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Responses of aquatic macrophytes to natural, land-use, and climatic changes in the Amazon: a functional approach

Respostas das macrófitas aquáticas às mudanças ambientais naturais, do uso da terra e climáticas na Amazônia: uma abordagem funcional

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Advisor: Prof^{a}. Dra. Thaísa Sala Michelan

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People are like plants: they grow towards the light. I chose science because science gave me what I needed – a home as defined in the most literal sense: a safe place to be.

Hope Jahren

Ces paysages d'eau et de reflects sont devenus une *obsession. C'est au*-delá de mes forces de *viellard, et je veux cepandant arriver à rendre ce que je ressens. J'en ai détruit... J'en recommence... Et j'espère que de tant d'efforts, il sortira quelque chose*

Claude Monet

Sou entre flor e nuvem, estrela e mar. Por que havemos de ser unicamente humanos, limitados em chorar? Não encontro caminhos fáceis de andar. Meu rosto vário desorienta as firmes pedras que não sabem de água e de ar.

Cecília Meireles

Respostas das macrófitas aquáticas às mudanças ambientais naturais, do uso da terra e climáticas na Amazônia: uma abordagem funcional

RESUMO

Durante as últimas décadas, os ecólogos têm utilizado abordagens baseadas em características funcionais para compreender a mais diversificada gama de questões ecológicas, incluindo a adaptação de indivíduos ao estresse, dinâmicas populacionais e respostas de comunidades a fatores bióticos e abióticos. As plantas têm sido o foco de muitos estudos de ecologia funcional ao redor do mundo. No entanto, apesar do grande número de estudos buscando entender as respostas das plantas a diversas variações em seu ambiente por meio da abordagem funcional, pouca atenção foi dada às plantas aquáticas. Portanto, esta tese teve como objetivo investigar as respostas de macrófitas aquáticas (no nível de indivíduo e comunidades) à variação ambiental natural e a múltiplos estressores (mudanças climáticas e uso da terra), utilizando a abordagem funcional. A tese está dividida em três seções. Na primeira, investigamos a variação intraespecífica de macrófitas em resposta à variação ambiental natural, enquanto na segunda, investigamos a variação intraespecífica sob estresse hídrico induzido por mudanças climáticas. Finalmente, na terceira seção, utilizamos medidas de diversidade funcional e filogenética para investigar as respostas em nível comunitário às mudanças no uso da terra. Demonstramos que indivíduos de macrófitas alteram características foliares ao longo de um gradiente de recursos ambientais, refletindo em estratégias ecológicas relacionadas à disponibilidade de nutrientes, oxigênio e composição do solo. A variação intraespecífica sob estresse hídrico revelou que algumas espécies podem ser tolerantes, enquanto outras são sensíveis, e estas respostas específicas de cada espécie podem ser cruciais para entender os impactos das mudanças climáticas nas comunidades de macrófitas e ecossistemas de água doce. Ao explorar as comunidades de macrófitas na Amazônia ao longo de um gradiente de uso da terra, revelamos um desacoplamento da diversidade funcional e filogenética. Sob pressão do uso da terra, a diversidade funcional aumenta, mas a diversidade filogenética é negativamente afetada. Essas mudanças devem ser consideradas ao escolher áreas prioritárias para a conservação, enfatizando a perda potencial de informação filogenética em comunidades de macrófitas devido às mudanças no uso da terra e enfatizando na importância de formular estratégias abrangentes para a preservação da biodiversidade diante dos desafios ambientais. Concluímos que é importante compreender as respostas ecofisiológicas de plantas aquáticas a estressores ambientais, bem como considerar aspectos funcionais e filogenéticos nos esforços de conservação, garantindo a preservação da biodiversidade e processos ecológicos em ecossistemas de água doce diante das contínuas mudanças ambientais, enquanto reforçamos que ainda há muitos padrões a serem explorados em relação às respostas das macrófitas a diversos estressores, especialmente em regiões ecologicamente significativas como a Amazônia.

Palavras-chave: ecologia de plantas aquáticas, estratégias funcionais de plantas, variação intraespecífica, biodiversidade aquática, adaptação às mudanças climáticas, resiliência ecológica

Responses of aquatic macrophytes to natural, land-use, and climatic changes in the Amazon: a functional approach

ABSTRACT

Over the last few decades, ecologists have focused on employing trait-based approaches to understand the most diverse array of ecological questions, including adaptation of individuals to stress, population dynamics, and community responses to both biotic and abiotic factors, and plants have been the focus group of many studies around the world. However, despite the enormous number of studies trying to understand plant responses to an assortment of variations in their environment through the trait-based approach, little attention has been paid to aquatic plants. Thus, this thesis aimed to investigate the responses of aquatic macrophytes (at individual and community levels) to natural environmental variation and multiple stressors (climate change and land use), using the trait-based approach. This thesis is divided into three sections. In the first section, we investigated the intraspecific variation of macrophytes in response to natural environmental variation, while in the second, we investigated intraspecific variation under climate-change-induced water stress. Finally, in the third section, we employed functional and phylogenetic diversity measures to investigate community-level responses to land-use change. We demonstrated macrophyte individuals altering leaf traits along a resource-use gradient, reflecting ecological strategies shaped by nutrient, oxygen, and soil composition. Intraspecific variation under water stress reveals some species may be tolerant, while others are sensitive, and this species-specific variation may be crucial for understanding climate change impacts on macrophyte communities and freshwater ecosystems. And, exploring Amazon macrophyte communities along a land-use gradient unveils a decoupling of functional and phylogenetic diversity. Under land-use pressure, functional diversity increases, but phylogenetic diversity is negatively affected. These shifts should be considered when choosing priority areas for conservation, emphasizing the potential loss of phylogenetic information in macrophyte communities due to land-use changes, and urging for formulation of comprehensive strategies for biodiversity preservation amidst environmental challenges. Thus, we conclude that it is important to understand the ecophysiological reactions of aquatic plants to environmental stressors, as well as consider both functional and phylogenetic aspects in conservation efforts, to ensure the preservation of biodiversity and ecological processes in freshwater ecosystems amidst ongoing environmental changes, while reinforcing that there are still many patterns to explore regarding macrophytes' responses to many constraints, particularly in regions as ecologically significant as the Amazon.

Keywords**:** aquatic plant ecology, plant functional strategies, intraspecific variation, freshwater biodiversity, climate change adaptation, ecological resilience

SUMMARY

1. INTRODUÇÃO GERAL

Durante as últimas décadas, os ecólogos têm se concentrado em utilizar a abordagem funcional para entender a mais diversa gama de questões ecológicas, incluindo a adaptação de indivíduos ao estresse, dinâmica populacional e respostas da comunidade a fatores bióticos e abióticos (de Bello et al., 2021). Um atributo funcional é definido como qualquer característica morfológica, fisiológica ou fenológica medida no nível individual, desde a célula até o organismo inteiro, que impacta a sua aptidão indiretamente por meio de seu efeito no crescimento, reprodução e sobrevivência (Violle et al, 2007). Devido à sua aplicabilidade a vários níveis organizacionais (de indivíduos a ecossistemas e biosfera), a abordagem funcional tornou-se crucial para compreender a montagem das comunidades, prever mudanças espaciais na distribuição de espécies e comunidades, e quantificar o impacto da composição da comunidade nos processos e serviços ecossistêmicos (de Bello et al., 2021). As características funcionais também servem como indicadores para avaliar os efeitos de estratégias de manejo sobre objetivos de conservação. Essas abordagens, incluindo outras não taxonômicas, são essenciais para entender padrões de diversidade e processos ecossistêmicos, especialmente em meio a impactos antropogênicos como mudanças climáticas e conversão do uso da terra (de Bello et al., 2021).

Dentre os organismos, as plantas têm sido amplamente utilizadas como organismos modelo para testar questões dentro da ecologia funcional (Garnier & Navas, 2012; Lavorel et al., 2007). Devido ao enorme esforço feito por muitos ecólogoss ao redor do mundo, possuímos um conjunto de protocolos destinados a medir atributos funcionais de plantas, que são acessíveis e foram reproduzidos em diferentes biomas e continentes (Bartlett et al., 2012; Pérez-Harguindeguy et al., 2013). Provavelmente, este é o conjunto mais unificado de teorias e estruturas visando entender a resposta funcional de um grupo de organismos à variação ambiental, espacial e temporal, junto com distúrbios impulsionados pelo homem (Díaz et al., 2016; Pierce et al., 2017; Reich, 2014; Wright et al., 2004). Alguns deles são o espectro econômico foliar (Wright et al., 2004), forma e função das plantas (Díaz et al., 2016; Wright et al., 2004), e o espectro econômico rápido-lento das plantas (Reich, 2014).

Além do espectro econômico das plantas, as plantas também podem ser classificadas em um conjunto de estratégias de acordo com a variação dos seus atributos. Além das estratégias aquisitivas-conservativas, um dos esquemas mais clássicos é o esquema CSR de Grime, que categoriza as estratégias das plantas em três tipos principais: competidoras (C), tolerantes ao estresse (S) e ruderais (R) (Grime, 1977; Grime & Pierce, 2012). Além disso, as plantas podem ser classificadas com base em sua capacidade de resistir a diferentes tipos de estresse, por exemplo, sob estresse hídrico, as plantas podem desenvolver estratégias para escapar, evitar ou tolerar a seca (Kooyers, 2015).

No entanto, apesar do enorme número de estudos tentando entender as respostas das plantas a uma variedade de variações em seu ambiente por meio da abordagem funcional, pouca atenção tem sido dada às plantas aquáticas. As macrófitas habitam ecossistemas de água doce, que são afetados por mudanças ambientais e climáticas decorrentes tanto de distúrbios naturais quanto de atividades antropogênicas (por exemplo, mudança no uso da terra, eutrofização e alterações nos regimes hidrológicos - períodos de secas e inundações extremas) (Dudgeon, 2019). Assim, as macrófitas desempenham um papel vital na preservação da biodiversidade aquática, possuindo diversas funções ecológicas em seus habitats (por exemplo, ciclagem de nutrientes, fornecimento de habitat e alimento para diversos organismos) (Bornette & Puijalon, 2011; Thomaz, 2021), e quaisquer mudanças ambientais e biológicas que afetem sua funcionalidade, padrões de ocorrência, dominância e sobrevivência nosecossistemas aquáticos podem ter efeitos em cascata sobre outros organismos.

No entanto, Dalla et al. (2020) mostraram em uma revisão que nos últimos anos aumentaram os estudos avaliando traços funcionais de macrófitas, mas ainda existem lacunas de conhecimento sobre os atributos fisiológicos, fenologia reprodutiva e atributos radiculares, e áreas prioritárias de biodiversidade de macrófitas, como o Brasil (e a Amazônia), foram pouco estudadas. Assim, o uso da abordagem funcional para entender questões relacionadas à variação intraespecífica, as estratégias, e padrões de diversidade (taxonômica, funcional ou filogenética) das maacrófitas é crucial, pois essa abordagem ajuda a compreender como as comunidades de macrófitas respondem a fatores constantes que afetam suas interações com outros organismos, bem como sua sobrevivência no ambiente (que está em constante mudança). Isso ajuda a entender como essas comunidades se adaptam e sobrevivem em seus habitats e como elas responderão às crescentes consequências do uso da terra impulsionado pelo homem e as mudanças climáticas.

Com base nisso, o objetivo desta tese foi investigar as respostas de macrófitas aquáticas (nos níveis individual e comunitário) à variação ambiental natural e a múltiplos estressores (mudanças climáticas e uso da terra), usando a abordagem funcional. Esta tese está dividida em três seções. Na primeira seção, investigamos a variação intraespecífica de macrófitas em resposta à variação ambiental natural, enquanto na segunda, investigamos a variação intraespecífica sob estresse hídrico induzido por mudanças climáticas. Finalmente, na terceira seção, empregamos medidas de diversidade funcional e filogenética para investigar respostas em nível comunitário à mudanças no uso da terra. Empregamos investigações de campo ou experimentos para contemplar os eixos de interesse das seções.

<u>Seção 1:</u> Variação intraespecífica de características foliares e estratégias evolutivas de três macrófitas ao longo de um gradiente ambiental na Amazônia Oriental (*“Intraspecific variation in leaf traits and evolutionary plant strategies of three macrophytes across an environmental gradient in Eastern Amazon”*).

Seção 2: A seca foi a pior de todas (para alguns): a variação intraespecífica de características funcionais e trade-offs de alocação de recursos sob estresse hídrico revelam estratégias de sobrevivência divergentes em macrófitas diante as mudanças climáticas (*"The drought was the very worst (for some): intraspecific trait variation and resource allocation trade-offs under water stress unveil divergent survival strategies in macrophytes amid climate change"*).

Seção 3: Funcionalmente favorecidas, filogeneticamente prejudicadas: diversidade funcional e estrutura filogenética de macrófitas ao longo de um gradiente de uso da terra (*"Functionally favored, phylogenetically impaired: functional diversity and phylogenetic structure of macrophytes along a land-use gradient"*).

1. GENERAL INTRODUCTION

Over the last few decades, ecologists have focused on employing trait-based approaches to understand the most diverse array of ecological questions, including adaptation of individuals to stress, population dynamics, and community responses to both biotic and abiotic factors (de Bello et al., 2021). A trait is defined as any morphological, physiological or phenological feature measured at the individual level, from the cell to the whole organism, that impact fitness indirectly via its effect on growth, reproduction, and survival (Violle et al, 2007). Because of its applicability to many organizational levels (from individuals to ecosystems and the biosphere), trait-based approaches have become crucial for comprehending community assembly, predicting spatial changes in species and community distribution, and quantifying community composition's impact on ecosystem processes and services (de Bello et al., 2021). Traits also serve as indicators for assessing the effects of management strategies on conservation goals. These approaches, including other non-taxonomic ones, are essential for understanding diversity patterns and ecosystem processes, especially amid anthropogenic impacts like climate change and land-use conversion (de Bello et al., 2021).

Among organisms, plants have been thoroughly used as model organisms to test questions under functional ecology (Garnier & Navas, 2012; Lavorel et al., 2007). Because of the enormous effort made by many ecologists around the world, we now have a set of protocols aimed to measure plant functional traits, that are accessible and have been reproduced across different biomes and continents (Bartlett et al., 2012; Pérez-Harguindeguy et al., 2013). Probably the most unified set of theories and frameworks aiming to understand a group’s functional response to environmental, spatial and temporal variation, along with human-driven disturbances (Díaz et al., 2016; Pierce et al., 2017; Reich, 2014; Wright et al., 2004). Some of them are the leaf economics spectrum (Wright et al., 2004), plant form and function (Díaz et al., 2016; Wright et al., 2004), and the fast-slow plant economics spectrum (Reich, 2014).

In addition to the plant economics spectrum, plants may be classified into a set of strategies according with their trait value variation. Besides acquisitive-conservative strategies, one of the most classical frameworks is Grime's CSR scheme, which categorizes plant strategies into three main types: competitors (C), stress-tolerators (S), and ruderals (R) (Grime, 1977; Grime & Pierce, 2012). Moreover, plants can be classified based on their ability to withstand different types of stress, for instance, under water stress, plants may develop strategies to escape, avoid or tolerate drought (Kooyers, 2015).

However, despite the enormous number of studies trying to understand plant responses to an assortment of variations in their environment through the trait-based approach, little attention has been paid to aquatic plants. Macrophytes inhabit freshwater ecosystems, which are affected by environmental and climatic changes stemming from both natural disturbances and anthropogenic activities (e.g., land-use change, eutrophication, and alterations in hydrological regimes – periods of extreme droughts and floods) (Dudgeon, 2019). Thus, macrophytes play a vital role in the preservation of aquatic biodiversity, serving various ecological functions in their habitats (e.g., nutrient cycling, providing habitat and food for diverse organisms) (Bornette & Puijalon, 2011; Thomaz, 2021), and any environmental and biological changes that affect their functionality, occurrence patterns, dominance, and survival in aquatic ecosystems can have cascading effects on other organisms.

However, Dalla et al. (2020), showed in a review that the recent years increased studies assessing macrophyte functional traits, but there are still gaps of knowledge regarding physiological, reproductive phenology and root traits, and hotspots of macrophyte biodiversity, such as Brazil (and the Amazon), have been thoroughly understudied. Thus, the use of the trait-based approach to understand issues related to macrophyte intraspecific variation, ecological strategies, and diversity patterns (taxonomic, functional, or phylogenetic) is crucial, as this approach helps comprehend how macrophyte communities respond to constant factors affecting their interactions with other organisms as well as their survival in the environment (which is constantly changing). It aids in understanding how these communities adapt and survive in their habitats and how they will respond to the increasing consequences human-driven land use and climate change.

Based on this, the aim of this thesis was to investigate the responses of aquatic macrophytes (at individual and community levels) to natural environmental variation and multiple stressors (climate change and land use), using the trait-based approach. This thesis is divided into three sections. On the first section, we investigated the intraspecific variation of macrophytes in response to natural environmental variation, while on the second, we investigated intraspecific variation under a climate-change induced water stress. Finally, on the third section, we employed functional and phylogenetic diversity measures to investigate community-level responses to land-use change. We employed either field investigations or experiments to contemplate the axes of interest of the sections.

Section 1: Intraspecific variation in leaf traits and evolutionary plant strategies of three macrophytes across an environmental gradient in Eastern Amazon.

Section 2: The drought was the very worst (for some): intraspecific trait variation and resource allocation trade-offs under water stress unveil divergent survival strategies in macrophytes amid climate change.

Section 3: Functionally favored, phylogenetically impaired: functional diversity and phylogenetic structure of macrophytes along a land-use gradient.

2.Section I

Intraspecific variation in leaf traits and evolutionary plant strategies of three macrophytes across an environmental gradient in Eastern Amazon

The first section of this thesis was prepared and formatted according to the norms of the scientific publication Hydrobiologia, available at: https://link.springer.com/journal/10750

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Intraspecific variation in leaf traits and evolutionary plant strategies of three macrophytes across an environmental gradient in Eastern Amazon

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2.1. Abstract

This study explores the intraspecific trait variation and ecological strategies of three macrophyte species in the Amazon region, focusing on leaf traits and CSR strategies (competitors, stress-tolerators, and ruderals). Individuals of *Eleocharis interstincta*, *Fuirena umbellata*, and *Nymphaea rudgeana* were examined across 22 sampling sites. Traits including leaf area, specific leaf area, and leaf dry matter content were measured, along with environmental variables. Results demonstrated significant differences in leaf traits among the species, highlighting their distinct strategies. Individuals of *F. umbellata* exhibited the lowest leaf dry matter content values, indicating a conservative and stress-tolerator strategy. *N. rudgeana* had the highest leaf area values, reflecting an acquisitive strategy, and varied from a S to S/CS strategy, while *E. interstincta* showed intermediate trait values and a stress-tolerator strategy. Furthermore, intraspecific variation was observed within each species, influenced by environmental factors (nutrient availability, water conductivity, dissolved oxygen, and soil composition). Our findings contribute to understanding the intraspecific trait variations and ecological strategies of macrophytes in the Amazon region, providing insights into plant adaptation and response to environmental changes. Future research should incorporate additional traits and encompass different macrophyte life forms, further enhancing our understanding of their strategies and responses to ongoing environmental change.

Keywords: aquatic plants, plant economics, plant strategies, stress-tolerators.

2.2.Introduction

Community composition and structure are influenced by a complex interplay of various processes, such as dispersal, biotic interactions, and the abiotic environment (Cadotte & Tucker, 2017), but also impacting the variation among individuals within a population (Violle et al., 2012; Dalla Vecchia & Bolpagni, 2022). Environmental filters, which are intrinsically related with resource availability, play a crucial role in shaping communities (affecting the distribution of organisms through survival and change in competition patterns) (Cadotte & Tucker, 2017), and can lead to diverse responses within individuals based on the level of intraspecific variation (Violle et al., 2012; Sides et al., 2014). Indeed, intraspecific variation can have a significant impact on community structure and ecosystem functions, as much as interspecific variation (Siefert et al., 2015; Des Roches et al., 2018).

Plants can adapt their development, morphology, and physiology in response to environmental conditions and biotic factors, which is reflected in their high phenotypic variability (Sultan, 2000; Nicotra et al., 2010). These adaptations involve resource allocation (trade-offs) (Weiner, 2004), and trait variations among genotypes, populations, and species (Violle et al., 2007). In resource-limited situations, these strategies are crucial for plant survival (Grime, 1977). So, studying trait variations, both at the species (interspecific) and individual (intraspecific) level, can provide insights into physiological, ecological, and evolutionary aspects (Bennett et al., 2016; Des Roches et al., 2018; Dalla Vecchia et al., 2020).

Intraspecific variation manifests in multiple plant functional traits. Among these traits, three key traits are commonly used to define ecological strategies (Wright et al., 2004; Pierce et al., 2017): leaf area (LA), which represents the investment in photosynthetic capacity and gas exchange

(Wright et al., 2004; Baird et al., 2021); specific leaf area (SLA), which reflects carbon investment in leaves, nutrient cycling, resource competition, and environmental tolerance (Wright et al., 2004; Díaz et al., 2016); and leaf dry matter content (LDMC), which represents carbon investment in leaf (tissue density), longevity, and resistance to physical damage and extreme events (e.g., grazing, wind) (Hodgson et al., 2011; Pérez-Harguindeguy et al., 2013). Plant size and leaf economics, along with stem and root traits, work together to shape a plant's survival strategy in the environment. These traits influence and may be indicative of each other, shaping the plant economics spectrum (Reich, 2014). Overall, global plant functional variation is predominantly defined by plant size and economics (Díaz et al., 2016; Pierce et al., 2017).

For the plant economics spectrum, environmental conditions may also trigger a growth vs. survival trade-off, in which a plant invest either in acquiring resources to grow fast or invest slowly in organs that are more resistant and have longer lifespan (Reich, 2014). This is associated with the leaf economics spectrum acquisitive and conservative plant strategies, in which plants with acquisitive strategies invest in growing fast, by producing short-lived leaves with little tissue density (less carbon and more nitrogen) and a high photosynthetic rate (presenting high leaf area and specific leaf area, and low leaf dry-matter content) (Wright et al., 2004; Donovan et al., 2011; Reich, 2014). In contrast, plant exhibiting a conservative strategy grow at slower rates, investing in leaves with a long lifespan, that are structurally expensive (more carbon), and a low photosynthetic rate (presenting a low leaf area and specific leaf area, and high values of leaf dry-matter content) (Wright et al., 2004; Donovan et al., 2011; Reich, 2014).

Through a comprehensive analysis of multiple traits, plant ecological strategies can be identified, offering insights into the phenotypic outcomes of natural selection within specific environments (Pierce et al., 2013, 2017; Estarague et al., 2022). Grime's CSR scheme further classifies plant strategies into three main categories: competitors (C), stress-tolerators (S), and ruderals (R) (Grime, 1977, 2002; Grime & Pierce, 2012; Pierce et al., 2017). Competitors invest in rapid growth to outcompete for resources, stress-tolerators focus on resource retention for survival in harsh environments, and ruderals prioritize the production of propagules for regeneration after disturbance events (Grime & Pierce, 2012; Pierce et al., 2017). However, such variation in ecological strategies may vary not only among species, but also between individuals in a given population, or among populations across contrasting environments (May et al., 2017; Vasseur et al., 2018; Estarague et al., 2022), especially in plants with high phenotypic plasticity. Indeed, attention has been given to intraspecific variation in ecological strategies, since it has significant effects on community patterns, species adaptations and ecosystem functions (Des Roches et al., 2018; Vasseur et al., 2018; Dalla Vecchia & Bolpagni, 2022).

Aquatic plants are characterized by their highly acquisitive strategies compared to their terrestrial counterparts (Poorter et al., 2009; Pierce et al., 2012; Pan et al., 2020). Macrophytes exhibit a diverse range of leaf sizes and morphologies, ranging from small-leaved Lemnids and even leafless *Utricularia* spp. to large-leaved Nympheaids (Barrett et al., 1993; Poorter et al., 2009; Pierce et al., 2012). Overall, aquatic plants display higher SLA and nitrogen content, and lower LDMC in comparison to terrestrial plants (Poorter et al., 2009; Pierce et al., 2012; Pan et al., 2020). These traits reflect their adaptation to water-saturated environments, with a reduced investment in structural tissues (lower carbon content in leaves) and an emphasis on maximizing photosynthetic surface area, either by producing large leaves (as seen in Nymphaeaceae) or numerous small ones (as observed in *Myriophyllum* spp.) (Díaz et al., 2016; Pan et al., 2020). However, it is important to consider that environmental variations, such as different physical structures of aquatic systems (e.g., lotic or lentic, perennial or temporary ecosystems, and harsh environments) and the associated biomes, can influence the strategies employed by aquatic plants (Lacoul & Freedman, 2006; Fu et al., 2023; Gao et al., 2023). Additionally, different life-forms within the aquatic plant community may exhibit distinct strategies, leading to variations in acquisitive or conservative traits among species inhabiting contrasting environments (Pierce et al., 2012; Albuquerque et al., 2020).

Macrophytes also exhibit similar patterns in their ecological strategies. Most aquatic plants are classified closer to the ruderal side of the ecological strategy scheme (but see (Lacoul &

Freedman, 2006) for a classification of macrophytes in other strategies) due to the frequent disturbances encountered in freshwater environments, such as flooding, trampling, and drought (Pierce et al., 2012; Albuquerque et al., 2020). Consequently, many species invest heavily in producing propagules and have a rapid regeneration capacity after biomass loss (Grime & Pierce, 2012; Pierce et al., 2017). However, certain macrophytes display a more competitive strategy by allocating resources towards faster growth, as seen in some species from the Nymphaeaceae family (Pierce et al., 2012; Albuquerque et al., 2020). While it is argued that no macrophyte species would exhibit a strong conservative strategy to be classified as a true stress tolerator (Pierce et al., 2012), there are exceptions (Lacoul & Freedman, 2006), especially among sedges (Cyperaceae). Some sedges (e.g., *Eleocharis* spp) are considered true macrophytes, and the global patterns for the Cyperaceae family indicate strong stress-tolerant characteristics (Pierce et al., 2017).

Therefore, further research on the variation in ecological strategies of macrophytes is necessary, since macrophyte communities have been understudied when it comes to assessing functional traits and ecological strategies, and because the evidence of intraspecific variation of ecological strategies (especially CSR strategies) at terrestrial ecosystems are at the beginning stages of research. In particular, investigation of these strategies in tropical forests like the Amazon is of interest, as tropical forests have a very heterogenous environments, and could show clear patterns. These regions exhibit high nutrient cycling but severe soil nutrient deficiency (Yu et al., 2015; Figueiredo et al., 2018), in addition to more acidic waterbodies (Ríos-Villamizar et al., 2013), and species in these environments tend to exhibit more competitive and stress-tolerant strategies (Pierce et al., 2017; Araujo da Costa et al., 2020; Aragón et al., 2023). Furthermore, these environmental conditions may lead to macrophyte strategies differing from the global pattern, potentially shifting dominance from ruderal species to competitors and stress-tolerant species.

In this study, we aimed to investigate the variation of leaf traits (LA, SLA and LDMC) and ecological strategies in individuals of three macrophyte species: *Eleocharis interstincta* (Vahl) Roem. & Schult. (Cyperaceae), *Fuirena umbellata* Rottb. (Cyperaceae) and *Nymphaea rudgeana* G.Mey.

(Nymphaeaceae), across an environmental gradient in Eastern Amazon. Our hypotheses are that 1) in resource-rich environments (with higher water temperature and conductivity, and higher concentrations of soil P, K and Mg), individuals of all species have a more acquisitive strategy (high SLA and LA, and low LDMC). In contrast, individuals in more resource-poor environments exhibit a conservative strategy (low SLA and LA, and high LDMC). However, we expect individuals of *N. rudgeana* to have higher LA and SLA than *E. interstincta* and *F. umbellata*, which have a more conservative strategy (high LDMC) due their tolerance to environmental stress. 2) There is a contrasting intraspecific variation in ecological strategies of macrophyte species. More specifically, a variation in the strategies of *N. rudgeana* from ruderal to competitors as resource availability increases due to their variability in leaf traits (more acquisitive strategy) in response to environmental change. In contrast, we expect individuals of *E. interstincta* and *F. umbellata* to be more conservative and vary little in their functional strategies, remaining stress-tolerators, as it is a tendency of the Cyperaceae family worldwide (Pierce et al., 2016). This study is, so far, the first one the investigate the intraspecific variation of CSR strategies in aquatic plant communities.

