UNIVERSITY OF AMSTERDAM

MSc Biological Sciences
Freshwater and Marine Biology

How does pore water H_2S affect mangrove restoration?

Matteo Bravo
ID student: 13186841

March 2022
42 ECTS Credits
August 2021 – April 2022

Supervisor:
drs. Sabine Engel

Assessor:
Prof. dr. Gerard Muijzer

Examiner:
dr. Arie Vonk

Abstract

Despite their critical importance and the numerous efforts to protect, conserve and restore mangrove forests, these ecosystems are still disappearing at worrying rates around the world. Alongside these losses, many restoration projects fail to achieve their target. This is often a consequence of ignoring why the natural recovery has not occurred in the first place. In this regard, hydrogen sulfide (H_2S) and its typical rotten egg smell are omnipresent components in mangrove sediments. Nevertheless, too little attention has been given to its influence on the mangrove seedlings when transplanted to an area with a different sulfide concentration compared to their original habitat. Thus, this study aims to determine the sulfide tolerance of the red mangrove (*Rhizophora mangle*) and black mangrove (*Avicennia germinans*) using experimental nurseries in areas with high and low sulfides. The results reveal that high sulfide levels (> 20 mM) have serious effects on the growth of *R. mangle*, while having no significant effect on *A. germinans*, unveiling its ability to tolerate higher concentrations. Replanting *A. germinans* seedlings can facilitate success when restoring degraded and highly sulfidic areas. Additionally, this study provides a novel analysis of how microbial communities of the rhizosphere of *A. germinans* affect the survivability of mangrove seedlings in restoration projects.

Keywords: Mangrove · Hydrogen sulfide · Restoration potential · Microbial communities

---

Table of contents

1. Introduction

1.1 Context

Mangroves are flood- and salt-tolerant trees that inhabit intertidal areas such as seashores, riverbanks, shallow water lagoons and estuaries between approximately 30° North and South of the Equator (Tomlinson, 1986). Such areas are usually inhospitable and even hostile for most plants due to a synergy of stressful environmental factors, including storms, heavy rains, high levels of salinity, waterlogged soils, shifting sediments and air exposure (Spalding, 2010), but not for mangroves. These plants and shrubs have evolved special adaptations to survive and cope with numerous physiological challenges. In ideal conditions, mangrove forests can extend along many tropical and subtropical regions for thousands of square kilometres. As of 2016, mangroves were found in 123 countries, covering a total area of 136.714 km^2 (Worthington and Spalding, 2018), about the size of Greece. Despite being a relatively minor forest cover (less than 0.4% of the world's forests – FAO, 2007), mangrove forests are a fundamental ecosystem, home to a plethora of other plants, animals, and microorganisms. Mangrove forests also supply a plenitude of priceless ecosystem services to more than 100 million people that inhabit coastal areas (United Nations Environment Programme (UNEP), 2014). Finally, mangroves play an important role in mitigating climate change in terms of sequestering carbon dioxide from the atmosphere and storing it in their biomass and soils for many thousands of years (Worthington and Spalding, 2018).

Like any other land or marine ecosystem, the dynamics and the basic structure of a mangrove ecosystem cannot be unravelled and understood simply by looking at its main component – plants. Related to mangroves and their occurrence is the soil (or sediment depending on the morphology of area) in which they grow. Mangrove soils, like other wetland soils, are typically known for the rotten egg odour which permeate the air of these environments. The smell is due to the presence of hydrogen sulfide (H_2S), which is naturally produced by sulfate-reducing bacteria (SRB) through sulfate reduction of organic matter under anaerobic conditions. Indeed, the typical characteristics of mangrove soils make them the perfect substrate to enhance the production of H_2S. In such conditions, the newly formed sulfides can also react and precipitate metal ions to form a variety of metal sulfides (El Bayoumy et al., 1999; Kryger and Lee, 1995): for instance, FeS, which gives many anoxic sediments their blackish colouration. The distribution of soil's sulfides is regulated by a multitude of factors such as tidal movements, weather, bioturbation, organic matter content, sediment ageing, ground elevation, and oxygen fluxes from the roots to the rhizosphere (Lugo & Snedaker, 1974; Alongi et al., 1992, 1993; Krauss et al., 2008; Wang et al., 2011). Alone and combined, all these agents play a role in the occurrence of mangroves, and thus, the plant's responses are as diverse as the environments in which they grow. To cope with these factors, mangroves have developed many adaptations that give them a wide range of tolerance. In all plants, including mangroves, H_2S has versatile functions that can cause direct or indirect effects, but only recently researchers have assessed its importance in various physiological processes. At high concentrations, H_2S acts as a phytotoxin, inhibiting aerobic respiration and nutrients uptake (Zhang et al., 2017; Fakhari et al., 2019; Li et al., 2021). At low concentrations, H_2S is relevant in promoting root growth, plant development, and controlling biotic and abiotic stress responses (Chen et al., 2011; Jin et al., 2013; Fu et al., 2018; Luo et al., 2020).

