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## Nutrient and water addition effects on day- and night-time conductance and transpiration in a C<sub>3</sub> desert annual

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**Abstract** Recent research has shown that many C<sub>3</sub> plant species have significant stomatal opening and transpire water at night even in desert habitats. Day-time stomatal regulation is expected to maximize carbon gain and prevent runaway cavitation, but little is known about the effect of soil resource availability on night-time stomatal conductance ( $g$ ) and transpiration ( $E$ ). Water (low and high) and nutrients (low and high) were applied factorially during the growing season to naturally occurring seedlings of the annual *Helianthus anomalous*. Plant height and biomass were greatest in the treatment where both water and nutrients were added, confirming resource limitations in this habitat. Plants from all treatments showed significant night-time  $g$  ( $\sim 0.07 \text{ mol m}^{-2} \text{ s}^{-1}$ ) and  $E$  ( $\sim 1.5 \text{ mol m}^{-2} \text{ s}^{-1}$ ). In July, water and nutrient additions had few effects on day- or night-time gas exchange. In August, however, plants in the nutrient addition treatments had lower day-time photosynthesis,  $g$  and  $E$ , paralleled by lower night-time  $g$  and  $E$ . Lower predawn water potentials and higher integrated photosynthetic water-use efficiency suggests that the nutrient addition indirectly induced a mild water stress. Thus, soil resources can affect night-time  $g$  and  $E$  in a manner parallel to day-time, although additional factors may also be involved.

**Keywords** Desert dunes · Great Basin Desert · *Helianthus* · Plant water potential · Soil water

### Introduction

During daylight hours, C<sub>3</sub> and C<sub>4</sub> plants open their stomata to take up CO<sub>2</sub> for carbon fixation through photosynthesis. As an unavoidable cost, plants lose water at the same time. In general, stomatal regulation is expected to maximize CO<sub>2</sub> uptake and minimize water loss as well as prevent cavitation (Cowan 1977; Sperry 2000). Thus, stomatal conductance ( $g$ ) and transpiration ( $E$ ) are expected to be minimal at night. However, significant night-time  $g$  and  $E$  have been observed in at least 40 C<sub>3</sub> and C<sub>4</sub> species, with both instantaneous gas exchange and integrated sap flux methods, suggesting that this may be a widespread phenomenon (Green et al. 1989; Iritz and Lindroth 1994; Hogg and Hurdle 1997; Benyon 1999; Donovan et al. 1999, 2003; Musselman and Minnick 2000; Oren et al. 2001; Tanner and Beevers 2001; Snyder et al. 2003; Bucci et al. 2004). A survey of instantaneous rates for 17 species from western US habitats showed that night-time  $E$  was high for 11 desert and riparian species, and that night- and day-time  $E$  were correlated suggesting that they may be similarly regulated (Snyder et al. 2003). However, only a few studies have experimentally investigated the effect of soil resource availability on night-time  $g$  and  $E$  (Rawson and Clark 1988; Donovan et al. 1999, 2003).

Soil water limitations and water potential declines often decrease day-time  $g$  and  $E$ , and might be expected to decrease night-time  $g$  and  $E$  as well. This expectation held true for droughted wheat plants, where night-time  $g$  decreased as compared to well-watered controls (Rawson and Clark 1988). However, results have been contradictory for two cold desert shrub species. In greenhouse studies where soil water potential was lowered by increasing soil salinity, night-time  $g$  decreased significantly for *Chrysothamnus nauseosus* but not for *Sarcobatus vermiculatus* (Donovan et al. 1999). In

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natural population studies where irrigation increased water availability, night-time  $g$  increased for *C. nauseosus* only early in the growing season, and for *S. vermiculatus* only later in the season (Donovan et al. 2003). Thus, an understanding of night-time  $g$  and  $E$  responses to manipulations in water availability requires further investigation.

The expectation for nutrient effects on day- and night-time  $g$  and  $E$  are less clear. For example, Toft et al. (1989) showed that nutrient addition can cause a small decrease in day-time  $g$  for cold desert shrubs. However, irrigation of the desert shrub *Larrea tridentata* increased day-time  $g$ , but adding extra N to irrigated shrubs had no additive effects on day-time  $g$  (Meinzer et al. 1988). To our knowledge, the effects of nutrient addition on night-time  $g$  have not been previously studied.

