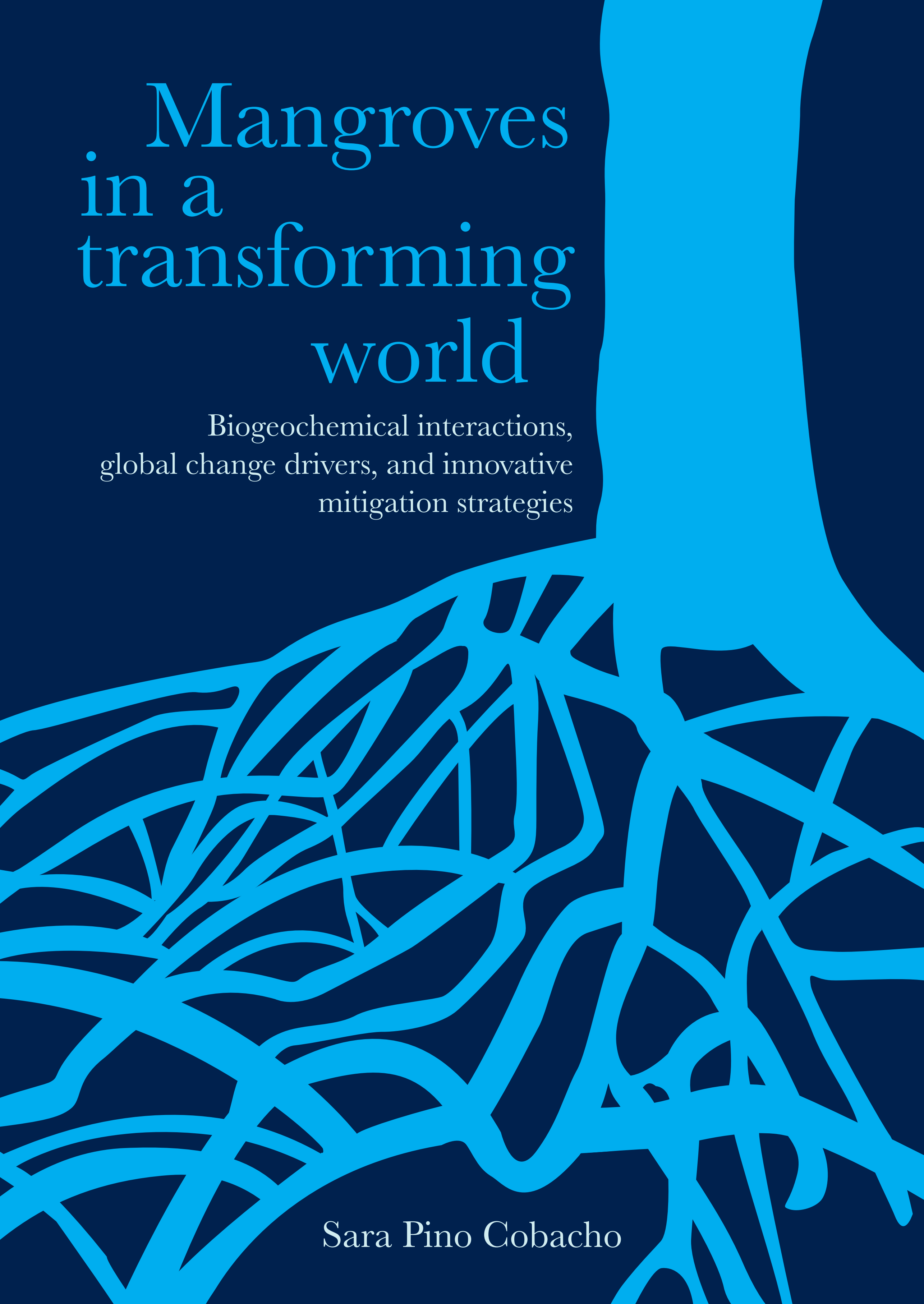

Mangroves in a transforming world

Biogeochemical interactions, global change drivers, and innovative mitigation strategies

Sara Pino Cobacho

Propositions

1. Mesocosm experiments are powerful tools for validating hypotheses and contributing to scientific consensus on ecological phenomena.
   (this thesis)
2. Lethal sargassum inundations on mangroves accelerate climate change and create a feedback loop of mangrove loss.
   (this thesis)
3. Effective leadership could eradicate unethical behaviour in academia and science.
4. A positive feedback loop occurs when individuals bring out the best in each other.
5. The key to solving a problem often lies in disengaging from it.
6. "Being too busy" is just a reflection of our selective prioritization.

Propositions belonging to the PhD thesis, entitled

Mangroves in a transforming world: biogeochemical interactions, global change drivers, and innovative mitigation strategies

Sara Pino Cobacho

Wageningen, 5 December 2023

Mangroves in a transforming world

Biogeochemical interactions, global change drivers, and innovative mitigation strategies

Sara Pino Cobacho

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Mangroves in a transforming world

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Sara Pino Cobacho

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Prof. Dr A. P. J. Mol,
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Contents

Chapter 1

General introduction

Sara Pino Cobacho

1.1 Mangrove ecosystems

Mangroves are halophyte trees growing in the interface between land and sea along tropical and subtropical coasts, where they form large habitats (Kathiresan & Bingham, 2001). Traditionally, mangroves had a significant cultural and spiritual value, and were seen by aboriginal peoples in Australia as places where ancestral spirits reside, representing the interconnectedness between land, water, and life (Friess, 2016). Decades of scientific research have demonstrated the immense ecological value of mangroves, extending well beyond their role in supporting high biodiversity (Dorenbosch et al., 2004; Nagelkerken et al., 2001) or mitigating climate change (Donato et al., 2011; Taillardat et al., 2018). Mangroves have great value for human and ecological communities, which can be assessed in the form of ecosystem services (Mitra, 2020; Ruslan et al., 2022; Warren-Rhodes et al., 2011). Afonso et al. (2021) identified a total of 33 different ecosystem services generated by mangroves, within both the marine and the terrestrial realms. Some of the regulating ecosystem services of mangroves such as “Natural hazards regulation”, and “Disease regulation” will be explored in this thesis. The functionality of mangroves is not yet fully understood, especially concerning their interactions with their surrounding environment and adjacent habitats. The influence of mangroves on their surroundings is well known when it comes to serving as a physical barrier against various disturbances (Kamil et al., 2021; Kathiresan & Rajendran, 2005; Menéndez et al., 2020) and acting as a highly efficient atmospheric carbon sink (Alongi, 2020a; Murdiyarso et al., 2015). However, the functionality of mangroves that determines ecosystem processes and the underlying biogeochemical fluxes contains several knowledge gaps. Furthermore, mangrove species exhibit distinct natural adaptations to the environmental conditions, resulting in different functions and ways of interaction with their surroundings (Feller et al., 2010). They have the ability to influence both the water column and the sediment as they can grow in aquatic environments while being anchored to the seabed by their root systems. Mangrove roots drive a variety of biogeochemical processes in their soils such as nutrient uptake, salt absorption and exclusion, soil oxygenation, carbon accumulation (Kitaya et al., 2002; Reef et al., 2010; Srikanth et al., 2016), and various other reactions that, while being conducted as a product of mangrove metabolism, can also influence their surroundings (Feller et al., 2010). In this thesis, I focused on two species of mangroves: the black mangrove *Avicennia germinans* and the red mangrove *Rhizophora mangle* (Figure 1.1).

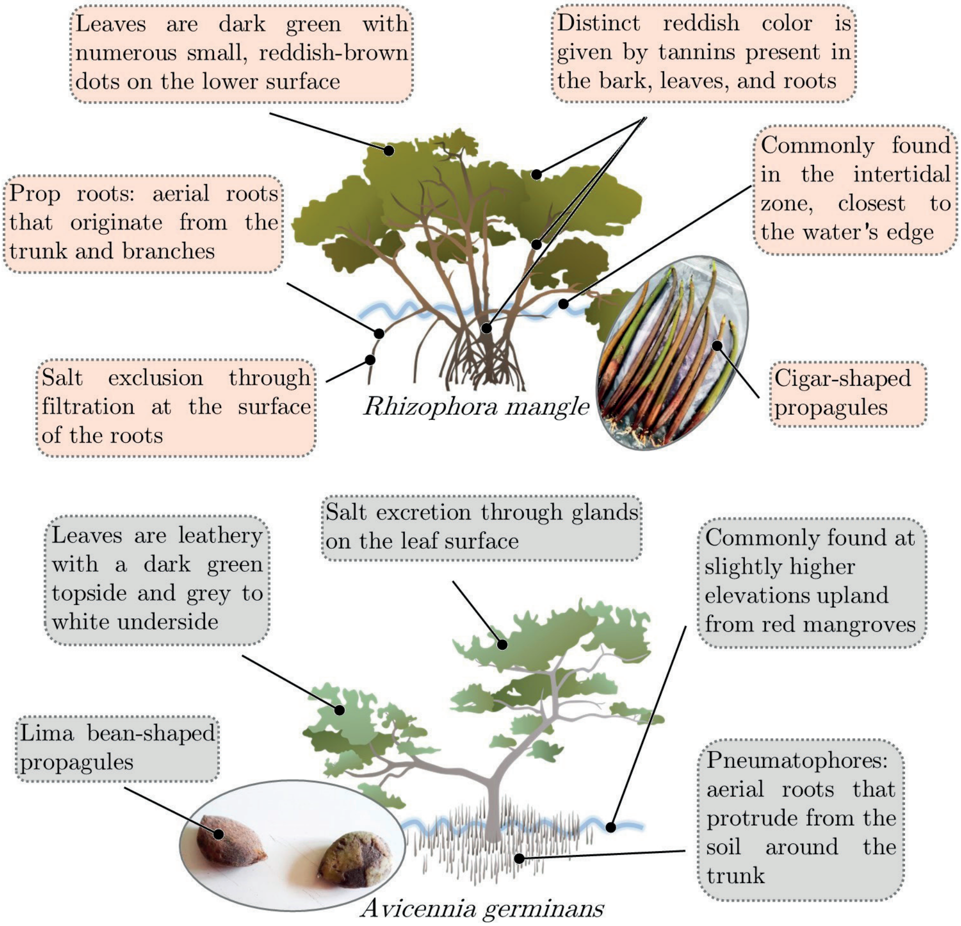

Figure 1.1 The red and the black mangrove are the two main species studied in this thesis.

The wide geographical distribution of these mangrove species (Dodd et al., 2002; Numbere & Camilo, 2017) implies that the outcomes derived from this thesis have broad applicability and can be used across numerous locations worldwide.

Furthermore, understanding mangrove functionality must be done by taking into consideration the broader context of connectivity between mangroves and their environment, including their surrounding habitats (Gillis et al., 2014, 2017; Guannel et al., 2016; Kathiresan & Alikunh, 2011). The concept of connectivity is further explained in section 1.2.

1.2 Mangrove interactions and connectivity

Mangroves interact with their surroundings through the flow of organisms and natural processes forming highly connected marine ecosystems (Gillis, Jones, et al., 2017; Huxham et al., 2018; Nagelkerken et al., 2001; van de Koppel et al., 2015). This connectivity describes the degree of linkage between ecosystem components, supporting their productivity and health (Fang et al., 2018; Sheaves, 2009). Mangrove forests, seagrass meadows, and coral reefs (hereinafter referred to as mangroves, seagrasses, and corals) coexist in tropical coasts, forming adjacent habitats (Figure 1.2) and influencing each other's living conditions (Earp et al., 2018; Gillis et al., 2014). These interactions exist not only between tropical coastal habitats, but also in multiple terrestrial ecosystems such as forests (Uezu et al., 2005) and grasslands (Soons et al., 2005), as well as between terrestrial and marine ecosystems (Adame et al., 2012; Fang et al., 2018).

Interactions within tropical coastal ecosystems may lead to higher productivity and functionality of the individual habitats (Huxham et al., 2018), and contribute to ecosystem functions (Berkström et al., 2012). On the other hand, the dependency between mangroves, seagrasses, and corals also means that disturbances to one habitat may trigger cascading effects, potentially resulting in the large-scale degradation of the entire ecosystem (de Fouw et al., 2018; Rocha et al., 2018).

While this thesis does not directly quantify connectivity or fluxes between mangroves and adjacent habitats, the newly gained insights into mangrove functionality are contextualized within the broader context of connectivity whenever possible, as it is a crucial component of the understanding of mangroves.

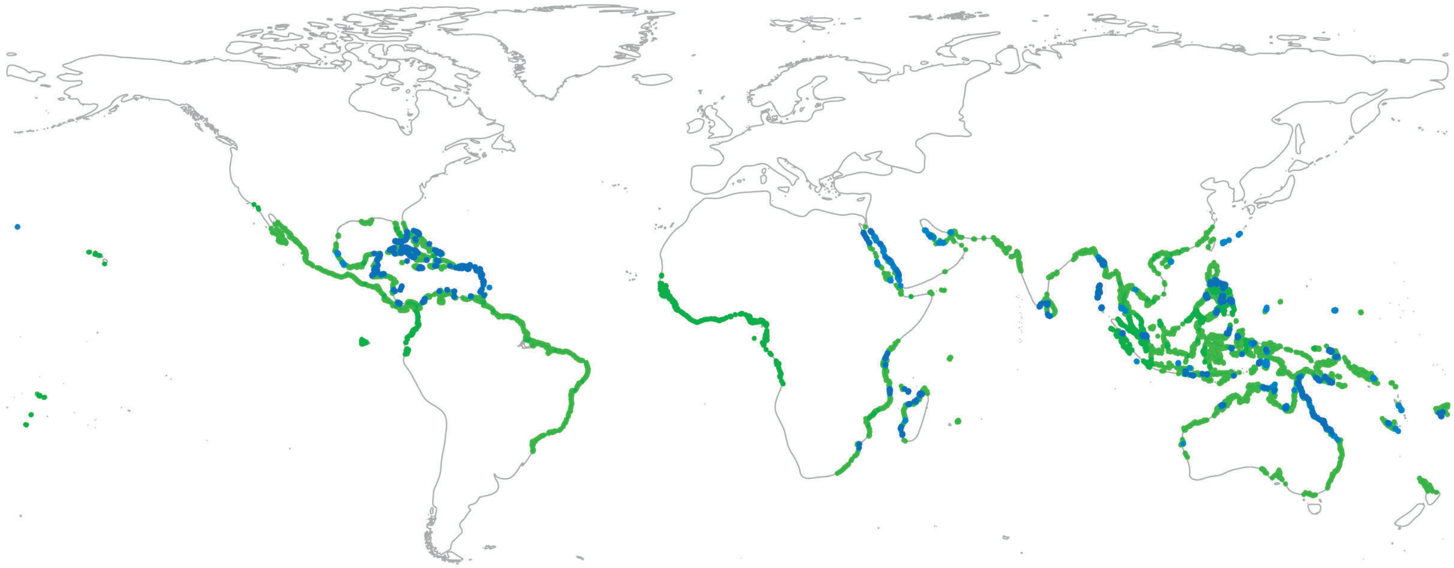

Figure 1.2 World map showing the global mangrove distribution in green. Additionally, blue dots indicate places where mangroves, seagrasses, and corals co-occur within a 3-km radius. Global distribution maps of mangroves, seagrasses, and corals were retrieved from the online Ocean Data Portal by the World Conservation Monitoring Centre (UNEP-WCMC) and subsequently combined using ArcGIS Pro 2.5. First, a 3-km buffer was created around the polygons corresponding to seagrass meadows. The intersection of this buffer with both seagrass and mangrove polygons was then calculated. The choice to create the buffer from the seagrass polygons was based on the common occurrence of these habitats between mangroves and corals, in a direction perpendicular to the shoreline (Akhand et al., 2021). However, the position of the seagrass and corals may not always follow this pattern, or they could be closely intertwined (Lohr et al., 2017). To address this variability, a similar analysis was conducted with the corals as the center of the calculations. Duplicate areas were subsequently removed. Choosing a buffer of 3 km was based on the idea that the fluxes dissipate as they travel long distances, causing the individual habitat signatures to become less distinct or negligible (Hemminga et al., 1994).

1.3 Mangroves under the impacts of global change

Mangroves are facing a worldwide decline, with 11.700 km^2 of mangroves lost over the past 27 years due to various anthropogenic and environmental factors (Leal et al., 2022), and an average annual loss rate of 0.13 % (Goldberg et al., 2020). While mangroves and human communities have co-existed for over 200,000 years, the escalating demand for mangrove resources and other services that entail their conversion have also resulted in extensive mangrove habitat fragmentation (Bryan-Brown et al., 2020). Currently, 40 % of the world population resides within 100 km from the coast, thereby intensifying the direct human pressures and impacts on coastal ecosystems such as mangroves (Maul & Duedall, 2019). These impacts encompass urbanization and land conversion to aquaculture, which lead to coastal squeeze, as well as eutrophication and pollution from industrial activities affecting mangrove forests. Furthermore, climate change drivers exacerbate the challenges faced by mangroves, including the increased frequency of hurricanes, global warming, and rising sea levels (Polidoro et al., 2010; Xie et al., 2020). However, the conservation of mangroves and the recovery of populations that have already been lost is difficult without understanding how these stressors interact and how mangroves respond to the combined effects of multiple stressors (Gillis et al., 2019). Among the multiple global change drivers threatening the survival of mangroves, sargassum brown tides, global warming, and coastal eutrophication are explored in this thesis.

Sargassum brown tides result from the combination of human and climate change impacts to marine systems that indirectly affect mangroves. The uncontrolled proliferation of sargassum is caused by the excessive nutrients discharged by continental rivers into the Atlantic Ocean as a result of anthropogenic deforestation of terrestrial forests, agro-industrial and urban activities, as well as African atmospheric dust deposition (Djakouré et al., 2017). Furthermore, climate change contributes to sargassum brown tides by causing sea surface temperature anomalies, as well as disrupting wind patterns and ocean currents that push the seaweed against the coast (Johns et al., 2020; Louime et al., 2017; Sissini et al., 2019; M. Wang et al., 2019). The accumulation and decay of stranded sargassum masses poses a significant concern for the health of mangroves (Hernández et al., 2022; León-Pérez et al., 2023). Furthermore, coastal eutrophication, the phenomenon of excessive nutrient inputs into coastal waters (Nixon, 1995), disrupts the nutrient balance of coastal ecosystems and impacts the resilience of mangroves against various disturbances (Feller et al., 2015; Lovelock et al., 2009). Overall, the impacts of these global change drivers on mangrove functionality remain largely unknown, creating a knowledge gap concerning specific mangrove responses to these drivers.

1.4 Study approach

While fieldwork-based studies are crucial for protecting and preserving mangroves, they also entail a level of complexity caused by several factors and interactions that may make the identification and understanding of specific processes difficult. While such complexity can reflect real-world conditions, it also reduces our ability to isolate the process under study. In this thesis, all experiments on mangroves are conducted through mesocosm studies (Box 1.1). These experimental mesocosms were designed as controlled environments to study the specific processes that explain mangrove functionality. Many of these processes and variables are hard to detect and measure directly *in-situ* due to natural variability, but these data can be collected under standardized conditions in mesocosm experiments by isolating the process or variable under study.

Box 1.1 The mangrove nursery

Over the course of this PhD, I developed a mangrove nursery that provided me with the opportunity to learn about mangroves and conduct experimental research with red and black mangrove specimens in captivity. The development of the nursery, where mangroves shared a greenhouse compartment with seagrass (Figure B 1.1.1), took between 6 months and a year and involved designing and setting up the necessary infrastructure and materials to ensure the long-term, healthy growth of mangroves. The main goals of this nursery were 1) to support the growth of mangroves by providing the optimal living conditions for their long-term survival, and 2) to develop various experimental designs for *ex-situ* mesocosm studies. To recreate the appropriate living conditions for mangroves in the greenhouse compartment (~ 38 m^2), an average air temperature of 25 ºC was maintained throughout the year, and artificial lighting was provided to stimulate mangrove growth (~ 300 $\mu mol\ m^{-2}\ s^{-1}$). All mangroves were planted in individual pots consisting of a sandy substrate (grain size of 0.1-0.3 mm) and enriched with slow-release fertilizer for aquatic plants (NPK 15:9:11), as well as Mg and Fe supplements. All pots were submerged in artificial seawater at varying levels of salinity, depending on their life stage and desired growth rate.

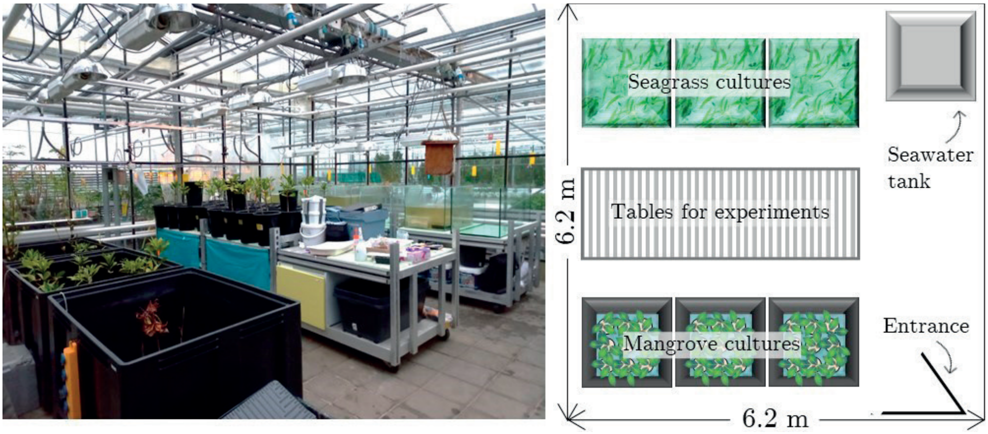

Figure B 1.1.1 Picture (left) and top view illustration (right) of the nursery in the greenhouse compartment with dimensions.

The nursery supported the development of red and black mangroves during their different life stages, from germination and establishment to the sapling stage (Figure B 1.1.2).

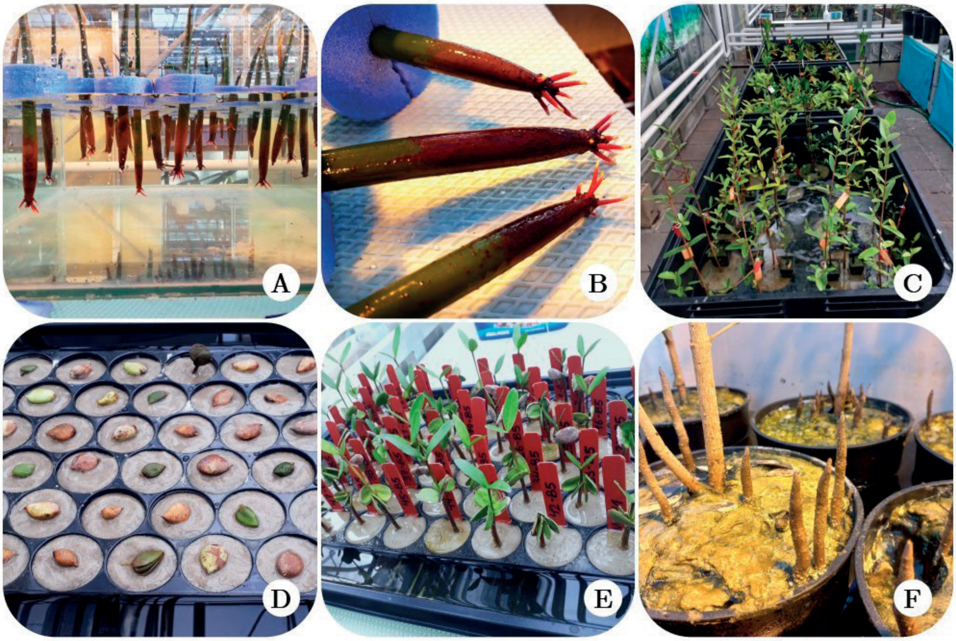

Figure B 1.1.2 Pictures showing red (A, B) and black mangrove (D) propagules at the emergence stage. Black mangroves rapidly grow their first roots and leaves (E), followed by the development of pneumatophores at 1 to 2 years old (F). Mangrove cultures in the nursery were kept in individual pots and submerged in large containers (C).

The conditions required for successful germination (low salinity, low nutrient demand, and heated water) varied widely from those needed, for instance, for fast growth or long-term maintenance of the saplings (high nutrient and light demands, as well as broad salinity and temperature tolerances). This experience provided valuable hands-on learning, as I had to adapt and respond to the specific needs of the mangroves. This included troubleshooting various aspects such as pest prevention and management, minimizing stress caused by re-potting and handling, and addressing other related concerns.

1.5 Scope and outline of this thesis

This thesis aims to study the impacts of global change on mangrove functionality, with a primary focus on biogeochemical processes. Through this thesis, my goal is to enhance our understanding of the intricate dynamics governing mangroves and their significance in the face of global challenges, while also hypothesizing about the potential implications of these new findings for ecosystem connectivity.

In **chapter 2**, I start with a literature review to determine the framework for this thesis and create a link between mangroves and the concept of biogeochemical connectivity. This helps define the scope of the following chapters and serves as a connecting point between mangroves, biogeochemical connectivity, and global change. In this chapter, I give an overview of the connectivity between tropical coastal habitats and identify the various biogeochemical interactions between mangroves, seagrasses, and corals. This chapter also identifies the various ways through which humans interfere with connectivity by disrupting or modifying biogeochemical fluxes in tropical coastal ecosystems. Chapter 2 looks at tropical coastal ecosystems from a holistic perspective, identifies knowledge gaps and discusses future research pathways in the field of connectivity between mangroves, seagrasses, and corals.

Chapter 3 takes the idea from chapter 2 that mangroves might be able to mitigate the detrimental effects of sargassum brown tides and prevent the decline of adjacent habitats. In this chapter, the capacity of mangrove soils to buffer the sulfide produced by the decomposition of sargassum is explored. Sulfide is a soil phytotoxin that accumulates in the water and soil of tropical coasts as a result of sargassum brown tides. The addition of iron to mangrove soils is proposed as a bioengineering solution for sulfide mitigation. The health and photophysiological responses of mangroves to sargassum and iron is monitored in a mesocosm experiment, as well as the capacity of iron to ameliorate sulfide in the sediment porewater.

Another form of interaction of mangroves with their environment through which they can ameliorate unfavourable conditions for adjacent habitats is by regulating water pH. As a consequence of climate change, the phenomenon of ocean acidification threatens the survival of sensitive marine organisms, like corals. Thus, it is vital to identify habitats that can act as ocean acidification refugia. However, the role of mangroves in ameliorating low pH is a source of scientific debate as the available body of scientific evidence stems from in-situ studies, where the local environmental conditions play a major role. In **chapter 4**, I conduct a mesocosm experiment where I identify the direct effect of black mangrove seedlings on water carbonate chemistry without the interference common to field situations and assess how mangrove metabolism under different temperatures and nutrient levels influences such effect.

During the experiment for chapter 4, growth and production data of black mangrove seedlings under different temperature and nutrient regimes were collected. Active restoration using seedlings can counteract the global loss of mangroves. However, restored mangrove populations must overcome the impacts of multiple stressors that may determine mangrove seedling establishment and restoration success. Therefore, in **chapter 5** I assess the impacts of a global stressor such as global warming, and a local stressor such as eutrophication on mangrove seedling biomass and nutrient allocation and discuss the implications of these findings for their establishment. These findings could inform management decisions concerning restoration efforts and ultimately improve restoration success.

When facing environmental changes such as those described in chapter 5, mangroves may make use of different reproductive strategies to ensure their expansion and continuity of the population. In **chapter 6**, I present the discovery of polyembryony in black mangroves, which came in the form of two seedlings from a single propagule and discuss the potential causes of polyembryony.

Lastly, in **chapter 7** I provide a reflective analysis of the findings presented in the previous chapters, discussing their potential implications for connectivity in tropical ecosystems. I also discuss the practical applications of these findings, as well as addressing their limitations. Additionally, I present data that lie beyond the scope of the chapters and could potentially lead to new research pathways.

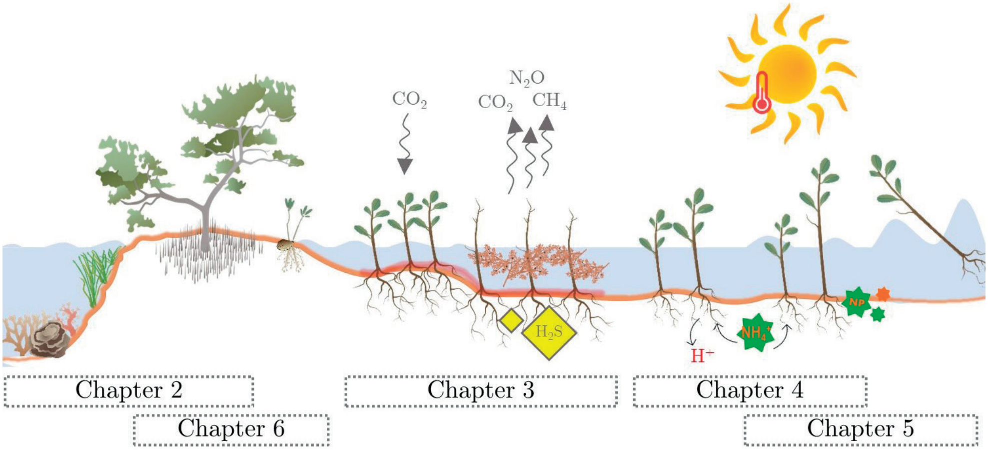

Figure 1.3 Illustration of the different chapters included in this thesis.

Chapter 2

The role of biogeochemical fluxes between connected habitats in tropical seascapes

Sara P. Cobacho, Ingrid A. van de Leemput, Milena Holmgren, Marjolijn J. A. Christianen

This chapter is under review at *Estuarine, Coastal and Shelf Science*

Abstract

Tropical seascapes rely on the feedback relationships among mangrove forests, seagrass meadows, and coral reefs, as they mutually facilitate and enhance each other's functionality. Biogeochemical fluxes link tropical coastal habitats by exchanging material flows and energy through various natural processes that determine the conditions for life and ecosystem functioning. However, little is known about the seascape-scale implications of anthropogenic disruptions to these linkages. Despite the limited number of integrated empirical studies available (with only 11 out of 81 selected studies focusing on the integrated dynamics of mangroves, seagrasses, and corals), this review emphasizes the importance of biogeochemical fluxes for ecosystem connectivity in tropical seascapes. It identifies four anthropogenic drivers that can disturb these fluxes, including nutrient pollution, pH imbalance, disease outbreaks, and other forms of environmental degradation. This review also highlights significant knowledge gaps in our understanding of biogeochemical fluxes and ecosystem responses to perturbations in tropical seascapes. Addressing these knowledge gaps is crucial for developing practical strategies to conserve and manage connected seascapes effectively. Integrated research is needed to shed light on the complex interactions and feedback mechanisms within these ecosystems, providing valuable insights for conservation and management practices.

2.1 Introduction

2.1.1 Tropical seascapes and their habitats

Tropical seascapes often consist of mangroves, seagrass, and coral reefs, with mangrove forests at the land-sea interface. The value of these habitats is frequently expressed by their capacity to provide ecosystem goods and services (de Groot et al., 2012). For example, these habitats play a significant role in nutrient cycling, carbon sequestration, erosion control, coastal protection, acting as nursery ground for marine organisms, and food production (Christianen et al., 2013; Donato et al., 2011; Taillardat et al., 2018; Williams et al., 2013). On a global scale, their presence is considered essential for addressing and mitigating climate change due to their disproportionately large contribution to global atmospheric carbon sequestration (IPCC, 2022). Furthermore, the ecological functioning of each of these habitats (e.g., primary production, carbon sequestration, nursery and feeding habitat) is considered higher in connected seascapes due to facilitative interactions. Facilitative interactions refer to positive interactions that improve conditions by alleviating abiotic stress (Gillis et al., 2014; van de Koppel et al., 2015), which will be described in detail in sections 2.1.3 and 2.1.4.

2.1.2 Anthropogenic challenges faced by tropical seascapes at a global scale

Mangrove forests, seagrass meadows, and coral reefs are among the most threatened habitats in the world (Polidoro et al., 2010). Their deterioration due to human activities is intense and rapidly expanding (Jouffray et al., 2020). Approximately 35% of mangroves, 50% of coral reefs, and 30% of seagrass have been lost or degraded worldwide in the last fifty years (Bruno et al., 2018; Donato et al., 2011; Hoegh-Guldberg et al., 2017; Orth et al., 2006; Waycott et al., 2009). Human activities along the coasts are driving intensified efforts to meet the growing demand for resources (Jouffray et al., 2020), often without adhering to sustainability standards. These activities include fishing (Shantz et al., 2019), mining (Asir et al., 2020), beach and diving tourism (Giglio et al., 2020), and infrastructure development (Alharbi et al., 2017). While mangrove forests can adaptively migrate landwards in response to sea level rise, the presence of urban infrastructure in the coastal zone limits their ability to relocate, resulting in coastal squeeze (Gilman et al., 2007). Coastal squeeze and deforestation of mangrove forests may cause a generalised disruption of the interaction network (Gillis et al., 2017a). Similarly, the fragmentation of one connected habitat can trigger cascading degradation effects on the other habitats due to the loss of facilitative effects. For example, fragmentation of seagrass meadows may negatively affect the remaining fraction of the meadow as it disrupts sediment stabilisation patterns (El Allaoui et al., 2016), seed dispersal (Livernois et al., 2017), and organic matter fluxes (Ricart et al., 2015). The degradation of one habitat, such as mangroves, can also impact the remaining habitats, e.g., corals, due to the lack of nutrient buffering the increased flow of nutrients into the water, which inhibits the success of coral settlement (Keyes et al., 2019).

2.1.3 Cross-ecosystem connectivity

Different cross-ecosystem interactions are described between coral reefs, seagrass, and mangroves. These interactions include animals migrating between these habitats at different life stages (Bastos et al., 2022), altering the physical environment, such as wave energy dampening (Odériz et al., 2020), and biogeochemical fluxes (Briand et al., 2015; Camp et al., 2016). The ecosystem gradient from mangroves to seagrass to corals is formed based on an environmental gradient dictated by the combination of ecological (e.g., nutrient tolerance and availability) and hydrological (e.g. tidal regimes, wave action, freshwater inputs) factors in tropical coasts. Cross-ecosystem interactions involving various substances and fluxes (Figure 2.1) improve the ecological performance of coral, seagrass, and mangrove systems (Earp et al., 2018; Gillis et al., 2014; van de Koppel et al., 2015).

