

include species' traits, water and nutrient availability. *Triodia* has not been studied in this respect, and it is unknown how Australia's arid zone vegetation will respond to changing environmental conditions.

This project aimed to advance understanding of *Triodia* responses to increase the predictive capacity of how these grasslands will fare in the future. We chose controlled-environment and field-based experimentation to study the responses of *Triodia* to water, nutrients and CO₂. While controlled-environment cabinets or glasshouses allow controlling water, nutrients and CO₂, they do not fully replicate natural conditions due to difference in light intensity, biotic interactions, soil depth, etc. To provide context for controlled-environment experiments, *Triodia* species were also studied in the field along a rainfall gradient from in north-west Queensland in dry and wet seasons.

We present results of three representative species, epistomatous ('hard') *T. longiceps* and *T. basedowii* (both are widespread) and amphistomatous ('soft') *T. pungens* (widespread resinous species that does not have abaxial stomata). We document functional responses along a rainfall gradient in the field including photosynthesis rates throughout the day (gas exchange), plant water relations, carbon and nutrient relations, spectral reflectance (comparison of 'green' biomass and derived water index) and temperature. In controlled environment chambers, multifactorial experiments exposed seedlings to ambient (380 ppm) or future elevated (600 ppm) CO₂ concentrations, a range of water and nutrient availabilities. Plants were grown for five months with similar measurements conducted as in field-based research.

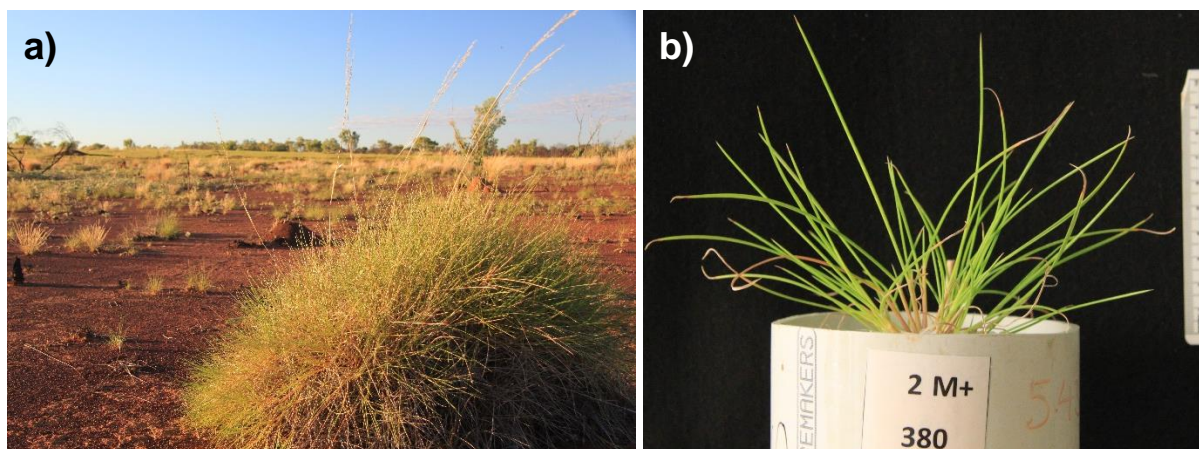


Figure 1. Mature (>5years) *T. pungens* at Camooweal in north-western Queensland (a) and seedling at 5 months grown in pot in controlled growth conditions (b).

Results

1. Gas exchange of 'hard' and 'soft' Triodia species in the natural environment

An 'intermediate' rainfall site at Dajarra (mean annual rainfall 350 mm) allowed comparing the two *Triodia* types under the same environmental conditions. The main finding was that both *Triodia* species were quasi dormant during the dry season displaying negative or very low net CO₂ fixation rates (Figure 2a). Water availability in the wet season elicited a strong response with considerable increases in net photosynthesis and transpiration rates (Figure 2a, b). In the wet season, most gas exchange occurred in the morning, followed by declining rates throughout the day and concomitant decrease in stomatal conductance and transpiration in the soft species (Figure 2). Transpiration of hard species show little decrease from its highest rate at ~9 am until the evening but stomatal conductance followed decreasing pattern of photosynthesis from approximately 9 am to 3 pm (Figure 2b, c). We estimated daily photosynthesis using a linear model based on the five daily photosynthesis measurements, and total hours of photosynthesis. Over the course of a day, hard species assimilated a total of 0.502 mols CO₂ m⁻² compared to soft species total of 0.332 mols CO₂ m⁻². The hard species transpired an estimated 216 mols H₂O m⁻², 2-fold more than the soft species with a daily total of 108 mols H₂O m⁻². There was no net carbon gain in the dry season for either species (-0.037 mols CO₂ m⁻² for hard and -0.003 mols CO₂ m⁻² per day for soft species) and continued water loss (110 mols H₂O m⁻² and 38 mols H₂O m⁻² per day for hard and soft species, respectively) at approximately half the rate measured in the wet season. Transpiration positively correlated with photosynthesis in the wet season, with hard species generally showing higher rates of transpiration per unit photosynthesis (Figure 3a), however there was no correlation between transpiration and photosynthesis in the dry season for either species (Figure 3b).