We examined plant night-time gas exchange responses to water and nutrient additions in a desert dune habitat. Desert sand dunes are very low in nutrients and shallow soil layers dry out quickly during summer droughts, although soil water often remains available in deeper soil layers (Noy-Meir 1973; Rosenthal et al. 2005). Both soil water and nutrient availability can limit plant growth in this desert dune habitat, similar to many other arid habitats (Noy-Meir 1973; Danin 1996; Snyder et al. 2004; James et al. 2005). The study species, *Helianthus anomalous*, is an annual endemic to actively moving desert sand dunes in the southwestern US, germinates April–May, and grows throughout long dry summers (Schwarzbach et al. 2001; Ludwig et al. 2004). In the greenhouse, *H. anomalous* can have a high night-time  $g$  ( $0.22 \text{ mol m}^{-2} \text{ s}^{-1}$ ) that is much higher than cuticular conductance ( $0.02 \text{ mol m}^{-2} \text{ s}^{-1}$ ; A. Howard, personal communication). Additionally, a closely related species, *H. annuus*, has high night-time  $g$  and  $E$  rates in natural populations ( $0.10 \text{ mol m}^{-2} \text{ s}^{-1}$  and  $1.8 \text{ mmol m}^{-2} \text{ s}^{-1}$ , respectively; Snyder et al. 2003). Thus, *H. anomalous* is an ideal species to investigate nutrient and water availability effects on night- and day-time  $g$  and  $E$ . In the natural desert dune habitat, we tested whether water and nutrient additions affected plant growth and day- and night-time  $g$  and  $E$ , and if so then whether day- and night-time  $g$  and  $E$  respond in a similar manner.

## Materials and methods

### Study site and species

The research was conducted on actively moving sand dunes in Little Sahara Recreation Area, UT, US (lat.  $39^{\circ}44'N$ , long.  $112^{\circ}18'W$ , elevation 1646 m asl). The climate is typical of the Great Basin Desert with the majority of precipitation occurring as snow or rain during the cold winter and cool spring months (Smith et al. 1997). The mean annual precipitation is 298 mm. Precipitation was below average for the nine months prior to the study (178 mm, September 2002–May 2003,

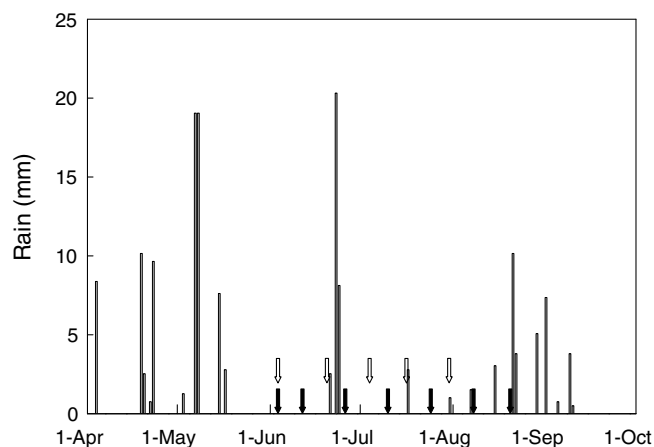
73% of average). There was 67 mm of rain during the experiment, which is similar to the long-term average (57 mm) for June–August (Fig. 1). At the study site, vegetation cover was very low, only 12%. Other common species in addition to *H. anomalous* are *Psoraleidium lanceolatum* (perennial shrub), *Salsola iberica* (annual) and *Stipa hymenoides* (perennial bunchgrass) (Rosenthal et al. 2005). The soils at the sites were slightly alkaline (pH=8.5), with little organic matter (0.70%), and few nutrients (total N=0.09%; total P=0.03%) (Rosenthal et al. 2005; B. Brouillette, personal communication).

### *H. anomalous* rooting depth

To get an indication of root distribution of *H. anomalous*, five isolated individuals were excavated on 8 September. The average rooting depth was 76 cm, and the maximum was 130 cm. Plants had on average more than 88% of their root biomass in the top 25 cm. There was a good correlation between aboveground and belowground biomass ( $r=0.98$ ,  $P<0.01$ ), but no correlation between root or shoot biomass and rooting depth ( $P>0.05$ ).