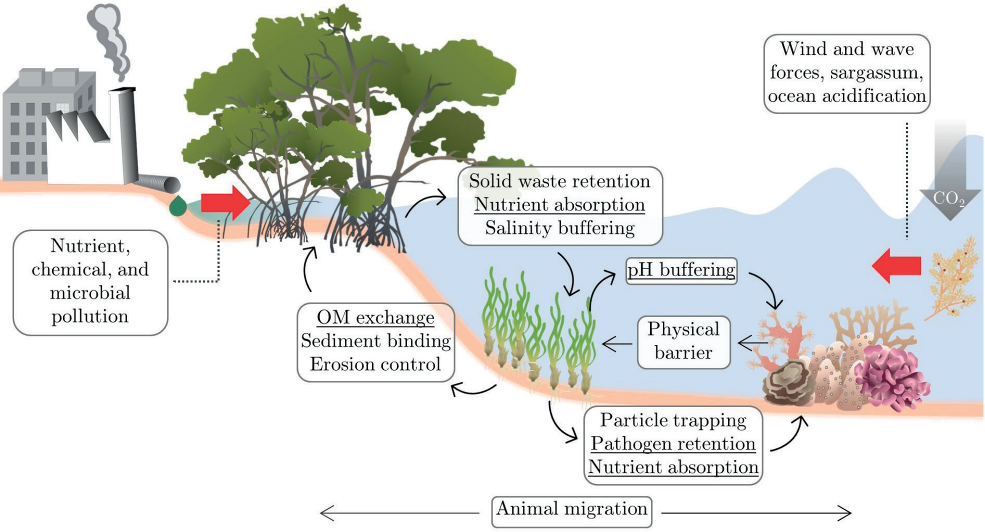

Figure 2.1 Synthesizing conceptual scheme showing the interactions known so far in connected seascapes. Human pressure (red arrows) gives rise to a number of drivers that enter and modify the interaction network. Black arrows indicate the source and receptor of the service provided. Biogeochemical fluxes are underlined.

For instance, mangroves trap nutrients from land sources and create a salinity gradient, lowering these pressures on seagrass. Vice versa, seagrass plays a role in erosion control and sediment binding, which results in land accretion and more stable ground for mangroves. At the same time, retain sediment particles and nutrients for the benefit of corals. Corals reefs influence seagrass beds positively by acting as barriers for hydrodynamic forces such as waves and wind. Although connectivity can refer to the movement of any material from one spatial point to another, this article explicitly focuses on biogeochemical connectivity, i.e., biogeochemical fluxes, further explained in section 2.1.4 and also included in Figure 2.1.

2.1.4 Biogeochemical connectivity

Research on the effects of connectivity in coastal seascapes has been disproportionally focused on biotic and physical interactions, likely due to easier monitoring, leaving biogeochemical fluxes relatively understudied. Biogeochemical fluxes are also included in Figure 2.1. Van de Koppel et al. (2015) defined biogeochemical fluxes as “a supply of non-living substances moving from one habitat to another”, making them intrinsic links within the interaction network. In other words, biogeochemical materials move between the three habitats, acting as connections. These fluxes not only occur in one direction, but they form a network. Due to the limited understanding of larger-scale implications and the crucial role that organic matter, nutrients, and various chemical species play in the tropical coastal interaction network, this study focuses on biogeochemical fluxes with and without human disturbance. While a few reviews have discussed the role of facilitative interactions (e.g., Earp et al., 2018), none have explicitly focused on the understudied biogeochemical fluxes involved in cross-ecosystem connectivity in tropical seascapes, such as nutrient removal and pH buffering. Therefore, the objectives of this study are 1) to quantify the empirical studies and provide an overview of biogeochemical fluxes affected by anthropogenic pressures and their consequences for cross-ecosystem interactions between mangroves, seagrass, and corals, and 2) to identify knowledge gaps and new research opportunities that arise from anthropogenic disruptions to connected tropical seascapes.

2.2 Literature review

To provide an overview of biogeochemical fluxes relevant to cross-ecosystem connectivity in tropical seascapes, anthropogenic pressures, and their impacts, a literature search was conducted. The selection of studies was a multi-step process that began by identifying the most relevant terms and concepts (“mangrove”, “seagrass”, “coral”, “tropical”, “connected/connectivity”, “flux”, “export”, “import”), which were combined to create search strings. Alternative search terms that are frequently used synonymously to ecosystem connectivity such as “interaction”, “link(age)”, “long distance effect”, “pathway”, “exchange”, and “cascade” were also considered. During the initial screening phase, several grouping themes were recognised, thus allowing us to refine our search for each of the different fluxes investigated, i.e., carbonate chemistry and pH, solid waste, microbial pollution, chemical pollution, and carbon stocks and nutrients. This research was performed using Scopus and Google Scholar databases. Articles resulting from the search were then screened, and those empirical studies consisting in the exchange of fluxes between the habitats of interest, or the import/export of biogeochemical material from a single habitat were included. Additional papers included in this study were identified through a subsequent forward search. Therefore, the literature reviewed in this article included, but was not limited to, that resulting from the search strings. A final number of 70 peer-reviewed articles in English were analysed and listed in Table 2.1.

Articles selected for review were categorized by habitat, type of fluxes studied, and year of publication. Those articles that included the study of several types of fluxes were cross classified (i.e. classified within more than one category). We provide a state of knowledge about biogeochemical fluxes in tropical seascapes (section 2.3), as well as the dominant anthropogenic drivers of change and their impacts (section 2.4). These anthropogenic impacts were not targeted by the literature review, but they were identified from the selected studies. Lastly, novel research pathways are identified (section 2.5).

2.3 Results and discussion of main findings

We found that research interest in biogeochemical fluxes in tropical seascapes has been relatively low, but it has exponentially increased over the last decade. In 2019 alone, there were a total of 13 studies and the number has gradually decreased until the present time (Figure 2.2A). However, out of the 80 articles reviewed, only 11 included all three habitats of interest, namely mangroves, seagrass and corals (Figure 2.2B). Our results also reveal that mangroves have been the most intensively studied habitat among the three in relation to biogeochemical fluxes, followed by seagrass and corals. However, studies focused on more than one habitat are significantly lacking (Figure 2.2B). The different types of biogeochemical fluxes have not been investigated to the same extent. In particular, the role of mangroves and seagrass in pathogen removal is poorly understood (Figure 2.2C, “microbial pollution”). On the other hand, organic carbon and nutrient fluxes have been the most thoroughly studied, closely followed by carbonate chemistry and pH, as well as chemical pollution (Figure 2.2C).

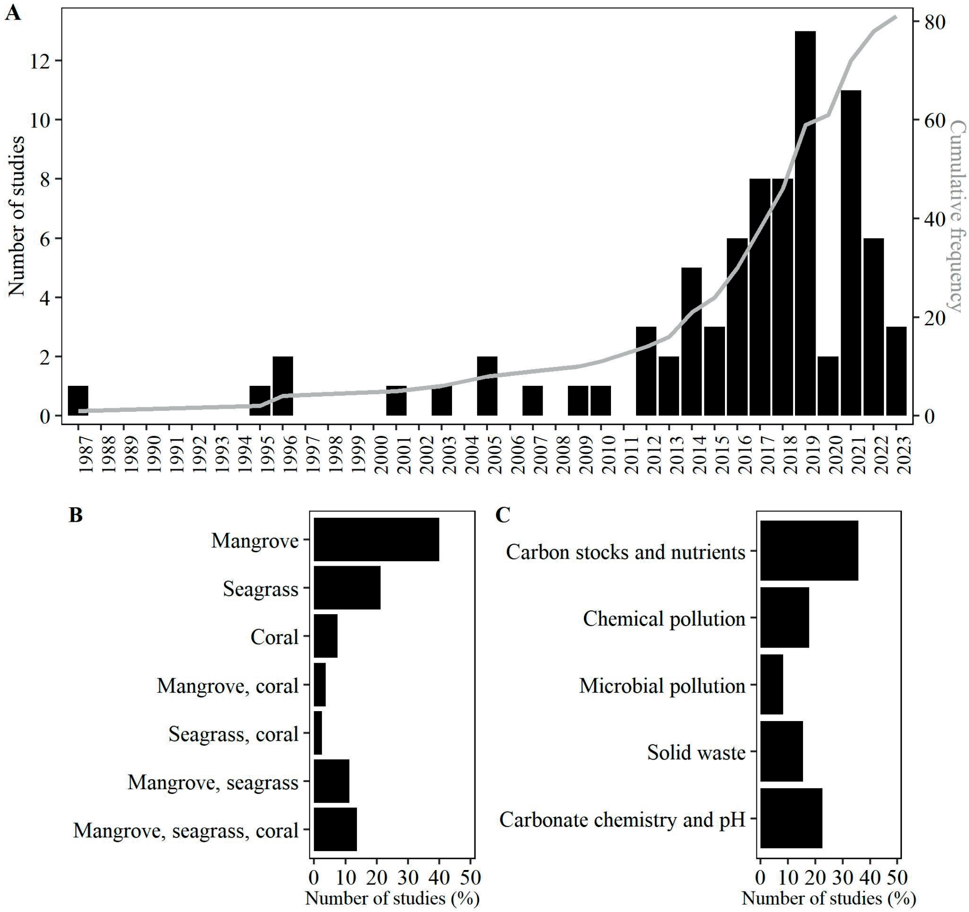

Figure 2.2 Number of studies included in this review classified by (A) year of publication from 1987 up until 2023, (B) habitat of study and (C) type of fluxes investigated.

From our literature survey, it becomes evident that empirical studies that include all three components of the tropical seascape, i.e., mangroves, seagrass, and corals are still scarce. Similarly, studies focusing on any combination of two habitats are also limited (Figure 2.2B). Out of 11 “integrated” studies identified, 5 were related to carbonate chemistry and pH across the seascape, 5 studies focused on carbon stocks and nutrients, and 1 study included both types of fluxes. A comprehensive overview of the complete literature survey can be found in Table 2.1.

Table 2.1 Summary overview of empirical studies on biogeochemical fluxes in tropical seascapes included in the literature survey.

Fluxes	Habitats of study	References
Carbon stocks and nutrients	Mangrove, seagrass, coral	(Adame et al., 2012; Briand et al., 2015; Hernández et al., 2022; León-Pérez et al., 2023; Middelburg et al., 1996; Signa et al., 2017)
	Mangrove, seagrass	(Asplund et al., 2021; Dahl et al., 2022; Hidayah et al., 2022; Kammann et al., 2022; A. K. Mishra et al., 2023; Sullivan et al., 2021; Walton et al., 2014)
	Mangrove, coral	(Keyes et al., 2019)
	Mangrove	(Agraz-Hernández et al., 2018; Analuddin et al., 2021; Feller et al., 2015; Gillis et al., 2016; Moroyoqui-Rojo et al., 2015; Vaiphasa et al., 2007; Williams et al., 2013)
	Seagrass	(Apostolaki et al., 2012; Evrard et al., 2005; Govers et al., 2014; S. Liu et al., 2018; Stapel et al., 1996; Sulawesi et al., 2001; Van Tussenbroek et al., 2017)
	Coral	(D'Angelo & Wiedenmann, 2014)
	Other (macroalgae, bacterial communities, etc)	(Djakouré et al., 2017; S. Liu et al., 2018)
Chemical pollution	Mangrove	(Analuddin et al., 2017; Arumugam et al., 2018; Bhattacharya et al., 2003; Chai et al., 2019; Dudani et al., 2017; Duke et al., 2005; Ngole-Jeme et al., 2016; Proffitt et al., 1995; Qiu et al., 2019; Santos et al., 2019; Shete et al., 2009; Shi et al., 2019; Teas et al., 1987)
	Seagrass	(Fonseca et al., 2017; Yadav et al., 2021)
Microbial pollution	Mangrove	(Ameen & Al-Homaidan, 2021; Zhao et al., 2019)
	Seagrass	(Deng et al., 2021; S. Liu et al., 2023)
	Coral	(Lamb et al., 2018)
	Seagrass, coral	(Lamb et al., 2017)
	Seagrass, bacterial communities	(S. Liu et al., 2018)
Solid waste	Mangrove	(Cordeiro & Costa, 2010; Garcés-Ordóñez et al., 2019; Ivar do Sul et al., 2014; Li et al., 2022; Martin et al., 2019, 2020; van Bijsterveldt et al., 2021; Wilson & Verlis, 2017)
	Coral	(Corona et al., 2020; Hankins et al., 2018; Lamb et al., 2018; Rotjan et al., 2019)
	Others (beach ecosystem, bacterial communities, etc)	(J. Li et al., 2018; Wilson & Verlis, 2017)

Table 2.1 Continued

Fluxes	Habitats of study	References
Carbonate chemistry and pH	Mangrove, seagrass, coral	(Akhand et al., 2021; Camp et al., 2016; George & Lugendo, 2022; Macklin et al., 2019; Middelburg et al., 1996; Saderne et al., 2019)
	Mangrove, seagrass	(Salma et al., 2022; Sandoval-Gil et al., 2016)
	Mangrove, coral	(Camp et al., 2019; Yates et al., 2014a)
	Seagrass, coral	(Unsworth et al., 2012)
	Mangrove	(Chen et al., 2021; Sippo et al., 2016)
	Seagrass	(Barry et al., 2013; Bergstrom et al., 2019; Cyronak et al., 2018; Koweek et al., 2018; Ow et al., 2016; Ricart et al., 2021)

2.4 Anthropogenic pressures that impact biogeochemical fluxes

Despite the scarcity of integrated studies, we have conducted a review of the available literature on biogeochemical fluxes and the the connectivity between mangroves, seagrass, and coral reefs, both in natural state and under anthropogenic disruption. In the following sections, we describe and discuss the impacts of four major anthropogenic drivers that we have identified in our literature review. These drivers are nutrient enrichment (section 2.4.1), chemical pollution (section 2.4.2), microbial contamination (section 2.4.3), and solid waste accumulation (section 2.4.4). These drivers have been found to disrupt all five types of biogeochemical fluxes (Figure 2.2C, Table 2.1) between mangroves, seagrass, and corals.

2.4.1 Nutrient enrichment

In the tropical seascape, mangrove, seagrass, and coral species are often nutrient-limited, with their tolerance to nutrient loads decreasing from mangroves to corals (Earp et al. 2018). Both mangroves and seagrasses act as nutrient filters, removing a considerable fraction of the nutrient load (Evrard et al., 2005; Moroyoqui-Rojo et al., 2015; Sandoval-Gil et al., 2016; Stapel et al., 1996; Sulawesi et al., 2001), potentially preventing excess nutrients from reaching the coral reef and seagrass from the land-side (Gillis et al., 2016; Keyes et al., 2019). Mangrove forests, located at the land-sea interface, receive and uptake nutrients and organic matter of terrestrial sources (Adame et al., 2012; Agraz-Hernández et al., 2018) although these inputs often buffered by terrestrial ecosystems (C. O. Williams et al., 2013). Therefore, their spatial co-occurrence can form a protective cascade against coastal eutrophication (Gillis et al., 2014). As the organic matter load from mangroves is context and site-dependent (Signa et al., 2017), they may serve as subsidiaries of organic matter and nutrients to seagrass and corals (Adame et al., 2012; Briand et al., 2015; Mishra et al., 2023; Sullivan et al., 2021), while the input of seagrass material also supports mangrove production (Walton et al., 2014). Furthermore, a study on a connected mangrove-seagrass system concluded that higher sediment nutrient concentrations benefit seagrass density and coverage, and consequently their carbon accumulation potential (Kammann et al., 2022). On the other hand, high phosphorus concentrations in soil can decrease mangrove root complexity (as phosphorus is readily available), which negatively affects carbon storage (Kammann et al., 2022). Therefore, the critical nutrient load in connected seascapes not only influences the health and productivity of individual habitats but also the carbon storage potential of the biome.

Anthropogenic disruptions to nutrient connectivity between tropical coastal habitats occur through the introduction of excess nutrients into the system. From the land side, the most common sources of anthropogenic nutrient enrichment are urban wastewater and agricultural effluents (B. L. Jones et al., 2018). These agricultural fertilizers and manure flow into the sea due to physical factors like precipitation, leaching, and river transport. Other anthropogenic impacts such as mangrove deforestation cause the mobilization and flow of large nutrient and organic matter loads that were previously retained by mangroves (Asplund et al., 2021; Dahl et al., 2022). Continuous exposure to nutrient enrichment significantly reduces the resilience of mangroves to physical disturbances (Feller et al., 2015). Feller et al. (2015) observed slower recovery rates of trees after the impacts of a hurricane in areas previously subjected to anthropogenic nutrient loading, as abnormal growth made them more vulnerable to wind damage. As the ecological performance of mangroves declines due to nutrient enrichment, they lose their capacity to filter excess nutrients, allowing more nutrients from land to reach seagrass and corals. Under eutrophic conditions, seagrasses and corals lose their competitive advantage against micro and macroalgae (D'Angelo & Wiedenmann, 2014; Evrard et al., 2005; Govers et al., 2014) which thrive on these nutrients and reproduce rapidly. Seagrasses commonly suffer from overgrowth by epiphytes, resulting in shading and death of the plants (Apostolaki et al., 2012). Similarly, these algae may outcompete corals for light resources and lead to their decline (D'Angelo & Wiedenmann, 2014).

From the sea side, organic carbon and nutrients can also be imported by seaweed (Hidayah et al., 2022), or in the form of sargassum brown tides resulting from large-scale anthropogenic enrichment of continental rivers and offshore areas (Djakouré et al., 2017). This phenomenon, which began in 2011 and has occurred yearly since then, involves the influx of sargassum algae (Oyesiku et al., 2014; Sissini et al., 2019; Van Tussenbroek et al., 2017). The nutrients imported by sargassum brown tides are released into the water column as the algae wash up on the shore begin to decompose (Van Tussenbroek et al., 2017). Algae decomposition leads to high nutrient turnover rates, thereby exacerbating the eutrophication effects. In addition to the large amounts of nutrients imported by sargassum, its decomposition also results in the accumulation of sulphide and ammonium in sediments and water, causing toxicity to animals (Rodríguez-Martínez et al., 2019) and plants such as mangroves (Hernández et al., 2021), seagrass, and corals (Van Tussenbroek et al., 2017). Mangrove basins and channels are particularly prone to sargassum accumulation (Hernández et al., 2021; León-Pérez et al., 2023) possibly due to being trapped in their complex mangrove root systems. The broader implications of sargassum brown tides for connected seascapes are not yet fully understood, nor is the natural capacity of mangroves, seagrass and corals acting in association to counteract their impacts.

2.4.2 Chemical pollution

Chemical pollution in coastal areas originates from various human activities, including agriculture (Duke et al., 2005; Santos et al., 2019), industrial water discharge (Shete et al., 2009; Vaiphasa et al., 2007), and solid waste disposal (Garcés-Ordóñez et al., 2019). High concentrations of heavy metals in coastal areas are often associated with the presence of industrial activities (Ngole-Jeme et al., 2016). The ability of mangroves to act as biofilters and retain pollutants in their sediments has been extensively studied (e.g. Chai et al., 2019; Shi et al., 2019). Mangroves can also uptake and translocate these pollutants within their tissues, leading to their bioaccumulation (Analuddin et al., 2017; Arumugam et al., 2018; Dudani et al., 2017). Moreover, seagrasses have the capacity to absorb heavy metals and can be used for phytoremediation in polluted water bodies, although their exposure to these substances severely impacts their health (Yadav et al., 2021), and that of grazers by bioaccumulation (Wilkinson et al., 2022).

Pesticides and herbicides are also common pollutants present in mangrove sediments (Bhattacharya et al., 2003) and they have been linked to massive mangrove dieback events (Duke et al., 2005). In sub-lethal concentrations, pesticides can be taken up by mangroves and bioaccumulated in leaf and other tissues (Shete et al., 2009). Moreover, maritime traffic and offshore activities increase the risk of oil spills, and the fuels can be absorbed by mangrove roots, causing tree mortality within a few days of exposure (Teas et al., 1987). A 29-year-long study on the effects of oil pollution on a mangrove-seagrass-coral continuum revealed significant long-term damage and slow recovery of mangroves, whereas no dramatic impacts on seagrass were observed, and corals were documented to fully recover after ten years (Renegar et al., 2022). Other experimental exposures of mangrove pods to oil have proven to be toxic and detrimental to their early development (Proffitt et al., 1995).

2.4.3 Microbial pollution

Due to structural and chemical mechanisms, mangroves and seagrasses provide several protective services to corals, such as buffering sediment outflows and trapping suspended particles (Christianen et al., 2013; Keyes et al., 2019). These mechanisms enable tropical coastal habitats to reduce exposure to microbial contamination, including pathogenic bacteria (Lamb et al., 2017) and antibiotic-resistant bacteria (H. Zhao et al., 2019), while also acting as sinks for chemical pollution (Analuddin et al., 2017) thus protecting the overall biome. Although microbes are living substances and do not fall under the definition of "biogeochemical fluxes" (section 2.1.4), they are included in this review as their occurrence is governed by biogeochemical dynamics. The main sources of microbial pollution are municipal wastewater discharge and aquaculture activities (Lamb et al., 2017; S. Liu et al., 2023).

A study by Lamb et al. (2017) demonstrated that the incidence of coral disease caused by sewage-derived bacterial pathogens in the vicinity of seagrass meadows was 50% lower compared to reefs without adjacent seagrass. Tall and dense seagrass meadows are more effective at capturing bacterial pathogens by retaining particles compared to fragmented seagrass meadows (S. Liu et al., 2023). The specific mechanism by which seagrass remove pathogens from the trapped particles is not fully understood, but Deng et al. (2021) suggested that seagrass segregates anti-bacterial phytochemicals as a stress response to anthropogenic pressure. The increased abundance of microbial pathogens in tropical coasts, often associated with eutrophication (section 2.4.1), could lead to a higher occurrence of infectious diseases in seagrass meadows (Hughes et al., 2018) and associated species (S. Liu et al., 2018). Furthermore, mangrove sediments have been proven to remove antibiotic-resistant bacteria from sewage effluents (H. Zhao et al., 2019), and compost inoculated with mangrove-associated fungi reduces plant disease incidence (Ameen & Al-Homaidan, 2021).

2.4.4 Solid waste

Marine plastic pollution is a globally concerning issue that poses a serious threat to marine ecosystems. Plastic items have been found in high densities in some of the world's largest connected seascapes (Wilson & Verlis, 2017). While plastics can be retained for long periods (Ivar do Sul et al., 2014), their persistence depends on plant density and topography (Cordeiro & Costa, 2010). In a Colombian mangrove lagoon, the highest concentrations of microplastics were found near populated areas (Garcés-Ordóñez et al., 2019), highlighting that improper waste disposal is a source of plastics in the tropical seascape.

Furthermore, the study by Wilson & Verlis (2017) identifies tourism as the primary cause of marine plastic debris in the Great Barrier Reef, with areas closest to tourism infrastructure being the most polluted. The complex 3D structure and intricate root systems of mangroves play a key role in retaining and trapping solid debris from land and ocean-based sources (Cordeiro & Costa, 2010). Some negative effects of these accumulations include the clogging of tidal channels (Figure 2.3A), which increases salinity in the lagoons (Bulow & Ferdinand, 2013) and suffocates the trees by covering their aerial roots (van Bijsterveldt et al., 2021). According to Martin et al. (2020) mangroves are experiencing an exponential increase of plastic burial in their sediments. Over time, retained plastic items degrade into microplastics while remaining trapped and accumulating within the vegetation (Li et al., 2018). Furthermore, mangroves have been found to retain marine microplastics with their leaves through adsorption and adhesion, acting as a coastal microplastic sink (Li et al., 2022). A recent study found that seagrass meadows can influence water flow and depositional dynamics, favouring the accumulation of plastics in their sediment (Unsworth et al., 2021), which could hinder their free movement.

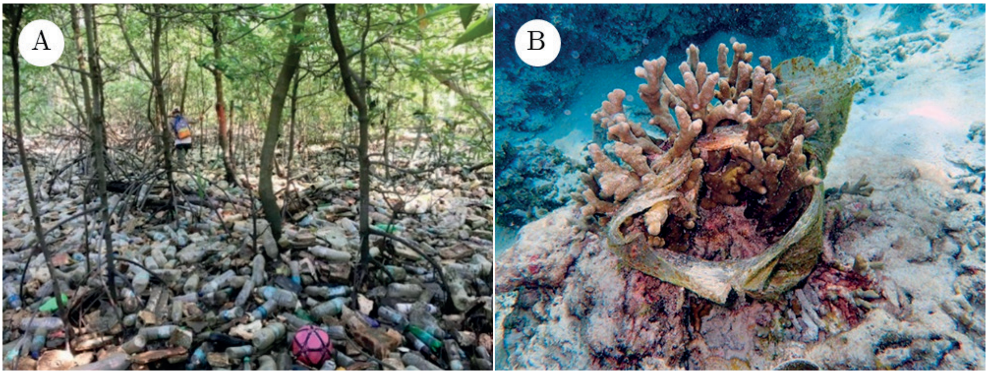

Figure 2.3 Mangroves retain plastics that would otherwise be transported by rivers to the sea. A) Plastic waste accumulation in Sungai Klang mangrove basin, Malaysia and covering of aerial roots (Aldrie Amir, 2018). B) plastic bag wrapped around coral (Bourzac, 2018).

Further out at sea, corals, with their filter and suspension feeding capacity, are highly exposed to marine microplastics. Numerous coral species have been shown to remove microplastics from the water column through ingestion (Hankins et al., 2018; Rotjan et al., 2019) and adhesion to the surface of these particles (Corona et al., 2020). The presence of large amounts of floating plastic could potentially reduce the photosynthesis of coral symbionts and negatively impact colony health by depriving them of light (Osinga et al., 2008). Additionally, the study by Lamb et al. (2018) concluded that the probability of corals experiencing infectious diseases is 20 times higher when they come into contact with plastics (Figure 2.3B). Plastics cause mechanical damage and abrasion to coral tissue, creating entry points for pathogens. These authors also found that corals ingest microplastics colonized by microbes, which can lead to disease. Lamb et al. (2018) estimated that approximately 11.1 billion plastic items (diameter $>$ 50 mm) are polluting the Asia-Pacific coral reefs, and these number is expected to increase by 40% in 2025.

This exponential increase in plastics and microplastics in mangrove, seagrass, and coral habitats affects and degrades all three components of the seascape. It directly impacts each habitat individually, hampering their natural functions and, consequently, disrupting cross-ecosystem connectivity. However, limited knowledge exists regarding the larger-scale resilience of the connected seascapes to solid waste pollution, and to (micro)plastics in particular.

2.4.5 pH and carbonate unbalance

According to recent studies, there is ongoing debate regarding the potential influence of seagrasses and mangroves on seawater pH adjacent to coral reefs, especially regarding mangroves.

The extent to which seagrasses can effectively remove CO_2 and have a significant positive effect on water pH depends on factors such as their photosynthetic efficiency, which is influenced by light availability and intensity (Ow et al., 2016), as well as water temperature and salinity (George & Lugendo, 2022). Diel variations in light, resulting in fluctuations between day and night, lead to variations in the net primary productivity of seagrass. This productivity is not only directly relates to their pH buffering capacity but is also influenced by the local hydrodynamic conditions (Barry et al., 2013). Model-based (Unsworth et al., 2012), field (Ricart et al., 2021), and laboratory experiments (Bergstrom et al., 2019; George & Lugendo, 2022) have confirmed the significant contribution of seagrasses to increasing water pH (Figure 2.4), potentially benefiting adjacent reef-forming species. Unsworth et al. (2012) applied a theoretical approach using data from several publicly available sources to examine seagrass productivity and estimated that seagrass could enhance coral calcification by up to 18% due to localized increase in water pH. On the other hand, the modelling approach of Koweek et al. (2018) concluded that seagrass could only temporarily increase water pH, suggesting that they could not provide a long-term solution. However, Bergstrom et al. (2019) conducted an ex-situ experiment and found that calcification rates of reef species in the presence of the seagrass *Halodule wrightii* were approximately twice as high as in its absence, and Ricart et al. (2021) observed that seagrass ameliorated low pH in 65 % of the sampled locations.

An integrative study conducted by Camp et al. (2016) examined mangrove, seagrass, and coral reef ecosystems at three different sites in the Pacific, Indian and Atlantic. The study confirmed the pH buffering capacity of seagrass, as they increased the mean water pH, supporting coral calcification in the adjacent reef. Similarly, a recent study in a connected tropical seascape in the western Indian Ocean found that seagrass ecosystems considerably raised water pH and potentially mitigated low pH in adjacent mangrove and coral ecosystems (George & Lugendo, 2022). The mean pH in mangrove areas was consistently lower than that in seagrass and coral reef sampling points (Camp et al., 2016; George & Lugendo, 2022). Biogeochemical processes occurring in mangrove areas such as plant and microbial respiration, and organic matter mineralization, contributed to increased levels of CO_2. While mangroves may not act as a buffer for ocean acidification in coexisting mangrove-coral habitats, the generally lower pH levels (Stewart et al., 2022) could pre-condition adjacent corals to future acidic waters (Camp et al., 2016, 2019; Yates et al., 2014a). This evidence emphasizes the importance of maintaining healthy seagrass meadows as a buffer zone between mangroves and corals. A more recent empirical study on the capacity of connected mangroves, seagrasses, and corals as sources or sinks of CO_2 indicated that mangroves and corals (to a lesser extent) acted as sources, while seagrasses played an uptake role (Macklin et al., 2019). According to Chen et al. (2021), mangroves globally export an estimated 83 ± 50 Tg C yr^{-1} of dissolved CO_2 to the open ocean, which is a larger amount of carbon than they sequester in sediment. On the other hand, an integrated study by Akhand et al. (2021), showed that the mangroves in a mangrove-seagrass-coral continuum acted as net sources of total alkalinity. Furthermore, a study of six different locations along the Australian coast by Sippo et al. (2016) demonstrated that mangroves exported DIC and alkalinity to adjacent waters (Figure 2.4) thus creating a buffer against ocean acidification

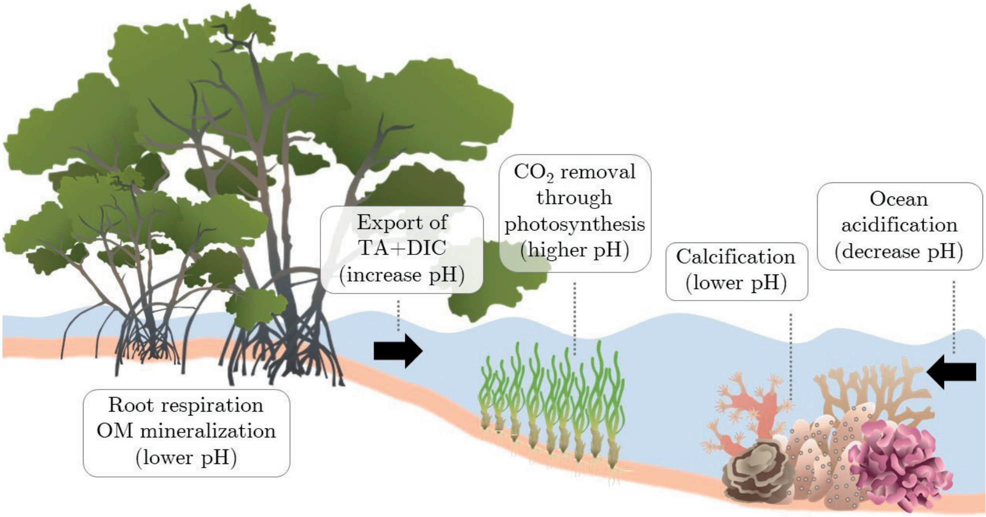

Figure 2.4 Conceptual model showing the potential contribution of tropical coastal habitats to water pH regulation. TA: total alkalinity. DIC: dissolved inorganic carbon.

Water pH and alkalinity exhibit high variability within seasons, days, and even hours due to tidal variation, temperature, light intensity, and local hydrodynamic conditions, as well as biotic factors (Cyronak et al., 2018; Saderne et al., 2019). Salma et al. (2022) found no significant differences in water pH between mangrove-dominated sites, seagrass-dominated sites, and sites where mangroves and seagrass co-occur. Consequently, the results regarding the relative contribution of cross-ecosystem connectivity to ocean acidification buffering are often contradictory and highly dependent on local conditions. It remains uncertain whether these habitats can buffer ocean acidification to benefit coral calcification or, conversely, precondition corals to higher stress levels, potentially losing this service due to anthropogenically-induced pH imbalances. When excess nutrients enter the system, decomposition rates, microbial activity, and sulfate production increase, leading to acidification (Middelburg et al., 1996). Therefore, a key knowledge gap exists regarding the extent to which nutrient enrichment of tropical coastal habitats may create a pH unbalance that, disrupts the existing alkalinity/acidity fluxes.

2.5 Impacted systems give way for potential new research pathways

Following the identification of knowledge gaps in section 2.4, we highlight what we consider the main research questions that need to be addressed regarding biogeochemical connectivity in tropical seascapes:

- Can the interactions between tropical habitats mitigate the effects of eutrophication and sulphide accumulation from sargassum brown tides? (section 2.5.1)
- Does the retention of sargassum by mangrove roots increase the exposure of adjacent seagrass and coral habitats, intensifying its impact to the biome? (section 2.5.1)
- Can mangroves reduce bacterial loads in seawater through litter fall and export to adjacent seagrass and coral habitats, thus reducing disease incidence? (section 2.5.2)
- Can connected mangrove-seagrass systems serve as refugia for sensitive organisms against ocean acidification or alleviate low pH in the benefit of adjacent coral reefs? (section 2.5.3)
- Can bioaccumulated contaminants in mangrove leaves be transferred to adjacent habitats through litter fall and export?
- How does plastic waste entanglement in mangrove roots and microplastic retention in seagrass sediment restrict the free movement of (micro)plastics within the biome?

Mechanistic understandings of processes driving the potential pH buffering by mangroves or the capacity of their leaf phytochemicals to reduce pathogen load can be obtained through relatively simple mesocosm experiments. These experiments may provide us with answers to significant remaining questions, such as the ones described in sections 2.5.2 and 2.5.3. Furthermore, citizen science may aid in documenting and assessing changes in tropical seascapes affected by sargassum (section 2.5.1), which could contribute to our understanding of whether these habitats can individually or collectively counteract the effects of sargassum. In the sections below (2.5.1 – 2.5.3), we elaborate on some of these questions and provide suggestions on how to best apply this knowledge.

2.5.1 Resistance to brown tides

The spatial co-occurrence of mangroves, seagrasses, and corals might also play a vital bioremediation role when it comes to sargassum brown tides. The accumulation and decomposition of sargassum lead to eutrophication, dissolved oxygen depletion, and toxic levels of sulphide and ammonium, causing the loss of flora and fauna (Rodríguez-Martínez et al., 2019; Van Tussenbroek et al., 2017). The impacts of sargassum accumulation and toxicity to coastal habitats have been documented in recent years (Hernández et al., 2021; León-Pérez et al., 2023; Rodríguez-Martínez et al., 2019) but the capacity of ecosystem connectivity to dampen subsequent eutrophication and sulphide toxicity is yet to be determined. A recent review highlights the potential of the three connected systems to mitigate the effects of hypoxia through their interactions and self-rescue mechanisms (Altieri et al., 2021). According to these authors, the photosynthetic production, storage, absorption, and redistribution of oxygen within their tissues can counteract hypoxia in their environment. Furthermore, mangroves and seagrasses are well-known natural biofilters (Agraz-Hernández et al., 2018; Sandoval-Gil et al., 2016). It could be hypothesized that these habitats, acting in association, could more rapidly dampen other impacts of sargassum than each individual habitat in isolation, and positively influence their recovery after disturbance (Gillis et al., 2014) thus preventing the decline of the entire biome. On the other hand, mangrove root systems are prone to sargassum accumulation due to their spatial complexity, leading to mangrove decline (Hernández et al., 2021; León-Pérez et al., 2023). It could also be hypothesized that the accumulation of sargassum in mangrove root systems increases the exposure time of neighbouring seagrass and corals to toxic compounds, leading to the collapse of the biome. We identify two important future research directions: 1) determining the relative contribution of connected mangroves, seagrass, and corals in mitigating the impacts of sargassum, compared to their contributions in isolation, and 2) understanding the negative effect of sargassum retention by mangroves on the connected seascape.

