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GRAZIELLE SALES TEODORO

EXTREME DROUGHT EFFECTS ON THE PHENOLOGY, GROWTH AND ECOPHYSIOLOGY PERFORMANCE OF CAMPOS RUPESTRES SPECIES

EFEITOS DE SECAS EXTREMAS NA FENOLOGIA, CRESCIMENTO E DESEMPENHO ECOFISIOLÓGICO DE ESPÉCIES NATIVAS DE CAMPO RUPESTRE

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ABSTRACT

In the past years, extreme droughts have been frequently recorded in several tropical regions. Plants in tropical mountains are particularly sensitive to changes in climate little is known about the plant physiological adjustments and responses to changes in rainfall patterns. Our objective was to evaluate the phenological and ecophysiological responses of six dominant species with contrasting life forms in *campos rupestres* under an extreme drought condition. We studied two rosettes species (*Vellozia nivea* and *Vellozia* aff. *variabilis*) and four shrubs (*Campomanesia pubecens, Eremanthus seidelii, Mimosa clausenii* e *Vernonia warmingiana*). We implemented a rainwater exclusion experiment with 12 plots (4x4 meters), in which eight was considered drought treatment and four control treatment. We monitored several aspects of the plant ecophysiology, such as gas exchange using instantaneous measurements and stable isotope as integrators of leaf functioning, pressure-volume curves, vulnerability curves, non-structural carbohydrates (NSC) in roots, growth, biomass and leaf phenology. Species showed a continuum of water use strategies, varying from anysohydric (*C. pubecens*) to completely isohydric (*E. seidelii*) species. The relationship between $\Delta^{18}O_{CELL}$ and gs_{MAX} was negative and significant only for isohydric species. The use of dual isotopic approach also was valid only for isohydric species. Across species, we found a strong positive relationship between $\delta^{18}O_{CELL}$ and NSC% for individuals under drought treatment, suggesting that the use of NSC% reserves in water stress conditions can affect leaf isotope composition. Our results suggest that in communities dominated to species with a great diversity of hydraulic strategies, such as the *campos rupestres*, $\delta^{18}O_{CELL}$ should be used with caution to infer physiological responses. We evaluated a congeneric pair of Velloziaceae species with contrasting strategies to cope with the erratic water availability in campos rupestres. One species is desiccation tolerant (DT) and the other is drought resistance (non-DT). The DT species showed high gs_{MAX} and low WUEi during the rainny season, showing a profligate water use. By contrast, the non-DT showed a conservative water use throughout the year. The DT species also increased the %NSC storage in roots during the prolonged drought, presumably a key mechanism that allow survival to desiccation in this species. The three shrub species (*C. pubecens*, *E. seidelii* and *M. clausenii*) showed different P_{50} (water potential that corresponds 50% of conductivity loss) and Ψ_{TLP} (water potential at turgor loss point). The biomass, in general, was little affected by our drought treatment. This three species showed a trade-off between the vulnerability to cavitation and NSC storage, in which, species more resistant to cavitation (more negative P50) showed lower NSC% storage in roots. Our results illustrate the diversity of strategies that plants might present to deal with drought and bring important contributions to understand the functioning of water-limited environments.

Keywords: Drought, hydraulic, non-structural carbohydrates, desiccation tolerance, stable isotope.

RESUMO

Eventos de secas extremas têm ocorrido com frequência nos últimos anos em regiões tropicais. Plantas em montanhas tropicais são particularmente sensíveis a mudanças no clima e pouco se sabe sobre a resistência e resiliência de comunidades vegetais ricas em espécies, como os campos rupestres, a condições climáticas extremas. Nosso objetivo foi avaliar as respostas fenológicas e ecofisiológicas e o crescimento de seis espécies abundantes de campo rupestre sob condições de seca extrema, sendo duas rosetas (*Vellozia nivea* e *Vellozia* aff. *variabilis*) e quatro arbustivas (*Campomanesia pubecens, Eremanthus seidelii, Mimosa clausenii* e *Vernonia warmingiana*). Para simular eventos de seca extrema, realizamos um experimento de exclusão de chuva, constituído por 12 parcelas (4x4 metros), sendo quatro controle e oito de exclusão, em uma area de campo rupestre no Parque Nacional da Serra da Canastra. Avaliamos o desempenho ecofisiológico das plantas submetidas a condições climáticas contrastantes mediante um conjunto de atributos morfofisiológicos, incluindo: trocas gasosas, composição isotópica do carbono ($\delta^{13}C_{CELL}$) e oxigênio ($\delta^{18}O_{CELL}$) da celulose foliar (medidas integradoras da assimilação de carbono e condutância estomática), curvas de vulnerabilidade à cavitação, curvas pressão-volume, carboidratos não-estruturais (CNE), crescimento e fenologia foliar. As espécies estudadas mostraram-se bastante resistente à seca, pois após 17 meses de exclusão de chuva não encontramos diferenças de crescimento e mortalidade entre populações nas parcelas controle e experimentais. As espécies apresentaram um contínuo de estratégias de uso de água, variando de espécies isohídricas (*E. seidellii*) à anisohídricas (*C. pubecens*). O uso da abordagem isotópica dupla ($\delta^{13}C_{CELL}$ e $\delta^{18}O_{CELL}$) foi válida para avaliar e predizer as respostas fotossintéticas e estomáticas à seca apenas para as espécies isohídricas. Além disso, a composição isotópica da celulose da folha pode ser influenciada pela mobilização de reservas de CNE nas raízes. As duas espécies congenéricas de Velloziaceae apresentaram estratégias constrastantes para lidar com a seca: uma espécie é tolerante à dessecação (TD) (*V. nivea*) e a *Vellozia* aff. *variabilis* é não-TD. A espécie TD apresentou maiores taxas de trocas gasosas durante a estação chuvosa e durante a seca prolongada entrou em estado de “dormência”, exibindo um comportamento oportunista de uso de água. Já a espécie não-DT apresentou uma estratégia conservadora de uso de água. Durante a seca, observamos um aumento no estoque de CNE% nas raízes na espécie TD, o que pode representar um mecanismo chave para sobrevivência dessa espécie durante os ciclos de dessecação e rehidratação. As três espécies arbustivas (*C. pubecens*, *E. seidellii*, *V. warmingiana*) apresentaram sistemas hidráulicos contrastantes, com diferentes pontos de perda de turgor (Ψ_{TLP}) e vulnerabilidade à cavitação (estimada mediante o P_{50} - potencial hídrico que corresponde à 50% de perda de condutividade). As espécies mais resistentes à cavitação (maior P_{50}) apresentam menores reservas de CNE nas raízes, sugerindo um trade-off entre atributos hidráulicos e de estoque de carbono. Nossos resultados trazem importantes contribuições para o entendimento de funcionamento de ecossistemas sazonais limitados por água e ilustram a diversidade de estratégias hidráulicas que conferem resiliência à seca.

Palavras-chave: seca, hidráulica de plantas, carboidrato não estrutural, tolerância à dessecação, isótopos estáveis.

ÍNDICE

"O sertão é do tamanho do mundo.
Sertão é dentro da gente.
O sertão é sem lugar.
O sertão não tem janelas, nem portas...
...Sertão: quem sabe dele é urubu, gavião, gaivota, esses pássaros: eles estão sempre no alto, apalpando ares com pendurado pé, com o olhar remedindo a alegria e as misérias todas."

(João Guimarães Rosa)

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Há pessoas que nos falam e nem as escutamos,
há pessoas que nos ferem e nem cicatrizes deixam,
mas há pessoas que simplesmente aparecem em nossas vidas
e nos marcam para sempre (Cecília Meireles).

LISTA DE ABREVIAÇÕES

Símbolo	Meaning	Significado
Ψ_{PD}	leaf pre-dawn water potential	potencial hídrico da madrugada da folha
Ψ_{MD}	leaf mid-day water potential	potencial hídrico do meio-dia da folha
Ψ_{TLP}	water potential at turgor loss point	potencial hídrico no ponto de perda de turgor
Ψ_{sFL}	osmotic potential at full turgor	potencial osmótico no turgor máximo
ε	elasticity modulus	módulo de elasticidade
$\delta^{13}C_{CELL}$	carbon isotopic composition from leaf cellulose	composição isotópica do carbono da celulose da folha
$\delta^{18}O_{CELL}$	oxygen isotopic composition from leaf cellulose	composição isotópica do oxigênio da celulose da folha
δD_{XYL}	deuterium isotopic composition from xylem water	composição isotópica do deutério da água do xilema
$\delta^{18}O_{XYL}$	oxygen isotopic composition from xylem water	composição isotópica do oxigênio da água do xilema
$\Delta^{18}O_{CELL}$	oxygen isotopic composition above source water	composição isotópica do oxigênio removendo a composição isotópica da fonte de água
A	CO_2 assimilation	fotossíntese mensurada sob saturação luminosa
gs	stomatal conductance	condutância estomática
WUEi	intrinsic water use efficiency	eficiência intrínsica de uso de água
P_{50}	water potential at which plants loss 50% of conductivity	potencial hídrico no qual a planta perde 50% da sua condutividade
P_{88}	water potential at which plants loss 88% of their conductivity	potencial hídrico no qual a planta perde 88% da sua condutividade
SM	safety margin	margem de segurança
NSC	non-structural carbohydrates	carboidratos não estrutural
VPD	vapor pressure deficit	déficit de pressão de vapor da atmosfera
PAW	plant available water	água disponível para as plantas
PAR	photosynthetically active radiation	radiação fotossinteticamente ativa
T	temperature	temperatura
RH	relative humidity	umidade relativa

INTRODUÇÃO GERAL

Secas extremas têm sido amplamente documentadas em regiões tropicais nos últimos anos (McDowell et al. 2008, McDowell et al. 2011, IPCC 2013). Apesar da incerteza em relação aos padrões futuros de precipitação, há previsões de aumento na freqüência e magnitude de eventos extremos, como secas severas nas regiões tropicais (IPCC 2013), o que virá a intensificar o estresse hídrico de plantas em vários ecossistemas naturais. A disponibilidade de água afeta a distribuição de espécies vegetais, a produtividade primária e a ciclagem de nutrientes (Engelbrecht et al. 2007, Brenes-Arguedas et al. 2009). Estudos mostram que secas sazonais e interanuais podem causar reduções no crescimento, aumento nas taxas de mortalidade de plantas e aumento na vulnerabilidade a queimadas em muitos ecossistemas (Korner 2003, Engelbrecht et al. 2007, Brenes-Arguedas et al. 2009, Bier et al. 2012).

Montanhas tropicais apresentam microclimas peculiares e altamente vulneráveis à mudanças do clima (Korner 2003, Nogués-Bravo et al. 2007, Colwell et al. 2008). No domínio Cerrado uma fisionomia bastante peculiar é o campo rupestre. Essas áreas apresentam flora bastante rica em espécies e com alto grau de endemismo (Walter et al. 2008, Negreiros et al. 2009). Estruturalmente são caracterizados pela predominância de um componente herbáceo-arbustivo com a presença eventual de arvoretas pouco desenvolvidas de até 2 metros de altura. Ocorrem em formações de afloramentos de quartzitos ou arenitos com solo pouco profundo, caracterizado por baixa disponibilidade de água e nutrientes (Jacobi et al. 2007). Climaticamente apresentam grande sazonalidade em relação à radiação solar, precipitação, evaporação e acentuadas oscilações térmicas diárias, principalmente nos meses de inverno (Giulietti et al. 1987; Jacobi et al. 2007).

A flora dessa fisionomia evoluiu submetida a uma grande variação sazonal de disponibilidade de água no solo e déficit de pressão de vapor atmosférico, além da baixa disponibilidade de nutrientes e frequência de queimadas. Sob essas condições, as plantas desenvolveram várias maneiras de lidar com a limitada e errática disponibilidade de água, surgindo, assim, inúmeras respostas adaptativas relacionadas à tolerância ao estresse hídrico, variando de espécies resistentes à seca – que mantém o metabolismo operando em condições sub-ótimas ao crescimento – até aquelas espécies fugitivas – que entram em estado de dormência nos períodos desfavoráveis ao crescimento, como as espécies tolerantes à dessecação, conhecidas como ressurgentes (Porembski & Berthlott 2000, Scarano 2002). A tolerância à dessecação é definida como a habilidade de alguns organismos em equilibrar seu potencial hídrico com o ambiente e, após a reidratação retornam às funções normais (Alpert 2000). Plantas ressurgentes são encontradas em habitats com precipitação esporádica, incluindo os campos rochosos e zonas áridas dentro de áreas tropicais e subtropicais (Porembski & Berthlott 2000; Rascio & La Rocca 2005). Os campos rupestres do Brasil são um centro de diversidade de espécies vasculares que apresentam tolerância à dessecação (Porembski & Bertholott 2000; Oliveira et al. 2005). Pouco se sabe sobre os limites de tolerância de plantas com diferentes estratégias ecológicas a condições climáticas ainda mais secas.

Plantas precisam controlar a perda de água para a manter homeostase do sistema hidráulico e se manter funcionalmente ativas (Oliveira et al. 2014). Elas dependem de um transporte de água eficiente e seguro pelo xilema da raíz até as folhas. No entanto, esse sistema hidráulico pode sofrer falhas, como a embolia, que é o resultado da entrada de ar nos condutos do xilema reduzindo a habilidade da planta em mover a água do solo até as folhas (Choat et al. 2012). A resistência ao embolismo representa um importante atributo relacionado à resistência à seca (Choat et al. 2012) e o ponto em que plantas perdem 50% da sua condutividade (P_{50}) tem sido utilizado como um atributo chave para definir a resistência de espécies à seca (Choat et al. 2012). No nível tecidual um outro importante atributo relacionado a resistência de plantas à seca é o potencial hídrico na qual a espécie perde o turgor (Bartlett et al. 2012). Espécies com potencial mais negativo de ponto de perda de turgor são consideradas mais resistantes à seca (Sack et al. 2003, Mitchell et al. 2008, Blackman et al. 2010, Bartlett et al. 2012).

O tempo e a magnitude de resposta das plantas a eventos de seca podem variar entre espécies e entre as estratégias de uso de água apresentada por elas (McDowell et al. 2011; et al. 2012). Tem-se dois extremos de estratégias de uso de água, desde plantas isohídricas, que mantém constante o potencial hídrico em diferentes condições de conteúdo de água no solo, contudo apresentam uma forte regulação estomática; até aquelas espécies com um comportamento anisohídrico, as quais suportam potenciais hídrico bastante negativos, mantendo as trocas gasosas (West et al. 2007; West et al.

2012; McDowell et al. 2011). Espécies anisohídricas são consideradas mais resistentes à cavitação (Plaut et al. 2012; Plaut et al. 2013).

Em relação às respostas das plantas a eventos extremos de seca, espécies resistentes ao estresse, que ocorrem em ambientes pobres em recursos, como é o caso dos campos rupestres, geralmente apresentam baixas taxas intrínsecas de crescimento e respostas principalmente fisiológicas à variação na disponibilidade de recursos (Grime & Mackey 2002, Alpert & Simms 2002). Geralmente, as primeiras respostas das plantas as mudanças na disponibilidade de recursos são ajustes fisiológicos e tem sido relacionados com a habilidade de persistência de uma espécie em ambientes heterogêneos (Xu et al. 2009). O controle da condutância estomática é talvez o principal mecanismo de ajuste hidráulico, modulando as trocas de vapor de água com a atmosfera e o fluxo de carbono (Schuldt et al. 2011).

Uma importante ferramenta que tem sido utilizada para entender as respostas fisiológicas a seca apresentada pelas espécies e a relação dessas espécies com o uso de recursos são os isótopos estáveis (Dawson et al. 2002, Grams et al. 2007, Moreno-Gutiérrez et al. 2012). Em contraste com as técnicas pontuais de medidas de trocas gasosas, a análise da composição isotópica do carbono da celulose da folha ($\delta^{13}C_{CEL}$) integra à atividade fotossintética por todo o período em que os tecidos foliares foram sintetizados (Dawson et al. 2002). A análise da composição isotópica do oxigênio da celulose foliar ($\delta^{18}O_{CEL}$) associada a $\delta^{13}C_{CEL}$ tem propiciado o entendimento do funcionamento foliar em longo prazo, sendo possível inferir sobre a fotossíntese máxima (A), condutância estomática (gs) e eficiência intrínseca do uso de água (EUAi=A/gs) (Grams et al. 2007). Esta é uma abordagem recente e promissora para entender como a A e gs respondem a variações nas condições ambientais, podendo-se inferir sobre o balanço de carbono e balanço hídrico (Grams et al. 2007). As razões isotópicas do C e do O da celulose foliar refletem melhor as estratégias de uso de água em ecossistemas fortemente sazonais, limitados por água, no qual o controle estomático tem um importante papel no controle do balanço hídrico e de carbono (Moreno-Gutierrez et al. 2012).

Nessa tese o nosso objetivo foi avaliar experimentalmente as respostas a seca apresentada por seis espécies abundantes de campo rupestre *in situ* (Figura 1 e 2). Para isso, utilizamos uma série de atributos preditores da resposta à seca, como o P_{50} e o potencial de perda de turgor e monitoramos o efeito da exclusão de água da chuva nas trocas gasosas, dinâmica fenológica e de crescimento. Abaixo apresentamos o objetivo de cada um dos capítulos:

*No primeiro capítulo discutimos a diversidade de estratégias de uso de água apresentada por seis espécies abundante no campo rupestre e o uso de isótopos estáveis (composição isotópica do carbono ($\delta^{13}C_{CELL}$) e oxigênio ($\delta^{18}O_{CELL}$) da celulose da folha como preditores das estratégias hidráulicas e as respostas fisiológicas.

*No segundo capítulo trabalhamos com duas espécies congenéricas de Velloziaceae com estratégias hidráulicas contrastantes. Uma espécie é tolerante a dessecação enquanto a outra é resistente a seca. O nosso objetivo foi avaliar o efeito do atributo ressurgência no desempenho ecofisiológico do gênero em condições contrastante de disponibilidade hídrica.

*No terceiro capítulo nosso objetivo foi avaliar a influência de atributos hidráulicos relacionados à tolerância a seca no crescimento de três espécies arbustivas em condições contrastantes de disponibilidade hídrica.

Figura 1. Experimento de exclusão de água da chuva em vegetação de campo rupestre no Parque Nacional da Serra da Canastra. Parcelas com o telhado coberto representam as parcelas para exclusão de água da chuva e aquelas com o telhado aberto representam as parcelas controle.

Figura 2. Espécies abundantes de vegetação de campo rupestre que foram selecionadas para o estudo. A) *Campomanesia pubecens*; B) *Eremanthus seidelii*; C) *Vernonia warmingiana*; D) *Mimosa clausenii*; E) *Vellozia* aff. *variabilis*; F) *Vellozia nivea*

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CHAPTER 1

Is the dual isotopic approach useful to infer physiological responses of isohydric and anysohydric species to changes in water availability in a neotropical savanna?

Is the dual isotopic approach useful to infer physiological responses of isohydric and anysohydric species to changes in water availability in a neotropical savanna?

ABSTRACT

Plants from water-limited environments have evolved several morphological and physiological adaptations to maximize carbon gain and maintain metabolic homeostasis during periods of low water availability. A functional spectrum of stomatal regulation strategies is expected in natural communities, from isohydric to fully anisohydric behaviors. The simultaneous analysis of $\delta^{13}C$ and $\delta^{18}O$ leaf cellulose (dual isotopic approach) has been suggested as robust tool to determine the diversity of stomata and photosynthetic responses to drought in natural communties. This study aims at evaluating the diversity of leaf-level responses of dominant *campos rupestres* species to drought and the usefulness of the dual isotope model to infer species physiological responses. To impose extreme drought conditions, we implemented a rainwater exclusion experiment, and monitored several aspects of the ecophysiology of six abundant species of contrasting life-forms. Changes in leaf gas exchange, especially stomatal conductance, as a function of variation in mid-day water potential (Ψ_{MD}) revealed a continuum of water use strategies, from anysohydric to completely isohydric species. We found a negative relationship between $\Delta^{18}O_{CELL}$ and gs_{MAX} only for isohydric species. The use of dual isotopic approach was also valid only for isohydric species. We found a strong positive relationship between $\delta^{18}O_{CELL}$ and NSC% for individuals in the drought treatment, suggesting that the use of non-structural carbohydrates (NSC%) reserves in water stress conditions can affect leaf isotope composition. Our results suggest that in communities dominated by species with many water use strategies, such as the *campos rupestres*, $\delta^{18}O_{CELL}$ should be used with caution to infer physiological responses.

Key words: water use strategy, non-structural carbohydrates, oxygen, stomatal conductance

INTRODUCTION

Extreme drought events have become more common in tropical regions (IPCC 2013, McDowell et al. 2008, Beier et al. 2012) and some climate models predict droughts of greater magnitude and duration for the next century (IPCC 2013). Tropical mountains are amongst the most susceptible environments to climate change in the world (Nogués-Bravo et al. 2007; Beier et al. 2012). They have an unquestionable role in promoting regional and global diversity due to the discontinuous distribution of edaphic and climatic variations (Burke 2003). Of particular interest are the *campos rupestres* in Central Brazil, a savanna vegetation mosaic located above 1000 m that harbors enormous plant diversity and lots of endemic species, perhaps the most diverse region within the Cerrado biodiversity hotspot (Burke 2003; Jacobi et al. 2007). High seasonality of rainfall, high evaporative demand, and impermeable soils with low water retention characterizes the abiotic conditions at this environment (Porembski & Barthlott 2000; Scarano 2002; Jacobi et al. 2007) and we know little about the diversity of water use strategies of dominant species.

Plants from water-limited environments have evolved several morphological and physiological adaptations to improve water acquisition/use and maintain their homeostasis during periods of low water availability (Porembski & Barthlott 2000, Oliveira et al. 2005, Jacobi et al. 2007, Peñuelas et al. 2011, Oliveira et al. 2014). Drought resistant species keeps their metabolism operating at sub-optimal conditions for growth, while fugitive species enter a state of dormancy during periods unfavorable to growth (Larcher 2000, Porembski & Bertholott 2000). Stomatal regulation plays a central role in controlling plant water use under varying conditions of water availability (Schwinning & Ehleringer 2001, Damour et al. 2010). Stomata work as pressure regulators that modulate the water vapor exchange and carbon flux between the plant and the atmosphere, preventing xylem tension from reaching runaway cavitation thresholds (Damour et al. 2010, Schuldt et al. 2011). Plants exhibit a continuum of stomatal regulation strategies, from isohydric to fully anisohydric behavior (Tardieu & Simonneau, 1998; McDowell et al. 2008, McDowell et al. 2011, Plaut et al. 2013). Isohydric species have a strong regulation on stomatal conductance and maintain nearly constant water potential despite of reductions in soil water potential. Typically, isohydric plants exhibit a high stomatal sensitivity to evaporative demand. In contrast, anisohydric plants allow midday water potential to decline and maintain gas exchange even in drought conditions (Tardieu & Simonneau, 1998, McDowell et al. 2008, Plaut et al. 2012). These water use strategies are related to species capacity to survive during drought in many ecosystems (Meinzer et al. 2009, West et al. 2012, Plaut et al. 2012, McDowell et al. 2013).

The use of a dual isotopic approach (carbon - $\delta^{13}C$ and oxygen - $\delta^{18}O$ isotopic composition from leaf cellulose) has been regarded as a useful tool to determine the diversity of plant responses to drought in water-limited environments (Scheidegger et al. 2000, Dawson et al. 2002). The carbon isotopic composition ($\delta^{13}C_{CELL}$) of plant cellulose provides an integrative estimate of the ratio of internal to atmosphere CO_2 concentration (ci/ca) (Farquhar et al. 1982). The ci/ca is a function of the leaf intercellular air space CO_2 supply by stomatal conductance - gs (CO_2 influx) and the demand for CO_2 by photosynthetic capacity within the leaf (CO_2 consumption) (Farquhar et al. 1989, Cernusak et al. 2013). Variation in $\delta^{13}C$ can be caused by changes on gs, on photosynthetic rate, or on both of them (Scheidegger et al. 2000, Grams et al. 2007), with the dual isotopic approach, the combined analyses of C with O allow us to infer the causes of variation in $\delta^{13}C$ (Scheidegger et al. 2000, Grams et al. 2007). Stable oxygen isotope composition of plant cellulose ($\delta^{18}O_{CELL}$), on the other hand, represents an integrated record of environmental and stomata regulation of water loss during the period of plant photosynthesis and cellulose formation (Barbour 2007, Cernusak et al. 2009, Kahmen et al. 2010, Song et al. 2014). Generally, the $\delta^{18}O_{CELL}$ is negatively related with the gs (Scheidegger et al. 2000, Grams et al. 2007, Barbour 2007, Moreno-Gutiérrez et al. 2012). However, recent studies have shown that the interpretation of $\delta^{18}O_{CELL}$ in uncontrolled conditions can be challenging, especially in plants with a highly dynamic of non-structural carbohydrates (NSC) pools (Barnard et al. 2012, Song et al. 2014). The pool size of NSC can influence the oxygen atoms exchange during the cellulose synthesis, reflecting in leaf $\delta^{18}O_{CELL}$ (Song et al. 2014, Ellsworth & Sternberg 2014). This influence of the NSC pool on $\delta^{18}O_{CELL}$ is particularly important under the climate change scenario, in which different responses (increases, decreases and no changes) on the NSC storage are expected under drought stress (Sala et al. 2010, Piper & Fajardo

2011, Galvez et al. 2011, Mitchell et al. 2013). Is the dual isotope model useful to predict physiological responses for species with contrasting water use strategies (isohydric x anisohydric) and contrasting NSC dynamics?

To evaluate the diversity of drought responses of dominant species in *campos rupestres* and the usefulness of the dual isotope model to infer species physiological responses, we implemented a rainwater exclusion experiment, and monitored several aspects of the ecophysiology of six abundant species of contrasting life-forms. We addressed the following questions: 1) Is the iso/anihydric framework a useful scheme to predict drought response/resistance in *campos rupestres*? 2) Can we use the dual isotopic approach ($\delta^{13}C_{CELL}$ and $\delta\ ^{18}O_{CELL}$) to infer changes in gas exchanges in isohydric and anisohydric species? 3) Is the variation across species on $\delta\ ^{18}O_{CELL}$ a reliable indicator of stomatal regulation? 4) Is the NSC% storage in roots related to leaf $\delta\ ^{18}O_{CELL}$ across species? We expect to find a diversity of water use strategies among the studied species, varying from isohydric species to anisohydric, with differential drought resistance in this rich environment. The dual isotopic approach is a reliable indicator of gas exchange for species in both extremes of water use strategies. We also expected a high amount of NSC% storage in roots of studied plants, in which may influence the $\delta\ ^{18}O_{CELL}$.