2.3.Material and Methods

2.3.1. Study area

Data sampling was performed in September 2022, across 22 sites, at the Capim River Basin, located in the municipalities of Paragominas (Lat: 02º 59' 45" S; Long: 47º 21' 10" W) and its surroundings, in the northeastern portion of the State of Pará, Brazil (Fig.1). The sites comprise mostly lentic ecosystems, such as lakes and ponds. The climate is wet and hot (mean annual temperature of 26º mean air humidity of 81%, and mean annual precipitation of 1.800 mm; (Pinto *et al.* 2009). The vegetation of the area consists of tropical rainforest.

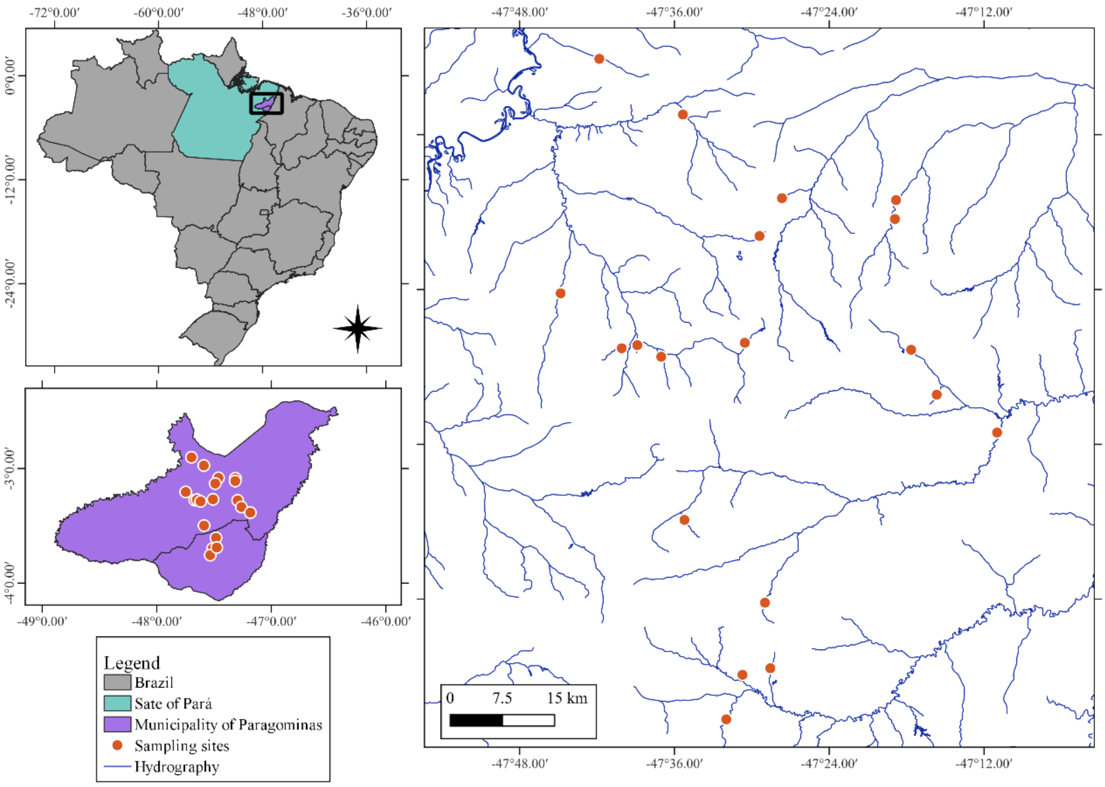

Fig. 1: Sampling sites of the study (Municipality of Paragominas and surroundings, State of Pará, Brazil).

2.3.2. Sampling Design

2.3.2.1.Environmental variables

At each site, we sampled environmental variables, which comprise water and soil parameters, and physical aspects of the sites. For the water variables, we measured temperature (ºC), conductivity (µs/cm), pH, and dissolved oxygen (mg/L), using a multiparameter probe (Model Akso AK48). We measured water depth using a meter, and the inclination of the shoreline using a digital clinometer. These measurements were taken trice in each the site.

For the measurement of soil variables, we collected composed soil samples. This consists of 15 to 20 simple samples collected randomly along each site. These samples were taken using a shovel at a 20cm depth. Then, the simple samples are placed in a tray, homogenized and stored in plastic bags. We collected 400g of soil for each site. Soil samples were then taken to the Laboratory of Soils of the EMBRAPA Amazônia Oriental located in the city of Belém, Pará Brazil, where the chemical (amount of nutrients and elements present in the soil) and physical (distribution of particles – granulometry) parameters of the soil were assessed. We obtained the following measures: Chemical parameters – amount of Phosphorous (P), Potassium (K) and Sodium (Na) (mg/dm^3), and amount of Aluminum (Al), Calcium (Ca) and Calcium + Magnesium (Ca+Mg) (cmol$_c$/dm^3); Physical parameters: amount of coarse and fine sand, silt and total clay (g/kg). The procedure was performed following the protocol proposed by (Teixeira et al., 2017).

2.3.2.2.Biological sampling

For the collection of macrophyte species, we selected three species that were representative of the macrophyte community in the region:

1) *Eleocharis interstincta* (Cyperaceae): emergent, perennial herb, with rhizomes, leaves are underdeveloped, and the colms perform photosynthesis (Lorenzi, 2008; Pott & Pott, 2000). Is native from tropical America, occurs in all regions of Brazil (Nunes et al., 2023). Normally inhabits wetlands and other stillwater habitats, forming stands closer to the shorelines (Lorenzi, 2008).
2) *Fuirena umbellata* (Cyperaceae): amphibious, perennial herb, rhizomatous, reaching from 40 to 100cm (Lorenzi, 2008). Inhabits waterlogged and flooded environments, at the shorelines (Lorenzi, 2008). Species native from America, it is present in all regions of Brazil (Alves et al., 2023), and reproduces by rhizome and seeds (Pott & Pott, 2000).
3) *Nymphaea rudgeana* (Nymphaeaceae): Floating-leaved herb, rooted in the soil with flexible petioles below the water column (Moreira & Bove, 2017). Inhabits more lentic freshwaters (personal observation). In Brazil, it is present in all regions except for the central-western region (Pellegrini, 2023)

The selection followed two criteria: these species were the most frequent ones in the field (*E. intersticta* – 16 occurrences; *F. umbellata* – 12, and *N. rudgeana* – 10 occurrences), and, when found, they were the most abundant ones. However, they rarely occurred at the same site together, normally there was a co-occurrence of two species at a time. Because of that, we performed the analyses separately for each species, in order to truly assess the intraspecific variation in them.

For the sampling, we selected three individuals of each species in the sites. From those individuals, we selected three middle leaves (for *E. interstincta*, we considered as 'leaves' the unfertile stems, following the recommendation of Pérez-Harguindeguy et al. (2013) for special cases), that were nor too young or too old, and that had no signs of herbivory or any other damage. We took photographs of these leaves, and then hydrated them in trays filled with water to measure the water-saturated fresh mass, using a digital scale (0.001g). The leaves were then oven-dried at 65ºC for 72h, and weighted again to obtain the dry mass. With these measurements, we were able to calculate the following functional traits, as described by the protocol proposed by Pérez-Harguindeguy et al. (2013):

a) Leaf area (LA): measures leaf size, and is the one-sided area of a leaf (mm^2). It is associated with the investment of plants in the photosynthetic surface. We used the photos of the leaves to measure LA, using de package BiocManager (Morgan, 2022) in the R program (R Core Team, 2022).

b) Specific leaf area (SLA): is the one-sided area of a fresh leaf divided by its oven-dried mass (SLA = leaf area / dry mass). We used the area calculated in the same leaves used to measure LA. It expresses the amount of carbon invested in the photosynthetic area of a leaf.

c) Leaf dry matter content (LDMC): is the dry mass of a leaf divided by its water-saturated mass (mg/g). LDMC measures the average density of leaf tissues.

We considered these leaf traits because they are more accessible to measure in field conditions and they are suitable to test our hypotheses, as these traits vary with environmental change, and are efficient in expressing plant strategies (i.e. more conservative or acquisitive). Additionally, these traits are required to calculate the CSR functional strategies as proposed by Pierce et al (2017).

2.3.3. *Statistical Analysis*

Prior to the hypothesis-testing analyses, we assessed multicollinearity among predictors, using a Pearson Correlation Matrix. We considered the correlation coefficient value of $r \geq \pm 0.65$ as high (Table S1). Whenever two variables were correlated, we selected one to be retained based on the literature concerning the macrophyte community, where we chose the variables to be retained based on what elements or components would be important or limiting to plant establishment and growth in freshwaters (Lacoul & Freedman, 2006; Akasaka et al., 2010; Bornette & Puijalon, 2011; Aoki et al., 2017).

For the hypothesis-testing analyses, we considered the individuals as the sample unit, and used the mean values of the three leaves of each individual. To test the difference in trait variation among species, we performed a Permutational multivariate analysis of variance (PERMANOVA), using the species as predictors, and the trait values as response variables (Anderson et al., 2008). As PERMANOVA tests only if there is a variation among the groups but does not tell which groups are significantly different from one another, We tested the pairwise difference between species using the Pairwise Adonis analysis (Martinez Arbizu, 2017). For this analysis, we included the trait values of all species in one matrix, and the traits matrix was standardized prior the analysis. In addition, to better visualize the distribution of the traits of individuals of each species, we performed a Principal Component Analysis (PCA).

To test the effects of these environmental variables on the trait variation of individuals in each species, we performed a Redundancy analysis (RDA; (Gotelli & Ellison, 2012), using the variables selected after the Pearson correlation analysis as predictors, and the functional traits (LA, SLA and LDMC) as response variables. We performed one model for each species, as they did not occur in all the sampling sites. Before running the RDA models, we performed a model selection with the best predictors based on the functional traits and environmental variables. Environmental variables were standardized prior to the analysis, and the functional trait matrix was Hellinger-transformed to ensure linearity among predictors and response variables (Gotelli & Ellison, 2012). We validated the models using a permutation test at 10000 permutations, and model adjustment was assessed using the Adjusted R^2.

Finally, to assess the intraspecific variation of CSR strategies (Competitor, Stress-tolerator and Ruderal), we calculated the relative proportions of each component of CSR strategies of each individual, based on their trait (LA, SLA, LDMC) values using the StrateFy tool (Pierce et al., 2017). Then, to better represent and visualize this variation, we produced a ternary plot using the proportions of each strategy for each individual.

All the analyses were performed in RStudio version 4.2.0 (R Core Team, 2022). Pearson correlation was performed using the ‘rcorr’ function of the ‘Hmisc’ package (Harrell Jr, 2023). PERMANOVA was performed using the ‘adonis2’ function, and PCA was performed using the ‘prcomp’ function, also from the ‘vegan’ package (Oksanen et al., 2022). Pairwise Adonis comparison between groups was performed using the ‘pairwise.adonis’ function from the ‘pairwiseAdonis’ package (Martinez Arbizu, 2017). RDA was performed using the ‘rda’ function, model validation was performed using the ‘anova.cca’ function, and the adjusted R^2 was calculated using the ‘RsquareAdj’ function, all from the ‘vegan’ package. Model selection for functional traits of each species was performed using the ‘forward.sel.par’ function from the adespatial package (Dray et al., 2022). Finally, all graphs were plotted using the ‘ggplot2’ package (Wickham, 2016), except from the ternary plot of the CSR strategies which was built using the ‘ggtern’ package (Hamilton & Ferry, 2018).

2.4.Results

2.4.1. Environmental variables

The environmental variables that were retained to be used in the models to test our hypothesis were water temperature, pH, conductivity, dissolved oxygen and depth, the amount of coarse sand and total clay of the soil and the amount of phosphorus, potassium and calcium + magnesium in the soil. Regarding of them, water temperature varied from 24.8 to 32.1 ºC. pH varied from 3.47 (acidic) to 9.96 (basic). Conductivity varied from 15.900 to 87.600 µs/cm. Dissolved oxygen varied from 1.100 to 4.800 mg/L. Water depth varied from 5 to 44cm. For the soil variables, the amount of coarse sand varied from 34 to 554g/kg, while total clay varied from 60 to 480g/mg. Phosphorus concentration varied from 0.450 to 9.350 mg/dm^3. Potassium concentrations varied from 4.610 to 64.890 mg/dm^3, while sodium varied from 0.750 to 48.580 mg/dm^3. Finally, the amount of Calcium + Magnesium concentrations varied from 0.100 to 2.870 cmol$_c$/dm^3 (Table 1).

Table 1: Environmental variables investigated in the 22 sampling sites collected and their range (minimum and maximum), mean values and standard deviations (SD). Variables in bold are the ones retained for the further analyses after correlation.

Variable	**Unit**	**Range (Min-Max)**	**Mean ± SD**
Water			
Temperature	**ºC**	**24.8 - 32.1**	**28.145 ± 2.054**
pH		**3.47 - 9.69**	**4.738 ± 1.332**
Conductivity	**µs/cm**	**15.9 - 87.6**	**37.800 ± 17.321**
Dissolved Oxygen	**mg/L**	**1.1 - 4.8**	**3.480 ± 0.929**
Physical structure			
Water depth	**cm**	**5.0 - 44.0**	**23.391 + 12.525**
Shoreline inclination	º	7.267 - 36.000	19.5143 ± 9.268
Soil			
Coarse sand	**g/kg**	**34.0 - 554.0**	**216.100 ± 134.421**
Fine sand	g/kg	64.0 - 758.0	359.100 ± 152.138
Silt	g/kg	10.0 - 622.0	229.800 ± 167.879
Total clay	**g/kg**	**60.0 - 480.0**	**195.000 ± 116.732**
Phosphorus	**mg/dm^3**	**0.45 - 9.35**	**1.821 ± 2.203**
Potassium	**mg/dm^3**	**4.61 - 64.89**	**25.708 ± 17.106**
Sodium	**mg/dm^3**	**0.75 - 48.58**	**11.921 ± 11.473**
Aluminum	cmol$_c$/dm^3	0.04 - 1.16	0.458 ± 0.365
Calcium	cmol$_c$/dm^3	0.06 - 2.31	0.477 ± 0.509
Calcium + Magnesium	**cmol$_c$/dm^3**	**0.11 - 2.87**	**0.796 ± 0.665**

2.4.2. Variation in functional traits and differences among species

We analyzed three leaves of 117 individuals (a total of 351 leaves) of three macrophyte species. *Eleocharis interstincta* had the highest number of individuals, 48, followed by *Fuirena umbellata* (39) and *Nymphaea rudgeana* (30). Overall, leaf area varied from 54.065 to 5462.524 mm^2, leaf dry matter content varied from 3.955 to 92.019%, and specific leaf area varied from 0.568 to 14.749 mm^{2}mg^{-1} (for more information, see table S1).

The PERMANOVA results showed that there is a difference in the trait values (Pseudo-F = 40.722; p = 0.001) among all species (Table 2). The first axis of the PCA represented 55.95% of total data variance, while he second axis represented 27.30% (Fig. X). On average, *E. individuals* had the lowest SLA values, while *F. umbellata* individuals presented the highest LDMC values and the lowest LA values, and individuals of *N. rudgeana* showed the opposite pattern (highest LA values and lowest LDMC values) (Fig. 2).

Table 2: Result of the pairwise Adonis among all pairs of species. Values with an asterisk (*) indicate significant relationships among pairs.

Pairs	Df	Sums of Squares	Pseudo-F	R^2	P	Adjusted-p
E. interstincta vs *N. rudgeana*	1	4.954	61.222	0.449	0.001	0.003*
E. interstincta vs *F. umbellata*	1	1.522	29.955	0.261	0.001	0.003*
N. rudgeana vs *F. umbellata*	1	7.249	93.612	0.586	0.001	0.003*

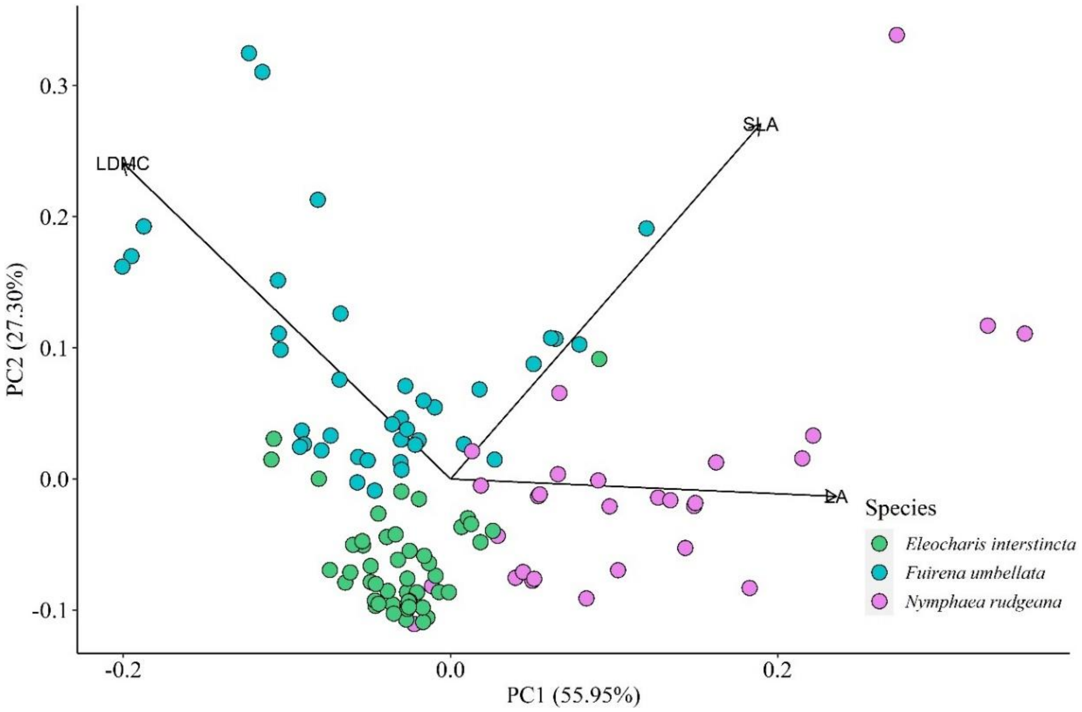

Fig. 2: Principal Component Analysis performed with the functional traits (Leaf area - LA, Specific leaf area - SLA and Leaf dry matter content - LDMC) of three macrophyte species.

2.4.3. Effect of environmental variables on individuals' functional traits

The result of the RDA models showed that different variables drove the intraspecific trait variation of the studied species. More specifically, the model for *E. interstincta* showed water conductivity, dissolved oxygen and Calcium+Magnesium explained 62.013% of variance (F = 26.575, p = 0.001, df = 3, Adjusted R^2 = 0.62). The first RDA axis explained 99.502% (p > 0.001) of the fitted variance in *E. interstincta* and was positively related with Ca+Mg concentrations (0.51; p > 0.001), and negatively correlated with water conductivity (-0.57; p > 0.001) and dissolved oxygen (-0.514; p > 0.001). The second axis explained 0.04% (p = 0.854) of the fitted variation and was negatively associated with Ca+Mg (-0.701) and dissolved oxygen (-0.690). LDMC values increase with increased conductivity and dissolved oxygen concentrations, and decrease with high soil Ca+Mg concentrations, while LA values increase with low water conductivity and increased with an increase in Ca+Mg concentrations (Fig 3A).

The model for *F. umbellata* indicates water conductivity, dissolved oxygen and soil Phosphorus explained 39.912% of total trait variance (F = 9.414; $p = 0.001$, df = 3; Adjusted $R^2 = 0.40$). The first axis explained 99.324% ($p > 0.001$) of the fitted variance, and was positively correlated with water conductivity (0.49; $p = 0.002$) and dissolved oxygen (0.60; $p > 0.001$), and negatively related with soil phosphorus concentrations (-0.481; $p = 0.013$). The second axis explained 0.030% ($p = 0.925$) of the fitted variation and was negatively associated with soils phosphorus (-0.534) and dissolved oxygen (-0.41). LDMC values of individuals increase with high water conductivity and dissolved oxygen, and decrease with high concentrations of soil P, while LA values decrease in those conditions (Fig. 3B).

Furthermore, the model for *N. rudgeana* explained 50.729% of trait variance, and was explained by the amount of total clay, coarse sand and water depth (F = 10.61, $p = 0.001$, df = 3, Adjusted $R^2 = 0.51$). The first axis explained 99.754% ($p > 0.001$) of the fitted variance, and was positively correlated with coarse sand (0.34; $p = 0.040$) and negatively related with water depth (-0.84; $p > 0.001$). The second axis explained 0.244% ($p = 0.983$) of the fitted variation and was positively associated with total clay (0.93), and negatively correlated with water depth (-0.43). SLA decrease with increased water depth and total clay, while LDMC decrease in sites with decreased water depth and amount of clay (Fig. 3C).

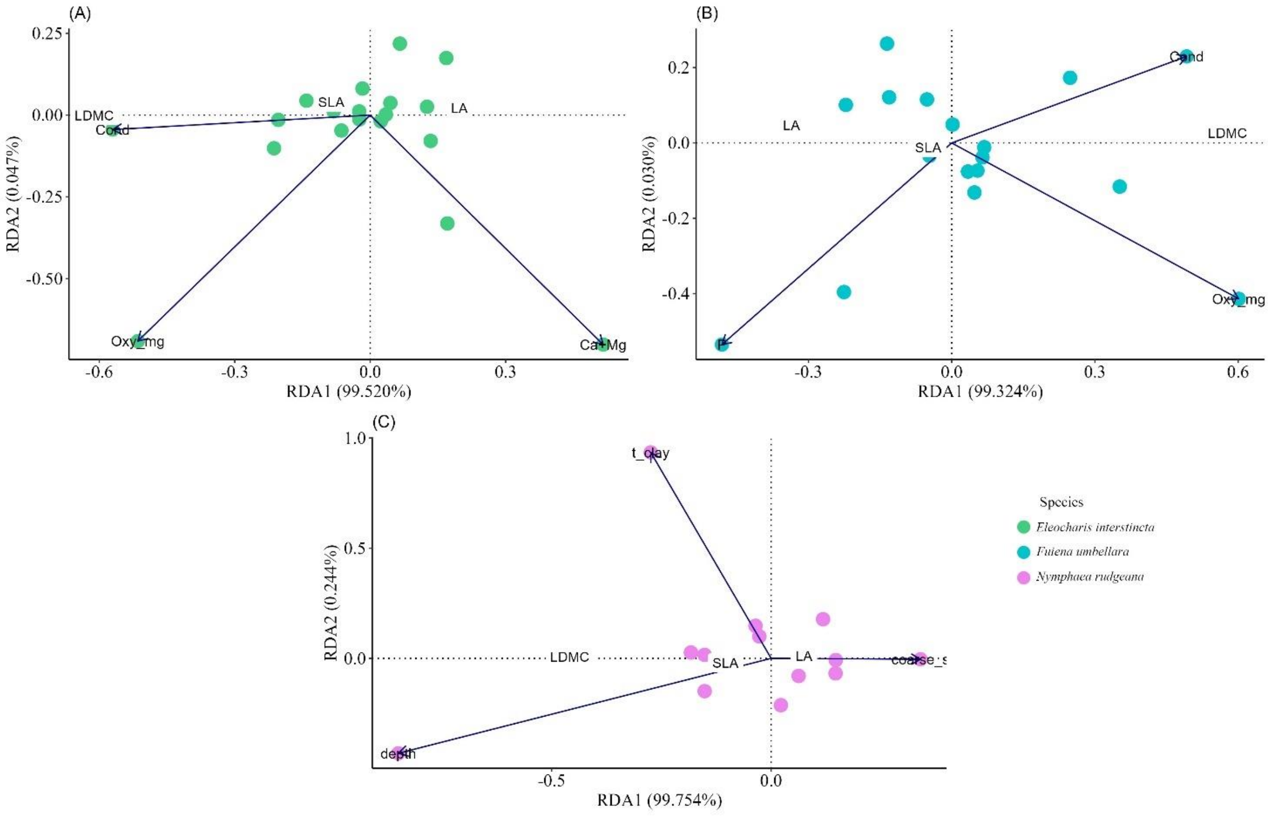

Fig. 3: Redundancy Analysis performed between the traits (LA, SLA and LDMC) of individuals of three macrophyte species (*Eleocharis interstincta*, *Fuirena umbellata* and *Nymphaea rudgena*) and environmental variables: Calcium+Magnesium (Ca+Mg), soil Phosphorus (P), Coarse sand (coarse_s), Total clay (t_clay), water conductivity (Cond), depth (depth)(depth), and dissolved oxygen (Oxy_mg).

2.4.4. *CSR strategies*

We did not find large variation in individuals CSR strategies of the macrophyte species along the resource availability gradient (Fig. 3). All individuals of *E. interstincta* and *F. umbellata* remained stress-tolerators (S >80% of this strategy) in all sites. While individuals of *N. rudgeana* varied from stress tolerators to stress-tolerators/competitors (S/CS) across the sites. Additionally, only one individual of *N. rudgeana* exhibited a small percentage of ruderal strategy (Fig.4).

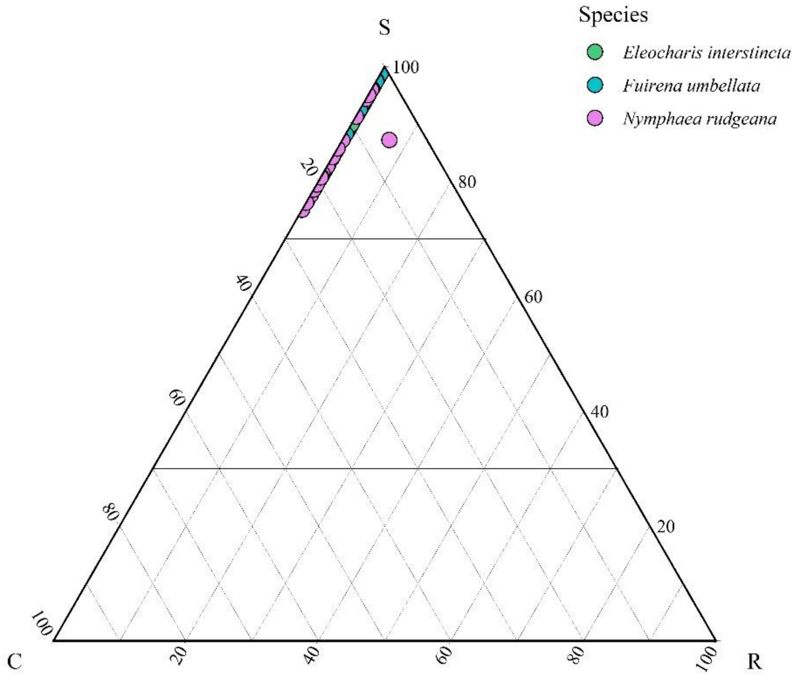

Fig. 4: Ternary plot showing the intraspecific variation in the CSR strategies of three macrophyte species *Eleocharis interstincta*, *Fuirena umbellata* and *Nymphaea rudgeana*.

2.5.Discussion

According with the results, our first hypotheses, that in resource-rich environments individuals of all species have a more acquisitive strategy was partially corroborated, because there was a variation in traits between all species. The intraspecific variation in *E. interstincta* and *F. umbellata* was driven by a combination of variables associated with nutrient availability (water conductivity, and concentrations of phosphorus and calcium+magnesium). However, *N. rudgeana* was associated with variables that are not directly related with resource availability: amount of coarse sand, total clay and water depth. In addition, our second hypothesis that there is contrasting intraspecific variation in ecological strategies of macrophyte species was also partially corroborated, as individuals of *E. interstincta* and *F. umbellata* were all stress-tolerators, however, individuals of *N. rudgeana* presented a slight variation of strategies from competitors to stress-tolerators, contrary with what we expected (a variation from ruderal to competitor).

2.5.1. Trait differences among species

There was a variation in traits among all species (Figure 1; Table 2). *E. interstincta* had lowest SLA, while *F. umbellata* had the highest LDMC and *N. rudgeana* had the highest LA and lowest LDMC. This is an indicative of the contrasting strategies of the species if compared with one another. *N. rudgeana* is the most acquisitive of them, this means that this species invests more in growing fast, by producing larger leaves that are richer in nitrogen than carbon (less structural tissue) (Díaz et al., 2016) and their size vary greatly with environmental conditions. *F. umbellata* was the most conservative, this species invests in leaves that are more resistant (more carbon), with long lifespan, meaning they are more resistant to desiccation, and can resist drought periods (Rodríguez-Alarcón et al., 2022). *E. interstincta* exhibited an intermediate strategy, tending to be more conservative than *N. rudgeana*, with the lowest SLA. This species also invests in having photosynthetic parts that are more resilient, being also resistant to desiccation (Albuquerque et al., 2020).