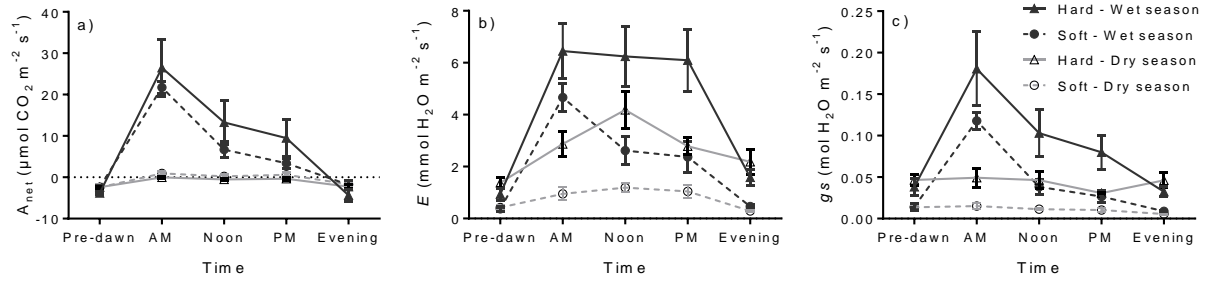


Figure 2. a) Net photosynthesis (net CO_2 fixation, A_{net}), b) transpiration (E) and c) stomatal conductance (g_s) of soft *T. pungens* and hard *T. longiceps* species in dry and wet season at Dajarra (NW Qld) from pre-dawn to evening (pre-dawn = complete darkness, AM= \sim 9am, PM = \sim 3pm and evening = before dark). Error bars are standard errors of 5 replicate (same leaves of each plant measured at each sampling time). For statistical analysis please see Table 1.

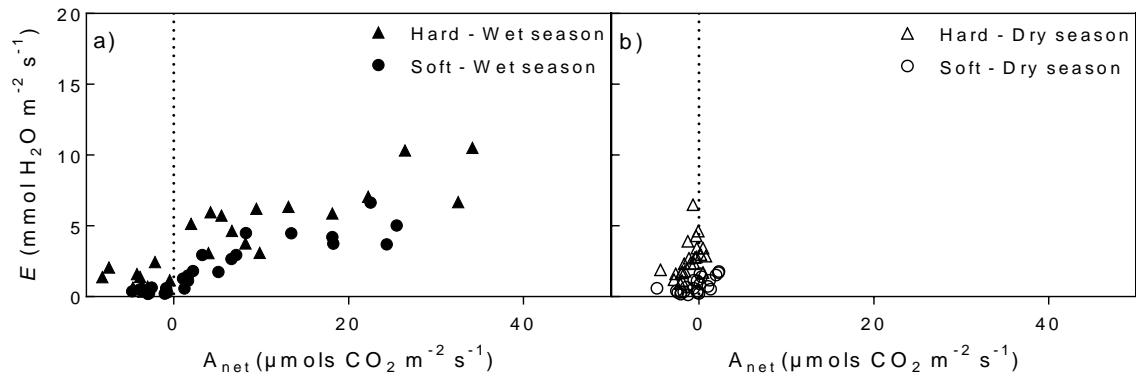


Figure 3. Response of net photosynthesis (A_{net}) compared to transpiration (E) for hard *T. longiceps* and soft *T. pungens* during the a) wet and b) dry seasons at intermediate rainfall site Dajarra (NW Qld).

