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Permalink

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Journal

Plant and Soil, 427(1-2)

ISSN

0032-079X

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Publication Date

2018-06-01

DOI

10.1007/s11104-018-3657-y

Peer reviewed

Response of a facultative CAM plant and its competitive relationship with a grass to changes in rainfall regime

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Received: 5 February 2018 / Accepted: 16 April 2018 / Published online: 21 April 2018
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Abstract

Background and aims We investigated the response of a model facultative CAM plant (*Mesembryanthemum crystallinum*) and its competition with a C₃ grass (*Bromus mollis*) to changes in rainfall regime. **Methods** Seedlings of *M. crystallinum* and *B. mollis* in both monoculture and mixtures growing in shallow and deep pots were subjected to three levels of intra-seasonal rainfall variability and rainfall seasonality in both high water and low water conditions. Response of plants were evaluated by nocturnal carboxylation and biomass. **Results** A high rate of water drainage beneath root zones in coarse soil led to a negative response of

M. crystallinum and *B. mollis* in monoculture under increased intra-seasonal rainfall variability. Seasonal rainfall shifts to later dates during the growing season generally favored the growth of *M. crystallinum* and *B. mollis* in monoculture, with the exception of high water stress conditions whereby drought-intolerant species *B. mollis* was disfavored. Rainfall seasonality but not intra-seasonal rainfall variability affected nocturnal carboxylation by *M. crystallinum* in monoculture. **Conclusions** We suggest that soil texture, root depth, and rainfall gradient are important mediators of plant growth under increased intra-seasonal rainfall variability. Drought severity and the ability of a plant to tolerate drought and can greatly affect its response to the seasonal timing of rainfall. Nocturnal carboxylation by *M. crystallinum* in response to rainfall variability depends on the timescale.

Responsible Editor: Rafael S. Oliveira

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11104-018-3657-y>) contains supplementary material, which is available to authorized users.

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Keywords Intra-seasonal rainfall variability · Rainfall seasonality · Crassulacean acid metabolism · Competition · Soil texture · Drought severity · Drought tolerance

Introduction

Climate change studies have documented an increase in the extreme precipitation patterns with fewer, larger rainfall events (hereafter called intra-seasonal rainfall variability) (Easterling et al. 2000; Trenberth 2011) and changes in monthly rainfall distribution (hereafter called rainfall seasonality) (Feng et al. 2013). Climate

models predict that this trend will continue in the decades to come, especially in arid and semiarid regions (Tebaldi et al. 2006). Changes in rainfall regimes lead to significant changes in ecological processes such as interspecies interactions and ecosystem productivity (Knapp et al. 2008; Kulmatiski and Beard 2013; Zeppel et al. 2014; Li et al. 2016; Yu et al. 2016, 2017a; Chen et al. 2018).

Previous studies have extensively investigated the response of C_3 and/or C_4 plant communities to changes in rainfall regimes. Focusing on grasslands, Knapp et al. (2008) suggests that increases in intra-seasonal rainfall variability reduces soil evaporation and plant water stress and thus may lead to a positive response in xeric ecosystems, in contrast to mesic ecosystems. This framework has been verified in some grasslands (e.g., Heisler-White et al. 2008, 2009; Thomey et al. 2011) but not others (e.g., Fang et al. 2005; Ross et al. 2012; Zhang et al. 2013). Studies in the Southwestern United States have focused on the effects of increased winter rainfall on increased deep soil water, thus favoring woody plants (deep rooted) over grasses (shallow rooted) (Brown et al. 1997; Gao and Reynolds 2003; Munson et al. 2012; Sponseller et al. 2012). However, little is known about the response of plants with crassulacean acid metabolism (CAM) and its competitive relationship with other functional groups (i.e., grasses) to changes in rainfall regime. By performing water storage used for drought avoidance and a temporal separation of C_3 and C_4 photosynthesis, CAM plants are suggested to have a different response from C_3/C_4 communities in an increasingly variable climate (Borland et al. 2009; Bartlett et al. 2014). In particular, it also remains uncertain how CAM plants may respond to changes in monthly rainfall distribution (rainfall seasonality) during the growing (summer) season. Plants in drylands typically experience intense water limitations and thus are expected to be sensitive to changes in monthly rainfall distribution during the growing season (Schwinning and Sala 2004).

Studies of CAM plants are engendering scientific interest, particularly in recent years, because of their ability to adapt to drought through photosynthetic plasticity which may allow them to increase their dominance in a future (drier and warmer) climate (Drennan and Nobel 2000; Lüttge 2004; Borland et al. 2009; Yu et al. 2017b, 2017c). This study investigates the response of a model facultative CAM species (*Mesembryanthemum crystallinum*, occasional crop species) and its

competitive relationship with a C_3 grass (*Bromus mollis*) to changes in rainfall regime (i.e., intra-seasonal rainfall variability and rainfall seasonality). *M. crystallinum* and *B. mollis* are both invasive species and co-occur in coastal California sand dunes (McCown and Williams 1968; Vivrette and Muller 1977). In facultative CAM plants, the phase of CAM photosynthesis (in which stomata was open at night for CO_2 uptake to form malic acid) depends on environmental conditions (stresses). For instance, *M. crystallinum* typically performs C_3 photosynthesis in winter/spring wet season and then switches to nocturnal carboxylation (C_4 photosynthesis) to adapt to subsequent summer dry season (Osmond 1978; Cushman and Borland 2002; Winter and Holtum 2007, 2014). This photosynthetic plasticity performed by *M. crystallinum* was found to increase its fitness and reproduction in dry conditions (Winter and Ziegler 1992; Cushman et al. 2008). A recent study indicates that *M. crystallinum* could also adapt to biotic stress (i.e., moderate competition from grasses) because of its photosynthetic plasticity (Yu et al. 2017b). It remains unclear, however, how changes in rainfall regime (i.e., increased intra-seasonal variability and rainfall seasonality) may affect nocturnal carboxylation in *M. crystallinum*.