The variation found in this study agrees with what is expected for aquatic plants: specially the differences in strategies according with life-form (Lacoul & Freedman, 2006; Pierce et al., 2012). However, the pattern found is slightly different from the global one: specific leaf area was not inversely proportional to leaf dry-matter content (Pierce et al. 2017; Figure 1). This may be due to the overall low investment in tough tissue structure of aquatic plants (Poorter et al., 2009; Pierce et al., 2013; Pan et al., 2020), that, despite showing a variation among *E. interstincta* and *F. umbellata* (that invested in more resistant leaves with lower leaf area) and *N. rudgeana* (that invested more in high leaf area and less in tissue density), were still low in proportion if compared, for example, with terrestrial plants (Poorter et al., 2009; Pan et al., 2020). Additionally, it is suggested that macrophytes have distinct functional strategies to surpass the stressors present in freshwater ecosystems and inhabit them (Pan et al., 2020). Furthermore, another way the better understand this variation would be to assess other traits such as leaf thickness and N and P content, as they would give insights in the plant's investments to survive in a water-saturated environment (Pan et al., 2020). Also, there are few studies assessing the variation in traits of aquatic macrophytes and we emphasize that these are the initial results of environmental effects on the foliar traits of macrophytes in the Amazon, so in order to consolidate the comparison with a global pattern, it is necessary to include more species and more traits.

2.5.2. Intraspecific variation in response to environmental conditions

The intraspecific variation in *E. interstincta* is influenced by water conductivity, dissolved oxygen, and the amount of soil Calcium+Magnesium. Similarly, in *F. umbellata*, conductivity and oxygen levels affect leaf traits. The positive relationship between dissolved oxygen and leaf dry matter content (LDMC) in plants can be attributed to the essential role of oxygen in plant metabolism and growth (Sousa & Sodek, 2002). When soil lacks sufficient oxygen, microbial activity shifts, and certain substances become scarce (like nitrite, sulphide, iron and manganese), potentially acting as phytotoxins and imposing stress on plants (Armstrong et al., 2006). This can reach a point where plant survival becomes compromised. Aquatic plants have evolved morpho-anatomical adaptations to overcome anoxic conditions prevalent in their habitats (Lemoine et al., 2012). For instance, *E. interstinca*, a rooted macrophyte, features long colms with segmented air cavities (Gil & Bove, 2007), while *F. umbellata* possesses aerenchyma in its roots and stems (Pott & Pott, 2000). These adaptations facilitate the transfer of oxygen from well-oxygenated parts to hypoxic regions (e.g. from leaves to roots), enabling plants to endure anoxic conditions. In sites with higher oxygen availability, individuals can maintain metabolic processes, promote growth, and allocate more carbon towards building denser leaf tissues, resulting in higher LDMC. Conversely, conditions with limited oxygen pose challenges for plant survival, as they impede vital physiological functions.

The changes observed in *E. interstincta* individuals, with an increase in leaf area and a decrease in leaf dry matter content corresponding to higher levels of soil Calcium and Magnesium, and in *F. umbellata* individuals, with an increase in leaf area and a decrease in leaf dry matter content associated with soil phosphorus concentrations, indicate a strategic shift in response to nutrient availability (Vance et al., 2003; Wright et al., 2004; Grassein et al., 2010). Magnesium is an element present in the chlorophyll molecule, being important to the photosynthetic activity of a plant, calcium is important for plant growth (especially at cell levels), and phosphorus is a macronutrient required many fundamental processes of a plant's metabolism (Grusak et al., 2016). Thus, when those nutrients are scarce, the individuals tend to exhibit a more conservative strategy, investing in producing more long-lived leaves (higher LDMC) so they can retain these nutrients as much as possible and avoid losing them for the environment (Wright et al., 2004; Reich, 2014). In contrast, when these elements are abundant in the system, the individuals invest in growing faster to be more competitive (higher LA and lower LDMC), exhibiting a more acquisitive strategy (Wright et al., 2004; Reich, 2014).

Water conductivity is related with the concentrations of ions in water, which is either related with nutrient availability, like nitrogen (Lacoul & Freedman, 2006). However, some of those ions (such as ammonia and ammonium) can be harmful for plants (Kinsman-Costello et al., 2015; Esteban et al., 2016). For instance, the diversity of macrophyte can either increase (Rolon & Maltchik, 2006) or decrease with water conductivity (Murphy et al., 2003). We believe the positive relationship between *E. interstincta* and *F. umbellata*'s individual leaf dry matter content, and the negative relationship with leaf area may be indicative to resistance to a minor ammonia stress. A possible cause for this increase in nitrogen-based compounds may be the presence of agricultural and livestock activities in the surrounding areas, which leads us to believe that this phenomenon occurs due to the anthropogenic nature of the sites (Obi et al., 2016).

Moreover, contrary to our hypothesis, the variation in the functional traits of individuals of *N. rudgeana* were affected by variables more related to the structure of freshwater ecosystems: water depth and the amount of clay. Nympheids have an intrinsic relationship with water depth and sediment due to their life-form (they are rooted but their leaves float in the water surface), and are very sensitive to water level fluctuation, as they can quickly elongate their petioles to keep their leaves above water (Richards et al., 2012; Dalla Vecchia & Bolpagni, 2022).

Dalla Vecchia and Bolpagni (2022) found that the leaf area and petiole area of a *Nuphar lutea* (Nympheaceae) increases with water depth, which they associated with the cost in investing in building the petiole: the plant is rewarded with a higher photosynthetic surface. Our findings, of a negative relationship between specific leaf area and water depth, and a positive relationship with leaf dry matter content, may indicate a trade-off in investment on petiole mass vs leaf area (Li et al., 2008). In sites with increased water depth, the individuals tended to invest in petiole length, to increase height, in order to reach the water surface (and then be able to photosynthesize), in detriment of having a high photosynthetic surface (Titus & Sullivan, 2001; Richards et al., 2012). An alternative hypothesis is that the individuals in deeper sites invest in having a higher number of leaves that are smaller in area, to increase overall photosynthetic surface while avoiding losing them completely in case the plant gets fragmented or cut (Richards et al., 2012). In addition, individuals in deeper sites invest in more structural tissue (both in leaves and petioles), so the leaves can support the stress caused by the increased water level (e.g. trampling and tidals) and not perish easily under such conditions (Titus & Sullivan, 2001; Dalla Vecchia & Bolpagni, 2022).

Furthermore, the negative relationship between *N. rudgeana* SLA and the amount of clay, and the positive relationship between LDMC and clay are indicative of the importance of the sediment type to the establishment and growth of individuals of this species. Since the aquatic environment is prone to disturbance caused by currents (flooding, trampling, tidals), sediments that are good for root anchorage, such as the ones rich in clay, are advantageous to the establishment of some macrophyte life forms (such as submersed, emergent and floating leaved species) (Schwarz et al., 2015; De Wilde et al., 2017). Additionally, soils richer in clay retain more water and elements, including nutrients for plants, which can be good for the growth of rooted macrophytes (De Wilde et al., 2017). Thus, in sites with more clay, the individuals invest in more tissue density, while in sites with less clay, the plants invested in increasing their specific leaf area.

2.5.6. Intraspecific variation in CSR strategies

Our analysis regarding the variation in CSR strategies among individuals showed that there was no variation in the ecological strategies of *E. interstincta* and *F. umbellata*, who remained stress-tolerators, but *N. rudgeana* varied slightly from stress-tolerator to competitive strategy. This result partially corroborated our hypothesis, as *F. umbellata* and *E. interstincta* followed the global pattern for the Cyperaceae family (be closer to the stress-tolerator axis) (Pierce et al., 2017; Albuquerque et al., 2020). However, we expected some individuals of *N. rudgeana* to exhibit a more ruderal strategy, as most macrophytes are expected to be ruderals (Pierce et al., 2012; Albuquerque et al., 2020), due to the characteristics of the aquatic environment to be more prone to constant disturbance (e.g. flooding, trampling, and drought periods). Indeed, Albuquerque et al. (2020), in a study performed in temporary pools in the Brazilian semi-arid region (Caatinga), found that *E. interstincta* exhibited a ruderal strategy, while several species from the *Nymphaea* genus exhibited a R/CR strategy, which they concluded that the communities' strategies were structured by regional disturbance (e.g. changes in evapotranspiration and precipitation) which is quite contrasting from our results. Furthermore, the variation in individuals of *N. rudgeana* from stress tolerators to competitors (S to S/CS strategy) may be to the high diversity of macrophytes found in some sites. In those conditions, where resource availability is not a limiting factor, these individuals invest in increasing their biomass to acquire more resources and outgrow other plants. Species from the Nymphaeaceae family are known to be more competitive for resources in the environments they inhabit, which reflects in their traits: investment in high leaf area, moderate relative growth rate, limited vegetative dispersal and seeds that sink immediately (Pott et al., 2011; Pierce et al., 2012). Moreover, some individuals may exhibit a more stress-tolerator strategy in an environment where some resources are limited, but they are also excellent competitors where resources are more abundant and species diversity is high. Therefore, the pattern observed in this study aligns more closely with the patterns found in tropical forests than with other macrophyte communities worldwide. However, it is important to note that (Lacoul & Freedman, 2006) classified macrophyte species inhabiting infertile, acidic, alkaline, and saline habitats as stress tolerators, suggesting that they exhibit a different ecological strategy. Our results indicate that individuals of the same species can modify their ecological strategies to thrive in diverse environments, highlighting the phenotypic plasticity observed in macrophytes.

2.6.Conclusions

This study is the first one assessing the intraspecific variation of aquatic plant CSR strategies, revealing intraspecific variation in functional traits and ecological strategies among the studied species, despite the local scale. Nutrient availability influenced the functional traits of *E. interstincta* and *F. umbellata*, while their ecological strategies remained relatively conservative as stress tolerators. However, it was observed that the strategies exhibited by these species in the study area differed from those found in another biome (ruderal), suggesting potential intraspecific variation at a regional or global scale. Those species in our study exhibited a strategy that align more with the niche requirements of tropical forests, such as the Amazon.

In contrast, *N. rudgeana* demonstrated intraspecific variation along the resource availability gradient, transitioning from stress tolerators to competitors. This species exhibited high sensitivity to environmental changes and a highly acquisitive strategy, even at a local level, but certain individuals adopted a more conservative strategy when resources were limited. The findings highlight the adaptability and phenotypic plasticity of aquatic plants at both the species and individual levels. Future research should incorporate other macrophyte life forms (such as submerged and free-floating) and additional traits (e.g. petiole length, mass and area, leaf thickness, leaf phosphorus and nitrogen content, and root traits) to further comprehend their strategies, life history, and responses to environmental changes driven by climate and land-use shifts, which pose ongoing challenges to biological communities.

2.7.Supplementary Material

Intraspecific variation in leaf traits and ecological strategies of macrophytes across an environmental gradient in Eastern Amazon

Ana Luísa Biondi Fares, Thaisa Sala Michelan

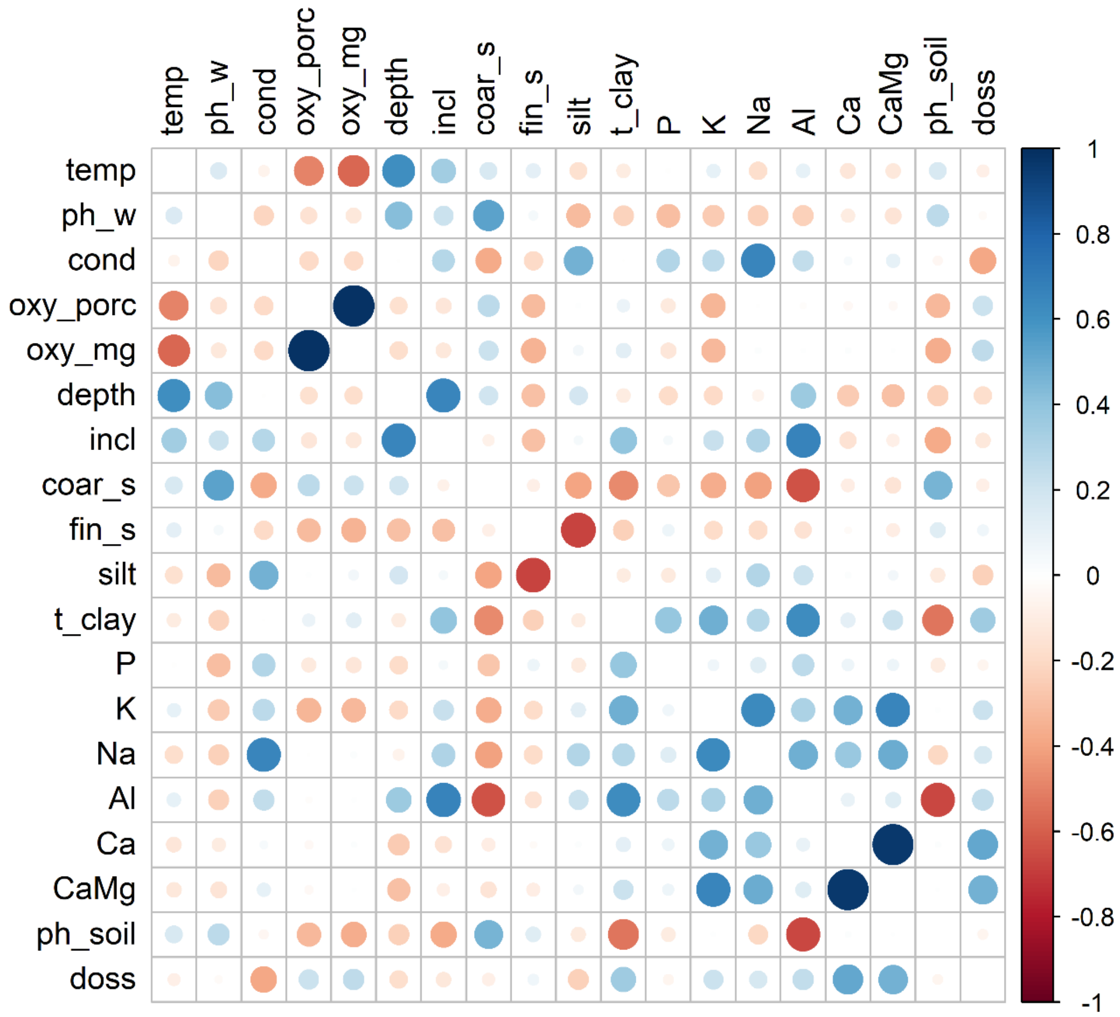

Fig. S1: Correlation among environmental (water, physical structure and soil) factors. Blue circles indicate a positive relationship, while red circles indicate a negative relationship. The bigger and the darkest the color, the strongest the relationship. Water: temp = water temperature (°C), ph_w = water pH, cond = water conductivity (µs/cm), oxy_porc = dissolved oxygen (in %), oxy_mg (in mg/L), depth = water depth (cm); Physical strucutre: incl = inclination of the shoreline (°); Soil: coarse_s = coarse sand (g/kg), fin_s = fine sand (g/kg), silt = silt (g/kg), t_clay = total clay (g/kg), P = amount of phosphorus (mg/dm^3), K = amount of Potassium (mg/dm^3), Na = Sodium (mg/dm^3), Al = Aluminum (cmol$_c$/dm^3), Ca = Calcium (cmol$_c$/dm^3), CaMg = amount of calcium + magnesium.

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3. Section II

The drought was the *very worst* (for some): intraspecific trait variation and resource allocation trade-offs under water stress unveil divergent survival strategies in macrophytes amid climate change

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The drought was the *very worst* (for some): intraspecific trait variation and resource allocation trade-offs under water stress unveil divergent survival strategies in macrophytes amid climate change

3.1.Abstract

1. Human-induced climate change poses a dual threat to natural ecosystems, impacting water availability and causing extreme climatic events that affect plant responses. Examining intraspecific trait variability in macrophytes under water stress amid climate change is crucial for predicting community shifts and preserving biodiversity in freshwater ecosystems. Trait variation, including biomass allocation and reproductive strategies, reflects in adaptations to stress.

2. Our study focused on evaluating intraspecific variation in aquatic macrophytes under drought and flood conditions, exploring biomass allocation trade-offs from aboveground to belowground parts. We hypothesize that, under drought conditions, individuals exhibit a conservative strategy, and we expect a trade-off involving biomass allocation from above to belowground parts, coupled with a strategy of early flowering to produce offsprings that will germinate when conditions are more favorable.

3. For this, we performed a greenhouse experiment where we submitted individuals of two species (*Limnocharis flava* and *Pontederia cordata*) to water stress extremes, and we examined physiology (leaf water relations, stomatal conductance), morphology (roots, leaves), phenology (flower onset), and biomass allocation. Results revealed species-specific strategies: *L. flava* displayed a drought scape-avoidance strategy and flood tolerance, allocating biomass belowground under drought and aboveground under flooding.

4. Surprisingly, *P. cordata* exhibited osmotic adjustment under drought while maintaining high stomatal conductance, indicating the individuals were able to maintain (and even increase) their metabolism under water limitation, due to the osmotic adjustment. Additionally, individual flowering onset was not affected by water stress, implying individuals invested more in vegetative reproduction (especially under drought conditions, as expressed by the higher values of

aboveground biomass allocation under this conditions), while they allocated more biomass to roots under flooding, indicating this species is both drought and flood tolerant.

5. Our findings highlight the role of intraspecific variability in species survival under contrasting environments, offering insights into population dynamics and community assembly amid climate change. However, we stress the importance of considering adaptive factors triggering plant responses and recognizing certain plants' sensitivity, facing extinction risks as the consequences of climate change worsens. Thus, acknowledging intraspecific variability within populations and communities is crucial for safeguarding global species diversity.

Keywords: anisohydric species, drought escape, hydraulic traits, Amazon freshwaters, plant size and economics, trait coordination

3.2.Introduction

Human-induced climate change poses a dual threat to natural ecosystems, with long-term impacts such as rising global temperatures and ocean acidification, as well as acute events like storms, floods, heatwaves, and droughts (Malhi et al., 2020). The increasing frequency of extreme climatic events includes shifts in precipitation and hydrological regimes, such as prolonged droughts or flooding (IPCC, 2023). These changes significantly influence water availability, potentially disrupting entire communities and causing ecosystem imbalance (Dudgeon, 2019; Flitcroft et al., 2019; Malhi et al., 2020). Given their pivotal roles in biogeochemical cycles, primary productivity, carbon fixation, and climate regulation, plants emerge as crucial entities in mitigating the impacts of climate change on ecosystems, and understanding how they will respond to the shifting climate is of extreme importance (Feeley et al., 2020; Jasechko et al., 2013; Phillips et al., 2009; Tavares et al., 2023).

While the investigation of species-level traits has revealed the broad effects of environmental disturbances on communities and ecosystems, it is vital to recognize that species traits are dynamic across space and time, varying among populations, individuals, and genotypes (Moran et al., 2016). Consequently, exploring intraspecific variation and phenotypic plasticity becomes paramount (Matesanz & Ramírez-Valiente, 2019; Merilä & Hendry, 2014), offering insights into a species' adaptability across distinct environments amid ongoing environmental shifts induced by climate change (Arnold et al., 2019; Garzón et al., 2011; Mimura et al., 2017; Song & Li, 2023). Furthermore, in tropical ecosystems (such as the Amazon) that experience extreme hydrological regimes (prolonged periods of drought or flooding) (Marengo et al., 2011), the ability to modify some characteristic in response to water stress can provide the necessary conditions for the survival of a species, or cause the loss of others (Oliveira et al., 2021; Valladares et al., 2007).

Intraspecific variability in plant functional traits, which reflects in plant size and economics, can provide advantages or disadvantages in extreme environmental conditions, like resource limitations (Doudová & Douda, 2020; Mimura et al., 2017; Sultan, 2000). The most successful species are those with sufficient variability in life history strategies to optimally acquire and utilize available resources (Merilä & Hendry, 2014; Mimura et al., 2017; Valladares et al., 2007). For example, under water stress, some plants can develop different strategies by decreasing their water loss to reduce dehydration, changing their reproductive period by anticipating their life cycle and reproductive cycle as a way of 'escaping' from adverse conditions, or simply maintaining metabolism even with a decrease in tissue water potential in response to adverse conditions (avoidance, escape and tolerance strategies, respectively) (Kooyers, 2015; Verslues et al., 2006). Additionally, plants can have different physiological pathways to tolerate water stress: either by closing stomata to prevent transpiration and cells from reaching more negative water potentials, thus maintaining cell turgor and metabolism but also reducing photosynthetic rates (isohydric plants), or by decreasing water potentials (through lower turgor loss point and osmotic potential) to maintain osmotic adjustment even as stomata remain open and photosynthetic rates are high (anisohydric plants) (Chen et al., 2023; Sade et al., 2012). These strategies align with the fast-slow plant economics spectrum, where traits across all plant parts (roots, stem, leaves, and reproductive parts) are coordinated (via synergy or trade-offs) to enable a faster (more acquisitive) or slower (more conservative) response to the surrounding environment (Díaz et al., 2016; Oliveira et al., 2021; Reich, 2014; Xu et al., 2021).

For instance, under drought conditions, plants may be more conservative, and prioritize root investment for rapid resource acquisition, resulting in the production of fewer but more resilient and long-lasting leaves, characterized by increased leaf dry matter content and low specific leaf area (Carrascosa et al., 2023; F. Liu & Stützel, 2004; Wellstein et al., 2017a). Similarly, in flood conditions where soil anoxia and high-water levels limit light penetration, plants may allocate more resources to their aboveground parts (Chen et al., 2023), emphasizing growth and high leaf area to elevate leaves above the water column and enhance photosynthetic efficiency (Mommer et al., 2006; Winkel et al., 2016; Wright et al., 2017). Drought stress also affect plant physiological traits, as stomata close under these conditions (low stomatal conductance) to avoid more water loss, reducing photosynthesis (Chen et al., 2023; Osakabe et al., 2014), and plants may reduce leaf turgor loss point and osmotic potential in order to provide osmotic adjustment to avoid wilting and death (Chen et al., 2023; Maréchaux et al., 2015; Oliveira et al., 2021; Sueltenfuss et al., 2020). Moreover, plants may also alter their phenology, by anticipating or delaying the flower onset under water stress (Satake et al., 2019; Valladares et al., 2007; Welles & Funk, 2021).

Furthermore, there may be a trade-off in biomass allocation between plant portions, such as favoring belowground (roots) over aboveground parts (stems and leaves) under severe drought stress (Loreti & Oesterheld, 1996; Poorter et al., 2012; Welles & Funk, 2021) and the reverse under flooding (Chen et al., 2023; Loreti & Oesterheld, 1996; Poorter et al., 2012). In addition, there is the underlying cost in investing in reproduction under stress conditions, which may cause production of unfertile seeds and compromise future reproductive cycles and generations (Obeso, 2002). These trade-offs highlight the complex interplay among plant parts in response to environmental stress, showcasing coordinated traits to ensure individual survival (de la Riva et al., 2016; H. Liu et al., 2019; Martínez-Vilalta et al., 2023; Xu et al., 2021).

In this context, aquatic plants (also known as macrophytes) can be used as models to investigate how plants can respond to adverse conditions such as water stress (drought and flood). Macrophytes inhabit freshwater ecosystems, which are some of the most affected by various environmental and climatic changes (originating from both natural disturbances and human activities), such as eutrophication (Dudgeon, 2019; Zhang et al., 2017), sedimentation (Dudgeon, 2019), and changes in the hydrological regime (extreme drought and flood periods) (Aldous et al., 2011; Bond et al., 2008). In spite of being considered sensitive to water limitation (Short et al., 2016; Touchette et al., 2014), some macrophytes can sustain considerable water constrains (Sueltenfuss et al., 2020; Touchette et al., 2007) and even other types of stress (e.g. salinity and heavy-metal contamination) (Nguyen et al., 2021; Touchette et al., 2014), in addition to being adapted to waterlogged conditions (via aerenchyma in roots, petioles and leaves). It is believed that environmental changes are impacting macrophyte species composition globally, leading to anticipated shifts in future communities (Short et al., 2016). These changes may result in reduced diversity of life forms (e.g., free-floating, submerged, and floating-leaved), with a potential dominance of species adapted to altered conditions, particularly emergent and amphibious varieties (Alahuhta et al., 2011; Zhang et al., 2017).

In addition, macrophytes have many ecological roles in the environments they inhabit, such as nutrient cycling, water oxygenation, sedimentation of matter, as well as serving as habitat and food for microorganisms, macroinvertebrates and fish (Bornette & Puijalon, 2011; Thomaz, 2021). They are important components for the preservation of aquatic biodiversity, and environmental changes that affect their survival in the aquatic environment cause a cascading effect, affecting the other communities of organisms that depend on them in some way (Hossain et al., 2017; Thomaz, 2021). Understanding the interplay between environmental change, functional traits, and intraspecific variability is crucial to decipher how macrophytes traits may respond amid continuous environmental fluctuations. This adaptation is vital for survival in natural habitats and predicting responses to escalating human-induced climate change consequences (Bartlett et al., 2012). Furthermore, intraspecific variation can be as strong as variation between species, affecting community structure and ecosystem functions just as much as interspecific variation (Des Roches et al., 2018; Doudová & Douda, 2020; Siefert et al., 2015). Consequently, studies on intraspecific variation yield valuable insights not only into climate change impacts on individual species but also provide inferences about broader effects on communities and their ecosystem services (Des Roches et al., 2018; Doudová & Douda, 2020).

In light of this, the aim of study is to evaluate the intraspecific variability of aquatic macrophytes in response to water stress conditions (drought and flood) and possible biomass allocation trade-offs from the aboveground to the belowground. We investigated that in all plant parts: morphology (roots and leaves), physiology (leaf water relations and stomatal conductance) and phenology (flower onset and reproductive biomass). We hypothesize that under drought conditions, individuals exhibit avoidance a conservative strategy, by developing longer-lived leaves (high dry matter content and low specific leaf area). This adaptation includes lower turgor loss point, osmotic potential, and stomatal conductance values, aimed at minimizing water loss. Additionally, we expect a trade-off involving resource allocation from above to belowground parts, coupled with a strategy of early flowering to produce offspring that will germinate when conditions are more favorable. Conversely, under flood conditions, we anticipate an acquisitive strategy in individuals, reflected in higher leaf and specific leaf area values. This involves a trade-off favoring aboveground biomass allocation, with delayed or absent flowering, in a strategy to overcome the water column, seeking optimal conditions for photosynthesis and flowering. These questions will be tested experimentally.

3.3.Material and Methods

We selected two macrophyte species belonging to the emergent life-form. We chose this particular life-form because they normally survive more in waterbodies during the dry season (when there is few or no water availability), and also because this is the type of aquatic vegetation that is becoming dominant in ecosystems that have suffered from disturbance due to climate change (Alahuhta et al., 2011). The species selected were:

- *Limnocharis flava* (L.) Buchenau (Alismataceae) (Fig. 1A)

A species of macrophyte native to the Americas. A perennial, glabrous, lactiferous herb that grows up to 60cm in height and produces yellow flowers. It reproduces by seed or vegetative propagation. This plant inhabits shallow areas, such as marshes, ditches, pools, among others (Pott & Pott, 2000). This species has commercial value as an ornamental plant, is an ingredient in the cuisine some Asian countries and has phytoremediation potential. It is considered an invasive species in countries such as India, Australia and Sri Lanka (Ranawakage et al., 2013).

- *Pontederia cordata* L. (Pontederiaceae) (Fig. 1D)

Perennial aquatic herb, native to the Americas. It grows from 25 to 100 cm tall. It has simple, cordate to oval, alternate, glabrous leaves and flowers that vary in color from lilac to bluish. It inhabits shallow waters and moist soil (Pott & Pott, 2000). This species has heterostyly, a floral polymorphism in which the same population can have up to three floral morphologies, with different pistil and anther sizes (Gettys & Wofford, 2008). Like *L. flava*, this species has commercial value mainly because it is used as an ornamental plant, is considered an invasive species in South Africa (Wansell et al., 2022), as well as having phytoremediation potential.

3.1.1. Experimental design

The experiment was conducted at the AQUA greenhouse, located in the Universidade Federal do Pará (Brazil; 1°28'33.10"S, 48°27'26.24"O). In august 2023, plants were collected near the university for propagule reproduction, where we cut all leaves and removed the roots so the individuals would grow again in similar conditions. After 15 days, forty identically sized individuals of each species were selected and placed separately in 90 transparent buckets (20L) with approximately 4kg of a mixture of organic soil and sand in a 5-to-3 proportion (2.5kg of soil and 1.5kg of sand).