The many threats that mangroves face have made them, together with coral reefs and tropical rainforests, among the most threatened ecosystems. Although rates of loss have declined considerably (from 1.04% per year in the 1980s, 0.72% in the 1990s and 0.21% between 1996 and 2016, data from Worthington & Spalding, 2018), in just over twenty years, mangrove forest cover decreased from 181.000 km^2 (Twilley, 1995) to approximately 136.714 km^2 (Worthington and Spalding, 2018) worldwide. These losses are largely attributable to human activities, which reclaim indiscriminately the land once occupied by thriving forests for urban development, shrimp farms, aquaculture, and overexploitation of timber (Romañach et al., 2018). Consequently, deforestation for anthropogenic uses is likely to have long-term and adverse consequences for the entire ecosystem functions and both the natural and local human communities that mangals support. To mitigate this, national governments, NGOs, and groups of volunteers have started leading local initiatives for their protection, including plans for conservation, rehabilitation, and restoration. For clarity, the concept of "restoration" is intended here as any process that aims to revert a system to pre-existing conditions (*sensu* Lewis, 1990), including the natural recovery of the system itself following the principle of secondary succession (which depends on mangrove propagule availability). Over the last fifty years, numerous efforts have been performed in many parts of the globe to set up efficient restoration plans using innovative techniques when a natural recovery does not seem possible (Lewis, 2005). Traditional techniques for mangrove restoration follow the same guidelines for any other terrestrial forest, including collecting seeds, planting them in suitable areas usually referred to as "nurseries" and transplanting the small seedlings to the desired location after a definite period. Yet, finally restoring an ecosystem is a difficult achievement. The causes that account for restoration failures are numerous, highlighting the complexity of the restorative path (Kodikara et al., 2017). That is because a combination of physical and chemical factors must be considered in advance while many restoration projects move immediately into replanting mangroves without determining why the natural recovery has not occurred. Past restoration attempts have considered changes in the abiotic environment including salinity, soil properties and waterlogging (Macnae, 1969; Thom, 1967; Odum 1975; Krauss et al., 2008; Cheng, 2015), propagule dispersal characteristics (Rabinowitz, 1978), interspecific competition (Ball, 1980), propagules predation (Smith, 1987), seasonal changes in flood duration (Carlson et al., 1983) and hydrological regime (Lewis, 2005). However, no major implementations have been made, to date, to consider H_2S as a relevant factor for failing in mangrove restoration plans and its complete influences still are to be elucidated.

1.2 Objectives

The main target of this research is to investigate the effects of pore water H_2S on the growth of two different mangrove species, *Rhizophora mangle* and *Avicennia germinans*, and obtain experimental evidence of the relative importance of sulfides on their zonation. On the island of Bonaire, current restoration plans foresee replanting *R. mangle* in low-saline, open-ocean settings, while *A. germinans* in intertidal, highly saline areas that no longer support the presence of *R. mangle* stands. Thus, this study aims to support these restoration projects and clarify the distribution patterns of the two mangrove species based on sulfides distribution. Additionally, including *A. germinans* in the outline of future restoration plans of degraded areas - or where conditions are no longer suitable for the survival of *R. mangle* - could potentially represent the solution for their restoration. Finally, this research aims to explore the difference in the composition of the microbial community in the

rhizosphere of the two mangrove species and in particular the presence of sulfate-reducing and sulfide-oxidizing bacteria in the sediments.

