

Effects of competition on induction of crassulacean acid metabolism in a facultative CAM plant

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Abstract Abiotic drivers of environmental stress have been found to induce CAM expression (nocturnal carboxylation) in facultative CAM species such as *Mesembryanthemum crystallinum*. The role played by biotic factors such as competition with non-CAM species in affecting CAM expression, however, remains largely understudied. This research investigated the effects of salt and water conditions on the competition between *M. crystallinum* and the C₃ grass *Bromus mollis* with which it is found to coexist in California's coastal grasslands. We also investigated the extent to which CAM expression in *M. crystallinum* was affected by the intensity of the competition with *B. mollis*. We found that *M. crystallinum* had a competitive advantage over *B. mollis* in drought and saline conditions, while *B. mollis* exerted strong competitive effects on *M. crystallinum* in access to light and soil nutrients in high water conditions. This strong competitive effect even outweighed the favorable effects of salt or water additions in increasing the biomass and productivity of *M. crystallinum*

in mixture. Regardless of salt conditions, *M. crystallinum* did not switch to CAM photosynthesis in response to this strong competitive effect from *B. mollis*. Disturbance (i.e., grass cutting) reduced the competitive pressure by *B. mollis* and allowed for CAM expression in *M. crystallinum* when it was grown mixed with *B. mollis*. We suggest that moderate competition with other functional groups can enhance CAM expression in *M. crystallinum*, thereby affecting its plasticity and ability to cope with biological stress.

Keywords Crassulacean acid metabolism · Competition · Drought · Soil salinity · Grass cutting · *Mesembryanthemum crystallinum* · *Bromus mollis* · California's coastal grasslands

Introduction

Plants with crassulacean acid metabolism (CAM) perform a temporal separation of the dark and light reactions of photosynthesis (Lüttge 2004; Borland et al. 2011). At night, CAM plants use phosphoenolpyruvate carboxylase (PEPC) to fix CO₂ into 4-C organic acids, while during the day these 4-C organic acids are decarboxylated to release CO₂ for the Calvin cycle by means of the enzyme Rubisco. CAM plants feature water storage and exhibit a higher water use efficiency relative to C₃ and C₄ plants (Lüttge 2004; Ogburn and Edwards 2010). These characteristics make crassulacean acid metabolism an effective adaptive strategy in water-limited environments (Ranson and Thomas 1960; Lüttge 2004; Borland et al. 2009). Some studies show that CAM plants are increasing their abundance in many drylands around the world (Borland et al. 2009, 2011) and a variety of drivers have been invoked to explain this phenomenon, including changes in climate

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(e.g., drought intensification, warming) and/or increasing atmospheric CO₂ concentrations (Drennan and Nobel 2000; Borland et al. 2009; Reyes-García and Andrade 2009).

Competitive interactions between coexisting species have long been considered major determinants of plant community structure and function (Tilman 1982; Connell 1983; Suttle et al. 2007). Research in ecology in the last few decades has often focused on the role of competition on shifts in plant dominance between C₃ and C₄ species under environmental change (Huxman and Monson 2003; Niu et al. 2008; van der Waal et al. 2009; Fay et al. 2012), while the response of mixed communities of CAM and non-CAM plants remains largely understudied (but see Yu and D'Odorico 2015; Yu and Foster 2016). Climate change studies predict more frequent heat waves, increased occurrences of extreme rainfall events, and increased risk of prolonged drought especially in dryland regions (Easterling et al. 2000; Held and Soden 2006). Drought and sea level rise can also favor salt accumulation and enhance soil salinity in coastal areas with shallow water tables or affected by coastal flooding (Lynn and Simpson 1987; Morris et al. 2002; Herppich et al. 2008; Charles and Dukes 2009). To date, it is still unclear how these drivers of environmental change affect CAM plant dominance by altering their competitive relations with other species.

This study evaluates how water and salt conditions affect the competition between CAM plants and grasses, and the emergence of CAM photosynthesis in a facultative CAM plant. We focus on two species, *Mesembryanthemum crystallinum* (a halophytic annual in the Aizoaceae; facultative CAM) and *Bromus mollis* (a C₃ grass), which co-occur in coastal California (McCown and Williams 1968; Vivrette and Muller 1977; D'Antonio and Vitousek 1992). *M. crystallinum* is a model facultative CAM species which can switch from C₃ photosynthesis to CAM expression as it transitions from juvenile to adult under water stress conditions (Osmond 1978; Cushman and Borland 2002; Winter and Holtum 2007, 2014). A high salt tolerance of *M. crystallinum* was previously thought to be a main mechanism for its successful invasion of coastal California (Vivrette and Muller 1977). These characteristics combined with water storage imply that *M. crystallinum* would win over *B. mollis* under low water and saline conditions. In wet conditions, *B. mollis* (C₃ grasses) could have a high growth rate (McCown and Williams 1968; Teuling et al. 2010; Collins and Xia 2015), which suggests that *B. mollis* would have a strong competitive effect on *M. crystallinum* in access to light and soil nutrients. *M. crystallinum*, however, may acclimate to competition from *B. mollis* thanks to the physiological plasticity resulting from its ability to switch from C₃ to CAM photosynthesis (Herrera 2009; Borland et al. 2011). These hypothetical mechanisms, however, still need to be tested and to date empirical evidence is still missing.

A better understanding of these dynamics would shed light on how CAM plants can adapt to both abiotic and biotic stress and thus potentially explain the increased competitive advantage by CAM plants relative to non-CAM species under global environmental change.

The role of biotic factors in the expression of CAM in facultative CAM species has been emphasized, but empirical studies are still rare (Herrera 2009). Mycorrhization can modify host water relations and thus affect CAM expression in facultative CAM species (*Clusia minor*) in response to droughts (Maiquetía et al. 2009). Kuzniak et al. (2010) indicated that when switching to CAM metabolism, *M. crystallinum* became less sensitive to the attacks by its fungal pathogen (*Botrytis cinerea*). We hypothesize that the emergence of CAM photosynthesis in *M. crystallinum* would depend on the competition intensity of *B. mollis*. In fact, biotic stress may induce CAM expression in *M. crystallinum* only in the presence of sufficient carbohydrates as substrates. In general, starch/sugars need to be degraded at night to generate the 3-carbon acceptor phosphoenolpyruvate (PEP) for nocturnal carboxylation (Antony and Borland 2008; Antony et al. 2008). CAM expression in starch-deficient mutants of *M. crystallinum* was suppressed in response to salts (Haider et al. 2012). Thus, a strong competition of *B. mollis* on *M. crystallinum* would lead to a substantial suppression of photosynthesis and consequently impede the accumulation of a sufficient amount of carbohydrates to induce CAM expression in *M. crystallinum*. In contrast, in the case of moderate competition, CAM expression is more likely to occur because the plant would be able to accumulate a sufficient amount of carbohydrates, while being exposed to biotic stress.