Initially, all buckets were watered during the first 34 days to ensure acclimatation of the plants. After these days, the samples were divided into 3 treatments for each species: thirteen buckets were watered regularly to reach a constant water level of 3-4cm above the ground (control treatment), 13 buckets were regularly watered until the plant was completely submerged (flood treatment), and 13 received minimal water doses (400ml spaced at a time interval that varied from every 2 to three days, depending on how hot the day was – we have an extra sampling unit in this treatment as there was a greater risk of losing individuals in this situation) during the experiment only to avoid lethal consequences on the plants (drought treatment), totaling 78 samples (2 species x 3 treatments x 13 replicates). The buckets were randomly arranged in the greenhouse (Fig. 1D).

Figure 1: Studied species and phases of the experiment. A) Limnocharis flava; B) Experimental design; C) Measurement of water parameters; D) Pontederia cordata; E) Measurement of pressure-volume curves; F) Measurement of leaf morphological traits.

Before the start of the experiment, six buckets of each species were selected to quantify the aboveground height, as well as being dried in an oven to measure their biomass. This ensured that the responses obtained are the effect of the treatments applied, and also used to estimate the initial biomass, a number that was used to calculate some traits (see the section 2.2 below).

After 106 days, we took the leaves of selected individuals to perform pressure volume-curves (see the section of hydraulic traits for more information), and all individuals were removed to measure the remaining functional traits. We applied 1g per bucket of fertilizer (solid macro-micro nutrient mix) four times through all the duration of the experiment. Throughout the experiment, we monitored water physicochemical (temperature, pH, and conductivity): using a multiparameter probe (Akso model AK48), we measured these variables at the beginning, the middle and at the end of the experiment time (Fig. 1C). Additionally, we used a datalogger (Akrom model AK430) to measure parameters of the air (temperature and humidity) every thirty minutes of the day until the end of the experiment.

3.1.2. Measurement of functional traits

In order to investigate the intraspecific variation in response to water stress, in various aspects of the plants (plat water relations, leaf morphological traits, plant phenology and biomass allocation) as well as possible trade-offs and change in strategies according to the water stress, we measured the following functional traits, which we divided in five main categories:

3.1.2.1. Leaf water relation traits and stomatal conductance

To assess individuals' physiological responses, we measured one gas exchange trait:

i) Stomatal conductance (gs; mmol m-2 s-1), in one leaf per individual, using a Leaf porometer (Model Meter SC-1). Stomatal conductance indicates the degree to which the stomata are open, which influences the leaf's gas exchange and leaf transpiration, implying the leaf's state of hydration. Low stomatal conductance values imply water stress in the plant. We measured the individuals three times during the experiment: at the beginning, the middle, and the end of the experiment, on sunny days, between 7:30 and 10:30AM, to guarantee we got the full spectrum of change in response of the treatments.

Additionally, using the pressure-volume curve method, we evaluated leaf water potential traits that are associated with tolerance to water stress (Bartlett et al., 2012). These measures were obtained by the pressure-volume curve method, using the bench-drying methodology, where we measured leaf water potential and mass until the plant reached below -5 MPA or was completely dry (Fig. 1E). Curves were constructed using a pressure chamber (Scholander Pump, Model 1000, PMS, USA). For this measurement, we selected five to six individuals of each species per treatment (the ones with the most leaves), and we measured one to four leaves per individual to make the curves, depending on the number of leaves available. After measuring the water potential, the leaves were oven-dried at 60°C for 72 hours, to calculate the relative water content. From the curves, it was possible to calculate, for each individual:

ii) Turgor loss point (Ψ_{TLP}; MPa), which indicates the potential at which the leaf cells lose turgor, becoming flaccid and losing their function.

iii) Osmotic potential at maximum turgor (Ψ_o; MPa), which indicates the potential with the maximum content of solutes in the leaf, and is related to the plant's osmotic adjustment.

iv) Bulk elastic modulus or Elasticity (ε; MPa): is the ratio between the change in cell turgor and the relative cell volume (Bartlett et al., 2012), and indicates how the cell walls can sustain change in volume as the leaf dehydrates or rehydrates (Bartlett et al., 2012). Low values of leaf elastic bulk modulus indicate more elastic cell walls, while high values indicate cell walls that are stiffer (Nadal et al., 2023).

We followed the recommendations of Pérez-Harguindeguy et al. (2013) and Bartlett et al. (2012) for measuring these traits.

3.1.2.2.Leaf morphological traits

In order to investigate the intraspecific variation in plant leaf traits and strategies, we selected three leaves of all the individuals that were alive at the end of the experiment, and measured (Fig. 1F):

v) Leaf area (LA; mm^2): measures leaf size, and is the one-sided area of a leaf. It is associated with the investment of plants in the photosynthetic surface. We scanned the leaves to measure LA, using de package BiocManager (Morgan, 2022) in the R program (R Core Team, 2022).

vi) Specific leaf area (SLA; $mm^2.mg^{-1}$): It expresses the amount of carbon invested in the photosynthetic area of a leaf. After the leaves were scanned and leaf area was determined, we dried them in an oven (at 65ºC for 72 hours) and then weighed on an electronic scale. The SLA was calculated after determining the dry mass using the formula: SLA= Leaf area / dry mass.

vii) Leaf thickness (L_{Th}; mm): plays an important role in determining leaf resistance. We measured the thickness of the limbus (in the central portion of the limbus, avoiding the main vein) using a digital caliper (Pérez-Harguindeguy et al. 2013).

viii) Leaf dry matter content (LDMC $mg.g^{-1}$): this was be measured on the same leaves used to calculate SLA. LDMC is the dry mass of a leaf divided by its water-saturated fresh mass (Pérez-Harguindeguy et al., 2013). This measure is an average of leaf tissue density. The water-saturated fresh mass was measured on a precision scale after the leaves have been hydrated, and then the oven-dried mass (65ºC for 72 hours) was determined.

3.1.2.3.Phenological trait

We recorded the **ix)** flower onset of each individual, expressed in days, which is the time when each individual began flowering in order to assess whether water stress affects the plants' reproductive phenology.

3.1.2.4.Investment in aboveground, belowground, and reproductive parts

On all individuals, we measured the following metrics that show the investment in plant parts: **x)** Plant height (cm), which is the complete length starting from the stem base to the apex of the tallest leaf. After that, we separated the plant parts and oven-dried them until they reached constant weight, and then weighted them to obtain the **xi)** final aboveground biomass (stems+leaves; g), **xii)** final belowground biomass (roots; g) and **xiii)** final reproductive biomass (flowers+fruits; g).

Additionally, we used the oven-dry weight to calculate the **xiv)** Relative growth rate (RGR; g.g.-1): calculated with the whole plant biomass, using the initial and final biomass values, by the formula: TCR= (lnM1-lnM2)/(t1-t2), where M1 represents the initial biomass, M2 the final biomass, and t1 represents the collection time of the initial biomass, and t2 the collection time of the final biomass (expressed in days).

3.1.2.5.Trade-offs between plant parts: biomass allocation

Finally, in order to investigate the biomass allocation trade-offs in response to water stress, we used the individual's oven-dried biomass to calculate three metrics:

xv) Aboveground mass fraction (AMF; g-1): is the ratio between the aboveground biomass and the total plant biomass, showing how much of the plant biomass was allocated to produce leaves and stems.

xvi) Belowground mass fraction (BMF; g-1): is the ratio between the belowground biomass and the total plant biomass, showing how much of the plant biomass was allocated to the production of roots.

xvii) Reproductive mass fraction (RMF; g-1): is the ratio between the reproductive biomass and the total plant biomass, showing how much of the plant biomass was allocated to produce flowers and fruits.

3.1.3. Data analysis

In order to visualize the strategies against water stress of each species, reflected in the variation on their traits, and the coordination of the traits, we performed a Principal Component Analysis (PCA), using the Broken-stick criterion as a stopping rule for the ordination (Jackson, 1993).

To test our hypothesis, we used Analysis of Variance (ANOVA), using the water stress treatments (control, drought and flood) as the predictor variable, and the traits (stomatal conductance , turgor loss point, osmotic potential, elasticity, leaf area, specific leaf area, leaf thickness, leaf dry matter content, flower onset, plant height, aboveground, belowground, and reproductive biomass, and, finally, relative growth rate) and trade-off measures (aboveground, belowground and reproductive mass fraction) as response variables. When the assumptions of normality and homoscedasticity of the models were not met, we performed a logarithmic or square-root transformation on the response variable. Variation between pairs of groups on ANOVA models was tested using Tukey's post-hoc test. When the model assumptions were not met by data transformation, we performed a Kruskall-Wallis test, and the pairwise variation was tested using Duun's test with Bonferroni correction (Dinno, 2015).

The analyses were carried out using the R program version 4.2.0 (R Core Team, 2022). We performed PCA with the 'prcomp' function, both from the 'vegan' package (Oksanen et al., 2022). For the analyses testing our hypothesis, we tested homogeneity of variances using the 'leveneTest' function from the car package (Fox & Weisberg, 2019), and normality of residuals was tested with the Shapiro-Wilk Normality Test, using the 'shapiro.test' function from the stats package, which is part of R (R Core Team, 2022). ANOVAS were performed using the 'aov' function, and the post-hoc test was performed using the 'tukeyHSD' function (R Core Team, 2022). Finally, we performed Kruskal-Wallis test using the 'kruskal.test' function (R Core Team, 2022), the post-hoc test was performed using the 'dunnTest' function from the FSA package (Ogle et al., 2023). We also used boxplots of all significant relationships to better visualize the results.

3.4.Results

3.1.4. Trait variation among species

At total, we analyzed 80 individuals, 40 of each species. We measured total biomass and allocation measures of all individuals, however, some individuals died during the experiment (in drought treatment), and we were not able to measure some leaf traits on all of them. While there were consistencies in some trait responses on both species, the results substantially diverged in other evaluated metrics. Overall, individuals of *L. flava* demonstrated the highest specific leaf area under controlled conditions (Mean = 2.862 g), as well as the highest values for belowground (0.515 g^{-1}) and reproductive (0,126 g^{-1}) mass fractions, leaf dry matter content (12.232 $mg.g^{-1}$), stomatal conductance (79.220 mmol $m^{-2}s^{-1}$), turgor loss point (-1.272 Mpa), and osmotic potential (-0.871 Mpa) in drought conditions. Additionally, they exhibited superior height (78.023 cm), aboveground (17.543 g), belowground (8,075 g), and reproductive (3.631 g) biomass, relative growth rate (3.198 $g.g^{-1}$), aboveground mass fraction (0.686 g^{-1}), leaf area (1021.822 mm^2), leaf thickness (0.302 mm), elasticity (10.739 Mpa), and longest flowering onset (54 days) in flooding conditions. Conversely, individuals displayed the lowest stomatal conductance (70.410 mmol $m^{-2}s^{-1}$) and flowering onset (30 days) values under control conditions, as well as reduced height (31.843 cm), aboveground (3.53 g), belowground (4,538 g), and reproductive (1.219 g) biomass, relative growth rate (1.880 $g.g^{-1}$), aboveground mass fraction (0.485 g^{-1}), leaf area (355.173 mm^2), leaf thickness (0.165 mm), and elasticity (3.428 Mpa) values in drought conditions. Furthermore, they exhibited the lowest values for belowground (0.314 g^{-1}) and reproductive (0,098 g^{-1}) mass fractions, specific leaf area (2.652 mm^2 g^{-1}), leaf dry matter content (10.623 mg g^{-1}), turgor loss point (-2.64 Mpa), and osmotic potential (-1.848 Mpa) under flooding conditions.

Meanwhile, *P. cordata* individuals exhibited the highest relative growth rates (3.964 $g.g^{-1}$), leaf area (774.696 mm^2), specific leaf area (2.604 mm^2 g^{-1}), osmotic potential (-0.596 Mpa), and elasticity (9.017 Mpa) under controlled conditions. In drought treatment, they showed the highest aboveground mass fraction (0.821 g^{-1}), leaf dry matter content (18.570 mg g^{-1}), and stomatal conductance (82.298 mmol $m^{-2}s^{-1}$), while in flooding conditions, they displayed the highest height (95.054 cm), aboveground (40.888 g), belowground (17.110 g), and reproductive biomass (1.557 g), along with elevated belowground (0.288 g^{-1}) and reproductive (0.260 g^{-1}) mass fractions, leaf thickness (0.251 mm), and turgor loss point (-0.973 Mpa). Conversely, under controlled conditions, *P. cordata* had the lowest stomatal conductance (40.538 mmol $m^{-2}s^{-1}$), and flowering onset (35 days). In drought conditions, they exhibited the lowest turgor loss point (-1.979 Mpa), osmotic potential (-1.389 Mpa), height (47.757 cm), aboveground (16.532 g), belowground (3.215 g), and reproductive biomass (0.182 g), as well as reduced belowground (0.179 g^{-1}) and reproductive mass fractions (0.008 g^{-1}), leaf area (508.632 mm^2), specific leaf area (2.438 mm^2 g^{-1}), and leaf thickness (0.206 mm). Finally, in flooding conditions, they showed the lowest elasticity (3.642 Mpa), leaf dry matter content (16.504 mg g^{-1}), and aboveground mass fraction (0.712 g^{-1}).

The first axis of the PCA represented 29.73% of total data variation, while the second axis represented 24%, showing a clear separation between the species (Fig. 2). Individuals of *L. flava* had the highest belowground and reproductive mass fractions, the highest values of specific leaf area and reproductive biomass, and were related with the flowering onset and leaf elasticity, indicating this species allocated more biomass to the root and reproductive parts. In contrast, individuals of *P. cordata* had the highest values of plant height, root biomass, relative growth rate, aboveground biomass, aboveground mass fraction, and were related with turgor loss point and osmotic potential.

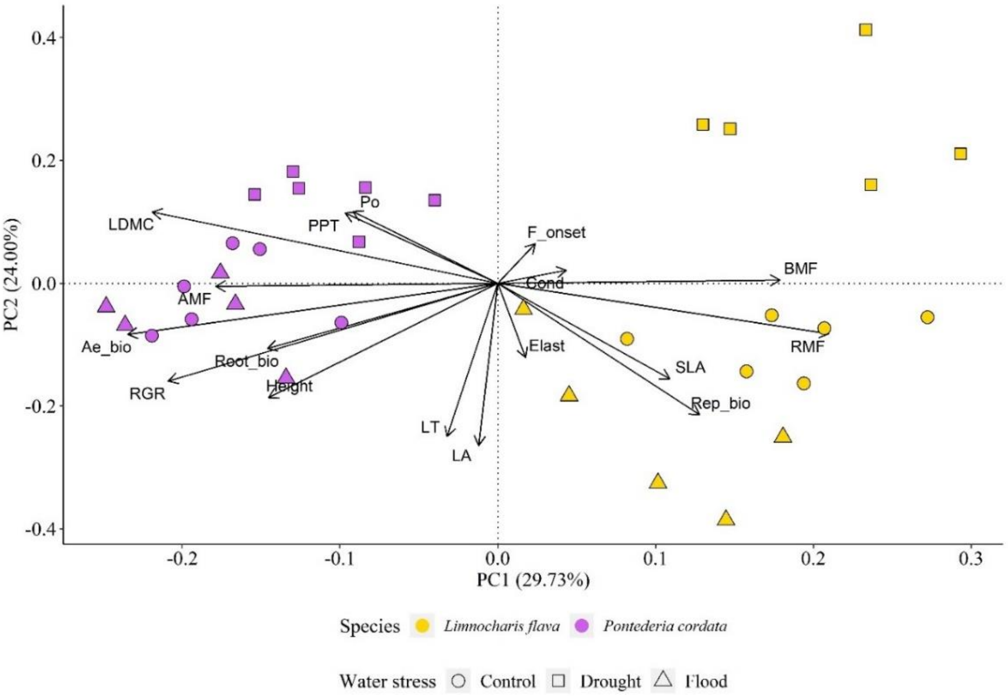

Figure 2: Principal Component Analysis performed with the water relations, stomatal conductance, morphological and phenological functional traits of individuals of *Pontederia cordata* and *Limnocharis flava*. Po = Osmotic potential; PPT = turgor loss point; Elast = elasticity; LA = leaf area; SLA = specific leaf area; LDMC = leaf dry matter content; LT = leaf thickness; F_onset = flower onset; Height = plant height; Ae_bio = aboveground biomass; Root_bio = belowground biomass; Rep_bio = reproductive biomass; RGR = relative growth rate; AMF = aboveground mass fraction; BMF = belowground mass fraction; RMF = reproductive mass fraction.

3.1.5. Leaf water relation traits and stomatal conductance

Individuals of the species showed a different strategy regarding their ability to retain water. On *L. flava*, of all hydraulics (turgor loss point, osmotic potential and elasticity) and gas exchange (stomatal conductance) traits, only elasticity was significantly related with the water stress treatments (Table 1). Specifically, elasticity differed between flood and drought conditions, being higher in the former (Fig. 3).

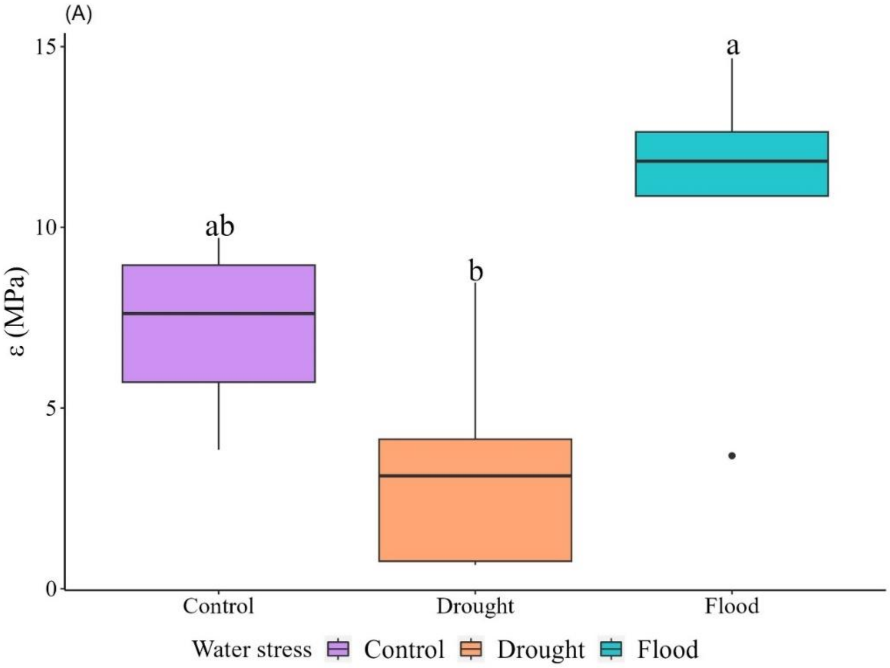

Figure 3: Result of models showing the relationship between leaf elasticity of individuals of *Limnocharis flava* and the water stress treatment (control, drought and flood). Different upper letters indicate statistical differences between groups tested with post hoc tests. Ɛ = Elasticity.

Table 1: Results of the models performed to predict the effects of water stress (control, drought and flood) in the intraspecific functional trait variation of *Limnocharis flava*.

Statistical model	**Response Variable**	**Data transformation**	**Predictive Variable**	**Degree of freedom**	**Sum of squares**	**Mean squares**	**F/x^2**	**P**
Kruskall-Wallis	Stomatal conductance (g_s)	-	Water stress	2	-	-	0.0518	0.974
Kruskall-Wallis	Turgor loss Point (Ψ_{TLP})	-	**Water stress**	**2**	**-**	-	**5.729**	**0.05***
ANOVA	Osmotic potential (Ψ_o)	-	Water stress	2	2.392	1.196	2.824	0.096
		-	Residuals	13	5.506	0.424		
ANOVA	Elasticity (ε)	-	**Water stress**	**2**	**133.7**	**66.83**	**6.299**	**0.012***
		-	Residuals	13	137.9	10.61		
Kruskall-Wallis	Leaf area (LA)	-	**Water stress**	**2**	**-**	-	**22.023**	**<0.001***
ANOVA	Specific leaf area (SLA)	-	Water stress	2	0.300	0.150	2.294	0.117
		-	Residuals	32	2.093	0.065		
ANOVA	Leaf thickness (L_{th})	-	**Water stress**	**2**	**0.101**	**0.051**	**41.18**	**<0.001***
	Residuals	-		32	0.039	0.001		
ANOVA	Leaf dry matter content (LDMC)	Log	**Water stress**	**2**	**0.125**	**0.062**	**15.53**	**<0.001***
		-	Residuals	32	0.128	0.004		
ANOVA	Flowering onset	Log	**Water stress**	**2**	**2.27**	**1.135**	**4.156**	**0.024***
		-	Residuals	37	10.11	0.273		
ANOVA	Plant height	-	**Water stress**	**2**	**14420**	**7210**	**129.8**	**<0.001***
		-	Residuals	37	2056	56		
Kruskall-Wallis	Aboveground biomass	-	**Water stress**	**2**	**-**	-	**29.759**	**<0.001***
Kruskall-Wallis	Root biomass	-	**Water stress**	**2**	**-**	-	**13.085**	**0.001***
Kruskall-Wallis	Fruit biomass	-	**Water stress**	**2**	**-**	-	**7.5021**	**0.02***
Kruskall-Wallis	Relative growt rate (RGR)	-	**Water stress**	**2**	**-**	-	**24.335**	**<0.001***
ANOVA	Aboveground mass fraction (AMF)	-	**Water stress**	**2**	**0.296**	**0.148**	**14.7**	**<0.001***
		-	Residuals	37	0.372	0.010		
ANOVA	Root mass fraction (RMF)	-	**Water stress**	**2**	**0.296**	**0.148**	**14.7**	**<0.001***
		-	Residuals	37	0.372	0.010		
ANOVA	Fruit mass fraction (FMF)	-	Water stress	2	0.039	0.020	2.477	0.098
		-	Residuals	37	0.292	0.008		

However, individuals of *P. cordata* showed the opposite pattern. For this species all models were significant, except for Elasticity (Table 2). Stomatal conductance was higher in drought conditions in comparison to flooding (Fig. 4A), while turgor loss point and osmotic potential did not differ between control and flood conditions, but was lower at drought conditions, indicating individuals were more resistant to water scarcity ate the drought (Fig. 4B and 4C)

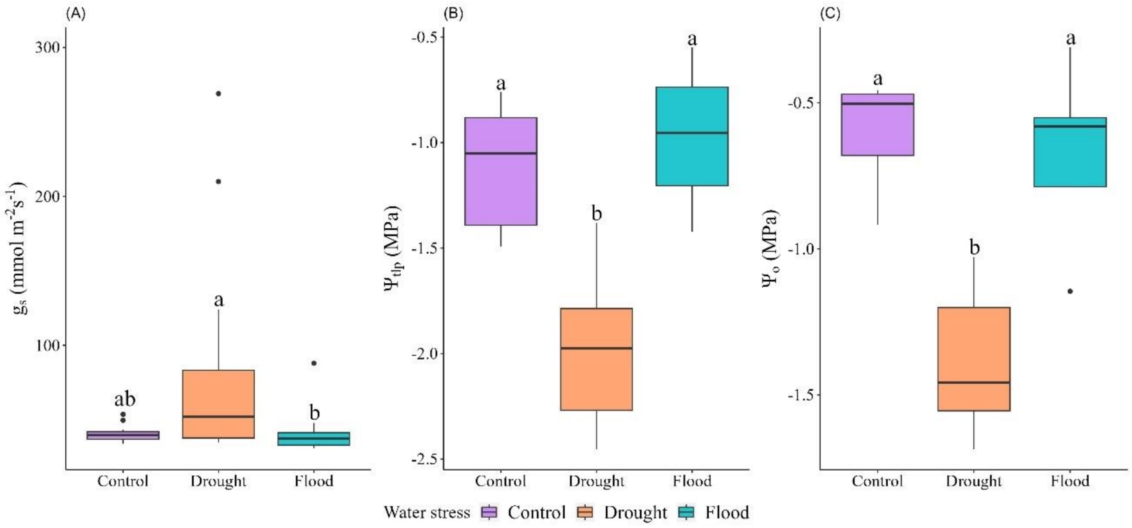

Figure 4: Result of models showing the relationship between leaf gas exchange and water relation traits of individuals of *Pontederia cordata* and the water stress treatment (control, drought and flood). Different upper letters indicate statistical differences between groups tested with post hoc tests. g_s = stomatal conductance; Ψ_{tlp} = turgor loss point; Ψ_o = osmotic potential.

Table 2: Results of the models performed to predict the effects of water stress (control, drought and flood) in the intraspecific functional trait variation of *Pontederia cordata*.

Statistical model	**Response variable**	**Data transformation**	**Predictive Variable**	**Degree of freedom**	**Sum of squares**	**Mean squares**	**F/x2**	**p**
Kruskall-Wallis	Stomatal conductance (g_s)	-	**Water stress**	**2**	**-**	-	**6.3647**	**0.041***
ANOVA	Turgor loss Point (Ψ_{TLP})	-	**Water stress**	**2**	**3.405**	**1.702**	**13.48**	**<0.001***
		-	Residuals	14	1.768	0.126		
ANOVA	Osmotic potential (Ψ_o)	-	**Water stress**	**2**	**2.239**	**1.120**	**17.43**	**<0.001***
		-	Residuals	14	0.899	0.064		
ANOVA	Elasticity (ε)	Log	Water stress	2	3.716	1.858	3.334	0.065
		-	Residuals	14	7.802	0.557		
ANOVA	Leaf area (LA)	-	**Water stress**	**2**	**503954**	**251977**	**7.788**	**0.002***
		-	Residuals	35	1132409	32355		
Kruskall-Wallis	Specific leaf area (SLA)	-	Water stress	2	-	-	2.4683	0.291
ANOVA	Leaf thickness (L_{th})	-	**Water stress**	**2**	**0.015**	**0.007**	**10.33**	**<0.001***
		-	Residuals	35	0.025	0.001		
ANOVA	Leaf dry matter content (LDMC)	-	**Water stress**	**2**	**27.49**	**13.743**	**5.404**	**0.009***
		-	Residuals	35	89.01	2.543		
ANOVA	Flowering onset	-	Water stress	2	2143	1071.3	3.095	0.060
		-	Residuals	37	12806	346.1		
ANOVA	Plant height	-	**Water stress**	**2**	**15110**	**7555**	**28.19**	**<0.001***
		-	Residuals	37	9915	268		
ANOVA	Aboveground biomass	-	**Water stress**	**2**	**5223**	**2611.7**	**32.7**	**<0.001***
		-	Residuals	37	2955	79.9		
ANOVA	Root biomass	Log	**Water stress**	**2**	**22.26**	**11.132**	**30.54**	**<0.001***
		-	Residuals	37	13.49	0.365		
ANOVA	Fruit biomass	Log	**Water stress**	**2**	**4.735**	**2.3676**	**59.67**	**<0.001***
		-	Residuals	37	1.468	0.0397		
ANOVA	Relative growt rate (RGR)	-	**Water stress**	**2**	**12.207**	**6.104**	**34.92**	**<0.001***
		-	Residuals	37	6.467	0.175		
ANOVA	Aboveground mass fraction (AMF)	-	**Water stress**	**2**	**0.082**	**0.041**	**4.627**	**0.016**
		-	Residuals	37	0.326	0.009		
ANOVA	Belowground mass fraction (BMF)	-	**Water stress**	**2**	**0.082**	**0.041**	**4.627**	**0.0161***
		-	Residuals	37	0.326	0.009		
ANOVA	Reproductive mass fraction (RMF)	-	**Water stress**	**2**	**0.003**	**0.001**	**38.83**	**<0.001***
		-	Residuals	37	0.001	0.000		

3.1.6. *Leaf morphological traits*

Both species showed a similar response regarding leaf morphological traits. For them, all morphological traits models were significant, apart from Specific leaf area (Table 1, Table 2), and they showed a higher investment in leaf area in control and flooding conditions, higher leaf thickness in the flood, and more investment in leaves with longer lifespan (high LDMC) in the drought. For individuals of both species, leaf area was similar in flooding and control conditions, differing from drought conditions (Fig 5A; Fig. 6A). For *L. flava*, leaf thickness was also higher in flooding conditions, but differed across all treatments (Fig 5B), and for *P. cordata* they were similar in control and flood conditions, differing from the drought (Fig. 6B). Furthermore, leaf dry matter content was higher in drought conditions in individuals of both species, differing from control and flooding conditions (Fig 5C, Fig 6C).

