NATIONAL INSTITUTE OF AMAZONIA RESEARCH - INPA
GRADUATE PROGRAM IN TROPICAL FOREST SCIENCE - CFT

ECOPHYSIOLOGY OF FABACEAE TREE SPECIES DURING FOREST RESTORATION IN THE BALBINA HYDROELETRIC DAM IN AMAZONAS STATE

ROBERTO KIRMAYR JAQUETTI

Manaus, Amazonas
June 2018

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Thesis presented to the National Institute of Amazonia Research as part of the requisite to acquisition of Philosophy Doctor in Tropical Forest Science, concentration area: Tropical Forestry.

Approved June 27, 2018.

<table>
<tr><td>Dr. Bala Rathinasabapathi
Committee member
University of Florida</td><td>Dr. Lincoln Zotarelli
Committee member
University of Florida</td></tr>
<tr><td>Dr. Jair Max Fortunato Maia
Committee member
Amazonas State University</td><td>Dr. Newton Paulo de Souza Falcão
Committee member
INPA</td></tr>
</table>

Dr. Diego Pereira de Souza
Committee member
INPA

Dr. José Francisco de Carvalho Gonçalves
Academic advisor
INPA

Synopsis:

      The response of functional traits of tropical tree legumes species were studied in a forest restoration plantation in Central Amazon under low vs. high water and nutrient availabilities. Functional traits such as growth, gas exchange, chlorophyll fluorescence, leaf nutrients, non-structural carbohydrates and photosynthetic pigments contents were evaluated under multivariate analysis.

Key-words: biomass allocation, growth, fertilization treatment, chlorophyll fluorescence photosynthesis, plant nutrition, seasonality effects

DEDICATION

My thesis, as all of my victories in life, I dedicate to my grandfather, to my grandmother, and my beloved Mom. They have been and will always be my inspiration in life!

ACKNOWLEDGMENTS

First I am grateful to God as he filled my life with blessings, thanks for being so protective giving me energy and strength to move forward in these amazing journey.

I thank all the The National Institute of Amazonia Research – INPA and the Graduate Program in Tropical Forest Science – (PPG-Ciências de Florestas Tropicais) for the opportunity and support and for the crew members who were always so gentle and kind.

Appreciate to Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES) and National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq) for financial support.

I thank my family for being always present unconditionally, and we have gone through some though times, but I am sure that we are heading towards something bigger when we will be united in love and friendship. Love you so much.

I am grateful for my beloved friends and brothers that life has given me, for you I dedicate this achievement. I thank every day for having you all in my life.

I appreciate the effort and the patience of my academic advisor Dr José Francisco de Carvalho Gonçalves, and for keeping me focused throughout this experience. The support has been giving since I got to Amazonia as he showed me how to become a better and organized student. I also thank for allowing me the use of the Laboratory of Plant Physiology and Biochemistry - LFBV, with reagents and equipment for the complete collection and analysis of data.

I thank to whole crew of Balbina for the constant support with materials and workers for the condiction of the experiment trial. In special I thank Elessandra, Redman, Andrey, Daniela e Juliana for the great moments we spent together. I also appreciate the field workers who worked very hard during biomass collection. It was such a great and special moment.

Thanks to my friends of the LFBV for the emotional support and the friendship during this trajectory in special for Karen, Larissa, Rafaela, Adamir, Josiane, Rose, Diego, Vinicius, Sabrina and Victor. Also appreciate Dr Jair for the constant support during my post graduation process.

Appreciate my colleagues from the graduate programs of INPA for the support, love and respect.

Appreciate the professors of INPA for the dedication and patience, and for teaching how the incredible Amazon forest works. Also thank the committee members for the valuable revisions and suggestions.

I am grateful to be and to work in this amazing forest that has been so welcoming. Its beauty is incomparable, and I hope to contribute to preserve its natural resources much more throughout the years.

Gratefully, I THANK all of you from the bottom of my heart!

ABSTRACT

Plants can adjust functional traits according to the variations in the availability of resources. Under low soil water and nutrient availability, as found in degraded areas, the growth of tree species can be drastically reduced. Tree legumes evolved different strategies to efficiently uptake and use nutrients, securing high survival and growth rates, thus facilitating the restoration of important biogeochemical cycles during forest restoration. Early successional species may increase fast (aquisitive) traits under high resources, while slow (conservative) traits can be enhanced under limited resources and for late successional species. Variations in functional traits such as growth, gas exchange, water potential, nutrients, non-structural carbohydrates (soluble sugars and starch) (NSC), photosynthetic pigments and chlorophyll fluorescence of six tree legumes species were studied according with low vs. high water and nutrient availabilities during a forest restoration plantation in Hydroelectric dam Balbina. The species selected were five native species from Amazonia *Cenostigma tocantinum*, *Senna reticulata*, *Dipteryx odorata*, *Clitoria fairchildiana* and *Inga edulis*, and one alien species from Australia *Acacia* sp.. The individuals were either fertilized or remain unfertilized throughout the 24 month experiment. Three main hypotheses were tested: a) economics spectrum; b) functional equilibrium; and c) NSC dynamics and source-sink allocations. Economics spectrum was mostly confirmed under high water and nutrient availabilities increasing transpiration (E), photosynthesis (P_n), relative growth rates in diameter and performance index of chlorophyll fluorescence (PI_{ABS}). Traits from the slow spectrum such as dissipation energy flux (DI_0/RC), intrinsic water use efficiency ($IWUE$) and root mass fraction (RMF) were enhanced under limited conditions. Functional equilibrium hypothesis was also confirmed as unfertilized plants increased twice the RMF as compared with high nutrient plants. Further RMF was positively correlated with starch and soluble sugars in root tissues indicating an active process of increased root fraction involving energy costs. *Acacia* sp. which respond differently than economics spectrum had significant growth under severe nutrient limitations, increasing nutrient and energy use efficiency, and the pool of NSC in leaf biomass. *S. reticulata* also diverge from economics assumptions being unable to adjust traits such as gas exchange and showing responses to drought stress which were related with increased leaf calcium and starch content. The fast acquisition of resources according (increased E and P_n) was considered the main strategy of species in a degraded and fertilized environment, as adjustments in energy use efficiency and NSC pools were essential for plants acclimation. Tree legumes especially N_2-fixing species are proven to be well succeeded in forest restoration plantations producing eight times more biomass than non N_2-fixing. Photosynthesis was the most responsive variable to evaluate species responses to resource variations in our study, nonetheless chlorophyll fluorescence appears to be an alternative efficient technique to evaluate the performance and responses to abiotic stress factors. This study makes a significant contribution to the understanding of the ecophysiology of tropical tree legumes and also to provide data to enhance Dynamic Global Vegetation Models. Additionally, from the conceptual view this thesis provide data to enhance climate change predictions models on the interface of soil-plant-atmosphere (during 2015/16 El Niño event) in Amazon forest. In conclusion, the mixing of different successional ecological groups and including biological N_2 fixation process appears to be an efficient way to achieve ecological forest restoration goals in nutrient limited soils such as in Amazon basin.

RESUMO

Diferentes espécies são capazes de ajustar suas características funcionais de acordo com as variações na disponibilidade de recursos do ambiente. Sob baixa disponibilidade de água e nutrientes no solo como em áreas degradadas, o crescimento das plantas pode ser drasticamente reduzido. Por outro lado, espécies leguminosas arbóreas desenvolveram evolutivamente diferentes estratégias para adquirir e utilizar nutrientes de maneira eficiente, garantindo altas taxas de sobrevivência e crescimento, facilitando a restauração de importantes ciclos biogeoquímicos em plantios de restauração florestal. De acordo com a hipótese do espectro econômico espécies iniciais podem aumentar respostas relacionadas as características do rápido espectro sob alta disponibilidade de recursos, enquanto respostas de crescimento lento (conservacionistas) são encontradas em condições limitantes e em espécies tardias. As variações das características funcionais como crescimento, trocas gasosas, nutrientes foliares, potencial hídrico, carboidratos não estruturais (açucares solúveis e amido), pigmentos fotossintetizantes e fluorescência da clorofila de seis espécies leguminosas arbóreas foram estudados de acordo com a alta X baixa disponibilidade de agua e nutrientes em um plantio de restauração florestal na UHE Balbina. Foram selecionadas cinco espécies nativas da região *Cenostigma tocantinum*, *Senna reticulata*, *Dipteryx odorata*, *Clitoria fairchildiana* e *Inga edulis*, e uma espécie nativa da Austrália *Acacia* sp. as quais foram fertilizadas ou permaneceram sem adubação durante os 24 meses de experimento. Três principais hipóteses foram avaliadas: a) espectro econômico; b) equilíbrio funcional; e c) dinâmica de carboidratos e relações fonte-dreno. A hipótese do espectro econômico foi confirmada em condições de alta disponibilidade de água e nutrientes com o aumento da transpiração (E), fotossíntese (P_n), taxa de crescimento relativo em diâmetro e índice de desempenho da fluorescência da clorofila (PI_{ABS}). Características do espectro lento como dissipação do fluxo de energia (DI_0/RC), eficiência intrínseca no uso de água ($IWUE$) e fração de biomassa de raízes (RMF) foram incrementadas em condições limitantes. A hipótese do equilíbrio funcional também foi confirmada com um aumento de 100% dos valores de RMF em plantas não adubadas. Além disso, o aumento da RMF foi correlacionado com os teores de amido e açúcares solúveis nas raízes indicando um carregamento ativo com gastos de energia durante o processo. *Acacia* sp. contrariando o espectro atingiu um crescimento significativo mesmo não fertilizada, através do ajuste de suas características funcionais com eficiência no uso de energia (PI_{ABS}) e nutrientes, além do alto conteúdo de carboidratos e produção de biomassa de folhas. *S. reticulata* também divergiu das respostas do espectro, apresentando sinais de estresse fisiológico como o aumento dos teores de cálcio e amido nas folhas. A rápida aquisição de recursos como o aumento da P_n e da E foi a principal estratégia das espécies nos ambientes degradados e fertilizados, por outro lado o ajuste na eficiência do uso de energia e na dinâmica de carboidratos tem papel fundamental na aclimatação das espécies. Espécies leguminosas arbóreas principalmente fixadoras de N_2 são comprovadamente bem sucedias em plantios de restauração produzindo oito vezes mais biomassa do que espécies não fixadoras. A fotossíntese foi a variável mais responsiva para avaliar as respostas das espécies em relação às variações ambientais, no entanto a fluorescência da clorofila parece ser uma técnica alternativa robusta e eficaz para avaliar a desempenho e as respostas aos fatores abióticos de estresse. Concluindo, a inclusão de espécies de diferentes grupos sucessionais e do processo de fixação biológica de nitrogênio parece ser uma técnica eficiente de restauração ecológica em áreas com fortes limitações nutricionais como em solos da bacia Amazônica.

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(ET_0/TR_0); F_{2ms}, Fluorescence intensity at 2 ms; M_0, net rate of PSII closure [$4(F_{300\mu s}$ - $F_{50\mu s})/(F_m - F_{50\mu s})$]; V_j, relative variable fluorescence at J step $(F_{2ms} - F_0)/(F_m - F_0)$....75

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1. GENERAL INTRODUCTION

The increased pressure for natural resources in a planet where the population constantly rises is becoming each time more challenging over time. Through forest degradation huge amounts of greenhouse gases mainly CO_2, are released to the atmosphere by the burn or decomposition of forest biomass (Houghton *et al*., 2000; Achard *et al*., 2002). The ecosystem degradation can disrupt important biogeochemical cycles thus enhancing the global climate change (Malhi *et al*., 2008; Moss *et al*., 2010). Therefore, Amazon forest plays an important role in regulating climate change and in maintaining the global carbon (C) cycle as biomass is constituted around 50 % by carbohydrates (Favaretto *et al*., 2011; Houghton 2012; Thomas e Martin 2012).

Annual deforestation rates which depend strongly on political decisions, still fluctuate significantly (Nepstad *et al*., 2009; Soares-Filho *et al*., 2014). Estimates on Brazilian Amazon showed that 20 % of the initial forest cover has been completely degraded (Davidson *et al*., 2012; Nobre *et al*., 2016). Additionally, climate change has altered the precipitation regime in some regions of Amazonia increasing the intensity and frequency of drought periods mainly during El Niño events (Phillips *et al*., 2009; Jiménez-Muñoz *et al*., 2016).

Forest restoration on the other hand, can increase the biodiversity and the functionality of ecosystems providing goods and ecological services (Rodrigues *et al*., 2009). One of the main objectives of ecological restoration is to reestablish the C cycle of aboveground biomass increasing soil organic matter, thus favoring the establishment of more complex and resilient communities (Chaer *et al*., 2011; Suding *et al*., 2015). In this sense forest restoration plantations can be considered an efficient method for C sequestration as established by the Kyoto protocol, having important role in the maintenance of natural forest ecosystems (Lamb *et al*., 2005; Li *et al*. 2012).

Degraded ecosystems undergo major changes in abiotic factors composition, increasing irradiance while reducing water and nutrients in the soil, which may cause physiological stress to the least plastic species (Gonçalves and Santos Junior 2005; Santos Junior *et al*., 2006; Jaquetti *et al*., 2014). Plant responses are variations of a certain trait along the gradient of low to high resource or early to late successional groups increasing fast traits from low to high resources or early to late species (Wright *et al*., 2004; Poorter *et al*., 2014; Reich 2014). Limited water and nutrients in the soil decreases primary productivity in most

terrestrial ecosystems (Elser *et al*., 2007; Fisher *et al*., 2012). The low availability of nitrogen (N), phosphorous (P), calcium (Ca), magnesium (Mg) and even potassium (K) of some degraded soils in the Amazon basin can cause multiple nutrient limitations being the main limiting factor for plant growth in some highly degraded areas (Jaquetti *et al*., 2016; Jaquetti and Gonçalves 2017).

Soil nutrient limitation effects usually involve a reduction in photosynthesis and growth in forest plantations and secondary forests (Davidson *et al*., 2004; Kant *et al*., 2011; Li *et al*., 2016). Indeed, the primary productivity of natural forests in Amazonia is strongly related with the total soil P concentrations (Quesada *et al*., 2012). Therefore, it is expected that plants have evolved different strategies adjusting traits to enhance the efficiency of uptake, transport and use of nutrients (Mommer *et al*. 2016; Castro-Rodríguez *et al*., 2017). Under limited N and P conditions plants can increase root mass fraction (RMF), caused by the increased transportation of soluble sugars which can be used, or stored as starch (Wissuwa *et al*., 2005; Hammond and White 2008).

In such N and P poor environments the ability of biological N_2 fixation (BNF) in nodules, is an important strategy of N_2-fixing species to overcome the nutrient limitations, increasing greatly the biomass production and the N use efficiency (Hughes and Denslow 2005; Morris *et al*., 2011). N_2-fixing species from Fabaceae family (legumes) are able to enhance soil fertility, reducing the need for external inputs and therefore connecting important biogeochemical cycles (Macedo *et al*., 2008; Chaer *et al*., 2011; Behie and Bidochka 2014; Batterman 2018; Good 2018). Nonetheless BNF process can be drastically reduced under low P availabilities (Tesfaye *et al*., 2007; Sulieman *et al*., 2013). Despite the importance of tree legumes in the ecological restoration little is know about its ecophysiological responses and strategies in degraded ecosystems (Khamzina *et al*., 2009; Adams *et al*., 2010).

Fertilization treatments increase nutrient availabilities in the soil thus enhancing photosynthesis and growth of agricultural and tree species (Cooke *et al*., 2005; Luo *et al*., 2006; Jaquetti *et al*., 2014). Moreover, under high soil nutrient availabilities plants can increase aboveground biomass consequently increasing shoot-to-root ratio (S:R) (Novaes *et al*., 2009). Under drought plants may reduce photosynthesis and growth, which is mainly caused by the stomatal closure with the accumulation of abscisic acid and the efflux of K and

carbohydrates of the guard cell (Flexas *et al*., 2004; Rolland *et al*., 2006; Cramer *et al*., 2011; Osakabe *et al*., 2014; Kuromori *et al*., 2018).

Aiming to understand the strategies of tree legumes during forest restoration in Amazonia and how responses vary along economics spectrum axes, individuals were studied according to the variations in the functional traits (gas exchange, nutrients, non-structural carbohydrates (NSC), photosynthetic pigments and chlorophyll fluorescence) under low vs. high water and nutrient availabilities.

Based on grounded ecological theories three main hypothesis were tested using multivariate data analysis:

a) Different ecological groups and species will increase fast traits and growth under high resource availabilities in accordance with the economics spectrum, while increasing slow (conservative) traits under low resources availabilities;

b) Plants will increase root biomass fraction under nutrient limitations at the expense of growth accordingly to the functional equilibrium theory which imply an active process of biomass allocation;

c) Acclimation responses of the species under limited resources will be influenced by the NSC dynamics and the allocations of different pools (leaves, stems and roots).

This thesis was separated in two chapters (papers). The first chapter evaluate the fertilization effects of 10 growth variables in addition to leaf P content and root and stem N and P content of species and effects of functional groups (early vs. late successional and N_2-fixing vs. non N_2-fixing). Growth variables were sensitive to detect the fertilization effects, whereas low leaf P content in addition to increased leaf mass fraction (LMF) separated unfertilized *Acacia* sp. from the other species. Root and stem nutrient content was insensitive to variations in species and ecological groups.

In the second chapter results were more complex as 59 functional traits were analyzed, including seven growth variables. As physiological traits allow us data collection during different periods, it was also possible to evaluate seasonality effects. The photosynthesis, transpiration, growth and energy use efficiency were mostly enhanced under high resources. Whereas slow traits such as intrinsic water use efficiency, energy dissipation and RMF were enhanced under low resources availabilities. Throughout our study we aim to enhance the knowledge of tree legumes ecophysiology to increase the productivity of these species, and to

make forest restoration plantations in Amazonia more competitive with others worldwide, thus providing goods and energy security to the region.

2. OBJECTIVES

2.1 GENERAL OBJECTIVE: The main objective of this thesis was to investigate the ecofunctional traits of six tree legumes species in a forest restoration plantation conditions in Central Amazonia, growing under fertilization treatments in both drought and wet periods. Conducted in a degraded area near of Balbina Hydroelectric Dam our study also aimed to understand the responses and strategies of native and one oligotrophic alien species under low and high water and nutrient availabilities. Using multivariate analysis there was an effort to find the most responsive variables to environmental changes, with the objective to develop a nondestructive and practical technique, to evaluate the growth performance and stress responses of species in degraded environments. Notwithstanding, this experiment intended to probe the knowledge of ecofunctional responses of stressed and well adapted species with potential use on restoration of degraded forest ecosystems.

2.2 SPECIFIC OBJECTIVES

1). Investigate the effects of low and high water and nutrient availabilities on gas exchange (photosynthesis, transpiration, dark respiration and stomatal conductance) and on photosynthetic water and nutrient use efficiency of the studied species.

2). Determine leaf nutrient contents of macronutrients (N, P, K, Ca, Mg), micronutrients iron (Fe) and zinc (Zn) and of photosynthetic pigments of tree legumes.

3). Investigate the seasonality and fertilization effects on NSC dynamics in leaf and root tissues.

4). Investigate the variations in the energy use efficiency parameters, such as chlorophyll fluorescence, aiming to find robust variables to assess photosynthetic status of species in the field condition.

Chapter 1

Fertilization and biological N$_2$ fixation effects on growth traits of tropical tree legumes during forest restoration in Amazonia

Fertilization and biological N$_2$ fixation effects on growth traits of tropical tree legumes during forest restoration in Amazonia[1]

Roberto K. Jaquetti[2], José Francisco C. Gonçalves[2,*], Henrique E. M. Nascimento[2]

[1] National Institute of Amazonian Research - INPA, Laboratory of Plant Physiology and Biochemistry, Ave. André Araújo, 2936, Aleixo, 69011-970 - Manaus, AM, Brazil. *corresponding author: jfc@inpa.gov.br

Abstract

Restoring degraded landscapes will be the major task of the new millennium. Biological N$_2$ fixation (BNF) root traits can support the ecosystem-level restoration of disrupted carbon (C) and nitrogen (N) cycles in nutrient-poor degraded environments. However, the effectiveness of BNF in the field remains poorly understood. Here, we hypothesized that BNF may be an acquisitive strategy of tree legumes that enhances the biomass growth of N$_2$-fixing species. We also speculate whether growth, allocation and nutritional traits represent strategies for managing different nutrient availabilities for different ecological groups. Twenty-four months after the forest restoration (FR) experiment was established, the high nutrient availability (HN) treatment increased growth rates, mostly in their aboveground structures, whereas plants under low nutrient treatment (LN) primarily adjusted their C allocation to root biomass as a conservative strategy. BNF greatly enhanced the biomass growth rates of N$_2$-fixing species with mean values 8 times greater than those of non-N$_2$-fixing species, providing evidence of a fast acquisitive strategy, although this strategy heavily depends on the nutritional status of the individuals. Not all species were constrained by the extreme nutrient-poor soil conditions, *Acacia* sp. was found to have evolved functional strategies to cope under severe nutrient limitations. Accordingly, mixing different ecological strategies and including the BNF process in FR may have considerable impact on resilience and C sequestration in low nutrient degraded tropical forest ecosystems.

Key words: C allocation, Degraded area, Economics spectrum, Fabaceae, Plant nutrition

1. Introduction

Nutrients are essential for biomass growth and directly affect the ecosystem-level carbon (C) biogeochemical cycles. Nitrogen (N) has important functions in organic compounds and in various processes, such as protein and gene regulation, and strongly affects root architecture and leaf expansion (Krouk et al., 2010). Phosphorous (P) is essential to energy conservation and carbon assimilation during photosynthesis (Ticconi and Abel, 2004). In general, nutrient availabilities strongly influence metabolism and energy homeostasis, thereby affecting the N and P relationships (N:P_{ratio}) of plant species, but both N and P can be considered major limiting nutrients for productivity in many natural ecosystems worldwide (Güsewell, 2004; Hedin et al., 2009; Quesada et al., 2010).

In response to nutrient limitations, plants may adjust their architecture and allocation of carbohydrates to root tissues production, increasing the root mass fraction (RMF), thus enhancing soil foraging (Hermans et al., 2006; Poorter et al., 2012; Fort et al., 2015). However, in extremely nutrient-poor environments, such as degraded tropical environments, both metabolism and growth rates can be impaired by co-limitations of several nutrients (Kleczewski et al., 2010). Only a few species may have evolved different strategies to cope under extreme low P and N availabilities in the soil by adjusting entire plant functional traits (Mommer et al., 2016).

Different fertilization treatments with N and P as well as with potassium (K), calcium (Ca), magnesium, sulfur and micronutrients performed in forest restoration (FR) strongly influence the concentrations and diffusion rates of nutrients in the soil, affecting the stoichiometric relationships and individual metabolic processes with considerable impacts on growth (Cernusak et al., 2010; Ågren and Weih, 2012; Chapman et al., 2012). Additionally, simultaneous nutrients fertilization can have a synergistic effect on biomass growth because the status of the nutrient in question will influence the uptake and use efficiency of other nutrients and vice versa (Ågren et al., 2012). However, different species may respond differently to nutrient starvation and fertilization treatments in degraded environments (Jaquetti et al., 2016; Jaquetti and Gonçalves, 2017).

Degraded tropical forest ecosystems undergo extreme changes in abiotic factors (Santos Junior et al., 2006; Silva et al., 2008). High solar irradiance can cause oxidative stress to the photosynthetic apparatus, reducing photosynthesis and growth (Gonçalves and Santos Junior, 2005; Dias and Marenco, 2006). Additionally, drought may also constrain productivity in previously unthreatened regions of the Amazon Basin during and after El Niño events (Davidson et al., 2012; Kintisch, 2016; Phillips et al., 2009). Nevertheless, nutrient-poor soils may be the major limiting factor for seedling growth and establishment during FR because of nutrient stoichiometric relationships (Sardans et al., 2015; Jaquetti et al., 2016). In such environments N_2-fixing tree legumes species can be considered key species for the restoration of N and C stocks (Siddique et al., 2008; Justino et al., 2017).

Symbiotic rhizobia interactions with N_2-fixing hosts can convert available atmospheric dinitrogen (N_2) gas, increasing fertilizer use efficiency and soil fertility and thereby lowering the need for external energy inputs; therefore, N_2-fixing species are strongly recommended for use in FR (Jaquetti and Gonçalves, 2017; Sprent et al., 2017). Further, non-structural carbohydrates (NSC) are crucial in the BNF process as they sustain the high metabolic requirements in the nodule region (El Msehli et al., 2011; Sachs et al., 2018). BNF root traits may increase biomass growth in the field (Chaer et al., 2011), but basic information is lacking on the effects of BNF on biomass growth under different environmental conditions (Omena-Garcia et al., 2015).

The economics spectrum (ES) hypothesis indicates that species present different strategies for the fast acquisitive or slow conservative use of resources (Reich, 2014). The former strategy presents high growth rates of the early successional species, which are correlated with higher photosynthetic rates among other functional traits (Wright et al., 2004). Recently, mycorrhizal symbiosis have been noted as a conservative strategy (Weemstra et al., 2016). In contrast, BNF may be considered an acquisitive strategy as N_2-fixing species tend to have higher photosynthesis rates, leaf N concentration and growth rates (Jaquetti et al., 2014), but this has not been evaluated.