### Experimental design

On 3 June 2003, within a 500x500 m area, we marked 20 2x2 m plots that each had at least ten naturally occurring *H. anomalous* seedlings. In each plot, ten randomly selected seedlings were individually marked. Other vegetation and extra *H. anomalous* individuals were removed from the plots. Given the low vegetation cover, only a few other plants grew adjacent to the experimental plots. Four plots formed a block for a total of five blocks, and within a block the plots were randomly assigned one of the four treatments: (1) control, (2) water, (3) nutrients, and (4) water + nutrients. Each watered plot received 100 l per plot on seven dates (3 and 10 June and then every 2 weeks until 18 August) for an added precipita-



**Fig. 1** Daily rainfall during the experiment at Little Sahara Sand Dunes. Dates of nutrient treatment are indicated by light arrows and water treatment by dark arrows

tion equivalent of 175 mm over the summer (59% of annual precipitation). Nutrient plots received a total of 200 kg/ha N (as  $\text{NO}_3\text{NH}_4$ ) and 87 kg/ha P, applied in five equal doses starting 3 June and then every 2 weeks until 31 July, in alternate weeks from water additions. At each application, nutrients for one plot were dissolved in 8 l of water, for a total of 40 l of water over the summer or 10 mm precipitation equivalent (3% of annual precipitation).

## Measurements

Plants were checked weekly for mortality and date of first flower. Stem diameter 2 cm above the soil surface and plant height (from ground to top of stem or base of bud) were measured on 3 June, every 2 weeks until 29 July, and finally on 21 August. On 8 September, the number of buds and flowers were counted, aboveground biomass was harvested, and one soil core was taken from each plot to determine soil water content in 25 cm layers to 100 cm depth. Soil samples were collected only at the end of the experiment because of the large disturbance caused by coring in the unstable sandy soils.

On 7 July, one leaf was sampled from each live plant with more than four leaves and at least one fully expanded mature leaf. The youngest mature leaf was collected from each plant and measured for leaf area (CID, Pullman, Washington, US). Thereafter, leaves were dried at 70°C for at least 48 h and dry biomass was determined. Specific leaf area (SLA) was calculated by dividing leaf area by leaf dry weight.

Two plants per plot were randomly selected for day and night-time gas exchange measurements in July, done over one day (9 July) and two nights (9–10 July, one plant per plot each night), 1–2 days after the most recent irrigation. Measurements were repeated on 6–7 August, 2–3 days after the most recent irrigation. Gas exchange rates were measured on recently matured fully expanded leaves with a LI-6400 (Li-Cor, Lincoln, NE, USA) between 0200 and 0400 hours (night) and between 1000 and 1400 hours (day). PPFD was set at 0 at night and at 2,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during the day. Cuvette temperature, humidity and  $\text{CO}_2$  concentrations tracked ambient. Leaf area was measured with a CID portable meter. At night, frequent empty closed chamber measurements served as a control for equipment performance (see Snyder et al. 2003). Plants' night-time  $g$  and  $E$  rates were significantly higher than empty closed chamber measurements in July and August ( $P < 0.001$ , all comparisons).

Leaves collected after August gas exchange measurements were dried, individually ground, and analyzed for N content (Carbo Erba NA 1500 CN analyzer, Milan, Italy). Leaf carbon isotopic composition ( $\delta^{13}\text{C}$ ) was determined using a continuous flow mass spectrometer (Finnegan MAT, Bremen, Germany). Leaf  $\delta^{13}\text{C}$  provides an integrated measure of leaf intercellular  $\text{CO}_2$  concentration ( $c_i$ ) over the lifetime of the leaf. Integrated  $c_i$  is, in turn, a relative measure of seasonally integrated

photosynthetic water use efficiency (WUE), provided leaf temperatures and photosynthetic characteristics are similar (Farquhar et al. 1989; Ehleringer et al. 1992). Greater (less negative) leaf  $\delta^{13}\text{C}$  reflects greater WUE.

