



The interplay between leaf water potential and osmotic adjustment on photosynthetic and growth parameters of tropical dry forest trees

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Abstract *Mimosa tenuiflora* and *Piptadenia stipulacea* are commonly accepted as drought-tolerant species but little is known about their response to drought followed by rehydration. Therefore, the interplay between leaf water potential and osmotic adjustment on photosynthetic and growth parameters of these species was examined. A greenhouse study was conducted in a split-plot design with two water conditions in the main plots (control; drought followed by rehydration), and eight sampling times in the subplots (1, 4 and 7 days of drought, and 1, 3, 6, 12, and 17 days of rehydration). Plant water status and biochemical changes were assessed as well as leaf gas exchange and subsequent growth. Under drought stress, both species maintained a low leaf water potential throughout the day by accumulating compatible solutes, thus allowing a rapid and full recovery of water status when rehydrated. Although these plants minimized water loss by closing their stomata, neither showed stomatal

limitations to photosynthesis. The inhibition of this process during drought was possibly related to mesophyll limitations as well as to a reversible downregulation of photosystems, along with adjustments of their stoichiometry. Water deficits also triggered morphological adaptations at the whole plant level, leading to reduced growth, mainly of the shoots in *M. tenuiflora* and the roots in *P. stipulacea*.

Keywords Drought stress · Rehydration · Gas exchange · Plant biomass

Introduction

Drought is one of the main factors causing tree mortality and forest decline, thus altering the carbon balance of terrestrial ecosystems (Choat et al. 2018; Serra-Maluquer et al. 2018; Jiao et al. 2021). Therefore, further information on the ability of trees to survive low water availability is of paramount importance to predict changes in carbon cycling (Santiago et al. 2016; Pritzkow et al. 2020). Drought stress triggers physiological, biochemical and molecular alterations in plants (Ashraf and Harris 2013). Unfavourable water status induces turgor loss and consequently reduces cell growth (Fox et al. 2018). Furthermore, it usually promotes stomatal closure as well as non-stomatal limitations to photosynthesis, inhibiting carbon assimilation (Gomes et al. 2008; Duan et al. 2020; Antezana-Vera and Marenco 2021). Under field conditions, drought may also lead to several other abiotic stresses such as light, temperature and nutrient stress.

Similar to what is observed in other tropical dry forests, drought events are frequent in the Brazilian Caatinga (Sampaio 1995). The annual rainfall ranges from 250 to 750 mm and is distributed over three to four months, followed by a dry season that lasts the rest of the year (Silva et al. 2010;

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Barros et al. 2020). In addition to low water availability, Caatinga plants face high irradiance and temperatures, making water deficits a common phenomenon (Silva et al. 2010; Dombroski et al. 2014), especially because the potential evapotranspiration exceeds 1500 mm a^{-1} (Sampaio 1995). Nevertheless, water deficits will intensify in the following years, with impacts on flora characteristics (Frosi et al. 2016). In fact, Campos et al. (2020) reported an increase in tree mortality from 2009 to 2019, along with a decrease in biomass production of a Caatinga fragment.

Mimosa tenuiflora (Willd.) Poir. (Mimosaceae) and *Piptadenia stipulacea* (Benth.) Ducke (Mimosaceae) are woody species widely distributed in tropical dry forests from Mexico to Brazil. These pioneer species play a key role in the ecological succession of the Caatinga dry forest vegetation (Alves and Freire 2019; Barros et al. 2019). However, it is unclear how they cope with the extreme conditions of this semi-arid habitat, particularly low and erratic rainfall (Sampaio 1995). To thrive in such environment, species depend on the ability to withstand droughts and on the capacity to recover (Gallé et al. 2007; Xu et al. 2010). This is especially important when considering the occurrence of short but frequent drought events. Yet there is far more information available on drought stress than on stress recovery, although the latter may determine survival (Santiago et al. 2016; Choat et al. 2018).

Despite being regarded as drought-tolerant little is known about the responses of *M. tenuiflora* and *P. stipulacea* to drought followed by rehydration. Lima and Meiado (2018) assessed the effect of hydration and dehydration cycles on the germination of *M. tenuiflora* and concluded that seedlings benefited from a discontinuity in the imbibition process, showing increased shoot heights, stem diameters and total dry weight. Alves and Freire (2019) evaluated the physiological response of one-year-old seedlings to water deficits and rewatering and found that drought-induced changes in relative water content and gas exchange parameters were normalized within three days of rehydration. As for *P. stipulacea*, apart from germination tests, there are few studies on biomass production and allocation as affected by drought (Barros et al. 2019; Campos et al. 2020), with no information on biochemical and physiological responses of this species nor on its recovery from drought stress.

Drought-triggered tree mortality can result in vegetation shifts with unknown environmental consequences (Serra-Maluquer et al. 2018; Thammanu et al. 2021). There is a wide variation in the response of plants to this stress as well as in their recovery dynamics on rewatering (Yordanov et al. 2000; Taiz et al. 2015; Niinemets 2016). Understanding these intrinsic characteristics may be useful in the implementation of management practices to ensure the resilience of tropical dry forests under climate change conditions (Xu et al. 2010; Serra-Maluquer et al. 2018; Stan et al. 2021).