Most studies on plant nutrition and BNF are performed under greenhouse or laboratory conditions, whereas under natural conditions, plants may be exposed to multiple stress factors (Cramer et al., 2011; Prasch and Sonnewald, 2015). However, it is not known how species differing in successional status subjected to FR will respond to variations in fertilization in open fields. Additionally, how different ecological groups influence ES is not completely understood, and this information is important for enhancing global model responses to future climate change (Poorter et al., 2009, Freschet et al., 2015). Furthermore, forest plantations, particularly in degraded areas, can play determinant roles in deforestation by accelerating natural regeneration and can reduce pressure on native forests (Lamb et al., 2005).

Here, we tested the hypothesis that growth can be differently enhanced under high nutrient availability or reduced under nutrient starvation for the tree legumes in relation to their ecological groups. Second, we tested the hypothesis that the growth, biomass allocation and nutrient concentration traits of species can be adjusted as a strategy to different environmental conditions. In addition, finally we hypothesized that BNF is an acquisitive strategy enhancing biomass growth rates of N_2-fixing species under nutrient-poor environments. Therefore, the objective of the present study was to understand the strategies of tree legumes under resource limited degraded environment, investigating the potential of these species to C sequestration and climate change mitigation.

2. Materials and methods

2.1. Study site, leguminous species and fertilization treatments

The experiment was conducted in a degraded area of the Forest Restoration Program of the Balbina Hydroelectric Dam (FRP Balbina) (01°55'S and 59°27'W, elevation 100 m) in Presidente Figueiredo Municipality, Amazonas State, Brazil, which is nearly 170 km northwest of Manaus City. The area used for this study is 3 ha in size, and it degraded during the dam construction in 1983, in which all natural forest was cut while the topsoil was left in place. This area has remained abandoned, and natural regeneration has not occurred. The surrounded matrix is a natural well-preserved non-flooded *terra firma* dense forest. The climate of the region is Af (tropical humid) based on the Köppen climate classification system, and the area presents a mean annual temperature above 25 °C and annual precipitation of 2300 mm. During the second year after the experiment installation, a strong El Niño Southern Oscillation (ENSO) event occurred (Figure 1), which allowed us to evaluate the establishment of the species in a climate change scenario. The soil in the area is a yellow Oxisol with a pH near 5 and extremely low natural fertility. The most dominant genus found in the surrounding natural forest are from *Pouteria*, *Protium*, *Inga*, *Ocotea*, *Hymenaea*, *Eschweilera* and *Dipteryx*, and the mean canopy height is 30 m.
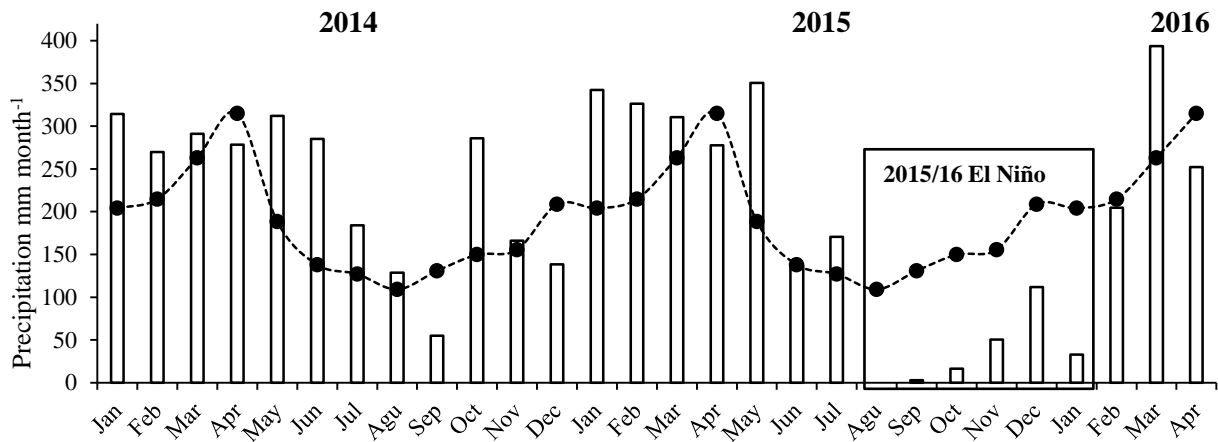
Figure 1: Monthly precipitation near the experimental area measured in the Balbina Hydroelectric Dam climatic station in Presidente Figueiredo, Brazil. The period influenced by the 2015/16 El Niño is highlighted by the square. The dotted line represents the precipitation mean from 1980-2015.

Five native leguminous species, *Cenostigma tocantinum* Ducke, *Senna reticulata* (Willd.) H.S. Irwin & Barneby, tonka bean *Dipteryx odorata* (Aubl.) Willd., orchid tree *Clitoria fairchildiana* R.A. Howard., ice-cream bean tree *Inga edulis* Mart., and alien species *Acacia* sp., were selected for this study. Thus, this study covered a number of species representative of each of the three subfamilies / clades of the Fabaceae family, the Caesalpinioideae and Faboideae subfamilies, and two are from the mimosoid clade. An important aspect in terms of the evolutionary history of Fabaceae is whether the species are N_2 fixing. Therefore, three of the species are N_2-fixing species (*C. fairchildiana*, *I. edulis* and *Acacia* sp.), and three are non-N_2-fixing species (*C. tocantinum*, *S. reticulata* and *D. odorata*). *D.*

odorata has great potential for use in FR because it is a late successional species well adapted to degraded environments, and it produces high-quality hardwood (Jaquetti et al., 2016). *I. edulis* is an early successional facilitative species that is primarily used in agroforestry systems and mixed-species plantations (Nichols and Carpenter, 2006). *Acacia* sp. has been used worldwide for numerous purposes and represents a facilitative species in *Eucalyptus* mixed-plantations; currently, it is the second most planted genus in forestry activities (Forrester et al., 2006). *C. tocantinum* and *C. fairchildiana* are far less studied species but both may have great ecological potential, with the latter apparently an extraordinary facilitative species.

Seedlings from a greenhouse were selected based on the criteria of uniform size and individual health within the same species: height of 25 cm for *C. tocantinum*, 35 cm for *S. reticulata*, 40 cm for *D. odorata*, 20 cm for *C. fairchildiana*, 30 cm for *I. edulis*, and 20 cm for *Acacia* sp. A complete randomized block experimental design was used. Nine blocks were placed across the 3-ha degraded area. Each block, which consisted of two planting lines, covered 432 m^2 (6 m x 72 m) and contained all six studied species, which were either fertilized (high nutrient – HN treatment) or remain unfertilized (low nutrient – LN treatment) throughout the course of the experiment. Each treatment, which represented a combination of fertilization and species, was replicated four times within each block, which resulted in 48 seedlings per block and 432 individuals in the total area. We began the site preparation in February 2014 by opening the planting lines with subsoil tillage to a depth of 30 cm. The distance between lines within each block was 3 m. The distance between seedlings within each planting line was also 3 m, resulting a 3 x 3 m spacing. We opened the 30-cm square x 40-cm deep tree-planting holes manually using a straight spade with an iron cable.

To provide nutrient relief, the fertilization for the HN treatment was initially incorporated into the soil at the time of the tree-planting hole preparation, and this treatment subsequently received two fertilizer additions each year (Table 1), with one in the beginning of the rainy (December) season and other in the beginning of the dry season (May-June). The fertilizer was distributed around the crown projection area of each plant. Fifteen days after planting, the N$_2$-fixing species were inoculated with the following strains generated in the Laboratory of Microbiology of the National Institute of Amazonian Research (INPA): *C. fairchildiana* 944, 949, and 959; *I. edulis* 831, 841, 842, 844, and 852; and *Acacia* sp. 563. A cocktail with *Rhizobium* bacteria was applied to the field sites in two applications of 10-ml each, with 15 days between the first and the second applications.

Table 1: Type and quantities of fertilizer applied individually during the HN treatment.

Application	Date	Organic fertilizer (chicken manure)	Lime	NPK	FTE BR-12[®4]
Fertilization pits	Dec/2014	3 kg dry mass	100 g	200 g (04.14.08)[1]	20 g
1ª post planting	Jun/2014		100 g	200 g (10.10.10)[2]	20 g
2ª post planting	Dec/2014		100 g	300 g (15.15.15)[3]	20 g
3ª post planting	May/2015		100 g	300 g (15.15.15)[3]	20 g
4ª post planting	Dec/2015		100 g	300 g (15.15.15)[3]	20 g

[1]NPK 4-14-8[®] Bunge (São Paulo, SP, Brazil 4% N, 14% P and 8% K), [2] NPK 10-10-10[®] Tocantins (Porto Nacional TO, Brazil; 10% N. 10% P and 10% K), [3]NPK 15-15-15[®] Yara (Porto Alegre RS, Brazil; 15% N, 15% P and 15% K), [4]FTE BR-12[®] Nutriplant (São Paulo, SP, Brazil 7.1% Ca. 5.7% S. 1.8% B. 0.8% Cu. 2.0% Mn. 0.1% Mo and 9.0% Zn).

2.2. Relative growth rates in diameter, height and biomass

The growth of each planted individual was calculated by determining the relative growth rates (RGRs) of its height, diameter, and total biomass, including the roots. The collar diameter (CD) and stem length (SL) were measured immediately after the seedlings were planted and 24 months later. For both RGR variables, we randomly selected one replicate of each treatment (species and fertilization) in the nine blocks. The SL was measured with a hypsometric stick, and the CD was measured using a digital caliper rule.

For the initial biomass data, it was used the average of the nine seedlings. These seedlings were separated into three main compartments: leaves, woody parts (stems + branches) and roots. The harvested material was placed in a forced-ventilation oven at 65 °C until it reached a constant weight (approximately 3 days). The dry material was then weighed using a digital scale with a precision of 0.01 g. By the end of the experiment (24 months), we harvested and weighed the same selected individuals used for the estimates of CD and SL. To collect the whole root system, we manually excavated the soil starting at a distance of 20-30 cm from the base of the plant. The main roots were cut using an ax, pulled from the soil to their ends, and then the area was excavated to separate the soil from the roots. The roots were carefully washed using a high-pressure washing machine until all the soil particles were removed prior to weighing. Samples for all compartments were taken to obtain the wet-to-dry mass conversions and perform a carbon concentration analysis. The individual wet-to-dry mass conversion factors were used to convert the total wet weight to total dry weight in each compartment.

The RGRs for diameter (RGR_D), height (RGR_H), and biomass (RGR_{Bio}) were determined as described by Hunt, (2012): $RGR_D = LogD_2 - LogD_1/t_2 - t_1$, where $LogD_1$ = initial diameter, $LogD_2$ = final diameter, t_1 = initial time, and t_2 = final time.

2.3. Biomass allocation

The biomass allocation, which consists of the leaf mass fraction (LMF), stem mass fraction (SMF), and RMF, was determined as follows (Poorter et al., 2012): LMF = leaf biomass/total plant biomass, SMF = stem biomass/total biomass, and RMF = root biomass/total biomass.

2.4. N and P concentrations

Samples of the leaves material were taken in each treatment, oven dried at 65 °C and ground prior to the analysis of N and P concentrations. Samples from stem and root compartments were also analyzed in the HN treatment. The N concentration for each compartment was assessed with the 2400 Series II CHNS/O Organic Elemental Analysis (PerkinElmer Inc., Waltham, MA, USA). The P concentration was determined by spectrophotometry at 725 nm according to Vitti and Ferreira, (1997).

2.5. Data analysis

The interrelationships among the 10 growth and biomass variables were assessed using the non-metric multidimensional scaling (NMDS) ordination method, which reduces the dimensionality of the original matrices (McCune and Mefford, 1995). NMDS was performed to evaluate the effects of the fertilization treatment, species and BNF root trait. The number of ordination axes was determined by examining the relationship between stress and number of axes, and randomization tests were performed to confirm that each axis in the final analysis explained significantly more variation than expected by chance. All variables were standardized by the relativization maximum method (Noy-Meir et al., 1975) prior to analysis. Sorensen's distance measure was used (comparable results were obtained with different distance metrics and ordination techniques, and they indicated that the observed patterns were insensitive to the methods used).

Product-moment correlations were used to assess the influences of fertilization (fertilized and unfertilized), species and the BNF root trait on the ordination axes and each original variable. When conducting multiple simultaneous correlations, Bonferroni corrections were employed to reduce the likelihood of spurious correlations using an experiment-wide error rate of 0.15 (cf. Chandler, 1995). At a probability level of $P < 0.05$, pairwise t-tests were performed to evaluate the actual significances of the fertilization and BNF root trait, and a one-way ANOVA was performed to assess differences between species. All the analyses were run using PC-Ord (McCune and Mefford, 1995) for the NMDS and STATISTICA 7.0 (StatSoft Inc., Tulsa, OK. USA) for the inferential tests.

3. Results

3.1. Fertilization effect

For the NMDS, a randomization test (n = 50 runs) revealed that the first two axes explained a significant portion of the variation ($P = 0.02$). The final stress values (Standardized Residual Sum of Squares = 0.21) indicated that the ordination matrix distances reflected those in the original distance matrix. When the planted seedlings were arrayed in ordination space (Figure 2), an obvious separation

occurred between fertilized seedlings and unfertilized seedlings, mainly along NMDS axis 1. The ES hypothesis was supported when the ordination scores were compared for fertilized vs. unfertilized seedlings, and a highly significant difference was observed for axis 1 (t = 10.24, $P < 0.001$). This axis explained 49.2% of the total variance in the original matrix and was positively correlated with the RGR_D and RGR_H and negatively correlated with the RMF, thus indicating a gradient among seedlings with greater RGRs (HN availability) and those with a greater RMF (LN availability). The second NMDS axis, which explained 22.3% of the original variation, was positively correlated with the LMF and negatively correlated with the leaf P (P_L) concentration (see Table 2 for the product-moment correlations). There was a discrete separation when fertilized vs. unfertilized seedlings were compared along NMDS axis 2 (t = 2.12, P < 0.05) caused mainly by the low P_L and increased LMF traits responses of the *Acacia* sp. under the LN treatment.

3.2. Species responses to LN and HN treatments

The species were assessed with respect to their separate responses to LN and HN treatments. For both cases, the NMDS ordinations revealed two major gradients in the dataset. Randomization tests (n = 50 runs) revealed that the first two axes explained significant amounts of variation ($P < 0.04$). The final stress values (< 0.25) indicated that the ordination matrix distances reflected those in the original distance matrix. When the seedlings were arrayed in the ordination spaces (Figure 3), a clear separation was not observed between species subjected to the LN treatment (Figure 3A), whereas a more obvious separation was observed for those with the HN treatment (Figure 3B). Indeed, significant differences were observed only between *Acacia* sp. and the other species (Tukey's test, $P <$ 0.01 for all comparisons) for NMDS axis 1 in the LN treatment, indicating that the species respond differently to ES. This axis captured 37.3% of the total variation in the original matrix and was negatively correlated with RGR_H and RGR_D and positively correlated with the RMF, suggesting that in such degraded unfertile sites, i) *Acacia* sp. seedlings are able to invest in aerial growth and ii) the other species primarily need to invest in the root system. Significant differences along NMDS axis 2 (F = 4.34, $P < 0.01$), were found only between *C. tocantinum* vs. *D. odorata* and *C. fairchildiana*. This axis explained 22.4% of the total variation, capturing higher values for P_L concentrations and RMF in the unfertilized *C. tocantinum* seedlings.

Conversely, highly significant differences were observed among the species for both NMDS axis 1 (F = 80.71, $P < 0.001$) and axis 2 (F = 40.33, $P < 0.001$) in the HN treatment. This result partially confirmed our second hypothesis stating that species assume different strategies from each other (Tukey's test, $P < 0.018$ for all comparisons) except *C. fairchildiana* vs. *I. edulis* ($P = 0.86$) and *C. tocantinum* vs. *D. odorata* ($P = 0.82$) for NMDS axis 1. This axis captured 45.7% of the total variation and was positively correlated with the RGR_{Bio}, stems and leaf tissue production (*Acacia* sp.)

and negatively correlated with the RMF (*D. odorata*). *D. odorata* and *S. reticulata* differed from the other species along NMDS axis 2 ($P < 0.02$ and $P < 0.004$, respectively, for all comparisons). This axis explained 31.2% of the total variation and was positively correlated with the LMF and leaf N:P (N:P_L) ratio (*D. odorata*) and negatively correlated with P_L concentrations and the SMF (*S. reticulata*) (Table 2). Our results also indicate the correlation between LMF and N:P_L ratio traits, suggesting a strategy of late successional *D. odorata*, while P_L and SMF traits are correlated to a response to stress conditions in *S. reticulata*. For the HN treatment, the differentiation in species growth was mainly related to the BNF root trait as revealed by the NMDS ordination, which showed a clear separation between N_2-fixing and non-N_2-fixing species along NMDS axis 1 (Figure 3B). Indeed, the two groups differed significantly along axis 1 (t = 12.70, $P < 0.001$) but not along axis 2 (t = 0.015, $P = 0.37$). However, for the LN treatment, a clear separation was not observed between these two ecological groups (Figure 3A). According to the ES, the BNF appears to be a fast acquisitive trait, although its effects were dependent on the individual nutritional status.

Table 2: Product-moment correlations between 10 growth, allocation and nutrient variables and NMDS for the fertilization, species and BNF effects for the six species tested 24 months after planting. The relative growth rates in diameter (RGR_D), height (RGR_H) and biomass (RGR_{Bio}); biomass allocations of the leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF); shoot-to-root ratio (S:R); biomass growth of the leaf (Leaves), stem (Stems) and root (Roots) tissues; phosphorous (P), nitrogen (N) concentrations and nitrogen-to-phosphorous ratio (N:P) of leaves ($_L$), stem ($_S$) and root ($_R$) tissues, under high (HN) and low nutrient (LN) availability.

Variables	Units	Fertilization treatment		Species and BNF - HN		Species and BNF - LN	
		r		*r*		*r*	
Growth variables		Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
RGR_D	mm mm^{-1} year^{-1}	**0.94**	-0.07	0.82	-0.50	**0.86**	-0.17
RGR_H	cm cm^{-1} year^{-1}	**0.93**	0.06	0.85	-0.14	**0.91**	-0.20
RGR_{Bio}	kg kg^{-1} year^{-1}	0.74	-0.06	**0.92**	0.09	0.57	-0.25
LMF	kg kg^{-1}	0.21	**0.75**	-0.07	**0.84**	0.72	0.25
SMF	kg kg^{-1}	0.72	-0.20	0.42	-0.64	0.32	**0.32**
RMF	kg kg^{-1}	**-0.85**	-0.36	**-0.65**	-0.19	**-0.81**	**-0.43**
S:R	ratio	0.75	0.29	0.67	0.25	0.75	0.03
Leaves	kg dry mass	0.74	0.02	**0.91**	0.18	0.62	-0.24
Stems	kg dry mass	0.73	-0.07	**0.91**	0.07	0.54	-0.24
Roots	kg dry mass	0.76	-0.15	0.90	-0.04	0.56	-0.27
Nutrient variables							
N_L	g kg^{-1}	-	-	0.60	-0.14	-	-
P_L	g kg^{-1}	0.55	**-0.66**	0.03	**-0.90**	-0.18	**-0.68**
$N:P_L$	ratio	-	-	0.26	**0.79**	-	-
N_S	g kg^{-1}	-	-	0.49	0.24	-	-
P_S	g kg^{-1}	-	-	0.53	0.25	-	-
$N:P_S$	ratio	-	-	-0.10	0.02	-	-
N_R	g kg^{-1}	-	-	0.25	0.05	-	-
P_R	g kg^{-1}	-	-	0.26	-0.25	-	-
$N:P_R$	ratio	-	-	0.07	0.42	-	-
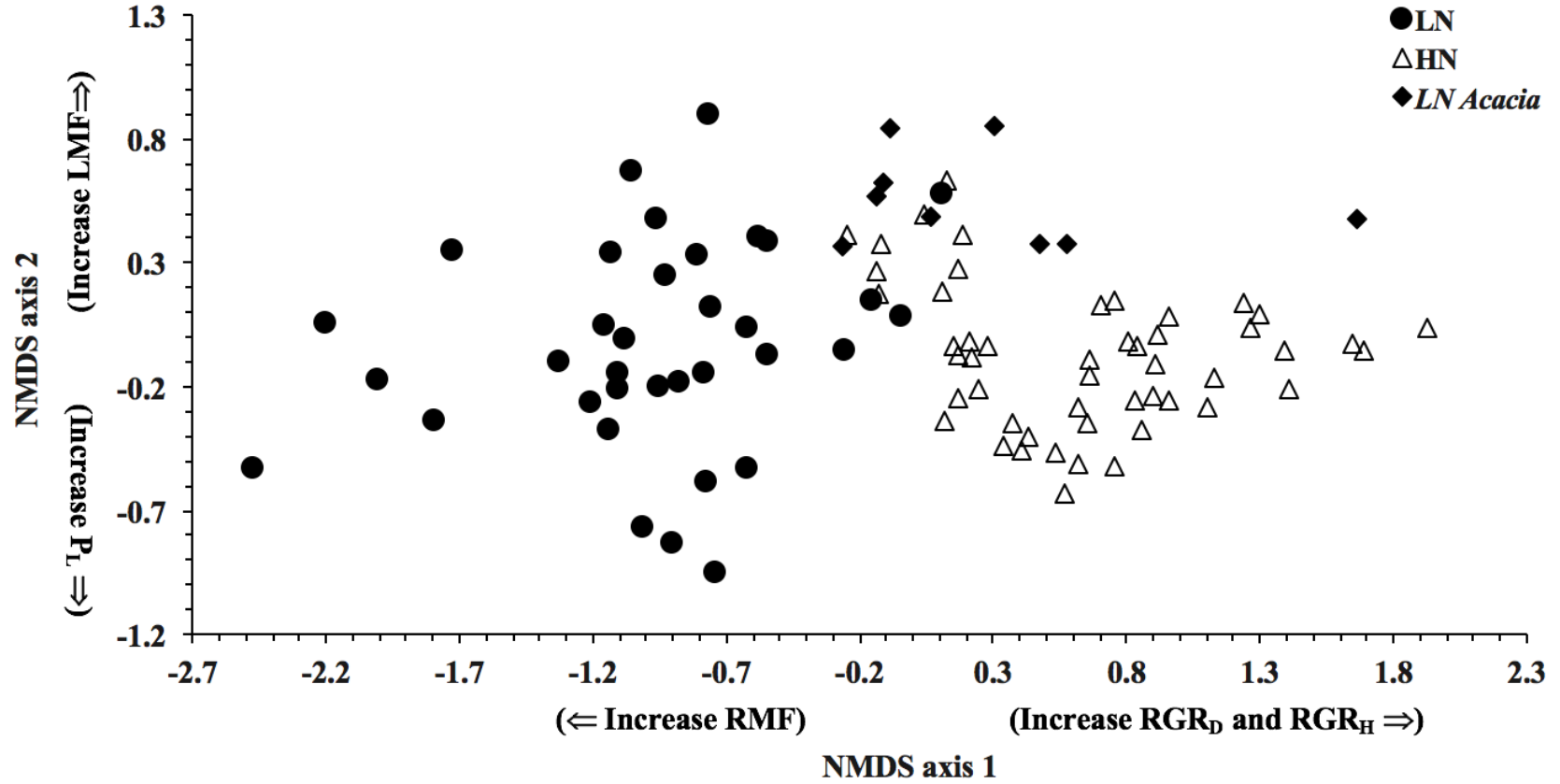
Figure 2: NMDS ordination diagram of 94 seedlings in the low nutrient (LN) and high nutrient (HN) treatments based on 10 individual growth and biomass allocation variables; relative growth rates in terms of diameter (RGR_D) and height (RGR_H); and mass fractions of the root (RMF) and leaf (LMF) and leaf P (P_L) concentration.
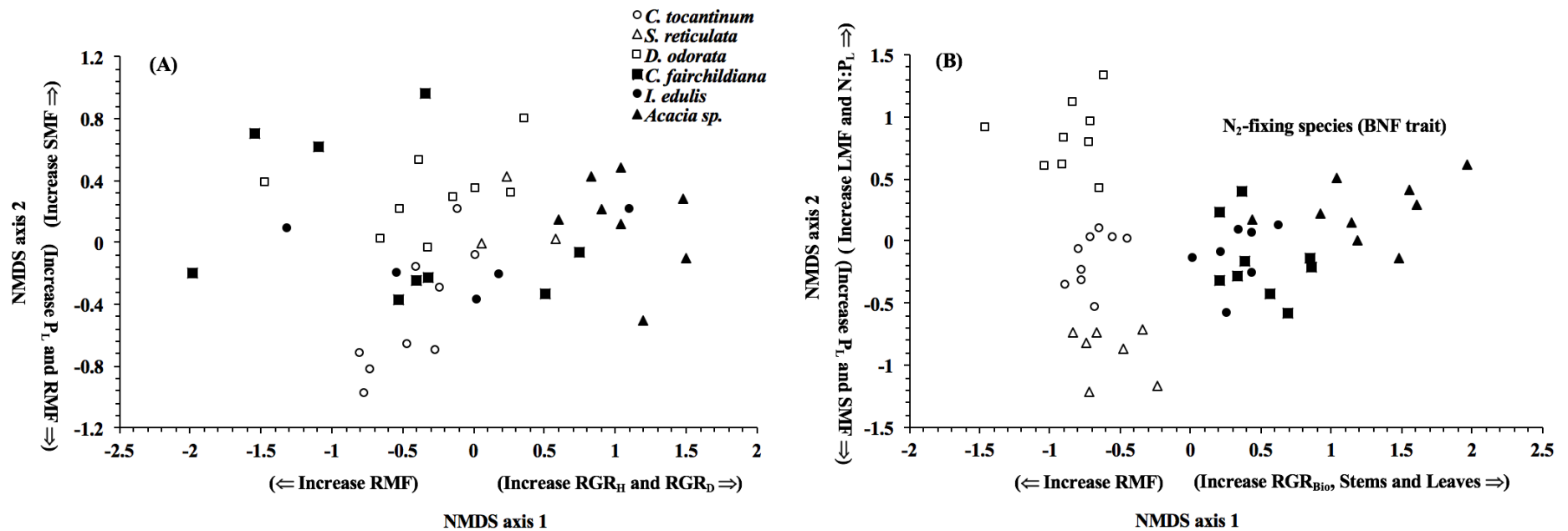
Figure 3: NMDS ordination diagrams of 94 seedlings based on 10 individual growth and biomass allocation variables; relative growth rates in the biomass (RGR$_{Bio}$), diameter (RGR$_D$) and height (RGR$_H$); mass fractions of the root (RMF); leaf (LMF) and stem (SMF); stem (Stems) and leaf (Leaves) tissues production and; leaf P (P$_L$) concentration and nitrogen-to-phosphorous (N:P$_L$) ratio for all six species in the LN (A) and HN (B) treatments. Open symbols correspond to the non-N$_2$-fixing species, and closed symbols correspond to the N$_2$-fixing species.