Plant predawn water potentials ( $\psi_{\text{pd}}$ ) were measured with a pressure chamber (PMS Instruments, Corvallis, Oregon, USA) on 10 August, between 0230 and 0430 hours, on the same plants used for gas exchange measurements. Although night-time  $E$  can affect plant  $\psi_{\text{pd}}$  (Donovan et al. 2001, 2003), preliminary comparisons of bagged and unbagged *H. anomalous* seedlings indicated that predawn disequilibrium due to night-time  $E$  was only 0.06 MPa. Thus,  $\psi_{\text{pd}}$  of unbagged plants likely provides a good estimate of soil water potential accessed by the roots for this species.

## Data analyses

The effects of water and nutrient additions on plant survival, growth, and leaf traits were analyzed as mixed model analysis of variance (Proc Mixed; SAS 2001). Water and nutrient additions were fixed factors and block was a random factor. Survival, calculated for each plot as the percent of the ten original seedlings alive at final harvest, was arcsine transformed prior to analysis. For the measurements on individual plants (biomass, morphology, leaf traits), traits were averaged per plot to avoid pseudoreplication ( $n = 5$  replicates per treatment). Aboveground vegetative biomass, reproductive biomass, flower number, and date of first flower were log transformed as necessary to meet ANOVA assumptions, and their analysis included initial plant height (3 June) as a covariate. Differences between treatment lsmeans were determined with Tukey's test (SAS 2001). Stem height and diameter, measured on six consecutive dates, were analyzed as a repeated measures. Soil core water content was also analyzed as a mixed model, with soil depth as a covariate.

Combining data for all plots, Pearson correlation coefficients were calculated for day- and night-time rates of  $g$ , and day- and night-time rates of  $E$ , for both July and August. For each of the August gas exchange traits (photosynthesis, and day- and night-time  $g$  and  $E$ ), a multiple regression was used to relate the response of the gas exchange traits to leaf traits related to resource status ( $\psi_{\text{pd}}$ , N, and  $\delta^{13}\text{C}$ ).

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## Results

Plants in water + nutrient treatment had the greatest vegetative and reproductive biomass, and tended to have the most flowers and buds (Table 1). Better plant performance in this treatment was also supported by repeated measures analysis of plant height (Fig. 2,  $W \times N \times \text{time}$  effect,  $F_{1,80} = 3.17$ ,  $P < 0.05$ ). Stem diameters showed the same trend as height, but the resource addition effects were not significant. For the water,

**Table 1** Water and nutrient addition effects on mortality, aboveground vegetative biomass, reproductive biomass, numbers of buds and flowers, and date of first flower in *H. anomalus* plants in a natural population

	Water + nutrients	Nutrients	Water	Control	df	Initial height covariate	Water effect (W)	Nutrient effect (N)	W × N effect
Survival (%)	68 <sup>a</sup>	70 <sup>a</sup>	58 <sup>a</sup>	56 <sup>a</sup>	1,12	–	2.12	0.11	0.00
Vegetative biomass (g)	14.04 <sup>a</sup> (23.6–8.4)	2.98 <sup>b</sup> (5.0–1.8)	3.18 <sup>b</sup> (5.3–1.9)	4.41 <sup>b</sup> (7.4–2.6)	1,11	<b>7.06*</b>	2.19	<b>4.83*</b>	<b>4.83*</b>
Reproductive biomass (g)	2.28 <sup>a</sup> (2.6–2.0)	1.46 <sup>b</sup> (1.7–1.3)	1.58 <sup>b</sup> (1.8–1.4)	1.78 <sup>b</sup> (2.1–1.5)	1,11	<b>3.71*</b>	1.81	1.91	<b>5.34*</b>
Number of buds and flowers	4.47 <sup>a</sup> (5.4–3.7)	2.55 <sup>a</sup> (3.1–2.1)	2.90 <sup>a</sup> (4.0–2.4)	3.37 <sup>a</sup> (4.05–2.8)	1,11	4.15	1.47	0.14	4.21 <sup>(<i>P</i>=0.06)</sup>
Date of first flower	25 Aug <sup>a</sup>	1 Sep <sup>b</sup>	1 Sep <sup>b</sup>	1 Sep <sup>b</sup>	1,11	<b>6.03*</b>	<b>4.76*</b>	<b>7.70*</b>	<b>4.88*</b>

Biomass components and numbers of buds and flowers required log transformation to meet ANOVA assumptions, and means and SE are back-transformed to original units (results in asymmetric upper and lower bounds for SE presented). Additionally presented are degrees of freedom (*df*), and *F* values for covariate (initial height), main effects (water, nutrients), and the interaction (water × nutrients), from a mixed model analysis, with block as random. *F*-values in **bold** indicate statistical significance (\* *P* < 0.05). For each trait, significantly different means are denoted by different *lower case letters*

nutrient, and control treatments, plant growth and biomass were very similar.

