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MARIANA SANTOS DE SOUZA GONÇALVES

DISTRIBUIÇÃO DOS AÇÚCARES E DESEMPENHO ECOFISIOLÓGICO DE ESPÉCIES LENHOSAS EM RESPOSTA AO DÉFICIT HÍDRICO

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Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de doutora em Biologia Vegetal.

Área de concentração: Ecologia e conservação.

Orientador: Prof. Dr. Mauro Guida dos Santos

Co-orientador: Drª. Gabriella Frosi Albuquerque Figueirôa Faria

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DEDICATÓRIA

À Márcia Santos, Socorro Santos, João Pedro Santos e aos meus amigos e amigas, que são meus amores e minha base, dedico.

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23.
Tenho o privilégio de não saber quase tudo.
E isso explica
o resto.
(BARROS, Manoel de. Menino do Mato: Caderno do aprendiz.
Campo Grande, 2010)

RESUMO

O investimento eficiente em carbono (C) pode determinar o desempenho de espécies lenhosas em ambientes com recursos limitados, como a disponibilidade de água em Florestas Tropicais Secas (FTSS). A habilidade de particionar e armazenar os compostos de C pode ser crítica para o sucesso de plantas decíduas de regiões como a Caatinga, que experienciam eventos seca ao longo de sua história de vida. Dessa forma, o presente estudo avaliou a variação no particionamento de carboidratos não estruturais (CNE) e Custo de Construção Foliar (CCF), em dez espécies lenhosas nativas da Caatinga que apresentam distribuição distintas em áreas com média de precipitação anual (MPA) extremas: (1) maior distribuição sob alto MPA (WA); (2) maior distribuição sob baixa MPA (DA) e distribuição semelhante em ambas as áreas (BA), bem como, os aspectos ecofisiológicos de uma dessas espécies submetidas a ciclos recorrentes de déficit hídrico. Foram avaliados trocas gasosas, conteúdo hídrico foliar (CHR), conteúdo de CNE e seus componentes (açúcares solúveis (AST) e amido) em folha, caule e raiz e CCF. Sob ciclo recorrente de déficit hídrico, *Cenostigma micrphyllum*, espécie escolhida devido a sua ampla distribuição na Caatinga, apresentou estratégias distintas nos dois ciclos. No ciclo 1, o CHR foi mantido à custa de uma rápida queda nas trocas gasosas e leve acúmulo de AST no caule e raízes, em detrimento do crescimento em altura e diâmetro do caule. No ciclo 2, o CHR permaneceu 40% superior ao menor nível medido no primeiro déficit hídrico, e a assimilação de CO_2 permaneceu duas vezes maior nas plantas previamente estressadas. O conteúdo de AST dos caules e raízes foi fortemente correlacionado com o CHR antes do amanhecer. Em campo, a partição de CNE nas plantas variou de acordo os grupos de espécies, mas não diferiu entre as áreas. WA apresentou 73% do conteúdo de CNE nas folhas, enquanto BA e DA apresentaram investimentos também em raiz 33% e 40% respectivamente. De modo geral, as espécies DA apresentam maiores concentrações de CNE comparado a WA e BA, e em áreas de baixa MPA armazenam maiores conteúdos de amido, em relação às áreas de alto MPA. Espécies BA mantem concentrações menores e mais estáveis de CNE mas respondem à sazonalidade do ano em questão, aumentando a concentração de CNE em raízes durante o ano mais seco. O CCF não foi relacionado com o acúmulo de CNE para nenhum dos grupos. Dessa forma, observamos que o acúmulo de CNE sob condições de menor disponibilidade hídrica, é uma via prioritária no investimento de C para espécies arbóreas decíduas de FTSS, e pode favorecer o desempenho dessas em condições de déficit hídrico.

Palavras-chave: Carboidratos não estruturais, açúcares solúveis, amido, Floresta Tropical Seca

ABSTRACT

Efficient investment in carbon (C) can determine the performance of woody species in resource-limited environments, such as water availability in Tropical Dry Forests (TDF). The ability to partition and store C compounds may be critical to the success of deciduous plants from regions such as the Caatinga, which experience drought events throughout their life history. Thus, the present study evaluated the variation in the partitioning of non-structural carbohydrates (NSC) and Leaf Construction Cost (LCC), in ten woody species native to the Caatinga that have different distribution in areas with average annual precipitation (MPA) extreme: (1) greater distribution under high MPA (WA); (2) greater distribution under low MPA (DA) and similar distribution in both areas (BA), as well as the ecophysiological aspects of one of these species subjected to recurrent cycles of water deficit. Gas exchange, leaf water content (RWC), NSC content and its components (soluble sugars (SS) and starch) in leaf, stem and root and LCC were evaluated. Under recurrent cycle of water deficit, *Cenostigma microphyllum,* chosen due to its wide distribution in the Caatinga, presented different strategies in the two cycles. In cycle 1, RWC was maintained at the expense of a rapid drop in gas exchange and a slight accumulation of AST in the stem and roots, at the expense of growth in height and stem diameter. In cycle 2, RWC remained 40% higher than the lowest level measured in the first water deficit, and CO2 assimilation remained twice as high in previously stressed plants. The SS content of stems and roots was strongly correlated with pre-dawn RWC. In the field, the partition of NSC in the plants varied according to the species groups, but did not differ between the areas. WA presented 73% of the NSC content in the leaves, while BA and DA also presented 33% and 40% investments in root, respectively. In general, DA species showed higher concentrations of NSC compared to WA and BA, and in low MAP areas store higher starch contents, in relation to high MAP areas. BA species maintain lower and more stable concentrations of NSC but respond to the seasonality of the year in question, increasing the concentration of NSC in roots during the driest year. LCC was not related to NSC accumulation for either group. Thus, we observed that the accumulation of NSC under lower water availability, is a priority route in C investment for deciduous tree species of TDF, and can favor their performance in conditions of water deficit.

Keywords: Non-structural carbohydrates, soluble sugars, starch, Tropical Dry Forest

SUMÁRIO

1. APRESENTAÇÃO

A disponibilidade de água é um fator que influencia fortemente a distribuição e biodiversidade de espécies vegetais (CHAVES et al., 2016). Nas Florestas Tropicais Sazonalmente Secas (FTSS), o desempenho das espécies durante um período de seca, é um fator que pode determinar o estabelecimento destas nos mais variados ecossistemas, sugerindo que a seca age como um filtro ambiental, influenciando os padrões de diversidade dentro de um gradiente de aridez, por exemplo (ENGELBRECHTETAL et al., 2007; ADAMS et al. 2017). Entretanto, apesar de sua origem natural, o aumento da severidade e intensidade dos eventos de seca, previstos para as condições climáticas eminentes, podem causar grandes impactos ambientais, levando a mortalidade em massas de árvores (HARTAMANN et al., 2015), alterando desde a composição de comunidades vegetais à serviços ecossistêmicos e função da floresta (CHAVES et al., 2016), convertendo as florestas de grandes sumidouros líquidos de carbono (C) em grandes fontes de C (ANDEREGG et al., 2013; WANG, ZHUANG, & LÄHTEENOJA, 2018; PELTIER & OGLE, 2019)

Já sob condições naturais, durante o seu ciclo de vida, as espécies arbóreas perenes experienciam eventos recorrentes de déficit hídrico, como acontece em FTSS. Essa condição exige que as plantas desenvolvam mecanismos de resposta e aclimatação, de maneira a tolerar essas perturbações. A aclimatação a estresses subsequentes é denominada memória ao estresse (*stress imprint*) e caracteriza-se por respostas mais rápidas e eficientes das plantas durante um novo estresse (WOJTYLA et al., 2016). Essas alterações ocorrem à nível molecular e epigenético (BRUCE et al., 2007; WALTER et al., 2010), e recentemente, tem sido sugerido traços de memória ao nível metabólico (SCHWACHTJE et al., 2019), através da alteração no acúmulo de aminoácidos, como a prolina, e compostos primários como os carboidratos (MOYANKOVA et al., 2014; WEDEKING et al., 2018), tendo em vista que grande parte do metabolismo da planta é alterado durante um evento de perturbação. Entretanto, pouco se sabe como esse mecanismo influência a tolerância de espécies arbóreas, nativas de ambientes secos, sob estresse hídrico.

Durante um período de déficit de água o metabolismo do carbono (C) é alterado nas plantas devido, principalmente, à diminuição das taxas fotossintéticas, causada pela redução da abertura do poro estomático. Atualmente, as duas hipóteses que baseiam a mortalidade de árvores sob seca, (1) hipótese de falha hidráulica e (2) depleção de C, estão diretamente relacionadas com a redução das plantas ao acesso de compostos de C, como os carboidratos não-estruturais (CNE). Entretanto, é também evidenciado na literatura que os CNE facilitam a adaptação de espécies arbóreas à seca por atuarem como osmoprotetores, eliminadores de espécies reativas de oxigênio (ROLLAND et al. 2006; REGIER et al. 2009) e na sinalização molecular (SECCHI e ZWIENIECKI, 2011; EVELAND e JACKSON, 2012). Em espécies nativas de ambientes secos, o déficit hídrico induz o acúmulo de açúcares, mas as respostas metabólicas diferem entre os órgãos (PIPER et al. 2011; SANTOS et al., 2021). Sendo assim, durante eventos de seca, onde o balanço de C é alterado, o particionamento eficiente de CNE, pode determinar a performance da espécie frente a um evento de seca.

Os traços foliares também são importantes indicadores para avaliar as respostas das plantas às questões ambientais (VIOLLE et al., 2007). O custo de construção foliar (CC) determina o quanto de energia/recurso é utilizado na obtenção de cada folha, está altamente correlacionado com as taxas fotossintéticas das plantas, tendo em vista que são C-dependentes, bem como está relacionado à disponibilidade hídrica (WILLIAMS et al., 1987). Entretanto, um estudo recente, usando uma espécie modelo e altamente representativa na de FTSS no Brasil, mostrou que o CC não está relacionado com o regime de chuvas, sugerindo que, nesse caso, o CC, esteja relacionado com a alocação de recursos na planta (FALCÃO et al., 2017).

As mudanças no investimento do C de acordo com as condições ambientais, são estratégias importantes para as espécies vegetais, principalmente nos ecossistemas secos (PRENTICE et al., 2014). Para espécies de FTSS, onde o habito decidual é um mecanismo evolutivo, sob as condições climáticas eminentes é possível que essas plantas passem períodos ainda mais longo sem folhas, principal órgão fonte de C. Diante disso, se faz muito importante entender os padrões de investimento de C em espécies com diferente habilidade de tolerar ambientes mais secos. O presente estudo avaliou a variação no particionamento de carboidratos não estruturais (CNE) e Custo de Construção Foliar (CCF), duas principais vias de investimento de C, em onze espécies lenhosas nativas de FTSS no Brasil, que apresentam diferentes distribuição, em dois extremos de precipitação. Bem como, os aspectos ecofisiológicos de uma dessas espécies submetidas a ciclos recorrentes de déficit hídrico.

2. FUNDAMENTAÇÃO TEÓRICA

2.1. CONDIÇÃO DE SECA E AS PLANTAS

2.1.1 Geral

A seca é considerada um fenômeno natural, que ocorre nos mais diversos tipos de clima e em várias regiões por todo o globo. Essa condição é resultante da combinação de fatores, incluindo precipitação, temperatura, demanda e uso da água e umidade do solo (VICENTE-SERRANO et al., 2015). Em Florestas Tropicais, os episódios de seca são um fator determinante na estrutura, diversidade e funcionamento de ecossistemas (BRANDO et al., 2006) atuando como um filtro ambiental, do nível molecular às comunidades (ADAMS et al., 2017). Sob condições naturais, as plantas experenciam curtos ou longos períodos de seca, mas, apesar de sua origem, esse fenômeno pode causar impactos ambientais de forma considerável, levando a mortalidade em massas de árvores (HARTAMANN et al., 2015), alterações na composição das espécies vegetais e na estrutura e função da floresta (CHAVES et al., 2016). O aumento das pressões antropogênicas sobre a vegetação, incluindo a agricultura e populações crescentes, interagem de forma adicional aos efeitos da seca à medida que as florestas vão ficando cada vez mais fragmentadas (NOBRE et al., 2016; BRANDO et al. 2019). Adicionalmente, as consequências das mudanças climáticas aceleram esses distúrbios antrópicos e naturais, e é possível que os eventos de perturbação resultantes da seca se intensifiquem, de forma que o processo de regeneração natural das florestas tropicais seja prejudicado (TRUMBORE et al., 2015).

A nível de organismo, a seca afeta as relações hídricas das plantas, causando estresse hídrico. Esse estresse é estabelecido quando a absorção de água pelo sistema radicular não atende as demandas necessárias (FAN et al., 2006) forçando-as a alterar o seu metabolismo. Essas alterações variam desde a expressão de genes a mudanças no crescimento e na produtividade das plantas (SONI et al., 2014). Contudo essas respostas serão moduladas de acordo com a intensidade e severidade do estresse a que essas espécies são acometidas (CHAVES: PEREIRA, 1992).

Evolutivamente, as espécies vegetais desenvolveram diferentes estratégias para suportar os efeitos do estresse hídrico. Essas estratégias podem ser dividias em três mecanismos: (1) - escape, relacionada às espécies anuais, que apresentam uma estratégia de “fuga”, alto grau de plasticidade e um rápido desenvolvimento fenológico, completando todas as fases do seu ciclo de vida antes que o déficit hídrico se torne severo, causando danos fisiológicos; (2) retardo, que consiste no atraso da desidratação dos tecidos, mantendo o volume e turgor celular por meio da absorção de água através de um sistema radicular bem desenvolvido e redução da perda por transpiração, através do fechamento estomático; (3) tolerância, ligada às espécies perenes, que mantem suas taxas metabólicas mesmo com redução do potencial hídrico, devido principalmente ao acúmulo de solutos compatíveis e/ou osmólitos e um sistema antioxidante eficiente. Essas espécies apresentam altas taxas metabólicas durante um estresse moderado e redução dessas taxas durante um estresse mais severo (KOOYERS et al., 2015; SOUZA, et al., 2015). Em regiões de Florestas Topicais Sazonalmente Secas (FTSS), há a predominância do comportamento decíduo das espécies no período de estiagem, e no curto período de chuvas, as plantas rebrotam as folhas e desenvolvem o processo de brotamento e floração (SOUZA et al., 2010).

Entretanto, apesar do sucesso evolutivo dessas estratégias, a performance dessas espécies frente as mudanças climáticas ainda não são bem definidas. Os cenários climáticos, de acordo com o Painel Intergovernamental de Mudanças Climáticas (IPCC 2014), preveem aumentos na temperatura global e alteração no regime de chuvas para determinadas regiões. Para as FTSS, que experimentam temperatura média anual de 25°C, com precipitação variando entre 700 a 2000mm, estudos a preveem aumento da aridez devido ao aumento da temperatura e diminuição das chuvas (IPCC 2014). Dentro desse cenário, são esperados eventos de seca ainda mais recorrentes e ainda não está bem compreendido como as espécies arbóreas, nativas dessas regiões mais secas, respondem a sucessivos eventos de estresse hídrico.

2.1.2 Ciclos recorrentes de déficit hídrico

A maiorias dos eventos de estresse são transientes, como limites extremos de temperatura, períodos de seca e ataques por herbívoros. As espécies vegetais ao longo do seu ciclo de vida experienciam eventos recorrentes desses estresses bióticos e abióticos e precisam ser capazes de responderem e aclimatarem-se a esses eventos (TARDIF et al., 2007). A aclimatação a estresses subsequentes é denominada memória ao estresse (stress imprint) e caracteriza-se por respostas mais rápidas e eficientes das plantas durante um novo estresse (WOJTYLA et al., 2016). Essas respostas são controladas à nível molecular, por mudanças na expressão de alguns genes (BRUCE et al., 2007). Existem dois mecanismos bem compreendidos de memória das plantas ao estresse, um por meio de acúmulo de proteínas sinalizadoras e outro por acúmulo de fatores de transcrição (CONRATH et al., 2006). Estudos clássicos documentaram também traços de memória em plantas através da alteração do acúmulo de pigmentos fotossintéticos, prevenindo danos ao fotosistema (JIANG et al., 2005) e mudanças epigenéticas (BRUCE et al., 2007; WALTER et al., 2010).

Entretanto, durante um evento de estresse grande parte do metabolismo das plantas é alterado e é evidenciado que as respostas metabólicas estão na linha de frente para mitigar os efeitos de perturbações nas plantas (como será abordado no tópico seguinte). Partindo desse princípio, tem sido sugerido também, que a memória ao estresse pode ser dada ao nível metabólico das plantas (memória metabólica), caracterizada pelo acúmulo permanente e/ou crescente de metabólitos nos tecidos das plantas previamente estressadas (SCHWACHTJE et al., 2019). Concentrações permanentes no conteúdo de prolina em plantas tem sido reportado para estresses recorrentes de seca (SHARMA E VERSLUES, 2010; AN et al., 2013) e congelamento (BENINA et al., 2013; ZUTHER et al., 2015). Metabólitos primários como açúcares e ácidos orgânicos também mantiveram características de memória ao estresse em plantas previamente submetidas à seca (MOYANKOVA et al., 2014; WEDEKING et al., 2018). Apesar do exposto, a maioria dos estudos realizados avaliaram as evidências de memória metabólica em espécies herbáceas e de interesse econômico. Pouco se sabe sobre a relação desse mecanismo com a tolerância de espécies lenhosas, que passam por inúmeros períodos de estresse ao longo do seu ciclo de vida.

O estresse hídrico recorrente é um dos eventos mais comuns aos quais as plantas são acometidas e seus efeitos tem sido amplamente estudado. Em espécies arbóreas, secas recorrentes de curto prazo podem melhorar as respostas das plantas ao estresse (WALTER et al., 2013), por meio de mudanças nas taxas fotossintéticas e melhoria na eficiência do uso da água (EUA) (RIVAS et al., 2013; NOIA et al., 2020), mudanças nos pigmentos de conteúdo fotossintético, reduzindo fotodanos (Rivas et al., 2013), aumento na produção de biomassa e eliminação de espécies reativas de oxigênio (ABDALAH et al., 2017). Alguns desses estudos também relacionaram a melhoria no estado da água de plantas lenhosas previamente estressadas em função de ajustes no reservatório de carboidratos (ABDALAH et al., 2013; TOMASSELHA et al., 2017; NOIA et al., 2020). Por exemplo, TOMASSELHA et al. (2017) conduziram um experimento controlado usando uma espécie lenhosa de uma floresta temperada, onde foi observado que as plantas sob déficit hídrico recorrente apresentam capacidade de recuperação aos danos causados no xilema, quando a disponibilidade de água do solo aumentou, e foi apoiado pela alteração do reservatório carboidratos nos tecidos do caule.

2.1.3 Traços de tolerância ao déficit hídrico

Segundo LARCHER (1987), o estresse possui elementos tanto destrutivos como construtivos. É considerado um fator de seleção, bem como pode direcionar e melhorar a resistência e o processo de evolução adaptativa. Devido à natureza multidimensional, o estresse hídrico reflete uma gama de respostas interligadas, que ocorrem nos diversos níveis de organização, desde o morfológico ao nível de alteração no metabolismo celular (SALEHILISAR et al., 2016). Dessa forma, plantas nativas de regiões mais secas, como as FTSS apresentam características mais expressivas voltas à tolerância, que refletem no fitness desses indivíduos, alterando o crescimento e sobrevivência (POLLE et al., 2019).

Características morfológicas e estruturais das plantas estão relacionadas e podem ser consideradas de grande importância na tolerância das espécies ao déficit hídrico. Alterações morfoanatômicas como a redução do tamanho da folha, espessamento das paredes dos tecidos foliares, redução no número dos estômatos, cutinização da superfície foliar aumentam a capacidade de manutenção do conteúdo de água na planta, através da redução das taxas de transpiração (LISAR et al., 2012; SIMOVA-STOILOVA et al., 2016). Aumentando, dessa forma, a eficiência do uso da água dessas espécies. As raízes, por serem o principal órgão responsável pela captação de água e nutrientes do solo, são componentes chaves durante o déficit hídrico. Estratégias efetivas para coordenar as complexas demandas metabólicas e estruturais vão determinar as respostas de desenvolvimento das raízes durante um período seco. Algumas espécies, tolerantes ao déficit hídrico, apresentam um aumento no crescimento do tecido radicular, com raízes mais profundas e mais finas (auxiliares), afim de melhorar a captação de água em camadas mais internas do solo. Entretanto, outras espécies, também consideradas tolerantes, reduzem o alongamento do tecido radicular, investindo em raízes mais superficiais, na tentativa de captar a água nas camadas mais rasas do solo (SIMOVA-STOILOVA et al, 2016).

Na ótica fisiológica, as plantas respondem à condição de seca, em primeiro caso, na tentativa de manutenção da turgescência celular. A exposição da planta à deficiência hídrica, diminui o potencial hídrico foliar, seja pela desidratação dos tecidos ou redução da hidrólise dos compostos de reserva (NOGUEIRA; TÁVORA, 2005). Essa diminuição afeta a turgescência celular, além de alterar as relações hídricas e composição das células. Com o objetivo de atenuar esses efeitos, as plantas aumentam a produção e acúmulo de solutos orgânicos, a fim de diminuir o potencial hídrico celular para a entrada de água e preservar os processos metabólicos (ATHAR; ASHRAF, 2005, BEN AHMED et al., 2006, ASHRAF; FOOLD, 2007) Esse processo é denominado ajuste osmótico e é caracterizado pelo acúmulo de uma misturas de solutos de várias classes: açúcares solúveis como sacarose, frutose e glicose, póliois como monitol e sorbitol e aminoácidos como a prolina (SPOLLEN; NELSON, 1994; DAWOOD et al.

2016; HELD; SADOWISK, 2016). Esses solutos compartilham a propriedade de permanecerem estáveis em pH neutro e também por serem altamente solúveis em água (BALLANTYNE; CHAMBERLIN, 1994).

Na tentativa de manutenção do status hídrico durante um período seco, as plantas adotam estratégias em vários níveis. A redução da abertura estomática, evita o processo acelerado de dessecação, por meio da redução da transpiração (TANG, 2002; FLEXAS et al., 2006). Esse ajuste permite que a planta aumente a eficiência do uso da água (EUA), que está relacionada ao número de moléculas de H2O que é perdida durante a assimilação de CO2. Durante um período de estresse hídrico, espécies tolerantes conseguem manter altas taxas de EUA (FROSI et al., 2017; BARROS et al., 2018). O fechamento estomático é dado, principalmente, pelo rápido aumento dos níveis do fitorregulador ácido abiscisico (ABA), que é regulado por uma rede de transdução de sinais (HSIE, 2016; DARYANTO et al., 2016). Entretanto, o acumulo de açúcares, como a glicose, podem induzir o fechamento estomático (HU et al., 2012), apresentando-se como uma alternativa de baixo custo energético para a planta, aumentando a adaptabilidade das plantas à seca.

Devido a redução das taxas fotossintética, o baixo consumo de NADPH e ATP no ciclo de Calvin, reduz a disponibilidade de NDP+, molécula que recebe os elétrons da cadeia transportadora, levando ao aumento de energia livre no sistema. Esse aumento leva a uma produção excessiva de espécies reativas de oxigênio (ROS), causando danos à membrana celular, devido à produção de substâncias envolvidas na peroxidação lipídica, degradação de proteínas e rompimento de filamentos de DNA e RNA (UZILDAY et al., 2002; AYALA et al., 2014). As plantas possuem sistemas antioxidantes eficientes, que desempenham um papel importante na manutenção da homeostase através da eliminação de ROS. Essas moléculas interagem com as ROS, e modulam os processos bioquímicos e fisiológicos das plantas sob secam, reduzindo os efeitos do estresse oxidativo (SINHG, 2019). Estudos também reportam um papel importante dos açúcares da atenuação dos efeitos negativos na acumulação de ROS. Açúcares solúveis, como a glicose, estão associados tanto ao anabolismo como catabolismo de ROS, como as via das pentoses-fosfato, que estão envolvidas na produção de NADPH e na eliminação de ROS (HU et al., 2012; BORIBOONKASET et al., 2012). Além disso, outros açúcares como sacarose, frutose e trealose atuam como moléculas osmoprotetoras, regulando o ajuste osmótico, fornecendo proteção para membrana e eliminação de moléculas tóxicas de ROS (SINGH et al., 2015; KEUNEM et al., 2013).