While both *Triodia* species shared similar overall net photosynthesis rates in both seasons, the hard species consistently had higher transpiration rates and stomatal conductances than the soft species (Table 1). Gas exchange over the course of the day was more strongly correlated to atmospheric condition of vapour pressure deficit (Vpd) than relative humidity or temperature during the wet season only; there was no significant correlation with Vpd, relative humidity or temperature was found in the dry season (Table 2).

Table 1. Mean daily values (average of AM, noon and PM values) \pm standard error (SE) for gas exchange variables, including net photosynthesis (A_{net}), transpiration (E), and stomatal conductance (g_s) of soft *T. pungens* and hard *T. longiceps* at the end of dry and wet seasons. Different letters represents significant differences between means (2-way ANOVA, $P < 0.05$) of soft and hard species in both seasons.

Species	Dry Season		Wet Season	
	<i>T. pungens</i>	<i>T. longiceps</i>	<i>T. pungens</i>	<i>T. longiceps</i>
A_{net} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.59 ± 0.26^b	-0.30 ± 0.17^b	10.61 ± 2.3^a	16.40 ± 3.6^a
E ($\text{mmol m}^{-2} \text{s}^{-1}$)	1.06 ± 0.13^c	3.28 ± 0.33^b	3.22 ± 0.41^b	6.26 ± 0.61^a
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	0.01 ± 0.00^c	0.04 ± 0.01^b	0.06 ± 0.01^b	0.12 ± 0.02^a

Table 2. Reported Pearson product-moment correlation coefficient (R) from correlation analysis of net photosynthesis (A_{net}), transpiration (E), stomatal conductance (g_s) with vapour pressure deficit (Vpd), relative humidity (RH) and air temperature (Air temp) for dry and wet season of soft and hard species combined. Data used in analysis obtained from AM-PM measurements. Correlation strength scales from -1 to 1 where 0 equates to no association between variables and ± 1 indicates either absolute positive/negative association. Highlighted values in bold denote significant correlation at $P < 0.05$, $n=30$.

Atmospheric variable	Dry Season			Wet Season		
	Vpd	RH	Air temp	Vpd	RH	Air temp
A_{net} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	-0.060	0.092	-0.094	-0.781	0.635	-0.602
E ($\text{mmol m}^{-2} \text{s}^{-1}$)	0.028	-0.018	0.186	-0.411	0.161	-0.123
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	-0.323	0.279	-0.181	-0.747	0.573	-0.539

2. Effect of elevated atmospheric CO_2 ($e\text{CO}_2$)

CO_2 concentrations did not affect leaf-level photosynthesis (Figure 4a), or biomass accumulation (Figure 5c, d) of soft species *T. pungens* grown with replete nutrient levels, but $e\text{CO}_2$ resulted in reduced transpiration and associated greater instantaneous water use efficiency (WUE_i) as a result of lower stomatal conductance (Figure 4b-d). Small leaves prevented measurement of leaf-level gas exchange (*T. pungens* with low nutrients and *T. basedowii* with low or high nutrient supply). There was no effect of CO_2 on total biomass while

replete nutrient supply significantly increased total biomass of both species (Figure 5, $P < 0.001$).