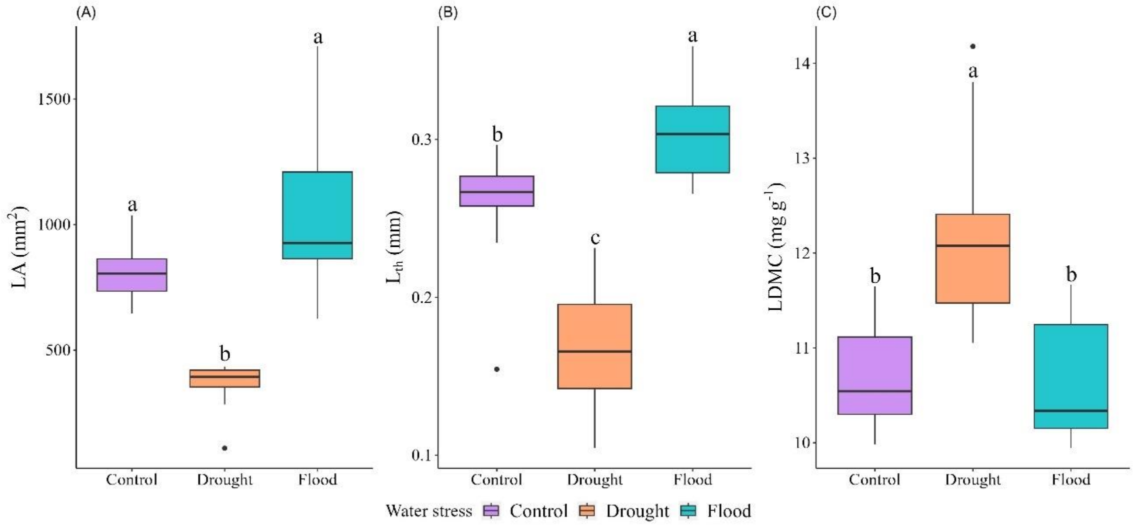

Figure 5: Result of models showing the relationship between leaf morphological traits of individuals of *Limnocharis flava* and the water stress treatment (control, drought and flood). Different upper letters indicate statistical differences between groups tested with post hoc tests. LA = Leaf area; Lth = Leaf thickness; LDMC = leaf dry matter content.

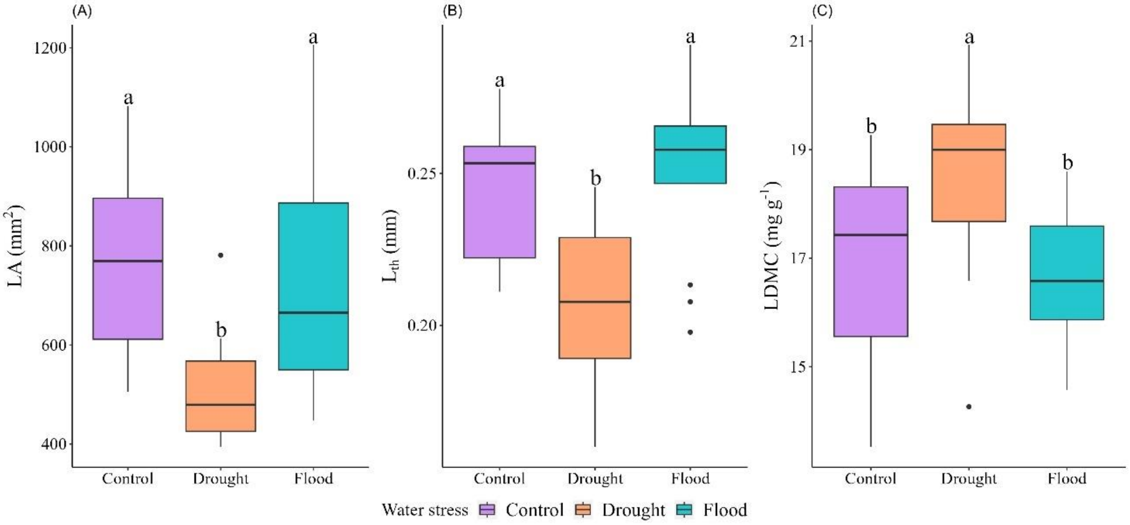

Figure 6: Result of models showing the relationship between leaf morphological traits of individuals of *Pontederia cordata* and the water stress treatment (control, drought and flood). Different upper letters indicate statistical differences between groups tested with post hoc tests. LA = Leaf area; Lth = Leaf thickness; LDMC = leaf dry matter content.

3.1.7. Phenological trait

There was a difference in the flower onset of *L. flava* individuals between drought and flood conditions. It took more days for individuals to flower under flood conditions than in the drought condition (Fig. 7). On the other hand, there was no significant difference among treatments for the flowering onset of individuals o *P. cordata* (Table 2). We also observed that, on average, individuals of *P. cordata* invested much more in vegetative biomass (root+stems+leaves) than *L. flava*, across all treatments (*P. cordata* mean vegetative biomass: control = 54.915g, drought = 19.747g and flood = 57.998g; *L. flava* mean vegetative biomass: control = 15.747g; drought = 8.047 and flood = 25.618), which crossing over with the results regarding the phenological traits, made us believe that *P. cordata* is investing in vegetative reproduction (via clumps) rather than sexual reproduction, and that's why we did not observe a significant difference on the flowering onset of this species.

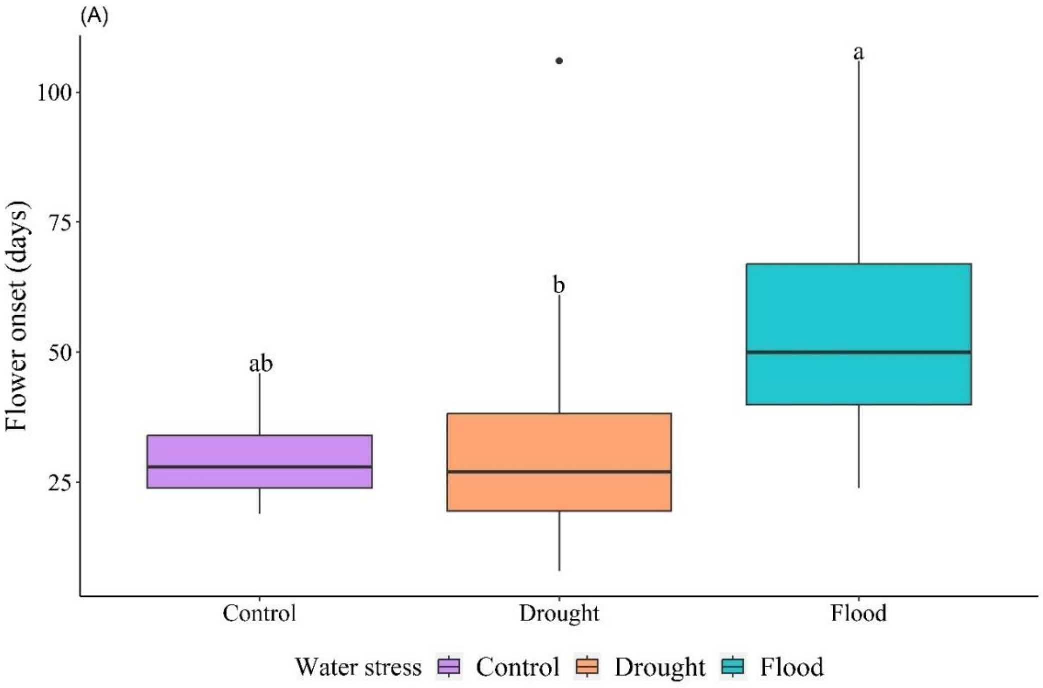

Figure 7: Result of models showing the relationship between the flowering onset of individuals of *Limnocharis flava* and the water stress treatment (control, drought and flood). Different upper letters indicate statistical differences between groups tested with post hoc tests.

3.1.8. Plant height and biomass

The model results showed that both species invested in more biomass and growth in flood conditions. Regarding the intraspecific variation of *L. flava*, all models assessing the effects of water stress treatments on height, biomass, relative growth rate, all yielded significant results (Table 1). Plant height and aboveground biomass varied across all treatments, with higher values in flooding conditions and lower values in drought conditions (Fig 8A and 8B, respectively). Belowground biomass showed similarity between control and flooding conditions, both surpassing levels observed in drought conditions (Fig 8C). Reproductive biomass only differed between control and drought conditions, being lower in the latter (Fig 8D). While relative growth rate was higher in flooding compared to control and drought conditions, with no significant difference observed between control and drought conditions (Fig 8E).

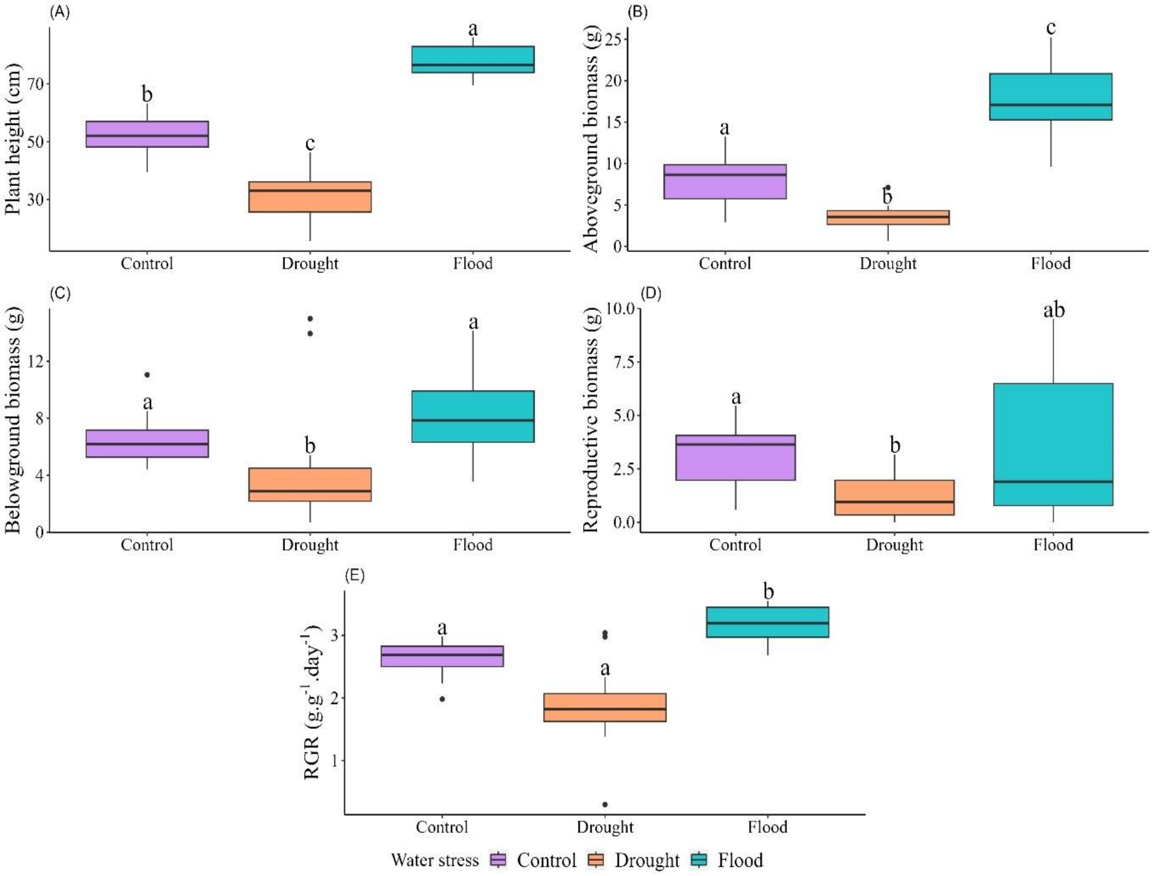

Figure 8: Result of models showing the relationship between biomass and relative growth rate of individuals of *Limnocharis flava* and the water stress treatments (control, drought and flood). Different upper letters indicate statistical differences between groups tested with post hoc tests. RGR = Relative growth rate.

Similarly, the models assessing the effects of water treatment in the height, biomass and relative growth rate of *P. cordata* were all significant (Table 2). Plant height varied across all treatments, being higher in flood conditions, same as *L. flava* (Fig 9A). Aboveground biomass was higher in flood conditions, and differed from drought and conditions, and it was also significantly different between drought and control treatments (Fig. 9B). Belowground biomass, reproductive biomass, and relative growth rate were similar in control and flooding conditions, but both differed from the drought treatment (Fig. 9C, 9D and 9E, respectively).

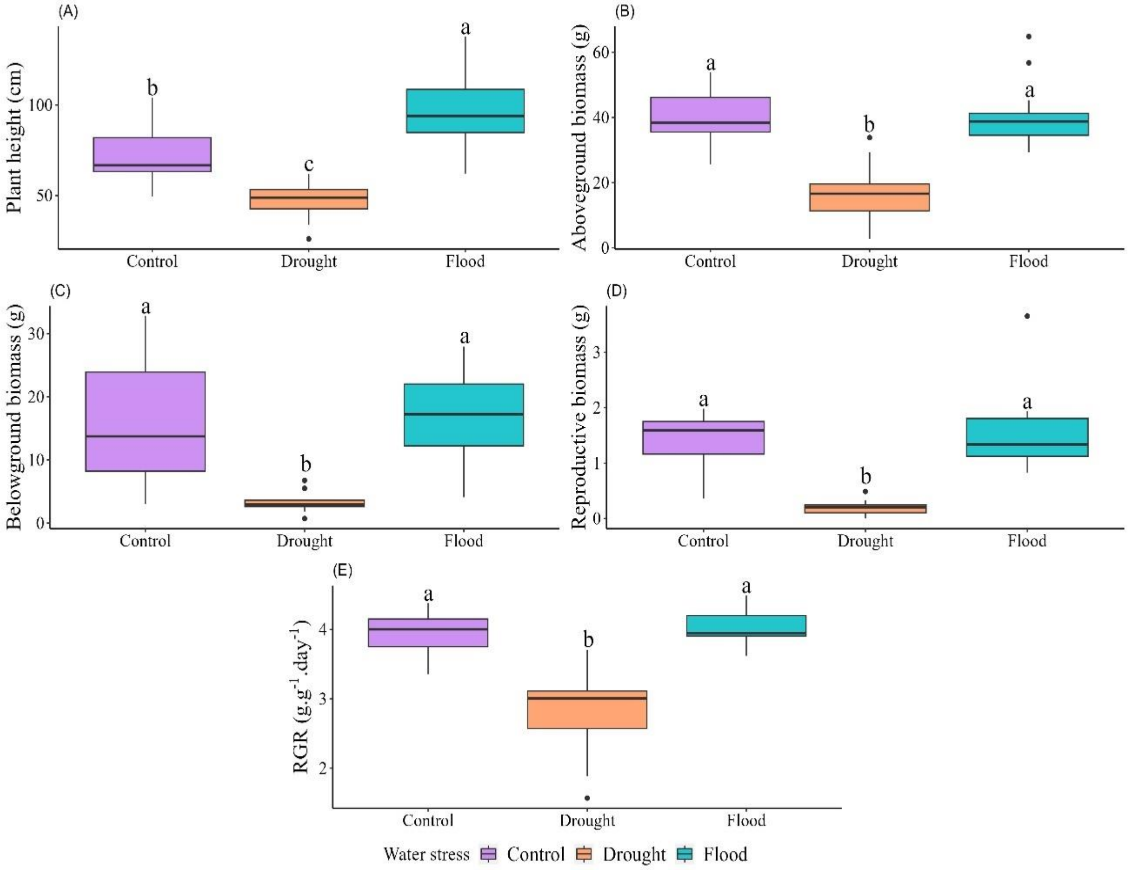

Figure 9: Result of models showing the relationship between biomass and relative growth rate of individuals of *Pontederia cordata* and the water stress treatments (control, drought and flood). Different upper letters indicate statistical differences between groups tested with post hoc tests. RGR = Relative growth rate.

3.1.9. Trade-offs between plant parts: biomass allocation

When regarding biomass allocation, and the possible trade-off between belowground, aboveground, and reproductive parts, the species showed contrasting results. For *L. flava*, all models had significant results, except for reproductive mass fraction (Table 1). Individuals of these species showed more investment in aboveground mass in flooding conditions, which was significantly different from control and drought conditions, while it was similar in drought and control conditions (Fig. 10A). While individuals invested more in the belowground mass in drought conditions, differing from both control and flooding conditions, with no difference between control and flood treatments (Fig. 10B).

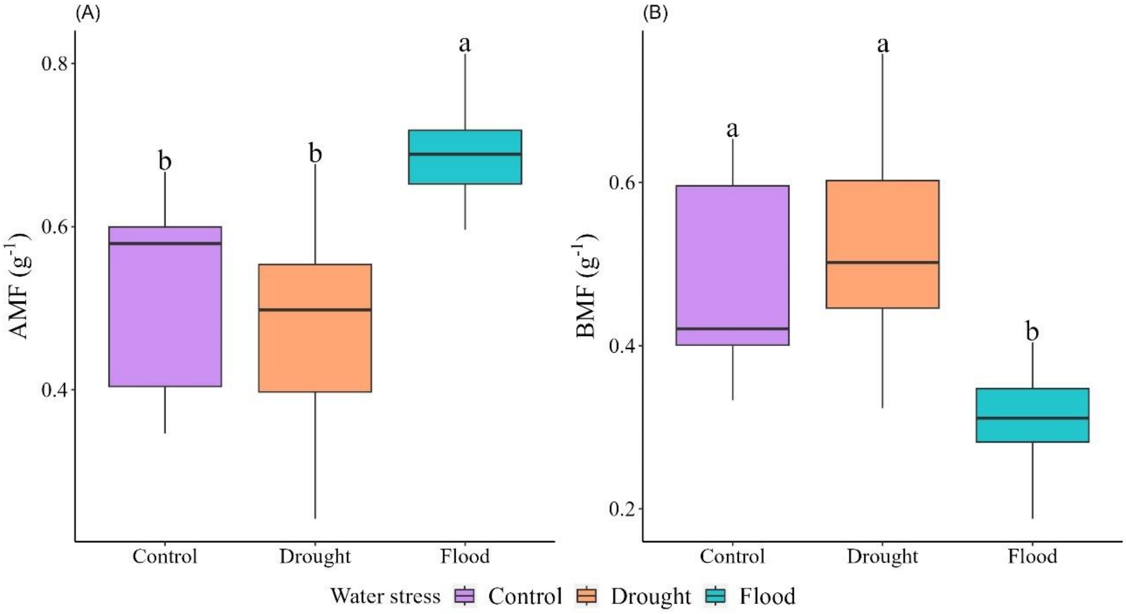

Figure 10: Result of models showing the biomass allocation (represented by the aboveground and belowground mass fractions) of individuals of *Limnocharis flava* along the water stress treatments (control, drought and flood). Different upper letters indicate statistical differences between groups tested with post hoc tests. AMF = Aboveground mass fraction; BMF = Belowground mass fraction.

Finally, for *P. cordata*, all models had significant results (Table 2). Individuals invested more in aboveground mass in the drought than in flood (Fig. 11A), and more in belowground mass in flood conditions than in drought conditions (Fig. 11B). While the reproductive mass fraction was similar in control and flood conditions, but both differed from drought (Fig. 11C).

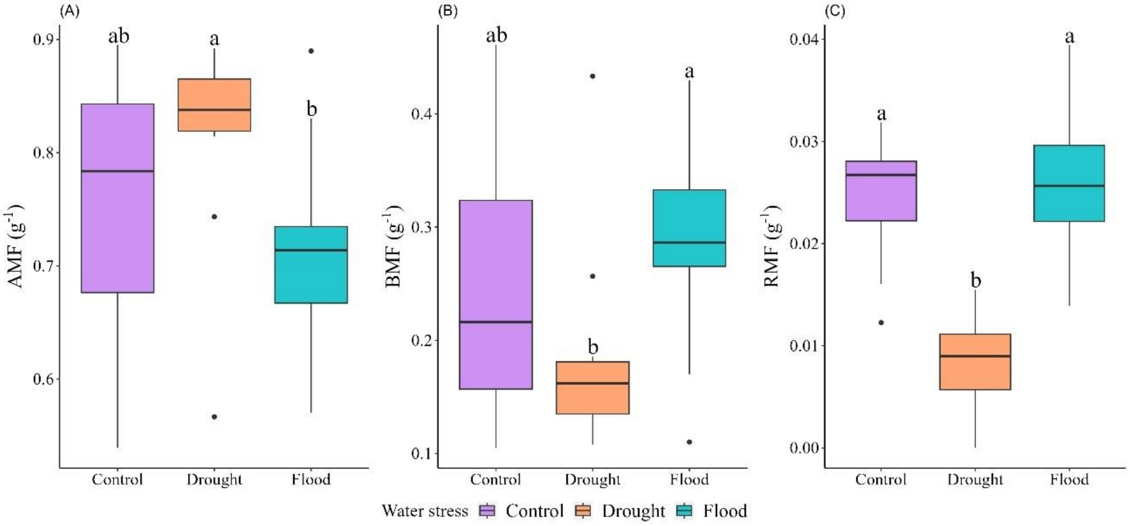

Figure 11: Result of models showing the biomass allocation (represented by the AMF = Aboveground mass fraction; BMF = Belowground mass fraction; RMF = Reproductive mass fraction) of individuals of *Pontederia cordata* along the water stress treatments (control, drought and flood). Different upper letters indicate statistical differences between groups tested with post hoc tests.

3.5.Discussion

Our hypothesis, that individuals would exhibit more conservative and resistance-escape strategy in drought conditions (which would reflect in investment in longer-lived leaves, a better osmotic adjustment and closed stomata to minimalize water loss, early flowering, and more overall investment in root biomass), and a more acquisitive and tolerant strategy under flood conditions (reflecting in more investment in leaf surface area than longevity, late or inexistant flowering, and a trade-off favoring aboveground biomass allocation), was partially corroborated. The response depending on the evaluated plant parts, individuals within a species responded to water stress in similar manners, such as leaf morphological traits but also in different directions, like leaf water relations and biomass allocation.

For instance, on *L. flava* individuals, osmotic adjustment remained unaffected by treatments. Moreover, elasticity decreased in drought and increased in floods. Elasticity is indirectly related to drought tolerance, but it mostly depends on its interaction with other parameters, like osmotic potential and capacitance (Bartlett et al., 2012). Low elasticity implies flexible cell walls, storing more water, while high values indicate stiffer walls with lower water storage. High elasticity may also work in coordination with lower osmotic potential to maintain a constant relative water content at low turgor loss points and prevent severe cell dehydration (Bartlett et al., 2012; Griffin-Nolan et al., 2023). In this study, it seems low elasticity in drought may represent a water storage attempt, and an adaptation to water fluctuations/drawdowns in freshwater ecosystems (Romanello et al., 2008; Touchette et al., 2007, 2014), while high values in floods indicate individuals could sustain cell turgor without bursting under hyperhydration (because the cell walls are stiffer and more resistant). Despite non-significant differences, individuals of *L. flava* showed higher turgor loss point and osmotic potential under drought, suggesting potential sensitivity to low water availability, and that this species is more vulnerable to drought conditions.

Meanwhile, *P. cordata* individuals exhibited contrasting, but still coordinated, responses under drought and flooding. In drought, lower turgor loss point and osmotic potential, coupled with higher stomatal conductance, indicate osmotic adjustment (Da Sois et al., 2024; Hessini et al., 2009; Onyemaobi et al., 2021; Sourour, 2017). This suggests their leaves can reach lower water potentials while maintaining turgor (and cell functioning), showcasing drought tolerance (Chen et al., 2023; Hessini et al., 2009). (Hartmann et al., 2021; Onyemaobi et al., 2021). Unexpectedly, under drought, individuals maintained open stomata, likely facilitating high photosynthetic rates through osmotic adjustments (more negative values of turgor loss point and osmotic potential), meaning this species is anisohydric (Da Sois et al., 2024; Onyemaobi et al., 2021; Sade et al., 2012). However, under intense drought, this strategy might be dangerous and cause leaf injury and plant mortality (Martin-StPaul et al., 2017; Sade et al., 2012). In floods, the strategy to close stomata and increase turgor loss point and osmotic potential emerged to prevent water excess in tissues (Aslam et al., 2023; Pezeshki, 2001; Voesenek et al., 2006). Thus, the ability of *P. cordata* to modulate the osmotic adjustment pathway in response to various forms of water stress is a highly valuable feature, enabling individuals to thrive amidst diverse pressures induced by climate change such as those observed in this study.

In terms of leaf morphological traits, both species employed similar strategies. Under drought, individuals exhibited higher leaf dry-matter content, and lower leaf area and thickness, reflecting a conservative strategy. Conversely, in floods, a more acquisitive strategy emerged with higher leaf area and thickness and lower leaf dry-matter content. This means that, during drought, individuals invested in physical resistance and low photosynthetic rates to reduce water loss, favoring leaf structures over numerous leaves (Anjum et al., 2026; Hessini et al., 2009; Parolin et al., 2010; Wellstein et al., 2017b). However, *P. cordata* did this while maintaining high photosynthetic rates (high stomatal conductance) and osmotic adjustment, indicating individuals of this species used all their (low) photosynthetic surface (leaf area) to their full potential, while *L. flava* had more elastic cell walls and was able to sustain more wilting (low elasticity) under drought. In floods, they invested less carbon to develop leaves with aerenchyma for oxygen transportation and to prevent complete submergence (which is explained by low values of leaf dry matter content and high leaf thickness), an adaptation to flooding stress (Aslam et al., 2023; Mommer et al., 2006), and invested in expansive photosynthetic surfaces to acquire more resources and stimulate growth (Jung et al., 2010; Mommer et al., 2006). These results show both species here present plasticity in in the traits associated with the leaf economic spectrum, and despite their distinct physiological strategies, individuals of each species can navigate between more acquisitive or conservative strategies depending on the level of water stress.

Concerning intraspecific variation in plant phenology, *L. flava* individuals exhibited early flowering onset during drought and delayed flowering under flooding conditions. This aligns with hydrological trait results, indicating the species' sensitivity to drought. The early flowering in drought supports their inclination toward a drought avoidance-escape strategy rather than tolerance (Kooyers, 2015). Many aquatic angiosperms synchronize flowering with the hydrological period, investing more in vegetative growth during the wet season, and preferring onset at the beginning of the dry season (Eckert et al., 2016). This adaptation aligns with the evolutionary history of their terrestrial ancestors, as their reproductive parts are still geared for water-independent reproduction (in fact, water often hinders sexual reproduction in macrophytes) (Eckert et al., 2016). Early flowering serves not only as a drought avoidance strategy for *L. flava* but is a common approach among macrophytes. Under climate change, plants are expected to anticipate/accelerate flowering (Becklin et al., 2016; Bykova et al., 2019; Song et al., 2020), impacting not only future generations by affecting seed germination capacity due to the investment of scarce resources and the speed at which fruits are produced, which are typically more sensitive, but also the mutualism with the pollinators who depend on them (Becklin et al., 2016; Flores et al., 2023).

Unexpectedly, the flowering onset of *P. cordata* individuals remained unaffected by water treatments but was consistent with hydrological trait results for this species. The ability of these individuals to maintain osmotic adjustment under drought, indicative of a drought tolerance strategy (Sade et al., 2012), allows normal metabolic function and, consequently, no impact on the reproductive cycle. Furthermore, to adapt to freshwater ecosystems, aquatic angiosperms invested more on asexual reproduction, relying on a broader array of vegetative propagules compared to terrestrial plants (Eckert et al., 2016; Philbrick & Les, 1996). This adaptation may have evolved across different lineages due to 1) reduced water stress, making these plants more susceptible to breakage and detachment of parts (e.g., fragments, stolons, rhizomes), and 2) the prevalence of monocots among aquatic plants, with rhizomes being a common mode of vegetative propagation in this group (Eckert et al., 2016; Li, 2014). In the case of *P. cordata*, we speculate that individuals invested in vegetative reproduction (via clumps), especially under drought conditions, in parallel with sexual reproduction, possibly explaining the absence of a significant difference in flowering onset for this species.

Moreover, under flood conditions, individuals of both species exhibited elevated values across all biomass portions (aboveground, belowground, and reproductive), increased height, and higher relative growth rates, supporting our hypothesis. When the plants have the right adaptations, abundant water availability in floods promotes cell growth, maintaining turgidity and encouraging investment in the growth of all plant parts (Colmer & Voesenek, 2009; Pan et al., 2022; Voesenek et al., 2006), while the opposite happens under drought conditions (Romanello et al., 2008; Sourour, 2017; Touchette et al., 2007). This also reflects in the trade off of biomass allocation (Chen et al., 2023; Voesenek et al., 2006), the *L. flava* individuals exhibited a trade-off by investing more in belowground biomass over aboveground biomass during drought, and the reverse under flooding, aligning with our hypothesis. Root investment in drought is a strategy to enhance absorption surface and access deeper soil layers in search of additional water resources, while reducing respiration and photosynthetic rates in aboveground parts, conserving water and energy (Hessini et al., 2009; Zhao et al., 2023) – an established strategy in plants. Conversely, during floods, water availability is not a concern, but other environmental disruptions (e.g., soil anoxia, nutrient availability, water turbidity) may pose stress to plants (Chen et al., 2023). In response, individuals allocate investment to increase aboveground biomass (Chen et al., 2023; Romanello et al., 2008). These findings align with the earlier results: under drought, individuals invest in more long-lived leaves with reduced leaf area, exhibit early flowering, and allocate less to aboveground height, thus reducing allocation to the aboveground biomass; under flooding, they invest in increased height and a high photosynthetic surface over leaf structure, apporting more biomass to this portion.