To elucidate some of the mechanisms behind the drought tolerance of *M. tenuiflora* and *P. stipulacea*, the interplay between leaf water potential and osmotic adjustment on photosynthetic and growth parameters of these plants was studied by evaluating their responses to drought followed by rehydration.

Material and methods

Plant material and experimental conditions

A greenhouse study was conducted in Mossoró, Brazil ($5^{\circ} 12' 16'' \text{ S}$, $37^{\circ} 19' 29'' \text{ W}$) where *M. tenuiflora* and *P. stipulacea* were grown in polyethylene bags (1.9 L). A Thermo Recorder TR-72U (T&D Corporation, Matsumoto, Nagano, Japan) monitored air temperature and relative humidity, which had average values of 28.8° C and 62.4%, respectively. Two independent experiments, one for each species, were performed in a split-plot design with two water conditions in the main plots (1 – control and 2 – drought followed by rehydration) and eight sampling dates in the subplots (1, 4 and 7 days of drought, and 1, 3, 6, 12, and 17 days of rehydration). Treatments were laid out in a randomized complete block design, replicated 10 times, with eight bags per experimental unit.

M. tenuiflora and *P. stipulacea* seeds were immersed in hot water at 100° C for 4 min to break dormancy (Benedito et al. 2017, 2019), sown in a substrate of topsoil (Table S1) mixed with $200 \text{ mg P}_2\text{O}_5 \text{ dm}^{-3}$ and subjected to five top dressings (40, 50, 60, 70 and 80 days after sowing), totalling 500 mg N dm^{-3} and $250 \text{ mg K}_2\text{O dm}^{-3}$. Thinning was carried out 21 days after sowing to one seedling per bag. The plants were watered to field capacity in the early mornings and late afternoons for six months. Half were then subjected to the treatment shown in Fig. 1. While control plants were

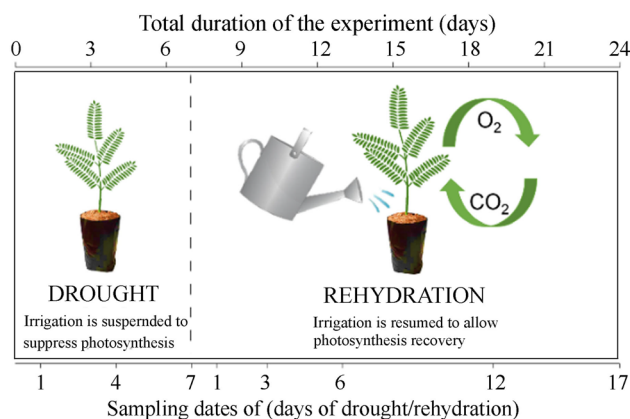


Fig. 1 Schematic of the drought followed by rehydration; the dash line is the transition between the two periods

kept well-watered, drought was imposed by suspending irrigation for seven days, causing net photosynthesis of both species to be almost completely suppressed (Suresh et al. 2012; Freitas et al. 2018). Drought-stressed plants were then rehydrated until their photosynthetic activity returned to control levels (Gomes et al. 2008), which took 17 days for *M. tenuiflora* and 12 days for *P. stipulacea*.

Water potential (Ψ_w) measurements

To assess plant water status, fully expanded leaves from the middle-third (Schimpl et al. 2019) of one plant per treatment were used for measurements with a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA). Predawn and midday Ψ_w (leaf water potential) were recorded immediately after excision (Karimi et al. 2015), from 4 to 6 a.m. and from 11 am to 1 pm

Biochemical assay

On the seventh day of drought, the highest stress level, newly expanded leaves of plants from five replications were collected and stored at -18°C for subsequent analysis of proline, soluble sugars and amino acids and photosynthetic pigments (chlorophyll *a* and *b*). To ensure the accuracy of the results, data were collected in triplicate and expressed on a dry weight basis. Proline accumulation was determined after reaction with acid-ninhydrin solution (Bates et al. 1973). Soluble sugars and amino acids were estimated using the phenol-sulphuric acid method (DuBois et al. 1956) and the ninhydrin method (Yemm et al. 1955), respectively. Chlorophyll (Chl) *a* and *b*, and their ratio, were calculated from absorbance (A) values at 645 and 663 nm following acetone extraction (Lichtenthaler 1987), where: $\text{Chl } a = 12.25 \times A_{663} - 2.79 \times A_{645}$ and $\text{Chl } b = 21.5 \times A_{645} - 5.1 \times A_{663}$.