Soil water content measured at the end of the experiment was affected by water addition ( $F_{1,88} = 29.0$ ,  $P < 0.001$ ), nutrient addition ( $F_{1,88} = 25.6$ ,  $P < 0.001$ ), and a water by nutrient interaction ( $F_{1,88} = 5.5$ ,  $P < 0.05$ ). The water and water + nutrient treatments increased soil

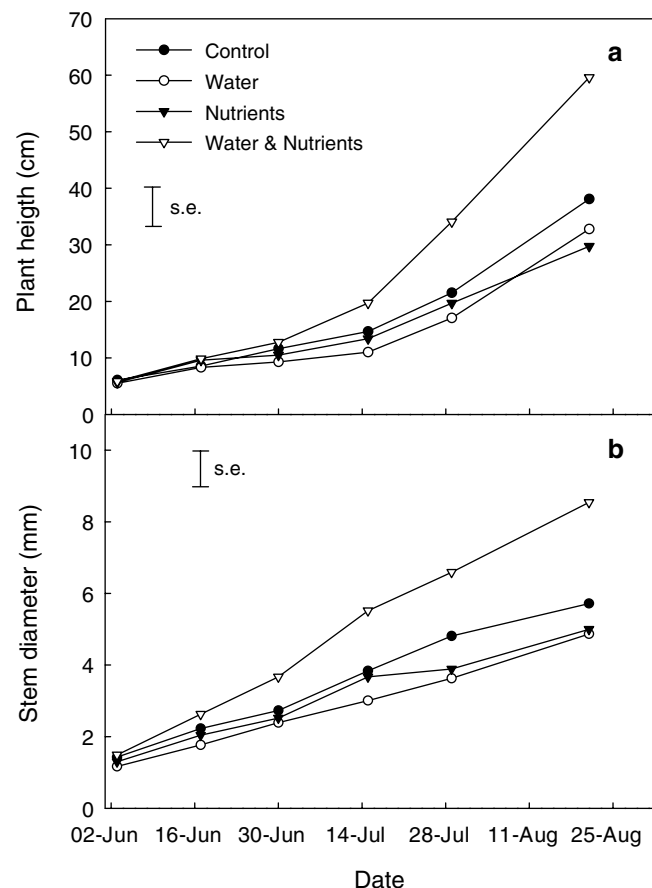
water content, especially at a 25 cm depth (Fig. 3). At deeper soil depths, water content was higher only in the water treatment. The remaining treatments (control, nutrient, water + nutrient) showed similar water contents at 50 and 100 cm depths.

For recently matured leaves sampled on 7 July, leaf area, biomass or SLA did not differ with treatment (Table 2). Additionally, SLA was not correlated with leaf area ( $r = 0.27$ ,  $P = 0.25$ ).

In July, the resource addition affected only day-time *E*, which was higher in the water addition treatments ( $28.6 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) than in the nutrients and control treatments ( $24.0 \text{ mmol m}^{-2} \text{ s}^{-1}$ , water  $F_{1,12} = 4.77$ ,  $P < 0.01$ ). Resource additions had no effect ( $P > 0.05$  for all water, nutrient, and water × nutrient effects) on photosynthesis ( $42.3 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ), day- and night-time *g* ( $0.78$  and  $0.08 \text{ mol m}^{-2} \text{ s}^{-1}$ , respectively), and night-time *E* ( $1.26 \text{ mmol m}^{-2} \text{ s}^{-1}$ ). There was no correlation between day- and night-time *g* ( $r = 0.31$ ,  $P = 0.18$ ) or day- and night-time *E* ( $r = 0.42$ ,  $P = 0.07$ ) in July.

In August, nutrient additions (nutrient and water + nutrient treatments) reduced photosynthesis, day- and night-time *g*, and night-time *E* (Table 3). There was also an effect of water addition, but only for day-time *E*. There was no correlation between day- and night-time *g* ( $r = 0.16$ ,  $P = 0.51$ ) or day- and night-time *E* ( $r = 0.23$ ,  $P = 0.32$ ) in August.