Dessa forma, fica evidenciado a importância dos mais diversos açúcares na tolerância de espécies submetidas à períodos de estresse. São moléculas de baixo custo, que podem assumir papeis semelhantes a moléculas como fitorreguladores e enzimas. Entretanto, poucos estudos quantificam e analisam esses açúcares ao longo de um período de estresse. Estudos que busquem estratégias de partição desses compostos e a relação com níveis tolerância das espécies se fazem bastante importantes.

2.2 FISIOLOGIA DO CARBONO (C) EM PLANTAS

2.2.1 Dinâmica de C nas plantas e os carboidratos não-estruturais (CNE)

A fração do carbono assimilado que será utilizado pelas plantas, depende da eficiência do uso de CO2, na divisão entre o incremento de biomassa (i.g crescimento primário e secundário) e a respiração (DANNOURA et al., 2016) A fotossíntese é o processo que reduz o carbono inorgânico atmosférico em compostos orgânicos. Esse processo consiste em duas fases: (1) fotoquímica, que ocorre na membrana dos tilacóides e utiliza a energia absorvida pelos pigmentos fotossintéticos (i.g clorofila e carotenoides) para oxidar a molécula de água. Seus produtos finais são a redução da molécula do dinucleotídeo fosfato de nicotinamida adenina (NADP+) a NDPH e a fosforilação da molécula de adenosina difosfato (ADP) em adenosina trifosfato (ATP) e a fase (2) bioquímica, que ocorre no estroma dos cloroplatos. É a fase que incorpora o CO2 absorvido, para formar ácidos orgânicos, reduzindo esses compostos à carboidratos, utilizando o NADPH e ATP produzidos pela fase fotoquímica. Os carboidratos são os produtos finais do processo fotossintético. Essas moléculas fornecem substratos para construção das estruturas das plantas (carboidratos estruturais), bem como a energia necessária para as reações biológicas das células e a síntese de outros compostos orgânicos (carboidratos não estruturais). (DANNOURA et al., 2016).

Os carboidratos não estruturais (CNE), são os principais substratos para o metabolismo primário e secundário das espécies vegetais. São divididos em classes que vão de acordo com a natureza das moléculas. Os monossacarídeos são moléculas simples, que contém entre 3 e 5 átomos de carbono (i.g glicose, frutose, arabinose e galactose). Esses carboidratos, como a glicose e frutose, funcionam principalmente como osmólitos e substratos para respiração celular. A glicose é principal produto da fotossíntese, é convertida para a síntese de muitas outras moléculas. Já a frutose, é também um importante CNE na respiração celular e atua como molécula de reserva energética rápida em algumas plantas. Os dissacarídeos e oligossacarídeos (i.g sacarose, rafinose, estaquiose), são moléculas compostas, por três a nove açúcares simples, em média. Podem ser hidrolisados a moléculas menores e assim, utilizados como substratos. A sacarose é um dos principais açúcares de transporte nas plantas (HARTMANN; TRUMBORE, 2016). Já os polissacarídeos são cadeias longas de monossacarídeos, seus representantes mais comuns são a amilopectina e amilose, que formam o amido, os carboidratos de reserva mais comum em plantas. A natureza osmoticamente inativa desse carboidrato, permite que sejam armazenados em grandes quantidades pelas plantas (HARTMANN; TRUMBORE, 2016).

Os CNE estão presentes em todos os tecidos vegetativos vivos das plantas. Entretanto, a oferta desses compostos ao longo da planta é feita de forma assincrônica entre os órgãos mesmo sob condições tidas como favoráveis (STITT et al., 2012). Nas folhas, durante o dia, os carboidratos excedentes da fotossíntese são armazenados nos cloroplastos na forma de amido. Durante a noite, para a manutenção dos processos vitais, o amido é hidrolisado em glicose ou exportado para os outros órgãos na forma de sacarose. O floema é o principal tecido de condução e devido as suas propriedades anatômicas e funcionais, conseguem controlar a quantidade de carboidratos diários a serem alocados para os tecidos dreno (EPRON et al., 2016). O tecido radicular, recebe cerca de 31% da produção primária, e suas funções são fortemente dependentes dos recursos fotossintéticos (LITTON et al., 2007). Além de serem alocados pra diversos compartimentos, a depender da espécie, outra parte desses carboidratos, pode ser transferido pra simbiontes microbianos.

Os CNE produzidos pela fotossíntese somam metade do carbono assimilado pelas plantas. Entretanto, a alocação do C podem ser alterados de acordo com vários fatores como: clima, temperatura, conteúdo de água no solo, luminosidade, idade da planta e é também espécie-específico, mostrando que a prioridade do investimento de compostos de C não é constante, mas dinâmica e pode ser determinante para a sobrevivência das plantas frente à diversas perturbações (EPRON et al., 2012).

2.2.2 Os CNE e o estresse hídrico

Os CNE possuem papel importante na manutenção de toda a estrutura vegetal. Eles são regulados nas plantas de forma complexa, devido à alta especificidade local de cada carboidrato (LEMOINE et al., 2013). Essas moléculas são altamente sensíveis a estresses ambientais, e durante um período adverso, atuam ativamente na regulação do crescimento, fotossíntese, partição de C, homeostase osmótica, síntese de proteínas, expressões de genes e também como estabilizadoras d membrana (ROSA et al., 2009; ROCH et al., 1996; HOEKSTRA et al., 2001).

Durante um estresse hídrico, a regulação da abertura estomática, que visa a manutenção do conteúdo de água na planta, afeta diretamente a assimilação de C (TANG et al., 2002; FLEXAS et al., 2006).

Hipóteses mecanicistas, relacionam a morte de plantas sob déficit hídrico, à redução da disponibilidade de C, quer seja pela redução da produção de CNE (hipótese depleção de carbono) ou pela dificuldade de alocação desses compostos para os tecidos da planta (hipótese de falha hidráulica) (SALA et al., 2010; MILLARD et al., 2007). Entretanto, nos últimos dez anos, estudos tem mostrado que os CNE possuem um papel importante na tolerância das plantas contra vários estresses abióticos (ROSA et al., 2000; RIGIER et al., 2009; PIPER, 2011). Evidenciando os CNE não como produtos finais do estresse, mas como moléculas importantes na modulação dos processos fisiológicos durante um período de estresse.

O estresse hídrico afeta significativamente o balanço de água na planta. Para amenizar os efeitos, as plantas adotam estratégias para manter os níveis de água suficientes para a manutenção das atividades. O acumulo de glicose induz o fechamento estomático, regulando a perda de água (OSAKABE et al., 2013), bem como o acúmulo de outros açúcares solúveis como frutose, sacarose. O acúmulo de CNE nas células, auxilia a manutenção da turgescência foliar e do ajuste osmótico das espécies sob déficit hídrico, evitando a desidratação de membranas e proteínas (CROWE et al., 1999; SAWHNEY; SINGH, 2002). A redução das taxas fotossintéticas aumenta a produção de ROS, que causam o estresse oxidativo. Os NSC podem atuar como moléculas antioxidantes, tendo papeis importantes na proteção contra a oxidação de membranas e atuando na eliminação de ROS (KRASENSKY; JONAK, 2012). Os açúcares podem atuar também sincronicamente com fitoreguladores. Tanto a glicose como a sacarose estão associadas aos aumentos da expressão de genes de biossíntese do ácido abcisico, principal regulador no fechamento estomático. (DEKKERS et al., 2008; FINKELSTEIN; GIBSON, 2001). A percepção do estresse e como isso será traduzido para a planta vai determinar o nível de tolerância da espécie diante de um estresse. Os NSC, assim como os fitoreguladores, podem atuar também como moléculas sinalizadoras, regulando a expressão de vários genes, envolvidos principalmente na fotossíntese, metabolismo de sacarose e sínteses de osmólitos (PEGO et al., 2000; ROLLAND et al., 2006; CHEN et al., 2007).

É importante ressaltar que os diferentes órgãos das plantas percebem e respondem de forma diferente o estresse, assumindo funções distintas na tolerância das espécies. Grande parte da rede de respostas ao estresse é iniciada nas raízes e não nas folhas (BRUNNER et al., 2015;

WEEMSTRA et al., 2016). Por estar em contato direto como o solo, as raízes respondem primeiro à falta de água. Entretanto, é escasso o número de estudos que quantificam e analisam as respostas dos açúcares num período de escassez hídrica, em toda a planta. Esses estudo são ainda mais escassos para espécies lenhosas. Entretanto, estudos bioquímicos recentes mostram que, plantas lenhosas nativas de FTSS apresentam uma dinâmica específica de NSC (RIVAS et al., 2013; FROSI et al., 2016, BARROS et al., 2018). Evidências que vão de encontro às hipóteses de depleção de carbono e falha hidráulica, mostram que espécies tolerantes à seca apresentam níveis mais altos de NSC ao final de um período de déficit hídrico, em relação à espécies não tolerantes (RIGIER et al., 2009; PIPER, 2011).

Fica evidenciado que as repostas a nível de alocação de recursos de C são muito específicas e podem variar de acordo com muitos fatores. Dessa forma, a avaliação dos ajustes o investimento de C em resposta à condições déficit hídrico é uma ferramenta importante para elucidar como essas estratégias refletem na performance da espécie frente à um estresse por déficit hídrico.

2.3 CUSTO DE CONSTRUÇÃO FOLIAR

Outra via de investimento do C assimilado, é a produção dos carboidratos estruturais (CE). Os CE, são moléculas de cadeias longa, utilizados na construção e enrijecimento das estruturas e componentes da biomassa da planta (i.g. parede celular, caule, ramos). Os representantes principais são a celulose e a hemicelulose. As plantas não possuem enzimas que degradem a celulose, portanto, o C investido na construção das suas estruturas não estará mais disponível para a planta no futuro (HARTMAN; TRUMBORE, 2016). Dessa forma, as plantas precisam investir de forma eficiente os compostos de C, afim de suprir todas as demandas, tanto ao longo do ciclo de vida, quanto à aclimatação das alterações ambientais. Essa capacidade de aclimatação pode ser determinada pelos atributos funcionais de cada espécie. Os atributos funcionais são características morfológicas, fisiológicas ou fenológicas que vão determinar a performance de um indivíduo frente à modificações no ambiente (McGILL et al. 2006).

O custo de construção foliar é considerado um importante atributo funcional, relacionado com o investimento de C na planta. Pode ser definido pela quantidade de glicose necessária para fornecer os esqueletos de carbono, poder redutor da forma de NADPH e energia para a síntese de compostos orgânicos, como os carboidratos estruturais (WILLIAMS et al. 1987).

Plantas com alto custo de construção foliar, tendo uma maior longevidade das folhas, podem investir seus recursos na produção de metabólitos de defesa, que demandam uma alta utilização de energia (WESTOBY et al., 2002). Em contrapartida, plantas com baixo custo de construção foliar, investem energia na obtenção de folhas novas ao invés de manter as folhas antigas, sendo bioquimicamente e estruturalmente mais econômicas. (ZHU et al., 2013). Essas espécies apresentam, também, altas taxas fotossintéticas e uma alta concentração de nutrientes, características de plantas de ambientes áridos (WEI et al., 2016). Em estudos conduzidos em FTSS, no Brasil, Franco (2005), observou estratégias diferentes no investimento foliar, entre espécies decíduas e sempre-verdes, relacionado também aos investimentos da parte subterrânea. Estudos relacionando o custo de construção, com forma de crescimento e o estágio sucessional de espécies de ambientes áridos, começam a elucidar o papel do investimento do carbono na construção foliar dessas espécies (FALCÃO et al., 2015; FALCÃO et al., 2017).

No Brasil, a Caatinga é um domínio de FTSS, considerada uma das maiores áreas de florestas secas do mundo (MILES et al., 2006), abrangendo cerca de 900.000km^2 do território do país. Apresenta períodos de até 11 meses de seca, e uma irregularidade nas chuvas, que variam entre 240 a 700mm anuais (SANCHEZ-AZOFEIFA et al., 2005). A vegetação é caracterizada por um mosaico de espécies de porte arbustivo-arbóreo e não se apresenta fisionomicamente uniforme. Os fatores ambientais (i.g solo, precipitação, altitude) que afetam as plantas se distinguem de tal modo que é possível identificar áreas com predominância de características particulares e uma mudança progressiva até os limites com outro tipo de vegetação (SAMPAIO; RODAL, 2000). Essa condição caracteriza a Caatinga como um importante e potencial modelo de estudo, no que diz respeito às estratégias de espécies tolerantes à condições ambientais adversas.

2.4 CONSIDERAÇÕES GERAIS DAS ESPÉCIES VEGETAIS

2.4.1 Fabaceae

A família Fabacea possui uma distribuição cosmopolita e é uma das maiores famílias de plantas encontradas no mundo. Inclui cerca de 720 gêneros e mais de 19 mil espécies, que possuem vários tipos de hábitos e portes (LEWIS et al., 2005). No Brasil, Fabaceae é a família que possui maior riqueza de espécies na Floresta Amazônica e na Caatinga (BGF, 2015). Para a Caatinga, a família apresenta importância ainda mais significativa, é também a mais abundante em termos de indivíduo, apresenta cerca de 320 espécies, distribuídas em 86 gêneros. O sucesso da distribuição da família, está em parte, associado à associações com simbiontes como bactérias fixadoras de nitrogênio e na Caatinga, também com os Fungos micorrizicos arbusculares (LEWIS et al., 2005; QUEIROZ, 2009). Assim, a dominância da família Fabaceae na Caatinga, e seus mais diversos hábitos faz das espécies, bons indicadores de diversidade regional de uma FTSS.

A *Poincinella microphylla* foi recentemente classificada como *Cenostigma microphyllum* (Mart. ex G. Don) E. Gagnon & G.P. Lewis. É uma espécie endêmica da Caatinga e está distribuída entre os estados da Bahia, Pernambuco e Piauí. Possui porte arbustivo-arbóreo denso, e hábito semidecidual, permanecendo com folhas ao longo de todo o ano (IBGE 1992). É uma espécie típica de solos arenosos e ocorre em altitudes inferiores a 600m (Queiroz 2009). Apresenta potenciais uso forrageiro e medicinal, e foi considerada em estudos anteriores, como uma espécie facilitadora (CARRIÓN et al., 2017).

A *Pytirocarpa moniliformis* (Benth) Luckon & R.W. Jobson é uma espécie típica de Caatinga. Está distribuída nos estados do Maranhão, Piauí, Ceará indo até a Bahia. Possui porte arbustivo-arbóreo, sem espinhos. É uma planta associada a solos arenosos e distróficos (QUEIROZ, 2009) Pode ser conhecida popularmente como “angico de bezerro”, surucucu, canzenzo e carrasco (BENEDITO et al., 2010). Apresenta potenciais uso na apicultura, apresentando-se como uma das plantas melíferas mais importantes da Caatinga. (SILVA et al., 2004). Possui potencial forrageiro tanta para bovinos e caprinos. É uma espécie de crescimento rápido sendo indicadas em estudos anteriores, para reflorestamentos (AZEREDO et al., 2010)

A *Senegalia bahiensis* (Benth.) Seigler & Ebinger é uma espécie nativa do Brasil. Apresenta porte arbustivo-arbóreo pequeno e hábito decidual (QUEIROZ, 2009). Está amplamente distribuída no Brasil, e na Caatinga pode ser encontrada em áreas de transição para florestas estacionais, e com grande efetividade no estabelecimento em áreas degradadas, devido à sua alta adaptabilidade à vários tipos de solo. Possui potenciais uso forrageiro, medicinal e madeireiro (BARROS, 2011b; QUEIROZ, 2009).

Senegalia piauhiensis (Benth.) A.Bocage & L.P.Queiroz é endêmica do Brasil e restrita à fitofisionomia de Caatinga. Está distribuída nos estados de Alagoas, Bahia, Ceará, Pernambuco, Piauí e Sergipe (Flora do Brasil, 2019). Apresenta porte arbustivo-arbóreo, e pode ser encontrada em solos arenosos e profundos (BOCAGE, 2005; CÓRDULA et al., 2008). Possui potenciais uso madeireiros, sendo utilizada para construção de cercas e residências (SILVA et al., 2015)

Peltogyne paucilflora (Benth) Mart. Fl.Bras. é uma espécie nativa do Brasil e está distribuída em todos os domínios fitogeográfico. Na Caatinga, estende-se por todo o seu território, até os estados da Bahia e Minas Gerais. Apresenta porte arbóreo. É popularmente conhecida como coração-de-nêgo e possui potenciais uso para fins madeireiros (ESTATISTICA FLORAL DA CAATINGA, 2015).

Piptadenia stipulacea (Benth.) Ducke é endêmica da Caatinga, ocorre em todos os estados do Nordeste brasileiro, exceto o Maranhão (AMORIM 2014). É uma espécie de porte arbustivo-arbóreo pequeno e hábito decidual, perdendo suas folhas durante 4-6 meses durante o ano (BEZERRA, 2008). Ocorre em diferentes tipos de solo, em elevações inferiores a 700 m (QUEIROZ 2009). É conhecida popularmente como Jurema branca, cassaco e saia-velha (FLORENTINO et al., 2007). Possui potencial forrageiro para caprinos e bovinos e é considerada uma espécie pioneira, que geralmente ocupa áreas com altos níveis de perturbação (BEZERRA, 2008).

Trischidium molle (Benth.) H. E. Ireland é uma espécie de porte arbustivo, que possui hábito sempre-verde (GOMEZ-APARICIO et al, 2004). Tem sua distribuição relacionada à solos arenosos, principalmente na Caatinga, com altitudes de 200 a 500m, sendo encontrada também em áreas costeiras e no domínio de Cerrado (QUEIROZ, 2009). A manutenção das suas folhas ao longo de todo o ano é uma característica ecológica importante, caracterizando a espécie com enfermeira.

Bauhinia acuruana é uma espécie endêmica do Brasil. Possui distribuição nos estados da Bahia, Ceará, Goiás, Maranhão Minas Gerais, Pernambuco e Piaui. (VAZ, 2001). Geralmente encontrada em áreas com altitudes de 600 a 1.100m. Possui porte arbustivo e sub arbustivo. Seu hábito e potenciais uso não são descritos em literatura disponível.

2.4.2. Euphorbiaceae

A família Euohorbiaceae tem ampla distribuição e maior diversidade nas regiões tropicais. É representada por cerca de 307 gêneros e aproximadamente 6.900 espécies. Os representantes possuem hábitos e habitats variados, difundidos nos mais variados tipos fitofisionômicos. No Brasil, ocorrem 72 gêneros e 1.100 espécies, e na Caatinga é a segunda família mais representativa, com 17 espécies endêmicas nesse domínio (SAMPAIO et al., 2002), caracterizando a família como importante modelo de estudos para regiões secas.

Croton heliotropifolius Kunth é uma espécie de porte arbustivo, com comportamento decíduo. É conhecida popularmente como velame (SILVA et al., 2010; QUEIROZ et al., 2014) e quebra-faca (NUNES et al., 2015). Tem ampla distribuição pelo Nordeste do Brasil, estendendo-se até o estado de Minas gerais (SILVA et al., 2010). Tem alto potencial econômico, especialmente na indústria farmacêutica (SILVA et al., 2009) e forrageiro (SAMPAIO et al., 2002).

A *Jatropha mutabilis* (Pohl) Baill é uma espécie endêmica da Caatinga (SANTOS et al., 2005), sua distribuição abrange os estados. Bahia, Pernambuco, Piaui e Sergipe (Flora do Brasil, 2019). É comumente encontrada em terrenos arenosos e áreas perturbadas (SANTOS et al., 2005) Caracteriza-se -pelo seu porte arbustivo suculento e possui comportamento decíduo. É popularmente conhecida como “pinhão-de-seda” e suas folhas e flores tem potencial uso na medicina popular (ALBUQUERQUE et al., 2007).

2.4.3 Burseraceae

A familia Burseraceae tem cerca de 20 gêneros e 600 espécies descritas. Estão distribuídas pelas regiões tropicais em todo mundo, com maior diversidade na América e África. No Brasil, ocorrem sete gêneros e cerca de 60 espécies, a maioria delas nativa da Amazônia, entretanto a família ocorre nos mais diversos domínios fitogeográficos do país (SOUZA; LORENZI, 2005). Na Caatinga, a maioria dos estudos relatam a ocorrência de apenas uma espécie (PINNHEIRO et al., 2010).

Commiphora leptopholeos (Mart.) J.B. Gillet é uma árvore de pequeno porte, de comportamento decíduo (BRAGA, 1996). Apresenta ocorrência no Brasil, Bolívia, Colômbia, Venezuela (ITIS, 2015). É típica de ambientes secos e florestas secundárias, e é uma das plantas mais abundantes na Caatinga (ALVES; NASCIMENTO, 2010). É conhecida popularmente como imburana de cambão, umburana e imburana (CABRAL et al, 2014). Suas folhas possuem potencial forrageiros tento verdes como secas (AGRA et al., 2007).

3. MANUSCRITO I

Dynamics of non-structural carbohydrates in a deciduous woody species from tropical dry forest under recurrent water deficit

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ANEXO (A)

Dynamics of non-structural carbohydrates in a deciduous woody species from tropical dry forest under recurrent water deficit

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Abstract

In tropical dry forests, both the dry season and the short rainy season has become increasingly irregular. This study replicated these conditions to investigate the effects of two water deficit cycles on *Cenostigma microphyllum* seedlings. Impacts were assessed by measuring growth traits, water relations, gas exchange, and dynamics of non-structural carbohydrate (NSC) content in the whole plant under greenhouse conditions in potted plants. In the first water deficit cycle, the relative water content (RWC) was maintained at the expense of a rapid drop in gas exchange. Furthermore, there was a slight accumulation of NSC, mainly soluble sugars (SS) in the stem wood and roots, to the detriment of height and stem diameter growth. In the second cycle, the RWC remained 40% higher than the lowest level measured in the first water deficit, and CO_2 assimilation remained twice as long in previously stressed plants. The SS content of the stems and roots was strongly correlated with the predawn RWC. The bark NSC content was not used in the experiment, and no strong reduction was observed in this bark stock even with the gradual increase of SS in the wood. Our data suggest that under recurrent water deficit prior to leaf drop, CO_2 assimilation is maintained, with the highest possible RWC, under reduced stomatal conductance. This assists in SS transport to wood and root, which is no longer used to support the growth of the aboveground parts.

Keywords: drought stress, non-structural carbohydrates, semiarid, soluble sugars, starch

1 INTRODUCTION

Water deficit is one of the most discussed abiotic stresses in the literature because of the intensity and severity of its impacts on forest systems (Allen et al. 2010, Santos et al. 2014, Martínez-Vilalta et al. 2019, Lima et al., 2021, Signori-Muller et al. 2021). In woody species, water deficit events directly affect water transport and carbon dynamics (Santos et al. 2021). These mechanisms are interconnected and can strongly influence species survival (Rosell et al. 2020). The complexity of these responses and the impact of water deficit on vegetation can vary considerably according to the ecosystem; the degree of adaptation and tolerance of the species rendering makes predicting future scenarios difficult (Sala et al. 2010, Choat et al. 2018). In regions such as dry forests, climate change forecasts predict more frequent and longer water deficit episodes (Marengo and Bernasconi 2015, IPCC 2021), subjecting plants to recurrent water deficit events.

Water deficit affects the carbon metabolism of plants through factors that directly affect the production and transport of non-structural carbohydrates (NSC) (Tomasella et al. 2019; Signori-Muller et al. 2021). The reduction in stomatal closure limits CO_2 assimilation, consequently decreasing carbohydrate production and water deficit-related damage to plant hydraulics. This, in turn, affects photoassimilate transport (McDowell et al. 2008, Sala et al. 2010, Chuste et al. 2020). NSCs are the most important carbon reserves in deciduous and evergreen trees and can be used as a carbon source to satisfy the demands of metabolism, defense, and growth (Hoch et al. 2003, Schoonmaker et al. 2021). Moreover, NSCs play pivotal roles in plant tolerance to water deficit because of their ability to act as signaling molecules (Secchi and Zwieniecki 2011), osmoprotectors, and reactive oxygen species scavengers (Rolland et al. 2006, Regier et al. 2009). Thus, the strategic allocation of carbohydrates in plants can favor acclimatization to the effects of water deficit (Sala et al. 2012, Rosell et al. 2020, Santos et al. 2021).

