University of Puerto Rico
Faculty of Natural Sciences
Department of Environmental Sciences
Rio Piedras Campus

Ecophysiological responses of plant functional types to environmental conditions in a coastal urban wetland, Ciénaga Las Cucharillas in Northeastern Puerto Rico

By

Elix Hernández Figueroa

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Doctor in Philosophy

Elvira Cuevas, Ph.D. (Advisor)

Ernesto Medina, Ph.D. (Co-Advisor)

Mei Yu, Ph.D. (Committee member)

Eduardo Nicolau, Ph.D. (Committee member)

Eugenio Santiago, Ph.D. (Committee member)

Jess Zimmerman, Ph.D. (Director)

Department of Environmental Sciences

May 20, 2022

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Abstract

Coastal wetlands are influenced by their terrestrial-marine connectivity. Anthropogenic changes in these ecosystems have added additional stressors to plants, like the presence of heavy metals in soils. We evaluated ecophysiological responses of predominant-plant functional types in an urban coastal wetland to local and temporal conditions such as local rainfall, spatio-temporal dynamics of water quality (salinity, pH) regulated by the input of fresh inland water or seawater, and variations in soil concentrations and availability of metals derived from anthropogenic disturbances. In addition, we assessed temporal changes in land and vegetation cover in the wetland after the passage of hurricane Maria and how it affected plant responses. Depending on the local rainfall regimen, wetlands are either a source or sink of available elements and heavy metals. It works as a sink during wet periods with waterlogged conditions, changing to a nutrient source during the dry period with aerobic soils. We found that *Laguncularia* can accumulate Pb in leaf biomass and *Acrostichum* can accumulate Pb in roots, making them both good candidates for phytoremediation practices. Plant functional types differed in their photosynthetic capacity which showed temporal and species variability. According to precipitation, water level, and salinity, they displayed leaf morphology plasticity. We found that plant functional types proved to be resilient to the initial hurricane effect and subsequent changes. These studies have shown that vegetation communities in coastal urban wetlands seem to be adapted to large-scale anthropogenic changes to the ecosystem. Future studies should focus on how exponential increases in human activities paired with sea-level rise and extremes of precipitations will impact the overall long-term responses of these ecosystems.

Keywords: urban coastal wetlands, plant ecophysiology, Cienaga Las Cucharillas

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Introduction

Abiotic features and related biotic processes define the variations within the landscape matrix and the associated ecosystem processes (Fig. 1: Chapin et al., 2011).

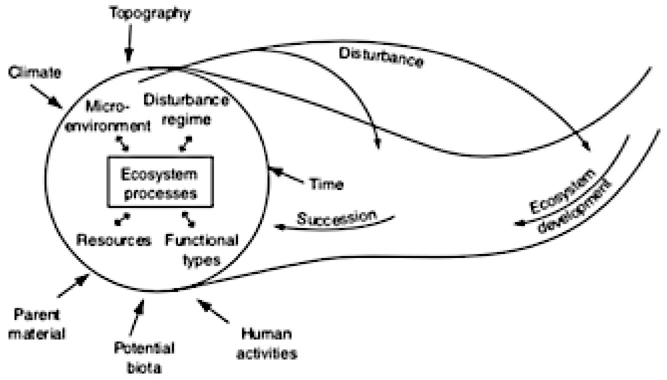


Figure 1. State factors, interactive controls, and ecosystem processes (From Chapin et al. 2011)

State factors (such as topography and climate) and interactive controls (such as disturbance, plant functional types and resource availability) govern variability in ecosystem processes: e.g., productivity, decomposition, and elemental cycling (Cuevas & Medina, 1998; Chapin, 2011). Plant functional types are nonphylogenetic groupings of species that show close similarities in their resource use and response to environmental and biotic controls (Wilson, 1999). Functional grouping is a trait-based approach based on the hypothesis that plant species within functional groups possess similar traits, such as life forms *sensu* Raunkiær (1934), and act in ecologically similar ways (Lavorel & Garnier, 2002).

Because of the complexity of their nature, there is no single definition for wetlands. The Ramsar Convention (2018) defines wetlands as “areas of marsh, fen, peatland, or water, whether natural or artificial, permanent, or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters. They intercept incoming freshwater, mitigating possible negative effects to marine ecosystems (Meuser, 2010). Runoff from different land use areas can be significant sources of nutrients – mostly nitrogen and phosphorus, heavy metals and other contaminants (Carpenter 1998). Nutrient and heavy metal immobilization in the wetland occurs via soil adsorption, bacterial immobilization, vacuole sequestration and vegetation uptake (Fulda & Kretzschmar, 2013; Viehweger, 2014).

Coastal wetlands, transitional areas between terrestrial and marine ecosystems, are influenced by their terrestrial-marine-terrestrial connectivity. They exhibit prolonged periods of water saturation and plants show varying degrees of adaption to physicochemical conditions (Cowardin et al., 1979; Mitsch & Gosselink, 2015). Current wetland distribution is dictated by landscape transformations from either Pleistocene-era glacial events and subsequent rapid deglaciation at the beginning of the current geological epoch - the Holocene 11,500 BP (Toscano & Macintyre, 2003) or, in tropical landscapes, sea level rise and terrestrial inputs - 4,550-5,000 years BP in northeastern Caribbean (Cohen et al., 2016).

Global climate change and increased regional climate variability are driving changes in coastal wetland processes and operations through sea level rise, changes in tropical storm intensity, and longer periods of extreme weather conditions, such as drought and flooding (IPCC 2022). The expected changes in precipitation at the end of the century for Puerto Rico point to a considerable decrease in annual precipitation that varies between -10 to -30% (Girvetz, 2009), -25% to -50% (Campbell 2011), and -30% in the spring and summer (Biasutti, 2012) with overall drier wet seasons and drier periods in the dry season (Uyarra et al., 2005).

Global sea level has risen by an average of 120 meters in the last 18,000 years (Bindoff et al., 2007). The average increase over the last 3,000 years has been 1 to 2 cm per century and the estimate for the 20th century alone is 15- 23 cm (IPCC, 2022). Since 1901, there has been an average global sea level rise of 20 cm and in 1993 alone the sea level rose by 3.1 mm on average (IPCC ,2022). The estimated increase for the Caribbean has been 10 cm per century or 1.4 mm per year (Jacobs et al., 2013). Recent data has increased the rate of sea level rise to 2.5 mm per year in the San Juan Bay and 1.9 mm per year in the Southwestern part of Puerto Rico (Fig 2. Mercado-Irizarry, 2019). This may lead to a change in wetland structure and displacement of species because of hydrological changes in the ecosystem (Brinson et al., 1995).

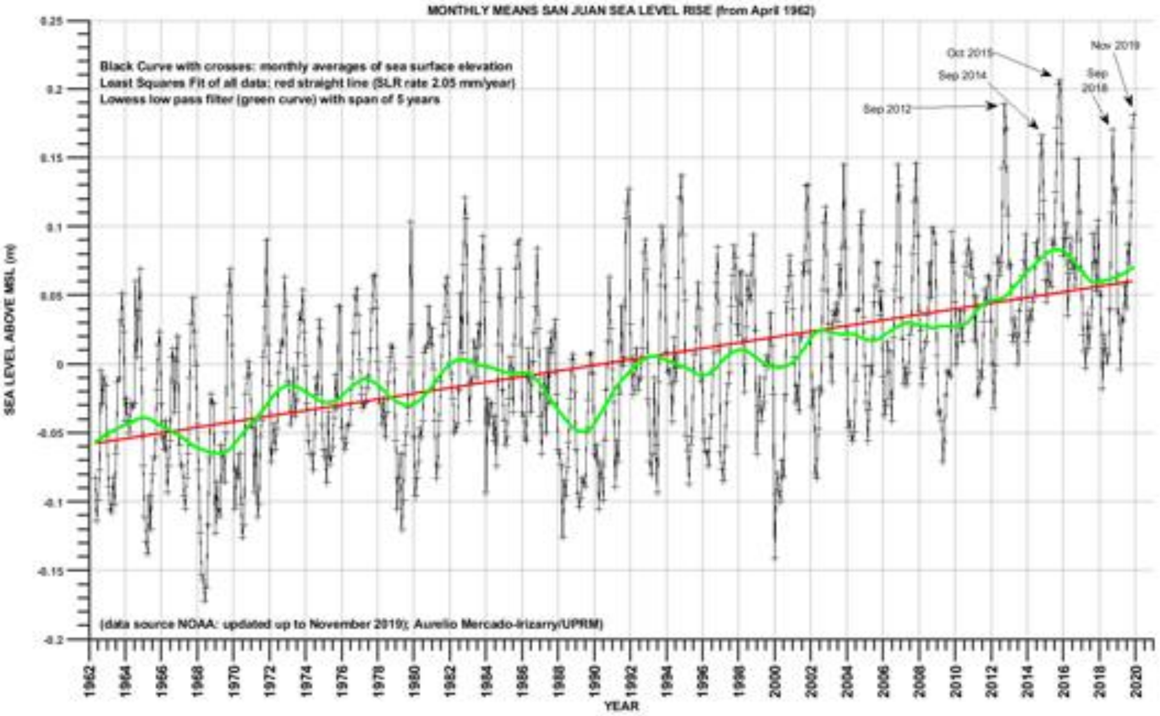


Figure 2. Monthly mean sea level in San Juan (From Aurelio Mercado-Irizarry, 2019). The red line represents (overall trend) and the green line represents five-year trends).

Factors influencing the functioning and productivity of coastal wetlands

Hydrological conditions are the fundamental factors for the function and productivity processes in coastal wetlands (Mitsch & Gosselink, 2015). It varies at both temporal and spatial scales based on a) climate b) topographic gradient, c) natural and anthropogenic disturbances such as storms and channelization, d) persistent events (e.g., sea-level rise), e) groundwater subtraction, and f) agricultural, industrial, and urban development.

Hydrologic movement in a coastal wetland can be explained as a four-dimensional matrix: 1) *in situ* precipitation, 2) fresh water inputs by surface or shallow subsurface and deep subsurface flows (aquifers, basin drainage via channels, rivers and streams), 3) sea water, either via deep subsurface flow or tidal ebb and flows (Kim et al., 2005), and 4) spatio-temporal dynamics of water logging, regulated physicochemical parameters such as aquifer level, salinity, pH and redox (Fig. 3)

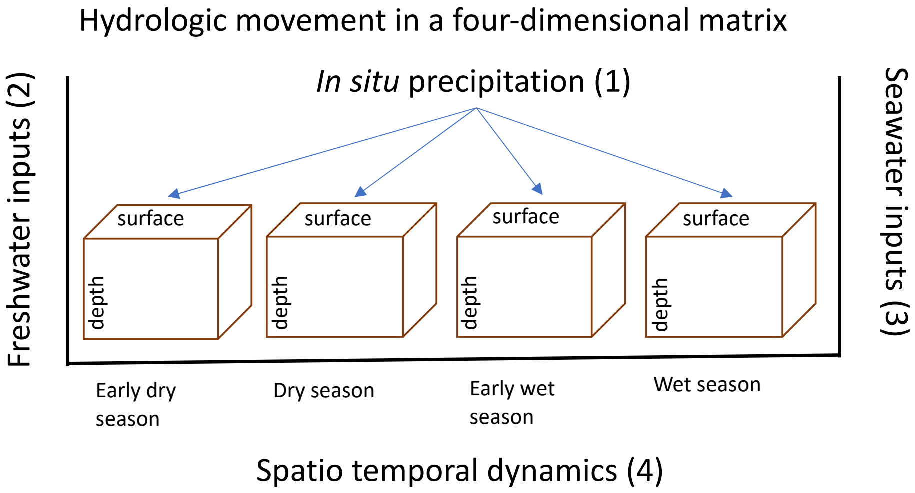


Figure 3 Hydrologic movement in coastal wetlands

Soil salinity, redox potential and pH are major factors determining mobilization of nutrients and heavy metals in flooded soils and their availability to plants (Gambrell, 1994). Whether nutrients and heavy metals are mobilized or immobilized will depend on oxidation-reduction conditions, soil pH, cation exchange capacity, amount of organic material in the soil, salinity content and microbial activity (Gambrell, 1994).

Wetlands on mineral substrate tend to have a neutral-acid range (pH of 6-7) whereas wetlands on organic substrates are more acidic due to the formation of humic substances during the microbial decomposition of plant material (Gambrell, 1994;

Brady, 1996). Soils in temporarily flooded wetlands exhibit hypoxic characteristics. The relative concentrations of nutrients found in the soil solution which can penetrate by diffusion into the apparent free root space is what mainly determines the rates of nutrient acquisition by plants, by diffusion (Brady, 1996; Fritz 2007).

In waterlogged soils, the air spaces are occupied by water, preventing, or delaying the passage of oxygen (Pezeshki & DeLaune, 2012). This leads to anaerobic or reduced conditions resulting in an accumulation of dissolved inorganic carbon (Greenway et al. 2006), followed by decreased oxygen availability for roots and microorganism respiration (Pezeshki & DeLaune, 2012). This causes a drop in the redox potential (going from positive values to negative values) leading to chemical transformations. The reactions under major anoxic conditions are summarized by Mitsch & Gosselink (2015) as follows:

- Denitrification (400 to 200 mV)
- Manganese reduction (200 to 100 mV)
- Reduction of ferric Fe^{3+} to ferrous Fe^{2+} (+100mv to -100mV)
- Reduction of sulphate to sulphite (-100 to -200 mV)
- Reduction of organic matter by producing low molecular weight organic compounds and methane

Reduced conditions in flooded soils, such as in wetlands, lead to changes in the availability and concentrations of dissolved essential nutrients (N, Fe) and production of compounds that can have negative effects to non-adapted plants, such as reduced forms of Fe, Mn, Pb. (Pezeshki & DeLaune, 2012).

Alkaline and earth alkaline metals such a K, Ca and Mg have a wider pH range of availability (Maathius & Diatloff, 2013). For example, at positive redox values (oxidation) and more acidic pH, greater mobility of Pb (a heavy metal) and Zn occurs, while for Fe mobilization to occur, the redox potential conditions must be low and acidic (pH 4 to 7). In reduced soils (low redox conditions or negative values), Zn is found as water insoluble sulfide (ZnS.) Zn plant availability increase slightly at pH<7 and positive redox potentials. (Fig. 4).

The solubility of Pb salts is dependent on the pH, more alkaline soils promoting the formation of $PbSO_4$ and $PbCO_3$ while an increase in acidity under oxidized conditions increase solubility by the substitution of H causing the release of Pb^{2+} ions. As for Cd, under acid and oxidized conditions, Cd is found in cationic form (Cd^{2+}) available for plants, but in a negative redox potential (reduction) it is found in an insoluble inorganic form, bound to sulfur (CdS). In oxygen-rich soils, such as wet soils that have been dried out, sulfates of metals such as CdS and ZnS oxidize becoming available again to plants (Hooda, 2010) (Fig. 4)

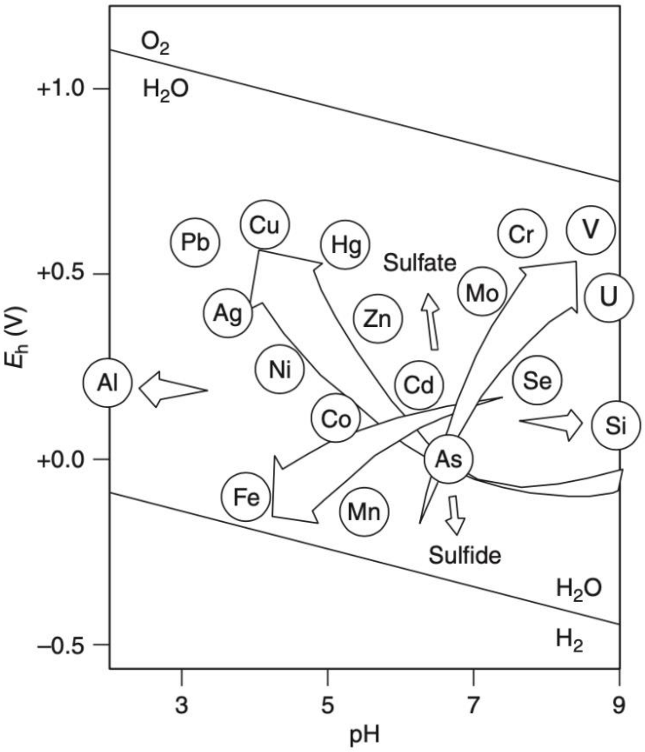


Figure 4 Physicochemical relationships between element availability in soils and the interaction between redox potentials and pH (From Hooda, 2010).

The hydrological four-dimensional matrix (saltwater and freshwater inputs) has an associated impact not only on the biotic makeup of the wetland (Pezeshki & DeLaune, 2012), but also the biogeochemical processes such as decomposition, soil organic matter formation, elemental uptake, immobilization, and availability in the system (Fig 3; Gambrell, 1994).

Soil salinity is considered one of the main plant stressors in wetlands (Mitsch & Gosselink, 2015). Salinity increases has been found to affect nutrient uptake and salt accumulation yielding decreases in photosynthesis (Watzka & Medina, 2018). As coastal wetlands are an intermediate ecosystem between marine and terrestrial ecosystems, water quality parameters such as salinity, and conductivity are indicators of this connectivity, as freshwater from terrestrial sources, precipitation and seawater from marine sources determine wetland phreatic water composition (Fig. 5).

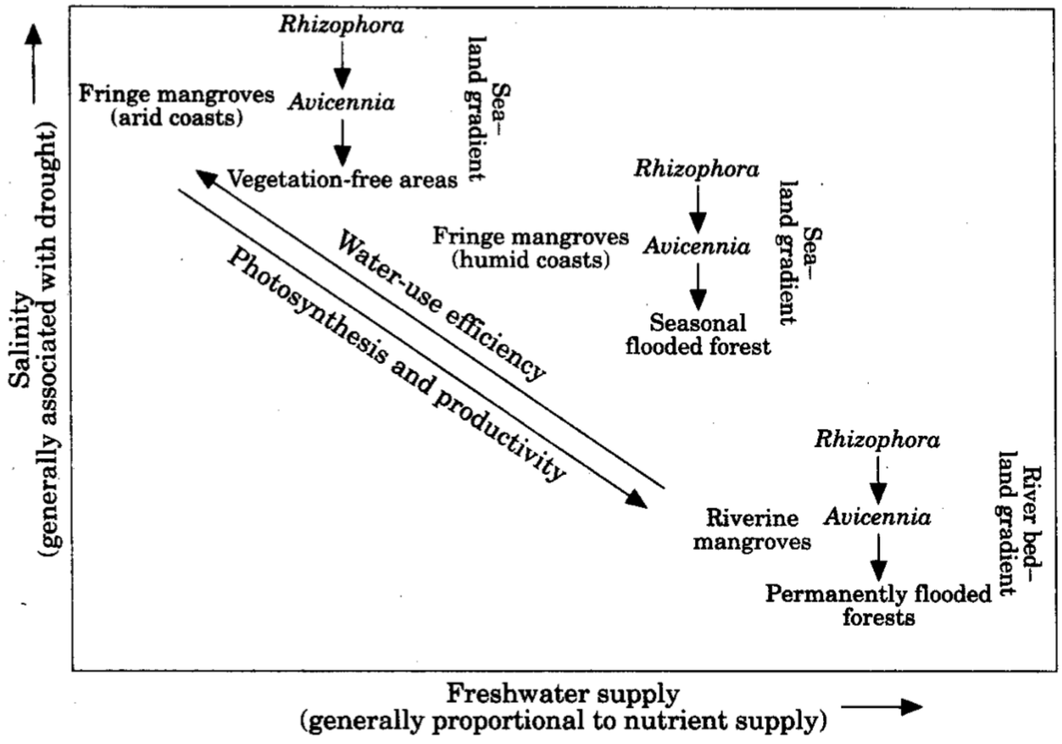


Figure 5 Hypothetical mangroves distribution based on salinity and freshwater inputs. (Retrieved from Medina & Francisco 1997)

Effects of landscape transformation in coastal wetlands

Changes in landscape for urban and industrial development and the infrastructure used to control natural hydrological processes in coastal wetlands has created novel ecosystems. For example, channeling rivers for flood control reduces flooding in the short term, but alters water flows and sediment movement, increasing the rate of coastal erosion. Similarly, filling in wetlands to establish urban areas eliminates spatial connection, resulting in flooding and land subsidence, mostly due to climatic events.

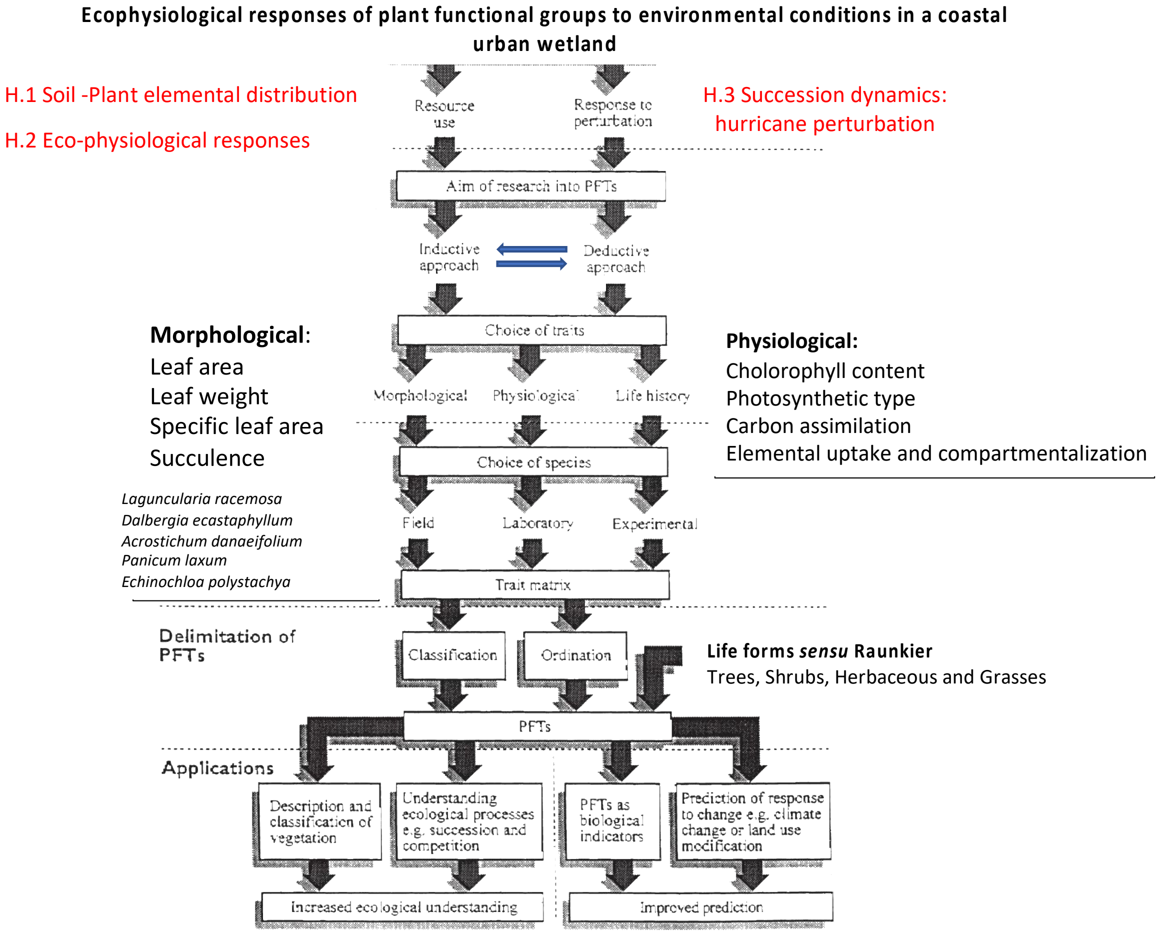
Urban, agricultural, and industrial runoff to streams, reservoirs and channels are one of the most significant contributors of nutrient inputs into coastal wetlands (NRC, 2009).

Urban soils present higher amounts of heavy metals because of their previous land use such as agriculture, atmospheric deposition of particulates, dumping of waste and man-made materials, and discharges of industrial waste to streams (Meuser, 2010; Olivares, 2003). Elements with an atomic mass higher than 50 or density higher than 5 g cm^{3} are considered "heavy metals" (Alloway, 2012). Some heavy metals are essential for plant normal growth and have low concentrations in plant tissues (i.e. Iron, Manganese, Nickel and Zinc), whereas others may be even toxic in small concentrations (Cadmium, Arsenic, Lead, Chromium) (Hooda, 2010).

Heavy metals, when in high concentrations and water-soluble form, can affect entire trophic webs by changing soil alkalinity (pH), causing senescence in plants as the case of some plants by inducing Glutamate Hydrogenase (GDH) in response to Cd (Boussama, 1999; Furini, 2012), inhibiting photosynthesis, decreasing soil microbial dynamics, and altering nutrient cycling throughout the ecosystem (Prasad and Strzalka, 1999; Kelly et al., 2019).

Heavy metal resistant plants exhibit three patterns: a) excluders or avoiders, that maintain metal concentrations in tissues at low levels over a broad range of soil available metal concentration, binding them at the root external cell walls; b) indicators, in which internal concentrations reflects soil availability levels; and c) accumulators, in which metals concentration in plant tissues are not related directly to their soil availability (Baker, 1981).

Functional types of the vegetation in tropical and subtropical coastal wetlands based in life forms include mangroves, terrestrial halophytic and non-halophytic trees, shrubs, herbs, and herbaceous monocots: grasses, sedges, and rushes (Raunkiær, 1934; Ellenberg & Mueller- Dombois, 1967). Adding to this set of functional types based on life form and morphological and physiological traits provides a more ecologically integrated approach to the analysis of ecosystem function (Fig. 6).



Modified from Duckworth et al., 2000

Figure 6 Eco-physiological responses of plant functional types to environmental conditions

Acclimation versus adaptation

Living organisms to establish to new environments and survive must adjust their physiology to their surroundings. Adaptation is the genetic process which a population change in response to environmental factors (Whitehead, 2012) and is a long-term process. Acclimation is based at the organism level, and is established on the physiological, anatomical, or morphological adjustments that improve performance or survival in response to acute environmental changes such as droughts, and long-term changes as sea level rise. Coastal wetlands plants have a great plasticity and have adapted to the constant abiotic changes in their environment including salinity changes, high wind energy and fluctuating water levels. However, in urban areas, the ongoing landscape transformations, as discussed before, puts additional stressor to these plant communities which are in constant acclimation.

Objectives

The Ciénaga Las Cucharillas Natural Reserve wetland provides the opportunity to study physiological responses and patterns of leaf trait variation of several co-occurring plant functional types (trees, grasses, ferns, shrubs) along a wide range of soil salinity, water table elevation, and pH under the same meso-climate. The area has been submitted in the decades to strong changes in hydrology and was use as a landfill for industrial residues rich in chemical elements potentially toxic to plants. We differentiate plants in based in plant growth forms (Fig.6): woody dicot plants, encompassing 1) trees, woody vegetation with a trunk, supporting branches, and leaves with a height surpassing more two meters. 2) Shrubs, woody vegetation that does not exceed 2 meters in height. Herbaceous monocot plants as grasses, which can be easily identified by hollow stems and narrow alternate leaves and ferns, nonflowering vascular plants that reproduce by spores. We investigated morphological traits: Leaf area and weight, specific leaf area and succulence (Fig. 6). Physiological traits investigated were photosynthesis, nitrogen acquisition, element uptake and heavy and post-transition metal accumulation and exclusion (Fig.6).

The main objectives of this research are:

To measure the eco physiological response of predominant-plant functional groups to local and temporal conditions in an urban coastal wetland: a) local rainfall, b) spatio-temporal dynamics of water quality (salinity, pH) regulated by inputs inland fresh water or sea water, and c) variations in soil concentrations and availability of metals derived from anthropogenic disturbances.

Questions and hypotheses.

How does elements allocation compare between plant functional tyoes associated to spatial and temporal variations of substrate (or soil) elemental composition?

It was hypothesized that plant functional types would respond differently to soil salinity and pH temporal and spatial variability in their element uptake and accumulation.

It answered to this question by

1. determining spatial-temporal distributions and availability for macronutrients and metals in soils
2. Determining relative accumulation or exclusion from plant tissues.

How do plant functional types differ in physiological ecological responses to temporal variability in water level, soil salinity, and interstitial salinity?

It was hypothesized that they would respond differently according to life forms. It was answered this question by

1. Measuring leaf morphological traits (leaf area, fresh mass, dry mass, water content, and calculated succulence and specific leaf area indices) at an intra and interplant level inter and intraspecific plant level for one year.
2. Determining photosynthetic performance, by measuring net CO^2 assimilation, and chlorophyll fluorescence of the selected plant functional types.

What were the spatial-temporal regeneration dynamics of plant cover and composition during the two years after Hurricane María?

It was hypothesized that vegetation cover and composition were going to be altered due to direct and indirect effects of the disturbance and the differences between vegetation cover composition (trees, shrubs, grasses, and ferns), and regeneration was determined by abiotic conditions (soil texture and elemental composition), water chemistry and water logging of the wetland. It was responded to this question by:

1. Assessing temporal changes in land and vegetation cover in the wetland
2. Assessing plant regeneration dynamics using ecophysiological traits of the vegetation.

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Study Area:

The Ciénaga Las Cucharillas natural reserve (18º26’25.27” N, 66º08’08.39” W) is an urban coastal palustrine/estuarine wetland located on the western side of the San Juan Bay in the northern metropolitan area of Puerto Rico (Lugo et al., 2011). San Juan Bay was naturally formed during the last million years in the tertiary period as a result of eustatic sea level and tectonic changes (Seguinot, 1983). Ciénaga Las Cucharillas was believed to be a swamp formed in the quaternary period over the alluvial deposits of the Holocene due to the periodic flooding of these areas by the Bayamon River (Kaye, 1959; Seguinot, 1983).

The remains of the original wetland cover an area of 500 hectares, the rest having been filled for urban and industrial use during the 20th century (Lugo et al., 2011; Martinuzzi et al., 2009). The wetland is part of the Cucharillas Drainage basin, located in between two large basins that also drain to the bay: the Bayamon basin and the Rio Piedras basin which starts in the karstic area in Caparra, Guaynabo at an approximately 60 meters msl, the middle part of the basin is located at Fort Buchanan (army facilities), and ending in the Ciénaga Las Cucharillas surrounded by Juana Matos, Cucharillas and Puente Blanco communities (Fig. 7).

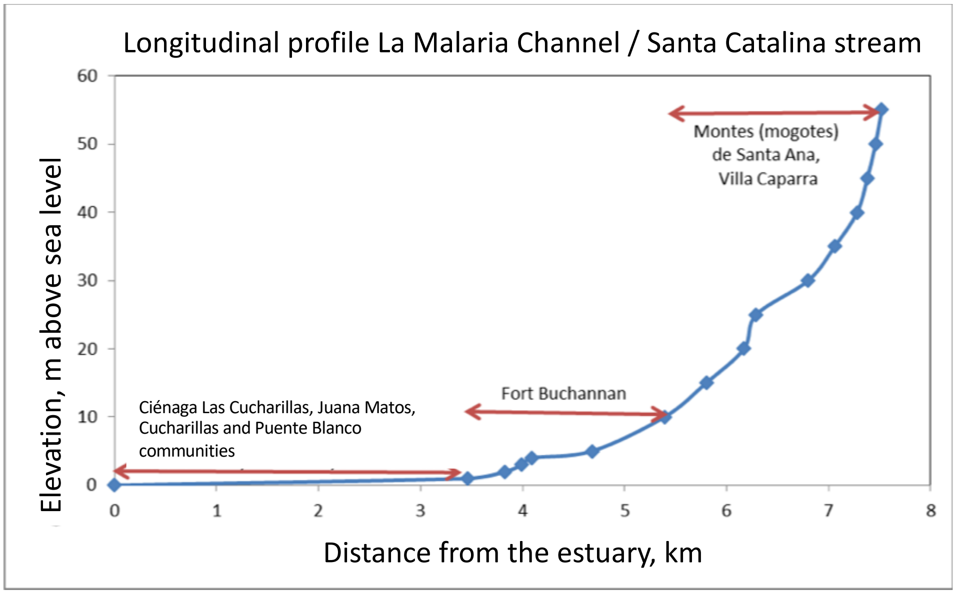


Figure 7 Longitudinal profile of Ciénaga las Cucharillas and Channel Malaria (Reproduced with permission from Jorge Ortiz, Elvira Cuevas 2019).