MATERIAL AND METHODS

Study area and species

The study was carried out in a *campo rupestre* community, located in Serra da Canastra National Park (Parque Nacional da Serra da Canastra - PNSC), São Roque de Minas, Minas Gerais, Brazil. The altitude in PNSC varies from 800 to 1500 meters. The maximum average temperature varies from 18ºC to 31ºC and the minimum average varies from 9.5ºC to 17.5ºC. In the last twenty years the annual average temperature was 21.62ºC and the average of annually accumulated precipitation was 1239.3 mm (data from CEMIG, São Roque de Minas meteorological station). The climate is typically seasonal: the winter is extremely dry, with low or no rain from June to September; and the summer is wet. Soils are derived from weathering of quartzite-sandstone and are generally shallow, coarse textured, and nutrient-poor (Benites et al. 2007).

We studied six abundant species in the area that belong to different functional groups (Table 1). We measured gas exchanges, water potential, and δ^{13}C and δ ^{18}O of leaf cellulose to evaluate the diversity of water use strategies in this community.

Table 1. Characteristics of the focal studied species inside the plots. Root systems were classified by Monteiro (2013).

Specie	Family	Code	Growth form	Root system	Average height*
Campomanesia pubecens Mart.	Myrtaceae	Cam.pub	Small Subshrubs	Shallow roots	52
Eremanthus seidelii MacLeish & Schumacher	Asteraceae	Ere.sei	Tall shrubs	Dimorphic roots	80
Mimosa clausenii Benth	Fabaceae	Mim.cla	Subshrubs	Dimorphic roots	11
Vellozia nivea L.B.Sm & Ayensu	Velloziaceae	Vel.niv	Rosettes	Fasciculate roots	44
Vellozia aff. *variabilis* Mart. ex Schult.	Velloziaceae	Vel.var or Vel.sp	Rosettes	Fasciculate roots	46
Vernonia warmingiana Baker	Asteraceae	Ver.war	Subshrubs	Shallow roots with lignotuber	43

*Average height (cm) corresponds to plants measured during the study.

Experimental design

We implemented 12 experimental plots (4 controls and 8 rain-out shelters) to evaluate plant response to extreme dry conditions. Rainfall was excluded from April 2011 to August 2012 (16 months of rainfall exclusion). Rain-out shelters were constructed with metalon iron and the roofs were covered with transparent polycarbonate, following the designed used in West et al. (2012). In the experimental plots, roofs were closed, while in the controls, roofs were maintained open. The height of the structures was 2 meters above soil level. Around each plot, we dug trenches (50 cm deep) to avoid plants from accessing water from outside the plots and we impermeabilized the trenches with plastic. One lateral side of the plot was closed with transparent plastic to minimize the wetting effect of horizontal precipitation. We used rocks as foothold to take the measurements and avoid trampling inside the plots (Figure 1).

Climate characterization and soil moisture measurements

Soil moisture was monitored by eight soil water content sensors (TDR - Campbell Scientific CS616-L150 13932-17), four in each condition (control and rain water exclusion plots). Soil water sensors were installed only in the superficial layer (1-30 cm), due to the high amount of rocks in the soil. A complete meteorological station with temperature and air humidity (Campbell Scientific - Vaisala), photosynthetic active radiation (Campbell Scientific LI190SB-L15 8721-45), and pluviometer (Campbell Scientific TB4-L15) was installed in the area in December 2011. We also installed sensors of temperature and air humidity sensors (HOBO Pro Series - ONSET) inside the experimental plots. Before the implementation of micrometeorological station we monitored the environmental conditions of the study area and inside the plots by installing a group of sensors of temperature and air humidity (HOBO Pro Series - ONSET) and soil water content (Soil Smart Sensor - ONSET).

We used temperature (T) and relative humidity (RH) data to calculate the atmosphere vapor pressure deficit (VPD, kPa), according to the following equation: VPD=0,611375e^(t*(1-RH/100)), where t=[917.502*(T)/(240.97+T)] (Jones 1992). Plant available water (PAW) was calculated as the difference between the soil water content (SWC) and minimum soil water content (SWC_{MIN}) registered by the TDR sensors, according to the equation: $PAW=SWC-SWC_{MIN}$.

Figure 1. Study area: vegetation of *campos rupestres* at Serra da Canastra National Park. Plots with closed roofs represent the drought treatment and the plots with open roofs represent the control treatment.

Leaf water potential and gas exchange

We measured pre-dawn water potential (Ψ_{PD}); the mid-day water potential (Ψ_{MD}); the stomatal conductance (gs) in period that correspond the maximum CO_2 assimilation (A). The leaf water potential was measured with a Pressure Chamber (PMS Instruments, Corvallis, OR, USA) and the gas exchange with a portable IRGA system (CIRAS 2, PP Systems, Inc., Amesbury, MA, USA). The gas exchange (A and gs) was measured during clear and sunny days, between 8:30 and 11:00 a.m. We performed curves of CO_2 assimilation in response to the photosynthetic active radiation and we used the radiation that corresponds to the maximum assimilation (A_{MAX}) (PAR=1200 µmol quanta $m^{-2}s^{-1}$ - LED light source). The air CO_2 concentration was maintained at 380 ppm; and we kept the leaf temperature and humidity the same at the ambient.

These measurements were taken during five different periods: in the rainy season (February 2011/2012); in the transition between the rainy and dry season (April 2012 and June 2012, completing 12 months of rain exclusion treatment); and in the dry season (August 2012), when we ended the rain exclusion experiment. We measured five individuals per species in each condition. Before the implementation of rainwater shelters (February 2011), individuals/species in all plots showed similar gs, A, and Ψ.

To compare the minimum values observed of gs among species, we relativized the gs (gs_{REL}). For this we used the gs obtained in dry season (minimum values) and it was relativized based in maximum values obtained for each species during the study. Species with low values of gs_{REL} were those with huge variation between minimum and maximum values. We performed this standardization due to different minimum values of gs among species.

To determine the water use strategy (iso/anisohydric) of the six species, we plotted all Ψ_{MD} data versus $gs_{MAX.}$ Species with an anysohydric behavior showed a marked decrease in $\Psi_{MD,}$ while isohydric species maintained Ψ_{MD} nearly constant with a decrease in gs (Tardieu & Simonneau, 1998). In addition, to confirm the water use strategies we evaluated the stomatal sensitivity to changes in air vapor pressure deficit (VPD) and $\Psi_{PD,}$ as a proxy of soil water potential (Oren et al. 1999). Anisohydric species generally exhibit less stomatal response to VPD and Ψ_{PD}, allowing large fluctuations in Ψ_{MD} (Franks & Farquhar 2007).

Dual isotopic approach: carbon ($\delta^{13}C_{CELL}$) and oxygen ($\delta^{18}O_{CELL}$) isotopic composition from leaf cellulose

Cellulose is the major structural carbohydrate in plants, this single and purified component of plant tissue is most often recommended for isotopic analysis of organic matter (Flanagan & Ehleringer 1991, Grams et al. 2007). The combined analysis of $\delta^{13}C_{CELL}$ and $\delta^{18}O_{CELL}$ is an integrative approach to infer leaf functioning (photosynthetic capacity and stomatal conductance) and integrate all the environmental effects during the period in which leaf tissues were synthetized and maintained functioning (Scheidegger et al. 2000, Grams et al. 2007).

We collected leaves for isotopic analyses in three different periods: rainy season, before the start of the drought experiment (February 2011), transition between rainy to dry season (June 2012), peak of dry season and end of experiment (August 2012). In these periods we also measured instantaneous leaf gas exchange and leaf water potential. Leaves collected during June and August 2012 were produced after the implementation of the rainout shelters. Leaf samples were dried at 65°C for 24-48 hours and ground to extract the alpha-cellulose. Alpha-cellulose was extracted from leaf-homogenized material using the method described by Gaudinski et al. (2005). The cellulose extraction was carried out at the Functional Ecology Laboratory at the University of Campinas, Brazil.

To analyze the $\delta^{13}C_{CELL}$, we weighted the samples in tin capsules (0.3 to 0.6 mg), placed them in an elemental analyzer (ANCA/SL elemental analyzer) and carried into a mass spectrometer (Finnigan MAT Delta PlusXL IRMS). For $\delta^{18}O_{CELL}$ analysis, we weighted the samples in silver capsules (0.6 to 0.9 mg), and placed them in varioPYRO cube Elementar connected to a mass spectrometer (Delta V Plus Isotope Ratio Mass Spectrometer (IRMS)). We performed the isotopic analyses at the Center for Stable Isotope Biogeochemistry, University of California-Berkeley, USA. The relationship between the rare isotopes and the most abundant isotope is expressed by R notation ($^{13}C/^{12}C$ or $^{18}O/^{16}O$). The international standard for carbon is V-PDB and oxygen is V-SMOW.

To determine the isotopic composition enrichment above that of the source of water used by plants ($\Delta^{18}O_{CELL}$), we evaluated the isotope composition of hydrogen (δD_{XYL}) and oxygen ($\delta^{18}O_{XYL}$) of xylem water in non-photosynthetic plant tissues. We collected two or three segments of suberized stems in three different periods: during the wet season (November 2011), transition between seasons (June 2011) and the dry season (August 2012). We collected those stem segments from five individuals/species per conditions. The segment samples were immediately placed in screw-capped vials, wrapped with parafilm and stored in freezer until water extraction to prevent possible evaporation. We used the cryogenic distillation method (Ehlenringer et al. 2000) to extract the water from soil and plant material, at the Laboratory of Isotopic Ecology at CENA, USP, Piracicaba, SP, Brazil. The δD and $\delta^{18}O$ analyses were carried-out at the

Stable Isotope Ratio Facility for Environmental Research (SIRFER), in University of Utah, USA. The technique consists in measuring the ratio of oxygen ($^{18}O/^{16}O$) and deuterium ($^2H/H$) using an elementar analyzer convertor at high temperature coupled to a mass spectrometer (Thermo Finnigan Delta Plus XL). We considered the monthly average of $\delta^{18}O_{XYL}$ using data for individuals both in control and under the rainwater exclusion treatment. We calculated the $\Delta^{18}O_{CELL,}$ using the equation $\Delta^{18}O_{CELL}=\delta^{18}O_{CELL}-\delta^{18}O_{XYL}$ (Barbour et al. 2004, Barbour 2007, Moreno-Gutiérrez et al. 2012). Barbour et al. (2004) and Barbour (2007) recommend the removal in source water ($\delta^{18}O_{XYL}$) from $\delta^{18}O_{CELL}$ because this simplifies interpretation and enables identification of variation due to leaf water enrichment and isotopic exchange. The use of $\Delta^{18}O_{CELL}$ is particularly important in situations that the $\delta^{18}O_{XYL}$ vary among species, and this is the case for the investigated species.

To investigate the temporal dynamic of leaf gas exchange, by evaluating the $\delta^{13}C_{CELL}$ and $\Delta\ ^{18}O_{CELL}$ trajectory, we used the dual isotopic approach proposed by Scheidegger et al. (2000) and modified by Grams et al. (2007). To do that, we used data from the beginning (February 2011) and the end of the experiment (August 2012). We calculated the variation in the isotope values between February 2011 and August 2012; and the values of February were considered the reference (point zero). To test the applicability of the model output (Grams et al. 2007), we traced the trajectory of changes in our instantaneous measurements of gas exchange in this period using the same procedure performed for the isotopic data. We plotted the trajectories for all six studied species, under the control and the water limited conditions. For *Campomanesia pubecens* we did not have data from February 2011, so we used data from June 2012 as the point zero.

Non-structural carbohydrates pool in roots

We quantified the pool of non-structural carbohydrates per dry mass in roots (NSC%) to relate to $\delta^{18}O_{CELL,}$ because recent evidence suggests that NSC pool affects $\delta^{18}O_{CELL}$ (Song et al. 2014). We collected roots from five individuals per treatment per species to quantify the NSC% storage. We followed the enzymatic method proposed by Sevanto et al. (2014) to analyze the NSC content. Here, NSC is defined as free, low molecular weight sugars (glucose, fructose and sucrose) and starch. Immediately after the collection, samples were microwaved to stop the enzymatic activity. After that, samples were oven-dried at 70°C for 24–48 h and ground to fine powder. We prepared approximately 12 mg of plant material with 1.6 mL of distilled water to the analyzes. We used amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich) to digest total NSC to glucose; and invertase, glucose hexokinase kits (GHK) and phosphorus glucose (Sigma-Aldrich) to quantify the low molecular weight sugars. The concentration of free glucose was determined photometrically in a 96-well microplate spectrophotometer (BioTek, Epoch). NSC values are expressed as percent of dry matter. For method details, see Sevanto et al. (2014).

Statistical analysis

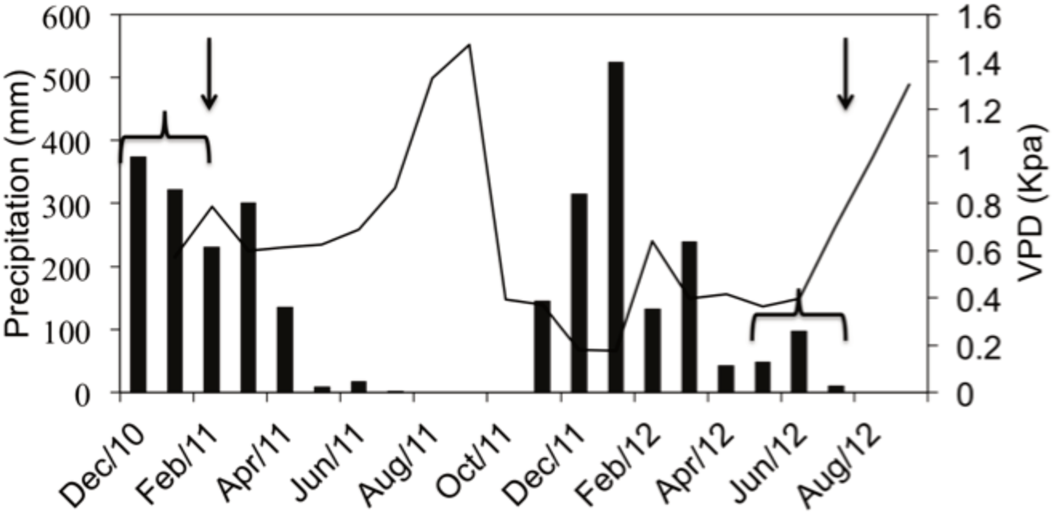
To determine differences in leaf gas exchange and water potential variables, and plant available water between treatments control and drought we evaluated the effect size (ES) as used by West et al. 2012, using the equation: $ES = \frac{(MC-MD)}{\sqrt{e^2 C+e^2 D}}$, where MC and MD represents the mean values in control and drought treatments, respectively. e represents the standard errors in control (C) and drought (D) treatments. Values above +or-2 represent significant differences between treatments.

To test the relationship between VPD or Ψ_{PD} and gs_{MAX} we performed a multiple regression. To compare the effects of each variable, the treatment and interaction between the variable and treatment on gs_{MAX} we used ANCOVA. Linear regression was used to investigate the relations between $\Delta^{18}O$ and gs_{MAX} for each species. We also used linear regressions to evaluate the relations between $\delta^{18}O$ and gs_{MAX}, NSC% and gs_{REL} (gs relativized) across species. The statistical analyses were prepared with the open-source program R (R Core Team 2012, http://www.R-project.org) and Statistica software (version 7.0).

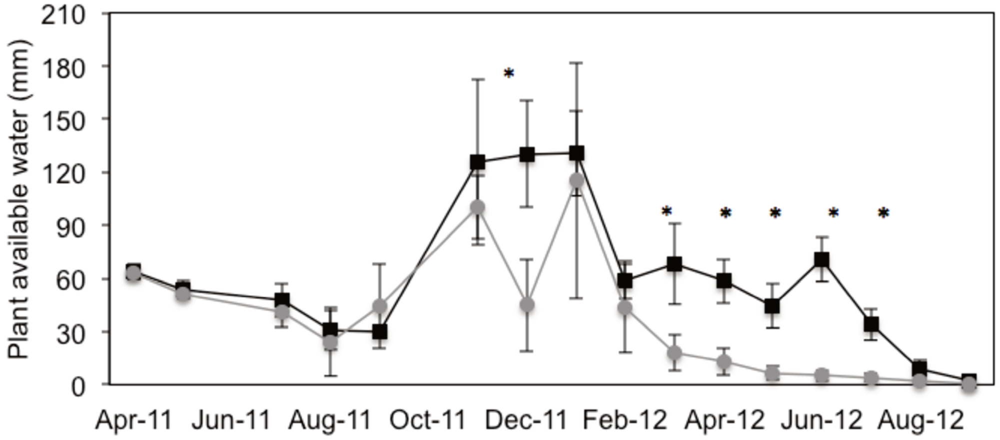
RESULTS

Environmental conditions

The first year of study (2011) was characterized by an intense dry season, with no precipitation and high VPD from May until October. The second year showed a similar pattern, but 96.52 mm rainfall fell in June (Figure 2A). The rainout shelters significantly reduced PAW in December 2011 and from March until August 2012 (Figure 2B). From December 2011 to August 2012 there was a decrease of 34% on PAW in the exclusion plots compared to the control ones.



A



B

Figure 2. A) Total precipitation per month (bars) and monthly average of atmosphere vapor-pressure deficit (VPD) (lines) during the study in *campo rupestre* at Serra da Canastra National Park. The arrows mark the month that we collected leaves to isotopic composition for dual isotopic approach and bracket mark the period that those leaves were produced. B) Plant available water (PAW-mm) per condition from April 2011 to August 2012 for plots in both treatments, control and drought. Black squares represent average in control plots (n=4) and gray circle indicate average in rain exclusion plots

(n=4); bars represent the standard error. The asterisks indicate significant differences ($p<0.05$; test t-student) between conditions.

Leaf gas exchange and water potential revealing patterns of water use strategies: isohydric x anysohydric

Our drought treatment caused reductions on gs, A, Ψ_{PD} and Ψ_{MD} in some months for most studied species (Table S1 - supl. material). Changes in leaf gas exchange, especially gs, as a function of variation in Ψ_{MD} unravel a continuum of strategies, from anysohydric to completely isohydric species. *Campomanesia pubecens* showed an anysohydric strategy, as the Ψ_{MD} fell to negative values while gs_{MAX} was maintained relatively constant. This species, reached the most negative value of Ψ_{MD} during the study (Ψ=-5.2 MPa). At the other end, *E. seidelii*, maintained constant $\Psi_{MD,}$ while showing strong reductions on $gs_{MAX,}$ both in drought and control treatments, representing an isohydric strategy. The others species showed intermediate responses between these two extreme of strategies. *V. warmingiana* and *M. clausenii* tended to the anisohydric behaviour, while the two *Vellozia* species tended to an isohydric strategy (Figure 3).

The results of the multiple regressions between VPD and Ψ_{PD} versus gs confirmed the water use strategies described above. In control treatment significant effects of VPD and Ψ_{PD} on gs were observed only in *E. seidelii* and in *V. nivea*. In drought treatment, VPD influenced gs_{MAX} in *C. pubecens* and *E. seidelii.* The Ψ_{PD} have significant effects (positively related) on gs in *E. seidelii*, *M. clausenii*, *V. nivea* and *V. warmingiana* (Table 2). The relationship between Ψ_{PD} versus gs and VPD versus gs did not differ between treatments and in the interaction between the variable (Ψ_{PD} or VPD) and treatments (Table S2 - suppl. material).

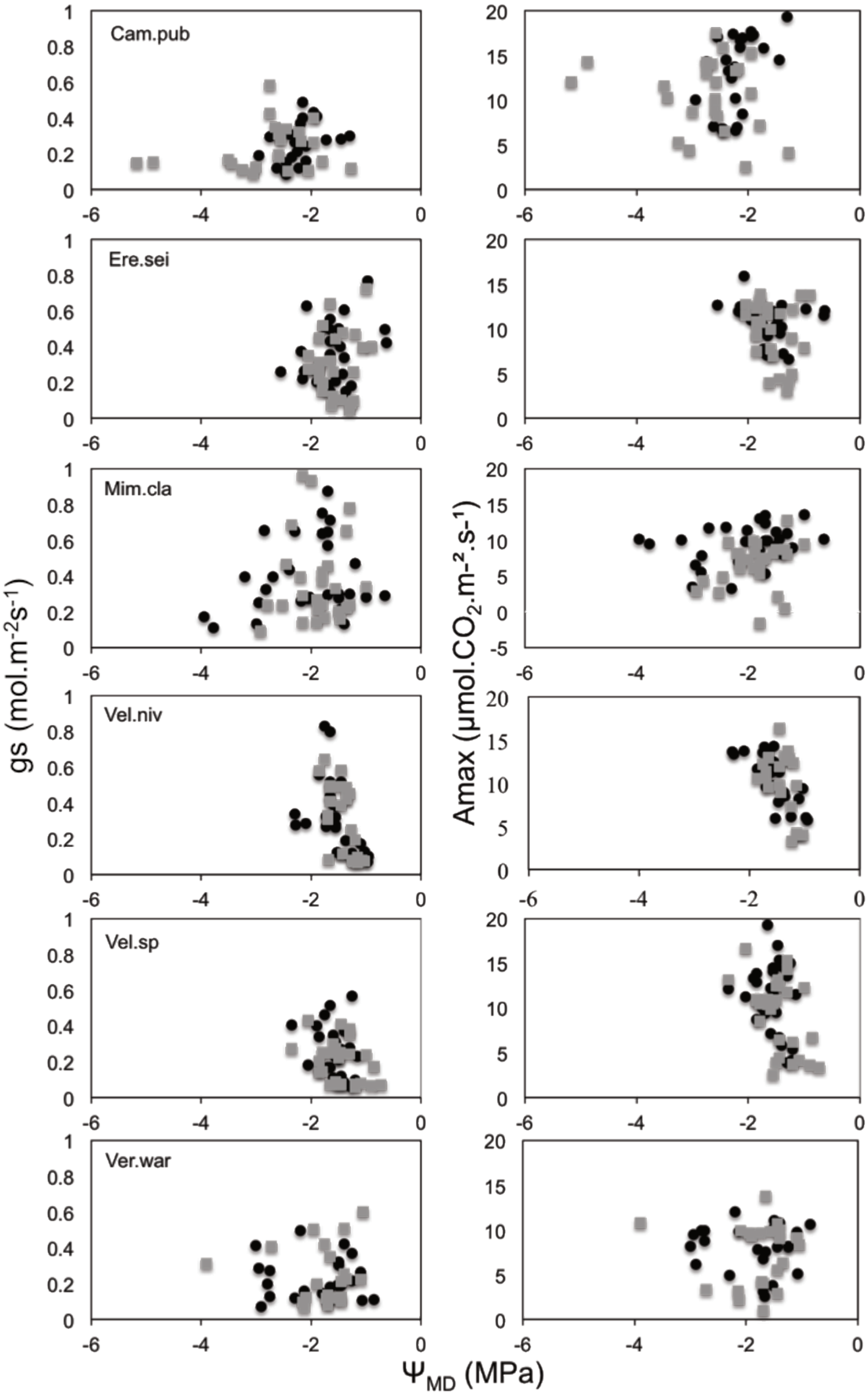


Figure 3. Maximum stomatal conductance (gs$_{MAX}$ - mol.m^{-2}.s^{-1}) versus mid-day water potential (Ψ_{MD} - MPa) for six abundant species in *campos rupestres* at Serra da Canastra

National Park. Black circles represent individuals in control treatments and grey squares indicate individuals in drought treatments. Species codes are listed on table 1.

Table 2. Multiple regression between stomatal conductance (gs) and vapor pressure deficit (VPD), pre-dawn water potential (Ψ_{PD}) for six species in control and drought treatments in a *campo rupestre* vegetation. gs is in mol.m^{-2}.s^{-1}, VPD is kPa and Ψ_{PD} in MPa. Significant differences are marked in bold. The p-value represents the significance of the multiple regression; the p-Ψ_{PD} represents the significance of the relationship between pre-dawn water potential versus gs_{MAX} and, the p-VPD the significant for the relationship between VPD versus gs.

	Control treatment				**Drought treatment**			
Species	**R2**	**p-value**	**p-Ψ_{PD}**	**p-VPD**	**R2**	**p-value**	**p-Ψ_{PD}**	**p-VPD**
Cam.pub	0.07	0.59	0.32	0.81	0.42	**0.01**	0.73	**<0.01**
Ere.sei	0.44	**0.01**	**0.04**	**<0.01**	0.64	**<0.01**	**<0.01**	**<0.01**
Mim.cla	0.02	0.86	0.92	0.59	0.35	**0.04**	**0.01**	0.57
Vel.niv	0.50	**<0.01**	**<0.01**	0.13	0.78	**<0.01**	**<0.01**	0.11
Vel.var	0.06	0.67	0.41	0.78	0.15	0.34	0.19	0.29
Ver.war	0.15	0.45	0.36	0.84	0.35	**0.04**	**0.01**	0.44

Dual isotopic approach

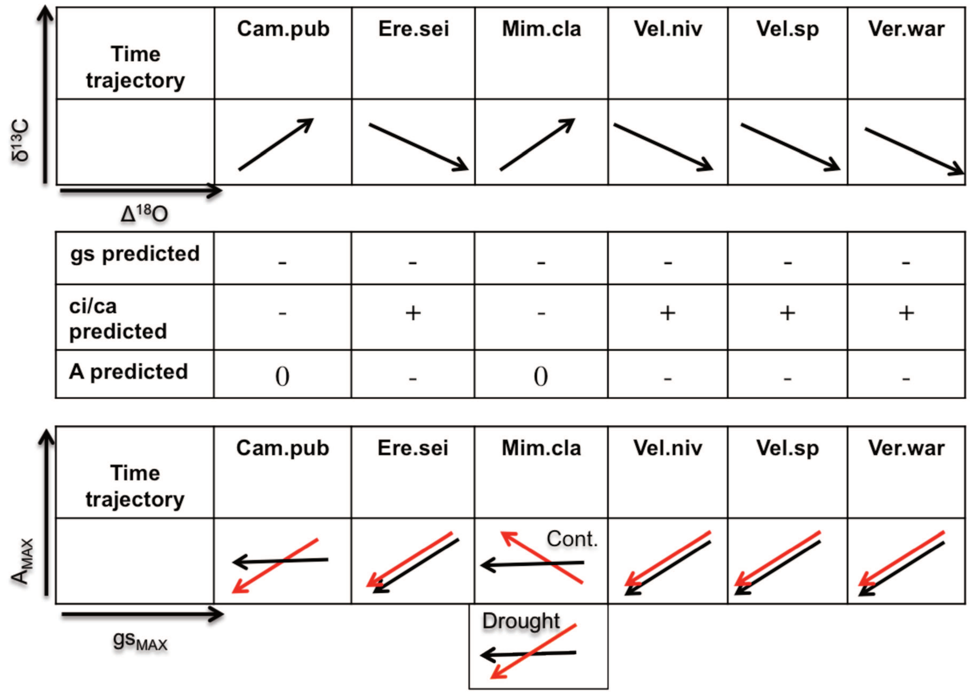
Before the beginning of the experiment (February 2011), the $\delta^{13}C_{CELL}$ and $\Delta^{18}O_{CELL}$ were similar between control and exclusion plots. The exception was *V. warmingiana* that showed more enriched ^{18}O in the exclusion plots. In the dry season (August 2012), there was no difference in $\delta^{13}C_{CELL}$ and $\Delta^{18}O_{CELL}$ between treatments. The exception was *V. nivea,* in which individuals under water exclusion plots exhibited more enriched ^{13}C and ^{18}O.