Recurrent short-term water deficit events can improve plant responses to stress (Walter et al. 2013) by altering photosynthetic rates, improving water use efficiency (Rivas et al. 2013), changing photosynthetic pigment content, reducing photodamage (Rivas et al. 2013), increasing biomass production, and scavenging of reactive oxygen species (Abdallah et al. 2017). Moreover, in previously stressed woody plants, water status improved as a function of adjustments in the NSC pool (Abdallah et al. 2017, Tomasella et al. 2019). As such, the stem NSC pool can support the recovery of xylem damage once soil water availability increases again (Tomasella et al. 2017). Indeed, stem NSC reserves are an important response to increase plant embolism resistance. The conversion of starch to soluble sugars in xylem cells generates an osmotic effect that supplies the vessels (Secchi and Zwieniecki, 2011). Under current and future climate scenarios, an increase in the frequency and duration of droughts can lead to the progressive loss of NSC reserves due to the shortening of recovery time of pools, particularly in trees in tropical dry forests (Niinemets et al. 2010).

Specifically, woody species in tropical dry forests display high resilience following drought periods (Chazdon et al. 2016, Frosi et al. 2017, Santos et al. 2021). Under these conditions, most woody species are deciduous during the dry season. Thus, throughout the short rainy season, NSC production needs to be sufficient to maintain growth, biomass, and respiration, and during the transition to the dry season. Here, leaf senescence contributes to drought stress tolerance (Munné-Bosch and Alegre, 2004), and the produced NSC must be translocated to roots (Santos et al. 2021). Even in the absence of carbon assimilation due to species deciduousness, the NSC pools stocked in the stem and roots can support biomass during the dry season and regrowth once water availability increased. As delayed growth in the dry season drastically reduces carbon demand, sugars stored during the rainy season are sufficient to support survival (Sala et al. 2010, 2012). Moreover, there are large variations among species in terms of the stored NSC components and among years in terms of possible storage amount (Davidson et al. 2021). However, little is known about the consequences of short water deficit events during the rainy season, which may affect the sugar dynamics of deciduous woody species in tropical dry forests (Santos et al. 2021). According to a recent study, among the 82 tree species in the tropical rainforests of Amazon, only those that were more tolerant of water deficit showed adjustments in NSC distribution during the dry season, and there was very little variation among species during the wet season (Signori-Muller et al. 2021).

In this study, we investigated how deciduous woody species distribute their NSC at the whole-plant level under successive cycles of water deficit using *Cenostigma microphyllum*, a widespread tree in tropical dry forests. We evaluated changes in NSC partitioning among leaves, stem segments (wood and bark), and roots under water-deficit cycles and their relationships with three key functional traits that support sugar dynamics (water status, stomatal conductance, and net CO_2 assimilation rate). We addressed the following questions: (1) How do *C. microphyllum* seedlings perform during water deficit events, mainly in terms of water status, gas exchange, and NSC dynamics, and (2) how do seedlings respond to a subsequent water deficit event, with possible links between NSC dynamics and shoot water status? We hypothesized that, because *C. microphyllum* presents intrinsic strategies to tolerate water deficit, the species will respond more readily to subsequent stress episodes, improving gas exchange and water status by increasing NSC reserves in the root tissue.

2 MATERIAL AND METHODS

2.1 Plant material, growth conditions, and experimental conditions

The present study was conducted from July to September 2019 in the Botanical Department of the Federal University of Pernambuco, Brazil (8°08′58″S, 34°56′55″W). The seeds of *Cenostigma microphyllum* (Tul.) E. Gagnon & G. P. Lewis were collected from Catimbau National Park, located in the Caatinga, a Brazilian dry forest region (8°35′22.11″S, 37°14′49.83″W). The seeds were disinfected with sodium hypochlorite (0.5%) and germinated in 3 L plastic pots, 27.0 cm deep and 12.5 cm in diameter, containing 4.5 kg of soil from the areas where the species occurred. During the growing period (six months), all seedlings were irrigated daily between 4:00 and 4:30 pm, according to the pot capacity (100 mL). No additional fertilizer solution was used in the soil. The average air temperature was 30 °C; and the relative humidity was 60% during the daytime.

After six months of development, 60 seedlings of similar size were randomly divided into two experimental groups: control plants (n= 30), at soil field capacity, and stressed plants (n= 30), subjected to total water reduction (Figure S1). Following the introduction of the treatment, the plants were separated into two sets: (1) the first water deficit (WD) cycle, comprising stressed plants subjected to a single WD cycle (n=15) and their control plants (n= 15), and (2) the second WD cycle (recurrent water deficit), comprising stressed plants subjected to the first WD cycle, rehydrated, and subjected to the subsequent WD cycle (n= 15) and their respective controls (n=15) (Figure S1).

After two months of development, we followed the seedling growth weekly until the end of the experiment. We measured the height of the plants (n= 10 per treatment) using a ruler and the diameter of the stem at ground level (n= 10 per treatment) using a digital caliper (Figure S2). The experiment lasted for 71 days; the first WD cycle lasted 28 days, and the second cycle lasted for 32 days. The stomatal conductance (g_s) and soil water content were measured every five days, on average, throughout the experiment in five plants per treatment. The g_s was used to determine the maximum stress (DM) in plants under water deficit when the values reached a value close to zero. The water content in the soil was used to determine the stage of water deficit for collecting the material used for NSC quantification (Figure 1). Thus, for both cycles, Stage I of water deficit was reached when soil moisture (SM) values dropped to 50% relative to the control, stage II when levels dropped to 70%, and stage III when SM dropped 80% relative to the control. (Figure 1). In the period between the cycle of water deficit, the period of rehydration of seedlings under water deficit was determined when the mean value of gs was similar to that of control plants for 3 follow days.

On each day of sampling, to determine the water content, leaf discs of approximately 0.6 cm in diameter were collected from five seedlings per treatment between 06:00 and 06:30 h. Thereafter, gas exchange, environmental parameters, and SM were measured between 08:30 and 10:00 h. On the days determined for collection of the material used in the quantification of sugars, we collected, between 15:30 and 16:00 h, the first fully expanded and healthy leaf pair, stem, and fine roots, which were immediately taken to the laboratory and placed in a microwave oven to stop the enzymatic activity (more details below).

2.2 Soil moisture and leaf RWC

Soil moisture was measured at a depth of 20 cm in five plants per treatment using Falker HFM 2030 m. Soil moisture measurements were obtained between 08:30 and 10:00 h, concomitantly with gas exchange measurements (detailed below). The measuring ruler was held close to the vessel wall to avoid damage to the roots.

The leaf relative water content (RWC) was determined following the methodology described by Barrs and Weatherley (1962). At five plants per treatment, one disk of standard size 0.6 cm in diameter was collected predawn and weighed immediately on a precision scale (A&D HR-200) to obtain a fresh weight (FW). Thereafter, the leaf discs were soaked in deionized water for 24 h and weighed to obtain the turgid weight (TW). Finally, the leaf discs were maintained in a forced ventilation oven at 80°C for 48 h to obtain the dry weight (DW). RWC was calculated using the following formula: RWC $(\%) = (\mathrm{FW} - \mathrm{DW} / \mathrm{TW} - \mathrm{DW}) \times 100$. At the beginning of each WD cycle, the RWC was measured every seven days from the beginning of water withholding. As the water deficit progressed, RWC was measured more frequently to assist in MS determination.

2.3 Gas exchange parameters

Gas exchange was measured in the third healthy and fully expanded leaf pairs using an infrared gas analyzer (IRGA-ADC, model LC-pro; Hoddesdon), using an open system with a 6 cm^2 clamp-on leaf cuvette to obtain the net CO_2 assimilation rate (A), stomatal conductance (g_s), and transpiration rate (E). The gas exchange system was calibrated daily using CO_2-free air. Measurements were taken at the same air temperature, controlled by the IRGA, and the same PPFD of the greenhouse (800 μmol m^{-2} s^{-1}) and air CO_2 concentration (400 μmol mol^{-1}). Measurements were obtained in the morning, between 8:30 and 10:00 h, in five plants per treatment. During the two WD cycles, measurements were performed every five days and intensified with an increase in the degree of water deficit. During the rehydration period, after 1 week of recovery, two measurements were obtained to determine the levels of plant recovery in the first cycle.

Vapor pressure deficit (VPD) was calculated using the formula $e_s - e_a$, where e_s is the saturated vapor pressure and e_a is the ambient vapor pressure (Campbell and Norman 1998), obtained using temperature and relative humidity with a digital thermo-hygrometer (Termo-Higro SH 122, J Prolab. São José dos Pinhais, BR). Throughout the experiment, the VPD varied between 1 and 2 kPa.

2.4 Leaf, stem segment (wood and bark), and root NSC analyses

For each water deficit stage in both cycles, we collected leaves that were healthy and well-developed, stem segments (bark (cambium included) and wood (similar thickness)), and roots (similar thickness) from the control (n = 5) and stressed (n = 5) plants at 15:00 h. The stem segments were separated using a razor blade, and both were treated as separate samples. The samples were placed in a microwave oven at 700 W for 30 s to cease all enzymatic activity (Quentin et al., 2015) and subsequently stored in a freezer at -20 °C.

The soluble sugar (SS) content was quantified using 25 mg of dry fine material mass obtained using a mortar and pestle, suspended with 1.200 µL of 80% ethanol, vortexed, and incubated for 90 min in a 70 °C water bath. Thereafter, the material was centrifuged at 12.500 × *g*, and the supernatant was collected. The procedure was repeated using 600 µL of 80% ethanol for 30 min (Dubois et al. 1956). The pellets were used for starch quantification. The pellet was suspended in 800 µL of 0.2 M KOH, vortexed, and incubated for 2 h in a 95 °C water bath. Next, 200 µL of acetic acid was added to restore the pH to 5.5; the samples were centrifuged at 12.500 × *g*, and the supernatant was collected. The samples were then hydrolyzed using 10 units of amyloglucosidase (A1621, Sigma-Aldrich) for 1 h in a 55 °C thermal bath. SS and starch concentrations (measured as glucose equivalents) were measured at 487 nm using a double-beam spectrophotometer (Geneses 10S UV-Vis, Thermo Scientific, Waltham, USA), according to the methodology described by Dubois et al. (1956). The NSC content of leaves, stem segments, and roots of each plant was calculated by adding SS and starch concentrations. The value was applied to the relationship between the concentration of the compound in the sample (mol) and the weight of 25 mg of dry mass for each organ, obtained after the samples had been dried in a forced ventilation oven for 48 h.

2.5 Data analysis

Statistical analyses were performed using Statistica 8.0 (StatSoft, Inc., Tulsa, OK, USA). Data were tested according to the assumptions of normality of residuals using the Shapiro test and homogeneity of residuals using the Levene test. The dataset that violated the premises was log- or square-root transformed. Data presented as percentages were logit-transformed (Warton and Hui, 2011).

For each WD cycle level and RH, RWC, and gas exchange (A and g_s) data were analyzed using a *t*-test at 5% significance to assess the differences between the control (n= 4) and water deficit (n= 4) treatments. The data on total NSC content were obtained by adding the NSC content of leaves, stem segments, and roots for each sample for each collection date at each water deficit level in both cycles. Factorial analysis of variance (ANOVA) was used to assess the differences in SS and starch concentration in different tissues between the control (n= 4) and water deficit (n= 4) treatments. In the graphs, the mean values are reported with error bars. One-way analysis of variance (ANOVA) was performed to identify differences in total NSC between organs for each treatment (n= 4) during ME of the two WD cycles. When necessary, means were checked using the Student–Newman–Keuls test at 5% significance. In the graphs, the mean values are reported with standard error bars.

Pearson correlation coefficients were calculated between RWC (n=12) and SS content in wood (n=12) and root (n=12) were calculated for stressed plants in both WD cycles.

3 RESULTS

3.1 Water relations and growth in response to recurrent water deficit

The RWC of plants subjected to the first WD cycle remained constant when the soil moisture level exceeded 66% (24 days of water withholding), but decreased dramatically thereafter (by 49.9%, p = 0.0144) compared with that of the control plants (Figure 2A). During rehydration, the water levels of stressed plants were fully recovered, with no significant differences compared with controls after 8 days.

In the second WD cycle, plants previously subjected to WD reduced the RWC by 25.8% (p = 0.0461) relative to the control plants when the SM was around 50%. This response was DC different to that of plants from the first WD cycle. Along the WD, the RWC values in plants under water deficit were slightly reduced until the day of DM (Figure 2A).

Since the beginning of the first WD cycle, the stressed plants reduced their growth rate, relative to the control plants, reducing height and the diameter of the main stem (Figure S2).

3.2 Gas exchange in response to recurrent water deficit

During the first WD cycle, no effect on leaf gas exchange parameters (A and g_s) was observed, whereas soil moisture was greater than 50%. Stressed plants showed reductions in g_s and A (g_s: 54%, $p < 0.0001$ and A: 57%, p = 0.0027, respectively) only when soil water levels decreased by 66% (Figure 2C, D), with drastic reductions until MS day. Rehydration increased g_s and A levels after 8 days, but the gas exchange parameters of stressed plants recovered to levels like those of control plants after 12 days (Figure 2C, D).

In the second WD cycle, despite a previous reduction in RWC, the gas exchange parameters were reduced only when soil moisture levels were decreased by 66% (g_s: 60%, p = 0.0004 and A: 62%, p = 0.0025), like that observed in the first WD cycle (Figure 2B, C). In plants that had been previously stressed, g_s was reduced to the same values as in the first WD cycle, but a different trend for RWC and A was observed. During the second WD cycle, leaf RWC was reduced earlier compared to the first WD cycle and was only 20% lower than that in control plants, while A was kept twice as high as that during the first WD cycle (arrow) (Figure 2A, B).

3.4 Effects of recurrent water deficit on NSC reserves and dynamics

In our study, we sought to evaluate the changes in NSC dynamics in plants subjected to recurrent water deficit. Plants subjected to previous stress responded more quickly by accumulating NSC in conductive tissues when soil water levels were above 50%, when compared to WD first cycle plants. In the first WD cycle, the soil water level >50% (stage I) did not change the concentration of NSC components (SS and starch) in any tissue (Figure 3A-F). Thereafter, the accumulation of SS and starch in wood and that of SS in roots increased according to the stages of water deficit. At the end of the first WD cycle (stage III), stressed plants accumulated twice as much SS in the wood and roots than control plants (Figure 3B, D).

In the WD recurrent cycle, even during stage I, the previously stressed plants already accumulated SS in the wood when compared to the control (Figure 3B). At the end of the second WD cycle (stage III), the SS concentration in the wood and root tissues of stressed plants was five-fold higher than that of the control plants (Figure 3B, D). During the first WD cycle, the wood starch content of stressed plants was approximately 50% higher than that of control plants, but the values decreased to the control levels under recurrent water deficit (Figure 3F). In roots, the critical water deficit at the end of the second WD cycle increased starch accumulation and decreased SS accumulation in stressed plants, with 90% higher concentrations than the control values (Figure 3H).

Monitoring the water status showed that as they went through the second WD cycle, the plants reduced their RWC rates early, keeping it stable for a longer time when compared to the first WD cycle. To make this physiological acclimatization more explicit, we determined the relationship between SS content in wood and roots and the RWC of the plants in each cycle (Figure 4). In the first WD cycle, the SS content in wood showed a negative correlation with RWC in plants, and root SS was not related to RWC variation (Figure 4A). In the second WD cycle, a negative relationship between wood and root content and RWC was observed (Figure 4B).

3.5 Principal components analysis (PCA)

The PCA was performed for the maximum stress days for each WD cycle (Figure 5). The analysis showed that the ordering of the two variables represented 68.02% of the total data variation. The treatments were separated in the PC1 water regime and WD cycles in PC2. For PC1, the variables RWC, *A*, g_s NSC total, and SS wood were more important to distinguish between hydrated and water-deficient plants, while SS root and starch wood were more important in distinguishing plants from the first and second WD (Figure 5). During the first WD cycle, the plants kept the RWC high until the end of stage II (-70%); thus, it was possible to accumulate starch in the wood; in the subsequent cycle, due to the anticipated reduction in RWC, the plants accumulated much more SS in wood and no significant accumulation in starch content.

4 DISCUSSION

Our study suggests that recurrent water deficit led *C. microphyllum* seedlings to respond more intensely to NSC allocation. Our prediction partially occurred; the water deficit stimulated the seedling storage SS in root tissues in both WD cycles, but similar concentrations were also observed in wood. During recurrent WD, the plants were able to maintain leaf RWC and CO_2 assimilation for twice as long, and at a level higher than that observed in the first WD cycle (Figure 6). Stressed plants had reduced aboveground growth following the first WD cycle, leading to greater water savings and lower carbon consumption, providing SS accumulation in the stem wood and root, which is known to be important for the survival of woody species under water deficit.

Different responses of woody species subjected to WD cycles have already been documented (Abdallah et al. 2017, Tomasella et al. 2017, Tombesi et al. 2018, Chuste et al. 2020). During a water deficit, the plant changes the use and dynamics of C, leading to an increase in the NSC pool. The ability to alter the NSC dynamics of trees under different degrees of water stress is an important mechanism for plant adaptation to water deficit (Davidson et al. 2021, Santos et al. 2021, Signori-Muller et al. 2021). Recurrent water deficit events can modulate NSC partitioning and investment responses in tree species. Some authors have reported an increase in SS in different organs during subsequent stress, improving the water status of plants (Abdallah et al. 2017, Tomasella et al. 2017). In fact, SS are the main osmotically active components of the NSC (Martínez-Vilalta et al 2016) and are important regulators of plant water balance, promoting osmotic protection for cell turgor maintenance and xylem viability (Sevanto et al. 2014, Sapes et al. 2019). Starch stock is considered an important first-line strategy for reducing water potential and preventing xylem embolism (Bucci et al. 2003, Tomasella et al. 2019). Reduction in the starch content of wood with a consequent increase in the SS content of marginal xylem cells can take up water and air from the vessels. However, the allocation of NSCs may vary according to the species (Piper et al. 2011), the degree of water deficit (Martínez-Vilalta et al. 2016, Santos et al. 2021), and the ability of the plant to store and transport these metabolites. Previous studies on the hydraulics or carbon metabolism of woody species in temperate (Palacio et al. 2007a, 2018, Martínez-Vilalta et al. 2014, Nardini et al. 2016, Tomasella et al. 2017, 2019, Resco de Dios et al. 2020, Schoonmaker et al. 2021) and tropical (Bucci et al. 2003, Rivas et al. 2013, Rosell et al. 2020, Santos et al. 2021, Signori-Muller et al. 2021) climates have shown that their responses are far from standard, being closely dependent on study conditions, selected species, and traits measured (Souza et al. 2020).

4.1 Recurrent water deficit and NSC content in wood and root tissues

In the present study, although higher root NSC allocation was expected (Santos et al. 2021), in both cycles, seedlings also accumulated high concentrations of SS in wood tissue. In woody species, carbon starvation due to decreased photosynthetic rates or reduced sugar dynamics from source to sink tissues has been widely discussed in climate change scenarios (Sala et al. 2010, Tomasella et al. 2019, Chuste et al. 2020, Resco de Dios et al. 2020). Here, even under significant reductions in water content (-80% than control plants) conditions, plants showed strong increases in root and wood SS content, without storage in leaves, which might be the typical response of a tropical deciduous woody species (Santos et al. 2021). The common water deficit at the end of the rainy season in seasonally dry tropical forest induces senescence and leaf abscission, this characteristic of deciduous woody species will support the translocation of nutrients to the rest of the tissues that will cross the dry season. In addition, it will provide a favorable water balance as the plant decreases its transpiration rate (Munné-Bosch and Alegre, 2004). However, the recurrent WD cycle increased the availability of SS in both wood and root tissues (~5 times greater than control plants), whereas starch concentration decreased only in the wood tissue. These results suggest that the roots continued to receive SS from aboveground tissues, even under severe water deficit conditions. Similar results were observed by Tomassella et al. (2019) in *Fagus sylvatica* L. plants subjected to two WD cycles. NSC dynamics can improve plant responses during consecutive water deficit events (Tomassella et al. 2017, 2019, Abdallah et al. 2017, Chuste et al. 2020) and can integrate plant signaling responses to stress (Secchi and Zwieniecki, 2011). Indeed, plants can have metabolic impressions that would prepare tissue responses to subsequent stresses (Schwachtje et al. 2019). This could explain the faster responses of carbohydrate storage in wood and roots in *C. microphyllum* seedlings in the second WD cycle.

4.2 Relative water content and gas exchange under recurrent water deficit

During the first WD cycle, the plants strongly decreased gas exchange, which allowed them to maintain higher leaf RWC levels at the beginning of the treatment. This behavior is typical of isohydric species (Martínez-Vilalta et al. 2017), and although it is not the focus of our study, this response pattern was also recently found for woody species in tropical dry forest environments (Souza et al. 2020). Here, plants under recurrent WD had stomatal conductance with strong control during both water deficit cycles, which, together with reduced biomass production, would have saved water in the leaf tissues and led to a higher rate of CO_2 assimilation in the second WD cycle for a longer period. A possible explanation is that *C. microphyllum* has a high wood density (Zoger et al. 2019), which would have allowed the plant to maintain its photosynthetic rate even with a low water potential in tissues (Lima et al. 2012). According to Sapes et al. (2019), the RWC measure reflects the water status of plants and can integrate the hydraulic failure and carbon depletion processes. During the second WD cycle, *C. microphyllum* seedlings maintained RWC at a threshold, allowing the transportation of SS among different plant organs, since starch degradation was low (Martínez-Vilalta et al. 2019). Previous studies have shown that the best measure of whole plant water status is water potential, due to the translation of thermodynamic properties and the demonstration of water status along the soil-plant-atmosphere continuum (Slatyer and Taylor 1960; Kramer and Boyer 1995). On the other hand, several studies have also shown that the plant's water status can be measured through other variables, such as RWC, gas exchange, turgor pressure, and hydraulic conductance, all of which have their relevance and limitations (Passioura 1988; Schulze et al. 1988; Jones, 2007; Martínez-Vilalta et al. 2019). The water status was measured through RWC predawn and in independent plants for each measured day. At this time of day, water status is likely to be the same across all plants (Kramer and Boyer 1995); therefore, minimizing the risk of error or confusion. The RWC is perhaps the most used variable to demonstrate the level of hydration of different organs in plants (Jones 2007), especially when working with seedlings of woody species in pots, where biomass is restricted and the use of independent plants is essential.

Previous studies have shown that recurrent water deficits improve woody plant responses at the photosynthetic level (Rivas et al. 2013, Barros et al. 2018) and water performance (Abdallah et al. 2017, Tomasella et al. 2019). Thus, the rapid adjustment to NSC allocation level would have allowed the plants to maintain stable stomatal conductance and CO_2 assimilation rates for 7 days longer and with 80% drier soil, leading to the highest NSC storage at the end of the experiment (31% compared to plants from the first WD cycle). Under field conditions, with short-term water deficit events (i.e., during the rainy season), this behavior may be advantageous for deciduous tropical dry forest species by increasing the availability of NSC for tissue storage and sustainability during the dry period without CO_2 assimilation. It is important, however, to consider that under field conditions, soil desiccation is slower than the experimental substrate in pots. Furthermore, although the NSC storage time in the plants differed between the WD cycles, the wood starch storage was the main variable that differentiated the two groups (Figure 5). *C. microphyllum* plants showed decreased secondary growth, as measured by stem diameter (Figure S2), since the first WD cycle, which probably led to an increase in starch concentration in the stem. In the second WD cycle, the starch content in the wood decreased, while SS in the same tissue increased. Thus, there was a change of strategy in the wood tissue. During the first WD cycle, the storage of SS was parallel to starch accumulation. On the other hand, during the second WD cycle, the intensification of stored SS was intensified. Reducing the growth of above-ground parts may have allowed for more NSC storage and decreased water consumption (Tomasella et al. 2019).