4. Discussion

4.1. Fertilization treatment effects

Species increased the growth mainly in diameter and height under fertilization treatments as has also been found in many trees species corroborating to the ES hypothesis (Bown et al., 2009; Xia and Wan, 2008). The effect of higher growth under fertilization is due to the increased concentrations and diffusion rates of nutrients in the soil, affecting the metabolism and stoichiometric relationships (Ågren and Weih, 2012; Cernusak et al., 2010; Chapman et al., 2012). The effect of high N availability on growth can be correlated to the increase in photosynthesis and leaf area (Li et al., 2012; Li and Korpelainen, 2015). Although less documented P addition effects on growth may be related to higher photosynthetic rates and P-use efficiency (Li et al., 2016). K addition can also stimulate growth as observed in *Populus*, *Pinus* and *Eucalyptus* genus (Ache et al., 2010; Laclau et al., 2009; Smethurst et al., 2007).

Our findings also support the wood ES hypothesis as the increased height and diameter growth under high nutrient availability can be negatively correlated with the RMF and wood density (Chao et al., 2008; Chave et al., 2009). The diameter-height relations in trees are influenced by the environmental conditions such as edaphic factors and can be sustained by mechanical features (Henry and Aarssen, 1999; Niklas, 2007). Apparently, the shoot-to-root (S:R) ratio is a parameter of the nutrient status of plants with a decreased allocation of NSC to root tissues under fertilization also observed in *Betula* and *Salix* genus (Glynn et al., 2007; Kleczewski et al., 2010).

In our study, aboveground growth traits were more responsive to fertilization treatments than the S:R ratio, although N_2-fixing *C. fairchildiana* and *I. edulis* showed approximately three-fold increases in S:R ratio values under fertilization. Additionally, higher responses to fertilization have been found in early successional and non-legume vs. late successional and N_2-fixing species respectively (LeBauer and Treseder, 2008; Xia and Wan, 2008), our results were inconclusive in this regard. Nevertheless, the late successional *C. tocantinum* (non-N_2-fixing) species was the most responsive to the fertilization and produced approximately 200 times more biomass under HN availability suggesting a synergistic effect of the addition of multiple nutrients (Appendix 1).

4.2. Nutrient stress (unfertilized treatment effects)

In extreme LN availability, both metabolism and growth rates are drastically reduce caused by an exceedingly limited energy (Kleczewski et al., 2010). Nutritional limitations on growth may be related to a decrease in amino acids, proteins, chlorophyll and carbohydrate contents (Ågren et al., 2012; Fisher et al., 2012; Morcuende, 2007). The growth seedlings of LN availability was impaired supposedly by reduced metabolic and biosynthetic processes, including BNF and gene expression (Baena-González and Sheen, 2008; Usadel et al., 2008).

LN availability may cause a series of changes in the morphological and functional traits of species and ecosystems (Quesada et al., 2010). One of the strongest responses to nutrient starvation is the increased allocation of NSC (e.g., starch and soluble sugars) to root tissues with a consequent increase in the RMF, enhancing soil foraging and nutrient uptake (Hermans et al., 2006; Poorter et al., 2012; Turner, 2008; Valladares et al., 2007). The mean RMF values for the six tested species were two-fold higher in LN vs. HN plants (Figure 4). Consistent with the ecosystem ES, the increased RMF under LN availability indicates a conservative strategy that is negatively correlated with the RGR_D and RGR_H (Reich, 2014). Common plant responses to either N and P starvation also include increased reactive oxygen species (ROS) and root exudates and reduced biological interactions and shoot growth (Kannenberg and Phillips, 2017).

Acacia sp. responses to LN availability somehow diverged from the ES assumptions with significantly high growth rates under nutrient starvation (Figure 5). By mixing above and belowground acquisitive and conservative strategies the species was able to overcome multiple nutrient starvation similarly to *Larix* species (Withington et al., 2006). Our analysis suggests that high LMF and RGR_D is a fast strategy while low P_L concentration is a slow strategy for *Acacia* sp. under LN treatment as LMF and P_L are negatively correlated. Other acquisitive strategies of the species may be related to increased photosynthesis and BNF, root production, foraging and exudate release, whereas conservative traits may be related to the enhanced nutrient remobilization, catabolism, leaf lifespan and AMF interactions (Chaer et al., 2011; Faucon et al., 2017; Raven and Andrews 2010).

4.3 Species and BNF effects

Confirming our second hypothesis, different ecological groups (early successional vs. late successional and N_2-fixing vs. non-N_2-fixing species) influenced the growth traits in fertilized individuals. In contrast, the pioneer species *S. reticulata* did not respond according to ES, which we assume to be related to drought effect caused during and after 2015/16 El Niño event. Evidence from the NMDS analysis indicates that the stressed traits of *S. reticulata* are correlated to the increased P_L concentration and SMF. Unfertilized individuals also did not support the ES hypothesis as no effects of species or ecological groups were observed in the LN treatment.

Considering the HN treatment, the early successional species acquisitive strategies were positively correlated with increased RGR_{Bio}, stem and leaf tissues production, favoring increased resource uptake (Hodge et al., 1999). The higher biomass growth of the fast-growing species may be correlated to the increased gas exchange rates (e.g., photosynthesis and transpiration), light- and nutrient-use efficiency (Ashton et al., 2010; Freschet et al., 2015; Jaquetti et al., 2016). Adjustments in root architecture by acquisitive species enhance root proliferation in nutrient-rich patches favoring

nutrient forage and uptake, thus supporting continuous growth under fluctuating environments (De Smet et al., 2012; Ma et al., 2017; Trewavas, 2005).

Extremely productive N_2-fixing species such as *C. fairchildiana*, *I. edulis* and *Acacia* sp., may also have access to different nutrient pools in the soil through increased root exudates (Lambers et al., 2011). In terms of absolute growth, the individual *Acacia* sp. biomass growth rates (53.1 kg year^{-1} plant^{-1}) were approximately 2.5-times higher than other N_2-fixing species and 66-times higher than that of late successional *D. odorata* two years after establishment (Appendix 1).

Slow conservative strategies of late successional species were positively correlated with RMF and negatively correlated with the RGR_{Bio} as a trade-off for longevity (Chambers et al., 1998; Wright et al., 2004). The low photosynthesis and transpiration along with energetically expensive long-lived leaves can partially explain the reduced growth of conservative species (Donovan et al., 2011). As slow-growing species have great ecological relevance in restoring biodiversity and long-term C and N cycles, they should be considered to provide both ecosystem services and economic benefits in FR (Lima and Vieira, 2013; Rodrigues et al., 2009; Suding et al., 2015). Additionally, increased LMF and $N:P_L$ ratio which were positively correlated indicate a strategy of late successional *D. odorata* and early successional *Acacia* sp. for managing the degraded and nutrient-variable environment.

Corresponding to an acquisitive strategy, BNF root trait strongly increased the biomass growth rates of N_2-fixing species, which may be related to increased photosynthetic rates and N_L concentrations and a lower C:N ratio, and these traits would have great ecological relevance (Faucon et al., 2017; Jaquetti et al., 2014). Additionally, increased sink strength caused by root nodule metabolism may also be related with the increased growth of N_2-fixing species. In our study, N_2-fixing species increased N_L concentration and $N:P_L$ ratios (Appendix 2), although these traits were less responsive than growth traits (see Table 2). In contrast to P addition, under high N availability, N_2-fixing species can downregulate N_2 fixation, which may provide an advantageous strategy for N homeostasis in a fluctuating environment during and after fertilization (Gentili and Huss-Danell, 2003; Hedin et al., 2009; Murray et al., 2017; Omena-Garcia et al., 2015). Additionally, allocating more NSC to efficient nodules N_2-fixing species may enhance the N uptake and N-use efficiency (El Msehli et al., 2011). Evidence also suggests that AMF may interact synergistically with rhizobia to increase N_2 fixation rates and growth (Andrade et al., 2004; Bulgarelli et al., 2017).

Nonetheless, under LN availability, BNF effects were unclear due to energy deficiency since the nodule region represents a strong sink for carbohydrates (Khamzina et al., 2009; Jeudy et al 2010). Additionally, the expression of most genes involved in nodule organogenesis and development may be downregulated under Ca, boron and molybdenum deficiencies (Redondo-Nieto et al., 2012; Reguera et al., 2009).

4.4 Ecological and economic considerations of N$_2$-fixing species and FR

One of the primary objectives of restoring degraded ecosystems is to increase soil organic matter and plant biomass to support more complex and diverse communities (Chaer et al., 2011; Six et al., 2002; Suding et al., 2015). Indeed, in extreme LN environments, evidence indicates that restoring the natural C cycle may be accomplished only if accompanied by an increase in the N stocks via stoichiometric relationships as FR failures are frequently caused by nutrient limitations (Hungate et al., 2003; Siddique et al., 2008; Yang et al., 2011).

The C sequestration rates of N$_2$-fixing species were estimated approximately 18.30 Mg C ha^{-1} year^{-1} and may be traded in the emerging C market (Khamzina et al., 2009; Visseren-Hamakers et al., 2012). Including the BNF process in FR can greatly reduce the time required to rebuild the C and N stocks and may also reduce the need for external inputs when implementing forestry management (Coskun et al., 2017; Macedo et al., 2008). N$_2$-fixing species can also influence the restoration of the P cycle through the release of nitrogen-rich extracellular phosphatase enzymes in the rhizosphere (Houlton et al., 2008). *Inga* and *Acacia* are well-established nursing species that improve the early growth of interplanted species, such as *Eucalyptus* spp. and *Terminalia amazonia* (Forrester et al., 2006; Nichols and Carpenter, 2006; Nouvellon et al., 2012; Pfautsch et al., 2009; Yang et al., 2009).

The crown architecture and high productivity of *Acacia* sp. indicate its considerable potential for use in energy plantations, and this species can become established on marginal degraded landscapes or in legal reserve areas as predicted by Brazilian's law enhancing energy security in the Amazon region (Adams et al., 2010; Nepstad et al., 2009; Soares-Filho et al., 2014). However, the use of alien species such as *Acacia* sp. in FR must be carefully considered because these species may become invasive in certain open landscapes, such as savannahs and grasslands; therefore, legislative criteria and monitoring must be properly implemented (D'Antonio and Meyerson, 2002; Emms et al., 2005).

The approach presented here constitutes a key tool for understanding the impact of nutrient starvation on the carbon balance in FR in Amazonia, a region that is being increasingly subjected to stress from deforestation and climatic changes. In view of this, perhaps the major challenge is to deepen the understanding of the establishment and growth of tree species in the open field with an emphasis on species that present high performance for growth and high functional plasticity, as displayed by some tree legumes. Additionally, including functional traits such as nutrients enhances the resolution of multivariate analyses and therefore should be encouraged to refine Dynamic Global Vegetation Models (DGVM). Stem and root N and P concentrations were less responsive traits, indicating a lower metabolic functional activity of these organs compared to that in the leaf site. Lastly, we show that functional leaf, root and wood traits are influenced by different Leguminosae tree

species and environmental conditions and demonstrate that a strong foundation of tree ecophysiology in plantations can be established, which has many practical applications for the FR in the Amazon basin and elsewhere.
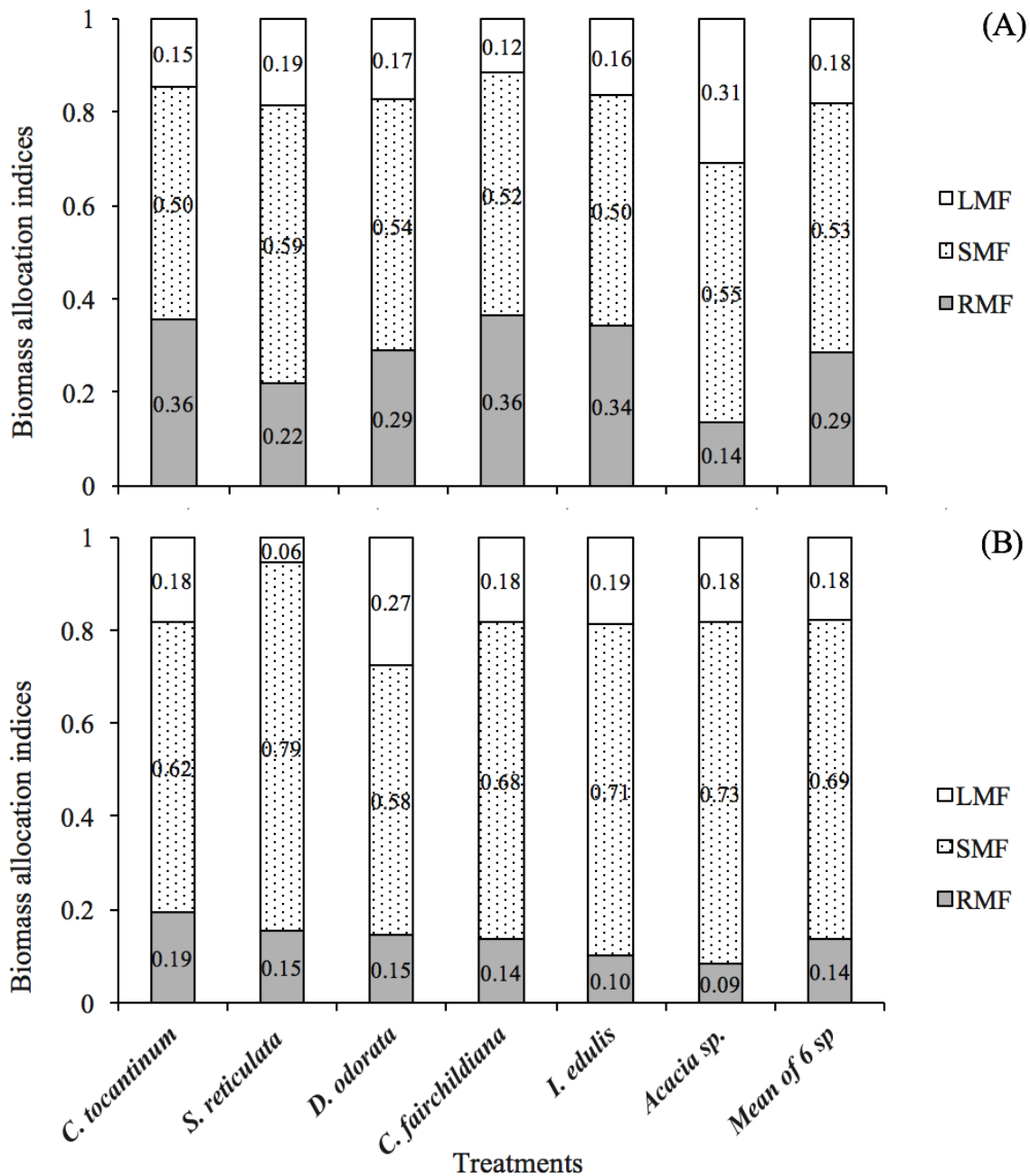
Figure 4: Biomass allocation indices of the leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF) of all six studied species in the LN (A) and HN (B) treatments.
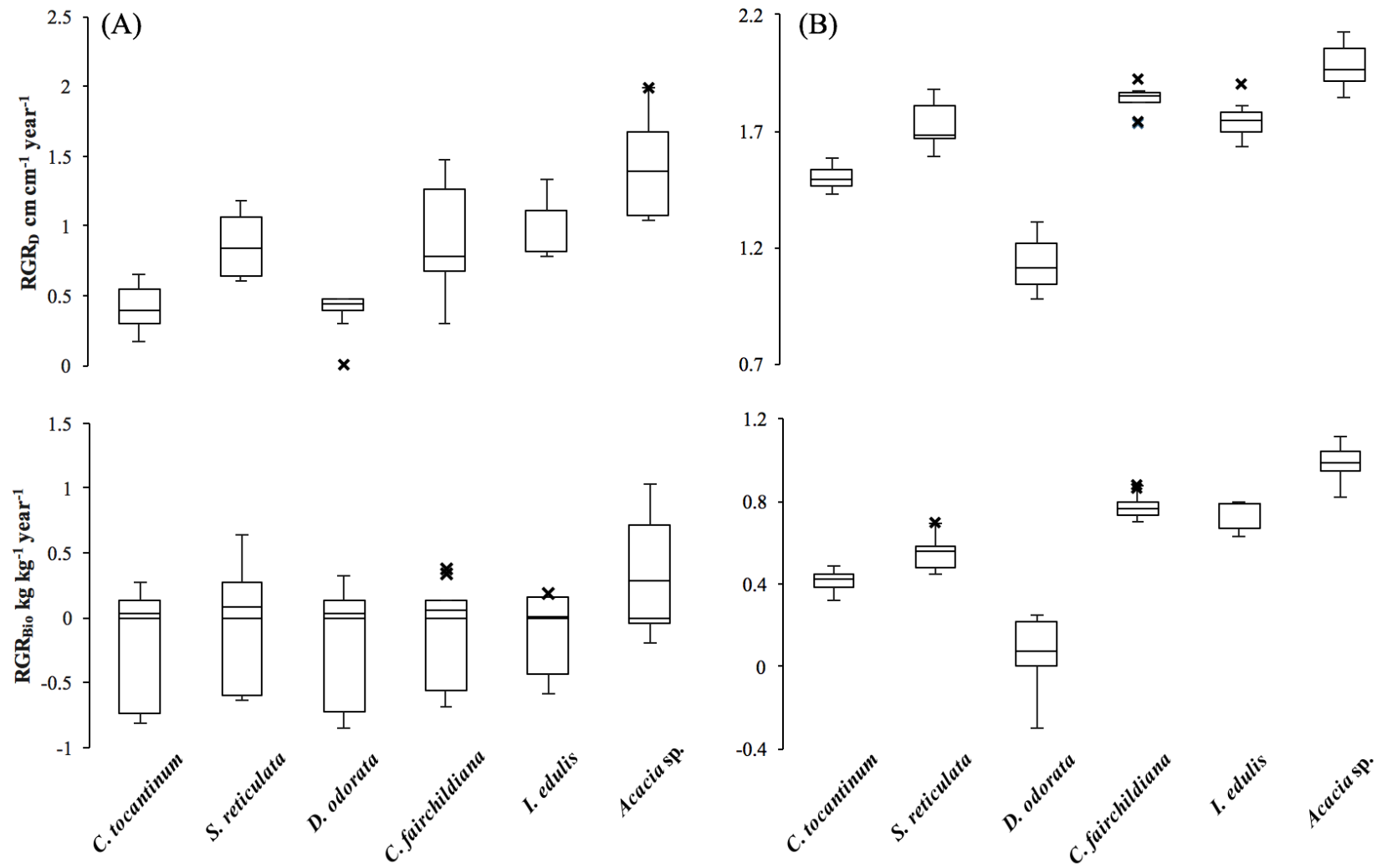
Figure 5: Box plot based on the relative growth rates in the diameter (RGR_D) and biomass (RGR_{Bio}) of all six studied species in the LN (A) and HN (B) treatments.

4. Conclusions

Nutrients determine the establishment and growth of tree legumes in the open field. Plant growth was impaired under extreme LN availability in the open field. In response to nutrient starvation, most species increased C allocation to root tissues, and such allocation greatly increased the RMF. *Acacia* sp. somehow evolved fast and conservative strategies in root and shoot traits, exhibiting significant growth under harsh conditions. Increased RGR_H and RGR_D was an acquisitive strategy of species under HN availability, while RGR_{Bio} and woody biomass production was correlated to fast-growing species. The BNF root trait is a fast acquisitive strategy that is positively correlated to RGR_{Bio} and negatively with RMF. Therefore, for *Acacia* sp./ mimosoid clade, the hypothesis of the efficiency of the early successional species, fixing nitrogen in open field with low nutrient was confirmed. But, it does not appear to be a universal strategy for all legume trees. Finally, N_2-fixing species should be thoroughly investigated because they may play a crucial role in important global biogeochemical cycles when applied on a short or large scales.

Abbreviations: forest restoration (FR), biological N_2 fixation (BNF), arbuscular mycorrhizal fungi (AMF), economics spectrum (ES), leaf mass fraction (LMF), stem mass fraction (SMF), root mass fraction (RMF), relative growth rates in diameter (RGR_D) height (RGR_H) and biomass (RGR_{Bio}), collar diameter (CD), stem length (SL), shoot-to-root ratio (S:R), high nutrient (HN) and low nutrient (LN) treatments, phosphorous concentration in leaves (P_L) stems (P_S) and roots (P_R), nitrogen concentration in leaves (N_L) stems (N_S) and roots (N_R), nitrogen-to-phosphorous ratio in leaves ($N:P_L$) stems ($N:P_S$) and roots ($N:P_R$), non-metric multidimensional scaling (NMDS).

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Appendix 1: Stem, leaf and root tissues production, absolute growth rate in biomass (AGR_{Bio}), stem length (SL), collar diameter (CD) and survival for the six species tested in the low nutrient (LN) and high nutrient (HN) treatments 25 months after planting. Mean of nine plants (± SD).

Species	Treatment	Stems	Leaves	Roots	AGR_{Bio}	SL	CD	Survival
		...............(kg plant^{-1})...............			kg year^{-1} plant^{-1}	m	cm	%
C. tocantinum		0.02 ± 0.0	0.01 ± 0.0	0.01 ± 0.0	0.02 ± 0.0	0.63 ± 0.2	1.89 ± 2.2	91.7
S. reticulata		0.22 ± 0.3	0.07 ± 0.1	0.08 ± 0.1	0.18 ± 0.3	1.40 ± 0.7	2.25 ± 1.2	16.7
D. odorata	LN	0.03 ± 0.0	0.01 ± 0.0	0.01 ± 0.0	0.02 ± 0.0	0.70 ± 0.2	1.14 ± 0.2	83.3
C. fairchildiana		0.75 ± 1.5	0.26 ± 0.5	0.37 ± 0.7	0.64 ± 1.2	1.12 ± 0.9	3.00 ± 2.4	66.7
I. edulis		0.36 ± 0.7	0.17 ± 0.4	0.09 ± 0.1	0.29 ± 0.5	1.29 ± 0.4	2.50 ± 1.3	22.2
Acacia **sp.**		13.75 ± 29.1	4.50 ± 7.1	1.61 ± 2.3	9.53 ± 18.0	3.33 ± 0.2	6.92 ± 5.6	86.1
C. tocantinum		5.12 ± 1.7	1.42 ± 0.4	1.60 ± 0.6	3.76 ± 1.1	2.88 ± 0.5	6.88 ± 0.7	100
S. reticulata		11.84 ± 5.6	0.78 ± 0.3	2.23 ± 0.9	7.13 ± 3.2	3.75 ± 1.1	11.66 ± 2.7	77.8
D. odorata	HN	1.05 ± 0.7	0.47 ± 0.3	0.24 ± 0.2	0.81 ± 0.5	2.68 ± 0.8	3.37 ± 0.7	91.7
C. fairchildiana		33.00 ± 12.5	8.53 ± 3.6	6.56 ± 2.9	23.08 ± 7.7	4.54 ± 1.1	14.33 ± 1.9	100
I. edulis		24.50 ± 8.0	6.73 ± 2.9	3.50 ± 1.4	16.67 ± 6.3	4.47 ± 0.8	11.94 ± 2.6	80.5
Acacia **sp.**		81.84 ± 34.9	20.04 ± 8.3	8.82 ± 3.6	53.14 ± 21.8	8.19 ± 1.3	19.96 ± 4.0	97.2

Appendix 2: Phosphorous and nitrogen concentrations in leaves (P_L and N_L), stems (P_S and N_S) and roots (P_R and N_R); nitrogen-to-phosphorous ratio in leaves (N:P_L), in the high nutrient (HN) treatment and phosphorous concentrations in leaves (P_L) in the low nutrient (LN) treatment.