$\Delta^{18}O_{CELL}$ and gs were negatively correlated for *E. seidelii*, *V. nivea* and *V.* aff. *variabilis* for individuals in both treatments (Table 3). The other species did not show a significant relationship between these two traits. *M. clausenii* exhibited a significant negative relation between $\Delta^{18}O_{CELL}$ and gs_{MAX} only for individuals in drought treatment plots (Table 3).

As we did not find differences in trajectories of variation in isotopic data and gas exchange measurements between treatments (except for *M. clausenii*), the trajectories of variation in $\delta^{13}C_{CELL}$ and $\Delta^{18}O_{CELL}$ were calculated using the average data from control and drought treatments together. The variation in $\delta^{13}C_{CELL}$ x $\Delta^{18}O_{CELL}$ from wet (February 2011) to dry season (August 2012) was similar among species (Figure 4). *E. seidelii, V. nivea*, *V.* aff. *variabilis* and *V. warmingiana* became more depleted in ^{13}C and more enriched in ^{18}O compared with initial time; *C. pubecens* and *M. clausenii* became more enriched in both isotopes (Figure 4). The gas exchange trajectories for most species confirmed the expected by Grams et al. (2007) model output, the exception were *C. pubecens* and *M. clausenii*. The reduction on gs_{MAX} was the main response for all species (Figure 4).

Table 3. Relationship between stomatal conductance (gs) and leaf cellulose isotopic composition ($\Delta^{18}O_{CELL}$) for each species in control and drought treatments in a *campo rupestre* vegetation. Significant differences are marked in bold.

Species	**Control treatments**			**Drought treatments**		
	Equation	**R^2**	**P**	**Equation**	**R^2**	**P**
Cam.pub	$\Delta^{18}O=-5.3008gs+37.7568$	0.33	0.23	$\Delta^{18}O=-10.059gs+37.972$	0.36	0.11
Ere.sei	**$\Delta^{18}O=-30.141gs+51.767$**	**0.51**	**0.01**	**$\Delta^{18}O=-26.055gs+49.9.84$**	**0.58**	**<0.01**
Mim.cla	$\Delta^{18}O=-5.268gs+42.724$	0.09	0.36	**$\Delta^{18}O=-22.806gs+45.476$**	**0.30**	**0.05**
Vel.niv	**$\Delta^{18}O=-15.181gs+34.848$**	**0.36**	**0.08**	**$\Delta^{18}O=-51.620gs+39.079$**	**0.72**	**<0.01**
Vel.sp	**$\Delta^{18}O=-38.591gs+42.066$**	**0.69**	**<0.01**	**$\Delta^{18}O=-34.432gs+38.804$**	**0.66**	**<0.01**
Ver.war	$\Delta^{18}O=13.331gs+28.166$	0.22	0.14	$\Delta^{18}O=-6.371gs+32.945$	0.17	0.27



	Cam.pub	Ere.sei	Mim.cla	Vel.niv	Vel.sp	Ver.war
gs predicted	-	-	-	-	-	-
ci/ca predicted	-	+	-	+	+	+
A predicted	0	-	0	-	-	-

Figure 4. A) Trajectory of mean (n=8-10) variation of the isotopic composition of carbon (δ^{13}Ccel) and oxygen above water source ($\Delta^{18}O_{CELL}$) of leaf alpha-cellulose (Grams et al. 2007 model) between February 2011 and August 2012 for six species in *campos rupestres*. *Mimosa clausenii* showed differences in gas exchange parameters in August 2012 between conditions; due to this, the trajectories in instantaneous measurements were separated by conditions (control treatment "Cont.", and rain-out treatment "Drought"). The first table represents the trajectory of variation in $\delta^{13}C_{CELL}$ and $\Delta^{18}O_{CELL}$; the second table represents the predicted response based in Grams et al. 2007 model; the third one represents the model predicted response (black arrows) and our trajectories of variation on instantaneous measurements of gas exchange. (-) indicates decrease in rates; (+) indicates increase in rates and, (0) represents no changes.

NSC, gs and $\delta^{18}O_{CELL}$ across species

Across species, $\delta^{18}O_{CELL}$ was positively related to NSC% in roots. *M. clausenni* showed the highest values of $\delta^{18}O_{CELL}$ and NSC% and *V.* aff. *variabilis*, the lowest values (Figure 5, Table 4). Contrary to our expectation, $\delta^{18}O_{CELL}$ was positively related to gs across species. In one extreme of the spectrum, *M. clausenni* has high gs $\delta^{18}O_{CELL}$, while *V. nivea* was located in the opposite side. Species that maintained high rates of gs were those with high content of NSC% in roots. The relationship between $\delta^{18}O_{CELL}$ and gs relative in dry season (gs_{REL}) was not significant across species in both conditions (Table S3, Figure 5).

Table 4. Simple linear regression for leaf cellulose oxygen isotopic composition ($\delta^{18}O_{CELL}$) and non-structural carbohydrate per dry mass (NSC%), maximum stomatal conductance (gs) and stomatal conductance standardized based in high rates of gs during the study across species from biodiversity hotspot, *campos rupestres*. All data corresponds to samples collected in dry season. Significant relations are marked in bold.

Control			**Experimental**		
Equation	**R^2**	**P**	**Equation**	**R^2**	**P**
$\delta^{18}O_{CELL}$=0.67NSC+28.53	0.4789	0.1277	$\delta^{18}O_{CELL}$=0.45NSC+28.84	**0.73**	**0.03**
$\delta^{18}O_{CELL}$=34.16gs+24.33	**0.75**	**0.025**	$\delta^{18}O_{CELL}$=27.38gs+26.86	**0.93**	**0.001**
$\delta^{18}O_{CELL}$=0.10grel+27.30	0.22	0.35	$\delta^{18}O_{CELL}$=0.058gsrel+28.92	0.18	0.39

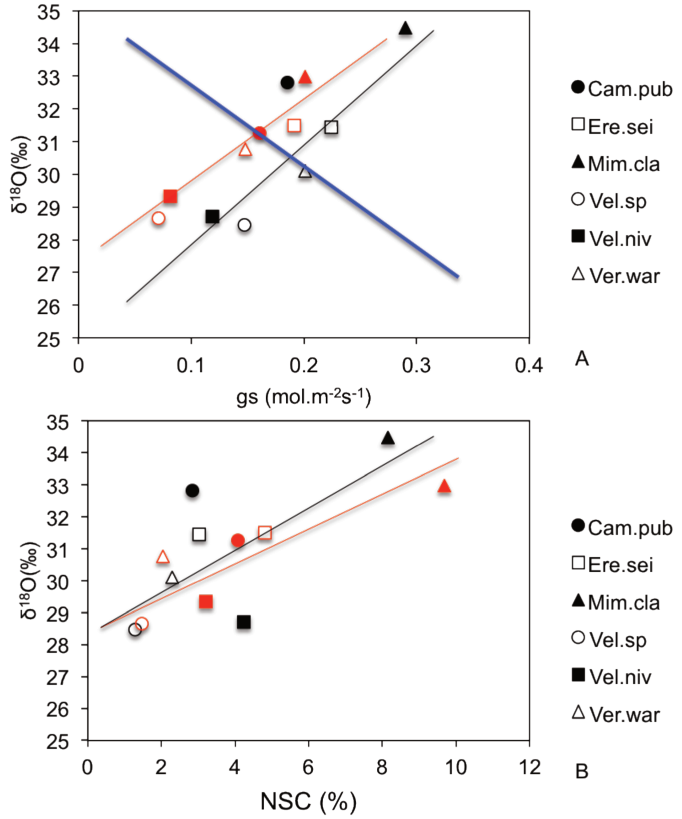


Figure 5. Linear regressions across species in *campo rupestre* vegetation between leaf cellulose oxygen isotopic composition ($\delta^{18}O$) and: A) stomatal conductance (gs - $mol.m^{-2}s^{-1}$). Black symbols represent the average per species in control treatments, while red symbols represent the average per species in drought treatments. The blue line shows the expected relationship between these two attributes; B) non-structural carbohydrate per dry mass (NSC%).

DISCUSSION

Our structures to exclude rainwater caused a strong reduction in PWA in shallow soils (0-30 cm) in the second year of study; this resulted in a prolonged drought in upper soils layers for plants. Our studied species showed a continuum of water use strategies, varying from isohydric to anisohydric species. This water status regulation may be a critical factor related to survival and mortality of species during drought, with anysohydric species pointed as being more resistant to drought than isohydric plants (Meinzer et al. 2009, Plaut et al. 2012, Plaut et al. 2013). Usually, anisohydric species occur in drought-prone habitats, as they usually possess a more cavitation-resistant vascular system (Brodribb & Holbrook 2004, McDowell et al. 2008), in which the hydraulic failure occurs at a much more negative water potential (West et al. 2007, McDowell et al. 2008). Under optimal conditions and mild-to-moderate drought conditions, anisohydric species usually maintain higher gs and CO_2 assimilation than isohydric plants (Tardieu & Simonneau 1998, McDowell et al. 2008). However, regulation on stomatal conductance, even in anisohydric plants, is needed to avoid hydraulic failure (McDowell et al. 2008, Meinzer et al. 2009, Plaut et al. 2012, Plaut et al. 2013). Our anisohydric species, *C. pubecens* showed a strongly regulation on gs_{MAX} in drought treatment, but maintained high rates of A when compared to the other studied species.

The isohydric species, *E. seidelii, V.* sp. *variabilis* and *V. nivea* exhibited a strong stomatal regulation during dry season and maintained the Ψ_{MD} nearly constant; we also observed a significant relationship between VPD, Ψ_{PD} and gs. Our observations corroborate the prediction that stomatal regulation in isohydric species depends on climatic demand, estimated by VPD and soil water content (Ψ_{PD}) (Ogle & Reynolds 2002, Damour et al. 2010). To avoid high rates of transpiration due to high VPD, the strong stomatal regulation (partial closure) minimizes the decrease in plant water potential, preventing plants from excessive dehydration and physiological damage (Oren et al. 1999). The Velloziaceae species also showed an isohydric strategy. Those species are rosette and seem to be very adapted to this drought-prone environment. The Velloziaceae family is one of the most abundant in *campos rupestres* in Brazil (Mello-Silva 1995, Conceição et al. 2007), and many species from this family have a fugitive strategy to cope with drought, a desiccation tolerance (Porembski & Barthlott 2000, Alcantara et al. 2014 submitted manuscript, Teodoro et al. unpublished manuscript - "Chapter 2 in this thesis"). Comparing the shrubs species, the anisohydric species (*C. pubecens* and *V. warmingiana*) seem to be more drought resistance than *E. seidelii*, the main isohydric species. A preliminary analysis in vulnerability curves for studied species (data not shown, Teodoro et al. unpublished manuscript) showed that *C. pubecens* and *V. warmingiana* have a more negative water potential when 50% of the hydraulic conductivity is lost due to cavitation (P50) than *E. seidelii*.

Isotopic data and water use strategies

The $\Delta^{18}O_{CELL}$ is expected to reflect variation on gs (Barbour & Farquhar 2000, Barbour 2007, Grams et al. 2007) therefore, a negative relationship is expected between these traits based in the steady-state leaf water ^{18}O enrichment theory. The theory shows that $\delta^{18}O$ is mainly determined by the isotopic composition of the soil water, the leaf water enrichment due to transpiration, isotopic exchange between organic molecules and plant water, and water biochemical fractionation during incorporation (for details in theory see Farquhar & Lloyd 1993, Scheidegger et al. 2000, Barbour 2007). Considering each of our studied species, we found a significant negative relationship between $\Delta^{18}O_{CELL}$ and gs for the isohydric species (*E. seidelii*, *V. nivea* and *V.* aff. *variabilis*) and no relationship for anisohydric species. Cernusak et al. (2009) studied three tropical trees species under two different water and nutriments conditions, and the relationship between $\delta^{18}O$ of stem dry matter and gs varied from a strongly negative for one species to no relations for other one. They were unable to explain the strong relation in one species, the weak, and the absence in the other, once the three species showed similar range of variation in gs (Cernusak et al. 2009). For our species, the presence or absence of relationship between $\Delta^{18}O_{CELL}$ and gs was related to water use strategies, as only isohydric species that showed a strong stomatal regulation (in response to variation in VPD and Ψ_{PD}) exhibited the expected relationship. There are some evidence that the $\delta^{18}O_{CELL}$ is a robust and sensitive indicator of VPD in tropical ecosystems (Kahmen et al. 2010) and only the isohydric species showed significant relations between gas exchange and VPD. This reinforces the negative relationship between $\delta^{18}O_{CELL}$ and gs found to our isohydric species. According with Cernusak et al. (2009) and our results, we concluded that $\Delta^{18}O_{CELL}$ is a reliable predictor of gs and VPD only in isohydric species and caution needs to be taken in using this attribute to make inferences about the stomatal behavior in anisohydric plants.

The dual isotopic approach was useful to predict and understand the gas exchange trajectory (gs and A_{MAX}) only for our isohydric studied species and one anisohydric (*V. warmingiana*), in which, we found a reliable negative correlation between $\Delta^{18}O_{CELL}$ and gs as we discussed above. For these species, the instantaneous measurements of gas exchange were the expected by the model output (Grams et al. 2007). Based in the dual isotopic model and in our instantaneous measurements of gas exchange, the main controller of drought response in this water-limited ecosystem species was the stomatal regulation, even in some anisohydric species. Most studied species showed a decrease on gs from February 2011 to August 2012. Stomatal regulation is pointed as a key mechanism that limits water loss and avoids extremely negative water potentials, which could result in high level of xylem embolism and hydraulic failure (Oren et al. 1999, Mitchell et al. 2008, McDowell et al. 2008).

Isotopic data across species

We found a positive relationship between $\delta^{18}O_{CELL}$ and NSC%, for individuals/species under our rainout shelters, indicating that NSC pool has an influence in O isotopic data. Recently, Song et al. (2014) showed that the turnover time of carbohydrate pool available for cellulose synthesis has a strong influence on $\delta^{18}O_{CELL}$. The proportion of organic oxygen exchange that occurs with local water during cellulose synthesis (known as p_{ex}) can change with the pool of NSC%. The p_{ex} can increase whether increase the available pool of NSC, reflecting in an enriched composition of ^{18}O (For details in isotopic theory see Song et al. (2014) and Ellsworth & Sternberg (2014)). In an experiment with salinity in *Arabidopsis*, the pool of soluble sugars and consequently the p_{ex} increased with salinity and were reflected in high $\delta^{18}O_{CELL.}$ In Song et al. (2014) and Ellsworth & Sternberg (2014) studies, the pools of NSC analyzed were those present in leaves and stems, respectively (the tissues that the isotopic analyses were performed). In our case, we analyzed the storage of total NCS% in roots and isotopic composition in leaves and we also found a reliable and strong relation for individuals/species subjected to our drought treatment, indicating the importance of NSC storage in roots for isotopic composition in leaves, since this reserves can be remobilized to maintain the plants homeostasis and the leaf functioning.

The positive relationship between leaf $\delta^{18}O_{CELL}$ and gs observed in our study contradicts the expected pattern (Figure 5A), showed by Moreno-Gutiérrez et al. (2012) in a Mediterranean community, in which species showed a strongly negative relation among these traits, indicating the existence of isotopic niches for each evaluated specie. Perhaps, our results can be explained by the differences in water use strategies showed by species and by the influence of NSC reserves in the leaf isotope composition, as species with high $\delta^{18}O_{CELL}$ also had high content of NSC% and high rates of gs on dry season. High rates of gs observed in dry season can be an intrinsic characteristic of the species, since these species showed a strong reduction when we compared the minimum value with the maximum gs found during the study. Song et al. (2014) recommends a field examination of p_{ex} in ecophysiological studies to better understand the factors that determined $\delta^{18}O_{CELL.}$ This is particularly important when interpretations of $\delta^{18}O_{CELL}$ variability involve comparisons among species. In our study, although we have not quantified p_{ex}, the NSC% in roots can be a proxy of this variability among species and might have an important effect on $\delta^{18}O_{CELL.}$

CONCLUSION

The six studied species represent a continuum of water strategies, from anisohydric to isohydric species. In all species, despite of water use strategy, the stomatal conductance seems to be a key controller of water balance. The relations between $\Delta^{18}O_{CELL}$ and gs were negative and significant only for isohydric species; likewise, the use of dual isotopic approach was valid only for isohydric species. Across species, we found a strong relationship between $\delta^{18}O_{CELL}$ and NSC% for individuals under drought treatment, suggesting that the use of NSC% reserves under water stress conditions can affect leaf isotope composition. In communities dominated by species with a great diversity of hydraulic strategies, such as the *campos rupestres*, $\delta^{18}O_{CELL}$ should be used with caution to infer physiological responses, mainly stomatal condutance. Also, the analyses of NSC pools should be included in studies with isotopic data, helping to elucidate the factors that influence $\delta^{18}O_{CELL}$ and tracing drought response in plants.

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SUPPLEMENTARY MATERIAL

Table S1. Mean and standard error of stomatal conductance (gs), maximum assimilation (A), pre-dawn water potential (Ψ_{PD}) and mid-day water potential (Ψ_{MD}) in each condition control and drought treatment for six species in *campo rupestre* in five months. The drought treatment effect (effect size - ES) indicates the differences between conditions, in which, values above +or-2 represent significant differences between treatments. The equation to calculated the ES is $ES = \frac{(MC-MD)}{\sqrt{e^2C+e^2\,D}}$, where MC and MD represents the mean values in control and drought treatments, respectively. e represents the standard errors in control (C) and drought (D) treatments. February 2011: n=3 per specie/condition; others: n=5 per specie/condition.

Species	**Control treatment**				**Drought treatment**				**Drought treatment effect**			
	gs	**A**	Ψ_{PD}	Ψ_{MD}	**gs**	**A**	Ψ_{PD}	Ψ_{MD}	**gs**	**A**	Ψ_{PD}	Ψ_{MD}
February 2011												
Ere.sei	0.46±0.06	9.29±2.37	-0.43±0.04	-1.28±0.16	0.41±0.03	10.06±2.32	-0.45±0.03	-1.18±0.15	0.63	-0.23	0.52	-0.50
Mim.cla	0.27±0.06	7.44±0.73	-0.25±0.05	-1.05±0.2	0.35±0.11	8.90±1.18	-0.25±0.05	-1.20±0.1	-0.59	-1.05	0.00	0.67
Vel.niv	0.39±0.08	9.93±0.5	-0.28±0.02	-1.63±0.02	0.22±0.03	9.06±0.63	-0.22±0.04	-1.70±0.01	1.87	1.09	-1.41	4.00
Vel.var	0.35±0.07	11.02±1.31	-0.27±0.07	-1.83±0.04	0.33±0.03	10.00±1.74	-0.33±0.02	-1.45±0.09	0.23	0.47	0.97	-3.94
Ver.war	0.29±0.08	8.70±1.81	-0.54±0.07	-1.38±0.07	0.35±0.09	7.91±1.16	-0.70±0.18	-1.40±0.2	-0.42	0.37	0.82	0.08
February 2012												
Cam.pub	0.42±0.01	17.13±0.23	-0.19±0.05	-2.07±0.09	0.35±0.02	14.88±0.88	-0.84±0.22	-2.48±0.18	2.70	2.47	2.84	2.00
Ere.sei	0.52±0.03	10.06±0.12	-0.23±0.04	-1.42±0.2	0.56±0.05	9.47±0.79	-0.24±0.04	-1.57±0.15	-0.56	0.74	0.10	0.58
Mim.cla	0.45±0.08	7.31±1.29	-0.13±0.02	-2.10±0.3	0.79±0.2	7.24±0.17	-0.21±0.03	-1.69±0.24	-1.58	0.05	2.57	-1.06
Vel.niv	0.65±0.07	11.64±0.18	-0.17±0.03	-1.67±0.07	0.56±0.03	10.76±0.54	-0.12±0.01	-1.63±0.08	1.12	1.53	-1.77	-0.38
Vel.var	0.43±0.04	14.08±0.3	-0.30±0.08	-1.72±0.16	0.35±0.07	13.86±1.27	-0.50±0.15	-1.76±0.21	1.09	0.18	1.19	0.15
Ver.war	0.31±0.06	9.40±0.74	-0.90±0.03	-2.44±0.28	0.31±0.08	5.80±2.54	-0.95±0.16	-2.01±0.25	0.08	1.36	0.30	-1.16
April 2012												
Cam.pub	0.33±0.05	11.85±1.54	-0.25±0.05	-2.23±0.03	0.22±0.03	9.82±0.84	-0.81±0.13	-2.31±0.18	2.01	1.16	4.03	0.44
Ere.sei	0.37±0.04	12.21±0.14	-0.68±0.03	-1.80±0.12	0.30±0.02	11.94±0.36	-0.43±0.01	-1.93±0.05	1.75	0.68	-7.01	1.07
Mim.cla	0.71±0.07	11.74±0.75	-0.22±0.03	-1.72±0.04	0.43±0.1	8.69±0.38	-0.20±0.01	-1.78±0.2	2.35	3.64	-0.78	0.31
Vel.niv	0.31±0.02	11.55±0.88	-0.34±0.05	-1.55±0.01	0.43±0.01	13.13±0.32	-0.48±0.03	-1.35±0.01	-5.47	-1.69	2.37	-1.41
Vel.var	0.31±0.09	12.36±1	-0.29±0.02	-1.40±0.06	0.26±0.06	10.58±0.1	-0.50±0.01	-1.74±0.06	0.51	1.78	2.04	3.30
Ver.war	0.22±0.08	9.47±0.84	-0.72±0.02	-1.77±0.03	0.18±0.03	7.11±1.46	-1.04±0.1	-1.54±0.14	0.40	1.40	3.30	-0.40
June 2012												
Cam.pub	0.28±0.01	17.43±0.58	-0.59±0.17	-1.77±0.19	0.33±0.03	15.00±0.79	-0.99±0.17	-2.44±0.11	-1.92	2.50	1.64	3.10
Ere.sei	0.31±0.04	12.19±0.2	-0.41±0.04	-1.59±0.35	0.35±0.06	11.29±1.3	-0.49±0.03	-1.51±0.16	-0.44	0.67	1.71	-0.20
Mim.cla	0.71±0.25	7.36±1.46	-0.40±0.03	-2.99±0.29	0.35±0.05	2.94±1.7	-0.42±0.02	-2.07±0.22	1.44	1.97	0.63	-2.57
Vel.niv	0.30±0.02	13.79±0.21	-0.78±0.13	-2.03±0.13	0.18±0.03	12.06±0.38	-1.02±0.1	-1.34±0.06	3.57	3.95	1.54	-4.89
Vel.var	0.29±0.01	14.15±1.05	-0.62±0.05	-1.45±0.05	0.19±0.03	10.90±1.61	-0.39±0.08	-1.29±0.15	3.49	1.70	-2.46	-0.99
Ver.war	0.16±0.02	8.08±0.83	-1.61±0.08	-1.48±0.19	0.20±0.03	8.78±1.23	-1.57±0.07	-1.36±0.16	-1.31	-0.47	-0.42	-0.46

August 2012												
Cam.pub	0.19±0.06	12.62±1.53	-0.35±0.01	-2.54±0.15	0.16±0.03	12.06±0.85	-1.45±0.34	-4.36±0.37	0.39	0.32	3.23	4.60
Ere.sei	0.22±0.02	10.97±0.68	-0.44±0.06	-1.78±0.15	0.19±0.02	10.65±1.09	-0.68±0.03	-1.73±0.05	1.01	0.25	3.48	-0.28
Mim.cla	0.29±0.04	9.57±0.91	-0.37±0.06	-2.30±0.3	0.20±0.05	4.45±0.86	-0.49±0.06	-2.23±0.28	1.44	4.08	1.34	-0.17
Vel.niv	0.12±0.01	7.54±0.67	-0.53±0.06	-1.33±0.09	0.08±0.01	6.40±1.13	-1.84±0.44	-1.44±0.14	5.23	0.86	2.92	0.65
Vel.var	0.15±0.02	10.24±0.99	-0.44±0.09	-1.82±0.07	0.07±0.01	5.85±1.35	-0.60±0.05	-1.41±0.09	4.50	2.62	1.52	-3.56
Ver.war	0.20±0.05	7.45±1.92	-1.65±0.35	-1.63±0.35	0.14±0.02	8.83±0.86	-2.12±0.19	-1.92±0.1	1.12	-0.71	1.17	0.77

Table S2. Analysis of co-variance (ANCOVA) to test the effects of pre-dawn water potential (Ψ_{PD}), vapor pressure deficit (VPD) and conditions (control vs drought treatment) on stomatal conductance (gs) for six studied species in a *campo rupestre* vegetation. Significant differences are marked in bold.