Soil texture affects rainfall infiltration rates. More extreme rainfall events are expected to have a high rate of drainage (water loss) beneath the root zone in coarse soils (i.e., sands) in coastal California (Macdonald and Barbour 1974) where *M. crystallinum* and *B. mollis* interact, especially for shallow roots (Tietjen et al. 2009; Zeppel et al. 2014; Yu and D'Odorico 2015). This may even lead to a negative response of plants to increase in intra-seasonal rainfall in xeric ecosystems, a hypothesis which needs to be tested. CAM plants typically have shallow roots (Drennan and Nobel 2000; Lüttge 2004). In coarse soil increased intra-seasonal rainfall variability increases deep soil moisture and thus may favor plants with deeper roots (i.e., grasses) than CAM plants (see Munson et al. 2012; Kulmatiski and Beard 2013 for competition between deep rooted woody plants and shallow rooted grasses). However, other studies indicated that the response of some species to climate drivers or CO_2 enrichment could be outweighed by their competition with other species. For instance, species interactions were found to strongly affect and even overturn direct climatic effects (i.e., rainfall seasonality and intensity) on natural grassland communities in California (Suttle et al. 2007). The positive response

of C₃ and C₄ grasses when grown alone to CO₂ enrichment was not observed when grown in natural (mixed) communities (Morgan et al. 1998, 2004). Changes in rainfall seasonality (i.e., seasonal rainfall shifts to later dates) during the growing season are expected to lead to early seasonal drought with a negative impact on drought intolerant species (i.e., grasses), thus favoring CAM plants. Tests of these hypotheses contribute to our understanding of potential shift in species composition and changes in ecosystem productivity in a future and more variable climate.

This study developed greenhouse experiments in which the seedlings of *M. crystallinum* and *B. mollis* in both monoculture and mixtures growing in two types of square pots with varying depth (shallow and deep) were subjected to three levels of intra-seasonal rainfall variability and three levels of rainfall seasonality in both high water and low water conditions. This study asked: (i) how do *M. crystallinum* and *B. mollis* grown alone in coarse soils (i.e., sands) respond to increased intra-seasonal rainfall variability and rainfall seasonality during the growing season? (ii) how does competition between *M. crystallinum* and *B. mollis* respond to increased intra-seasonal rainfall variability and rainfall seasonality during the growing season? (iii) how do changes in rainfall regimes (i.e., intra-seasonal rainfall variability and rainfall seasonality) affect nocturnal carboxylation in *M. crystallinum*?

Materials and methods

Experimental design

Seeds of *B. mollis* and *M. crystallinum* were germinated in plastic trays on June 6th 2016 and June 16th 2016, respectively, in the greenhouse facility at University of Virginia. Seedlings of *B. mollis* and *M. crystallinum* were transported to Blandy Experimental Farm in Northern Virginia, and then transplanted into two types of square pots (shallow: 12.5 cm in width, 18 cm in depth, 1.6 L in capacity; deep: 12.5 cm in width, 30 cm in depth, 4 L in capacity) on June 19–20th 2016 and June 26th 2016, respectively. Two types of pots (deep and shallow) were used to examine the effects of root depth on competition between *B. mollis* and *M. crystallinum* in response to changes in rainfall regime (Munson et al. 2012; Kulmatiski and Beard 2013). The seedlings were constructed as both monoculture (10

individuals of *B. mollis* or 1 individual of *M. crystallinum*) and in polycultures (10 individuals of *B. mollis* and 1 individual of *M. crystallinum* with *M. crystallinum* located in the middle of the pots). A relatively high number (10 individuals) of *B. mollis* was used to investigate whether the strong competition from *B. mollis* was able to outweigh the effects of altered rainfall regimes (Suttle et al. 2007). Soil was a mixture of Canadian sphagnum peat moss and Calcined Kaolin (Turface MVP) (6:5) (hydraulic conductivity = $102 \pm 37 \text{ mm h}^{-1}$) to simulate the sand dunes in coastal California where *M. crystallinum* and *B. mollis* naturally interact (Macdonald and Barbour 1974).

A randomized block experiment design was implemented in which seedlings of *M. crystallinum* and *B. mollis* in both monoculture and polycultures (6 replicates) were subjected to three levels of intra-seasonal rainfall variability (treatments V1, V2, and V3) and three levels of rainfall seasonality (treatments S1, S2, and S3) in both high water (HW) and low water (LW) conditions. The treatments lasted for 48 days from July 5th to Aug 21th, 2016; plants were well-watered (i.e. watered daily) before treatments began. Plants under intra-seasonal rainfall variability V1 (control) were watered every 2 days with an intensity of 8 mm per event in high water conditions and were watered every 4 days with an intensity of 8 mm per event in low water conditions. To investigate the effects of intra-seasonal rainfall variability in both high water and low water conditions, watering intensity per event in V2 and V3 was 2 times and 3 times the value (i.e., 8 mm) used in the case of the control while maintaining the same total amount of water application (in other words the frequency of water application was divided by 2 and 3, respectively).

To investigate the effects of rainfall seasonality during the growing season (treatments S1, S2, S3) in both high water and low water conditions, the experimental period (48 days) was divided into two stages (early: 24 days and late: 24 days). S₂ represents the case with rainfall shifted to early in the growing season, whereby plants in high water conditions were watered every day with an intensity of 6 mm per event until July 28th, 2016 (total amount of watering: 24 events × 6 mm per event = 144 mm) and were then watered once every 8 days with an intensity of 16 mm per event (total amount of watering: 3 events × 16 mm per event = 48 mm) until Aug 21th, 2016. By comparison, in low water conditions plants for the case of S₂ were watered every 2 days

with an intensity of 6 mm per event until July 28th, 2016 (total amount of watering: 12 events \times 6 mm per event = 72 mm) and were then watered every 8 days with an intensity of 8 mm per event (total amount of watering: 3 events \times 8 mm per event = 24 mm) until Aug 21th, 2016. Therefore, compared to control (V1) total amount of water in early stage in S2 in both high water and low water conditions is 50% higher while maintaining the same total amount of water additions in the two treatments. To investigate the case of a rainfall shift to late stage during the growing season (S3), the water treatment in S2 was reversed between early and late stages. Each pot was fertilized with 15 mg N every 12 days; the form of fertilizer is Peters Professional 20–20–20 (i.e., 20% total N including 3.2% ammoniacal nitrogen, 5.3% nitrate nitrogen, and 11.5% urea nitrogen, 20% P₂O₅, 20% K₂O, as well as other micronutrients).