Although Rosell et al. (2020) showed that, in 45 tropical forest species, the bark is an important tissue for NSC storage, our seedlings showed no variation in the stored content between treatments. However, the total NSC in bark was 23% and 14% in the first and second cycles, respectively, which may reflect the decrease in growth. Alternatively, the seedlings were too young to have such a developed bark; Rosell et al. (2020) found strong variation in the inner bark, the portion closest to most living region. Our imposed water deficit may also be not severe enough for the seedlings to start using the stock present in the bark, as it still managed to translocate SS to wood and root tissues.

On the other hand, in root tissue, the increase in SS content was correlated with a decrease in RWC in plants during the second WD cycle. Stored carbon in the roots can determine the response of the whole plant during the dry period (Basu et al. 2016), since woody plants can achieve osmotic adjustment to maximize water uptake (Bucci et al. 2003, Sala et al. 2010, Resco de Dios et al. 2020). However, our data suggest that the storage of SS in the roots occurred via the translocation of an organ above ground, as there was no significant modification in the starch content. This behavior in the pre-senescent period could favor the regrowth of leaves during rehydration or the next rainy season under field conditions.

Our set up water deficit cycles in potted plants may not faithfully simulate natural conditions because of the volume of substrate and limitation of roots; however, during irregular rainy seasons, plants have experienced a similar situation in the semiarid region. Our results suggest that under water deficit, *C. microphyllum* reduces aboveground growth and maintains water status for more time, moving NSC during successive WD cycles under controlled conditions. While the plants still retained their leaves, we could measure intense storage of SS in the wood and root tissue, to the detriment of a strong decrease in the growth rate of above-ground organs. After going through the first WD cycle, *C. microphyllum* seedlings that were rehydrated and later subjected to another WD cycle intensified their strategy of increasing NSC storage in wood and root tissues. The results of this study may be advantageous in the face of increasingly irregular conditions of semiarid tropical climates. The present work encourages further research into woody plant behavior under dry and rainy seasons; this would generate important data to develop predictive models for the behavior of vegetation in tropical dry forests under predicted climate change.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

MS and MGS conceived and designed the experiments. MS and JN performed the experiments. MS and JN performed the laboratory analyses. MS and MGS wrote the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

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LEGENDS

Figure 1. Soil moisture (SM) during the experiment. The soil water deficit stages were followed up in purchase with control plants of *C. microphyllum* (n = 10 started row), irrigated to pot capacity, and determined to collect NSC material. For both cycles, stage was considered with SM in water deficit plants (n = 5) dropped to 50%, stage II when SM dropped to 70% values and stage III when values dropped to 80% in relation to control. Stages I, II and III of each water deficit (WD) cycle are presented (vertically dashed lines) and their respective dates, and the rehydration period.

Figure 2. Water status, and gas exchange conditions during the two water deficit cycles Relative water content (RWC) **(A)**, CO_2 assimilation (A) **(B)**, stomatal conductance (g_s) **(C)** of seedlings *Cenostigma microphyllum* plants submitted to two water deficit (WD) cycles and rehydration (dotted lines) under controlled conditions (drought and control plants). Each point represents the mean (n = 5 plants per treatment) and the trait ± SE value. Significant differences between treatments (*, $P<0.05$).

Figure 3. Soluble sugar (SS) and starch content of leaves **(A-E)**, wood **(B-F)**, bark **(C-G)**, and root **(D-H)** of seedlings *Cenostigma microphyllum* plants submitted to two water deficit (WD) cycles and rehydration (dotted lines) under controlled conditions (control and water deficit plants). Each point represents the mean (n = 4 plants) and trait ± SE value. Significant differences between treatments (*, $P<0.05$).

Figure 4. Effects of variations in SS content in wood and root in relative water content (RWC) for the first water deficit (WD) cycle **(A)** and the second WD cycle **(B)** of seedlings *Cenostigma microphyllum* plants submitted to two water deficits cycles and rehydration. Regression line refers to stressed plants (n = 12), full lines refer to wood and dotted lines refer to root. Pearson correlation coefficient (r), and respective P-value are reported.

Figure 5. Principal component analysis (PCA) based on the whole dataset of the study of *C. microphyllum* seedlings submitted to two water deficit (WD) cycles and respective plants control under greenhouse conditions (n = 4). Treatments were separated as water regime in PC1 and as water deficit cycle 1 and 2 on PC2.

Figure 6. Related major traits to the different strategies adopted by *Cenostigma microphyllum* submitted to two recurrent water deficit cycles. The color highlighted organs refer to the total concentration of non-structural carbohydrates (NSC) at the end of each water deficit cycle and the boxes refer to the components of the NSC with greater change over the cycles (Soluble sugars - SS and starch). The

framework represents the behavior of the variables measured during the two water deficit cycles. Relative water content (RWC); CO_2 assimilation (A) and stomatal conductance (g_s).

Figure 1 Supplementary. Experimental design of the study. In the first water deficit cycle, the *C. mycriphyllum* seedlings were randomly organized into two groups: control (n = 30) and water deficit (n = 30). The first water deficit cycle lasted 27 days and 15 plants were collected per treatment. During rehydration, all remaining plants were kept well watered for 12 days. In the second water deficit cycle, the stressed plants (n = 15), previously subjected to water deficit, were again subjected to water withholding. The second water deficit cycle lasted 32 days. Periods of water deficit are outlined in grey.

Figure 2 Supplementary. Height **(A)** and stem diameter **(B)** before and during the 1st and 2nd water deficit cycle in control and water deficit treatment plants. Each point represents the mean (n = 5 plants per treatment ± SE value). (*) indicates differences between treatments ($P < 0.05$).

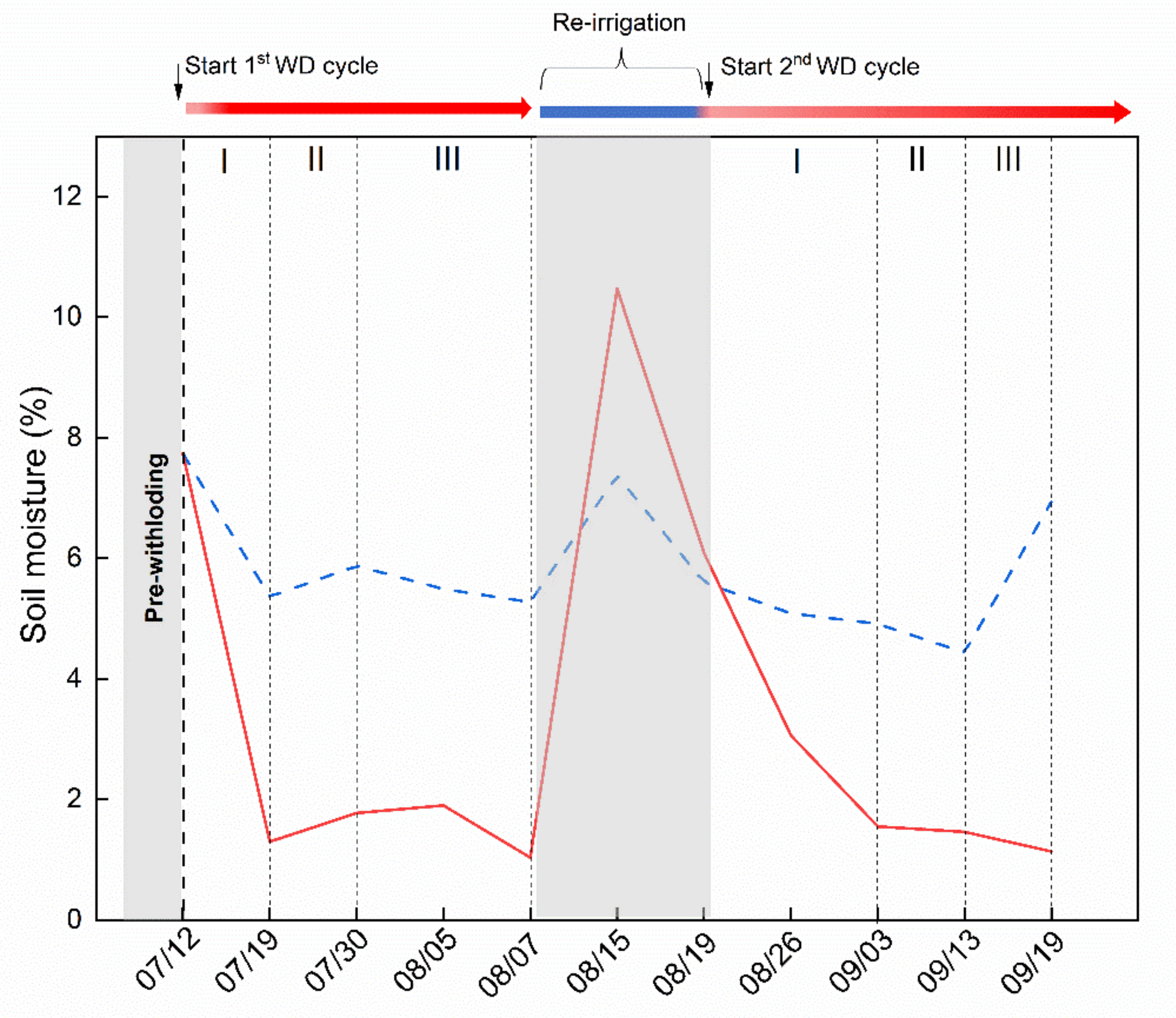
Re-irrigation
Start 1st WD cycle
Start 2nd WD cycle
I
II
III
I
II
III
Pre-withloding
Soil moisture (%)
0
2
4
6
8
10
12
07/12
07/19
07/30
08/05
08/07
08/15
08/19
08/26
09/03
09/13
09/19

Figure 1

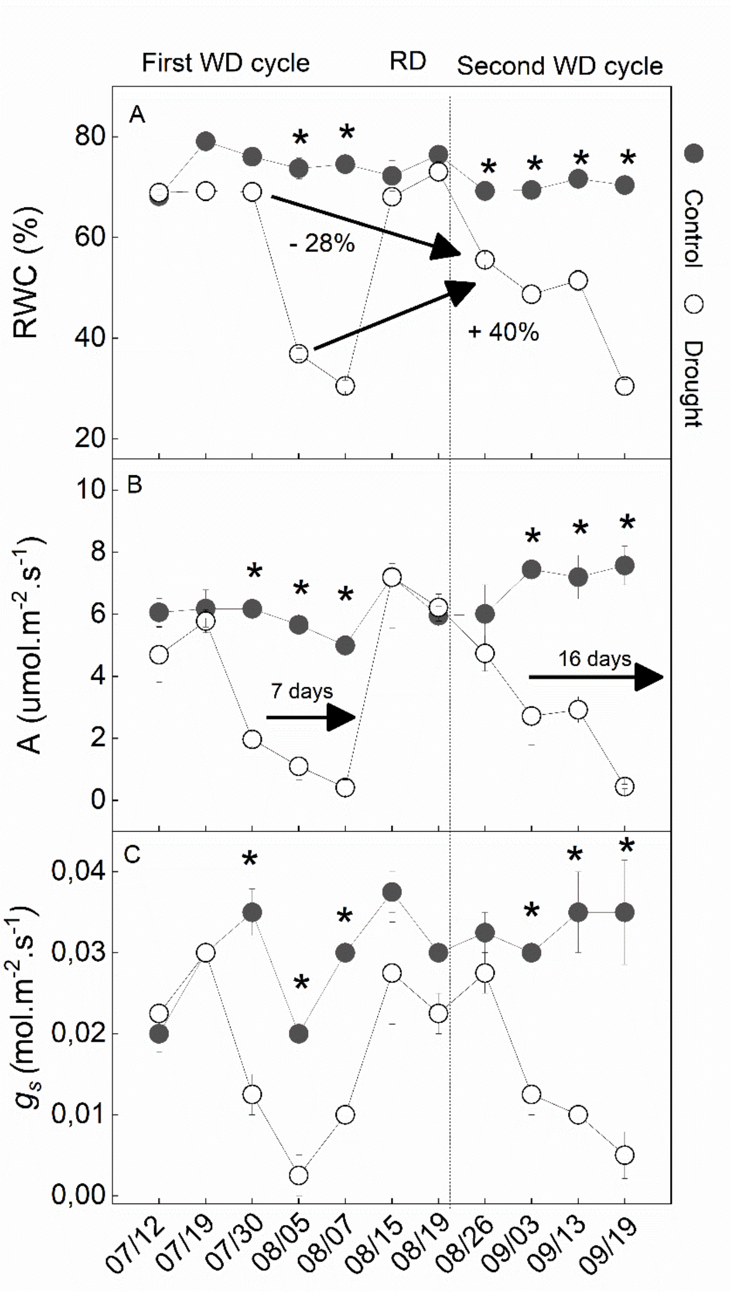
First WD cycle
RD
Second WD cycle
A
RWC (%)
80
60
40
20
- 28%
+ 40%
Control
Drought
B
A (umol.m-2.s-1)
10
8
6
4
2
0
7 days
16 days
C
gs (mol.m-2.s-1)
0,04
0,03
0,02
0,01
0,00
07/12
07/19
07/30
08/05
08/07
08/15
08/19
08/26
09/03
09/13
09/19

Figure 2

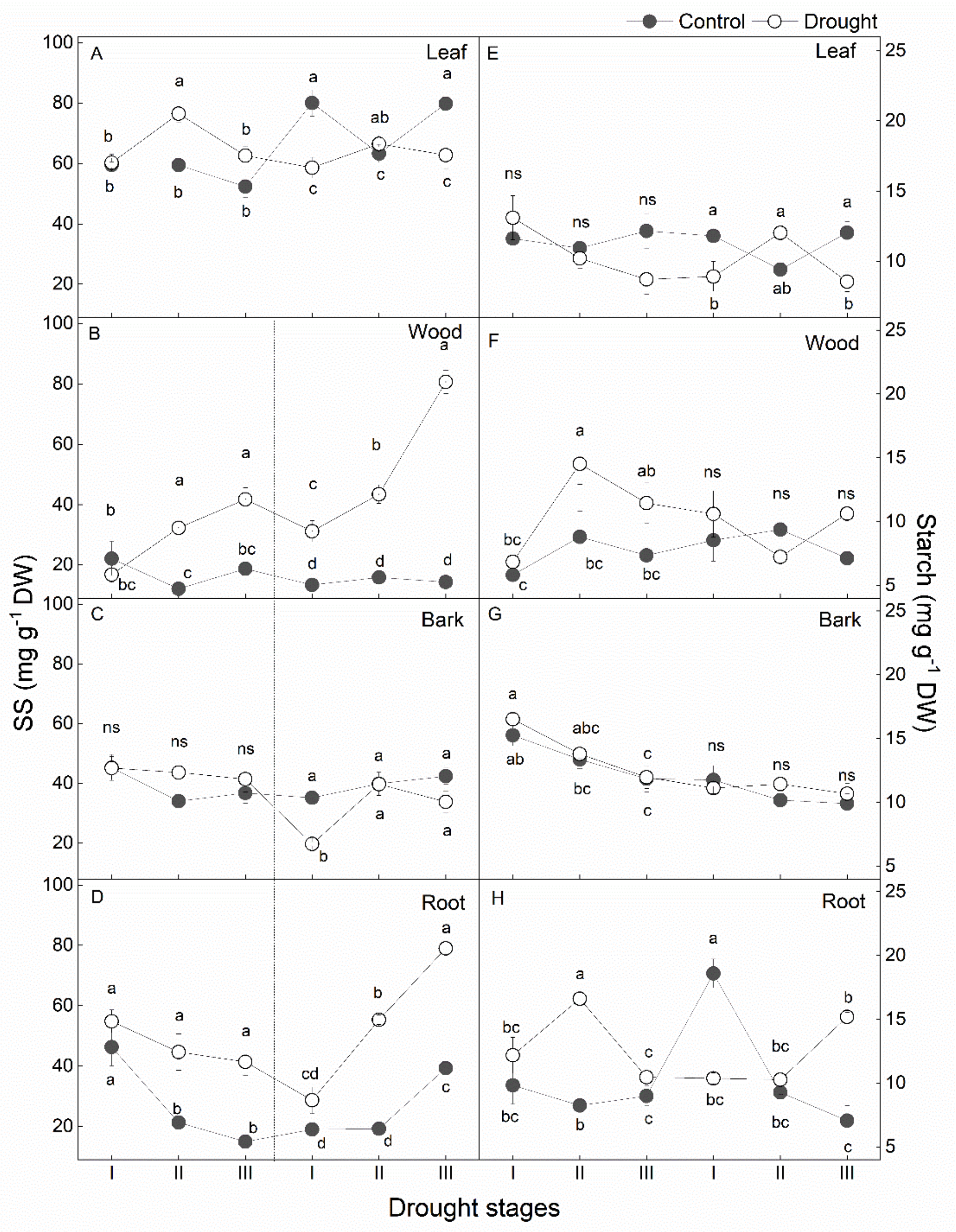
● Control ○ Drought
A
Leaf
B
Wood
C
Bark
D
Root
E
Leaf
F
Wood
G
Bark
H
Root
SS (mg g^{-1} DW)
Starch (mg g^{-1} DW)
Drought stages
100
80
60
40
20
25
20
15
10
5
I
II
III
I
II
III
I
II
III
I
II
III
a
b
c
ab
abc
bc
cd
d
ns

Figure 3

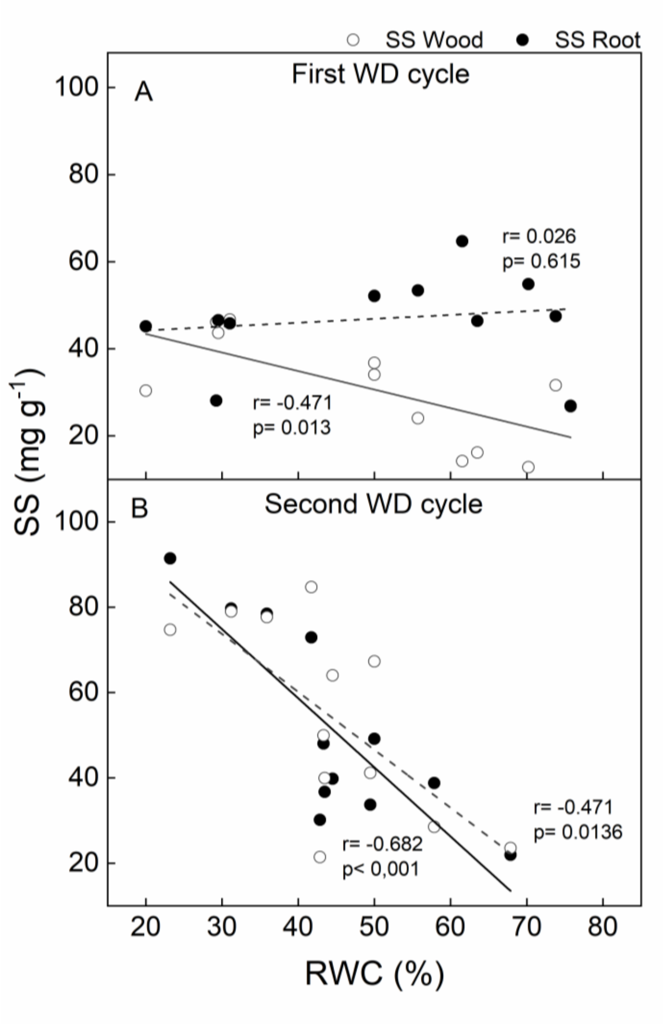
○ SS Wood
● SS Root
A
First WD cycle
r= 0.026
p= 0.615
r= -0.471
p= 0.013
B
Second WD cycle
r= -0.471
p= 0.0136
r= -0.682
p< 0,001
SS (mg g^{-1})
20
40
60
80
100
RWC (%)
20
30
40
50
60
70
80

Figure 4

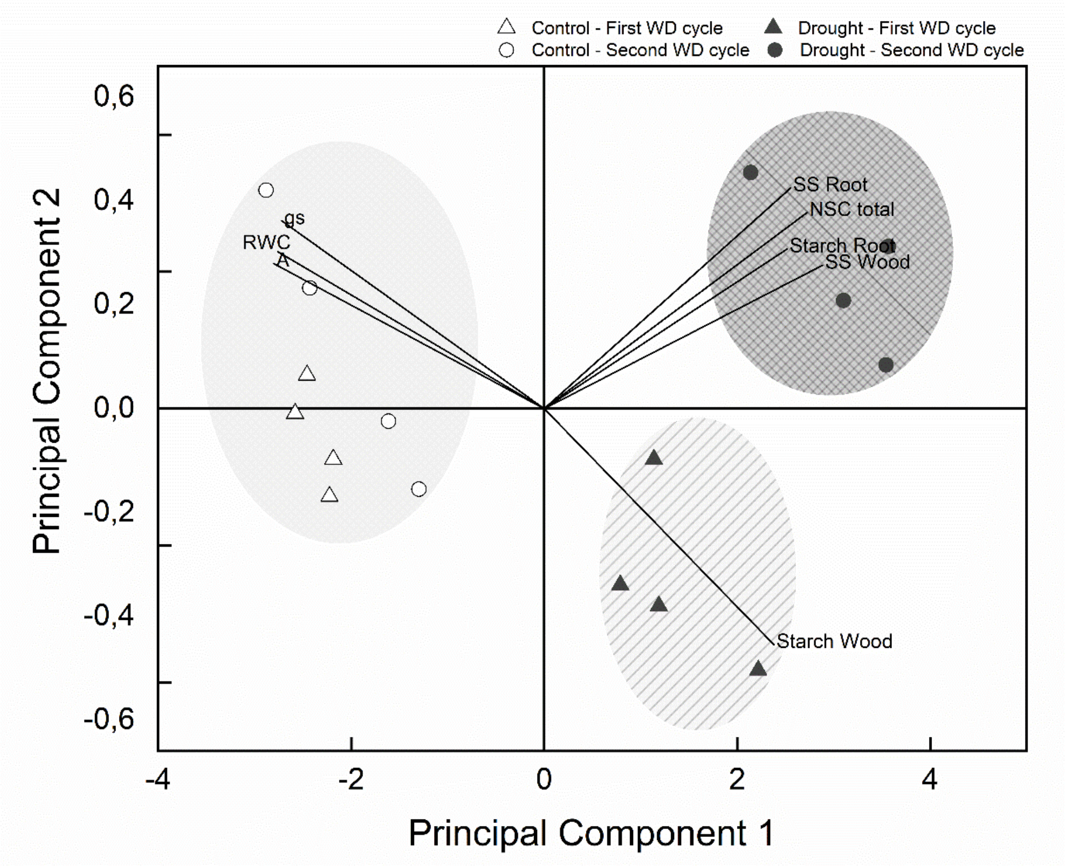
△ Control - First WD cycle
○ Control - Second WD cycle
▲ Drought - First WD cycle
● Drought - Second WD cycle
Principal Component 2
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
-4
-2
0
2
4
Principal Component 1
gs
RWC
A
SS Root
NSC total
Starch Root
SS Wood
Starch Wood