The present hydrological regime has been modified since colonial times, including drainage channels for agricultural use until mid-20th century (Kennaway & Helmer, 2007), the construction of the Malaria Channel in in the 1940s bringing direct flow of freshwater to the wetland from the upper and middle parts of the basin (Pumarada, 1997), and restricted seawater exchange (Webb & Gomez, 1998). Currently, it is surrounded by highly urbanized and industrialized area where discharges from septic tanks, grey waters, illegal industrial intermittent flows and seepages, and atmospheric emissions from power generating plants of the Electric Power Authority (AEE) effect the wetlands in addition to legacy effects from incidents like the Caribbean Petroleum Corporation explosion and clandestine landfills (EPA, 2003, 2020; UCSHI, 2009; Subramanian et al., 2018). The previous anthropogenic changes transformed the wetland into a novel ecosystem with distinctive hydrological and edaphic characteristics not normally seen in these ecosystems.

Climate conditions:

Ciénaga Las Cucharillas is humid, with an annual temperature average of 25.2 C and annual precipitation sum of 1575 mm. The daily maximum temperature reported for the period of this study was 31.2 C and the lowest temperature recorded was 16.8 C (Fig.8). Rainfall in the northern Caribbean has a bimodal pattern (Taylor et al., 2002). Puerto Rico has two wet seasons: a peak in July-December, another in May-June, and a dry season from January to April (Torres-Valcarcel et al., 2014).

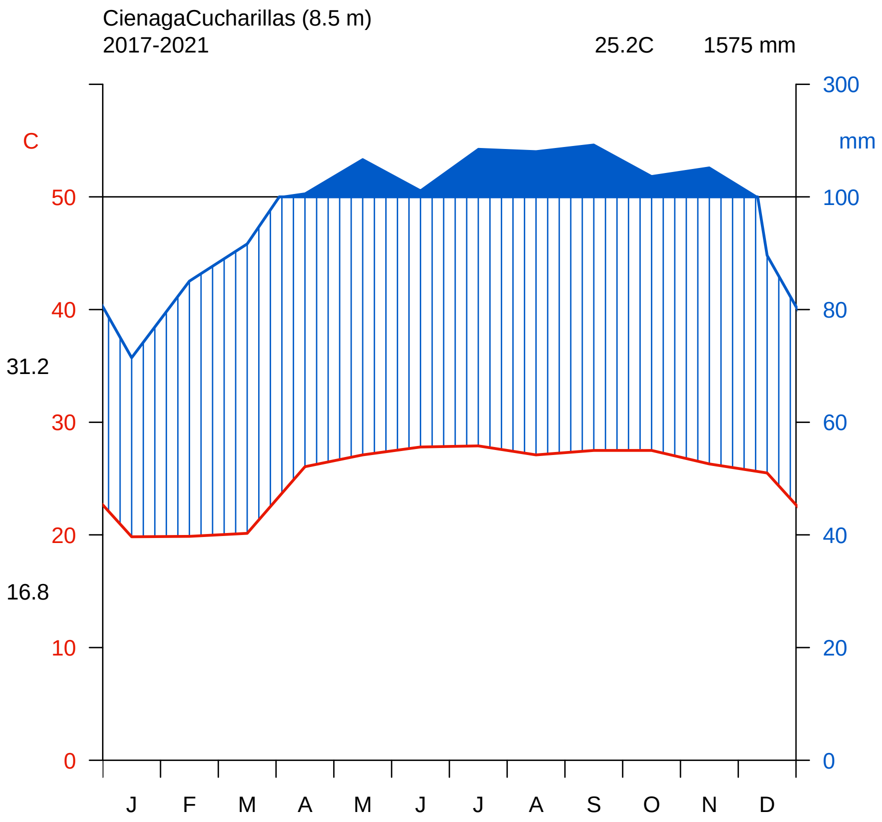


Figure 8. Walter - Leith climate diagram for Ciénaga Las Cucharillas covering 2017-2021 time period.

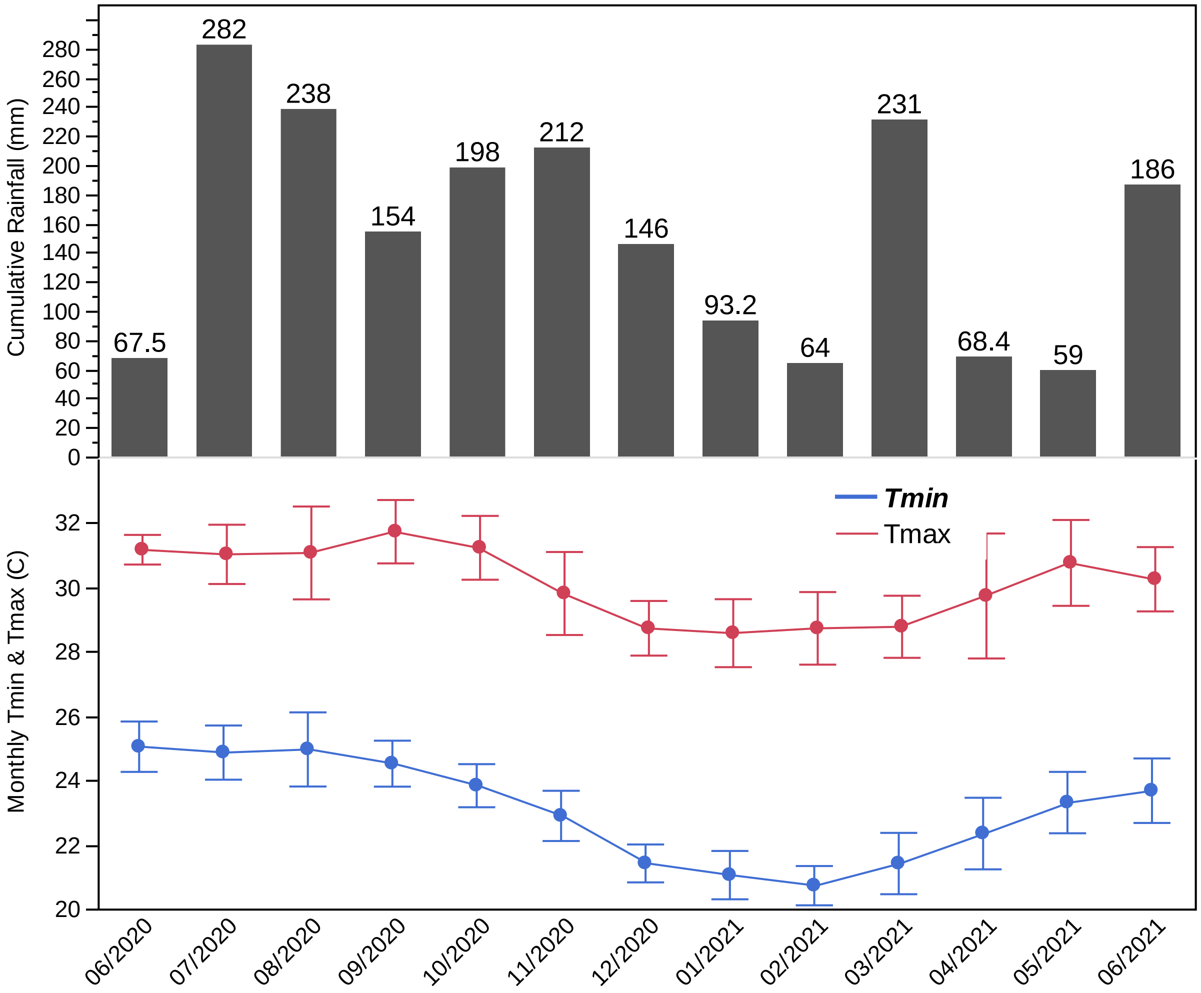


Figure 9 Monthly Rainfall and Temperature for Cienaga Las Cucharillas. Bars denote standard error

Through the year our study took place, rainfall data exhibited the bimodal pattern for the island, where the months of July – December exhibited months with higher rainfall (Fig. 9), however, during the dry period, in the month of march, 168 mm of rain were reported on a single day (March 21, 2021), causing temporary floodings in the wetland. The island climate bimodality was seen in the monthly temperatures as the colder months correlated with the dry period, and hotter months with the wet period.

Hydrology:

Lugo et al. (2011) described Cucharillas wetlands as a “large swamp dominated by freshwater herbaceous vegetation with euryhaline transition to mangroves and saline vegetation”. Based on this general description, in 2018, at a 5-ha area which is co-managed and under rehabilitation by Department of Natural Resources and the Corredor del Yaguazo, a community NGO, we took measurements in 10 monitoring wells of water level and salinity and deployed water level data loggers (Fig.10).

Figure 10 Ciénaga las Cucharillas natural reserve delimitation and location of long-term monitoring wells (red dots)

Salinity:

Since 2014 the Processes and Function in Tropical Ecosystems laboratory based in the University of Puerto Rico – Rio Piedras campus, (Ecolab) have been carrying out abiotic measurements in the study area. For our research purposes we are presenting the values from June 2020 - June 2021 (Hernandez et al 2021). Conductivity values ranged from 0.4 to 51 mS/cm for the whole wetland which was influenced by the precipitation regime. At the phreatic level, we found higher conductivity values closer to the coastline with a sharp drop around 1 km inland, with conductivity values ranging spatially from 10 to 35 mS/cm (Fig.11). We found marine intrusion at a depth of 2.5 meters in most of the wells. Water levels also reflected rainfall bimodality, as the water levels dropped in June 2020 and May 2021, and the other months had waterlogging conditions. Saline conditions at the phreatic level fluctuated from oligohaline to mesohaline conditions (Fig.12). This can be evidence of the subsurface maritime-terrestrial connectivity of the wetland, because of the saltwater intrusion in the water column which increases with depth.

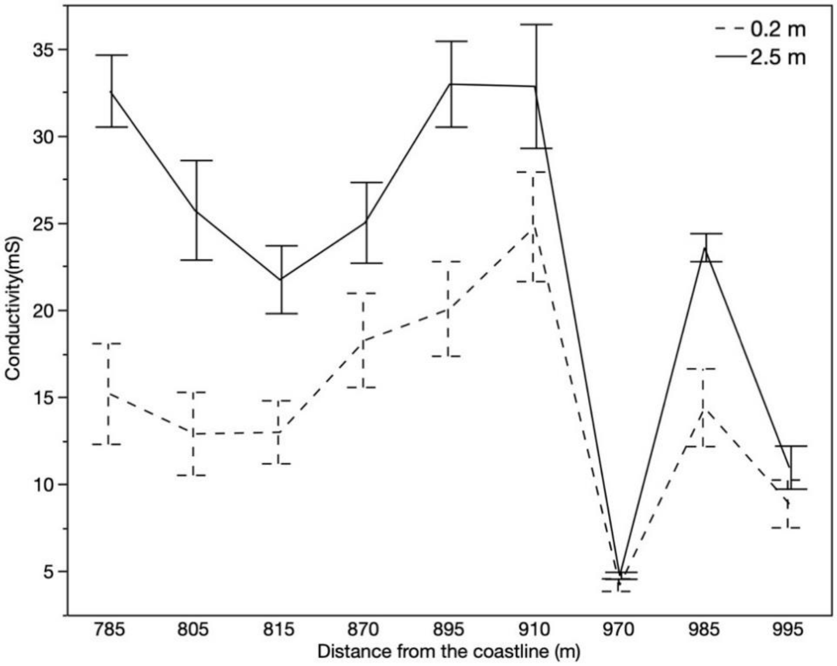


Figure 11 Average conductivity (mS/cm) at the phreatic level and 2.5-meter depth in reference to the coastline (Hernandez et al 2021)

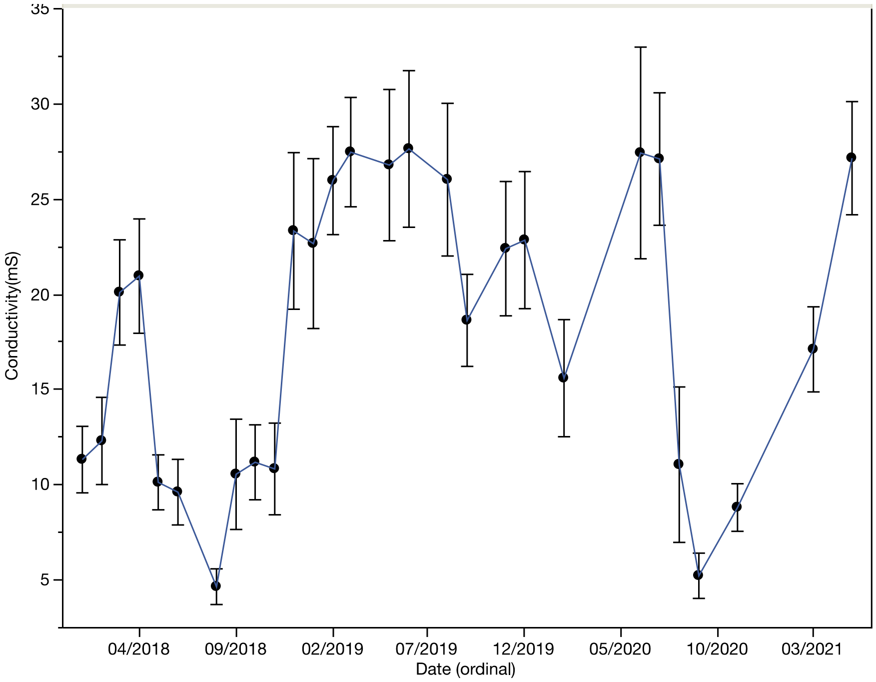


Figure 12 Average and standard deviations for monthly conductivity values (mS/cm) at the phreatic level

Waterlogging conditions:

Over the course of 2020- 2021, the depth to the water table ranged from -1.5 meters (minus sign denoting below surface) to 1.1 m (flooding conditions) (Fig.13). Over the period of one year, from June 2020 to June 2021, the wetland was flooded for 175 days over a period of 6 months from August 2020 to January 2021 and was dry for 179 days (Fig.14). We selected four plots that represented the different conditions of the wetland for further considerations. These plots differed from each other in mean water levels however temporal trends were similar. Differences in mean water level and salinity within plots can be related to edaphic conditions and previous land uses (Figs.15,16 & 17).

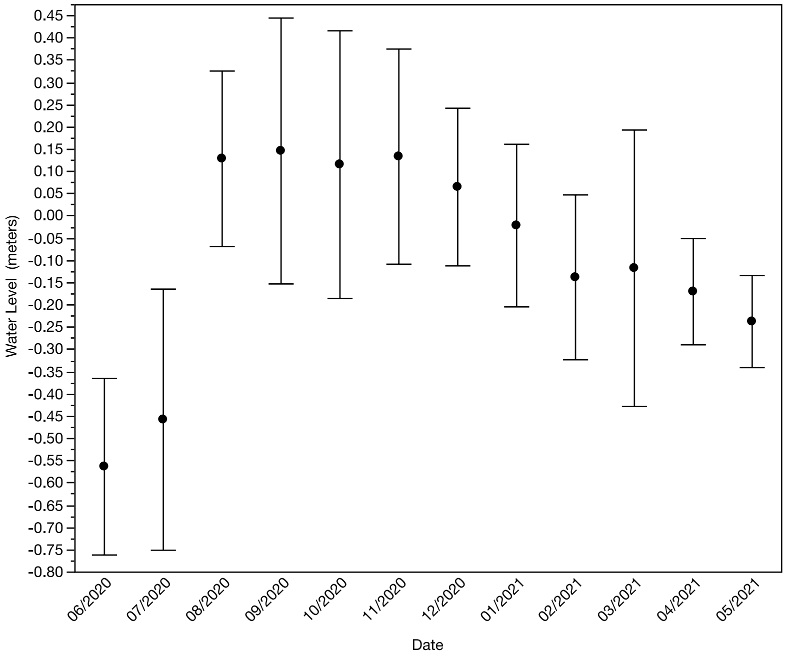


Figure 13 Monthly average ± standard deviation of water level at Cienaga Las Cucharillas n= 24

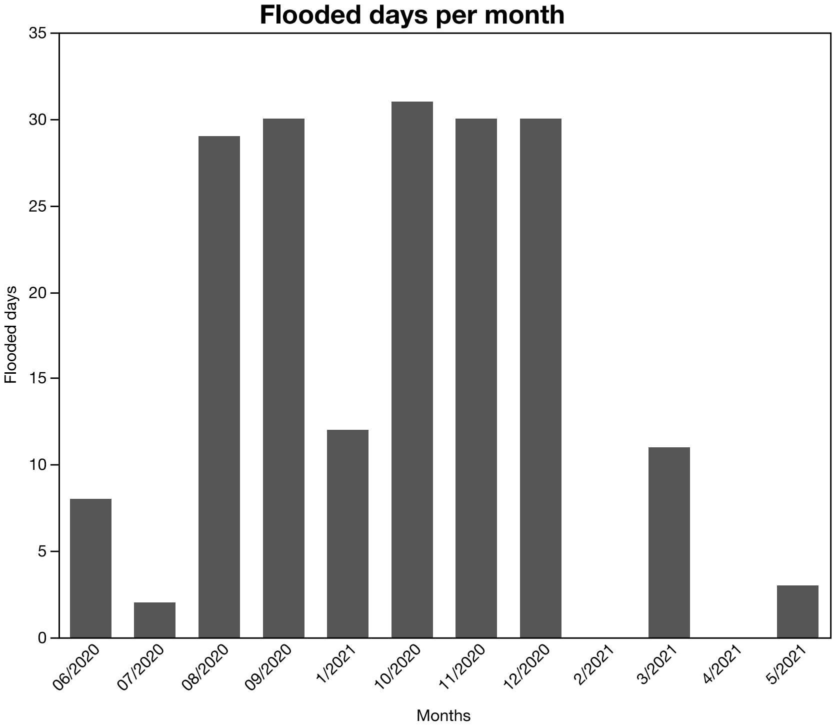


Figure 14 Flooded days per month at Cienaga Las Cucharillas. (Flooded are defined as days with water table above surface for a period of 24 hours)

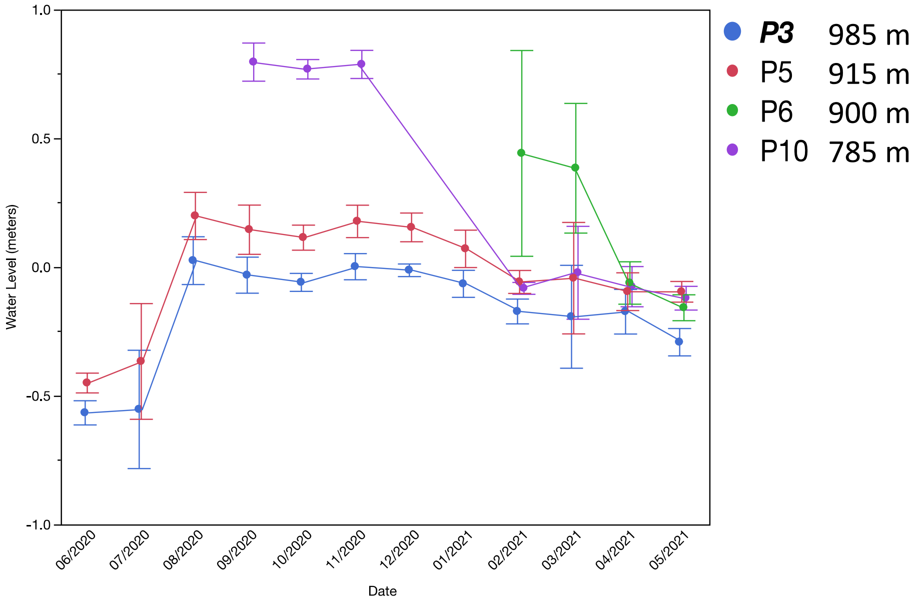
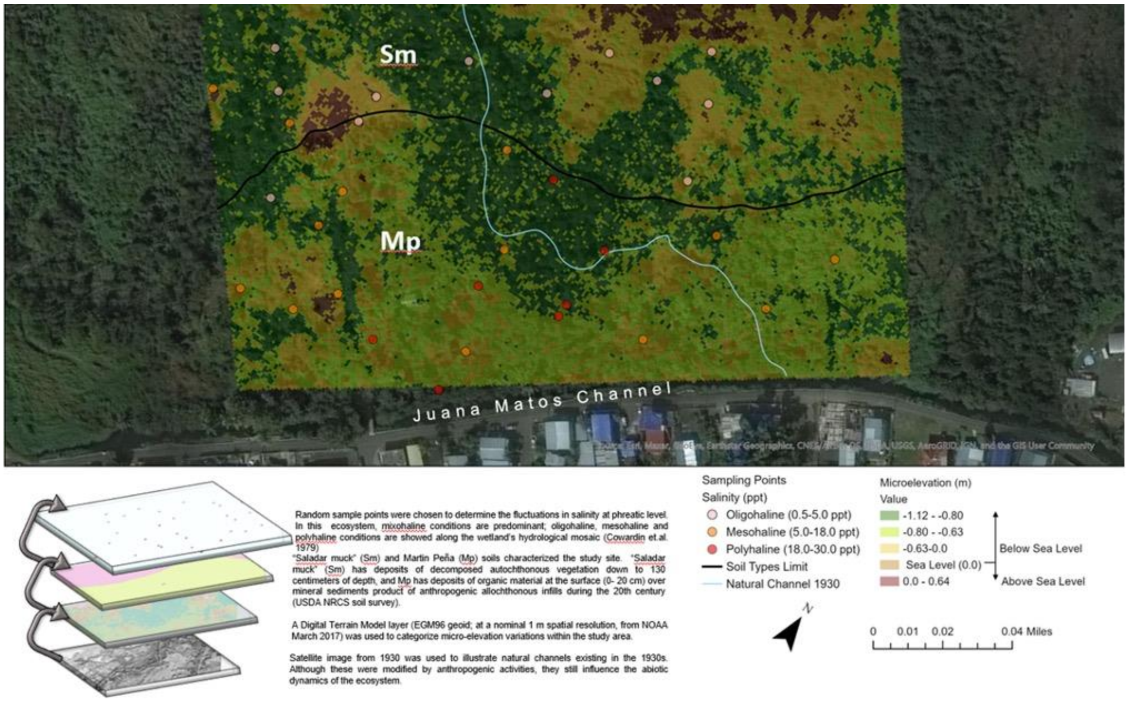


Figure 15 Monthly averages (in meters) for water levels at four monitoring wells. In the legend is the distance from the coastline

Edaphic factors:

According to the USDA NRCS soil survey, Ciénaga las Cucharillas is characterized by “Saladar muck” (*Sm*), Martin Peña (*Mp*) and hydraquents saline (*Hy*). *Sm* soils are made up of mostly deposits of decomposed autochthonous herbaceous vegetation down to 130 centimeters. *Mp* soils usually have a more complex profile – close to the surface (0-20 cm) there is organic material and allochthonous mineral soil embedded in the organic matrix, deeper, there is more silty clay loam (20-45 cm) and clay (45 - 160 cm). *Hy* soils are characterized by saturated soils composed of mineral sediments. These mineral sediments layers are product of anthropogenic allochthonous infills from upper terrestrial sources for urban and industrial use during the 20th century, whereas *Sm* organic layers were derived from autochthonous natural plant decomposition.



Random sample points were chosen to determine the fluctuations in salinity at phreatic level. In this ecosystem, mixohaline conditions are predominant; oligohaline, mesohaline and polyhaline conditions are showed along the wetland's hydrological mosaic (Cowardin et.al. 1979)

"Saladar muck" (Sm) and Martin Peña (Mp) soils characterized the study site. "Saladar muck" (Sm) has deposits of decomposed autochthonous vegetation down to 130 centimeters of depth, and Mp has deposits of organic material at the surface (0- 20 cm) over mineral sediments product of anthropogenic allochthonous infills during the 20th century (USDA NRCS soil survey).

A Digital Terrain Model layer (EGM96 geoid; at a nominal 1 m spatial resolution, from NOAA March 2017) was used to categorize micro-elevation variations within the study area.

Satellite image from 1930 was used to illustrate natural channels existing in the 1930s. Although these were modified by anthropogenic activities, they still influence the abiotic dynamics of the ecosystem.

Figure 16 Spatial soil salinity (in ppt) and micro elevation (m). Used with permission from Ortiz G, & Pinto-Pacheco, S. & Cuevas,E.

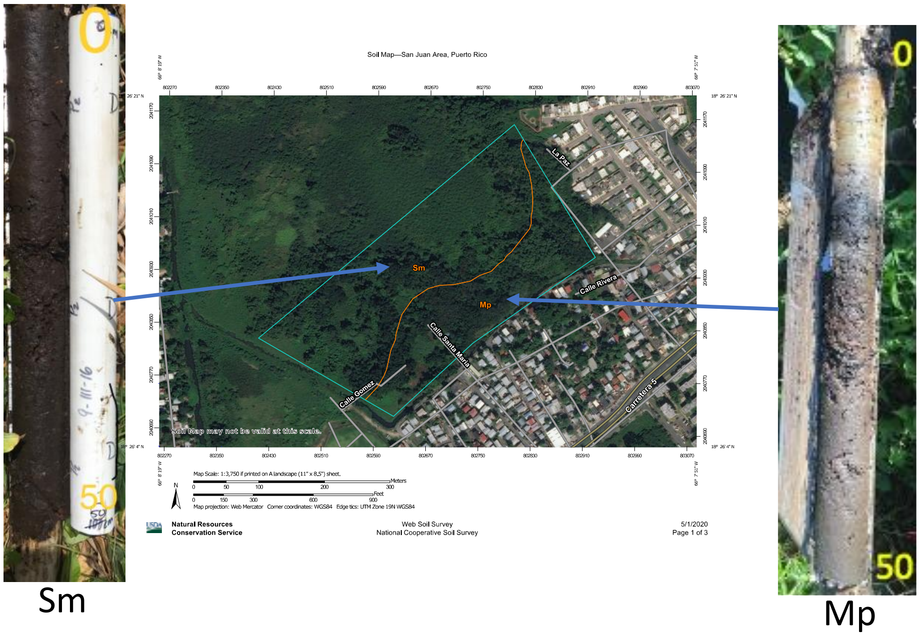
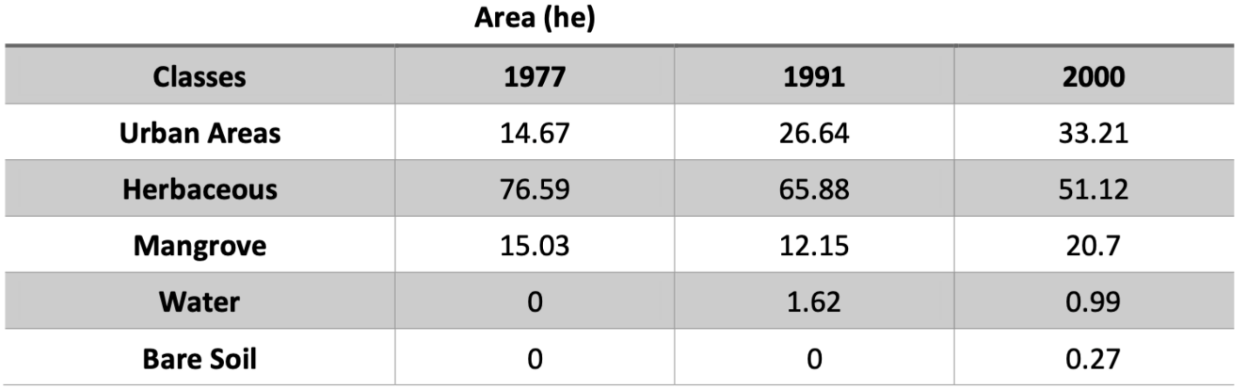


Figure 17 . Contrasting soil samples and spatial distribution Saladar Muck (Sm) soils – left image. Martin Peña (Mp) – right image - first 0-20 cm there is organic material and a allochthonous mineral soil embedded in the organic matrix.

Plant cover and composition: Past and present

Paleobotany records suggested that original vegetation for Ciénaga Las Cucharillas was composed of hydrophytic vegetation of fresh water and lagoons with *Typha domingensis* and *Cladium jamaicensis* as dominant species (Hollick, 1928), a vegetation cover along the borders of the swamp area dominated by *Scleria hirtella*, *Fimbristyllis dichotoma* and *Aeschynomene sensitive* (Seguinot, 1983). According to Lugo et al. (2011), from 1508 to 1980, Cucharillas wetland lost 90% of its mangroves. Based in classifications made by Ramos & Lugo (1994) and Helmer et al (2002) for the years 1977, 1991, and 2000 (Kennaway & Helmer, 2007), I present Cienaga Las Cucharillas coastal modifications from 1977 to the present. For the year 1977 of 106 hectares, urban areas occupied 14.67 he, Herbaceous species occupied 76.59, mangroves areas 15.03 and no permanent water reported. For 1991, Urban areas represented 26.64 hectares of the totality, herbaceous 65.88 he, mangroves areas 12.15 he and present water (in form of ponds or floods) 1.62 ha. For the year 2000, 33.21 hectares were urban areas, herbaceous occupied 51.12, mangrove 20.7 and water 1 hectare (Table 1).

Table 1. Land use/Land cover for the study area (ha)



Area (he)

Classes	1977	1991	2000
Urban Areas	14.67	26.64	33.21
Herbaceous	76.59	65.88	51.12
Mangrove	15.03	12.15	20.7
Water	0	1.62	0.99
Bare Soil	0	0	0.27

At present Ciénaga las Cucharillas is dominated by an herbaceous freshwater vegetation and in a lesser spatial extent mangroves and associated vegetation, in a mosaic-like pattern. In 2003, a botanical census in Cucharillas done by Axelrod F. (http://herbario.uprrp.edu/Bol/uprrp/Results) recorded 28 species distributed across 19 families. The predominant plant life form was herbaceous species (65%), 26 out 28 species are labeled as native (92%) and 21 of them as perennials.