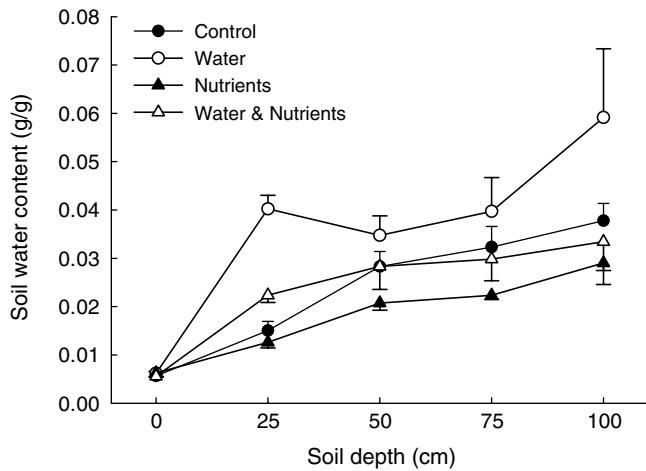
There was an interaction of water and nutrient addition on leaf N, which was highest in the water + nutrient treatment (Table 3). Leaf  $\psi_{pd}$  was more negative and leaf  $\delta^{13}\text{C}$  was less negative in the nutrient and nutrient + water treatments. Leaf  $\delta^{13}\text{C}$  explained more of the variation in day-time *g* and *E* than leaf  $\psi_{pd}$  or leaf N, but did not explain any of the variation in night-time *g* and *E* (Table 4).



**Fig. 2** Water and nutrient addition effects on plant height (a) and stem diameter (b) of *H. anomalus* plants growing on desert dunes. Data are lsmeans from repeated measures analysis

## Discussion

*H. anomalus* grew faster and produced more biomass only when substantial amounts of both water and



**Fig. 3** Soil water content at the end of the experiment on 8 September in plots with nutrients and water added to soil. Data are means  $\pm$  SE ( $n=5$  replicate plots per treatment)

nutrients were added to the soil. This suggests that water and nutrients co-limit *H. anomalous* growth in this desert dune habitat. Adding only water did increase soil water content, and thus water availability, but this did not translate into consistently higher plant  $\psi_{pd}$ , gas exchange rates, or growth. The lack of growth response to water additions was likely due to the relatively deep rooting of *H. anomalous*. In the dune habitat, water remains available in deeper soil layers due to an inverse texture effect and lower plant cover (Noy-Meir 1973; Rosenthal et al. 2005). A similar lack of growth response to summer

irrigation has also been found for desert shrubs (Hodgkinson et al. 1978; Gebauer et al. 2002; Snyder et al. 2004). Adding nutrients in minimal additional water (nutrient treatment) increased nutrient content in at least part of the soil, but did not increase leaf N or growth, suggesting that the added nutrients were not taken up by the plant. However, the response of August leaf traits to the nutrient treatment (lower gas exchange rates, lower  $\psi_{pd}$ , and higher WUE) indicates that the nutrients were impacting the plants roots in a manner consistent with an indirect water stress effect. It is hard to imagine how roots could detect the nutrient concentration as a water stress, but then not be able to take up those nutrients, unless some of the nutrient concentrations were approaching toxicity, which is possible for high ammonia concentrations. Plant growth could also have been constrained by conflicting environmental signals perceived by different portions of the root system. So, while it is clear that water and nutrients co-limited growth for the plants in this experiment, it is less clear whether increased nutrient availability alone would increase growth if it occurred throughout the deeper and moister soil layers.