Species	Variables							
	P_L	N_L	P_L	N:P_L	N_S	P_S	N_R	P_R
	g kg^{-1}	.............(g kg^{-1})............		ratio	...............................(g kg^{-1})..............................			
Treatment	**LN**				**HN**			
C. tocantinum	3.26 ± 1.6	17.14 ± 2.9	1.41 ± 0.3	12.46 ± 2.7	4.30 ± 0.9	0.32 ± 0.1	4.71 ± 0.6	0.32 ± 0.0
S. reticulata	1.18 ± 0.4	18.83 ± 2.9	1.73 ± 0.2	10.89 ± 1.6	2.83 ± 0.5	0.25 ± 0.1	3.26 ± 0.5	0.37 ± 0.1
D. odorata	0.72 ± 0.2	18.11 ± 1.2	0.73 ± 0.1	25.62 ± 5.0	4.43 ± 1.2	0.37 ± 0.1	4.10 ± 0.9	0.26 ± 0.1
C. fairchildiana	1.22 ± 0.3	29.41 ± 2.3	1.52 ± 0.2	19.01 ± 1.2	5.91 ± 1.1	0.54 ± 0.1	5.58 ± 0.9	0.42 ± 0.1
I. edulis	0.84 ± 0.4	29.83 ± 1.8	1.42 ± 0.1	21.16 ± 2.2	5.53 ± 1.7	0.44 ± 0.1	5.69 ± 1.0	0.41 ± 0.1
Acacia **sp.**	0.83 ± 0.4	22.32 ± 1.2	1.06 ± 0.1	21.31 ± 2.4	5.51 ± 0.7	0.43 ± 0.0	4.17 ± 0.6	0.25 ± 0.2

Functional responses of tree legumes to low vs. high water and nutrient: Linking economics with carbohydrates dynamics

Functional responses of tree legumes to low vs. high water and nutrient: Linking economics with carbohydrates dynamics[1]

Running title: Fertilization and seasonality effects of tree legumes

Highlight: Plant strategies are important for the establishment in degraded environment. Species respond to high resources increasing photosynthesis, growth and light use efficiency whereas increasing energy dissipation under limited resources.

Roberto K. Jaquetti[2], José Francisco C. Gonçalves[2],*,

[2]National Institute of Amazonian Research - INPA, Laboratory of Physiology and Biochemistry, Ave. André Araújo, 2936, Aleixo, 69011-970 - Manaus, AM, Brazil. *corresponding author: jfc@inpa.gov.br, tel: +55 92 3643-1938; jaquettiroberto@gmail.com

Abstract

Plants growth is majorly constrained by water and nutrients whereas species may adjust functional traits to fluctuating and stressful conditions. Under high resource availabilities traits related to the fast side of economics spectrum axes are enhanced and so do sink strength in growth tissues. Adjustments in functional traits including growth, water potential, nutrient content, gas exchange, non-structural carbohydrates (NSC), photosynthetic pigments and chlorophyll fluorescence of six tree legumes species were studied with reference to high vs. low water and nutrients treatments carried out in a degraded area in central Amazon. The high resource economics spectrum axes were mostly confirmed in principal component analysis (PCA) axis 1 with increased photosynthesis, transpiration, relative growth rate in diameter and performance index of chlorophyll fluorescence (PI_{ABS}). Slow traits were positively correlated with intrinsic water-use efficiency, dissipation energy flux (DI_0/RC), root mass fraction and starch in root tissues. PCA axis 2 captured the stressed condition of one species as increased leaf calcium was related to drought stress. Our results demonstrate a tight correlation between important functional process through regulation of gas exchange, light use-efficiency and NSC allocation. Source-sink partitioning seems an important strategy for growth of species despite that sink strength apparently play a major role.

Key words: biomass allocation, chlorophyll fluorescence, drought stress, economics spectrum, fertilization treatment, forest restoration, photosynthesis, rainy season

Abbreviations: NSC, non-structural carbohydrates; PCA, principal component analysis; RGR_D, relative growth rate in diameter; PI_{ABS}, performance index of chlorophyll fluorescence; DI_0/RC, effective dissipation of an active reaction center; N, nitrogen; P, phosphorous; K, potassium; Ca, calcium; Mg, magnesium; C, carbon; P_n, net photosynthetic rates; g_s, stomatal conductance; *WUE*, water use efficiency; BNF, biological N_2 fixation; *E*, transpiration rates; RMF, root mass fraction; SS, soluble sugars; *LA*, leaf area; S:R, shoo-to-root ratio; ES, economics spectrum; *ABS/RC*, effective antenna size of an active reaction center; Zn, zinc; *IWUE*, intrinsic water use efficiency; N:P, leaf nitrogen-to-phosphorous ratio; $SS:Starch_L$, leaf soluble sugar-to-starch ratio; F_0, initial fluorescence; Ψ_L, leaf water potential; *PCaUE*, photosynthetic calcium use efficiency; *PMgEU*, photosynthetic magnesium use efficiency; $NSC_L:NSC_R$, leaf-to-root NSC ratio; R_d, dark respiration rates; LMF, leaf mass fraction.

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[1]Chapter formatted according with *Journal of Experimental Botany* instructions

Introduction

Resource availabilities determine growth rate of plant species in the field. The plasticity of species is regulated by the perception to environmental cues adjusting functional traits to obtain appropriate uptake and use of resources (Freschet et al., 2015; Satbhai et al., 2015). Abiotic factors such as irradiance, temperature, water and nutrients can severely constraint growth of plants. Low soil water availability reduces the productivity of most ecosystems, and about 16-28% of global terrestrial plant productivity is estimated to be reduced by nutrient limitations, particularly nitrogen (N) and phosphorous (P), and to some extent potassium (K), calcium (Ca), magnesium (Mg) and other micronutrients affecting the carbon (C) cycle (Vitousek and Howarth, 1991; Elser et al., 2007; Heimann and Reichstein, 2008; Fisher et al., 2012).

After reducing growth, the down regulation of net photosynthetic rates (P_n) is usually the first response to drought (Flexas et al., 2004; Cramer et al., 2011). Additionally, the decreased photosynthesis and transpiration rates (E) under mid-drought are related to the reduced stomatal conductance (g_s) and the consequent decreased CO_2 diffusion from the atmosphere to the carboxylation site (Pinheiro and Chaves, 2011). Stomatal responses to drought are controlled by K, abscisic acid, osmotic and secondary metabolites signaling (Rolland et al., 2006; Osakabe et al., 2014; Kuromori et al., 2018). Under mid-drought soluble sugars (SS) tend to increase caused by the reduced growth thus maintaining metabolic activity, meanwhile starch content may decrease caused by the depletion of storage pools and repression of starch synthesis (Lawson, 2009; Pinheiro and Chaves, 2011).

Drought can also affect tree nutritional status by decreasing nutrient availability and uptake (Kreuzwieser and Gessler, 2010). Many lines of evidence suggest that N_2 fixation rates of nodules can also be reduced under drought reducing the sink strength of N_2-fixing species (Streeter, 2003; Naya et al., 2007). Drought effects on photosynthesis and growth, may be alleviated under high N, P, and/or K availability, by enhancing the stomatal control responses thus increasing water use efficiency (*WUE*) (Ripullone et al., 2004; Waraich et al., 2011; Zahoor et al., 2017). The drought factor is especially important in the tropics because in areas where rainfall has seasonality like in the Amazon forest, it suggests direct relation between water deficiency and the carbon balance (Bonal et al., 2016).

Negative effects of N and P deficiencies on plants have been demonstrated and photosynthesis always decreases (Kant et al., 2011; Warren, 2011; Li et al., 2016). Transpiration may increase under low nutrient as observed in *Ficus insipida* and other species (Cernusak et al., 2007; Cramer et al., 2008; Garrish et al., 2010). Combined N and P limitations have shown to decrease productivity of Amazonian secondary forests (Davidson et al., 2004). Nonetheless, the primary productivity of natural Amazonia forest is mainly controlled by soil P availability (Quesada et al., 2012). Therefore, species have evolved different mechanisms to acquire, transport and use nutrients increasing nutrient use-efficiency (Castro-Rodríguez et al., 2017).

Photosynthetic gas exchange studies has been developed for some native Amazonian species but little is known about the nitrogen use efficiency in such tropical emergent species like some tree legumes (Omena-Garcia et al. 2011; Justino et al., 2017). Biological N_2 fixation (BNF) converts available atmospheric dinitrogen (N_2) gas to N mineral increasing fertilizer use efficiency and soil fertility, therefore N_2-fixing species are strongly recommended for use in forest restoration due to the connection with carbon metabolism and growth of plants (Jaquetti and Gonçalves, 2017; Sprent et al., 2017; Batterman, 2018; Good, 2018). However, when carbohydrates accumulate in leaves, photosynthesis is suppressed. Probably, low nitrogen content is related with this negative effect (Araya et al. 2010). Despite the importance of the issue, the interaction between carbohydrate and nitrogen limitation in leaf photosynthesis has not been examined intensively in tree legumes.

Under low N and P availability a greater allocation of non-structural carbohydrates (NSC) to roots often increases root mass fraction (RMF) due to the facilitated movement of SS from the phloem to the root which can then be converted to starch (Wissuwa et al., 2005; Hermans et al., 2006; Hammond and White, 2008). According to with the "multiple limiting resources hypothesis" the combined low soil availabilities of nutrients such as N, P, Ca and Mg can mainly constraint plant growth in degraded environments (Ågren et al., 2012; Jaquetti et al., 2016). In such N- and P-poor soils biological N_2 fixation of some tree legumes may be an effective strategy to overcome nutrient limitations (Hughes and Denslow, 2005; Morris et al., 2011), however N_2 fixation can be strongly limited under low P (Tesfaye et al., 2007; Sulieman et al., 2013). High nutrient availability promoted by fertilization increases tree growth and biomass production, caused by increased P_n and leaf area (*LA*) (Cooke et al.,

2005; Luo et al., 2006; Jaquetti et al., 2014). High nutrient availability may also increase aboveground while decrease root biomass, thus increasing the shoot-to-root ratio (S:R) (Novaes et al., 2009). Fertilization may also enhance sink strength accelerating NSC export from source (Li et al., 2012; De Schepper et al., 2013; Guo et al., 2016).

The whole-plant economics spectrum (ES) presents a set of functional traits grounding ecological theories (Lambers and Poorter, 1992; Chapin et al., 1993). These traits are variables related to physiology, growth, biomass allocation, biochemical composition, as well as morphology of different organs (e.g. leaves, stems and roots), linking plant strategies and ecological preferences (Poorter et al., 2014; Fort et al., 2015). Together they constitute an axis of slow (conservative) vs. fast (acquisitive) growth, varying in accordance to the resource availability and species responses (Wright et al., 2004; Reich, 2014). The NSC source-sink hypothesis dynamics is based on the availability and demand from source and sink organs and the partitioning between metabolism, growth and storage (Chapin et al., 1990; Kozlowski, 1992). The variations in NSC pools will be determined by photosynthetic rates, and the sinks activities with the depletion of pools when demand exceeds the production (Mooney, 1972; Martínez-Vilalta et al., 2016). Alternatively, Fatichi et al., (2014) has suggested that source-sink relations may be driving by sink strength determined by the intrinsic capacity of a specific sink to attract assimilates which were supported by experiments by Guo et al., (2016).

Here we hypothesized that functional traits will vary according to the economics spectrum axes of low/slow vs. high/fast resources and species responses. Our second hypothesis assumes that plants will increase root biomass fraction under nutrient limitations at the expense of growth accordingly to the functional equilibrium theory, which implies an active process of biomass allocation. Finally, the NSC dynamics and allocation to different pools (leaves, stems and roots) were investigated aiming to understand the acclimation responses of species and how sink strength can enhance the individual performance under limited resources. These hypotheses were evaluated by analyzing the variations in growth, gas exchange, nutrients content, NSC and chlorophyll fluorescence of six tree legumes species with reference to low vs. high water and nutrients treatments carried out in a degraded area in the Central Amazonia, using a multivariate matrix analyses. This research represents one of the first studies in Amazonia to examine functional traits of tree legumes for restoration of

degraded areas during the greatest drought in recent years (2015/16 El Niño event), which frame our results into a perspective response of these species to future climate change events.

Materials and methods

Study site, leguminous species and fertilization treatments

    The experimental trial consisted of 108 studied plants from six tree legume species grown in a combination of four treatments as drought and wet periods under low and high nutrient availabilities. The experiment took place in a degraded area of the Balbina Hydroelectric Dam Forest Restoration Program (01°55'S and 59°27'W, elevation 100 m) in Presidente Figueiredo Municipality, Amazonas State, in Brazil. The climate of the region is Af (tropical humid) based on the Köppen climate classification system, with a mean annual temperature above 25 °C and annual precipitation of 2300 mm. The area used for this study was degraded as all natural forest was removed while the topsoil was left in place. Surrounding is a natural well-preserved non-flooded terra firma dense forest with dominant species from the genera *Pouteria*, *Protium*, *Inga*, *Ocotea*, *Hymenaea*, and *Dipteryx*, with mean canopy height of 30 m. The soil in the area is a yellow *Oxisol* with a pH near 5 and extreme low fertility for N (0.16 g kg^{-1}), P (0.14 mg dm^3), Ca and Mg (0.3 cmol$_c$ dm^3). Five native tree legumes species, *Cenostigma tocantinum* Ducke, *Senna reticulata* (Willd.) H.S. Irwin & Barneby, tonka bean *Dipteryx odorata* (Aubl.) Willd., orchid tree *Clitoria fairchildiana* R.A. Howard., ice-cream bean tree *Inga edulis* Mart., and one alien *Acacia* sp. from Australia, were selected for this study. Thus, this study covered species of each of the three subfamilies/clades of the Fabaceae family, three of the species are N$_2$-fixing species (*C. fairchildiana*, *I. edulis* and *Acacia* sp.), and three are non-N$_2$-fixing species (*C. tocantinum*, *S. reticulata* and *D. odorata*). Seeds were collected in adjacent areas whereas *D. odorata* seedlings were acquired in the local greenhouse of Manaus city. Seedlings were selected based on the criteria of uniform size and individual health within each species: height of 25 cm for *C. tocantinum*, 35 cm for *S. reticulata*, 40 cm for *D. odorata*, 20 cm for *C. fairchildiana*, 30 cm for *I. edulis*, and 20 cm for *Acacia* sp. A complete randomized block experimental design was used with nine blocks (n = 9) placed across the 3-ha degraded area. Each block covered 432 m^2 (6 m x 72 m) and contained all six studied species that were either fertilized (high nutrient) or remain unfertilized (low nutrient) throughout the course of the experiment. Each species was replicated four times within each block and fertilization

treatment, which resulted in 48 seedlings per block and 432 individuals in the total area.

The installation of the experiment began with the tree-planting hole preparation in a spacing of 3 x 3 m when high nutrient plants were fertilized with 3 kg dry mass of organic fertilizer (chicken manure) + 200 g NPK 4-14-8[®] Bunge (São Paulo, SP, Brazil 4% N, 14% P and 8% K) + 100 g of lime + 20 g of micronutrients FTE BR-12[®] Nutriplant (São Paulo, SP, Brazil 7.1% Ca. 5.7% S. 1.8% B. 0.8% Cu. 2.0% Mn. 0.1% Mo and 9.0% Zn) in the pits. The high nutrient treatment received four additional amendments throughout the 2-year experiment period performed in the beginning of the rainy (December) and of the dry season (May-June) of each year. In each individual the first post fertilization was performed through the addition of 200 g NPK 10-10-10[®] Tocantins (Porto Nacional TO, Brazil; 10% N. 10% P and 10% K), and the three subsequent with 300 g of NPK 15-15-15[®] Yara (Porto Alegre RS, Brazil; 15% N, 15% P and 15% K), additional 100 g of lime + 20 g micronutrients FTE BR-12[®] Nutriplant were added in each time. The low nutrient treatment plants received no fertilization through the whole experiment. After planting the N_2-fixing species were inoculated with the following strains generated in the Laboratory of Microbiology of INPA: *C. fairchildiana* 944, 949, and 959; *I. edulis* 831, 841, 842, 844, and 852; and *Acacia* sp. 563. In *S. reticulata* and *I. edulis* it was considered four blocks (n = 4) in the low nutrient and seven blocks (n = 7) in the high nutrient treatments due to mortality of all four replicates during the experiment period. Additionally, some variables such as growth and NSC in root tissues were only performed at final harvest and therefore were not considered in the seasonality effects analysis. A list of all functional traits collected and used in the text including the symbols, units and formulae is presented in the Supplementary Table S1 at *JXB* online.

Growth analysis and biomass allocation

The growth of each planted individual was determined by the relative growth rates (RGRs) of its height, diameter, and the absolute growth rate (AGR) of total biomass including root tissues. The collar diameter (CD) and stem length (SL) were measured immediately after the seedlings were planted and the final measurement was done 24 months later. One replicate of each fertilization treatment and species was randomly selected in the nine blocks for growth measurements. The SL was measured with a hypsometric stick, and the CD was measured using a caliper rule. For the initial biomass data, we selected nine additional

seedlings. By the end of the experiment (24 months), we harvested and weighed the same selected individuals used for the measurement of the other growth variables. Plants were sorted into three main compartments: leaves, stems and roots. Samples for all compartments were taken to obtain the wet-to-dry mass conversions. The material was placed in a forced-ventilation oven at 65 °C until it reached a constant weight. The dry material was weighed with a digital scale with a precision of 0.1 g. The RGRs for diameter (RGR_D), height (RGR_H), and biomass (RGR_{Bio}) were determined as described by Hunt, (2012): $RGR_D = LogD_2 - LogD_1/t_2 - t_1$, where $LogD_1$ = initial diameter, $LogD_2$ = final diameter, t_1 = initial time, and t_2 = final time. The biomass allocation, leaf mass fraction (LMF), stem mass fraction (SMF), and root mass fraction (RMF), were calculated as proportions of the total biomass (Poorter et al., 2012).

Gas exchange, leaf area, specific leaf area, leaf water potential, water use-efficiency and intrinsic water use-efficiency

Gas exchange was measured between 8:00 and 11:30 h in nine selected plants per treatment in the drought and wet periods. A completely expanded healthy leaf fully exposed to the sun was selected on each plant from the middle third of the plants. The net photosynthetic rate (P_n), dark respiration rate (R_d), transpiration rate (E) and stomatal conductance (g_s) were measured using a portable photosynthesis system (Li-6400, Li-Cor Inc., Lincoln, NE, USA) as described by Santos Junior et al. (2006). Each measurement for the above variables was determined based on photosynthetic photon-flux densities (PPFD) of 0 and 2000 $\mu mol\ m^{-2}\ s^{-1}$, with the foliar chamber adjusted for a CO_2 concentration, temperature and water-vapor concentration of $400 \pm 4\ \mu mol\ mol^{-1}$, $31\ °C \pm 1\ °C$ and 21 ± 1 $mmol\ mol^{-1}$, respectively. Leaf area (*LA*) was measured with a portable laser leaf area meter (CI-202, CID Bio-Science, WA, USA). Specific leaf area (*SLA*) was determined by the ratio between the leaf area and the leaf dry mass at 70 °C from leaf discs of a known area. Mid-day leaf water potential (Ψ_L) was determined with a pressure chamber between 11:30 and 13:00 h (Scholander et al., 1964). Water use-efficiency (*WUE*) was determined by the ratio between P_n/E and intrinsic water use-efficiency (*IWUE*) by the ratio between P_n/g_s.

Nutrients and photosynthetic nutrient use-efficiency

Macro and micronutrients concentrations were determined in each treatment after oven-dried at 65 °C for 72 h. The C, N and H concentration for each compartment was assessed

with the 2400 Series II CHNS/O Elemental Analyzer (PerkinElmer Inc., Waltham, MA, USA). The phosphorus concentration was determined by spectrophotometry at 725 nm according to Vitti and Ferreira (1997). The macronutrient (K, Ca and Mg) and micronutrient (Fe and Zn) concentration were determined using spectrophotometric atomic absorption (Perkin-Elmer 1100B, Uberlingen, Germany) (Embrapa, 2009). The instantaneous photosynthetic nutrient use efficiency [P(element)UE] was calculated as the net CO_2 assimilation per unit of the element and time based on P_n, the *SLA*, and the nutrient content of leaves (Li et al., 2012).

Non-structural carbohydrates

Fully expanded leaves of nine plants were harvested for NSC assays at each treatment. Soluble sugars and starch were determined by the colorimetric method according to Blackig et al., (1996) with modifications. The 200 mg oven-dried (65 °C for 72 h) fine powder samples were extracted with ethanol (80%), after incubating in a water bath set at 75 °C for 30 min and centrifugation at 10.000 rpm for 5 min, which was repeated more 3 times. Starch was extracted posteriorly with perchloric acid (35%). SS and starch were quantified by phenol-sulfuric with spectrophotometry at 490 nm (Ultrospec 2100 pro UV/visible, Amersham Biosciences, Cambridge, UK) using glucose (Sigma) as a standard (Dubois et al. 1956).

Photosynthetic pigments

The fully expanded healthy sun exposed leaves criteria was also used for chlorophyll *a* (Chl *a*) and *b* (Chl *b*) and carotenoids (Car) pigment analysis. The material was collected in the field, covered with aluminum paper, and conditioned in icebox for transportation to the Laboratory of Physiology and Biochemistry of INPA. The analysis was performed using 0.1 g of fresh material ground in 10 ml of 80% acetone with magnesium carbonate ($MgCO_3$) and 10 ml of 100% acetone added immediately following the initial grinding step. The suspension was filtered, and the absorbance was read at 663 nm (Chl *a*), 645 nm (Chl *b*) and 480 nm (Car) using a spectrophotometer (Ultrospec 2100 pro UV/visible, Amersham Biosciences, Cambridge, UK) (Lichtenthaler and Wellburn, 1983).

Chlorophyll fluorescence

The chlorophyll fluorescence was measured with a portable fluorometer (Handy PEA, MK2-9600-Hansatech, Norfolk, UK) on the same individuals and leaves used for the gas exchange measurements between 8:30 and 11:00 h. The leaves selected were dark-adapted to

a period of 30 min and then were exposed to a 5 s excitation pulse of intensely saturating light (3000 μmol m^{-2} s^{-1}) with a wavelength of 650 nm. A performance index, which combines the parameters of energy conservation from photons absorbed by the PSII antenna and the reduction of quinone Q_B, was calculated on absorption basis (PI_{ABS}) as described by Gonçalves et al. (2007).

Data analysis

The interrelationships among the 59 growth and functional traits variables were assessed using the principal components analysis (PCA) ordination method, which reduces the dimensionality of the original data (James and McCulloch, 1990). PCA was performed to evaluate the effects of the seasonality and fertilization treatment. All variables were standardized by the relativization maximum method (Noy-Meir et al., 1975) prior to analysis. Product-moment correlations were used to assess the influences of seasonality (drought and wet) and fertilization (fertilized and unfertilized) on the ordination axes and each original variable. At a probability level of $P < 0.05$, pairwise t-tests were performed to evaluate the actual significances of the seasonality and fertilization effects. All the analyses were run using PAST-UiO 3.0 (Hammer and Harper, Oslo, NO) for the PCA and STATISTICA 13.0 (TIBCO Software Inc., CA, USA) for the inferential tests.