Drainage and concentration of titratable acidity

Drainage for all the pots in all the treatments were collected by a 0.27 m² container and then measured using 200 ml graduated cylinder on Aug 20–21th, 2016. During the phase of CAM photosynthesis, CAM plants open their stomata at night and fix carbon dioxide into four-carbon (4-C) acids using phosphoenolpyruvate carboxylase. To assess CAM activity, before plant harvest on Aug 21–22th, 2016, one leaf of *M. crystallinum* alone or *M. crystallinum* in mixture was sampled from each plant in each pot at 7–8 am and 5–6 pm, respectively. These samples were then stored in a –20 °C freezer for measurements of concentration of titratable acidity. Concentration of titratable acidity was measured using the typical acid base titration method in which leaf discs (2.5 cm²) were boiled in 1.5 mL H₂O for 5 min in a microfuge tube and the solution was then added by freshly made 10 mM NaOH with 20 μ L of a 1/5 dilution of phenolphthalein as indicator (Von Caemmerer and Griffiths 2009). The amount of NaOH added was used to calculate the concentration of titratable acidity (mmol m⁻²) (Von Caemmerer and Griffiths 2009).

Biomass

Plants were harvested on Aug 22–25th, 2016. *M. crystallinum* and *B. mollis* in mixture were separated. Roots, and root debris were separated and attributed to *M. crystallinum* or *B. mollis* based on root color, diameter and shape; these root debris were insignificant

(<5%) compared to total root biomass. 0.1-mm mesh sieves were used to wash roots free of soil. Plants were dried at 60 °C for 72 h and then weighted. Total biomass and the biomass ratio between aboveground and belowground biomass were calculated; the fresh *M. crystallinum* sampled alone or in mixture for measurements of titratable acidity was weighted and then converted to dry biomass according to fresh/dry ratio.

Statistical analysis

The effects on total biomass (TB) of water amounts, intra-seasonal rainfall variability (RV), rainfall seasonality (RS), pot size, competition, and species as well as their interactions were analyzed by a five-way ANOVA with block (greenhouse bench) as a random factor. The effects of water, intra-seasonal rainfall variability and rainfall seasonality, pot size, and competition on overnight titratable acidity accumulation (ACC) by *M. crystallinum* alone or in mixture were analyzed by four-way ANOVA with block as a random factor. In general, the most interesting effects were found in multiway interactions. To explore these interactions, we constructed pairwise orthogonal contrasts to detect differences between individual pairs of means. All statistics were performed in SAS 9.4 while plots were made in R.

Results

Total biomass as affected by intra-seasonal rainfall variability

Water amounts, intra-seasonal rainfall variability (RV), pot size, competition, and species all had significant effects on total biomass (TB) (all $P < 0.0001$, Appendix Table S1). Total biomass of all vegetation types except *M. crystallinum* in mixture (FCM) in high water conditions were significantly greater than that in low water conditions, regardless of pot size and intra-seasonal rainfall variability ($P \geq 0.369$ for FCM; all $P < 0.0001$ for others, Fig. 1). A similar pattern was found for the effect of pot size with deep pots having higher total biomass than shallow pots regardless of water conditions (all $P \geq 0.5162$, all $P < 0.0001$ for others Fig. 1). Total biomass of *M. crystallinum* in mixture was significantly lower than *M. crystallinum* alone (FC) regardless of pot size and water conditions (all $P < 0.0323$, Fig. 1).

Difference in total biomass of *M. crystallinum* in mixture was not significant among treatments (all $P \geq 0.5849$, Fig. 1), which indicated that competition outweighed the effects of water, intra-seasonal rainfall variability and pot sizes

The effects of intra-seasonal rainfall variability on total biomass depended on other conditions (i.e., water, pot size, competition, and species) ($P < 0.0001$ for Water \times RV, $P < 0.0001$ for RV \times Pot, $P < 0.0001$ for RV \times Competition, $P < 0.0001$ for RV \times Species, Appendix Table S1). In particular, the increase in intra-seasonal rainfall variability significantly reduced total biomass of

all vegetation types except *M. crystallinum* in mixture in high water conditions regardless of pot size (all $P \leq 0.006$ for others, Fig. 1a and c). In comparison, in low water conditions a moderate increase in intra-seasonal rainfall variability (scenario V2) did not lead to significant changes in the total biomass of all vegetation types (except *M. crystallinum* in mixture) with respect to the control (V1), regardless of the pot size (all $P \geq 0.4216$, Fig. 1b and d). In low water and shallow pot conditions a stronger increase in intra-seasonal variability (scenario V3) led to a reduction in the total biomass of all vegetation types (except *M. crystallinum* in mixture) with