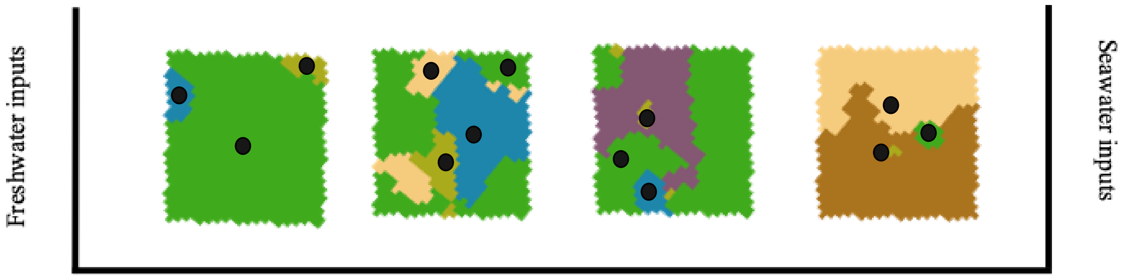
Table 2 Species listed for Cucharillas; Group: M (Monocots) D (Dicots); Substrate: T (Terrestrial) A (Aquatic); PT (Photosynthetic Type) (Sage 2011), 2. Salt tolerance: http://www.sussex.ac.uk/affiliates/halophytes/ 3. N-Fixing (Tedersoo, 2018)

Family (-ceae)	***Species***	**Group**	**Life form**	**Lifespan**	**Substrate**	**Origin**	**PT[1]**	**Salt Tolerance[2]**	**N-Fixing[3]**	**13C**
Alismata	*Sagittaria Intermedia*	M	Herb	Perennial	T	Native	C_3	non-halophyte	none	
Annona	*Annona glabra*	D	Tree	Perennial	T	Native	C_3	non-halophyte	none	
Apocyna	*Asclepias curassavica*	D	Herb	Perennial	T	Native	C_3	non-halophyte	none	
Arac	*Lemna aequinoctialis*	M	Herb	Perennial	A	Not Native	C_3	non-halophyte	none	
Astera	*Mikania cordifolia*	D	Vine	Perennial	T	Native	C_3	non-halophyte	none	
Astera	*Cyanthillium cinereum*	D	Herb	Annual	T	Not Native	C_3	non-halophyte	none	
Astera	*Pluchea odorata*	D	Herb	Annual	T	Native	C_3	halophyte	none	
Astera	*Sphagneticola trilobata*	D	Vine	Perennial	T	Not Native	C_3	non-halophyte	none	
Astera	*Eclipsa prostata*	D	Herb	Annual	T	Native	C_3	non-halophyte	none	
Astera	*Symphyotrichum expansum*	D	Herb	Perennial	T	Native	C_3	non-halophyte	none	
Avicennia	*Avicennia germinans*	D	Tree	Perennial	T	Native	C_3	halophyte	none	-30.14
Bignonia	*Amphitecna latifolia*	D	Tree	Perennial	T	Native	C_3	halophyte	none	
Cleoma	*Gynandropsis gynandra*	D	Herb	Annual	T	Not Native	C_4	non-halophyte	none	
Combreta	*Terminalia catappa*	D	Tree	Perennial	T	Not Native	C_3	halophyte	none	
Combreta	*Laguncularia racemosa*	D	Tree	Perennial	T	Native	C_3	halophyte	none	-28.99
Combreta	*Conocarpus erectus*	D	Tree	Perennial	T	Native	C_3	halophyte	none	
Commelina	*Commelina diffusa*	D	Herb	Perennial	T	Native	C_3	non-halophyte	none	
Convolvula	*Ipomoea setifera*	D	Vine	Perennial	T	Native	C_3	non-halophyte	none	
Convolvula	*Ipomoea triloba*	D	Vine	Perennial	T	Native	C_3	non-halophyte	none	
Convolvula	*Ipomoea tiliacea*	D	Vine	Perennial	T	Native	C_3	non-halophyte	none	
Convolvula	*Aniseia martinicensis*	D	Vine	Perennial	T	Native	C_3	non-halophyte	none	
Cypera	*Cyperus difformis*	M	Herb	Annual	T	Not Native	C_4	non-halophyte	none	
Cypera	*Cyperus elegans*	M	Herb	Perennial	T	Native	C_4	non-halophyte	none	
Cypera	*Cyperus distans*	M	Herb	Perennial	T	Native	C_4	non-halophyte	none	
Cypera	*Cyperus ligularis*	M	Herb	Perennial	T	Native	C_4	non-halophyte	none	
Cypera	*Eleocharis mutata*	M	Herb	Perennial	T	Native	C_4	non-halophyte	none	
Faba	*Dalbergia ecastaphyllum*	D	Shrub	Perennial	T	Native	C_3	halophyte	nodulated	-28.80
Faba	*Senna alata*	D	Shrub	Perennial	T	Not Native	C_3	non-halophyte	none	
Faba	*Vigna luteola*	D	Vine	Perennial	T	Native	C_3	non-halophyte	nodulated	
Faba	*Stahlia monosperma*	D	Tree	Perennial	T	Native	C_3	non-halophyte	none	
Lythra	*Cuphea strigulosa*	D	Herb	Perennial	T	Native	C_3	non-halophyte	none	
Malva	*Malachra alceifolia*	D	Herb	Perennial	T	Native	C_3	non-halophyte	none	
Malva	*Malachra capitata*	D	Herb	Perennial	T	Native	C_3	non-halophyte	none	
Malva	*Thespesia populnea*	D	Tree	Perennial	T	Not Native	C_3	halophyte	none	-30-03
Muntingia	*Muntingia calabura*	D	Tree	Perennial	T	Native	C_3	non-halophyte	none	
Onagra	*Ludwigia octovalvis*	D	Shrub	Perennial	T	Native	C_3	non-halophyte	none	
Passiflora	*Passiflora foetida*	D	Vine	Annual	T	Native	C_3	non-halophyte	none	
Plantagina	*Bacopa monnieri*	D	Herb	Perennial	T	Native	C_3	non-halophyte	none	

Plots selection and sampling methods:

In September 2020, we used a quadcopter drone for an aerial image of the plots. Using the picture, we marked the extent of the species sampled in each plot and estimated the cover in squared meters. Plots were arranged perpendicular to the coastline, each one a square of 10 x 10 meters and a distance of 100 meters in between them (Fig.18).

Figure 18 Locations of plots for field measurements



		985 (P3)	915 (P5)	910 (P6)	785 (P10)
🟩	**Laguncularia**	92.6	59.9	46	2.2
🟨	**Acrostichum**	3.23	1.97	7.85	0.42
🟧	**Dalbergia**	0	0	13.3	40.4
🟦	**Panicum**	4.21	4.2	32.8	0
🟫	**Echinochloa**	0	0	0	56.9
🟪	**Herbs**	0	33.8	0	0

Figure 19 . Plant cover (in %) for the selected plots. Column headers are distance from the coastline in meters (plot identifiers in parenthesis. Black dots denotes where soil sampling was done, one sample per specie, three – four at each plot.

The closest plot to the coastline, was composed mostly of *Echinochloa* (~57 m^2) and *Dalbergia* (~40 m^2), where *Laguncularia* and *Acrostichum* had little presence (Fig. 19). Only two individuals of *Laguncularia* were present at this plot. Plot 6 was transitioning to a dominant tree cover, *Laguncularia* (46 m^2) followed by *Panicum* (~32 m^2) and *Dalbergia* (~13 m^2). Plot 5 had a dominant cover of *Laguncularia* (~60m^2) followed by herbaceous species (33.8 m^2) which were not sampled for this study. Some of the herbaceous species belong to the Cyperaceae, Vitaceae and Polygonacae families. At Plot 3, *Laguncularia* covered ~92.6 m^2 and the rest was composed of *Acrostichum* and *Panicum* species (Fig.19).

Dominant plant functional types:

Based on the plant life forms currently present in the wetland, I selected the dominant species of the following groups (Fig. 20), to be described later.

- In representation of trees, *Laguncularia* was the most abundant, and occurred in the four plots.
- For the shrubs, *Dalbergia,* which was present in two plots (P6, P10).
- As herbaceous species *Acrostichum was* present in the four plots
- As grass two different species were selected:
    - A stoloniferous, fibrous rooting, invasive grass*,* *Panicum* was present in 3 plots (P3, P5, P6)
    - The aquatic and semiaquatic *Echinochloa grss* was present in P10

Figure 20 Examples of vegetation at the study plots

Laguncularia racemosa, a representative species of mangroves*,* is *a* tree with solitary or clustered trunk, gray bark. Leaves elliptic to oblong, margin entire, fleshy, with two glands at the adaxial part in the petiole. Inflorescence with terminal panicles, flowers unisexual 4-5 mm in diameter (Tomlinson, 1986). It has a superficial tap root system with large roots expanding horizontally, which includes a "peg-roots" bearing pneumatophores radiating from the stems at a depth of 10-20 cm (Fig. 21; Jeník, 1969).

Figure 21 Understory of a *Laguncularia* tree during the wet period, seedlings cover most of the soil and, in the left side (inside red square) near tree trunk, pneumatophores can be seen.

Laguncularia is considered a salt-tolerant specie, succulence and salt-secreting adaptations being the main mechanisms (Fig. 22), allowing tolerance for a wide range of soil salinity (Parida and Jha, 2010). Lugo & Snedaker (1974) biomass estimates for mangroves stands in Puerto Rico, are about 49,970 kg dry mass/ ha for subsurface roots, and 62,850 kg dry mass/ ha for above ground biomass (without litter). Recent studies have confirmed this specie as a bioindicator of anthropic pollution (Souza et al. 2014).

Figure 22 Close-up of *Laguncularia* succulent leaves and flowers.

Dalbergia ecastaphyllum, is a decumbent shrub, with branches reaching from 1-5 m in length. Its branched, with alternate leaves which could be composed of one or exceptionally three ovate, coriaceous leaflets (Fig. 23; Acevedo-Rodriguez, 2005). It grows in non-forested areas, forming monospecific stands. It develops a taproot and a lateral root system; A common characteristic is the nitrogen-fixing roots nodules growing on the superficial roots (Sauer et al. 2000).

Figure 23 Dalbergia ecastaphyllum flowers and seeds.

Acrostichum danaeifolium (Fig. 24) and *A. aureum (Fig. 25)* are herbaceous rhizomatous species from the ferns family (Pteridaceae) common in brackish swamps, tolerant to soil saline conditions up to 30 ppt but require freshwater for establishment. High levels of soil salinity and light conditions (understory vs open canopy) will produce changes in plant size and density (Medina et al. 1990). At Ciénaga las Cucharillas both species are present, however *Acrostichum* is more abundant and is the one that will be used in this study.

Figure 24 *Acrostichum danaeifolium* fern with all the pinnae fertile are covered by sporangia in the entire fronds.

Figure 25 *Acrostichum aureum* fern with fertile pinnae in the distal portion of the lamina~~e~~. *Acrostichum* has entire fertile fronds. Also *A. aureum* is smaller than *Acrostichum*

Grasses are herbaceous species, with fibrous root systems, like *Panicum laxum* a C3 photosynthetic pathway perennial grass (Fig. 26) with stoloniferous growth, where a horizontal stem above surface produce adventitious roots and stems arising from the nodes. It grows in wet soils and tolerates periodic floorings. It could reach 1-2 tons of biomass per 0.4 hectare, but droughts decrease productivity (Más & Garcia-Molinari, 2006) *Echinochloa polystachya* a C4 photosynthetic pathway perennial grass with decumbent erects stems that could reach 2 m height. (Fig. 27; Más & Garcia-Molinari, 2006). It is commonly found in flooding areas, and vegetative propagation is the common form of distribution.

Figure 26 Panicum inflorescence

Figure 27 *Echinochloa* inflorescence and leaves

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Plant elemental dynamics: Spatial and temporal distribution

Introduction:

Changes in the landscape for urban and industrial development and the infrastructure used to control natural hydrological processes in coastal wetlands have created novel ecosystems, with the anthropogenic imprint in edaphic conditions such as higher amounts of heavy metals because of their previous land use such as agriculture, atmospheric deposition of particulates, dumping of waste and artificial materials, and discharges of industrial waste to streams (Meuser, 2010; Olivares, 2003). Also, changes to the plant community, as urban wetlands have a higher plant diversity, by non-indigenous species related to the residential areas (Ackerman et al. 2016). These novel ecosystems exhibit various ecological traits, sometimes divergent from non-perturbed ecosystems (Pickett et al., 2001; Abelleira & Lugo, 2008).

The bioremediation technique uses hyperaccumulators for contaminant extraction of soils. A study was done by Srivastava et al. (2005) with three ferns species (*Pteris vittata*, *Pteris ensiformis,* and *Nephrolepis exaltata*) found arsenic accumulation in them (highest in *P. vittata*) without signs of toxicity. However, they had different responses in terms of antioxidative response. In the Poaceae family, *Gynerium sagittatum,* a native species of the West Indies, has been cataloged as metal accumulator~~s~~ (Madera-Parra et al., 2014), while *Avicennia germinans*, a mangrove from the Acanthaceae family, accumulates cadmium in roots and leaf tissues (Soto et al. 2020; Marchand et al., 2006). *Typha domingensis,* an herbaceous plant from the Typhaceae family, is known for its high tolerance to toxic concentrations of Al, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and

Zn and restricted translocation of Hg and Ni from sediments to leaf tissues (Bonanno & Cirelli, 2017).

Heavy metals can be present in the soil but not necessarily available to plants; thus, determination of heavy metal availability under natural conditions in the soil and the effectiveness of the differential uptake and storage in plants is vital. High concentrations of heavy metals can cause senescence in plants, affect CO_2 assimilation, induce changes in soil faunal assemblages and decrease the productivity of the soil and nutrient cycling through the ecosystems (Riffat, 2009). Some plant species accumulate heavy metals without being adversely affected (Baker, 1981). however, little is known about the ecophysiology of uptake and compartmentalization of plants under the combined parameters mentioned above; for example, mangroves like such as *Avicennia germinans* and *Rhizophora mangle* can take up and accumulate Hg, Ni, Co (Marchand et al.,2016, Soto et al. 2020) to stems and roots.

Under reduced conditions, toxic levels of some nutrient and heavy metals concentrations could occur due to the element becoming available in higher quantities; for example, the accumulation of reduced forms of Fe and Mn could reach levels that cause stress and even injury in plants (Pezeshki & DeLaune, 2012). Under aerobic conditions, Cd is found in cationic form (Cd2+), but in a negative redox potential (reduction), it is found in an insoluble inorganic form, bound to a sulfur (CdS). In oxygen-rich soils, such as wet soils that have been dried out, sulfates of persistent metals such as CdS and ZnS oxidize to form CdSO4 and ZnSO4 (Hooda, 2010).

The four-dimensional matrix hydrological conformation in wetlands: a) *in situ* precipitation, b) freshwater inputs by surface or shallow subsurface and deep subsurface flows (aquifers, basin drainage via channels, rivers, and streams), c) seawater, either via deep subsurface flow or tidal ebb and flows (Kim et al. 2005), and d) spatio-temporal dynamics of waterlogging, regulate physicochemical parameters such as phreatic level, salinity, pH and redox. This impacts the wetland's biotic composition and provokes changes in biogeochemical processes, such as decomposition, soil organic matter formation, nutrients, heavy metals uptake, immobilization, and availability to plants (Pezeshki & DeLaune, 2012).

Soto et al. (2016) measured soil heavy metal concentrations from 2013-to 2015 in three areas in the wetland parallel to the Juana Matos community, where soil material from the petrochemical refinery, located in the mid basin, was presumably used for infill and in areas with mid and no infill effects in the wetland. Heavy metal and metalloids concentrations varied among years, and infill effects with lead (Pb) ranged from 620 - 20 mg/kg), cadmium (Cd) from100 - 0 mg/kg) and arsenic (As) from120 - 0 mg/kg). These changes were related to oxidation-reduction conditions in soil because of a drought. Groundwater values at 30cm depth for Cd, As, and Pb: 2 µg/L, 8 µg/L, and 13 µg/L, respectively, were three to two orders of magnitude less than in the soil; however, they exceed the values allowed by island water quality regulations (EQB, 2016).

In a tropical urban coastal wetland, how do nutrients and heavy metals allocation compare between plant functional groups based on the spatial and temporal variations of soil elemental composition regulated by a) local rainfall, b) spatio-temporal dynamics of water quality (salinity, pH) and availability of metals derived from anthropogenic disturbances. It is hypothesized that the five species will in the area will differ in elemental concentrations to temporal and spatial variability of state factors such as soil salinity, pH, and redox in their nutrient and heavy metal uptake and accumulation. To test the hypothesis, plant-available elements, total nutrients, and heavy metals were determined during dry and wet seasons in surficial soils and plant tissues: roots and leaves for the following elements: C, N, P, K, Ca, Mg; Transition metals Fe, Mn, Cu, Zn, Pb, Cd, and post-transition metal Al.

Methods:

Based on the two types of soils present in the study area: "Saladar muck" (*Sm*), which is mostly organic, undisturbed soils, and Martin Peña (*Mp*) soils (Fig. 17), which are organic soils embedded with mineral soils from allochthonous origin; we chose two plots in each soil type, one close to the Malaria channel, a freshwater input and another one closer to the coast, influenced by saltwater intrusion. The four plots had differences in mean water levels, however they followed similar temporal trends (see Fig 15.) At each plot there were present at least three species of interest (Fig 19). Plots P3 and P5 are considered Mp soils, as this were disturbed soils, where a shantytown was established during the last century. P6 and P10 are considered Saladar muck “Sm” soils, are these soils were minimally disturbed and are composed mostly of organic material in decomposition.

We assessed at each plot: *Panicum ,* perennial grass with intensive root growth in Plots 3,5,6; *Echinochloa*, a C4 grass only present in Plot 10*; Acrostichum,* an herbaceous, rhizomatous species present in all plots (P3, P5, P6, P10); *Laguncularia* a halophytic tree present in all plots (P3, P5, P6, P10) *;* and *Dalbergia* only present in the organic autochthonous soils *(*P6, P10*)* a scandent or decumbent shrub. We chose these species as representatives of the plant functional groups in each plot based on their abundance.

Sampling

To determine differences between the two soil types and the two plots within each soil type present in the study area, three soil samples from the first 10 cm were used for posterior analyses. In each plot, one soil core down to 10 cm using a Russian peat borer was collected near each plant functional type. The cores were placed in halved same diameter PC pipes, wrapped tightly in plastic wrap to prevent desiccation, and stored at 4°C in the laboratory until processed. Soil salinity for the first 10 centimeters, was measured in the Process and Functioning of Tropical Ecosystems laboratory at the University of Puerto Rico, with a conductivity meter using air dried soil in a 1:1 soil-to-water ratio mixture, and pH was measured with a pH meter (Orion instruments) using air dried soil from the first 10 cm. Soil pH was averaged using the following formula: $-\log10[(\Sigma_{\mathrm{Ci}})/(n)]$, where C is the concentration of hydrogen ions and n is the number of measurements. Laboratory procedures were done by graduate student Gloria Ortíz.

Depth of the water table was measured hourly in a monitoring well located in the plot using HOBO water level data loggers (Onset corp), which recorded absolute pressure, barometric pressure, and temperature, giving water level measurements with a typical error of ± 0.1 % or 0.4 cm. Hourly measurements were taken continuously from 2017 to the present, this study only presenting from 2020 to 2021; gaps in the data product from equipment malfunction. Phreatic water conductivity was measured hourly in a monitoring well located in the plot using a HOBO Saltwater conductivity logger (Onset Corp); measuring conductivity, temperature, and specific conductance at 25C (calculated) with a range of 5000 to 55,000 uS/cm with a resolution of 2 uS/cm and an accuracy of 5% of reading. Hourly measurements were taken continuously, and gaps in the data was caused by equipment malfunction.

Based on the preliminary chemical results, the average water depth (7 cm, Fig 10) and previous fine root depth studies - where it was found that 56% of biomass of fine live roots are present in the top 10cm of soil (Weber 2014, unpublished); we only analyzed the upper 10 cm of soil around the assessed species; at the peak of the wet period (September 2020) and during the dry period (May 2021). Three to five adult fully expanded leaves were collected in two wet periods (September 2020 and November 2020), humid (March 2021), and dry (May 2021). Climate variability was expressed in the sampling dates, as March, normally the peak of the dry season, was humid, and May, which normally is the beginning of the rainy season (humid), was dry.

Soil sample preparation and analyses

Soil samples for exchangeable element analysis of Na, Mg, Al, K, Ca, P, Mn, Fe, Cu, Zn, Cd, and Pb were sent to the ICP-MS Metals Lab at the Department of Geology and Geophysics, University of Utah. A leaching procedure was performed where 200 mg of air-dried soil was weighed and added 4 mL of 1 molar ammonium acetate buffered to pH 7, stirred, and left overnight. Samples were centrifuged at 5000 rpm for 5 minutes and the supernatant was withdrawn for analysis. This procedure allows the extraction of water-soluble elements and displacement of adsorbed cations from the soil fine particles. Samples were run in a quadrupole inductively coupled plasma mass spectrometer (ICP-MS). Standard Reference Material (SRM) 1643e Trace elements in water of the National Institute of Standards and Technology, USA) was used. (Goodman et al., 2019). Soil available elements were expressed in mg/kg and converted to mmol/kg using the molar mass of each element.

Carbon and Nitrogen isotope analyses ($\delta^{13}C$, $\delta^{15}N$) and C (%) and N (%), of soil and plant samples were done at the Stable Isotope Ratio Facility for Environmental Research at the Department of Geology and Geophysics, University of Utah. Plant samples were grounded with a planetary ball mill (Retsch) and soils were grounded with a mortar and pestle until passing over a 20-mesh sieve, a 5.00 mg subsample for each soil sample was taken for analyses. The Primary Standard used for $^{13}C/^{13}C$ reference was the Vienna Pee Dee Belemnite (VPDB) with a molar ratio of 0.0112372 and for $\delta^{15}N$ was atmospheric air. Soil Carbon was used to estimate soil organic matter using the following formula: SOM = C x 1.72. Cation exchange capacity was estimated by summing the cations extracted Ca, Mg, K and Na values and converting to meq/g. Conversion factor is 1 mmol/kg = 1 meq/kg. Effective Cation exchange capacity was estimated by summing the cations extracted Ca, Mg, K, Na and Al values and converting to meq/kg.

Plant sample preparation and measurements

Elemental analysis of Na, Mg, Al, K, Ca, P, Mn, Fe, Cu, Zn, Cd, and Pb in leaf samples were conducted at ICP-MS Metals Lab at the Department of Geology and Geophysics, University of Utah. Leaf tissues (0.5g dry mass) in porcelain crucibles were and burned in a muffle furnace for 8 hours at 550 C, after cooling ashes were weighed and dissolved in 4mL of 5 % HNO_3. Crucibles were rinsed with 5% HNO_3 and added to a final volume of 10 mL of 5% HNO_3. Samples were centrifuged at 3000 rpm for 5 min, and an aliquot of 0.5 mL of the supernatant diluted to 10mL. Samples were run in quadrupole inductively coupled plasma mass spectrometer (ICP-MS). Standard Reference Material (SRM) 1643e Trace elements in water of the National Institute of Standards and Technology, USA was used (Bitter et al., 2020). Plant total elements were expressed in mg/kg and converted to mmol/kg using the molar mass of each element.

Leaf/Soil concentration ratios (LSR) in mmol/kg were calculated to determine relative plant accumulation or exclusion (Medina et al., 2007). A leaf/soil ratio over >1 would mean relative accumulation. Leaf/soil elements concentration ratios were calculated using the equation:

$$\text{LSR} = \text{Cation}_{\text{Leaf}} / \text{Cations}_{\text{Available Soil}} \qquad (1)$$

Selective cation uptake was calculated to determine salt-resistant species in the wetland by using the K/Na cation selectivity (S) trait (Tsialtas et al. 2017):

$$S= (K/Na)_{\text{Leaf}} / (K/Na)_{\text{Available Soil}} \quad (2)$$

If K/Na is higher than 1, indicates preferential absorption of K over Na and it is directly related to resistant to salinity. Soil Calcium: Magnesium ratios were calculated to determine soil parent material influences in the wetland using the Ca/Mg ratio in the first 10 cm of soil. (Medina et al. 2007).

Temporal ion adsorption dynamics using Plant Root Simulator (PRS®) probes

Ions measured by PRS probes reflect availability in the soil solution and are reported as µg/10cm2/24hrs because the rate of ion sorption to the membranes is asymptotic, not allowing it to be divided into subunits of time. In each plot, two sets (with duplicates at different places inside the plot) of Plant Roots Simulator (PRS®) probes are ion exchange resin membranes (Western Ag Innovations, Saskatoon, SK) consisting of three pairs of anion probes with a positively charged membrane and cation probes with a negatively charged membrane that measure ions supply in soils. PRS® probes were buried at 5 cm with a burial period of 24 hours to assess membranes ion adsorption dynamics in time. After exposure in soil, PRS probes were taken out, cleaned with a soft brush, and distilled deionized water, separated in sealed plastic bags with labels, and sent for analysis at Western AG Innovations in compliance with return protocol. Ammonium (NH_4) and nitrate (NH_3) were determined colorimetrically using automated flow injection analysis with a Skalar San++ Analyzer (Skalar Inc., Netherlands) and P, K, S, Ca, Mg, Al, Fe, Mn, Cu, Zn, and B were measured using inductively coupled plasma (ICP) spectrometry (Optima ICP-OES 8300, PerkinElmer Inc., USA) at the Western Ag Innovations facilities.

Results:

Table 3 Soil characterization, for the plots during wet and dry periods. Soil differences between plots were assessed using ANOVA if p value was less than 0.05 a post-hoc test Tukey HSD was performed. Soil differences between date periods were assessed using a t-test, if p value < 0.05. Values are displayed as average ± standard deviation.

	Mineral/organic (*Mp*)				**Organic soils (*Sm*)**					
	Plot 3		**Plot 5**		**Plot 6**		**Plot 10**		**p-val plots**	**p-val date**
Period	**Wet *n*=3**	**Dry *n*=3**	**Wet *n*=3**	**Dry *n*=3**	**Wet *n*=4**	**Dry *n*=3**	**Wet *n*=4**	**Dry *n*=3**		
SOM %	35.3±15	na	31.3± 7	na	36.7± 11	na	32.9± 15	na	0.92	na
pH (1:1)	4.76	6.1	4.64	5.2	4.6	5.6	4.8	6.1	0.54	<0.01
CEC mequiv/g	13896 ± 3885	11195 ± 314	9209± 2341	8029± 1221	1198± 7568	10666± 5604	8226± 1115	9383± 1693	0.13	0.50
ECEC mequiv/g	13907 ± 3882	11198 ± 3882	9215 ± 2342	8031 ± 1220	11986 ± 7567	10668 ± 5603	8231 ± 1113	9386 ± 1694	0.13	0.49
Soil Salinity (1:1) ppt	3.4±2	4±0.4	2.8± 1	12± 2	2.4± 2	11± 5	2.1±2	7±1	0.62	<0.01
$\delta\ ^{13}C$ ‰	-30.6± 4	na	-23.3± 2	na	-26.5± 0.2	na	-26.8 ± 0.67	na	<0.01	na

δ ^{15}N $^{0}/_{00}$	3.8± 0.8	na	5.1± 2	na	1.9± 1	na	3.3± 1	na	<0.03	na
Total C mg/g	20.5± 9	na	18.2 ± 4	na	21.3 ± 6	na	19.2± 9	na	0.92	
Total N mg/g	1.3± 0.6	na	1± 0.2	na	1 ± 0.2	na	1.1± 0.7	na	0.85	
Available Na mmol/kg	944 ± 359	545 ± 272	636 ± 193	481± 77	785± 559	610 ± 392	457 ± 59	487 ± 124	0.35	0.15
Available P mmol/kg	0.46 ± 0.22	0.36 ± 0.10	0.20 ± 0.09	0.53 ± 0.21	0.14 ± 0.03	0.45 ± 0.16	0.57 ± 0.43	0.76 ± 0.06	0.08	0.12
Available K mmol/kg	13.1 ± 3	11.6 ± 1	12.9 ± 3	14.4 ± 10	10.7 ± 1	13.9 ± 4	12.3 ± 2.4	12 ± 2.9	0.92	0.74
Available Ca mmol/kg	209 ± 20	226 ± 12	115 ± 20	168 ± 47	194 ± 66	267 ± 104	201 ± 57	265 ± 18	0.14	<0.05
Available Mg mmol/kg	222 ± 50	147 ± 53	155 ± 34	138 ± 16	208 ± 130	174 ± 80	151 ± 19	173 ± 27	0.55	0.30
Available Al mmol/kg	1 ± 0.4	0.18 ± 0.06	0.5 ± 0.1	0.3 ± 0.06	0.5 ± 0.1	0.17 ± 0.02	0.55 ± 0.26	0.29 ± 0.06	0.44	<0.01
Available Mn mmol/kg	1.1 ± 0.2	0.6 ± 0.2	0.55 ± 0.3	0.42 ± 0.2	0.82 ± 0.4	0.53 ± 0.1	1 ± 1	0.45 ± 0.07	0.54	<0.03

Available Fe mmol/kg	2.2 ± 1	0.42 ± 0.33	0.36 ± 0.28	1.2 ± 0.7	0.6 ± 0.3	0.6 ± 0.1	0.76 ± 0.16	0.56 ± 0.12	0.35	0.33
Available Cu mmol/kg	0.02 ± 0.002	0.02 ± 0.003	0.04 ± 0.02	0.12 ± 0.11	0.01 ± 0.009	0.02 ± 0.002	0.004 ± 0.002	0.01 ± 0.003	<0.04	0.23
Available Zn mmol/kg	0.35 ± 0.13	0.15 ± 0.02	1 ± 0.56	0.47 ± 0.04	0.40 ± 0.16	0.09 ± 0.04	0.19 ± 0.08	0.1 ± 0.01	<0.003	<0.03
Available Cd mmol/kg	0.001	0	0.002	0.001	0.002	0	0	0	0.06	<0.01
Available Pb mmol/kg	0.05 ± 0.02	0.001 ± 0.0006	0.11 ± 0.1	0.03 ± 0.05	0.026 ± 0.03	0.001 ± 0.0006	0.015 ± 0.01	0.001 ± 0.0006	0.09	<0.03

Spatial and temporal elemental variability in soils**:**

Soil organic matter (SOM) ranged from 31.3 to 35.3 within plots and dates without statistical differences (Table 3). Soil salinity averaged ten ppt during the dry period and March 2021 and 2 ppt in May 2021 (Table 3) with statistical significance and relates to the complex hydrological conditions of the area. During the wet period, this water comes not only by rainfall but also tidal influences in the phreatic water, when waterlogging conditions are present. Exchangeable Na correlated positively with Exchangeable Mg ($r2=0.36$ $p<0.05$) and Exchangeable Ca (Fig. 28) during the dry period (March 2021) but not calcium. During the wet period (July 2021), Exchangeable Na correlated positively with Ca and Mg. We found a negative correlation between Exchangeable Na and Exchangeable P (Fig. 29).

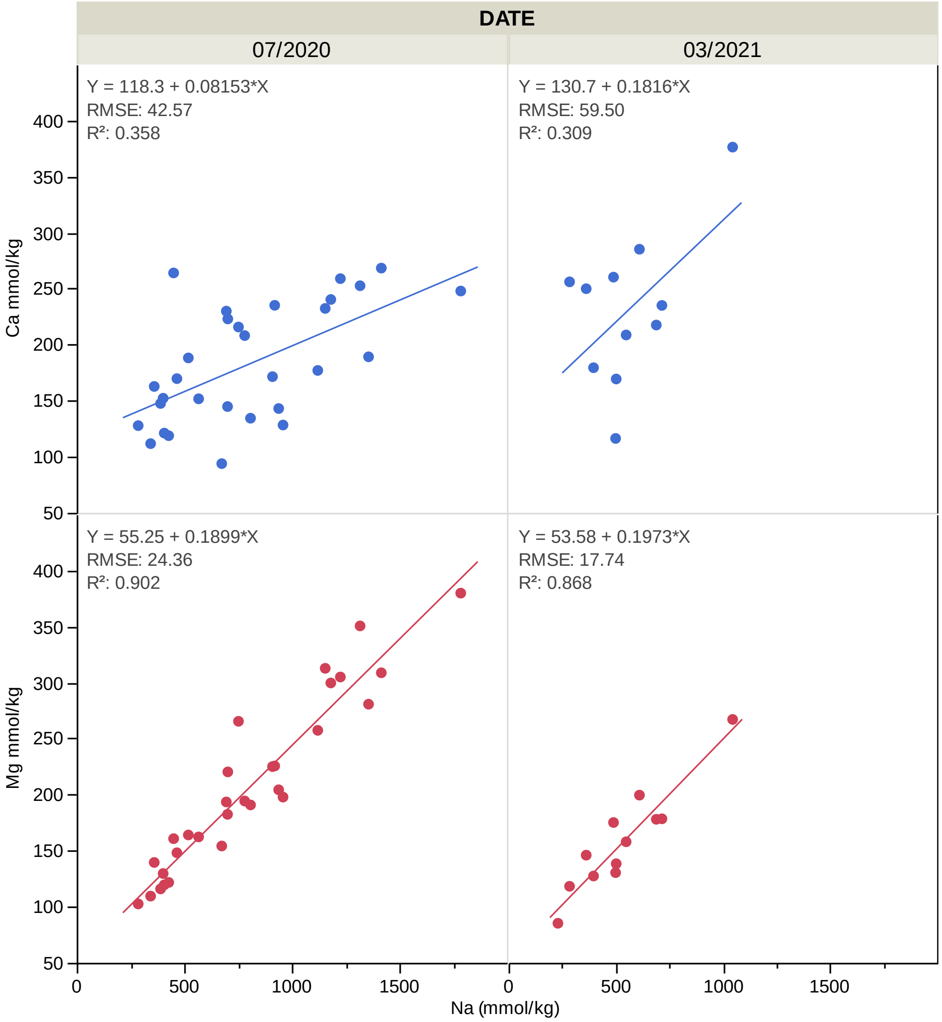


Figure 28 Soil exchangeable Na) in X axis correlations with a) Soil Exchangeable Ca and c) Soil Exchangeable Mg. Equations and r-square inside graphs.

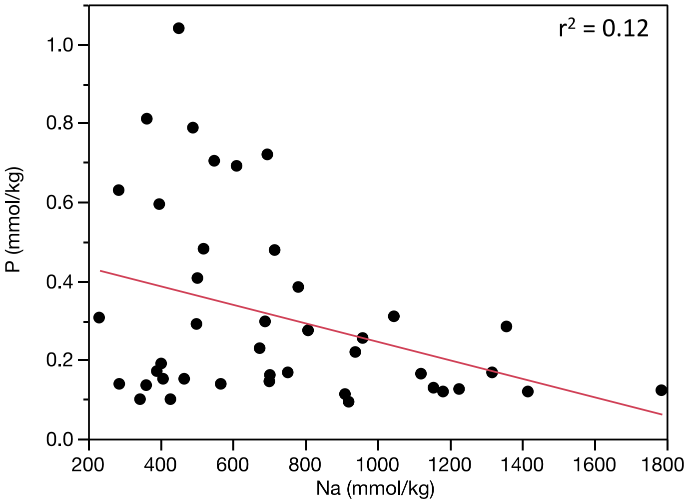


Figure 29 Soil Exchangeable P (Y-axis) correlation with Exchangeable Na (mmol/kg).