2. Materials and methods

2.1 Study area

Bonaire (12°12'6.8" N, 68°15'44.5"W) is part of the Dutch Caribbean and it is located about 80 kilometers north of Venezuela. The island has a semi-arid climate and receives roughly 500 mm of precipitation annually (Observation Station: Flamingo Airport (Lat: 12.1333 | Long: -68.2833). The area chosen for the experimental setup was a natural and unvegetated pool (12°01'48.7''N, 68°15'14.9''W) of around 1.330 m^2 on the South coast of Bonaire (Fig. 1). Due to its vicinity to the place of interest known as "Red Slave", the pool will also be called "Red Slave (RS)" in this report. Bonaire's South coast displays a minor and non-homogeneous cover of *R. mangle* (and very scattered specimens of *A. germinans* and *Languncularia racemosa*) and other mangroves-related and not-related shrubs. The natural pool is not directly exposed to the tidal movement, but the water circulation is guaranteed by the semi-open connection to the ocean (personal observation) and, potentially, it could run dry at exceptionally dry periods. Additionally, the pool presents a peculiar layout because, within it, two very distinct conditions can be found: although not separated by any natural barrier. The side further from the ocean is characterized by high sulfides concentration (marked as Red Slave A - "RSA"), while the one closer to the ocean displays a lower sulfide concentration (marked as Red Slave B - "RSB"). The presence of sulfides can be easily detected by the characteristic rotten egg smell. The second difference between the two areas lies in the sediment composition: although the base was solid limestone in both areas, at RSA the rocky layer was covered with thick and homogeneous mud, while at RSB the sediment above was looser, composed of a heterogeneous layer of coral rubble with fine mud on top. In both areas, the sediment was occasionally black, indicating a strong presence of metal sulfides. Unidentified small mollusks, shrimps and fish were also inhabiting the pool. In addition, a moderate presence of the algae *Dasycladus vermicularis* was observed at RSB. Green algae like *D. vermicularis* are known to oxygenize the sediments by means of root leaking (Chapman, 2013). Besides the cited agents and because of the physical-chemical layout of the area, I was able to test the two species across an array of different environmental conditions and, simultaneously, to focus the research primarily on the effects of H_2S on the plants, avoiding confounding factors. The choice of the experimental area was further motivated by the outcomes of previous replantation attempts: during a 2019 experiment to assess the survival of mangrove seedlings along Bonaire's South coast, *R. mangl*e (~ 1 or 2 years old) did not survive when planted in high sulfide areas, while, still today, are flourishing in areas with low sulfides.

Figure 1. **Location of the study area**. The satellite images (**1A** and **1B**) show the island of Bonaire and its location in the Caribbean Sea (orange mark). Image (**1C**) is the magnification of the small orange square in (**1B**) and shows the natural pool (Red Slave) divided into two areas: **RSA,** in orange**,** with **high sulfide concentration** and **RSB,** in light-blue, with **low sulfide concentration.** The black arrows stand for the location of the four experimental nurseries: RA & RB = *R. mangle***;** BA & BB = *A. germinans*. (**1D**) is a picture of the natural pool at Red Slave "RS". North of the pool there is a protected bird's nesting site, while in the south it is an open-ocean setting. The area closer to the ocean is RSB, while on the opposite side, where I took the photo, was RSA. Satellite images provided by Google Earth® .