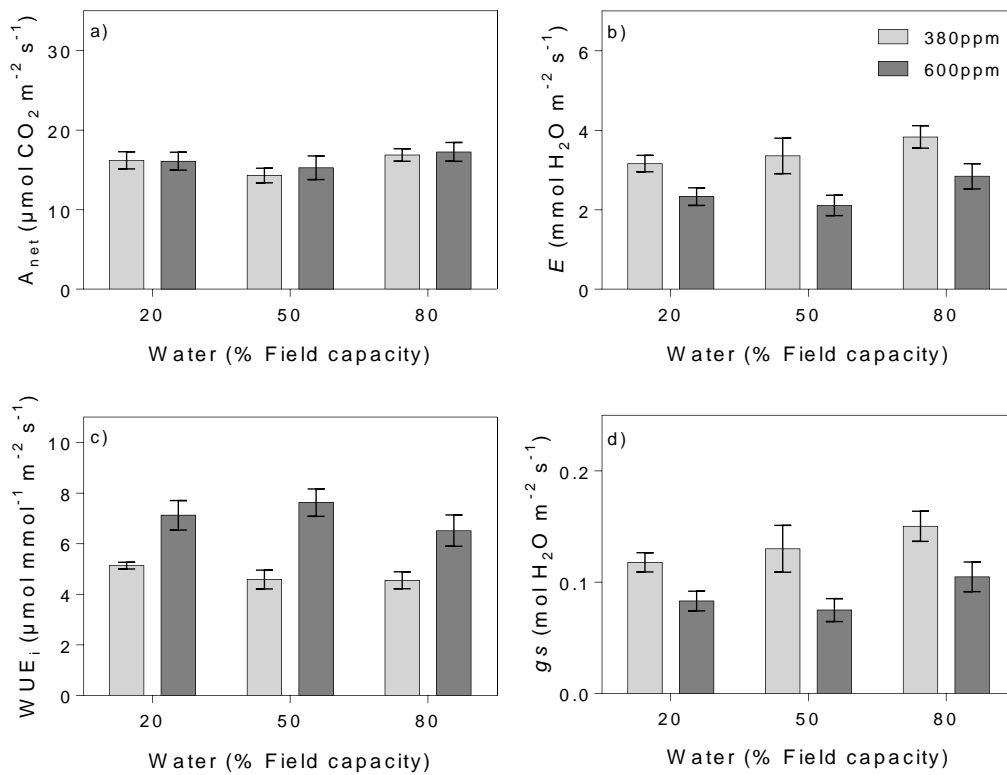


Figure 4. a) Net photosynthesis (A_{net}), b) transpiration (E) c) instantaneous water use efficiency (WUE_i , μmols of CO_2 assimilated per mmol of H_2O) and d) stomatal conductance (g_s) of soft species *T. pungens* of youngest fully expanded leaves. Plants were grown at replete levels of nutrient in a multifactorial design with water supply of 20, 50 and 80% field capacity and CO_2 concentrations of 380 and 600 ppm. Error bars represent standard errors of eight plants except for 20% field capacity treatment with CO_2 of 380 ppm had $n=7$, and 600 ppm $n=6$.

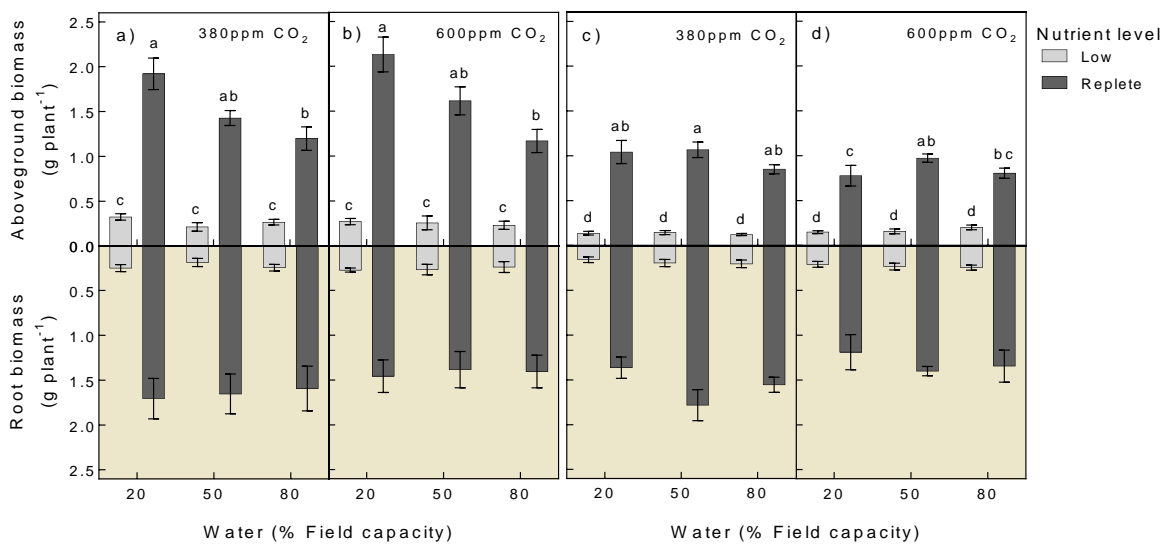


Figure 5. Biomass accumulation aboveground and belowground of hard species *T. basedowii* (a, b) and *T. pungens* (c, d) grown with low and replete nutrient concentrations and CO₂ concentrations of 380 ppm (a, c) and 600 ppm (b, d) across water levels of 20, 50 and 80% field capacity. Experiments for both species were conducted at separate times but under the same conditions. Error bars are standard error, n=8. Lowercase letters represent statistical differences in total biomass between treatments for each species.