During July 2021 soil pH averaged (6.1±0.3) and March 2021 (5.8±0.6) with no statistical difference, possibly related to the rainfall inputs. It is helpful to remind that pH measurements in March were performed last week after the copious rainfall reported, explaining the variability in pH values, as this period usually has lower precipitation. During the May season, as precipitation decreased, soils became more acidic, averaging 4.7 (Fig.30).

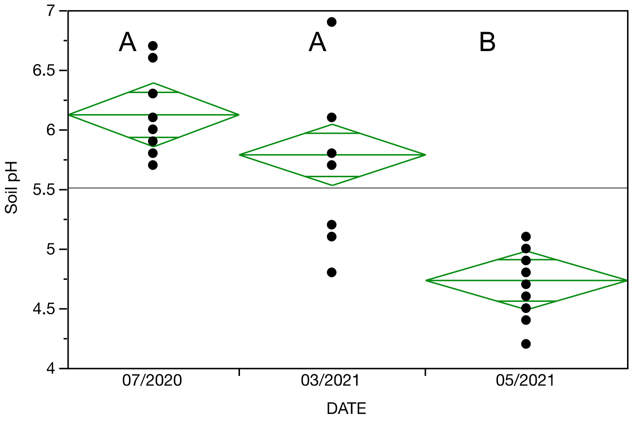


Figure 30 Soil pH temporal variability. ANOVA with post hoc Tukey HSD analysis was performed. Letters denote statistical differences

Soil Carbon Concentration in the first 10 cm ranged from 18 mg/g to 20.5 mg/g within plots without significant differences. The δ13C isotopic signature ranged from -23.3 $^{0}/_{00}$ in P5 to -30.6 $^{0}/_{00}$ in P3. δ13C was higher in plot 5 than in plot 3 (Table 4). Values from P3 are more negative than those reported from Brazilian mangroves soils (Tognella et al. 2016) and brackish wetlands in Venezuela (Medina et al. 2007). They could reflect the present and previous non-mangrove vegetation in the sites, which includes *Laguncularia* (-27 to -29 $^{0}/_{00}$) *Acrostichum* (-28 $^{0}/_{00}$), and *Panicum* (-27 $^{0}/_{00}$). More positive values points to C4 vegetation such as *Echinochloa* with its isotopic signature closer to -14 $^{0}/_{00}$. Isotopic signatures of N in soils were positive but with high variability ranging from 0.9 $^{0}/_{00}$ in P6 to 6.5 $^{0}/_{00}$ in P5. It was significantly higher in P5 than in P6 and P10, and P3 was between (Fig.29). Upper soil layers (first 10 cm) have high ^{15}N fractionation, possibly meaning higher decomposition rates due to mineralization and denitrification. Higher δ^{15}N values also are related to anthropogenic inputs (Medina et al. 2007), as this seems to be the case in P5, the area closest to the urban areas. Nitrogen concentration ranged from 1 to 1.3 mg/g within plots without significant differences.

Sodium in soils exhibited no statistical difference between dates/sites, ranging from 457 mmol/kg at the plot closest to the coastline to 944 mmol/kg at the plot further inland. Phosphorus was available in low concentrations in the soil, ranging from 0.14 to 0.76 mmol/kg, with no statistical difference between dates or sites. It was found a slight correlation between soil exchangeable-P and Ca (Fig. 31). Available P in the soil solution as measured by PRS ® probes, P correlates with Fe (Fig. 31).

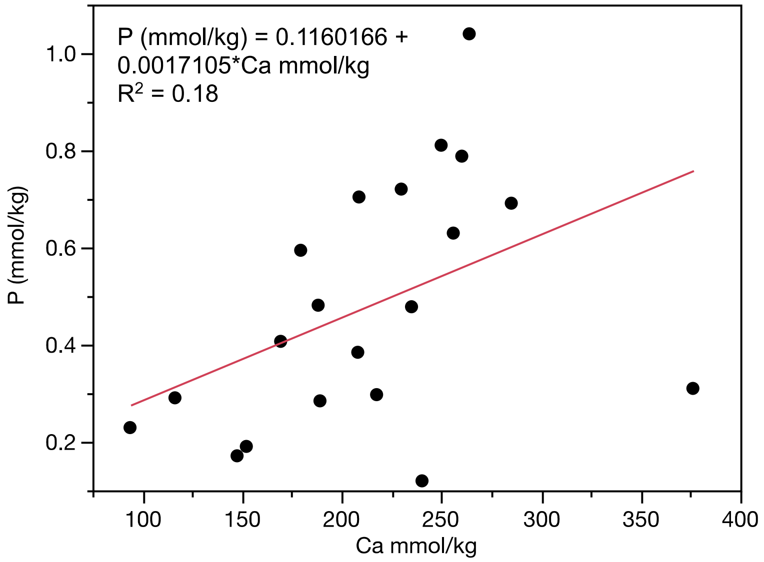


Figure 31 Soil Exchangeable P correlation with Soil Exchangeable Ca (mmol/kg).

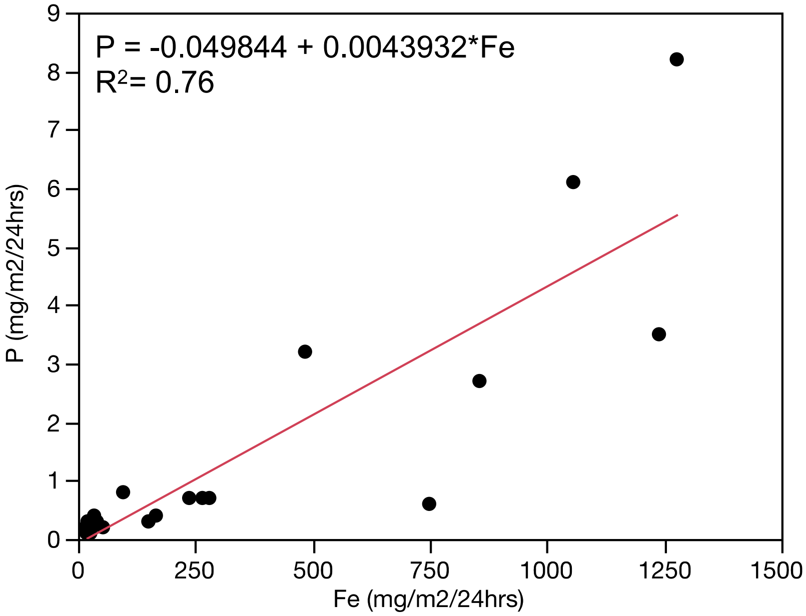


Figure 32 Available P (PRS Probes) correlation with Available Fe (PRS Probes).

Potassium ranged from 10.7 to14.4 mmol/kg without differences within plots or sites. Exchangeable Calcium was significantly lower in Plot 5 than in P6 and P3, possibly reflecting the soil parental material influence (Table 4). Exchangeable Ca was lower during the wet period (mean = 178 mmol/kg) than in the dry period, which averaged 232 mmol/kg; but it was not statistically significant, much variability within plots can be seen (Fig.33). In most soils, Ca is adsorbed to colloids in soils, but a small fraction goes to the soil solution (Maathius 2012). However, in high pH soils, Ca becomes more available to plants, as observed in this study.

Table 4 Spatial differences of δ 13C, δ 15N, Cu and Zn in soil samples among study plots.

	Organic/mineral soils (*Mp*)		**Organic soils (*Sm*)**		**Statistical test**
	P3	P5	P6	P10	
	n=6	n=6	n=8	n=8	
δ 13C $^{0}/_{00}$	-28.35 ±0.78 **b**	-23.05 ±0.5 **b**	-26.45 ±0.07 **a**	-27.35 ±0.35 **b**	Anova/Tukey
δ 15N $^{0}/_{00}$	4.3 ±0.3 **ab**	6.2 ±0.07 **a**	2.6 ±1.5 **b**	4.45 ±0.8 **ab**	Anova/Tukey
Cu (mmol/kg)	0.02 ±0.00 **b**	0.11 ±0.10 **a**	0.02 ±0.01 **b**	0.01 ±0.01 **b**	Anova/Tukey
Zn (mmol/kg)	0.25 ±0.14 **ab**	0.46 ±0.04 **a**	0.21 ±0.19 **b**	0.14 ±0.07 **b**	Anova/Tukey

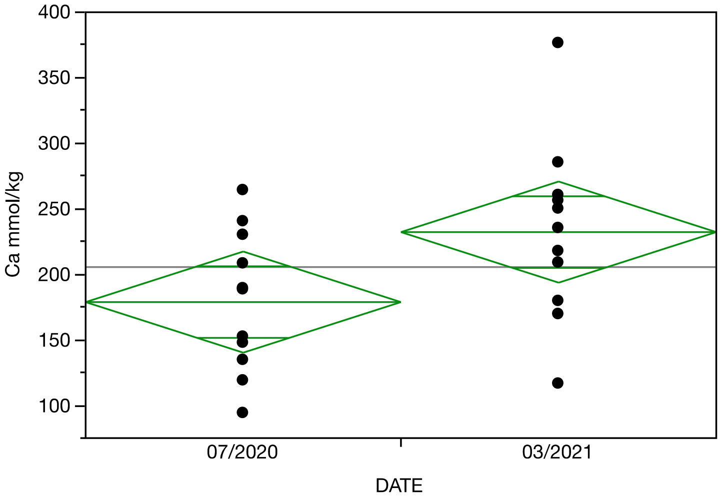


Figure 33 Exchangeable Calcium (mmol/kg) in soils in two periods. A student t-test was performed to determine statistical differences

Magnesium ranged from 151 to 267 mmol/kg without differences within plots or sites.

Copper was significantly higher in P5 than in P3 and P10 (Table 4).

Zinc is one of the metals most abundant in soils worldwide, with available concentrations of 0.15 to 1.53 mmol/kg (10-100 mg kg^{-1} total concentration), and the main anthropogenic inputs are atmospheric depositions and the use of fertilizers in agricultural practices (Mertens & Smolders, 2013). Zinc was higher during the wet period than in the dry period; however, it had a high intra-variability within plots (Table 4). Zinc was significantly higher in P5 than in the other plots.

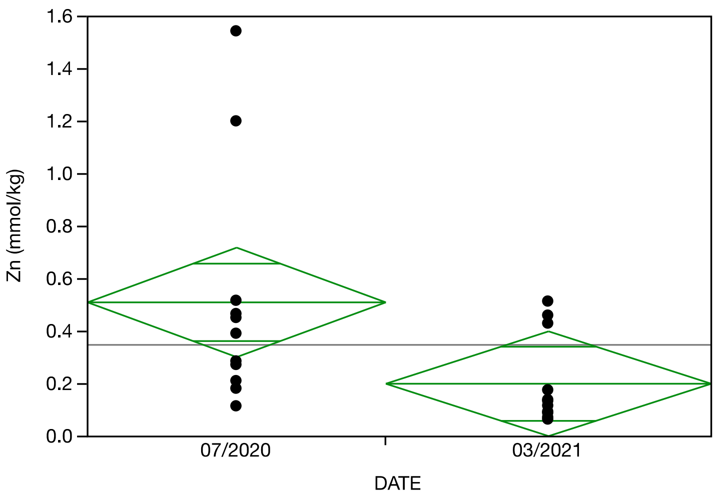


Figure 34 Soil Exchangeable Zn (mmol/kg) averages in the first 10 cm per date

Exchangeable Aluminum was significantly higher during the wet period, reflecting the high availability of this metal compared to the dry period (Fig. 35).

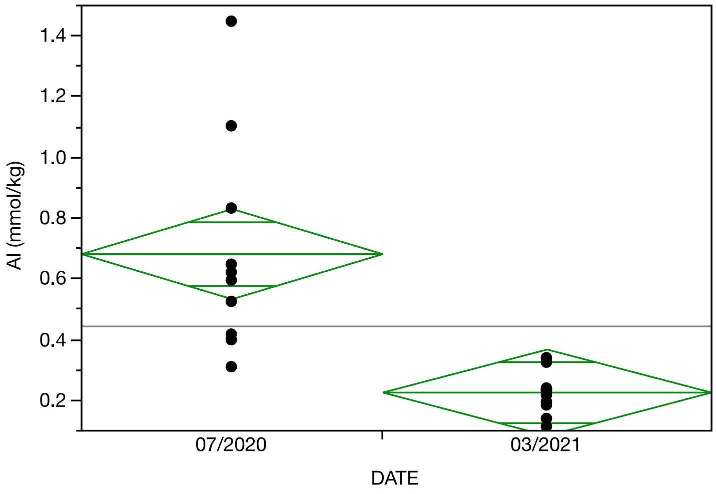


Figure 35 Soil exchangeable Al during wet and dry periods. A student t-test was performed to determine statistical differences

Exchangeable Manganese average during the wet period was significantly higher than during the dry period (Fig. 36).

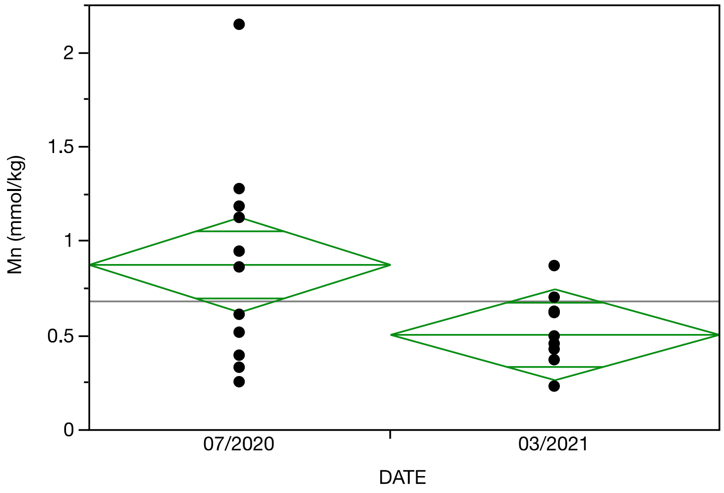


Figure 36 Soil Exchangeable Mn (mmol/kg) averages in the first 10 cm per date. A student t-test was performed to determine statistical differences

Exchangeable Cadmium, a heavy metal, differs significantly between periods, being statistically higher in the wet period, but the concentrations are relatively small (Fig. 37).

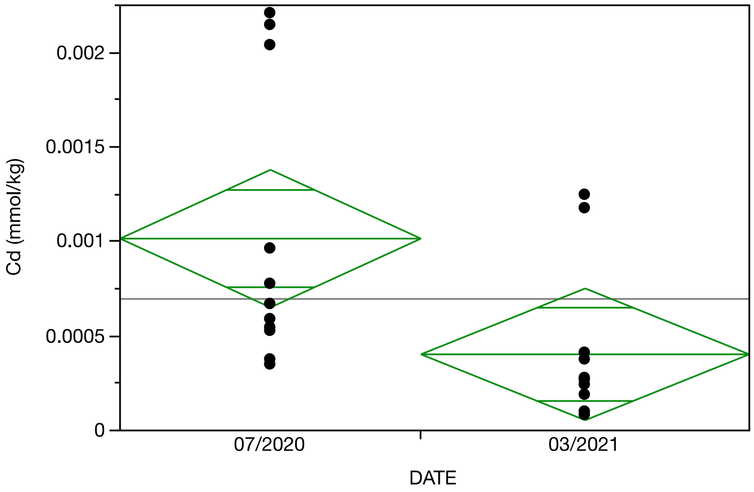


Figure 37 Soil Exchangeable Cd (mmol/kg) averages in the first 10 cm per date A student t-test was performed to determine statistical differences

Exchangeable lead also was statistically higher during the dry period (Fig. 38). Our results show higher concentrations of Pb in Cucharillas, with a mean of 0.24 ranging from 0.006 to 0.96 mmol/kg. Our site's most polluted area is Plot 5, with an urban allochthonous infill. Available Pb had a median of 0.235mg m^2 in average from the three sampling dates, ranging from 0 to 1.8 mg m^2 in a 24-hour burial period. Under oxidized and low pH soil conditions Pb becomes mobile as Pb^{2+} ion.

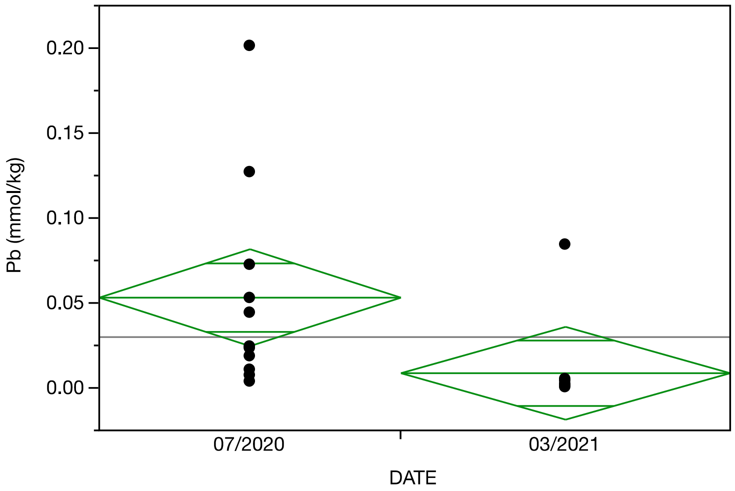


Figure 38. Soil Exchangeable Pb (mmol/kg) averages in the first 10 cm per date A student t-test was performed to determine statistical differences

Exchangeable Iron did not differ significantly between plots or dates.

Soil ratios:

Na/K ratios averaged 50 within all plots, similar to seawater ratio (~46) (Tognella et al. 2016), with no significant differences within plots or dates. This indicates although the wetland is in a humid climate zone, seawater influences the soil chemistry, and the rainfall does not significantly counteract Na accumulation in the superficial soil layer. Ca/Mg ratio averaged 2 in all plots ranging from 1.6 in P5 to 2.4 in P10 without a statistical difference. However, it was statistically higher in the dry period (~2) than in the wet period (ratio ~ 1.4). Soil Ca/Mg ratio is used widely as a proxy of soil fertility, whereas values lower than 1 could mean poor nutrient soils (Schulte and Keling 1985; Stevens et al. 2005)

Table 5 Readily Available Nutrient and spatial supply patterns (PRS Probes)

Nutrient	Median adsorption (range) (mg/m^2/24hrs)	p-value site	p-value date	Interaction
Nitrate (NO_3-N)	3.6 (0 to 27.8)	<0.01	<0.0006	<0.0001
Ammonia (NH_4-N)	6.4 (1 to 36.7)	<0.035	<0.001	<0.0001
Calcium (Ca)	722.5 (206 to 1431)	0.68	<0.001	<0.0001
Magnesium (Mg)	402.5 (142 to 686)	0.52	<0.001	<0.0001
Potassium (K)	42.5 (9 to 124)	0.78	<0.001	<0.001
Phosphorus (P)		0.20	<0.007	<0.009
Iron (Fe)	174 (16 to 1277)	0.16	<0.001	<0.001
Manganese (Mn)	4.9 (0.4 to 36.7)	0.07	<0.001	<0.004
Copper (Cu)	0.2 (0 to 2.48)	<0.001	<0.0001	<0.0001
Zinc (Zn)	1.7 (0.4 to 11.2)	<0.01	<0.003	<0.0022
(B)	0.6 (0.1 to 12.7	0.68	0.15	0.39
Sulfur (S)	177 (33 to 359)	0.39	<0.0001	<0.001
Lead (Pb)	0.235 (0 to 1.8)	<0.005	0.08	<0.002
Aluminum	13.35 (7 to 63.8)	0.74	0.24	0.54
Cadmium (Cd)	0	ns	ns	ns

Spatial and temporal elemental variability in plants:

Intraspecific variability

First, it will be described the intraspecific variability within each specie and then interspecific comparisons among species. *Acrostichum* C (mg/g) was significantly higher during March and May 2021 than in July and September 2020 without differences between plots (Table 7). Leaf concentrations of N were also higher during March and May 2021 than in July 2020. Leaf Na averaged 456±142 mmol/kg without statistical differences between dates or sites. Leaf K Averaged 399 ± 172 without statistical differences between dates or sites. Leaf Ca differed between dates, in March 2021 is significantly higher (154 mmol/kg) than May 2021 (74 mmol/kg). Magnesium averaged 103± 25 mmol/kg without statistical differences between dates or sites. Aluminum averaged 4.5± 11 mmol/kg without statistical differences between dates or sites. Phosphorus was significantly higher during November 2020 (96.6 ±33 mmol/kg) than in March 2021 (54.3 ± 10 mmol/kg). Manganese averaged 1.18 ± 0.48 mmol/kg without statistical differences between dates or sites. Iron averaged 5.6± 12 mmol/kg without statistical differences between dates or sites. Leaf Iron concentrations were significantly higher during March 2021, averaging 17 ± 24 mmol/kg. Copper leaves concentration averaged 0.19± 0.11 mmol/kg without statistical differences between dates or sites. Zinc leaf concentrations were significantly higher in Plot 5 (0.8±0.4 mmol/kg) than in the rest of the sites, reflecting the soil composition, as soil samples also had significant amounts of Zinc in this plot. There were insignificant amounts of Cd in leaf concentrations. Lead, albeit in small concentrations, was statistically higher in leaves during March 2021, averaging 0.01± 0.01 mmol/kg.

Table 6 *Acrostichum* average leaf element concentrations. (n= 4 individuals per date), p-value <0.05 denotes statistical difference between averages.

Acrostichum danaeifolium														
	P3			P5			P6			P10			p-val date	p-val sites
	Nov-20	Mar-21	May-21	Nov-20	Mar-21	May-21	Nov-20	Mar-21	May-21	Nov-20	Mar-21	May-21		
mg/g														
C	**39.6**	**36.3**	**45.5**	**37.5**	**44.1**	**46.4**	**41.3**	**48.6**	**48.5**		**46.4**	**48.4**	<0.001	0.23
N	2.6	2.3	3.5	2.7	3.1	2.9	2.8	3.4	3.7	na	3.3	**2.6**	<0.001	0.35
mmol/kg														
Na	396.58	818.41	633.93	423.33	447.55	628.41	354.97	475.91	423.10	440.52	522.06	320.40	0.1	0.52
K	449.23	635.81	501.25	372.54	323.39	418.48	403.04	372.06	493.86	230.73	239.63	469.66	0.66	0.37
Ca	95.08	158.98	38.02	97.18	194.24	123.93	87.24	92.47	62.33	132.63	170.33	62.65	<0.04	0.55
Mg	107.17	120.06	69.49	97.91	163.34	107.43	105.99	88.75	69.49	115.55	117.05	72.17	0.06	0.77
Al	1.99	2.26	1.78	1.63	1.41	2.45	0.93	51.70	1.48	1.76	5.67	0.93	0.1	0.54
P	88.23	62.71	73.01	92.85	39.07	76.62	125.08	54.92	74.88	71.88	60.45	64.87	<0.03	0.44
Mn	1.24	1.38	0.66	0.87	2.29	1.53	1.27	1.55	0.96	1.27	0.78	0.53	0.22	0.77
Fe	2.80	3.77	1.73	3.05	4.73	1.84	2.67	53.92	1.49	3.01	6.73	1.26	0.07	0.52
Cu	0.19	0.13	0.32	0.14	0.10	0.14	0.28	0.32	0.20	0.13	0.15	0.13	0.96	0.2
Zn	0.36	0.36	0.25	0.55	1.37	0.99	0.43	0.55	0.41	0.33	0.49	0.25	0.27	<0.01
Cd		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00	0.35	0.47
Pb	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.02	0.00	<0.002	0.64
K/Na	1.15	0.78	0.79	0.91	0.72	0.67	2.02	0.78	1.17	0.52	0.46	1.47		
Ca/Mg	1.41	2.18	0.90	1.59	1.96	1.90	1.31	1.72	1.48	1.82	2.40	1.43		

Dalbergia C (mg/g) was statistically higher during March 2021 than in June 2020 (Table 8). Leaf concentrations of N were significantly higher during March 2021 and June 2020 than in November 2020 and was higher in P6 (2.8 ± 0.5 mg/g) than P 10 (2.2 ± 0.4 mg/g). Leaf Na concentration was higher during May 2021, averaging 74± 51 mmol/kg than September 2020 (21± 10 mmol/kg). Leaf K was statistically higher in Plot 6, averaging (221 ±30 mmol/kg than P10 (151±20 mmol/kg). Both plots are autochthonous organic soils, but P10 is closer to the shoreline. Leaf Ca differed between dates, where it was significantly higher during March (291 ± 10 mmol/kg and May 2021 (276 ± 88 mmol/kg) and lower during September (85 ± 17 mmol/kg) and November 2020 (164 ± 46 mmol/kg). Leaf concentrations of Magnesium were significantly higher during November 2020 (97± 6 mmol/kg) and May 2021 (95 ± 21 mmol/kg) than in September 2020 (63 ± 8 mmol/kg). Aluminum leaf concentrations were slightly higher during September (1.5 ± 0.4 mmol/kg) and May 2020 (2.2 ± 0.5 mmol/kg) than November 2020

(0.6 ± 0.1 mmol/kg) with no differences within sites. Phosphorus was slightly higher in P6 (49 ± 5 mmol/kg) than P10 (41 ± 3 mmol/kg) without differences within dates. Manganese averaged 7.2 ± 2 mmol/kg without statistical differences between dates or sites. Iron averaged 2.9 ± 2 mmol/kg without statistical differences between dates or sites. Copper leaf concentration was slightly higher in P10 (0.11 mmol/kg) than P6 (0.07 ± 0.008 mmol/kg) Zinc leaf concentration averaged 1.2 + 0.4 mmol/kg without statistical differences between dates or sites. There were insignificant amounts of Cd in leaf concentrations averaging (0.001 mmol/kg). Lead averaged 0.001 ± 0.001 mmol/kg without statistical differences between dates or sites.

Table 7 *Dalbergia* average leaf element concentrations. (n= 3 individuals per date), p-value <0.05 denotes statistical difference between averages.

Dalbergia ecastaphyllum									
	P10				**P6**			**pval date**	**pval sites**
	Sep-20	**Nov-20**	**Mar-21**	**May-21**	**Sep-20**	**Mar-21**	**May-21**		
mg/g									
C	46.7	45.7	52.2	52.1	43	51.9	53.3	<0.006	0.36
N	**2.4**	**1.9**	**2.8**	**2.7**	**3**	**2.9**	**3**	<0.001	<0.004
mmol/kg									
Na	30.43	25.10	81.65	110.27	12.99	53.85	37.76	<0.02	0.27
K	158.89	137.09	163.79	160.52	228.50	171.80	249.42	0.21	<0.0004
Ca	83.07	164.01	284.16	213.50	88.61	299.38	339.05	<0.002	0.65
Mg	61.18	97.15	71.51	80.56	64.86	86.81	111.17	<0.004	0.99
Al	1.22	0.64	0.93	2.56	1.78	1.56	1.89	<0.006	0.06
P	40.64	45.09	39.13	38.91	50.88	40.36	52.73	0.6	<0.007
Mn	5.58	6.43	8.39	6.92	6.67	10.99	11.30	0.13	0.07
Fe	2.69	1.91	9.34	2.46	2.32	3.75	1.86	0.07	0.57
Cu	0.101	0.124	0.115	0.094	0.077	0.066	0.071	0.3	<0.01
Zn	0.985	1.551	1.126	1.060	0.821	1.731	1.548	0.07	0.74
Cd			0.00044	0.00053		0.00071	0.00098	0.56	0.12
Pb	0.00250	0.00040	0.00150	0.00058	0.00184	0.00048	0.00043	0.13	0.93
K/Na ratios	5.33	5.65	2.01	1.46	19.32	3.19	6.61		
Ca/Mg	2.22	2.76	6.55	4.37	2.23	5.69	5.03		

Laguncularia leaf C (mg/g) was higher during March (44 ± 3 mg/g) and May 2021(44 ± 3 mg/g) than June (39 ± 3 mg/g) and September 2020 (39 ± 3 mg/g) (Table 9). Leaf concentrations of N were slightly higher during March 2021 (1.6 ± 0.2 mg/g) than the other dates, without plot differences. Leaf Na concentration was higher in P5 (421 ± 121 mmol/kg) than P10 (192 ± 49 mmol/kg). Leaf K was higher in P6 (308 ± 151 mmol/kg) than in P10 (148 ± 27 mmol/kg). Both plots are autochthonous organic soils, but P10 is closer to the shoreline. Leaf Ca differed between dates, significantly higher during March 2021 (541 ± 205 mmol/kg) than in September 2020 (267 ± 88 mmol/kg). Leaf concentrations of Magnesium were significantly higher during November 2020 (191 ± 24 mmol/kg) than in September 2020 (153 ± 20 mmol/kg). Phosphorus concentrations were highest during November 2020 (52 ± 7 mmol/kg) and lower during May 2021 (31 ± 5 mmol/kg). Aluminum leaf concentrations averaged 1.2 ± 1 mmol/kg with no differences within sites or dates. Manganese averaged 1 ± 0.38 mmol/kg without statistical differences between dates or sites. Leaf iron concentrations were higher during November 2020 (16 ± 4 mmol/kg) and May 2021 (18 ± 5 mmol/kg) than September 2020 (6 ± 2 mmol/kg) and May 2021 (7 ± 10 mmol/kg). Copper leaf concentration was slightly higher in P5 (0.14 ± 0.02 mmol/kg). Zinc leaf concentrations also were significantly higher in P5 (1.02 ± 0.18 mmol/kg). Leaf Cadmium concentrations averaged 0.001 ± 0.0004 mmol/kg without statistical differences between dates or sites. Leaf Pb was slightly higher in P5 (0.01 ± 0.006 mmol/kg).

Table 8 *Laguncularia* average leaf element concentrations. (n= 4 individuals per date), p-value <0.05 denotes statistical difference between averages.