2.2 Description of the two mangrove species used in the experiment

Two of the three mangrove species that commonly occur in the Caribbean have been used for this experiment: *R. mangle* and *A. germinans*. *R. mangle* (or red mangrove, Fig. 2A) is an evergreen tree that is generally 5-10 m tall usually growing right along the water's edge and therefore subject to fluctuations in the water level and salinity, ranging from 0 to 90 ppt (Hill, 2001). In the adult plant, its aerial roots system stretching up to 4 m long is fundamental (Fig. 2B), creating a stable substrate that consolidates the soil, giving extra support and protection to the plants, and shaping enough room for organisms to live within, such as fish, molluscs, and crabs. Covered in lenticels, these small holes on the roots are crucial to help the tree withstand the risk of hypoxia by allowing a direct supply of oxygen during low tide (Duke, 1983). Additionally, *R. mangle* is a viviparous plant and its fruit – the propagule – is a small living mangrove that can float for extended periods (over a year) in saltwater without rooting if conditions are not suitable. *A. germinans* (or black mangrove, Fig. 2C) is generally shorter than *R. mangle* (2-3 m) and it is distributed more inland, in subtidal zones, in which it thrives thanks to its peculiar root structures, the pneumatophores, which vertically spring up from the ground (Fig. 2D). A single tree may have more than 10.000 pneumatophores, allowing the plant to breathe when water levels are high and in very hypoxic soils. In contrast to *R. mangle* which copes with high salinity excluding the salt at the root-substratum interface, *A. germinans* uptakes the seawater through its roots, excreting excess salt through salt glands, or pores, located on the surface of leaves, usually forming salt crystals. Like *R. mangle*, *A. germinans* is viviparous, but its seed is smaller, rounded and enclosed in two fleshy leaves which open when ripe. On Bonaire, occurring almost exclusively at the water-land interface, *R. mangle* seedlings thrives in areas with lower sulfide concentrations compared to *A. germinans*, which naturally exist in intertidal areas with generally lower water flow regime, anoxic conditions, and a higher decomposition rate.

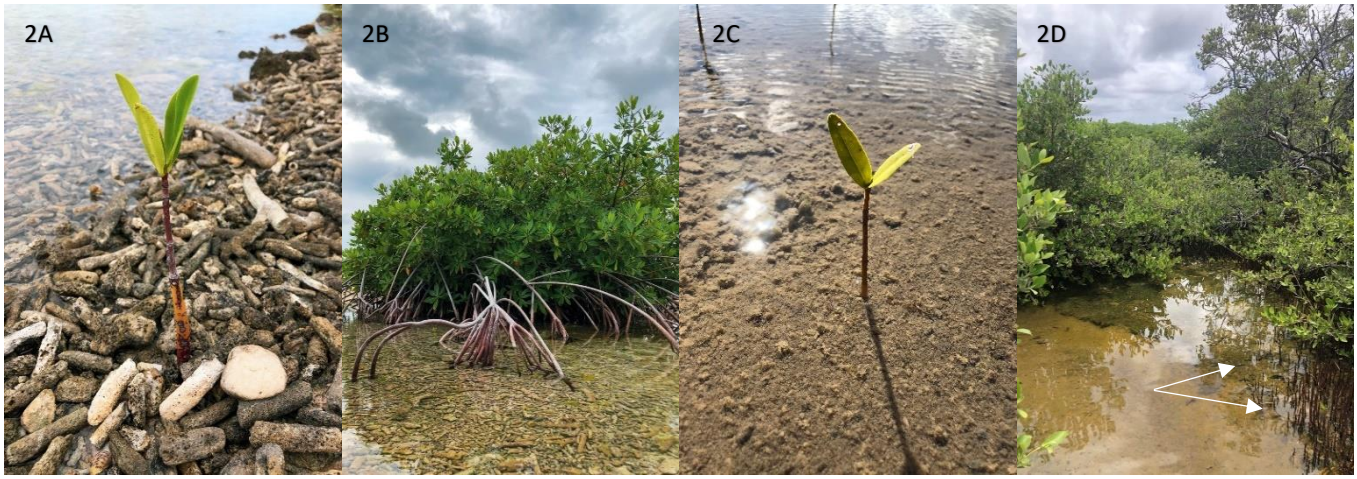


Figure 2. (**2A**) A *R. mangle* seedling that has naturally sprouted on a coral-rubble shore on Bonaire's south coast; (**2B**) a stand of adults *R. mangle* with the typical branching root system. (**2C**) *A. germinans* seedling and (**2D**) a group of *A. germinans* with the visible pneumatophores coming up from the sediment (bottom right, white arrows).