Conversely, individuals of *P. cordata* exhibited the opposite pattern, allocating more biomass to aboveground biomass and less to belowground and reproductive parts under drought conditions. In flooding conditions, they invested more in belowground and reproductive biomass and less in aboveground biomass, contrary to our hypothesis. We posit that, given the species' tolerant strategy, individuals could maintain metabolism even under water-limiting conditions (Sade et al., 2012). This, coupled with higher stomatal conductance and osmotic adjustment under drought (Onyemaobi et al., 2021), suggests stored resources were directed towards aboveground growth. In contrast, under flooding, individuals invested more in belowground biomass (despite increased plant height) due to abundant water. Surprisingly, biomass allocation to reproductive parts was higher under flooding, potentially reflecting increased water resources for larger flowers and heavier fruits and seeds (Mony et al., 2010), while individuals opted to invest more in vegetative growth under drought conditions. Considering the high intraspecific variation and tolerance to various water stress levels, we deem this species a strong candidate to resist/survive amidst the escalating impact of climate change. Moreover, there is a potential risk of it becoming a dominant species in the freshwater ecosystems it occupies, leading to a potential decrease in diversity and causing imbalances within these ecosystems.

3.6.Conclusion

As the climate crisis intensifies, it becomes imperative to comprehend how organisms will respond to the many environmental constrains triggered by it. It is important to understand the ecophysiological response of aquatic plants, especially their strategy to survive under water stress, because they are key organisms to keep the functionality of freshwater ecosystems, notably on tropical regions. Our results reinforce the growing notion that, despite different response between species, intraspecific variation can be the key to understand how species survive under contrasting environments, and to understand how population dynamics and community assembly will adjust to climate change. In addition, we show that the coping mechanisms to stress in different plant parts and levels are coordinated, as these responses triggered by it can be very complex and this depend on each species' responses. Nevertheless, we emphasize on the significance to consider adaptative factors that trigger the responses of plants to climate change. Beyond the array of strategies for coping with water stress, which may differ both between species and within individuals of the same species, it is essential to recognize that certain plants are just sensitive and may face the risk of extinction as the consequences of climate change worsen.

Therefore, we recommend that future research focus in understanding how the various functional groups of macrophytes will respond to different aspects of climate change (such as increasing temperature, CO_2 concentration and water turbidity) and their interactions. It is equally vital to account for the varying responses that individual species within each functional group might exhibit, as even though they may seem morphologically similar, they might have pathways to endure stress that are singular to their evolutionary history. Moreover, acknowledging the intraspecific variability within populations and communities is crucial, as these factors play a pivotal role in safeguarding species diversity on our planet.

3.7.References

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4. Section III

Functionally favored, phylogenetically impaired: functional diversity and phylogenetic structure of macrophytes along a land-use gradient

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Functionally favored, phylogenetically impaired: functional diversity and phylogenetic structure of macrophytes along a land-use gradient

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4.1.Abstract

1. Land-use changes pose a threat to freshwater ecosystems, impacting the composition and the stability of the aquatic macrophytes communities. This study investigated the effect of different land-use regimes on the functional and phylogenetic diversity of these communities in the Amazon.
2. To do that, we sampled 29 Amazonian freshwater sites to include a wide range of pressures linked to changes in land use estimated by landscape structure analysis. Then we measured macrophytes communities' taxonomic richness, functional diversity, and phylogenetic diversity and test their relationship with the land-use variables.
3. This work demonstrates that macrophyte assemblages in altered environments occupied a larger functional space, exhibited greater trait divergence, and displayed greater functional dispersion than those in less altered environments, suggesting communities' composition modification in response to land-use changes. That could be due to the strong light limitation (caused by increased canopy cover) and low pH in more preserved environments which act as strong filters, selecting species that can support those conditions.

4. In contrast, communities in less altered environments were more distant phylogenetically, potentially driven by competition for light in more preserved habitats. In more altered sites, the community consists of species that are more closely related.

5. *Synthesis:* Our work demonstrates that land-use changes impact in a different way functional and phylogenetic diversity of aquatic macrophytes. This point should be considered for the selection priority areas for macrophyte conservation, and the actions planed in these areas. It is essential to consider multiple diversity aspects to avoid overlooking the evolutionary history of these assemblages.

4.2.Introduction

Human activities have directly or indirectly disrupted the functioning of most of Earth's ecosystems (Malhi et al., 2014) leading to increased unpredictability in ecosystem structure and processes (Corlett, 2015). These disturbances are critical for freshwater ecosystems, which despite their small size host a rich biodiversity (Román-Palacios et al., 2022). Anthropogenic-driven changes in freshwater ecosystems, such as alterations in hydrological regimes, pollution, erosion, the introduction of non-native species, and land conversion (Dudgeon, 2019; Flitcroft et al., 2019) have massive effects on freshwater biota. These disturbance result in biodiversity loss and altered ecosystem functioning, and compromised ecosystem services (Dudgeon, 2019).

The Amazon basin, the world’s largest fluvial system, harbors a biota that is diverse, specialized, and unique, but it is threatened by land-use pressure (Castello & Macedo, 2016). In these ecosystems, aquatic macrophytes function as primary producers, in nutrient processing, and provide habitat and food to the animal and microbial communities (Bornette & Puijalon, 2011). Moreover, they are sensitive and efficient bioindicators of freshwater ecological conditions (Alahuhta et al., 2014; Bleich et al., 2015). Species diversity may increase with canopy openness, provided by the loss of riparian vegetation and increased nutrient input (Bleich et al., 2015; Elo et al., 2018; Mackay et al., 2010). On the other hand, the same conditions plus others (e.g. increased turbidity), may decrease species richness (Akasaka et al., 2010).

Most studies assessing the effects of human activities and disturbance on aquatic macrophytes still use classical measures of (alpha, beta and gamma) diversity, such as species richness and abundance (Bleich et al., 2015; Elo et al., 2018; Fernández-Aláez et al., 2020). However, species richness or diversity alone are insufficient for assessing anthropogenic impacts. Other aspects of diversity are useful for investigating biodiversity patterns at different scales (e.g.: trait-based and phylogenetic approaches) (Chmara et al., 2015; Delatorre et al., 2020; García-Girón et al., 2020; Paz et al., 2021). These measures have indicated assemblage changes that have previously been overlooked, such as functional group substitution, and evolutionary dispersion or clustering along environmental, spatial and temporal filters. To better understand community diversity, we must consider different aspects of taxonomic, functional, and phylogenetic diversity (Mammola et al., 2021; Tucker et al., 2017). It is useful to explore the richness, divergence, and evenness of the assemblages (Pavoine & Bonsall, 2011; Tucker et al., 2017). We can gain a more comprehensive understanding of the different facets of assemblage functional and phylogenetic diversity by considering these diverse dimensions.

Functional, and phylogenetic diversity offer a complementary interpretation for community assembly. In the phylogenetic approaches it was generally assumed that ecological differences between organisms may be proportional to the divergence time from a common ancestor (that is, the traits show strong phylogenetic signal – a trait conservatism). On the other hand, functional traits which are related to species ecology may have no correlation with the position of each species in the phylogenetic tree (weak phylogenetic signal) (de Bello et al., 2017). Trait-based and phylogenetic diversity approaches, together, help to reveal the multitude of processes driving community assembly, and aid understanding ecological questions, and enable better decision-making for conservation strategies (Winter et al., 2013). However, there is much to learn about such patterns regarding aquatic macrophytes, because these approaches have been employed mostly in terrestrial ecosystems (Alahuhta et al., 2019).

The objective of this work is to assess the effect of a land-use gradients on the functional and phylogenetic diversity of aquatic macrophyte assemblages. We hypothesized that macrophyte assemblages would exhibit greater functional diversity and distinctiveness as land use intensity increases. This is because higher land-use intensity creates more heterogeneous environments, which provides more suitable habitats for species with diverse ecological niches. If phylogenetic diversity were closely related to functional diversity, it should follow the same pattern of variation. However, adaptations to low light availability, which is the major constraint in undisturbed landscape, have evolved in different phyla, which can lead to significant phylogenetic diversities in undisturbed communities.

4.3.Material and Methods

4.1.1. Study area and sampling design

We sampled 20 streams, 5 lakes and 4 ponds, in the municipality of Paragominas, Pará, Brazil, within the Capim River basin in July 2017 (Figure 1). The natural vegetation is tropical rainforest. We measured environmental and biological data during the dry season. All sites were at least 1 km apart from each other.

4.1.2. Environmental variables

Local environmental variables were measured by using a multiparameter probe (Horiba U-50) and included conductivity (µS/cm), dissolved oxygen (mg/L), temperature (ºC), total dissolved solids (g/L), turbidity (NTU) and pH. Additionally, we measured percent canopy cover on the sites using a convex densitometer (Peck et al., 2006).

We used satellite image processing to determine land use and land cover classes present at the sampling sites. We identified four classes. (a) Primary vegetation consisted of old natural tropical rainforest. (b) Secondary vegetation is defined as a young tropical natural forest. (c) Pastures are used for intensive and/or extensive livestock grazing. (d) Bare soil consisted of mining areas and roads. The percent of each land use/land cover class was quantified by using a 300 m circular buffer around each sampling site.

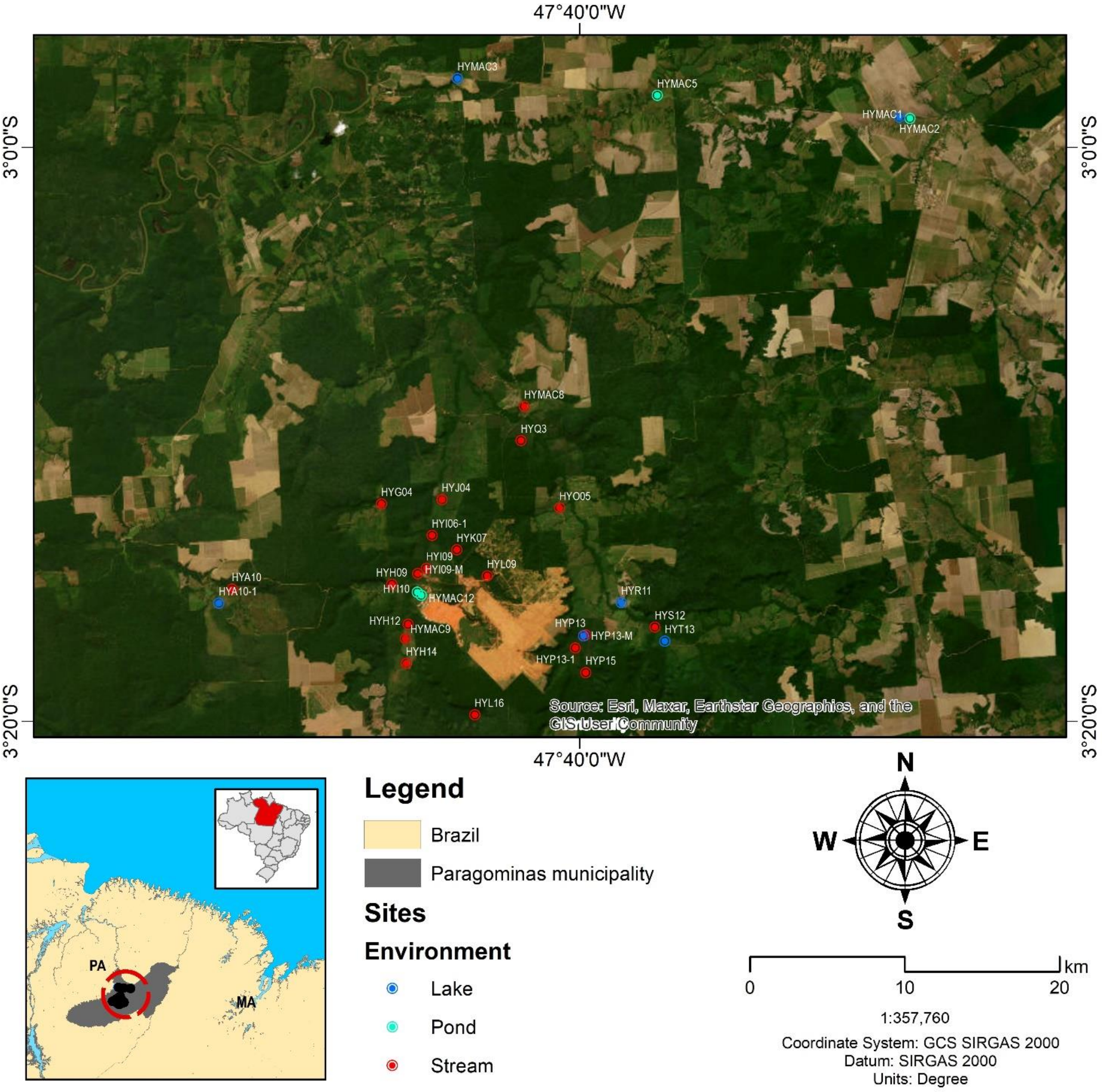

Figure 1: Sampling locations and ecosystem type.

We used the preceding percentages to calculate the amount of disturbance in each site using an adaptation of the Catchment Disturbance Index (CDI; Ligeiro et al., 2013). In this index, lower values represent more pristine areas, and high values indicate a highly disturbed site. Each land-use class was given a weight based on the degree of anthropogenic change caused in the natural environment. This index was divided by 300 to standardize 75% of the maximum value, giving the following formula:

CDI = (4 x % bare soil + 2 x % pasture + 1 x % degraded forest) /300.

4.1.3. Biological Sampling

We sampled macrophyte species and abundance, by placing a 1 m2 quadrat twice in each site. Species richness was measured as the number of species found inside the quadrats. A percentage of cover (%) was assigned to each species inside the quadrats and quantified as macrophyte composition. Voucher specimens were deposited in the João Murça Pires (MG) Herbarium, at the Museu Paraense Emílio Goeldi (Belém, Brazil).

4.1.4. Measurement of functional traits

For functional diversity, we selected 8 traits obtained in the literature and in an online database (TRY – (Kattge et al., 2020)): Life-form, Plant height, Competitive architecture, Foliar arrangement, Dispersal mode, Vegetative propagation, Life-history and Leaf area. These traits were chosen because they indicate macrophyte ecophysiological adaptations to their environment, competitive ability, growth strategies, resource-use efficiency, dispersal ability and survival to disturbance (Delatorre et al., 2020; Pérez-Harguindeguy et al., 2013; Pozzobom et al., 2021). For more information on the significance of all traits, see the supplementary material, table S1.

We used dried herbarium leaves to measure leaf area, acknowledging shrinkage during drying, because they offer extensive spatial and temporal coverage in functional trait ecology (Heberling, 2022; Perez et al., 2020). Previous studies have demonstrated that herbarium material can reliably represent leaf area when fresh samples are unavailable, particularly when shrinkage is minimal (Heberling, 2022; Perez et al., 2020). Approaches like rehydration methods or correlation analysis with fresh material or using solely dried or fresh material have been effective in error mitigation (Perez et al., 2020). Images were sourced from the SpeciesLink database (https://specieslink.net/), and leaf area was calculated using ImageJ software, prioritizing images with scale references and measuring all available leaves per individual when fewer than five were present.

4.3.4. Building the phylogenetic tree

To analyzes phylogenetic diversity and structure, we had to first build a phylogenetic tree of the assemblages found in the study. For that, we used the megatree GBOTB.extended in the V.Phylomaker package (Jin & Qian, 2019) of R. We built the tree by using scenario 3, where a new genus is linked to the midpoint of the family branch (Jin & Qian, 2019). The tree was plotted using FigTree v.1.4.4 software.

4.3.5. Statistical analyses

We performed a Principal Component Analysis (PCA) to assess the environmental variation among the sites. We used all environmental variables in this analysis. All of them were standardized prior the analysis, except for pH. We used the Broken stick criterion as a stopping method for the ordination (Jackson, 1993).

To calculate functional diversity indexes, we used the species-by-trait matrix. Because it has both categorical and quantitative data, it was transformed into a mixed-variable coefficient of distance based on the Gower distance (Pavoine et al., 2009). We applied a square root correction to the dissimilarity matrix (Laliberté et al., 2014).

After that, we calculated four functional diversity indexes. (1) Functional richness (FRic) quantifies the functional space that the species occupy in the assemblage. (2) Functional Divergence (FDiv) measures the changes in the proportion of total species abundances based on the most extreme trait. (3) Functional Evenness (FEve) calculates the regularity of species distribution and abundance of species in the functional space. (4) Functional dispersion (FDis) measures the volume of functional space that two species share in the assemblage, using the differences between traits (Laliberté & Legendre, 2010).

We calculated five phylogenetic diversity and structure indexes, using the phylogenetic tree and the species abundance matrix. (1) Phylogenetic diversity (PD) calculates the sum of the lengths of the branches of the phylogenetic tree of the species in an assemblage. (2) Mean Pairwise Distance (MPD) measures the mean phylogenetic distance of all the species within the assemblage (3) Mean Nearest Taxon Distance (MNTD) quantifies mean phylogenetic distance between each species and its closest relatives (Webb et al., 2002). The standardized effects of (4) MTD and (5) MNTD (sesMPD and sesMNTD) are the size effect of MPD and MNTD values compared to a null model (Webb, 2000; Webb et al., 2002). We used the 'taxa.labels' as the null model for both preceding measures, which randomizes the taxon labels from the matrix across the phylogeny (10,000 randomizations). To test whether the functional traits had a phylogenetic signal, we performed an EM-Mantel test with 999 permutations (a Mantel test followed by a test based on a Brownian motions evolutionary model) (Debastiani & Duarte, 2017).

To test our hypothesis that functional and phylogenetic diversity were affected by land-use, we performed Linear Models, and tested for model normality and homogeneity of variance. When the relationship between predictor and response variables was non-normal, we performed Generalized Linear Models (GLMs) with the Gamma distribution family (Dunn & Smyth, 2018). We also tested the relationship between taxonomic richness and environmental variables by performing a GLM with the negative binomial family, which is best suited for the model, because the response variable was count data with overdispersion (Dunn & Smyth, 2018).

We performed a model selection based on multimodel inference. We built a global model using species richness, functional diversity, and phylogenetic diversity indexes as the response variables, and the environmental variables as predictors, and performed a model selection with the best set of predictive variables. We removed variables with correlation > 0.6 (Figure S1). We selected models by using a modified function of the 'dredge' function from *MunIn* package (Bartoń, 2022), 'dredge_mc' (https://github.com/rojaff/dredge_mc), followed by the 'model.avg' function. After that, we performed another multimodel inference (using the 'dredge' function) this time including only the predictive variables that were previously selected and the type of ecosystem (lotic and lentic). Finally, we selected the model with the lowest Akaike Information Criterion for small sample sizes (AICc) and delta scores, and the highest weight for representing the response-environment relationships. Model diagnostics were performed by analyzing standardized residuals against fitted values plots (Dunn & Smyth, 2018).

All analyses were performed in R (R Core Team, 2020). For functional diversity, the trait dissimilarity matrix was calculated using the 'dist.ktab' function (*ade4* package; (Dray et al., 2007) and index calculation were made using the 'dbFD' function (FD package; (Laliberté et al., 2014). For phylogenetic diversity, we used the picante package (Kembel et al., 2010), in particular the functions 'PD', 'mpd', 'mntd', 'ses.mpd' and 'ses.mntd'. GLMs were performed using the 'glm' function, while Linear Model diagnostics was performed using the 'shapiro.test' (performs the Shapiro-Wilk test of normality) and by analyzing model residual plots, (*stats* package (R Core Team, 2020)). Gamma GLM diagnostics was performed using the *statmod* package (Dunn & Smyth, 1996). Finally, the significant relationships of the models were plotted using the function 'plot_model' (*sjPlot* package; (Lüdecke, 2022).

4.4.Results

4.4.1. Site environmental variation

The first axis of the PCA explained 95.500% of environmental variation and was negatively related with canopy cover (correlation r = -0.759), water conductivity (r = -0.633) and total dissolved solids (r = -0.657), and positively related with the catchment disturbance index (r = 0.736) and water temperature (r = 0.724; Figure 2). This shows that sites presented high environmental variation, mostly related with loss of canopy cover and increased values of the catchment disturbance index.

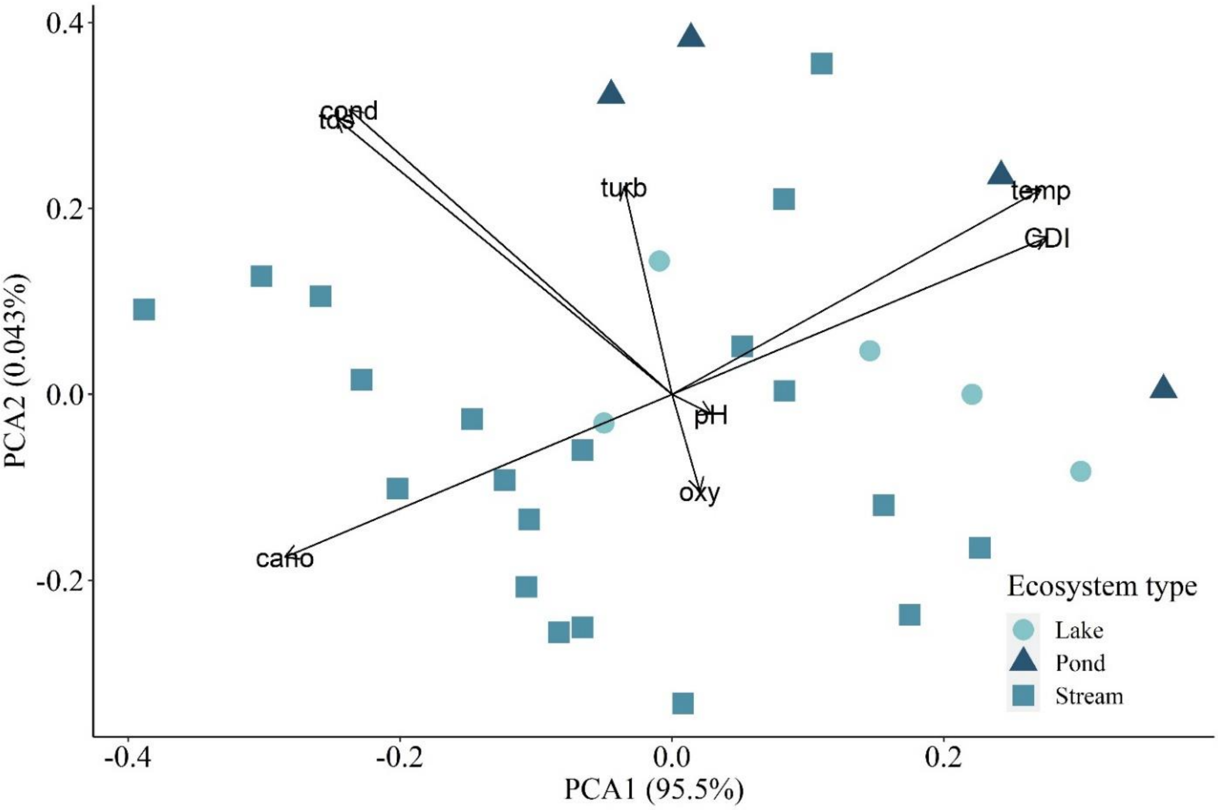

Figure 2: Principal component analysis showing environmental variation among sites. cano = canopy cover, CDI = catchment disturbance index, cond = conductivity, oxy = dissolved oxygen, temp = temperature, turb = turbidity, and pH.

4.4.2. Assemblage structure, functional traits, and phylogenetic signal

Among the 29 sites we collected 43 species, these species contain Ferns (nine species), Basal Angiosperms (two species), Monocots (23 species) and Eudicots (nine species) (Figure 3). We collected information for eight functional traits of these species (Table S2).

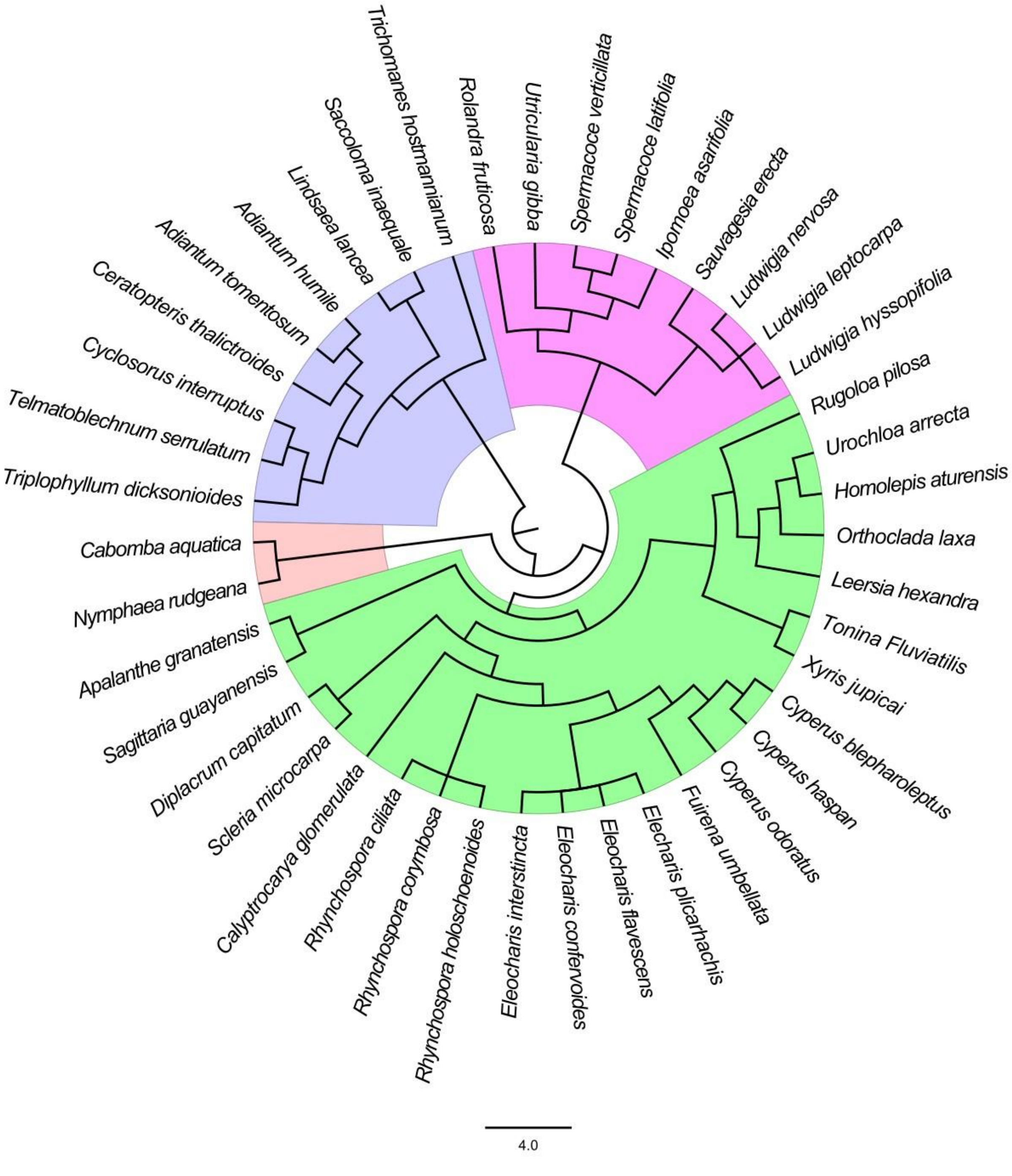

Figure 3: Phylogenetic tree based on the classification of the Angiosperm Phylogeny Group (APG IV; Byng et al., 2016) and the Pteridophyte Phylogeny Group (PPG I; Schuettpelz et al., 2016) containing the study species. Blue = ferns, salmon = basal angiosperms, green = monocots and pink = eudicots.

No phylogenetic signal was found in the functional traits (matrix with all traits: r = 0.310, p = 0.817, life-form: r= -0.065, p = 0.873; plant height: r= -0.046, p = 0.99; competitive architecture: r=0.212, p=0.343; foliar arrangement: r = 0.176, p = 0.32; dispersal mode: r = 0.389, p = 0.11;

vegetative propagation: r = - 0.029, p = 0.939; leaf area: r = 0.733, p = 0.141; life-history: r = -0.036, p = 0.566).