2.5.2 Reduction of bacterial pathogens

Like seagrass, mangroves might have the ability to protect adjacent systems from bacterial pathogens (Ameen & Al-Homaidan, 2021; H. Zhao et al., 2019). Mangrove tissues contain a wide range of phytochemicals with biocidal properties. Preparations of mangrove bark, root and leaf material have been used for centuries in traditional medicine to cure infections (Bandaranayake, 1998) and are now a novel source of antimicrobial compounds widely used by the pharmaceutical industry.

Phytochemical screenings indicate that mangrove leaves are particularly rich in secondary metabolites, such as steroids, triterpenes, saponins, flavonoids alkaloids, tannins, and phenolic compounds (Amaral-Zettler et al., 2015; Eswaraiah et al., 2020; Santhi & Sengottuvel, 2016; Shi et al., 2010). These phytochemicals can naturally leach from mangrove leaves after only a few hours in seawater (Figure 2.5). Up to 23% of mature leaf dry mass can consist of these bioactive phytochemicals (Kandil et al., 2004). When chemically extracted from mangrove leaves, phytochemical compounds exhibit strong antimicrobial activity against various human, animal, and plant pathogens (Bandaranayake, 1998; Eswaraiah et al., 2020; Manilal et al., 2009; P. Mishra & Sree, 2007; Prabhakaran et al., 2012). However, is it yet to be determined whether untreated, raw mangrove leaves have a comparable effect to their laboratory-created extracts in inhibiting the growth of bacteria. It could be hypothesized that large amounts of mangrove litter regularly fall from the trees and are subsequently exported to adjacent systems due to local hydrodynamic conditions, thus releasing their phytochemicals elsewhere, for instance at the nearby seagrass meadows and coral reefs (Figure 2.5).

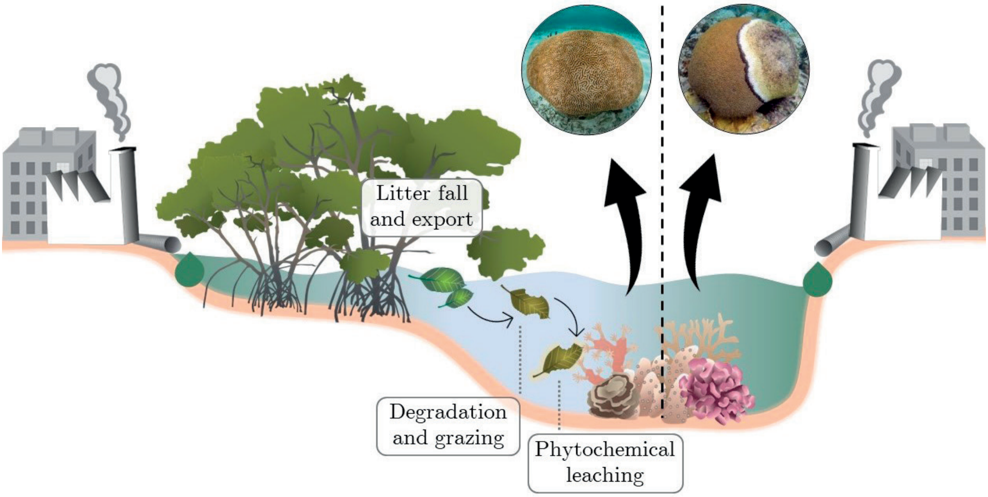

Figure 2.5 Conceptual model showing the hypothetical fate of mangrove phytochemicals and their potential antimicrobial service to coral reefs. Healthy brain coral in connected seascape on the left (Photo credit: Oceana), and coral suffering from black band disease in seascape without mangroves on the right (Photo credit: Wikipedia).

If this is the case, mangroves may act as cleansing agents, particularly in areas where adjacent habitats are affected by diseases related to pathogens due to the absence of well-functioning urban sanitation systems. Therefore, research priorities include understanding how naturally extracted mangrove phytochemicals influence microbial pathogen abundance and whether, in combination with adjacent seagrass meadows, they function as protective cascades against microbial pathogens for the benefit of coral reefs.

2.5.3 Ocean acidification refugia

Lastly, the capacity of mangroves and seagrasses to act as refugia for coral reefs against ocean acidification, as shown in Figure 2.4, still carries some uncertainty due to conflicting evidence discussed in section 2.4.5, particularly regarding the role of mangroves (Camp et al., 2016; George & Lugendo, 2022; Macklin et al., 2019; Ricart et al., 2021; Saderne et al., 2019; Sippo et al., 2016). Although many of these discrepancies may be attributed to the local environmental conditions where the studies were conducted and the mangrove species under investigation, a key priority is to determine the extent to which tropical seascapes that include mangroves and seagrass can serve as as refugia for coral reefs in the face of ocean acidification. If confirmed, the close proximity to seagrass and mangroves as potential long-term shelters could be considered in the decision-making process for future coral reef restoration programs.

2.6 Final recommendations

Future conservation and restoration efforts in tropical seascapes need to consider cross- ecosystem connectivity and address any topic biases in their approach. It is crucial to gather robust empirical evidence that can effectively guide conservation practices in relation to cross-ecosystem connectivity. Simple techniques, such as chlorophyll fluorescence, can be utilized for regular monitoring of mangrove, seagrass, and coral stress, serving as an indicator of human pressure in tropical seascapes. The application of remote sensing techniques that can assess the quality of large seascapes from aerial or satellite images, combined with ecological knowledge, as well as citizen science projects, can greatly enhance data availability and break down barriers to understanding anthropogenic impacts on biogeochemical fluxes at a global scale. The knowledge provided by this review and the suggested research priorities call for future integrative work that can offer appropriate guidance for adaptive management strategies.

Chapter 3

Addition of iron does not ameliorate sulfide toxicity by sargassum influx to mangroves but dampens methane and nitrous oxide emissions

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Abstract

The proliferation of drifting Sargassum spp. and its accumulation along Caribbean coastlines have detrimental effects on the native ecological communities, such as mangroves, due to the high sulfide concentrations in sediment porewater. Although sargassum is being investigated as a carbon sink, methane and nitrous oxide are produced during sargassum decomposition. Iron has proven effective in detoxifying sulfide in sewage and freshwater systems, as well as inhibiting methane production. In a mesocosm experiment, we assessed the capacity of iron(III)(hydr)oxides to mitigate sulfide accumulation resulting from sargassum decay and alleviate sulfide toxicity to red mangroves (*Rhizophora mangle*), as well as potentially reducing carbon dioxide, methane, and nitrous oxide fluxes. Our results showed that sulfide levels in the porewater increased rapidly upon sargassum addition, reaching highly toxic concentrations (15000 µmol l^{-1}) within 14 days, unlike ammonium. However, the iron levels employed in this experiment did not prevent mangrove mortality caused by sargassum-induced sulfide toxicity. Although iron reduced sulfide accumulation in sediment porewater by 42%, the quantity required to lower the sulfide levels sufficiently for mangrove survival renders this technique unfeasible for field application. Our results suggest that early removal within two weeks could potentially prevent mangrove death. Methane and nitrous oxide emissions increased 13 to 16-fold in sargassum-impacted mesocosms, while carbon dioxide emissions strongly increased 39-fold, resulting in the production of approximately 1 g CO_2-equivalents m^{-2} h^{-1} during daylight hours. Mangrove systems turned from sinks to sources of greenhouse gasses. Iron reduced methane emissions by 62% and nitrous oxide emissions by 57% in sargassum-impacted mangrove mesocosms, while carbon dioxide emissions remained unaffected. Our study not only shows that the adverse effects of sargassum strandings are hard to tackle but also provides novel insights into the chemical and ecological mechanisms within mangrove ecosystems following sargassum strandings, including the emission of greenhouse gases.

3.1 Introduction

3.1.1 Brown tides: mechanisms and impacts on coastal ecosystems

Holopelagic Sargassum (*Sargassum fluitans* and *Sargassum natans*) (hereafter sargassum) is a genus of macroalgae from the Phaeophyceae class, historically found as free-floating rafts near the North Atlantic subtropical gyre, forming the Sargasso Sea (Butler, 1983). Pelagic sargassum constitutes a marine ecosystem of high ecological value as it provides physical shelter, food, and nursery habitat for marine fauna (Huffard et al., 2014; Witherington et al., 2012). Over the last decade however, the abnormal proliferation and growth of sargassum have led to massive, periodic influxes of this seaweed to the Caribbean and West African shores (Fidai et al., 2020). The exact causes for these changes in the abundance and distribution of sargassum have not fully been determined, but a combination of wind, current patterns, and nutrient sources along sargassum's trajectory are suspected. Nutrient sources are upwelling, nutrient enrichment of continental rivers such as the Amazon, and atmospheric deposition, mainly of Sahara sand (Skliris et al., 2022). Furthermore, increasing climatic variations affecting sea surface temperatures may also promote the proliferation of sargassum (M. Wang et al., 2019).

The massive sargassum strandings have become a nuisance not only for residents and businesses (Bartlett & Elmer, 2021; Fraga & Robledo, 2022) but also cause irreversible impacts on the native ecological communities (Rodríguez-Martínez et al., 2019; Van Tussenbroek et al., 2017). While small amounts of sargassum fortify the coast by stimulating plant growth and dune formation (A. Williams & Feagin, 2010), the accumulation and anoxic decomposition of large stranded sargassum masses leads to organic matter leachates and oxygen depletion, followed by sulfide and ammonium production (Rodríguez-Martínez et al., 2019). Porewater sulfide concentrations increase enormously in affected beaches and wetlands (Rodríguez-Martínez et al., 2019), which is concerning because sulfide is a strong phytotoxin capable of affecting ecosystem health and functioning (Lamers et al., 2013).

3.1.2 Effects of brown tides on mangroves

Mangrove ecosystems are particularly exposed to sargassum impacts due to its accumulation around their complex root systems and in tidal channels (Hernández et al., 2022; León-Pérez et al., 2023). Consequently, the mangrove trapping of sargassum hampers its natural or artificial removal. The impacts of sargassum brown tides on coastal ecosystems such as mangroves, seagrass, and corals have been previously documented (Hernández et al., 2022; Van Tussenbroek et al., 2017), but effective mitigation is lacking due to low feasibility of existing measures (Fraga

& Robledo, 2022). Current mitigation measures involve the mechanical removal of beached sargassum using manual labour and heavy machinery (Hinds et al., 2016). Still, efforts are often exceeded by the large volumes of accumulated seaweed and long response times. A recent study by Leemans et al. (*in preparation*) linked sulfide accumulation in porewater to the increased mortality of mangroves following a sargassum beaching event on Bonaire in the Dutch Caribbean.

3.1.3 Iron as sulfide mitigation: would it work for mangroves?

Mangroves with a high sediment iron content have complex sulfide dynamics compared to low-iron sediments (Reithmaier et al., 2021). Under conditions of no or low oxygen, ferric iron (Fe(III)) is reduced to ferrous iron (Fe(II)) by bacteria or archaea. Like sulfide, free (dissolved) iron may be toxic to plants, especially in wetlands and flooded soils (Schmidt et al., 2020). However, the reaction between iron and sulfide in low oxygen conditions results in their mutual detoxification and subsequent removal from the water as they precipitate as iron sulfides (Reithmaier et al., 2021; Smolders & Roelofs, 1996; Van Der Welle et al., 2006; Xiao et al., 2022). Iron-rich minerals are commonly found as a natural component of mangrove sediments (Pan et al., 2019), where mangrove leaf phytochemicals and associated fauna facilitate iron dissolution, thus increasing the bioavailability of iron (Hinokidani & Nakanishi, 2019). The addition of different forms of iron to counteract the detrimental effects of sulfide has been successful in various systems such as sewage and freshwater wetlands (Smolders et al., 1995; Van Der Welle et al., 2006; van der Welle et al., 2008), but has not been tested in mangroves.

The role of sargassum brown tides in global warming

Besides causing ecological and socio-economic damage to coastal communities, sargassum blooms play diverse and contrasting roles regarding global warming. According to Gouvêa et al. (2020), the amount of blue carbon contained in floating sargassum is comparable to that of well-known blue carbon ecosystems such as seagrass meadows and mangrove forests on a global scale, although this has been disputed (Hu et al., 2021). Furthermore, sargassum produces recalcitrant dissolved organic carbon (DOC) (Hu et al., 2021) (estimated at 6% of fixed carbon) and calcite (Bach et al., 2021) (from 4.3 up to 21.4% of wet weight, the latter value from slow-growing sargassum in the Sargasso Sea). Due to its efficient carbon sequestration into biomass, sargassum has been suggested as a prime candidate for CO_2 removal by ocean afforestation (Bach et al., 2021).

3.1.4 GHG emissions from mass sargassum decay

Decaying sargassum under anoxic conditions releases greenhouse gasses (GHGs), including methane (CH_4) (Milledge et al., 2020) and potentially nitrous oxide (N_2O), which are 27 and 273 times more potent than CO_2 in terms of global warming, respectively (IPCC, 2021). A study by Nielsen et al. (2021) measured CH_4 production in a sargassum mesocosm, simulating beach decay for six months. Furthermore, the more potent GHG, N_2O can be generated by archaea, bacteria, and fungi, as well as through abiotic processes (Otte et al., 2019). Drifting macroalgal blooms can contribute to significant N_2O emissions in coastal habitats (Wong et al., 2021).

The addition of iron as a mitigation for sulfide can also inhibit CH_4 production, according to a study by Zhang et al. (2009) in a sulfide-rich sewage system. Evidence about the application of different forms of iron to inhibit or stimulate methanogenesis in natural systems was reviewed by Baek et al. (2019), who, for example, discussed six studies that added ferric hydroxides, which inhibit methanogenesis in varying degrees. Additionally, high reduced iron concentrations can buffer N_2O emissions from flooded rice paddy soils by facilitating efficient denitrification (M. Wang et al., 2016).

3.1.5 Aims of this study

In the present study, we assess the capacity of iron(III)(hydr)oxide to ameliorate sulfide toxicity in mangroves and its effects on porewater chemistry. As a second goal, greenhouse gas emissions from mangrove mesocosms were measured, and the effect of sargassum and iron on such fluxes was assessed. The research questions are the following: 1) What are the effects of sargassum decomposition on porewater sulfide and ammonium levels? 2) What is the effect of iron(III)(hydr)oxide on porewater sulfide concentrations? 3) How do sargassum and/or the addition of iron affect the health of sargassum-impacted mangroves with regard to leaf loss, photosynthetic efficiency, and leaf elemental content? 4) How does sargassum and/or the addition of iron affect CH_4, CO_2, and N_2O fluxes from a mangrove mesocosm? To answer these questions, sargassum and iron(III)(hydr)oxide were applied to a mangrove mesocosm in a full factorial, controlled experiment to gain insights into the biogeochemical processes and their potential effect on mangrove vitality following sargassum brown tides and the potential mitigating role of iron addition.

3.2 Materials and methods

3.2.1 Experimental set-up

To test the capacity of iron to ameliorate sulfide concentrations in the sediment porewater and reduce greenhouse gas emissions caused by sargassum decomposition, a mangrove mesocosm experiment was conducted in which the effects of sulfide and iron on red mangrove Rhizophora mangle saplings were studied over time. The iron source used in this experiment was iron(III)(hydr)oxide, an iron-rich sludge material generated as a by-product of drinking water production, also known as 'waterijzer' in Dutch. The accessibility and affordability of iron(III)(hydr)oxide make it particularly advantageous for developing countries and remote areas affected by sargassum brown tides, as it eliminates the need for expensive imports, reducing financial constraints associated with its acquisition. The mesocosm experiment was conducted at Nergena greenhouse, Unifarm, Wageningen University and Research, the Netherlands for 35 days, from February 25 to April 1, 2022. Red mangrove saplings were 1.5 years old, collected from the Caribbean island of Guadalupe as unrooted propagules, and were subsequently nursery-raised in the Netherlands. Fresh sargassum seaweed was collected from Bonaire on January 21, 2022, sun-dried, and transported to the Netherlands. Although the sargassum used in this experiment was rehydrated, it was expected to contain and release large quantities of organic components such as nitrogen compounds (Zhao et al., 2022). Sand and water content were calculated from a representative sample of the bulk sargassum.

We included control treatments that did not contain a mangrove tree. In these treatments, the mangrove tree was removed from the sediment before the experiment to ensure that the sediment contained a stable mangrove-associated microbial community. Thus, the experimental setup consisted of three factors: sargassum addition, iron addition, and mangrove presence, as well as their combinations following a full-factorial completely randomized design with five replicates (Figure 3.1). All experimental units consisted of a nursery pot with 4.1 litres of quartz sediment previously washed and ashed of 0.3-0.1 mm grain size and submerged in containers with 7.5 litres of artificial seawater at ~ 32 ppt (Aquaforest Reef Salt, Aquaforest sp. O. o., Poland). Mangroves were planted in these pots three months before the experiment. They were fertilized with 10 g of an NPK 15:9:11 slow-release fertilizer (Nutri Caps with Osmocote ®, Colombo B. V., the Netherlands) for aquatic plants that contained ~ 6.6 % NO_3^--N, 8.4 % NH_4^+-N, 9 % P_2O_5, 11 % K_2O, 2 % MgO, 0.03 % B, 0.05 % Cu, 0.045 % Fe, 0.06 % Mn, 0.02 % Mo, and 0.015 % Zn. All experimental units were acclimated for one week before the start of the experiment. The greenhouse compartment was kept at a constant temperature of

~ 24 ºC, and lighting was provided by a 400 W metal halide grow light (Hortilux, the Netherlands) 1 meter above the plants and set on a 12-hour light cycle.

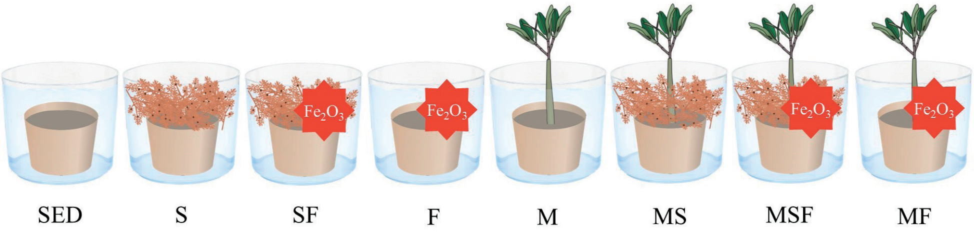

Figure 3.1 Conceptual figure of experimental design with 8 treatments to which sargassum and/or iron(III)(hydr)oxide were added to pots with or without a mangrove tree, following a complete block design with treatment names below each figure. Each treatment consisted of 5 replicates. Abbreviations: SED (control, only sediment), M (mangrove), S (sargassum), and F (iron). Iron(III)(hydr)oxide is represented as red stars.

The experiment consisted of several additions of dry sargassum previously mixed with or without iron(III)(hydr)oxide to the mangrove containers (Figure 3.1). Waterijzer was supplied by Aquaminerals (AquaMinerals B.V., the Netherlands), with an iron content of ~ 4.7 mol kg^{-1} or ~ 26.3 % (see supplementary Table A 3.1 for elemental content of waterijzer and sargassum). A total of 500 g of dry sargassum (including sand and moisture) was added to the containers, an amount similar to those found between mangroves in Bonaire, the Dutch Caribbean (personal observation by authors). Sargassum was added to the pots twice to resemble multiple beaching events and more realistic field conditions. Thus, 137 g and 206 g of dry sargassum (after accounting for sand and moisture content) were added on days 0 and 6 of the experiment, respectively. Regarding iron(III)(hydr)oxide, 5 g, 9.5 g, and 15 g dry weight were added to the pots on days 0, 6, and 20, of the experiment, respectively. Analyses of the dry sargassum and waterijzer concluded that the actual iron-to-(sargassum-derived) sulfur molar ratio was 0.95 (Table A 3.1). This ratio was selected based on a pilot experiment, the results of which showed iron reduced sulfide to non-toxic levels (Figure A 3.1).

The water layer above the sediment in the containers was stirred weekly, and the total volume of water was refreshed on two occasions throughout the experiment. Mangroves were misted twice per week with demineralized water to ensure appropriate humidity levels.

3.2.2 Porewater analysis

Twice a week, 10 ml of porewater samples were collected using a 5 cm Rhizon pore water sampler with a 0.15 µm pore size connected to a 30 ml syringe. We sampled the pore water between 5 and 10 cm depth in the quartz sediment. Part of the sample was diluted and preserved for elemental analysis, and part used for sulfide analysis. Porewater elemental composition was determined at the facilities of Radboud University, Netherlands, using colorimetric methods (Auto Analyser III, Bran and Luebbe GmbH, Norderstedt, Germany) for NH_4^+ and NO_3^- and inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fischer Scientific, Bremen, Germany) for the rest. Samples were kept out of direct sunlight and sulfide was measured within 5 hours after collection using a Hanna Instruments HI4115 sulfide ion-selective electrode. In accordance with the recommended protocols as specified in the probe manual, fresh sulfide anti-oxidation buffer was used and electrode slope was regularly checked to ensure correct functioning. This ensured that probe wear did not cause a deviation in sulfide values of more than 5 % since the last electrode calibration.

3.2.3 Mangrove dynamics

To monitor mangrove health, their photosynthetic efficiency and leaf number were measured four days after the start of the experiment, and repeated on a weekly basis over a period of 35 days. The photosynthetic efficiency (F_v/F_m) was determined from the mangroves as a proxy of stress (Bhagooli et al., 2021), which is reflected by F_v/F_m values below 0.79 µmol m^{-2} day^{-1} indicating a low photophysiological performance (Maxwell & Johnson, 2000). A continued decline in plant photosynthetic performance may eventually result in plant death (Bhagooli et al., 2021). F_v/F_m was measured as the chlorophyll fluorescence on the surface of mangrove leaves by a non-invasive technique, i.e. Pulse-Amplitude-Modulation (PAM) fluorometry using a PAR-FluorPen FP 110 (Photon System Instruments, Czech Republic) after a 30-minute dark adaptation period. The dark adaptation was achieved by placing a white-black polyethylene plastic sheeting below the grow lights and approximately 0.5 meters above the mangrove canopies with small lateral perforations to allow for air circulation. The F_v/F_m was measured on the four fully expanded leaves closest to the apical meristem (Panda et al., 2006). The F_v/F_m is a widely recognized proxy for plant photophysiological activity (Guidi et al., 2019) and the most popular chlorophyll fluorescence indicator for assessing stress photophysiology in mangroves (Bhagooli et al., 2021).

Green and yellow leaf samples were collected upon completion of the experiment and dried at 60 ºC for 48 h, or until they had reached a constant weight. Samples were ground to powder using a MM200 ball mill (Retsch GmbH, Haan, Germany).

Elemental content of tissue samples was determined by microwave-assisted digestion of ~ 200 mg of material with 4 ml 65% HNO_3 and 1 ml 30% H_2O_2 followed by analysis using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fischer Scientific, Bremen, Germany) at the facilities of Radboud University, the Netherlands.

3.2.4 Gas flux measurements

On day 21 of the experiment (March 18, 2022), CO_2, CH_4, and N_2O emissions from the mangrove mesocosms were determined. A 40 cm long closed, transparent, cylindrical chamber (Perspex, 15 cm diameter) was carefully placed above the experimental pots to avoid pressure differences or causing disturbance to the mangroves. The chamber was stacked on top of the sediment or the sargassum layer (depending on the treatment), thus making a seal with the water to create a gas-tight chamber. The chamber was connected to a G2508 gas concentration analyser with cavity ring-down spectroscopy (G2508 CRDS Analyzer, Picarro, Santa Clara, USA). A small fan was installed inside the chamber to homogenize the air and avoid overheating. To calculate the diffusive fluxes of CO_2, CH_4 and N_2O, the linear change in the concentration of every gas over time was used (Vroom et al., 2018). In the case of ebullition (abrupt increase in gas concentration), the chamber was removed from the mesocosm and vented before repeating the measurement (Almeida et al., 2016; Oliveira-Junior et al., 2018). Headspace volume was determined for every experimental unit, as well as water temperature (Greisinger GTH 175/PT thermometer, GHM Messtechnik GmbH, Regenstauf, Germany).

3.2.5 Statistical analysis

Mangrove health and greenhouse gas data analyses were conducted using R, version 4.0.2 (R Core Team, 2021). Shapiro-Wilk and Levene's tests were used to assess normality and homogeneity of variance prior to analysis. Outliers were identified and excluded using the built-in *boxplot.stats* function in R. When normality and homoscedasticity assumptions were not met, non-parametric tests were conducted. For the analysis of discrete variables such as CH_4, CO_2, N_2O and CO_2-equivalents, generalized linear models (GLMs) were conducted to detect differences between treatments, with sargassum addition, iron addition, and mangrove presence as predictors. To present the comparisons of GHG emissions between the different treatments, we expressed the effect sizes of these comparisons as a percentage change or a -fold change when the percentage change exceeded 100 %. These effect sizes were only calculated when the denominator did not approach zero or when the denominator was a negative number. To account for temporal differences between treatments in continuous variables, generalized linear mixed models (GLMMs) were applied to F_v/F_m and leaf number. In these models, sargassum addition, iron addition, and mangrove presence were treated as fixed factors, while time was considered a random factor. The Anova function from the car package was used to report Chi-squared, F, and p-values from the GLMMs and GLMs. Tukey pairwise comparison tests were conducted to identify specific treatment differences. The results were presented as mean ± standard error, and a significance value of $p \leq 0.05$ was considered statistically significant. Porewater and tissue content data was analysed using R version 4.2.3 (R Core Team, 2021). For the porewater data, GLMMs were used with the *lme4* package. Mangrove presence, iron addition, sargassum addition, and time were treated as fixed effects, while pot ID was considered a random effect. All interactions were included in the model. The data were modelled untransformed, square root transformed and log transformed, after which a Shapiro–Wilk test of normality was conducted on the model residuals, accompanied by visual inspection of histograms and QQ-plots. The model with the most normally distributed residuals was selected, and a Tukey post-hoc test was performed. To calculate the denominator degrees of freedom (df2) in GLMM, the Satterthwaite approximation was used. Surface water data were measured only once, and thus ANOVAs were used. For leaf tissue elemental content, ANOVAs were also used, with sargassum addition, iron addition and leaf colour (green or yellow-brown) as fixed effects. A Tukey test was subsequently conducted.

3.3 Results

3.3.1 Porewater chemistry

The best-fitting transformations, determined based on the outcome of Shapiro-Wilk tests on model residuals, were the square root transformation for sulfide ($W = 0.983$, $p < 0.001$) and the logarithm transformation for NH_4^+ ($W = 0.956$, $p < 0.0001$), Fe ($W = 0.967$, $p < 0.0001$), P ($W = 0.916$, $p < 0.0001$), and Mn ($W = 0.963$, $p < 0.0001$).

For porewater sulfide, the effect of time was significant ($F = 1345.8$, $p < 0.0001$), as were the interactions between mangrove and time ($F = 10.8$, $p < 0.01$), sargassum and time ($F = 511.6$, $p < 0.0001$), iron and time ($F = 18.2$, $p < 0.0001$), mangrove, sargassum, and time ($F = 13.5$, $p < 0.001$), and sargassum, iron, and time ($F = 4.3$, $p < 0.05$). After the second addition of sargassum, sulfide concentrations gradually increased, reaching a peak of around 15000 μmol l^{-1} in the absence of iron (Figure 3.2A). The addition of iron reduced sulfide levels by 42 % when mangroves were present, and by 33 % when mangroves were not present. Without sargassum, iron treatments resulted in a 77 % reduction in sulfide levels with mangroves and a 27 % reduction without mangroves.

For dissolved iron (Fe), the effects of sargassum ($F = 7.24$, $p < 0.01$) and time ($F = 64.54$, $p < 0.0001$) were found to be significant. At the beginning, there were high concentrations of Fe. However, after day 6 (sargassum addition, iron addition, and surface water change) the concentrations were low across all treatments, then peaked again, and subsequently decreased. The third addition of iron resulted in a gradual increase of Fe in treatments without sargassum (Figure 3.2B).

For ammonium (NH_4^+), the main effects of mangrove ($F = 23.9$, $p < 0.0001$), sargassum ($F = 21.9$, $p < 0.0001$), and time ($F = 28.0$, $p < 0.0001$) were significant, as was the interaction between mangrove and time ($F = 14.3$, $p < 0.001$). Iron did not have an influence on NH_4^+ concentrations. Towards the end of the experiment, there was a gradual increase in NH_4^+ across all treatments (Figure 3.2D). Nitrate (NO_3^-) concentrations were very low due to the strongly anoxic conditions, and no significant differences were found between the treatments.

Phosphorus (P) was strongly associated with sargassum decomposition and not influenced by mangroves or iron addition (Figure 3.2C). The effects of sargassum ($F = 10.44$, $p < 0.01$) and time ($F = 22.21$, $p < 0.0001$) were significant, as was the interaction between sargassum and time ($F = 27.44$, $p < 0.0001$).

For manganese (Mn), the effects of mangrove ($F = 34.89$, $p < 0.0001$), sargassum ($F = 4.98$, $p < 0.05$), and time ($F = 46.83$, $p < 0.0001$) were found to be significant. Furthermore, significant interactions were observed between mangrove and time ($F = 23.02$, $p < 0.0001$), as well as between iron and time ($F = 5.13$, $p < 0.05$). Mangroves consistently maintain lower Mn concentrations compared to treatments without mangroves (Figure A 3.2) regardless of sargassum addition.

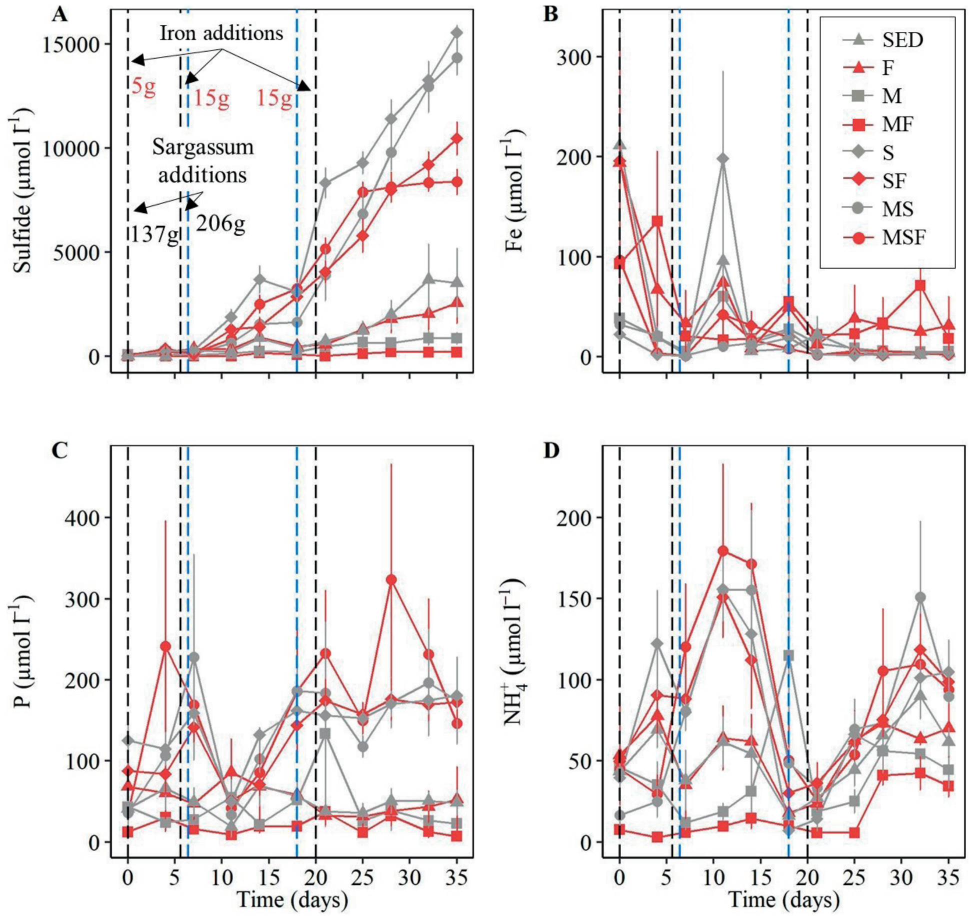

Figure 3.2 Temporal dynamics of sulfide (A), iron (B), phosphorus (C), and ammonium (D) in sediment porewater over the experimental period. Sargassum and iron(III)(hydr)oxide additions are represented as black dashed lines with their corresponding quantities indicated at the top of the figure. Surface water in the experimental containers was refreshed on two occasions, represented as blue dashed lines. Letters depicted in the legend indicate treatment; SED: soil only, F: iron, M: mangrove, MF: mangrove and iron, S: sargassum, SF: sargassum and iron, MS: mangrove and sargassum, MSF: mangrove, sargassum, and mangrove. Treatments containing iron are represented in red (see figure legend).

The surface water data collected on the final day of the experiment (Table A 3.2) shows that the addition of iron increased NH_4^+, as supported by the ANOVA (untransformed, Shapiro > 0.05), which revealed a significant effect of sargassum ($F = 15.62$, $p < 0.001$) and a significant interaction between sargassum and iron ($F = 4.61$, $p < 0.05$). Mn and P concentrations did not show significant differences between treatments, and Fe was slightly higher in sargassum treatments ($F = 4.86$, $p < 0.05$), albeit at low levels. Fe concentrations were similar to those in the pore water measured on the same day, while Mn was slightly higher than in the pore water but still low. Both P and NH_4^+ were lower compared to the pore water. Sulfide, which was measured a week earlier on day 28, showed a strongly significant main effect of sargassum ($F = 398.1$, $p < 0.0001$) and was slightly lower than in the pore water.

3.3.2 Mangrove health

Sargassum exposure reduced F_v/F_m in mangroves (X^2 (1, N = 133) = 4.05, $p < 0.05$) by 25 to 50 %. Additionally, the effects of sargassum became more pronounced over time (Figure 3.3), yielding a significant interaction between sargassum and time (X^2 (1, N = 133) = 13.88, $p < 0.05$). F_v/F_m in sargassum-impacted mangroves experienced a sharp decline after 15 days and continued to decrease towards the end of the experimental period. The combination of sargassum and iron resulted in a more substantial decline in F_v/F_m, reaching stress levels of 0.4 ± 0.3 $\mu mol\ m^{-2}\ day^{-1}$ after 35 days of exposure (Figure 3.3A). However, the interaction term between sargassum and iron on the F_v/F_m of mangroves was found to be non-significant (X^2 (1, N = 133) = 0.53, $p > 0.05$), as was the main effect of iron (X^2 (1, N = 133) = 0.64, $p > 0.05$). Similar to F_v/F_m, the interaction term between sargassum and time had a significantly negative effect on leaf number (X^2 (1, N = 133) = 61.87, $p < 0.001$). Mangroves exposed only to iron experienced a 12 % loss in leaf number, but the main effect of iron on leaf number was non-significant (X^2 (1, N = 133) = 1.27, $p > 0.05$). Mangroves in sargassum-iron treatments lost 20 % more leaves by the end of the experiment compared to those in the only-sargassum treatments (Figure 3.3B). No significant interaction was found between sargassum and iron with respect to leaf number (X^2 (1, N = 133) = 10.28, $p > 0.05$).