									Laguncularia racemosa									
	P10				**P3**				**P5**				**P6**				**pval date**	**pval sites**
	Sep-20	**Nov-20**	**Mar-21**	**May-21**	**Sep-20**	**Nov-20**	**Mar-21**	**May-21**	**Sep-20**	**Nov-20**	**Mar-21**	**May-21**	**Sep-20**	**Nov-20**	**Mar-21**	**May-21**		
									mg/g									
C	**35.9**	**39.2**	**45.8**	**49**	**39.6**	**39.6**	**38.5**	**41.6**	**40.9**	**38.5**	**44.4**	**45.3**	**40.2**	**39.8**	**44.2**	**41.4**	<0.001	0.59
N	**1.1**	**1.2**	**1.9**	**1.8**	**1.4**	**1.6**	**1.5**	**1.4**	**1.2**	**1.2**	**1.5**	**1.6**	**1.4**	**1.2**	**1.8**	**1.9**	<0.002	0.08
									mmol/kg									
Na	236.4	142.8	170.1	224.2	305.9	407.8	233.5	258.8	426.3	384.5	501.9	397.7	386.6	287.0	203.0	281.0	0.81	0.02
K	166.8	126.3	123.1	181.1	255.9	266.3	214.5	338.6	282.6	293.8	319.3	324.5	438.6	232.7	259.0	193.7	0.52	0.02
Ca	291.6	550.3	456.6	577.8	267.0	445.4	844.7	474.7	265.3	372.7	471.8	457.3	255.4	321.4	392.0	280.3	0.03	0.19
Mg	133.9	195.8	155.7	162.8	157.4	195.9	170.5	120.7	163.4	180.0	155.3	167.5	151.6	190.7	214.8	178.8	0.007	0.81
Al	0.63	0.91	0.41	0.89	1.16	0.95	3.04	1.11	1.09	4.13	0.44	0.59	1.73	1.09	0.52	0.48	0.61	0.41
P	39.59	43.35	28.61	32.19	46.36	54.48	42.91	32.06	41.49	56.28	33.90	38.04	51.14	54.38	44.69	24.44	<0.0006	0.26
Mn	0.67	1.49	1.00	1.53	0.86	1.47	1.49	1.18	0.79	1.06	0.95	0.82	0.84	0.72	0.82	0.64	0.16	0.06
Fe	8.4	17.7	21.1	23.3	5.3	19.7	23.7	3.9	4.5	12.8	11.6	1.2	6.8	16.5	17.8	3.2	<0.0001	0.17
Cu	0.11	0.09	0.08	0.05	0.11	0.09	0.09	0.12	0.13	0.14	0.14	0.19	0.12	0.07	0.08	0.03	0.54	0.0001
Zn	0.23	0.34	0.41	0.35	0.34	0.43	0.41	0.44	0.87	1.22	1.14	1.00	0.37	0.34	0.31	0.22	0.95	<0.0001
Cd			0.0004	0.0003			0.0004	0.0004			0.0008	0.0015			0.0003	0.0004	0.64	0.07
Pb	0.013	0.004	0.002	0.003	0.008	0.009	0.005	0.003	0.016	0.025	0.014	0.011	0.004	0.004	0.002	0.001	0.45	<0.001
K/Na	0.71	0.89	0.72	0.81	0.97	0.83	0.92	1.31	0.70	0.76	0.64	0.82	1.12	0.95	1.28	0.69		
Ca/Mg	3.5845	4.6022	4.8351	5.8509	2.7850	3.8653	8.1682	6.4843	2.6575	3.3702	5.0109	4.5032	2.8150	2.7919	3.0102	2.5857		

Grasses: *Panicum* and *Echinochloa*

Panicum had higher N (3.1 ± 0.6 mg/g) than *Echinochloa* (2.8 ± 0.5 mg/g). *Echinochloa* did not have differences between dates; *Panicum* N concentrations were higher in November 2020 (2.8 ± 0.5 mg/g) than in September 2020 (2.5 ± 0.3 mg/g). C leaf concentrations for *Echinochloa* were higher during March 2021 (48 ± 3.3 mg/g) than the rest of the dates with no differences within plots (Table 10). *Panicum* Na concentrations averaged 126.65 ± 103 mmol/kg without statistical differences between dates or sites. Potassium concentrations in *Panicum* leaves averaged 405.5 ± 59 mmol/kg without statistical differences between dates or sites. Ca leaves concentrations averaged 102 ± 24 mmol/kg without statistical differences between dates or sites. Mg leaves concentrations averaged 150 ± 27 mmol/kg without statistical differences between dates or sites. Al in *Panicum* leaves averaged 24. 7 ± 35 mmol/kg without statistical differences between dates or sites. Phosphorus averaged 55.6 ± 12.6 between dates or sites. Mn in leaves averaged 7.7 ± 3.7 mmol/kg without statistical differences between dates or sites. Fe in leaves averaged 19.6 ± 23 mmol/kg without statistical differences between dates or sites. Cu concentration in leaves averaged 0.14 ± 0.03 without statistical differences between dates or sites. Zn concentrations in *Panicum leaves* was significantly higher in P5 (2.7 ± 0.17 mmol/kg) than the other plots *Panicum* was present (P3, P6). Cd was significantly higher in P5 (0.11 ± 0.004 mmol/kg) than the other plots *Panicum* was present (P3, P6). Lead in leaves averaged 0.005 ± 0.005 without statistical differences between dates or sites. *Echinochloa* was only found in one plot (P10), hence no analyses between plots. Temporal differences couldn't be statistically tested because of the small amount of sample per period (n=3). *Panicum* had higher Zn concentration (1.5 ± 0.9 mmol/kg) than *Echinochloa* (0.47 ± 0.04 mmol/kg). *Panicum* had higher Cd concentration (0.005 ± 0.004 mmol/kg) than *Echinochloa* (0.0002 mmol/kg). Pb averaged 0.005 mmol/kg without statistical differences between dates or sites.

Table 9 *Panicum* and *Echinochloa* average leaf element concentrations. (n= 4 individuals per date, except for C, N analyses. *Echinochloa* n = 2 per date), p-value <0.05 denotes statistical difference between averages.

Grasses											
	P. laxum							***E. polystachya***			
	P3			**P5**		**P6**		**P10**		**pval dates**	**pval sites**
	Sep-20	**Mar-21**	**May-21**	**Mar-21**	**May-21**	**Mar-21**	**May-21**	**Mar-21**	**May-21**		
mg/g											
C	40.1	40.3	na			41.8	48.5	48.3	43.4	0.7	0.47
N	2.6	3.3	na	na	na	2.4	3.5	3.3	2	0.82	0.9
mmol/kg											
Na	68.3	84.0	270.1	62.8	77.9	40.7	282.8	195.3	213.1	0.16	0.65
K	484.2	335.3	375.3	408.0	489.3	380.0	368.6	252.7	559.3	0.19	0.92
Ca	49.9	114.6	127.8	101.8	107.8	101.8	110.5	154.5	120.1	0.26	0.53
Mg	98.7	136.3	140.0	169.2	174.2	165.5	167.0	101.2	114.5	0.36	0.01
Al	7.19	91.25	4.97	3.63	4.08	58.45	3.45	6.04	2.67	0.29	0.7
P	79.04	46.30	57.06	47.76	44.37	49.56	65.03	47.11	72.26	0.17	0.7
Mn	1.11	9.48	11.76	5.57	6.63	8.61	11.05	4.95	7.64	0.17	0.74
Fe	14.98	65.46	5.90	5.81	3.62	38.35	2.91	5.73	2.33	0.29	0.6
Cu	0.12	0.18	0.14	0.13	0.12	0.19	0.10	0.11	0.14	0.35	0.84
Zn	0.39	1.09	1.03	2.79	2.54	1.21	1.17	0.50	0.45	0.63	0.001
Cd		0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.94	0.001
Pb	0.01	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.2	0.63
K/Na	7.1	4.0	1.4	6.5	6.3	9.3	1.3	1.3	2.6		
Ca/Mg	0.8	1.4	1.5	1.0	1.0	1.0	1.1	2.5	1.7		

Interspecific comparisons

For simplicity plant species will be indicated by their genus. *Dalbergia* leaves had in average a higher total C (46.8 mg/g) concentration than the other species (Table 11). Leaves N ranged from 3.2 mg/g in the C3 grass *Panicum* to 1.4 mg/g in *Laguncularia.* The order goes as follows: *Panicum > Echinochloa* ≥ *Acrostichum* ≥ *Dalbergia > Laguncularia.* Total Na was highest in *Acrostichum* (456 mmol/kg*)* and lowest in *Dalbergia* (37.6 mmol/kg). Na concentrations in leaves followed the order *Acrostichum > Laguncularia* ≥ *Echinochloa > Panicum* ≥ *Dalbergia.* Total K concentrations ranged from 405 mmol/kg in *Panicum* to 178.4 in *Dalbergia*. Total K in leaves followed the order *Panicum* ≥ *Acrostichum* ≥ *Echinochloa* > Laguncularia ≥ *Dalbergia*. Calcium was significantly higher in *Laguncularia* (380 mmol/kg) than other species. Mg in leaves ranged from 169 mmol/kg in *Laguncularia* to 78.4 mmol/kg in *Dalbergia.* K leaves concentration followed the order: *Laguncularia > Panicum* ≥ *Echinochloa >Acrostichum* ≥ *Dalbergia. Panicum* had higher Al concentrations (24 mmol/kg) than the rest of the species. P concentration was significantly higher in *Acrostichum* (82.5 mmol/kg) than other species. P in leaves followed the order *Acrostichum >Echinochloa >Panicum* ≥ *Laguncularia* ≥ *Dalbergia*. Leaves Mn followed the order Panicum ≥ *Dalbergia* ≥ Echinochloa > *Acrostichum* ≥ Laguncularia. Leaves Fe concentration ranged from 19.7 mmol/kg in *Panicum* to 2.9 mmol/kg in *Dalbergia*. Fe concentrations followed the order: *Panicum* ≥ *Laguncularia > Acrostichum* ≥ *Echinochloa* ≥ *Dalbergia.* Cu leaves concentration ranged from 0.18 in *Acrostichum* to 0.09 mmol/kg in *Dalbergia*. Cu leaves concentrations follow the order: *Acrostichum* ≥ *Panicum* ≥*Echinochloa > Laguncularia* ≥ *Dalbergia.* Zn concentrations ranged from 1.46 mmol/kg in *Panicum* to 0.47 mmol/kg in

Echinochloa. Zn concentrations followed the order: *Panicum* ≥ *Dalbergia* > *Laguncularia* ≥ *Acrostichum* ≥ *Echinochloa.* Cd leaves concentrations were significantly higher in *Panicum* (0.005 mmol/kg) than the other species. Pb leaves concentrations ranged from 0.008 mmol.kg in *Laguncularia* to 0.001 mmol/kg in *Dalbergia*. Pb leaves concentrations followed the order: Laguncularia > *Panicum* ≥ *Acrostichum* ≥ *Echinochloa* > *Dalbergia.*

Table 10 Comparison of leaf element concentrations between species (columns), considering all dates and plots. Differences were tested with an ANOVA and Tukey HSD. Letters denote statistical significance, p<0.05. Values are displayed as average ± standard deviation.

	Acrostichum	***Dalbergia***	***Echinochloa***	***Laguncularia***	***Panicum***
	n=20	*n=12*	*n=3*	*n=29*	*n=9*
C mg/g	40.2 ± 4.9 **b**	46.8 ± 5.2 **a**	40.7 ±3.9 **b**	40.07 ± 3.8 **b**	41.4 ± 2.6 **b**
N mg/g	2.66 ±0.54 **b**	2.53 ± 0.56 **b**	2.79 ±0.55 **b**	1.39 ±0.29 **c**	3.17 ± 0.6 **a**
(mmol/kg)					
Na	456.60 ±143 **a**	37.62 ±29 **c**	204.20 ±12 **abc**	318.56 ±139 **b**	126.65 ±103 **c**
K	399.47 ±172 **a**	178.38 ±43 **b**	405.98 ±217 **ab**	260.56 ±110 **b**	405.83 ±60 **a**
Ca	105.62 ±50 **b**	164.85 ±95 **b**	137.31 ±24 **b**	380.97 ±152 **a**	102.05 ±25 **b**
Mg	103.79 ±25 **c**	78.43 ±18 **c**	107.88 ±9 **bc**	169.76 ±28 **a**	150.13 ±27 **ab**
Al	4.465 ±11 **b**	1.374 ±0.60 **b**	4.355 ±2 **ab**	1.272 ±1 **b**	24.715 ±35 **a**
P	82.57 ±31 **a**	44.69 ±5 **b**	59.69 ±18 **ab**	44.79 ±10 **b**	55.59 ±12 **b**
Mn	1.178 ±0.48 **b**	7.204 ±2.04 **a**	6.298 ±1.9 **a**	0.995 ±0.4 **b**	7.744 ±3.7 **a**
Fe	5.633 ±11.8 **b**	2.935 ±2.0 **b**	4.032 ±2.41 **ab**	11.800 ±7.2 **ab**	19.575 ±23.8 **a**
Cu	0.187 ± 0.115 **a**	0.096 ± 0.028 **b**	0.126 ± 0.018 **ab**	0.104 ± 0.036 **b**	0.141 ±0.035 **ab**
Zn	0.493 ± 0.279 **c**	1.195 ± 0.385 **ab**	0.473 ± 0.040 **bc**	0.513 ± 0.313 **c**	1.460 ± 0.871 **a**
Cd	0.00010 ±0.00003 **b**	0.00067 ± 0.00024 **b**	0.00022 ± 0.00006 **b**	0.00054 ± 0.00043 **b**	0.00572 ± 0.00446 **a**
Pb	0.00349 ± 0.00726 **ab**	0.00132 ± 0.00125 **b**	0.00181 ± 0.00106 **ab**	0.00834 ± 0.00690 **a**	0.00567 ± 0.00582 **ab**

Leaf soil concentration ratios (LSR)

During the wet period, Na was excluded in all species (Table 12); all the ratios were lower than 1, possibly meaning salinity was not a stressing factor. During the Dry period (March 2021) *Acrostichum* showed some accumulation (LSR ~ 1), whereas the other species did not. It should be noted that soil salinity in March averaged 10 ppt. Potassium, an essential nutrient, was accumulated in all species in both periods. Calcium was accumulated only by *Laguncularia* during July 2020; in March 2021 was accumulated by *Acrostichum* and *Dalbergia* in high quantities (LSR ~ 64 and 143 respectively) and double from July 2020 by *Laguncularia*. All species showed some degree of exclusion to Magnesium during the wet period (July 2020) but accumulated during March 2021 except C4 grass *Echinochloa*. All species showed Aluminum accumulation in both seasons but mostly *Panicum* (LSR ~382) during the dry season and *Acrostichum* (LSR ~72). All species showed accumulation to P, a limited nutrient, significantly higher during July 2020, specially *Acrostichum* (LSR ~ 442). *Acrostichum* showed some exclusion to Mn during July 2020 and *Laguncularia* during May 2021, while the rest of the species showed relative accumulation. All species accumulated Fe, but it was significantly higher during March 2021. *Dalbergia* and *Laguncularia* showed relative Zinc accumulation during July 2020, but in March 2021, all species showed relative accumulation of Zn. Leaf-Soil concentration ratios were not able to do for Available Cadmium due to was below the detection limit in most of the samples. In those which did not, it was insignificant when expressed by molar mass. *Laguncularia* showed some accumulation of Pb (LSR ~ 0.3±0.45) in July 2020 but exclusion during March 2021.

Table 11 Leaf-Soil concentration ratios (LSR) per dates. At each plot a soil sample was taken next to each specie. It was used soil exchangeable concentrations.

Leaf-Soil concentration ratio							
	July 2020						
	Acrostichum		***Dalbergia* (n=6)**		***Laguncularia***		***Panicum***
	(n=4)		(*n=6)*		*(n=11)*		*(n=1)*
Na	0.65	±0.34	0.05	±0.02	0.36	±0.2	0.10
K	21.80	±21	19.60	±5.9	18.94	±15	31.20
Ca	0.66	±0.25	0.56	±0.1	0.99	±0.6	0.22
Mg	0.54	±0.15	0.44	±0.1	0.48	±0.3	0.51
Al	1.98	±0.94	2.16	±0.4	1.78	±1	4.96
P	442.00	±135	306.42	±116	229.92	±165	109.78
Mn	0.32	±0.23	11.16	±13	1.87	±1	2.47
Fe	9.50	±6.4	5.81	±5	23.27	±39	4.58
Cu	6.90	±5.9	8.00	±1	7.67	±6	6.00
Zn	0.85	±0.5	6.82	±7	1.79	±2	0.85
Cd	0.00	±0	na		0.00	±0	na
Pb	0.00	±0	0.00	±0	0.30	±0.45	0.50

Leaf-Soil concentration ratio							
	July 2020						
	Acrostichum		***Dalbergia* (n=6)**		***Laguncularia***		***Panicum***
	(n=4)		(*n=6)*		*(n=11)*		*(n=1)*
Na	0.65	±0.34	0.05	±0.02	0.36	±0.2	0.10
K	21.80	±21	19.60	±5.9	18.94	±15	31.20
Ca	0.66	±0.25	0.56	±0.1	0.99	±0.6	0.22
Mg	0.54	±0.15	0.44	±0.1	0.48	±0.3	0.51
Al	1.98	±0.94	2.16	±0.4	1.78	±1	4.96
P	442.00	±135	306.42	±116	229.92	±165	109.78
Mn	0.32	±0.23	11.16	±13	1.87	±1	2.47
Fe	9.50	±6.4	5.81	±5	23.27	±39	4.58
Cu	6.90	±5.9	8.00	±1	7.67	±6	6.00
Zn	0.85	±0.5	6.82	±7	1.79	±2	0.85
Cd	0.00	±0	na		0.00	±0	na
Pb	0.00	±0	0.00	±0	0.30	±0.45	0.50

Based on the leaf soil ratios above, roots from *Laguncularia* and *Acrostichum* were processed in July 2020 for analyses (Table 13). Pb was accumulated in the roots of both species. It was found that both species accumulate Pb at the root level (Fig. 39).

Table 12 Root-Soil concentration ratios (RSR)

Root – soil concentration ratios (RSR)		
	Acrostichum (n= 4)	***Laguncularia (n=3)***
Na	0.17 ± 0.11	0.52 ± 0.07
K	1.49 ± 1.50	8.85 ± 2.21
Ca	2.28 ± 0.98	2.33 ± 0.03
Mg	0.62 ± 0.18	0.30 ± 0.02
Al	93.57 ±103.40	83.29 ±16.53
P	149.81 ± 28.73	78.40 ± 2.60
Mn	3.09 ±2.44	2.58 ±0.64
Fe	1231.62 ± 926.25	64.33 ± 4.21
Cu	24.69 ±18.58	15.18 ± 3.64
Zn	4.79 ± 2.66	1.15 ± 0.33
Cd	na	na
Pb	1.03 ±1.06	2.64±1.22

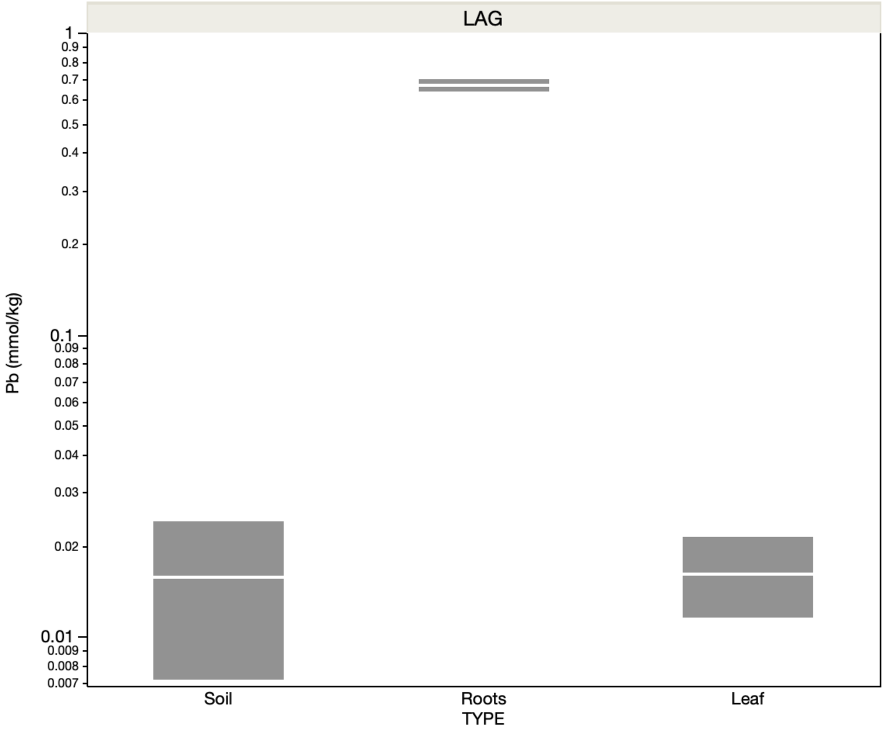


Figure 39 Pb (mmol/kg) concentration in different plant tissues from *Laguncularia*. Note Logarithm scale in Y-axis.

Leaves Molar Ratios K/Na, K/Ca, and Mg/Ca

Acrostichum had K/Na molar ratios lower than one, meaning preference of K in leaves (Table 14). *Laguncularia* had a K/Na ratio higher than one as expected for halophytes (Medina, 1999). *Laguncularia had* Ca/Mg molar ratios over 1 with some temporal variabilities (Table 15). *Acrostichum had a K/Ca ratio of 5.6, Dalbergia* had a ratio of 1.5 *Echinochloa* had a ratio of 3.1, *Laguncularia* had the lowest K/Ca ratio an *Panicum* had a K/Ca ratio of 4.4. The sequence goes as follow: *Acrostichum > Panicum > Echinochloa >> Dalbergia>> Lr.* Mg/Ca molar ratios ranged from 0.68 in *Panicum* to 2 in *Dalbergia.* The sequence goes as follow:

Panicum>Acrostichum>Echinochloa>Dalbergia>>Laguncularia.

Table 13 Averages ± standard deviations molar ratios for the five species.

	n	K/Na	K/Ca	Ca/Mg
Acrostichum	20	1.8 ±1.74	5.61 ±5.4	0.98 ±0.28
Panicum	9	8.72 ±5.13	4.45 ±2.4	0.68 ±0.14
Echinochloa	3	3.33 ±1.6	3.14 ±2.1	1.29 ±0.34
Dalbergia	12	13.6±12.4	1.48 ±0.9	2.02 ±0.94
Laguncularia	29	1.5 ±0.52	0.89 ±0.9	2.26 ±0.91

Table 14 Species ratios (Average ± standard deviation): K/Na, K/Ca and Ca/Mg per plots

			P3			P5			P6			P10	
	n	K/Na	K/Ca	Ca/Mg	K/Na	K/Ca	Ca/Mg	K/Na	K/Ca	Ca/Mg	K/Na	K/Ca	Ca/Mg
A. danaeifolium	*18*	1.001 ± 0.5	7.136 ± 4.7	0.89 ± 0.3	0.826 ± 0.2	3.739 ± 2.1	1.05 ± 0.24	1.602 ± 1.9	7.77 ± 8.9	0.87 ± 0.3	0.742 ± 0.5	3.353 ± 3	1.13 ± 0.3
P. laxum	*8*	4.158 ± 2.85	5.187 ± 3.9	0.75 ± 0.2	6.389 ± 0.2	4.274 ±0.38	0.61 ± 0.01	5.318 ± 5.7	3.533 ± 0.3	0.64 ± 0.03	na	na	na
E, polystachya	*4*	na	na	na	na	na	na	na	na	na	1.959 ± 0.94	3.146 ± 2.1	1.29 ± 0.34
D. ecastaphyllum	*13*	na	na	na	na	na	na	13.552 ± 9.4	1.859 ± 1.2	2.11 ± 1.0	4.551 ± 1.9	1.244 ± 0.7	1.96 ± 0.9
L. racemosa	*28*	0.952 ± 0.43	0.747 ± 0.4	2.62 ± 1.3	0.723 ± 0.11	0.951 ± 0.44	2.1 ± 0.62	1.022 ± 0.3	1.377 ± 1.5	1.7 ± 0.6	0.789 ± 0.12	0.367 ± 0.2	2.73 ± 0.57

Isotopic signatures in plants and sources

The C3 species sampled had carbon isotope ratios ranging from -35.3 to -26.2‰ (Average –28.66 ‰). The C4 grass, *Echinochloa* had carbon isotope ratios from -12.3 to -13.4‰ (Average -13.67 ‰).

The highest values corresponded in the C3 species to *Acrostichum,* collected from sun-exposed areas (Table 16). The more-negative values were from *Laguncularia* sun-exposed leaves. The species sampled had nitrogen isotope ratios ranging from -3 to 10 ‰ (Average 3.1 ‰).

Table 15 13C and 15N Averages and ± standard deviations per each plot for dry and wet periods. (n=3 plants, 2-5 leaves per plant)

		δ13C ‰		**δ15N ‰**	
PLOT	**Plant**	**Dry Period**	**Wet**	**Dry**	**Wet**
P3	ACR	-27.96 ± 0.81	-28.28 ± 0.82	2.77 ± 1.24	4.72 ± 0.94
P3	LAG	-29.00 ± 0.64	-28.69 ± 0.58	1.61 ± 0.82	2.98 ± 0.81
P3	PAN	-27.40 ± 0.63	-29.49 ± 0.16	3.77 ± 0.85	6.06 ± 1.95
P5	ACR	-28.60 ± 0.89	-27.08 ± 0.67	8.13 ± 0.32	5.82 ± 0.76
P5	ECH	na	-13.08 ± 0.30	na	9.02 ± 1.27
P5	LAG	-27.53 ± 0.82	-28.61 ± 0.20	1.14 ± 0.65	2.96 ± 0.21
P6	ACR	-27.77 ± 0.12	-28.85 ± 0.42	5.08 ± 0.38	5.47 ± 0.41
P6	DAL	-27.77 ± 0.23	-29.78 ± 0.31	-0.27 ± 0.25	0.62 ± 0.12
P6	LAG	-29.61 ± 0.93	-28.80 ± 0.00	2.25 ± 0.52	2.70 ± 0.14
P6	PAN	na	-29.38 ± 0.41	na	5.96 ± 3.18
P10	DAL	-28.20 ± 0.88	-29.86 ± 0.20	-0.72 ± 0.15	-0.70 ± 0.17
P10	ECH	-13.47 ± 0.54	-12.82 ± 0.32	2.66 ± 1.73	3.44 ± 0.41
P10	LAG	-29.55 ± 0.94	-30.25 ± 0.57	2.87 ± 0.78	1.77 ± 0.62

Averages for each plot (n=3 plants, 2-5 leaves per plant)

Dalbergia was the only nodulated specie sampled, evidenced by the negative δ^{15}N values, indicating a strong fixation of atmospheric N. The other species sampled had positive values, an indication of the use of mineral nitrogen in soils. Salinity at the water table in plots 6 and 10 was similar both during wet and dry periods, it has been reported that salinity decreases root nodulation in legumes, but we did not detect changes in δ^{15}N values (Pacheco et al., 2021).

Discussion

Spatial and temporal elemental variability in soils:

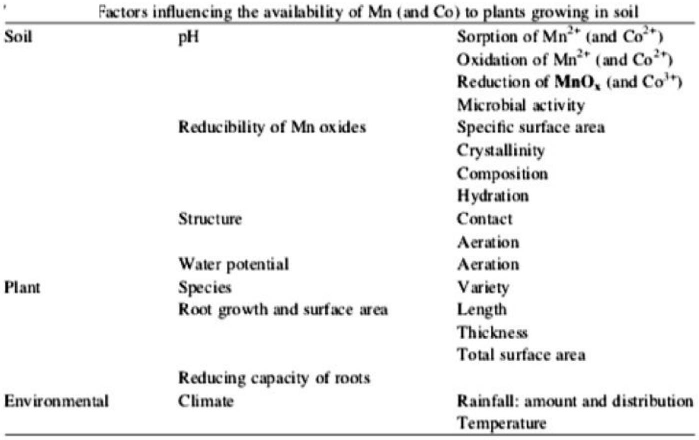
In coastal wetlands, a negative relationship between salinity and SOM has been reported (Morrisey et al., 2014); however, with minimal changes in salinity, as presented in this study, the response is non-significant (Osland et al., 2018). Organic matter also has a role in the availability of nutrients and heavy metals (Gambrell, 1994); however, based in this study, SOM did not correlate with the availability of nutrients, as changes between sites were minimal. Soil Na correlations with Mg but no Ca, could be an indication that the wetland is saltwater influenced (Medina et al., 2007; Mitsch & Gosselink., 2015. We found a negative correlation between soil salinity and Exchangeable P (Fig. 25), which backs up previous mesocosm studies that with increasing salinity, P decreases (Servais et al., 2021). The high variability in the soil carbon concentration could result from Hurricane María's aftermath, where flooding and strong winds could have mixed soils throughout (Griffiths et al., 2021). In general, C concentrations at Cucharillas fell within concentrations reported for other wetlands on the island with different levels of hydrological disturbances (Griffiths et al. 2021) and other wetlands worldwide (Embers et al. 1987). Carbon isotopes values were found more negative than those reported from Brazilian mangroves soils (Tognella et al. 2016) and brackish wetlands in Venezuela (Medina et al. 2007). They could reflect the present and previous non-mangrove vegetation in the sites, which includes *Laguncularia* (-27 to -29 $^{0}/_{00}$) *Acrostichum* (-28 $^{0}/_{00}$), and *Panicum (*-27 $^{0}/_{00}$). Higher δ^{15}N values in the study area could be related to anthropogenic inputs (Medina et al. 2007), as this seems to be the case in the plot closest to the urban areas. These results also suggest that the δ^{15}N accumulation in the first 10 cm stems from the open Nitrogen cycle usually found in tropical climates (Martinelli et al. 1999).

Phosphorus availability in wetland soils is mostly affected by interactions with other elements (Mitsch & Gosselink, 2018). Phosphorus becomes insoluble when it precipitates with Fe and Al under aerobic conditions in acid soils, and it is bound with Ca and Mg in alkaline soils (Mitsch & Gosselink, 2018). P becomes bioavailable under slightly acidic to neutral pH if it is bound to Ca (Mitsch & Gosselink,2018). Potassium concentrations are lower than values reported for coastal wetlands (Tognella et al., 2016; Medina et al., 2007). Calcium concentrations were in the range of values reported for coastal wetlands (Tognella et al., 2016); Ca is a relatively abundant mineral in soils, averaging 3.5 % (by weight) of the earth's lithosphere (Maathius 2012). Soils whose parent materials are limestone, dolomite apatite, and gypsum are high in Ca. Also, in humid areas with constant precipitation, Ca supplies are less than in areas with lower rainfall (Pritchett & Fisher, 1987). Mg concentrations were in the range of values reported for coastal wetlands (Tognella et al., 2016; Medina et al., 2007).

Copper soil concentrations range from 0.03 to 0.79 mmol/kg (2 to 50 mg kg ^{1}) globally, and main anthropogenic inputs are atmospheric depositions and the use of fertilizers and agricultural practices; hence higher concentrations of Cu in P5, can be related to urban areas. Copper is mainly fixed to the organic matter in soil, and its availability and sorption is regulated by soil pH and organic matter. In low pH conditions, low organic matter content becomes soluble in the soil solution (Oorts,2013). Cu toxicity will depend on soil characteristics depending on the bioavailability of it. Based in Eh-pH diagrams, we can decipher that the main form of $CuFeS_2$. Zn variability between study plots can be explained by soil pH and organic matter, with concentrations in soil solution increasing up to five times per unit pH decrease (Mertens & Smolders, 2013). We found that Al, which has a range of 370 to 11118 mmol/kg (10,000 to 300,000 mg kg^{-1}) in soils with an average of 2631 mmol/kg (71,000 mg kg$^{-1)}$, (Lindsay, 1979) increased during the wet period. According to USEPA 2003, total concentrations in soils are not correlated with toxicity but instead with soluble Aluminum, which plants can uptake.

Manganese (Mn), closely associated with Cobalt in soils because of its similarity in chemical properties, has a mean available concentration in soils of 15.47 mmol/kg (850 mg kg^{-1} total concentration), and it's one of the elements most studied (Uren, 2013). Mn soil availability is dependent on Mn oxides, structure, and pH, being present in soil solution as M$^{2+,}$ an exchangeable cation on low pH values. Table 4 modified from Alloway (2013), summarizes factors that influence the availability of Mn to plants.

Table 16 Factors influencing Mn availability to plants. Modified from Alloway (2013)



Factors influencing the availability of Mn (and Co) to plants growing in soil

Soil	pH	Sorption of Mn^{2+} (and Co^{2+})
		Oxidation of Mn^{2+} (and Co^{2+})
		Reduction of $\mathbf{MnO_x}$ (and Co^{3+})
		Microbial activity
	Reducibility of Mn oxides	Specific surface area
		Crystallinity
		Composition
		Hydration
	Structure	Contact
		Aeration
	Water potential	Aeration
Plant	Species	Variety
	Root growth and surface area	Length
		Thickness
		Total surface area
	Reducing capacity of roots	
Environmental	Climate	Rainfall: amount and distribution
		Temperature

Cadmium (Cd) concentration in non-polluted soils range between 0.0009 to 0.009 mmol/kg (0.1-1 mg kg ^{1}) but could be higher in organic soils. Cadmium retention in the soil is mostly controlled by pH. In waterlogging conditions, soil pH increases and probably immobilizes Cd. Although it is found in trace levels, its high toxicity could affect ecosystem functioning (Smolders & Mertens, 2013). Anthropogenic activities augment soil Cd via atmospheric deposition and the use of fertilizers and sewage. Soil organic matter, Fe, Al, and Mn hydroxides clay minerals are the main Cd adsorbents in soil (Fulda et al. 2013). In studies done with rice paddies, plant Cd concentrations increase with total Cd and decreasing pH, whereas also Cd plant concentration increases when soil organic matter decreases (Fulda et al. 2013). Soil salinity is thought to increase plant Cd concentrations. Acevedo-Figueroa et al. (2006) reported values of 0.016±0.009 mmol/kg (1.8± 1 mg kg $^{-1}$) for Cd in lagoon sediments at San Jose Lagoon in San Juan Northwestern P.R., an area highly urbanized, and 0.0009± 0.005 (0.1±0.06 mg kg^{-1}) in Joyuda Lagoon a fairly undisturbed natural reserve located at southwestern Puerto Rico.

Lead is considered one of the most harmful heavy metals, with deleterious effects on human health (Furini, 2012). It is also one of the first metals extracted for human uses dating back to Roman empire. According to (Nriagu,1978) mean concentration of Pb in uncontaminated soils globally is estimated to be 0.08 mmol/kg (17 mg kg $^{-1)}$.
Iron is one of the most common elements in soils and constitutes an essential micronutrient for plant growth in small amounts, 1.79 mmol/kg (~100 mg kg^{-1} total concentration) (Marschner, 1986). Typical soil total concentrations range from 20,000 to 550,000 mg kg^{-1} (Bodek et al., 1988). Iron to be found in a soluble and interchangeable form, potential redox conditions must be low (most negative values -100 to -150 mV) and acid pH values - 4 to 7. (Gotoh & Patrick, 1974).