Leaf gas exchange

Gas exchange parameters were measured between 9 and 10 a.m. in one newly expanded leaf of each experimental unit using a LI-6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA). After setting light to $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$, CO_2 concentration to $400 \mu\text{mol mol}^{-1}$ and flow rate to $400 \mu\text{mol s}^{-1}$, the following parameters were simultaneously recorded: stomatal conductance, transpiration rate, net photosynthesis, intercellular CO_2 concentration and carboxylation efficiency. Net photosynthesis was used to establish the length of the drought and rehydration periods, as proposed by Freitas et al. (2018), and it was also plotted against stomatal conductance and intercellular CO_2 concentration data to assess their relationships.

Growth traits

Once considered recovered from drought (after rewatering *M. tenuiflora* for 17 days and *P. stipulacea* for 12 days), all plants were irrigated to field capacity for a further two months. This allowed for the evaluation of the effects of drought stress on subsequent biomass production and allocation within the plant. Leaves, stems and roots of two seedlings per treatment were harvested and placed in a forced-air drying oven at $65 \pm 2^\circ\text{C}$ for three days. Based on their dry weights, the total dry weight and the shoot/root ratio were calculated, where: shoot = leaf + stem.

Statistical analysis

Data were analysed by Student's *t*-test at the 5% level, comparing control and drought stress conditions at each sampling date using the Sisvar software, version 5.6 (Federal University of Lavras, Lavras, MG, Brazil). Regression analysis was also performed when assessing the relationships between photosynthetic parameters.

Results

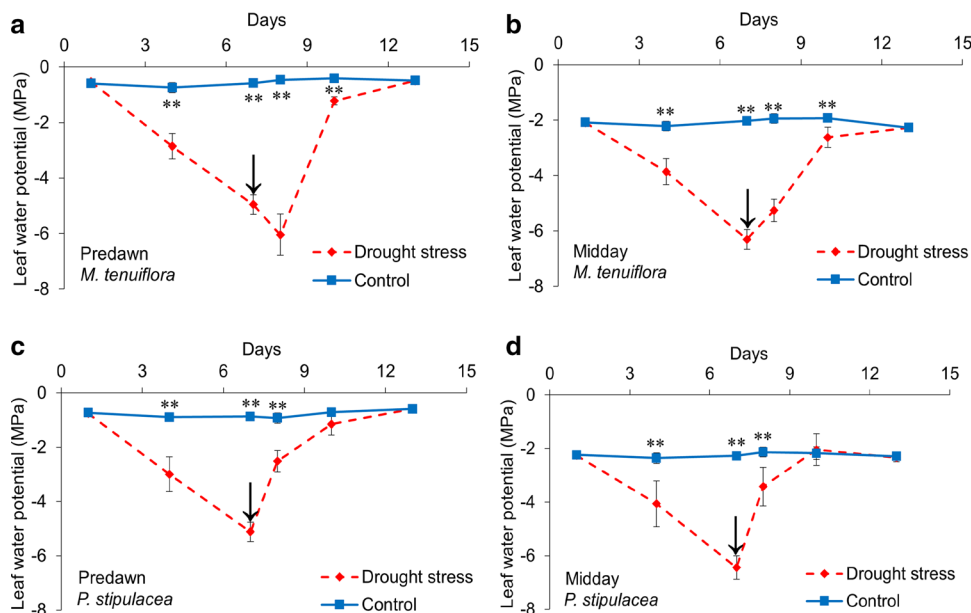
Predawn and midday Ψ_w

Leaf measurements revealed that these pioneer species had a high ability to adjust leaf water potential (Ψ_w) to cope with decreasing soil water availability. In general, the recorded values were naturally lower at midday than at predawn (as observed for control plants), but suspending irrigation resulted in much greater reductions over time. After seven days of drought, the Ψ_w of *M. tenuiflora* ranged from -5.0 MPa at predawn (Fig. 2a) to -6.3 MPa at midday (Fig. 2b). Intriguingly, the former decreased even further to -6.0 MPa on the first day following rehydration, suggesting a lingering response to drought. It was only on the sixth sampling date that leaf Ψ_w returned to control levels. At maximum stress, compared with well-watered plants, there were 5.9 and 2.8-fold decreases in predawn (Fig. 2c) and midday (Fig. 2d) Ψ_w of *P. stipulacea*, respectively. Nevertheless, this species rehydrated relatively rapidly, and water status was normalized three days after rewatering.

Biochemical changes

Suspending irrigation for seven days resulted in significant accumulation of compatible solutes by augmenting the levels of proline, soluble sugars and soluble amino acids in the seedlings. The highest differences between control and treated plants were in proline content, with 30.7 and 32.6-fold increases for *M. tenuiflora* and *P. stipulacea*,

Fig. 2 Predawn (a, c) and mid-day (b, d) leaf water potentials of *M. tenuiflora* and *P. stipulacea* over time as a function of drought and rehydration; arrows indicate the beginning of the recovery period; values are means \pm SD ($n=10$); asterisks denote significant differences from controls ($P<0.01$)



respectively (Fig. 3a). Unlike free proline, the accumulation of soluble sugars (Fig. 3b) and amino acids (Fig. 3c) was proportionally higher in the former species. After the drought treatment, chlorophyll *a* of *M. tenuiflora* went from 1.12 ± 0.20 to 2.15 ± 0.87 mg g⁻¹ ($P<0.05$) and there was also a significant increase of chlorophyll *a/b* ratio (Fig. 3d). The other photosynthetic pigments analysed did not differ statistically and their contents were as follows (mg g⁻¹): *M. tenuiflora* – chlorophyll *b* of 0.62 ± 0.13 ; *P. stipulacea* – chlorophyll *a* of 2.33 ± 0.66 and chlorophyll *b* of 0.39 ± 0.07 .