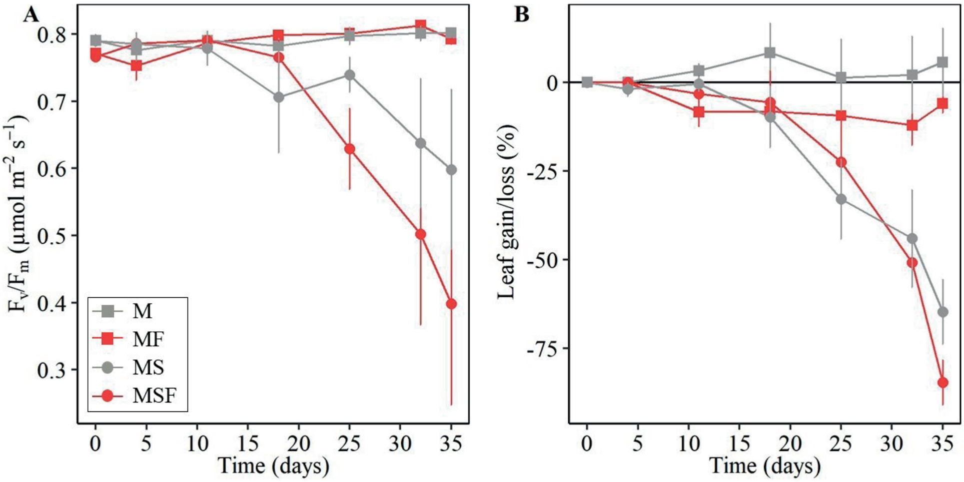

Figure 3.3 Temporal dynamics of photosynthetic efficiency (F_v/F_m) (A) and leaf number (B) in mangroves over the experimental period. Letters depicted in the legend indicate treatment; M: mangrove, MF: mangrove and iron, MS: mangrove and sargassum, MSF: mangrove, sargassum, and mangrove. Treatments containing iron(III)(hydr)oxide are represented in red (see figure legend).

All mangroves exposed to sargassum died over the course of the experiment, irrespective of iron addition, while the control trees remained alive. Toxicity symptoms became evident a few days after the first sargassum exposure, characterized as a downward change in the leaf orientation angle, in contrast to the upward-facing leaves of healthy mangroves in the non-sargassum treatments. After 18 days, severe chlorosis of the whole leaves became noticeable, followed by necrosis and defoliation (Figure 3.4). Initially, these symptoms were restricted to the bottom leaves but rapidly progressed from the bottom up, affecting the entire plant. Subsequently, the terminal buds would wither and detach from the branches, while still showing a green coloration (Figure 3.4).

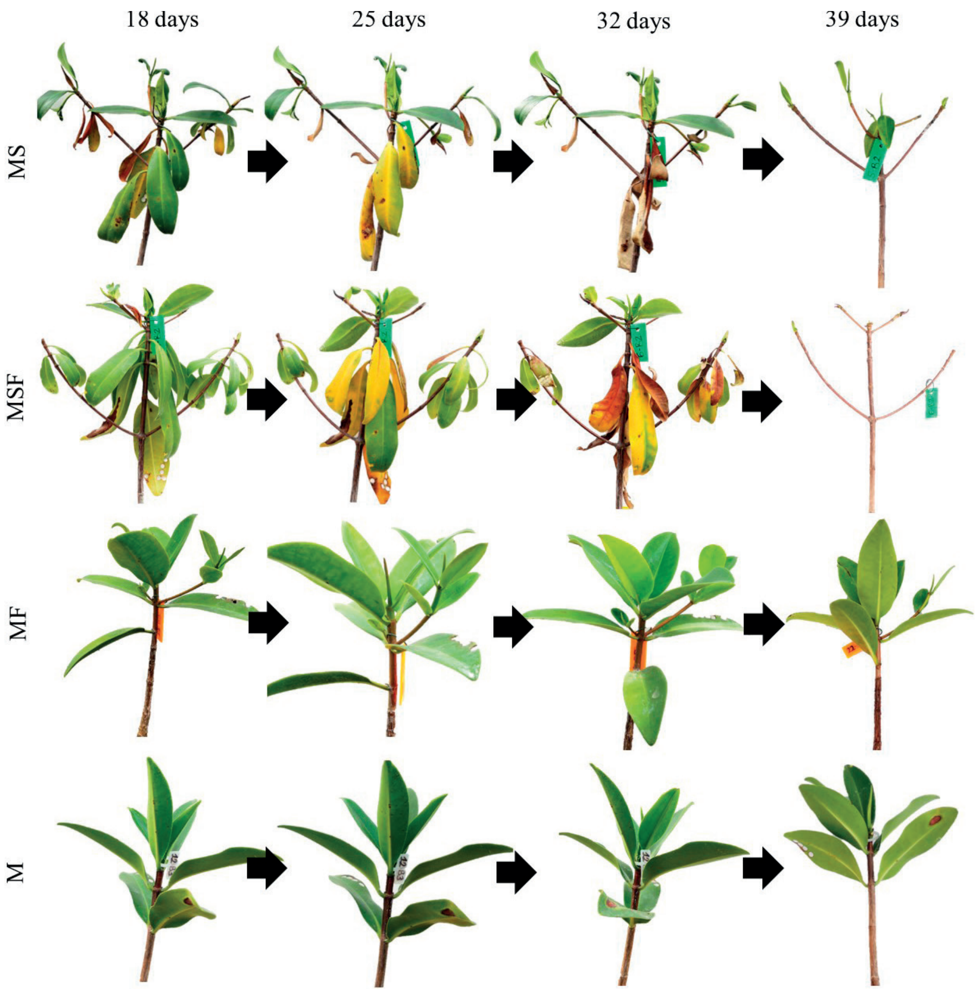

Figure 3.4 Visual symptoms of toxicity in mangrove leaves under the different experimental treatments after 18, 25, 32, and 39 days from the first sargassum and iron exposure. Letters depicted on the left side indicate treatment; M: mangrove, MF: mangrove and iron, MS: mangrove and sargassum, MSF: mangrove, sargassum, and mangrove.

The leaf tissue content on the final day of the experiment (Table A 3.3) did not show significant differences between treatments for Fe, Mn, and S. However, significant differences were observed for P (Figure 3.5) in the interactions between sargassum and iron ($F = 4.88$, $p < 0.05$), as well as sargassum and leaf colour ($F = 5.024$, $p < 0.05$). A Tukey test indicated a significant difference between sargassum:iron:yellow_brown and sargassum:no iron:green in the three-way interaction (p adjusted < 0.05), as well as between sargassum:yellow_brown and sargassum:green in the two-way interaction (p adjusted < 0.05). When considering only green leaves in the analysis, the main effect of sargassum was significant (p

adjusted < 0.01). Significant differences were also observed between sargassum:no iron and no sargassum:no iron (p adjusted < 0.01), as well as between no sargassum:no iron and sargassum:iron (p adjusted < 0.05).

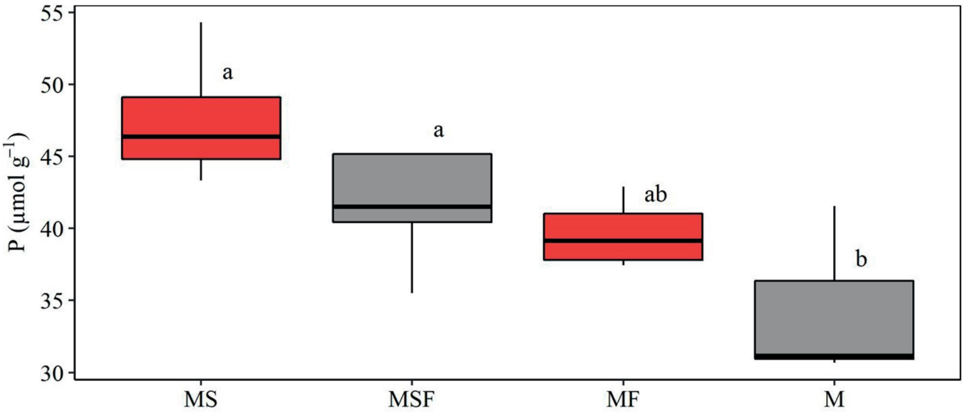

Figure 3.5 Phosphorus content measured in mangrove leaves from the different treatments at the end of the experimental period. Letters depicted in the x-axis indicate treatment; M: mangrove, MF: mangrove and iron, MS: mangrove and sargassum, MSF: mangrove, sargassum, and mangrove. Treatments containing iron are represented in red. Letters above boxes indicate significant differences by Tukey's test at $p = 0.05$.

3.3.3 Greenhouse gas fluxes

Sargassum was found to strongly increase all three greenhouse gas fluxes measured in this experiment (Figure 3.6), CH_4 (t (38) = 2.81, $p < 0.01$), CO_2 (t (39) = 8.76, $p < 0.001$), and N_2O (t (39) = 2.80, $p < 0.01$). Besides the significant main effect of sargassum, the interaction term between sargassum and iron also yielded a significant effect on CH_4 fluxes (t (38) = 2.26, $p < 0.05$) indicating that the sargassum effect was lower when Fe had been added. Sargassum alone caused on average a 16.5-fold increase of CH_4 emissions compared to non-sargassum treatments, and a 13.5-fold increase in combination with a mangrove, although the interaction term between sargassum and mangrove on CH_4 fluxes was non-significant (t (38) = 0.56, $p > 0.05$). Sargassum-impacted treatments that contained iron produced on average 10.4 % less CH_4 emissions than those containing only sargassum but the main effect of iron was non-significant (t (38) = 0.16, $p > 0.05$). When a mangrove was present, the combination between iron and sargassum led to 61.7 % less CH_4 emissions ($p < 0.05$ compared to same treatment without Fe; Figure 3.6). CO_2 fluxes were not only significantly increased upon sargassum addition, but also by the interaction between sargassum and mangrove (t (39) = 2.81, $p < 0.05$), indicating that the sargassum effect on CO_2 emissions was 53 % greater in the presence of a mangrove. Regarding N_2O, no factors other than sargassum (t (39) = 2.80, $p < 0.01$) yielded a significant effect on N_2O emissions. Although the main effect of iron was found non-significant for N_2O, there were significant differences between treatments. The presence of iron in sargassum treatments with and without a mangrove reduced N_2O fluxes by 57 % ($p < 0.05$ compared to same treatment without Fe) and 35 % (n.s.), respectively.

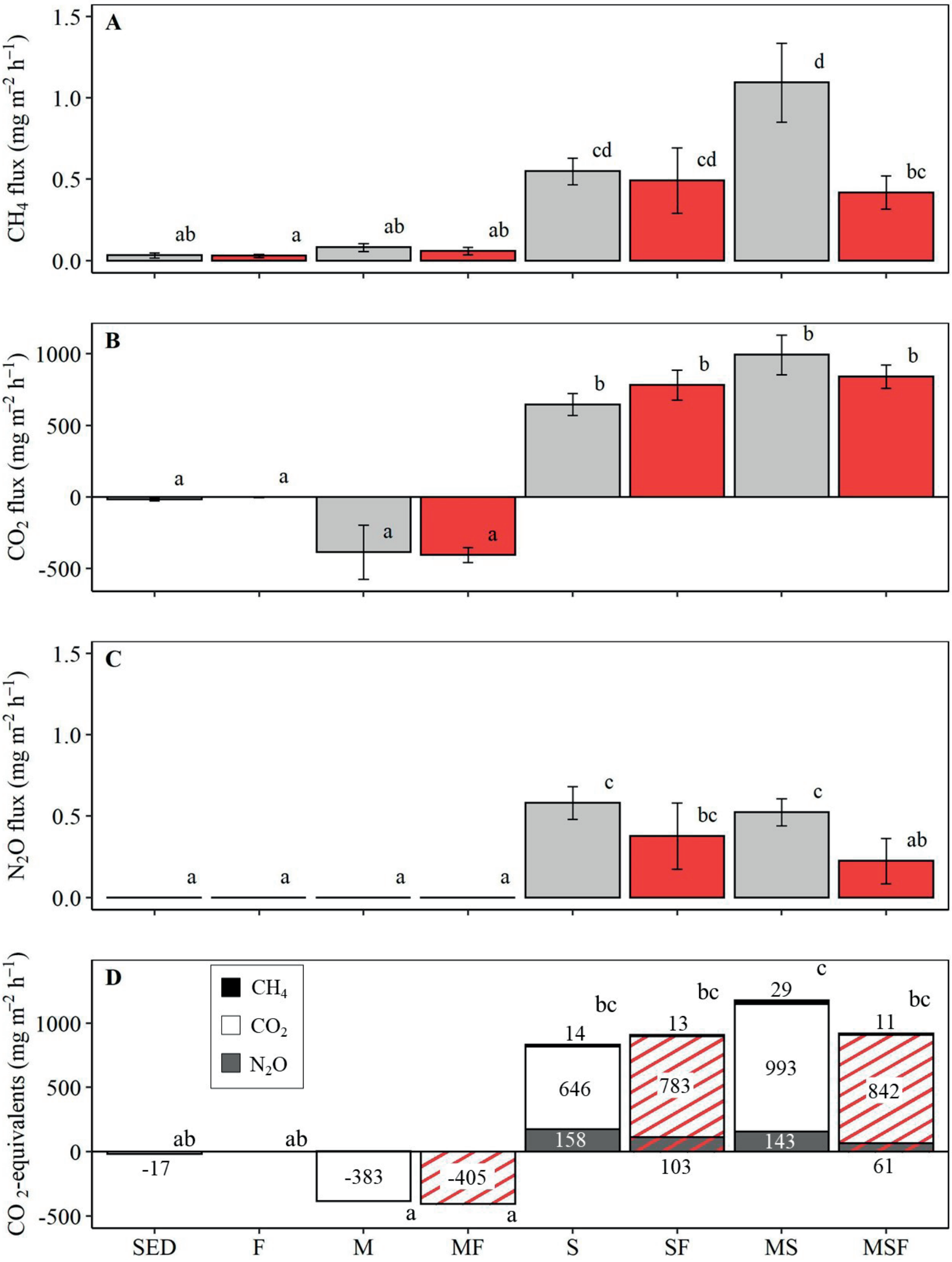

Figure 3.6 Fluxes of CH_4 (A), CO_2 (B), and N_2O (C) measured from the different treatments after 22 days from the start of the experiment, as well as the total CO_2-equivalents (D). Letters above bars indicate significant differences between treatments by Tukey's test at $p = 0.05$. Capital letters depicted in the x-axis indicate treatment; SED: soil only, F: iron, M: mangrove, MF: mangrove and iron, S: sargassum, SF: sargassum and iron, MS: mangrove and sargassum, MSF: mangrove, sargassum, and mangrove. Iron treatments are represented in red.

After converting each gas to CO_2 equivalents (CO_2e) using their Global Warming Potential value (IPCC, 2022), the relative contribution of N_2O to net emissions becomes substantial, 6.7 % to 19.3 % in sargassum-impacted treatments, the relative contribution of CH_4 becomes negligible and CO_2 remains the major contribution to net emissions (Table A 3.4, Figure 3.6D). CO_2e was significantly increased in the sargassum treatments ($p < 0.001$), exceeding 1 g CO_2e m^{-2} h^{-1}, whereas treatments without sargassum showed no or negative emissions. The presence of mangroves resulted in negative fluxes of approximately -400 mg CO_2e m^{-2} h^{-1}.

3.4 Discussion

Very high sulfide concentrations in sediment porewater resulting from sargassum decay caused mangrove death due to toxicity. Although the addition of iron lowered sulfide levels by 62%, it was insufficient to prevent mangrove death. We argue that the addition of even higher amounts of iron(III)(hydr)oxides than the 1 kg m^{-2} eventually used in this experiment would not be feasible as a field mitigation measure. The low solubility of iron(III) in seawater, poor mixing of iron(III)(hydr)oxides with sargassum, and likely the lack of iron reduction in the short term limit the effectiveness of this approach. The presence of mangroves resulted in negative CO_2 fluxes, while the addition of sargassum increased the production of CH_4, N_2O, and CO_2, transforming mangrove mesocosms from sinks to sources of GHGs. The addition of iron effectively reduced N_2O and CH_4 fluxes from sargassum-impacted mesocosms.

3.4.1 Sulfide toxicity due to sargassum kills mangroves

Our experimental results indicated that the mangroves died as a consequence of sulfide toxicity, rather than ammonium toxicity, following the rapid accumulation of sulfide in the sediment porewater, with concentrations reaching up to 15000 µmol l^{-1} at the end of the experiment (Figure 3.2A). The decaying top layer of sargassum in our experimental pots formed a dense, gel-like, tacky layer that facilitated sulfide accumulation in the sediment porewater and effectively blocked oxygen intrusion from the atmosphere. Sulfide concentrations reaching up to 15000 µmol l^{-1} in our experiment exceeded previously documented mangrove tolerance thresholds, typically ranging from 1000 to 4000 µmol l^{-1} (McKee, 1993).

Ammonium concentrations in sediment porewater peaked at 200 µmol l^{-1} in sargassum-impacted treatments (Figure 3.2E). Ammonium toxicity to mangroves was unlikely since it typically occurs at concentrations higher than 100-500 µmol l^{-1} (Britto & Kronzucker, 2002). Furthermore, mangroves are well adapted to high soil ammonium concentrations and often utilize it as their primary nitrogen source (Reef et al., 2010). Thus, it is improbable that ammonium had a detrimental effect on mangroves in this experiment, and the observed decline in mangrove health was most likely caused by sulfide.

The severity of the sargassum effect on mangroves increases over time, as evidenced by a notable decline in health indicators, such as F_v/F_m and leaf number. This decline signifies a point of irreparable damage occurring after 15 to 18 days from the first sargassum exposure (Figure 3.3). This suggests that to enhance the effectiveness of mitigation measures involving mechanical removal of sargassum and improve the chances of survival for mangrove saplings, the removal time should not exceed 15 days from the first sargassum beaching event. Based on visual observations, signs of sulfide toxicity in mangroves progressed from the lower parts of the plants towards the upper leaves (Figure 3.4), which aligns with field observations of adult mangroves (Leemans et al., *in preparation*).

Sargassum enhanced mangrove uptake of P in our mesocosms, potentially serving as a significant nutrient source in tropical coastal systems, particularly in P-limited systems with calcium carbonate sediments. The observed 38 % increase in P content in mangrove tissues compared to controls (Figure 3.5) is likely attributed to the additional P from decaying sargassum, as indicated by higher pore water values. When iron and sargassum were added together, mangrove tissue P remained significantly higher than in controls, although slightly less. The addition of iron alone showed a trend of increased tissue P. It could be hypothesized that mangroves were able to uptake more P due to lower sulfide levels compared to controls and improved availability of Fe, an important micronutrient.

3.4.2 Iron reduces sulfide insufficiently to prevent mangrove die-off

The addition of iron significantly dampened the rapid sulfide accumulation in the sediment porewater. However, this effect was insufficient to decrease the existing sulfide concentrations or prevent the mangroves from dying due to sulfide toxicity. The coexistence of dissolved iron and sulfide in the sediment porewater points to simultaneous processes of iron reduction and sulfate reduction. This interaction led to the formation of insoluble iron sulfides, resulting in decreased sulfide concentrations observed in the iron treatments. By the 15-day mark, it was evident that significantly more sulfide was produced than reduced iron, as indicated by the lower levels of porewater Fe in the sargassum-iron treatments (Figure 3.2A, Figure 3.2B). This reaction is further highlighted in the sargassum-iron treatments, where the concentrations of Fe in the sediment porewater dropped from 100-200 µmol l^{-1} initially to less than 5 µmol l^{-1} by the end of the experiment.

Iron(III) in mangrove sediments acts as the preferred electron acceptor (Kristensen et al., 2008). However, when it undergoes reduction and binds with sulfide, both iron and sulfide are excluded from the pool, hindering their reoxidation and recycling as electron acceptors. This depletion is particularly problematic for iron, as it is less abundant compared to sulfur and becomes depleted during the experiment. Generally, Fe(II) would be reoxidized around mangrove roots through processes such as bioturbation, radial oxygen loss, drought or strong tidal effects. However, once fully reduced to pyrite (FeS_2), it becomes highly stable.

It was only after the third addition of iron that the accumulation of sulfide was dampened. However, by that stage of the experiment, the mangrove health had already been severely affected after 21 days of exposure to extremely high sulfide levels. As a result, the mangroves did not benefit from the dampening of sulfide by the addition of iron in an approach that is feasible for field application. Furthermore, the remaining 8000 µmol l^{-1} of sulfide was still extremely high. Our results do not support the presence of iron toxicity effects, as Fe was found to be non-significant for either health indicator. Additionally, the interaction term between iron and sargassum was also non-significant, suggesting that the addition of iron(III)(hydr) oxide does not alleviate the toxic effects of sulfide on mangroves at the levels used in our experiment.

3.4.3 Greenhouse gasses from decaying sargassum are mitigated by iron

Mesocosms containing only bare sediment exhibited minimal to negligible emissions of CH_4, CO_2, and N_2O. As our measurements were conducted during daytime, the presence of mangroves resulted in negative CO_2 fluxes (Figure 3.6B), indicating their role as a natural carbon sink. Although CH_4 emissions doubled in the presence of mangroves, this increase was not statistically significant. Estimates for CH_4 and N_2O fluxes from mangrove forests are highly variable, ranging from −1.1 to 1169 mg CH_4 m^{-2} d^{-1} and from −0.2 to 6.3 mg N_2O m^{-2} d^{-1} (Rosentreter et al., 2021). In our mesocosm experiment, N_2O fluxes remained below the detection limit (0.16 mg m^{-2} h^{-1}) in treatments without sargassum (Figure 3.6C). CH_4 emissions from these treatments were relatively low compared to existing literature, but since we did not simulate a forest, the relative differences are more informative.

In contrast to bare sediment, the addition of sargassum resulted in not only high CO_2 emissions but also a 16-fold increase in CH_4 emissions. It is generally accepted that SO_4^{2-} reducers tend to outcompete methanogens for acetate and H_2 (van Erk et al., 2020). In our experiment, the localized depletion of SO_4^{2-} may have contributed to an increase in CH_4 emissions. Although the emission of CO_2 resulting from sargassum stranding primarily represents the release of photosynthetically fixed CO_2, a portion of this substantial CO_2 release would typically be fixed as blue carbon (see section 3.4.5). The addition of sargassum also led to an unexpectedly large flux of N_2O being released from the mesocosms (Figure 3.6). Our findings demonstrate that N_2O emissions can occur during sargassum decomposition, accounting for 19 % of the total greenhouse gas emissions in our study. To our knowledge, this study is the first to measure N_2O emissions from decaying sargassum.

Iron significantly reduced CH_4 and N_2O emissions from sargassum decay in the presence of a mangrove, reducing them by 62% and 57%, respectively (Figure 3.6). The higher CH_4 emissions observed in the mangrove treatment could be attributed to the aerenchyma in mangroves, which not only transports O2 into the sediment but also facilitates the release of CH_4 into the atmosphere (Jeffrey et al., 2019). The plant-mediated transport of methane to the atmosphere is a major pathway in wetlands (Vroom et al., 2022).

The specific reduction in CH_4 emissions attributed to iron in the presence of mangroves could indicate a decrease in CH_4 production or a reduction in CH_4 transport facilitated by the mangroves. It has been documented that iron plaques forming on roots, as observed in R. mangle (Machado et al., 2005), could obstruct CH_4 transport, a phenomenon also noted in mangroves (Pi et al., 2010). These iron plaques can also harbour methane-oxidizing bacteria, as observed in rice paddies (Maisch et al., 2020). In our experiment, we observed an increase in iron plaques on roots in both iron and iron-sargassum treatments, as evidenced by the blackening of the roots (Figure A 3.3). The decreased CH_4 production observed after the addition of iron could potentially be caused by the presence of added iron, which acts as a more favourable electron acceptor than CO_2.

In all replicates of the sargassum treatment without iron, we observed consistent N_2O fluxes. In contrast, N_2O was detected in only 40% of the replicates (2 out of 5) in the iron-sargassum treatments. N_2O production typically occurs under sub-oxic conditions and is facilitated by fluctuating oxygenation, resulting in considerable oxygen gradients where “intermediate” electron acceptors like oxidized metals and nitrate/nitrite are prevalent. In addition, localized nitrification (e.g. in the rhizosphere) can also lead to N_2O production (Wrage et al., 2001). Despite the lack of hydrodynamic forces (see section 3.4.4), numerous reactions leading to N_2O production can be envisioned in this experiment. Mixing iron oxides with sargassum creates a mosaic of microenvironments where NO_3^- from sargassum decomposition, Fe(III) at the iron oxide interface, and SO_4^{2-} from seawater are exposed to high loads of dissolved organic matter. These reactions occur within diffusive microgradients ranging from the most oxidized to the most reduced species, driven forward by precipitation of solids and diffusion (and ebullition) of gases. Within the heterogenous matrix of decaying sargassum, localized areas may emerge where N_2O evades complete denitrification and escapes to the atmosphere. Summing the CO_2, CH_4, and N_2O emissions as CO_2-equivalents, the sargassum treatments resulted in approximately 1 g CO_2e m^{-2} $h^{\ 1}$ emissions, whereas treatments without sargassum yielded either zero or negative values. It is important to note that hourly emissions cannot be directly extrapolated to daily emissions due to significant variations between day and night fluxes (Roth et al., 2022).

3.4.4 Limitations

Compared to natural mangrove basins, our laboratory experiment did not include tides, bioturbation, or water mixing due to hydrodynamic forces. To mimic the latter, artificial mixing of the sargassum, iron and surface water layer was carried out, as well as a complete refreshment of the full volume of water in the mesocosms. Although these circumstances may still have contributed to further sulfide accumulation and limited the solubility of iron oxide, the sulfide levels found in this experiment align with those found in the field following sargassum brown tides on Bonaire in the Dutch Caribbean (Leemans et al. *in preparation*). Moreover, our results regarding the impacts of sargassum on mangrove health also align with the study by Trench et al. (2022) who found very high mortality and rapid demise of R. mangle growing in waterlogged soil consisting of 50 and 100 % sargassum compost.

The protocol used in this experiment for F_v/F_m measurements, which focuses on the measurement of top leaves (Panda et al., 2006) does not allow for early detection of sulfide toxicity in mangroves as the chlorophyll fluorescence in the top leaves reflects the severity of the damage last, thereby delaying the detection of sulfide toxicity. Although monitoring leaf colour and leaf loss would provide a better understanding of the severity of sulfide toxicity, it would not serve as a reliable early detection method either, as sulfide is already present within the mangrove tissue.

Lastly, GHG were only measured once over the course of our experiment. Although our results show the GHG production potential of decaying sargassum, longer measurements that capture the diurnal and seasonal fluctuations (Roth et al., 2022) would generate more insights into the temporal dynamics and peaks of greenhouse gas production by sargassum.

3.4.5 Implications for coastal management

Unlike sewage and freshwater systems where sulfide mitigation by iron has been successfully implemented in the past (e.g., van der Welle et al., 2008), the application of iron(III)(hydr)oxides to coastal sediments affected by sargassum brown tides does not appear to be feasible. In this study, we used 29.4 g of iron(III)(hydr) oxide (26 % Fe content, Table A 3.1) per mesocosm, with each mesocosm having a surface diameter of 19 cm. Our calculations indicate that deploying this approach to the natural environment would require approximately 1 kg of iron(III)(hydr) oxide per m2 of sediment. Moreover, the levels of iron used in this study were still not sufficiently high to mitigate the production and accumulation of sulfide nor did they prevent the death of the mangroves, but only dampened the rapid increase of sulfide. Therefore, larger quantities of iron(III)(hydr)oxide would be necessary to achieve a mitigation effect. Coastal areas experiencing frequent influxes of fresh sargassum would require even greater quantities of iron and regular interventions, creating additional logistical and financial barriers for implementing this measure. Despite the good accessibility and affordability of iron(III)(hydr)oxide, these factors present barriers to its practical implementation. There are also additional disadvantages to introducing iron into a natural system, including the potential for phytoplankton blooms. These blooms can trigger changes in the composition of phytoplankton species, which can lead to cascading effects on ecosystem dynamics and food web structure, as well as the depletion of other nutrients from the increased primary production resulting from iron addition. Furthermore, the absence of an effective iron-reducing microbial population likely prevents the replication of this method from its applications in freshwater systems, at least when it concerns massive sargassum inundations or similar scenarios.

Rather than relying on iron addition, our results suggest that mangrove health could perhaps be safeguarded through a shorter exposure time to sargassum, (e.g., early removal within two weeks). This approach could potentially improve the survival and rebounding capacity of mangrove saplings as the cause for their decay is tackled before reaching a point of no recovery.

Sargassum is considered an atmospheric carbon sink in the oceans that can later be transferred to the deep ocean in the form of dead seaweed wracks (e.g. Krause-Jensen & Duarte, 2016). The uncontrolled proliferation of sargassum, leading to the formation of brown tides and subsequent beach stranding, results in the export of large amounts of carbon in the form of seaweed biomass to the shore. The decomposition of sargassum on land releases the CO_2 back into the atmosphere. In this experiment, CO_2 fluxes measured from sargassum may offset the original CO_2 uptake by the seaweed during biomass production. The significance of macroalgae such as sargassum as blue carbon ecosystems is gaining recognition, although the ultimate fate of the carbon they sequester remains uncertain. While the deep ocean effectively sequesters the produced carbon (Krause-Jensen et al. 2016), sargassum strandings on shallow coasts and beaches are likely to release the produced carbon back into the atmosphere. As sargassum events become more frequent and globally significant, our findings contribute to understanding the role of sargassum blooms in greenhouse gas dynamics.

3.5 Conclusions

Sargassum undergoes anoxic decomposition, leading to the rapid production of sulfide, which accumulates in the sediment porewater. Our experimental results indicate that the death of the mangroves is caused by sulfide toxicity, rather than ammonium or a combination of both as the concentration of ammonium in sediment porewater does not reach toxic levels. The sulfide concentrations in sediment porewater exceed the tolerance threshold of mangroves, and the addition of iron does not prevent their death. While the addition of iron can partially mitigate the rapid sulfide accumulation by sequestering it in the sediment, implementing this measure on a larger scale is not feasible in practical field applications. Furthermore, our study revealed that sargassum enhances CH_4 and N_2O emissions from mangrove mesocosms, resulting in a 13 to 16-fold increase in fluxes. The total emissions for sargassum treatments amounted to approximately 1 g CO_2e m^{-2} h^{-1}, whereas treatments without sargassum addition showed zero emissions, and the presence of a mangrove resulted in negative emissions of approximately -400 mg m^{-2} h^{-1}. This transformation of mangrove habitats from carbon sinks to sources of greenhouse gases contributes to climate change due to the high global warming potential of these gases. The addition of iron effectively reduced CH_4 emissions from sargassum by 62 % and N_2O emissions by 57%, reducing total GHG emission by 21 % of CO_2-equivalents.

3.6 Appendix

Table A 3.1 Iron(III)(hydr)oxide and sargassum elemental content in mmol kg^{-1} dry weight, with standard deviation and n=3

Element	Iron(III)(hydr)oxide (mmol kg^{-1} DW)	Sargassum (mmol kg^{-1} DW)
Al	58.69 ± 4.23	16.33 ± 2.99
Ca	777.79 ± 7.32	1907.39 ± 432.71
Fe	4705.21 ± 403.56	17.07 ± 3.16
K	9.41 ± 1.06	865.77 ± 173.3
Mg	59.5 ± 2.74	499.54 ± 22.31
Na	5.7 ± 0.97	2012.52 ± 169.04
P	22.02 ± 1.52	19.43 ± 1.77
S	39.61 ± 2.44	440.46 ± 9.27
Si	240.2 ± 46.25	19.32 ± 5.22

Table A 3.2 Surface water concentrations on day 35 (April 1, 2022) showing mean values of sulfide, S, Fe, Mn, NH_4^+, NO_3^-, P, and S^{2-} in µmol l^{-1}.

Treatment	S	Fe	Mn	NH_4^+	NO_3^-	P	S^{2-}
MF	28465	3.23	2.22	6.40	1.58	7.89	22.02
M	26622	0.92	2.63	10.41	2.20	13.90	52.03
SED	26676	1.43	2.21	6.54	2.25	5.85	168.63
F	31076	0.98	2.76	6.85	2.16	9.16	62.47
S	22686	2.25	2.09	8.88	2.83	9.46	7554.91
SF	22962	3.73	3.10	16.64	2.67	17.48	6303.89
MSF	24405	3.51	3.48	17.03	3.28	26.15	6147.32
MS	25265	1.60	2.77	11.42	2.64	10.91	9265.36

Table A 3.3 Fe, Mn, P, and S elemental content in mangrove leaves collected at the end of the experiment, if still present, with sample sizes (n). Values represent mean ± SD in µmol kg^{-1} of dry weight.

Treatment	Leaf colour	S	Fe	Mn	P	n
M	green	91535 ± 22727	652 ± 182	1322 ± 3039	34478 ± 5601	3
MF	green	73730 ± 61659	727 ± 375	1820 ± 7278	39667 ± 16245	4
MS	green	66739 ± 207818	725 ± 253	4816 ± 2228	47589 ± 8688	5
MSF	green	60721 ± 206973	715 ± 6217	2307 ± 2777	46342 ± 9734	3
M	yellow/ brown	79571 ± 22727	830 ± 182	2774 ± 3039	42442 ± 5601	2
MF	yellow/ brown	195904 ± 61659	1456 ± 375	8925 ± 7278	46665 ± 16245	3
MS	yellow/ brown	248264 ± 207818	1362 ± 253	3947 ± 2228	40453 ± 8688	4
MSF	yellow/ brown	185998 ± 206973	3920 ± 6217	6148 ± 2777	29723 ± 9734	5

Table A 3.4 Relative contribution of CH_4, CO_2, and N_2O fluxes (%) to Net CO_2e emissions measured from the different experimental treatments Fluxes are shown as CO_2-equivalents (CO_2e), calculated using the Global Warming Potential value of each gas (IPCC, 2022).

Treatment	CO_2e fluxes from CH_4 as % of total emissions	CO_2e fluxes from CO_2 as % of total emissions	CO_2e fluxes from N_2O as % of total emissions
MF	0.39	99.60 (−)	~ 0
M	0.53	99.43 (−)	~ 0
SED	6.06	94.94 (−)	~ 0
F	52.18	47.82	~ 0
S	1.80	78.86	19.33
SF	1.47	87.09	11.43
MSF	1.23	92.08	6.68
MS	2.53	85.21	12.25

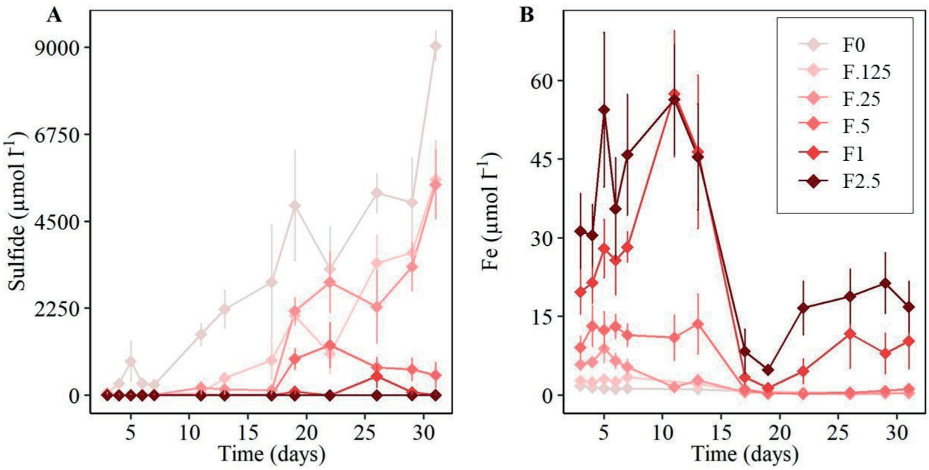

Figure A 3.1 Temporal dynamics of sulfide (A) and iron (B) in pilot incubation experiment where different iron-to-(sargassum-derived) sulfur molar ratios were tested. Iron-to-sulfur molar ratios ranged from 0 to 2.5 (see figure legend). 30 g of dry sargassum were incubated in close-lid glass jars containing 250 ml of artificial seawater and varying concentrations of iron(III)(hydr)oxide. Jars were inoculated with mangrove sediment to ensure the presence of sulfide-reducing bacteria and incubated in the dark at 30 ºC, simulating tropical temperatures.