Species	**Ψ_{PD}**		**Cond**		**Ψ_{PD}:cond**		**VPD**		**Cond**		**VPD:cond**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Cam.pub	4.73	**0.04**	0.39	0.54	0.32	0.58	5.65	**0.02**	1.32	0.25	4.5	**0.04**
Ere.sei	0.28	0.60	1.87	0.18	0.02	0.89	13.1	**<0.01**	0.42	0.52	0.61	0.44
Mim.cla	3.89	**0.05**	0.07	0.79	2.90	0.09	0.06	0.81	0.69	0.41	0.28	0.6
Vel.niv	34.1	**<0.01**	0.01	0.9	0.41	0.53	4.3	**0.04**	0.25	0.62	1.87	0.18
Vel.sp	2.25	0.14	0.69	0.41	0.03	0.86	0.28	0.6	1.87	0.18	0.02	0.89
Ver.war	8.57	**<0.01**	0.02	0.96	0.32	0.57	1.35	0.25	0.04	0.95	0.03	0.85

Table S3. Values of stomatal conductance in the dry season (gs absolute - mol.m^{-2}.s^{-1}) and the value standardized by the maximum value (gs relative to maximum) obtained for each species in control and exclusion plots.

Species	**Control treatment**		**Drought treatment**	
	gs absolute	**gs relative to maximum**	**gs absolute**	**gs relative to maximum**
Cam.pub	0.18 ± 0.09	44.69	0.16 ± 0.07	46.44
Ere.sei	0.22 ± 0.05	42.73	0.19 ± 0.05	34.36
Mim.cla	0.29 ± 0.08	38.38	0.20 ± 0.11	25.58
Vel	0.15 ± 0.03	34.07	0.07 ± 0.03	20.32
Vel.niv	0.12 ± 0.01	18.41	0.08 ± 0.01	18.81
Ver.war	0.20 ± 0.10	49.26	0.15 ± 0.04	41.39

CHAPTER 2

Water availability affects function, phenology and reproduction in congeneric desiccation-tolerant and non-tolerant plants of central Brazil

Water availability affects function, phenology and reproduction in congeneric desiccation-tolerant and non-tolerant plants of central Brazil

ABSTRACT

Desiccation tolerance (DT) is the ability of a plant to tolerate extremely low tissue water content from which it can recover and equilibrate its internal water potential to a fully functionally state when water becomes available. A global center of endemism of vascular plant DT species is within the vegetation of *campos rupestres*, located in Central Brazil. The Velloziaceae is a prevalent family in these areas and contains both DT and non-DT species, representing an ideal system to evaluate the costs and benefits of resurrection traits for plant performance under contrasting water availabilities. We investigated the effects of changes in water availability in the ecophysiology, growth performance and reproductive output of two congeneric species of *Vellozia* with contrasting hydraulic strategies that coexist in *campos rupestres*. *Vellozia nivea* is a DT species and *Vellozia* aff. *variabilis* is a non-DT species. We measured instantaneous gas exchange and determined the stable oxygen composition ($\delta^{18}O_{CELL}$) of leaf cellulose as an integrative method to evaluate stomata regulation and leaf carbon isotope composition ($\delta^{13}C_{CELL}$) to determine stomatal regulation (gs) and intrinsic water-use efficiency (WUEi). We evaluated growth and reproductive performance by measuring leaf demography, leaf life span and reproductive and vegetative phenology. These measurements were made in rainfall exclusion and control plots. The DT species showed high gs (profligate water-use) and low WUEi during the narrow windows available to growth. In contrast, the non-DT species showed conservative water-use throughout the year. The DT species also increased the %NSC storage in roots during the prolonged drought, presumably a key mechanism that allow survival to desiccation in this species. Individuals of DT species had lower biomass and higher reproductive output under drier conditions than the non-DT species. These data suggest that DT tolerance is a complex phenomenon that affects several life-history traits and allow species to occupy very specialized and desiccating habitats such as rock outcrops.

Key-words: Desiccation tolerance, *campos rupestres*, carbon balance, stable C and O isotope ratios, leaf monthly balance, carbon starvatio

INTRODUCTION

Desiccation tolerance, or the ability of plants to endure extremely low tissue water content from which they can recover and equilibrate their internal water potential to a fully functionally state when water becomes available, is one of the most remarkable adaptations to extreme drought (Alpert 2000). During the desiccation process, the vegetative tissues of desiccation tolerant plants (hereafter DT) are able to survive extremely low water contents without suffering irreparable and irreversible damage (Alpert 2000, Gaff & Oliver 2013). DT plants are also known as "resurrection" or resurgent plants. These plants generally occur in habitats subjected to long periods with no rainfall and often inhabit substrates with low water retention capacity such as rocky outcrops, shallow and sandy soils (Sherwin & Farrant 1996, Porembski & Barthlott 2000). In Brazil, the *campos rupestres* (rock outcrops fields) are considered a center of endemism for DT plants (Porembski & Barthlott 2000, Oliveira et al. 2005). The *campos rupestres* occurs in high altitudes (*i.e.* above 900 meters) in Cerrado Domain that are characterized by shallow and impermeable soils, low soil water retention, strong daily thermal variation, high evapotranspiration and constant winds (Porembski & Barthlott 2000, Jacobi et al. 2007). In this habitat, the Velloziaceae is an important plant family, showing its center of diversity in the *campos rupestres* of southeastern Brazil (Mello-Silva 1995, Porembski & Barthlott 2000). This family includes drought resistant (non-DT) and DT species (Alcantara et al. 2014, submitted paper). While DT and non-DT congeneric plant species co-exist in this ecosystem, we have never quantified the physiological limits of DT and non-DT plants in relation drought conditions. Such knowledge is critical because climate change projections for tropical mountains predict that drought events of increasing magnitude, duration and frequency, will be commonplace in the *campos rupestres* in future decades (IPCC 2013). The existence of DT and non-DT species within the Velloziaceae represents an ideal system to evaluate the costs and benefits of resurrection traits on plant performance under contrasting water availabilities.

In water-limited environments, plants have to regulate the water loss through stomata while simultaneously having to obtain CO_2 for photosynthesis and growth. Plants have evolved several responses to cope with this conflicting demand of water loss regulation and carbon uptake, varying from conservative to profligate/opportunist water users (Filella & Peñuelas 2003, Peñuelas et al. 2011, Moreno-Gutiérrez et al. 2013). Conservative species tend to use resources more efficiently, optimizing water-use efficiency. In contrast, profligate/opportunist species tend to maintain very high metabolism and only when water is plentiful in the environment. For example, drought-deciduous species in tropical dry forests tend to have higher transpiration rates in the wet season but shorter windows of opportunity for carbon gain during the rainy months of the year, when compared to evergreen species (Peñuelas et al. 2011).

Phenological traits are expected to be one of the most responsive traits to climate and also one of the most easily observable traits in nature (Badeck et al. 2004, Glick et al. 2011). The timing of growth, reproduction and functional events reflects the strategy of plants to cope with favorable and unfavorable conditions in alternate seasons (Castro-Díez et al. 2003). Leaf phenological events may be very important for resurrection plants that go through cycles of leaf dehydration and rehydration that limit the window of opportunity for carbon gain. These phenological events are in turn strongly influenced by temperature, air-humidity and soil water availability (Chuine et al. 2010, Laube et al. 2014). DT species seem to have evolved in harsh environments with substrates (soils) that may contain very low nutrient concentrations and low water retention (Porembski & Barthlott 2000). Because of this, we predict that their growth performance and foliar dynamics are sensitivity to both, variations in soil availability and atmospheric conditions (*i.e.* VPD - vapor pressure deficit). Further, some Velloziaceae species show structures to absorb water directly from the atmosphere, such as adventitious roots covered by velamen in the stems (Porembski & Barthlott 2000, Oliveira et al. 2005).

Plant water status is also thought to be an important factor influencing the variation in timing of plant reproductive processes (Borchert et al. 1994, Willians et al. 1997, Brando et al. 2006, Laube et al. 2014). Changes in reproductive phenology have important consequences on the reproductive success of populations, and consequently, in their dynamics and future plant establishment (Sherry et al. 2007). Little is known about the reproductive phenophases in DT plants and some authors suggest a physiological trade-off where the mechanisms of tolerating dehydration and rehydration may conflict with resource acquisition resulting in fewer resources available to be allocated to reproduction (Alpert 2006, Tuba 2008). Thus, we wonder whether extremes drought events in areas of rock fields could influence differently the timing of flowering for DT and non-DT species.

Another key aspect of the carbon metabolism of perennial plants in seasonally dry environments is how they allocate the carbon fixed towards long-term storage (non-structural carbohydrate - NSC) that can be drawn upon and used either during drought condition or for regrowth after drought subsides (Chapin et al. 1990, Sala et al. 2010). Drought modulates plant carbon (C) dynamics through a complex cascade of events (see McDowell 2011). As soil dries, plants tend to close stomata and consequently, photosynthesis is reduced. In DT plants, during the desiccated period, the photosynthetic activity can cease completely. However, carbon reserves must be consumed to maintain metabolic processes during drought, despite the lack of photosynthesis, which in turn may result in a negative C balance. This process causes a decrease in NSC storage of plants leading to C depletion or reduction to a lower probability of survival and, eventually, could lead to plant mortality (carbon starvation hypothesis) (McDowell & Sevanto 2010, Sala et al. 2010, Galvez et al. 2011, Gaff & Oliver 2013). However, evidence is mixed and therefore the widespread existence of carbon starvation resulting from drought is still controversial, as in some species NSC storage can increase (Muller et al. 2011, Korner 2013, Fatichi et al. 2014). In DT plants, the concentration of NSC%, especially soluble sugars (*e.g.* sucrose, glucose), can increase (Müller et al. 1997, Ghasempour et al. 1998, Xu et al. 2010), or decrease or remain at low levels (Bianchi et al. 1991) during the course of dehydration.

Faced with the real possibility of increased drought events in the *campos rupestres*, the main question we addressed in this study was: how do DT and non-DT species respond to drier conditions? To address this question, we implemented a drought experiment *in situ* and in a greenhouse to evaluate different aspects of plant responses to drought, including gas exchange dynamics, carbon balance, growth and leaf and reproductive phenology. Our specific questions were: 1) Do the DT-species have higher rates of carbon assimilation and stomatal conductance during the wet season than non-DT species? 2) Do NSC reserves decrease during extreme drought events, affecting the carbon balance in *Vellozia* species? 3) How does an extreme drought affect leaf phenology of species with contrasting hydraulic strategies and what are the environmental variables influencing leaf phenology and leaf desiccation process? 4) Is the reproductive phenology of the two species equally affected by an extreme drought? We predict that the DT species would maintain high rates of gas exchange during the wet season in order to maximize NSC storage and to maintain a positive C balance through the period of desiccation during the dry season. In contrast, we predict a conservative water use for non-DT species during the whole year, since it remains evergreen during the dry season. We predict greater drought sensitivity in leaf phenology in DT compared to non-DT species. As a result, we predicted that prolonged drought events would deplete NSC storage in DT species severely, with more negative consequences for DT than non-DT plants. Moreover, the allocation of resources for leaf recovering/production in DT species would be higher than in non-DT, leading to fewer resources available for reproduction in DT plants than in non-DT species.

MATERIAL AND METHODS

Study area and focal species

Our study was carried out in a *campos rupestres* area, located in Serra da Canastra National Park (Parque Nacional da Serra da Canastra - PNSC), São Roque de Minas, Minas Gerais, Brazil. The vegetation is dominated by small shrubs, rosette-form, herbs and grasses, growing over a rocky substrate, mainly quartzite-sandstone. Mean annual temperature is 21.6ºC and mean annual precipitation is 1240 mm (data from CEMIG, São Roque de Minas meteorological station). The climate is typically seasonal, in which the winter is extremely dry, with low or no rain from June until September (mean accumulated in dry season is 61.5mm), while the summer corresponds to rainy season. The study area is located at 1200 m of altitude. The central point in study area has the geographic coordinate 20º15’40”S and 46º25’25”W.

The *campos rupestres* vegetation is a center of diversity and endemism of plants (Burke 2003; Jacobi et al. 2007). The family Velloziaceae is an important group in this vegetation (Mello-Silva 1995; Conceição et al. 2007). The two species studied, *Vellozia nivea* L. B. Sm. & Ayensu and *Vellozia* aff. *variabilis* Mart. ex Schult. & Schult. f.

(Velloziaceae), were chosen due to the high abundance in the areas of *campos rupestres* at Serra da Canastra National Park. *V. nivea* is a DT species, poikilochlorophyllous (*i.e.*, plants that dismantle the internal chloroplast structure by an ordered deconstruction process during drying, and resynthesize them upon rehydration; Tuba et al. 1998, Proctor & Tuba 2002). *Vellozia* aff. *variabilis* is a non-DT species, and both are restricted to *campos rupestres* areas. This congeneric pair of species with contrasting hydraulic strategies offers an excellent model system for studying the costs and benefits of resurrection traits on plant performance under contrasting water availabilities.

Field experimental design

We implemented rainwater exclusion plots to impose extreme dry conditions to *campos rupestres* vegetation. We installed 12 plots in the study area; four were used as control plots and eight as rainwater exclusion (experimental condition). The structures used to exclude rainfall in the plots were implemented in April 2011 and were kept for 17 months, excluding about 1580 mm of rainfall (total rainfall amount during the study). This experimental design follows that of West et al. (2012). The structures were constructed of iron and roofs were covered with polycarbonate to allow radiation to reach the vegetation. In the experimental plots, roofs were closed, while in the control ones, the roofs were kept open. The height of the structures above each plot was 2 meters. We dug trenches (50 cm deep) around each plot to minimize plants from accessing water outside the plots. In December 2011, we also closed one side of the plots to keep possible horizontal precipitation away from the plots. We used rock stepping-stones to move around within the plots and from which we took our measurements so as to avoid trampling of any kind inside the plots.

Climate characterization and soil moisture measurements

We monitored the environmental conditions inside the plots with sensors of temperature and air humidity (HOBO Pro Series - ONSET) and soil water content (Soil Smart Sensor - ONSET). At the end of 2011, we installed a complete meteorological station with temperature and air humidity sensors (Campbell Scientific - Vaisala), photosynthetic active radiation sensor (Campbell Scientific LI190SB-L15 8721-45), and pluviometer (Campbell Scientific TB4-L15). We also installed eight soil water content sensors (TDR - Campbell Scientific CS616-L150 13932-17), four in each condition (experimental and control plots) and two soil temperature sensors (Campbell Scientific 107-L150 10755-219), one in each condition. The soil water content sensors were installed only in the superficial soil (1-30 cm), due to the high amount of rocks in the soil. The values obtained with sensors in the meteorological station were used to calibrate the data obtained with the previous sensors in the area.

We used the temperature (T) and relative humidity (RH) to calculate the atmosphere vapor pressure deficit (VPD, KPa), according to the following equation: $VPD = 0.611375e^{t\left(1-\frac{RH}{100}\right)}$, where $t = \frac{917.502T}{240.97+T}$ (Jones 1992). Plant available water (PAW) was calculated as the difference between the soil water content (SWC) and minimum soil water content (SWC_{MIN}) registered by the TDR sensors, according to the equation: $PAW=SWC-SWC_{MIN}$.

Gas exchange and isotopic measurements

We constructed light-response curves for both species to determine the radiation that corresponds to the maximum CO_2 assimilation (A) (PAR=1200 µmol quanta $m^{-2}s^{-1}$ - LED light source). CO_2 concentration was maintained at 380 ppm and leaf temperature and humidity were kept the same at the ambient. Rates of stomatal conductance (gs) and the CO_2 assimilation (A) were determined from 8:30 until 11:00 am (correspondent to the hours of maximum photosynthetic rates) under light saturation. We measured gas exchange on leaves from five individuals per treatment during five different periods in the rainy season (February 2011, 2012; April 2012), in transition between seasons (June 2012) and in dry season (August 2012). We calculated the instantaneous intrinsic water use efficiency as WUEi=A/gs (Seibt et al. 2008).

We measured the stable oxygen composition ($\delta^{18}O_{CELL}$) of leaf cellulose as an integrative method to evaluate stomatal regulation and carbon isotopic composition ($\delta^{13}C_{CELL}$) to infer WUEi during the period of cellulose synthesis (Dawson et al. 2002, Grams et al. 2007). We collected leaves for isotopic analyses in the three periods that we also measured gas exchange: 1) the rainy season (February 2011), before the rainwater exclusion experiment implementation; 2) the transition between rainy to dry season (June

2012); and 3) the dry season (August 2012). Leaf samples were dried at 65°C for 24-48 hours and ground to extract the alpha-cellulose used to performed the isotopic analyses. We performed alpha-cellulose extraction using the method described by Gaudinski et al. (2005), at the Functional Ecology Laboratory at the University of Campinas, Brazil. To analyze the $\delta^{13}C_{CELL}$, we weighted the samples in tin capsules (0.3 to 0.6 mg), placed them in an elemental analyzer (ANCA/SL elemental analyzer) and carried in a mass spectrometer (Finnigan MAT Delta PlusXL IRMS). To the analysis of $\delta^{18}O_{CELL}$, we weighted the samples in silver capsules (0.6 to 0.9 mg), and placed them in varioPYRO cube Elementar connected to a mass spectrometer (Delta V Plus Isotope Ratio Mass Spectrometer (IRMS)). The isotopic analyses were made at the Center for Stable Isotope Biogeochemistry, University of California-Berkeley, USA. The relationship between the rare isotopes and the most abundant isotope is expressed by R notation ($^{13}C/^{12}C$ or $^{18}O/^{16}O$).

To relate $\delta^{18}O_{CELL}$ with plant gas exchange we calculated the isotopic composition as an enrichment above of the plant's source of water ($\Delta^{18}O_{CELL}$) which we determined from the isotope composition of xylem water ($\delta^{18}O_{XYL}$) and using the equation: $\Delta^{18}O_{CELL}=\delta^{18}O_{CELL}-\delta^{18}O_{XYL}$ (Barbour et al. 2004, Barbour 2007, Moreno-Gutiérrez et al. 2012). Barbour et al. 2004 and Barbour 2007 suggest the removal in source water $\delta^{18}O_{XYL}$ signal from $\delta^{18}O_{CELL}$ because this simplifies interpretation and enables identification of variation due to leaf water enrichment and isotopic exchange. To characterize the $\delta^{18}O_{XYL}$, we collected non-green plant tissues from five individuals per condition in three different periods corresponding to the seasons and transitions of seasons described above. The segment samples were immediately placed in screw-capped vials, wrapped with Parafilm and stored in freezer until water extraction to prevent possible evaporation. We used the cryogenic distillation method (West et al. 2006) to extract the water from plant material, at the Laboratory of Isotopic Ecology at CENA, USP, Piracicaba, SP, Brazil. The $\delta^{18}O$ analyses were made in the Stable Isotope Ratio Facility for Environmental Research (SIRFER), at the University of Utah, USA. The technique consists of measuring the ratio of oxygen ($^{18}O/^{16}O$) and deuterium ($^{2}H/H$) using an elemental analyzer (TCEA) held at high temperature (1450 ºC) coupled to a mass spectrometer (Thermo Finnigan Delta Plus XL).

Non-Structural Carbohydrates analyses

We evaluated the percentage of non-structural carbohydrates per dry mass (NCS%) storage in the roots in the end of dry season (September 2012). We followed the enzymatic method proposed by Sevanto et al. (2014) to analyze the NSC content. NSC is defined in this work as free, low molecular weight sugars (glucose, fructose and sucrose) and storage (starch). Immediately after collection, samples were microwaved to stop enzymatic activity. After that, samples were oven-dried at 70°C for 24–48 h and ground to fine powder. We prepared approximately 12 mg of plant material with 1.6 ml of distilled water to the analyses. We used amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich) to digest total NSC to glucose; and invertase, glucose hexokinase kits (GHK) and phosphorus glucose (Sigma-Aldrich) to quantify the low molecular weight sugars. The concentration of free glucose was determined photometrically in a 96-well microplate spectrophotometer (BioTek, Epoch). Starch was calculated as the difference between total NSC and low molecular weight sugars (see details in Sevanto et al. 2014). All NSC values are expressed as percent of dry matter.

Biomass, leaf phenology, leaf live span and reproductive phenology

To evaluate the biomass per species, we collected 30 individuals per species outside the plots. We evaluated leaf demography (to determine leaf monthly balance (LMB)), leaf longevity, and reproductive phenology for both species. In *V. nivea* we studied 21 individuals in control and 18 in drought plots, while in *Vellozia* aff. *variabilis* we studied 17 individuals in control and 35 in drought treatments. For each individual we tagged three rosettes, counted the initial number of leaves and recorded the growth of new leaves. We repeated this procedure on a monthly basis from February 2011 to September 2012. The LMB was calculated as the total number of leaves per month, including the previous leaves in rosette plus the new ones produced. As the initial number of leaves was different among individuals, we standardized this attribute considering the first studied month as reference (the values found in February 2011 was considered 100%). During the study period, we monitored an average of the 979 ± 140.7 leaves per month in *V. nivea* and an average of the 2055.7 ± 340.9 leaves in *Vellozia* aff. *variabilis*.

Greenhouse experiment with *Vellozia nivea* - the resurgent species

We also conducted a greenhouse experiment to gain a more detailed understanding of how leaf desiccation affects the dynamics of leaf gas exchange and NSC% in roots, stems, and leaves for the resurgent species. We measured gas exchange during the entire desiccation process, while in field we only measured A and gs from green leaves that were functionally active. We collected 12 individuals of *V. nivea* in Serra da Canastra National Park and acclimated them in a greenhouse for two months before the experiment. Five individuals were used as control, irrigated twice per day, and seven individuals were subjected to a non-irrigation treatment. We measured the stomatal conductance (gs) and the assimilation rates (A) from 8:30 until 9:30 am in two fully-expanded leaves per individual. The gas exchange attributes were measured daily for 20 days follwing the same procedures outlined in the "Gas exchange and isotopic measurements" section. We did not follow the rehydration process because our sampling for NSC was destructive.

To determine soil water content, we weighed the pots daily. The weight of the pots in first day of measurements was considered as our reference to standardize the following measurements.

To understand the NSC dynamics during the desiccation process, we quantified the percentage of non-structural carbohydrates per dry mass (NSC%) in plant tissues (leaf, stem and roots). We analyzed all individuals in control (five) and drought: 1) three individuals were sampled immediately after they reached a complete desiccated appearance; 2) four individuals were kept in desiccated state for additional eight months, to simulate a drought event longer in duration than the usual dry season experienced by the individuals in field conditions, which is around six months.

Statistical analyses

To evaluate the temporal variation on LMB, new leaves, gs, A, WUEi, $\Delta^{18}O_{CELL}$ and $\delta^{13}C_{CELL}$ during the study and the treatment effect, we fitted a linear-mixed model. We specified random intercepts per individual to account for the temporal pseudo replication in our design. In the model used to assess LMB, we considered treatment as a fixed factor with two levels (control and drought); time was also considered a fixed factor and corresponded to 21 months that we measured the variables in the field. In the model to evaluate the stable isotopes ($\Delta^{18}O_{CELL}$ and $\delta^{13}C_{CELL}$), the term time corresponded to seasons that we performed the measures (wet season (February 2011), transition wet to dry (July 2012) and dry (August 2012). For NSC% we used treatment (control vs. drought) and tissue (leaves, stems and roots) as fixed factors. We used 95% confidence intervals estimated from the model least square means to evaluate differences between pairs. To evaluate the relationship between variables ($\Delta^{18}O_{CELL}$ and gs/ gs and PAW) we performed a linear regression for each species per treatment. After that, we compared the treatments using analysis of covariance, testing the significance for each parameter studied and the interaction between then.

To test the relationship of VPD, PAW and PAR on the LMB and new leaves production we performed a multiple regression and used AIC (Akaike Information Criterion) to select the best model. For all regressions, the best model considered only the VPD and PAW without the interaction between them. Due to this, PAR was excluded from the model. To compare the NSC% and leaf longevity per treatment (control and drought treatments) we used a t-test. To compare the observed frequency of reproductive individuals per treatment by the expected frequency we used a Chi-square analyzes (χ^2).

To compare number of leaves, rosette length, biomass per rosette, gas exchange and isotopic data between species, we used a t-test. The statistical analyses were performed with the open-source program R (R Core Team 2012, http://www.R-project.org) and Statistica software.

RESULTS

Field experiment: affects of rainwater exclusion on ecophysiological performance

Our drought treatment caused a decrease of 34% in plant available water (PAW) in shallow soil layers in December 2011 and from March until July 2012 (Figure 1).

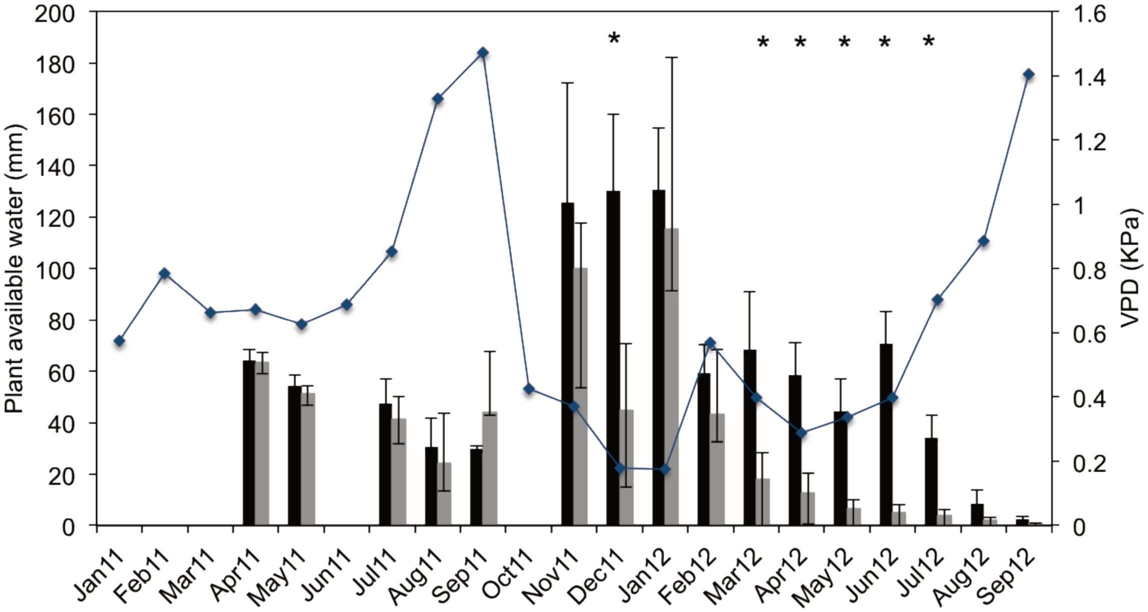

Figure 1. Plant available water (PAW-mm) for plots in both treatments, control and drought. Black bars represent average in control plots (n=4) and gray bars indicate average in rainwater exclusion plots (n=4); bars represent the standard desviation. The asterisks indicate significant differences ($p<0.05$; test t-student) between conditions in PAW. The blue line represents the average of VPD (kPa) in control and drought treatments.

