitation and subsequent rewatering. Mean concentrations with the same letters are not statistically significantly different (* $p < 0.05$) from one another

Treatment		Chlorophyll a	Chlorophyll b	Total chlorophyll	Anthocyanin	Carotenoids
15 days water limitation	Water limited	4.05 ± 0.486 ^b	0.17 ± 0.043 ^b	4.22 ± 0.526 ^b	0.03 ± 0.004 ^a	0.02 ± 0.016 ^a
	Water limited	0.73 ± 0.172 ^a	0.02 ± 0.007 ^a	0.75 ± 0.178 ^a	0.16 ± 0.006 ^c	0.27 ± 0.022 ^c
	Recovery	2.70 ± 0.683 ^b	0.15 ± 0.029 ^b	2.85 ± 0.711 ^b	0.12 ± 0.008 ^b	0.13 ± 0.014 ^b
	$F_{(2,9)}$	11.477	7.654	11.245	72.706	48.506
	p	0.009	0.022	0.009	< 0.001	< 0.001
30 days water limitation	Well watered	1.29 ± 0.084 ^{ab}	0.05 ± 0.006 ^a	1.34 ± 0.090 ^b	0.07 ± 0.013 ^a	0.08 ± 0.006 ^a
	Water limited	1.12 ± 0.134 ^a	0.02 ± 0.001 ^a	1.15 ± 0.133 ^a	0.23 ± 0.033 ^c	0.20 ± 0.002 ^b
	Recovery	2.29 ± 0.141 ^b	0.27 ± 0.018 ^b	2.56 ± 0.077 ^b	0.13 ± 0.001 ^b	0.19 ± 0.008 ^b
	$F_{(2,9)}$	27.447	119.904	39.644	26.104	42.500
	p	0.002	< 0.001	0.001	0.002	0.001
45 days water limitation	Well watered	1.97 ± 0.246 ^b	0.12 ± 0.008 ^b	2.09 ± 0.242 ^b	0.05 ± 0.008 ^a	0.09 ± 0.006 ^a
	Water-limited	1.27 ± 0.271 ^a	0.04 ± 0.009 ^a	1.31 ± 0.280 ^a	0.18 ± 0.023 ^b	0.20 ± 0.008 ^b
	Recovery	2.45 ± 0.080 ^b	0.12 ± 0.004 ^b	2.57 ± 0.077 ^b	0.12 ± 0.014 ^b	0.19 ± 0.006 ^b
	$F_{(2,9)}$	7.582	59.111	8.523	19.174	73.357
	p	0.023	< 0.001	0.018	0.002	< 0.001
60 days water limitation	Water limited	2.30 ± 0.307 ^b	0.14 ± 0.010 ^b	2.44 ± 0.311 ^b	0.02 ± 0.007 ^a	0.03 ± 0.003 ^a
	Water limited	0.61 ± 0.129 ^a	0.06 ± 0.007 ^a	0.68 ± 0.123 ^a	0.16 ± 0.020 ^b	0.27 ± 0.020 ^c
	Recovery	2.61 ± 0.148 ^b	0.13 ± 0.025 ^b	2.74 ± 0.132 ^b	0.12 ± 0.008 ^b	0.17 ± 0.015 ^b
	$F_{(2,9)}$	26.339	6.254	28.940	28.896	77.782
	p	0.001	0.034	0.001	0.001	< 0.001

Table 2: Photosynthetic pigment content in three months old *Calobota sericea* plant leaves subjected to different durations of water limitation and subsequent rewatering. Mean concentrations with the same letters are not statistically significantly different (* $p < 0.05$) from one another.