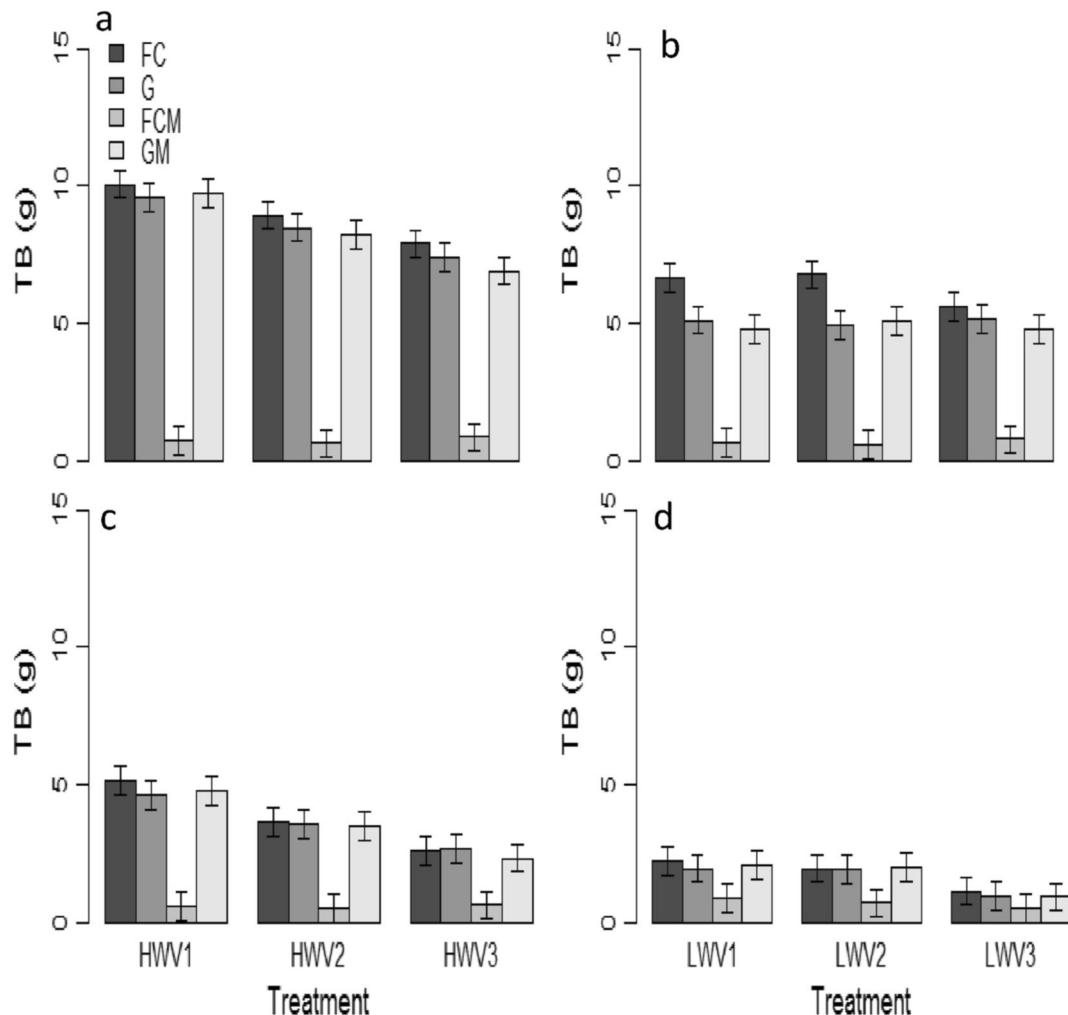


Fig. 1 Total biomass (TB) in *Mesembryanthemum crystallinum* alone (FC), *M. crystallinum* in mixture (FCM), *Bromus mollis* alone (G), *B. mollis* in mixture (GM) as affected by intra-seasonal rainfall variability in conditions of high water (HW) and deep pots (a), low water (LW) and deep pots (b), high water and shallow pots (c), low water and shallow pots (d). V1 refers to

control while V2 and V3 refer to the cases in which the amount of water applied in each watering event is doubled (V2) or trebled (V3) with respect to the control, while maintaining the same total amount of water additions by decreasing the frequency of watering events accordingly. Each bar represents the mean of 6 values while error bars indicate 95% confidence intervals

respect to the V2 scenario (all $P < 0.0001$, Fig. 1d), while in low water and deep pot conditions this result was found only in the case of *M. crystallinum* alone ($P = 0.0004$, Fig. 1b). In general, these results indicated that increased intra-seasonal rainfall variability decreased growth of both *M. crystallinum* alone and *Bromus mollis* alone (G), especially in high water and/or shallow pot conditions.

Total biomass as affected by rainfall seasonality during the growing season

Water, rainfall seasonality during the growing season (RS), pot size, competition, and species all had significant effects on total biomass (TB) (all $P < 0.0001$, Appendix Table S1). Similar to the case of intra-seasonal rainfall variability, total biomass of all vegetation types – except *M. crystallinum* in mixture – was significantly higher in high water (or deep pots) conditions than in low water (or shallow pots) conditions, regardless of rainfall seasonality (all $P \leq 0.0005$, Fig. 2). A strong competition from *B. mollis* led to a significantly lower total biomass in *M. crystallinum* in mixture than *M. crystallinum* alone (all $P < 0.0001$, Fig. 1); this strong competition outweighed the effects of water amounts, rainfall seasonality, and pot sizes, thus generally leading to no significant difference in the total biomass in *M. crystallinum* in mixture between those treatments and the control (Fig. 1). In general, a delay of rainfall occurrences until the late stage of the growing season (scenario S3) increased the total biomass of all vegetation types except *M. crystallinum* in mixture, in contrast to the case of rainfall shift to early stage during the growing season (scenario S2), which showed the opposite pattern (Fig. 2). However, this pattern was not observed in low water and shallow pot conditions in which total biomass in *B. mollis* alone (G) and in mixture (GM) was significantly higher in S2 than the control (V1) (both $P \leq 0.0006$, Fig. 2) and was significantly lower in S3 than V1 (both $P \leq 0.0214$, Fig. 2). The disadvantage of *B. mollis* (as shown by a lower biomass) under rainfall shift to the late stage of the growing season (scenarios S3) in low water and shallow pot conditions reduced its competitive effects on *M. crystallinum* in mixture, thus leading to a higher biomass in *M. crystallinum* in mixture with respect to the control ($P = 0.0019$; Fig. 2).