Another factor potentially affecting between CAM plants and grasses is disturbance. Studies in savanna systems have shown how grazing greatly reduces grass biomass and fire frequency and thus favors woody plant encroachment (Scholes and Archer 1997; Anderies et al. 2002). Likewise, grazing could reduce the competitive effects of grasses and thus favor CAM plants if CAM plants are less edible to grazers. In fact, *M. crystallinum* typically has Chenopod salt bladders to deter its insect herbivores (i.e., caterpillar) (LoPresti 2014). It is unclear, however, whether the reduced competitive effects of *B. mollis* could induce/increase CAM expression in *M. crystallinum*. Previous studies have shown that CAM expression in *M. crystallinum* increases reproduction rate and plant fitness (Winter and Ziegler 1992; Cushman et al. 2008). Can grazing favor CAM plants over grasses not only by reducing the competitive effects of grasses but also by inducing/increasing CAM expression in CAM plants?

We developed a set of greenhouse experiments and investigated the response of a facultative CAM species and a C₃ grass—alone as well as in association with one

another—to water and salt conditions. We hypothesized that: (1) *M. crystallinum* has a competitive advantage over *B. mollis* in low water and high salinity conditions, while *B. mollis* has a competitive advantage over *M. crystallinum* in high water conditions; (2) *M. crystallinum* may transition to CAM to compete with *B. mollis*, and this behavior depends on the intensity of the competition; and (3) simulated grazing could favor *M. crystallinum* by the direct effect of reduced competitive pressure with *B. mollis* and the indirect effect of inducing/increasing CAM expression in *M. crystallinum*. To test these hypotheses, seedlings of *M. crystallinum* and *B. mollis* were planted both in monocultures and in polycultures and subjected to two water (high and low) and salt (without and with) treatments. The effect of disturbance was evaluated by clipping half of the aboveground biomass of *B. mollis* grown in polyculture with *M. crystallinum*.

Materials and methods

Experimental design

On 6 September 2015, seeds of *M. crystallinum* were germinated in plastic trays, while seeds of *B. mollis* were germinated in plastic pots (14.5 cm in diameter and 10.5 cm in height with a capacity of 1.3 L) to avoid the subsequent transplantation. These seeds were covered with 1–2 mm of mineral soil during germination. On 21 September 2015, seedlings of *M. crystallinum* and *B. mollis* were planted both in monoculture (1 individual of *M. crystallinum* or 20 individuals of *B. mollis*) and in polycultures (1 individual of *M. crystallinum* and 20 individuals of *B. mollis* with *M. crystallinum* located in the middle of these 1.3 L pots). Here a relatively high number (20) of *B. mollis* individuals was used to investigate the case in which *B. mollis* could have a strong competitive effect on *M. crystallinum* in access to light and soil nutrients. Conversely, only one individual of *M. crystallinum* was planted both in monocultures and polycultures because the focus of this study is on the effects of interspecific rather than intraspecific competition on the emergence of crassulacean acid metabolism in *M. crystallinum*.

A randomized block experiment design was used in this study with seedlings of *M. crystallinum* and *B. mollis* in both monoculture and mixture subject to two water levels (high and low) and two salt levels (control without salt additions and with salt additions). In the salt addition treatment, 65 ml of 400 mM NaCl solution was added to each pot four times at an interval of two days beginning on 13 October 2015. With respect to the water treatment, plants in high water conditions were watered every two days with an intensity of 8 mm per event during the whole

experiment. Plants in low water conditions were watered every two days with an intensity of 8 mm per event until 19 October 2015 and then they were watered every 8 days with an intensity of 8 mm per event until 6 December 2015, when the water treatment returned to high water conditions until the end of experiment (5 January 2016); thus, the low water treatment was indeed in the form of “wet-drought-wet” in which “drought” was imposed to induce CAM expression in *M. crystallinum*, while the subsequent “wet” period was studied to verify whether *M. crystallinum* may switch back from CAM to C₃ photosynthesis (Vernon et al. 1988; Schmitt 1990; Winter and Holtum 2014). To investigate the effects of disturbance in inducing/increasing CAM expression in *M. crystallinum*, in high water conditions, half of the aboveground biomass of *B. mollis* grasses (i.e., 10 individuals both in monoculture and mixed with *M. crystallinum*) was removed on 6 December 2015, leaving the remaining 10 individuals untouched. Each pot was fertilized with 15 mg N every 8 days applied in the form of Peters Professional 20-20-20 fertilizer (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). Plants were kept in the greenhouse with well-controlled light (i.e., 12 h light with photosynthetically photon flux density (PPFD) of 700–800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from 0700 to 1900 h EDT) and temperature (i.e., 25 °C during the day and 20 °C during the night).

Gas exchange and concentration of titratable acidity

Gas exchange for *M. crystallinum* and *B. mollis* in both monoculture and mixture was measured using the regular leaf chamber (2 × 3 cm) in a Licor 6400 gas analyzer (LiCor Inc., Lincoln, NB). These measurements were usually taken two days before plant harvest at each phase of the experiment. During the day, gas exchange was measured once between 10:00 am and 12:00 pm with photosynthetic active radiation (PAR) set at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while during the night gas exchange was measured once every two hours with PAR = 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Leaf temperature was set to be 23 °C both during the day and at night (Li et al. 2016).

After the gas exchange measurements were performed, one leaf of *M. crystallinum* was sampled from each plant in each pot at 0700 and 1700 h, respectively. These samples were stored at –20 °C for measurements of concentration of titratable acidity using the acid–base titration method (von Caemmerer and Griffiths 2009). Leaf disks (4 cm²) were boiled in 1.5 mL H₂O for 5 min in a microfuge tube and freshly made 10 mM NaOH was added into the boiled solution with 20 μL of a 1/5 dilution of phenolphthalein as indicator; the amount of NaOH added was used to calculate concentration of titratable acidity.

Light availability, soil salinity, and soil moisture

To investigate the potential light limitation *B. mollis* exerts on *M. crystallinum* in mixture, light intensity was measured with HOBO Pendant® Temperature/Light sensor both above and below the canopies (approximately at ground level). Following Sun et al. (2016), relative light intensity (RLI, %) was calculated as the ratio of light intensity under and above the canopies. Salt additions increase soil electrical conductivity; therefore, soil electrical conductivity is used as an indicator of soil salinity. Soil electrical conductivity was measured using FieldScout Direct Soil EC Meter. Soil moisture content was determined gravimetrically by drying samples at 60 °C for 72 h after plants were harvested.

Biomass measurements

Plants were harvested during the first (30–31 October 2015), second (4–5 December 2015), and third (4–5 January 2016) phases of the experiment. The biomass of *M. crystallinum* and *B. mollis* harvested from mixed pots was separated. Likewise, the roots of these two species were separated and root fragments were classified using color, diameter, and shape to distinguish roots of *M. crystallinum* from those of *B. mollis*; these root fragments accounted for <5% in total root biomass. Roots were washed free of soil through 0.1-mm mesh sieves. Plants were dried at 60 °C for 72 h and weighed for the calculation of the total biomass and the belowground to aboveground biomass ratio.