Photosynthetic responses

The response patterns of stomatal conductance and net photosynthesis to drought were similar, suggesting that photosynthetic activity was driven by changes in stomatal aperture and perhaps limited by a low CO₂ uptake. After only four days without irrigation, the stomatal conductance of *M. tenuiflora* seedlings decreased by 90.1% relative to the controls (Fig. 4a), which also led to a 79.3% decrease in net photosynthesis (Fig. 4b). This last parameter was almost completely suppressed within seven days of drought,

Fig. 3 Proline (a), soluble sugar (b) and soluble amino acid (c) contents and chlorophyll *a/b* ratio (d) in leaves of *M. tenuiflora* and *P. stipulacea* after seven days of drought; values are means \pm SD ($n=5$) and different letters indicate significant differences from controls ($P<0.05$)

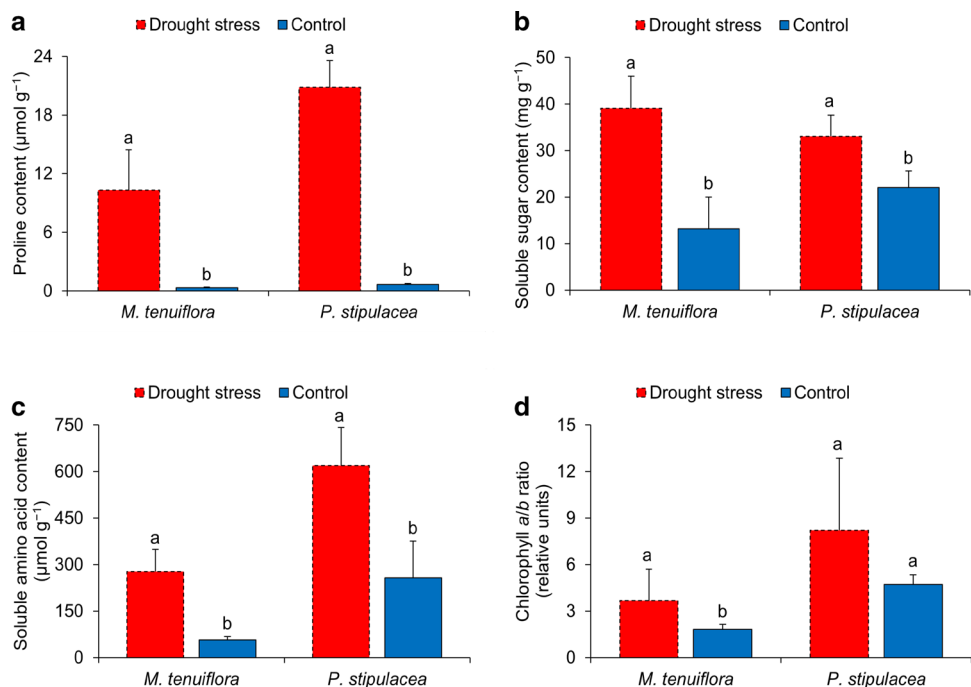
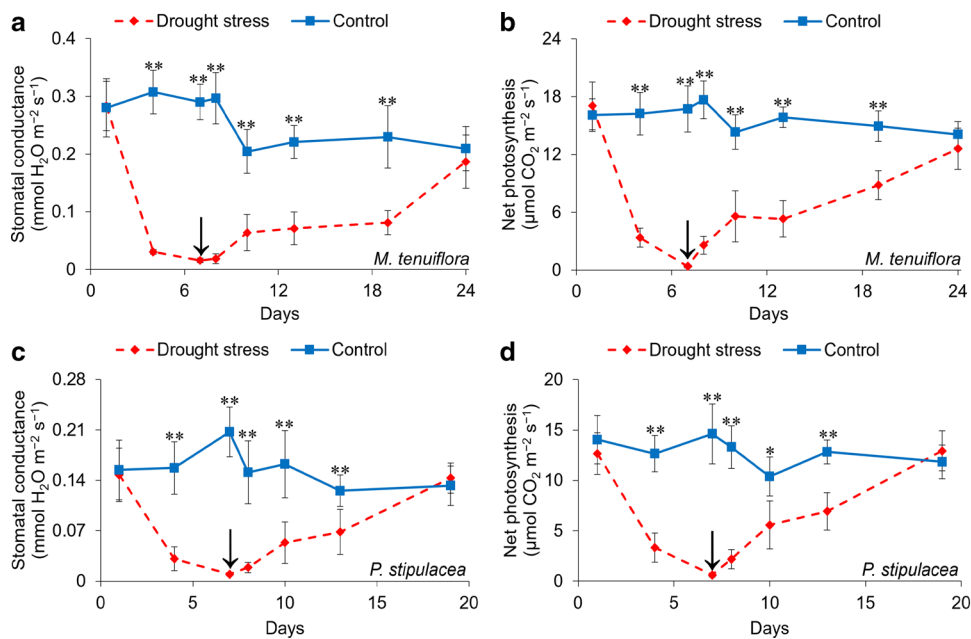