CAM activity: Overnight accumulation of titratable acidity

There were significant effects of water, pot size, and competition but not intra-seasonal rainfall variability on concentration of overnight titratable acidity accumulation (ACC) in *M. crystallinum* (Appendix Table S2). The effects of water amounts in the water treatments on ACC depended on pot size ($P = 0.0009$ for Water \times Pot, Appendix Table S2). ACC of *M. crystallinum* alone in low water conditions was significantly higher than that in high water conditions in deep pots (all $P \leq 0.0066$, Fig. 3a and b), which was in contrast to the case of shallow pots (Fig. 3c and d). ACC of *M. crystallinum* alone in deep pots was significantly higher than shallow pots in low water conditions (all $P < 0.0001$, Fig. 3), while in high water conditions this pattern was only seen with a stronger increase in intra-seasonal variability (scenario V3) ($P = 0.0015$ for V3; both $P \geq 0.2011$). *M. crystallinum* in mixture had significant titratable acidity accumulation overnight (ACC ≈ 100 – 130 mmol m⁻²) regardless of other treatments, which indicated nocturnal carboxylation (Fig. 3; Fig. 4), while there was generally no difference of ACC among intra-seasonal rainfall treatments (Fig. 3). In contrast to the case of intra-seasonal rainfall variability, rainfall seasonality significantly affected ACC by *M. crystallinum* ($P < 0.0001$; Appendix Table S2). Regardless of water and pot size, rainfall shift to the early stage of the growing season (S2) significantly increased ACC in *M. crystallinum* alone (all $P < 0.0001$) while rainfall shift to late stage (S3) significantly reduced ACC in *M. crystallinum* alone (all $P < 0.015$, Fig. 4).

Discussion

Rainfall variability is predicted to increase at different time scales under a warming climate and will have important impacts on ecological processes (Knapp et al. 2008; Smith 2011). Its effects on ecological processes such as interspecies interactions and ecosystem productivity have been primarily investigated in C₃ and/or C₄ plant communities (Knapp et al. 2008; Kulmatiski and Beard 2013; Zeppel et al. 2014). This study investigated response of a model facultative CAM plant (*M. crystallinum*) and its competitive relationship with a C₃ grass (*B. mollis*) to rainfall variability at intra-seasonal and seasonal time scales. We found that rainfall

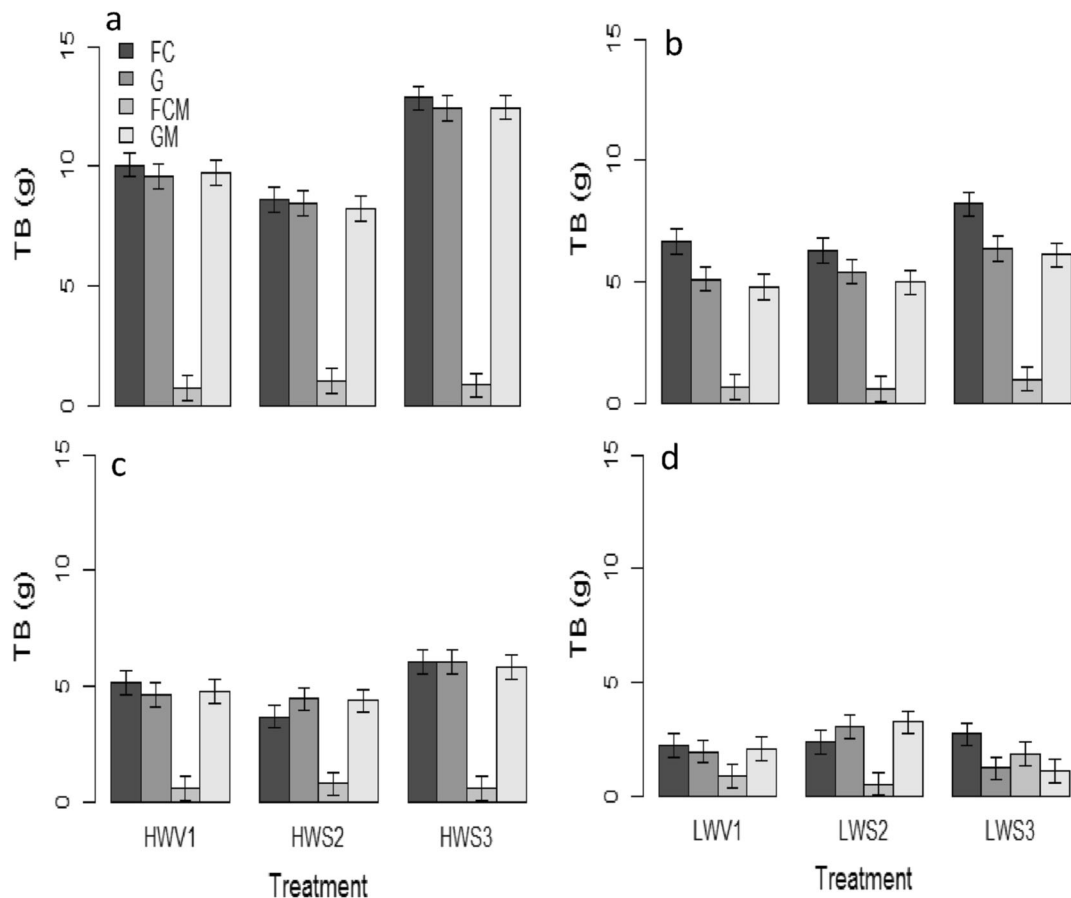


Fig. 2 Total biomass (TB) in *Mesembryanthemum crystallinum* alone (FC), *M. crystallinum* in mixture (FCM), *Bromus mollis* alone (G), *B. mollis* in mixture (GM) as affected by rainfall seasonality during the growing season in conditions of high water (HW) and deep pots (a), low water (LW) and deep pots (b), high water and shallow pots (c), low water and shallow pots (d). V1

amount, soil texture, and root depths mediated plant response to intra-seasonal rainfall variability, while drought severity and plants' tolerance to drought largely affected plant response to rainfall seasonality during the growing season. The performance of nocturnal carboxylation, a typical strategy adopted by *M. crystallinum* to adapt to abiotic and biotic stress, was affected by rainfall seasonality during the growing season but not intra-seasonal rainfall variability.