4.4.3. Functional diversity and taxonomic richness

Several models were significant for functional diversity indexes. Functional richness had a negative relationship with canopy cover (Figure 4A; Table S4 and S5). Functional divergence had a positive relationship with the CDI (Figure 4B, Table S5). Functional evenness was negatively associated with pH (Figure 4C), and positively associated with turbidity (Figure 4D, Table S5). Functional dispersion had a negative relationship with canopy cover (Figure 4E, Table S5). Unlike functional diversity, no variable explained data variation in species richness. The model inference selected the null model as the best model to predict species richness variation (Table S3).

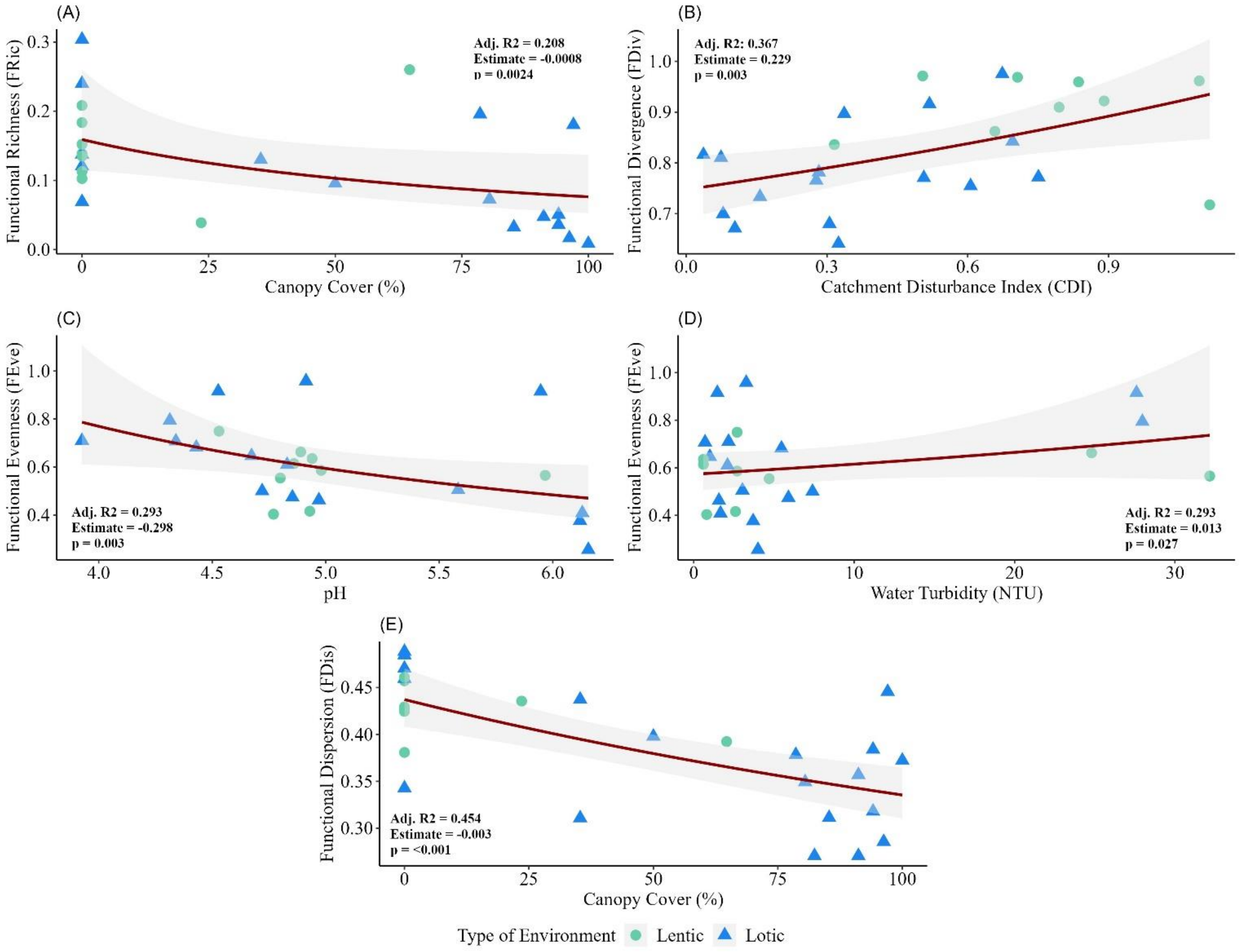

Figure 4: Relationship between Functional Richness, Dispersion and Functional evenness of aquatic macrophytes versus environmental predictor variables. Red lines indicate the direction of the response (upwards – positive relationship, downwards – negative relationship) and the shaded area around them indicate the confidence interval.

4.4.4. Phylogenetic diversity

All the phylogenetic indexes, except for phylogenetic diversity, had a significant relationship with environmental variables (Figure 5; Tables S6 and S7). For mean and standardized effects of Pairwise Distance (MPD and sesMPD) and Mean Nearest Taxon Distance (MNTD) all had a positive relationship with the canopy cover (Table S7; Figure 5A, 5B and 5C, respectively). The standardized effects of MNTD had a positive relationship with the type of ecosystem (Figure 5D).

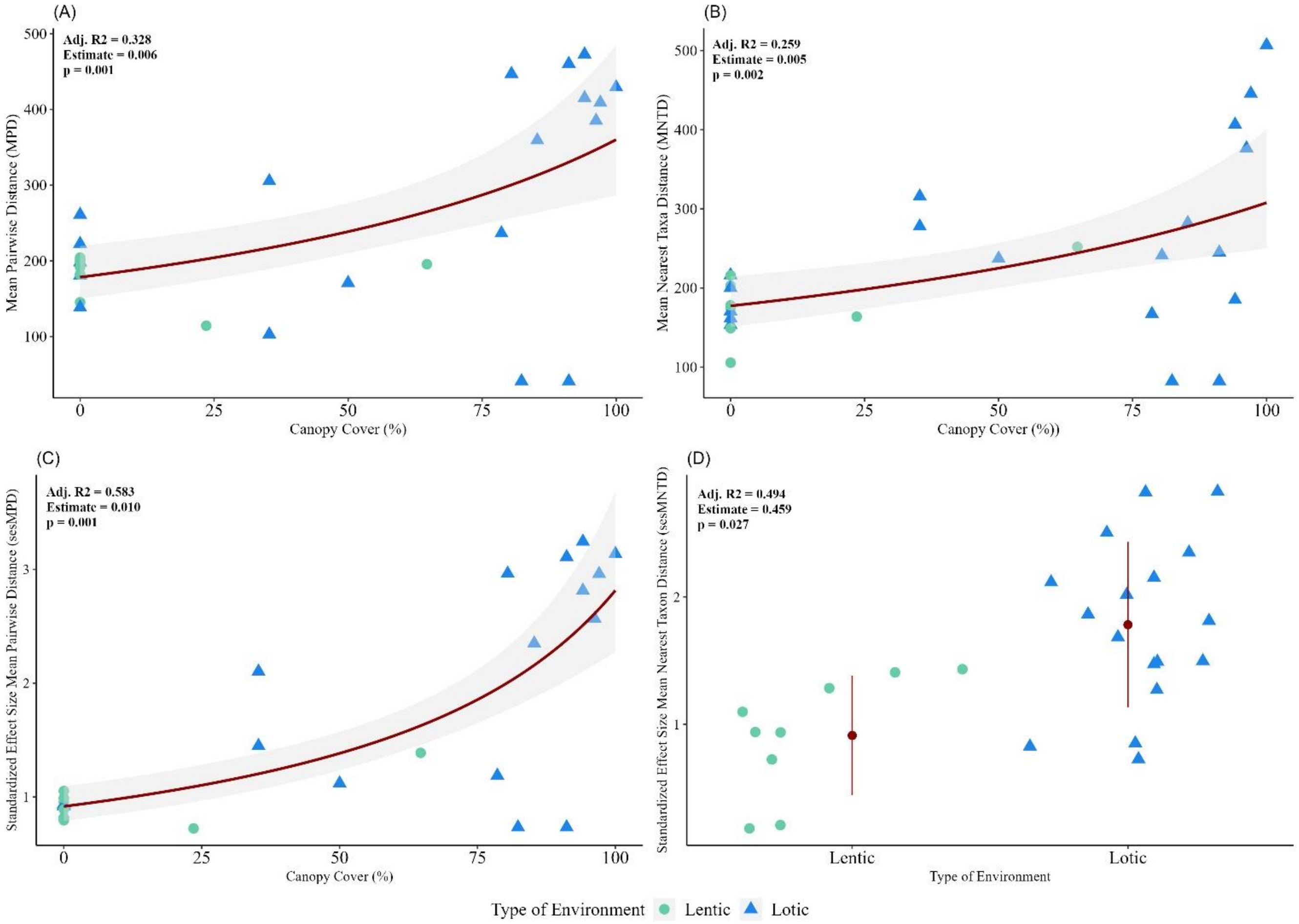

Figure 5: Relationship between the Mean Pairwise Distance, Mean Nearest Taxa Distance, Standardized Effect Size Mean Pairwise Distance, Standardized Effect Size Mean Nearest Taxa Distance of aquatic macrophytes and environmental variables and type of ecosystem. Red lines indicate the direction of the response (upwards – positive relationship, downwards – negative relationship) and the shaded area around them indicate the confidence interval. Blue triangles = lotic environments; Green circles = lentic environments

4.5.Discussion

Functional and phylogenetic structure of the macrophyte communities were impacted in opposite ways by land use degradation. Environmental factors associated with the anthropization of the landscape had mostly positive effects on all aspects of macrophyte functional diversity while various aspects of phylogenetic structure were negatively influenced by theses variables.

This work demonstrates that aquatic plant assemblages of the Amazonian basin are diverse and encompass phylogenetically distant groups, including algae, ferns, and flowering plants, resulting in different morphologies, physiologies, and phenologies (Chambers et al., 2008). García-Girón et al.

(2020) also found no support for phylogenetic signals in global lake macrophyte phylogenies, suggesting independent evolution of ecological niches and high phenotypic plasticity. As we did not observe significant relationships between ecological niches (represented by functional traits) or the evolutionary history of co-occurring plants, we interpreted functional and phylogenetic diversity indexes separately. This suggests decoupling between ecological niches and the evolutionary history of aquatic plant species of the Easten Amazonian streams.

As for the diversity measures, species richness had no relationship with the studied environmental variables, this differs from results previously found in the same area (Fares et al., 2020). Clearly taxonomic metrics alone are insufficient to quantify assemblage diversity patterns (Döbert et al., 2017; Pavoine & Bonsall, 2011). Conversely, all functional diversity indexes showed significant responses to environmental variables. Functional richness decreased with increasing canopy cover, indicating reduced functional space in more altered sites. Similarly, functional divergence increased with disturbance, suggesting greater functional differences among species in response to human-induced impacts. Despite evidence pointing to decreased functional diversity with human-induced disturbance (Mouillot et al., 2013; Paz et al., 2021; Ribeiro et al., 2019), we found the opposite pattern for macrophyte assemblages. This is likely because decreased light caused by the dense riparian vegetation in more preserved sites is a limiting factor for macrophyte occurrence. We this is an example of the intermediate disturbance hypothesis (high diversity is expected at moderate disturbance levels (Biswas & Mallik, 2010).

In our study functional evenness was positively related to turbidity, and negatively with pH. (Paz et al., 2021). These result contrast with the results of Chmara et al. (2015) which reported that macrophyte functional diversity decrease along an acidity gradient. In their study, undisturbed waters are basic, they associated the results to the different types of carbon acquisition of macrophyte groups, which they considered a key factor shaping assemblages' diversity. Similarly, our results indicate trait filtering and functional adaptation of the native macrophytes to a natural environmental condition (low pH), as most Amazon basin waters are naturally acidic (Ríos-Villamizar et al., 2013).

The more commonly found pattern indicates decreased functional dispersion with human disturbance (Ribeiro et al., 2019; Schellenberger Costa et al., 2017). However, in our case Functional dispersion increased with decreased canopy cover. Döbert et al. (2017) also found increased functional diversity with increased human impact, likely linked to changes in light availability that act as a strong filter on species composition. Our results suggest that assemblages in more altered habitats exhibited greater trait diversity among species (e.g., different competitive strategies and dispersal abilities), possibly because a post-disturbance increase of the number of available niches.

We presume the observed functional diversity patterns result from the characteristics of natural sites in the region, these sites exhibit characteristics such as shading, elevated banks, and high flow, thereby instigating a process of trait filtering. In response to these conditions, the stable macrophyte assemblages are less taxonomically rich and composed of shade-tolerant coexisting species (Junk & Howard-Williams, 1984). Those species have similar traits (amphibious, perennials, intermediate height, high leaf area), showing a trait convergence in more preserved sites (Wiens et al., 2010). This is likely driven by a combination of environmental and biotic filtering (Cadotte & Tucker, 2017; Germain et al., 2018). That is, species with similar niche-related traits compete symmetrically for light, facilitating coexistence among competitors (Gerhold et al., 2015). In contrast, more-disturbed sites displayed variation in light availability facilitating habitat colonization by species with different traits adapted to either avoid or sustain other limiting factors, resulting in increased functional diversity with increased deforestation. Additionally, increased sunlight facilitates the spread of a non-native invasive species in the area (Urochloa arrecta), that decreases all kinds of diversity (Evangelista et al., 2017; Thomaz & Michelan, 2011).

For phylogenetic diversity, Mean Pairwise Distance, its standardized effect size and Mean Nearest Taxa Distance were all positively affected by increased canopy cover, whereas the standardized effect size of Nearest Taxa Distance was affected by ecosystem type. Contrary to our predictions, the assemblages were more phylogenetically dispersed both at the whole assemblage level and among phylogenetically closer relatives in less altered sites.

This positive relation between increased canopy cover and phylogenetic diversity could be due to the fact that assemblages in less altered sites are mainly composed of ferns versus monocots (Cyperaceae and Poaceae), which are phylogenetically distant, thus harboring more phylogenetically dispersed assemblages. In more altered sites, even though species diversity is high, the species are phylogenetically close, e.g. Cyperaceae, Nympheales, Alismatales, Lentibulariaceae, and Onagraceae, meaning that there is a phylogenetic clustering in more altered sites (Cavender-Bares et al., 2009; Webb et al., 2002). The congruent directional responses observed in Pairwise and Nearest Taxa Distance suggest that assemblages within more disturbed sites exhibit greater dispersion as a collective entity and among phylogenetically close relatives (Webb et al., 2002). This inference is further supported by the standardized effect size of Mean Pairwise Distance outcome, which reinforces the trend of phylogenetic dispersion in macrophyte assemblages within less altered sites and clustering in ecosystems subjected to land-use disturbance (Toyama et al., 2020). Moreover, the presence of *U. arrecta* exerts a discernible influence on the phylogenetic diversity of assemblages, inducing shifts in patterns from clustering to dispersion (Kusuma et al., 2018) or vice versa.

Furthermore, lotic assemblages appeared to be more phylogenetically dispersed than lentic ones. This relates to our other findings because of the different characteristics of lotic and lentic ecosystems. Lotic ecosystems have increased discharge and less light, whereas lentic ecosystems have still waters, increased nutrients, and more light, similar to conditions found in less- and more-altered sites, respectively (Castello & Macedo, 2016). Lentic ecosystems could be driving the assemblages to be more phylogenetically clustered than lotic assemblages. These patterns could be explained by the fact that the same combination of filters selected species with the same niche requirements and survival ability in shaded environments, but these species were phylogenetically distant due to the overdispersion of the shade tolerance capacity among various phylogenic groups, resulting in coexistence among distant relatives (Cadotte & Tucker, 2017; Gerhold et al., 2015).

4.6.Conclusion

Species inhabiting less altered sites were more functionally similar, but phylogenetically dispersed, reinforcing the lack of a phylogenetic signal in the assemblages. Assemblages in more altered sites, despite being functionally richer, are less stable, more generalist, and are experiencing non-native invasions, which could induce a decrease of the diversity after competitive exclusion of native species. The relationships between assemblage patterns and environmental and biotic filters are complex to understand, especially in such a diverse landscape as Amazonia. A loss of functional or phylogenetic information may hinder the ability of macrophyte assemblages to survive intensive land-use change over time, thus changing assemblage and community patterns. Temporal monitoring, investigation of beta diversity patterns, and ecosystem assessments (including macrophyte effect traits that can influence ecosystems) are needed for a better understanding of the macrophyte assembly processes in highly altered landscapes, now and in the future.

4.7.Supplementary material

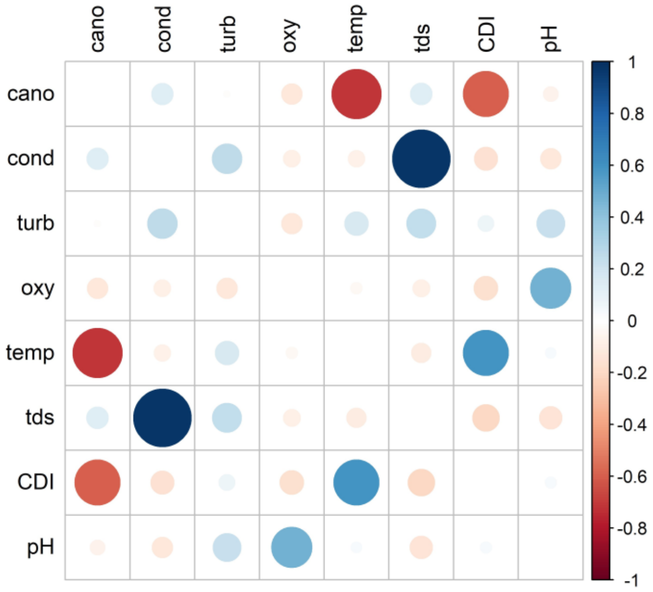

Fig. S1: Spearman correlation performed with environmental variables. The color of the circles represents whether the relationship is positive (all the blue shades) or negative (all the red shades). The size and tones of the circles represent how strong the relationship is. The bigger the circle and the more saturated the color, the stronger is the relationship. cano = canopy cover, cond = water conductivity, oxy = dissolved oxygen, temp = water temperature, tds = total dissolved solids, turb = water turbidity, CDI = catchment disturbance index.

Functional Trait measurement

For the functional trait analysis, we used a combined effort of literature search in specialized guides, e.g. (Amaral et al., 2008; Cook et al., 1974; Lorenzi, 2008; Pott & Pott, 2000; Zuquim et al., 2008), and in taxonomic keys for the species) collection in a plant traits online database (TRY – (Kattge et al., 2020), and a measurement of quantitative data in order to gather all the information available for the following traits: (1) Life-form, (2) Plant height, (3) Competitive architecture, (4) Foliar arrangement, (5) Dispersal mode, (6) Vegetative propagation, (7) Life-history, and (8) Leaf area (Table S2 and Table S3).

Regarding plant height, the most recorded class was class 2 (species between 51-99 cm), with 12 species (27.91%), followed by class 1 (spp ≤50cm) and class 3 (spp between 100-199 cm) both with 10 species each (23.26%). Class 0 (submerged species) had 6 species (13.95%), and Class 4 (spp ≥200 cm) had 5 species (both with 6 species (11.66%). Species with the erect competitive architecture were the most recorded ones, with 26 species (60.77%), followed by prostrated plants (11 species; 25.58%), and submersed species, with 6 records (13.95%). Caulescent plants were the most recorded on the study, with 19 species (44.19%), followed by cespitose, with 17 species (39.53%), and floating (7 species, 16.28%).

For the dispersal mode, the most recorded one was autochory, with 23 species (58.489%), followed by anemochory, with 13 species (30.23%), zoochory, with 5 records (11.63%) and hydrochory, with 2 records (4.65%). The most-common vegetative propagation was propagation by rhizome, with 25 species (58.14%), followed by stolon, with 7 species (16.28%), propagation by fragmentation (5 species, 11.63%), cuttings (4 species, 9.30%), and sprouts (2 spp; 4.65%). For life history, the majority of species recorded were perennials (33 species; 76.74% of all species), followed by species that presented both cycles (annual-perennials, with 6 species; 13.95%), and annuals (4 species; 9.30%). Finally, the mean dry leaf area was 35.314 cm², the species with the highest mean dry leaf area was *Adiantum tomentosum*, with 304.046 cm², while the species with the lowest leaf area was *Utricularia gibba*, with 0 cm².

Table S1: Significance of the plant functional traits used on the study.

Trait name	Qualitative/Quantitative	Attribute	Functional relevance/Ecological significance
Life-form	Qualitative	Emergent, floating-leaved, rooted-submerged, free-submerged, floating, epiphyte	Ecophysiological adaptations of the species to the environment, fine-scale view of establishment and survival strategy (Catian et al., 2018; Delatorre et al., 2020; Pérez-Harguindeguy et al., 2013; Pozzobom et al., 2021).
Plant Height	Qualitative	Class 0 (submerged species); Class 1 (spp ≤50cm); Class 2 (spp between 51-99 cm); Class 3 (spp between 100-199 cm), Class 4 (spp ≥200 cm)	Competitive ability for light capture (Delatorre et al., 2020).
Competitive architecture	Qualitative	Submerged, erect, prostrate	Competitive ability and survival strategy to disturbance (Delatorre et al., 2020).
Folia arrengement	Qualitative	Floating, cespitose, caulescent	Competitive ability (Delatorre et al., 2020).
Dispersal mode	Qualitative	Hydrochory, autochory, anemochory, zoochory	Dispersal ability: consequences for the distances the species can cover, the routes they can travel and the places it can end up in (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013).
Vegetative propagation	Qualitative	Stolon, rhizome, fragmentation, cuttings, sprout	dispersal ability, suvival to disturbance (Delatorre et al., 2020; Pozzobom et al., 2021).
Life-history	Qualitative	Annual, Perennial	Population persistence, growth time strategy, linked w/ fast-slow economic spectrum, resource use efficiency (Catian et al., 2018; Fu et al., 2015; Pérez-Harguindeguy et al., 2013).
Leaf surface area	Quantitative	-	Photosynthetic surface (light capturing surface area), plant growth, evapotranspiration surface (Pérez-Harguindeguy et al., 2013).

Table S2: List of macrophyte species found in the study along with their traits (Life-form, plant height, competitive architecture, foliar arrangement, photosynthetic pathway, dispersal mode, vegetative propagation, life history and dry leaf area).

Order	Family	Genus	Species	Life-form	Plant Height	Competitive architecture	Foliar arrengement	Dispersal mode	Vegetative propagation	Life history	Leaf area
Polypodiales	Pteridaceae E.D.M.Kirchn.	*Adiantum* L.	*Adiantum humile*	Amphibious	Class 1	Prostrate	Caulescent	Anemochory	Rhizome	Perennial	86.514
Polypodiales	Pteridaceae E.D.M.Kirchn.	*Adiantum* L.	*Adiantum tomentosum*	Amphibious	Class 3	Prostrate	Caulescent	Anemochory	Rhizome	Perennial	304.046
Alismatales	Hydrocharitaceae Juss.	*Apalanthe* Planch.	*Apalanthe granatensis*	Rooted-submersed	Class 0	Submerged	Floating	Autochory	Fragmentation	Annual-perennial	0.104
Nymphaeales	Cabombaceae Rich. ex A.Rich.	*Cabomba* Aubl.	*Cabomba aquatica*	Rooted-submersed	Class 0	Submerged	Floating	Zoochory	Fragmentation	Perennial	3.647
Poales	Cyperaceae Juss.	*Calyptrocarya* Nees	*Calyptrocarya glomerulata*	Emergent	Class 1	Erect	Cespitose	Autochory	Rhizome	Perennial	9.365
Polypodiales	Pteridaceae E.D.M.Kirchn.	*Ceratopteris* Brongn.	*Ceratopteris thalictroides*	Emergent	Class 2	Prostrate	Caulescent	Anemochory	Rhizome	Annual	27.642
Polypodiales	Thelypteridaceae Pic.Serm.	*Cyclosorus* Link	*Cyclosorus interruptus*	Amphibious	Class 3	Prostrate	Caulescent	Anemochory	Rhizome	Perennial	161.064
Poales	Cyperaceae Juss.	*Cyperus* L.	*Cyperus blepharoleptos*	Emergent	Class 2	Erect	Caulescent	Autochory	Rhizome	Perennial	2.419
Poales	Cyperaceae Juss.	*Cyperus* L.	*Cyperus haspan*	Amphibious	Class 2	Erect	Cespitose	Autochory	Rhizome	Perennial	2.072
Poales	Cyperaceae Juss.	*Cyperus* L.	*Cyperus odoratus*	Amphibious	Class 2	Erect	Cespitose	Autochory	Rhizome	Annual-perennial	6.231
Poales	Cyperaceae Juss.	*Diplacrum* R.Br.	*Diplacrum capitatum*	Amphibious	Class 1	Erect	Cespitose	Autochory	Rhizome	Perennial	3.780
Poales	Cyperaceae Juss.	*Eleocharis* R.Br.	*Eleocharis confervoides*	Rooted-submersed	Class 0	Submerged	Floating	Autochory	Rhizome	Perennial	33.250

Cont.

Order	**Family**	**Genus**	**Species**	**Life-form**	**Plant Height**	**Competitive architecture**	**Foliar arrengement**	**Dispersal mode**	**Vegetative propagation**	**Life history**	**Leaf area**
Poales	Cyperaceae Juss.	*Eleocharis* R.Br.	*Eleocharis flavescens*	Emergent	Class 1	Erect	Cespitose	Autochory	Stolon	Perennial	0.965
Poales	Cyperaceae Juss.	*Eleocharis* R.Br.	*Eleocharis interstincta*	Emergent	Class 3	Erect	Cespitose	Anemochory	Rhizome	Perennial	13.447
Poales	Cyperaceae Juss.	*Eleocharis* R.Br.	*Eleocharis plicarhachis*	Emergent	Class 2	Erect	Cespitose	Autochory	Stolon	Perennial	4.871
Poales	Cyperaceae Juss.	*Fuirena* Rottb.	*Fuirena umbellata*	Emergent	Class 2	Erect	Cespitose	Autochory	Rhizome	Perennial	10.814
Poales	Poaceae Barnhart	*Homolepis* Chase	*Homolepis aturensis*	Amphibious	Class 1	postrate	Cespitose	Zoochory	Stolon	Perennial	8.016
Solanales	Convolvulaceae Juss.	*Ipomoea* L.	*Ipomoea asarifolia*	Amphibious	Class 3	Prostrate	Caulescent	Autochory	Stolon	Perennial	22.604
Poales	Poaceae Barnhart	*Leersia* Sol. ex Sw.	*Leersia hexandra*	Amphibious	Class 1	Prostrate	Cespitose	Autochory	Rhizome	Perennial	7.579
Polypodiales	Lindsaeaceae Pic.Serm.	*Lindsaea* Pic.Serm.	*Lindsaea lancea*	Amphibious	Class 2	Prostrate	Caulescent	Anemochory	Rhizome	Perennial	108.633
Myrtales	Onagraceae Juss.	*Ludwigia* L.	*Ludwigia hyssopifolia*	Amphibious	Class 4	Erect	Caulescent	Autochory	Fragmentation	Annual	2.394
Myrtales	Onagraceae Juss.	*Ludwigia* L.	*Ludwigia leptocarpa*	Amphibious	Class 4	Erect	Caulescent	Autochory	Fragmentation	Annual-perennial	4.888
Myrtales	Onagraceae Juss.	*Ludwigia* L.	*Ludwigia nervosa*	Emergent	Class 4	Erect	Caulescent	Anemochory	Sprout	Perennial	3.287
Nymphaeales	Nymphaeaceae Salisb.	*Nymphaea* L.	*Nymphaea rudgeana*	Floating-leaved	Class 0	Submerged	Floating	Zoochory	Rhizome	Perennial	100.528
Poales	Poaceae Barnhart	*Orthoclada* P.Beauv.	*Orthoclada laxa*	Amphibious	Class 4	Erect	Cespitose	Zoochory	Stolon	Perennial	13.745
Poales	Cyperaceae Juss.	*Rhynchospora* Vahl	*Rhynchospora ciliata*	Emergent	Class 1	Erect	Cespitose	Anemochory	Rhizome	Perennial	2.647 Cont.
Poales	Cyperaceae Juss.	*Rhynchospora* Vahl	*Rhynchospora corymbosa*	Emergent	Class 3	Erect	Cespitose	Autochory	Rhizome	Perennial	6.722
Poales	Cyperaceae Juss.	*Rhynchospora* Vahl	*Rhynchospora holoschoenoides*	Amphibious	Class 2	Erect	Cespitose	Anemochory	Rhizome	Perennial	2.834 Cont.