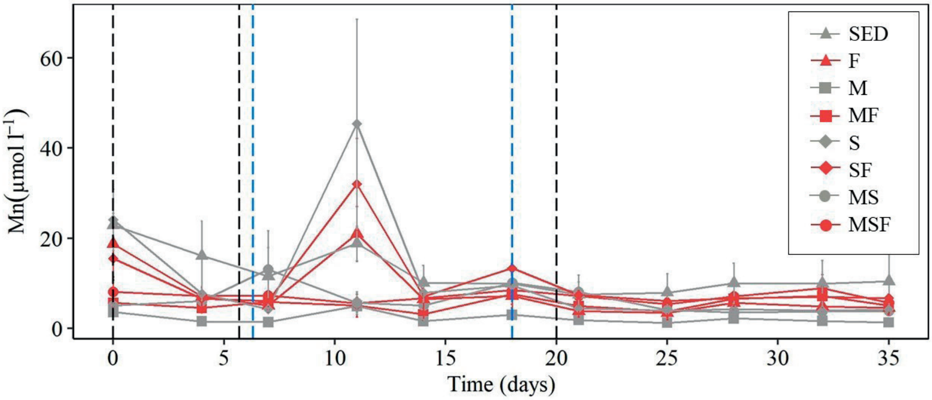

Figure A 3.2 Temporal dynamics of manganese in sediment porewater over the experimental period. Sargassum and iron(III)(hydr)oxide additions are represented as black dashed lines. Surface water in the experimental pots was refreshed on two occasions, represented as blue dashed lines. Letters depicted in the legend indicate treatment; SED: soil only, F: iron, M: mangrove, MF: mangrove and iron, S: sargassum, SF: sargassum and iron, MS: mangrove and sargassum, MSF: mangrove, sargassum, and mangrove. Treatments containing iron are represented in red (see figure legend).

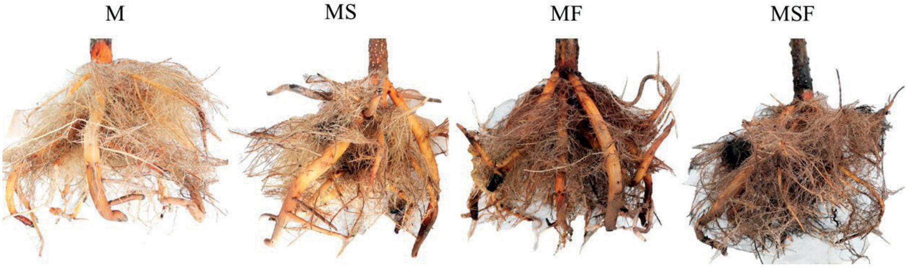

Figure A 3.3 Iron plaque formation on red mangrove roots. Letters depicted above the photographs indicate treatment; M: mangrove, MF: mangrove and iron, MS: mangrove and sargassum, MSF: mangrove, sargassum, and mangrove. Photographs were taken at the end of the experimental period.

Chapter 4

Black mangrove seedlings have a direct dampening effect on increasing porewater pH: a mesocosm study

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Abstract

Ocean acidification poses a significant threat to the survival of numerous marine species, particularly those dependent on the formation of calcium carbonate structures such as corals. Therefore, it is crucial to identify habitats that can locally mitigate ocean acidification to benefit sensitive organisms. *In-situ* studies exploring the impact of mangroves on water carbonate chemistry have produced contrasting results due to species-specific variations and the influence of environmental factors such as temperature and soil nutrient levels. To determine whether mangroves can alter water carbonate chemistry or if these effects arise from interactions with the environment, we conducted an experimental mesocosm study. This study aimed to examine the influence of black mangrove seedlings on pH, alkalinity (TA), and dissolved inorganic carbon (DIC), while also assessing how elevated temperature and nutrient levels influenced this phenomenon. Our results showed a direct negative impact of black mangroves on water carbonate chemistry. Over a ten-week period, the presence of black mangrove seedlings, in combination with high temperature and nutrient enrichment, led to a 6.5 % reduction in pH, a 38 % decrease in TA, and a 31 % decline in DIC. These results provide valuable insights into the role of mangroves in water carbonate chemistry. However, establishing scientific consensus on the direct effects of mangroves in mitigating ocean acidification necessitates longer-term, controlled experiments involving mangroves of varying species and age groups.

4.1 Introduction

The global increase in atmospheric carbon dioxide concentrations as a result of anthropogenic climate change is the main driver behind the phenomenon of ocean acidification (Sabine et al., 2004). It is estimated that the oceans and associated ecosystems have absorbed a third of all global anthropogenic CO_2 emissions since the industrial revolution, becoming a vital carbon sink (Fabry et al., 2008; Gattuso et al., 2015). Mangroves have been broadly recognized for their ability to capture and store large amounts of carbon in their soils, playing a pivotal role in the battle against climate change (Alongi, 2012). However, the role that mangroves play in buffering ocean acidification is site and context-dependent. Often, a lower pH is observed within mangrove habitats compared to adjacent waters (Camp et al., 2016; Middelburg et al., 1996) due to microbial degradation of the large amount of organic matter produced and retained by mangroves (Senger et al., 2021; Wallace et al., 2014). Therefore, organic matter and nutrient loads into coastal zones often result in the intensification of microbial activity and subsequent acidification of water. Nevertheless, mangroves may still be able to buffer ocean acidification through the production and export of dissolved inorganic carbon (DIC) and alkalinity (TA) (Senger et al., 2021; Sippo et al., 2016; Yates et al., 2014b). In seawater, alkalinity is generated through the weathering and dissolution of carbonate minerals (Middelburg et al., 2020). Additionally, in coastal environments such as mangrove sediments, alkalinity is also produced by microbial organic matter cycling (Sippo et al., 2016). These processes, along with dissolved inorganic carbon (DIC), contribute to the water's resistance against acidification (Middelburg et al., 2020). Moreover, mangrove metabolic functions are also influenced by other slow onset impacts of climate change such as global warming (Krauss et al., 2008).

Ocean acidification is threatening the survival of numerous marine species that rely on the availability of carbonate for calcification or are sensitive to pH changes due to narrow tolerance limits (Fabry et al., 2008). Thus, it is vital to identify those organisms capable of maintaining favourable conditions, such as pH, and how external drivers such as nutrients and temperature influence these processes. The pioneer black mangrove *Avicennia germinans* is characterized for colonizing harsh environments and establishing positive interactions with other plant species (Friess et al., 2012; Osborne & Berjak, 1997; Panapitukkul et al., 1998). *Avicennia* mangroves are a popular candidate for mangrove restoration using seedlings, but the effect of these seedlings on local water pH is still unknown. Therefore, the goals of this study are 1) to determine whether, and if so, how black mangroves can alter water carbonate chemistry, and 2) to assess how higher temperature and nutrient concentrations influence these effects. We conducted a mesocosm experiment to examine the influence of black mangrove seedlings on pH, alkalinity (TA), and dissolved inorganic carbon (DIC). Since temperature and soil nutrients affect soil chemistry and mangrove metabolism (Feller et al., 2003a; Gillis et al., 2019; Reef et al., 2016), our experiment included two different levels of nutrients and temperature. We hypothesise that black mangrove seedlings can increase water pH via TA and DIC production, but high temperature and nutrient enrichment will dampen this effect.

4.2 Materials and methods

We used black mangrove seedlings that originated from an adult tree in captivity at Royal Burgers' Zoo, Arnhem, the Netherlands, and were reared from propagules in a greenhouse (Nergena, Unifarm) at Wageningen University, the Netherlands. Six weeks prior to the experiment, each seedling was potted in an 8x8x8 cm nursery pot containing 0.39 l of quartz sand with a gran size of 0.1 - 0.3 mm. They were fertilised with 0.5 g of an NPK 15:9:11 slow-release fertiliser for aquatic plants (Nutri Caps with Osmocote Ⓡ, Colombo B. V., the Netherlands). The seedlings were transported and placed in two different climate rooms at 25 ºC and 30 ºC and submerged in 1.7 l of artificial seawater (Aqua Medic Reef Salt, AB Aqua Medic GmbH, Germany) at the Aquatic Ecology and Water Quality Management laboratory (AEW), Wageningen University, the Netherlands, where they underwent a one-week acclimation period. The seedlings were four months old at the start of the acclimation period. The salinity was set to 32 g l^{-1}, and light intensity was 300 µmol m^{-2} s^{-1}, provided by a metal halide grow light (Hortilux, the Netherlands), following a 12-hour light cycle. The water level was maintained at 2 cm above the sediment surface and adjusted regularly to compensate for evaporation using artificial seawater or demineralized water as required. The experimental design comprised four different treatments, combining two temperature levels (25 ºC and 30 ºC) and two nutrient levels (enriched and non-enriched). Consequently, the resulting treatments were Non-enriched 25 ºC, Non-enriched 30 ºC, Enriched 25 ºC, Enriched 30 ºC. Seedlings subjected to nutrient enriched conditions received 0.41 g slow-release fertiliser for aquatic plants (Nutri Caps with Osmocote Ⓡ, Colombo B. V., the Netherlands) at the root level. The experiment also included a similar series of treatments that did not contain a mangrove seedling. Seedlings were regularly misted to provide moisture and increase air humidity. The experiment ran for a period of ten weeks. All three variables pH, TA, and DIC, were measured weekly from the sediment porewater and surface water. Porewater samples were collected using soil moisture samplers with a 0.15 µm pore size (Rhizon SMS, Rhizosphere Research Products B.V., the Netherlands) connected to 20 ml Luer-lock syringes. Surface water samples were collected using a 20 ml syringe and subsequently filtered through a 0.45 µm syringe filter. Total alkalinity (TA) was measured by the endpoint acid-titration method, with 0.1N hydrochloric acid as a titrant (Sharp & Byrne, 2020), and pH was measured electrochemically using a pH electrode (3110 meter, WTW, Xylem Analytics Germany Sales GmbH & Co, Germany). DIC was measured as the equivalent to total CO_2 using a Segmented Flow Analyzer (SAN++, SKALAR Analytical B.V., the Netherlands) at the Soil Chemistry Laboratory (CBLB) of Wageningen University & Research.

Data analysis was conducted in R, version 4.0.2 (R Core Team, 2021). Prior to analysis, normality and homoscedasticity checks were performed using the Shapiro-Wilk and Levene's tests, respectively, as well as QQ-plots for visual assessment. Outliers were identified and excluded using the built-in *boxplot.stats* function in R. Generalized linear mixed-effects models were applied, with time as a random effect, to examine the effect of seedling presence, temperature, and nutrient enrichment on pH, DIC, and TA. Three separate models were fitted using the *glmer* function from the *lme4* package (Bates et al., 2015). Along with *glmer* procedure, the *Anova* function from the *car* package was used to report Chi-squared and p-values. Results were presented as mean ± standard error, and a significance value of $p \leq 0.05$ was considered statistically significant.

4.3 Results

Black mangrove seedlings significantly affected porewater pH (X^2 (1, N = 420) = 86.83, $p < 0.001$), TA (X^2 (1, N = 409) = 71.22, $p < 0.001$), and DIC (X^2 (1, N = 180) = 58.55, $p < 0.001$), resulting in consistent decreases in all three variables. The extent of these changes was further influenced by the treatments and whether the seedlings were exposed to higher temperature, nutrient enrichment, or a combination of both. After ten weeks, the presence of a seedling led to 6.5 % lower pH, 38 % lower TA, and 31 % lower DIC in the porewater under the Enriched 30º C treatment (Figure 4.1). The main effects of nutrients and temperature were found to be significant for both TA (X^2 (1, N = 409) = 22.13, $p < 0.001$; X^2 (1, N = 409) = 39.54, $p < 0.001$) and DIC (X^2 (1, N = 180) = 14.16, $p < 0.001$; X^2 (1, N = 180) = 17.85, $p < 0.001$), respectively.

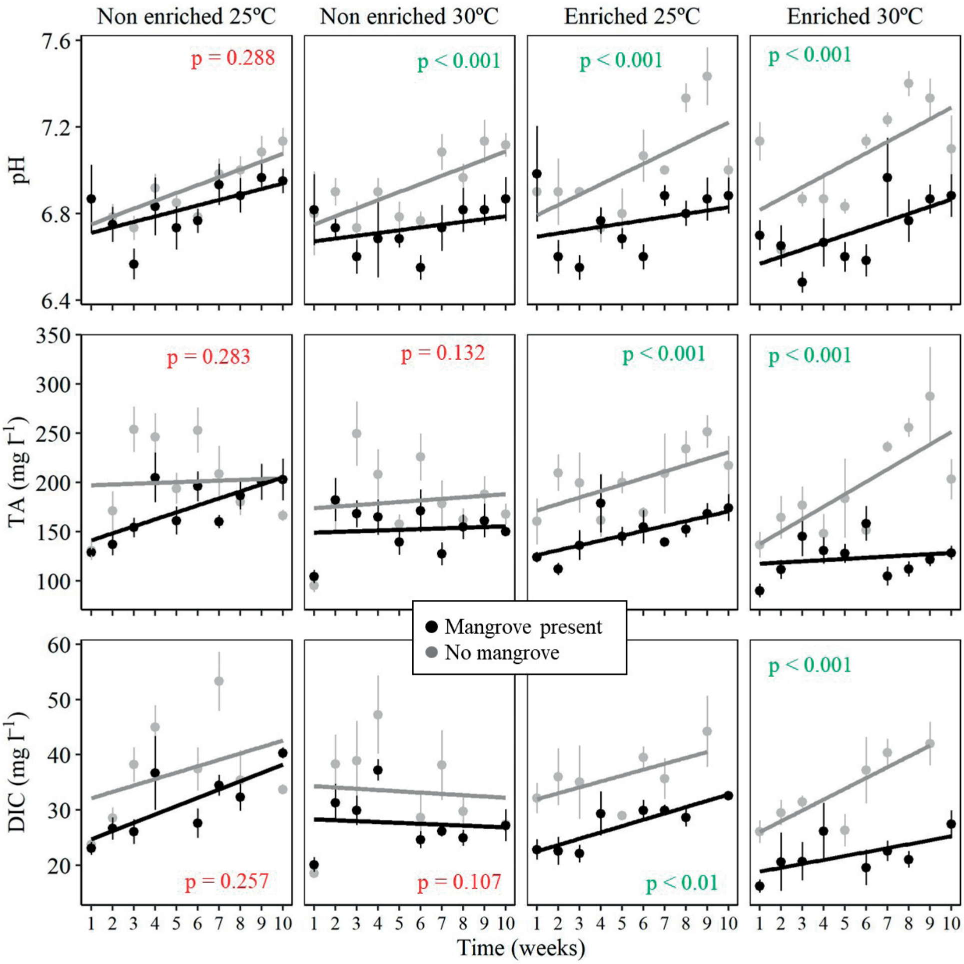

Figure 4.1 Porewater pH, total inorganic carbon (DIC), and total alkalinity (TA) were measured over the 10-week experimental period. Linear regression lines are shown for visualization purposes. The p-values depicted in the plot area indicate whether seedling presence had a significant influence on each treatment and variable.

Furthermore, we found a significant interaction between seedling presence and nutrient enrichment on all three porewater variables. This interaction indicates that the effect of seedlings is dependent on the level of nutrients in soil. In nutrient-enriched treatments, the seedlings led to a more pronounced decrease in pH (X^2 (1, N = 420) = 12.24, $p < 0.001$), TA (X^2 (1, N = 409) = 24.01, $p < 0.001$), and DIC (X^2 (1, N = 180) = 7.72, $p < 0.01$). Additionally, the interaction term between seedling and temperature on pH was also significant (X^2 (1, N = 420) = 5.36, $p < 0.05$). This suggests that in treatments with seedlings, higher temperatures had a stronger impact on water pH compared to treatments without seedlings. All porewater variables showed an increasing trend over time regardless of the treatment. A clear effect of the treatments and the presence of seedlings is observed in the temporal trend of pH, with an increasing divergence and magnitude of changes over time. However, the sharpest decrease between seedling and no seedling treatments was found in TA under the Enriched 30 ºC treatment (Figure 4.1).

Regarding surface water, the main effect of seedling did not have a significant effect on TA or DIC. However, the presence of a seedling significantly reduced surface water pH (X^2 (1, N = 420) = 103.04, $p < 0.001$), with seedlings maintaining a lower pH over time (Figure A 4.1). Temperature (X^2 (1, N = 420) = 193.57, $p < 0.001$), nutrient enrichment (X^2 (1, N = 420) = 13.57, $p < 0.001$), and the interaction terms between temperature and nutrients (X^2 (1, N = 420) = 8.55, $p < 0.01$), as well as seedling and nutrients (X^2 (1, N = 420) = 19.03, $p < 0.001$), also led to a significant increase in pH.

4.4 Discussion

Our results provide novel evidence that black mangroves have a direct effect on carbonate chemistry by contributing to acidification, measured for the first time under controlled conditions. The presence of black mangrove seedlings leads to lower pH, DIC, and TA levels in the sediment porewater. Although pH, TA, and DIC in the mesocosms increase over time regardless of treatment, our results indicate that black mangrove seedlings can dampen that increase. The concentration of all three chemical species in the porewater increased at a lower rate and reached lower values after ten weeks when a seedling was present. Thus, the presence of seedlings has a negative effect on pH, TA, and DIC.

Given that the slow-release fertilizer used un this experiment contained more NH_4^+ than NO_3^- (Figure A 4.2), NH_4^+ uptake by the mangroves seems to be the main cause of the acidification of the sediment porewater. Moreover, mangroves are highly adapted to high NH_4^+ concentrations in the soil, using it as their preferred nitrogen source (Reef et al., 2010). Our results also indicate that the effect of mangroves on carbonate chemistry was intensified by nutrient enrichment and higher temperature, likely caused by shifts in mangrove metabolism (Figure A 4.3). As an artifact of our experimental setup, pH, TA, and DIC increase over time regardless of treatment. This phenomenon was caused by the successive addition of artificial seawater containing bicarbonate and alkalinity to the mesocosm to compensate for surface water evaporation and NaCl uptake and secretion by the seedlings. As a consequence of DIC and TA additions, and given the strong influence of these variables on pH (García-Troche et al., 2021), the overall pH of the mesocosms increased over time.

Under field conditions, several processes such as animal burrowing, tidal flushing, and general coastal hydrodynamics govern porewater discharge and export to the ocean (X. Chen et al., 2021; George & Lugendo, 2022; Stieglitz et al., 2013). Although our surface water results are influenced by regular seawater addition to the mesocosms, these results also show a noticeable parallel between porewater and surface water trends with regard to pH (Figure A 4.1). This parallel is likely caused by diffusion processes and in the absence of interferences common in field situations. However, TA and DIC in the surface water do not show a clear trend, possibly due to the strong influence of seawater addition. While drawing definitive conclusions on the overall effect of black mangrove seedlings on carbonate chemistry is challenging based on our small-scale mesocosm experiment, our results suggest a potential negative effect of black mangrove seedlings on pH. Further studies, including long-term controlled experiments involving mangroves of varying species and age classes, as well as consideration of water turbulence, are sorely needed to confirm their direct effect on water carbonate chemistry and their potential role in mitigating ocean acidification.

4.5 Appendix

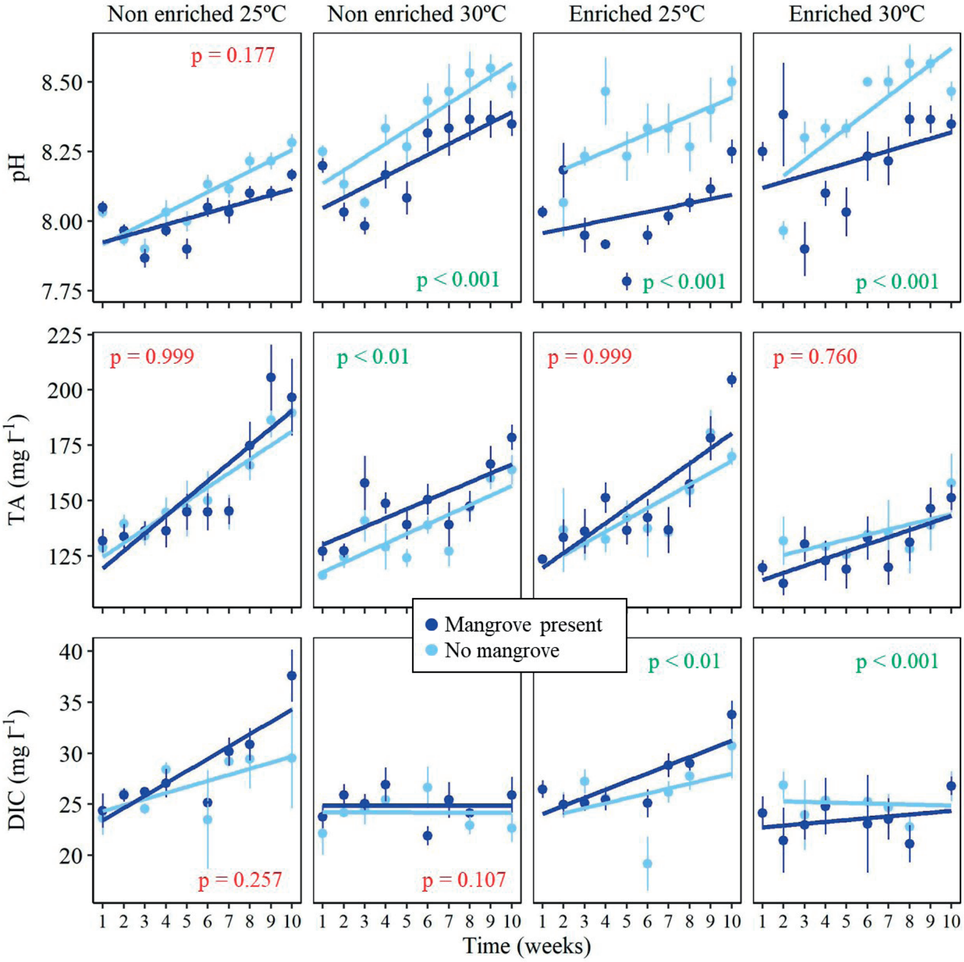

Figure A 4.1 Surface water pH, total alkalinity (TA), and total inorganic carbon (DIC) were measured over the 10-week period. Linear regression lines are depicted in the plot area for visualization purposes. The p-values depicted in the plot area indicate whether seedling presence had a significant influence on each treatment and variable.

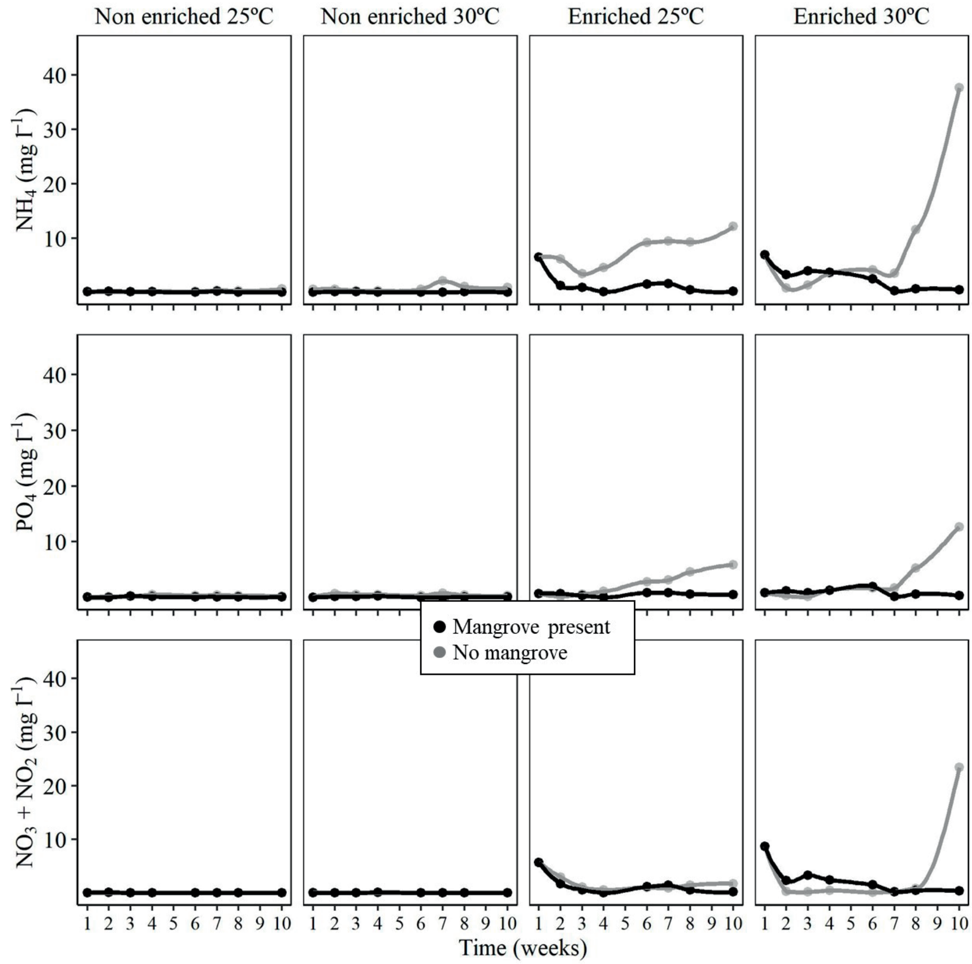

Figure A 4.2 Nutrient concentrations in the sediment porewater over time showing NH_4^+, PO_4^{3-}, and $NO_3 + NO_2$ for treatments with and without a mangrove.

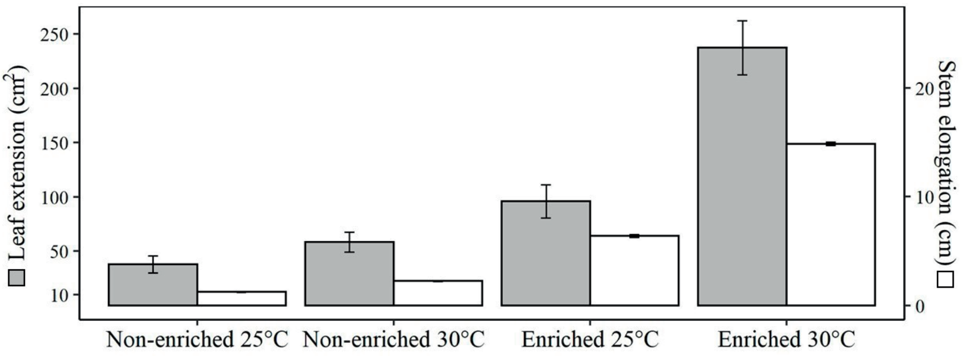

Figure A 4.3 Seedling growth shown as leaf extension and stem elongation over the experimental period, calculated as the end value minus the beginning value.

Chapter 5

High temperature and eutrophication alter biomass allocation of black mangrove (*Avicennia germinans* L.) seedlings

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This chapter is under review at *Marine Environmental Research*

Abstract

Mangrove restoration is taking place along tropical coastlines to combat their rapid worldwide decline. However, restoration success is limited due to local drivers such as eutrophication, and global drivers such as climate change, yet their interactions remain unclear. We used a mesocosm experiment to assess the impact of increased nutrients and temperature on the photosynthetic efficiency and development of black mangrove seedlings. Seedlings exposed to high temperature and eutrophication showed reduced root growth and disproportionally long stems, with lower net assimilation rates. This architectonical imbalance between root and stem growth may make them more susceptible to physical disturbances and dislodgement. Notably, none of the experimental seedlings displayed signs of photophysiological stress, and those exposed to increased nutrients and temperature exhibited robust photosynthetic performance. The disbalance in biomass allocation highlights the importance of considering local hydrodynamic conditions and nutrient status in restoration projects, ensuring the effective anchorage of mangrove seedlings and restoration success under a warming climate.

5.1 Introduction

Mangrove forests are located at the interface between land and sea of tropical and subtropical coastlines. They provide a wide range of resources and often occur near high human population densities (Friess et al., 2019). These ecosystems not only provide climate change mitigation through the capture and storage of carbon (Lagomasino et al., 2019), but they also play a role in flood protection and erosion control (Blankespoor et al., 2017; Horstman et al., 2014), as well as acting as buffers of nutrients and sediment run-off from land sources (Agraz-Hernández et al., 2018; Moroyoqui-Rojo et al., 2015; Wadnerkar et al., 2019). Over the past decades, however, increasing local human pressures and climate change, including rapid-onset events such as hurricanes, have led to a sustained loss of mangrove forests (Goldberg et al., 2020). A recent study on the extent of change mangroves indicates that 3.4% of mangrove forests were lost between 1996 and 2020 worldwide (Bunting et al., 2022).

Once mangroves have been lost, their reestablishment is difficult unless the proper environmental conditions are met (Kamali & Hashim, 2011; Macy et al., 2021; Pérez-Ceballos et al., 2017), which makes restoration a vital tool to counteract the global loss of mangroves (Macy et al., 2021; McKee & Faulkner, 2000; Ram et al., 2021; Toledo et al., 2001). However, mangrove restoration is currently prone to failure due to lack of understanding of the cause for decline (thus the cause was not tackled), poor site selection (Hai et al., 2020), choosing areas with unsuitable topography or hydrology (Kodikara et al., 2017), failing to implement positive ecosystem interactions (Gillis et al., 2014; Gillis, Jones, et al., 2017), and potentially the interactive effects between local (e.g. nutrients) and global (e.g. increasing temperature) drivers. Increasing air and sea surface temperatures by global warming can impact newly restored mangroves by raising leaf temperatures and consequently inhibiting photosynthesis (Arifanti, 2020). High temperature also leads to larger evaporation rates, which increases salinity and affects mangrove structure and growth (Hai et al., 2020). Additionally, it causes the seedlings and propagules to dry out faster (Elster, 2000). A study that encompassed all mangrove restoration sites in Vietnam found that dry and arid zones had the lowest survival rates of restored seedlings, highlighting the importance of climate and global drivers in restoration (Kodikara et al., 2017). The same study determined that nutrient imbalance and availability in the soil are also causes for poor seedling survival (Kodikara et al., 2017), indicating that local drivers also play a role in restoration success. Furthermore, eutrophication in mangrove soils makes them more vulnerable to physical disturbances such as tropical storms (Feller et al., 2015).

Mangrove restoration projects mostly use nursery-reared seedlings grown to a suitable age for replanting (Toledo et al., 2001). When these seedlings are deployed to the natural environment, they have already overcome the seed and emergence phases, and usually consist of a small root system, a stem, and a few leaves. The transplanted seedlings must then overcome the establishment phase in their new environment, which is often limited by physicochemical stress factors such as nutrient regimes (McKee, 1995). The successful establishment of seedlings in the new environment increases their resilience against dislodgement and is therefore key to restoration success. However, this crucial phase could be slowed or even hindered by stressors present in the new environment such as high temperature and eutrophication. Despite the importance of using seedlings in restoring/re-planting mangroves, information on the establishment phase of nursery-reared mangroves is generally lacking. In addition, most scientific studies focus on adult, well-established mangrove trees or early life stages (seed survival and germination).

Previously, the impacts of temperature and eutrophication on mangroves were investigated separately. Mangrove seedling development can be affected by excess nutrients (Agraz-Hernández et al., 2018; Moroyoqui-Rojo et al., 2015), often resulting in lower root/shoot biomass ratios (McKee, 1995; Reef et al., 2016). High temperature causes inhibition of root growth (Krauss et al., 2008). The interactive effects of temperature and eutrophication were tested for the first time in the orange mangrove *Bruguiera sexangula* (Gillis et al., 2019). This study found antagonistic effects between these two stressors on the above-ground growth, i.e. stem length, and limited root architecture. When exposed to high nutrients and temperature, orange mangrove seedlings grew less than when exposed to the two stressors separately (Gillis et al., 2019). Limited root development as a consequence of high nutrients and temperature suggested a lower resistance of seedlings to withstand their dynamic environment, which could potentially impact mangrove resilience to physical disturbance and, thereby, anchorage. Since these findings are only reported for the orange mangrove, the effects of high nutrients and temperature on other mangrove species remain unknown. Thus, it is important to investigate the response of other mangrove species to multiple stressors such as temperature and eutrophication as these responses are likely to differ based on species characteristics and ecological niches.

The widespread black mangrove *Avicennia germinans* commonly inhabits intertidal zones and is considered an important pioneer tree. Pioneer species, such as the black mangrove, can colonise bare sediment and establish positive plant interactions that facilitate later colonisation by other plant species (Friess et al., 2012). Restoration projects use black mangrove seedlings around the world, with varying temperatures and nutrient conditions (Macy et al., 2021; McKee & Faulkner, 2000; Ram et al., 2021), although mangrove restoration, in general, has not yielded high success (Lovelock et al., 2022). To increase restoration success and improve site selection, a key yet unanswered question is how nutrients and temperature impact black mangrove seedlings' health and development, and whether they exhibit a similar growth response as the orange mangrove. Moreover, previous studies ran for relatively short periods of time i.e. up to six weeks, thus failing to detect long-term responses or did not consider below-ground biomass, which is closely linked to above-ground biomass dynamics.

Growth and development responses are often linked to changes in photosynthetic efficiency, and these responses are valuable indicators of the health of restored mangroves (Rovai et al., 2013). Temperature changes can cause stress (Duke et al., 2017; J. Liu & Wang, 2020) and marked reductions in the photosynthetic efficiency of several mangrove species (L. Chen et al., 2017). Furthermore, excess nutrients result in higher photosynthetic electron transport (Feller et al., 2003b). Assessing the photosynthetic efficiency of mangroves provides a deeper insight into their tolerance and adaptation capacity to temperature and eutrophication pressures. Moreover, it is unknown to what extent black mangrove seedlings can experience changes in response to increased nutrients and temperature.

In this study, we aimed to assess the effect of temperature and nutrient conditions on the photosynthetic efficiency and development of black mangrove seedlings. We addressed the following questions: How do increased nutrient loads and temperature impact 1) biomass allocation, growth, and nutrient ratios in tissue, 2) photosynthetic efficiency, and 3) net assimilation rates of black mangrove seedlings? To answer these questions, we conducted an *ex-situ* experiment using black mangrove seedlings that were four months old, a typical age for out-planting. The seedlings were subjected to two different levels of nutrients and temperature and their combined effect under controlled conditions for a period of ten weeks.