Statistical analysis

The effects of salt, water, species, competition, and time as well as their interactions on diurnal photosynthetic assimilation (A_D), total biomass (TB), and belowground to aboveground biomass ratio (BA) were analyzed by means of a five-way ANOVA with block as a random factor. The effects of salt, water, species, and time as well as their interactions on soil moisture (S) and soil electrical conductivity (SC) were analyzed through a four-way ANOVA with block as a random factor. The effects of salt, water, and time as well as their interactions on relative light intensity (RLI) in the two-species mixture were analyzed by three-way ANOVA with block as a random factor. The effects of disturbance (grass cutting) on RLI, S, TB of *M. crystallinum* in mixture were analyzed through a one-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

Diurnal photosynthetic assimilation (A_D)

The effects of salt additions on diurnal photosynthetic assimilation (A_D) depended on competition (Table 1, $P = 0.003$ for salt \times competition). In the first phase of the experiment, salt additions significantly suppressed A_D in *M. crystallinum* (facultative CAM, FC) alone and *B. mollis* (grass, G) alone in high water conditions ($P = 0.0021$ for FC, $P < 0.0001$ for G, Fig. 1a), but A_D

Table 1 Results (P values) of five-way factorial ANOVA on total biomass (TB), biomass ratio of belowground to aboveground (BA), and photosynthetic assimilation during the day (A_D)

	df	TB	BA	A_D
Salt	1	<0.0001	<0.0001	<0.0001
Water	1	<0.0001	<0.0001	<0.0001
Species	1	<0.0001	<0.0001	<0.0001
Competition	1	<0.0001	0.0004	–
Time	2	<0.0001	<0.0001	0.001
Salt \times water	1	–	–	–
Salt \times species	1	<0.0001	<0.0001	0.0466
Salt \times competition	1	–	0.0225	0.0003
Salt \times time	2	<0.0001	<0.0001	0.0002
Water \times species	1	<0.0001	<0.0001	<0.0001
Water \times competition	1	<0.0001	–	<0.0001
Water \times time	2	<0.0001	<0.0001	<0.0001
Species \times competition	1	<0.0001	0.0024	–
Species \times time	2	0.0001	<0.0001	<0.0001
Competition \times time	2	<0.0001	<0.0001	<0.0001
Salt \times water \times species	1	0.0044	<0.0001	0.0181
Salt \times water \times competition	1	0.0272	–	0.0289
Salt \times water \times time	2	0.0444	<0.0001	0.0022
Salt \times species \times competition	1	–	–	0.0007
Salt \times species \times time	2	–	<0.0001	<0.0001
Salt \times competition \times time	2	0.0132	–	–
Water \times species \times competition	1	<0.0001	0.0012	<0.0001
Water \times species \times time	2	<0.0001	<0.0001	<0.0001
Water \times competition \times time	2	<0.0001	–	0.0136
Species \times competition \times time	2	<0.0001	0.0089	<0.0001
Salt \times water \times species \times competition	1	–	–	–
Salt \times water \times species \times time	2	0.0002	0.0006	–
Salt \times water \times competition \times time	2	–	–	–
Salt \times species \times competition \times time	2	<0.0001	–	–
Water \times species \times competition \times time	2	<0.0001	0	0.0065
Salt \times water \times species \times competition \times time	2	0.0215	–	–

“–” Means no significance ($P > 0.05$)

of *M. crystallinum* in mixture (FCM) with *B. mollis* after salt additions was significantly greater than in *M. crystallinum* alone (FC) in high water conditions ($P = 0.0271$, Fig. 1a). This result suggests that salt additions suppressed A_D in *B. mollis* with the indirect effect of reducing the competition of *B. mollis* with *M. crystallinum*, and this indirect effect outweighed the direct negative effects of salt additions on A_D in *M. crystallinum* in the first phase. Because of gradual flushing (leaching) of salts with time (Online Resource Fig. A1), the effects of salt additions on A_D of *B. mollis* [both alone (G) and in mixture with *M. crystallinum* (GM)] were not significant in the second and third phases (Fig. 1b, c). Reduction in

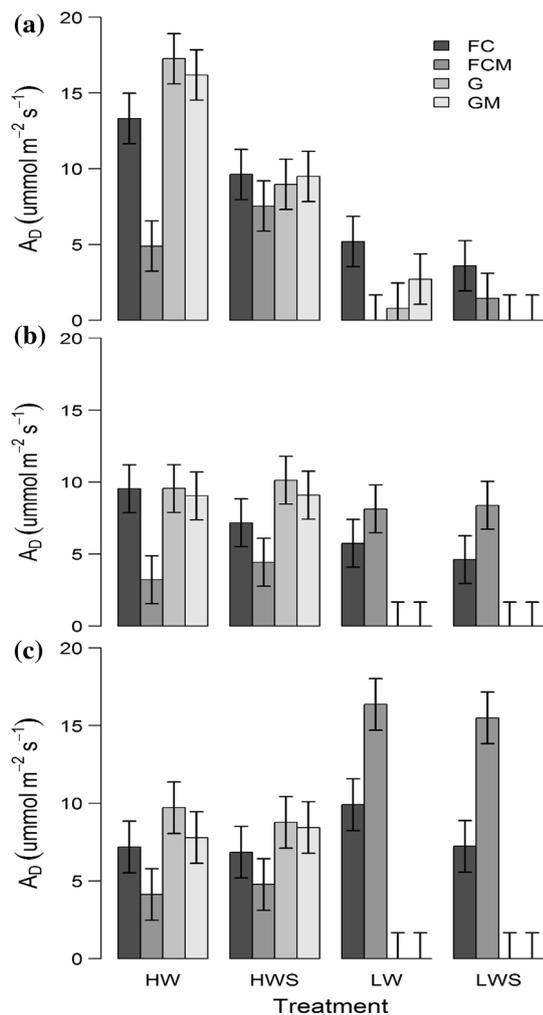


Fig. 1 Photosynthetic assimilation during the day (A_D) in *Mesembryanthemum crystallinum* alone (FC), *Mesembryanthemum crystallinum* in mixture (FCM), *Bromus mollis* alone (G), *Bromus mollis* in mixture (GM) as affected by salt and water conditions in the first (a), second (b), and third (c) phases. HW refers to high water conditions and without salt addition; HWS refers to high water conditions with salt addition; LW refers to low water conditions and without salt addition; LWS refers to low water conditions and with salt addition. Each bar represents the mean of six values while error bars indicate 95% confidence intervals

water applications significantly decreased A_D in all the vegetation types (FC, FCM, G, GM) in the first phase of the experiment (all $P < 0.0001$, Fig. 1a). In the second and third phases, however, A_D of *M. crystallinum* in mixture (FCM) was significantly higher in low water than in high water conditions, regardless of the salt treatment (all $P < 0.0001$, Fig. 1b, c); this result was due to the fact that *B. mollis* died (both alone and in mixture), thereby eliminating the competitive effects of GM on FCM. Conversely, in high water conditions, when grasses were alive, A_D of *M. crystallinum* was significantly lower in mixture (FCM) than in the case of *M. crystallinum* alone (all $P \leq 0.0104$, Fig. 1) because of the competitive effects of *B. mollis* on *M. crystallinum* in access to light (Online Resource Fig. A2) and soil moisture (Online Resource Fig. A3).