Order	**Family**	**Genus**	**Species**	**Life-form**	**Plant Height**	**Competitive architecture**	**Foliar arrengement**	**Dispersal mode**	**Vegetative propagation**	**Life history**	**Leaf area**
Asterales	Asteraceae Bercht. & J.Presl	*Rolandra* Rottb.	*Rolandra fruticosa*	Emergent	Class 2	Erect	Caulescent	Zoochory	Cuttings	Perennial	12.698
Poales	Poaceae Barnhart	*Rugoloa* Zuloaga	*Rugoloa pilosa*	Emergent	Class 2	Erect	Cespitose	Autochory	Stolon	Perennial	4.937
Polypodiales	Saccolomataceae Doweld	*Saccoloma* Kaulf.	*Saccoloma inaequale*	Amphibious	Class 3	Erect	Caulescent	Anemochory	Rhizome	Perennial	115.727
Alismatales	Alismataceae Vent.	*Sagittaria L.*	*Sagittaria guayanensis*	Floating-leaved	Class 1	Submerged	Floating	Autochory	Rhizome	Perennial	8.809
Malpighiales	Ochnaceae DC.	*Sauvagesia* L.	*Sauvagesia erecta*	Amphibious	Class 1	Erect	Caulescent	Autochory	Cuttings	Annual-perennial	0.962
Poales	Cyperaceae Juss.	Scleria P.J.Bergius	*Scleria microcarpa*	Amphibious	Class 3	Erect	Caulescent	Autochory	Rhizome	Perennial	8.624
Gentianales	Rubiaceae Juss.	*Spermacoce* L.	*Spermacoce latifolia*	Amphibious	Class 2	Erect	Caulescent	Autochory	Cuttings	Annual	5.026
Gentianales	Rubiaceae Juss.	*Spermacoce* L.	*Spermacoce verticillata*	Amphibious	Class 3	Erect	Caulescent	Autochory	Cuttings	Perennial	1.206
Polypodiales	Blechnaceae Newman	*Telmatoblechnum*	*Telmatoblechnum serrulatum*	Amphibious	Class 3	Erect	Caulescent	Anemochory	Rhizome	Perennial	139.297
Poales	Eriocaulaceae Martinov	*Tonina* Aubl.	*Tonina fluviatilis*	Rooted-submersed	Class 0	Prostrate	Floating	Hydrochory	Sprout	Annual	0.302
Polypodiales	Hymenophyllaceae Gaudich.	*Trichomanes* L.	*Trichomanes hostmannianum*	Amphibious	Class 1	Erect	Caulescent	Anemochory	Rhizome	Perennial	32.527
Polypodiales	Tectariaceae Panigrahi	*Triplophyllum* Holttum	*Triplophyllum dicksonioides*	Amphibious	Class 2	Prostrate	Caulescent	Anemochory	Rhizome	Perennial	222.115
Poales	Poaceae Barnhart	*Urochloa* P.Beauv.	*Urochloa arrecta*	Emergent	Class 3	Prostrate	Cespitose	Autochory	Stolon	Perennial	6.298
Lamiales	Lentibulariaceae Rich.	*Utricularia* L.	*Utricularia gibba*	Free-submersed	Class 0	Submerged	Floating	Hydrochory	Fragmentation	Annual-perennial	0.000
Poales	Xyridaceae C.Agardh	*Xyris* Gronov. ex L.	*Xyris jupicai*	Amphibious	Class 4	Erect	Cespitose	Autochory	Rhizome	Annual-perennial	5.875

Table S3: Result of model selection including all the possible models for species richness. The best models are selected based on the lowest Delta values and the highest weight values. Selected models that were discussed in the paper are in bold.

Predictive Variable	Models	df	logLik	AICc	Delta AICc	Weight
Species Richness	**Null**	**2**	**-72.75**	**149.96**	**0**	**0.32**
	Canopy cover	3	-71.76	150.47	0.51	0.25
	Water conductivity	3	-71.9	150.77	0.8	0.22
	Catchment Disturbance Index (CDI)	3	-71.93	150.82	0.86	0.21

Table S4: Result of model selection including all the possible models for functional diversity measures (FRic, FDiv, FEve, FDis). The best models are selected based on the lowest Delta values and the highest weight values. Selected models that were discussed in the paper are in bold.

Family (link function)	Predictive Variable	Models	df	logLik	AICc	Delta AICc	Weight
Gamma(link = log)	FRic	**Canopy cover**	**3**	**32.670**	**-58.190**	**0.000**	**0.150**
		Canopy cover + Water Conductivity	4	33.870	-57.730	0.460	0.120
		Canopy cover + Dissolved Oxygen	4	33.780	-57.550	0.640	0.110
		Canopy cover + Water Conductivity + Dissolved Oxygen	5	35.210	-57.260	0.930	0.100
		Catchment Disturbance Index (CDI)	3	32.160	-57.180	1.010	0.090
		Canopy cover + Catchment Disturbance Index (CDI)	4	33.570	-57.140	1.050	0.090
		Canopy cover + Water conductivity + Water turbidity	5	34.990	-56.820	1.370	0.080
		Catchment Disturbance Index (CDI) + Water conductivity	4	33.400	-56.800	1.390	0.080
		Canopy cover + Catchment Disturbance Index (CDI) + Water conductivity	5	34.830	-56.500	1.690	0.070
		Canopy cover + Water conductivity + pH	5	34.820	-56.480	1.710	0.060
		Canopy cover + Water turbidity	4	33.150	-56.290	1.900	0.060
Gamma(link = log)	FDiv	**Cathment Disturbance Index (CDI) + Dissolved Oxygen**	**4**	**27.170**	**-44.350**	**0.000**	**0.290**
		Type of Ecosystem	3	25.700	-44.270	0.080	0.280
		Cathment Disturbance Index (CDI) + Dissolved Oxygen + pH	5	28.180	-43.210	1.140	0.160
		Cathment Disturbance Index (CDI) + Type of Ecosystem	4	26.470	-42.940	1.410	0.140 Cont.

Family (link function)	Predictive Variable	Models	df	logLik	AICc	Delta AICc	Weight

		Cathment Disturbance Index (CDI)	3	24.980	-42.820	1.530	0.130
Gamma(link = log)	FEve	**pH + Water turbidity**	**4**	**14.280**	**-18.570**	**0.000**	**0.590**
		Canopy cover + pH + Water turbidity	5	15.490	-17.820	0.750	0.410
Gamma(link = log)	FDis	**Canopy Cover**	**3**	**44.350**	**-81.690**	**0.000**	**0.430**
		Canopy cover + Catchment Disturbance Index (CDI)	4	45.650	-81.560	0.130	0.400
		Canopy cover + Dissolved Oxygen	4	44.770	-79.810	1.890	0.170

Table S5: Result of the Generalized Linear Models performed with Macrophyte functional diversity measures (Functional Richness – FRic, Functional Divergence – FDiv, Functional Evenness – FEve, and Functional Dispersion – FDis) and local environmental variables (canopy cover, water conductivity, turbidity, and dissolved oxygen), CDI and type of ecosystem, in Paragominas, Pará, Brazil. Variables in bold and with an asterisk (*) indicate statistically significant relations.

Family (link function)	**Predictive Variable**	**Explanatory Variable**	**Estimate**	**Std. Error**	**t**	**p**	**Adjusted R^2**	**AIC**
Gamma (link = log)	FRic	Intercept	-1.827	0.194	-9.404	0.000	0.208	-59.333
		Canopy cover	**-0.008**	**0.003**	**-2.412**	**0.024***		
Gamma (link = log)	FDiv	Intercept	-0.528	0.118	-4.480	0.000	0.367	-46.348
		CDI	**0.229**	**0.069**	**3.315**	**0.003***		
		Dissolved Oxygen	0.031	0.015	2.073	0.050		
Gamma (link = log)	FEve	Intercept	0.881	0.438	2.011	0.057	0.293	-20.570
		pH	**-0.298**	**0.088**	**-3.375**	**0.003***		
		Water Turbidity	**0.013**	**0.006**	**2.376**	**0.027***		
Gamma (link = log)	FDis	Intercept	-0.829	0.036	-23.252	0.000	0.454	-82.691
		Canopy Cover	**-0.003**	**0.001**	**-4.452**	**<0.001***		

Table S6: Result of model selection including all the possible models for phylogenetic diversity measures (PD, MPD, MNTD, sesMPD, sesMNTD). The best models are selected based on the lowest Delta values and the highest weight values. Selected models that were discussed in the paper are in bold.

Predictive Variable	Models	df	logLik	AICc	Delta AICc	Weight
PD	**Dissolved Oxygen**	**3**	**-206.28**	**419.55**	**0**	**0.23**
	Type of ecosystem + Dissolved Oxygen	4	-205.11	419.95	0.4	0.19
	Type of ecosystem	3	-206.58	420.16	0.61	0.17
	Catchment Disturbance Index + Dissolved Oxygen	4	-205.23	420.19	0.64	0.17
	Null	2	-208.08	420.64	1.09	0.13
	Canopy Cover + Dissolved Oxygen	4	-205.66	421.06	1.51	0.11
MPD	**Canopy Cover**	**3**	**-170.26**	**347.52**	**0**	**0.71**
	Canopy Cover + pH	4	-169.8	349.34	1.81	0.29
MNTD	**Canopy Cover**	**3**	**-161.759**	**330.5**	**0**	**1**
sesMPD	**Canopy cover**	**3**	**-17.69**	**42.41**	**0**	**0.42**
	Canopy cover + Water conductivity	4	-16.82	43.46	1.05	0.25
	Canopy Cover + pH	4	-17.12	44.07	1.65	0.18
	Canopy Cover + Type of Ecosystem	4	-17.29	44.4	1.98	0.15
sesMNTD	**Canopy Cover + Type of Ecosystem**	**4**	**-23.26**	**56.43**	**0**	**0.4**
	Type of Ecosystem	3	-24.7	56.49	0.06	0.39
	Canopy Cover	3	-25.27	57.64	1.21	0.22

Table S7 : Result of the Generalized Linear Models performed with Macrophyte phylogenetic diversity measures (Phylogenetic diversity – PD, Mean Pairwise Distance – MPD, Mean Nearest Taxa Distance – MNTD and the standardized effects of the Mean Pairwise Distance and the Mean Nearest Taxa Distance – sesMPD and sesMNTD, respectively) and and local environmental variables (canopy cover, water conductivity, turbidity, and dissolved oxygen), CDI and type of ecosystem, in Paragominas, Pará, Brazil. Variables in bold and with an asterisk (*) indicate statistically significant relations.

Family (link function)	Predictive Variable	Explanatory Variable	Estimate	Std. Error	t	P	R^2/Adjusted R^2	AIC
Gaussian (link=identity)	PD	Intercept	1570.710	390.900	4.018	0.000	0.121	418.550
		Dissolved Oxygen	-100.750	53.240	-1.892	0.070		
Gamma(link = log)	MPD	Intercept	5.194	0.101	51.410	0.000	0.328	346.520
		Canopy Cover	**0.006**	**0.002**	**3.776**	**0.001***		Cont.

Family (link function)	**Predictive Variable**	**Explanatory Variable**	**Estimate**	**Std. Error**	**t**	**P**	**R^2/Adjusted R^2**	**AIC**
Gamma(link = log)	MNTD	Intercept	5.176	0.092	56.310	0.000	0.259	329.520
		Canopy Cover	**0.005**	**0.002**	**3.430**	**0.002***		
Gamma(link = log)	sesMPD	Intercept	-0.105	0.086	-1.216	0.235	0.583	41.370
		Canopy Cover	**0.010**	**0.001**	**7.410**	**<0.001 ***		
Gamma(link = log)	sesMNTD	Intercept	-0.138	0.136	-1.020	0.319	0.494	54.524
		Canopy Cover	0.004	0.002	1.929	0.066		
		Type of Ecosystem (lotic)	**0.459**	**0.194**	**2.363**	**0.027***		

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5. CONSIDERAÇÕES FINAIS

O uso da abordagem funcional tem ajudado os ecólogos a compreender melhor como os indivíduos, populações, comunidades e ecossistemas funcionam, os mecanismos que os fazem habitar locais com alta variedade ambiental ao qual estão constantemente submetidos (sejam fatores ambientais, espaciais e/ou temporais), e sua adaptação a gradientes naturais, mas também como responderão às perturbações humanas em seus ecossistemas. No entanto, ainda há uma infinidade de padrões em relação à resposta de alguns organismos em diversas condições e áreas inteiras do mundo que ainda não foram completamente compreendidas. Esta tese teve como objetivo reduzir a lacuna de conhecimento em relação às respostas mediadas por atributos funcionais de plantas aquáticas à variação ambiental, tanto natural quanto humana, em uma região que é muito importante para a biodiversidade global, processos ecológicos e regulação do clima: a Amazônia.

Ao longo de cada seção, mostramos que alguns indivíduos de macrófitas podem alterar as suas características foliares em resposta a um gradiente ambiental natural, indo de mais conservativas a mais aquisitivas em resposta à disponibilidade de nutrientes e oxigênio e à mudança na composição do solo, o que se reflete em suas estratégias ecológicas, e que essas estratégias podem variar em outros biomas. Além disso, algumas espécies da mesma forma de vida podem apresentar uma alta variação intraespecífica quando submetidas a estresse hídrico (seca e inundação), e algumas delas podem ser tolerantes a esse estresse hídrico, enquanto outras podem ser mais sensíveis, o que também se reflete em suas estratégias ecológicas. Isso é interessante para entender como as espécies operarão em resposta às consequências das mudanças climáticas (por exemplo, eventos extremos de seca e inundação), que há uma coordenação nos atributos funcionais dos indivíduos para sustentar tal estresse, mas essas respostas estão intrinsecamente relacionadas com a história evolutiva de cada espécie e suas adaptações. Na última seção, nos mergulhamos nas respostas das comunidades de macrófitas ao longo de um gradiente de uso da terra na Amazônia, examinando-as através das lentes da diversidade funcional e filogenética. Nossas descobertas revelaram um desacoplamento da diversidade funcional e filogenética nesta região, com cada aspecto da comunidade respondendo em direções diferentes. Sob pressão de uso da terra, a diversidade funcional aumentou, enquanto a diversidade filogenética sofreu impactos negativos devido à mudança no uso da terra. Comunidades sob essas condições alteradas mostraram maior similaridade filogenética. Essas percepções devem ser levadas em consideração ao designar áreas prioritárias para conservação, pois as consequências da mudança no uso da terra podem resultar em uma perda significativa de informações filogenéticas dentro das comunidades de macrófitas.

Nossas descobertas reforçam a importância de considerar aspectos funcionais e filogenéticos nos esforços de conservação, para garantir a preservação da biodiversidade e dos processos ecológicos em ecossistemas de água doce em meio às mudanças ambientais em curso. Assim, mostramos que ainda há muitos padrões a serem explorados em relação às respostas das macrófitas a muitos estressores, particularmente em regiões tão ecologicamente significativas quanto a Amazônia. Os próximos passos a serem dados devem incluir a exploração da variação intraespecífica nas respostas dos atributos radiculares a essas condições, dinâmicas populacionais e também respostas comunitárias a longo prazo em condições naturais e em resposta às consequências das atividades humanas e mudanças climáticas.

5. CONCLUDING REMARKS

The use of the trait-based approach has helped ecologists understand more how individuals, populations, communities, and ecosystems function, the mechanisms that make them inhabit an environment under the array of variation they are constantly submitted to (be it environmental, spatial and/or temporal factors), and their adaptation to natural gradients but also how they will respond to human-driven disturbance in their ecosystems. However, there is still a plethora of patterns regarding the response of some organisms in various conditions and whole areas of the world that have still not been completely understood/investigated. This thesis aimed to reduce the knowledge gap regarding the trait-mediated responses of aquatic plants to both natural and huma-driven environmental variation, in a region that is very important to global biodiversity, ecological processes and climate regulation: the Amazon.

Throughout each section, we showed that some macrophyte individuals can suffer change their leaf trait values in response to a natural environmental gradient, going from more conservative or acquisitive in response to nutrient and oxygen availability and change in soil composition, which reflects in their ecological strategies, and that these strategies may vary across other biomes. In addition, some species from the same life-form may present a high intraspecific variation while submitted to water stress (drought and flooding), and some of them can be tolerant to such water stress, and others may be more sensitive, which also reflects in their ecological strategies. This is interesting to understand how species will operate in response to the consequences of climate change (e.g. extreme events of drought and flooding), that there is a trait coordination among individuals' traits in order to sustain such stress, but these responses are intrinsically related with each species evolutionary history and their adaptations. In the final section, we delved into community-level responses of macrophytes along a land-use gradient in the Amazon, examining them through the lenses of functional and phylogenetic diversity. Our findings revealed a decoupling of functional and phylogenetic diversity in this region, with each aspect of the community responding in different directions. Under land-use pressure, functional diversity exhibited an increase, while phylogenetic diversity suffered negative impacts due to land-use change. Communities under these altered conditions showcased higher phylogenetic similarity. These insights should be taken into account when designating priority areas for conservation, as the consequences of land-use change may result in a significant loss of phylogenetic information within macrophyte communities.

Our findings reinforce the importance of considering both functional and phylogenetic aspects in conservation efforts, to ensure the preservation of biodiversity and ecological processes on freshwater ecosystems amidst ongoing environmental changes. Thus, we showed that there are still many patterns to explore regarding macrophytes' responses to many constrains, particularly in regions as ecologically significant as the Amazon. Further steps to be taken should include the exploration of intraspecific variation in root trait responses to these conditions, population dynamics and as well as long-term community responses under natural conditions and in response to the consequences of human activities and climate change.

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7. COMPLEMENTARY ACTIVITIES

Published Papers:

- Carvalho, Raquel L. ; Resende, Angelica F. ; Barlow, Jos ; França, Filipe M. ; Moura, Mario R. ; Maciel, Rafaella ; Alves-Martins, Fernanda ; Shutt, Jack ; Nunes, Cassio A. ; Elias, Fernando ; Silveira, Juliana M. ; Stegmann, Lis ; Baccaro, Fabricio B. ; Juen, Leandro ; Schietti, Juliana ; Aragão, Luiz ; Berenguer, Erika ; Castello, Leandro ; Costa, Flavia R.C. ; **Fares, Ana Luisa B.** . Pervasive gaps in Amazonian ecological research. Current Biology, v. 33, p. 1-9, 2023.

- Bomfim, Francieli F. ; **Fares, Ana Luísa B.** ; Melo, Dilene G. L. ; Vieira, Elaine ; Michelan, Thaisa S. . Land use increases macrophytes beta diversity in Amazon streams by favoring amphibious life forms species. Community Ecology, v. 24, p. 1-12, 2023.

- Carmo, Rayssa S.; **Fares, Ana Luísa B.**; Pereira, Gizelia F. M.; Michelan, Thaisa S.. Does the structure of riparian vegetation affect the diversity of macrophytes in eastern amazonian streams?. Biologia, v. 78, p. 79-89, 2022.

- Fares, Ana Luisa B.; Carvalho, W. V. ; Michelan, Thaísa Sala ; Teodoro, G. S. . Which Variables Influence The Herbivory Amount On Montrichardia Spp. (Araceae) In Aquatic Ecosystems?. Revista De Biologia Tropical, V. 70, P. 263-276, 2022.

- Mendes, T. P. ; Montag, L. F. A. ; Michelan, T. S. ; Ferreira, G. C. ; Torres, N. R. ; Guterres, A. P. ; Fares, **Ana Luísa B.** ; Calvão, Lenize Batista ; Maia, C. ; Paiva, C. K. S. ; Barbosa, T. A. P. ; Santos, L. L. ; Begot, T. O. ; Luiza-Andrade, A. ; Cardoso, M. N. ; Juen, L. . Recovery Processes In Areas Affected By Mining: A Scienciometric Review. Ciência E Natura, V. 43, P. 1-22, 2021

- Fares, Ana Luísa B.; Sousa, R. L. M. ; Gurgel, Ely Simone C. ; Gil, A. S. B. ; Silva, C. A. S. ; Michelan, Thaísa Sala . Diversity Of Macrophytes In The Amazon Deforestation Arc: Information On Their Distribution, Life-Forms And Habits. Rodriguesia, V. 72, P. 1-9, 2021.

- Fares, Ana Luísa B.; Calvão, Lenize Batista ; Torres, Naiara Raiol ; Gurgel, Ely Simone C. ; Michelan, Thaísa Sala . Environmental Factors Affect Macrophyte Diversity On Amazonian Aquatic Ecosystems Inserted In An Anthropogenic Landscape. Ecological Indicators, V. 113, P. 106231, 2020.

- Fares, Ana Luísa B.; Nonato, Flávia Alessandra Da Silva ; Michelan, Thaísa Sala . New Records Of The Invasive Macrophyte, Urochloa Arrecta Extend Its Range To Eastern Brazilian Amazon Altered Freshwater Ecosystems. Acta Amazonica, V. 50, P. 133-137, 2020.

- Calvão, Lenize Batista ; Paiva, C. K. S. ; Brito, J. S. ; **Fares, Ana Luísa B.** ; Maia, C. ; Michelan, T. S. ; Montag, L. F. A. ; Juen, L. . Influence Of Biotic And Abiotic Factors On Adult Odonata (Insecta) In Amazon Streams. Animal Biology, V. 12, P. 1-18, 2020.

Articles under review:

- **Carmo R. S.;** Franco N. L.; **Fares A. L. B.;** Nonato F. A. S.; Bomfim F. F.; Michelan T.S. Desempenho de *Pistia stratiotes* sobre interferência de uma espécie de macrófita nativa e uma invasora. Scientia Plena

- Cabral, Guilherme Sampaio; Sousa, Raimundo Luiz Morais; Costa, Antônio Augusto Souza; Louback-Franco, Nayara; **Fares, Ana Luísa B.;** Carmo, Rayssa Silva; Jesus, Alexandro Monteiro; Lins, Alba Lucia Ferreira de Almeida; Pott, Vali Joana; Michelan, Thaisa Sala. "First record of *Landoltia punctata* (G.Mey) Les & D.J.Crawford (Araceae, Lemnoidae) from Legal Amazonia". Acta Amazonica.

- Costa, Antônio Augusto Souza; Raimundo Luiz Morais; Costa; Louback-Franco, Nayara; **Fares, Ana Luísa B.**; Carmo, Rayssa Silva; Nonato, Flavia; Michelan, Thaisa Sala. "Aquatic macrophytes in Amazon: review, knowledge, gaps and perspectives". Discover Environment.

Book chapter, in process:

Chapter: Macrófitas. 2024. In: Monitorando a Biota Aquática no contexto do Licenciamento Ambiental.

Doctoral internship abroad:

In 2021, I did a 6-month internship (CAPES-PDSE) at the Centre d'Écologie Fonctionnelle et Évolutive in Montpellier, France, under the supervision of Dr. Florian Fort. It was a very enriching experience for both my personal and professional development, and I bring back great friendships and academic collaborations from this period.

Coordination of Projects:

On 2021, I had a project approved by the Brazilian Biodiversity Fund entitled " *Functional response of aquatic macrophytes to water stress conditions caused by climate change: evidences of* *intraspecific variability and trade-offs* (Resposta funcional de macrófitas aquáticas às condições de estresse hídrico causado por mudanças climáticas: evidências sobre variabilidade intraespecífica e trade-offs)", from which I produced two chapters of this thesis, the first and second one.

Participation in research projects:

I was involved in three research projects developed by the Ecology of Primary Producers laboratory (ECOPRO-UFPA): i) Effects of an invader on the macroecological patterns of native communities: a comparison between its area of origin (Brazil) and an invaded area (China); ii) Effects of soil use on diversity and ecophysiology on the riparian vegetation, aquatic macrophytes and plankton in streams and lagoons in mining areas of Paragominas SA, Pará, Brazil and iii) Aquatic biota monitoring and assessment upstream and downstream of bauxite pipeline Norsk Hydro Paragominas - Barcarena (Pará, Brazil) an instream and riverscape approach.

I am also a collaborator in research networks in the Amazon: the SYNERGYZE project, as well as the recently-approved SinBiAm - Syntheses of Amazonian Biodiversity – INCT.

Supervision:

During my doctorate, I supervised four undergraduate students on scientific initiation internships linked to the Laboratory of Primary Producers (ECOPRO/UFPA). Two have already finished their internship and two are still working on the laboratory:

- Flávia Lourrany Gonçalves Ribeiro. Macrófitas aquáticas como bioindicadoras: testando a congruência de resolução taxonômica e grupo morfológico na região de Barcarena, Pará, Brasil. 2022. Iniciação Científica. (Graduando em Ciências Biológicas) - Universidade Federal do Pará, Fundação Amazônia Paraense de Amparo à Pesquisa. (concluded).

- Rayssa Silva do Carmo. A estruturação da vegetação ripária afeta a diversidade de macrófitas aquáticas em riachos amazônicos?. 2020. Iniciação Científica. (Graduando em Ciências Biológicas) - Universidade Federal do Pará, Conselho Nacional de Desenvolvimento Científico e Tecnológico. (concluded).

- Jéssica Thamires Corrêa Damasceno. Efeito multifatorial do ambiente nas características funcionais de Nymphaea rudgeana G.Mey. (Nymphaeaceae) na Amazônia Oriental, Brasil. Início: 2023. Iniciação científica (Graduando em Ciências Biológicas) - Universidade Federal do Pará, Fundação Amazônia Paraense de Amparo à Pesquisa. (under supervision)

Ana Luiza Soares Gomes. Mudanças climáticas e seu efeito nos atributos funcionais de macrófitas aquáticas. Início: 2023. Iniciação científica (Graduando em Ciências Biológicas) - Universidade Federal do Pará, Fundação Amazônia Paraense de Amparo à Pesquisa. (under supervision).

Attendance at scientific events:

Participation in the Brazilian Botanical Congress at the Federal University of Pará - 2023. Scientific event where I attended and presented 3 conference Papers as first author, and was also co-author of five other works:

- Fares, Ana Luisa; Cunha, E. J. R.; Michela, T. S.. Fatores ambientais direcionam a diversidade β-funcional de características reprodutivas de macrófitas aquáticas. In: 73ª Congresso Nacional de Botânica, 2023. Livro de Resumos, 2023. v. 1.

- Fares, Ana Luisa; Fort, Florian ; Michelan, T. S. . Mixed responses of functional diversity and phylogenetic structure of macrophytes along a land-use gradient in Eastern Amazon. In: 73ª Congresso Nacional de Botânica, 2023, Belém. Livro de Resumos, 2023. v. 1.

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- Ribeiro, F. L. G.; Fares, Ana Luisa ; Michelan, T. S. . Macrófitas aquáticas como bioindicadoras: testando a congruência de resolução taxonômica e grupo morfológico na região de Barcarena, Pará, Brasil. In: 73ª Congresso Nacional de Botânica, 2023, Belém. Livro de Resumos, 2023. v. 1.

- Damasceno, J. T. C.; Silva, J. M. X. T.; Fares, Ana Luisa ; Michelan, T. S. . Efeito multifatorial do ambiente nas características funcionais de Nymphaea rudgeana G.Mey. (Nymphaeaceae) na Amazônia Oriental, Brasil. In: 73ª Congresso Nacional de Botânica, 2023, Belém. Livro de Resumos, 2023. v. 1.

- Carmo, R. S.; Franco, N. L. ; Bomfim, Francieli F. ; Fares, Ana Luisa ; Michelan, T. S. . Desempenho de Pistia stratiotes L. sobre interferência de compostos químicos de uma espécie nativa e uma invasora. In: 73ª Congresso Nacional de Botânica, 2023, Belém. Livro de Resumos, 2023. v. 1

- Cabral, G. S.; Sousa, R. L. M. ; Costa, A. A. S. ; Franco, N. L. ; Fares, Ana Luisa ; Carmo, R. S. ; Jesus, A. M. ; Pott, V. J. ; Michelan, T. S. . Primeiro registro de Landoltia punctata (G.Mey) Les & D.J.Crawford (Araceae, Lemnoidae) para a Amazônia Legal. In: 73ª Congresso Nacional de Botânica, 2023, Belém. Livro de Resumos, 2023. v. 1.

Fieldwork

Finally, during my doctorate I went to three fieldtrips to collect aquatic macrophytes in various projects: Paragominas, Barcarena and Tailândia, all at the state of Pará, Brazil. Some of them were part of my project and were the data used in some chapters from this thesis, while another was as a collaborator. They helped me to improve my knowledge in the field, in collection methodology and in the identification of aquatic macrophytes, as well as other aquatic groups (e.g. insects and fish).