Results

Seasonality effects on low and high nutrient treatment

When ordination scores were compared for dry vs. wet season, there was a highly significant difference for high nutrient plants in PCA axis 1 (t = -6.80, p < 0.001) but no differences were found in PCA axis 2 (t = -1.07, p = 0.28) (Figure 1). Meanwhile, in low nutrient plants slight differences were found in PCA axis 1 (t = 2.79, p < 0.01), and again no differences were found in PCA axis 2 (t = 0.18, p = 0.18) (Figure 2). In accordance with ES when high nutrient plants were arranged in ordination space, a separation between drought vs. wet period was evident on PCA axis 1 with increased E, P_n and g_s in the wet period whereas under drought most species increased $IWUE$. PCA axis 2 separated stressed *S. reticulata* with increased values of leaf Ca and starch content in both drought and wet periods (Figure 1a), while well adapted species increased PI_{ABS} and electron transport probability (ET_0/TR_0). The seasonality effect was less clear under low nutrient (Figure 2b), however plants tend to increased P_n and E in wet period, while increasing the effective dissipation of an active RC

(*DI_0/RC*), the effective antenna size (*ABS/RC*) and leaf zinc concentrations (Zn) during drought.
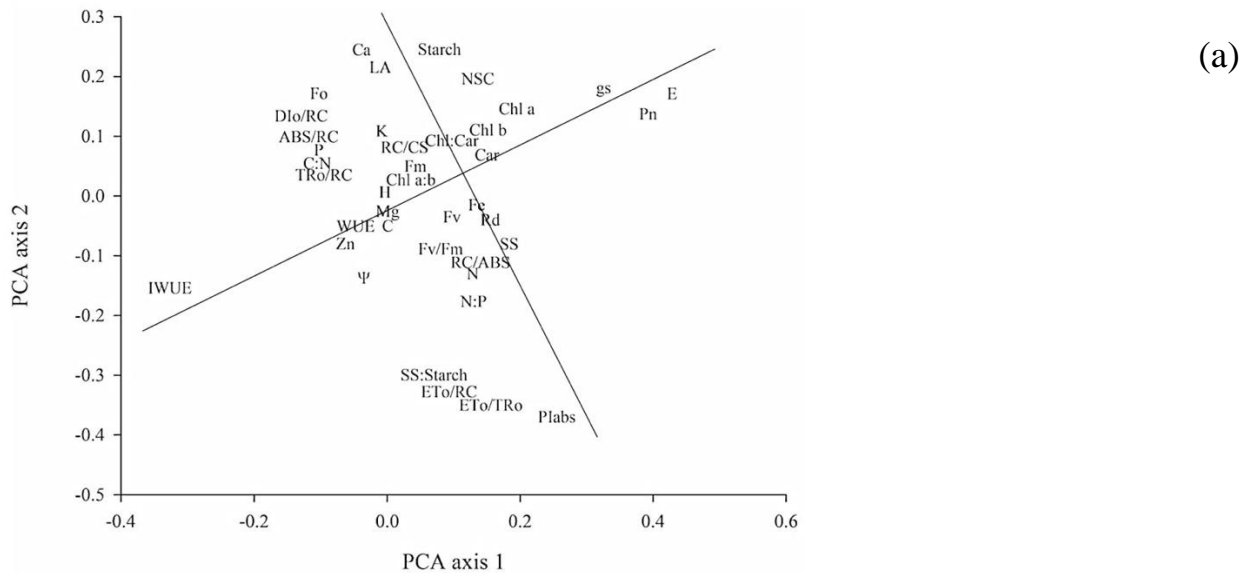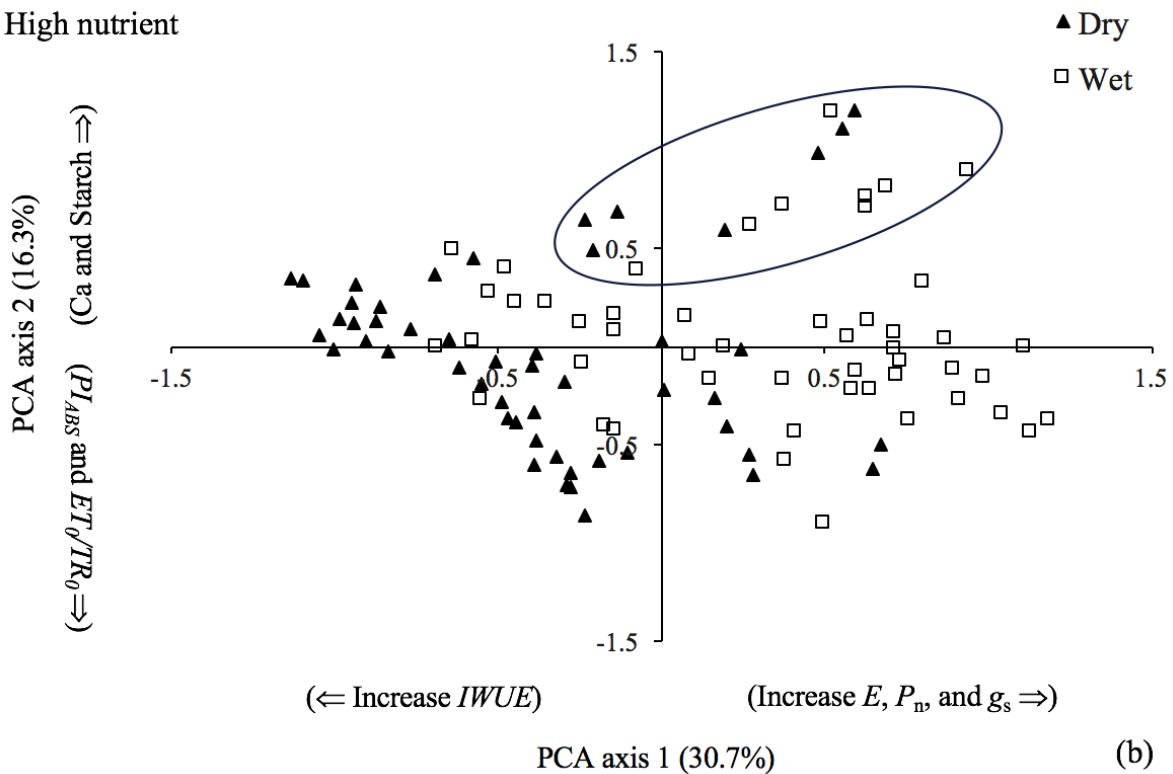
Figure 1: Seasonality effects with the (a) component loadings graph and (b) PCA ordination diagram of 102 seedlings in the Drought and Wet period based on 39 functional traits variables of high nutrient treatment; net photosynthetic rates (P_n); transpiration (*E*); stomatal conductance (g_s); intrinsic water use-efficiency (*IWUE*); leaf calcium content (Ca); leaf starch content ($Starch_L$); performance index (PI_{ABS}) and electron transport probability (ET_0/TR_0). Circulated are increased Ca and $Starch_L$ values of *S. reticulata* in the drought and wet periods.
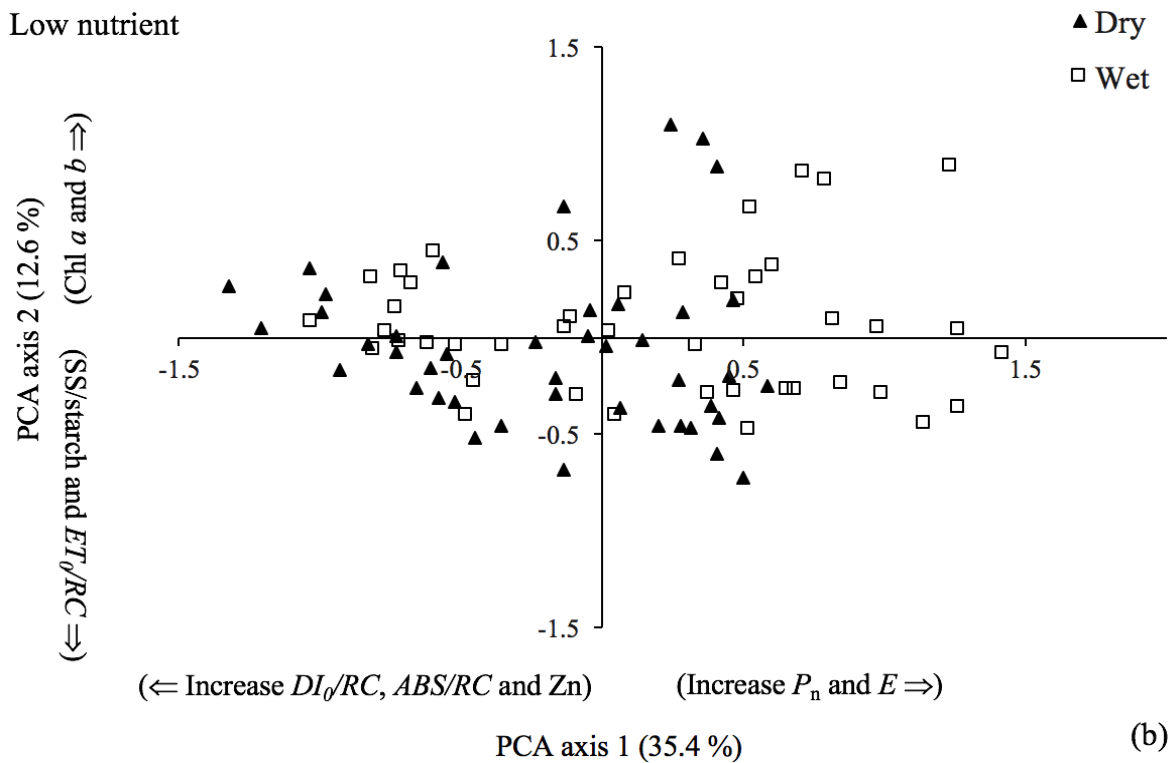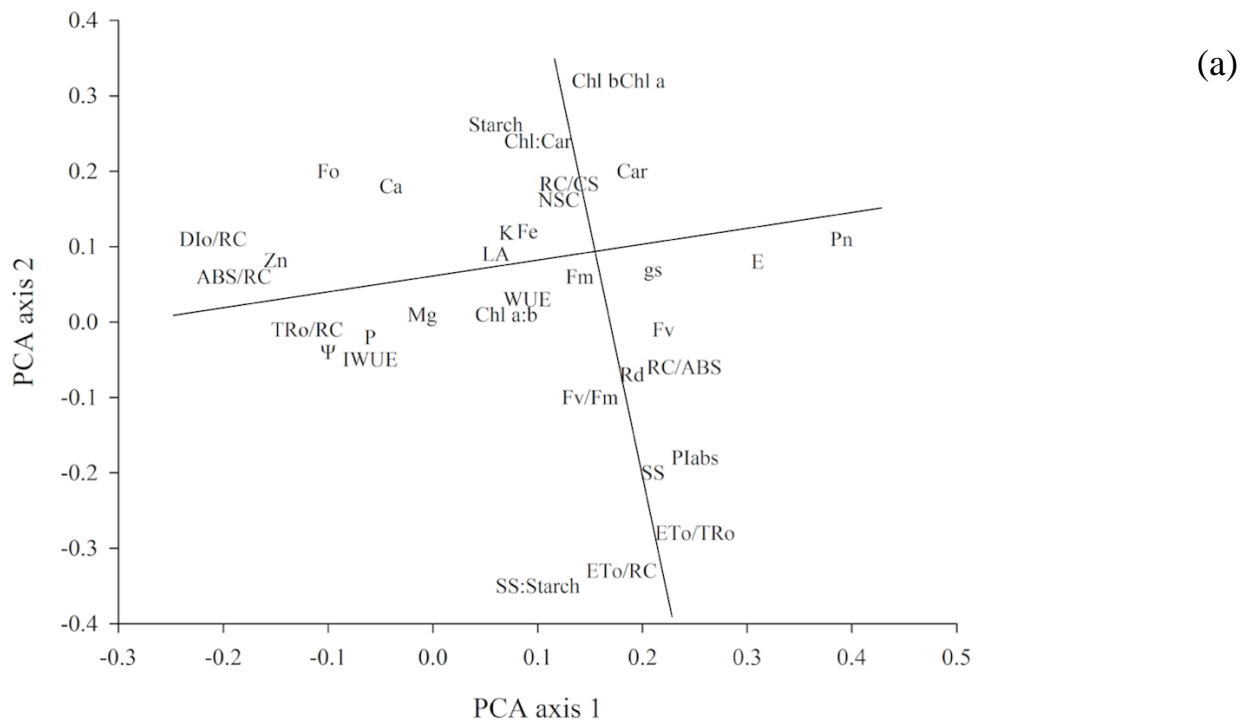
Figure 2: Seasonality effects with the (a) component loadings graph and (b) PCA ordination diagram of 86 seedlings in the Drought and Wet period based on 34 functional traits variables of low nutrient treatment; net photosynthetic rates (P_n); transpiration (E); stomatal conductance (g_s); intrinsic water use-efficiency ($IWUE$); leaf calcium content (Ca); leaf starch content ($Starch_L$); performance index (PI_{ABS}) and electron transport probability (ET_0/TR_0).

Fertilization effects on drought and wet period

Confirming ES there was a separation between low nutrient vs. high nutrient plants in the wet period along both PCA axis 1 (t = -5.34, p < 0.001) and PCA axis 2 (t = 2.16, p < 0.05) (Figure 3). However, *Acacia* sp. responds mostly as fertilized plants even under low nutrient treatment (Figure 3b). During drought the fertilization treatment effects were diffuse but slight differences were found on both PCA axis 1 (t = -2.56, p < 0.05) and PCA axis 2 (t = -2.64, p < 0.01) (Figure 4). In the wet period most variation comparing low vs. high nutrient plants including low nutrient *Acacia* sp. occur within PCA axis 1 with increased P_n, E, g_s, under high nutrient while low nutrient plants increased DI_0/RC. Along the PCA axis 2 increased values of RGR_D and PI_{ABS} of high nutrient plants were negatively correlated with increased values for RMF, $Starch_R$ and leaf Zn under low nutrient treatment. Our results indicate that *Acacia* sp. species was able to adjust its functional traits to low nutrient increasing mostly RGR_D, P_n and PI_{ABS}, corroborating with its oligotrophic characteristics.

Seasonality effects on high nutrient functional traits

For a better understanding of the seasonality responses of the species functional traits of high nutrient plants were grouped in gas exchange, nutrients, NSC, and chlorophyll fluorescence variables. Considering the nutrient variables, the separation between dry vs. wet seasons was evident in the PCA axis 1 (t = 9.52, p < 0.001) but the differences were not significant in the PCA axis 2 (t = 0.66, p = 0.51). Among the PCA 1 axis plants increased leaf P content during drought period which was negatively correlated with increased nitrogen-to-phosphorous ratio (N:P) values in the wet period (Figure 5a).

For the carbohydrate variables the separation between seasons was not evident in PCA axis 1 (t = -1.38, p < 0.18), while differences in PCA axis 2 were significant (t = -4.52, p < 0.001) (Figure 5b). Along PCA axis 1 most species increased leaf starch content ($Starch_L$) in the wet period while leaf SS-to-starch ratio ($SS:Starch_L$) increased during drought, despite *S. reticulata* had extremely high values for $Starch_L$ during drought. Chlorophyll fluorescence traits varied along PCA axis 1 in high nutrient plants with increased PI_{ABS}, and ET_0/TR_0 in the wet season while DI_0/RC and initial fluorescence (F_0) was increased during drought (Figure 5c). Our result indicates that *Acacia* sp. is highly efficient in light energy use increasing values for PI_{ABS}, ET_0/TR_0, and ET_0/RC (Figure 5d).
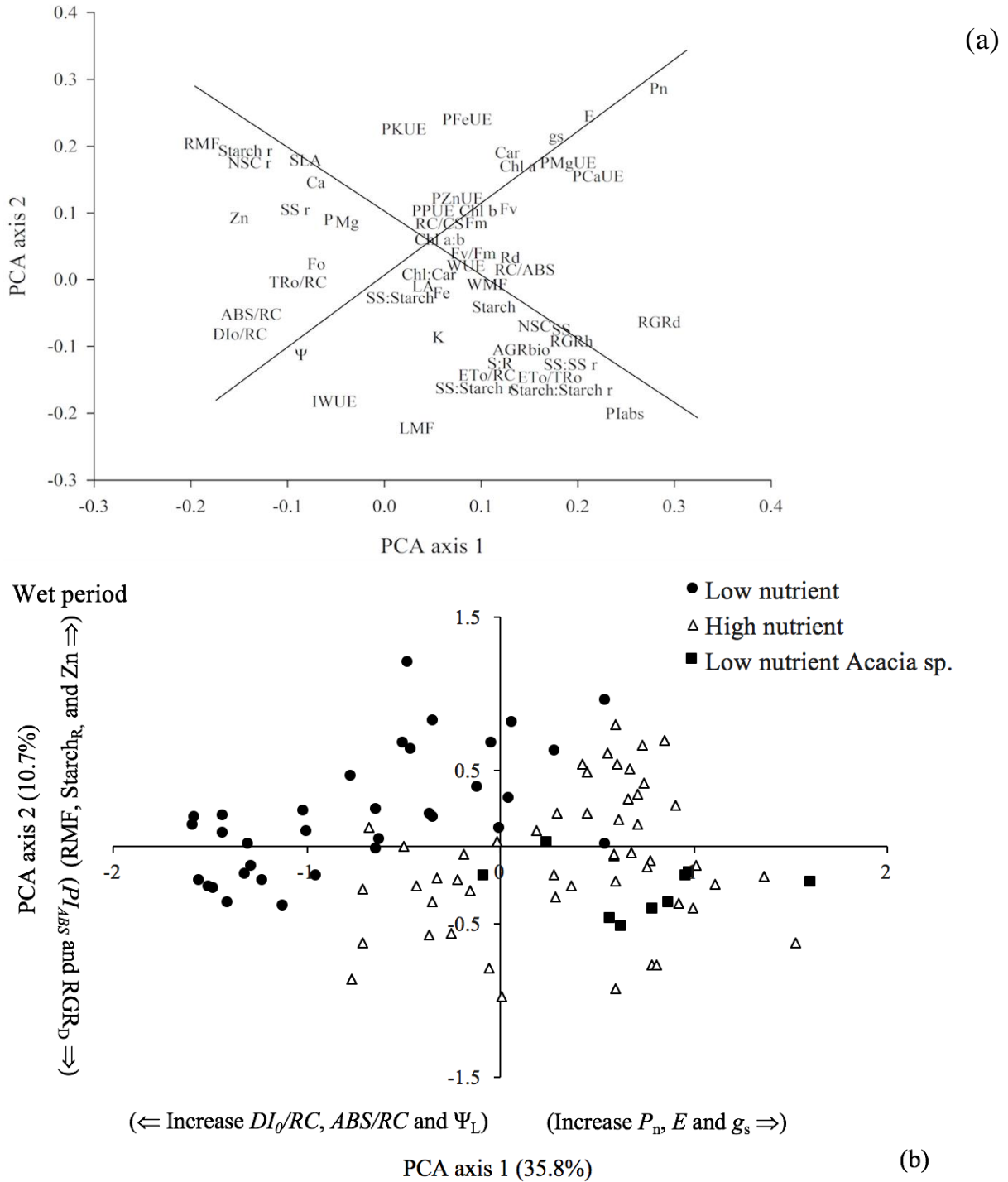
Figure 3: Fertilization effects with the (a) component loadings graph and (b) PCA ordination diagram of 94 seedlings in the low nutrient and high nutrient treatments and low nutrient *Acacia* sp. based on 54 functional traits variables on the wet period; net photosynthetic rates (P_n); transpiration (E); stomatal conductance (g_s); effective dissipation of an active RC (DI_0/RC); effective antenna size of an active RC (ABS/RC); leaf water potential (Ψ_L); root mass fraction (RMF); root starch content ($Starch_R$); leaf zinc concentration (Zn); performance index of chlorophyll fluorescence (PI_{ABS}) and relative growth rates in diameter (RGR_D).
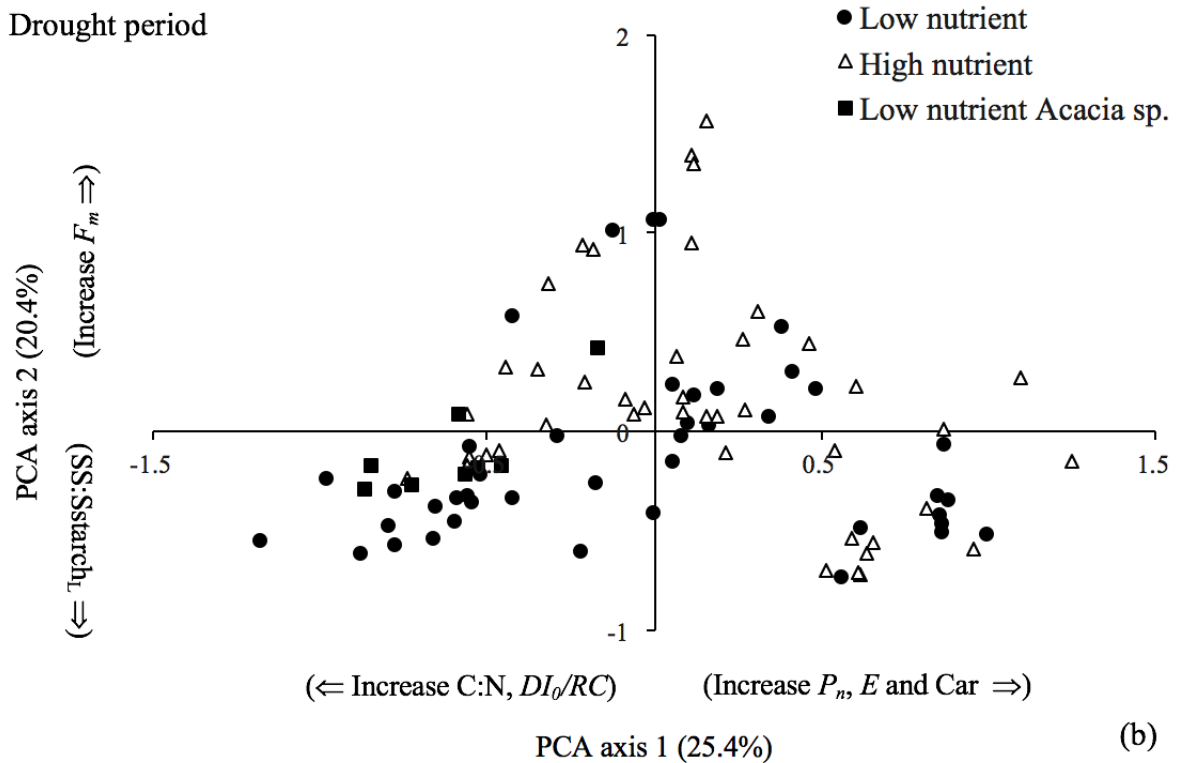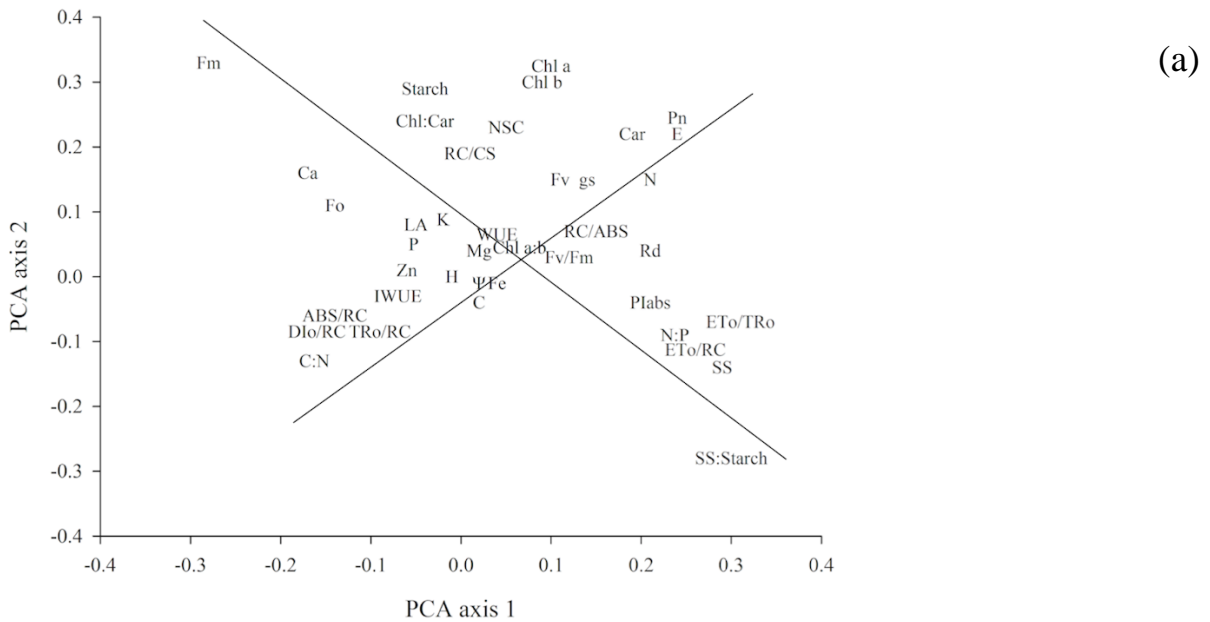
Figure 4: Fertilization effects with the (a) component loadings graph and (b) PCA ordination diagram of 94 seedlings in the low nutrient and high nutrient treatments and low nutrient *Acacia* sp. based on 39 functional traits variables on the drought period; net photosynthetic rates (P_n); transpiration (E); leaf carotenoids content (Car); carbon-to-nitrogen ratio (C:N); effective dissipation of an active RC (DI_0/RC); maximum fluorescence (F_m); leaf soluble sugars-to-starch ratio (SS:Starch$_L$).
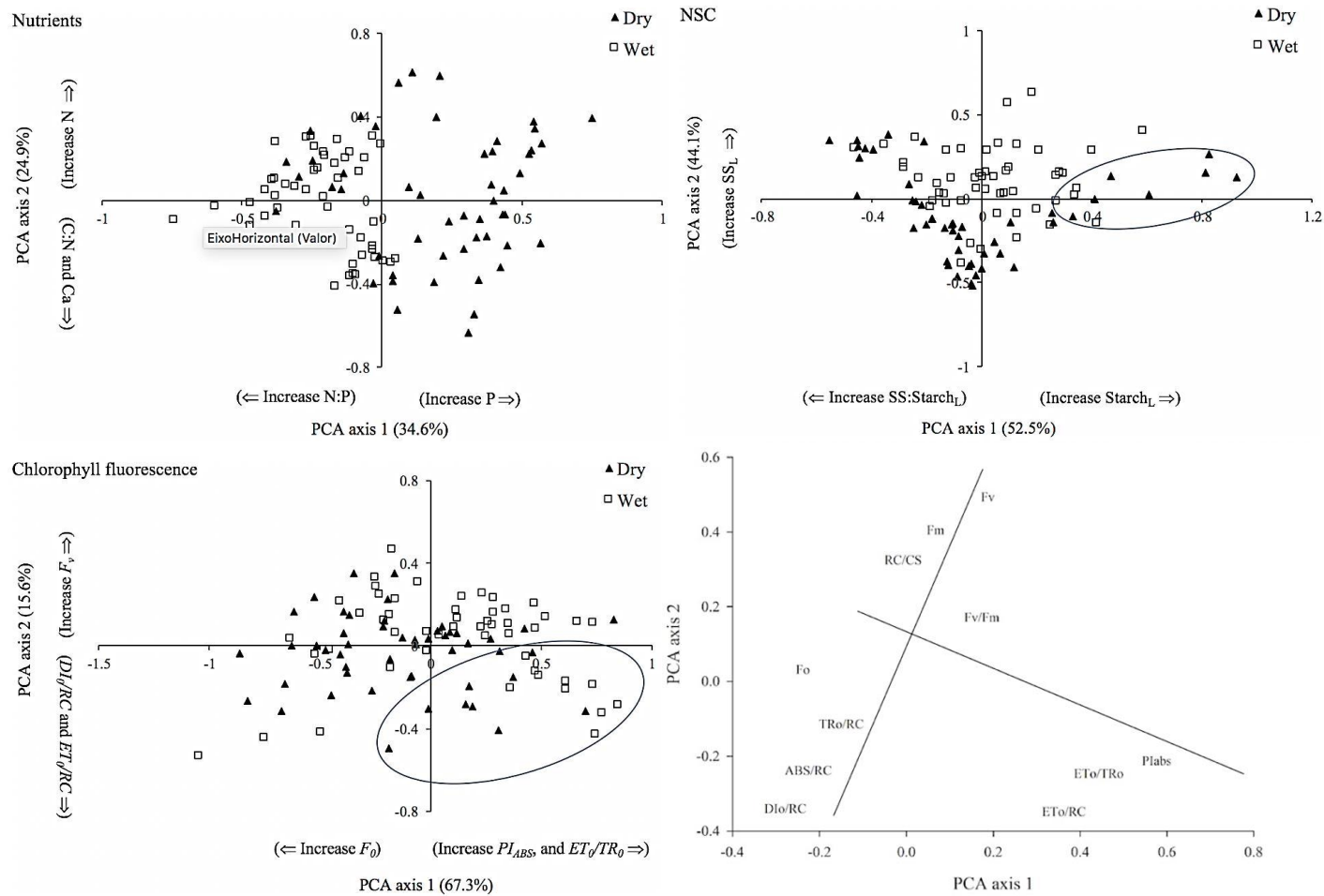
Figure 5: Seasonality effects with the PCA ordination diagram of nutrients, non-structural carbohydrates (NSC) and chlorophyll fluorescence of 102 high nutrient plants and the chlorophyll fluorescence component loadings graph; leaf nitrogen-to-phosphorous (N:P) and carbon-to-nitrogen (C:N) ratios; leaf phosphorous (P), nitrogen (N), calcium (Ca) and zinc (Zn) contents; leaf soluble sugars (SS_L) and starch ($Starch_L$) contents and soluble sugars-to-starch ratio ($SS/Starch_L$); initial fluorescence (F_0), variable fluorescence (F_v), performance index (PI_{abs}), electron transport probability (ET_0/TR_0), electron transport in an active RC (ET_0/RC), effective dissipation of an active RC (DI_0/RC). Circulated are increased values of *S. reticulata* in the drought period plants and increased PI_{abs} and ET_0/TR_0 of *Acacia* sp. during drought and wet periods.