From an ecohydrological perspective, plant response to changes in intra-seasonal rainfall variability depends on its influence on components of soil water loss (i.e., soil evaporation, runoff, drainage beneath root zones) and thus soil water availability for plant establishment and growth (Rodríguez-Iturbe et al. 2001; Knapp et al. 2008; Ross et al.

refers to control while S2 and S3 refer to the cases in which rainfall occurrence has been shifted to the early stage (S2) and late stage (S3) of the growing season, while maintaining the same total amount of water additions. Each bar represents the mean of 6 values while error bars indicate 95% confidence intervals

2012). In fine soils increased intra-seasonal rainfall variability was found to increase runoff (water loss) and thus plants responded negatively (i.e., in terms of carbon assimilation and growth) to this treatment (i.e., Arora et al. 2001; Tietjen et al. 2009; Tietjen et al. 2010). This study found the negative response of *M. crystallinum* and *B. mollis* in monoculture to increased intra-seasonal rainfall variability (Fig. 1), which was likely due to the substantial water losses by drainage beneath the root zone in coarse soil (i.e., sand) (Appendix Fig. S1) (Zeppel et al. 2014; Yu and D'Odorico 2015), especially in the case of shallow pots (roots) and high water conditions. In fine-coarse soils, runoff and drainage beneath the root zone are relatively insignificant in xeric ecosystems and thus increased intra-seasonal rainfall variability

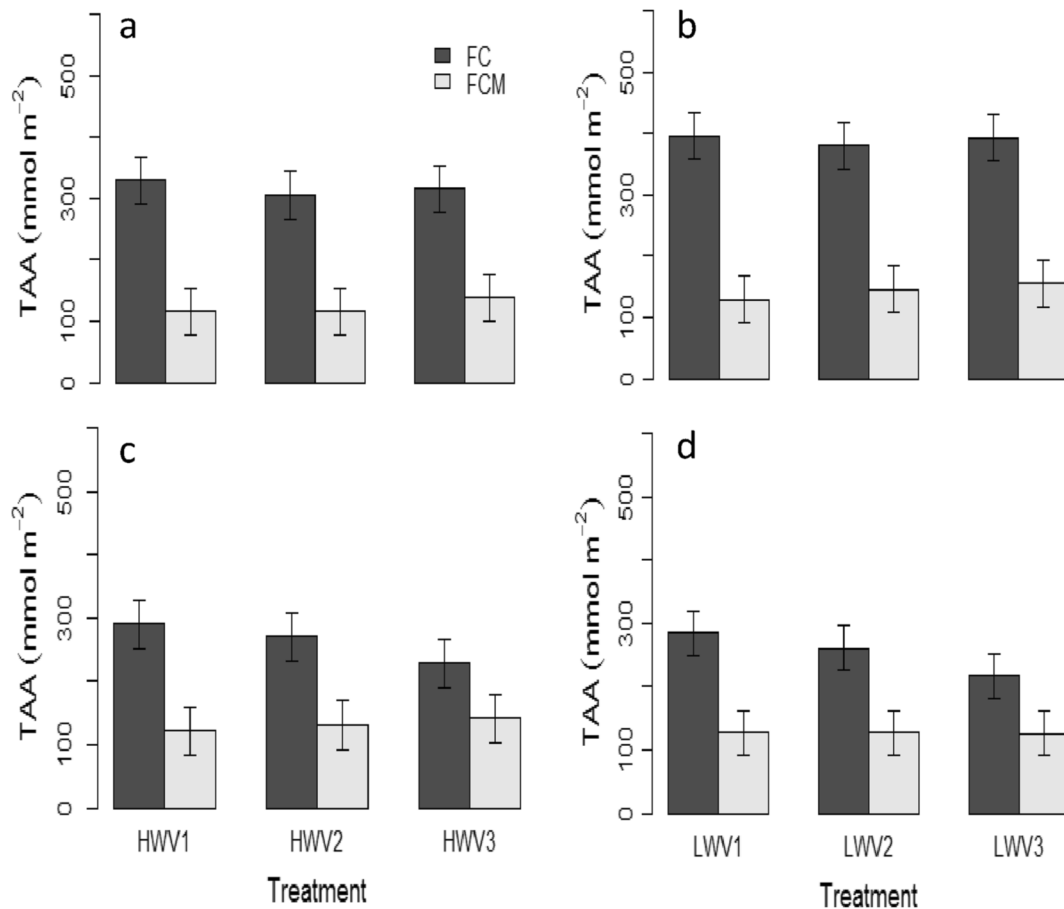


Fig. 3 Titratable acidity accumulation overnight (ACC) in *Me-sembryanthemum crystallinum* alone (FC) and *M. crystallinum* in mixture (FCM) as affected by intra-seasonal rainfall variability in conditions of high water (HW) and deep pots (a), low water (LW)

and deep pots (b), high water and shallow pots (c), low water and shallow pots (d). Symbols for each treatment are the same as Fig. 1. Each bar represents the mean of 6 values while error bars indicate 95% confidence intervals

generally reduces water loss (soil evaporation) and benefits plants (Knapp et al. 2008; Heisler-White et al. 2008, 2009; Thomey et al. 2011).

Based on these results, we conclude that total rainfall amount, soil texture, and plant traits (i.e., root depth) are major factors controlling plant responses to intra-seasonal rainfall variability (Fig. 5a). Very fine soils exhibit high runoff rates and plant response is therefore not affected by the root depth. In coarse texture soils plant available moisture is affected by drainage losses beneath the root zone, especially in the case of shallow roots. Both of these pathways of water loss (i.e., runoff and drainage) lead to a negative plant response to increased intra-seasonal rainfall variability (see shaded zones in Fig. 5a). An increase in total rainfall corresponding to a shift from arid to semi-arid environments increases water losses by either drainage or runoff, thus

expanding the domain (Fig. 5a) in which a negative plant response to increased intra-seasonal rainfall variability is observed. We note that, however, other plants' ecophysiological traits that are crucial to adapting to rainfall fluctuations (i.e., drought tolerance and growth rate) may also play important roles in the responses to intra-seasonal rainfall variability (Ogle and Reynolds 2004; Reyer et al. 2013; Zeppel et al. 2014). For instance, under increased intra-seasonal rainfall variability a species with high vulnerability to drought associated with increased time intervals of plant water stress between rainfall events would have a negative response, while a species with a high growth rate may benefit from the window opportunity associated with increased intensity of rainfall events (Davis et al. 2000; Yu et al. 2016, 2017a). These difference in plants' ecophysiological strategies may account for the observed difference