The nutrient addition treatments affected all gas exchange parameters in August. Photosynthesis, day-time  $g$  and  $E$ , and night-time  $g$  and  $E$ , were lower in plants that received additional nutrients, with or without water. This is surprising since we expected nutrient additions to increase photosynthesis in this low nutrient sand dune habitat. However, the nutrient additions (nutrient and water + nutrient treatments) consistently

**Table 2** Water and nutrient addition effects on leaf biomass, area and the SLA of leaves sampled on 7 July

	Water + nutrients	Nutrients	Water	Control	SE for lsmeans	df	Water effect (W)	Nutrients effect (N)	W $\times$ N effect
Leaf area (cm <sup>2</sup> )	6.70	4.84	3.34	4.02	1.36	1,12	0.21	2.64	0.98
Leaf biomass (g)	0.068	0.053	0.033	0.044	0.013	1,12	0.03	3.27	1.10
SLA (g/cm <sup>2</sup> )	94.3	89.0	98.6	99.1	4.42	1,12	0.41	3.59	0.60

Presented are lsmeans and SE for each of the four treatments, degrees of freedom ( $df$ ), and  $F$  values for main effects (water, nutrients) and the interaction (water  $\times$  nutrients), from a mixed model analysis. None of the  $F$ -values indicated are of statistical significance ( $P > 0.05$ )

**Table 3** Water and nutrient addition effects on August leaf traits: photosynthesis, stomatal conductance ( $g$ ) and transpiration ( $E$ ), leaf predawn water potential ( $\psi_{pd}$ ), N concentration, and integrated photosynthetic water use efficiency ( $\delta^{13}C$ ) in *H. anomalous* plants in a natural population

	Water + nutrients	Nutrients	Water	Control	SE for lsmeans	df	Water effect (W)	Nutrients effect (N)	W $\times$ N effect
Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	37.03 <sup>B</sup>	34.59 <sup>B</sup>	40.92 <sup>A</sup>	38.06 <sup>A</sup>	$\pm 1.91$	1,12	2.53	<b>4.87*</b>	0.02
Day-time $g$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )	0.754 <sup>B</sup>	0.645 <sup>B</sup>	1.084 <sup>A</sup>	0.963 <sup>A</sup>	$\pm 0.151$	1,12	0.70	<b>5.51*</b>	0.00
Day-time $E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	21.13 <sup>b</sup>	19.21 <sup>b</sup>	27.16 <sup>a</sup>	22.93 <sup>b</sup>	$\pm 2.27$	1,12	<b>4.92*</b>	<b>11.76**</b>	0.86
Night-time $g$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )	0.057 <sup>B</sup>	0.044 <sup>B</sup>	0.072 <sup>A</sup>	0.083 <sup>A</sup>	$\pm 0.011$	1,12	0.01	<b>6.37*</b>	1.17
Night-time $E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	1.37 <sup>B</sup>	1.06 <sup>B</sup>	1.77 <sup>A</sup>	1.85 <sup>A</sup>	$\pm 0.18$	1,12	0.40	<b>10.34**</b>	1.07
Leaf $\psi_{pd}$ (MPa)	-0.48 <sup>B</sup>	-0.49 <sup>B</sup>	-0.37 <sup>A</sup>	-0.42 <sup>A</sup>	$\pm 0.36$	1,12	2.13	<b>15.21**</b>	0.77
Leaf N (mg/g)	31.2 <sup>a</sup>	23.6 <sup>b</sup>	25.8 <sup>a,b</sup>	28.9 <sup>a,b</sup>	$\pm 0.27$	1,12	0.83	0.00	<b>4.60*</b>
Leaf $\delta^{13}C$ (‰)	-27.89 <sup>B</sup>	-27.71 <sup>B</sup>	-29.01 <sup>A</sup>	-28.62 <sup>A</sup>	$\pm 0.38$	1,12	0.56	<b>8.15*</b>	0.22

Presented are lsmeans and SE for each of the four treatments, degrees of freedom ( $df$ ), and  $F$  values for main effects (water, nutrients) and the interaction (water  $\times$  nutrients), from a mixed model analysis.  $F$ -values in **bold** indicate statistically significant ( $*P < 0.05$ ,  $**P < 0.01$ ). For each trait, means with different letters are significantly different. *Capital letters* are used to denote contrasts of means when only one main effect is significant and *lower case letters* denote contrasts of all means when both main effects or the interaction is significant

**Table 4** Estimated partial regression slopes for the response of August gas exchange traits to leaf traits related to resource availability

	Leaf $\psi_{pd}$	Leaf N	Leaf $\delta^{13}C$
Day A	-1.06	3.24 ( $P=0.06$ )	-2.68*
Day $g$	-0.00	<b>0.33*</b>	-0.22*
Day $E$	-1.25	-1.46	-3.48*
Night $g$	-0.01	0.01	-0.01
Night $E$	-0.07	0.22	-0.24