Total biomass

There was a significant effect of salt and species interaction on total biomass (TB) (Table 1, $P < 0.0001$ for salt \times species). Salt additions significantly decreased TB of *B. mollis* both alone and in mixture (G and MG) in all the phases of the experiment (all $P \leq 0.0012$, Fig. 2), but TB of *M. crystallinum* alone (FC) had a positive response to salt additions in the second phase and third phases when plants were well-watered (all $P \leq 0.0161$) (Fig. 2b, c) despite the negative response observed in the first phase (both $P \leq 0.0348$) (Fig. 2a). Salt additions significantly increased the total biomass (TB) of *M. crystallinum* in mixture (FCM) only in the third phase of the low water treatment ($P = 0.0125$, Fig. 2); in the other cases, there were no significant effects of salt additions on the total biomass of FCM (all $P \geq 0.6587$, Fig. 2) likely because of the strong competitive effect of *B. mollis* on *M. crystallinum*. Moreover, because of such a competitive effect, there was no significant increase in the total biomass of FCM with time in high water conditions regardless of the salt additions (all $P \geq 0.5186$, Online Resource Fig. A4). Likewise, there was no significant effect of water additions on the total biomass of FCM in the first and second phases, regardless of the salt additions (all $P \geq 0.9056$, Fig. 2a, b). In the third phase, the removal of water stress by returning to high water conditions favored FCM in low water conditions regardless of salt conditions (both $P \leq 0.0001$, Fig. 2c) because competition from *B. mollis* was lacking in low water treatments. The total biomass of FC, G, and GM had significantly positive responses to water additions in all the phases (all $P \leq 0.0301$, Fig. 2). The effects of four-way or five-way interactions on TB are generally significant because of strong effects of each factor and dependence among factors (Table 1).

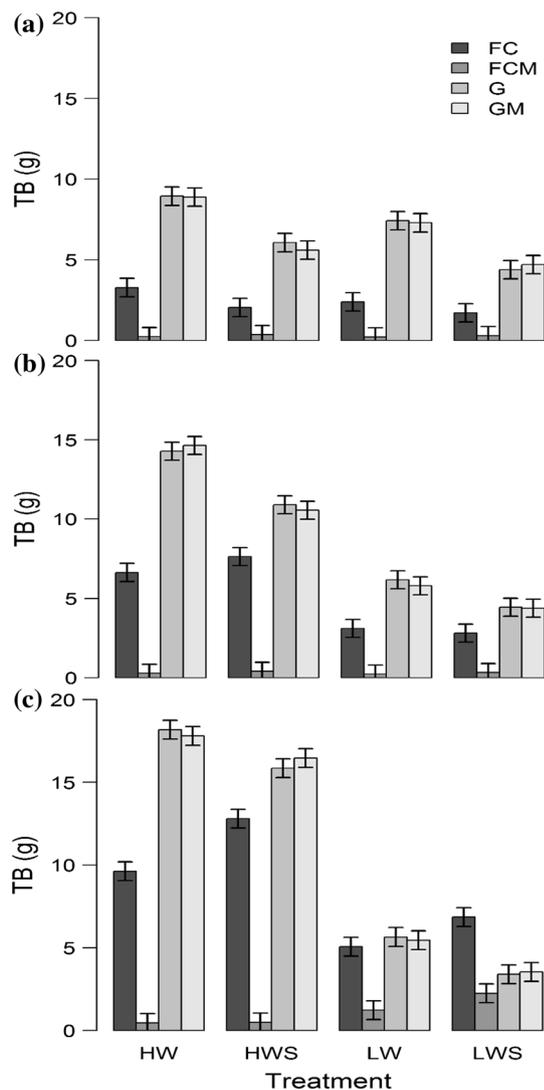


Fig. 2 Total biomass (TB) in *Mesembryanthemum crystallinum* alone (FC), *Mesembryanthemum crystallinum* in mixture (FCM), *Bromus mollis* alone (G), *Bromus mollis* in mixture (GM) as affected by salt and water conditions in the first (a), second (b), and third (c) phases. Symbols for each treatment are the same as Fig. 1. Each bar represents the mean of six values while error bars indicate 95% confidence intervals

Nocturnal photosynthetic assimilation (A_N) and titratable acidity (TA)

M. crystallinum performed CAM photosynthesis (nocturnal carboxylation) both alone (FC) and in mixture (FCM) in the second phase of the low water treatment experiment with no salt additions, as shown by the positive nocturnal photosynthetic assimilation ($A_N \approx 0.3\text{--}0.8 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 3a) and significant accumulation of titratable acidity (TA) overnight (both $P < 0.0001$, Fig. 3c). In contrast, in high water conditions with no salt additions, *M. crystallinum* did not perform CAM photosynthesis (Fig. 3b, d).

When salts were added, however, *M. crystallinum* alone had a significant nocturnal carboxylation ($A_N \approx 0.4\text{--}1.2 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 3b) and accumulation of triable acidity (TA) overnight in the second phase of the experiment even in high water conditions ($P < 0.0001$, Fig. 3d). Likewise, in low water conditions, salt additions significantly increased nocturnal carboxylation (A_N) (Fig. 3a) and accumulation of triable acidity (TA) at night in *M. crystallinum*, both alone and in mixture (SFC vs FC; SFCM vs FCM) in the second phase of the experiment (both $P \leq 0.0146$, Fig. 3c). Regardless of salt conditions, once induced, CAM photosynthesis was significantly lower in *M. crystallinum* in mixture (FCM and SFCM) than alone (FC and SFC), as shown by lower values of A_N and TA in the second phase (Fig. 3a, c). Because of the strong competitive effects of *B. mollis* on *M. crystallinum* in high water conditions, salt additions inducing CAM expression in *M. crystallinum* alone did not induce CAM expression by *M. crystallinum* in mixture (Fig. 3).

In the third phase (i.e., after reducing/removing water stress), *M. crystallinum* alone in high water conditions with salt additions (SFC) still performed CAM photosynthesis, as evidenced by the positive nocturnal A_N and accumulation of triable acidity (TA) (Fig. 4a, c). Conversely, in low water treatments, *M. crystallinum* alone and in mixture did not perform CAM photosynthesis during the third phase of the experiment regardless of salt additions (FC, SFC, FCM, and SFCM, see legend in Fig. 4), which indicated that CAM expression was reversed to C_3 photosynthesis after removing water stress. In high water conditions, *M. crystallinum* in mixture (FCM and SFCM) did not perform CAM photosynthesis, but grazing (grass cutting) significantly led to CAM expression in FCM and SFCM (Fig. 4a–d).

Synthesis of CAM expression in *M. crystallinum* as affected by environmental stress and competition

The results of salt and water manipulations are synthesized in Fig. 5: once induced, the expression of CAM behavior in *M. crystallinum* both alone and in mixture (FC and FCM) increased with environmental stress (i.e., water stress). Salts acted as “physiological drought,” thereby promoting CAM expression in *M. crystallinum* both alone and in mixture. Interestingly, the competitive effect of *B. mollis* on *M. crystallinum* in access to light (Online Resource Fig. A2) and soil moisture (Online Resource Fig. A3) increased the environmental stress experienced by *M. crystallinum* in mixture, but it significantly suppressed CAM expression (Fig. 5). This effect of competition even outweighed the direct effects of salt additions, i.e., the expression of CAM behavior observed in *M. crystallinum* alone (SFC); in fact, no CAM expression was detected in *M. crystallinum* in mixture in high water conditions after salt additions