Treatment		Chlorophyll a	Chlorophyll b	Total chlorophyll	Anthocyanin	Carotenoids
15 days water limitation	Water limited	1.43 ± 0.120 ^b	0.06 ± 0.004 ^a	1.49 ± 0.123 ^b	0.06 ± 0.010 ^a	0.12 ± 0.016 ^a
	Water limited	0.67 ± 0.138 ^a	0.08 ± 0.015 ^a	0.75 ± 0.140 ^a	0.16 ± 0.029 ^b	0.51 ± 0.018 ^c
	Recovery	1.37 ± 0.222 ^b	0.05 ± 0.006 ^a	1.42 ± 0.227 ^b	0.05 ± 0.005 ^a	0.32 ± 0.010 ^b
	$F_{(2,9)}$	6.731	3.652	5.914	12.371	180.123
	p	0.029	0.092	0.038	0.007	< 0.001
30 days water limitation	Water limited	1.44 ± 0.182 ^b	0.06 ± 0.006 ^a	1.50 ± 0.187 ^b	0.05 ± 0.010 ^a	0.09 ± 0.008 ^a
	Water limited	0.51 ± 0.163 ^a	0.07 ± 0.037 ^a	0.58 ± 0.200 ^a	0.12 ± 0.017 ^b	0.36 ± 0.024 ^c
	Recovery	1.50 ± 0.237 ^b	0.05 ± 0.010 ^a	1.55 ± 0.247 ^b	0.04 ± 0.016 ^a	0.16 ± 0.006 ^b
	$F_{(2,9)}$	8.047	0.312	6.655	12.511	95.550
	p	0.020	0.743	0.030	0.007	< 0.001
45 days water limitation	Water limited	1.76 ± 0.219 ^b	0.06 ± 0.010 ^a	1.82 ± 0.225 ^b	0.02 ± 0.006 ^a	0.09 ± 0.013 ^a
	Water limited	0.39 ± 0.056 ^a	0.02 ± 0.004 ^a	0.41 ± 0.060 ^a	0.14 ± 0.012 ^c	0.38 ± 0.027 ^c
	Recovery	1.53 ± 0.065 ^b	0.03 ± 0.018 ^a	1.56 ± 0.053 ^b	0.10 ± 0.006 ^b	0.19 ± 0.023 ^b
	$F_{(2,9)}$	29.443	2.400	29.221	62.312	46.452
	p	0.001	0.171	0.001	< 0.001	< 0.001
60 days water limitation	Water limited	1.73 ± 0.125 ^b	0.03 ± 0.024 ^a	1.76 ± 0.147 ^b	0.01 ± 0.004 ^a	0.13 ± 0.012 ^a
	Water limited	0.80 ± 0.044 ^a	0.04 ± 0.008 ^a	0.83 ± 0.052 ^a	0.17 ± 0.044 ^b	0.38 ± 0.013 ^c
	Recovery	1.52 ± 0.106 ^b	0.04 ± 0.010 ^a	1.56 ± 0.097 ^b	0.09 ± 0.017 ^{ab}	0.26 ± 0.004 ^b
	$F_{(2,9)}$	27.699	0.078	20.956	8.821	114.112
	p	0.002	0.926	0.004	0.023	< 0.001

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Table 3: Photosynthetic pigment content in four months old *Calobota sericea* plant leaves subjected to different durations of water limitation and subsequent rewatering. Mean concentrations with the same letters are not statistically significantly different (* $p < 0.05$) from one another

Treatment		Chlorophyll a	Chlorophyll b	Total chlorophyll	Anthocyanin	Carotenoids
15 days water limitation	Water limited	1.60 ± 0.073 ^b	0.04 ± 0.017 ^a	1.64 ± 0.090 ^b	0.003 ± 0.001 ^a	0.13 ± 0.018 ^a
	Water limited	0.74 ± 0.077 ^a	0.03 ± 0.007 ^a	0.77 ± 0.083 ^a	0.18 ± 0.039 ^b	0.44 ± 0.014 ^b
	Recovery	1.59 ± 0.098 ^b	0.04 ± 0.009 ^a	1.63 ± 0.089 ^b	0.18 ± 0.002 ^b	0.45 ± 0.001 ^b
	$F_{(2,9)}$	39.220	0.381	32.615	15.497	14.957
	p	0.001	0.701	0.001	0.007	< 0.001
30 days water limitation	Water limited	0.90 ± 0.088 ^b	0.04 ± 0.003 ^c	0.94 ± 0.087 ^c	0.02 ± 0.009 ^a	0.09 ± 0.003 ^a
	Water limited	0.21 ± 0.016 ^a	0.001 ± 0.001 ^a	0.21 ± 0.017 ^a	0.17 ± 0.0001 ^c	0.40 ± 0.007 ^c
	Recovery	0.53 ± 0.147 ^{ab}	0.02 ± 0.003 ^b	0.55 ± 0.150 ^b	0.08 ± 0.019 ^b	0.23 ± 0.020 ^b
	$F_{(2,9)}$	19.271	52.160	21.384	73.067	76.406
	p	0.004	< 0.001	0.004	< 0.001	< 0.001
45 days water limitation	Water limited	1.30 ± 0.268 ^b	0.05 ± 0.005 ^a	1.35 ± 0.270 ^c	0.02 ± 0.007 ^a	0.08 ± 0.011 ^a
	Water limited	0.38 ± 0.033 ^a	0.03 ± 0.012 ^a	0.41 ± 0.043 ^a	0.21 ± 0.019 ^c	0.29 ± 0.005 ^c
	Recovery	0.65 ± 0.045 ^{ab}	0.10 ± 0.008 ^b	0.74 ± 0.053 ^b	0.10 ± 0.003 ^b	0.18 ± 0.009 ^b
	$F_{(2,9)}$	8.940	12.652	8.806	63.700	141.857
	p	0.016	0.007	0.016	< 0.001	< 0.001
60 days water limitation	Water limited	1.18 ± 0.259 ^b	0.05 ± 0.002 ^b	1.23 ± 0.261 ^b	0.01 ± 0.002 ^a	0.16 ± 0.020 ^a
	Water limited	0.07 ± 0.039 ^a	0.01 ± 0.006 ^a	0.08 ± 0.041 ^a	0.15 ± 0.032 ^b	0.49 ± 0.004 ^b
	Recovery	0.02 ± 0.006 ^a	0.003 ± 0.002 ^a	0.02 ± 0.007 ^a	0.11 ± 0.009 ^b	0.50 ± 0.007 ^b
	$F_{(2,9)}$	18.715	29.845	20.017	14.181	26.500
	p	0.003	0.001	0.002	0.005	< 0.001

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