Time, treatment and the interaction between time and treatment had significant effects on stomatal conductance in *V. nivea* (Table 1). In February and June 2012 (transition between wet to dry season) individuals in our drought treatment had significantly lower rates of gs compared to the control plants. A and WUEi showed no treatment effect though both varied significantly in time (Table 1). In *V.* aff. *variabilis*, gs and A varied significantly in time and with treatment. Individuals subjected to the drought experiment showed a reduction in gs and A rates in June and August 2012 (Table 1). In the wettest month (February 2012) gs was higher in resurgent species than the non-DT in the wettest month and WUEi was higher in *Vellozia* aff. *variabilis*, the non-resurgent species. As we did not measure desiccated leaves in *V. nivea* in the drier months, gs, A, and WUEi were similar between species (Table 2).

Table 1. Linear Mixed-effects Model for stomatal conductance (gs), CO_2 assimilation (A) and intrinsic water use efficiency (WUEi). Condition refers to control and drought treatment and time refers to period that we collected the data (Feb11 and 12, Apr12, Jun12, and Aug12) for *Vellozia nivea*, the desiccation tolerant species and *Vellozia* aff. *variabilis* the non-desiccation tolerant species.

		gs		**A**		**WUEi**	
		F value	**p**	**F value**	**p**	**F value**	**p**
Vellozia nivea	Condition	4.77	0.04	1.48	0.23	<0.01	0.96
	Time	47.04	<0.01	22.24	<0.01	14.28	<0.01
	Condition:time	3.78	0.01	1.35	0.27	1.66	0.18
Vellozia* aff. *variabilis	Condition	4.68	0.04	6.82	0.01	0.001	0.97
	Time	9.63	<0.01	6.76	<0.01	2.72	0.04
	Condition:time	0.20	0.94	0.92	0.47	0.17	0.95

Table 2. Comparison (Student-t Test) of stomatal conductance (gs), CO_2 assimilation (A) and intrinsic water use efficiency (WUEi) between the species *Vellozia nivea* (the desiccation tolerant species) and *Vellozia* sp. (the non-desiccation tolerant species).

	gs				**A**				**WUEi**			
	t	**p**	**Vel. sp**	**V. nivea**	**t**	**p**	**Vel. sp**	**V. nivea**	**t**	**p**	**Vel. sp**	**V. nivea**
Feb11	-0.55	0.59	0.31± 0.13	0.34± 0.08	-1.01	0.34	10.51± 2.45	9.41± 0.97	0.89	0.4	32.09± 9.17	37.42± 10.39
Feb12	3.84	<0.01	0.38± 0.12	0.6± 0.12	-3.34	<0.01	13.94± 2.16	11.15±1	-7.66	<0.01	40.07± 6.77	19.64± 2.34
Jun12	0.12	0.90	0.24± 0.06	0.24± 0.07	0.35	0.73	12.52± 3.33	12.95± 1.63	0.61	0.61	51.56± 12.59	55.52± 15.59
Aug12	-0.46	0.65	0.11± 0.05	0.10± 0.02	-0.86	0.41	8.04+- 3.4	6.97+- 2.05	0.55	0.59	63.78+- 24.52	71.12+- 29.74

Leaves of *V. nivea* were more enriched in ^{18}O and ^{13}C in the dry season (Figure 2, Table S1). Neither treatment (control or drought) had a significant effect on $\Delta^{18}O_{CELL,}$ though the interaction between season and treatments did have a significant effect (Table S1). The $\Delta^{18}O_{CELL}$ differed significantly between treatments in transition from wet to dry (June 2012) and in dry season (August 2012), in which individuals in drought treatment showed highest values of ^{18}O (Figure 2A, C). The $\delta^{13}C_{CELL}$ also differed between treatments in June and August 2012. Individuals in the drought treatments had higher (less negative) $\delta^{13}C_{CELL}$ (Table 3). For *V.* aff. *variabilis*, the isotopic composition ($\Delta^{18}O_{CELL}$ and $\delta^{13}C_{CELL}$) was not affected by different treatments nor by the interaction between season and treatment. The isotopic composition differed only between seasons, in which, the new leaves produced showed higher $^{18}O_{CELL}$ and $\delta^{13}C_{CELL}$ as the moisture decreased (Table S1 - suppl. material) (Figure 2B, D). The $\Delta^{18}O_{CELL}$ and $\delta^{13}C_{CELL}$

differed between species in all seasons investigated (Table 3). *V. nivea* showed more depleted in ^{13}C and ^{18}O than *Vellozia* aff. *variabilis* (Table 3) (Figure 2A-D).

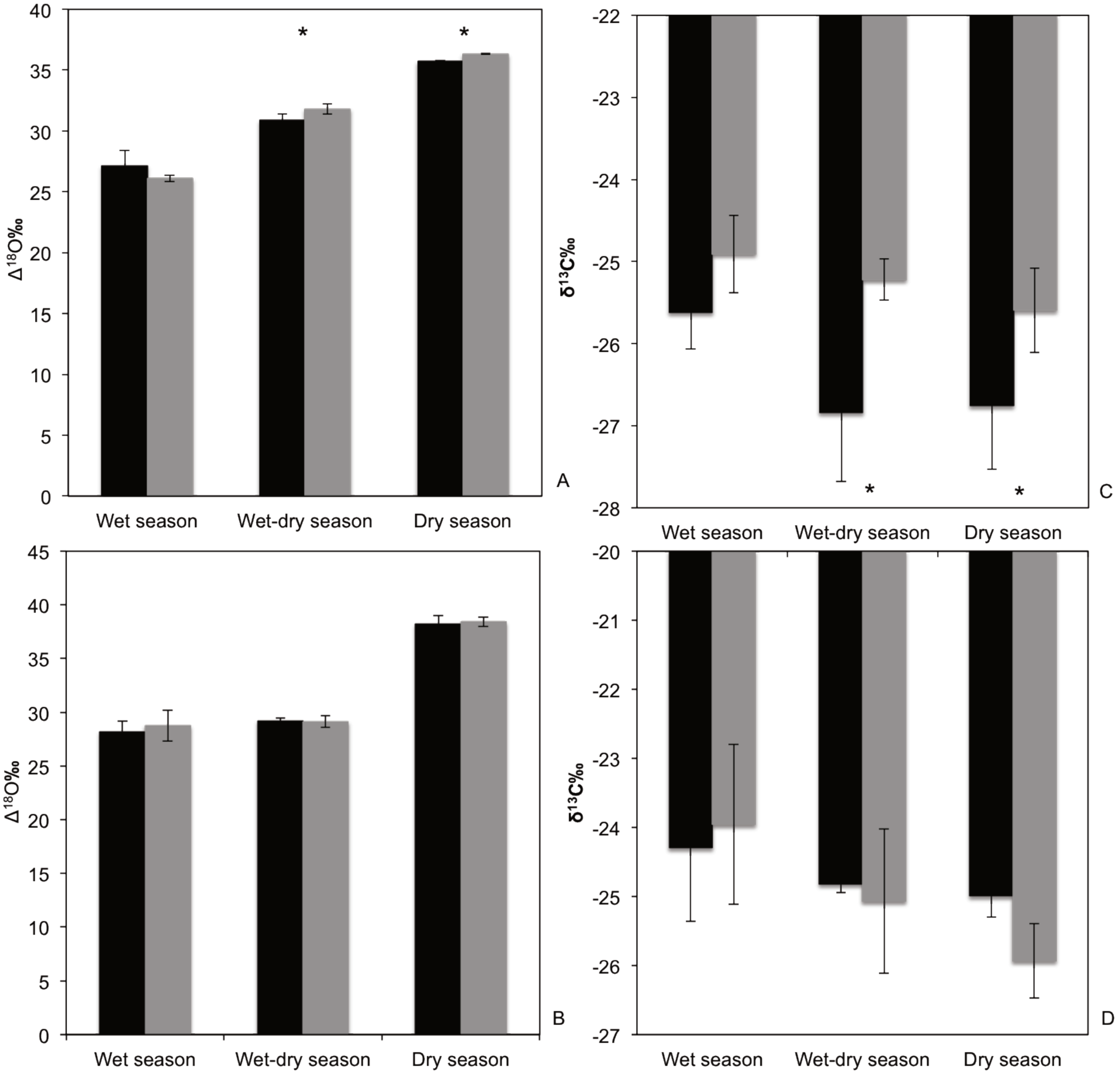

Figure 2. Comparison of leaf cellulose oxygen isotopic composition ($\Delta^{18}O_{CELL}$) and carbon isotopic composition ($\delta^{13}C_{CELL}$) per season and conditions for each species. A) $\Delta^{18}O_{CELL}$ in *Vellozia nivea*, the desiccation tolerant species; B) $\Delta^{18}O_{CELL}$ in *Vellozia* sp., the non-desiccation tolerant species; C) $\delta^{13}C_{CELL}$ in *Vellozia nivea*; D) $\delta^{13}C_{CELL}$ in *Vellozia* sp. Black bars represent individuals average in control plots (n=5ind) and gray bars indicate individuals average in drought treatments (n=5); bars represent the standard deviation; *represents significant differences between conditions (95% confidence

intervals estimated from the model least square means to evaluate differences between pairs);

Table 3. Comparison (Student-t Test) of leaf cellulose isotopic composition of oxygen ($\Delta^{18}O_{CELL}$) and carbon ($\delta^{13}C_{CELL}$) between the species *Vellozia nivea* (the desiccation tolerant species) and *Vellozia* aff. *peripherica* (the non-desiccation tolerant species) in February 2011, June 2012 and August 2012.

	$\delta^{13}C_{CELL}$				**$\Delta^{18}O_{CELL}$**			
	V.* aff. *variabilis	***V. nivea***	**t**	**p**	***V.* aff. *variabilis***	***V. nivea***	**t**	**p**
Feb11	-24.13±1.01	-25.26±0.56	-2.39	0.044	28.48±1.15	26.63±0.97	-3.02	0.01
Jun12	-24.94±0.7	-26.12±1.05	-2.74	0.02	29.16±0.38	31.29±0.68	7.99	2.457e-06
Aug12	-25.41±0.63	-26.18±0.87	-2.20	0.04	38.32±0.61	36.02±0.35	-9.57	1.611e-07

In *V. nivea,* gs was positively related to PAW only for individuals subjected to the drought treatment (control: $r^2=0.09$; p=0.21/ drought: $r^2=0.94$; p=2.434e-09). A (positively) and WUEi (negatively) were related to PAW for individuals in both treatments (Figure 3A-C). In *Vellozia* aff. *variabilis* the gs and A were positively related to PAW for individuals in both treatments. WUEi was not explained by variation in PAW (Figure 3D-F).

At the end of the dry season, the total NSC% did not differ between treatments for either species (*V. nivea*: control = 4.23% ± 2.84, drought treatment = 3.22% ± 1.96, t=0.5311, p=0.6281; *Vellozia* aff. *variabilis*: control = 1.27 ± 0.76, drought=1.47 ± 0.71, t=-0.33, p=0.75). Comparing the species, *V. nivea* (3.65% ± 2.22) showed higher storage of total NSC% than *V.* aff. *variabilis* (1.38%±0.66) in root tissues (t=2.58, p=0.035) in the end of dry season. In *V. nivea* we did not find stored starch in roots, only soluble sugars (Table S2 - suppl. material).

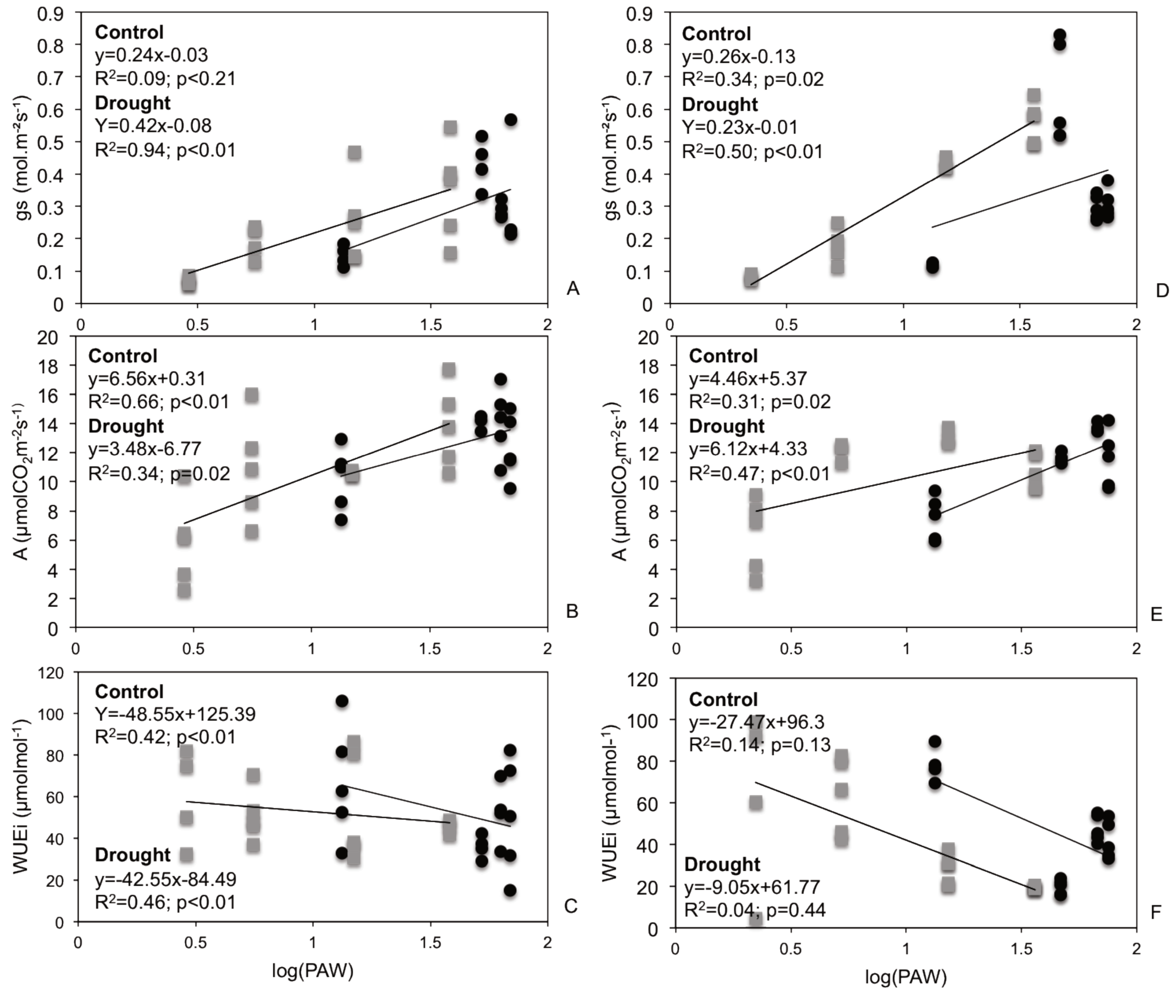

Figure 3. Linear regression between plant available water (PAW) and A) stomatal conductance (gs) in *Vellozia nivea*; B) CO_2 assimilation (A) in *Vellozia nivea*; C) intrinsic water use efficiency (WUEi) in *Vellozia nivea*; D) stomatal conductance (gs) in *Vellozia* sp.; E) CO_2 assimilation (A) in *Vellozia* sp.; F) intrinsic water use efficiency (WUEi) in *Vellozia* sp. *Vellozia nivea* is the desiccation tolerant species and *Vellozia* sp. is the non-desiccation tolerant species. The black circle represents individuals in control treatments while the grey square represents individuals in drought treatment.

Leaf and reproductive phenology

Individuals of *V.* aff. *variabilis* showed a greater number of leaves per rosette and rosettes were longer and total biomass was greater than individuals of *V. nivea*. Leaf longevity did not differ between species (Table 4).

Table 4. Comparison (Student-t Test) between species in number of leaves per rosettes, rosette length (cm), biomass per rosette (g) and leaf longevity (months). *Vellozia nivea* is the desiccation tolerant species and *Vellozia* aff. *variabilis* the non-desiccation tolerant species.

Traits	*Vellozia nivea*	*Vellozia* aff. *variabilis*	t	p
Number of leaves	8.98±2.58	16.83±5.96	-7.87	<0.01
Rosette length	30.64±5.71	40.38±6.46	-7.46	<0.01
Biomass per rosette	7.97±3.94	37.60±17.20	-10.52	<0.01
Leaf longevity	17.18±2.3	19.80±2.61	-6.85	0.55

In both species, leaf mensal balance (LMB) was negatively related to VPD and positively related to PAW. In *V. nivea,* VPD explained 59% and PAW 41% of the total variation explained by the relationship on LMB (Multiple R^2=0.61; p<0.01). In *V.* aff. *variabilis*, VPD explained 61% and PAW 39% of the total variation on LMB (Multiple R^2=0.56; p<0.01) (Table S3 - suppl. material).

Time (months) and the interaction between treatments and time had a significant effect on LMB in both species (Table 5), while the treatment alone did not have a significant effect on LMB. In *V. nivea* the proportion of desiccated leaves was higher in the drought treatment (Table 6). The months with highest proportion of desiccated leaves were August and September in both, 2011 and 2012. Individuals under drought treatment had a significant lower LMB in July 2011 and 2012, May 2012, August 2011 and 2012 and September 2012. In these months the proportion of desiccated leaves was higher in individuals under the drought treatment than in the controls (Table 6, Figure 4 A). In *V.* aff. *variabilis* the drought treatment did not cause a reduction on LMB (Figure 4 B).

Table 5. Linear Mixed-effects Model for leaf mensal balance (LMB) for species, *Vellozia nivea*, the desiccation tolerant species and in *Vellozia* aff. *variabilis*, the non-desiccation tolerant species. Condition refers to control and drought treatments and time to the months studied (from February 2011 until September 2012).

	Vellozia nivea		***Vellozia* aff. *variabilis***	
	F value	p	F value	p
condition	2.05	0.15	0.84	0.36
time	108.08	<0.01	51.81	<0.01
condition:time	6.64	<0.01	2.1	<0.01

Table 6. Proportion (%) of leaves desiccated per month in each condition, control and drought treatments for *Vellozia nivea*. In this table, we showed only the months that leaves dried out.

	Jun 11	**Jul 11**	**Aug 11**	**Sep 11**	**Oct 11**	**Nov 11**	**Mar 12**	**Apr 12**	**May 12**	**Jun 12**	**Jul 12**	**Aug 12**	**Sep 12**
Control	7.15	0.71	51	75.3	0.62	0.18	0.00	0.82	4.62	13.7	13.4	17.9	50.8
Drought	3.84	21.3	82.1	86.9	22.3	6.58	5.81	14.1	25.9	24.8	36.6	64.1	90.6

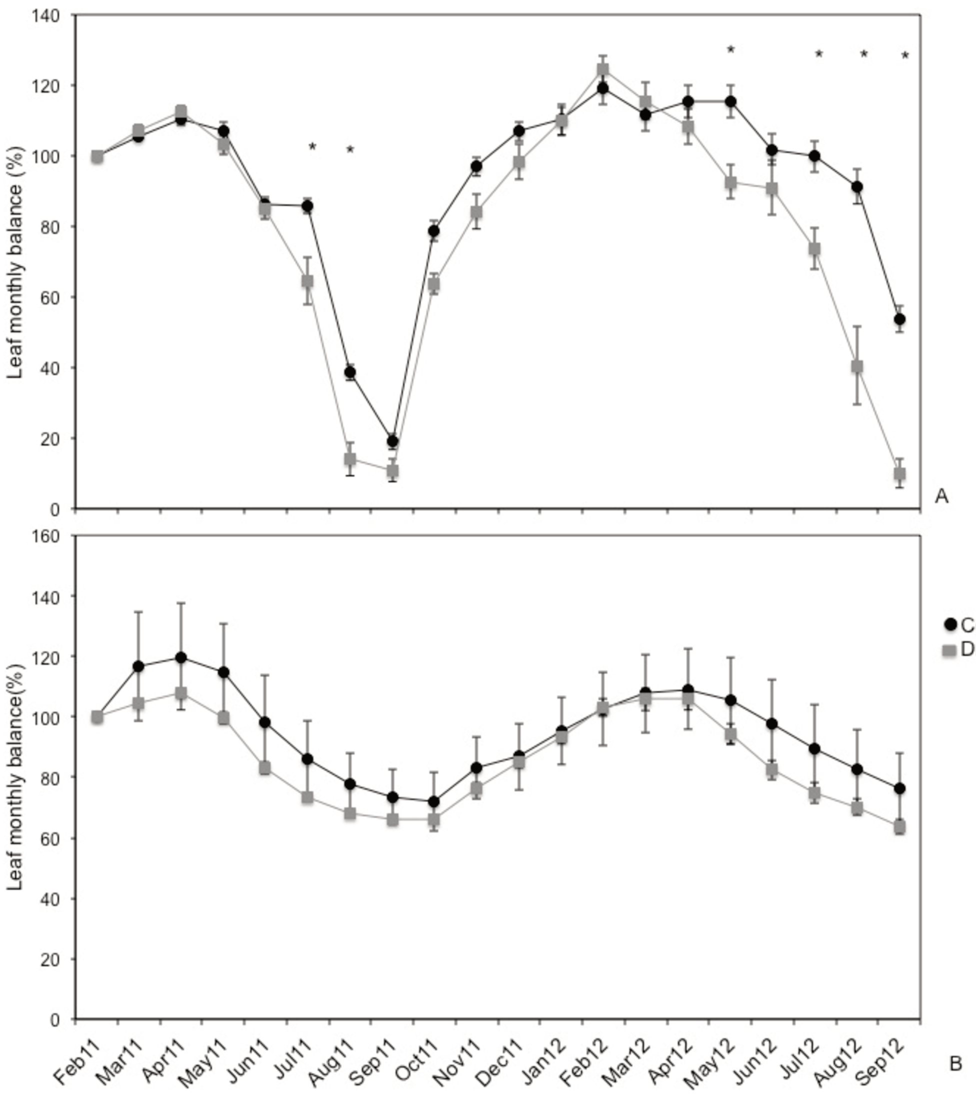

Figure 4. Leaf monthly balance % (LMB) for A) *Vellozia nivea* - the desiccation tolerant species for individuals in both conditions, control and drought treatments. The first month of study was considered 100% and the following were standardized based in the first one. The red line represents the LMB considering all leaves, including the desiccated ones; the green line represents the LMB considering only the green leaves, which remained functionally active. The circle represents individuals in control plots and square individuals in treatment condition; B) *Vellozia* sp. the non-desiccation tolerant species. The black circle represents individuals in drought treatment and grey square individuals in control treatments. The * represents differences between conditions from LMB considering only the green leaves (95% confidence intervals estimated from the model least square means to evaluate differences between pairs).

In *V. nivea* the frequency of individuals that reproduced (defined as individuals that produced floral structures) also differed between treatments (χ^2=181.83; p<0.00001) (Figure 5). In the drought treatment, 50% of the individuals (9 individuals) reproduced. In control plots, only 9% of individuals (2 individuals) reproduced. In addition to sexual reproduction, we also observed changes in vegetative reproduction with the rosettes that produced floral scapes also having produced new rosettes within the parental rosette concomitantly with scape production. For vegetative reproduction, 18 new rosettes were produced in the drought treatment and in the control treatment only one new rosette was produced. All reproductive individuals were completely desiccated during the seasonal drought (Figure 5). No individuals of *V.* aff. *variabilis* reproduced.

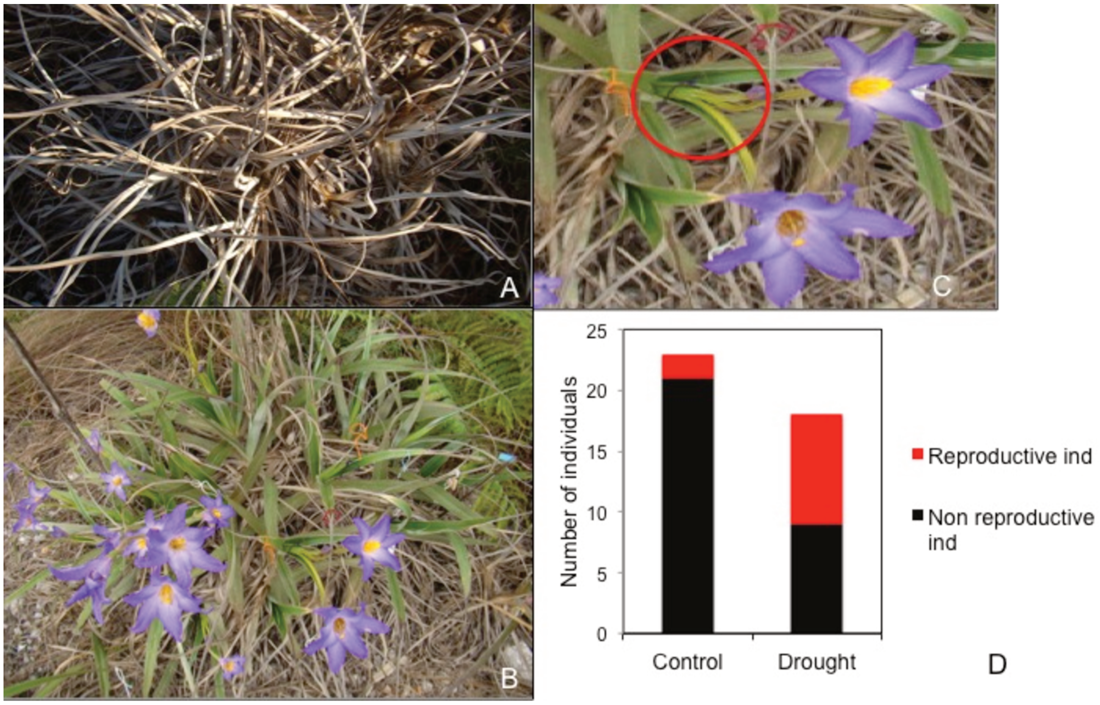

Figure 5. A) Individual of *Vellozia nivea* completely desiccated in drought treatments plots; B) Reproductive individual in drought treatments. This individual was completely desiccated in the previous dry season; C) Vegetative growth associated with reproductive events, the red circle shows the beginning of a new rosette produced inside the parental rosette; D) Number of reproductive *V. nivea* individuals in the control and drought treatment. There were no reproductive individuals for *Vellozia* aff. *variabilis.*

Drought experiment in the greenhouse for the resurgent species - *Vellozia nivea*

Soil water content (SWC) differed between treatments only one day after irrigation was withheld (Figure 6). Stomatal conductance was marginally related to SWC for individuals in control treatment and positively related for individuals in drought treatment (Figure 6). Individuals in the control treatment showed higher rates of gs than individuals under water exclusion treatment. Stomatal conductance reached zero after 11 days of no water. Photosynthesis also was related to SWC for individuals submitted to drought and was not related to SWC for control individuals. Individuals in the drought treatment were only respiring after 10 days without water.