Fig. 3 Nocturnal change of photosynthetic assimilation (A_N) of *Mesembryanthemum crystallinum* alone (FC) and in mixture (FCM) as affected by salt additions (S) in low water (a) and high water conditions (b) in the second phase. Values are mean \pm 1 SD ($n = 6$). Titratable acidity (TA) of FC and FCM as affected by salt additions (S) in low water (c) and high water conditions (d) in the second phase. Each bar represents the mean of six values while error bars indicate 95% confidence intervals. FC and FCM refer to FC and FCM without salt additions, while SFC and SFCM refer to FC and FCM with salt additions

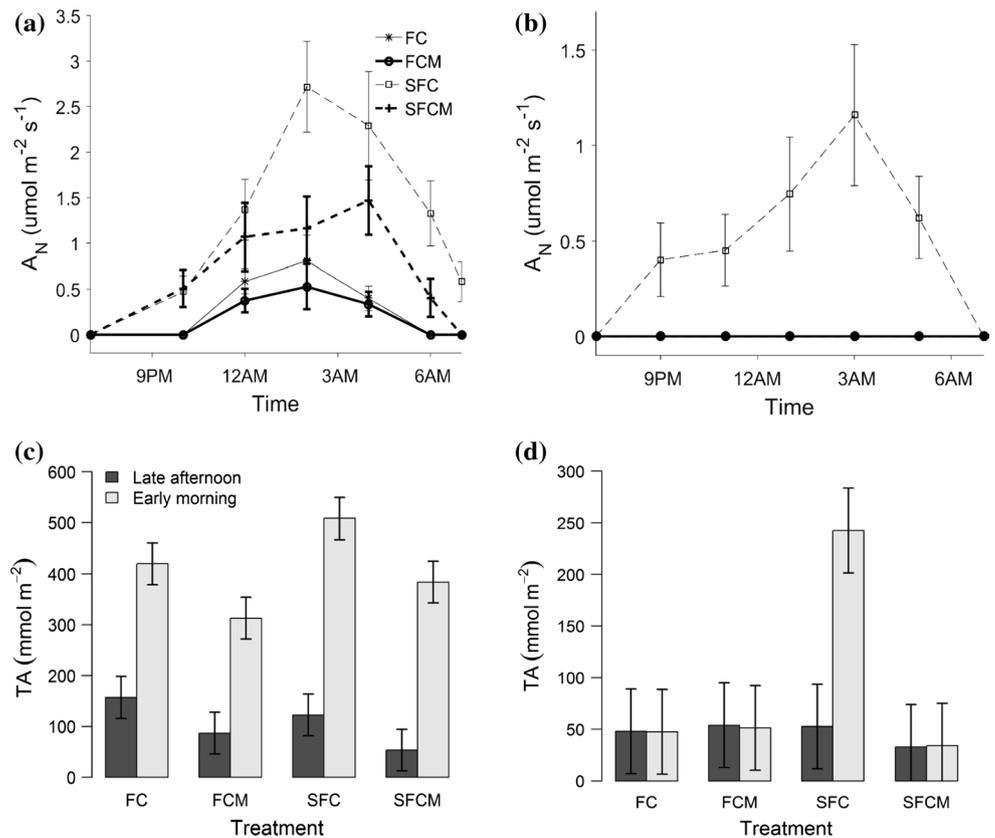
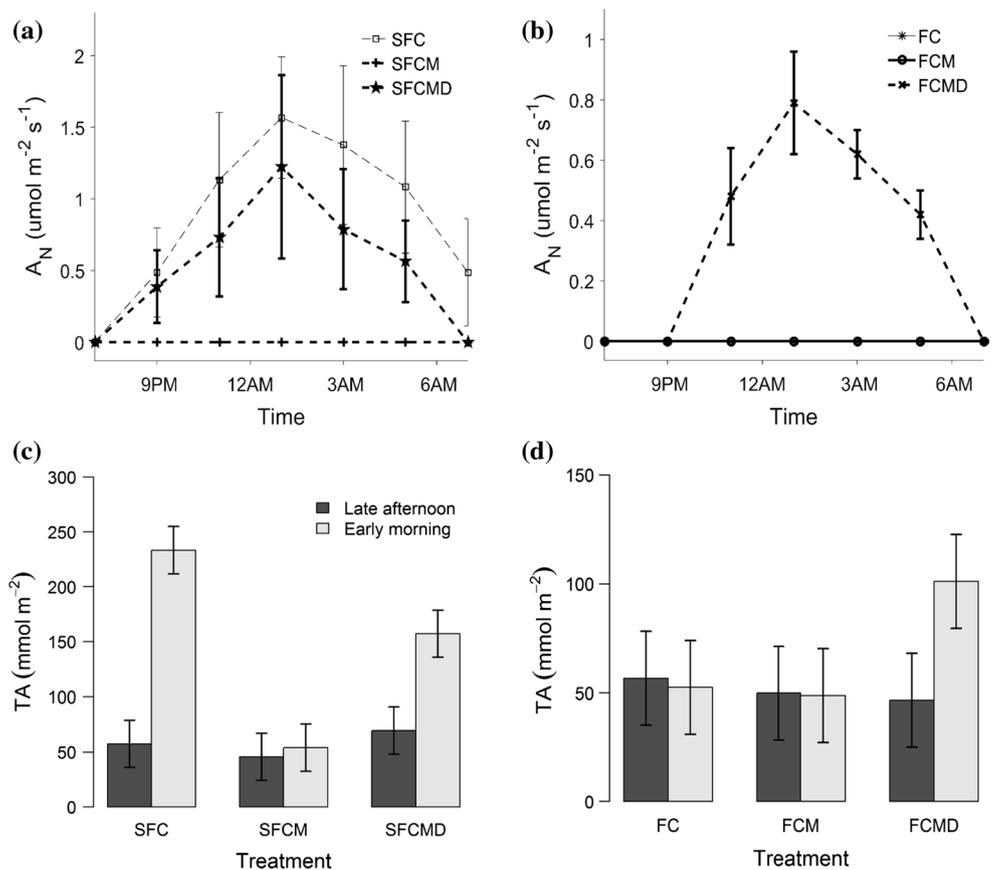


Fig. 4 a, b Nocturnal photosynthetic assimilation (A_N) of *Mesembryanthemum crystallinum* alone (FC) and in mixture (FCM) as affected by salt additions and grazing (grass cutting) in high water conditions in the third phase. Values are mean \pm 1 SD ($n = 6$). c, d Titratable acidity (TA) of FC and FCM as affected by salt additions and grazing (grass cutting) in high water conditions in the third phase. Each bar represents the mean of six values, while error bars indicate 95% confidence intervals. Symbols of FC, FCM, SFC, and SFCM are the same as in Fig. 3, while FCMD refers to FCM with grazing (grass cutting) and SFCMD refers to SFCM with grazing (grass cutting). In low water conditions (indeed well-watered in the third phase), FC and FCM had a negative A_N ($A_N \approx -1-2 \mu\text{mol m}^{-2} \text{s}^{-1}$) and did not have a significant accumulation/increase of TA; thus, they did not perform CAM expression