2.3 Experimental design

Two experimental nurseries containing ten mangrove seedlings each of similar age (5x *R. mangle* and 5x *A. germinans*) were set-up, respectively, at RSA and RSB. The one-year-old seedlings were retrieved from the nurseries that Mangrove Maniacs have created within the extensive network channels of Lac Bay - their natural habitat - and were all in good health. Before planting, the seedlings were carefully removed from the biodegradable bags in which they were growing. The average height for *R. mangle* was 33.3 cm at RSA and 30.2 cm at RSB, while for *A. germinans* was 13.8 cm and 13.6 cm. All the seedlings have been planted 50 cm away from each other to avoid interference during measurements.

2.4 Pore water H_2S sampling and analysis

Although it is relatively simple to detect the presence of sulfides due to their characteristic smell, the two areas were tested with a HACH® Hydrogen Sulfide Test Kit (Model HS-C, provided by CRC® – Centro Ricerche Chimiche (IT)), to ensure the presence of sulfides (Fig. 3). Pore-water samples were obtained using a Rhizon sampler (Rhizosphere Research Products, Wageningen) connected to a 10 mL plastic syringe and placed at around 10 cm depth below the soil-water interface and less than 3 cm next to the plants, where most of the roots of both species were found (Fig. 3). Additionally, pore water samples were also collected from the adjacent sediments as a control treatment (20 cm away from the plants), to assess if the presence of the nursery-raised seedlings would finally pose a significant difference in the sulfide concentrations in the two areas. The presence of *Dasycladus vermicularis* was controlled by manually removing the algae from the nursery areas to avoid additional oxygen diffusion from its roots. Before inserting the Rhizon into the soil, a small hole was made using a stick to prevent any damage to the micro filtering membrane. To start the suction, a vacuum was created using a piece of wood holding the piston, and each extraction took between 20 - 30 min. Between 8 and 10 mL of pore water were extracted from each sampling point and collected into 5 mL tubes which were already pre-loaded with 0.1 mL of a 2 M solution of Zinc (Zn) -acetate prepared on the first day of sampling and stored at 4 °C. This was of the uttermost importance to prevent oxygen from entering the samples and so oxidizing the sulfide (S^{2-}) to sulfur (S). Immediate shaking followed to allow sulfides to react with Zn to form insoluble Zinc sulfide (ZnS) which could be seen as a white suspended mucilage. A total of 90 pore water samples were collected during the three-week experiment (30 per week) and stored at 4°C until being carried to the University of Amsterdam for analysis. All the pore-water samples were transparent with zero or little presence of particulate residue in just a few samples. This was crucial for the later H_2S analysis since organic matter is absorbing the formed methylene blue. The protocol I was using to prepare the Zn-acetate solution (APAT IRSA CNR_4160) foresaw using 2 mL of solution per 1 L of the sample, so the correct aliquot would have been 0.01 mL for 5 mL tubes, but due to gear limitations, 0.1 mL was the lowest resolution I could obtain. Consequently, I used 10-times more Zn-acetate solution in the 5 mL tubes, but this did not pose any differences in the final results.

Before the sulfide analysis, each of the 90 samples was centrifuged for 2 min to separate the supernatant from the insoluble pellet of ZnS. After removing the supernatant, the final volume of the samples was 0.5 mL. The ZnS pellet was then resuspended for 30 sec using a Vortex-Genie 2 (Scientific Industries, Bohemia, NY, USA) and 100 µL of the homogeneous mix was injected into a 0.4 mL 10% (w/v) Zn-acetate solution preloaded into a 2.2 mL Eppendorf tube so that the final

volume was 0.5 mL. Then 1.28 mL of demi-water was added to the fixed samples. To develop the methylene blue, two sulfide reagents were added one after another to the samples. The first reagent consisted of 2 g of dimethylparafenyldiamine (oxalate salt) diluted in 200 mL of demi-water and 200 mL of concentrated H_2SO_4, the final volume adjusted to 1 L. The second reagent was obtained by diluting 10g of $Fe(NH_4)(SO_4)_2$ in 50 mL of demi-water and 2 mL of concentrated H_2SO_4, the final volume was adjusted to 100 mL with demi-water. Immediately after the injection and a little mixing, the blue colour started to form, showing the presence of the sulfides. H_2S was determined spectrophotometrically at 675 nm as methylene blue and the 10% (w/v) Zn-acetate solution as a blank.