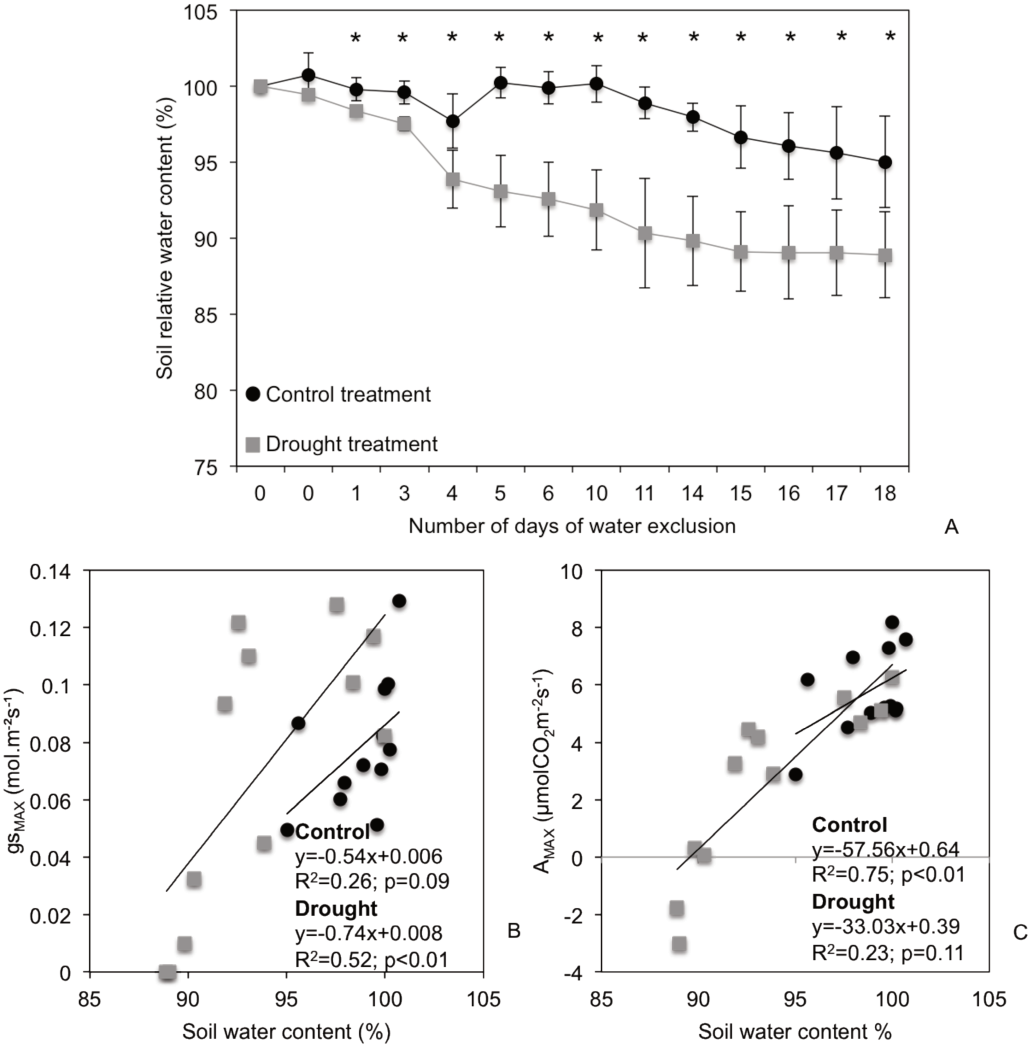

Figure 6. A) Soil relative water content (%) in the drought experiment performed to *Vellozia nivea*; B) Simple linear regression between soil relative water content (%) and

stomatal conductance and C) Simple linear regression between soil relative water content (%) and CO_2 assimilation. The black circles represent individuals under control condition (n=5) and grey square represents individuals under water exclusion treatment (n=7). * represents significant differences between conditions (t-test with $p<0.05$);

Although the total NSC% appeared to have increased under drought (Figure 7), the concentration did not differ significantly among tissues (leaves, roots and stem) (F=0.6289; p=0.543) nor in the interaction between tissues and treatments (F=5.1955; p=0.512). The differences between treatments were marginally significant (F=3.7391; p=0.08). Comparing pairs, NSC% was significantly higher in roots for individuals in the drought treatment (p=0.04) (Figure 7).

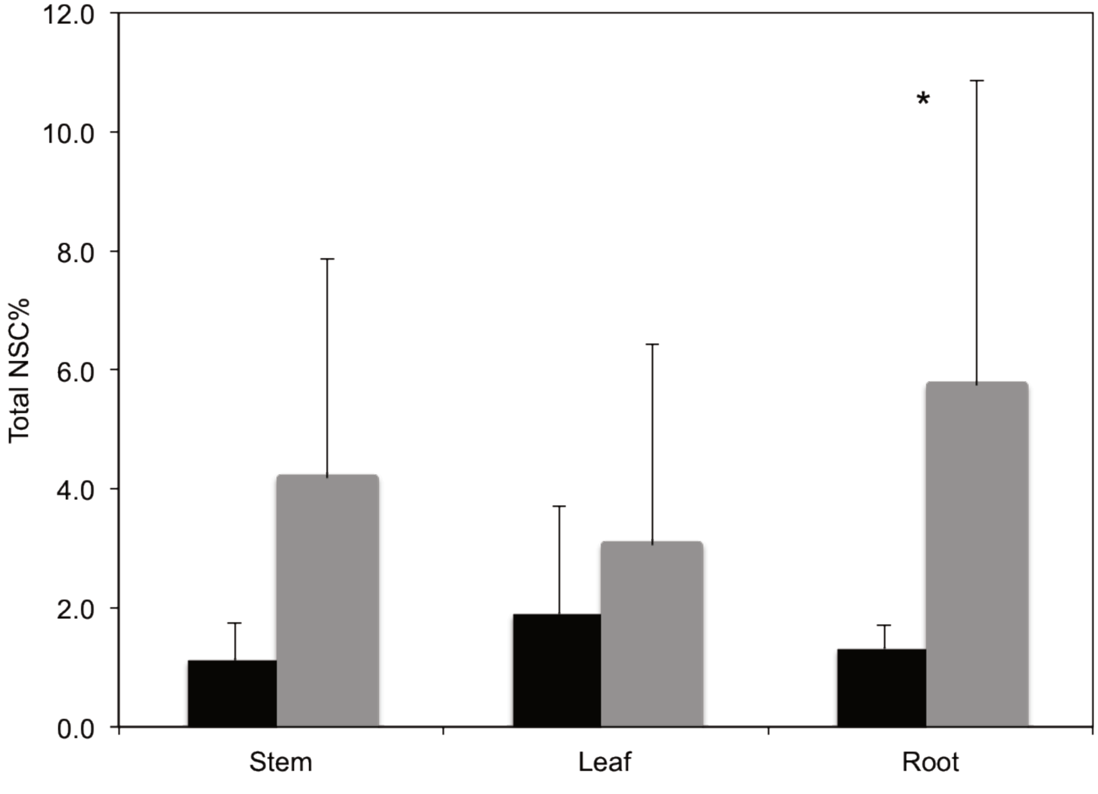

Figure 7. Comparison of total non-structural carbohydrates (NSC%) as percent of dry matter (%) per tissue between conditions. Black bars represent the average of individuals in control treatment and grey bars individuals under drought treatment. * represents significant differences between conditions (95% confidence intervals estimated from the model least square means to evaluate differences between pairs).

DISCUSSION

In this study, we show a comprehensive set of drought-responses related to plant performance, from detailed ecophysiology, to leaf dynamics and reproductive performance, for two species with contrasting hydraulic strategies. The desiccation tolerant species (*V. nivea*) showed a profligate water-use strategy when water was available. Gas exchange and growth (here represented by monthly changes) were highly responsive to changes in soil and atmospheric water availability, indicating an opportunistic strategy. In contrast, the non-DT species (*Vellozia* aff. *variabilis*) showed a conservative water-use strategy, with higher water-use efficiency in all months and no changes in growth when water availability decreased. As a result, the DT species produced less leaves and accumulated less biomass (when assessed over an entire year) compared with the non-DT species. Interestingly, the DT species did not show evidence of C depletion during drought, and instead increased NSC storage (see also Muller et al. 2011, Korner 2013). This response suggests that the DT species is likely better adapted to respond to water deficit via storage of C-expensive compounds that permit it to better cycle through periods of dehydration and rehydration. Furthermore, the DT species continued to invest in reproduction under water limitation (with more floral scapes as well as vegetative growth) compared to the non-DT species, *V.* aff. *variabilis*.

1. Ecophysiological performance

The $\delta^{13}C_{CELL}$ and $\Delta^{18}O_{CELL}$ differed between species under wet, dry and during the transition between wet to dry seasons (Table 3), indicating that these two species differ in carbon assimilation and stomatal conductance during all periods that cellulose was synthesized. The leaf cellulose isotope data are reliable indicators of changes in gas exchange, representing an important trait, since these isotope changes integrate the gas exchange during all period of cellulose synthesis (Scheidegger et al. 2000, Grams et al. 2007). The more enriched composition of ^{13}C in *Vellozia* aff. *variabilis* (non-DT) than in *V. nivea* also suggest higher WUEi for the non-tolerant species while the high $\Delta^{18}O$ reflect a clear down-regulation of gs to conserve water (Cernusak et al. 2003, Grams et al. 2007, Barbour 2007, Cernusak et al. 2013). This pattern was also evident in instantaneous measurements of gas exchange at the peak of the wet season (Feb12), in which *V. nivea* exhibited higher stomatal conductance and lower WUEi than *Vellozia* aff. *variabilis*. These differences in gas exchange between species reveal two contrasting water-use strategies, a profligate/opportunistic behavior showed by *V. nivea* and a conservative water-use in *Vellozia* aff. *variabilis*. The profligate/opportunistic water-use is showed by species that occurs in habitats with shallow or no soil (such as the rock outcrops), where fluctuation occurs more intensely and species show high amplitude of seasonal dynamics in gas exchange. Species in these harsh habitats tend to maximize stomatal conductance, photosynthesis and growth during the narrow windows of opportunity during rainy season (Schwinning 2010, Peñuelas et al. 2011, Moreno-Gutiérrez et al. 2012). This is the case of *V. nivea* that remains desiccated and, consequently, functionally inactive during the dry season. In contrast, the non-DT species showed a conservative water-use, maintained lower rates of stomatal conductance and high WUEi than the DT species. This pattern is consistent with the stomatal optimization theory (Cowan & Farquhar 1977) that predicts plants tend to maximize CO_2 assimilation for a determined amount of water used in the photosynthetic process or tend to minimize water loss for a fixed amount of CO_2 assimilation (Damour et al. 2010), indicating a drought resistance strategy.

Individuals of *V. nivea* (DT species) subjected to the drought treatment showed stronger and faster reductions in stomatal conductance as water availability in the soil decreased. The fast reduction in gas exchange of the DT species, in response to drier conditions, is indicative of an optimization WUEi in individuals under the drought treatment, and can be considered a drought-avoiding mechanism, consistent with the profligate/opportunistic behavior showed by this species (Proctor & Tuba 2000, Tuba 2008, Peñuelas et al. 2011).

In our greenhouse experiment, we observed a complete shutdown in metabolism for individuals subjected to drought treatment. Both A and gs reached zero after 10 days of no water, following which we observed high respiration rates and complete desiccation of the leaves (Figure 6). This result was similar to that observed in other DT plants (Tuba et al. 1996, 1997). For example, *Xerophyta scabrida* (Velloziaceae) leaves took 16 days to dry out completely (Tuba et al. 1996, 1997). The respiration rate, which we observed during the dry-out period in our greenhouse experiment, is common in early desiccated state in poikilochlorophyllous species (Tuba et al. 1998)

Alpert (2000, 2006) pointed out that DT species are less productive than NT species across their annual cycles of dehydration/rehydration. In addition, the carbon demand represented by the respiration rates in DT species in the desiccated period would indicate a higher carbon imbalance in DT species. This "desiccation respiration" should, in theory, lead to the use and possible depletion of NSC storage (Chapin et al. 1990, McDowell et al. 2008, Sala et al. 2010). One hypothesized mechanism of plant mortality during prolonged periods of drought is related to the carbon (C) starvation (McDowell et al. 2008; McDowell & Sevanto, 2010, Sala et al. 2010). Although many plants have mechanisms to down-regulate their C demands during drought, such as the mortality of fine roots and leaf shedding (Sala et al. 2010), the C costs of desiccation respiration in DT species may be a significant cost during drought. Additionally, the degree of root respiration during the desiccation phase, which could add considerably to the C costs of desiccation, is presently unknown.

At end of the dry season in the field, the NSC% in roots did not differ between control and drought treatments in both species. However, the NSC% was higher in DT species (*V. nivea*) than in non-DT (*V.* aff. *variabilis*). Interestingly, the DT species did not contain starch as storage, all NSC% were soluble sugars. Accumulation of sucrose and others soluble sugars has been observed in resurrection plants during the desiccated state (Hoekstra et al. 2001; Moore et al. 2009). Some enzymes of carbohydrate metabolism are activated during desiccation, resulting in a re-direction of carbon flow from reserve substance (such as starch) to soluble "saccharides" (sucrose) and this is fundamental to rehydration process (Moore et al. 2009, Whittaker et al. 2004, Xu et al. 2010). Starch accumulation early in drought is also an important process. Once a threshold of water deficit is reached, the stored starch can be used to maintain an adequate concentration of soluble sugars needed for osmoregulation (Chaves 1991). In the field, all the stored starch was probably remobilized as soluble sugars by the end of dry season when we measured the NSC%. In the greenhouse drought experiment, *V. nivea* were maintained for eight months without water, a longer period than the dry season duration in field. These individuals showed a tendency to increase the storage of NSC%, starch and soluble sugars in leaves, stem and significant increases in roots with drought (Figure 7). *Reaumuria soongorica,* a DT species in a salt desert in China, showed a pronounced increase in NCS% during drought. These NSC% stored appear to be necessary for regrowth when water became available (Xu et al. 2010). Due to the evidence of higher concentration of NSC% in DT than in non-DT species during drought, as well as similar NSC% between treatments (control and drought) in our field experiment, we suggest that this DT *Vellozia* do not die due C starvation during prolonged drought events.

2. Ecological performance

The two congeneric species co-exist in *campos rupestres* areas, however there is evidence of microhabitat specialization. The species are abundant in *campos rupestres* areas, with a marked association of *V. nivea* with rocks and outcrops, while *Vellozia* aff. *variabilis* is abundant in areas with deep soil. Alcantara et al. (submitted paper) showed that DT Velloziaceae species occur mainly in rocky outcrops, a habitat that few species of vascular plants can colonize due to the extremely harsh conditions and low availability of resources.

The LMB and growth performance in both species are dependent on variation in atmospheric and soil water content (e.g., VPD and PAW respectively; Table 5, Figure 4); however, the VPD explained the majority variation on LMB. The correlation of LBM with VPD was also accentuated in the DT species. In our drought treatment, the soil water content decreased continually (Figure 1), but the decrease in VPD during wet season, was enough to allow for leaf rehydration and the return to normal metabolism. Both studied species showed morphological and anatomical structures to absorb water directly into aboveground parts, such as the pseudostems covered by sheath and dead leaves with adventitious roots covered by velamen (Porembski & Barthlott 2000, Oliveira et al. 2005). This rapid water uptake by aboveground plant parts may be advantageous for those species, reducing water deficits in this water limited-ecosystem, in which, several shallow rooted species coexist close to each other (Porembski & Barthlott 2000, Oliveira et al. 2005).

In our two-year study, only the DT species reproduced. Studies on phenology that have been done in the context of climate change have shown that flowering times may be the most plastic trait in response to a change in environmental factors (Parmesan & Yohe, 2003, Kobayashi & Shimizu, Castro-Diez et al. 2012). Plants can show other reproductive responses to climate change related to reproductive phenology as either earlier flowering (Parmesan & Yohe 2003, Root et al. 2003, Menzel et al. 2006, Wolkovich et al. 2012) or a delay in flowering (Brando et al. 2006, Shimizu et al. 2011). Our results showed that all individuals that reproduced in November and December 2011 were completely desiccated during the last dry season (end of September 2012). Half of the individuals that were subjected to the rain water exclusion reproduced, while only two individuals in control plots reproduced (9%). The mostly Velloziaceae species have the reproductive phase synchronized with fire regimes (Conceição et al. 2007, Conceição et al. 2013). The drought stress can be enough to stimulate the reproductive output in V. nivea despite the non-occurrence of fire. Some DT species show a rapid sexual reproduction after desiccation, such as *Craterostigma plantagineum* (Linderniaceae) (Moore et al. 2009). This, did not seem to be the case with *V. nivea.* Many individuals in control plots also dried out completely in end of dry season and those individuals did not reproduce. Outside the control and drought treatments there were a high abundance of individuals and we did not observe flowering individuals of *V. nivea* during the two years of study outside the plots. Contrary to the idea of rapid reproduction after the desiccation phase, some mechanisms of desiccation tolerance seem to conflict with rate or time available for resource acquisition, such as the metabolism shutdown during desiccation state, metabolic cost associated to recovery and reduced threshold for cavitation, leading some trade-offs between tolerance and growth/reproductive rates (Alpert 2006).

CONCLUSIONS

Some models of climate change point that events of drought will became more frequent, increasing in magnitude and intensity in mountainous tropical sites (IPCC, 2013). The DT species shows a profligate water use, exhibiting high gs and low WUEi in the narrow windows available to growth, during the hydrated period. By contrast *V.* aff. *variabilis* showed a conservative water use. The carbon starvation hypothesis seems not be apply to DT species, once this plant showed evidences of increase the NSC% storage in prolonged drought. These two species seems to be very adapted to drought. The Velloziaceae already is an important group in *campos rupestres* areas and with the eminent climate change predictions; these species may increase the abundance in some microhabitats in *campos rupestres*, *V. nivea* can increase the cover area in extremely harsh micro-habitats, such as rocky outcrops, while *Vellozia* aff. *variabilis* may be more abundant in shallow soil.

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SUPPLEMENTARY MATERIAL

Table S1. Linear Mixed-effects Model for leaf cellulose isotopic composition of oxygen ($\Delta^{18}O_{CELL}$) and carbon ($\delta^{13}C_{CELL}$). Condition refers to control and drought treatment and season refers to period that we collected the data (wet - February 2011; transition wet to drought - July 2012 and dry - August 2012) for *Vellozia nivea* (the desiccation tolerant species) and *Vellozia* aff. *variabilis* (the non-desiccation tolerant species).

	Vellozia nivea				***Vellozia* aff. *variabilis***			
	$\Delta^{18}O_{CELL}$		$\delta^{13}C_{CELL}$		$\Delta^{18}O_{CELL}$		$\delta^{13}C_{CELL}$	
Source	F value	p	F value	p	F value	p	F value	p
Condition	1.07	0.32	23.09	<0.01	0.52	0.48	0.9	0.36
Season	454.58	<0.01	4.42	0.03	453.26	<0.01	7.24	<0.01
Condition:Season	7.67	0.007	1.02	0.38	0.32	0.73	1.55	0.25

Table S2. Comparison (Student-t Test) of non-structural carbohydrates between two Velloziaceae species. *Vellozia* aff. *variabilis*, the non-desiccation tolerant species and *Vellozia nivea*, the desiccation tolerant.

	V.* aff. *variabilis	***V. nivea***	**t**	**p**
Glucose/frutose	0.58±0.46	0.13±0.28	-2.05	0.07
Sucrose	0.24±0.21	3.52±2.36	3.66	0.01
Starch	0.55±0.47	0.00±0.00	-2.86	0.04
NSC%	1.38±0.66	3.65±2.22	2.58	0.04

Table S3. Minimum adequate model to describe the relationship between monthly leaf balance (pooling individuals from both treatments) with vapor pressure deficit (VPD) and plant available water (PAW) for each species: *Vellozia nivea*, the desiccation tolerant species and *Vellozia* sp., the non-desiccation tolerant species.

	Vellozia nivea		***Vellozia* aff. *variabilis***	
	t value	**p**	**t value**	**p**
Intercept	3.65	<0.01	10.27	<0.01
VPD	-4.49	<0.01	3.15	0.004
PAW	3.59	<0.01	-4.22	<0.01

CHAPTER 3

Coordination among hydraulic traits and growth performance in three shrub species in *campos rupestres* under contrasting water availability

Coordination among hydraulic traits and growth performance in three shrub species in *campos rupestres* under contrasting water availability

ABSTRACT

The maintenance of water transport is of utmost ecological importance to plants, mainly because this process is essential to allow carbon assimilation and growth. Dysfunction in the water transport can be deleterious to plant performance and survival. Xylem water potential at which plants loss 50% e 88% of conductivity ($P_{50,}$ P_{88}) and the leaf water potential at turgor loss (Ψ_{TLP}) are key functional traits commonly used to assess plant resistance to drought. In our study, we investigated how these hydraulic traits relate to growth, gas exchange and dynamics of non-structural carbohydrates (NSC) of three shrub species growing in contrasting water availability. To impose contrasting conditions of water availability, we implemented a rainwater exclusion experiment in *campos rupestres* vegetation. The $P_{50,}$ $P_{88,}$ and Ψ_{TLP} differed among studied species. Our drought treatment reduced biomass and relative growth performance in only one species (*Vernonia warmingiana*). This species is drought-deciduous and had the most resistant vascular system to cavitation. The other two studied species showed a trade-off between vulnerability to cavitation and NSC storage. *Eremanthus seidellii,* the species with the most vulnerable xylem to cavitation exhibits increases the amount of NSC% in its roots during drought periods. *Campomanesia pubecens* does the opposite, showing a very negative P_{50} and lower NSC% storage. As we observed that even plants with a hydraulic system relatively vulnerable to hydraulic failure might present compensatory mechanisms to deal with drought, as the NSC accumulation in roots.

Key-words: drought, vulnerability to cavitation, P_{50}, water potential at turgor loss (Ψ_{TLP}), trade-off, biomass

INTRODUCTION

The water transport is a key physiological process that determines plant survival under different rainfall regimes (Brodribb & Hill 1999; Hacke et al. 2001; Maherali et al. 2004; Kursar et al. 2009). This water pathway from roots to leaves must remain functional to avoid the stomatal closure and maintain carbon assimilation through photosynthesis. However, during periods of drought, hydraulic failure might interfere with this pathway of water transport (Johnson et al. 2011). Hydraulic failure occurs when water transport is interrupted by a large number of embolized vessels, created by air bubbles entering the water column via pit membranes (*i.e.* cavitation); this might lead to the desiccation of plant tissues (Zimmermann 1983, Tyree & Sperry 1989). The vulnerability to hydraulic failure is typically assessed as the water potential value that plants lose 50% and 88% of their hydraulic conductivity ($P_{50,}$ P_{88}).

Another important functional trait, that reflects plant adaptations to dry environments, is the leaf water potential at turgor loss (Ψ_{TLP}). The Ψ_{TLP} is recognized as a trait directly related with plant drought tolerance (Sack et al. 2003, Bartlett et al. 2012). Species with more negative Ψ_{TLP} showed greater tolerance of lower leaf water potentials and greater ecological amplitude in terms of minimum water availability (Lenz et al. 2006, Blackman et al. 2010). The Ψ_{TLP} reflects the leaf capacity to maintain its physiological function during drought and is correlated with other leaf drought tolerance traits, such as, the water potential in which leaf growth ceases and stomatal conductance decline (Sack et al. 2003, Mitchell et al. 2008, Blackman et al. 2010, Bartlett et al. 2012). Another important trait related to Ψ_{TLP} is the P_{50leaf} that indicates the water potential that leaves lose 50% of their stomatal conductance and also is related to drought resistance.

The maintenance of cell turgor, which is a hydraulically mediated process, has an important role regulating the carbon balance of plants. Growth is highly sensitive to changes in cell turgor and the decrease in xylem or leaf water potential due to drought implies in a reduction in cell turgor and in the capacity to transport sugars (Sala et al. 2010, Korner 2013, Fatichi et al. 2014). This lower cell turgor has the potential to limit cell wall expansion and synthesis (Hsiao 1973, Sala et al. 2010). While there is a decrease in sink activity (i.e. growth), C assimilation can continue which illustrates the decoupling between growth and photosynthetic dynamics (Tardieu et al. 2011, Muller et al. 2011, Korner 2013, Fatichi et al. 2014). In the beginning of drought stress the high decline in growth and the maintenance of photosynthesis can lead the conservation or increase of the non-structural carbohydrate reserves (NSC) (Sala et al. 2010, McDowell et al. 2011). Recently, Mitchell et al. (2014) proposed a trade-off between growth and C safety margin (*i.e.* the difference between leaf water potential when growth is zero and leaf water potential when photosynthesis is zero). In this trade-off, species with a narrow C safety margin (low C storage) were able to maintain growth during chronic drought at the expense of its C reserves and species with large C safety margin reduced growth rate under prolonged droughts and invest in storage. Thus, the accumulation of NSC is fundamental to maintain the integrity of the hydraulic system under drought (Sala et al. 2012). This evidence the interdependency of hydraulic and carbon metabolism in plant survival during drought conditions (McDowell et al. 2011).