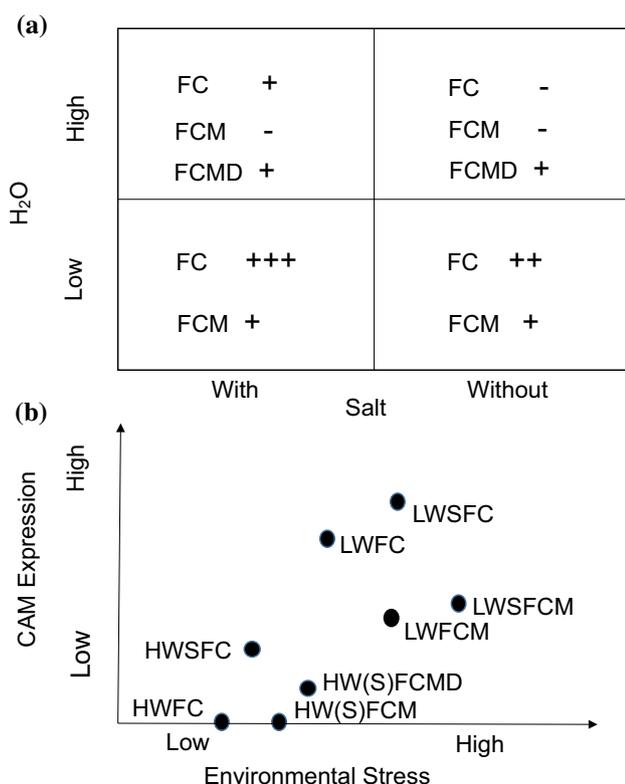


Fig. 5 **a** A synthesis of CAM expression by *Mesembryanthemum crystallinum* alone (FC) and in mixture (FCM) as affected by salt, grazing (grass cutting), and water conditions. “+” means CAM expression with the number of “+” indicating magnitude of CAM expression (as seen from the values of nocturnal photosynthetic assimilation and accumulation of triable acidity overnight), while “-” means a lack of CAM expression. **b** CAM expression by *Mesembryanthemum crystallinum* alone (FC) and in mixture (FCM) as affected by environmental stress, grazing (grass cutting), and competition. Symbols for each treatment are the same as Fig. 4; HW refers to high water and LW refers to low water; S refers to salt additions; (S) refers to the cases regardless of salt additions; D refers to grazing (grass cutting)

(SFCM) (Fig. 5). Disturbance (i.e., grass cutting) reduced the intensity of the competition of *B. mollis* on *M. crystallinum* in access to light (Online Resource Fig. A5a) and soil moisture (Online Resource Fig. A5b) and increased the biomass of FCM (Online Resource Fig. A5c), thus inducing CAM expression by *M. crystallinum* in mixture (FCM vs FCMD; SFCM vs SFCMD) (Fig. 5).

Discussion

Research in ecology in the past decade has investigated the response of C_3 and C_4 plants to global environment change (e.g., Huxman and Monson 2003; D’Odorico et al. 2012; Fay et al. 2012; Kulmatiski and Beard 2013). An empirical evaluation of competitive relationships between CAM

plants and other functional groups (i.e., C_3 or C_4 plants) under global environmental change, however, is still missing. This study investigates the effects of salt and water conditions on competition between a facultative CAM species, *M. crystallinum*, and its interacting C_3 species, *B. mollis*, found in California’s coastal grasslands. We found that *M. crystallinum* was in competitive advantage in low water and salinity conditions, while in high water conditions *B. mollis* exerted a strong competitive effect on *M. crystallinum* in access to light and soil nutrients. While *M. crystallinum* switched to CAM photosynthesis in response to moderate intensity of competition with *B. mollis*, stronger competition with *B. mollis* prevented CAM expression in *M. crystallinum*.

This study found evidence of a competitive advantage of the facultative CAM species *M. crystallinum* over the C_3 grass *B. mollis* under low water conditions. The high number of individuals (20) of *B. mollis*—which led to high biomass accumulated during prior well-watered conditions (Fig. 1a)—and high evapotranspiration led to high water stress and *B. mollis* mortality after drought was applied. Compared to *B. mollis*, *M. crystallinum* is more tolerant to droughts, likely because of its ability to adapt to water stress by relying on water storage (Lüttge 2004; Borland et al. 2011). Our findings are in agreement with other studies which show that CAM plants featuring water storage and a high water usage efficiency are increasing their abundance in many drylands worldwide (Borland et al. 2009; Reyes-García and Andrade 2009), which suggests that some marginal lands could be used for the cultivation of CAM plants (i.e., *M. crystallinum*) as food or bioenergy crops (Herppich et al. 2008; Borland et al. 2009; Owen et al. 2016).

Consistent with other studies (Vivrette and Muller 1977; Agarie et al. 2007), *M. crystallinum* exhibited a higher salt tolerance than *B. mollis*. The adaptation of *M. crystallinum* to salinity mainly involves development of epidermal bladder cells, accumulation of Na^+ , and compatible solutes (predominantly ononitol and pinitol) for osmotic adjustment (Adams et al. 1998; Oh et al. 2015) as well as the ability to switch to CAM photosynthesis in response to environmental stress such as salinity (Fig. 3) (Osmond 1978; Cushman and Borland 2002; Winter and Holtum 2007, 2014). In fact, in the case of *M. crystallinum*, a facultative CAM plant, the optimal growth conditions require moderate salinity for maintenance of turgor (Winter and Lüttge 1976; Winter and Holtum 2007). In this study, we found an increase of biomass and productivity of *M. crystallinum* with salt addition conditions in the second and third phases of the experiment (Fig. 2b, c), when the salt added in the first phase was diluted by subsequent water application (Fig. 2a; Online Resource Fig. A1). Increased risk of droughts and increased evaporation under a warming

climate are expected to lead to an increase in soil salinity (Lynn and Simpson 1987; Herppich et al. 2008; Charles and Dukes 2009). Overall, this study suggests that the joint effects of drought and salinity likely contribute to further invasion of *M. crystallinum* into California's grasslands under global climate change.

In high water conditions, grasses (*B. mollis*) have strong competitive effects on *M. crystallinum* in access to light and soil nutrients (Online Resource Fig. A2 and A3) and thus greatly suppress the photosynthesis and productivity/biomass of *M. crystallinum* when these two species are grown together (Figs. 1, 2). As expected, we did not find that *M. crystallinum* switched to CAM photosynthesis in response to water stress resulting from the strong competition with *B. mollis* (Figs. 3, 5). We interpret that the strong competition of *B. mollis* on *M. crystallinum* in high water conditions delayed plant maturity and prevented the accumulation of the carbohydrates required for CAM expression; these effects outweighed those associated with increased environmental stress, thereby suppressing CAM expression in *M. crystallinum* grown in mixture with *B. mollis*. In fact, generation of the 3-carbon acceptor phosphoenolpyruvate (PEP) by degradation of non-structural carbohydrates (i.e., starch/sugars) is needed for nocturnal carboxylation (Antony and Borland 2008; Antony et al. 2008); these non-structural carbohydrates are also required for other metabolic functions maintaining the growth of sink tissues (Borland and Dodd 2002). Haider et al. (2012) indicated that for starch-deficient mutants of *M. crystallinum*, the CAM expression was suppressed in response to salt additions because of the primary partitioning of carbohydrates into cyclitols and respiration. In the natural environments, *M. crystallinum* typically germinates, grows, and accumulates carbohydrates during the rainy season and then switches to CAM photosynthesis as an adaptive strategy to cope with water deficit during the (summer) dry season (i.e., Adams et al. 1998; Cushman and Borland 2002).