Fig. 4 Stomatal conductance (a, c) and net photosynthesis (b, d) of *M. tenuiflora* and *P. stipulacea* over time as a function of drought and rehydration; arrows indicate the beginning of the recovery period; values are means \pm SD ($n = 10$); asterisks denote significant differences from controls (**, $P < 0.01$; *, $P < 0.05$)

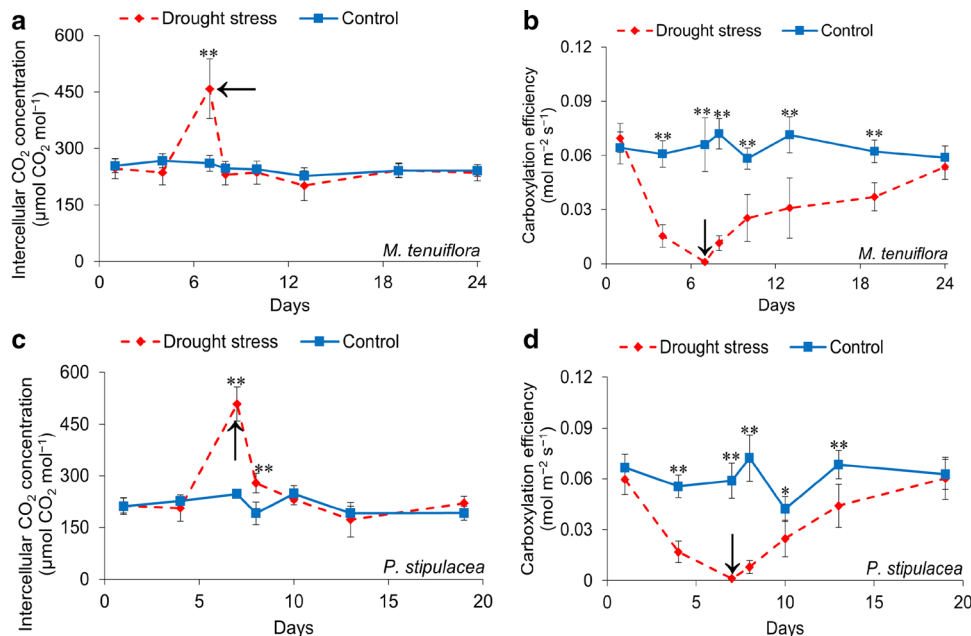


requiring a 17-day period to fully recover. The performance of *P. stipulacea* under stress was analogous to that of *M. tenuiflora* except that its photosynthetic activity was completely restored by the 12th day of rehydration (Fig. 4c, d). For both species, stomatal closure resulted in a decrease in transpiration rate (Fig. S1).

Even though by the third sampling date stomatal conductance was reduced to near zero, there were increases in intercellular CO_2 concentration, indicating that CO_2 was not a limiting factor for photosynthesis. Moreover, the fact that at that time net photosynthesis was suppressed,

despite a great availability of CO_2 , resulted in extremely low carboxylation efficiency values. At the highest stress level, intercellular CO_2 concentration of *M. tenuiflora* was 76.0% higher than that of control seedlings (Fig. 5a), and its carboxylation efficiency was normalized by the 17th day of rehydration (Fig. 5b). *P. stipulacea* showed essentially the same responses but the increase in intercellular CO_2 concentration was of 105.7% with a significant difference shortly afterwards (Fig. 5c), and it took only 12 days to recover its carboxylation efficiency (Fig. 5d).

Fig. 5 Intercellular CO_2 concentration (a, c) and carboxylation efficiency (b, d) of *M. tenuiflora* and *P. stipulacea* over time as a function of drought and rehydration; arrows indicate the beginning of the recovery period; values are means \pm SD ($n = 10$); asterisks denote significant differences from controls (**, $P < 0.01$; *, $P < 0.05$)



As indicated previously, drought-induced stomatal closure did not have a negative effect on intercellular CO₂ concentration. Nevertheless, by plotting net photosynthesis against stomatal conductance and intercellular CO₂ concentration data, it was confirmed that photosynthetic activity was indeed coupled with stomatal aperture but not limited by a low CO₂ influx into the sub-stomatal chamber. For both *M. tenuiflora* and *P. stipulacea*, there was a high correlation between the first two parameters, while the third was weakly and negatively correlated with the first one (data not shown). Regression analysis showed a quadratic relationship between net photosynthesis and stomatal conductance, where the photosynthetic activity increased with stomatal opening (Fig. 6a, c). On the other hand, most of the net photosynthesis occurred between 150 and 300 μmol CO₂ mol⁻¹, with basically no carbon assimilation above this optimal range (Fig. 6b, d).