Few studies have assessed how climate change, especially drought (see in Anderegg et al. 2012, Mitchell et al. 2013, 2014) might affect the relationships between stomatal behavior, growth and C balance with hydraulic traits (P_{50}, P_{88} and Ψ_{TLP}). This is a fundamental question to understand plants resistance/sensitivity to drought and to predict plant mortality and distribution. Mitchell et al. (2013) pointed that the capacity for rapid growth, characterized as high intrinsic growth rates, can reduce carbon allocation to hydraulic safety. This trade-off can make fast growing species more vulnerable to hydraulic failure (Mitchell et al. 2013). To evaluate the relationship and the possible trade-off between hydraulic traits, growth performance and NSC% storage in plants under contrasting water availability, we implemented a drought experiment *in situ* in rich and endemic vegetation in mountainous area in Cerrado Biome in Brazil, the *campos rupestres.* This vegetation is characterized by high seasonality of rainfall, high evaporative demand, and impermeable soils with low water retention (Porembski & Barthlott 2000, Jacobi et al. 2007). This vegetation, as the others mountainous vegetation, is pointed as a vulnerable ecosystem front of climate change and there is a scarcity of ecological and physiological data for plants in this physiognomy. Little is known about the physiological adjustments and tolerance limits showed by species in this environment. In this work, we aimed answer the following question: how stem vulnerability ($P_{50,}$ P_{88}) and leaf tissue (Ψ_{TLP}) affect to plant growth, gas exchange and water potential during drought? We hypothesize that species more vulnerable to drought (i.e. low P50 and Ψ_{TLP}) exhibiting higher reductions on gas exchange and lower growth rate when exposed to drought.

MATERIAL AND METHODS

Study area and focal species

Our study was carried out in a *campos rupestres* area (20º15'40"S 46º25'25"W), located in Serra da Canastra National Park (Parque Nacional da Serra da Canastra - PNSC), São Roque de Minas, Minas Gerais, Brazil. The vegetation at the site is dominated by small shrubs, rosettes, herbs and grasses, growing over a rocky substrate, mainly quartzite-sandstone. Mean annual temperature is 21.6ºC and mean annual precipitation is 1240 mm (data from CEMIG, São Roque de Minas meteorological station). The climate is typically seasonal, in which the winter is extremely dry, with low or no rain from June until September (mean accumulated in dry season is 61.5mm), and a rainy summer. The study area is located at *c.* 1200 m of altitude.

The *campos rupestres* vegetation is a center of diversity and endemism of plants (Burke 2003; Jacobi et al. 2007). We choose the three most abundant shrub species to conduct the study: *Campomanesia pubecens* (Myrtaceae), *Eremanthus seidelii* (Asteraceae) and *Vernonia warmingiana* (Asteraceae).

Field experimental design

We implemented rainfall exclusion plots to impose extreme dry conditions to *campos rupestres* vegetation. We installed 12 plots in the study area; four were used as control plots and eight as rainwater exclusion (experimental condition). The structures used to exclude rainfall in the plots were implemented in April 2011 and were kept 17 months, excluding about 1580 mm of rainfall. This experimental design follows the one proposed by West et al. (2012), and the structures were constructed with metalon iron and roofs were covered with polycarbonate to allow radiation to reach the vegetation. In the experimental plots, roofs were closed, while in the control ones, the roofs were kept opened. The height of the structures around each plot was 2 meters. We dug trenches (50 cm deep) and envolve with plastic around each plot to minimize plants from accessing water outside the plots. In December 2011, we also closed one side of the plots to keep possible horizontal precipitation away from the plots. We used rocks as foothold to take the measurements and avoid trampling inside the plots. In some plots we inserted more rocks to access all the studied individuals.

Climate characterization and soil moisture measurements

We monitored the environmental conditions inside the plots with sensors of temperature and air humidity (HOBO Pro Series - ONSET) and soil water content (Soil Smart Sensor - ONSET). In the end of 2011, we installed a complete meteorological station with temperature and air humidity sensors (Campbell Scientific - Vaisala), photosynthetic active radiation (Campbell Scientific LI190SB-L15 8721-45), and pluviometer (Campbell Scientific TB4-L15). We also installed eight soil water content sensors (TDR - Campbell Scientific CS616-L150 13932-17), four in each condition (experimental and control plots) and two sensors of soil temperature (Campbell Scientific 107-L150 10755-219), one in each condition. The soil water content sensors were installed only in the superficial soil (1-30 cm), due to the high amount of rocks in the soil. The values obtained with sensors in the meteorological station were used to calibrate the data obtained with the previous sensors in the area.

We used the temperature (T) and relative humidity (RH) to calculate the atmosphere vapor pressure deficit (VPD, KPa), according to the following equation: VPD=0.611375eˆ(t*(1-RH/100)), where t=[917.502*(T)/(240.97+T) (Jones 1992). Plant available water (PAW) was calculated as the difference between the soil water content (SWC) and minimum soil water content (SWC_{MIN}) registered by the TDR sensors, according to the equation: $PAW=SWC-SWC_{MIN}$.

Gas exchange and water potential measurements

We measured pre-dawn leaf water potential (Ψ_{PD}); the mid-day leaf water potential (Ψ_{MD}); the stomatal conductance (gs) and CO_2 assimilation (A). The water potential was measured with a Pressure Chamber (PMS Instruments, Corvallis, OR, USA) and the gas exchange with a portable system IRGA (CIRAS 2, PP Systems, Inc., Amesbury, MA, USA). The gas exchange (A and gs) was measured during clear and sunny days, between 8:30 and 11:00 a.m, under light saturation. We performed curves of assimilation in response to the photosynthetic active radiation and we used the radiation that corresponds to the maximum CO_2 assimilation (A_{MAX}) (PAR=1200μmol quanta $m^{-2}s^{-1}$ - LED light source). The air CO_2 concentration was maintained at 380 ppm; and we kept the leaf temperature and humidity the same at the ambient.

These measurements were taken during five different periods: in the rain season (February 2011/2012); in the transition between the rain and dry season (April 2012 and June 2012, completing 12 months of rain exclusion treatment); and in the dry season (August 2012), when we ended the rain exclusion experiment. We measured five individuals per species in each condition.

Leaf longevity, biomass, and SLA

We evaluated leaf demography monthly to determine the leaf longevity. We selected three stems to following monthly during the study; counted the initial number of leaves, recorded the burst of new leaves and measured the stem length. We repeated this procedure on a monthly basis from February 2011 to September 2012. To determine the plant and stems biomass, we collected plants in field (n=40-100 per species) and we took the following measurements: total height (cm), diameter in the stem base (mm), stem length (cm) and number of leaves per stem. The samples were dried for 72 h at 70°C and, after they were weighted to determined the mass (g). To determine the monthly biomass, we performed a linear regression using the plants that we collected in field and used this equation to estimate the monthly biomass. The biomass was strongly related to the product of stem length (cm) and number of leaves (stem length * number of leaves) for all three species (Figure S1 - suppl. material). As the initial number of leaves and length stems were different among individuals, we relativized this attribute considering the first studied month as reference (the values found in February 2011 was considered 100%).

We collected approximately 100 leaves per species to characterize the specific leaf area (SLA). The SLA was calculated as SLA=leaf area/dry mass, the unit is cm^2/g.

Stem vulnerability curves

To construct the vulnerability curves we use both, the double-ended pressure method (Sperry & Saliendra 1994) and the bench drying method (Sperry et al. 1988). We collected the plants in field, transplanted than to pots and transported to laboratory. In the double-ended pressure, the principle of the technique is to connect a branch segment in a special pressure chamber, pressurize the chamber to the desired water potential and measure the conductance afterwards. We pressurize the chamber for 10 minutes. We determined the vessel length and we used a stem larger than the maximum vessel length to avoid overestimate the percentage of loss conductivity (PLC) following the advices of Ennajeh et al. (2011). As our studied species was shorter (*C. pubecens*=52cm; *E. seidelii*=80cm; *V. warmingiana*=43cm), we used the entire individual to construct the curves (n=3 per species). To details in technique, see Sperry & Saliendra (1994) and Ennajeh et al. (2011).

To construct the vulnerability curves using the bench drying method, we leave the stems drying until they achieve the desired water potential and then, we wrapped them in plastic bags for 1-2h to equilibrate the water potential between leaves and stems. Two or three leaves from each branch were used to measure the water potential with a pressure chamber. We cut the stems segments (4-5 cm long) underwater and connected them to the hydraulic measurements apparatus to determine the flow rate using Ultra Flow meter. The PLC is a relative measurement, using a stem initial flow and the end flow after the embolism removal (flush). P_{50} and P_{88} were used to assess the xylem-cavitation resistance of our different shrub species. The safety margin (SM) is another trait related to plant resistance to drought, in which species with larger SM are considered less susceptible to hydraulic failure (Choat et al. 2012). We calculated the safety margin as SM=minimum Ψ_{MD} - P_{50} or $P_{88.}$

Pressure–volume curves

For each species, we collected the plants in May 2012 in field and transported them to the laboratory to construct pressure-volume curves (p-v curves; n=3-7 per species). We cut the petioles under water and we leave them rehydrating in dark for 12 hours, until Ψ_{leaf} was lower than 0.1 MPa. Before start the measurements we cut the petiole again and started the measures of leaf mass (g) and water potential (MPa). We used the bench drying technique (Turner 1988). The leaves were dried in a temperature of

70°C per 48h and weighted to determinate the relative water content (RWC). We calculated the water potential at turgor loss (Ψ_{TLP}), the relative water content in turgor loss point (RWC_{TLP}), osmotic potential in full turgor (Ψ_{sFT}) and the elasticity modulus (ε). Calculus details can be found in Turner (1988). We used the spreadsheet developed by Sack & Pasquet-Kok (2011), available in prometheuswiki (http://prometheuswiki.publish.csiro.au/tiki-index.php?page=Leaf+pressure-volume+curve+parameters).

Non-Structural Carbohydrates analyses

To evaluate the NSC storage in end of dry season (September 2012), we quantified the percentage of non-structural carbohydrates per dry mass (NSC%) in roots. We followed the enzymatic method proposed by Sevanto et al. (2014) to analyze the NSC content. NSC is defined in this work as free, low molecular weight sugars (glucose, fructose and sucrose) and starch. Immediately after collection, samples were microwaved to stop enzymatic activity. After that, samples were oven-dried at 70°C for 24–48 h and ground to fine powder. We prepared approximately 12 mg of plant material with 1.6 mL of distilled water to the analyses. We used amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich) to digest total NSC to glucose; and invertase, glucose hexokinase kits (GHK) and phosphorus glucose (Sigma-Aldrich) to quantify the low molecular weight sugars. The concentration of free glucose was determined photometrically in a 96-well microplate spectrophotometer. Starch was calculated as the difference between the total NSC and the low molecular weight sugars (see details in Sevanto et al. 2014). All NSC values are expressed as percent of dry matter.

Statistical Analyses

To assess the variation on leaf gas exchange and water potential traits, we calculated the seasonal variation in traits following the equation: Variation or Plasticity index = (maximum values of X - minimum values of X)/ maximum values of X. The X in equation represents the trait evaluated. Values next to 1 represents a high variation between maximum and minimum values for the trait studied.

To compare the average among species in leaf longevity, MFA, RGR in dry season, RGR in wet season, P_{50}, parameters extracts from p-v curves, gs and A we used ANOVA with post-hoc test (Tukey) to evaluate the differences between pairs. When necessary, data were transformed to satisfy the ANOVA assumptions of residual normality and homoscedasticity.

To evaluate the temporal variation on gas exchange, water potential, biomass and RGR during the study and the treatment effect, we fitted a linear-mixed effects model. We specified random intercepts per individual to account for the temporal pseudo replication in our design. In the model, we considered condition as a fixed factor with two levels (control and drought treatment); time was also considered a fixed factor and corresponded to months that we measured the variables in the field. We investigated differences between levels of the fixed factors by using 95% confidence intervals estimated from the model least square means.

RESULTS

Our drought experiment caused a strong reduction on soil water content in upper soil layers. From December 2011 until September 2012 we observed a reduction of 34% in soil water content in drought treatment plots (details are available in Teodoro et al. unpublishing results, Chapter 1 and 2 in this thesis).

MFA and leaf longevity differed among species; *C. pubecens* showed higher SLA and leaf longevity than *E. seidelii* and *V. warmingiana* (MFA: F=222.22; p<0.0001/ Leaf longevity: F=28.64; p<0.0001). The RGR in wet season did not differed among species (F=1.89; p=0.16), while the RGR in dry season *V. warmingiana* exhibited the lower RGR than the other two species (F=4.39; p=0.016). The Ψ_{TLP} also differed among species (F=3.95; p=0.048), *C. pubecens* showed values of Ψ_{TLP} more negatives than the other two species. The ε was similar among species (F=1.25; p=0.32). P_{50} differed among species, in which, *E. seidelii* showed the most positive P_{50}. The P_{88} was higher in *E. seidelii*, followed by *V. warmingiana* and *C. pubecens* showed the most negative value. The safety margin calculated using both, P_{50} and P_{88}, was higher in *V. warmingiana*, followed by *E. seidelii* and *C. pubecens* (Table 1).

Table 1. Average of traits and standard deviation for each studied species in *campos rupestres*. SLA - Specific leaf area ($g.cm^{-2}$); Leaf longevity (months); RGR - relative growth rate (% of increase in biomass); Ψ_{TLP} - Water potential in turgor loss point (MPa); ε - elasticity modulus (MPa); P_{50} - Water potential when plants loss 50% of conductivity (MPa); Safety margin - SM (MPa).

Species	**SLA**	**Leaf longevity**	**RGR in dry season**	**RGR in wet season**	$\mathbf{\Psi_{TLP}}$	**ε**	$\mathbf{P_{50}}$	**SM (P_{50})**	$\mathbf{P_{88}}$	**SM (P_{88})**
C. pubecens	333.24 ±114.6	11.55±4.6	-0.17±0.35	0.23±0.5	-2.81±0.17	4.76±1.92	-1.91±0.28	-2.45	-5.67	1.31
E. seidelii	139.94 ±46.5	8.8±4.26	-0.16±0.44	0.21±0.28	-2.48±0.32	2.01±0.92	-1.63±0.23	-0.31	-3.85	1.92
V. warmingiana	165.46 ±51.6	7.75±3.17	-0.76±1.14	0.57±0.9	-1.69±0.88	7.98±9.09	-3.22±0.16	+1.21	-4.89	2.89

Gas exchange and water potential under contrasting water availability

The maximum stomatal conductance (gs) observed in wet season differed among species, in which *E. seidelii* showed the highest rates (F=12.17; p=0.0002). The minimum gs, showed by species in dry season, did not differed among species (F=0.94; p=0.40) (Figure 1A). The maximum A differed among species, in which, *C. pubecens* showed the high assimilation and *V. warmingiana* the lowest rate (F=50.04; p=<0.0001). The minimum A did not differ among species (F=2.72; p=0.08) (Figure 1B).

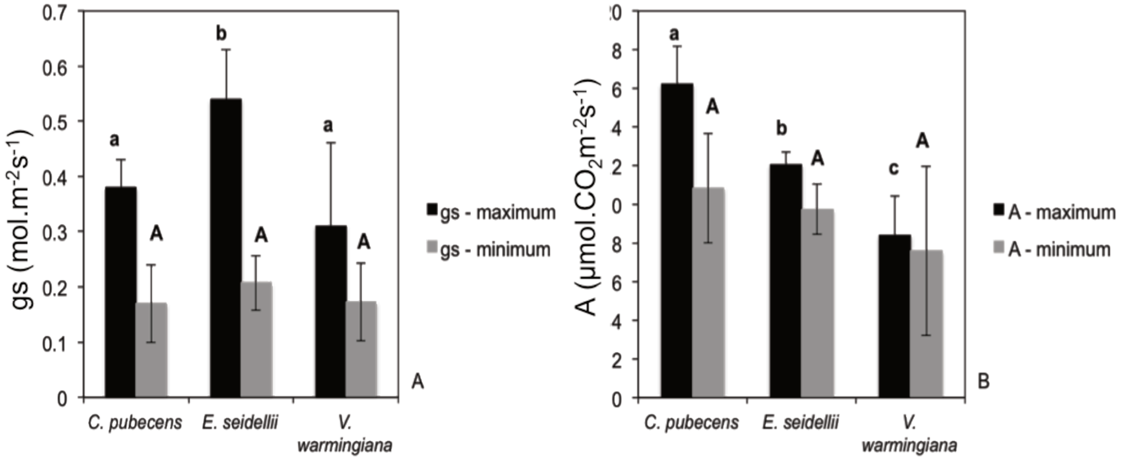

Figure 1. Comparison among three species in *campos rupestres*: A) stomatal conductance (gs - maximum: black color) observed in wet season and minimum stomatal conductance (gs - minimum: gray color) observed in dry season; B) CO_2 assimilation (A - maximum: black color) and minimum photosynthesis (A - minimum: grey color). The

lower case indicates similarity/differences in gs and A maximum and the capital case indicates similarity/differences in gs and A minimum.

The gs differed temporally in all studied species, in which the gs was higher in wet season and lower in dry season. Our drought treatment and the interaction between conditions and time had lower influence in gs rates (Table 2). Only *C. pubecens* showed significant reductions on gs rates under drought treatments in Feb and Apr 2012. The maximum assimilation (A) differed temporally, with higher rates in wet season than in dry one, and between conditions, with lower rates under drought treatment in *C. pubecens* (difference in Feb and Jun 2012) (Table2). *E. seidelii* and *V. warmingiana* showed similar rates of A along the two study year (no time effect). Treatment and the interaction between treatment and time also did not influence A in those two species (Table 2).

Table 2. Linear Mixed-effects Model for stomatal conductance (gs) and CO_2 assimilation (A) for three abundant species in *campos rupestres*. Condition refers to differences between control and drought treatments; Time corresponds to temporally variation in the attributes; Condition:Time refers to interaction between condition and time terms.

	gs						**A**					
Spp	**Condition**		**Time**		**Condition:Time**		**Condition**		**Time**		**Condition:Time**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
C. pubecens	2.9	0.09	**9.9**	**<0.001**	2.5	0.07	**6.1**	**0.02**	**11.2**	**<0.001**	0.3	0.8
E. seidelii	0.4	0.54	**20.8**	**<0.001**	0.7	0.6	0.2	0.64	2.1	0.11	0.18	0.94
V. warmingiana	0.1	0.99	**3.3**	**0.02**	0.4	0.83	0.9	0.35	0.1	0.98	1.1	0.41

Ψ_{PD} and Ψ_{MD} differed between conditions, time and interaction between condition and time in *C. pubecens* (Figure 2A). Individuals under drought showed most negative Ψ_{PD} in Jun11, Feb, Apr and Aug 12 and also showed reductions on the Ψ_{MD} in Jun and Aug12. *E. seidelii* showed temporal differences in Ψ_{PD} and Ψ_{MD}. The interaction between condition and time also had a significant effect on Ψ_{PD}, in which, individuals under drought treatment showed lower Ψ_{PD} in Jun 11, Apr and Aug 12 (Figure 2B). *V. warmingiana* showed only significantly temporal variations on Ψ_{PD} and Ψ_{MD}. The drought treatment and the interaction between condition and time did not have effects on water potential for this species (Figure 2C) (Table 3).

E. seidelii showed the highest variation between seasons in gs and it was accentuated for individuals subjected to drought treatment. *C. pubecens* exhibited the highest variation for A, Ψ_{PD} in control treatment and Ψ_{MD} drought treatment. *V. warmingiana* showed the highest variation for Ψ_{MD} in control treatments and Ψ_{PD} drought treatments (Table 4).

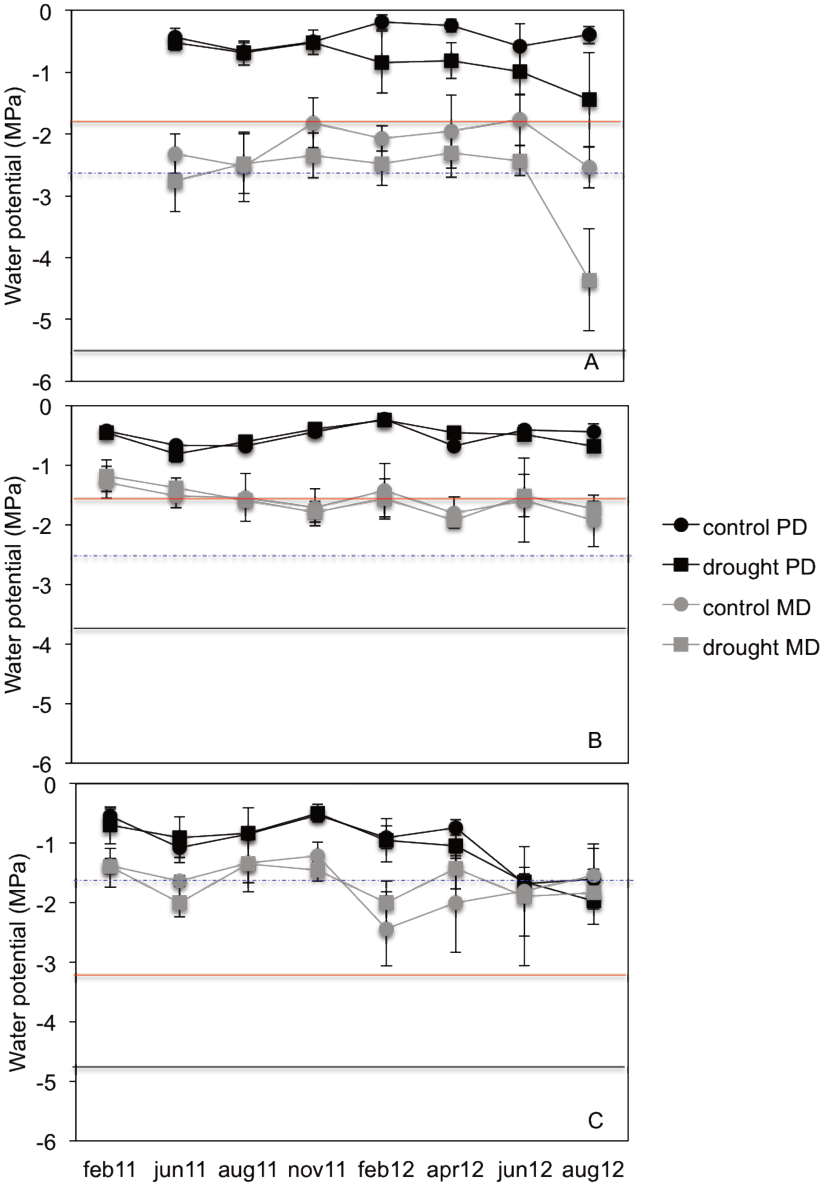

Figure 2. Temporal variation on pre-dawn water potential (Ψ_{PD}) and mid-day water potential (Ψ_{MD}) for individuals in control and drought treatments. The black color represents $\Psi_{PD,}$ while the gray color the Ψ_{MD}. The circle represents individuals in control treatments and square individuals in drought treatments. The red line indicates the water

potential in which plants loss 50% of their conductivity (P_{50}), the black line indicates the water potential in which plants loss 88% of their conductivity (P_{88}), and the blue one indicates the turgor loss point (Ψ_{TLP}). A) *Campomanesia pubecens*; B) *Eremanthus seidelii*; C) *Vernonia warmingiana*.

Table 3. Linear Mixed-effects Model for pre-dawn water potential (Ψ_{PD}) and mid-day water potential (Ψ_{MD}) for three abundant species in *campos rupestres*. Condition refers to differences between control and drought treatments; Time corresponds to temporally variation in the attributes; Condition:Time refers to interaction between condition and time terms.

Species	**Pre-dawn water potential**						**Mid-day water potential**					
	Condition		**Time**		**Condition:Time**		**Condition**		**Time**		**Condition:Time**	
	F	p	F	p	F	p	F	p	F	p	F	p
C. pubecens	**28.3**	**<0.001**	**2.9**	**0.015**	**3.8**	**0.003**	**29.3**	**<0.001**	**11.2**	**<0.001**	**4.1**	**0.002**
E. seidelii	0.9	0.33	**33.7**	**<0.001**	**7.3**	**<0.001**	0.05	0.81	**4.2**	**0.0008**	0.48	0.84
V. warmingiana	1.4	0.24	**24.9**	**<0.001**	1.05	0.41	<0.1	0.99	**2.5**	**0.03**	0.79	0.59

Table 4. Plasticity index (PI) for stomatal conductance (gs), maximum assimilation (A), pre-dawn water potential (Ψ_{PD}) and, mid-day water potential (Ψ_{MD}). Values next to 1 indicate high variation between maximum and minimum values for each trait.

Species	**Control treatment**				**Drought treatment**			
	gs	**A**	Ψ_{PD}	Ψ_{MD}	**gs**	**A**	Ψ_{PD}	Ψ_{MD}
C. pubecens	0.55	0.31	0.71	0.30	0.54	0.35	0.64	0.47
E. seidelii	0.57	0.24	0.66	0.34	0.66	0.21	0.71	0.39
V. warmingiana	0.51	0.21	0.68	0.39	0.59	0.34	0.75	0.32

Biomass and Growth performance under contrasting water availability

Our drought treatment had little effect on biomass in our studied species (Figure 3A-C, Table 5). Biomass differed significantly among months (time effect) in all species. It was strongly accentuated in *V. warmingiana* that showed a marked seasonal pattern. In dry season (Jul to Sep) most individuals lost their leaves, similarly to deciduous plants (Figure 3C). The interaction between condition and time had a significant effect only in *V. warmingiana* (Table 5). In *V. warmingiana*, the biomass was lower in individuals under drought treatment in January, February, March, April, May, June and July 2012. In *C. pubecens* individuals under drought treatment showed a significant increase in biomass in October 2011. In *E. seidelii* also there was an increase in biomass in individuals subjected to drought in March 2012 (Table 5).