Fertilization effects on wet season functional traits

Species responses to low and high nutrient effects on the wet period were better evaluated as functional traits were grouped in gas exchange, nutrients, NSC, and chlorophyll fluorescence. For the nutrients variables the separation between fertilization treatments was evident in the PCA axis 1 (t = -3.68, p < 0.001) but less clear in the PCA axis 2 (t = 2.45, p < 0.05). Most high nutrient plants increased photosynthetic nutrient use efficiency for Ca (*PCaUE*) and Mg (*PMgUE*), along PCA axis 1 which was negatively correlated with increased Zn and Ca in low nutrient plants (Figure 6a).

Considering the NSC variables a separation between low vs. high nutrient plants were found in the PCA axis 1 (t = -4.19, p < 0.001), with less clear differences in the PCA axis 2 (t = -3.17, p < 0.01) (Figure 6b). Along PCA axis 1 high nutrient plants and low nutrient *Acacia* sp. increased leaf-to-root NSC ratio (NSC_L:NSC_R) which was negatively correlated with increased root starch ($Starch_R$) and root SS (SS_R) content of low nutrient plants. The differences comparing low vs. high nutrient plants among the chlorophyll fluorescence variables emerge to be more evident if low nutrient *Acacia* sp. are analyzed together with the high nutrient plants (t = -5.04, p < 0.001). Along the PCA axis 1 there was a gradient of increased *PI_{ABS}*, and *ET_0/TR_0* in high nutrient plants and low nutrient *Acacia* sp. while low nutrient plants increased *DI_0/RC* and *ABS/RC* (Figure 6c, d).
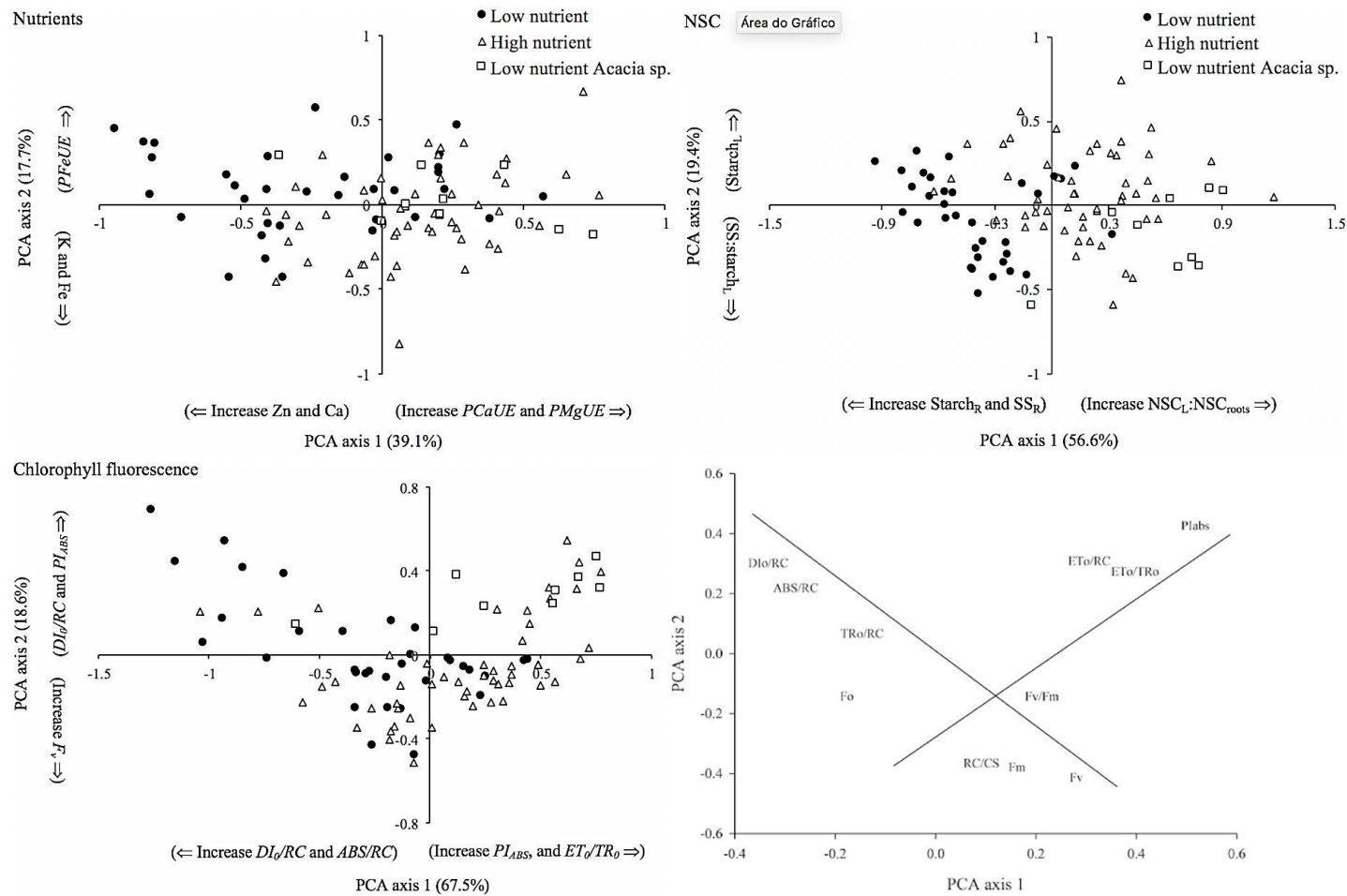
Figure 6: Fertilization effects with the PCA ordination diagram of nutrients, non-structural carbohydrates (NSC) and chlorophyll fluorescence of 94 in the wet period and the chlorophyll fluorescence component loadings graph; photosynthetic nutrient use efficiency for calcium (*PCaUE*), magnesium (*PMgEU*) and iron (*PFeUE*); leaf potassium (K), calcium (Ca), iron (Fe) and zinc (Zn) contents; leaf starch (Starch$_L$) content and soluble sugars-to-starch ratio (SS:Starch$_L$); root soluble sugars (SS$_R$) and starch (Starch$_R$); soluble sugars-to-starch ratio (SS:Starch$_R$); leaf-to-root non-structural carbohydrates (NSC$_L$:NSC$_R$); variable fluorescence (F$_v$), performance index (*PI$_{ABS}$*), electron transport probability (*ET$_0$/TR$_0$*), electron transport in an active RC (*ET$_0$/RC*), effective antenna size of an active RC (*ABS/RC*), effective dissipation of an active RC (*DI$_0$/RC*).

Discussion

How species do respond to resource availabilities according with the ES hypothesis?

Our results support ES hypothesis for both water and nutrient availabilities. Plants under high resource availability increase fast traits such as P_n, E in PCA axis 1 and RGR_D and PI_{ABS} in PCA axis 2. Under low resources availability plants increased slow traits such as *IWUE*, RMF and DI_0/RC. Individuals are spatially ordinated among PCA axes therefore if P_n for example increased in wet period it assumingly decreased during drought. Under low nutrient traits were poorly adjusted to drought due to the low energy status for acclimation and the poor stomatal adjustments under K deficiency (Mittler and Blumwald, 2010; Oddo et al., 2014). The lack of fertilization effects during drought was mostly caused by the down regulation of P_n of high nutrient plants. Hereafter the discussion will mostly focus on the seasonality effects of high nutrient plants and the fertilization effects in the wet period.

Under high water and nutrient availabilities plants increased mostly E (Figure 7) being an important cooling mechanism in degraded areas affecting the species whole-plant water balance (Lawlor and Cornic, 2002; Outlaw, 2003). The increased E which involves the transport of nutrients through xylem was positively correlated with P_n and g_s suggesting a strategy for high nutrient uptake in fertilized areas (McMurtrie and Näsholm, 2018). In our study plants increased leaf N:P ratios (N:P) in the wet period, despite that clear evidence of seasonality effects on N:P in terrestrial ecosystems is very scarce. Contrasting Mediterranean species show a negative correlation between N:P and water availability (Sardans et al., 2011; Sardans and Peñuelas, 2012). The increased N:P values in the wet period (mean N:P = 18.41) was caused by a rather decreased in leaf P than the increase in N content (see Supplementary Table S2 at *JXB* online). Additionally, the high N found in our species confirms the high N "lifestyle" of both N_2-fixing and non-N_2-fixing Fabacea species (Fyllas et al., 2009). *Inga* genus *is* known to have unusually high N, which was also found in N_2-fixing *C. fairchildiana*. Therefore, these species have great ecological importance to the restoration of degraded biogeochemical cycles (Jaquetti and Gonçalves, 2017).
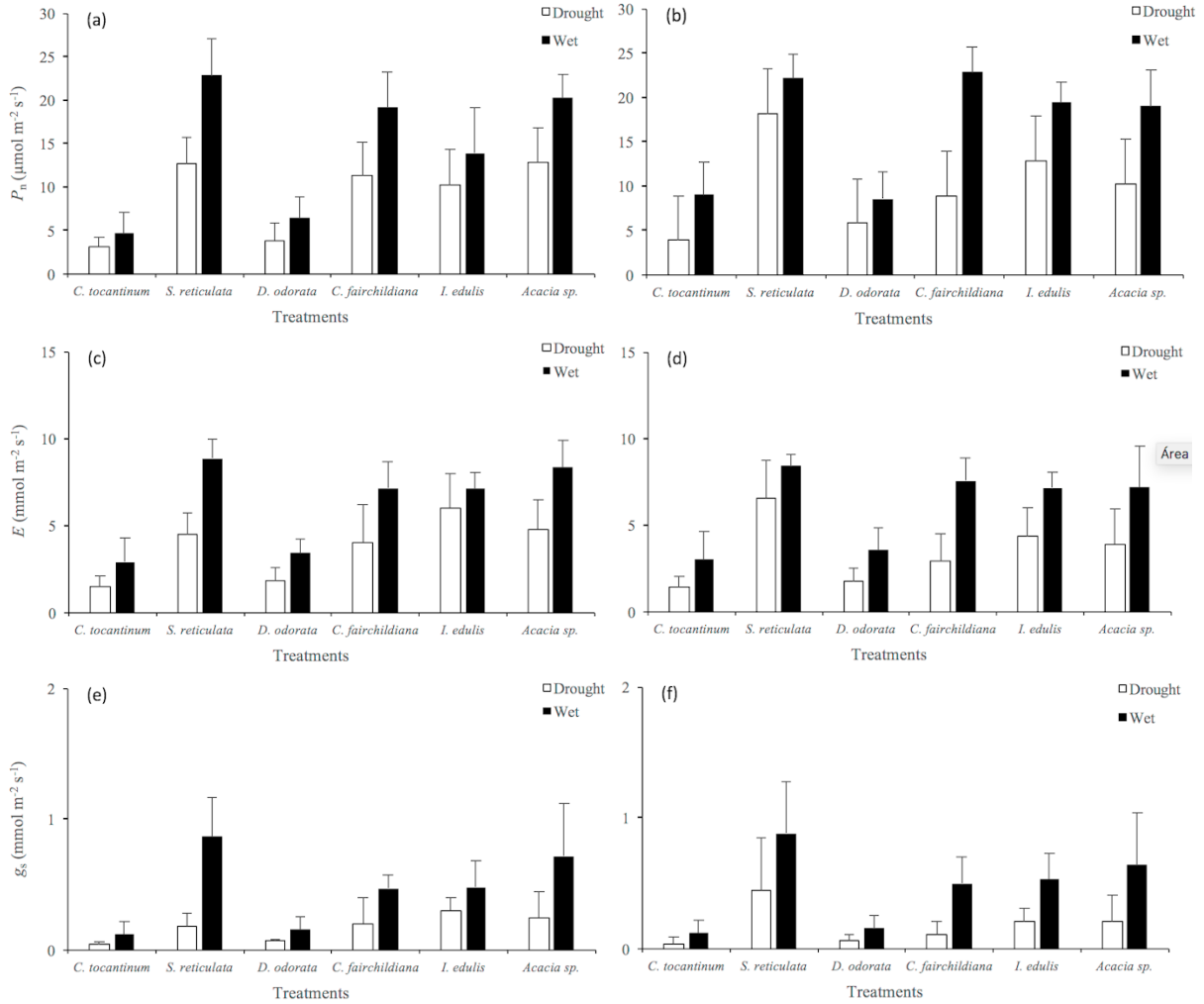
Figure 7: Net photosynthetic rate (P_n), transpiration rate (E) and stomatal conductance (g_s) of low (a, c and e) and high (b, d and f) nutrient in both drought and wet period for the 6 species studied.

The main response of species to drought was the increased *IWUE* as related to the slow traits spectrum. The increased *IWUE* under mid-drought is caused by the greater decrease in g_s compared to P_n (Figure 7) with protective effects against stress (Chaves et al., 2009). The reduced g_s also affect E restricting excessive loss of water thus enhancing acclimation to drought (Liberato et al., 2006; Lima et al., 2018). The stomatal closure is driven by a number of environmental cues, which is controlled by abscisic acid accumulation and the efflux of K^+ and malate^{2-} in the guard cell and the concentration of apoplastic sucrose (Lawson, 2009; Lawson and Blatt, 2014). After stomatal closure metabolism of species may be maintained by using storage starch as evidenced by the increased SS:Starch$_L$.

The reduced P_n under drought can be caused by the reduction in growth and sink strength and by the decreased CO_2 diffusion from the atmosphere to mesophyll due to stomatal resistance (Paul and Foyer, 2001; Grassi and Magnani, 2005; Nikinmaa et al., 2013). The positive correlation between g_s and P_n has been previously found under different nutrient availabilities (Hubbard et al., 2001, Hetherington and Woodward, 2003). Drought effects on dark respiration rates (R_d) are inconclusive with decrease, increase or negligible responses (Gimeno et al., 2010). Our results show a slight decreased in R_d during drought, as responses were smaller than the other gas exchange variables confirming previous findings (Flexas et al., 2006; Lawlor and Tezara, 2009). Additionally, increased leaf P content during drought corroborate with the increased *IWUE* due to improved stomatal movement (Singh et al., 2000; Waraich et al., 2011). Meanwhile the decreased mean N:P values (12.27) under drought (see Supplementary Table S3 at *JXB* online) may reflect the N mobilization and protein degradation (Gent and Forde, 2017; Guo et al., 2017).

According with ES different nutrient availabilities in the soil greatly affected species responses along both PCA axes. In the PCA axis 1 high nutrient plant increased P_n, which was positively correlated, with E while in the PCA axis 2 increased RGR_D was correlated with PI_{ABS}. The enhanced P_n may be explained by both increased availabilities of metabolic elements and alleviation of nutrient limitation (Ostertag, 2010; Pasquini and Santiago, 2012). N addition may increase chlorophyll content and proteins of the Calvin cycle, whereas P and K may increase the biochemistry efficiency of photosynthesis (Evans, 1989; Santiago, 2015). Moreover, N_2-fixing species are less dependent on external N inputs thus reducing the need for fertilizers (Murray et al., 2017).

Increased diameter and height growth rates under high nutrient can be partially explained by the higher nutrient availabilities in the soil and the enhanced nutrient use-efficiency of limited resources (Yavitt et al., 2011; Santiago et al., 2012). N and P addition increase growth because of its importance in most physiological and biochemical process such as photosynthesis, and as major components of proteins, amino acids, and membrane lipids affecting energy homeostasis (Shen et al., 2014; Santiago, 2015). Enhanced height growth under K addition can also be explained by the increased g_s and NSC export from source (Battie-Laclau et al., 2014; Liesche, 2015). Increased growth under multiple nutrients

fertilization has also been reported in pots and in field conditions (Cai et al., 2008; Yavitt and Wright, 2008; Jaquetti et al., 2014; 2016).

Chlorophyll fluorescence evaluate the photosynthetic responses to fluctuating resources assessing plant health and performance (Murchie and Lawson, 2013; Porcar-Castell et al., 2014). The energy fluxes of the PSII can be seen as the electron transport flux (ET), the trapping flux or energy converted to chemical components (TR), and the dissipation flux as energy is transferred or lost (DI) (Force et al., 2003). The performance index (PI_{ABS}) which correlates parameters of energy absorption, trapping and electron transport flux has been used to evaluate plant growth and photochemistry performance under stress (Gonçalves and Santos Junior, 2005; Strauss et al., 2006). Increased RGR_D and PI_{ABS} were positively correlated in high nutrient plants, confirming its potential use as proxy for productivity in plant studies. Increased PI_{ABS} was generally correlated with ET_0/TR_0, which is explained as the probability of an electron from quinone Q_A to enter the electron transport chain (Gonçalves et al., 2007). Additionally, species tend to increase both $PCaUE$ and $PMgUE$ under high nutrient, which can be associated to chemical similarities and the lack of selectivity for cation uptake (White, 2001).

Plants under low nutrient availability increased greatly the RMF as positively correlated with leaf Zn, $Starch_R$ and SS_R content. Additionally, plants under nutrient limitations increased DI_0/RC and ABS/RC. Chlorophyll fluorescence has been reported for the early detection of drought and nutrient stress (Kiani et al., 2008; Frydenvang et al., 2015). In degraded environments species may be photoinhibited caused by excess energy and the damage to the photosynthetic machinery which decreases photochemistry yield and growth (Demmig-Adams and Adams, 2000; Tyystjärvi, 2013). Accordingly, DI_0/RC seems more accurate to detect photoinhibition and stress levels than the commonly used F_v/F_m parameter which can be quite insensitive to environmental changes (Baker and Rosenqvist, 2004; Murchie and Niyogi, 2011). The increased DI_0/RC was negative correlated with P_n indicating a decreased consumption and transport of electrons (Saccardy et al., 1998; Tezara et al., 1999). Increased DI_0/RC was also observed in Amazonian species copaiba (*Copaifera multijuga*) and mahogany (*Swietenia macrophylla*) under high irradiance indicating a general response to stress (Gonçalves et al., 2007). The increased F_0 under drought may be associated with the inactivation of reaction centers (Haupt-Herting and Fock, 2000).

Functional equilibrium and biomass allocation adjustments as controlled by passive vs. active process

The 'optimal partitioning theory' or 'functional equilibrium' presumes that plants adjust biomass allocation to the uptake of the most limiting factor (Bloom et al., 1985; Kobe et al., 2010). As suggested by Poorter et al. (2012) low nutrient plants increased two-fold the RMF supporting the functional equilibrium hypothesis, however these responses were less responsive to detect the nutrient limitations than DI_0/RC. Currently there is debate if allocation is an active storage at the expense of growth or a passive process simply driven by the C supply and demand (Dietze et al., 2014). Our results corroborate with the active regulation of allocation as increased RMF was positively correlated with $Starch_R$ and SS_R and negatively correlated with RGR_D, since the conversion of SS-starch-SS is not a spontaneous reaction and involves energy costs. Increased RMF as a slow trait may be controlled by the sink root strength (Dietze et al., 2014). The positive correlation between increased Zn_L and RMF under low nutrient may be supported by symplastic root fluxes involving growth and S:R adjustments (White et al., 2002). The Zn action in stress responses needs to be better investigated, as it may be specific to certain families (Broadley et al., 2007).

Increased S:R ratio with nutrient addition has been observed in a variety of species (Bown et al., 2009; Li et al., 2016), however growth in diameter and height appears to be more sensitive to fertilization. The increased aboveground growth (RGR_D and RGR_H) also support the functional equilibrium as plants allocate less resources to root tissues as nutrient limitation is alleviated (Santiago et al., 2015).

How NSC source-sink (or sink-source) dynamics influence acclimation and performance of species under low resources?

According to NSC source-sink dynamics NSC pools are constantly adjusted by the production and consumption. Starch can be degraded to SS supporting demands for growth and metabolism under unfavorable conditions (Smith and Stitt, 2007). Additionally, water and nutrient availabilities can change the source-sink dynamics with a strong control in the sink activities such as growth, these specific responses however remain poorly understood (Sala et al., 2012). Even drought effects on NSC are inconclusive with increase, decrease or unchanged pools (Hartmann and Trumbore, 2016). Under mid-drought leaf NSC content may

increase due to a rather reduction in growth than photosynthesis (Muller et al., 2011; Woodruff and Meinzer, 2011).

Our results indicate an increase in $SS:Starch_L$ ratio rather than the variation in NSC content. Since SS synthesis may be strongly impaired during earlier drought the increased $SS:Starch_L$ indicate a mobilization of starch pools highlighting its major role in connecting metabolism and growth, which may be supported by increased inorganic P and changes in enzyme activities (Reddy et al., 2004; Sulpice et al., 2009; Stitt and Zeeman, 2012). Conversely, plants increased $Starch_L$ in the wet period supporting the sink strength hypothesis. The higher $NSC_L:NSC_R$ also indicate increased sink strength of high nutrient plants caused partially by the increased metabolic activities and the increased proportion of living cells (Sala et al., 2012; Sala and Mencuccini, 2014).

Ecofunctional strategies of stressed vs. acclimated species

Degraded environments usually expose plants to multiple stresses including drought, nutrient, heat and high light energy as the tolerance responses involve protective mechanisms (Suzuki et al., 2014; Prasch and Sonnewald, 2015). Accordingly, *S. reticulata* was not able to adjust the gas exchange traits during drought and may suffered from excessive loss of water. The stress responses of the species were correlated with the increased leaf Ca and $Starch_L$ contents. The high Ca values may be involved in the Ca^{2+} signaling responses to oxidative stress (Reddy et al., 2011; Kudla et al 2018). Increased $Starch_L$ during drought stress may be resulted by decreased growth and consequent sink strength (Paul and Pellny, 2003; Hartmann and Trumbore, 2016). Additionally, reduced accessibility of NSC pools may include storage mechanisms such as compartmentalization, limitations to the phloem transport and reduced enzymatic access to the inner nucleus of starch granules (Millard et al., 2007; Srichuwong and Jane, 2007; Sala et al., 2010). The $Starch_L$ values of *S. reticulata* under drought in high (335.9 g kg^{-1}) and low (430.3 g kg^{-1}) nutrient plants were four times higher than the other species (see Supplementary Tables S3-S4 at *JXB* online).