5.2 Materials and methods

Black mangrove seedlings had been reared from propagules produced by a single adult tree at Burgers' Zoo, Arnhem, collected in July 2021. The propagules (1 to 4 days old from the time of detachment) were transported to the nursery at Nergena greenhouse, Unifarm, WUR. They were horizontally placed on a nursery tray containing water-logged quartz sand under a 12 h natural light regime and ~ 25 ºC air temperature. No nutrients were provided at this stage. When the seedlings reached four months of age, those that appeared in good health were selected based on a visual examination of the colour and shape of their stem and leaves. Stem length was 19.5 ± 3.2 cm high, and the average number of leaves was 5.58 ± 1.0. Six weeks prior to the experiment, seedlings had been planted into 8 x 8 x 8 cm nursery pots containing ~ 390 cm^3 of quartz sand previously washed and ashed, of 0.1 - 0.3 mm grain size. The sediment was fertilised with 0.5 g of an NPK 15:9:11 slow-release fertiliser for aquatic plants (Nutri Caps with Osmocote ®, Colombo B. V., the Netherlands) by placing the fertiliser granules at root level during repotting. The fertiliser composition was ~ 6.6 % NO_3^--N, 8.4 % NH_4^+-N, 9 % P_2O_5, 11 % K_2O, 2 % MgO, 0.03 % B, 0.05 % Cu, 0.045 % Fe, 0.06 % Mn, 0.02 % Mo, and 0.015 % Zn. One week prior to the start of the experiment, seedlings were transported from the nursery to climate-controlled rooms set to 25 ºC and 30 ºC.

5.2.1 Experimental design

To test the interactive effects of nutrients and temperature on black mangrove seedlings, a 10-week mesocosm experiment was conducted in two climate-controlled rooms without natural daylight set at different temperatures at Wageningen University & Research (WUR) from 26 November 2021 to 4 February 2022. Pots with seedlings were placed in individual containers filled with 1.7 l of artificial seawater (Aqua Medic Reef Salt, AB Aqua Medic GmbH, Germany) and a salinity of 32 g l^{-1} (Figure 5.1), following a completely randomised design. Each container (mesocosm) contained one experimental unit (mangrove seedling). The experimental setup consisted of four treatments, resulting from the combination of 2 factors with 2 levels each (Figure 5.1). Thus, the experiment included the following treatments: nutrient-enriched 25 ºC (n = 6), non-enriched 25 ºC (n = 6), nutrient-enriched 30 ºC (n = 6), and non-enriched 30 ºC (n = 6). The lower temperature level represents the mean air temperature in mangrove forests (Fazlioglu et al., 2020). The upper-temperature level resembles a hypothetical global warming scenario of 5 ºC that would be reached, for instance, in Australia and the Red Sea region under the SSP5-8.5 scenario by 2081-2100 (Gutiérrez et al., 2021). Nutrient-enriched conditions were achieved by adding 0.4 g of slow-release fertiliser by pushing a thin stick down into the sediment and inserting the granules into the resulting hole at the root level to resemble a continued supply of nutrients over time. The experiment included negative control treatments, i.e. without a seedling to monitor nutrient concentrations in the porewater over time without the seedling's influence. The water level in the containers was set approximately 2 cm above the sediment surface and was restored every two days by adding demineralised water to compensate for evaporation, thus avoiding salinity fluctuations. Lighting was provided by a 400 W metal halide grow light (Hortilux, Monster, the Netherlands) 80 cm above the canopies and set on a 12-hour light cycle, with a light intensity of 300 μmol m^{-2} s^{-1} measured at leaf height. To reduce potential spatial variations within the experimental area, mesocosms were redistributed randomly on a weekly basis across the experimental surface. In our experience, roots grow out of the drainage holes on the bottom of the pot when the pot size is too small. In this experiment, roots stayed inside the pot, therefore it was assumed that pot size did not restrain seedling root growth. Furthermore, the time span of the experiment would suffice for the roots to grow (Gillis et al., 2019). Seedlings were regularly misted with demineralised water to provide moisture and increase air humidity.

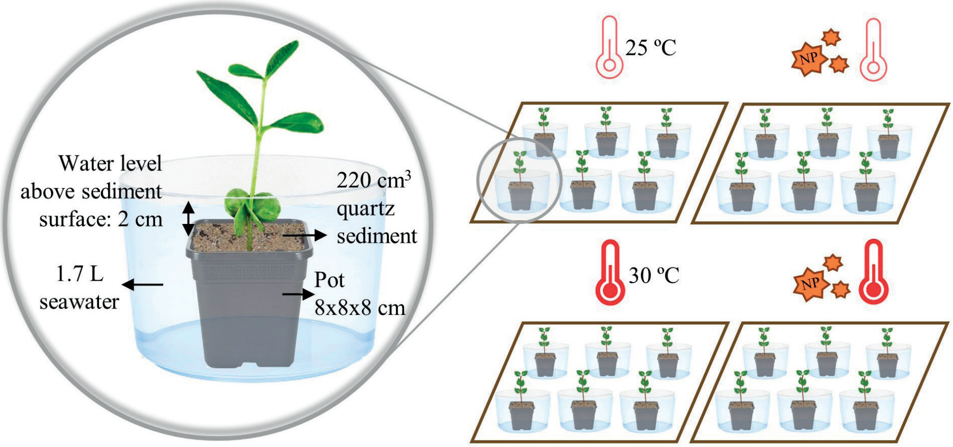

Figure 5.1 Black mangrove seedlings were grown in individual containers submerged in artificial seawater under different temperature and nutrient conditions.

5.2.2 Growth, biomass, and elemental content

The first measurements took place after one week of acclimation, at the start of the experiment and were repeated weekly for leaf number, leaf length, leaf width, stem length, and photosynthetic efficiency (F_v/F_m). At the end of the experiment, leaves were sampled for total nitrogen (TN), total phosphorous (TP), and total iron (TFe) analysis, and a subsample of three randomly selected seedlings per treatment was harvested for above-ground biomass and below-ground biomass measurements. TN and TP were also determined from above-ground and below-ground tissues to get a clear overview of nutrient allocation within the different plant parts. Leaf length, leaf width, and leaf area were measured from 30 randomly selected leaves of varying sizes across all treatments to fit an equation to predict leaf area. Leaf length and width (predictors) were measured by hand using an analogue calibre, and leaf area (response) was determined using ImageJ, version 1.52 (Schneider et al., 2012) from a white-background photograph of the leaves, which served as input to calculate leaf area. Above- and belowground parts were segmented at the hypocotyl and dried at 60 ºC for 48 h, or until they had reached a constant weight. Leaf samples followed the same procedure. Dry weight was recorded for above- and below-ground parts, and samples were grounded into powder using a ball mill (MM200, Retsch GmbH, Haan, Germany). Finely ground samples were transformed into single digests following the sulfuric acid–selenium digestion method at 330 ºC in a Kjeldatherm block digestion system and transferred to a Segmented Flow Analyzer (Skalar Analytical B. V., the Netherlands) for TN and TP determination (Novozamsky et al., 1983, 1984). Furthermore, only leaf digests were used for TFe determination using ICP-OES (Thermo iCAP-6500, Thermo Fisher Scientific) at Soil Chemistry Laboratory (CBLB, Wageningen, the Netherlands).

To monitor nutrient concentrations supplied by the slow-release fertiliser, weekly porewater samples were taken using a Rhizon for porewater sampling (Rhizon SMS, Rhizosphere Research Products B.V, the Netherlands) of 0.15 µm pore size and a length of 5 cm, connected to a 30 ml Luer-lock syringe. Porewater NH_4^+, PO_4^{3-}, and $NO_3 + NO_2$ were determined from each porewater sample using a Segmented Flow Analyzer (Skalar Analytical B.V., the Netherlands) at Soil Chemistry Laboratory (CBLB, Wageningen, the Netherlands).

5.2.3 Photosynthetic efficiency

Plant health was assessed by measuring mangrove seedlings' photosynthetic efficiency (F_v/F_m) using the Pulse Amplitude Modulated (PAM) fluorometry technique. The photosynthetic efficiency of mangroves, so-called F_v/F_m, is a widely recognised indicator of photophysiological activity and is measured as the chlorophyll fluorescence on a localised surface of their leaves. This parameter is defined as the maximum quantum yield of PSII, the most commonly used chlorophyll fluorescence parameter for studying stress photophysiology in mangroves (Bhagooli et al., 2021) and a powerful indicator of the plant's photosynthetic performance (Guidi et al., 2019). Seedlings were dark-adapted for 30 minutes prior to non-destructive F_v/F_m measurements on the four fully expanded healthy leaves per plant closest to the apical meristem (Panda et al., 2006) using a PAR-FluorPen FP 110 (Photon System Instruments, Czech Republic). These four measurements were subsequently used to calculate the mean F_v/F_m value for each plant, which provided a more accurate estimate than a single measurement. The F_v/F_m is calculated as $F_v/F_m=(F_m-F_0)/F_m$, where F_0 and F_m are the minimum and maximum fluorescence after dark acclimation, respectively.

5.2.4 Statistical analysis

Data analysis was conducted in R, version 4.0.2 (R Core Team, 2021). Variables were tested for normality and homogeneity of variance. When the relevant assumptions were not met, logarithmic or square root transformations were conducted prior to analysis, and statistical comparisons were only made between treatments unless stated otherwise. When homoscedasticity was not achieved throughout the normality transformations, variables were analysed using a generalised linear model (GLM). To account for the temporal dynamics of continuous variables such as the F_v/F_m, a generalised linear mixed model was used (GLMM), whereas end-point variables were analysed using two-way analysis of variance (ANOVA). Along with *glm* and *glmer* procedure, the *Anova* function from the car package was used to report Chi-squared and p-values. Results with a significance value of $\alpha \leq 0.05$ were considered statistically significant. To account for differences in nutrient concentration between leaves, above-ground biomass, and below-ground biomass, the plant part was included in the TN and TP ANOVA tests. N:P, N:Fe, and P:Fe ratios from the three different plant parts were tested separately. In addition to the measured variables, each seedling's Net Assimilation Rate (NAR) was also calculated to assess their efficiency in using resources under contrasting nutrient loads. Other authors referred to the net assimilation rate as E (Goodman, 1973; Vernon & Allison, 1963), and the formula used for its calculation has changed across studies and time (Anten & Ackerly, 2001; Eagles, 1971). In this study, NAR ($g\ m^{-2}\ day^{-1}$) was calculated according to the following formula:

$$NAR = W / (L^*t)$$

where W is the seedling's total dry weight (g), L is leaf area (m^2), and t is the time span of the experiment (days) (Hayes et al., 2017).

5.3 Results

Nutrients and temperature caused pronounced differences in seedling biomass allocation and photosynthetic efficiency over the 10-week experimental period. Seedling survival was 100 % during this period. The two-way ANOVA revealed a positive main effect of both nutrient enrichment (F (1, 20) = 50.6, $p < 0.001$) and temperature (F (1, 20) = 18.4, $p < 0.001$). The interaction effect was also significant (F (1, 20) = 9.3, $p < 0.01$), specifically, by the end of the experiment the mean stem length was 1.7-fold higher compared to the control (non-enriched 25 ºC) treatment (Figure 5.2). Around week seven, we observed bending in the seedling stems of the interaction treatment, indicating that the seedlings were becoming top-heavy. The main effects of both nutrient enrichment (F (1, 20) = 7.1, $p < 0.001$) and temperature (F (1, 20) = 23.9, $p < 0.001$) on leaf area were also significant, indicating that nutrient enriched conditions or a temperature of 30 ºC led to an increased mean leaf area. Although the interaction effect of nutrient enrichment and temperature was not significant, leaf area increased 2.7-fold compared to the control treatment (non-enriched 25 ºC) (Figure A 5.1). Temporal trends in growth parameters, such as leaf area and stem length, indicated that seedling growth stabilized after week eight in all treatments, except for the interaction treatment, where growth continued to increase at a relatively constant rate until the end of the experiment.

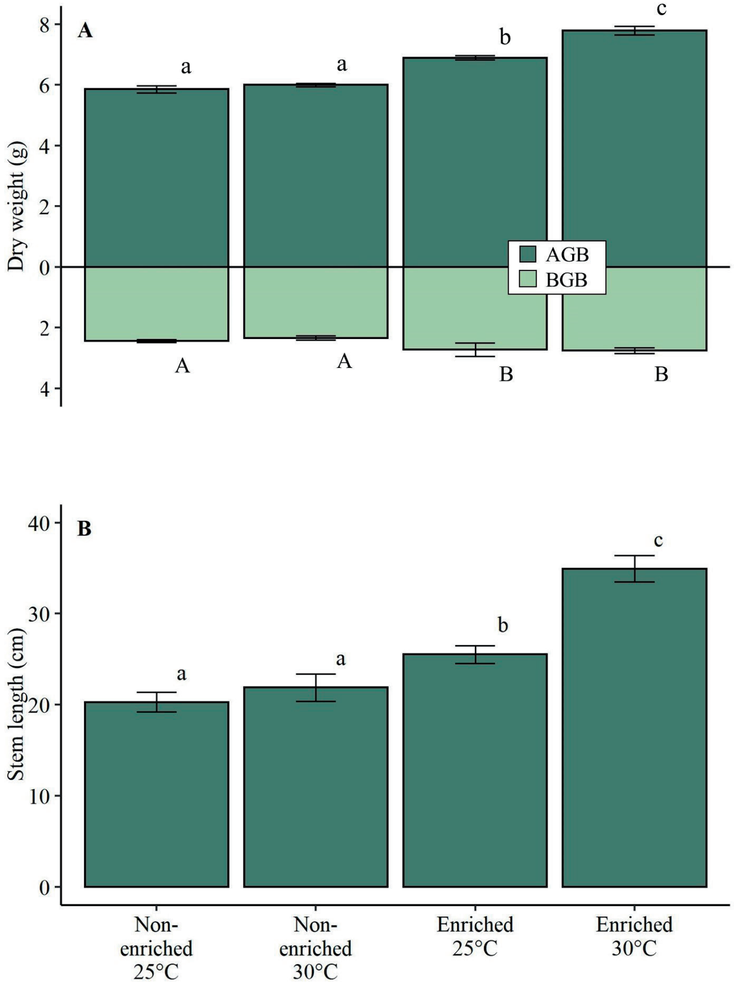

Figure 5.2 (A) Stem length and (B) dry weight of above-ground (ABG) and below-ground (BGB) parts of mangroves at the end of the experimental period. Means are presented with standard errors. Compact letter display next to bars indicates significant differences between treatments.

Results indicated that the mean above-ground biomass of seedlings was higher under nutrient enrichment (F (1, 8) = 183.5, $p < 0.001$) and temperature (F (1, 8) = 24.5, $p < 0.01$), but only the main effect of nutrient enrichment led to higher below-ground biomass (F (1, 8) = 7.6, $p < 0.05$). Furthermore, the interaction effect between nutrients and temperature (F (1, 8) = 12.8, $p < 0.01$) indicated that the temperature effect on above-ground biomass was greater under nutrient-enriched conditions, thus yielding 33 % higher above-ground biomass (Figure 5.2). Although the main effect of nutrient addition on below-ground biomass was found to be significant, it only resulted in 12 % higher biomass compared to the control (non-enriched 25 ºC) treatment.

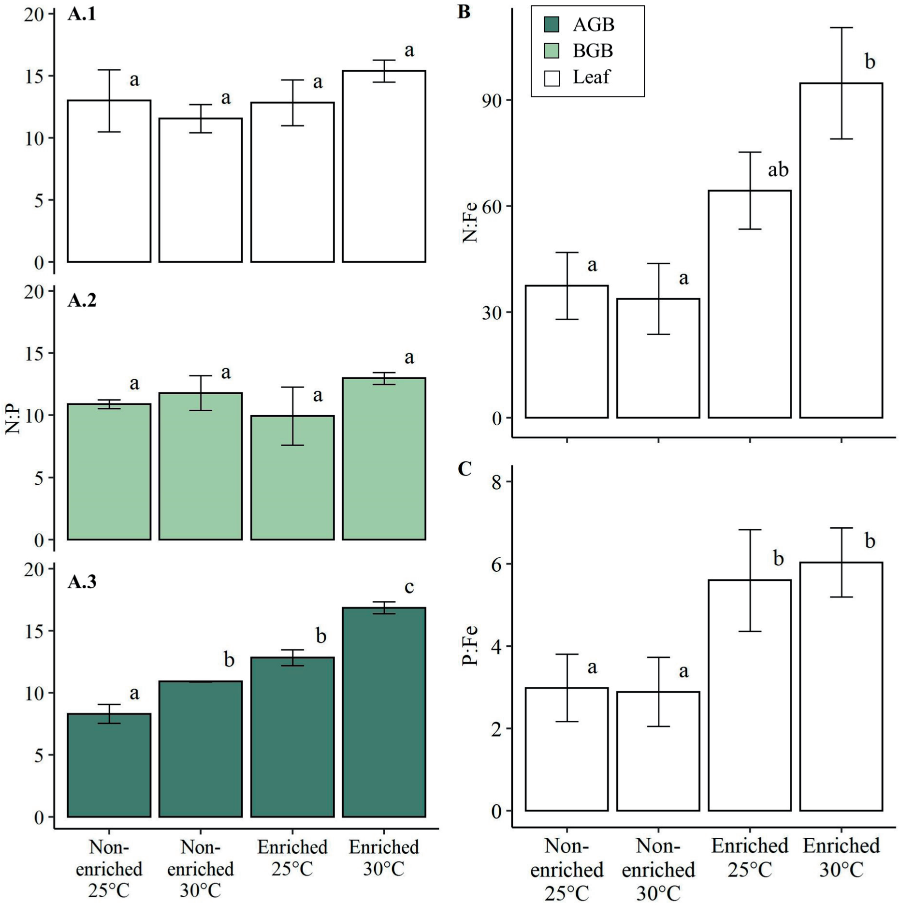

Figure 5.3 Seedling tissue nutrient ratios measured at the end of the experimental period; (A) N:P ratio measured from leaves (white bars), below-ground biomass (light green bars), and above-ground biomass (dark green bars); (B) N:Fe leaf tissue ratios; and (C) P:Fe leaf tissue ratios. Columns represent the mean values of the different treatments with standard error bars. Compact letter display depicts significant differences between treatments.

Overall, TN was highest in leaves (10.9 ± 4.7 mg g^{-1}), followed by below-ground biomass (10.71 ± 4.33 mg g^{-1}) and above-ground biomass (10.61 ± 4.16 mg g^{-1}), and TN results were markedly increased by nutrient enrichment (Figure A 5.3). The three independent two-way ANOVA tests, one for each plant part, showed a significant effect of nutrient enrichment on TN in leaves (F (1, 20) = 27.8, $p < 0.001$), TN in below-ground biomass (F (1, 8) = 23.2, $p < 0.01$), and TN in above-ground biomass (F (1, 8) = 71.7, $p < 0.001$). The main effect of temperature and the interaction between both factors were non-significant. Similarly, TP was found to be highest in leaves (0.92 ± 0.35 mg g^{-1}) followed by below-ground biomass (0.89 ± 0.32 mg g^{-1}) and above-ground biomass (4.16 ± 0.27 mg g^{-1}). TP in leaves was significantly higher under nutrient enriched conditions (F (1, 20) = 6.13, $p < 0.05$), but neither the main effect of temperature nor the combination between the two factors was found significant with regard to TP in leaves, but the pairwise comparisons with Tukey adjustment did not show any further differences between treatments (Figure A 5.3). Furthermore, TP in above-ground biomass was significantly increased by the main effects of nutrient enrichment (F (1, 8) = 18.4, $p < 0.01$) and temperature (F (1, 8) = 9.6, $p < 0.05$), but the interaction effect was not significant. The two-way ANOVA for TP in below-ground biomass did not yield a main effect for nutrient enrichment or temperature, neither was the interaction term significant. Overall, the Fe concentration in leaves ranged from 0.12 to 1.30 mg g^{-1}, with the lowest mean and dispersion of data in the enriched 30 ºC treatment (0.23 ± 0.04 mg g^{-1}) and the highest in the non-enriched 25 ºC treatment (0.45 ± 0.18 mg g^{-1}). The main effects of nutrient enrichment and temperature were found non-significant, as was the interaction term.

With regard to nutrient ratios, a two-way ANOVA yielded a main effect of nutrient enrichment (F (1, 8) = 89.3, $p < 0.001$) and temperature (F (1, 8) = 35.9, $p < 0.001$) such that N:P in above-ground biomass was higher under 30 ºC and nutrient-rich conditions. However, results indicated a non-significant effect of neither nutrient enrichment nor temperature on N:P of leaves and below-ground biomass (Figure 5.3A). The interaction effect of nutrient enrichment and temperature was not significant for N:P in any of the plant parts, i.e. leaves, above-ground biomass, and below-ground biomass. Leaf N:Fe was significantly higher in nutrient enriched treatments (F (1, 20) = 13.83, $p < 0.01$), with the highest values found under 30 ºC (94.76 ± 15.77) and the lowest in non-enriched 30 ºC (33.72 ± 10.05). Foliar P:Fe ranged from 2.89 to 6.03, where values in nutrient-enriched treatments were significantly higher than in non-enriched treatments (F (1,20) = 9.45, $p < 0.01$), with an overall high dispersion of data. Pairwise comparisons using a Tukey adjustment found no further differences between treatments due to its more conservative approach (Figure 5.3C).

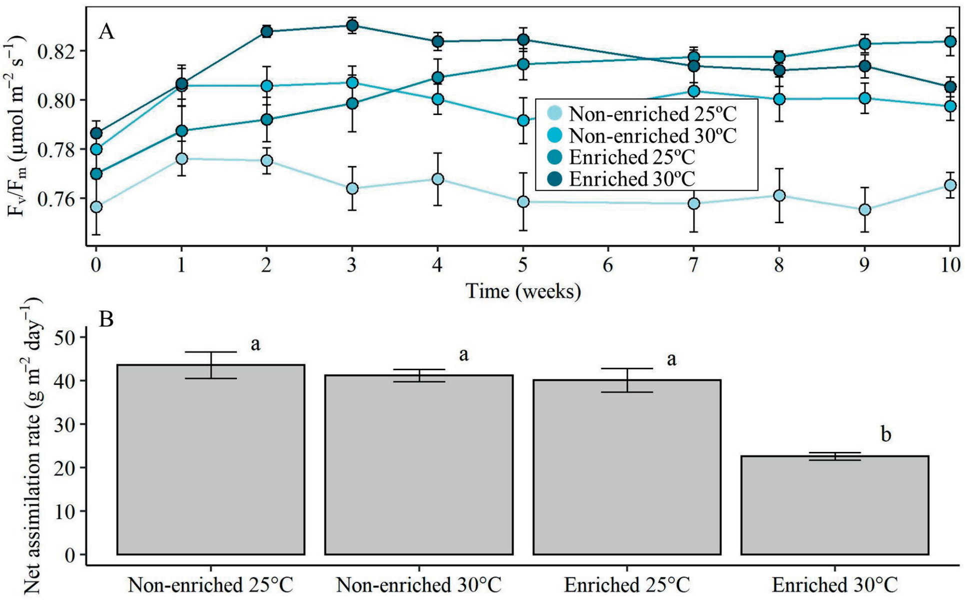

Figure 5.4 Photosynthetic efficiency (F_v/F_m) of seedlings over the 10-week experimental period. Marker shapes indicate mean values per treatment, and error bars represent standard error to the mean. Standard error bars are smaller than symbols in some cases. (B) Net Assimilation Rate (NAR). Columns represent mean values with standard error bars. The letters above the columns indicate significant differences between group means.

The mixed model (GLMM) applied to assess changes in the photosynthetic efficiency (F_v/F_m) of seedlings over time revealed a significant main effect of nutrient enrichment ($p < 0.001$) and temperature ($p < 0.001$), as well as a significant interaction between the two main factors and time ($p < 0.001$). F_v/F_m values ranged from 0.71 to 0.845 µmol m^{-2} s^{-1} (Figure 5.4A). The F_v/F_m of black mangrove seedlings increased by 4.1 %, 7.6 % and 5.2 % over the ten weeks in the non-enriched 30 ºC treatment, enriched 25 ºC treatment, and enriched 30 ºC treatment, respectively, compared to the non-enriched 25 ºC treatment. Nutrient addition caused a steeper increase in F_v/F_m values during the first half of the experiment, but all treatments reached a relatively stable trend after week seven. Despite the interaction treatment (enriched 30 ºC) treatment showing the sharpest initial increase, its final F_v/F_m values were lower than the enriched 25 ºC treatment by the end of the experiment (Figure 5.4A), indicating that such high photosynthetic performance could not be sustained in the long-term.

A generalized linear model (GLM) on the calculated NAR values showed that neither the main effect of nutrient enrichment nor temperature were significant.

The interaction between both main factors, however, was significant ($z = -2.6$, $p < 0.01$), indicating that the effects of nutrient enrichment on NAR were stronger under 30 ºC of temperature. After ten weeks, the interaction treatment (enriched 30 ºC) resulted in 48 % lower NAR than the control treatment (enriched 25 ºC). In contrast, the main effects of temperature or nutrient enrichment alone only caused a reduction in NAR by 5 % and 8 %, respectively (Figure 5.4B).

5.4 Discussion

High temperature in combination with nutrient enrichment enhanced black mangrove seedling growth but altered biomass allocation between above- and below-ground structures. The seedlings exposed to high nutrients and temperature showed a disproportional increase in above-ground biomass compared to their roots (33 % vs. 12 % dry weight gain). Additionally, this was accompanied by a decrease in Net Assimilation Rate (NAR), indicating lower carbon incorporation in photosynthesis per unit leaf area (Lambers et al., 2008). These results suggest that under high nutrients and high temperature seedlings allocated more carbon compounds to photosynthetically active organs such as leaves resulting in rapid growth of stems but potentially weaker cell walls and low fibre content (Lambers et al., 2008).

This study is the first to investigate the combined effect of high temperature and nutrient enrichment on black mangroves, examining impacts on both below and aboveground plant growth and development. Previous studies have focussed on the effects of single drivers on either above or belowground plant parts (with the exception of Gillis et al., 2019), with limited research on their combined effects. Lower biomass allocation to roots than shoots (Mckee, 1995) and increased leaf production (Simpson et al., 2013) had been previously reported separately as an effect of nutrient enrichment in black mangrove seedlings, as was the lower biomass allocation to roots when plants approach their optimum temperature (Lambers et al., 2008). By studying both drivers and plant parts together, we gain a more comprehensive understanding of their synergistic impacts. In this experiment, both temperature levels used (25 ºC and 30 ºC) appear to fall within the species' optimal temperature range, as indicated by the minor root growth observed.

Notably, the response of black mangroves to high temperature and nutrient enrichment differs from that of orange mangroves. Previous research on the above-ground growth of orange mangrove *B. sexangula* showed antagonistic interactions between high temperature and nutrient enrichment, negatively impacted root architecture (Gillis et al., 2019). In contrast, our findings suggest that black mangrove seedlings (Gillis et al., 2019). These contrasting responses are likely caused by differences in the temperature optimum of the two species, with the orange mangrove exceeding the optimum while the black mangrove does not reach it. When exposed to high temperature and nutrient enrichment, our seedlings do not experience substantial root growth, potentially impacting mangrove ecosystem traits such as carbon stock potential (Gillis et al., 2023). The bending observed in their stems may therefore be attributed to their inability to support their weight due to poor structural composition (Lambers et al., 2008).

The disproportionate and odd-shaped above-ground growth resulting from nutrient enrichment may increase vulnerability of mangroves to waves, wind and hurricane disturbance and hinder their recovery (Feller et al., 2015) or successful establishment. A study by Balke et al. (2011) showed that young mangroves make use of “windows of opportunity” in space and time for root growth and anchorage, and the penetration of roots several centimetres into the sediment could still lead to their dislodgement. Therefore, successful anchorage to soil by efficiently developing roots and moderate growth, e.g. stature and stem elongation have been proven crucial for mangrove resilience to physical disturbance (Balke et al., 2011; Feller et al., 2015). Recent experimental studies on the optimal seedling morphology to withstand physical disturbance revealed that small, short shoots and long, sturdy roots are the key factors for *Avicennia* seedlings’ stability (van Hespen et al., 2022). Thus, the fast-growing seedlings in our study, with long and unstable stems, may face challenges in withstanding hydrodynamic forces and experiencing establishment failure. Lastly, this phenomenon is not only applicable to mangrove ecosystems. It has been found that in aquatic plants exposed to eutrophication, their long, low-density stems are prone to breakage when exposed to mechanical stressors (Lamberti-Raverot & Puijalon, 2012).

Both temperature and nutrient enrichment influenced N:P in above-ground tissue (Figure 5.3), where only an effect of nutrient enrichment was to be expected, as it has been previously reported from short-term growth experiments (Güsewell, 2004). Thus, our findings first revealed an effect of temperature on N:P ratios in above-ground black mangrove tissues. Overall, the N:P values measured in this study were above 10, which is also the worldwide accepted value for N:P ratio in mangroves (Berrenstein et al., 2013). When looking only at the absolute N and P values, our leaf nutrient contents are generally higher compared to other plant parts, and their concentration increase under nutrient enrichment (Figure A 5.3). This nutrient allocation pattern may be due to to role of leaves in photosynthesis, protein synthesis, and chlorophyll production, processes that require significant amounts of nutrients. However, the combination of high nutrients and temperature had a negative impact on N and P concentrations in both above and below-ground tissue, likely due to the effects of temperature on photosynthesis and nutrient allocation.

Our results also indicate that the iron content in mangrove leaves is consistent with those reported in previous studies for other mangrove species. For instance, Bakshi et al. (2017) found that the iron concentration in *Avicennia marina*, *Avicennia alba*, and *Avicennia officinalis* leaves ranged between 0.15 to 0.3 mg g^{-1} of dry weight, which aligns with our findings of 0.12 to 1.3 mg g^{-1}. Similarly, Machado et al. (2005) reported leaf concentrations of iron in *Laguncularia racemosa* and *Rhizophora mangle* up to 0.15 mg g^{-1}, on the lower end of the concentrations found in this study (Figure A 5.3). Much higher concentrations were found by Thanh-Nho et al. (2019) in mangroves exposed to inputs from a heavily industrialized mega-city. Thanh-Nho et al. reported that the iron content in saplings of *Avicennia alba* leaves was around 2.28 mg g^{-1}, a 6.5-fold higher than the average value in the present study. Furthermore, we observed a decreasing trend in foliar Fe with treatment, exactly the opposite as what occurs with foliar N and P (Figure A 5.3). One possible explanation is that nutrient enrichment reduced the availability and mobility of iron in sediment, e.g. by increasing pH, which may result in lower solubility and availability of iron. Another possible explanation is that nutrient enrichment, and P in particular, altered the element and nutrient balance in mangrove tissues, which affected the uptake, transport, and accumulation of iron in leaves. Unlike other mangrove species prone to excluding metals at the root level, black mangroves are iron-excreting species, capable of taking up more metals and translocating them to their leaves where they are excreted (Maldonado-Román et al., 2012). Thus, nutrient enrichment may have affected Fe uptake, which was reflected as lower Fe concentrations in leaves (Figure A 5.3).

The photosynthetic efficiency of the seedlings indicated a high plant performance throughout the experiment (Figure 5.4A), i.e. above a maximum quantum yield of 0.71, and reaching up to ~ 0.83 (Murchie & Lawson, 2013). The interaction treatment showed the highest photosynthetic efficiency over time, although this trend was reversed at six weeks, reflected by a slight decline of F_v/F_m. This decline is believed to be an artifact of the experimental conditions, as the supply rate of nutrients by the slow-release capsule was lower than the seedlings' nutrient demand. This theory was supported by the accumulation of nutrients in the negative control treatment (without a seedling), showing that the slow-release granules continued to release nutrients over the entire experimental period (Figure A 5.2D). Still, these were automatically taken up by the seedlings (Figure A 5.2H). All seedlings exhibited a remarkably high photosynthetic efficiency throughout the experiment.

This may indicate that, at this life stage, black mangroves possess a high-stress tolerance (> 0.79 F_v/F_m, Maxwell and Johnson, 2000), making them a highly suitable candidate for restoration.

5.5 Implications for ecological restoration

Our experimental results showed that mangrove seedlings exposed to high nutrients and temperature exhibited biomass allocation patterns that may increase plant vulnerability to dislodgement. Restoring mangrove populations in coastal areas under high eutrophication and climate warming may come at the cost of a higher vulnerability to physical disturbances. Our mesocosm experimental results cannot be directly extrapolated to natural mangrove communities, however, they indicate how black mangroves respond to two timely relevant pressures that might play a key role in restoration success, highlighting the potential risks of mangrove restoration under eutrophication and global warming.

5.6 Conclusions

The combination of high temperature and nutrient enrichment accelerated black mangroves' above-ground growth and improved their photosynthetic yield. However, reduced root growth and disproportionally long, bent stems, with lower net assimilation rates, may introduce instability and suggest greater vulnerability to physical disturbance, e.g. waves and wind, that may eventually lead to mangrove dislodgement. Our results also point towards a potential synergistic effect caused by high temperature and nutrient enrichment. These outcomes may serve as an example that the negative consequences of global-scale stressors such as global warming can be dampened by implementing local policies, such as preventing localized coastal eutrophication. Our findings also highlight the importance of considering the local nutrient status and hydrodynamic conditions when executing mangrove restoration projects. Further work should verify whether these effects occur under in-situ mangrove restoration conditions and whether our results apply to other mangrove species.

5.7 Appendix

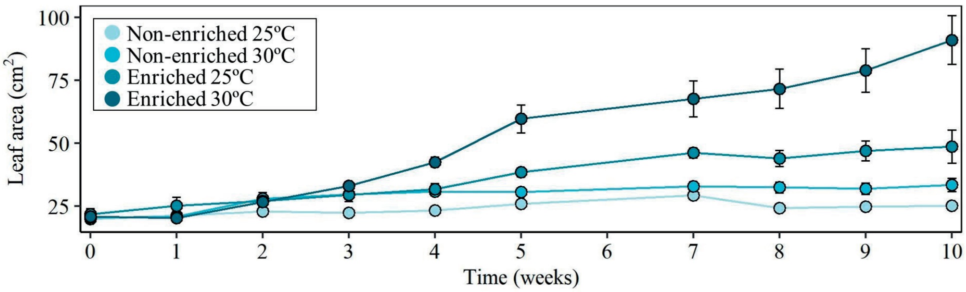

Figure A 5.1 Leaf area of seedlings over the 10-week experimental period. Marker shapes indicate mean values per treatment, and error bars represent standard error to the mean.