Biomass production and allocation

With regards to subsequent biomass production as compared to controls, treated plants invested considerably less in leaf and stem tissues and, in the case of *P. stipulacea*, also in root tissues. Consequently, the total dry weight of the two species was negatively affected by approximately 30%. Furthermore, exposure to a single drought period was enough to alter biomass allocation within the plant. Although the reductions in shoot and root dry weight (the latter not significant) of *M. tenuiflora* were relatively equivalent, they were quite different for *P. stipulacea*, where the shoot dry weight of previously stressed plants was 28.9% smaller than

that of well-watered seedlings, whereas the root dry weight was affected by 45.0%. Therefore, there was an increase in the shoot/root ratio, apparently favouring shoot growth at the expense of root growth (Table 1).

Discussion

One of the first responses of plants to drought stress is a decrease in leaf water potential (Ψ_w) (Haider et al. 2018), which allows for a rapid recovery of cell turgor and photosynthetic activity upon rewatering (Niinemets 2016; Ruehr et al. 2019). *M. tenuiflora* and *P. stipulacea* coped with the increasing water deficit by considerably reducing their Ψ_w throughout the day. In fact, their predawn and midday Ψ_w after seven days of drought were much lower than those recorded for six Caatinga trees in the dry season (Dombroski et al. 2011), as well as for other tropical species under drought stress, such as *Hevea brasiliensis* Müll. Arg., – 1.85 MPa at predawn (Falqueto et al. 2017), *Bertholletia excelsa* Bonpl., – 4.7 MPa at midday (Schimpl et al. 2019), and *Erythrina velutina* Willd., – 0.31 MPa at predawn and – 0.89 MPa at midday (Silva et al. 2010). However, despite showing similar responses, the rehydration of *P. stipulacea* was completed three days earlier than in *M. tenuiflora*.

The recovery of Ψ_w precedes, and is usually faster than, that of gas exchange parameters, occurring within hours to a few days (Ruehr et al. 2019; Duan et al. 2020). For this reason, water relations of drought-stressed trees can benefit even from short rainfall events (Dietrich and Kahmen 2019). This is particularly important in the Caatinga dry

Fig. 6 Relationships between net photosynthesis and stomatal conductance (a, c) and net photosynthesis rate and intercellular CO₂ concentration (b, d) of *M. tenuiflora* and *P. stipulacea* under drought and rehydration

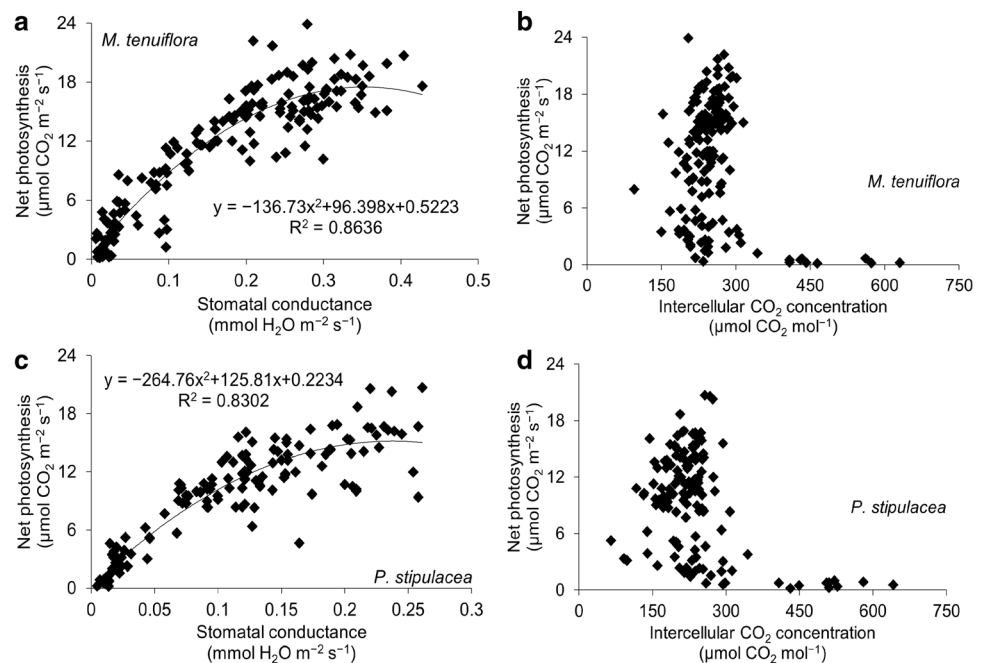


Table 1 Biomass production and allocation in *M. tenuiflora* and *P. stipulacea* two months after a drought period