Acacia sp. responses also diverge from ES as high plasticity facilitates traits adjustments maintaining growth even under severe nutrient limitations. The functional strategies of *Acacia* sp. involve increased fast traits such as RGR_D, P_n, PI_{ABS} ET_0/TR_0. Additionally, the species invest in slow and protective traits such as low leaf nutrient and increased Car content protecting photochemistry from oxidative damage (Demmig-Adams

and Adams, 2006). The origin of the sink strength resulted by the enhanced RGR_D of *Acacia* sp. under limited resources can be resulted by the increased synthesis of the cell wall components and consequent demand for NSC (Woodruff and Meinzer, 2011). The species also relay on a large NSC pools formed by the combination of increased LMF, leaf C content and NSC_L:NSC_R as coordinated with the increased sink strength (see Supplementary Table S5 at *JXB* online). The stress tolerance of *Acacia* sp. may also be attributed to the physiological characteristics of phyllodes, which are associated with arid environments (Pasquet-Kok et al., 2010; Sommerville et al., 2012). The species also had the lowest leaf Ca content during drought, which suggests that N_2 fixation of nodules remain active during mid-drought (O'Hara, 2001).

Conclusions

The whole-plant ES and functional equilibrium hypothesis were mostly confirmed in tree legume species. PCA axis 1 captured mostly the slow vs. fast traits spectrum while PCA axis 2 expresses the stress responses of species. Species responses to high water and nutrient availabilities were more general, with increased fast traits such as P_n, E, PI_{ABS}, and RGR_D. However, species responses vary quietly under water and nutrient limitations increasing slow traits such as DI_0/RC, *IWUE* and RMF. Gas exchange and chlorophyll fluorescence were the most responsive variables and seems to dictate species responses to resources variations. NSC and nutrients play important role in traits adjustments, but more importantly the nutrients stoichiometry and the ratio between NSC pools seems more responsive to environmental changes and therefore deserve to be better addressed. The increased RMF of low nutrient plants seems inefficient while increased NSC pools of *Acacia* sp. appear to be an efficient strategy under limited resources, corroborating with the sink-source hypothesis. Moreover, the capacity of species to assess the NSC pools during drought play important role for the acclimation of species. The multivariate principal component analysis is proven efficient to detect species responses and strategies under resources variations. Here we support that tree legumes species can adjust its functional traits to under fluctuating environments, and thus may tolerate future climate changes with great ecological impact on important biogeochemical cycles restoration of forest ecosystems such as Amazonia.

Supplementary data

Table S1. List of name, symbols and variable units.

Table S2. Functional traits mean results under high nutrient treatment in wet period.

Table S3. Functional traits mean results under high nutrient treatment in drought period.

Table S4. Functional traits mean results under low nutrient treatment in wet period.

Table S5. Functional traits mean results under low nutrient treatment in drought period.

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Supplementary materials to:

Functional responses of tree legumes to nutrient and water availabilities: Linking economics with carbohydrates dynamics

Roberto Jaquetti, José Francisco Gonçalves

Table S1 Name, symbols and variable units of leaf, stem and root functional traits and formulae used extracted in the fluorescence transient data calculation used in the multivariate principal component analysis. FM, fresh matter; φ_{P0}, (TR_0/ABS); ψ_0, (ET_0/TR_0); F_{2ms}, Fluorescence intensity at 2 ms; M_0, net rate of PSII closure $[4(F_{300\mu s} - F_{50\mu s})/(F_m - F_{50\mu s})]$; V_j, relative variable fluorescence at J step $(F_{2ms} - F_0)/(F_m - F_0)$.

Variables names	Symbols	Units and formulae
Relative growth rate in height	**RGR$_H$**	cm cm^{-1} month^{-1}
Relative growth rate in diameter	**RGR$_D$**	mm mm^{-1} month^{-1}
Absolute growth rate in biomass	**AGR$_{Bio}$**	kg kg^{-1} month^{-1}
Shoot-to-root ratio	**S:R**	ratio
Stem mass fraction	**SMF**	kg kg^{-1}
Leaf mass fraction	**LMF**	kg kg^{-1}
Root mass fraction	**RMF**	kg kg^{-1}
Net photosynthetic rates	P_n	μmol m^{-2} s^{-1}
Dark respiration rates	R_d	μmol m^{-2} s^{-1}
Stomatal conductance	g_s	mmol m^{-2} s^{-1}
Transpiration rates	E	mmol m^{-2} s^{-1}
Water use efficiency	WUE	μmol mmol^{-1}
Intrinsic water use efficiency	$IWUE$	μmol mmol^{-1}
Leaf water potential	Ψ_L	MPa
Leaf area	LA	m^2
Specific leaf area	SLA	cm^{-2} g^{-1}
Leaf carbon concentration	C	%
Leaf hydrogen concentration	H	%

Leaf nitrogen concentration	N	g kg^{-1}
Leaf carbon-to-nitrogen ratio	$C{:}N$	ratio
Leaf carbon concentration	P	g kg^{-1}
Leaf nitrogen-to-phosphorous ratio	$N{:}P$	ratio
Leaf potassium concentration	K	g kg^{-1}
Leaf calcium concentration	Ca	g kg^{-1}
Leaf magnesium concentration	Mg	g kg^{-1}
Leaf iron concentration	Fe	mg kg^{-1}
Leaf zinc concentration	Zn	mg kg^{-1}
Photosynthetic phosphorous use efficiency	$PPUE$	mmol mol^{-1} s^{-1}
Photosynthetic potassium use efficiency	$PKUE$	mmol mol^{-1} s^{-1}
Photosynthetic calcium use efficiency	$PCaUE$	mmol mol^{-1} s^{-1}
Photosynthetic magnesium use efficiency	$PMgUE$	mmol mol^{-1} s^{-1}
Photosynthetic iron use efficiency	$PFeUE$	mmol mol^{-1} s^{-1}
Photosynthetic zinc use efficiency	$PZnUE$	mmol mol^{-1} s^{-1}
Leaf soluble sugars concentration	SS_L	g kg^{-1}
Leaf starch concentration	$Starch_L$	g kg^{-1}
Leaf total non-structural carbohydrates	NSC_L	g kg^{-1}
Leaf soluble sugar-to-starch ratio	$SS{:}Starch_L$	ratio
Root soluble sugars concentration	SS_R	g kg^{-1}
Root starch concentration	$Starch_R$	g kg^{-1}

Root total non-structural carbohydrates	**NSC_R**	g kg^{-1}
Root soluble sugar-to-starch ratio	**$SS{:}Starch_R$**	ratio
Leaf-to-root SS ratio	**$SS_L{:}SS_R$**	ratio
Leaf-to-root starch ratio	**$Starch_L{:}Starch_R$**	ratio
Chlorophyll *a* content	**Chl *a***	mg g^{-1} (FM)
Chlorophyll *b* content	**Chl *b***	mg g^{-1} (FM)
Carotenoids content	**Car**	mg g^{-1} (FM)
Chlorophyll *a*-to-chlorophyll *b* ratio	**Chl *a:b***	ratio
Chlorophyll *a* + *b*-to-carotenoids ratio	**Chl:Car**	ratio
Initial fluorescence	**F_0**	Fluorescence intensity at 50 µs
Maximum fluorescence	**F_m**	Fluorescence intensity at 30 ms
Variable fluorescence	**F_v**	$(F_m - F_0)$
Maximum quantum efficiency of PSII photochemistry	**F_v/F_m**	(F_v/F_m)
Performance index of chlorophyll fluorescence	**PI_{ABS}**	$(RC/CS)[\varphi_{P0}/(1-\varphi_{P0})][\psi_0/(1-\psi_0)]$
Electron transport probability	**ET_0/TR_0**	$[1 - (F_{2ms} - F_0)/(F_m - F_0)]$
Maximal trapping rate of PS II	**TR_0/RC**	(M_0/V_j)
Effective antenna size of an active per reaction center	**ABS/RC**	$(TR_0/RC)/(TR_0/ABS)$
Electron transport in an active per reaction center	**ET_0/RC**	$(TR_0/RC){\cdot}(ET_0/TR_0)$
Effective dissipation of an active per reaction center	**DI_0/RC**	$(ABS/RC) - (TR_0/RC)$
Density of reaction centers per cross section	**RC/CS**	$(ABS/CS){\cdot}(RC/ABS)$

Table S2 Gas exchange, nutrients, non-structural carbohydrates, photosynthetic pigments and chlorophyll fluorescence variable of six tree legumes species under high nutrient treatment in wet period (n = 9; n = 7 for *S. reticulata* and *I. edulis*). The net photosynthetic rates (P_n); dark respiration (R_d); transpiration (E); stomatal conductance (g_s); water use efficiency (*WUE*); intrinsic water use efficiency (*IWUE*); leaf water potential (Ψ_L); leaf area (LA); specific leaf area (SLA); leaf carbon (C), hydrogen (H), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) concentrations; carbon-to-nitrogen (C:N) and nitrogen-to-phosphorous (N:P) ratios; photosynthetic nutrient use efficiency for phosphorous (*PPUE*), potassium (*PKUE*), calcium (*PCaUE*), magnesium (*PMgEU*), iron (*PFeUE*) and zinc (*PZnUE*); leaf soluble sugars (SS_L), starch ($Starch_L$), and total non-structural carbohydrates (NSC_L) concentration and soluble sugars-to-starch ratio ($SS:Starch_L$); root soluble sugars (SS_R), starch ($Starch_R$), and total non-structural carbohydrates (NSC_R) concentration and soluble sugars-to-starch ratio ($SS:Starch_R$); leaf soluble sugars-to-root soluble sugars ($SS_L:SS_R$); leaf starch-to-root starch ($Starch_L:Starch_R$); leaf chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid (Car) content; chlorophyll a-to-chlorophyll b ratio (Chl a:b), chlorophyll (a + b)-to-carotenoids ratio (Chl:Car); initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), maximum quantum efficiency of PSII photochemistry (F_v/F_m), performance index of chlorophyll fluorescence (PI_{abs}), electron transport probability (ET_0/TR_0), maximal trapping rate of PS II (TR_0/RC), effective antenna size of an active RC (ABS/RC), electron transport in an active RC (ET_0/RC), effective dissipation of an active RC (DI_0/RC) and density of reaction centers per CS (RC/CS) chlorophyll fluorescence parameters. Mean of nine plants (± SD); mean of seven plants for *S. reticulata* and *I. edulis* (± SD).

Variables	High nutrient species					
	C. tocantinum	*S. reticulata*	*D. odorata*	*C. fairchildiana*	*I. edulis*	*Acacia* sp.
	Wet period					
P_n	9.07 ± 3.6	22.21 ± 2.7	8.56 ± 3.1	22.84 ± 2.8	19.46 ± 2.3	19.05 ± 4.0
R_d	1.05 ± 0.2	1.59 ± 0.3	1.00 ± 0.4	2.02 ± 0.4	1.22 ± 0.3	2.21 ± 0.3
g_s	0.12 ± 0.1	0.88 ± 0.4	0.16 ± 0.1	0.50 ± 0.2	0.53 ± 0.2	0.64 ± 0.4
E	3.03 ± 1.6	8.42 ± 0.7	3.58 ± 1.3	7.57 ± 1.3	7.16 ± 0.9	7.18 ± 2.4
WUE	3.18 ± 0.6	2.65 ± 0.4	2.50 ± 0.7	3.05 ± 0.3	2.74 ± 0.3	2.59 ± 0.3
IWUE	92.52 ± 26.6	30.45 ± 14.9	66.07 ± 29.7	50.39 ± 16.1	39.88 ± 12.7	42.20 ± 26.1
Ψ_L	0.99 ± 0.4	0.69 ± 0.3	1.36 ± 0.4	0.82 ± 0.3	1.34 ± 0.3	0.96 ± 0.3
LA	218.00 ± 35.7	1.057.57 ± 414.6	920.00 ± 352.8	183.78 ± 18.5	615.38 ± 148.49	119.78 ± 27.0
SLA	179.16 ± 28.5	177.21 ± 33.7	110.84 ± 13.5	140.37 ± 15.5	128.57 ± 5.7	106.23 ± 9.1
C	47.04 ± 0.8	45.29 ± 0.6	48.86 ± 0.4	47.27 ± 0.3	47.90 ± 0.4	**51.20 ± 0.5**

H	6.32 ± 0.1	6.53 ± 0.0	6.97 ± 0.1	6.49 ± 0.1	6.25 ± 0.1	6.88 ± 0.1
N	17.14 ± 2.9	18.83 ± 2.8	18.11 ± 1.2	**29.41 ± 2.3**	**29.83 ± 1.8**	22.32 ± 1.2
C:N	28.06 ± 4.3	24.51 ± 3.1	27.08 ± 1.7	**16.17 ± 1.2**	**16.12 ± 0.9**	**22.99 ± 1.2**
P	1.41 ± 0.3	1.73 ± 0.2	**0.73 ± 0.1**	1.52 ± 0.2	1.42 ± 0.1	**1.06 ± 0.1**
N:P	12.46 ± 2.3	10.89 ± 1.6	**25.62 ± 5.0**	19.01 ± 1.2	21.16 ± 2.2	21.31 ± 2.4
K	7.03 ± 0.7	7.05 ± 0.3	**9.01 ± 3.2**	5.82 ± 1.7	6.38 ± 0.9	7.11 ± 1.0
Ca	12.09 ± 0.9	12.71 ± 1.8	5.23 ± 0.8	7.61± 1.1	7.14 ± 1.1	5.61 ± 1.2
Mg	0.92 ± 0.2	1.85 ± 0.2	0.79 ± 0.3	1.11 ± 0.2	1.13 ± 0.2	1.18 ± 0.1
Fe	71.63 ± 33.01	99.14 ± 15.3	99.33 ± 88.3	135.22 ± 76.1	108.00 ± 23.9	131.67 ± 38.4
Zn	5.78 ± 1.8	**4.14 ± 1.6**	5.89 ± 1.0	11.11 ± 3.1	8.29 ± 1.9	8.33 ± 1.0
PPUE	3561.85 ± 1380.0	7046.78 ± 1449.2	3999.20 ± 1128.4	6587.95 ± 1011.4	5505.09 ± 1082.2	5948.30 ± 1339.9
PKUE	889.81 ± 324.9	2158.01 ± 364.8	444.86 ± 201.0	2340.41 ± 778.7	1538.57 ± 212.6	1125.12 ± 287.8
PCaUE	579.41 ± 314.5	1256.39 ± 341.1	807.03 ± 371.6	1822.39 ± 566.7	1432.98 ± 257.0	1501.18 ± 497.8
PMgUE	4735.58 ± 2804.0	5189.03 ± 1198.2	3156.58 ± 1470.4	7389.95 ± 2118.6	5425.97 ± 977.9	4157.14 ± 882.0
PFeUE	144.08 ± 41.3	229.17 ± 75.9	74.45 ± 39.5	162.46 ± 68.8	133.32 ± 25.1	96.87 ± 44.0
PZnUE	1917.20 ± 871.4	7537.24 ± 4603.9	1047.07 ± 330.3	2074.95 ± 782.3	2057.08 ± 511.3	1588.92 ± 332.1
SS$_L$	59.67 ± 18.1	119.31 ± 24.3	93.53 ± 20.2	89.26 ± 13.0	101.94 ± 25.6	111.71 ± 22.2
Starch$_L$	170.12 ± 60.3	213.68 ± 38.9	159.85 ± 37.0	142.03 ± 79.3	120.65 ± 24.8	147.63 ± 52.7
NSC$_L$	229.79 ± 69.3	332.99 ± 47.3	253.38 ± 32.3	231.28 ± 87.7	222.59 ± 47.0	264.21 ± 52.2
SS:Starch$_L$	0.38 ± 0.1	0.57 ± 0.1	0.63 ± 0.3	0.73 ± 0.2	0.85 ± 0.2	0.87 ± 0.4
SS$_R$	47.02 ± 9.8	36.39 ± 7.3	40.52 ± 7.2	43.00 ± 15.8	37.96 ± 3.3	27.56 ± 4.0
Starch$_R$	147.83 ± 46.6	96.39 ± 16.4	108.13 ± 45.3	138.10 ± 28.1	87.02 ± 29.2	50.92 ± 6.3
NSC$_R$	194.85 ± 47.2	132.78 ± 22.3	148.65 ± 49.2	178.69 ± 35.4	127.57 ± 31.8	78.48 ± 8.9
SS:Starch$_R$	0.35 ± 0.1	0.38 ± 0.1	0.40 ± 0.1	0.32 ± 0.1	0.47 ± 0.1	0.54 ± 0.1
SS$_L$:SS$_R$	1.32 ± 0.5	3.33 ± 0.7	2.32 ± 0.4	2.52 ± 1.0	2.58 ± 0.6	4.11 ± 0.9
Starch$_L$:Starch$_R$	1.34 ± 0.8	2.31 ± 0.4	1.61 ± 0.5	1.07 ± 0.5	1.54 ± 0.5	3.03 ± 1.4

Chl a	0.82 ± 0.5	1.30 ± 0.5	0.67 ± 0.3	1.26 ± 0.3	1.23 ± 0.5	1.18 ± 0.3
Chl b	0.35 ± 0.3	0.43 ± 0.2	0.23 ± 0.1	0.42 ± 0.1	0.43 ± 0.2	0.39 ± 0.1
Car	0.43 ± 0.2	0.55 ± 0.2	0.27 ± 0.1	0.52 ± 0.2	0.50 ± 0.2	0.48 ± 0.1
Chl a:b	2.59 ± 0.6	3.02 ± 0.1	2.83 ± 0.3	3.07 ± 0.3	2.93 ± 0.4	3.05 ± 0.1
Chl:Car	2.57 ± 0.6	3.06 ± 0.4	3.37 ± 0.8	3.29 ± 0.5	3.25 ± 0.4	3.27 ± 0.3
F_0	562.78 ± 52.6	622.43 ± 119.9	709.23 ± 168.8	520.78 ± 59.2	571.06 ± 75.5	402.28 ± 80.8
F_m	2567.89 ± 266.7	2579.29 ± 208.9	2168.11 ± 342.9	2411.17 ± 160.7	2750.25 ± 228.2	2177.06 ± 240.2
F_v	2005.11 ± 298.5	1956.86 ± 302.0	1458.88 ± 413.0	1890.39 ± 195.2	2179.19 ± 180.8	1774.78 ± 180.1
F_v/F_m	0.78 ± 0.0	0.75 ± 0.1	0.65 ± 0.1	0.78 ± 0.0	0.79 ± 0.0	**0.82 ± 0.0**
PI_{ABS}	0.67 ± 0.3	**0.31 ± 0.1**	0.68 ± 0.6	1.15 ± 0.6	1.58 ± 0.4	**2.28 ± 0.5**
ET_0/TR_0	0.34 ± 0.0	0.22 ± 0.1	0.37 ± 0.1	0.41 ± 0.1	0.52 ± 0.1	0.56 ± 0.1
TR_0/RC	2.47 ± 0.2	2.50 ± 0.2	2.39 ± 0.3	2.12 ± 0.2	2.15 ± 0.1	2.21 ± 0.1
ABS/RC	3.19 ± 0.3	3.33 ± 0.4	3.98 ± 1.4	2.73 ± 0.3	2.71 ± 0.2	2.71 ± 0.1
ET_0/RC	0.84 ± 0.2	0.55 ± 0.1	0.85 ± 0.2	0.87 ± 0.1	1.10 ± 0.1	1.25 ± 0.1
DI_0/RC	0.72 ± 0.2	0.84 ± 0.3	1.59 ± 1.1	0.60 ± 0.2	0.56 ± 0.1	0.50 ± 0.1
RC/CS	176.94 ± 14.1	186.44 ± 20.4	190.50 ± 25.7	191.40 ± 7.9	211.12 ± 26.0	148.71 ± 31.4

Table S3 Gas exchange, nutrients, non-structural carbohydrates, photosynthetic pigments and chlorophyll fluorescence variable of six tree legumes species under high nutrient treatment in drought period (n = 9; n = 7 for *S. reticulata* and *I. edulis*). The net photosynthetic rates (P_n); dark respiration (R_d); transpiration (E); stomatal conductance (g_s); water use efficiency (*WUE*); intrinsic water use efficiency (*IWUE*); leaf water potential (Ψ_L); leaf area (LA); leaf carbon (C), hydrogen (H), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) concentrations; carbon-to-nitrogen (C:N) and nitrogen-to-phosphorous (N:P) ratios; leaf soluble sugars (SS_L), starch ($Starch_L$), and total non-structural carbohydrates (NSC_L) concentration and soluble sugars-to-starch ratio ($SS:Starch_L$); leaf chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid (Car) content; chlorophyll a-to-chlorophyll b ratio (Chl a:b), chlorophyll (a + b)-to-carotenoids ratio (Chl:Car); initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), maximum quantum efficiency of PSII photochemistry (F_v/F_m), performance index of chlorophyll fluorescence (PI_{abs}), electron transport probability (ET_0/TR_0), maximal trapping rate of PS II (TR_0/RC), effective antenna size of an active RC (*ABS/RC*), electron transport in an active RC (ET_0/RC), effective dissipation of an active RC (DI_0/RC) and density of reaction centres per CS (*RC/CS*) chlorophyll fluorescence parameters. Mean of nine plants (± SD); mean of seven plants for *S. reticulata* and *I. edulis* (± SD).