Our conclusion is also supported by the fact that while salt additions have been used to induce CAM expression in *M. crystallinum* alone (Osmond 1978; Cushman and Borland 2002; Winter and Holtum 2007, 2014), salt additions did not induce CAM expression in *M. crystallinum* in mixture in high water conditions when competition from *B. mollis* was strong (Figs. 3, 4). In fact, the strong competitive effects even outweighed the direct favorable effects of salt additions as well as the indirect effects associated with the salt intolerance in *B. mollis* (Vivrette and Muller 1977; Agarie et al. 2007) (Fig. 1), as evidenced by an insignificant increase of biomass/productivity by *M. crystallinum* in a mixture after salt additions (Fig. 2; Online Resource Fig A4). Moreover, we found that CAM expression in *M. crystallinum* alone induced by salt additions still occurs in high water conditions in the third phase (Fig. 4), which seems

to indicate that CAM expression was not reversed when environmental stress (salinity) was removed through gradual flushing (leaching) of salts with time (Online Resource Fig. A1). This is in contrast to other studies which proved reversibility of CAM expression by *M. crystallinum* after salts were instantly flushed (Vernon et al. 1988; Schmitt 1990). We interpret that salt additions promoted plant maturity and reduced the leaf span for opportunities of reversibility in high water conditions (Winter and Holtum 2007, 2014), in contrast to low water conditions (Fig. 4) in which leaves appeared to be young after returning to high water conditions.

While *M. crystallinum* in mixture did not switch to CAM expression in response to strong competition from *B. mollis*, disturbance (i.e., grass cutting) reduced the competitive effects of *B. mollis* on *M. crystallinum* in access to light and soil nutrients (Online Resource Fig. A5a and A5b) and increased biomass/productivity of *M. crystallinum* in a mixture (Online Resource Fig. A5c), thereby allowing for the occurrence of CAM expression in *M. crystallinum* in a mixture (Fig. 4). This further supports our conclusion about the importance of plant maturity and sufficient carbohydrates as determinants of CAM expression in *M. crystallinum*, regardless of the plant age. Previous studies indicated that grazing (grass cutting) reduces the competitive effects of grasses and fire frequency and thus promotes woody plant encroachment (Scholes and Archer 1997; Anderies et al. 2002). This study suggests that grazing (grass cutting) reduces the competitive effects of grasses and more importantly might increase reproduction rate and plant fitness of *M. crystallinum* by inducing the expression of CAM photosynthesis (Winter and Ziegler 1992; Cushman et al. 2008; Herrera 2009), thus favoring *M. crystallinum* invasion.

Crassulacean acid metabolism (CAM) featuring photosynthetic plasticity, water storage, and a high water usage efficiency was an evolutionary adaptation from C₃ photosynthesis to water-limited environments (Ranson and Thomas 1960; Lüttge 2004; Borland et al. 2009; Silvera et al. 2010). In this study, a set of experiments under salt, water, and disturbance conditions highlights the existence of a possible important link between C₃ photosynthesis used to accumulate carbohydrates in the earlier wet seasons and CAM expression in response to water limitation (i.e., dry seasons) in facultative CAM plants. This study also found that disturbance (i.e., grazing) as well as aridity and salinity, which are expected to increase in arid and coastal landscapes under future climate conditions, acts as an important driver for expansion of CAM plants, a phenomenon which has been largely ignored. The finding of a dependence of CAM expression in facultative CAM plants on competition intensity suggests that abiotic factors (i.e., aridity and/or salinity) thought to directly induce CAM expression may also indirectly affect CAM

expression in facultative CAM plants through their potential impacts on biotic stress. Understanding the eco-physiological strategies facultative CAM plants develop to adapt to both abiotic and biotic stress could be critical to explain the potential advantage of CAM plants relative to non-CAM species under global environmental change.

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References

- Adams P, Nelson DE, Yamada S, Chmara W, Jensen RG, Bohnert HJ, Griffiths H (1998) Growth and development of *Mesembryanthemum crystallinum* (Aizoaceae). *New Phytol* 138:171–190
- Agarie S, Shimoda T, Shimizu Y, Baumann K, Sunagawa H, Kondo A, Ueno O, Nakahara T, Nose A, Cushman JC (2007) Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladder-cell-less mutant of the common ice plant *Mesembryanthemum crystallinum*. *J Exp Bot* 58:1957–1967
- Anderies JM, Janssen MA, Walker BH (2002) Grazing, management, resilience and the dynamics of fire-driven rangeland system. *Ecosystems* 5:23–44
- Antony E, Borland AM (2008) The role and regulation of sugar transporters in plants with crassulacean acid metabolism. *Prog Bot* 70:127–143
- Antony E, Taybi T, Courbot M, Mugford S, Smith JAC, Borland AM (2008) Cloning, localization and expression analysis of vacuolar sugar transporters in the CAM plant *Ananas comosus* (pineapple). *J Exp Bot* 59:1895–1908
- Borland AM, Dodd AN (2002) Carbohydrate partitioning in crassulacean acid metabolism plants: reconciling potential conflicts of interest. *Funct Plant Biol* 29:707–716
- Borland AM, Griffiths H, Hartwell J, Smith JAC (2009) Exploiting the potential of plants with crassulacean acid metabolism for bioenergy production on marginal lands. *J Exp Bot* 60:2879–2896
- Borland AM, Barrera Zambrano VA, Ceusters J, Shorrocks K (2011) The photosynthetic plasticity of crassulacean acid metabolism: an evolutionary innovation for sustainable productivity in a changing world. *N Phytol* 191:619–633
- Charles H, Dukes JS (2009) Effects of warming and altered precipitation on plant and nutrient dynamics of a New England salt marsh. *Ecol Appl* 19:1758–1773
- Collins SL, Xia Y (2015) Long-term dynamics and hotspots of change in a desert grassland plant community. *Am Nat* 185:E30–E43
- Connell JH (1983) On the prevalence and relative importance of interspecific competition: evidence from field experiments. *Am Nat* 122:661–697
- Cushman JC, Borland AM (2002) Induction of crassulacean acid metabolism by water limitation. *Plant Cell Environ* 25:295–310
- Cushman JC, Agarie S, Albion RL, Elliot SM, Taybi T, Borland AM (2008) Isolation and characterization of mutants of common ice plant deficient in crassulacean acid metabolism. *Plant Physiol* 147:228–238
- D’Antonio CM, Vitousek PM (1992) Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annu Rev Ecol Evol Syst* 23:63–87
- D’Odorico P, Okin GS, Bestelmeyer BT (2012) A synthetic review of feedbacks and drivers of shrub encroachment in arid grasslands. *Ecohydrology* 5:520–530
- Drennan PM, Nobel PS (2000) Responses of CAM species to increasing atmospheric CO₂ concentrations. *Plant Cell Environ* 23:767–781
- Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate extremes: observations, modeling and impacts. *Science* 289:2068–2074
- Fay PA, Jin VL, Way DA, Potter KN, Gill RA, Jackson RB, Polley HW (2012) Soil-mediated effects of subambient to increased carbon dioxide on grassland productivity. *Nat Clim Change* 2:742–746
- Haider MS, Barnes JD, Cushman JC, Borland AM (2012) A CAM- and starch-deficient mutant of the facultative CAM species *Mesembryanthemum crystallinum* reconciles sink demands by repartitioning carbon during acclimation to salinity. *J Exp Bot* 63:1985–1996
- Held IM, Soden RJ (2006) Robust responses of the hydrological cycle to global warming. *J Clim* 19:5686–5699
- Herppich WB, Huyskens-Keil S, Schreiner M (2008) Effects of saline irrigation on growth, physiology and quality of *Mesembryanthemum crystallinum* L., a rare vegetable crop. *J Appl Bot Food Qual* 82:47–54
- Herrera A (2009) Crassulacean acid metabolism and fitness under water deficit stress: if not for carbon gain, what is facultative CAM good for? *Ann Bot* 103:645–653
- Huxman TE, Monson RK (2003) Stomatal responses of C₃, C₃-C₄, and C₄ Flaveria species to light and intercellular CO₂ concentration: implications for the evolution of stomatal behavior. *Plant Cell Environ* 26:313–322
- Kulmatiski A, Beard KH (2013) Woody plant encroachment facilitated by increased precipitation intensity. *Nat Clim Change* 3:833–837
- Kuzniak E, Kornas A, Gabara B, Ullrich C, Skłodowska M, Miszalski Z (2010) Interaction of *Botrytis cinerea* with the intermediate C₃-CAM plant *Mesembryanthemum crystallinum*. *Environ Exper Bot* 69:137–147
- Li HL, Yu KL, Ratajczak Z, Nippert J, Tondrob D, Xu DH, Li W, Du GZ (2016) When variability outperforms the mean: trait plasticity predicts plant performance in an alpine wetland. *Plant Soil* 407:401–415
- LoPresti EF (2014) Chenopod salt bladders deter insect herbivores. *Oecologia* 174:921–930
- Lüttge U (2004) Ecophysiology of crassulacean acid metabolism (CAM). *Ann Bot* 93:629–652
- Lynn RJ, Simpson JJ (1987) The California current system: the seasonal variability of its physical characteristics. *J Geophys Res* 92:12947–12966
- Maiquetía M, Caceres A, Herrera A (2009) Mycorrhization and phosphorus nutrition affect water relations and CAM induction by drought in seedlings of *Clusia minor*. *Ann Bot* 103:525–532
- McCown RL, Williams WA (1968) Competition for nutrients and light between the annual grassland species *Bromus Mollis* and *Erodium Botrys*. *Ecology* 49:981–990