Species	Water condition	LDW (g plant ⁻¹)	SDW (g plant ⁻¹)	RDW (g plant ⁻¹)	TDW (g plant ⁻¹)	S/R (relative units)
<i>M. tenuiflora</i>	Drought stress	8.57 ± 2.00 ^b	17.59 ± 3.29 ^b	6.67 ± 2.29 ^a	32.84 ± 5.81 ^b	4.38 ± 1.69 ^a
	Control	10.69 ± 1.47 ^a	26.15 ± 5.70 ^a	8.61 ± 2.13 ^a	45.46 ± 7.39 ^a	4.39 ± 0.77 ^a
	LSD	1.12	5.38	2.80	7.57	1.57
	CV (%)	11.49	24.32	36.27	19.10	35.37
<i>P. stipulacea</i>	Drought stress	5.12 ± 2.03 ^b	25.23 ± 5.79 ^b	6.63 ± 1.89 ^b	36.99 ± 8.46 ^b	4.69 ± 0.78 ^a
	Control	7.41 ± 1.60 ^a	35.28 ± 6.4 ^a	12.05 ± 3.33 ^a	54.74 ± 9.88 ^a	3.66 ± 0.65 ^b
	LSD	1.27	5.27	1.91	7.03	0.67
	CV (%)	19.99	17.23	20.17	15.16	15.89

LDW leaf, SDW stem, RDW root, TDW total dry weight, S/R: shoot/root ratio; values are means ± SD ($n = 10$) and different letters in the columns indicate significant differences from controls ($P < 0.01$)

forest, where plants experience low and irregular rainfall (Sampaio 1995; Dombroski et al. 2011). The sooner the plant water status is normalized, the faster the full recovery of photosynthetic activity will be (Yordanov et al. 2000), as demonstrated in this study for *P. stipulacea*. To withstand droughts, plants have evolved a series of adaptive strategies. An important mechanism is the accumulation of compatible solutes like proline, soluble sugars and amino acids, which helps to maintain cellular homeostasis and promotes osmotic adjustment (Liao et al. 2018). Therefore, the equal ability of *M. tenuiflora* and *P. stipulacea* to decrease their Ψ_w can be explained by the biochemical changes observed in these plants at the highest stress level.

Osmotic adjustment may take up to three weeks to achieve (Spieß et al. 2012). Nevertheless, after only seven days of drought, both species showed increased proline, soluble sugar and soluble amino acid contents. Proline had by far the highest increase, presumably because of its importance both as an osmolyte and as an antioxidant (Hu et al. 2015; Khaleghi et al. 2019). Plants under drought stress must contend not only with the loss of cell turgor, but also with the overproduction of reactive oxygen species, which increases the risk of irreversible damages to major macromolecules such as lipids, proteins and carbohydrates by oxidative stress (Gallé and Feller 2007). With regards to soluble sugars, short periods of drought induce the accumulation of readily metabolisable carbohydrates (Spieß et al. 2012). Thus, the increased contents observed here may indicate an important adaptation to prepare for recovery. During drought, soluble sugars are important for osmoregulation and as signalling molecules to induce stress responses. But once no longer needed for these purposes, they can be essential to supply carbon and energy for repair and regrowth (Khaleghi et al. 2019). This could also explain the accumulation of soluble amino acids, which would support subsequent protein synthesis (Taiz et al. 2015).

Decreases in photosynthetic pigments are commonly reported in drought-stressed plants and attributed to a slow

biosynthesis or a fast degradation of chlorophylls (Fox et al. 2018; Haider et al. 2018; Liao et al. 2018). Similar to our findings, there seems to be no major impact on the chlorophyll content of certain tree species (Frosi et al. 2016; Freitas et al. 2018; Duan et al. 2020), which suggests a lesser impact on their photosynthetic apparatus (Gallé et al. 2007). The increase in chlorophyll *a* and, consequently, in the chlorophyll *alb* ratio of *M. tenuiflora* under stress could also indicate an adjustment of photosystem (PS) stoichiometry towards a higher PSI/PSII ratio, reducing light harvesting and avoiding photoinhibition (Liu et al. 2011). In *P. stipulacea*, because there was no significant change in chlorophyll levels, and net photosynthesis recovered much earlier, it is presumed that these functional units may have been down-regulated but preserved during drought.

Altogether, the biochemical changes in *M. tenuiflora* and *P. stipulacea* contributed to the rapid and complete recovery of their photosynthetic activities after rehydration. Such recovery is an indicator of drought tolerance and demonstrates a high physiological plasticity (Schimpl et al. 2019), emphasizing the ability of these plants to withstand drought events. To prevent extreme water loss in leaf tissues during the stress period, both species reduced transpiration by closing their stomata, which in some circumstances affects CO₂ uptake (Yordanov et al. 2000; Haider et al. 2018; Liao et al. 2018). Peguero-Pina et al. (2018) observed that the reduction in stomatal conductance of *Quercus ilex* L. consisted in a limitation to carbon assimilation. Although it was initially considered the possibility of drought-induced stomatal limitations to photosynthesis, it was later confirmed that this was not the case in this study, indicating exclusively the occurrence of mesophyll or biochemical limitations.