Figure 5

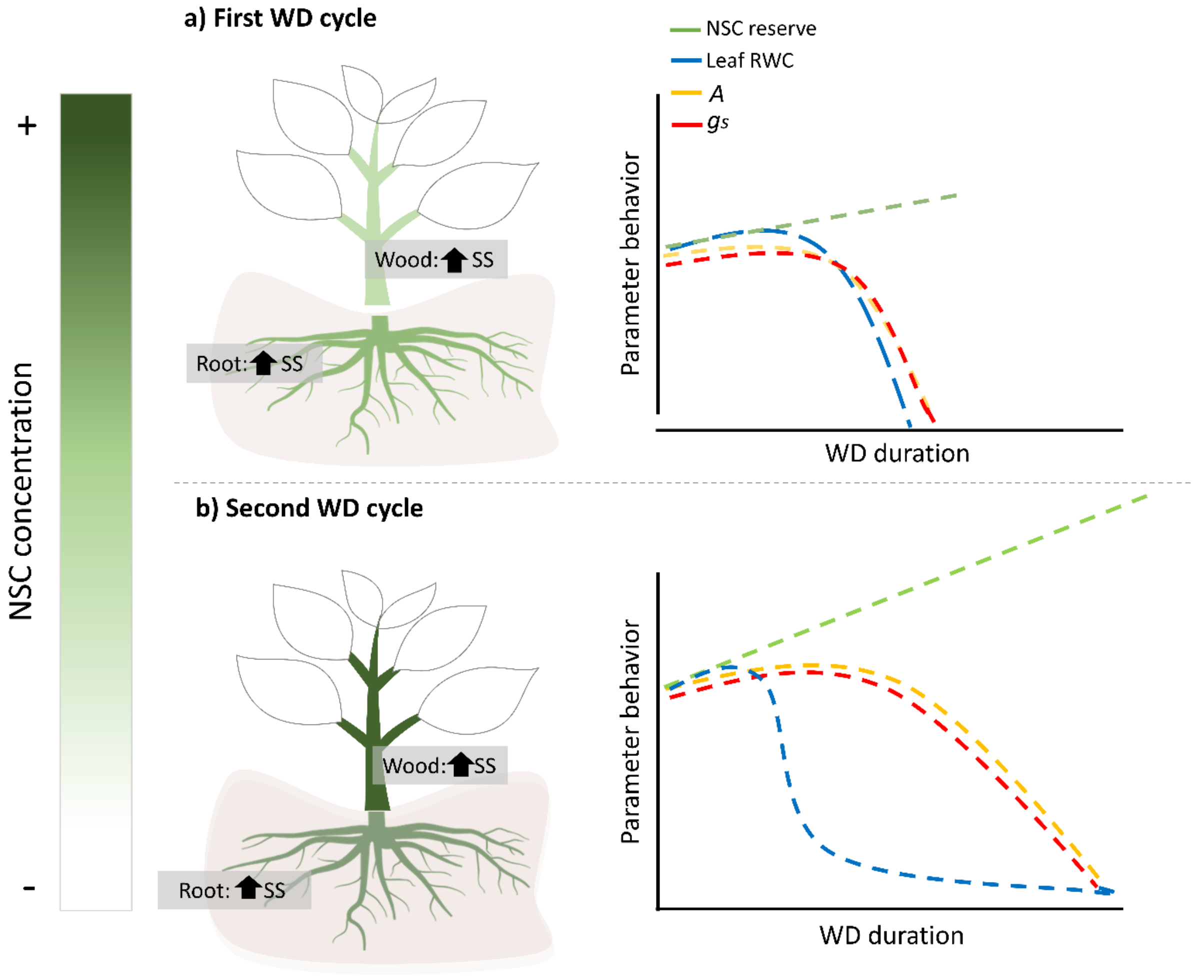
a) First WD cycle
NSC reserve
Leaf RWC
A
gs
Parameter behavior
Wood: ⬆ SS
Root: ⬆ SS
WD duration
b) Second WD cycle
Wood: ⬆ SS
Root: ⬆ SS
Parameter behavior
WD duration
+
NSC concentration
-

Figure 6

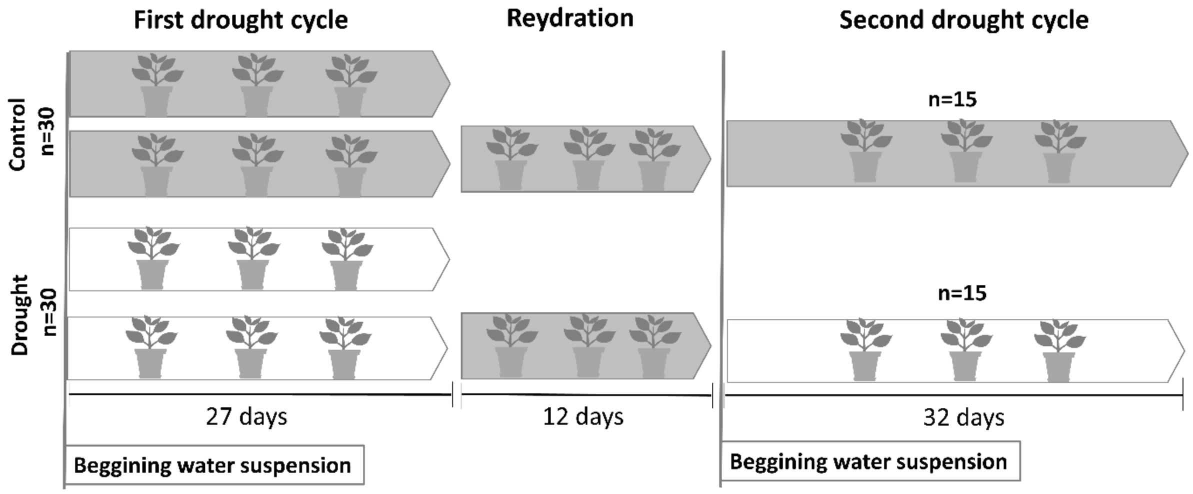
First drought cycle
Reydration
Second drought cycle
Control
n=30
Drought
n=30
n=15
n=15
27 days
12 days
32 days
Beggining water suspension
Beggining water suspension

Figure S1

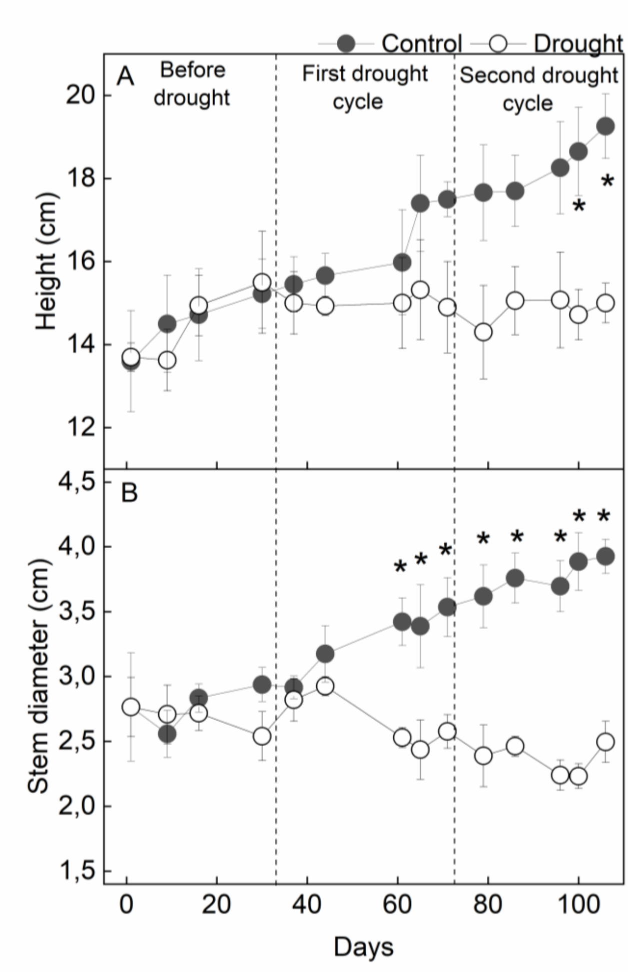
Control
Drought
A
Before drought
First drought cycle
Second drought cycle
Height (cm)
20
18
16
14
12
B
Stem diameter (cm)
4,5
4,0
3,5
3,0
2,5
2,0
1,5
0
20
40
60
80
100
Days
*

Figure S2

4. MANUSCRITO II

Dynamic allocation and of non-structural carbohydrates partitioning, a possible mechanism for tree species establishment in water deficit sites across the Brazilian Tropical Dry Forest)

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(ANEXO B)

Dynamic allocation and of non-structural carbohydrates partitioning, a possible mechanism for tree species establishment in water deficit sites across the Brazilian Tropical Dry Forest

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Key words: caatinga; drought tolerance; dynamic of sugars; semiarid; sugar metabolism

Running head: Ability to use sugars on woody species

ABSTRACT

Efficient carbon (C) investment can determine the performance of woody species in resource-limitans environments, such as water availability in Tropical Dry Forests. Thus, our main objective was to investigate how the allocation and partition of non-structural carbohydrates (NSC) and the investment in leaf construction cost (LCC) occur in species with different occurrences in high and low mean annual precipitation (MAP)extremes of precipitation. Ten species were selected, divided according to the degree of occurrence between the areas: (1) greater distribution under high MPA (WA); (2) greater distribution under low MPA (DA) and similar distribution in both areas (BA). We quantified the NSC and their components (SS and starch) in leaves, branches and roots in species sampled at the two extremes of precipitation, and also analyzed the LCC. We observed that the partition of NSC in the plants varied according to the species groups, but did not differ between the areas. WA presented 73% of the NSC content in the leaves, while BA and DA also presented 33% and 40% investments in root, respectively. In general, DA species showed higher concentrations of NSC compared to WA and BA, and in low MAP areas store higher starch contents, in relation to high MAP areas. BA species maintain lower and more stable concentrations of NSC but respond to the seasonality of the year in question, increasing the concentration of NSC in roots during the driest year. LCC was not related to NSC accumulation for either group. Thus, we observed that the accumulation of NSC under lower water availability, is a priority route in C investment for deciduous tree species of TDF, and can favor their performance in conditions of water deficit.

Keywords: caatinga; drought tolerance; dynamic of sugars; semiarid; sugar metabolism

Abbreviations:

BA	Species with similar distribution in both areas (high and low MAP)
C	Carbon
CCmass	Leaf construction cost per unit mass
DA	Species with greater distribution under low MPA
DW	Dry weight
GLMM	General linear mixed models
LCC	Leaf construction cost

LES	Leaf economic spectrum
MAP	Mean annual precipitation
NADPH	Nicotinamide adenine dinucleotide phosphate
NSC	Non-structural carbohydrates
PELD	Projeto Ecológico de longa duração
ROS	Reactive oxygen species
SLA	Specific leaf area
SS	Soluble sugars
STFD	Seasonal Tropical Dry Forest
WA	Species with greater distribution under high MPA
WD	Wood densit

INTRODUCTION

Non-structural carbohydrates (NSC) play an important role in carbon (C) metabolism in plants, as they are one of the main products of photosynthesis and substrate for growth and physiological functions such as respiration, osmotic regulation and defense of trees (Hartmann et al. 2013, Martinez- Vilalta et al. 2016). The pool of NSC in plants is mainly given in the form of soluble sugars and insoluble starch (Hartmann and Trumbore at al. 2016), and because they reflect the balance of carbon supply via photosynthesis, they present a high dynamic in the whole-plant (Doughty et al. 2015). Under resource-limiting conditions, such as water availability in Tropical Dry Forest regions, plants alter their C allocation in order to acclimate to these stressors (Piper et al. 2011, Hartmann et al. 2013). Thus, the dynamics of NSC in plants present themselves as an important response pathway to plant tolerance and survival. As a result, many studies have paid considerable attention to the role of NSCs in mediating tree tolerance during a drought period (O'Brien et al. 2014, Sala et al. 2011). However, there is still less understanding of how NSC investment can moderate the tolerance and distribution of mature trees across a geographic domain.

Sugar accumulation in different organs has been linked to a greater performance in situations of water deficit (Rolland et al. 2006, Mundim and Pringle 2018, Souden et al. 2020). This is due they can act as signaling molecules (Secchi and Zwieniecki 2011), osmprotector and reactive oxygen species scavenger (ROS) (Rolland et al. 2006, Regier et al. 2009). It is reported the relevance of mobilization of sugars to conductive tissues, in the refilling xylem vessels after an episode of embolism (Nardini et al. 2016), improving the plant's water status (Trugman et al. 2018), and increasing root sugar reserves for leaf regrowth (Hagedorn et al. 2019). NSC are the most important C reserves in deciduous and evergreen trees, and serve as a C source to satisfy demand during the leaf regrowth period (Fischer et al. 1991, Hoch et al. 2003).

Carbon investment in plants depends on the relationship between multiple leaf traits, which determine the C gain versus the environmental conditions in which they are exposed, this balance is known as leaf economic spetrum (LES). An important LES to assess C investment in leaf regrowth is the leaf construction cost (LCC), which is the expenditure of glucose and reducing power in the form of NADPH during the production of leaves (Williams et al. 1987). Thus, foliar CC can vary according to the NSC dynamics in the plant and can be seen as an important scorer of strategy type used by the plant to adjust its carbon investment to the environmental changes (Poorter and Villar 1997), especially in STDFs, which experience a high irregularity in water availability (Prentice et al. 2014). Especially for deciduous species, which depend on sugar reserves for leaf regrowth, NSC dynamics and energy investment in leaf construction, measured in this study through the LCC, may influence species tolerances in environments with large water availability fluctuations.

For woody species in semiarid environments, which growth under water limitations, a specific dynamic of the nature of sugars has been observed (Rolland et al. 2006, Rivas et al. 2013, Santos et al. 2021), along with a high resilience of carbon assimilation (Santos et al. 2014, Chazdon et al. 2016). Previous studies have also shown a strong relationship between the efficiency of carbon resource investments and the cost of foliar construction in native STDF tree species (Falcão et al. 2017, Barros et al. 2021).

Understanding the drives related to drought tolerance in woody species that determine the distribution within a precipitation gradient is important to predict the effects of longer periods of drought on vegetation composition in Tropical Forests. The objective of the study is to identify the strategies in C resources investment in woody species that present a different degree of distribution in relation to precipitation: areas with high and low mean annual precipitation (MAP). The main questions that guided this study were (1) How do species allocate and partition NSC according to their ability to tolerate drier locations? (2) Is there a relationship between

NSC investment strategies and LCC in plants? For this, we hypothesized that (1) species with greater capacity to establish themselves in drier places would present a greater NSC partitioning in branches and roots and have a lower LCC in areas of lower MAP (2) species that establish better in more wet places present a larger NSC partitioning in sheets and larger LCC in both areas, while, (3) species with similar distribution in high and low MAP areas show similar partitioning between tissues in the two areas.

MATERIALS AND METHOD

Study site

The study was carried out in the Catimbau National Park (8º24000″S, 37º14040″), a conservation unit located in Caatinga, in northeastern Brazil. The park has a total extension of 607km2, and its landscape is subjected to an average annual rainfall of 440mm to 1100mm, which varies within a gradient, and average temperatures of 23ºC, based on a historical average of 30 years (Rito et al. 2017). The region is exposed to a semi-arid Bsh climate with transition to tropical rainy As’, according to the K’open system. It presents predominantly a cover of sandy, well-drained and highly impoverished soils (Rito et al. 2017). The predominant vegetation is a dry forest with small trees dominated by species of Fabaceae, Euphorbiaceae and Cactaceae (Rito et al. 2017b, Souza et al. 2019). The park was the scene of a long-term ecological project (PELD-CNPq), with a network of predetermined plots along the precipitation gradient (Figure 1), where characteristic surveys of the areas were carried out, including a floristic survey for the arboreal/woody species (Rito et al. 2017).

Plant species

Within the floristic survey it was possible to observe that most tree species occur within the precipitation gradient. The study focus on species that are distributed in the extremes of mean annual precipitation (MAP): higher MAP sites (843-940mm) and lower MAP sites (510-640mm) in the park plots (Figure 1 and 2), as a proxy for drought sensitivity. We studied 10 species, within the most abundant families in the park (Table 1). Then we selected, (1) species with greater wet sites distribution (WA): *Senegalia bahiensis*, *Trischidium molle* and *Piptadenia stipulacea;* (2) species with greater dry sites distribution (DA): *Commiphora leptopholeos*, *Peltogyne paucilflora* and *Bauhinia acuruana*; (3) species with greater both sites distributions (BA): *Croton heliotropifolius, Pytirocarpa moniliformis, Cenostigma microphyllum* and *Senegalia piauhiensis*.

Leaf, branches and root NSC analyses

The campaigns were carried out in March - April (rainy season) in years 2018 and 2019 years (See Figure S1 for precipitation). The year 2018 was considered a rainy year, with an average rainfall, in the 12 months before the collections, of 132mm for areas of high MAP and 40mm for areas of low MAP. While 2019 was considered a drier year, with average rainfall of 43mm in high MAP areas and 28mm in low MAP areas.

Three plots were determined for each precipitation extreme (high and low MAP areas), totaling 6 plots in the sampling effort. In each plot, whenever possible, tissue samples (leaf, branches and roots) of 3 individuals of each species were collected, totaling 9 individuals per species in each extreme area (wet and dry). The sampling effort was determined according to Pérez-Harguindeguy et al (2016), for the analysis of nutrients in plant tissues.

We quantified the NSC in **leaf** (fully expanded, marginal to the crown area), **branches** (near the stem with same thickness) and **root** (tertiary with same thickness). Collections were carried out between 8 am and 12 am. The samples were microwaved (3min for each 100g of sample) to stop all enzymatic activity according to Quentin et al (2015). Subsequently, they were freezed and stored at -20°C. The soluble sugars (SS) extraction was performed using 25mg for each macerated tissue, through an ethanolic suspension at 80% according Dubois et al (1956). To starch quantification, the insoluble fraction of carbohydrates from ethanol extraction was used. The starch was hydrolyzed using 10 units of amyloglucosidase (Code – Sigma-Aldrich) for 1 hour in the 95°C thermal bath. SS and starch concentrations (measured as glucose equivalent) were measured at 487nm using a double-beam spectrophotometer (Geneses 10S UV-Vis, Thermo Scientific, Waltham, USA), according to the methodology of Dubois et al (1956) The quantification of NSC in leaves, branches and root of each plant was obtained by adding SS and starch concentration. The value was applied to the relationship between the concentration of the compound in the sample (mmol) and the dry mass (g).

Leaf construction cost analyses

The leaves were collected in conjunction with the material collection campaigns for the NSC analyses. Five individuals of each species were collected for each site. The leaves were placed in a forced ventilation oven at 40ºC for 4 hours and stored in organizer boxes containing silica, to avoid fungus. In the laboratory, were the leaves were oven dried at 60 °C for 72 to dry biomass. The ash content (g kg-1) of 250g of dry matter obtained through the muffle at 500ºC for 6h were quantified (Li et al. 2011), the calorific value (ΔHC kj g−1), obtained through the combustion of 500mg of dry matter in calorimeter (in calorimeter (C200, IKA, Heiters-Heim, DE) according to Villar and Merino (2001) using the formula: ΔHC=calories/ (1-ashes) and nitrogen quantification, through digestion in sulfuric acid solution (H2SO4) in a digester block at 350 °C to obtain the extract samples (Thomas et al. 1967). The total N content was determined by extract titration using HCl after adding boric acid and a colorimetric indicator (Thomas et al. 1967).

The leaf construction cost per unit mass (g glucose DW-1) was calculated using data from ash, nitrogen concentration and ΔHC according to Williams et al (1987): $CCmass = [(0.06968\ \Delta HC - 0.065)\ (1- Ash) + 7.5\ (kN/14.0067)]/GE$, where k is the oxidation state of nitrogen (+5 to −3 for nitrate and ammonium), and GE represents the growth efficiency estimated to be 0.87 m (De Vries et al. 1974).

Data analyses

We used general linear mixed models (GLMM) to assess the effect of each predictor variable: organ (partitioning within the group, Table 2) and species groups (NSC accumulation per organ, in each group, Table 2) controlling for the effect of collection years and different MAP sites, on the total NSC concentration through lme4 package in R. We adopted the Gamma distribution for the continuous data of NSC and q=0 (Crawley 2014). We tested the residuals for homoscedasticity using the Shapiro-Wilk test and no overdispersion of the data was observed.

As the NSC data for each species group (WA, BA and DA) were not normally distributed, non-parametric equivalent tests were performed. To assess whether species groups altered the allocation pattern of SS and starch between the two areas, we conducted a Mann–Whitney test at 5% of significance. Statistical analyzes were performed using Statistica 8.0 software (StatSoft, Inc., Tulsa, OK, USA). Data were tested according to the assumptions for normality of the residues using the Shapiro test and for data homogeneity using the Levene test. The dataset that violated the premises were -log or square-root transformed

GLMMs were also used to assess the effect of the predictor variable (1) species group, on LCC variation at high and low MAP sites (Table 3), controlling for the year effect. We used the same analysis to verify the effect of sites (high and low MAP) on the LCC variation in each species group, also controlling for the year effect (Table 3). We also tested the residuals for homoscedasticity using the Shapiro-Wilk test and no overdispersion of the data was observed.

RESULTS

Characteristics of NSC partition and accumulation between organs

NSC partitioning showed different patterns between organs for the three species groups (Figure 3, Table 2, p<0.001). Despite the interspecific variation (Figure S2) within each group, in BA and DA greater partitioning were observed in leaves and roots (46% and 40% in leaf and 33% and 40% in roots, respectively) (Figure 3b,c), while leaves in WA seem to be the organ with the highest investments, two times greater than stems and roots. (Figure 3a). BA species altered the pattern of partitioning of NSC between the organs according to the precipitation of the year of collection. During 2019 (driest year, Figure S1), these species partitioned higher amounts of NSC for leaves and roots, while in 2018 (wettest year), greater partitioning was observed in leaves (Figure S3).

At the organ level, the accumulation of NSC varied between the groups in leaf and root (Table 2). In leaf, groups differed significantly in NSC accumulation (Figure 4a), while only DA accumulates significantly more in roots (Figure 4c; Table 2, p<0.001). The accumulation and partitioning of NSC in stem did not differ between groups, as it is the tissue with the least investment in photosynthesis levels (Figure 3b, 4b). Among the groups, DA seems to accumulate more NSC, with 1,6 times higher in leaf and 2,2 times higher in root, than BA and WA (Figure 4). The BA group, on the other hand, has lower and stable levels of NSC, even regardless of the year of collection (Figure S4).

Characteristics of SS and starch concentrations according to precipitation

In general, for all species sampled in the study, SS was the most abundant NSC component in the three organs, with concentrations, on average, 6 times higher than starch (Figure 5). At the organ level, we found that the content of SS is changed to WA in conductive tissues, according to the precipitation of the areas, while BA has higher leaf SS contents under high MAP (Figure 5a,b,c).

For starch, DA species show variation in the content of conductive tissues (stem and root, significance shown) in relation to precipitation while WA shows reductions in root starch content in low MAP areas (Figure 5d,e,f).

Leaf construction cost

The leaf construction cost showed little variation between the studied species (Figure 6, Table S1), only *Croton heliotropifolius* showed a significant difference compared to the other species, with a lower LCC (20% lower) (Figure S6, Table S1). In areas of high MAP species groups did not show differences in LCC, whereas in areas of low MAP BA has less expensive leaves (ca 8% lower) when compared to WA and BA in these same areas (Figure 6, Table 3). None of the groups, however, change the LCC according to precipitation, showing similar LCC values in high and low MAP areas (Figure 6, Table 3). The LCC also did not show a significant relationship with the accumulation of NSC in any organ (Figure S5).

DISCUSSION

Main findings

Our results suggest that in areas of Caatinga the NSC partition pattern between organs in varies according to the ability of the species to establish itself better in naturally drier environments, but is not altered by the dryness factor in the areas. BA and DA present a partition of carbon resources, such as the accumulation of NSC in organs above and below ground during the wet season (i.e. growing season), highlighting the importance of roots for STDF species, while WA partitions higher concentrations to leaf tissues, which partially supports our 1st hypothesis. In general, WA and DA adjust the use of C by altering the content of SS and starch between the organs, depending on the precipitation in the area, while, in conditions of lower water availability, BA adjusts the energy investment in the construction of the leaves during the rainy season but this does not favor greater accumulations of NSC.