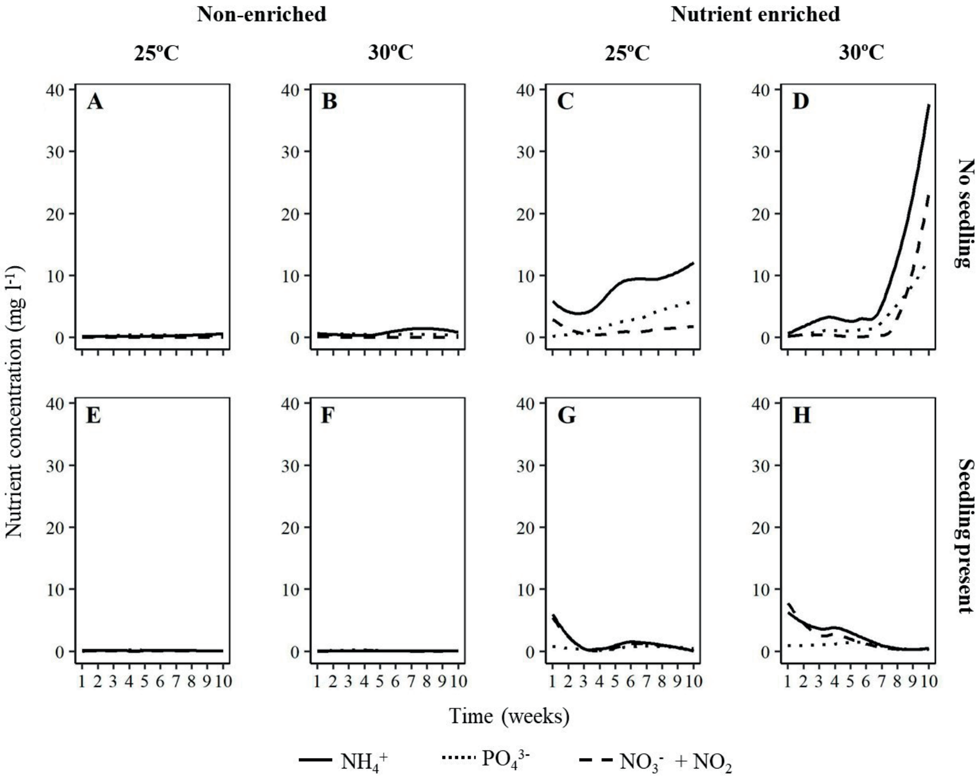

Figure A 5.2 Porewater NH_4^+,PO_4^{3-}, and NO_3 + NO_2 concentrations (see legend) over time for the four different treatments indicated above the graphs. Top row shows nutrient concentrations for experimental units without a seedling (A to D), and units containing a seedling are shown in the bottom row (E to H). Nutrient concentrations in the porewater were not statistically analysed and were only displayed for visual assessment.

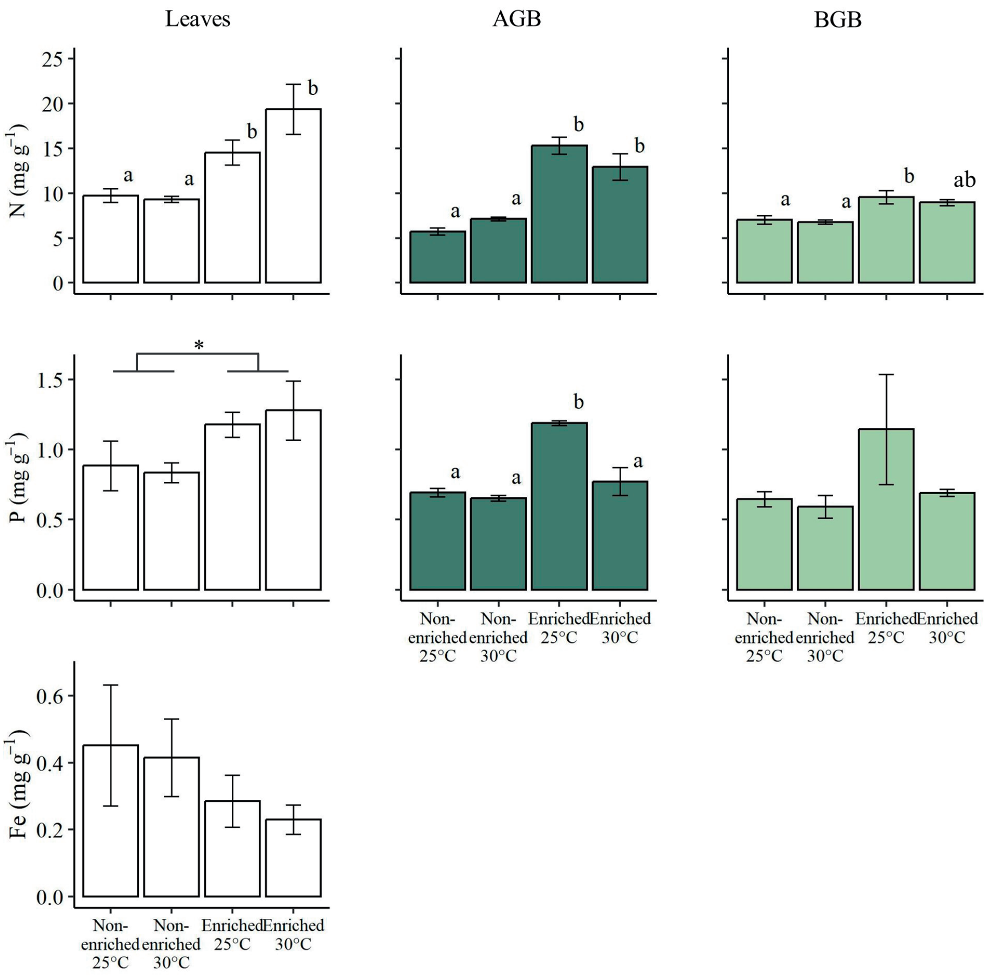

Figure A 5.3 Total nitrogen (N), total phosphorus (P), and total iron (Fe) in seedling tissue from the different plant parts: leaves (white), above-ground biomass (dark green), and below-ground biomass (light green). Columns represent mean values with standard error bars. The letters above the columns indicate significant differences between group means. When the overall F test comparing all four group means was significant but the more conservative Tukey test was not, asterisk signs were used to indicate significance values (*: $p<0.05$, **: $p<0.01$).

Chapter 6

First evidence of polyembryony in black mangrove *Avicennia germinans*

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Abstract

We provide the first documented case of the black mangrove, *Avicennia germinans*, producing multiple seedlings from a single propagule, a phenomenon called polyembryony, on several occasions. There is a lack of knowledge about polyembryony in mangroves, as it is an extremely rare phenomenon previously observed in only three other mangrove species. A higher weight of mature propagules may indicate polyembryony, thus enabling early detection. *Avicennia germinans* may make use of mixed reproductive strategies to ensure the continued survival of the species, or to increase its expansion under favourable environmental conditions. Potential causes and implications of polyembryony in *A. germinans* are discussed.

The black mangrove (*Avicennia germinans* (L.) L., Angiosperm, Acanthaceae) is a tropical mangrove species native to the American continent and West Africa (Dodd et al., 2002) that grows into a tree or shrub and reproduces by cryptovivipary (Alleman & Hester, 2011). As a common reproductive strategy in mangroves, A. germinans produces propagules that, once detached from the parent plant, are aquatically dispersed until they take root in sediment (Alleman & Hester, 2011). Once established, propagules develop into saplings, as shown in Figure 6.1.

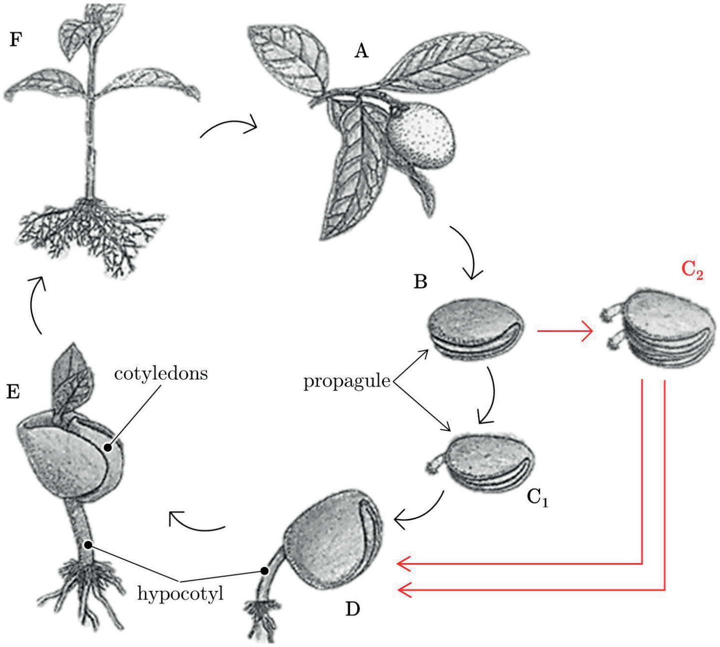

Figure 6.1 Reproductive cycle of *Avicennia germinans*. Propagules are produced by cryptovivipary thus remaining attached to the parent plant until germination (A, B). Propagules emerge by first developing an hypocotyl (C) and then roots (D). Epicotyl and first leaves emerge from the cotyledons (E), and subsequently the seedling develops into a sapling (F). Red arrows indicate deviations from the standard cycle as a result of polyembryony. Visible differences are the presence of two hypocotyls and a thicker, heavier mature propagule. Adapted from “The Mangrove Ecosystem- Adaptations” (available at: https://themangroveecosystem.weebly.com/adaptations.html (Accessed: 8 September 2021).

Polyembryony in plants is the result of multiple sexually or asexually produced embryos in one seed, resulting in twin (or multiple) seedlings (Batygina & Vinogradova, 2007). We collected propagules from a captive mangrove tree from the mangrove ecodisplay at Royal Burgers’ Zoo Arnhem, The Netherlands in 2 consecutive years, 2020 and 2021. Polyembryony was observed in one out of 46 propagules in 2021 (July 28) and 1 out of 15 propagules in 2020 (November 27).

Polyembryos were first recognised by the identification of two hypocotyls, one from each seedling, and two independent pairs of cotyledons (Figure 6.2).

The parent *A. germinans* tree of our polyembryonic propagules was collected in Cuba as a sapling, and grew into an adult tree in captivity. At Royal Burger's Zoo, the parent tree grows alongside another *A. germinans* individual blooming simultaneously, therefore our polyembryonic propagules could have resulted from either cross- or self-pollination. Resembling standard habitat conditions, humidity levels range between 60 and 85%, and water and air temperature are kept constant at 25 ºC all year round. Water salinity in the mangrove semi-diurnal tidal basin is 33. Nutrient analyses in the mangrove basin indicate a concentration of 0.018 mg l^{-1} of phosphate and 0.46 mg l^{-1} of nitrate in seawater.

The visual appearance of the 2021 polyembryonic, dry mature propagule before sprouting did not differ from the rest in any morphological parameter (Figure 6.2A). However, the polyembryonic propagule's initial weight on the day of collection was 2.2 g, 27.5% higher than the average of propagules in a similar development stage (1.7 g, n=46). This suggests that a higher weight of mature propagules could indicate the presence of multiple embryos, which may enable early detection of polyembryony. Propagules were grown in a greenhouse on waterlogged quartz sand, at 26 ºC, salinity of 20, and under a 12-h light regime of 300 µmol photons m^{-2} s^{-1}. All collected propagules sprouted after 48 h from the time of collection. After five days, hypocotyls were visible and the pericarp had decayed, exposing the cotyledons. In the case of our polyembryonic propagule, each seedling had their own pair of cotyledons and separate, independent hypocotyls from which more roots developed (Figure 6.2B). These four cotyledons presented an abnormal asymmetrical shape, whereas in normal seedlings it is usually symmetrical. This deviation is due to a limited amount of space under the seed coat that, under normal conditions, only accommodates one pair of cotyledons. This deformity was particularly visible in one of the seedlings (Figure 6.2C, right). One of the polyembryonic seedlings showed a delayed growth as no epicotyl or leaves had developed from the cotyledons as opposed to its polyembryonic counterpart. Six weeks after germination, an abnormal growth of the epicotyl and deformed leaves became evident in only one of the seedlings (Figure 6.2C, right). Over time, the abnormally-shaped seedling overgrew its original deformities and developed into a mangrove sapling (Figure 6.2D).

Figure 6.2 *Avicennia germinans*: polyembryonic, mature propagule (A) and different developmental stages of seedlings at (B) 5 days, (C) 6 weeks, and (D) 19 weeks after germination.

Polyembryony is an extremely rare phenomenon in mangroves that has only been observed in a few previous occasions and only three other mangrove species. Bowman (1917) reported the observation of polyembryony in *Rhizophora mangle* by other authors in the West Indies and Fiji and mentioned the existence of a heredity factor in certain trees of those regions to develop polyembryony. This phenomenon has also been reported in *Rhizophora mucronata* (Kumar et al., 1942) and *Lumnitzera racemosa* (Lakshmanan & Narmatha Bai, 1986). To our knowledge, there are no published reports of polyembryony in the mangrove species *A. germinans*.

The genotype of our polyembryonic seedlings of *A. germinans* is unknown. In almond seeds, a slow initial growth and poor development was observed in nondiploid organisms (Martínez-Gómez & Gradziel, 2003), however there could be several other causes for the stunting of mangrove seedlings. The fact that our observation corresponds to a propagule produced in captivity raises the question whether the production of polyembryos was due to chance or induced by the more favourable growing conditions provided in the zoo. In citrus fruits, the frequency of polyembryony was found to be increased by environmental factors such as plant nutrition, air temperature, environmental and soil humidity, and wind speed (Andrade-Rodríguez et al., 2005). We cannot completely rule out the possibility that these cases of polyembryony simply resulted from the anomalous development of the original cells. However, our multi-year observations indicate that polyembryony in *A. germinans* could be a genetically regulated character resulting in a deviation from the normal reproduction process. It cannot be determined whether this deviation is an artifact of captivity, but the fact that polyembryony has previously been observed in several natural mangrove communities (Bowman, 1917; Kumar et al., 1942; Lakshmanan & Narmatha Bai, 1986) provides evidence against the artifact hypothesis. In other words, *A. germinans* may make use of mixed reproductive strategies to ensure the continued survival of the species, or to increase its expansion under favourable environmental conditions.

The type of polyembryony determines the genetic diversity of the seeds (Batygina & Vinogradova, 2007; Michel et al., 2017). Lower genetic diversity can, in turn, determine the vulnerability of an ecological population to disturbance (Wernberg et al., 2018). Ample environmental tolerance boundaries in highly genetically diverse populations of mangroves could possibly provide an additional source of ecological resilience against the impacts of climate change. Among these impacts, increased evaporation and salinity, water temperature and sea level rise are determining factors for the survival of mangroves (Ward et al., 2016). Therefore, polyembryony could play an important role in the adaptive capacity of *A. germinans* to new, rapidly changing environmental conditions.

Chapter 7

Synthesis

Sara Pino Cobacho

This thesis aimed to study the impacts of global change on mangrove functionality, with a primary focus on biogeochemical processes. In this section, I shortly present the main findings and point out some limitations, show results that did not fall within the scope of the chapters, and discuss the practical implications of these findings. Lastly, I reflect on a number of points that were raised when I was writing this thesis.

7.1 Main findings

Mangrove functionality plays a crucial role in the connectivity of tropical coastal ecosystems (chapter 2). In this thesis, I have identified several stressors that hinder mangrove functionality through biogeochemical processes, such as sargassum brown tides (chapter 3), global warming, and eutrophication (chapter 5). Specific aspects of mangrove functionality such as their influence on water pH (chapter 4) or reproductive adaptations (chapter 6) were elucidated, primarily through mesocosm experiments. These experiments were useful to isolate the specific mechanisms underlying mangrove responses and fill the knowledge gaps raised in the different chapters. Consequently, the findings presented in this thesis provide valuable insights into various aspects of mangrove functionality and their responses to global change. I contextualize the insights gained from the chapters on mangrove functionality within the broader context of connectivity and speculate on the potential implications of this newfound knowledge for connectivity.

7.1.1 Impacts of global change stressors on mangroves: understanding the consequences

Mangroves are exposed to global change in various ways; therefore, identifying their vulnerabilities is key not only for preserving mangroves but also for restoring them.

Sargassum: from nutrient connector to lethal driver of change

In this thesis, I showed that exposure to sulfide released from the anoxic decomposition of sargassum is lethal for mangroves (chapter 3). These results corroborate previous studies on the impacts of sargassum brown tides (Hernández et al., 2022; Van Tussenbroek et al., 2017), confirming that the production and accumulation of sulfide in the sediment porewater result in sulfide toxicity for marine organisms (Leemans et al., *in preparation).* Initially, sargassum played a role as a nutrient connector, accumulating nutrients that enter the ocean via rivers (Lapointe et al., 2021), and later transporting them as macroalgae biomass from the open ocean to naturally oligotrophic tropical coasts (Gunter, 1979). However, presently, the uncontrolled proliferation of sargassum is a driver of change in tropical coasts due to climate and anthropogenic impacts (Johns et al., 2020; Louime et al., 2017; Sissini et al., 2019; M. Wang et al., 2019). Previous work had shown that iron addition could buffer the accumulation of sulfide in aquatic systems (Van Der Welle et al., 2006). Therefore, its application to mangrove sediments could potentially have a detoxification effect against sulfur (Lamers et al., 2013; Van Der Welle et al., 2006; van der Welle et al., 2008), although there was a risk of iron toxicity to the mangroves as shown by other authors (Alongi, 2010). However, we show that the effectiveness of iron addition was not sufficient to prevent mangrove death due to sulfide toxicity. Our findings indicate that mangrove soils with or without iron could not act as a buffer for sargassum brown tides and that iron addition to mitigate sargassum brown tides is not feasible for field application. Consequently, the idea that mangrove soils could detoxify sulfide and eliminate the risk of poisoning adjacent habitats has been debunked. Given that the main limitation of our study was the low solubility of iron, further steps could involve 1) introducing water mixing that enhances iron dissolution; 2) using different concentrations or forms of iron (Gutierrez et al., 2010); 3) investigating the effectiveness of alternative metals, such as manganese and copper (Cervi et al., 2021; Shea & Helz, 1988; F. Wang & Chapman, 1999), or other methods for sulfide removal where solubility is not heavily dependent on pH, unlike metal sulfides (Lewis, 2010). Furthermore, other authors have identified mangroves as extremely efficient traps for various forms of waste, including plastics, with their intricate root systems acting as traps for external objects (Horstman et al., 2017; Martin et al., 2019). This characteristic of mangroves is a form of connectivity, as they can retain waste and prevent its flow to rivers and seas (chapter 2). However, the effective retention of sargassum within these root systems may lead to the prolonged presence of sargassum, creating challenges for natural or artificial removal. This hypothesis gains support from the findings of León-Pérez et al. (2023), who observed that prolonged sargassum retention by mangroves resulted in elevated tree mortalities. Consequently, a knowledge gap remains regarding whether the retention capacity of mangroves presents a negative interaction of mangroves with adjacent habitats, causing damage due to the prologed exposure to sargassum.

Eutrophication and global warming as restoration roadblocks

Black mangrove seedlings at high temperature and nutrient concentrations exhibit a disbalance in their biomass allocation between above- and below-ground structures (chapter 5). The former shows synergistic growth as a consequence of combining the two factors, while the latter shows minimal growth. Previous work on a different species of mangrove, i.e., orange mangrove, showed that the combination of these two stressors triggers an antagonistic response in stem development (Gillis et al., 2019). The synergistic growth response found in our study concerning stem elongation in black mangroves is opposed to what Gillis et al. (2019) found for the orange mangrove. This contradiction may be explained by the two mangrove species having different temperature optima (Alongi, 2008), which was potentially surpassed by the orange mangrove but not yet reached by the black mangrove. Additionally, the indirect effects of temperature, such as vapor pressure deficit (Slot & Winter, 2017) which was not considered in our experiment may have also caused these differences between species. The main limitation of this study was the utilization of only two temperature levels instead of a temperature range with several levels, hindering the elucidation of the boundaries of the observed synergistic response. This point, as well as the differences observed between our results and those on the orange mangrove, indicate that further studies are needed to fill the remaining knowledge gaps: 1) investigate the tolerance range, optimum, and maximum temperature of black mangroves, and 2) identify the responses of mangrove species that have not been studied yet to multiple stressors, to ultimately identify the most suitable mangrove species to be matched with the future environmental conditions of the restoration sites (Sahana et al., 2022; Su et al., 2022).

7.1.2 Climate change and mangroves: a complex interplay

Rethinking climate change regulation: detrimental effects of impacted mangroves

In this thesis, I show that mangroves take up a significant amount of atmospheric CO_2 (chapter 3), making them crucial ecosystems for climate change mitigation, as previously demonstrated by other authors (Alongi, 2020a; Donato et al., 2011; Murdiyarso et al., 2015). Not only does this service disappear when mangroves are deforested (Atwood et al., 2017; Gillis, Belshe, et al., 2017), but in sargassum-impacted mangroves, the process of sargassum decomposition produces significant quantities of CO_2, CH_4 and N_2O that are released into the atmosphere, turning mangrove habitats from sinks to sources of greenhouse gases (chapter 3). With this greenhouse gas release and the increasing concentration of greenhouse gases in the atmosphere, sargassum brown tides generate a feedback loop of greenhouse gas emissions, where climate change is amplified, and its subsequent impacts exacerbate the environmental challenges faced by tropical coastal ecosystems (Figure 7.1).

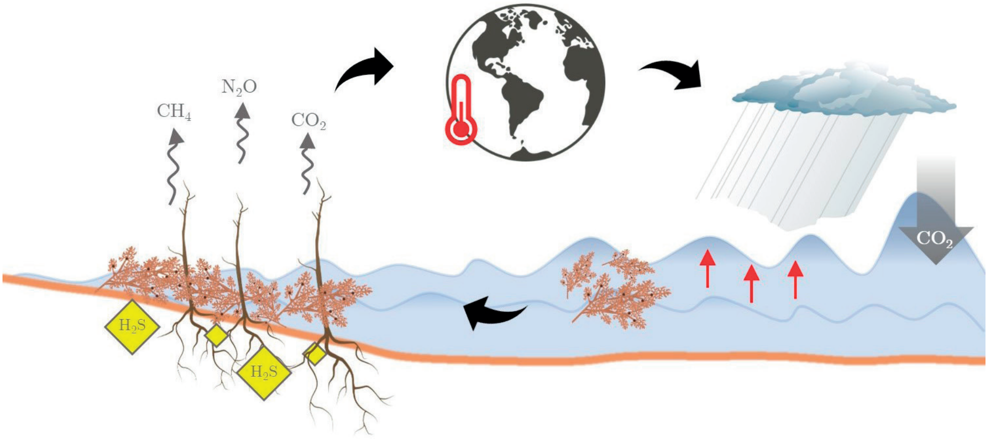

Figure 7.1 Illustration of the feedback loop caused by sargassum brown tides on mangroves. Greenhouse gasses produced as a result of sargassum decomposition amplify climate change, exacerbating the challenges faced by tropical coastal ecosystems.

Among the climate change impacts that may exceed the tolerance of mangroves are sea level rise, rising temperatures (whose effects were explored in chapter 5), and increased storm frequency and intensity (Jennerjahn et al., 2017; Saintilan et al., 2020; Ward et al., 2016).

From enhanced GHG emissions to increased vulnerability of mangroves

In this thesis, I report that an observed disbalance between root and stem development in black mangroves (chapter 5), caused by high temperature and nutrient concentrations, may potentially translate into greater vulnerability to physical disturbances such as storms and hurricanes. Other authors have reported that eutrophication weakens mangrove structures, and these populations cannot withstand the impacts of hydrodynamic forces, such as storms (Feller et al., 2015), which are expected to reduce mangrove biomass and cover area in the future (Friess et al., 2022). Moreover, a climate change impact such as global warming, in combination with a local stressor such as eutrophication (chapter 5), may drive the morphology of seedlings away from their most resilient form (van Hespen et al., 2022) to withstand the very impacts of climate change, such as storminess (Friess et al., 2022). Other authors also indicate that eutrophication in mangroves may also increase their vulnerability to other stressors, such as high salinity (Lovelock et al., 2009), which is also expected to rise as a consequence of a drier climate and reduced freshwater inputs (Friess et al., 2022; Jennerjahn et al., 2017; Ward et al., 2016).

Mangroves as pH regulators

In this thesis, I addressed the question of the direct impact of mangroves on pH when there are no interferences from the surrounding environment (chapter 4). This is a service that has already been confirmed for seagrass (Bergstrom et al., 2019; Unsworth et al., 2012), but it is still under debate for mangroves. Some authors reported that the mean water pH in mangrove habitats is lower compared to surrounding areas (Camp et al., 2016). Other studies, however, have shown that mangrove habitats can indirectly increase water pH through total alkalinity (TA) and dissolved inorganic carbon (DIC) production and export (Banerjee & Paul, 2022; Y. Liu et al., 2021; Sippo et al., 2016; Yates et al., 2014b) (Figure 2.4). In chapter 4, I approached this topic using black mangroves in a mesocosm experiment, where the interferences from other natural factors were minimized. In contrast to most field studies (Banerjee & Paul, 2022; Y. Liu et al., 2021; Sippo et al., 2016; Yates et al., 2014b), I found that black mangrove seedlings have a negative impact on water carbonate chemistry, confirming their direct influence on pH, alkalinity, and dissolved inorganic carbon by decreasing their concentration in seawater. Although this finding is highly relevant, it is also highly dependent on the fertilizer composition that was supplied to the mangroves. High ammonium uptake rates by plants lead to acidification (Brix et al., 2002), and ammonium was the most abundant component in the fertilizer, which other authors have reported as the primary nitrogen source for mangroves (Reef et al., 2010). However, it should be noted that under previously acidified conditions, ammonium uptake by higher plants is reduced (Brix et al., 2002). This would result in a lower influence of mangroves decreasing the pH by taking up ammonium. Therefore, the influence of mangrove metabolism on water pH is dependent on nutrient dynamics, in particular nitrogen abundance, fluxes, and enrichment (Alongi, 2020b; Gonçalves Reis et al., 2017).

It remains an open question whether mangroves experiencing the impacts of ocean acidification could transition from intensifying its effects to buffering them, thereby benefiting adjacent habitats. This is an example of why it is important to place mangrove connectivity research within a climate and global change context (as acidification may not only be caused by increasing CO_2 diffusion from the atmosphere (Sabine et al., 2004), but also by eutrophication (Wallace et al., 2014)), which factors may affect the state of the environment and determine the influence that mangroves exert in it. Therefore, the remaining knowledge gaps can be filled by investigating the influence of other mangrove species, various age classes, and different starting pH levels through long-term mesocosm experiments.

Impacts of climate-induced human migration

So far, this section has discussed specific aspects of mangrove functionality resulting from or contributing to climate change. Chapter 5 presented a situation where several stressors take place, which is a more realistic approach to reality, considering that climate and human impacts frequently occur in combination (Vinebrooke et al., 2004). Climate change not only affects natural ecosystems, but it is also disrupting the environmental conditions of the human climate niche (Xu et al., 2020), leading to the forcible displacement of up to 1.2 billion people worldwide by 2050 as a consequence of extreme weather events, sea level rise, and increasingly frequent natural disasters (McAllister, 2023). The consequences of the resulting human migratory movements are expected to cause severe impacts on the receiving ecosystems as they accommodate the sudden increase in livelihoods (Barua et al., 2018; Black, 1994; Jacobsen, 1997; Kerr et al., 2004; Mahmud et al., 2019).

Box 7.1. The impacts of refugee influx to tropical ecosystems, a remote sensing study of the Teknaf Peninsula (Bangladesh).

The Teknaf peninsula in Bangladesh was selected as the study region as it experienced a migratory influx of over 7 million refugees on the 25th of August, 2017, making it a suitable case study to identify the impacts of sudden refugee influxes on coastal ecosystems using remote sensing techniques. Moreover, this region was inhabited by mangroves with seagrasses and corals in the vicinity, as well as a rainforest in direct contact with the refugee camps established in August, 2017. This study aimed to detect changes to ecosystem health and their land cover area. Ideally, we would observe negative changes in terrestrial vegetation over time, that would extend to adjacent habitats (mangroves, seagrasses, and corals) that were not in direct contact with the perturbation (refugee camps), demonstrating their interdependency (connectivity). However, conducting a study that aims to collect data from both terrestrial and underwater ecosystems simultaneously comes with limitations and trade-offs. The resolution and quality of underwater remote sensing data may not match that of terrestrial data, and the former requires specific and advanced knowledge of underwater data collection techniques. Thus, the study was reduced to detecting spatio-temporal changes in the rainforest in direct contact with the refugee camps and the adjacent mangrove stands. Although direct perturbations from the rapidly established refugee camps were identified, the existing mangrove stands were not found to be in the immediate proximity of the rainforest. Other land uses, such as aquaculture and agriculture, had developed in between the two habitats, thus reducing the possibility of cascading disturbances that would provide information about the connectivity of the ecosystem and its implications. However, we observed a local shift in land use as a result of refugee influxes, including an overall decrease in vegetation health and cover area (Figure B 7.1.1).

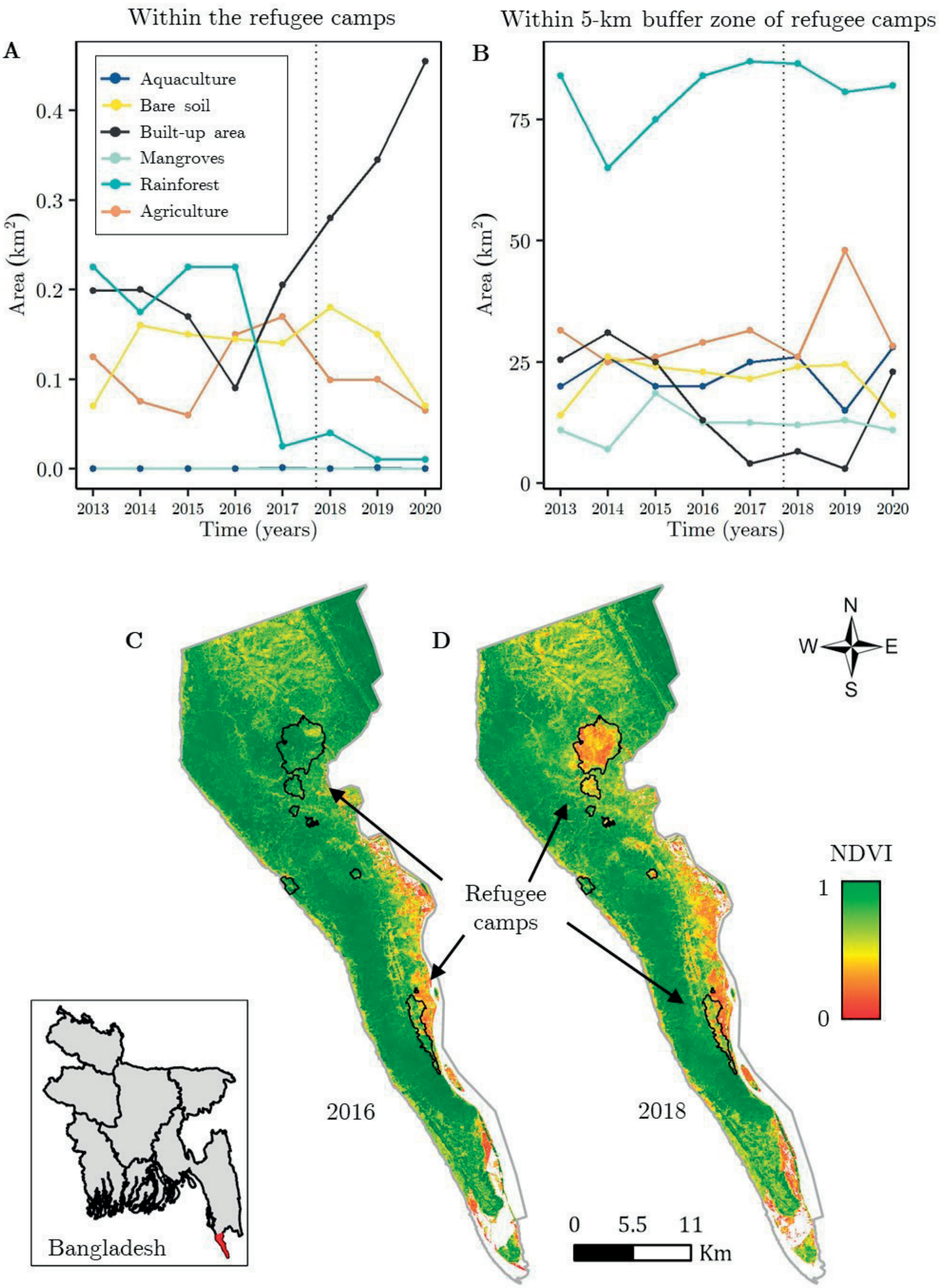

Figure B 7.1.1 Land use change within the refugee camps (A) and within a 5-km buffer of the camps (B) between 2013 and 2020. Vegetation health was assessed using the Normalized difference vegetation index (NDVI) before (C) and after the main refugee influx (D).

Although the perturbation caused by the rapid construction of refugee camps resulted in the conversion of rainforest to built-up areas (Figure B 7.1.1A), also evidenced by a decrease in vegetation health (Figure B 7.1.1D), these land cover changes had only a local effect. The land cover change dynamics within a larger radius did not exhibit a clear trend following the refugee influx (Figure B 7.1.1B). However, the vegetation health maps indicate an expansion of anthropogenic activities along the eastern border, supported by low NDVI values in 2018 compared to 2016 (Figure B 7.1.1C and D). These land use changes raise the question of whether the intensification and expansion of economic activities, such as agriculture and aquaculture, were intended to accommodate the larger population resulting from the refugee influx in the region.

Land-sea connectivity plays an important role in maintaining the health of mangroves and coastal ecosystems. However, this connectivity is significantly impacted by human activities, such as land-cover change, urban expansion, and industrialization (Fang et al., 2018). These impacts are exemplified in the outcomes presented in Box 7.1, which portray a prospective surge in human migration due to climate change influences (Lenton et al., 2023; Xu et al., 2020). Climate-driven displacements of human populations are already unfolding in Small Pacific Island States, Sub-Saharan Africa, South Asia, and Latin America (Clement et al., 2021). Consequently, the case study presented in Box 7.1 opens diverse considerations regarding land-sea connectivity and the forthcoming impacts of climate-induced human migration on coastal ecosystems.

7.1.3 Connectivity in tropical seascapes

The second chapter of this thesis contributed to the state of knowledge regarding the functioning and interconnectedness of tropical seascapes, and highlighted the role of biogeochemical fluxes between mangroves, seagrasses, and corals in ecosystem connectivity. One of the knowledge gaps identified in chapter 2 was whether mangroves, in a yet unexplored form of connectivity, could prevent the decline of adjacent habitats by reducing pathogen load in seawater though the release of their leaf phytochemicals.

Box 7.2. Mangrove phytochemicals and their potential antimicrobial properties in seawater.

As a result of their high productivity, mangroves generate large amounts of organic matter in the form of leaf fall and export (Efriyeldi et al., 2021). These mangrove leaves have a long history of applications due to their phytochemical composition, first in the traditional medicine (Bandaranayake, 1998), and nowadays in the pharmaceutical industry (Habib et al., 2018). When chemically extracted, mangrove leaf phytochemicals exhibit strong antimicrobial activity against various human, animal, and plant pathogens (Eswaraiah et al., 2020). Moreover, seagrasses are effective pathogen filters (Liu et al., 2023) that can reduce disease incidence in adjacent habitats such as coral reefs (Lamb et al., 2017). Therefore, to fill the knowledge gap identified in chapter 2 on whether mangroves can reduce pathogen load in seawater via leaf litter leachates, a comparative analysis of their leaf phytochemicals (Figure B 7.2.1) and a short experiment (Figure B 7.2.2) were conducted.