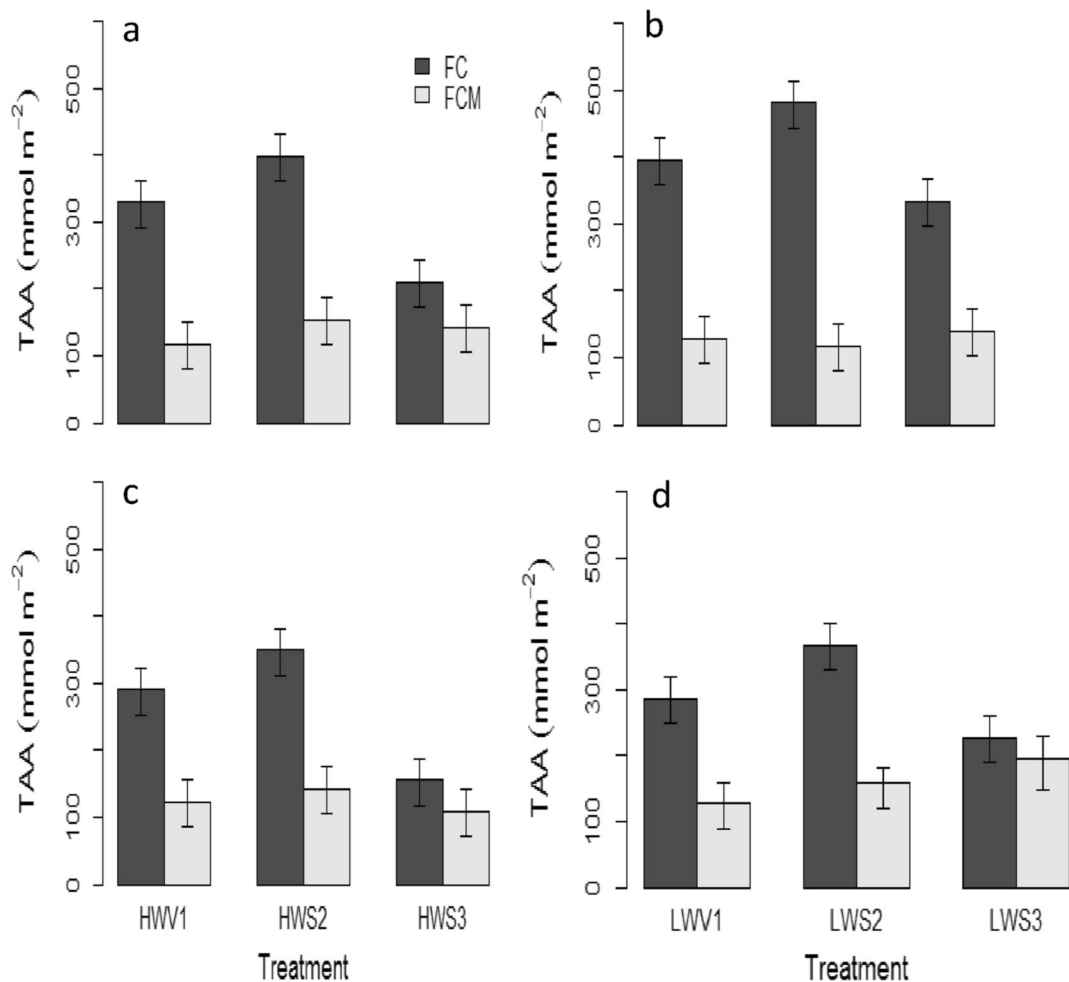


Fig. 4 Titratable acidity accumulation overnight (ACC) in *Meibryanthemum crystallinum* alone (FC) and *M. crystallinum* in mixture (FCM) as affected by rainfall seasonality in conditions of high water (HW) and deep pots (a), low water (LW) and deep pots

(b), high water and shallow pots (c), low water and shallow pots (d). Symbols for each treatment are the same as Fig. 2. Each bar represents the mean of 6 values while error bars indicate 95% confidence intervals

in the response of grasses or tree-grass compositions to intra-seasonal rainfall variability (Good and Caylor 2011; Kulmatiski and Beard 2013).

Our results showed that a delay in rainfall occurrences until later during the growing season had a positive effect on *M. crystallinum* and *B. mollis* alone, while a negative impact was observed in the case of rainfall occurrences shifting toward the early stage of the growing season (Fig. 2a, b, and c). These results can be explained by higher water demands associated with higher growth rate and/or higher biomass of older seedlings in the late stage than the early stage of the growing season (Reynolds et al. 1999; Jackson et al. 2005). In this sense, an increase in rain water inputs in the early stage of the experiment was neither efficiently used by

plants nor stored in coarse and shallow soils (<30 cm) and aboveground biomass, as evidenced by a higher rate of water drainage beneath root zones in early stage than late stage (all $P < 0.0001$; Appendix Fig. S2). Interestingly, the rainfall shift to the early stage of the growing season favored *B. mollis* in shallow pots and low water conditions, whereby the water stress was highest among treatments (Fig. 2d). In fact, *B. mollis* is not drought tolerant (Yu et al. 2017b, 2017c) and appeared to be severely damaged by early extreme droughts, thus restricting the ability of *B. mollis* to recover and benefit from later rainfall events (Walter et al. 2011). This “memory” of early droughts was not significant for *M. crystallinum* which as facultative CAM plants have higher plant water contents and are more drought