Values in *bold* indicate statistically significant ( $*P < 0.05$ )

lowered soil water content and plant  $\psi_{pd}$ , and increased integrated WUE ( $\delta^{13}C$ ) suggesting that nutrient addition treatments indirectly induced mild plant water stress. In the water + nutrient treatment, the large plants likely extracted more water from the soil, increasing plant water stress. A previous study, in 2002, where we measured leaf and total aboveground biomass of more than 200 plants showed a very good correlation ( $r > 0.98$ ,  $P < 0.001$ ) between total aboveground biomass and leaf biomass (Ludwig and Donovan, unpublished). This higher leaf biomass likely increased whole plant transpiration and plant water uptake, which could have subsequently lead to decreased soil water content and increased plant water stress. Reduced gas exchange in the larger plants of the water + nutrient treatment could also have been the result of a greater path length for water flow. In the nutrient treatment, where plants were not larger than in the control treatment, increased soil solute potential around at least part of the roots could have increased plant water stress.

For *H. anomalous*, August day- and night-time  $g$  and  $E$  declined in response to nutrient additions, lending support to the hypothesis that stomata respond similarly to habitat resource availability during day and night. Given the similar ranking of day- and night-time treatment responses, we asked whether there was a cross-treatment positive correlation between August day- and night-time  $g$ , or day- and night-time  $E$ , as was found by Snyder et al. (2003) in a cross-species analysis. However, in this study we found no correlation between day- and night-time  $g$ , or day- and night-time  $E$ . Additionally, seasonally integrated WUE ( $\delta^{13}C$ ), which is affected by both plant water and nitrogen status (Field and Mooney 1986; Wright et al. 2003), explained more of the variation in day-time gas exchange rates than for night-time rates. We offer several possible explanations. First, it may be that a narrow range of gas exchange rates, particularly at night, limits the statistical power for detecting such relationships. Second, the atmospheric VPD and the energy balance will be different for leaves during the day as compared to night. If the leaves are not aerodynamically well coupled with the atmosphere then this could contribute to the lack of correlation. However, the leaves are likely well coupled with the atmosphere, and thus close to air temperature, because they are relatively small (average  $\sim 4 \text{ cm}^2$ ), lanceolate in shape, and presented in a very open canopy. Third, the

sensitivity of the  $g$  to leaf-to-air VPD can differ for day and night-time, based on observational whole canopy data from Oren et al. (2001; data and references therein for three woody species). If this holds for *H. anomalous* as well, then the leaf-to-air VPD variation within the day and night measurement intervals could result in additional variation in  $g$ , possibly obscuring any soil-resource driven cross-treatment correlation for night and day  $g$  and for night and day  $E$ . Controlled-environment experiments will be needed to separate the roles of soil resource availability, plant resource status, and leaf-to-air VPD in regulating night-time  $g$  and  $E$ .

In addition to limited knowledge on what regulates night-time  $g$  and  $E$ , it is unknown if and how plants benefit from night-time  $E$ . In an analogous process of plant water loss at night, hydraulic redistribution (water leakage from roots to drier soil layers) provides a benefit of temporary water storage for greater transpiration the next day, and potentially provides a nutrient benefit by hydrating the rhizosphere in more nutrient rich soil layers (Caldwell et al. 1998; Querejeta et al. 2003). Similarly, one potential benefit of night-time  $E$  is increased nutrient uptake (Snyder et al. 2003). Higher daily plant water uptake increases mass flow of water and nutrients to roots, particularly mobile molecules such as  $\text{NO}_3^-$  (Barber 1995; Smith 1991; Nye and Tinker 2000). Plants limited by nutrients might open up their stomata to increase transpiration and mass flow of water and nutrient to their roots. For example, McDonald et al. (2002) found that plants took up more nitrogen if they transpired 24 h each day compared to only during day-time, and several additional studies suggest that transpiration is important for nutrient uptake (Masle et al. 1992; Pons and Bergkotte 1996; Polley et al. 1999). It remains to be tested how important rates of night-time  $E$  reported in this and other studies (Donovan et al. 1999, 2003; Musselman and Minnick 2000; Snyder et al. 2003; Bucci et al. 2004) are in terms of plant water, nutrient, and carbon relations.

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