Our study indicates that the NSC partition pattern, particularly for belowground tissues, may be linked to the ability of STDF deciduous tree species to establish themselves in drier locations. The importance of adjusting root NSC has been documented in other studies for species tolerant to reductions in water availability (Hagedorn et al. 2019), as well as in our recent studies using species native to this same region under controlled conditions (Santos et al. 2021; 2022). Although it is expected that more tolerant species present a greater ability to adjust the NSC partitioning (Piper et al. 2011; Hartmman et al. 2018, O'Brien et al. 2020), the species with a wider distribution (BA) presented smaller variations in the content of NSC and its components between the high and low MAP areas, however, modified the partitioning pattern according to the seasonality of the year in question. Recently, this same pattern was observed for species of Tropical Rainforest, which occur in areas with different levels of precipitation (Signore-Muller et al. 2020). At the geographic level, the NSC in our study presented a homeostasis between the areas, presenting little or no variation for the species, in general (Figure S3). These results are consistent for other Tropical Forests (Dickiman et al. 2018, Sigmore-Muller et al. 2020), but unprecedented for STDF. Although leaf traits can determine the use of C in plants (Edwards et al. 2014, Reich 2014), for the study in question the LCC was not altered in relation to precipitation, as observed in another study using a native tree species of STDF (Falcão et al. 2017), as well as it is not related to the dynamics of NSC in plants.

Although the precipitation of the areas does not seem to affect the NSC accumulation and partitioning pattern, for a better establishment of these species in driver areas it is necessary to tolerate greater fluctuations of water in the soil. It is known that NSCs are important regulators of plant water balance (Sapes et al. 2020, Sevanto et al. 2014) and that reserves stored in the previous rainy season can determine the performance of species in the next growing season (Hoch et al. 2003, Hagerdon et al. 2019). It is possible that BA and DA species present a more active root investment, such as SS, that favor water uptake through osmotic adjustment in this tissue (Wan et al. 2006) in rainy seasons, this would explain the higher values of root NSC in these species compared to WA, considering that the three groups have a similar degree of leaf photosynthetic activity (i.e. similar LCC values). The mechanisms that drive the homeostatic balance of NSCs, even under contrasting rainfall conditions, are still unknown but may include changes in the consumption and conversion of these photosynthates (i.e. starch to SS) (McDowell et al. 2011, Dickiman et al. 2018). In our study, for example, WA species accumulate more SS in conductive tissues in areas of low MAP, while accumulating more starch in roots under high MAP, suggesting osmotic adjustment in these tissues, linked to prevention of cavitation (Secchi and Zwieniecki 2011, Nardini et al. 2016). DA species, in turn, increase the starch content in stems under low MAP, suggesting a prioritization of storage over other processes and consistent with the idea that these plants, by adapting better to dry environments, are able to maintain high levels. of NSC under more extreme conditions (Wright et al. 2005).

Finally, the accumulation of NSC was not related to the LCC for any of the species' groups, as well as to the precipitation of the areas. It is possible that the taxonomic issue explains this lack of variation in the LCC of the species, considering that most of the species used in this study are from the same family, and only *Croton helhitopifolius* (Euphorbiaceae) had a lower LCC. Another explanation is that tree species, due to their greater local adaptation to more extreme conditions (Richter et al. 2012), show less plasticity to these leaf attributes. The latter is consistent with our data, when we observed differences in LCC for tree and shrub species (Figure S6). As well as the LCC, other life history characteristics of the species were not related to the accumulation of NSC (Figure S7). This lack of relationship suggests that, in all scenarios, NSCs play an important role and are maintained as an important pathway of C investment in these species (Chapin et al. 1990, Dietze et al. 2014, Martínez-Vilalta et al. 2014, Martínez-Vilalta et al. al. 2016, Hartmann et al. 2018). Although the BA species showed cheaper leaves in low MAP areas, differential accumulations of NSC were not observed. It is possible that the energy saved was allocated to other C pathways not addressed in this study, such as growth, production of exudates and associations with microorganisms which facilitate their establishment in these areas (Frosi et al. 2017, Pereira et al. 2021).

In an ecological context, driest areas in the study already have lower occurrences of WA species, our results add information for these species, but we know that the limitation of species occurrence can be given by a range of factors, including competition for resources and high rates of herbivory. For BA and DA species, a higher occurrence in naturally drier areas may be linked to two different strategies (1) for BA species, which show variations in NSC content more sensitive to seasonality (Figure S3) with greater accumulations in drier years; (2) for DA species, which present higher concentrations of NSC regardless of the scenario, favoring the accumulation of reserves in stem tissues under low MAP. Although distinct, the prioritization of NSC accumulation and its components under water deficit conditions suggests stress memory traces in plants (Schwachtje et al. 2019) that going through previous dry periods, these species adapted to maintain relatively high NSC levels under prolonged and severe drought periods (Tombesi et al. 2018, Tomasselha et al. 2019, Santos et al. 2022). In future climatic scenarios, with even longer dry periods, these plants will spend more time defoliated, so it is expected that species that have a greater capacity to accumulate NSC will have higher survival rates.

In summary, our results suggest that NSC partitioning and accumulation strategies in deciduous STDF species can determine their distribution in areas with different precipitation. On the other hand, more generally, the content of NSC proved to be homeostatic to the precipitation of the areas, suggesting that in the Caatinga, a STDF in Brazil, the dynamics of C, in terms of photosyntates, is not altered even under extreme conditions. Ultimately, this may be a deterministic factor for the composition of Tropical Dry Forests under continuous climate change, and as such, deserves investigation in the direction of seasonal responses to these species at broader organizational levels.

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LEGENDS

Figure 1. Location of sampled areas within PARNA-Catimbau limits and monthly rainfall. The map shows the predetermined plots of PELD-Catimbau in black dots, and the arranged gradient of distribution where the plots are. The collections were carried out in (1) high MAP: P17, P29 and P30 and (2) in low MAP plots: P08, P15 and P20.

Figure 2. Water balance (mm) over the years prior to the collections. In yellow the balance referring to areas of high MAP and red the balance referring to areas of low MAP for the year 2017 (prior to the 2018 collection) and 2018 (prior to the 2019 collection).

Figure 3. Groups-mean NSC partitioning between organs. Panel **a** represents the three tissues of the group of species with greater occurrence in areas of high MAP (WA), panel **b** represents the three tissues of the group of species with greater occurrence in both areas (BA) and panel **c** represents the three tissues of the group of species with greater occurrence in low MAP (DA) areas. Each box encompasses the 25th to 75th percentiles; the median is indicated by the horizontal line with each box while external horizontal lines indicate the 10th and 90th percentiles; dots indicate outliers. Number of species sampled in each groups are as follows: WSD=3, BSD=5 and DSD=3. Data obtained from general linear models (see table 2).

Figure 4. Groups-mean NSC accumulation between groups. Panel **a** represents leaf, panel **b** represents stem and panel **c** represents root. Blue boxes denote species with higher occurrence in high MAP areas (WA), purple boxes denote species with similar occurrence in both areas (BA) and red boxes denote species with higher occurrence in low MAP areas (DA). Each box encompasses the 25th to 75th percentiles; the median is indicated by the horizontal line with each box while external horizontal lines indicate the 10th and 90th percentiles; dots indicate outliers. Number of species sampled in each groups are as follows: WSD=3, BSD=5 and DSD=3. Data obtained from general linear models (see table 2).

Figure 5. Precipitation sites variation of soluble sugars (SS) and starch. Panels **a** and **d** represents leaves, **b** and **e** represents branches and **c** and **f** represent roots. Blue boxes denote the high MAP sites and red boxes denote low MAP sites. Each box encompasses the 25th to 75th percentiles; the median is indicated by the horizontal line with each box while external horizontal lines indicate the 10th and 90th percentiles; dots indicate outliers. Number of species sampled in each groups are follows as: +WA=3, BA=4 and +DA=3. To test for differences between site we used paired sample Wilcoxon tests, p values are shown.

Figure 6. Leaf construction cost for each cross-species group. Lines connect the LCC values for each group between high and low MAP areas. Significance value represented by asterisks, where **p* < .05; ***p* < .001; ****p* < .0001. Data obtained by glmm (see table 3).

Supplementary Figure 1. Historical monthly rainfall in the areas sampled in the study (y axis 1) and in the 12 months preceding the collection campaigns (y axis 2) in Catimbau National Park, Brazil (source: APAC).

Supplementary Figure 2. Mean non-structural carbohydrates (NSC), in leaf, stem and root in high and low MAP areas for all species sampled in the study. Each panel represents a tissue, as follows: a) leaf, b) stem, c) root. The colors represent the high MAP (red) and low MAP (blue) areas. Horizontal black bars denote one standard error of the mean.

Supplementary Figure 3. Groups-mean total NSC. Panel **a** and **c** represent high MAP sites and panel **b** and **d** represent low MAP sites. Green boxes denote the leaves, red boxes denote the braches and yellow boxes denote the roots. Each box encompasses the 25th to 75th percentiles; the median is indicated by the horizontal line with each box while external horizontal lines indicate the 10th and 90th percentiles; dots indicate outliers. Number of species sampled in each groups are follows as: WSD=3, BSD=5 and DSD=3. **Differences among organs for each groups (low cases letters)** were tested using Kruskal–Wallis. Sites with different letters are statistically distinguishable ($p < 0.05$, post hoc Mann–Whitney–Wilcoxon Rank Sum test. Only significants differences are shown.

Supplementary Figure 4. Groups-mean NSC accumulation between groups in the years 2018 and 2019. Panel **a** and **d** represent leaf, panel **b** and **e** represent stem and panel **c** and **f** represent root. Blue boxes denote species with higher occurrence in high MAP areas (WA), purple boxes denote species with similar occurrence in both areas (BA) and red boxes denote species with higher occurrence in low MAP areas (DA). Each box encompasses the 25th to 75th percentiles; the median is indicated by the horizontal line with each box while external horizontal lines indicate the 10th and 90th percentiles; dots indicate outliers. Number of species sampled in each groups are as follows: WSD=3, BSD=5 and DSD=3. Differences among groups for each organ and years (low case letters) were tested using Kruskal–Wallis. Groups with different letters are statistically distinguishable ($p < 0.05$, post hoc Mann–Whitney–Wilcoxon Rank Sum test. Only significant differences are shown.

Supplementary Figure 5. Relationship between non-structural carbohydrate content and LCC for leaf (**panel a**), stem (**panel b**) and root (**panel c**). As the NSC value does not vary between areas, the data used to construct this figure did correspond to the species NSC data in both areas (high and low MAP), as well as the LCC data. Values were log-transformed. The relationship between the NSC and LCC was fitted using a linear regression, with values of R^2 and p-value displayed in the figure.

Supplementary Figure 6. Comparison of leaf construction cost (LCC) between tree (*n=7*) and shrub (*n=3*) species in each area (high and low MAP). The comparison between the size of the species was calculated using the Kruskal-Wallis test. Each box encompasses the 25th to 75th percentiles; the median is indicated by the horizontal line with each box while external horizontal lines indicate the 10th and 90th percentiles; dots indicate outliers.

Supplementary Figure 7. Relationship between non-structural carbohydrate content in leaf, stem and root and life history traits of the species. This figure shows the relationship of NSC with: Specific Leaf Area (SLA, mean for each species; data from Zorger et al. 2019) and Wood density (DM, mean for each species, data from Zorger et al. 2019). The data used to build this figure are from the low MAP areas. The averages of NSC for each species in the two years of collection were used. The relationship between NSC and traits was fitted using linear regression, R^2 and *p values* are shown in the figure. To construct this figure and to test the relationship we log-transformed all NSC and traits values.

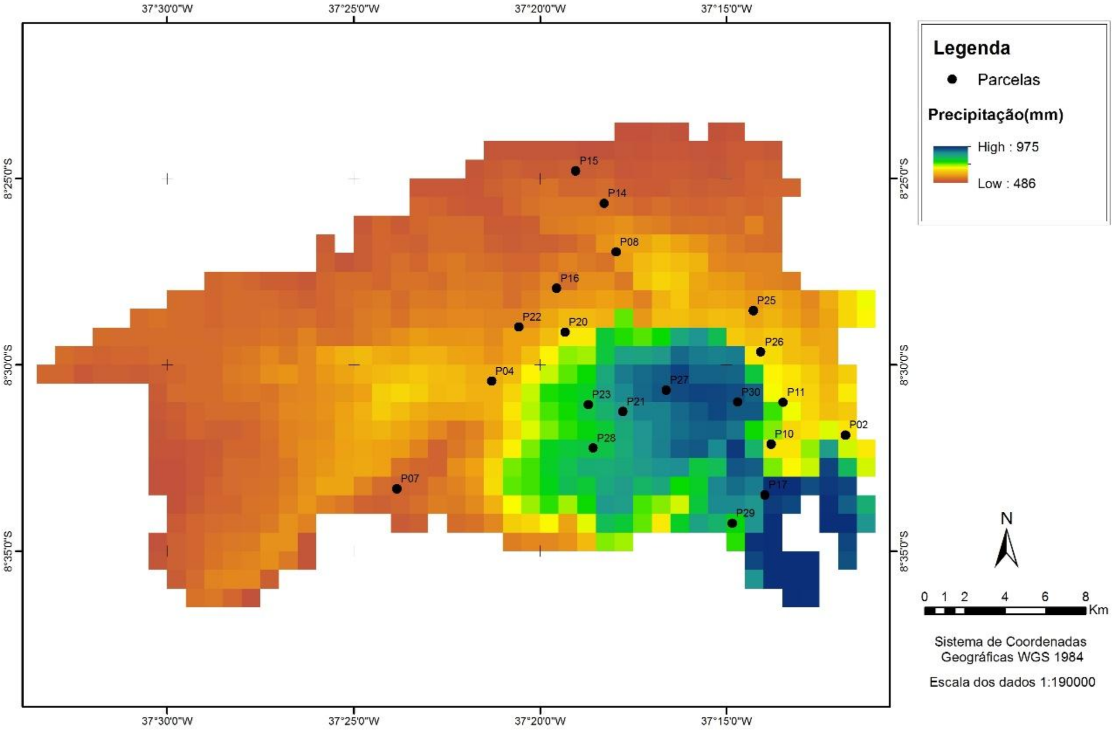
37°30'0"W
37°25'0"W
37°20'0"W
37°15'0"W
8°25'0"S
8°30'0"S
8°35'0"S
P15
P14
P08
P16
P25
P22
P20
P26
P04
P27
P23
P21
P30
P11
P02
P28
P10
P07
P17
P29
Legenda
Parcelas
Precipitação(mm)
High : 975
Low : 486
N
0 1 2 4 6 8
Km
Sistema de Coordenadas Geográficas WGS 1984
Escala dos dados 1:190000

Figure 1

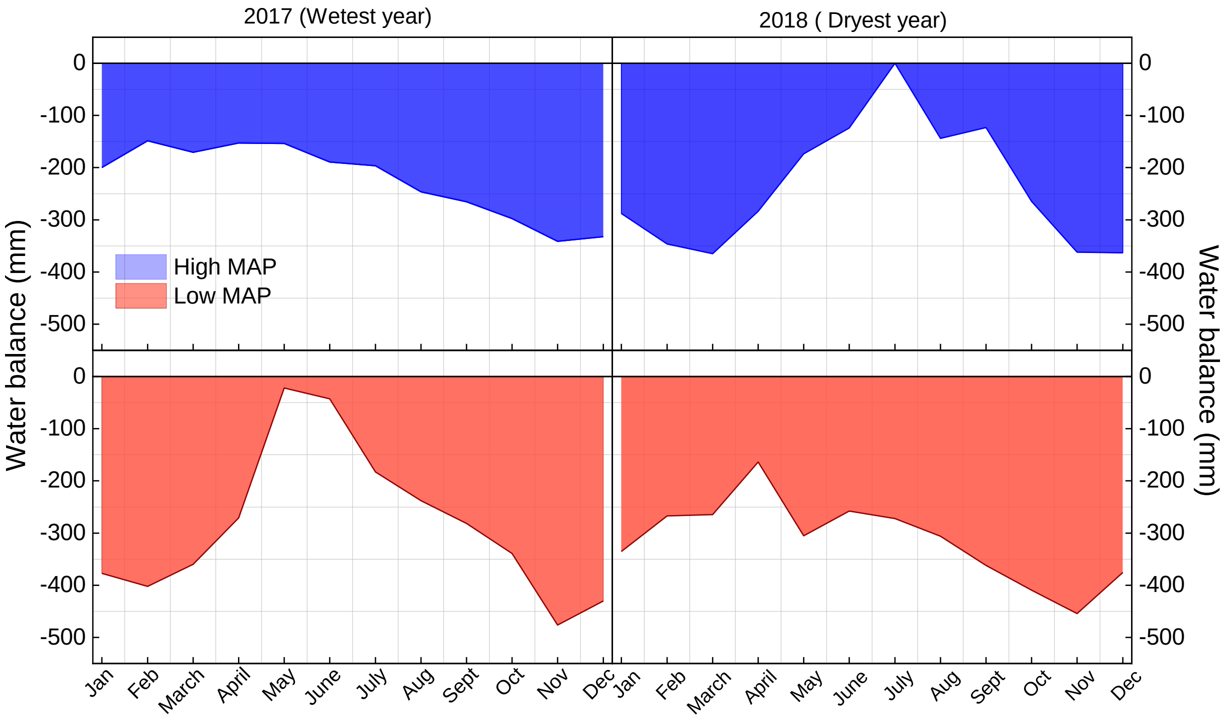
2017 (Wetest year)
2018 (Dryest year)
Water balance (mm)
Water balance (mm)
0
-100
-200
-300
-400
-500
High MAP
Low MAP
Jan
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec

Figure 2

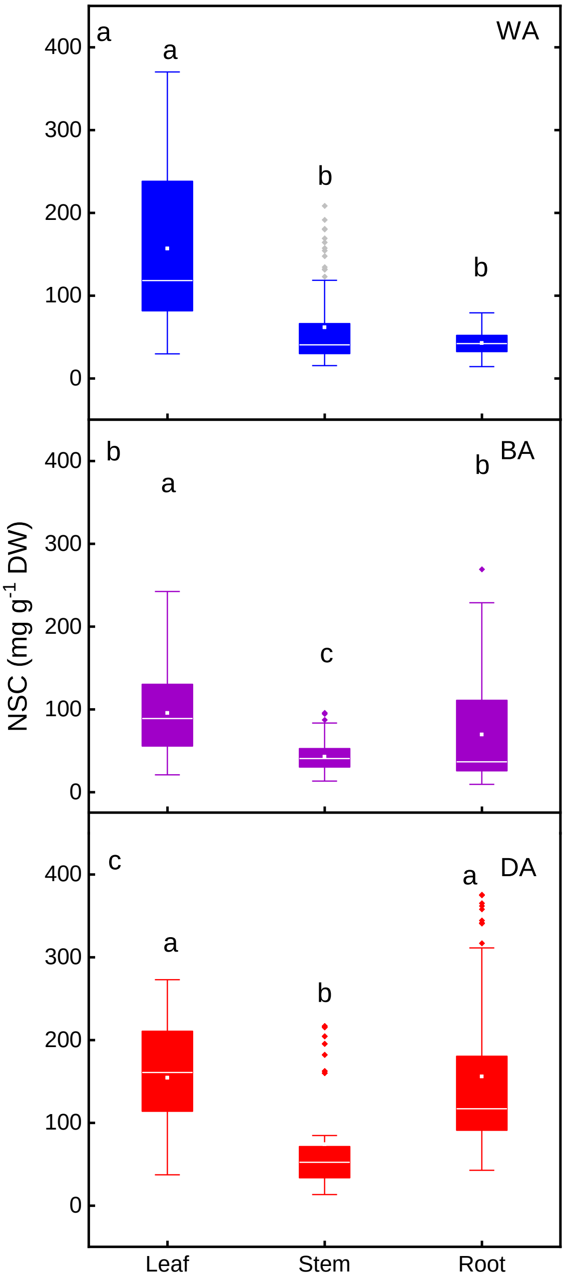
a
WA
a
b
b
b
BA
a
b
c
c
DA
a
a
b
NSC (mg g^{-1} DW)
400
300
200
100
0
400
300
200
100
0
400
300
200
100
0
Leaf
Stem
Root

Figure 3

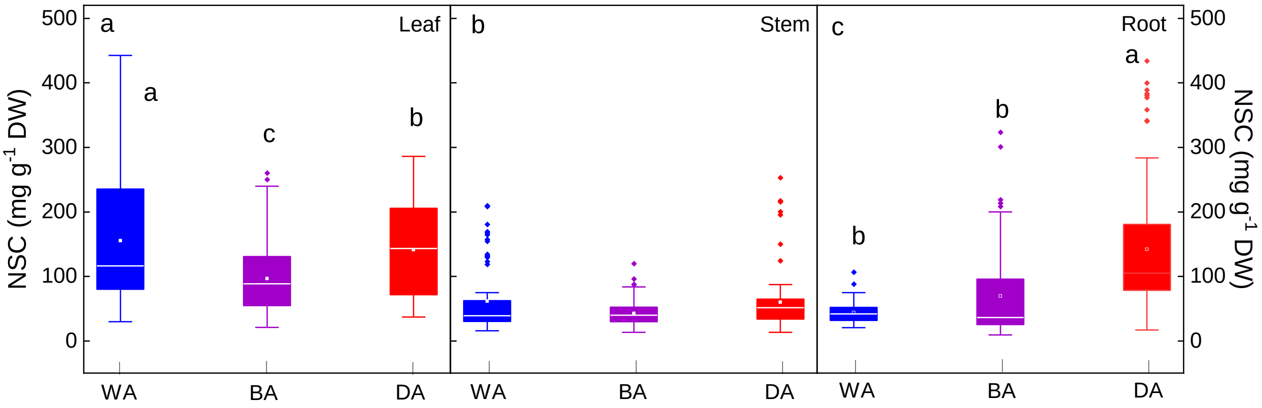
a
Leaf
b
Stem
c
Root
NSC (mg g^{-1} DW)
500
400
300
200
100
0
a
c
b
a
b
b
WA
BA
DA
WA
BA
DA
WA
BA
DA

Figure 4

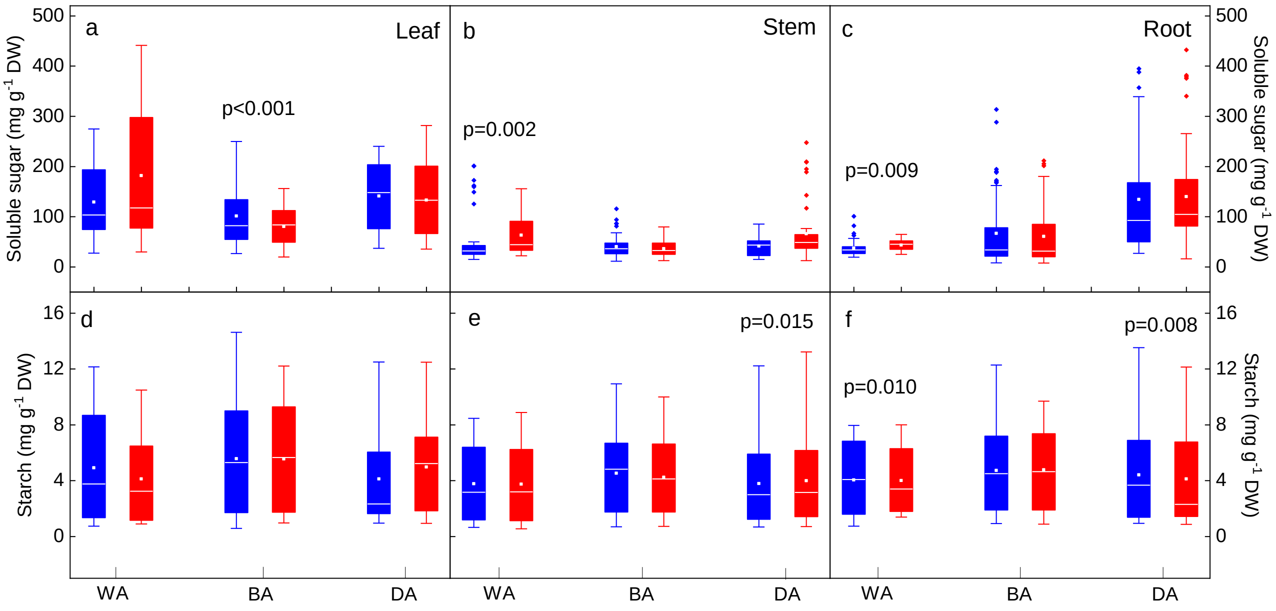
a
Leaf
p<0.001
b
Stem
p=0.002
c
Root
p=0.009
d
e
p=0.015
f
p=0.008
p=0.010
Soluble sugar (mg g^{-1} DW)
Starch (mg g^{-1} DW)
0
100
200
300
400
500
0
4
8
12
16
WA
BA
DA