	High nutrient species					
Variables	*C. tocantinum*	*S. reticulata*	*D. odorata*	*C. fairchildiana*	*I. edulis*	*Acacia* sp.
	Drought period					
P_n	3.93 ± 1.8	18.22 ± 7.3	5.87 ± 2.1	8.93 ± 4.1	12.91 ± 4.9	10.30 ± 4.1
R_d	1.01 ± 0.6	1.81 ± 0.4	0.84 ± 0.3	1.60 ± 0.4	1.06 ± 0.4	2.04 ± 0.7
g_s	0.04 ± 0.0	0.45 ± 0.4	0.06 ± 0.0	0.11 ± 0.1	0.21 ± 0.1	0.21 ± 0.2
E	1.45 ± 0.6	6.56 ± 2.2	1.80 ± 0.7	2.92 ± 1.6	4.40 ± 1.6	3.92 ± 2.0
WUE	2.75 ± 0.5	2.77 ± 0.5	3.34 ± 0.6	3.16 ± 0.3	2.94 ± 0.3	2.83 ± 0.6
IWUE	90.13 ± 16.8	57.60 ± 28.8	108.31 ± 16.6	97.08 ± 25.3	73.24 ± 25.2	75.11 ± 32.9
Ψ_L	0.84 ± 0.2	1.19 ± 0.3	1.37 ± 0.3	1.09 ± 0.2	1.86 ± 0.1	1.37 ± 0.2
LA	164.61 ±38.7	337.83 ±78.9	917.55 ± 344.5	115.69 ± 27.6	367.22 ± 73.5	111.24 ±17.2
C	47.27 ± 0.9	46.09 ± 1.6	48.84 ± 0.8	48.02 ± 0.6	48.54 ± 0.3	**50.03 ± 0.7**
H	6.42 ± 0.1	6.64 ± 0.2	6.82 ± 0.1	6.57 ± 0.1	6.23 ± 0.1	6.51 ± 0.1
N	16.79 ± 2.9	23.17 ± 1.85	16.71 ± 4.4	28.76 ± 3.3	**31.43 ± 2.9**	21.07 ± 2.7
C:N	28.85 ± 4.5	20.03 ± 2.1	31.25 ± 9.2	16.91 ± 2.0	15.56 ± 1.4	24.06 ± 2.7
P	1.67 ± 0.4	2.40 ± 0.4	2.77 ± 0.8	**3.53 ± 0.3**	2.02 ± 0.8	**1.27 ± 0.8**

N:P	10.49 ± 2.5	9.98 ± 2.1	**7.57 ± 6.7**	8.14 ± 0.7	17.48 ± 5.7	19.94 ± 7.0
K	6.87 ± 3.4	7.67 ± 3.3	**8.60 ± 2.7**	5.96 ± 1.0	4.23 ± 0.7	4.66 ± 1.4
Ca	15.96 ± 7.6	**18.29 ± 8.1**	5.74 ± 2.8	4.66 ± 1.7	6.31 ± 2.6	**2.82 ± 1.7**
Mg	1.54 ± 0.4	2.79 ± 0.6	0.95 ± 0.3	1.40 ± 0.3	**6.31 ± 2.6**	1.18 ± 0.3
Fe	18.22 ± 14.3	25.86 ± 5.3	51.22 ± 40.4	32.00 ± 11.1	41.25 ± 7.1	40.78 ± 11.0
Zn	10.67 ± 4.0	15.29 ± 3.6	13.22 ± 4.1	15.67 ± 5.8	**16.00 ± 4.0**	12.78 ± 2.7
SS_L	35.51 ± 11.7	57.02 ± 14.1	55.39 ± 12.4	71.88 ± 10.4	50.58 ± 21.6	**121.80 ± 11.9**
$Starch_L$	78.53 ± 11.2	**335.94 ± 91.1**	126.60 ± 40.5	81.90 ± 8.5	72.21 ± 12.7	88.99 ± 14.8
NSC_L	114.03 ± 21.8	392.95 ± 85.0	181.99 ± 42.6	151.12 ± 10.5	122.79 ± 27.2	210.78 ± 22.3
$SS:starch_L$	0.44 ± 0.1	**0.19 ± 0.1**	0.48 ± 0.2	0.81 ± 0.1	0.71 ± 0.3	**1.40 ± 0.2**
Chl a	0.40 ± 0.3	1.53 ± 0.5	0.70 ± 0.3	1.04 ± 0.4	0.96 ± 0.3	0.44 ± 0.2
Chl b	0.15 ± 0.1	0.47 ± 0.2	0.24 ± 0.1	0.36 ± 0.1	0.31 ± 0.1	0.16 ± 0.1
Car	0.27 ± 0.2	0.68 ± 0.2	0.33 ± 0.1	0.47 ± 0.1	0.53 ± 0.1	0.46 ± 0.1
Chl $a:b$	2.68 ± 0.3	3.26 ± 0.3	3.02 ± 0.2	2.93 ± 0.3	3.06 ± 0.5	2.92 ± 0.4
Chl:Car	1.93 ± 0.4	2.91 ± 0.3	2.81 ± 0.4	2.93 ± 0.3	2.37 ± 0.5	**1.38 ± 0.5**
F_0	737.00 ± 51.8	720.65 ± 65.5	737.44 ± 53.8	576.71 ± 34.5	600.25 ± 95.0	460.78 ± 67.7
F_m	2436.06 ± 302.8	2534.60 ± 172.6	2148.29 ± 262.1	2456.21 ± 100.0	2616.50 ± 197.8	1990.69 ± 204.2
F_v	1699.06 ± 339.3	1813.95 ± 194.4	1410.85 ± 290.5	1879.50 ± 97.4	2022.75 ± 233.1	1529.91 ± 220.5
F_v/F_m	0.69 ± 4.5	0.71 ± 2.1	0.64 ± 9.2	0.77 ± 2.0	0.77 ± 1.4	0.76 ± 2.7
PI_{ABS}	0.25 ± 0.2	**0.25 ± 0.1**	0.32 ± 0.2	0.66 ± 0.3	1.28 ± 0.7	1.09 ± 0.6
ET_0/TR_0	0.26 ± 0.1	0.23 ± 0.1	0.31 ± 0.1	0.38 ± 0.1	0.48 ± 0.1	0.48 ± 0.1
TR_0/RC	2.99 ± 0.2	2.53 ± 0.1	2.51 ± 0.1	2.54 ± 0.2	2.28 ± 0.2	2.59 ± 0.2
ABS/RC	4.35 ± 0.5	3.55 ± 0.3	3.92 ± 0.5	3.32 ± 0.2	2.97 ± 0.3	3.41 ± 0.4
ET_0/RC	0.76 ± 0.3	0.59 ± 0.2	0.76 ± 0.2	0.95 ± 0.1	1.10 ± 0.2	1.23 ± 0.1
DI_0/RC	1.36 ± 0.4	1.03 ± 0.2	1.41 ± 0.4	0.78 ± 0.1	0.69 ± 0.2	0.81 ± 0.3
RC/CS	170.71 ± 14.7	203.44 ± 13.2	190.08 ± 15.1	173.94 ± 5.3	202.55 ± 27.6	135.51 ± 14.6

Table S4 Gas exchange, nutrients, non-structural carbohydrates, photosynthetic pigments and chlorophyll fluorescence variable of six tree legumes species under low nutrient treatment in drought period (n = 9; n = 4 for *S. reticulata* and *I. edulis*). The net photosynthetic rates (P_n); dark respiration (R_d); transpiration (E); stomatal conductance (g_s); water use efficiency (*WUE*); intrinsic water use efficiency (*IWUE*); leaf water potential (Ψ_L); leaf area (LA); leaf carbon (C), hydrogen (H), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) concentrations; carbon-to-nitrogen (C:N) and nitrogen-to-phosphorous (N:P) ratios; leaf soluble sugars (SS_L), starch ($Starch_L$), and total non-structural carbohydrates (NSC_L) concentration and soluble sugars-to-starch ratio ($SS:Starch_L$); leaf chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid (Car) content; chlorophyll a-to-chlorophyll b ratio (Chl a:b), chlorophyll (a + b)-to-carotenoids ratio (Chl:Car); initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), maximum quantum efficiency of PSII photochemistry (F_v/F_m), performance index of chlorophyll fluorescence (PI_{abs}), electron transport probability (ET_0/TR_0), maximal trapping rate of PS II (TR_0/RC), effective antenna size of an active RC (*ABS/RC*), electron transport in an active RC (ET_0/RC), effective dissipation of an active RC (DI_0/RC) and density of reaction centres per CS (*RC/CS*) chlorophyll fluorescence parameters. Mean of nine plants (± SD); mean of four plants for *S. reticulata* and *I. edulis* (± SD).

Variables	Low nutrient species					
	C. tocantinum	*S. reticulata*	*D. odorata*	*C. fairchildiana*	*I. edulis*	*Acacia* sp.
	Drought period					
P_n	3.09 ± 1.1	**12.70 ± 3.0**	**3.89 ± 2.0**	**11.31 ± 3.8**	**10.20 ± 4.1**	**12.86 ± 4.0**
R_d	0.75 ± 0.1	1.65 ± 0.4	0.92 ± 0.4	1.48 ± 0.8	1.09 ± 0.4	2.16 ± 0.6
g_s	0.05 ± 0.0	0.18 ± 0.1	0.07 ± 0.0	0.20 ± 0.2	0.30 ± 0.1	0.25 ± 0.2
E	1.50 ± 0.6	4.51 ± 1.2	1.87 ± 0.7	4.04 ± 2.2	6.02 ± 2.0	4.82 ± 1.7
WUE	2.12 ± 0.5	2.88 ± 0.5	2.07 ± 0.7	3.16 ± 0.9	1.74 ± 0.6	2.56 ± 0.3
IWUE	63.51 ± 19.0	71.71 ± 9.2	59.72 ± 17.3	82.07 ± 37.5	36.30 ± 14.5	61.17 ± 20.3
Ψ_L	1.35 ± 0.6	1.00 ± 0.4	1.32 ± 0.4	0.88 ± 0.4	1.49 ± 0.3	1.01 ± 0.3
LA	97.49 ± 42.5	176.39 ± 151.4	113.47 ± 89.5	59.80 ± 26.5	258.56 ± 67.4	122.07 ± 13.7
C	46.22 ± 1.1	45.87 ± 1.0	49.89 ± 0.4	47.53 ± 0.6	48.17 ± 0.5	**50.75 ± 0.9**
H	6.28 ± 0.2	6.66 ± 0.2	6.94 ± 0.1	6.49 ± 0.1	6.11 ± 0.2	6.63 ± 0.1
N	14.64 ± 2.3	23.08 ± 4.2	12.67 ± 2.7	29.14 ± 2.9	24.50 ± 7.2	22.18 ± 2.5
C:N	32.15 ± 4.3	20.47 ± 4.3	41.34 ± 10.4	16.47 ± 1.9	21.22 ± 7.2	23.15 ± 2.8
P	2.49 ± 0.9	1.81 ± 0.6	2.20 ± 1.4	**3.91 ± 2.5**	3.33 ± 0.4	**0.84 ± 0.3**

N:P	6.60 ± 2.6	13.18 ± 2.0	8.90 ± 6.5	12.89 ± 8.4	7.32 ± 1.9	**28.45 ± 7.5**
K	2.58 ± 1.4	1.99 ± 4.0	6.50 ± 2.9	8.36 ± 1.7	1.89 ± 2.2	7.51 ± 2.6
Ca	14.24 ± 6.6	**14.25 ± 4.3**	6.50 ± 1.9	6.12 ± 1.6	4.97 ± 2.6	**3.84 ± 1.8**
Mg	2.30 ± 0.8	3.09 ± 0.5	1.20 ± 0.7	1.55 ± 0.4	**4.95 ± 2.6**	2.22 ± 1.8
Fe	24.00 ± 22.0	30.25 ± 8.7	42.88 ± 27.4	27.37 ± 13.7	35.25 ± 5.9	33.33 ± 6.5
Zn	17.22 ± 9.1	12.00 ± 4.8	22.25 ± 8.3	18.50 ± 4.5	14.25 ± 4.6	10.22 ± 3.1
SS	43.49 ± 9.3	51.89 ± 8.9	60.38 ± 6.5	106.75 ± 3.7	66.17 ± 10.0	**129.55 ± 16.0**
Starch	68.64 ± 6.0	**430.33 ± 21.1**	135.56 ± 23.1	103.44 ± 1.7	77.16 ± 20.7	96.58 ± 19.4
NSC	112.13 ± 15.2	482.22 ± 12.3	195.94 ± 27.6	210.19 ± 4.8	143.33 ± 29.4	226.13 ± 31.2
SS:Starch	0.62 ± 0.1	**0.12** ± 0.0	0.46 ± 0.1	1.03 ± 0.0	0.89 ± 0.1	**1.37 ± 0.2**
Chl a	0.31 ± 0.2	1.44 ± 0.5	0.39 ± 0.2	1.03 ± 0.3	0.60 ± 0.4	0.63 ± 0.2
Chl b	0.11 ± 0.1	0.45 ± 0.2	0.14 ± 0.1	0.34 ± 0.1	0.20 ± 0.1	0.19 ± 0.1
Car	0.22 ± 0.1	0.65 ± 0.2	0.24 ± 0.1	0.49 ± 0.1	0.38 ± 0.2	0.52 ± 0.1
Chl a:b	2.74 ± 0.4	3.20 ± 0.1	2.76 ± 0.3	3.01 ± 0.1	2.84 ± 0.7	3.20 ± 0.3
Chl:Car	1.85 ± 0.5	2.86 ± 0.2	2.26 ± 0.7	2.77 ± 0.3	1.96 ± 0.6	**1.67 ± 0.7**
F_0	561.63 ± 92.5	**674.13 ± 87.2**	**716.94 ± 146.8**	**716.93 ± 103.0**	**660.75 ± 173.4**	**437.21 ± 40.7**
F_m	1931.81 ± 348.7	2210.50 ± 133.4	1661.31 ± 412.9	2431.86 ± 143.7	1953.13 ± 308.0	1788.64 ± 252.9
F_v	1370.19 ± 329.0	1536.38 ± 144.6	944.38 ± 436.3	1714.93 ± 206.0	1292.38 ± 186.1	1351.43 ± 240.2
F_v/F_m	0.70 ± 0.1	0.69 ± 0.0	0.54 ± 0.1	0.70 ± 0.0	0.66 ± 0.1	0.75 ± 0.0
PI_{ABS}	0.25 ± 0.2	**0.21 ± 0.1**	0.18 ± 0.3	0.52 ± 0.3	0.27 ± 0.1	0.75 ± 0.3
ET_0/TR_0	0.26 ± 0.1	0.24 ± 0.1	0.22 ± 0.1	0.40 ± 0.1	0.35 ± 0.1	0.43 ± 0.0
TR_0/RC	3.11 ± 0.3	2.57 ± 0.2	3.06 ± 0.2	2.73 ± 0.2	3.02 ± 0.4	2.65 ± 0.1
ABS/RC	4.46 ± 0.7	3.72 ± 0.4	5.97 ± 1.6	3.91 ± 0.5	4.54 ± 0.3	3.52 ± 0.3
ET_0/RC	0.77 ± 0.3	0.61 ± 0.2	0.66 ± 0.4	1.08 ± 0.1	1.06 ± 0.4	1.14 ± 0.1
DI_0/RC	1.35 ± 0.5	1.14 ± 0.2	2.91 ± 1.5	1.18 ± 0.3	1.52 ± 0.2	0.88 ± 0.2
RC/CS	126.04 ± 11.9	181.77 ± 21.0	124.82 ± 26.7	183.41 ± 15.0	147.07 ± 42.4	124.93 ± 16.5

Table S5 Gas exchange, nutrients, non-structural carbohydrates, photosynthetic pigments and chlorophyll fluorescence variable of six tree legumes species under low nutrient treatment in wet period (n = 9; n = 4 for *S. reticulata* and *I. edulis*). The net photosynthetic rates (P_n); dark respiration (R_d); transpiration (E); stomatal conductance (g_s); water use efficiency (*WUE*); intrinsic water use efficiency (*IWUE*); leaf water potential (Ψ_L); leaf area (LA); specific leaf area (SLA); leaf phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) concentrations; photosynthetic nutrient use efficiency for phosphorous (*PPUE*), potassium (*PKUE*), calcium (*PCaUE*), magnesium (*PMgEU*), iron (*PFeUE*) and zinc (*PZnUE*); leaf soluble sugars (SS_L), starch ($Starch_L$), and total non-structural carbohydrates (NSC_L) concentration and soluble sugars-to-starch ratio ($SS:Starch_L$); root soluble sugars (SS_R), starch ($Starch_R$), and total non-structural carbohydrates (NSC_R) concentration and soluble sugars-to-starch ratio ($SS:Starch_R$); leaf soluble sugars-to-root soluble sugars ($SS_L:SS_R$); leaf starch-to-root starch ($Starch_L:Starch_R$); leaf chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid (Car) content; chlorophyll a-to-chlorophyll b ratio (Chl a:b), chlorophyll (a + b)-to-carotenoids ratio (Chl:Car); initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), maximum quantum efficiency of PSII photochemistry (F_v/F_m), performance index of chlorophyll fluorescence (PI_{abs}), electron transport probability (ET_0/TR_0), maximal trapping rate of PS II (TR_0/RC), effective antenna size of an active RC (ABS/RC), electron transport in an active RC (ET_0/RC), effective dissipation of an active RC (DI_0/RC) and density of reaction centres per CS (RC/CS) chlorophyll fluorescence parameters. Mean of nine plants (± SD); mean of four plants for *S. reticulata* and *I. edulis* (± SD).

Variables	Low nutrient species					
	C. tocantinum	*S. reticulata*	*D. odorata*	*C. fairchildiana*	*I. edulis*	*Acacia* sp.
	Wet period					
P_n	4.70 ± 2.4	22.86 ± 4.2	6.49 ± 2.4	19.19 ± 4.1	13.88 ± 5.3	20.27 ± 2.7
R_d	0.86 ± 0.2	1.47 ± 0.2	1.00 ± 0.3	1.71 ± 0.7	1.56 ± 0.3	2.22 ± 0.7
g_s	0.12 ± 0.1	0.87 ± 0.3	0.16 ± 0.1	0.47 ± 0.1	0.48 ± 0.2	0.72 ± 0.4
E	2.92 ± 1.4	8.88 ± 1.1	3.45 ± 0.8	7.17 ± 1.5	7.14 ± 0.9	8.34 ± 1.6
WUE	1.68 ± 0.5	2.56 ± 0.2	1.87 ± 0.5	2.69 ± 0.3	1.97 ± 0.8	2.46 ± 0.3
IWUE	46.29 ± 16.9	28.10 ± 7.0	45.31 ± 15.2	42.62 ± 8.2	32.57 ± 16.2	34.63 ± 15.7
Ψ_L	1.41 ± 0.4	0.88 ± 0.3	1.38 ± 0.3	0.81 ± 0.3	1.49 ± 0.2	0.85 ± 0.2
LA	1.26 ± 0.2	1.69 ± 0.2	1.11 ± 0.4	1.52 ± 0.3	1.65 ± 0.2	2.16 ± 0.2
SLA	188.05 ± 25.1	188.13 ± 30.8	154.64 ± 26.2	144.10 ± 27.4	144.11 ± 9.2	107.80 ± 8.9
P	3.26 ± 1.6	1.18 ± 0.4	**0.72 ± 0.2**	1.21 ± 0.3	0.84 ± 0.4	**0.83 ± 0.4**
K	3.19 ± 1.6	7.21 ± 1.4	6.26 ± 2.6	5.15 ± 2.4	4.32 ± 2.0	5.53 ± 1.4

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Ca	12.40 ± 3.8	13.50 ± 2.1	8.10 ± 4.0	10.07 ± 2.9	8.29 ± 2.7	7.94 ± 4.8
Mg	3.32 ± 2.7	2.70 ± 0.4	1.04 ± 0.7	1.73 ± 1.1	1.70 ± 1.2	1.11 ± 0.4
Fe	63.44 ± 17.9	117.00 ± 34.9	96.11 ± 56.9	122.62 ± 58.4	69.50 ± 19.2	94.44 ± 47.5
Zn	27.11 ± 14.4	**11.00 ± 4.3**	23.33 ± 4.5	18.62 ± 7.5	12.75 ± 6.1	6.78 ± 1.9
PPUE	1131.51 ± 1004.5	12492.71 ± 5966.5	4908.48 ± 2673.2	7357.30 ± 2646.9	7532.30 ± 711.8	10594.72 ± 7159.8
PKUE	1278.42 ± 679.4	2491.72 ± 1224.1	851.48 ± 674.9	2677.78 ± 1955.6	1976.87 ± 763.8	1621.98 ± 426.8
PCaUE	323.62 ± 173.7	1290.65 ± 284.5	634.80 ± 512.2	1201.19 ± 501.9	999.69 ± 257.4	**1420.99 ± 680.9**
PMgUE	851.95 ± 566.2	3836.15 ± 510.6	3103.71 ± 2247.5	4921.66 ± 2565.0	3774.01 ± 2158.5	**5268.00 ± 2029.0**
PFeUE	82.13 ± 35.9	210.13 ± 42.6	79.59 ± 48.9	144.68 ± 67.2	158.12 ± 27.7	157.02 ± 70.3
PZnUE	266.33 ± 210.9	1996.77 ± 1543.3	295.10 ± 135.2	1089.13 ± 454.6	1382.11 ± 1251.3	2221.86 ± 571.6
SS$_L$	42.62 ± 7.7	64.72 ± 20.2	51.21 ± 9.1	61.63 ± 22.1	84.21 ± 44.0	118.46 ± 35.0
Starch$_L$	70.91 ± 22.4	158.71 ± 25.5	57.83 ± 5.3	79.47 ± 28.8	68.37 ± 41.0	138.53 ± 48.5
NSC$_L$	113.53 ± 26.2	223.43 ± 42.8	109.04 ± 8.8	138.17 ± 41.9	152.59 ± 84.6	257.00 ± 72.6
SS:starch$_L$	0.67 ± 0.2	0.40 ± 0.1	0.90 ± 0.2	0.85 ± 0.4	1.27 ± 0.2	0.92 ± 0.3
SS$_R$	43.61 ± 9.8	39.82 ± 6.6	49.59 ± 9.8	53.42 ± 16.3	36.24 ± 3.8	26.77 ± 5.4
Starch$_R$	209.57 ± 44.9	93.51 ± 12.8	117.11 ± 33.1	221.48 ± 71.1	114.24 ± 44.3	52.36 ± 8.4
NSC$_R$	253.18 ± 42.0	133.33 ± 15.5	166.70 ± 36.1	274.90 ± 76.2	150.48 ± 47.7	79.12 ± 12.4
SS:Starch$_R$	0.22 ± 0.1	0.43 ± 0.1	0.44 ± 0.1	0.26 ± 0.1	0.34 ± 0.1	0.51 ± 0.1
SS$_L$:SS$_R$	1.03 ± 0.3	1.63 ± 0.4	1.07 ± 0.3	1.25 ± 0.6	2.32 ± 1.1	4.73 ± 1.9
Starch$_L$:Starch$_R$	0.35 ± 0.1	1.70 ± 0.2	0.53 ± 0.1	0.38 ± 0.2	0.66 ± 0.4	2.71 ± 1.0
Chl *a*	0.42 ± 0.1	1.56 ± 0.4	0.63 ± 0.2	1.03 ± 0.3	0.72 ± 0.3	0.82 ± 0.3
Chl *b*	0.19 ± 0.1	0.51 ± 0.1	0.28 ± 0.1	0.33 ± 0.1	0.25 ± 0.1	0.28 ± 0.1
Car	0.25 ± 0.1	0.66 ± 0.2	0.30 ± 0.1	0.41 ± 0.1	0.40 ± 0.1	0.40 ± 0.1
Chl *a:b*	2.46 ± 0.7	3.07 ± 0.2	2.59 ± 0.5	3.13 ± 0.4	2.84 ± 0.5	3.05 ± 0.4
Chl:Car	2.37 ± 0.3	3.09 ± 0.1	2.94 ± 0.4	3.26 ± 0.2	3.45 ± 0.6	2.71 ± 0.4
F_0	541.83 ± 77.2	623.75 ± 73.7	683.17 ± 155.6	564.18 ± 64.9	525.25 ± 42.2	406.17 ± 64.1

F_m	2006.44 ± 246.6	2541.00 ± 257.8	1651.78 ± 523.0	2359.94 ± 140.9	2508.75 ± 312.9	2006.78 ± 289.9
F_v	1464.61 ± 168.1	1917.25 ± 319.2	968.61 ± 485.5	1795.75 ± 192.9	1983.50 ± 271.9	1600.61 ± 347.4
F_v/F_m	0.72 ± 0.1	0.75 ± 0.1	0.55 ± 0.1	0.76 ± 0.0	0.79 ± 0.0	0.79 ± 0.1
PI_{ABS}	0.29 ± 0.2	0.47 ± 0.4	**0.12 ± 0.1**	0.82 ± 0.5	0.86 ± 0.2	**1.84 ± 1.0**
ET_0/TR_0	0.27 ± 0.0	0.29 ± 0.1	0.37 ± 0.1	0.41 ± 0.1	0.52 ± 0.1	0.56 ± 0.1
TR_0/RC	3.09 ± 0.3	2.37 ± 0.2	2.80 ± 0.3	2.35 ± 0.2	2.67 ± 0.3	2.32 ± 0.2
ABS/RC	4.31 ± 0.7	3.19 ± 0.5	5.32 ± 1.5	3.11 ± 0.4	3.38 ± 0.4	2.98 ± 0.5
ET_0/RC	0.84 ± 0.2	0.68 ± 0.2	0.58 ± 0.2	0.89 ± 0.2	1.14 ± 0.1	1.15 ± 0.2
DI_0/RC	1.21 ± 0.5	0.81 ± 0.3	2.52 ± 1.3	0.76 ± 0.2	0.71 ± 0.1	0.66 ± 0.4
RC/CS	127.70 ± 20.2	196.85 ± 16.2	135.77 ± 37.9	182.57 ± 12.5	157.22 ± 23.2	136.67 ± 4.4

GENERAL CONCLUSIONS

Amazonian forest species showed great plasticity during the great 2015/16 El Niño event. However, *Acacia* sp. native from drier ecosystems had higher tolerance to limited resources of degraded environments. In accordance with economics spectrum hypothesis under high water and nutrients availabilities plants increased significantly fast traits such as energy use efficiency and the photosynthetic, transpiration and growth rates. As for the ecological groups N_2-fixing species increased eight times the biomass growth compared to non N_2-fixing species, mostly in stems and leaves tissues, confirming BNF as a fast root trait. Under nutrient and water limitations species increase slow traits such as energy dissipation, water use efficiency and root fraction confirming the functional equilibrium hypothesis. Additionally, increased RMF was positively correlated with NSC (SS and starch) content in root also confirming an active process of allocation. However, biomass allocation to roots appears an inefficient strategy under multiple nutrient limitations, since unfertilized plants have growth rates close to zero. Some individuals of unfertilized *Acacia* sp. exceptionally cope under multiple nutrient limitations. Functional strategies of *Acacia* sp. involve traits adjustments such as increased growth, nutrient and energy use efficiency (PI_{ABS}), in addition to the enhanced NSC pools in leaf compartments. Drought stress responses found in *S. reticulata* along PCA axis 2 was related to increased leaf Ca and starch contents. Confirming our third hypothesis NSC dynamics was important to acclimation responses of species as pools of stored starch were used during drought period, according with the variations in the SS:Starch which was more sensitive than leaf NSC content alone. Additionally, the accessibility to NSC pools of the species will dictate acclimation responses. Moreover, the strategy of highly productive *Acacia* sp. is due to the increased NSC pools in LMF and the consequent increased sink strength.

The high leaf nutrient content, especially N, found in *C. fairchildiana* and *I. edulis*, indicates the potential use for restoration of N cycle, while the high biomass production and leaf C content of *Acacia* sp. are indicatives to use for the restoration of the C cycle in degraded environments.

The multivariate analysis of growth and functional traits such as gas exchange, chlorophyll fluorescence and NSC contents reveal to be efficient in the detection of plant responses to environmental cues. However, considering the practical, costs and complexity of responses the chlorophyll fluorescence appears quite effective to assess the performance and stress levels of species in particular the parameters DI_0/RC and PI_{ABS}. The specific responses in degraded areas represent extreme strategies; however, our results shall be to some extent, applicable to late successional species of natural forests. Finally, we recommend in-depth biochemical and ecofunctional studies with N_2-fixing species, as they play fundamental role in the restoration of important biogeochemical cycles in degraded areas in Amazonia.

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