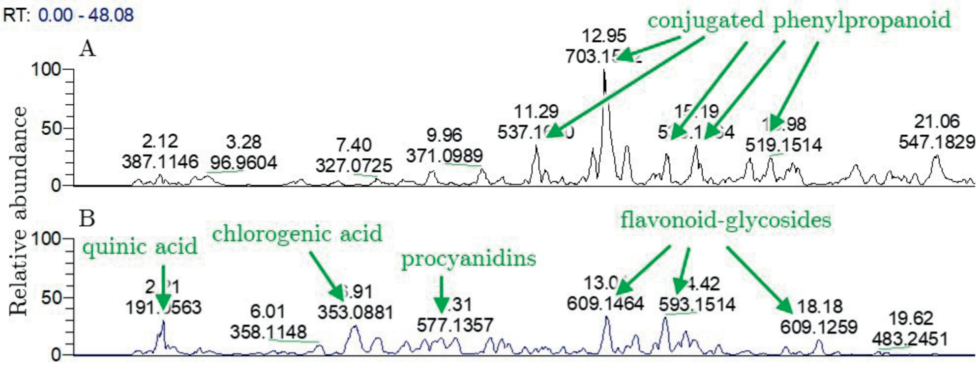

Figure B 7.2.1 Comparative analysis of black (A) and red mangrove leaves (B) using Liquid chromatography-mass spectrometry (LCMS).

The secondary metabolites of black and red mangrove leaves from saplings grown under similar environmental conditions were analysed and compared, revealing highly contrasting metabolite profiles. Black mangrove leaves contain more conjugated phenylpropanoids (Figure B 7.2.1A), while red mangrove leaves are enriched in mono-phenylpropanoids and flavonols (Figure B 7.2.1B). These results indicate that different mangrove species could potentially have different effects on water quality and pathogen load. Although organic matter from mangroves is exported to adjacent habitats through outwelling (Hemminga et al., 1994; Lee, 1995), we do not yet understand the fate of these phytochemicals when they are leached in seawater during the natural leaf decomposition process, whether they are active, and if so, for how long. Even though I realized that the topic of mangrove leaf phytochemicals and their potential for reducing pathogen loads in the seawater could be an entire subject for a PhD, I briefly explored this topic in a pilot experiment. Coral nubbins of *Pocillopora damicornis* (Figure B 7.2.2A) were incubated in mini-aquaria and exposed to fresh and pre-degraded red mangrove leaf fragments (Figure B 7.2.2B). The mini-aquaria were placed on a shaker at a constant circular motion to generate water turbulence, enhancing oxygen dissolution. The rationale behind this pilot experiment was that water quality in the mini-aquaria would be reduced within days as a result of, among other factors, bacterial growth, infecting the coral nubbins and reducing their health. The PSII yield of the coral nubbins was used as a health indicator (Ralph et al., 2015) and monitored over time, revealing a decline of coral health regardless of the treatment, simply as a consequence of reduced water quality. Although the presence of fresh and pre-degraded mangrove leaves did not prevent the decline in coral health, we observed an inhibition of green algae overgrowth in the mini-aquaria that contained pre-degraded mangrove leaf fragments (Figure B 7.2.1 C and D).

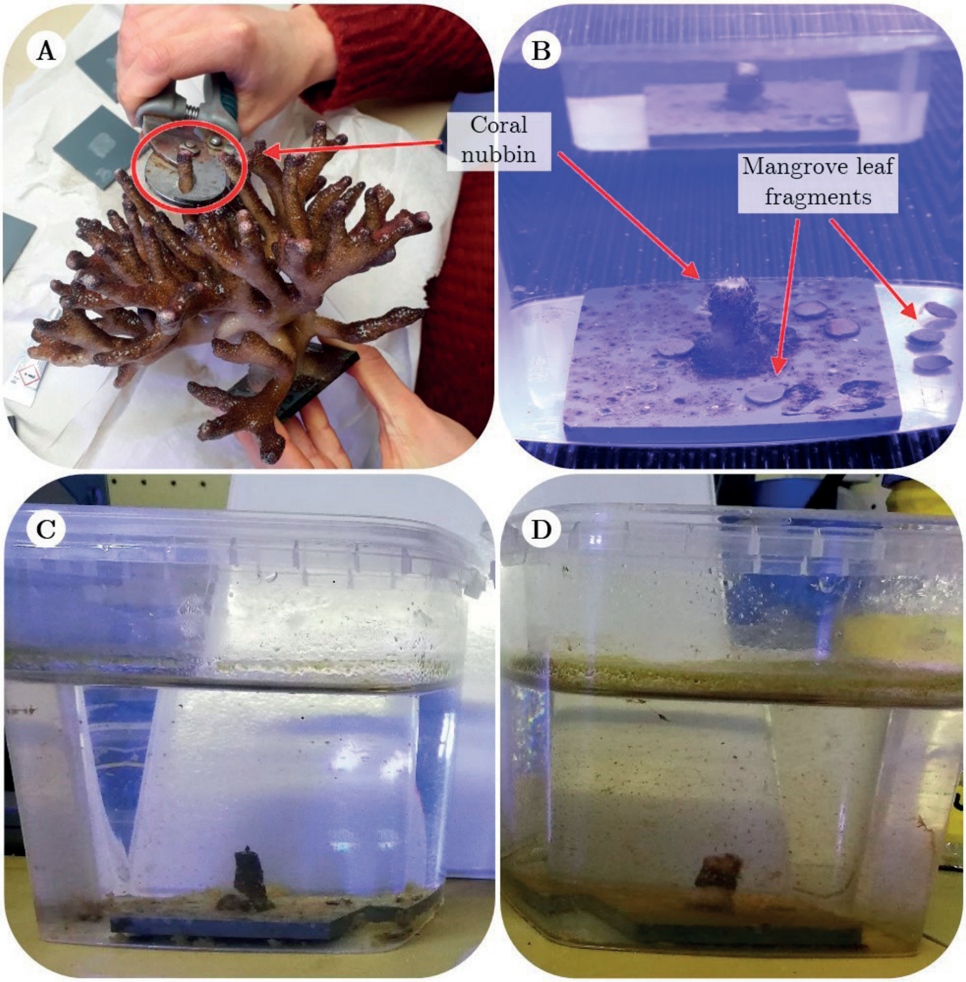

Figure B 7.2.2 Coral nubbins of *P. damicornis* (A) were incubated in mini-aquaria containing mangrove leaf fragments (B). Pre-degraded leaves inhibited the growth of green algae on the aquarium walls (C), whereas fresh leaves did not appear to influence the proliferation of green algae and developed a visual appearance similar to the control treatment (B).

These results point toward a negative allelopathic interaction between mangroves and green algae, a phenomenon also reported by other authors (Duan, 2012). This interaction is likely caused by the high content of mono-phenylpropanoid in red mangrove leaves (Figure B 7.2.1B).

Inhibition of growth by allelopathic effects from mangrove phytochemicals has been reported for crops (Desai et al., 2016), weeds (Dhaou et al., 2022; Othman et al., 2019), wetland plants (Lang et al., 2021), microalgae (Sun et al., 2012), and other mangrove species (Wang et al., 2009). However, there is currently no scientific evidence indicating that naturally released mangrove leaf phytochemicals can reduce pathogen load in seawater, leaving this as an open question. Nevertheless, the inhibition of green algae growth by mangrove leaf phytochemicals, as shown in Box 7.2, could enhance the resilience of corals when exposed to eutrophication and reduce their disadvantage when competing with macroalgae (van de Leemput et al., 2016; Zhao et al., 2021). This means that macroalgae would be less likely to displace the corals rapidly. Thus, this form of connectivity would be beneficial for corals, especially under high nutrient conditions.

Potential reasons for limited understanding

The scarcity of knowledge regarding biogeochemical connectivity in tropical coasts is also shown in chapter 2 of this thesis. Through a literature review, I identified 81 empirical studies concerning biogeochemical connectivity in tropical coasts, of which only 11 included the three habitats of interest: mangroves, seagrasses, and corals. The fact that only 11 studies encompassing the three habitats of interest fulfilled the criteria for review indicates a lack of holistic knowledge of tropical coastal ecosystems and poses a limitation to the conclusions that can be extracted from such a small pool of scientific knowledge. There are several potential reasons for the low availability of empirical studies: 1) the vast areas that need to be covered, which require long sampling times and extensive travel; 2) limited accessibility and the substantial financial, human, and material resources required to undertake large-scale projects of this kind; 3) the identification of ecosystem-wide patterns and cycles requires collecting data over long periods of time; and 4) knowledge of hydrodynamic conditions within each habitat is essential for understanding flows and interactions, but comparisons are challenging due to the unique nature of each habitat. However, technological advancements and scientific innovations, such as publicly available satellite data and more affordable drones with user-friendly systems, are currently bridging this gap and facilitating more efficient and integrated data collection (Amani et al., 2018; Bhatnagar et al., 2021; Lechner et al., 2020). Nevertheless, there are trade-offs to consider when applying, for instance, remote sensing techniques for data collection on terrestrial (mangroves) and aquatic systems (seagrasses, corals) simultaneously (Box 7.1). Additionally, these new technologies only offer the possibility of indirect measurements, as opposed to direct measurements of physical elements of the ecosystem, thus missing the information of detailed, localized field parameters. Therefore, the combination of both approaches is needed to develop a more robust understanding of the mechanisms and dynamics of connectivity in tropical coasts.

7.2 Implications for mangrove functionality and management

Besides the lethal effects of sulfide from sargassum on mangroves described in chapter 3, there are other ways in which sargassum brown tides can impact natural and restored mangrove populations. The greenhouse gases released during sargassum decomposition further intensify climate change, which, in turn, contributes to global warming (Figure 7.1). This leads to altered biomass allocation patterns in restored seedlings as demonstrated in chapter 5. Additionally, findings from chapter 3 suggest that early removal of sargassum may increase mangrove survival by limiting their exposure time to sulfide. However, chapter 5 reveals that newly restored seedlings exhibit poor root development, resulting in weak anchorage. Thus, while attempting to save mangroves by removing sargassum through human labour or machinery, such removal may unintentionally uproot poorly anchored seedlings. Therefore, sargassum removal might not be an effective solution in recently restored coasts under the impacts of eutrophication and global warming. Lastly, when restored seedling populations are not directly exposed to high sulfide concentrations (chapter 3), the nutrients released by sargassum decomposition can still have a harmful effect by causing eutrophication. This, in turn, contributes to altered biomass allocation patters and increased vulnerability of restored seedlings, as shown in chapter 5.

Furthermore, the decomposition of organic matter from sargassum decreases water pH (Van Tussenbroek et al., 2017). In higher plants, decreasing external pH reduces NH_4^+ uptake (Brix et al., 2002). In chapter 4, I showed that NH_4^+ uptake plays a significant role in the negative influence of mangroves on seawater pH. With a lower source seawater pH, which results in reduced NH_4^+ uptake, the capacity of mangrove seedlings to increase seawater pH may improve. However, the overall decline in water quality also caused by sargassum decomposition may hinder this service. The lower pH resulting from excessive decomposition of organic matter from sargassum may also act as an additional stressor for restored seedlings, affecting their growth dynamics and productivity (chapter 5).

Moreover, chapter 5 reveals that restored mangrove seedlings exposed to high nutrients and temperature exhibit poor root development. Considering that mangroves are globally valued for being vital carbon sinks (Afonso et al., 2021; Donato et al., 2011; Murdiyarso et al., 2015), poor root development can have consequences beyond stability and anchorage. Eutrophication and global warming may seriously impact soil carbon accumulation and below-ground carbon dynamics in restored mangrove coastlines. Mangrove root systems facilitate the trapping and accumulation of organic matter below-ground through root growth and decomposition, as well as the sequestration of atmospheric CO_2 into the soil (Donato et al., 2011; Taillardat et al., 2018). Thus, the interactive effects of eutrophication and global warming may affect the long-term carbon sequestration potential of restored mangroves and their climate regulation services (Afonso et al., 2021).

The allelopathic interactions between red mangroves and green algae described in Box 3 may also have implications for mangrove management and restored populations. The presence of mangrove allelopathic compounds inhibiting algae growth reduces their competition for nutrients and maintains water quality, giving mangroves a competitive advantage and favouring their establishment in restored areas. However, under eutrophication scenarios that impact seedling development (chapter 5), the absence of algae taking up the excess nutrients may result in persistent water eutrophication and loss of resilience in restored mangroves (Feller et al., 2015).

The discovery of polyembryony in black mangroves described in chapter 6 may also have implications for mangrove restoration. Under natural conditions, it can be assumed that two polyembryonic seedlings germinating and developing their root systems together provide added stability due to their combined roots forming a stronger base. This stronger base could help overcome vulnerabilities in anchorage, as revealed in chapter 5 for seedlings exposed to high temperature and eutrophication. Therefore, polyembryonic mangroves would be more resilient under the impacts of multiple stressors. Based on the theory that twin seedlings with combined roots and a stronger base are more stable, the idea arises of intentionally outplanting seedlings in pairs or clusters, so their combined root systems provide them with a stronger base and higher resilience against uprooting. The close proximity of outplants has demonstrated increased survival rates and overall restoration success (Gedan & Silliman, 2009; Silliman et al., 2015). However, further research is needed to determine whether this approach could effectively enhance the stability of restored mangrove populations, specially when faced with multiple stressors.

Furthermore, polyembryony (chapter 6) may also prove useful for monitoring the health of restored mangrove populations. The production of polyembryonic propagules could be used as an indicator for the successful development of the restored mangroves. However, the low genetic diversity of twin mangroves may be a disadvantage for restoration, as low genetic diversity may decrease the capacity of the mangrove population to adapt to changes and overcome environmental challenges. Genetic diversity is beneficial for ecological functions of mangroves, as a more diverse population creates a more robust ecosystem, capable of establishing varied interactions with adjacent habitats, thereby improving connectivity.

7.3 Afterthoughts

Finally, I reflect on three points that arose when I was writing this thesis.

7.3.1 What is the value of mesocosm studies?

All experimental studies in this thesis were conducted using mesocosm experiments (Figure 7.3). While both experimental mesocosms and numerical models aim to gain insight into underlying processes, mesocosms enable the collection of actual data from the organisms of interest. Mesocosms do not strive to recreate the complexity of a natural system on a smaller scale; instead, they can demonstrate the existence of a mechanism or process by isolating it from the interferences often observed in nature. The power of these mesocosms lies in their simplicity, making them potent tools for validating hypotheses previously developed by numerical models, for example.

Figure 7.3 Various mesocosms were set up over the course of this PhD project.

Mesocosm studies have both advantages and disadvantages compared to field and modelling studies as they differ in their scales and level of control. As mentioned before, artificial mesocosms cannot (nor they aim to) recreate the complexity observed in the field. Such complexity comes with financial constraints and the difficulties of replication. These aspects are not issues in mesocosm studies, as they are cheaper to conduct and much easier to replicate.

However, results from mesocosm experiments carry the risk of being misinterpreted as what happens in nature. The insights derived from mesocosm studies cannot be directly translated to field conditions. Instead, they show us the subsequent path and highlight areas that require attention. Mesocosm experiments offer guidance on what the next steps should be and steer us toward the types of scaled-up experiments that are needed. With this reflection I want to emphasize the need for combining the knowledge obtained from both mesocosms for mechanistic understandings and field studies for more realistic representations of ecological interactions.

7.3.2 Are we trying too hard?

The chapters presented in this thesis can inform the management and conservation of mangroves within connected ecosystems. In chapter 4, I demonstrate the fatal consequences of sargassum brown tides on the survival of mangroves, and in chapter 5, I show how mangrove restoration efforts may fail under multiple pressures. However, chapter 6 brings a surprise by suggesting that mangroves can adapt on their own, such as by producing twins to increase their chances of success and species continuity. The ability of mangroves to generate twice as many seedlings from the same energy investment has significant practical implications, as discussed earlier, and highlights their remarkable adaptability to their environment. A study by Jones et al. (2018) argues that allowing ecosystems to recover naturally following a disturbance and implementing active restoration only when it can lead to more complete or faster recovery would be a more efficient allocation of resources. However, the number presented in chapter 1, showing the loss of 11700 km^2 of mangrove forests in the past 27 years (Leal et al., 2022), provides clear evidence that mangroves cannot recover on their own, and adaptations like polyembryony (chapter 6) are not (fast) enough to prevent their decline. In the absence of anthropogenic disturbance, allowing natural mangrove systems to recover without human intervention could potentially be more effective, although preventing such disturbance (e.g., establishing exclusion zones) should always be the primary approach. However, the problem arises when the rate of mangrove ecosystem destruction exceeds the rates of natural recovery (Leal et al., 2022). In such cases, restoration through active intervention will always be more efficient and faster. This intervention does not always entail planting; it extends to tackling the root causes of decline to reinstate natural environmental conditions. There is no universal solution for every site; each location has its distinct characteristics. Site-specific assessments are crucial, as copy-paste approaches or generalizations across multiple restoration projects risk inefficacy. Hence, the no less than 34 ecosystem services identified by Afonso et al. (2021) are significant reasons to protect and restore mangrove ecosystems and develop the necessary knowledge to do so.

7.3.3 Are we using the knowledge correctly?

Some scientific interventions have unforeseen consequences for the environment. For instance, a notable example is the iron addition strategy for ocean fertilization in the Southern Ocean in 1999 (Boyd & Law, 2001). This intervention was proposed to increase CO_2 uptake by stimulating iron-limited phytoplankton growth, which removes carbon from the atmosphere. However, these iron-enrichment experiments in the open ocean resulted in shifts in the phytoplankton community composition, including an increase in toxic diatom abundance with detrimental impacts on the marine food web (Trick et al., 2010). The introduction of a micronutrient beyond its natural background concentration into an ecosystem may trigger unforeseeable cascading effects. In the specific context of iron addition, these effects may include algal blooms, nutrient depletion, shifts in the abundance and composition of primary and secondary producers, and long-term disruptions to the food web. While chapter 3 suggests an iron-based method to mitigate high sulfide concentrations in coastal waters, which typically contain higher nutrient concentrations compared to the open ocean, it is essential to acknowledge that this approach is not exempt from uncertainties. Hence, while scientific knowledge empowers us to evolve as a species and manage our planet more effectively, we should never lose sight of the ethical imperative when carrying out environmental manipulations that could interfere with natural processes, such as connectivity, and potentially cause more harm than they aim to solve.

When reflecting on the use of environmental interventions to tackle issues such as sargassum brown tides (chapter 3), I also find myself wondering why we allocate efforts towards resolving these issues rather than focusing our attention towards preventative measures. Using the example of introducing iron to mangrove sediments for the purpose of mitigation, the primary focus should be on preventing the occurrence of sargassum brown tides. This prevention could involve incorporating iron for nutrient removal at wastewater treatment plants (Gutierrez et al., 2010) when these are present. By doing so, the excessive nutrients that contribute to the growth of sargassum (Djakouré et al., 2017) can be removed before reaching rivers and subsequently the ocean. Although such technologies are already developed, there are obstacles for their implementation, and the permissibility of water legislation varies from country to country (Preisner et al., 2020; Rout et al., 2021).

7.4 Concluding remarks

The chapters presented in this thesis focus on various aspects of mangrove functionality and ecology such as their role in ecosystem connectivity, and responses to multi-scale drivers like eutrophication, sargassum events, and global warming. They explore the potential of mangroves for mitigating ocean acidification, and shed light on their reproductive strategies. Overall, this thesis highlights the challenges faced by mangroves within the broader context of connectivity and provides valuable insights for effective management strategies to ensure the long-term conservation and resilience of mangroves in a transforming world.

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Summary

Mangroves are essential components of interconnected tropical ecosystems, offering numerous benefits to nature and society. However, mangroves are facing the impacts of global changes due to shifts in climate and human activities, which are expected to intensify in the coming years. The world is changing, temperatures are rising, ocean pH is decreasing, water is being polluted. These factors, in combination with the conversion and overexploitation of mangroves lead to their rapid decline. To effectively preserve and restore mangroves, it is crucial to understand mangrove functionality and their responses to global change drivers. While their role in climate change mitigation and physical protection has been extensively studied, the biogeochemical processes driving mangroves' responses to global drivers remain poorly understood. Therefore, this thesis aimed to study the impacts of global change on mangrove functionality, elucidating their role within ecosystem connectivity, with a primary focus on biogeochemical processes.

This thesis starts by exploring the general topic of cross- ecosystem connectivity in tropical coasts, with a focus on biogeochemical connectivity between mangroves, seagrass, and corals, a perspective that is relatively unexplored. In chapter 2, I quantified the studies concerning biogeochemical connectivity and provided a deep understanding about the complex interactions and feedbacks that build up tropical coastal ecosystems. Furthermore, I showed that humans can directly or indirectly disrupt the biogeochemical interactions linking these habitats together by causing eutrophication, chemical pollution, microbial pollution, improper waste disposal, and breaking the water carbonate equilibrium. While analysing the selected literature, I found several knowledge gaps that were used as a stepping stone to develop the following chapters of this thesis.

In Chapter 3, I investigated the potential of mangrove systems to buffer the impacts of sargassum brown tides, which pose a significant and timely threat to tropical coastal ecosystems, including mangroves. The approach taken in this chapter was to investigate the effectiveness of iron in mitigating sulfide produced during the decomposition of sargassum, while also assessing the overall health of mangroves in this process. While mangroves are widely recognized as a frontline defense against various environmental and anthropogenic impacts, such as excessive nutrient input, Chapter 3 demonstrates that mangroves may not be effective in mitigating the effects of sargassum brown tides. While previous studies have shown the success of adding iron to mitigating high sulfide concentrations in sewage and freshwater systems, our findings suggest that this approach may not be feasible for marine systems. The presence of sargassum severely impacts mangrove health, and the levels of iron used

in our study did not prevent their death. Furthermore, the presence of sargassum on mangroves transforms these habitats from sinks to sources of greenhouse gas emissions, thus nullifying their crucial role in climate change mitigation. However, our study found that the addition of iron can reduce greenhouse gas emissions from stranded sargassum within mangrove systems.

Another form of connectivity in tropical coastal ecosystems is pH regulation, in particular, by buffering low pH conditions for the benefit of corals and other sensitive organisms. However, the role of mangroves in water pH regulation is still a subject of scientific debate. Chapter 4 presents the first *ex-situ* experiment that specifically examines the effect of mangroves on water carbonate chemistry, providing insights free from the complexities of field conditions. The results of Chapter 4 demonstrate that black mangrove seedlings do not rise water pH. In fact, their presence leads to lower pH levels, alkalinity, and dissolved inorganic carbon (DIC). This finding indicates that black mangrove seedlings have a negative impact on water pH. Moreover, these result emphasize the significance of healthy seagrass meadows located between mangroves and corals, as they can serve as a buffer, mitigating the influence of mangroves on pH and benefiting adjacent coral reefs.

Mangrove restoration plays a crucial role in counteracting the global loss of mangroves. However, restoration efforts often face setbacks due to a limited understanding of how mangroves respond to various drivers at different scales. Chapter 5 focuses on investigating the responses of mangroves to the combined effects of two stressors: global warming and eutrophication. The findings reveal that when these two drivers act together, there is a synergistic growth response in the above-ground parts of black mangrove seedlings. However, the growth of the root systems in these seedlings is minimal compared to their stems, leading to an imbalance between above and below-ground biomass allocation. Additionally, seedlings exposed to high temperature and eutrophication exhibit lower net assimilation rates, indicating reduced carbon incorporation into their rapidly growing tissues. These results suggest that weaker seedlings with small root systems and long stems are more susceptible to dislodgement due to lower stability in the sediment. Therefore, when selecting restoration sites for black mangroves under future global warming conditions, it is essential to consider the local nutrient status and hydrodynamic energy levels. Taking these factors into account will contribute to more effective mangrove restoration efforts.

In addition to the responses revealed in chapter 5 to multi-scale drivers, mangroves also exhibit adaptive reproductive strategies in response to their surroundings. Chapter 6 describes the discovery of polyembryony in black mangroves, a rare phenomenon where a single propagule produces two seedlings. This uncommon reproductive strategy in mangroves allows them to double their seedling production with the same energy investment, favouring their expansion and ensuring effective population continuity.

In the Synthesis, I conclude that mangroves are significantly impacted by global change drivers such as sargassum brown tides, rising temperatures, and local eutrophication. These impacts not only affect their functionality but also create a feedback loop of mangrove degradation. Additionally, mangroves play a role in decreasing local water pH and display various forms of reproduction as adaptive responses to their environment. The mesocosm experiments employed throughout this thesis have played a vital role in addressing key questions regarding mangrove functionality and the underlying biogeochemical processes. The knowledge generated in this thesis fills critical gaps in understanding mangrove ecology and can inform management practices for more efficient restoration and conservation of mangroves within connected tropical ecosystems.

Resumen

Los manglares son componentes esenciales de los ecosistemas costeros tropicales que aportan multitud de bienes y servicios ecosistémicos y contribuyen así al bienestar de la sociedad y la naturaleza. Sin embargo, el futuro de los manglares se ve amenazado por los impactos del cambio global, que se intensificará en los próximos años y que está a su vez provocado por las actividades humanas y el cambio climático. El mundo está cambiando, la temperatura aumenta, los océanos se acidifican, el mar se contamina. Estos factores, en combinación con la conversión y sobreexplotación de los manglares conducen rápidamente a su declive. Para conservar y reforestar los manglares de forma efectiva, es importante comprender los mecanismos que determinan su respuesta al cambio global. Si bien su papel en la mitigación del cambio climático y como barrera de defensa costera es bien conocido, los procesos biogeoquímicos que determinan las respuestas de los manglares al cambio global no se conocen en su totalidad, al igual que los mecanismos subyacentes de estas respuestas. Por tanto, esta tesis doctoral tiene como objetivo aumentar nuestro conocimiento sobre la funcionalidad de los manglares mediante el estudio de mecanismos biogeoquímicos en el contexto de la conectividad ecosistémica y el cambio global.

Esta tesis comienza con el tema de la conectividad en los ecosistemas costeros tropicales, en concreto con las interacciones biogeoquímicas entre manglares, praderas marinas y arrecifes de coral, una perspectiva que a día de hoy ha sido poco explorada. En el capítulo 2 cuantifico los estudios científicos publicados en el campo de la conectividad biogeoquímica y proporciono una visión de conjunto sobre las complejas interacciones y feedbacks que constituyen los ecosistemas costeros tropicales. También muestro que la acción humana altera directa o indirectamente las interacciones biogeoquímicas que conectan estos hábitats causando eutrofización, contaminación química, contaminación microbiana, acumulación de residuos (por efluentes urbanos e industriales, basura, plásticos) y rompe el equilibrio carbónico del agua de mar. Al analizar los artículos científicos seleccionados, me topé con varias preguntas sin responder que utilizo como punto de partida para los siguientes capítulos de la tesis.

En el capítulo 3 investigo el potencial de los manglares y sus suelos en la mitigación de las mareas de sargazo, las cuales representan una amenaza importante para los ecosistemas costeros tropicales, incluyendo los manglares. En este capítulo estudio la viabilidad de añadir hierro (Fe) al sedimento de manglar para formar un enlace químico con el sulfuro producido durante la descomposición anóxica del sargazo, a la vez que evalúo el impacto del sargazo en el estado de salud de los manglares. Si

bien los manglares están ampliamente reconocidos como primera línea de defensa contra desastres naturales (huracanes, tsunamis, etc.) y la acción humana (vertido de nutrientes, basuras, etc.), el capítulo 3 demuestra que los suelos de manglar no pueden mitigar los efectos de las mareas de sargazo, agregando hierro o sin él. Aunque existen experimentos anteriores que han demostrado la efectividad de agregar hierro al sedimento para mitigar las altas concentraciones de sulfuro en alcantarillados y ecosistemas de agua dulce, los resultados del presente estudio indican que este enfoque no es aplicable en ecosistemas marinos. Los niveles de hierro utilizados en este estudio no evitan la muerte de los manglares, en los cuales el sulfuro producido por sargazo tiene un impacto letal. Además, la presencia de sargazo en manglares transforma estos hábitats de sumideros en fuentes de gases de efecto invernadero, eliminando así el papel crucial de los manglares en la mitigación del cambio climático. Este estudio también demuestra que la emisión de dichos gases de efecto invernadero por parte del sargazo se reducen al agregar hierro de forma significativa.

Otra forma de conectividad en los ecosistemas costeros tropicales es la regulación del pH del agua de mar, en concreto, amortiguando el descenso del pH en beneficio de hábitats adyacentes como los arrecifes de coral. Sin embargo, el papel que juegan los manglares en la regulación del pH del agua mar sigue siendo motivo de debate científico. El capítulo 4 muestra el primer experimento *ex-situ* sobre el efecto de los manglares en el equilibrio químico del carbonato en agua de mar, aportando información sin las complejidades que conllevan los estudios de campo. Los resultados del capítulo 4 demuestran que las plántulas de manglar negro no aumentan el pH del agua de mar. De hecho, su presencia tiene el efecto contrario: los manglares dan lugar a una acidificación continua del agua, reducen la alcalinidad y la concentración de carbono inorgánico disuelto. Este hallazgo indica que las plántulas de manglar negro tienen un impacto negativo en el pH del agua de mar. A consecuencia de esto, los organismos sensibles a cambios en el pH del agua que viven dentro de los hábitats de manglares (como los corales) podrían estar preacondicionados a bajos niveles de pH.

La reforestación de manglares es una actividad esencial para contrarrestar su deforestación a nivel global. Sin embargo, existen varios obstáculos que limitan y dificultan la reforestación de manglares como puede ser la falta de conocimiento sobre la respuesta de los manglares al cambio global. El Capítulo 5 investiga la respuesta de los manglares a los efectos conjuntos de dos perturbaciones: el calentamiento global y la eutrofización. Los hallazgos de este estudio demuestran que ambas perturbaciones actuando simultáneamente dan lugar a un crecimiento sinérgico del tallo de las plántulas de manglar negro. Sin embargo, el crecimiento de las raíces no es proporcional al de los tallos, lo que causa un desequilibrio entre la biomasa destinada a las partes subterráneas y a las partes aéreas del manglar. Además, las plántulas expuestas a altas temperaturas y eutrofización exhiben tasas de asimilación más bajas, lo que indica una menor incorporación de carbono a sus tejidos de rápido crecimiento (tallos). Estos resultados parecen indicar que las plántulas de manglar expuestas a ambas perturbaciones son más débiles, tienen sistemas radiculares pequeños y tallos largos, lo que las hace más vulnerables a las fuerzas físicas costeras (olas, mareas, viento). Por tanto, se recomienda seleccionar áreas para la reforestación del manglar negro con una baja concentración de nutrientes y resguardadas del fuerte oleaje en base a las previsiones del futuro calentamiento global. Tener en cuenta estos factores contribuirá a un mayor porcentaje de éxito y también mayor efectividad de los proyectos de reforestación del manglar negro.

Además de la respuesta biológica de los manglares a las perturbaciones múltiples descritas en el capítulo 5, los manglares también exhiben distintas estrategias reproductivas como forma de interacción con el entorno. El capítulo 6 trata sobre el descubrimiento de la poliembrionía en el manglar negro, un fenómeno muy poco común que consiste en el desarrollo de dos plántulas independientes a partir de un único propágulo. Esta estrategia reproductiva, poco común en manglares, les permite duplicar la producción de plántulas invirtiendo la misma cantidad de energía y favoreciendo su proliferación.

En la Síntesis (capítulo 7) concluyo que los manglares se ven enormemente afectados por el cambio global, con factores concretos como las mareas de sargazo, el aumento de la temperatura por calentamiento global y la eutrofización de mares y costas. Los impactos del cambio global no solo afectan a la funcionalidad del manglar, sino que también generan in ciclo de procesos climáticos en cadena que dan lugar a la degradación continua del manglar. Los manglares desempeñan un papel en la regulación del pH del agua de mar y llevan a cabo varias estrategias reproductivas como respuesta adaptativa a las condiciones de su entorno. Además, los experimentos en mesocosmos empleados en los capítulos de esta tesis han jugado un papel imprescindible, permitiéndome abordar preguntas clave sobre los manglares y su papel en los ecosistemas costeros tropicales. La información presentada en esta tesis llena varios vacíos de conocimiento sobre los procesos subyacentes en la ecología del manglar. Este conocimiento se puede aplicar directamente a la gestión, reforestación y conservación de los manglares como un componente fundamental de los ecosistemas costeros tropicales.

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“*No pretendamos que el éxito sea una norma porque es una excepción.*”
- Rafael Nadal Parera

List of publications

Cobacho, S. P., Grol, N. A., Dros, T. A., Dekkers, D. T., & Christianen, M. J. (2022). First evidence of polyembryony in black mangrove Avicennia germinans. *Botanica Marina*, 65(5), 321-324.

Cobacho, S. P., Wanke, S., Konstantinou, Z., & El Serafy, G. (2020). Impacts of shellfish reef management on the provision of ecosystem services resulting from climate change in the Dutch Wadden Sea. *Marine Policy*, 119, 104058.

Cobacho, S. P., Navarro, L., Pedrol, N., & Sánchez, J. M. (2018). Shading by invasive seaweeds reduces photosynthesis of maerl from the Ría de Vigo (NW Spain). *Botanica Marina*, 61(5), 453-457.

SENSE

Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment

DIPLOMA

for specialised PhD training

The Netherlands research school for the
Socio-Economic and Natural Sciences of the Environment
(SENSE) declares that

Sara Pino Cobacho

born on the 28th of March 1994 in Zaragoza, Spain

has successfully fulfilled all requirements of the
educational PhD programme of SENSE.

Wageningen, the 5th of December 2023

Chair of the SENSE board
Prof. dr. Martin Wassen

The SENSE Director
Prof. Philipp Pattberg

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The SENSE Research School declares that **Sara Pino Cobacho** has successfully fulfilled all requirements of the educational PhD programme of SENSE with a work load of 41.0 EC, including the following activities:

SENSE PhD Courses

- Environmental research in context (2019)
- Research in context activity: 'Assisting with course AEW-51806 Introduction to Marine and Estuarine Ecology' (2020)

Other PhD and Advanced MSc Courses

- Remote Sensing, Wageningen University (2020)
- Brain friendly working and writing, Wageningen Graduate Schools (2019)
- Critical thinking and argumentation, Wageningen Graduate Schools (2019)
- Molecular Organic Biogeochemistry, Royal Netherlands Institute for Sea Research (NIOZ) (2020)
- Research Data Management, Wageningen University (2020)
- Basic statistics (2021), Linear Models and Generalized Linear Models, PE&RC (2021)
- Supervising MSc and BSc thesis students, Wageningen Graduate Schools (2021)
- Career Orientation, Wageningen Graduate Schools (2022)

Management and Didactic Skills Training

- Supervising 6 MSc students with thesis (2020-2022)
- Setting-up tropical marine plant nursery in Wageningen (2020-2021)
- Teaching in the B/MSc courses 'Marine Systems (2020) and 'Trending Topics in Biology and Chemistry of Soil and Water' (2021)
- Teaching in the BSc/MSc course 'Introduction Marine and Estuarine Ecology' (2021, 2022)
- Workshop preparation "Improving PhD supervision in AEW" (2022-2023)
- Talk at teacher conference: Nascholingsmiddag Klimaatverandering, NESSC, The Netherlands (2023)

Oral Presentations

- *Tropical Blue Carbon Cascades.* NESSC day 2020, 11 March 2020, Utrecht, The Netherlands
- *Facilitative interactions across tropical coastal habitats in a transforming world.* Netherlands Annual Ecology Meeting 9-10 February 2021, Online
- *Higher temperature and nutrient concentrations may decrease black mangrove seedling stability during coastline recovery.* European Geoscience Union Assembly, 23-28 April 2023, Vienna, Austria

SENSE coordinator PhD education

Dr. ir. Peter Vermeulen

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