Soil molar ratios:

Our results suggest that available nutrients, and heavy metals, capable of affecting plant establishment vary with rainfall frequency. During wet periods with waterlogging conditions serves as a sink with low nutrient availability, changing to a nutrient source in the dry period. Cienaga Las Cucharillas had a higher Ca/Mg ratio than Medina et al. 2007 reported for the Pterocarpus-*Laguncularia* forest in Sabana Seca (0.57), in the coastal plains of Toa Baja, but lower than the wetlands from the Punta Viento, Patillas (~5) influenced by the basin discharging from the mountains.

Spatial and temporal variation in plants

Al increased slightly in *Dalbergia* however no signs of stress were seen. Al toxicity in plants has been well documented, and effects include decreases in height and yield of crops and reduced root growth rates (Rees and Sidrak, 1961; Hutchinson et al., 1986). Plant tolerance differs greatly among species, ranging from 0.07 to 3.7 mmol/kg (2 to 100 mg kg ^{1}) without damage (USEPA 2003). *Dalbergia* also had a slight concentration of C in leaves but was in the low range reported for plant shoots. Cu concentration in plant shoots range from 0.06 to 0.23 mmol/kg (4 to 15 mg kg^{-1} Dry Matter (DM)) (Davis & Beckett, 1978). For iron (Fe), plants have been divided into two groups with different strategies according to how they interact with the rhizosphere to solubilize Fe, which includes increasing the reduction of Fe^{3+} to Fe^{2+} by acidifying the rhizosphere and producing chelating molecules - such as phyto siderophores (Marschner, 1986).

The mineral composition (N, P, Na, K, Mg, and Ca) for *Laguncularia* is consistent with reports for this genus (Lugo 1999, Medina et al. 2001, Medina et al. 2007).

Leaf Soil Ratios

We found Pb accumulation in *Laguncularia* leaves and roots; *Acrostichum* had accumulation only at root level, excluding it from aboveground biomass. Recent studies have shown the likelihood of using *Laguncularia* for harmful elements remediation and biomonitoring practices (del Refugio Cabañas-Mendoza et al. 2020; Da Souza et al. 2014) under varying degrees of salinity as was the case in our study area. More studies are needed to corroborate *Acrostichum* Pb concentration in roots since the literature is scarce in this sense.

Acrostichum had Na/K molar ratios higher than one, meaning accumulation in leaves (Table 12). This behavior is similar to *A. aureum* (Medina et al. 1990), tolerant to soil saline conditions up to 45–50 mS/cm but requires freshwater for establishment.

Laguncularia had a Na/K ratio higher than one as expected for halophytes (Medina, 1999). It can be inferred based in the K/Na ratios that *Dalbergia and Panicum* are salt excluders, and *Laguncularia, Acrostichum, and Echinochloa* can be considered salt tolerant.

Plants with a K/Ca molar ratio less (<1) than one are considered Calciotrophs, as they have high amounts of Ca (Kinzel, 1989). Although there was temporal variability, *Laguncularia* was the only species that can be considered calciotrophic *sensu* Kinzel or Ca accumulator. However, all the species had K/Ca ratios expected for higher plants (Epstein, 1965). Magnesium/ Calcium ratios reveals a different pattern, were woody plants - *Laguncularia* and *Dalbergia* favor Ca accumulation over Mg by a factor of two. *Acrostichum and Echinochloa* exhibits a neutral Mg/Ca ratio and *Panicum is* in the other side of the spectrum, excluding Ca and accumulating Mg. The Mg/Ca molar ratios of *Dalbergia* and *Laguncularia* were above the average reported (0.6) for higher plants (Epstein, 1965).

Conclusions

- There is moderately differences in soil element composition of Ca, Cu, Zn, Cd, as Martin Peña (Mp) soils (P3 and P5) had higher availability of these elements.
- There was high $\delta^{15}N$ values in soil samples in plots P3 and P5, posssibly related to anthropogenic inputs of N (Medina et al. 2007).
- $\delta^{15}N$ accumulation in the first 10 cm in soils reveals an open Nitrogen cycle usually found in tropical climates (Martinelli et al., 1999).
- Ciénaga Las Cucharillas wetland is most likely saltwater influenced, as seen in the changes in Available Na, P, Mg, and Ca were correlated with temporal changes in soil salinity.
- The wetland is a source or sink of plants available elements and "heavy metals", depending on the rainfall regimen. It works as a sink during wet periods with waterlogging conditions, changing to a nutrient source during the dry period and aerobic soils.
- Plant element uptake differed between wet and dry periods and could correspond to soil element availability.
- K/Na molar ratios are low in *Laguncularia and Acrostichum* as expected for halophytes (Medina, 1999)
- Sodium Leaf-soil ratio was higher than one in *Laguncularia* and *Acrostichum,* evidence of halophytic behavior.
- *Laguncularia* had LSR higher than one for Pb, suggesting it could be used for phytoremediation.
- Woody species (*Laguncularia, Acrostichum)* had higher Mg/Ca ratios than grasses (*Echinochloa, Panicum).*
- *Acrostichum* and *Laguncularia* had Pb accumulation at the root level, suggesting they could be used for phytostabilization.
- *Panicum* was the only species that did not had higher concentrations of Calcium in leaves than the available in soil.

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Plant Functional Types Ecophysiological Responses

Introduction

Plants responses will vary according to their capacity for acclimation to abiotic stressors such salinity, presence of heavy metals and low nutrient availability (Pezeshki, 2012). To survive these conditions, they have developed a series of traits, which in turn, affect ecosystem structure, processes, and function (Eviner & Chapin, 2003). Given the coastal wetlands dynamics, their plants and environmental traits are less known than those of other terrestrial ecosystems (Moor et al., 2017). Plant leaf and root traits are indicative of the physico-chemical environment including edaphic factors and climate patterns (Diaz et al., 2004; Wright et al., 2004).

Leaves are one of the main organs of vascular plants, harboring the structure for photosynthesis machinery. Specific leaf area (SLA), representing the cost of the photosynthetic area (leaf area / leaf mass) is a reliable index of plant resource allocation, strongly correlated with C and N concentrations, water use efficiency and photosynthesis (Wilson et al., 1999; Diaz et al., 2004). Leaf physical characteristics such as thickness, area and dry mass can be used as indicators of plant nutrients deficiency, and adverse climatic or soil abiotic conditions. Carbon-13 isotope analyses are used to estimate plant long-term water use efficiency (ratio of net photosynthesis to transpiration; Farquhar et al., 1982; Medina & Francisco, 1997). Leaf nutrients concentrations such as nitrogen and phosphorus, are correlated with maximum photosynthetic rate and SLA and with N soil availability and with leaf P (Hu et al. 2015). Photosynthetic rates can be affected by the soil reducing conditions in wetlands, as described by Pezeshki et al. (1996) for *Typha domingensis* and *Panicum hemitomos*.

Global correlations have been found between leaf-level traits and climate, termed “plant economic spectrum” (Wright et al., 2004), but at a local ecosystem level, patterns don’t seem robust enough and most of the relations are species-specific, although much of the research has been done in terrestrial ecosystems (non- wetlands) (Moor et al., 2017). Ciénaga las Cucharillas wetlands, with its heterogenous vegetation and contrasting soil salinity, depth of the water table, and pH values under the same mesoclimate, gives the opportunity to study plant responses and local patterns of leaf trait variation of several plant functional groups (halophytic trees, shrubs, herbs, and grasses). Field measurements were performed to examine eco-physiological responses of functional groups to key environmental conditions determinants of plant composition in wetlands: salinity, water table and pH conditions, and examine leaf-level trait variability. The goal of the study is to understand how plant functional groups differ in their physio-ecological responses to abiotic conditions in coastal wetlands, and how do they compare to terrestrial plant traits. It was asked: **Do plant functional groups differ in their photosynthetic capacity and water use efficiency responses? to seasonal changes in precipitation and salinity?** It was hypothesized that plant functional groups differ in their photosynthetic capacity, and water use efficiency, and respond differently to seasonal changes in precipitation, salinity and water levels.

Methods:

Four study plots (100m2) were chosen in the wetland based in edaphic factors, (especially those related to disturbance by deposition of industrial residues). Two plots were on typical organic wetland soil and the other two were located on soil whose mineral composition was influenced by anthropogenic allochthonous infills which varied from the coast to inland. Each plot included two or more of the species selected for functional comparisons. Plant species representative of distinct life forms that differs in their tolerance to saline conditions (halophytic trees, grasses, shrubs, and herbs) were chosen: (*Laguncularia (Abbreviated Lagunclaria), Dalbergia (Dalbergia), Acrostichum (Acrostichum), Panicum (Pl)* and *Echinocloa (Echinochloa).* Sampling dates for leaf gas exchange measurements were done in June 2020 and February 2021 in the same individuals. Light response curves were performed in May 2021. Samples collected for leaf morphology were in were June 2020, September 2020, November 2020, February 2021, March 2021, and May 2021.

Soil samples from the first 10 cm were taken near each specie with triplicates. A total of 12 samples were taken each date. Soil salinity was measured in the Process and Functioning of Tropical Ecosystems laboratory at the University of Puerto Rico, with a conductivity meter using air dried soil in a 1:1 soil-to-water ratio mixture. Laboratory measurements were done by graduate student Gloria Ortíz.

Morphological character traits

In each plot, three individual plants of the selected species (if present) were chosen. Three to five adult fully exposed leaves were collected and stored in a cooler until reaching the lab to prevent water loss. Leaf area, length, and width were measured with an LI-3100C Area Meter. Fresh leaf mass was determined before area measurements. All leaves per species were dried in a forced-air circulation oven at 60 C until constant mass for dry mass determination. For *Acrostichum*, individual leaflets were measured, and for the grasses (*Echinochloa* and *Panicum)*, the leaf sheath was excluded. Specific leaf area (the area of a fresh leaf divided by its oven-dry mass), water content, and succulence were calculated (Table 18). A total of 995 leaf measurements were done of the five selected species: *Acrostichum* (n=290), *Dalbergia* (n=171), *Echinochloa* (n= 71), *Laguncularia* (n=321) and *Panicum* (n=142) ranging 15-60 leaves per specie each sampling date. I sampled twelve individuals of *Acrostichum,* six *Dalbergia*, three individuals of *Echinochloa,* nine *Panicum,* and 12 individuals of *Laguncularia*.

Table 17 Formulas and units used for analyses

Leaf trait	Formula and units	Reference
Specific leaf area (SLA)	Leaf area/ dry mass (cm^2/g)	Cornelissen et al. (2003).
Leaf Water Content (LWC)	(Fresh mass – dry mass/ fresh mass) x100 (%)	Cornelissen et al. (2003).
Succulence	(Fresh mass – dry mass)/ area (g/m^2)	Mantovani, 1998
Water content: Dry mass ratio	(Fresh – dry mass g) / Dry mass (g)	Runyon, 1936

Leaf gas exchange measurements

Net assimilation

To measure net assimilation under field conditions, I chose three to five adult full sun exposure leaves in three species at each site. Light conditions were controlled with the PLC3 LED light unit (RGBW of the CIRAS 3 IRGA (PP Systems) to 800 m-2 s-1, replicating average irradiation values in situ as measured at the weather station. All measurements were done on the same day or two days before noon (7:00 – 11:00 am). We measured in June 2020 and February 2021.

Light response curves

We sampled fully expanded, sun exposed leaves from three individuals, one from each plot from the species: *Laguncularia (*tree)*, Acrostichum* (Fern)*, Dalbergia* (shrub)*, Echinochloa (*C_4 grass) *and Panicum* (C_3 grass). Light response curves were performed to measure changes in PAR, air and leaf temperature, air humidity and CO_2 concentration to estimate carbon assimilation (A), stomatal conductance (*gs*), transpiration rate (E), and water use efficiency (WUE) and ci using a CIRAS 3 IRGA connected to a PLC chamber (PP Systems). These were produced also with the CIRAS 3 with the PLC chamber with a LED source over 10 levels of light intensities (PAR): 2000, 1500, 1000, 800,500, 250, 100, 50,25, and 0 umol photons m-2 s-1. From the highest to lowest values with 2-5 minutes interval between each level. Ambient temperature averaged 33 C and leaf temperature averaged 34 C. Cuvette temperature was set to follow leaf temperature. Measurements were made at 400 umol mol-1 CO2 concentration. The light response curves were fitted to the data using JMP 13 (SAS) using the equation 1 (Prioul 1977) where: maximum photosynthetic capacity at saturating light intensity (A_{sat}), apparent quantum yield (φ), dark respiration rate (Rd), and curve convexity (q) were calculated.

$$\frac{\left(\left(\mathrm{Phi}\bullet\mathrm{PARi}+\mathrm{Asat}\right)-\sqrt{\left(\mathrm{Phi}\bullet\mathrm{PARi}+\mathrm{Asat}\right)^{2}-4\bullet\mathrm{theta}\bullet\mathrm{Phi}\bullet\mathrm{PARi}\bullet\mathrm{Asat}}\right)}{\left(2\bullet\mathrm{theta}\right)}-\mathrm{Rd} \qquad (1)$$

Chlorophyll fluorescence– Rapid Light curves

Fully expanded, sun exposed leaves from three individuals from the previous species were cut and kept in dark for 10 minutes. Rapid light curves measure φ PSII as a function of irradiance (PAR) providing information of leaf light use without reaching a steady state (Ralph & Gademann 2005). It consists of series of illumination steps (μmol m^{-2} s^{-1}) with a with a saturation pulse applied at the end of each step assessing chlorophyll fluorescence parameters: PS II quantum yield (Yield), the relative electron transport rate (ETR) and calculating coefficients of photochemical and nonphotochemical quenching. Relative electron transport rate (rETR) was calculated using the equation (1) based on Ralph & Gademann, 2005.

$$rETR = \Phi_{PSII} * PAR \quad (1)$$

Curves were fitted to the data using the nonlinear function in the statistical program JMP 13 (SAS) using the equation (2) based on Ralph & Gademann, 2005. The following parameters were estimated: a) electron transport rate, (ETR_{max}), which is the maximum rate at saturating irradiance; b) alpha, which is the initial slope of the curve; c) minimum saturating irradiance (Ek) was calculated as ETR max/ alpha (equation 3).

$$ETR_{max} = ETRs\ (\alpha / [\alpha + \beta][\beta / \alpha + b])^{\beta / \alpha}\ (2)$$

$$Ek = ETR_{max} / \alpha\ (3)$$

Chlorophyll content

Chlorophyll content was measured in three leaf discs per leaf (1.57 cm2) and extracted at the lab with 95% ethanol and measured its concentration with a spectrophotometer using the equations of Lichtenthaler (1987) and expressed by area (mg/cm2):

95% Ethanol = (13.36 * A664) - (5.19 * A649) (27.43 * A649) - (8.12 * A664):

- Chlorophyll a: (mg/mL) = (13.36* A664) – (5.19 * A649)
- Chlorophyll b: (mg/mL) = (27.43* A649) – (8.12*A664)
- Total Chlorophyll: (13.36 * A664) - (5.19 * A649) + (27.43 * A649) - (8.12 * A664)
- Total Carotenoids (xanthophyll and carotenes): (1000 * A470 – 2.13* Ca – 97.64* Cb)/209

Leaf morphology and nitrogen concentration

Adjacent leaves to those used for the previous measurements were collected and stored in a cooler until reaching the lab to prevent water loss, and leaf area was measured with a LI-3100C Area Meter. All leaves were dried in a forced air circulation oven at 60 C until constant mass for dry mass determination. Leaves then were grounded with a planetary ball mill (Retsch) until passing over a 20-mesh sieve and analyzed for C and N concentrations with Elementar vario cube.

Carbon isotopes

Carbon isotope analyses ($\delta^{13}C$) were performed in leaf samples to reveal plant long-term WUE which has been associated with salt tolerance (Ball and Passioura, 1995). Intracellular CO_2 and plant water use efficiency (WUE) was calculated based on the Farquar et al. (1989) equation: $\delta^{13}C_{leaf} = \delta^{13}C_{air} – a – (b\text{-}a)\ C_i / C_a$, where $\delta^{13}C_{air}$ is the carbon isotope ratio of the CO_2 in the air (around 8.2 ‰); *a* is the fractionation by slower diffusion of $\delta^{13}C$ to $\delta^{12}C$ (4.4 ‰); *b* is the fractionation by ribulose biphosphate carboxylase against ^{13}C (27 ‰); and C_a is the atmospheric CO_2 concentration which averaged 413 µmol/mol at the time of the study. Intrinsic WUE was derived from the Lambers et al. (2008) equation, where intrinsic WUE = (A_n/g_s).

Statistical analyses

Restricted maximum likelihood (REML) estimation is a multivariate statistic technique for estimating variance and covariance components in multi-classified data. It was used instead of maximum likelihood because it gives an unbiased variance estimator. It was used for describing the effects of water level and salinity in leaf morphology traits. The results shown here are the coefficient of determinations. Shapiro Wilk Test was used to test normality in distribution. Analysis of variance (ANOVA) was used when data fits normality, if not, nonparametric Wilcoxon/Kruskal Wallis test was used with the statistical program JMP Pro version 13. A slope correlation analysis with a pairwise correlation, was used to compare between Leaf area / weight slopes, with the fit model function in JMP Pro version 13.

Results

Soil salinity varied temporally according to local climate and precipitation inputs related to the complex hydrological conditions of the area (Table 19). During the wet period, flooding was caused by rainfall and the tidal influences in the phreatic water level.

Table 18 Soil salinity (in ppt)for the study plots. Soil was sampled from the first 10 cm, and air dried until analysis. Data provided by graduate student Gloria Ortiz.

Soil Salinity					
	n	Jun-20	Sep-20	Mar-21	May-21
P3	12	7.9 ± 1.5	1.4 ± 0.5	10.7 ± 5.4	4.0 ± 0.4
P5	12	13.9± 2.6	2.4 ± 0.5	11.9 ± 4.3	1.3 ± 0.4
P6	12	12.2 ± 4.7	0.9 ± 1.0	10.2 ± 5.1	2 ± 0.6
P10	12	5 ± 1.3	1.9 ± 0.6	6.6±2.1	1.17 ± 0.6

Leaf traits

Leaf area (cm^2) and dry mass (g) showed linear regressions for all species (Fig 40). A slope and intercept correlation analyses shows the sequence: *Panicum and Echinochloa* had similar slopes, followed by *Acrostichum, Dalbergia* and *Laguncularia (Table 20).* The collections at different times during the year in the same individuals allowed to assess temporal variability.

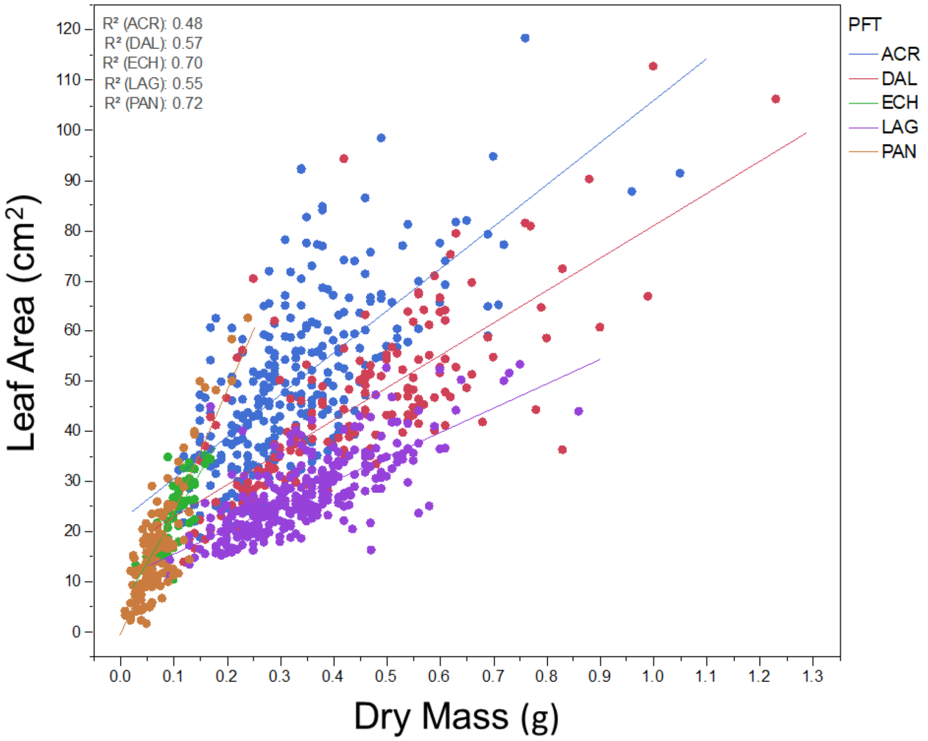

Figure 40 Leaf area and mass relationships regressions. r2 inside of the graph.

Leaf area followed the sequence *Acrostichum >Dalbergia >Laguncularia* ≥ *Echinochloa> Panicum.* On average, dry mass varied significantly among species, *Dalbergia* having the heaviest leaves (Table 15). Dry Mass followed the pattern *Dalbergia >Laguncularia* ≥ *Acrostichum >Echinochloa >Panicum*. As of specific leaf area, grasses were on one side of the spectrum with the highest values, *Echinochloa* (241 cm^2/g), *Panicum (*241 cm^2/g), followed by *Acrostichum* (164 cm^2/g), *Dalbergia* (110 ±4 cm^2/g), and *Laguncularia* the least (83 ±2 cm^2/g). There were no significant differences between specific leaf areas of *Echinochloa* and *Panicum*, both grasses.

Table 19 Equations for the leaf area/ dry mass correlation analyses.

Acrostichum	Leaf Area (cm2) = 22.132305 + 83.898188*Dry mass (g)	
Dalbergia	Leaf Area (cm2) = 16.41881 + 64.628369*Dry mass (g)	
Echinochloa	Leaf Area (cm2) = 4.6545653 + 181.3871*Dry mass (g)	
Laguncularia	Leaf Area (cm2) = 10.641212 + 48.588994*Dry mass (g)	
Panicum	Leaf Area (cm2) = -0.479225 + 243.31329*Dry mass (g)	a

Table 20 Leaf area/Dry mass slope pairwise correlations analyses. The first three letters of the genus were used as abbreviations.

Leaf Area/ Dry mass Slope Pairwise correlations							
PFT	**-PFT**	**Difference**	**Std Error**	**t Ratio**	**Prob>|t|**	**Lower 95%**	**Upper 95%**
ACR	DAL	11.4535	0.995293	11.51	<.0001*	8.7334	14.1737
ACR	ECH	-11.5620	6.442073	-1.79	0.3772	-29.1681	6.0440
ACR	LAG	22.0089	0.755510	29.13	<.0001*	19.9441	24.0737
ACR	PAN	-24.8747	4.543331	-5.47	<.0001*	-37.2915	-12.4578
DAL	ECH	-23.0156	6.475434	-3.55	0.0036*	-40.7128	-5.3183
DAL	LAG	10.5554	1.000865	10.55	<.0001*	7.8200	13.2907
DAL	PAN	-36.3282	4.590511	-7.91	<.0001*	-48.8740	-23.7824
ECH	LAG	33.5709	6.442936	5.21	<.0001*	15.9625	51.1794
ECH	PAN	-13.3126	7.847451	-1.70	0.4366	-34.7596	8.1343
LAG	PAN	-46.8836	4.544555	-10.32	<.0001*	-59.3038	-34.4634

Species sampled showed a certain variability in SLA values between collection dates which could be explained by changes in leaf age, as SLA decreases with leaf age (Medina et al. 2015) (Figs. 41 – 45). Leaf water content varied significantly among species, ranging from 75% in *Acrostichum*, to 58% in *Dalbergia*. The sequence goes as follow: *Acrostichum* ≥ *Echinochloa* ≥ *Panicum* > *Laguncularia* > *Dalbergia*. Length/width ratio varied across species, with the grasses *Panicum* and *Echinochloa* having the highest ratio, hence more linear leaves, followed by *Acrostichum*, whereas *Dalbergia* and *Laguncularia* presenting more elliptic to ovate leaves. Leaf succulence, which considers mass and area, varied between species, following the pattern *Laguncularia* ≥ *Echinochloa* > *Acrostichum* > *Panicum* ≥ *Dalbergia* (Table 22). Leaf water content by dry mass ratio varied significantly among species, the sequence goes as follows: *Echinochloa* > *Acrostichum* ≥ *Panicum Laguncularia* > *Dalbergia.*

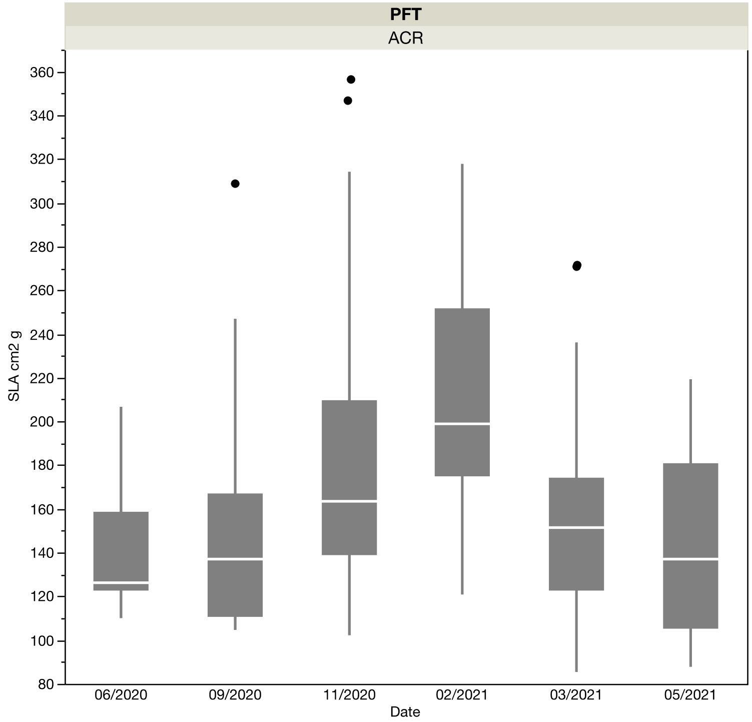

Figure 41 Specific leaf area (SLA cm2/g) for 1) Acrostichum over a period of one year.

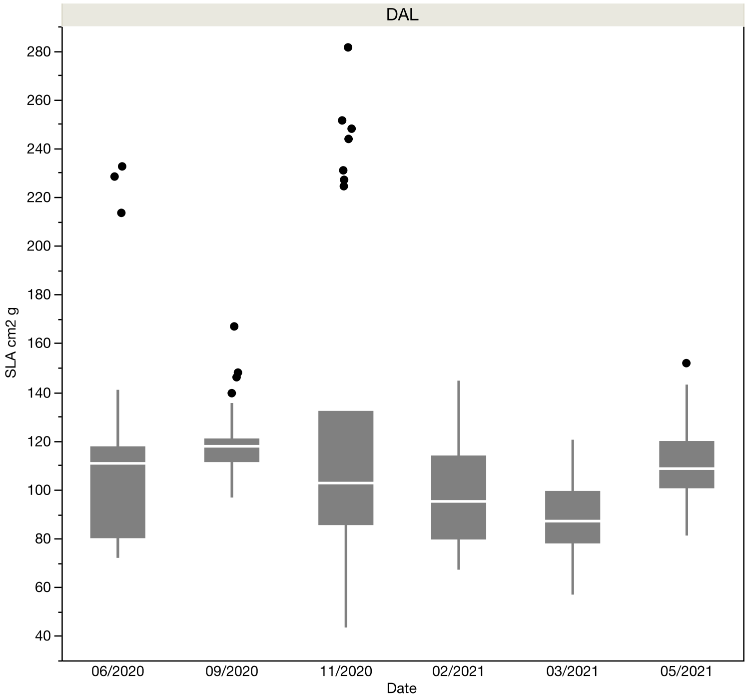

Figure 42 Specific leaf area (SLA cm2/g) for Dalbergia over a period of one year.

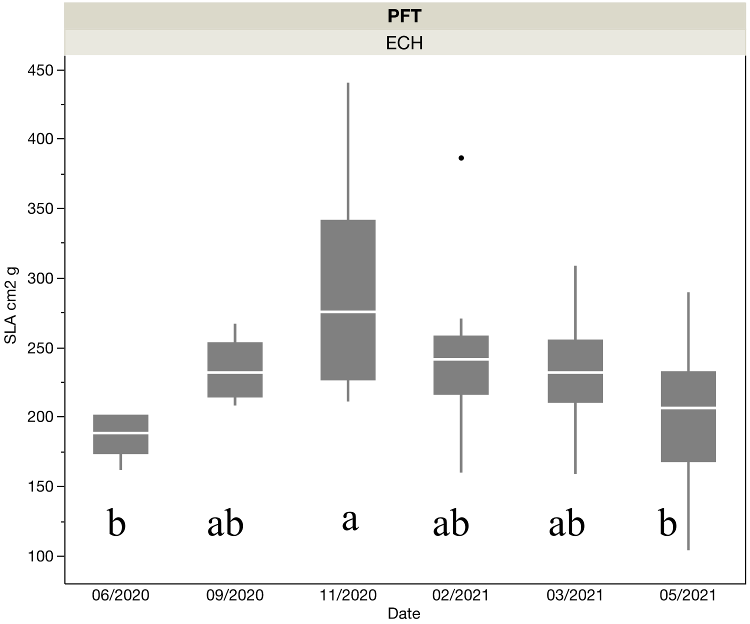

Figure 43 Specific leaf area (SLA cm2/g) for Echinochloa over a period of one year. ANOVA with post hoc Tukey HSD was used to determine differences between dates. Letters denote statistically differences between dates.

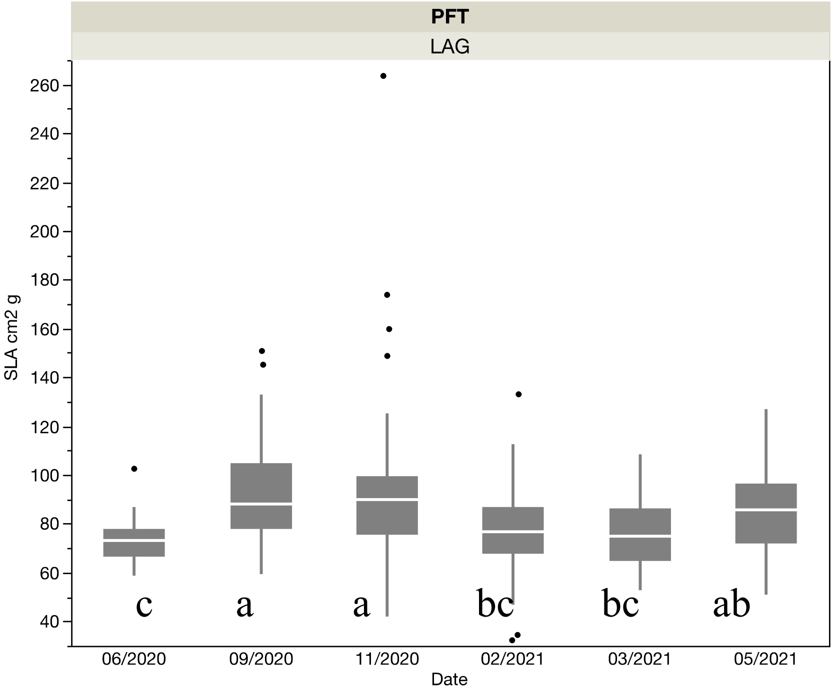

Figure 44 Specific leaf area (SLA cm2/g) for Laguncularia over a period of one year. ANOVA with post hoc Tukey HSD was used to determine differences between dates. Letters denote statistically differences between dates.

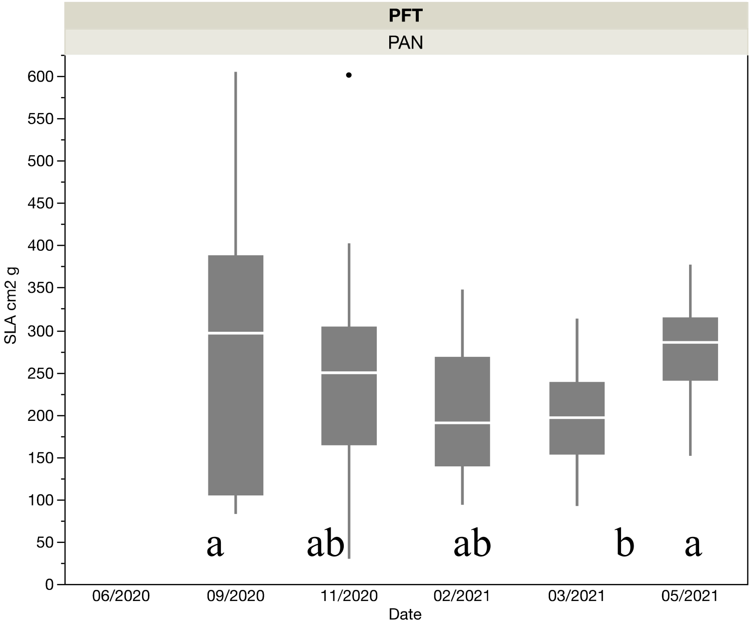

Figure 45 Specific leaf area (SLA cm2/g) for Panicum over a period of one year. ANOVA with post hoc Tukey HSD was used to determine differences between dates. Letters denote statistically differences between dates.