- Morris JT, Sundareshwar PV, Nietch CT, Kjerfve B, Cahoon DR (2002) Responses of coastal wetlands to rising sea level. *Ecology* 83:2869–2877
- Niu SL, Liu WX, Wan SQ (2008) Different growth response of C₃ and C₄ grasses to seasonal water and nitrogen regimes and competition in a pot experiment. *J Exp Bot* 59:1431–1439
- Ogburn RM, Edwards EJ (2010) The ecological water-use strategies of succulent plants. *Adv Bot Res* 55:179–225
- Oh D, Barkla B, Vera-Estrella R, Pantoja O, Lee S, Bohnert H et al (2015) Cell type-specific responses to salinity—the epidermal bladder cell transcriptome of *Mesembryanthemum crystallinum*. *N Phytol* 207:627–644
- Osmond CB (1978) Crassulacean acid metabolism: a curiosity in context. *Annu Rev Plant Physiol* 29:379–414
- Owen NA, Fahy KF, Griffiths H (2016) Crassulacean acid metabolism (CAM) offers sustainable bioenergy production and resilience to climate change. *GCB Bioenergy* 8:737–749
- Ranson SL, Thomas M (1960) Crassulacean acid metabolism. *Annu Rev Plant Physiol* 11:81–110
- Reyes-García C, Andrade JL (2009) Crassulacean acid metabolism under global climate change. *N Phytol* 181:754–757
- Schmitt JM (1990) Rapid concentration changes of phosphoenolpyruvate carboxylase mRNA in detached leaves of *Mesembryanthemum crystallinum* L. in response to wilting and rehydration. *Plant Cell Environ* 13:845–850
- Scholes RJ, Archer SR (1997) Tree-grass interactions in savannas. *Annu Rev Ecol Evol Syst* 28:517–544
- Silvera K, Neubig KM, Whitten WM, Williams NH, Winter K, Cushman JC (2010) Evolution along the crassulacean acid metabolism continuum. *Funct Plant Biol* 37:995–1010
- Sun XM, Yu KL, Shugart H, Wang G (2016) Species richness loss after nutrient additions as affected by N: C ratio and plant endogenous hormones in an alpine meadow. *J Plant Ecol* 9:201–211
- Suttle KB, Thomsen MA, Power ME (2007) Species interactions reverse grassland responses to changing climate. *Science* 315:640–642
- Teuling AJ, Senevirante SI, Stöckli R, Reichstein M, Moors E et al (2010) Contrasting response of European forest and grassland energy exchange to heatwaves. *Nat Geosci* 3:722–727
- Tilman D (1982) *Resource Competition and Community Structure*. Princeton University Press, Princeton, pp 1–296
- van der Waal C, de Kroon H, de Boer WF, Heitkönig IMA, Skidmore AK, de Knecht HJ, van Langevelde F, van Wieren SE, Grant CC, Page BR, Slotow R, Kohi EM, Mwakiwa E, Prins HHT (2009) Water and nutrients alter herbaceous competitive effects on tree seedlings in a semiarid savanna. *J Ecol* 97:430–439
- Vernon DM, Ostrem JA, Schmitt JM, Bohnert HJ (1988) PEPCase transcript levels in *Mesembryanthemum crystallinum* decline rapidly upon relief from salt stress. *Plant Physiol* 86:1002–1004
- Vivrette NJ, Muller CH (1977) Mechanism of invasion and dominance of coastal grassland by *Mesembryanthemum crystallinum*. *Ecol Monogr* 47:301–318
- Von Caemmerer S, Griffiths H (2009) Stomatal responses to CO₂ during a diel crassulacean acid metabolism cycle in *Kalanchoe daigremontiana* and *Kalanchoe pinnata*. *Plant Cell Environ* 32:567–576
- Winter K, Holtum JAM (2007) Environment or development? Lifetime net CO₂ exchange and control of the expression of crassulacean acid metabolism in *Mesembryanthemum crystallinum*. *Plant Physiol* 143:98–107
- Winter K, Holtum JAM (2014) Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. *J Exp Bot* 65:1–17
- Winter K, Lüttge U (1976) Balance between C₃ and CAM pathway of photosynthesis. In: Lange OL, Kappen L, Schulze ED (eds) *Water and plant life*. Springer, Berlin, pp 323–334
- Winter K, Ziegler H (1992) Induction of crassulacean acid metabolism in *Mesembryanthemum crystallinum* increases reproductive success under conditions of drought and salinity stress. *Oecologia* 92:475–479
- Yu KL, D’Odorico P (2015) Direct and indirect facilitation of plants with crassulacean acid metabolism (CAM). *Ecosystems* 18:985–999
- Yu KL, Foster A (2016) Modeled hydraulic redistribution in tree-grass, CAM grass, and tree-CAM associations: the implications of crassulacean acid metabolism (CAM). *Oecologia* 180:1113–1125