Figure 5

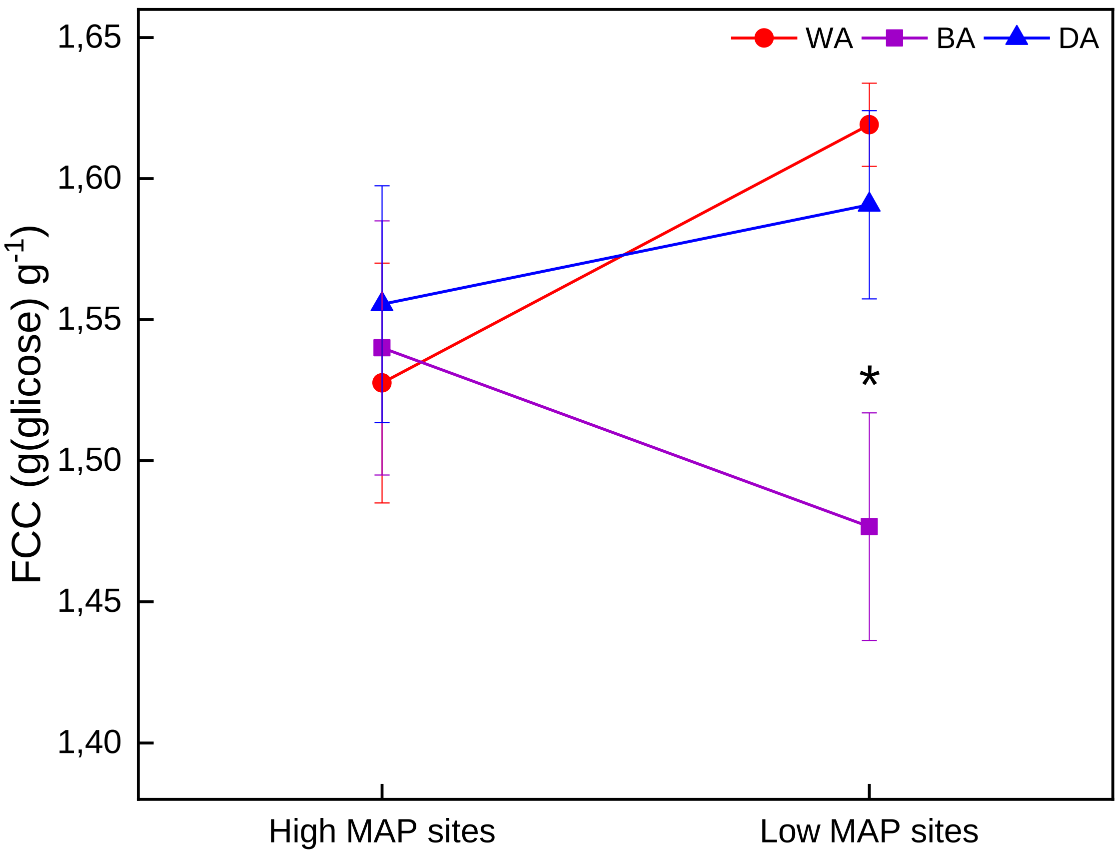
1,65
1,60
1,55
1,50
1,45
1,40
FCC (g(glicose) g^{-1})
WA
BA
DA
*
High MAP sites
Low MAP sites

Figure 6

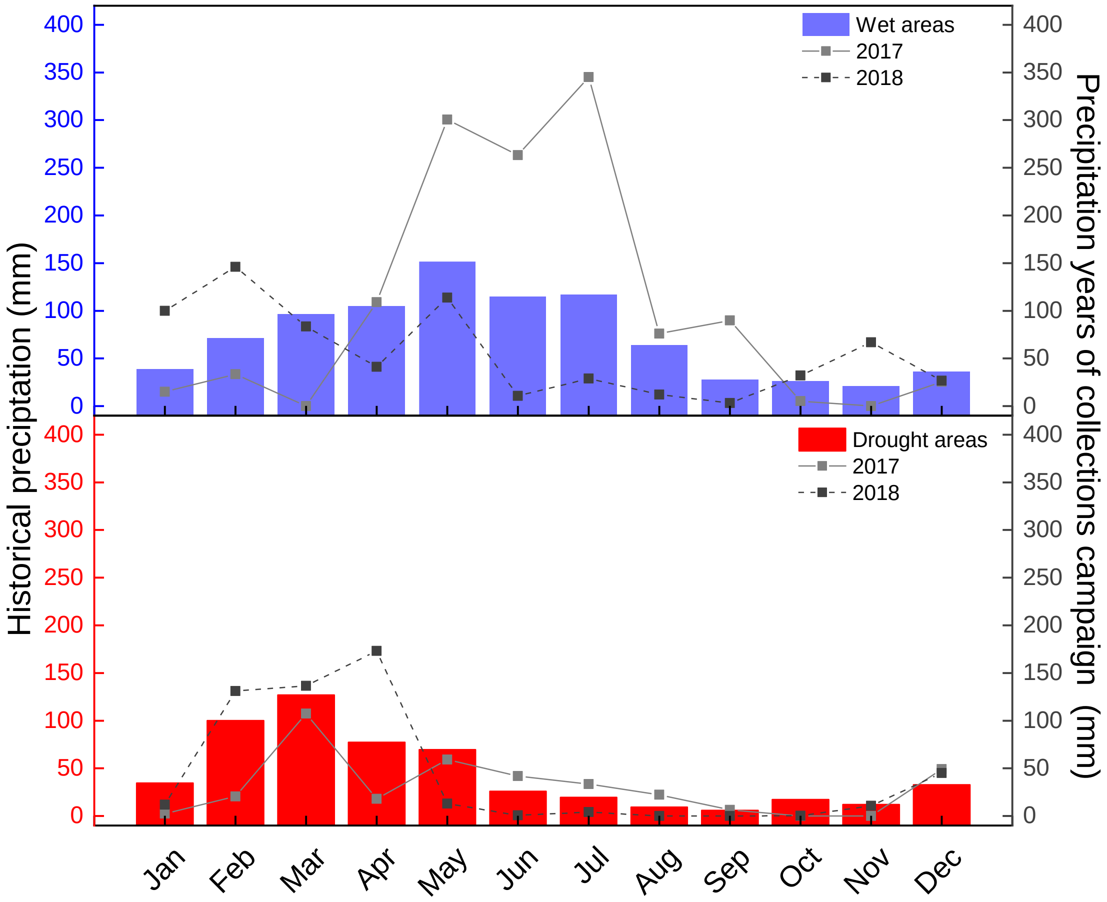
Historical precipitation (mm)
Precipitation years of collections campaign (mm)
Wet areas
2017
2018
Drought areas
2017
2018
0
50
100
150
200
250
300
350
400
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

Figure S1

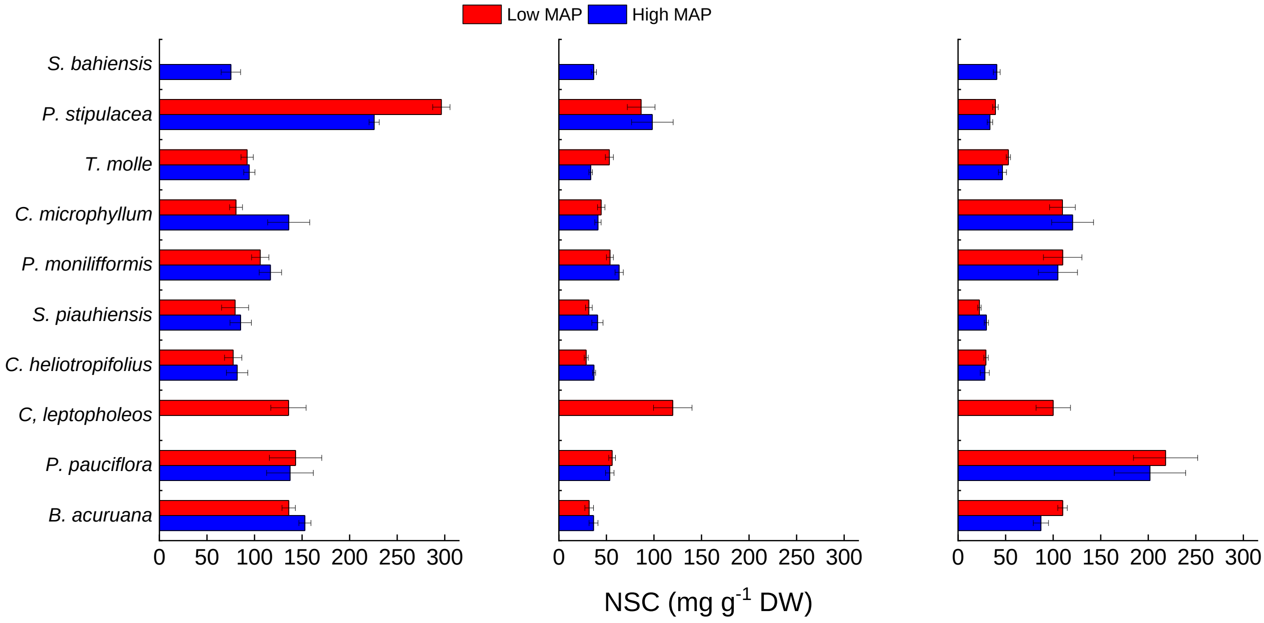
Low MAP
High MAP
S. bahiensis
P. stipulacea
T. molle
C. microphyllum
P. monilifformis
S. piauhiensis
C. heliotropifolius
C, leptopholeos
P. pauciflora
B. acuruana
0
50
100
150
200
250
300
NSC (mg g-1 DW)

Figure S2

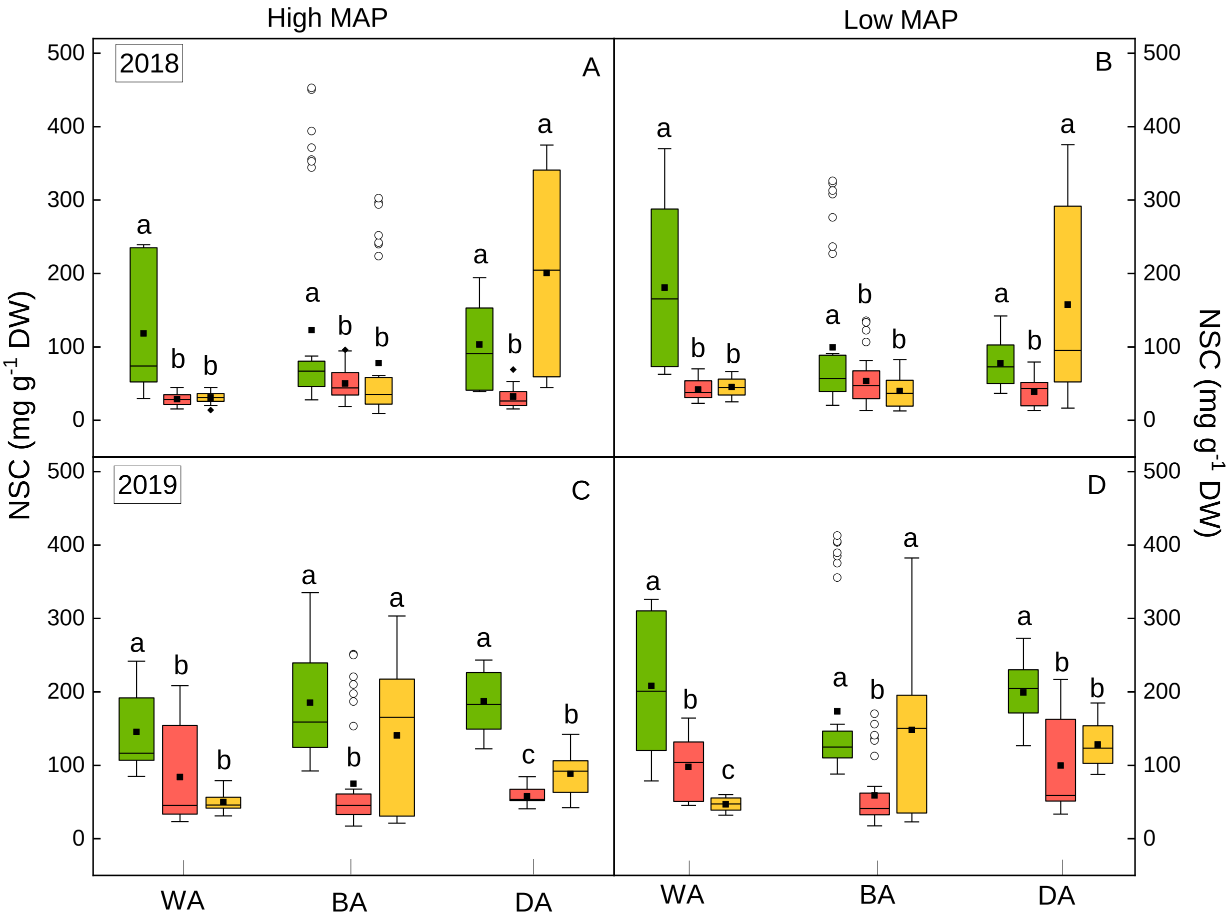
High MAP
Low MAP
2018
2019
A
B
C
D
NSC (mg g^{-1} DW)
500
400
300
200
100
0
a
b
WA
BA
DA

Figure S3

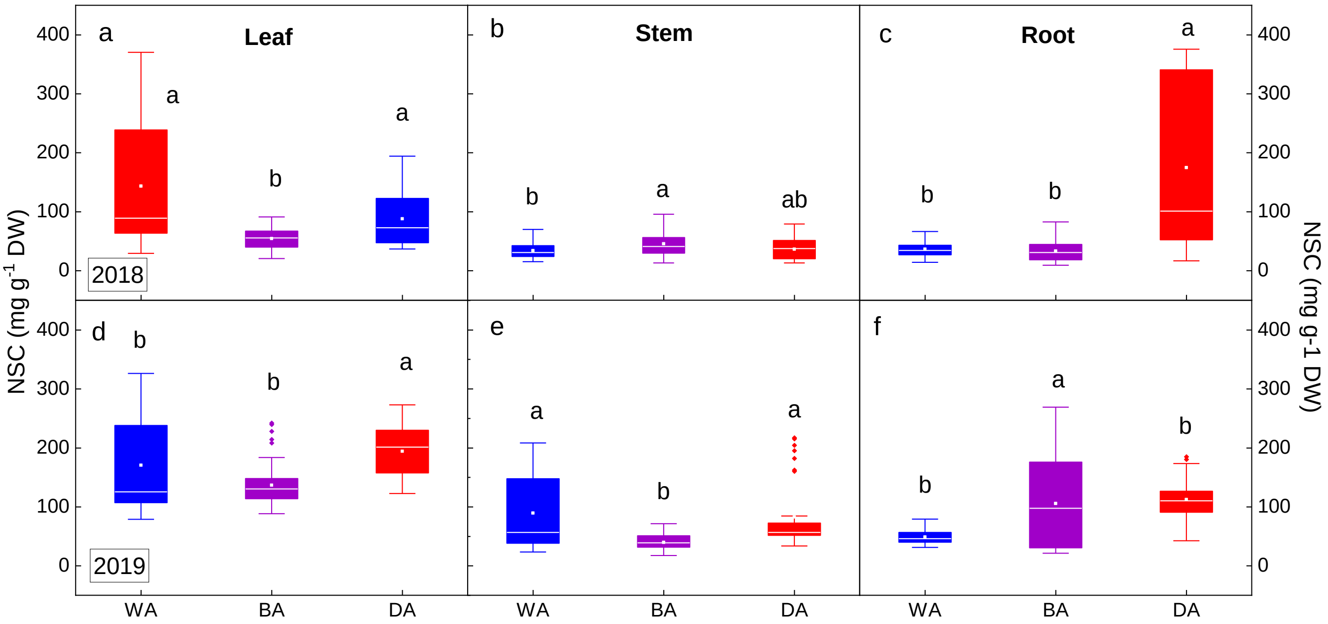
a
Leaf
b
Stem
c
Root
d
e
f
2018
2019
NSC (mg g^{-1} DW)
NSC (mg g-1 DW)
0
100
200
300
400
WA
BA
DA
a
b
a
b
a
ab
b
b
a
b
b
a
a
b
a
b
a
b

Figure S4

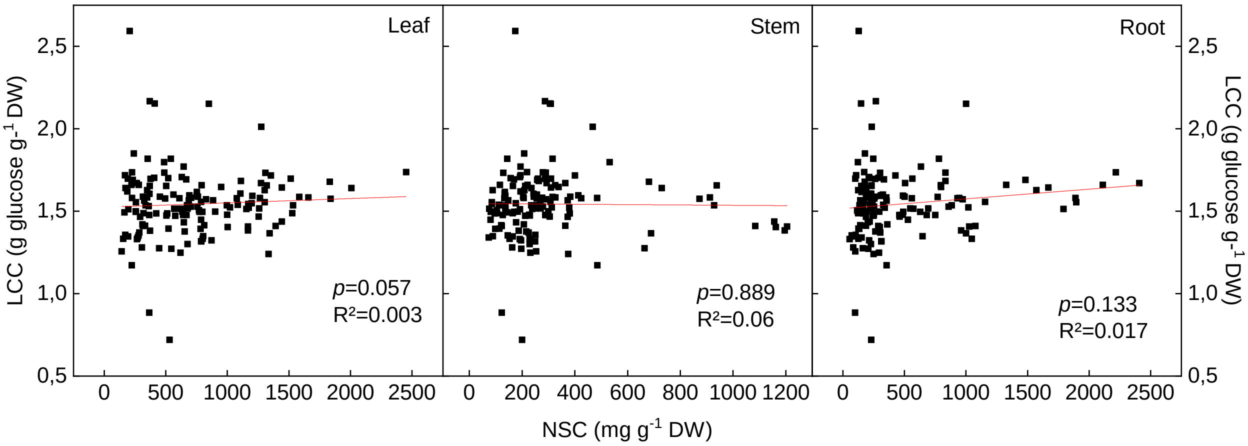
Leaf
Stem
Root
LCC (g glucose g-1 DW)
2,5
2,0
1,5
1,0
0,5
p=0.057
R²=0.003
p=0.889
R²=0.06
p=0.133
R²=0.017
0
500
1000
1500
2000
2500
0
200
400
600
800
1000
1200
0
500
1000
1500
2000
2500
NSC (mg g-1 DW)

Figure S5

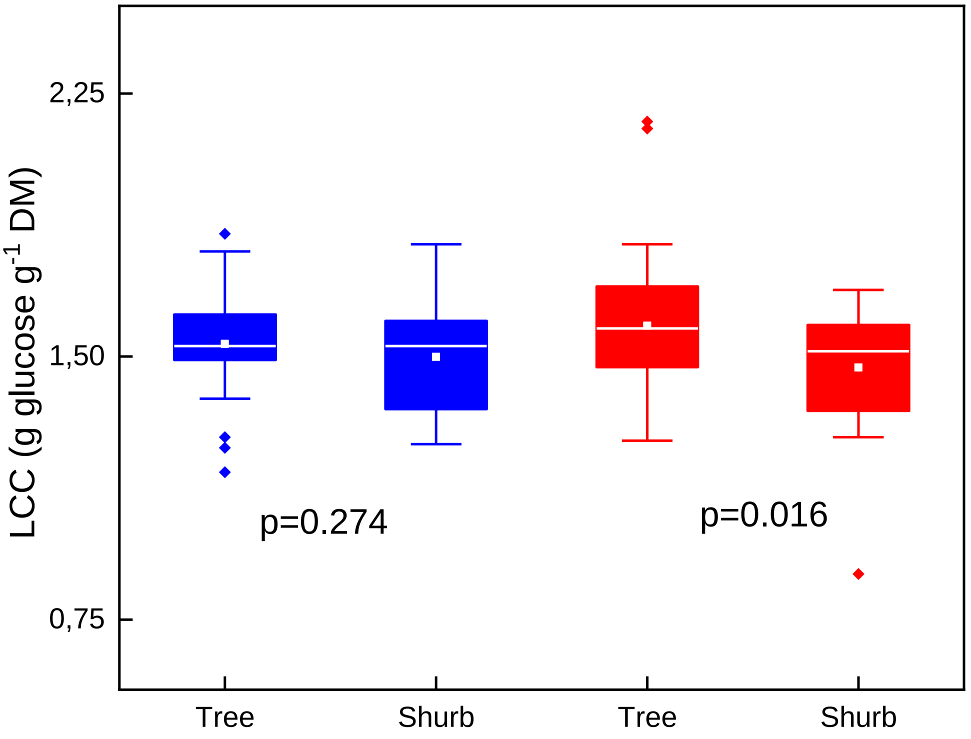
LCC (g glucose g^{-1} DM)
2,25
1,50
0,75
p=0.274
p=0.016
Tree
Shurb
Tree
Shurb

Figure S6

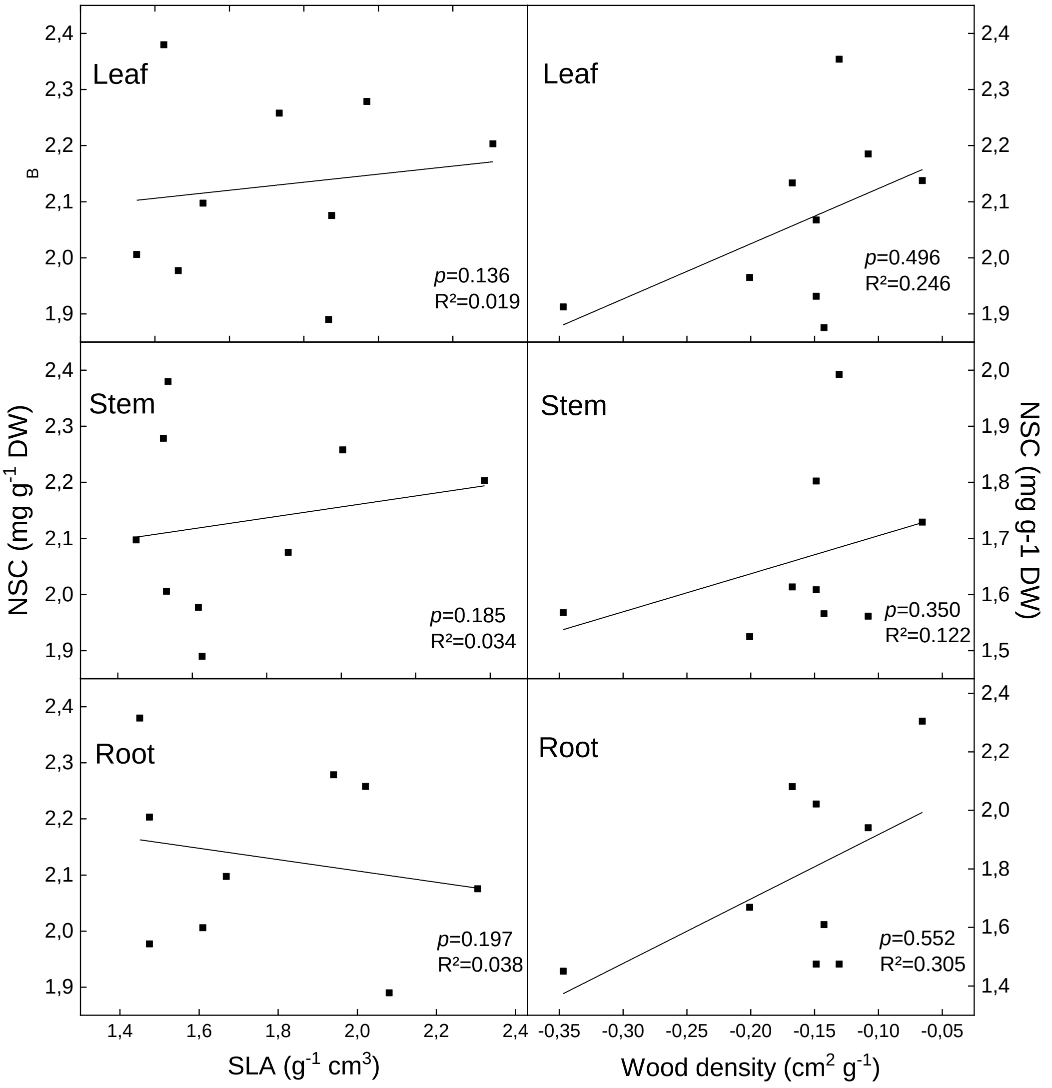
Leaf
p=0.136
R²=0.019
Leaf
p=0.496
R²=0.246
Stem
p=0.185
R²=0.034
Stem
p=0.350
R²=0.122
Root
p=0.197
R²=0.038
Root
p=0.552
R²=0.305
B
2,4
2,3
2,2
2,1
2,0
1,9
2,0
1,9
1,8
1,7
1,6
1,5
2,4
2,2
2,0
1,8
1,6
1,4
NSC (mg g-1 DW)
1,4
1,6
1,8
2,0
2,2
2,4
-0,35
-0,30
-0,25
-0,20
-0,15
-0,10
-0,05
NSC (mg g^{-1} DW)
SLA (g^{-1} cm^{3})
Wood density (cm^{2} g^{-1})

Figure S7

Table 1. Species, family, growth form and sites distribution of trees species sampled. Wood density (WD) and Specific Leaf Area (SLA) were taken from Zorger et al. 2019

Species and author	**Family**	**Growth form**	**Sites distribution**	**WD (cm2 g-1)**	**SLA (g^{-1} cm^3)**
Bauhinia acuruana	Fabaceae	Shurb	DA	0,78±0,07	189.93±0,07
Croton heliotropifolius Kunth	Euphorbiaceae	Shurb	BA	0.45±0.23	239.67±91.61
Jatropha mutabilis (Pohl) Baill	Euphorbiaceae	Shurb	BA	0.31±0.12	176.29±38.08
Trischidium molle (Benth.) H. E. Ireland	Fabaceae	Shurb	DA	0.63±0.10	125.13±44.43
Peltogyne paucilflora (Benth) Mart. Fl.Bras.	Fabaceae	Tree	DA	0.86±0.09	118.92±33.50
Piptadenia stipulacea (Benth.) Ducke	Fabaceae	Tree	WA	0.74±0.09	159.71±64.57
Pytirocarpa moniliformis (Benth) Luckon & R.W. Jobson	Fabaceae	Tree	BA	0.71±0.09	181.09±74.37
Commiphora leptopholeos (Mart.) J.B. Gillet	Burseraceae	Tree	DA	0.44±0.15	179.39±49.78
Cenostigma microphyllum (Mart. ex G. Don) E. Gagnon & G.P. Lewis	Fabaceae	Tree	BA	0.68±0.21	77.60±22.27
Senegalia piauhiensis (Benth.) A.Bocage & L.P.Queiroz	Fabaceae	Tree	BA	0.71±0.15	94.86±29.15
Senegalia bahiensis (Benth.) Seigler & Ebinger	Fabaceae	Tree	WA	0.72±0.13	101.31±46.88

Tabela 2. Results of general linear mixed models (GLM) examining combined group and agency influences on NSC variation; as well as the variation of NSC according to the group for each organ (leaf, stem and root). Coefficients of determination (r^2) and chi-square (χ^2) values are given.