Conclusions

Similar to what has been reported for other arid plants grown in field conditions, both *Triodia* types respond to increased water availability by increasing gas exchanges including rates of photosynthesis, while no or little net CO₂ assimilation in the dry season. Maximum net photosynthesis rates occurred in the morning and were similar in both *Triodia* type, and were comparable to other grass species in arid and semi-arid environments. Highest stomatal conductances coincided with net photosynthesis rates and were approximately 50% lower than those observed in other arid zone grasses, suggesting that *Triodia* is strongly conservative in its water relations. In line with the distribution of stomata, the ancestral hard type had similar photosynthesis rates at the expense of greater water loss than the more recently evolved soft type (stomata only on the upper leaf surface). It appears that the hard species has a strong ability to respond to favourable environmental conditions, but is more sensitive to a high availability of water under controlled conditions. It is unclear why hard species responded negatively to increased water availability but may be reflective of sensitivity to declining oxygen availability with increasing soil saturation; this requires further investigation.

Although net photosynthesis was not affected in elevated CO₂ concentrations, increased water use efficiency of the soft species suggest that *T. pungens*, and possibly other soft species, may be able to counteract reduced precipitation in future climates. Furthermore, the pronounced nutrient effect on biomass accumulation demonstrates that nutrient availability will have a significant impact on *Triodia* function, highlighting the need to consider nutrient availability together with other environmental variables.

The results here raise intriguing questions about the evolution and distribution of *Triodia* species. The more recently evolved soft species are more water efficient than the ancestral hard type studied here, most likely due to loss of the stomata on the lower leaf surface and possibly other adaptations not studied here. However, the distribution of the two types of *Triodia* is at odds with this notion, as ancestral and less water efficient hard species are distributed mostly in the drier regions of Australia, while the more recently evolved and more water efficient soft species prevail in wetter northern regions of the continent (Figure 6). Our data show that hard species are sensitive to the presence of water and perhaps this could explain the dominant distribution of hard species in the driest regions. We cannot explain the contrast between the species' ecophysiology and geographical distribution, and it is clearly a topic worthy of research.

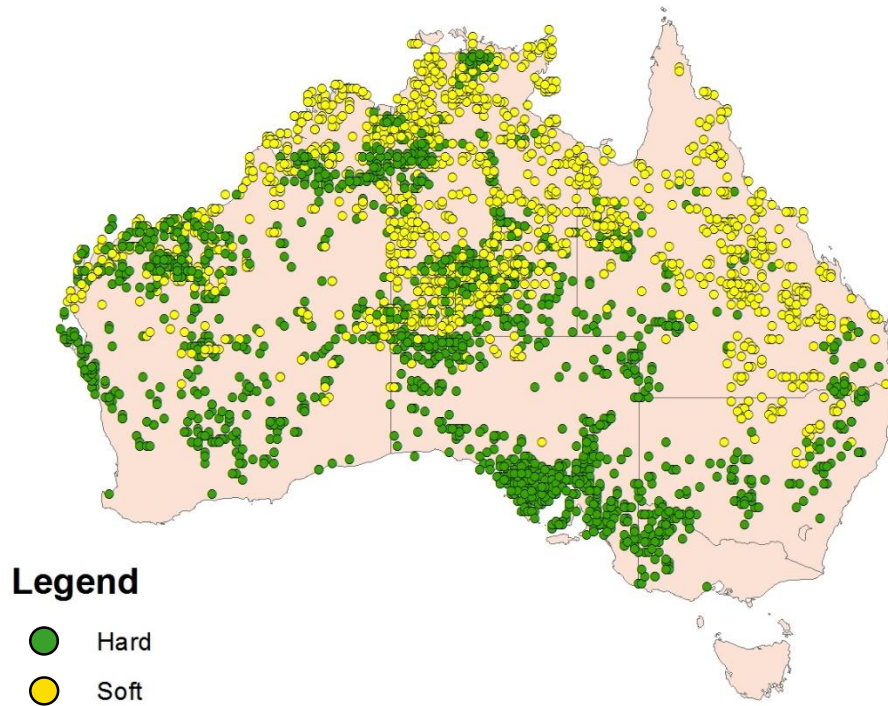


Figure 6. Current known locations of hard and soft types of *Triodia* based on herbarium collections. Map generated based on data from Australia's Virtual Herbarium (<http://avh.chah.org.au>).