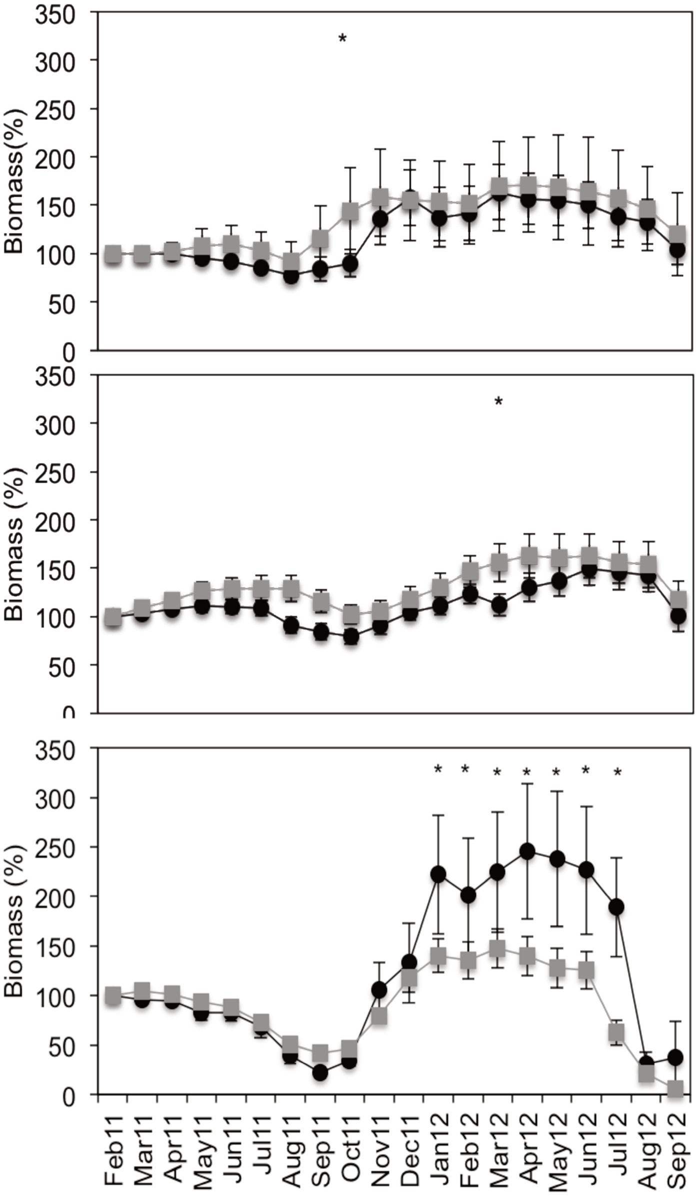

Figure 3. Relative biomass (%)per month per treatment. The black circle represents the control treatment and the grey square represents the drought treatment. A) *Campomanesia pubecens*; B) *Eremanthus seidelii*; C) *Vernonia warmingiana*.

Table 5. Linear Mixed-effects Model for biomass and relative growth rate (RGR) for three abundant species in *campos rupestres*. Condition refers to differences between

control and drought treatments; Time corresponds to temporally variation in the attributes; Condition:Time refers to interaction between condition and time terms.

Species	**Biomass**						**Relative Growth Rate**					
	Condition		**Time**		**Condition:Time**		**Condition**		**Time**		**Condition:Time**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
E. seidelii	1.54	0.22	7.57	<0.0001	0.56	0.93	0.25	0.61	10.31	<0.0001	1.34	0.15
C. pubecens	0.66	0.42	10.03	<0.0001	0.47	0.97	0.08	0.77	4.79	<0.0001	1.19	0.26
V. warmingiana	3.45	0.07	21.68	<0.0001	3.97	<0.0001	0.18	0.67	14.91	<0.0001	4.03	<0.0001

Table 6. Minimum adequate model to describe the relationship between biomass and relative growth rate (pooling individuals from both treatments) with vapor pressure deficit (VPD) and plant available water (PAW) for each species. RI represents the relative importance explained for each variable based in the r^2.

Species	**Data regression**		**VPD**			**PAW**		
	r^2	**p**	**t**	**p**	**RI**	**t**	**p**	**RI**
E. seidelii	0.55	<0.0001	-5.46	<0.0001	0.66	-4.43	0.0001	0.34
C. pubecens	0.34	0.003	-3.68	0.001	0.91	-1.45	0.16	0.09
V. warmingiana	0.6	<0.0001	-4.52	<0.0001	0.74	1.18	0.25	0.26

The starch, soluble sugars and, total NSC did not differ between treatments in roots at the end of dry season, while differing among species (Starch: F=7.35; p=0.004/ Soluble sugars: F=6.30; p=0.007/ total NSC: F=5.766; p=0.01). *C. pubecens* showed highest starch content and the lowest of soluble sugars. *E. seidelii* showed the highest amount of total NSC% (Figure 4).

The abiotic variables that mostly influenced the biomass were VPD and PAW (Table 6A-B). Those variables showed no relationship to PAR, due to this, it was excluded of model. The biomass was related to both VPD and PAW in *E. seidelii*, while in *C. pubecens* and *V. warmingiana* it was related only to VPD (Table 6).

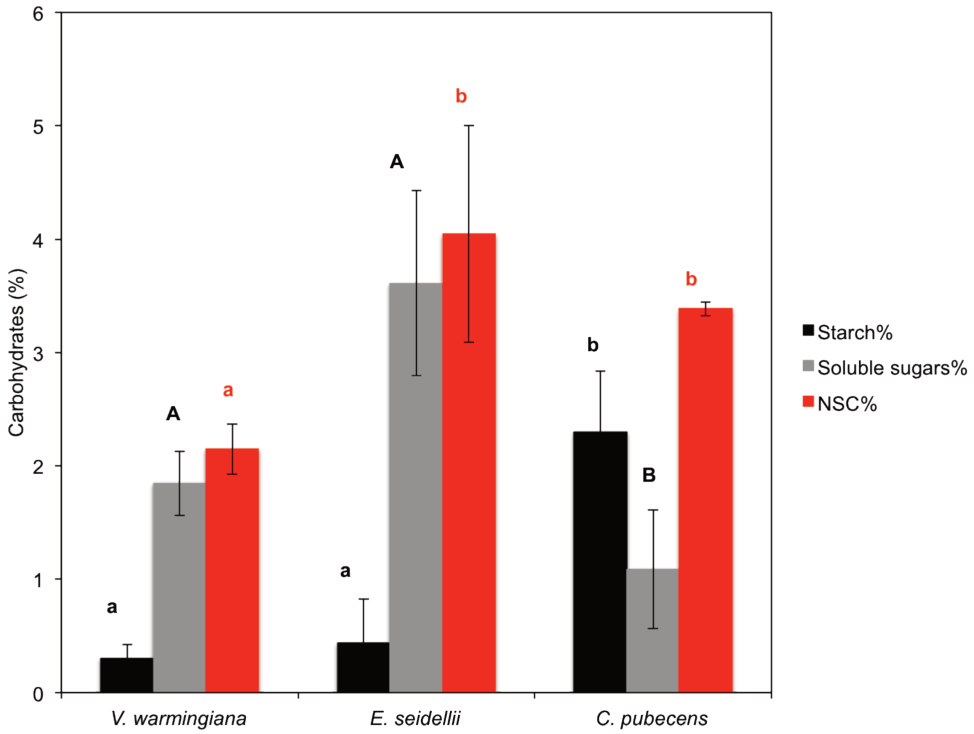

Figure 4. Starch, soluble sugars and total non-structural carbohydrates (NSC%) per dry mass in roots for three species in *campos rupestres* considering individuals in control and drought treatment. Different letters indicate significant differences between pairs of species. The lower case indicates similarity/differences in starch concentration; the capital case indicates similarity/differences in soluble sugars concentration, and the red letter indicates similarity/differences in NSC%.

Vulnerability curves and stomatal conductance

Individuals of *C. pubecens* and *E. seidelii* showed a strongly decrease in stomatal conductance in Ψ_{MD} more negatives than the P_{50}. Those species showed a reduction of 50% in gs under Ψ_{MD} more negatives than the P_{50} (Figure 5 A, B). Both species showed a negative safety margin (Table 1). In *V. warmingiana* we did not observe gas exchange in Ψ_{MD} more negative than P_{50}. This species exhibited a larger safety margin than the other two species (Figure 5 A-C). *C. pubecens* and *E. seidelii* showed the Ψ_{MD} along the years next to the P_{50} value (Figure 2 A-B). *C. pubecens* showed an accentuate decrease in Ψ_{MD} in individuals subjected to drought treatment, these individuals showed a Ψ_{MD} more negative than P_{50} with limited gas exchange (Figure 3A, 6A).

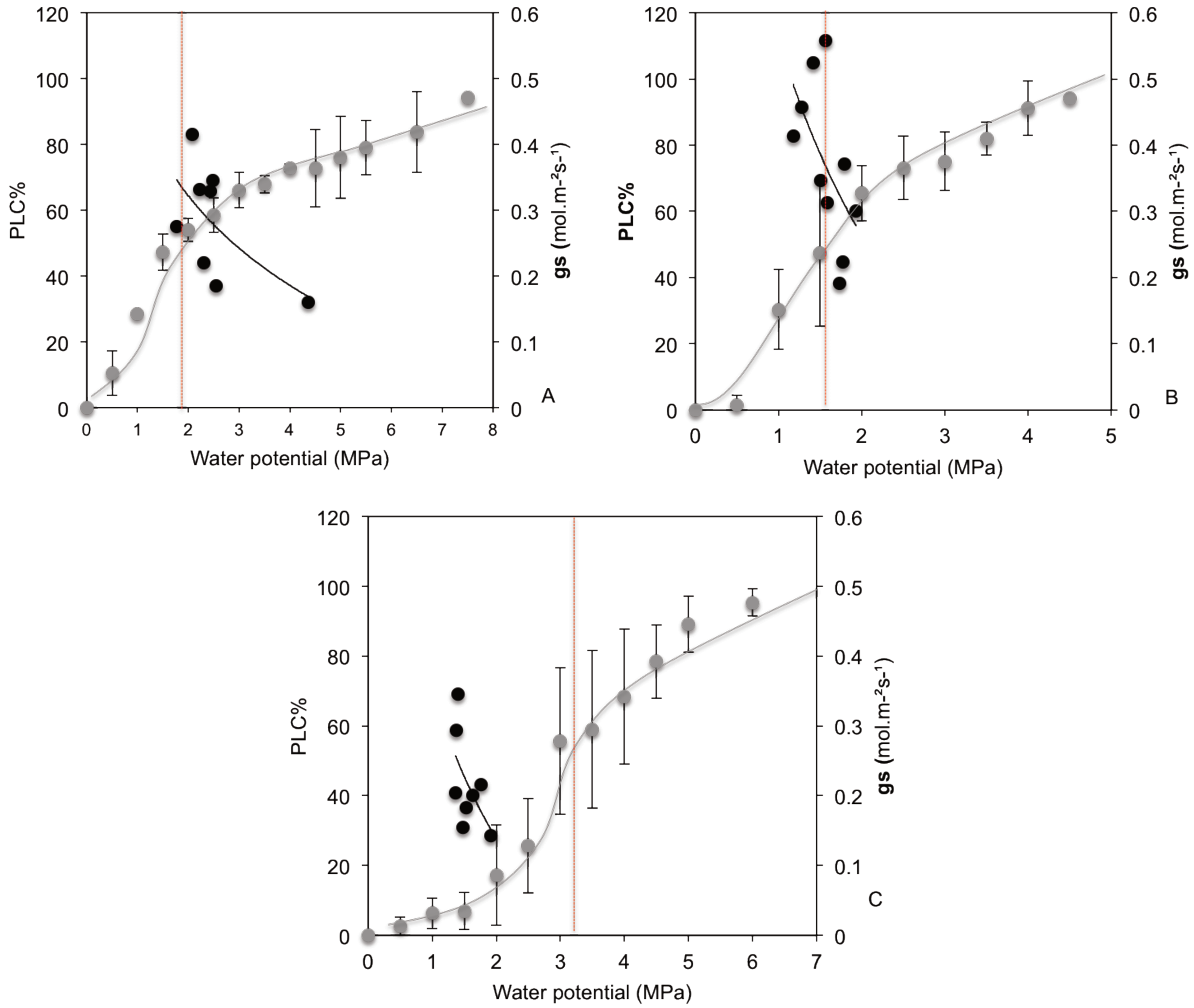

Figure 6. Percentage of loss conductivity (PLC) and stomatal conductance (gs) per water potential curves for three abundant species of *campos rupestres* vegetation. The red line represent the P_{50}, which represents the water potential that plants loss 50% of their conductivity. The vulnerabilty curves was constructed using the double-ended pressure method in three individuals per species. A) *Campomanesia pubecens*; B) *Eremanthus seidelii*; C) *Vernonia warmingiana*.

DISCUSSION

Plant strategies to cope with drought are the product of adaptive traits that enable plants to keep functioning under limited water supply and recovery when water deficit is relieved (McDowell et al. 2011). Our studied species showed different trade-offs related to vulnerability to cavitation, seasonal patterns of biomass and C balance that conferred a high resistance to drought. Even plants with a hydraulic system relatively vulnerable to hydraulic failure (lower P_{50} and Ψ_{TLP}) might present compensatory mechanisms to deal with drought, as the NSC accumulation in roots.

The differences in $P_{50,}$ P_{88} and in Ψ_{TLP} we observed (Figure 2) suggest different sensitivity to hydraulic failure among the studied species. In addition, our results showed different resistance to drought in the same plant considering each part of the pathway. Considering the stem P_{50}, *V. warmingiana* was the most resistant species, followed by *C. pubecens* and *E. seidelii*. However, in the leaf tissue hydraulic, *C. pubecens* showed the most negative Ψ_{TLP}, followed by *E. seidelii* and at least *V. warmingiana*. *Vernonia warmingiana* exhibited an almost deciduous phenology and this species showed the stem more resistant to cavitation among the three studied species. This species shows the lower leaf longevity among species (7.75±3.17 months). In general, deciduous tree species were more susceptible to water stress-induced embolism in stems than the co-existing evergreen tree species (Choat et al. 2003, 2005), the opposite that we found. This species lose their leaves in the seasonal dry season, but maintain the stems intact. The new leaves and stems are produced in the apex of the main stem. We did not measure the leaf vulnerability curves, but recent studies revealed a strong relation between Ψ_{TLP} and leaf P_{50} (Blackman et al. 2010, Bartlott et at. 2012). Considering valid this relation between Ψ_{TLP} and leaf P_{50}, the leaf in *V. warmingiana* is much more vulnerable to cavitation (Ψ_{TLP}=-1.69±0.88). Leaf embolism is considered a 'safety valve' to isolate and protect the upstream hydraulic pathway (Chen et al. 2010, Johnson et al. 2011). Johnson et al. (2011) pointed that leaves should be more 'disposable' due to their lower construction cost than branches and stems. Losing leaves may protect the stem from runaway xylem embolism and possible stem dieback (Sperry 1986, Chen et al. 2010, Johnson et al. 2011). In *campos rupestres* there are a lack of water and nutrients and due to the high construction cost of stems compared to leaves, *V. warmingiana* loss their leaves, but seem to maintain the stem integrity, avoiding the high cost to construct new vessels.

Campomanesia pubecens and *E. seidelii* are evergreen species. The stem vulnerability and the Ψ_{TLP} are coupled in these species, in which, *C. pubecens* seems to be more drought resistant than *E. seidelii*. These two species exhibited contrasting hydraulic strategies; *C. pubecens* showed anisohydric behavior, allowing strong reductions in water potential (Figure 2A) and maintained the gas exchange (Teodoro et al. unpublished manuscript - Chapter 1 in this thesis). Even showing anisohydric strategy, this species was the mostly drought responsive in relation to reductions on gs and A between the control and drought treatments. By oppositely, *E. seidellii* showed an isohydric strategy (Teodoro et al. unpublished manuscript - Chapter 1 in this thesis), maintained the Ψ_{MD} almost constant along the years (Figure 2B). This species did not show reduction on gs between treatments, however showed the highest variation on gs between seasons. In wet season, *E. seidelii* showed the highest rate of gs among the species and in peak of drought season the three species did not differ among them.

The difference between the minimum Ψ reached by species and the P_{50} is a measure of the safety margin (SM) and this is another trait indicating the plant's hydraulic strategy (Meinzer et al. 2009, Choat et al. 2012). This SM quantifies the degree of conservatism in a plant's hydraulic strategy, indicating that plants with low (or even negative) safety margins experience large amounts of embolism and therefore potential risk of hydraulic failure (Choat et al. 2012). Considering the P_{50} to calculate the SM, *Vernonia warmingiana* showed a positive larger SM, while *C. pubecens* and *E. seidelii* showed a negative SM, indicating that those species function with less than 50% of their conductivity. Recently, Choat et al. (2012) showed that 70% of 226 wood species from different environments operate with a narrow or even negative hydraulic safety margins. Anisohydric species are pointed to be more drought resistance and generally showed larger SM, in our study, this species seems to be more resistance, but it showed the most negative SM among the species (Meinzer et al. 2009, McDowell et al. 2011, Plaut et al. 2012, Plaut et al. 2013). The negative SM reached by two species can be an indicative that P_{50} is not the point of hydraulic failure for those species. However, this point has an important meaning for plants in *campos rupestres*. When these plants exceed the P_{50} water potential, there was a marked reduction on gas exchange, representing a loss of more than 50% of their maximum capacity of stomatal conductance (Figure 5). The SM calculated with the P_{88} value was positive for the three species. In field these species did not show Ψ more negative than the P_{88}, corroborant with the predictions that the P_{88} represents the point of hydraulic failure in an irreversible process, causing the plant mortality (Choat et al. 2012).

The biomass for all three species showed a seasonal pattern, with high increment in biomass and, consequently in growth performance during the wet season (Figure 3). Our drought experiment caused a strong reduction on biomass only in *V. warmingiana*. Individuals under drought treatment showed low biomass from January 2012 until July 2012 (Figure 3). Some plants in water limitation condition tend to reduce growth but increase C storage (Korner 2003, Sala et al. 2012, Fatichi et al. 2014, Mitchell et al. 2014). *V. warmingiana* was the only species that suffered the effects of our prolonged drought and the individuals subjected to drought treatment did not increase the amount of total NSC in roots in end of seasonal dry season. Mitchell et al. (2014) showed that there was not a generalized plant response to drought characterized by accumulation of NSC during moderate drought followed by depletion as drought intensified.

CONCLUSION

In this water-limited ecosystem the three studied plants showed different strategies and trade-offs to cope with the drought. The process of hydraulic failure and carbon dynamics are intimately linked through the balancing between minimize water loss and maximize the C uptake at the leaf surface (McDowell et al. 2011). This balance was acquired per different strategies. In *V. warmingiana*, the stem is very resistant to cavitation, but the leaves do not and the species shows a deciduous strategy. In this species leaf represents the major "bottle-neck", linking stem and leaf hydraulic systems (Sack et al. 2003, Johnson et al. 2011). Leaf abscission can be a strategy to avoid water loss by transpiring leaves. To avoid the extensive cost of stem production, *V. warmingiana* show a xylem resistant to drought and probably their integrity is maintained even without leaves. The others two species shows a trade-off between vulnerability to cavitation and NSC% storage in roots during drought. Both species did not showed reductions on biomass and RGR between conditions and the species with the xylem most vulnerable to cavitation and lower Ψ_{TLP} showed a compensatory mechanism, exhibiting a high amount of NSC storage in roots. This large storage can be very important to maintain the vessels integrality and can be used for refilling cavitated vessels (McDowell et al. 2011). By contrast, *C. pubecens*, the species with the xylem most resistant to cavitation showed lower reserves amount. Our results illustrate the diversity of strategies that plants might present to deal with drought. As we observed that even plants with a hydraulic system relatively vulnerable to hydraulic failure might present compensatory mechanisms to deal with drought, as the NSC accumulation. Due to this, we propose that an integrated investigation of the plant phenology, NSC dynamics and xylem hydraulics is needed to predict plant resistance to drought.

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SUPPLEMENTARY MATERIAL

Table S1. Comparisons between control and drought treatments of the total non-structural carbohydrates per dry mass (NSC), starch and, soluble sugars for three species in *campos rupestres*.

	Campomanesia pubecens		***Eremanthus seidelii***		***Vernonia warmingiana***	
	t	**p**	**t**	**p**	**t**	**p**
%NSC	-1.21	0.26	-1.10	0.32	0.42	0.69
%starch	-1.32	0.23	1.38	0.22	-0.42	0.69
%soluble sugars	1.06	0.32	-1.64	0.16	0.79	0.46

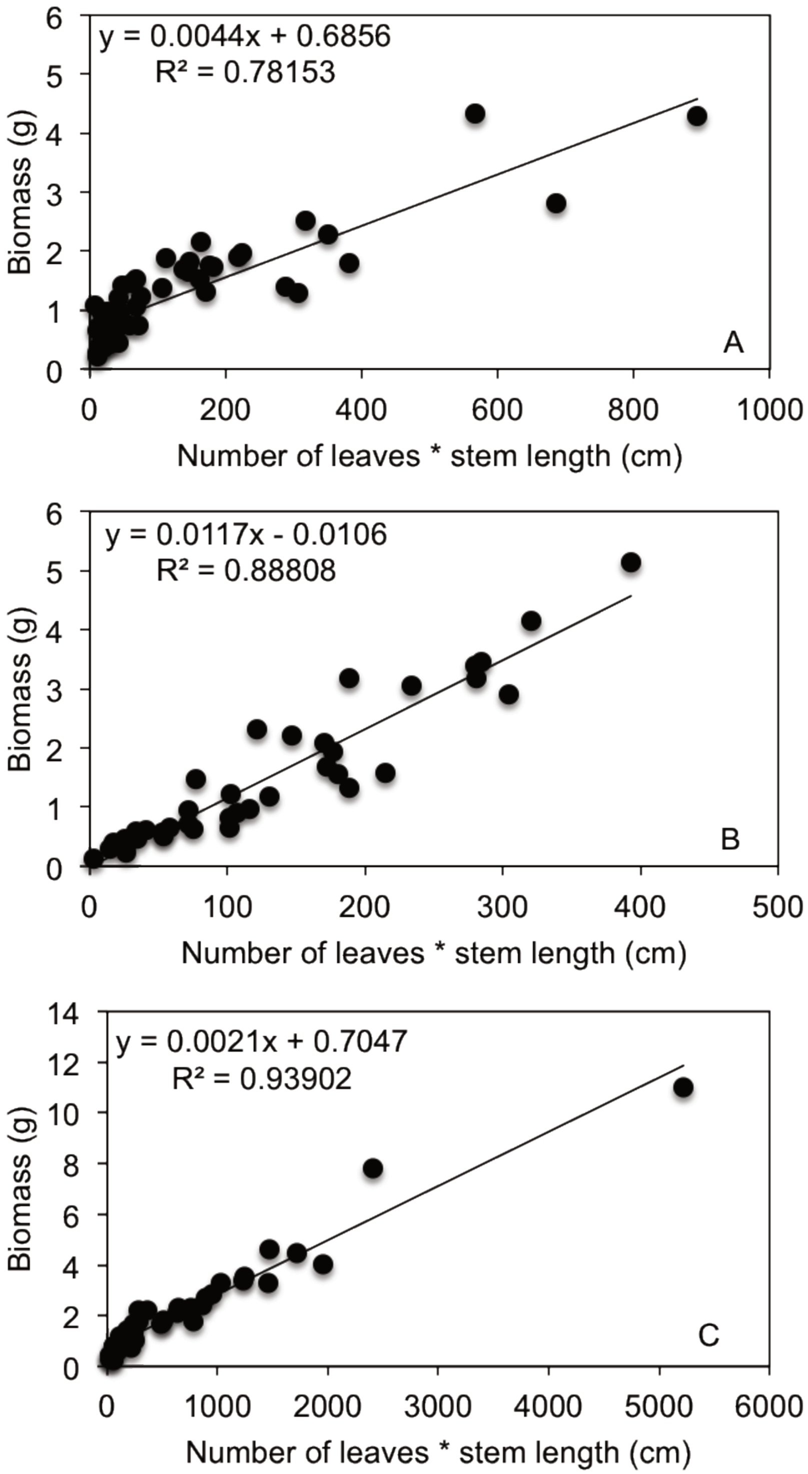

Figure S1: Relations between Biomass and (Number of leaves * stem length). These equations were used to calculate the monthly biomass per each species. A) *Campomanesia pubecens*; B) *Eremanthus seidelii*; C) *Vernonia warmingiana*.

CONSIDERAÇÕES FINAIS

Nossos resultados trazem importantes contribuições para o entendimento de funcionamento de ecossistemas sazonais limitados por água e ilustram a diversidade de estratégias hidráulicas que conferem resiliência à seca em espécies de campo rupestre. No Brasil, os poucos experimentos realizados em campo monitorando a resposta de plantas frente a mudanças climática (*e.g.* seca) localizam-se em ecossistemas florestais na Amazônia. Esse é o primeiro estudo experimental com seca realizado em formações do Domínio Cerrado, o campo rupestre.

As espécies de campo rupestre apresentaram diferentes *trade-offs* e estratégias para lidar com a limitada disponibilidade hídrica. As espécies podem tolerar completamente a dessecação, como a *Vellozia nivea* ou ser tolerante a seca como outras espécies congenéricas de *Vellozia* (e.g. *Vellozia* aff. *variabilis*). As espécies arbustivas também mostraram-se bastante resistentes à seca. As espécies diferiram nos seus atributos hidráulicos e apresentaram sistemas hidráulicos contrastantes, na qual algumas espécies são mais sensíveis a falha hidráulica e outras mais resistentes às secas. No entanto, as espécies mais sensíveis apresentaram maiores reservas de carboidratos não-estruturais nas raízes, sugerindo um trade-off entre os atributos hidráulicos e de estoque de carbono e evidenciando a interdependência entre esses mecanismos. Além disso, esse trabalho também trouxe importantes contribuições sobre o uso de isótopos estáveis (carbono - ^{13}C e oxigênio - ^{18}O) para inferir sobre respostas ecofisiológicas em plantas. O uso dessa abordagem foi útil para descrever as respostas de espécies que apresentaram uma determinada estratégia hidráulica (isohídricas). Essa tese evidenciou que os campos rupestres além de apresentar uma elevada riqueza de espécies também apresentam uma elevada diversidade funcional e de estratégias, nas quais, as espécies mais abundantes foram muito resistentes à seca.

Os campos rupestres apresentam sua formação vegetal semelhante a vários ecossistemas mediterrânicos, como os Fymbos na África do Sul e o Chaparral em várias regiões do mundo. Todos esses ecossistemas são apontados como sensíveis a mudanças no clima e as projeções climáticas indicam reduções na precipitação, aumentando a duração e a intensidade da estação seca. Diante disso, esse estudo passa ter uma abrangência muito maior, fornecendo subsídios para predizer a suceptibilidade das espécies desses outros ecossistemas à seca baseado em forma de vida, estratégias hidráulica e de uso de água e no estoque de carboidratos. De fato, esse trabalho traz importantes contribuições para o entendimento de ecossistemas limitados por água.