At the highest stress level, the reduction in net photosynthesis, despite increased CO₂ availability, could be attributed to a low gas use efficiency of chloroplasts (Ashraf and Harris 2013). Increased resistance in the mesophyll layer could reduce CO₂ diffusion to carboxylation sites (Elferjani et al. 2021). In addition, plants under increasing water deficits, if

still exposed to light, are likely to experience photoinhibition (Zargar et al. 2017). Drought can lead to the degradation of the D1 protein, a key subunit of PSII, thus causing the inactivation of its reaction centre (Ashraf and Harris 2013). In this case, the photosynthetic activity of drought-sensitive plants might not be fully recovered (Ruehr et al. 2019). Conversely, Gallé and Feller (2007) reported enhanced photoprotection in *Fagus sylvatica* L. after a reversible downregulation of PSII accompanied by an increase in the dissipation of excess excitation energy. This helped to maintain a functional photosynthetic apparatus, allowing the complete recovery upon rewatering, as observed here for both species.

While the magnitude of the effects of stomatal and non-stomatal limitations on photosynthetic capacity cannot be easily distinguished (Ashraf and Harris 2013), we found that *M. tenuiflora* and *P. stipulacea* are mainly affected by the latter, because at no time was photosynthesis limited by low CO₂ availability. Besides, there were no positive correlations between stomatal conductance and intercellular CO₂ concentration (data not shown). At the highest stress level, the fact that photosynthesis was almost completely suppressed explains, in part, the increases in CO₂ availability, given that this substrate was no longer being used. However, since stomatal conductance was restricted, this increase was probably due to respiration rather than a high influx of CO₂. In any case, the extremely low carboxylation efficiency observed at that point could be the result of declines in mesophyll conductance and/or in the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Zhou et al. 2014; Elferjani et al. 2021).

Based on several studies, Elferjani et al. (2021) estimated that stomatal, mesophyll and biochemical limitations accounted for 49%, 39% and 12%, respectively, of the reductions in photosynthesis of *Populus* spp. Nevertheless, the impacts of short-term drought stress on these proportions vary greatly among tree species (Zhou et al. 2014). Apparently, drought stress affects stomatal conductance and net photosynthesis at the same rate, leading to a high correlation between these parameters. However, the extent of stomatal closure was insufficient to limit CO₂ influx into the substomatal chamber, unlike that observed for another Caatinga species (Dombroski et al. 2014). Therefore, neither of the species studied here presented stomatal limitations to photosynthesis, which is in accordance with Alves and Freire (2019) for *M. tenuiflora*. Unfortunately, to the best of our knowledge, there are no studies on the effect of drought on photosynthesis of *P. stipulacea*, which highlights the need for further research.

In addition to altering photosynthetic activity, drought stress affected growth patterns. Changes in biomass production and allocation can play an important role in adaptation to future drought events (Gallé and Feller 2007; Niinemets 2016). We found that previously stressed plants invested

less in shoot (*M. tenuiflora* and *P. stipulacea*) and root (*P. stipulacea*) growth. Thus, even a relatively short drought period of seven days can induce morphological adaptations in these species. Despite reporting a strong compensation growth upon rewatering, Spieß et al. (2012) observed that long-term drought had a negative effect on the subsequent growth of *Quercus robur* L., affecting mainly the shoots. Curiously, root growth in *P. stipulacea* is more reduced than shoot growth, as suggested by Barros et al. (2019). A meta-analysis revealed that, under drought stress, roots are not as affected as leaves and stems, and their biomass generally increases to facilitate water uptake (Eziz et al. 2017). However, this is more common for herbaceous than woody species, and may not even occur under extreme water deficit (Xu et al. 2010; Eziz et al. 2017). Moreover, there are reports on the increase of shoot/root ratios during drought (Santiago et al. 2001; Bueno et al. 2021). In contrast, Barros et al. (2020) found no differences in the shoot/root ratio of four woody species after rehydration, as shown here for *M. tenuiflora*.

Conclusions

The drought tolerance of *Mimosa tenuiflora* and *Piptadenia stipulacea* is associated with their ability to respond quickly and effectively to this stress. These species maintain a low leaf water potential throughout the day by accumulating compatible solutes, thus allowing a rapid and full recovery of water status upon rewatering. Nonetheless, because the rehydration of *P. stipulacea* occurs at a faster rate, its photosynthetic activity recovers earlier. Although both species minimize water loss by closing their stomata, neither showed stomatal limitations to photosynthesis. The inhibition of this process during drought is probably related to mesophyll limitations as well as to a reversible downregulation of photosystems, along with, in the case of *M. tenuiflora*, adjustments of their stoichiometry. Moreover, water deficit triggers morphological adaptations in these species, leading to reduced subsequent growth, mainly of shoots in *M. tenuiflora* and roots in *P. stipulacea*. Future studies may help to elucidate the gene expression and antioxidant enzyme activity underlying this drought tolerance.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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