Variable/organ	*p*	χ^2	r^2
NSC			
Organ x group	**2.756e-14**	0.17	72.854
Folha			
NSC x group	**8.828e-10**	-0.55	129.83
Caule			
NSC x group	**0.002**	-0.2	7.68
Raiz			
NSC x group	**2.2e-16**	0.22	88.63

Tabela 3. Results of general linear mixed models (GLM) examining combined influences of the group and areas in the variation of CC; as well as the variation of LCC according to the area (high and low MAP) for each group separately (WA, BA and DA). Coefficients of determination (r^2) and chi-square (χ^2).

Variable/organ	*p*	χ^2	r^2
LCC			
group x high MAP	0.8746	0.003	0.268
group x low MAP	**0.0081**	0.131	10.33
WA			
LCC x preciptation areas	0.282	0.018	1.177
BA			
LCC x preciptation areas	0.122	-2.826	2.503
DA			
LCC x preciptation areas	0.486	0.012	0.495

Table Supplementary 1. Comparison of leaf construction cost (LCC) between areas (high and low MAP) for all species in the study. To test for differences between site we used paired sample Wilcoxon tests, p values and means with respective standard error (SE) are shown.

Species	*Leaf construction cost*	*p-value*
Senegalia bahiensis	1.53±0.02	-
Piptadenia stipulacea	1.59±0.03	0.6744
Trischidium molle	1.58±0.08	**0.0117**
Cenostigma microphyllum	1.55±0.04	1.000
Pytirocarpa moniliformis	1.60±0.08	0.6744
Senegalia piauhiensis	1.56±0.11	0.3269
Croton heliotropifolius	1.30±0.04	**0.0118**
Commiphora leptopholeos	1.58±0.04	-
Peltogyne paucilflora	1.63±0.05	**0.0117**
Bauhinia acuruana	1.52±0.02	0.9441

5. CONSINDERAÇÕES FINAIS

O presente estudo buscou entender a relação entre a dinâmica de C em espécies arbóreas, nativas da Caatinga, e a as respostas ao déficit hídrico. De forma geral essas espécies apresentaram alta capacidade de alterar a dinâmica e particionamento de carboidratos não-estruturais (CNE) sob condições de seca. Em condições controladas, sob ciclos recorrentes de déficit hídrico, a *Cenostigma microphyllum* apresentou ajustes em tecidos de caule e raiz, com maiores acúmulos de AST nesses tecidos, o que favoreceu a aclimatação do status hídrico nessas plantas e menores flutuações na assimilação liquida de CO_2. Em condições de campo, espécies com diferentes graus de tolerância à seca, apresentam um particionamento diferente de CNE entre os órgãos. Espécies que se estabelecem melhor em áreas úmidas particionam maiores concentrações de CNE nas folhas, enquanto para as espécies que se estabelecem melhor nas áreas e secas e em ambas as áreas, particionam CNE para folhas e raízes. Entretanto, a precipitação das áreas não afeta o padrão de particionamento de CNE para nenhum grupo de espécies. Espécies que se estabelecem em locais naturalmente mais secos acumulam mais NSC que os outros grupos, enquanto espécies mais generalistas acumulam menos CNE, mas alteram o padrão de particionamento a depender do ano, acumulando mais em anos mais secos.

Considerando as previsões climáticas futuras para a região semiárida da América do Sul ainda durante este século, com estações chuvosas mais curtas e irregulares, e consequentemente períodos secos mais longos, a capacidade de acumular ativamente mais reservas, como os CNE pode favorecer espécies arbóreas decíduas, que passarão mais tempos desfolhadas. Estudos de longo prazo e em níveis organizacionais maiores são necessários para compreender melhor as estratégias dessas espécies, na tentativa de prever possíveis padrões que determinarão a composição das comunidades vegetais em áreas de Florestas Secas.

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ANEXO A – Regras para submissão na revista Physiologia Plantarum

AUTHOR GUIDELINES

MANUSCRIPT SUBMISSION

All submissions need to be sent via Wiley's Research Exchange submission portal: https://wiley.atyponrex.com/journal/PPL.

During submission, you will be asked to provide information concerning your manuscript and its authors as well as potential conflicts of interest, choice of subject area (up to 2), Editors (up to 3), and names and e-mail addresses of 4 potential reviewers. Your choice of Editor and reviewers is indicative but may speed up the editorial process.

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Manuscripts accepted pending revision must be revised according to the *Physiologia Plantarum* format. Submit the text in DOCX or ODT format (do not submit the main text as PDF). Image files should be uploaded individually in JPEG, TIFF, PDF, or PNG formats.

Article Highlights

A separate Word document should be prepared highlighting the key findings, the novelty of the data, and how the results advance the field. If the species is not commonly used, the scientific rationale for its selection and insights gained must be explained. This summary may be used for social media promotion.

Cover Letter

A cover letter must accompany the manuscript. It should include background information relevant to the article's merit, previous correspondence with editors, and any changes in authorship between versions.

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Manuscripts may be written in British or American English, but usage must be consistent. Authors with language concerns are encouraged to use professional editing services, such as Wiley's.

Formats and organization

To qualify for Online Accepted publication, formatting guidelines must be strictly followed. While there is no page limit, concise manuscripts of around 6000 words (40,000 characters with spaces) and 3–7 data displays are recommended.

Layout

The manuscript must follow this structure:

- Title
- Full name(s) of author(s) (indicate the corresponding author)
- Address(es) (include email of corresponding author)
- Funding
- Abstract
- Introduction
- Materials and Methods
- Results and Discussion (combined or separate)
- Author Contributions
- Acknowledgements
- Data Availability Statement
- References
- Supporting Information
- Figure Legends (included in main text)
- Figures and Tables (submitted separately)

Title

Titles must be concise and engaging. Avoid long or overly descriptive titles.

Abstract

The abstract should be self-contained (max. 250 words, no paragraphs). It should:

- State the problem
- Indicate objectives
- Summarize methods
- Present main results
- Provide a conclusive statement

No unexplained abbreviations or references should be used. Spell out scientific names and cultivars. EC numbers must be provided for enzymes.

Provide a concise background, state-of-the-art context, rationale, and aims of the study.

This section must be detailed, including:

- Number of independent experiments and replicates
- Statistical methods
- Growth conditions (include trade names/manufacturers and irradiance in W m^{-2} or μmol m^{-2} s^{-1})
- Apparatus/chemical suppliers and trade names
- EC numbers for enzymes

For microarray/Affymetrix data, follow MIAME guidelines. Clarify what constitutes a replication. Repeat statistical and replication details in figure/table legends.

Results

Present results objectively in past tense. Tables and figures should be interpretable independently, with concise but informative legends.

Discussion

Interpret the results and demonstrate their contribution to scientific knowledge. Avoid listing results again. Write concisely and logically.

Author Contributions

Briefly describe contributions of each author. Follow COPE guidelines for ethical authorship.

Data availability statement

All manuscripts must include a data availability statement.

Examples:

Public repository
"The data supporting this study are openly available in [repository] at http://doi.org/[doi], reference number [number]."

Physical data not deposited
"The data supporting the findings are available from the corresponding author upon reasonable request."

Data in manuscript/supporting info
"All new data are contained within this article."
or
"Data are available in the supplementary material."

Derived from public data
"Data were obtained from public domain resources: [list URLs]."

No new data (reviews, opinions)

“No new data were created or analyzed in this study.”

Citations and references

Use Harvard style:

- One author: Gabriel (2000) or (Gabriel, 2000)
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Holding, M.Y., Saulino, M.F., Overton, E.A., Kornbluth, I.D. & Freedman, M.K. (2008) Interventions in chronic pain management. Archives of Physical Medicine and Rehabilitation, 89 (3): 38–40.

For early online articles, include DOI instead of volume/pages.

For preprints (e.g., bioRxiv):

Silas, P., Yates, J.R. & Haynes, P.D. (2008) Density-functional investigation... Arxiv. [Preprint] Available from: Available from: http://arxiv.org/abs/0810.1692. [Accessed: 23rd July 2010].

Supporting information

Additional data (datasets, tables, figures) may be submitted. These will be published online as supplied, without editorial formatting.

If possible, combine figures, tables, and legends into a single PDF. Movies and spreadsheets can be submitted separately.

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Submit each table on a separate page with headings. Fit within 80 or 166 mm width. Submit as editable text (DOCX or ODT). Avoid footnotes.

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Submit each figure as a separate file, named by number (e.g., Figure 1).

- Photographs: TIFF format, ≥300 dpi
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- Use LZW compression for TIFF
- Use greyscale for B&W, RGB for color

ANEXOS B – Regras para submissão na revista Tree Physiology

Sections

1. How we publish
2. Scope of the Journal
3. Topics Covered by Tree Physiology
4. Availability of data and materials
5. Publishing agreements and charges
6. Preparing your manuscript
7. Style Points
8. Submission Procedure

1) How we publish

Tree Physiology is a peer-reviewed journal publishing 12 issues per year online.

Once a paper is accepted and the publishing agreement is signed, the Journal will publish the Accepted Manuscript version of the paper (before copyediting and review of the final proof) within one week on the Advance articles page. The Accepted Manuscript will be removed from Advance articles when the Version of Record of the paper (after copyediting and proof review process) is published in the currently open issue. Substantial changes to the published Accepted Manuscript may require a correction notice.

2) Scope of the Journal

Tree Physiology publishes studies of tree functional processes, from the molecular to ecosystem scale. *Tree Physiology* welcomes submissions on wild and cultivated tree species as well as other woody and arborescent species (e.g. shrubs, vines, lianas, tree ferns, palms, bamboo). *Tree Physiology* promotes research in a framework of hierarchically organized systems, measuring insight by the ability to link different scales of biological organization. Investigations of tree physiological phenomenon should seek mechanistic explanation in finer-scale phenomena as well as seek significance in larger-scale phenomena (Passioura 1979). A phenomenon not linked downscale is merely descriptive; an observation not linked upscale lacks context of the plant in its surroundings.

With this emphasis on mechanistic understanding of functional processes, original research manuscripts submitted to *Tree Physiology* must have a focus on laboratory and/or field experimentation. Manuscripts that include in silico or modeling analyses must also contain laboratory or field experimentation to validate or extend the findings from computational analyses. Ecophysiological surveys (where randomization is impossible) should pay attention to the potential influence of confounding variables and include a clear hypothesis-formulation section. Submissions on newly-developed methods that improve our ability to quantify or model parameters important to plant physiological processes are also invited.

3) Topics Covered by *Tree Physiology*

Articles must provide new mechanistic insight into plant functional processes. A full range of physiological phenomena are covered by *Tree Physiology*. Areas of particular focus across the fields of Physiology, Ecophysiology, Molecular Biology and Genomics include:

- Resource acquisition and utilization—water relations, nutrient uptake, photosynthesis, respiration, primary metabolism, specialized (secondary) metabolism, allocation, partitioning
- Growth and Development—reproduction, morphogenesis, phenology, structure-function relationships
- Tree-Environment Interactions—acclimation and adaptation, biotic interactions, responses to abiotic cues

4) Availability of data and materials

Where ethically feasible, the Journal strongly encourages authors to make all data and software code on which the conclusions of the paper rely available to readers. Authors are required to include a data availability statement in their paper. When data and software underlying the research article are available in an online source, authors should include a full citation in their reference list. For details of the minimum information to be included in data and software citations see the OUP guidance on citing research data and software.

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Sections

Research papers, methods papers and letters comprise the following sections, in this order:

- Title Page
- Abstract
- Introduction
- Materials and Methods
- Results
- Discussion
- Acknowledgements
- Authors' Contributions
- Supplementary Data
- Funding
- Conflict of Interest
- Data Availability Statement
- References
- List of Figures
- Tables
- Figures

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The title page of the manuscript must include: title of paper, contributor names (first name, family name), key words, a running head of up to 50 characters and spaces, and the full address (including email) of the author designated to receive correspondence. The authorship of the paper should be limited to those who have made a significant, intellectual contribution to the design and execution of the work described. If two or more co-authors equally contributed to the paper, please add a statement to indicate this.

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The description of experimental procedures should be sufficient to ensure that experiments are reproducible. This includes details of any software or statistical procedures used in the study, including the version that was used for analyses. Details may be omitted where reference can be made to published procedures that outline methodologies in sufficient detail that experiments may be replicated. Except in the case of standard procedures, however, methods should be summarized even when reference is made to a published description. Procedures published in sources that are not peer-reviewed or in sources that are readily available to many readers of *Tree Physiology* should be described in full.

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In general, the Results and Discussion sections should not be combined.

Discussion

The Discussion should contain a distinct conclusions section in which the findings are used to support or refute the tested hypotheses. Ideally, the conclusion should also connect the findings of the study with larger and/or smaller scales of biological organization than those that were investigated in the study.

Acknowledgements

Acknowledgements and details of non-financial support must be included at the end of the text before references and not in footnotes. Personal acknowledgements should precede those of institutions or agencies.

Authors' Contributions

This section should succinctly summarize the contributions made by each author to the study conception and design, methodology implementation, experiment execution, data collection, data analysis/interpretation, and manuscript writing/revision. Please refer to each author by their initials.

Supplementary data

The Supplementary Data section should itemize each of the supporting documents that is provided as additional content. Please indicate clearly all material intended as Supplementary Data upon submission. Also ensure that the Supplementary Data is referred to in the main manuscript where necessary, for example as '(see Figure S1 available as Supplementary Data at *Tree Physiology* Online)'. Supplementary Data files should be used in lieu of Appendices.

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Details of all funding sources for the work in question should be given in this separate Funding section. The following rules should be followed: the full official funding agency name should be given, e.g. ‘National Institutes of Health’, not ‘NIH’; grant numbers should be given in brackets; multiple grant numbers should be separated by a comma; agencies should be separated by a semi-colon; no extra wording such as 'Funding for this work was provided by ...' should be used; where individuals need to be specified for certain sources of funding the following text should be added after the relevant agency or grant number 'to [author initials]'. An example is given here: 'National Institutes of Health (CB5453961 to C.S., DB645473 to M.H.); Funding Agency (hfygr667789).'

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Data and Materials Availability

Tree Physiology strives to meet best practice guidelines regarding data and materials accessibility to promote reproducibility of the studies at the core of your manuscript. To this end, a Data and Materials Accessibility statement should be included in your manuscript, either at original submission or at the revision stage. Additionally, *Tree Physiology* requires that authors agree at the publication stage to make experimental data and materials available to third party academic researchers upon reasonable request.

Data and materials archiving is key to ensuring long-term accessibility of these data and materials. The following provides a non-exhaustive list of expectations and recommendations regarding data and materials archiving.

Sequence data

At the time of manuscript acceptance, all sequence data must be deposited in a standard, acceptable format within a permanent, free-to-access, public repository, such as those available at the National Centre for Biotechnology Information (NCBI) or similar repository. This includes but is not limited to single nucleotide sequences (e.g. cDNAs), molecular markers (e.g. SNPs), protein/proteomic sequences, transcriptomes, transcriptomic comparisons (e.g. microarray or next-generation sequence datasets), and genomes. Unique identifiers such as accession numbers for sequence data must be provided prior to manuscript acceptance.

Other data, including ecoinformatic data

Tree Physiology strongly encourages all authors to submit datasets to an appropriate free-to-access public data repository, such as Dryad, FigShare or others that provide digital object identifiers (DOIs).

Computer code and scripts

In addition to being included in the manuscript as Supplemental Data, it is recommended that models, code and/or scripts be archived in a public repository providing DOIs such as Github, Dryad or others.

Microbial strains and molecular materials

Authors are encouraged to deposit bacterial and fungal strains, plasmids and similar resources in an appropriate Biological Resource Centre repository such as the CBS-KNAW. Accession numbers for deposited material should be included in the manuscript.

References

Authors are responsible for checking the accuracy of all references. At revision, please style and format according to the CSE 8th Edition. Articles cited in the text will be referred to by the name(s) of the author(s) with the year of publication in parentheses. If both are in parentheses, no punctuation separates the name(s) of the authors(s) and the year of publication. Consecutive citations in the text are placed in chronological order and separated by commas. If there are three or more authors, only the first author's name is given, and this is followed by the phrase et al., which should be in roman, not italic, type (e.g. Day et al. 2002).

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Journal article

Smart N, Fang ZY, Marwick TH. 2003. A practical guide to exercise training for heart failure patients. J Card Fail. 9(1):49–52. https://doi.org/10.1093/nar/gkab107.

Books

Wagner FH. 1980. Wildlife of the deserts. New York (NY): H.N. Abrams.

Smith ST, Johnston R, editors. 2009. Biodiversity: an introduction. 3rd ed. Cambridge (MA): Blackwell Science.

Chapters in books

Anderson RJ, Schrier RW. 2001. Acute renal failure. In: Braunwald E, Isselbacher KJ, Petersdorf RG, editors. Harrison's principles of internal medicine. 15th ed. New York (NY): McGraw-Hill. p. 1149–1155.

Shakelford RT. 1978. Surgery of the alimentary tract. Philadelphia (PA): W.B. Saunders. Chapter 2, Esophagoscopy; p. 29–40.

Websites

APSnet. 1994-2005. St Paul (MN): American Phytopathological Association. Plant pathologies; [accessed 2005 Jun 20]. http://www.apsnet.org/.

Biological Abstracts should be used as a guide to the abbreviation of journal titles. Personal communications, unpublished works, or papers in preparation or submitted for publication should not be listed as references, but incorporated in the text.

Tables

Please upload separate table files. If tables are submitted as non-editable vectors (e.g., PDF files) it is vital that they are linked to an editable format, such as a word document or an excel document.

Each table should be placed on a separate sheet and numbered in sequence using Arabic numerals. Tables should also have a title above and an explanatory footnote below, if required. Units in which results are expressed should be given in parentheses at the top of each column and not repeated in each line of the table. Ditto signs are not used. Avoid overcrowding the tables and the excessive use of words.

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Please use short, simple filenames when saving all your documents, and avoid special characters, punctuation marks, symbols (such as &), and spaces. If you are a Macintosh user, you must also type the extension at the end of the file name you choose (.doc, .rtf, .jpg, .gif, .tif, .xls, .pdf, .eps, .ppt, .mov or .qt).

Système International (SI) units of measure and their abbreviations should normally be used; although certain non-SI units are also acceptable including litre (l), minute (min), day, week and year, the last three of which should be spelled out in full.

When applicable, please use well-established abbreviations for concepts and variables. Non-standard abbreviations should be defined at the first occurrence. Such abbreviations should only be introduced in cases where multiple use is made (i.e. more than four times).

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The accuracy of a calculated result is limited by the least accurate measurement in the calculation. Please make sure that the presentation of the results in the text, tables, and figures reflects this principle. When presenting results from statistical tests, estimates of probability (P-value) should be given. Estimates of P-value and coefficients of determination (R2) should be expressed using no more than two and three decimal places, respectively.

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Common names of organisms may be used provided that in every case the Latin binomial and authority are given in parentheses following the first use in both the abstract and the body of the paper.

Tree Physiology follows international community guidelines regarding gene, transcript and protein nomenclature. These community standards are often based upon those developed for the model plant Arabidopsis thaliana, available on this page. Following these guidelines, three letter codes are preferable when designating gene nomenclature. In cases where gene nomenclature for genes, transcripts or proteins are assigned nomenclature based on similarity to Arabidopsis sequences, convincing phylogenetic evidence must be presented to support this nomenclature. Please use two- or three-letter prefixes to unambiguously indicate the taxon (species, hybrid) of the sequence. By convention, genes, transcripts and proteins representative of wildtype sequences are written in upper case (majuscule), while those of mutants are written in lower case (miniscule). Nucleotide sequences are italicized, while protein sequences are not.

8) Submission Procedure

Pre-submission enquiries

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Pre-submission enquiries should be sent to sari.palmroth@duke.edu.

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In partnership with Cactus, we are offering authors free usage of their Paperpal Preflight tool pre-submission. This is an AI-driven tool that performs technical checks tailored to *Tree Physiology*. Authors are given a markup of suggested corrections to match their paper to journal requirements. Before submission, we encourage authors to run their paper through this tool. Please note that this is not mandatory and suggested corrections are optional.

Authors also have the option to pay for additional language checking – again, this is not mandatory and all suggested corrections are optional.

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During online submission, please be prepared to provide the following information (n.b. this is not an all-inclusive list):

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- Three to four sentences outlining the novelty and significance of the findings to the field.
- The scales of biological organization directly addressed by the study (e.g., molecules, organ physiology, whole plant physiology, ecophysiology, and integration/synthesis)
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Cover letter

Each submission must be accompanied by a cover letter. The letter should include:

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- A statement to indicate that the authors have read the manuscript and agree to its submission
- The hypothesis/hypotheses addressed by the study
- Three or four sentences describing the novelty and significance of the finding)