Leaf traits – abiotic conditions correlations

Weak and moderate correlations were present between soil salinity, phreatic water salinity, water level, leaf area, dry leaf mass, leaf water content, and SLA (Table 23). The strongest positive correlation was leaf water content and water level of *Echinochloa*. The most robust negative relationship was the water level, and the length-width ratio of *Dalbergia* leaves. For *Acrostichum* leaflets, soil salinity, leaf area, and maximum width had moderate positive correlations. On the other hand, water level and water content had weak positive correlations, but water level, length, and leaf width-ratio had weak negative correlations in *Acrostichum*. Precipitation only correlated positively with leaf area and negatively with length-width ratio.

In *Dalbergia* leaves, soil salinity did not correlate with the parameters, but phreatic water salinity correlated positively with dry mass, length, and length-width ratio; a weak negative correlation was found with SLA. Water level correlated positively with maximum width and negatively with length and length-width ratio. Precipitation correlated negatively with length and length-width ratio.

Echinochloa leaves did not correlate with soil salinity, but phreatic water salinity had negative correlations with maximum width, water content, and succulence; length and length-width ratio had positive correlations. Water level correlated positively with water content, maximum width, and succulence but negatively with dry mass, length, and length-width ratio. Precipitation only correlated negatively with length and length-width ratio.

Laguncularia leaves showed positive correlations between soil salinity and length but negative weak correlations with water content and succulence. Phreatic water salinity correlated positively with length. Water levels and precipitation did not have any effect on *Laguncularia* leaves morphology.

Panicum leaves had a negative correlation between soil salinity and water content. Phreatic water salinity also had negative correlations with length, length-width ratio, water content, and succulence. Water levels and precipitation did not have any effect on *Panicum* leaves.

Table 21 Average leaf dimensions, all dates together. An analysis of variance was used to determine significal differences between species with a post hoc Tukey HSD test to determine where differences came from. Pvalue <0.05. Letters denote statistically significant differences in columns.

	Dry mass (g)	Leaf Area (cm2)	Max Width (cm)	Length (cm)	L/W (ratio)	Leaf water content (%)	SLA (cm2 g)	Succulence (g/m^2)	Leaf Water content / Dry mass(g) ratio
Acrostichum 12 plants, 286 leaves	0.32 b	49.23 a	7.02 a	14.07 c	4.88 c	74.97 a	164.3 b	196±38 b	3.16 ± 0.9b
Dalbergia 6 plants, 171 leaves	0.44 a	45.03 b	7.16 a	9.66 d	2.20 cd	58.25 d	110.2 c	137±3 c	1.5 ± 0.6 8 d
Echinochloa 3 plants, 69	0.10 c	22.66 c	3.16 c	18.79 b	17.41 b	73.9 ab	241.1 a	236±290 b	6.2± 9.1 a
Laguncularia 12 plants, 305 leaves	0.34 b	27.12 c	5.57 b	7.67 e	2.25 d	69.73 c	83.2 d	291±57 a	2.4± 0.4 c
Panicum 9 plants, 142 leaves	0.07 c	15.74 d	1.11 d	23.89 a	43.16 a	72.83 b	241.0 a	163±231 c	3.2 ± 1.9 b

Table 22 Coefficient of determinations (r2) of soil salinity, phreatic water salinity, water level, and precipitation and leave morphology parameters. A Multivariate analysis (by REML method) was used to determine correlations between Species characteristics and abiotic factors. NS denotes no significance, and signs inside parenthesis represent positive (+) or negative (-) coefficient correlations. All correlations $p<0.05$

r^2		Dry mass	Leaf area	Max Width	Length	L/W	SLA	Water content	Succulence
Acrostichum	Soil salinity (1:1 vv)	NS	0.22 (+)	0.44 (+)	NS	0.31 (-)	NS	NS	NS
	Phreatic water salinity	NS	NS	0.20 (-)	0.19 (+)	NS	NS	NS	NS
	Water Level	NS	NS	0.15 (+)	0.12 (-)	0.11 (-)	NS	0.11 (+)	NS
	Precipitation	NS	0.13 (+)	NS	NS	0.12 (-)	NS	NS	NS
Dalbergia	Soil salinity	NS	NS	NS	NS	NS	NS	NS	NS
	Phreatic water salinity	0.11 (+)	NS.	NS	0.28 (+)	0.18 (+)	0.10 (-)	NS	NS
	Water Level	NS	NS	0.40 (+)	0.15 (-)	0.44 (-)	NS	NS	NS
	Precipitation	NS	NS	NS	0.17 (-)	0.15 (-)	NS	NS	NS
Echinochloa	Soil salinity	NS	NS	NS	NS	NS	NS	NS	NS
	Phreatic water salinity	NS	NS	0.13 (-)	0.18 (+)	0.32 (+)	NS.	0.28 (-)	0.17 (-)
	Water Level	0.10 (-)	NS	0.28 (+)	0.42 (-)	0.59 (-)	NS	0.57 (+)	0.49 (+)
	Precipitation	NS	NS	NS	0.11 (-)	0.19 (-)	NS	NS	NS
Laguncularia	Soil salinity	NS	NS	NS	0.15 (+)	NS	NS	0.10 (-)	0.10 (-)
	Phreatic water salinity	NS	NS	NS	0.18 (+)	NS	NS	NS	NS
	Water Level	NS	NS	NS	NS	NS	NS	NS	NS
	Precipitation	NS	NS	NS	NS	NS	NS	NS	NS
Panicum	Soil salinity	NS	NS	NS	NS	NS	NS	0.10 (-)	NS
	Phreatic water salinity	NS	NS	NS	0.11 (-)	0.11 (-)	NS.	0.22 (-)	0.21 (-)
	Water Level	NS	NS	NS	NS	NS	NS	NS	NS
	Precipitation	NS	NS	NS	NS	NS	NS	NS	NS

Field conditions photosynthesis – net assimilation

Salinity in the phreatic water differed between dates (Table 24), as June 2020 (dry period) saline conditions were higher and water level lower in contrast to February 2021 (wet period). Net Assimilation differed significantly for all species in both dates, having lower values in the dry season (Table 25). Stomatal conductance also was significantly higher in species, except for the C_4 grass during February than in June. Leaf nitrogen content differed between dates apart from *Dalbergia*. Leaf Assimilation (An) at 800 (mmol m^{-2} s^{-1}) varied across species, *Echinochloa the C_4 grass* had the highest assimilation rates (23 ± 4 µmol (CO_2) m^{-2} s^{-1}) of all species and *Acrostichum* the lowest (6.2 ± 3 4 µmol (CO_2) m^{-2} s^{-1}). The sequence goes as follows *Echinochloa > Lag > Panicum ≥ Dalbergia > Acrostichum.* Leaf internal CO_2 concentration followed the sequence: *Panicum ≥ Laguncularia ≥ Acrostichum > Dalbergia > Echinochloa.* Transpiration rates (E) followed the sequence: *Panicum ≥ Laguncularia ≥ Acrostichum > Echinochloa > Dalbergia. Panicum had* the higher stomatal conductance (ci) (251± 88 mmol m-2 s-1), the sequences go: *Panicum > Laguncularia > Acrostichum ≥ Dalbergia ≥ Echinochloa.) Dalbergia* and *Echinochloa* had higher water use efficiency than the other species (11.3 ± 10 and 10.6 ± 2.3 mmol CO2 mol-1 H2O; respectively). The sequence goes: *Dalbergia ≥ Echinochloa > Acrostichum ≥ Laguncularia ≥ Panicum.* Long term water use efficiency (wue) did not differ between the time periods.

Table 23 Climate and hydrological conditions, Soil salinity was a one-time per month. Phreatic water salinity was measured monthly from monitoring wells at each plot. Water level were taken from a data logger located at the monitoring wells. Tide height was retrieved from (https://tidesandcurrents.noaa.gov) for the sampling day; it is reported as a height from the mean lower low water (MLLW) datum. Cumulative precipitation was from previous 30 days prior to sampling. All measurements for each month were done in 1-2 days. Daily maximum PAR was the highest value recorded for each day.

	June 2020	February 2021
Soil salinity (1:1) (ppt) n=12	9.9±4	NA
Phreatic water salinity (ppt) (n=4)	13.5±8	20.6±5
Water level (cm)	-0.51±0.2	0.03±0.2
Tide - during sampling period	Low (-0.12 m MLLW)	High (0.49 m MLLW)
Cumulative precipitation (previous 30 days) (mm)	62.7	103.3
Daily Max PAR (mmol m^{-2} s^{-1})	1808	1917
Average leaf temperature during measurements (^oC)	35.2±0.8	32.1±1
Daily average temperature (^oC)	28.1	24.7

Table 24 Photosynthetic parameters in situ for both dates. A T-test analysis was performed between dates. Asterisk denotes statistically significance between dates. Net Assimilation (An) is the assimilation rate at 800 mmol m^2 s^1.

	Acrostichum		***Dalbergia***		***Echinochloa***		***Laguncularia***		***Panicum***
	Jun-20	Feb-21	Jun-20	Feb-21	Jun-20	Feb-21	Jun-20	Feb-21	Feb-21
	12 plants n=28	*12 plants n=38*	*6 plants n=*15	*6 plants n =6*	*1 plant n=3*	*3 plants n=15*	*12 plants n =49*	*12 plants n=37*	*9 plants n =*12
PAR	800 (mmol m^{-2} s^{-1})								
An umol (CO$_2$) m^{-2} s^{-1}	6.2 ± 3	6.9 ± 2.3	5.7 ± 2.5	12.1± 3	11.1± 1	23.1± 4	11.2± 3	7.7±3	8.7±3
Ci umol m^{-2} s^{-1}	113.1± 78	257.3 ± 103	40.2 ± 59	292.5 ± 30	101 ± 8	-	188.5 ± 70	301 ± 40	312.8 ± 25
E mmol m-2 s-1	1.1±0.7	4.3±2	0.42± 0.3	5.8± 0.8	0.99 ± 0.16	2.32 ± 0.7	2.6 ± 0.9	5.5 ± 1.4	5.3 ± 1
Gs mmol m-2 s-1	43.4 ± 31	161.6 ± 98	19.3 ± 14	273.2 ± 81	65.3 ± 10	70.3 ± 24	116.5 ± 53	223.5 ± 101	251.9 ± 88
A/E mmol CO$_2$ mol H2O	6.9 ± 5	2.1 ± 1.7	15.1 ± 10	2.1 ± 0.6	11.2 ± 0.6	10.5 ± 2.5	4.74 ± 1.8	1.5 ± 0.6	1.7 ± 0.6
Long term WUE (mmol / mol)	2.2 ± 0.06	2.2 ± 0.11	2.19 ± 0.09	2.4 ± 0.02	0.53 ± 0.06	0.50 ± 0.04	2.33 ± 0.1	2.34 ± 0.09	2.39 ± 0.03
A/gs (mmol / mol)	0.22 ± 0.27	0.05 ± 0.05	0.41 ± 0.38	0.06 ± 0.03	0.17 ± 0.01	0.26 ± 0.83	0.11 ± 0.05	0.04 ± 0.03	0.04 ± 0.01
VPD (mb)	2.4 ± 0.4	3.2 ± 0.9	2.2 ± 0.4	2.4 ±0.3	1.47 ± 0.06	3.35 ± 0.5	2.35 ±0.4	2.8 ±0.4	2.3 ± 0.3

Light response curves

Maximum photosynthetic capacity at saturating light capacity (A_{sat}) varied among species (Fig. 46), the C4 grass *Echinochloa* having the highest (44.6 μmol (CO_2) m^{-2} s^{-1}) a a light intensity of 1500 umol photon m^2 s^1; 3 times higher as *Panicum* a C3 grass and 1.5 higher as *Laguncularia*. A_{sat} sequence goes as follow: *Echinochloa* > *Laguncularia* > *Acrostichum* ≥ *Dalbergia* ≥ *Panicum*.

Apparent quantum yield (Φ) didn't have a statistically significant difference (Wilcoxon / Kruskal-Walli’s test), but *Echinochloa* in average had a higher apparent quantum yield (0.045) mmol (CO_2) $μmol^{-1}$ and the lowest was *Dalbergia*. The order from highest to lowest is the following: *Echinochloa* ≥ *Laguncularia* ≥ *Acrostichum* ≥ *Panicum* ≥ *Dalbergia*. Dark respiration rates (Rd) went from 4.6 μmol (CO_2) m^{-2} s^{-1} in *Laguncularia,* 3 times higher than the lowest in *Acrostichum*. Descending order goes: *Laguncularia* > *Echinochloa* > *Dalbergia* > *Panicum* > *Acrostichum* (Table 26).

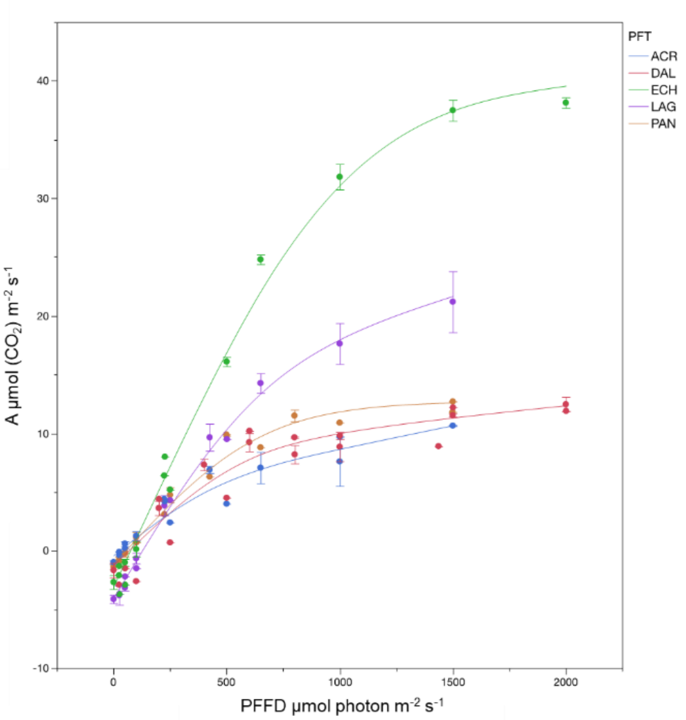

Figure 46 Light response curves regression. Three plants per curve were used during dry period. Error bars denote standard error

Table 25 Photosynthetic performance parameters. Averages and standard deviations n=3 for each specie. Letters denote significance in columns

	A sat µmol (CO_2) m^{-2} s^{-1}	θ	R_D mmol (CO_2) m^{-2} s^{-1}	**Φ** mmol (CO_2) $mmol^{-1}$ (photons)	**LCP** mmol (photons) m^{-2} s^{-1}
Acrostichum	13.5±0.9 c	0.53±0.2	1±0.2 c	0.03±0.002	18.5±5 b
Dalbergia	16±7.7 c	0.72±0.2	2±1.4 bc	0.02±0.007	62.7±36.8 ab
Echinochloa	45±1.6 a	0.93±0.02	3.6±1.2 ab	0.04±0.002	74.7±29 ab
Laguncularia	28±3 b	0.77±0.17	4.6±0.9 a	0.04±0.006	89.4±23.2 a
Panicum	14.5±1.1 c	0.88±0.11	1.5±0.3 bc	0.03±0.008	52.7±15 ab

Chlorophyll Fluorescence Rapid Light Curves

Maximum potential quantum yield (*Fv/Fm*) for all the species were high without any statistical differences, indicating no apparent stress at the time of measurement. Rapid light curves (RLC) were used to calculate ETR_{max} for each specie (Fig. 47). Electron transport rates (ETR_{max}) differed within species (Table 27), *Dalbergia* having the highest ETR_{max} (170± 51) and *Panicum* the lowest (77±17). The initial slope of the curve (α) was higher in *Echinochloa* than *Dalbergia* and *Panicum.* The minimum saturating irradiance (Ek) was significantly higher in *Dalbergia* than *Acrostichum*. qP, the proportions of open reaction centers, was different between species, with *Echinochloa* (a C_4 grass) having the highest ratio in contrast to *Panicum,* a C_3 grass which had the lowest. Non-Photochemical quenching (NPQ) was significantly higher in *Acrostichum* than the rest of the species.

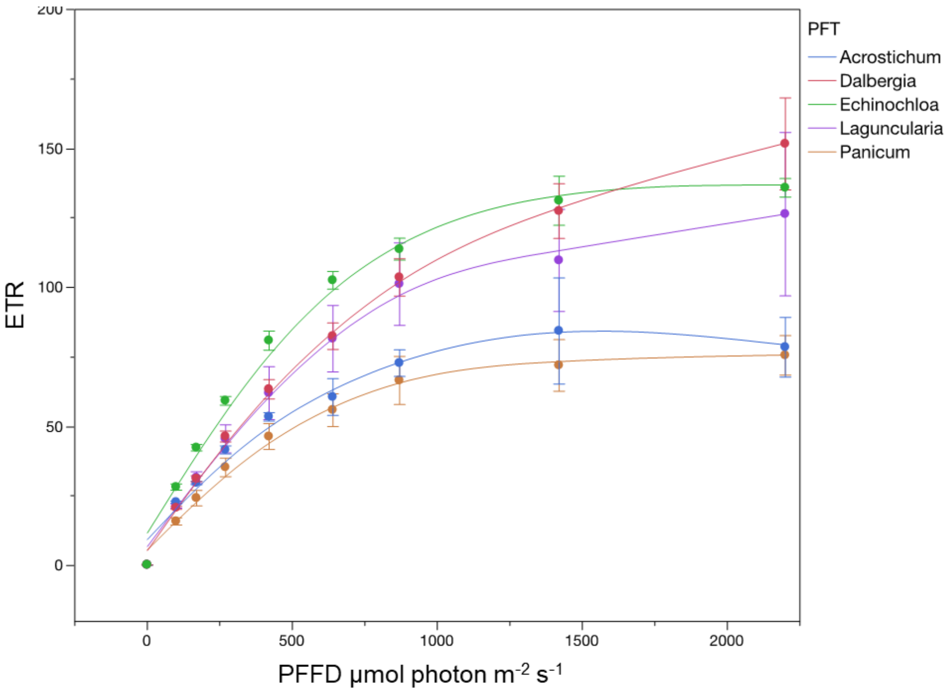

Figure 47 Rapid Light response curves. Error bars denote standard error. 3 curves per specie averaged. Measurements were done in the dry period.

Table 26 Chlorophyll fluorescence parameters averages and standard deviations, n=3 leaves for each specie. ANOVA with post-hoc Tukey analyses were used to determine statistical differences between species. Letters denote statistical significance in the same column.

	Fv/Fm	**ETR$_{max}$**	**α**	**Ek**	**qP**	**NPQ**
Acrostichum **n=3**	0.82± 0.04	81.1± 26.1 b	0.23±0.03 ab	372±168 b	0.52±0.03 ab	1.82±0.96 a
Dalbergia **n =3**	0.82± 0.01	170.6± 51.4 a	0.19±0.02 b	914±360 a	0.53±0.03 a	1.09±0.61 b
Echinochloa **n =3**	0.81± 0.01	137.0± 9.7 ab	0.30±0.03 a	467±71 ab	0.63±0.04 a	0.995±0.71 b
Laguncularia **n =4**	0.82± 0.02	131.6± 56.4 ab	0.22±0.02 ab	597±242 ab	0.52±0.03 ab	0.994±0.57 b
Panicum **n =3**	0.82± 0.01	77.0± 17.1 b	0.17±0.05 b	479±121 ab	0.38±0.03 b	0.73±0.53 b

Chlorophyll, carbon, and nitrogen concentrations

Chlorophyll a content per unit area was significantly higher in *Dalbergia* and lower in *Laguncularia*, the sequence goes as follows: *Dalbergia* > *Acrostichum* > *Echinochloa* ≥ *Panicum* > *Laguncularia* (Table 28). The species with the highest Clb were *Dalbergia* and *Acrostichum*, the sequence goes as follows: *Dalbergia* ≥ *Acrostichum* > *Laguncularia* > *Echinochloa* ≥ *Panicum*. Total Carotenoids contents had similar contents than Cla and Clb, content was higher *in Dalbergia* > *Acrostichum* ≥ *Echinochloa* ≥ *Laguncularia* > *Panicum*. There were not significant differences within species in the plots in Chlorophyll A and Chlorophyll B. There was a statistically significance difference in total carotenoids in *Acrostichum* within plots, as individuals in P6 had higher Cx contents than those in P3; the descending order goes as follow: P6 > P10 ≥ P5 > P3. (Fig. 49).

Table 27 Chlorophyll content, specific leaf area and nitrogen, carbon content averages and standard deviations.

	n	**Cl_a mg cm^2**	**Cl_b mg cm2**	**TCx mg cm2**	**SLA**	**% N**	**% C**
Acrostichum	21	6.1±1.4 b	2.6±0.6 a	1.53±0.4 ab	197±47 a	3.2±0.6 a	46±2.3 b
Dalbergia	6	7.7±1.4 a	3±0.6 a	1.91±0.3 a	121±25 b	2.9±0.8a	54±1.6 a
Echinochloa	11	5.5±1.3 bc	1.9±0.6 b	1.39±0.3 bc	213±31 a	3.5±0.04 a	44±0.4 b
Laguncularia	11	4.3±0.6 c	2.2±0.2 ab	1.19±0.1 bc	76±3 c	1.6±0.1 b	45±1.5 b
Panicum	7	5.1± 1.2bc	1.9±0.5 b	1.00± 0.2 bc	272±39	3.7±0.08 a	48.1± 0.69 b

Discussion

Leaf morphology

Abiotic conditions in the wetland fluctuated between oligohaline to mesohaline conditions. Neither soil salinity nor phreatic water salinity did not exceed 30 ppt. The wetland received constant inputs of freshwater through precipitation. Although water levels fell to 60 cm, no apparent stress was reflected in the leaf traits. The plot composition reflected a transition to *Laguncularia* inland, and closer to freshwater inputs. The area closest to seashore, were dominated by *Dalbergia* and grasses where inland plots were dominant by *Laguncularia*.

Acrostichum averaged 49.2±16 cm^2 pf leaf area across dates. We did not find previous studies regarding leaf measurements; we compared with the closest related specie, *A. aureum.* Medina (1990) reported leaf area of 44.8± 5.6 and 78.7± 6.4 growing under full sun and 119.5 ±7.7 of the shaded understory. Our values resemble full sun; the ferns sampled in our study were mainly in the understory but under a fragmented canopy after hurricane María's passage. Fonini et al. (2017) did not find differences between UV-treated plants and non-treated, suggesting the adaptations for full sunlight. Our SLA values are higher than Medina (1990) reported; however, the ferns under full sun in our sites have similar values. Leaf area, length, and width related positively to soil and phreatic water saline conditions, possibly indicating adaptations to constant salinity changes. Based on this information, it can be said that *Acrostichum* is not restricted to the understory in the wetland and has tolerance to saline conditions.

Dalbergia, a clamper shrub, had the widest leaves (7.1±2 cm) of the species sampled; this seems to be positively related to water levels and length and dry mass with phreatic water salinity. One possible cause is the shallow, superficial roots of *Dalbergia* as roots were abundant in the first 5 cm and decreased deeper. Increases in the water table by rainfall bring nutrient inputs (Medina & Francisco, 1997) hence the subtle increases in leaf size. *Dalbergia* exhibits a variable leaf morphology, as seen in the leaf length/width ratio, where two kinds of leaves can be distinguished: elliptical and ovate (deMorais et al., 2019). *Dalbergia (shrub)* and *Panicum (grass)* had similar succulence values but for different reasons, *Panicum had* low leaf area but small thickness, whereas *Dalbergia* has a thicker leaf because of sclerenchyma tissues, distinctive of plants resistant to water loss (Marques Das Neves et al., 2016).

Grasses had the highest SLA values for the species sampled. SLA and relative growth are positively correlated (Poorter & Remkes 1990). Leaves with high SLA usually have thinner leaves and decompose faster (Diaz et al., 2004). *Echinochloa*, considered one of the most productive plants (Sage 2016; Long 1999), correlated positively to water level, explaining almost 57% of increase of water content in leaves, possibly related to the need for water in photosynthesis. Baruch (1994), studying *Echinochloa,* found a decrease in leaf area with drought and lower SLA under flooding conditions, which were not seen in our study. *Echinochloa* produces longer linear leaves with increasing salinity and wider but shorter leaves with water availability. This is reflected in the water content as it decreases with salinity and increases with the water level. *Panicum* had the most extended leaves and the higher length-width ratio, meaning elliptical leaves, as with *Echinochloa*, have the highest SLA but a lower degree of succulence. Based on the negative correlations in length, water content, and succulence, it appears to be more susceptible to soil salinity and phreatic water salinity. *Echinochloa* and *Panicum* although both grasses, differed in their succulence values, this arises by anatomical differences resulting in thicker leaves in *Echinochloa (Silva-Correa & Maranho, 2021)* in comparison to *Panicum (*Zuloaga et al., 1992)

Laguncularia, dry mass, area, and SLA values are similar to those found by Lugo et al. (2007) in the basin of Jobos bay, although there is more variability in the present study, possibly to the freshwater availability. Low SLA values in *Laguncularia*, an halophytic tree, could mean more investment in structural components than investing in photosynthetic tissues (Franco et al., 2005). Decreases in water content and succulence with soil salinity contradict what is known of the physiology of *Laguncularia*, where increases in salinity could be related to solute accumulation, an adaptation to salt tolerance (Parida & Das, 2005). However, *Laguncularia* had the highest succulence of the species, I can infer those plants were neither under salt nor drought stress, hence the low correlations with soil and phreatic water salinities. We found that even species present in this coastal wetland have different trait variability between them and interspecific temporal variability responding in part to hydrological conditions (Maricle et al. 2007).

Field conditions photosynthesis performance

Laguncularia ranged from 11.2 to 7.7 fits with what Lugo et al. 2007 found in Jobos Bay between the fringe and basin which followed a salinity gradient. Lower An values, correlated with an increase in evaporation in February 2021 can be associated in an increase in salinity at the phreatic level. *Laguncularia* also exhibited lower water use efficiency during February, even though it had more saline conditions, it was flooded. It is thought that salinity and flooding are the main stressors for *Laguncularia*, but still is not well understood which exerts more influence over plant physiology (Hogan et al., 2021). In this case we believe that higher salinity values where the reason of lower A, because the flooding conditions and the low A/E compared to the dry season. However, there were no differences between periods in the integrated leaf water use efficiency, possibly meaning plant adjustments to humid conditions, as the hydrological conditions remained relatively constant throughout the year (Cardona-Olarte et al., 2009)

To the extent of our knowledge this is the first photosynthetic assessment of *Dalbergia*, a shrub in pantropical wetlands. *Dalbergia* increased An, Ci, E and gs during the wet period, while decreased WUE indicators of no apparent water stress even though it was flooded during this period, this was expected, as it is considered a flood-tolerant plant (Scarano, 1998). *Acrostichum* did not have significant differences in assimilation although rates between dates but did in the rest of the parameters all related to water use and more transpiration. In the grasses, *Echinochloa* had higher A rates than panicum and higher Evaporation rates, whereas *Panicum* had a higher WUE, possibly relating to the photosynthetic type. Flooding did not affect *Echinochloa*, following findings by Baruch (1994b).

The fact that there were discrepancies between long term and instant wue, could come from the fact that during the study period of one year, it was a humid climate and there was water available to plants, which led to no apparent stress in plants. The A/E and A/gs differences reflect daily acclimation to changes in water levels.

Correlation analyses determined that assimilation increased in *Dalbergia* with the phreatic water salinity and decreased in *Laguncularia*. The first case does not make sense particularly as it was not found a relationship with soil salinity. However, the correlation is stronger with *Laguncularia*, but it is stronger with water levels, reinforcing the hypothesis that water level, not interstitial salinity in this range (oligohaline), affect more photosynthetic performance of *Laguncularia*. In oligohaline environments, where salinity does not exceed 30 ppt, water level influences *Laguncularia* performance. This agrees with Krauss et al. (2006) which found intermittent flooding caused short term reductions in assimilation rates and with studies by Hogan et al. 2021 done with *Rhizophora mangle*.

Photosynthetic carbon assimilation (A) and Electron transport rate (ETR)

There were differences in the results between ETR_{max} and maximum assimilation rates (A) in the same leaves. This corroborates findings by Miyazama and Yahata (2006), that in field studies with spatial and temporal heterogeneity in environmental conditions, there are large differences in leaf temperature and other physical traits, rendering difficult correlation between these two parameters. Franklin and Badger 2001, working with macroalgae found a correlation between gross O_2 uptake and ETR under light-limited conditions, but not under saturating conditions, as it was in this study.

Apparent quantum yield seemed low in comparison to the theoretical value established for C_3 plants (~ 0.08 to 0.10). These low values could arise by errors in the methods during measurement which could underestimate quantum yield values; caused by the nonlinearity response of the photosynthetic light response (Singsaas et al., 2001)

The minimum saturating irradiance (Ek) for most of the species in the rapid light curves seems to be low fo tropical species in sun exposed leaves, these values are similar to those reported for *Rhizophora mangle* Cheeseman & Lovelock, 2004) and *Acrostichum* (Fonini et al., 2017).

Conclusion

- *Acrostichum* in urban setting was not restricted to the understory in the wetland and has tolerance to brackish conditions.
- *Dalbergia* exhibited plasticity in leaf morphology, as seen in the leaf length/width ratio, two kinds of leaves could be distinguished: elliptical and ovate (de Morais et al., 2019).
- *Echinochloa* was the specie more susceptible to soil salinity and phreatic water salinity.
- *Echinochloa leaves* were more succulent than those of *Panicum* due their thicker leaves as seen in the SLA differences and leaf water content.
- Our results suggests that water level, not interstitial salinity in brackish conditions, affects photosynthetic performance of *Laguncularia.*
- CO2 assimilation of the species sampled varied significantly during the measuring times
- Gas exchange and chlorophyll fluorescence measurements did not yield similar results in field saturating conditions curves.

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Vegetation Regeneration After Hurricane María Passed Over an Urban Coastal Wetland in Northeastern Puerto Rico

Introduction:

Coastal wetlands are transitional areas between terrestrial and marine ecosystems, with variable periods of water saturation (Cowardin et al., 1979). The presence and distribution of coastal wetlands result from eustatic sea-level rise and terrigenous sediment deposition and substrate development (Cohen et al., 2016). Plant species coexist in a dynamic state of change where eco physiological adaptations to waterlogging and saline conditions predict wetland structure and function (Medina and Francisco, 1997). It is possible to predict regeneration processes by considering these adaptations (Buma, 2015). Determining post-disturbance regeneration in vegetation cover is necessary for coastal urban wetlands which undergo constant stresses yet can establish floral and faunal communities (Perillo et al., 2019). Long-term studies suggest different pathways of succession, where ecosystem recovery can take from a couple years by plant resprouting or decades with shifts in vegetation (Fickert, 2018). Little is known about the factors involved in regeneration in these ecosystems where disturbance effects can be seen even after 30 years (Ferwerda et al., 2007).

The level of vegetation cover loss in wetlands caused by hurricane impacts depends directly on hurricane intensity, duration, forward speed of the storm, and wetland distance over which the storm passes (Morton and Barras, 2011). Indirect effects from hurricanes are prolonged retention of storm-surge seawater, flooding, and adverse physiochemical plant reactions to waterlogging and salinization (Morton and Barras, 2011). On September 20, 2017, at 10:00 am, category 4 hurricane María, with sustained winds of 69 m/s, crossed the island of Puerto Rico diagonally in the SE-NW direction. NOAA simulations estimate maximum flood levels up to 1 meter above mean sea level for the metropolitan area (Pasch et al., 2019). A tide gauge in San Juan reported up to 1.2 meters of swell before going out of service, and a total rainfall of 406 mm was recorded for the metropolitan area (Pasch et al., 2019). In Cucharillas, according to rapid post-hurricane assessment, freshwater flooding, seawater storm surges, and squalls from hurricane María resulted in substantial tree fall, tree decapitation, and extensive defoliation. How has the wetland regenerated after this event? In Branoff et al. (2018), we observed that previous cover and composition of functional plant groups in the wetlands were altered due to initial and subsequent changes in salinity, tidal effects, and light regime in the wetland.