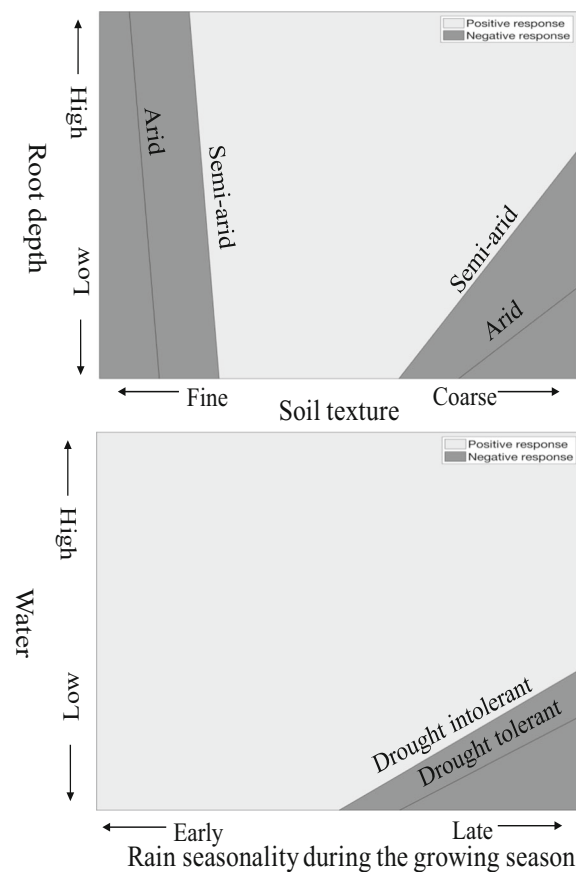


Fig. 5 A conceptual diagram showing (a) how rainfall gradient, soil texture, and root depth affect the response of plants to intra-seasonality rainfall variability; (b) how rainfall gradient and functional traits (i.e., vulnerability to drought) affect the response of plants to rainfall seasonality during the growing season

tolerant (Lüttge 2004; Borland et al. 2009; Yu et al. 2017b, 2017c).

Based on these results, drought severity and plant traits (i.e., tolerance to drought) appear to be major controls of plant response to rainfall seasonality during the growing season (Fig. 5b). This result suggests that plants generally benefit from an increase in late growing season rainfall (with total growing season precipitation remaining the same) because of their high water demands in the late growing season. However, species with high vulnerability to drought respond negatively (i.e., they exhibit reduced productivity and increased plant water stress), especially in low rainfall conditions because they tend to be severely damaged by extreme drought. Thus, a switch from drought tolerant species to drought intolerant species expands the domain in which plants respond negatively to a decrease in early growing

season precipitation and increase in late growing season precipitation (Fig. 5b). Previous studies largely showed the competitive advantage of woody plants (deep-rooted) over grasses (shallow-rooted) under conditions of increased winter rainfall and deep soil water (Brown et al. 1997; Gao and Reynolds 2003; Munson et al. 2012; Sponseller et al. 2012). This study investigates the effects of rainfall seasonality during the (summer) growing season when plants in drylands have high water stress (Schwinning and Sala 2004). It stresses that shifts in monthly rainfall distribution during the growing season could largely affect interspecific interactions depending on plants traits.

This study did not find empirical evidence for a competitive advantage of grasses (deep-rooted) over CAM plants (shallow-rooted) under increased intra-seasonal rainfall variability. This is in contrast to previous studies which show that increased intra-seasonal rainfall variability increased deep soil moisture, thus favoring deep-rooted plants (i.e., woody plants) over shallow-rooted plants (grasses) (i.e., Munson et al. 2012; Kulmatiski and Beard 2013). We interpreted that the strong competition of *B. mollis* may outweigh impacts of the environmental changes in rainfall regimes, as shown by lack of significant difference in total biomass and overnight titratable acidity accumulation by *M. crystallinum* in mixture among treatments. Consistent with these results, species interactions were found to outweigh the direct climate effects of rainfall seasonality and intensity on a California natural grassland (Suttle et al. 2007). The impact of competition was also found to outweigh other environmental change drivers (i.e., CO₂ enrichment) in natural (mixed) grass communities (Morgan et al. 1998, 2004).

Our study shows that intra-seasonal rainfall variability did not have significant effects on nocturnal carboxylation in *M. crystallinum*, in contrast to the case of rainfall seasonality (Fig. 3). In natural environments in coastal California where strong seasonal rainfall variability exists, *M. crystallinum* performs C₃ photosynthesis and accumulates carbohydrates (substrates required for nocturnal carboxylation) during the rainy (winter/spring) season and then switches to nocturnal carboxylation in response to the dry (summer) season (Adams et al. 1998; Cushman and Borland 2002; Antony et al. 2008; Haider et al. 2012; Yu et al. 2017b, 2017c). The role of carbohydrates in nocturnal carboxylation was also supported by examining the case of shallow pots with higher water stress in shallow pots, whereby C₃ photosynthesis and

accumulation of carbohydrates was suppressed and thus a lower nocturnal carboxylation by *M. crystallinum* alone was observed than in deep pots (Figs. 3 and 4). Overall, these results stress the role of the timescale of relevant environmental (rainfall) variability in plant response (i.e., nocturnal carboxylation by facultative CAM plants) to changes in environmental conditions.

Conclusion

This study found a negative response in the growth of *M. crystallinum* and *B. mollis* in monoculture, in particular in shallow pots and high water conditions, under increased intra-seasonal rainfall variability. This negative response resulted from a high rate of water drainage (loss) beneath root zones in coarse soil. Seasonal rainfall shifts to later dates during the growing season generally favored the growth of *M. crystallinum* and *B. mollis* in monoculture because of higher water requirement in later stage. The exception, however, was the case of high water stress conditions whereby drought-intolerant species *B. mollis* was disfavored. These results stress the role of soil texture, root depth, and rainfall gradient in determining plants' response to intra-seasonal rainfall variability. Drought severity and plant drought tolerance largely affect plants' response to rainfall seasonality. The different response of nocturnal carboxylation in *M. crystallinum* to intra-seasonal rainfall variability and rainfall seasonality highlights the role of the timescale of relevant environmental (rainfall) variability in plants' response to changes in environmental (rainfall) conditions.

Acknowledgements This research was funded through Blandy Summer Research Fellowship, Moore Research Grant and Graduate Environmental Resilience Fellowship through University of Virginia. We are grateful for help of experiments from Ashden Personius, Heng Huang, Yongli He, and Wendy Crannage.

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