This study determines the spatio-temporal regeneration dynamics of plant cover and composition during the first 2 years after hurricane María by determining current land cover and plant composition and assessing plant regeneration and succession dynamics by means of eco physiological traits of the vegetation.

Methods:

Post Hurricane Image Collection

A Phantom 3 (UAV) electrically powered quad copter (DJI Company) was operated with a Red-Green-Blue (RGB) camera in April 2018, October 2018, January 2019 and October 2019 to capture wet and dry season variability. Since the island of Puerto Rico and the Caribbean are located near the equator, the two seasons are defined as: “wet” season which comprises the months of May to October and “dry” season from January to April (Walter, 1971). Missions were planned around optimal weather conditions, which included no rain and low wind speed (less than 10 knots). The flight team consisted of three individuals – a certified remote pilot in command (remote PIC) and two visual observers (VOs). The UAV was programmed to fly a series of parallel transects over Cucharillas wetland. Surveys were divided in three zones inside the study area (total 60 ha). Each mission was flown at an altitude of 50 meters (2.2 cm/pixel resolution) in compliance with FAA regulations and each flight lasted about 18-20 minutes. Approximately 8 missions over a period of 3 days were needed to cover the 60 hectares of the Cucharillas wetland. A 2.2 ha area inside the study area was chosen for classifying land cover by functional groups.

The Map Pilot planning mobile application installed on an iPad Air was used to plan and fly the series of transects across each survey area. The collected images were sent to Maps Made Easy (web base aerial map processing) where they created a spatially corrected orthomosaic (tiff file) of the area with a resolution of 2 cm, therefore, land features as small as 2 cm were identified.

Ortho mosaic images were analyzed using the program ArcGIS Pro, the first analysis carried out was to create a vegetation index using only the visible RGB (red, green, blue) bands. Spectral vegetation indices are used for monitoring and analysis of spatial-temporal variations in vegetation structure, commonly based on infrared bands. The VARI (Visible Atmospherically Resistant Index) allows the calculation of vegetation indices using the visible bands (Gitelson et al, 2002; Kavoosi et al., 2018):

$$\text{VARI} = (\text{Bgreen} - \text{Bred})/ (\text{Bgreen} + \text{Bred} - \text{Bblue}).$$

After this, vegetation cover was identified manually creating feature classes, rasterized (converted from vector to a raster image) and adapted to the same spatial resolution for pixel-by-pixel comparison. Vegetation cover will be based on functional groups type. The use of functional groups allows us to describe vegetation groups focusing on physiological characteristics in response to environmental factors and ecosystem processes (Chapin et al., 1996). This gives an understanding of processes at an ecosystem level (Semenova, 2000). Functional groups could be based on similar life strategy, growth form, photosynthetic pathway or any kind of trait which relates to a same group of plants behaving similar. and cover categories were trees, herbs, shrubs, grasses, water, managed areas, and bare soil. The four vegetation-based categories represented plant growth forms. Using these land cover categories, the description of vegetation types focuses on physiological characteristics in response to environmental factors and ecosystem processes (Chapin et al., 1996) (Table 29).

Table 28 Land cover categories and definitions for spatial assessment

Category	Definition
Woody vegetation	Trees. Woody vegetation with a trunk, supporting branhes and leaves. e.g., *Laguncularia racemosa & Avicennia germinans*
Open water	No vegetation or soil seen
Managed areas	Infrastructure, recreation and educative areas. Green pruned grasses areas.
Grasses	Hollow stems and narrow alternate leaves
Emergent wetland	Ferns and herbaceous vegetation with no woody stems above ground.
Wetlands shrubs	Shrubs, woody vegetation which doesn't exceed 5 meters in height (e.g., *Dalbergia ecastaphyllum*)
Bare soil	No vegetation seen, includes educative and work trails

Species composition assessment

At the beginning and ending of the image collection missions in April 2018 and November 2019, random on-site vegetation assessments for plant cover were done to corroborate visual inspection of images. Prior to image collection, three 100m^2 quadrants were randomly selected and assessed and four 100m^2 quadrants were assessed post-image collection. For trees, diameter at breast height was measured, species were identified and counted, individuals were identified as dead or alive, and for herbaceous species, percent cover was measured and identified at the family taxonomic level. From 2018 to 2019, periodic census of species with flower or fruits within the wetlands were conducted to collect specimens for identification and classification. Individuals were pressed, dried, identified and grouped into functional groups based on life forms. Literature and herbarium research from New York Botanical Garden (http://sweetgum.nybg.org/pr/specimens.php), University of Puerto Rico, Rio Piedras (http://herbariodb.uprrp.edu/bol/) and Mayaguez campus (http://herbaria.plants.ox.ac.uk/bol/mapr) were used to determine previously identified species present in the wetland before hurricane to compare determine species that are not found in post- hurricane years.

Leaf traits

We assessed leaf area and dry mass at least once each dry and wet period for the three plant types representatives of the plant growth form (Table 30). At the end of the study, we measured δ13C and δ15N isotopes in the leaf tissue for assessing long-term water use efficiency (WUE). study. Intrinsic WUE was derived from the Lambers et al. (2008) equation, where intrinsic WUE = (An/gs). The leaves were stored in a cooler to prevent water loss until analysis. Leaf area was measured with an LI-3100C Area Meter and later dried in a forced-air circulation oven at 60◦C for dry mass determination. Specific leaf area (SLA; the area of a fresh leaf divided by its oven-dry mass) was calculated as an index of the construction cost of leaves. SLA tends to relate positively to relative growth among species (Pérez-Harguindeguy et al., 2016). In the leaf economics spectrum, SLA indicates habitat preferences and plant productivity in environments under stress (Medina et al., 1990b). The SLA is also related to light conditions, where high values indicate shaded leaves and low values suggest high light or open canopies.

Carbon isotope analyses (δ13C) were performed in leaf samples to reveal plant long-term WUE which has been associated with salt tolerance (Ball and Passioura, 1995). Intracellular CO2 and plant WUE was calculated based on the Farquhar et al. (1989) equation: $\delta 13Cleaf = \delta 13Cair - a - (b-a)\ Ci / Ca$, where δ13Cair is the carbon isotope ratio of the CO2 in the air (around 8.2 ‰); a is the fractionation by slower diffusion of δ 13C to δ 12C (4.4 ‰); b is the fractionation by ribulose biphosphate carboxylase against 13C (27 ‰); and Ca is the atmospheric CO2 concentration which averaged 413 μmol/mol at the time of the study.

Results:

Around 33% of the study area was devoid of standing vegetation (Figure 48), which decreased considerably in July 2018 and January 2019. In April, trees were the predominant cover with 8,221 m2, followed by grasses, 2,532 m2, shrubs, 1,545 m2, herbaceous vegetation, 1,368 m2, and managed areas with 778 m2 of the study area In July 2018, trees continued as the dominant cover with 10,929 m2, followed by grasses, 2,955 m2, herbaceous cover 22 2,763 m, shrubs 2,077, and managed spaces with 312 m (Fig. 49). In January 2019, trees occupied 10,345 m2, a decrease related to the removal of trees due to rehabilitation efforts, herbaceous cover 5,363 m2, shrubs 2,084 m2, grasses 1,767 m2, and managed areas 1,677 m2 within the study area (Fig.49, Table 30).

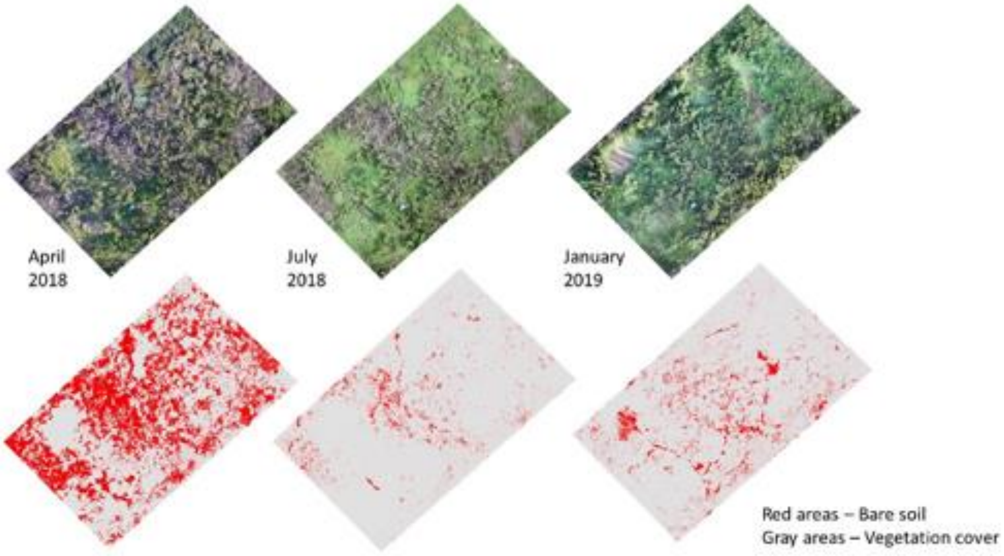


Figure 48 Aerial imagery of the study area. (A) Red-Green-Blue (RGB) drone images taken in April 2018, July 2018, and January 2019 are the raw images collected to determine (B) area of standing vegetation and bare soil within the study site in April 2018, July 2019; red color indicates bare soil and gray color indicates alive vegetation

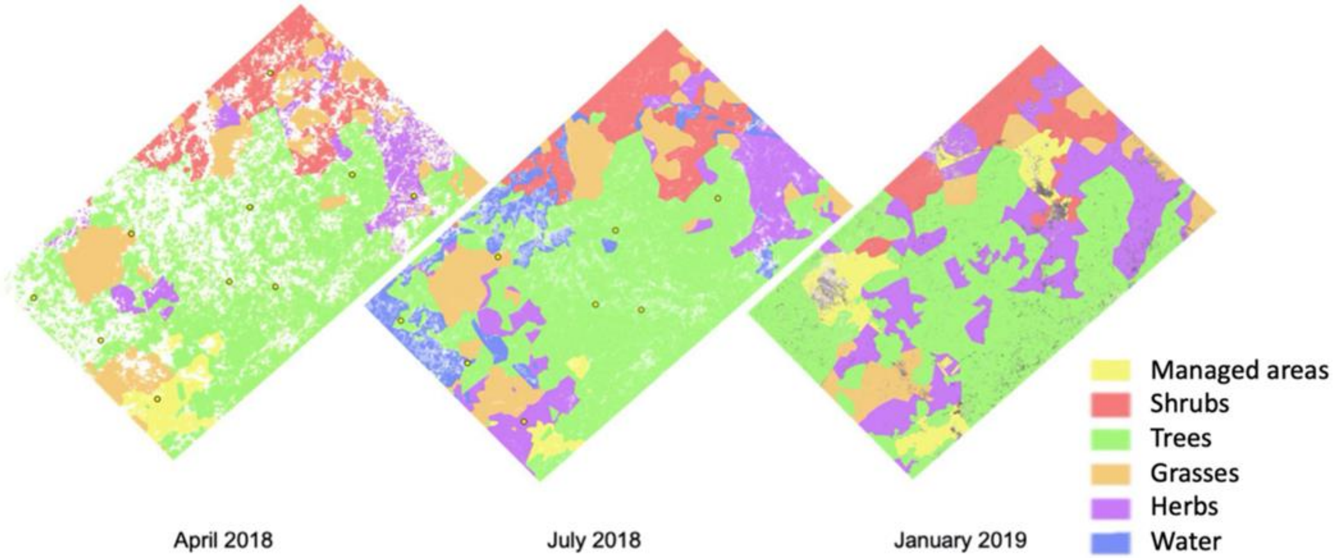


Figure 49 Spatial classification of land cover of each vegetation type in April 2018, July 2018, and January 2019. Yellow dots denote monitoring wells where phreatic water measurements were taken.

Table 29 Land cover area in m^2.

Class	Apr 2018 Cover (m^2)	July 2018 Cover (m^2)	January 2019 Cover (m^2)
Trees	8,221 (37.9%)	10,929 (48.5%)	10,435 (46.3%)
Managed areas	778 (3.6%)	312 (1.4%)	1,677 (7.4%)
Grass	2,532 (11.7%)	2,955 (13.1%)	1,767 (7.8%)
Emergent wetland	1,368 (6.3%)	2,763 (12.3%)	5,363 (23.8%)
Shrubs	1,545 (7.1%)	2,077 (9.2%)	2,084 (9.2%)
Surface Water	0	2,489 (11.0%)	0
Bare soil	7,217 (33.3%)	995 (4.4%)	1,208 (5.4%)
Total	21,661	22,520	22,534

Precipitation data exhibited the bimodal pattern for the island, June (316 mm) and August (281 mm) being the wettest months. The driest month was March (60.9 mm; Figure 4). At the phreatic level, we found higher conductivity values closer to the coastline with a sharp drop around 1 km inland, with conductivity values ranging spatially from 10 to 35 mS. We found marine intrusion at a depth of 2.5 meters in most of the wells (Figure 5). This trend prevailed through the wet period (September to November) and to the beginning of the dry period (January 2019; Fig. 50).

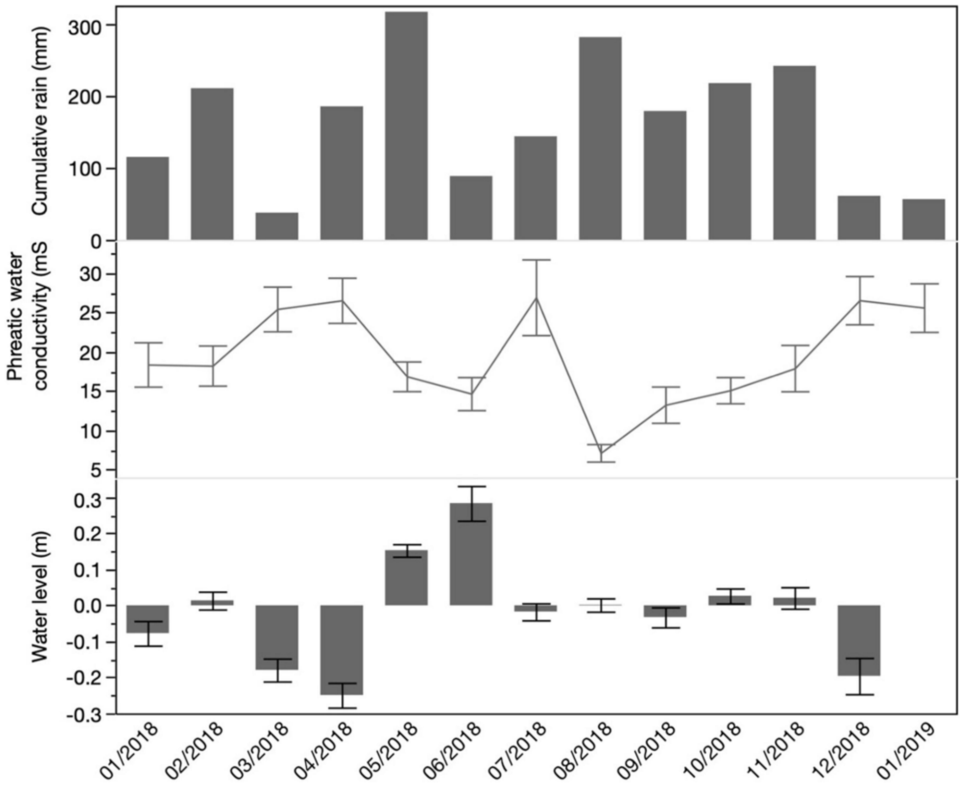


Figure 50 Total monthly rainfall accumulation (mm) and average monthly phreatic water conductivity (mS/cm) and water levels (negative values indicate below soil level, positive values are above soil level) in ciénaga Las Cucharillas. Error bars represent ± standard error

Ground truth and species composition assessment

From May 2018 to March 2019, we collected a total of 58 species which we categorized into 32 families. The predominant plant growth form was herbs with 34 species, followed by 10 species of trees, 10 vines, and 2 species of shrubs and 3 grasses. We found a proportion of 0.17 of woody species to non-woody (10 woody vs. 48 non-woody species), and 47 native species and 11 nonnatives (4.3 ratio). There were 42 dicotyledons and 12 monocotyledons. Of the collected species, 46 were perennial species, and 12 were annual (Table 2).

Leaf Traits

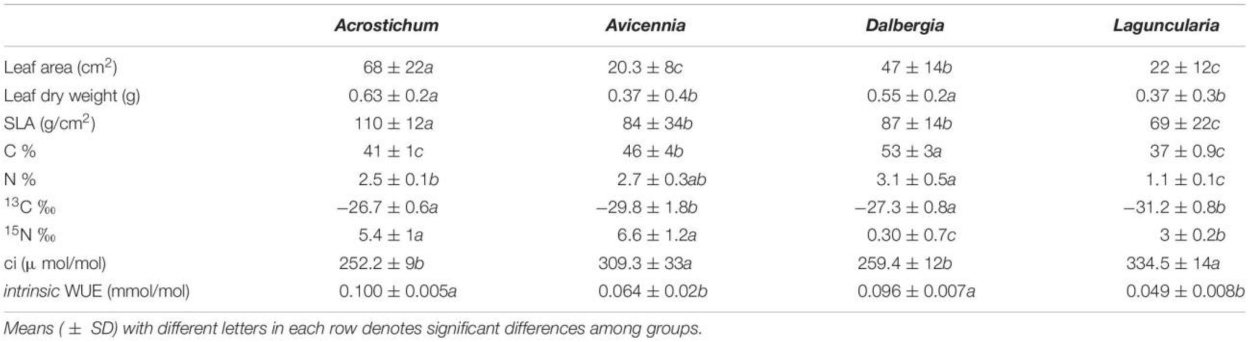
Trees had a lower leaf area than herbaceous and shrub species, where Avicennia and *Laguncularia* leaves were smaller than *Acrostichum* and *Dalbergia* (Table 31).

The heaviest leaves were from *Dalbergia*; *Laguncularia* and Avicennia trees had similar foliar weights to each other. Leaves for all species followed the same weight area relationship, indicating a uniform sampling of adult leaves, allowing for comparison within dates. SLA for species varied significantly between dates, showing decreasing patterns post-hurricane in Avicennia, and increasing SLA values in *Dalbergia*. *Laguncularia* remained constant, except for June 2019 which SLA values decreased significantly from the rest of the dates (average 47.2 g/cm2). The pattern of SLA was *Acrostichum* > *Dalbergia* ≥ Avicennia > *Laguncularia* (Table 31).

Leaf carbon (C) concentration varied from 36 to 58%, with a mean of 45 ± 7 % in all species. *Dalbergia* had the highest C concentration with 53 ± 3 %, and *Laguncularia* had the least with 37 ± 0.8 %. Tukey-Kramer comparison showed significant differences between species, except between *Acrostichum* and *Laguncularia*. Leaf nitrogen (N) concentration ranged from 0.91 to 3.95 %, with a mean of 2.4 ± 0.8 %. *Dalbergia* had the highest N concentration with 3 ± 3.1%, and *Laguncularia* had the least with 1.1 ± 0.1 %.

Leaf C isotope ratios spanned a range of over 6 $^{o}/_{oo}$ with a mean of −28.7 ± 2.1 $^{o}/_{oo}$. Avicennia showed a large range of values from −32.2 to −28.2 $^{o}/_{oo}$, (average −29.8 ± 1.9 $^{o}/_{oo}$) while the rest of the species had smaller ranges (standard deviations <0.8 $^{o}/_{oo}$). *Acrostichum* and *Dalbergia* C stable isotopes were similar and differed significantly from *Laguncularia* and Avicennia. N isotope ratios spanned a range of over 8 $^{o}/_{oo}$ with a mean of 3.79 ± 2.7 $^{o}/_{oo}$. Avicennia had the highest values with 6.6 ± 1.2 $^{o}/_{oo}$ and *Dalbergia* the lowest with 0.3 ± 0.7 $^{o}/_{oo}$. Plant WUE varied significantly among plant life-forms where trees had lower values than herbaceous species.

Table 30 Plant functional traits, carbon and nitrogen contents, isotopic signatures, intracellular CO_2concentrations (ci), and intrinsic water use efficiency (WUE) for the plant functional types.



	Acrostichum	***Avicennia***	***Dalbergia***	***Laguncularia***
Leaf area (cm^2)	68 ± 22*a*	20.3 ± 8*c*	47 ± 14*b*	22 ± 12*c*
Leaf dry weight (g)	0.63 ± 0.2*a*	0.37 ± 0.4*b*	0.55 ± 0.2*a*	0.37 ± 0.3*b*
SLA (g/cm^2)	110 ± 12*a*	84 ± 34*b*	87 ± 14*b*	69 ± 22*c*
C %	41 ± 1*c*	46 ± 4*b*	53 ± 3*a*	37 ± 0.9*c*
N %	2.5 ± 0.1*b*	2.7 ± 0.3*ab*	3.1 ± 0.5*a*	1.1 ± 0.1*c*
^{13}C ‰	−26.7 ± 0.6*a*	−29.8 ± 1.8*b*	−27.3 ± 0.8*a*	−31.2 ± 0.8*b*
^{15}N ‰	5.4 ± 1*a*	6.6 ± 1.2*a*	0.30 ± 0.7*c*	3 ± 0.2*b*
ci (μ mol/mol)	252.2 ± 9*b*	309.3 ± 33*a*	259.4 ± 12*b*	334.5 ± 14*a*
intrinsic WUE (mmol/mol)	0.100 ± 0.005*a*	0.064 ± 0.02*b*	0.096 ± 0.007*a*	0.049 ± 0.008*b*

Means (± SD) with different letters in each row denotes significant differences among groups.

Discussion

This study evaluated plant successional dynamics in coastal urban wetlands after a hurricane. The fast-slow continuum can explain these dynamics, where fast-growing but short-lived leaves are on one side of the spectrum, and slow-growth long-lived leaves are on the other, as leaf traits results suggest. Changes in hydrological conditions to more freshwater conditions will favor the establishment of herbaceous, non-halophyte species; however, as conditions stabilize to more saline, woody vegetation possibly bring nutrient inputs to the ecosystem, increasing the speed of the ecosystem's recovery. Hurricanes in coastal urban wetlands might initially affect plant cover but do not affect plant composition in the long term. Climate change, expressed as precipitation extremes, sea-level rise, and increased evapotranspiration due to

increased temperatures resulting in an increase in dry days (PRCCC, 2014), establishes the stressor baseline for coastal wetland ecosystems dynamics.
Wetland vegetation cover had a high recovery rate – 16 months after the hurricane, vegetation cover occupied 87 % of the study area, a 20 % increase in a year and a half post- hurricane. Regeneration dynamics were seen 7 months after the hurricane as barren substrate was occupied by polyhaline tolerant plants characterized by high SLA, fast growth, and short- lived leaves (Poorter and Remkes, 1990). Non-flooded conditions facilitated recolonization.

Leaf economics spectrums correlate with the plant fast-slow continuum, where plants that live longer invest more resources in leaf construction, whereas short-lived plants usually have fast growth leaves but are shorter-lived (Salguero-Gómez, 2016). We found that to be the case in this study, as the plant cover succession is related to the leaf investment of dominant plant species. Herbaceous cover doubled during the first 10 months, in contrast to tree cover, which had a moderate increase. Of the dominant species we assessed, *Acrostichum* (herbs) had a significantly higher SLA than *Laguncularia* and Avicennia.

As the wet season started, changes favored herbaceous species such as *Acrostichum*, tolerant to waterlogging, which doubled their plant cover. Sharpe (2009) reported increases in biomass rates and fertile leaf production of *Acrostichum* 8 months after a hurricane and a five-time increase in biomass rates after 2 years. This shift in vegetation could be due to changes in hydrological conditions, as has been previously reported in other coastal wetlands, where changes from saltwater conditions to freshwater inputs will favor the establishment of herbaceous wetland instead of woody vegetation (Ball, 1980; Clark and Csiro, 1988). The 20 % increase in tree cover observed in July 2018 can be explained as branching and canopy development of surviving trees. McKee et al. (2007) found positive interactions between herbaceous vegetation and mangroves, proposing the former as facilitators of mangrove recolonization in disturbed areas by trapping propagules and increasing survival and growth by enhancement of edaphic physiochemical factors. This proposed facilitation was not part of our study. Seedling distribution and survival should be included in future studies.

Hartman (1988) found that after disturbances in tidal saline wetlands, regeneration is controlled by vegetative propagation due to the scarcity of a seed bank. Based on field observations and image visual interpretation, the moderate increase in *Dalbergia* shrub cover in a saline area was due to vegetative propagation (Francis, 2004). Our results suggest the importance of vegetative propagation under constant salinity stress. After 16 months, a shift in vegetation cover was observed where species with long-lived, slow growth and low N content leaves (as in *Laguncularia*) had larger plant cover. WUE analysis seems to support these observations as species with higher WUE have higher chances of surviving and regrowing despite waterlogging or dry periods that intensify after hurricanes. High 15 N values in the sampled species point toward a eutrophic system where high nutrient availability increases the system’s regeneration speed. *Dalbergia* is an exception because of the symbiotic relationship with nitrogen-fixing bacteria (Saur et al., 2000). Another aspect to consider in urban coastal wetlands after disturbances is the arrival of non-indigenous species. Bhattarai and Cronin (2014) argued that hurricanes bring non-indigenous species to the ecosystem. We did not find this in our study area reserve, as native species present outnumbers non-indigenous species. This can be credited to several factors, such as biotic resistance or environmental factors in urban wetlands (Ehrenfeld, 2008; Ackerman et al., 2016). It was seen the introduction of several species after the hurricane. Some examples of these species are: *Muntingia calabura* ,*Lemna aequinoctialis*, and *Cissus verticillata*.

Hurricanes are a stress test to ecosystems where vegetation response will vary according to their severity (Lugo, 2000). The dynamic nature of coastal wetlands allows for resilience to stressors of different intensities. Hurricane María, an acute catastrophic stressor, severely affected the coastal wetland. Our results show that although the wetland was observably greatly affected initially, it was resilient in the continuous recovery of the vegetation cover despite the severity of the disturbance. How chronically stressed wetlands respond to catastrophic stressors will test the resiliency of these systems.

Conclusions

- Plant functional types proved to be resilient to the initial hurricane effect and subsequent changes in conductivity and freshwater conditions. Fast-slow continuum traits could help explain plant regeneration dynamics after a hurricane.
- High 15N values in the sampled species point toward a eutrophic system where high nutrient availability increases the system’s regeneration speed.
- Wetland vegetation cover had a high recovery rate without rehabilitation intervention. The succession dynamics and the hydrological conditions described in this study can help wetland managers prioritize rehabilitation (if necessary) efforts after disturbances.
- Future studies will expand how spatio-temporal conditions in the wetlands, sea-level rise, and extremes of precipitations and the degree and intensity of further atmospheric disturbances will affect long-term community dynamics.

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General Conclusions

Coastal urban wetlands have been a relevant subject of study in recent decades, but there have been few studies on the long-term plant responses in these ecosystems. Furthermore, these ecosystems are under constant pressure from anthropogenic sources, global climate change, and increased regional climate variability. We aimed to describe how plant functional groups acclimate their morpho-physiological ecological responses to past and present changes in the landscape for urban and industrial development. At an ecosystem scale, Ciénaga las Cucharillas has spatial variability in the mineral composition of the wetland soils as a result of legacy effects.

These differences in the soil composition bring higher concentrations of elements not typically found in wetlands such as Cu, Zn, Cd, and Pb; the latter two are not essential elements and could be harmful to plant species and the food web in general. Other anthropogenic effects on the coastal wetlands are the current vegetation composition, which showed some changes according to $\delta^{13}C$ values in the soil profile and our botanical census, and anthropogenic nitrogen inputs.

Even though Ciénaga las Cucharillas is surrounded by urban areas with constant pollution inputs, the ecosystem serves as both a source and sink of available nutrients and harmful elements depending on the rainfall regimen. During wet periods with waterlogging conditions, the wetland acts as a sink with low nutrient availability, changing to a nutrient source during the dry period and aerobic soils. Plants in coastal wetlands have remarkable plasticity and have adapted to these continuous abiotic changes. *Acrostichum* and *Laguncularia* can mitigate the harmful effects of certain heavy metals in the environment by bioaccumulating them in leaves and roots rather than remaining in the soils, suggesting they could be used for phytostabilization of wetlands. *Laguncularia* accumulated Pb in leaves, making it suitable for long-term bioremediation practices in coastal areas. Controlled experiments should follow to complement the data we observed under field conditions. Furthermore, we did not find that harmful soil elements affected any species photosynthetic performance. The vegetation in the ecosystem is opportunistic, establishing wherever space and resources are available; however, because of the constant changes in water level and conductivity, species composition is variable across relatively short temporal scales.

In a broader sense, we should focus on how spatio-temporal conditions in the wetlands, sea-level rise, and extremes of precipitations and the degree and intensity of further atmospheric disturbances will affect long-term community dynamics. The regional climate variability drives changes in coastal wetlands processes through sea-level rise, changes in tropical storm intensity, and more extended periods of extreme weather conditions, such as drought and flooding. The Ciénaga las Cucharillas topography is below mean sea level, and a flooding mitigation dam sustains current hydrological conditions. This makes it susceptible to minimal changes in sea-level rise, but if the barrier is removed, the nearby channel will cause a drastic change in the ecosystem, shifting to an estuarine area permanently flooded, composed primarily of mangroves, macrophytes, and shrubs, especially *Dalbergia* since the morphological plasticity exhibited in response to the abiotic changes in soil salinity and water levels leads us to infer that it is adapted for variable changes in hydrological conditions. It is not in near danger of displacement.

Dalbergia, often found on the landward side of mangrove swamps or coastal dunes, grows into dense monostands, preventing other species from establishing. This can be seen as a negative characteristic because of the possible loss of diversity; however, in perturbed ecosystems like those at Cucharillas this can help stabilize substrate and retain elements in soils. *Dalbergia* can be seen in historical air imagery at least 50 years back, withstanding several land-use changes.

If the system remains as is, with controlled water flows, sea-level rise will affect the marine intrusion at the phreatic level, as we reported in the present study. Changes in the water table are often overlooked in the context of sea-level rise, but they directly impact the vegetation. The water level changes and accompanying changes in salinity and the shortage of freshwater inputs will induce loss of vegetation, primarily grass species in the ecosystem, as it was seen that *Echinochloa* and *Panicum* were the species more susceptible to soil salinity and phreatic water salinity differences.

On the other hand, extreme flooding periods can affect plant processes as we observed that water level, not interstitial salinity was the main driver influencing the photosynthetic performance of *Laguncularia.* The morphological plasticity *Dalbergia* exhibited in response to the abiotic changes in soil salinity and water levels grant us to infer that it is adapted for variable changes in hydrological conditions. It is not in near danger of displacement. Based on our findings, these two species will dominate the ecosystem in a climate change scenario.

The passage of hurricane María allowed us to observe species' tolerance range to initial effects (such as winds and tidal surges) and subsequent changes in conductivity and freshwater conditions. High 15N values in the sampled species point toward a eutrophic system where high nutrient availability increases the system's regeneration speed. Although each plant functional type has different traits that respond to abiotic conditions, they all had a similar response in the recovery rate. In future atmospheric events, we expect similar post-hurricane responses if the controlled hydrological conditions are sustained.

Our study sets the basis for long-term studies in coastal plant ecophysiological responses using an approach that integrates knowledge from a multi-level scale (plant – ecosystem level) and provides affordable techniques to researchers and managers to provide continuous and reliable data. We found that species present in this coastal wetland have different trait variability and interspecific temporal variability that respond, in part, to hydrological conditions. Our final recommendations to the wetland managers are to:

1) Characterize hydrological conditions in the basin.
2) Systematize, based on hydrologic data, the management of pumps at the end of the Malaria Channel.
3) Restore surface marine-terrestrial connectivity in the wetland, which will help in the vegetative rehabilitation efforts.

4) Prioritize soil remediation in areas of high concentrations of heavy metals, considering abiotic conditions of the ecosystem and the use of phytoremediation techniques with native species already in the wetland, such as *Laguncularia* and *Acrostichum*.
5) Establish a periodic mapping scheme for vegetation composition changes.

Cienaga las Cucharillas wetland is an extraordinary case of plant responses to multiple stressors, including anthropogenic and climate-related changes. Currently, vegetation communities in this novel ecosystem seem to be adapted to the landscape transformations.