2.5 Microbial community: rhizosphere core sampling strategy and DNA extraction

A total of 8 rhizosphere cores (two per species per area) and 4 soil cores (two per area) were collected from a depth of 0-10 cm, representing the full spectrum of the root zone of the plants. To obtain the rhizosphere cores from the soil, a 20 mL plastic syringe from which the top was cut-off was used (Fig. 3). Once inserted in the sediment and retrieved the core, the latter was carefully emptied into a 25 mL tube and kept frozen until analysis. At the University of Amsterdam, the DNA from rhizospheres and the bare sediments was extracted using the DNeasy® PowerSoil® Pro Kit (QIAGEN), following the manufacturer's instructions. The quantity and quality of the extracted DNA were measured with a NanoDrop. The nearly complete 16S rRNA gene was amplified using the Oxford Nanopore kit SQK-16S024 containing 24-barcoded 16S rRNA primers. Subsequently, the PCR products were cleaned using AMPure XP magnetic beads and the concentration was determined with the NanoDrop. The PCR products were normalized, pooled, and sequenced on a MinION flongle flow cell (Oxford Nanopore). The sequences were first analysed using the software tool NanoClass developed by Evelien Jongepier (UvA, Amsterdam), and subsequently in R.

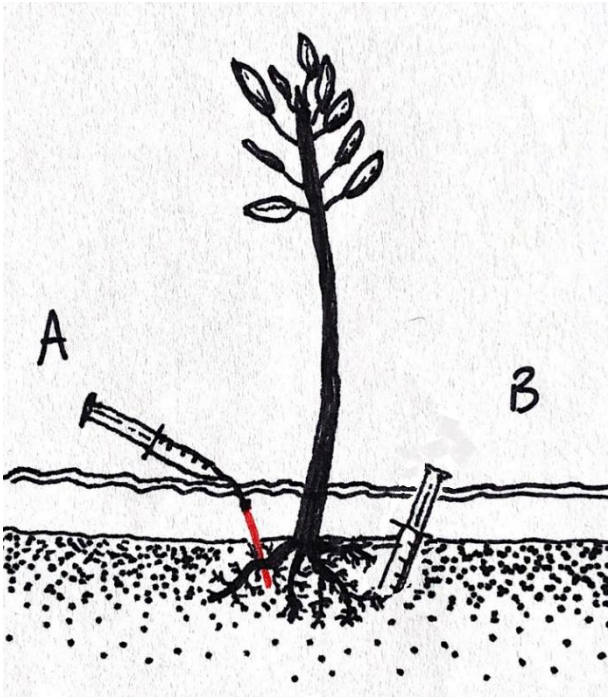


Figure 3. (A) Representation of the technique used to collect pore-water for H2S analysis, highlighting the Rhizon sampler in red; (B) The method used to collect rhizosphere core for microbial community analysis, using a plastic syringe which tip was cut off.

2.6 Additional measurements

Salinity, pH, dissolved oxygen (DO) concentrations and oxidation-reduction potential (ORP) were determined weekly using a Hanna® HI9829-00102 Multiparameter, while temperature and light intensity were constantly measured for three weeks using a HOBO® UA-002-64 Data Logger. The height of each seedling, the number of leaves and sediment/water level were also measured every week.

2.7 Statistical analysis

Statistical analyses included a series of paired t-tests used to evaluate the differences in the environmental parameters and the sulfide concentrations within and between the two areas. Logistic regression was also performed to determine the influence of environmental parameters, species, sulfide concentrations and the location of the two nurseries (RSA and RSB) on the leaf loss rate. Principal component analysis (PCA) revealed the distribution pattern of microbial communities of the rhizosphere of the two species and bare sediments. This analysis was performed in R with fviz_pca_biplot() function from the factoextra R package.

3. Results

3.1 Physico-chemical parameters

Despite being situated in the same natural pool, physico-chemical parameters were measured separately for the two nurseries for the best accuracy with the exception of light intensity and temperature. All the parameters are shown in Figure 4 and are listed in the supplementary Table 1. All the *in-situ* measurements (pH, salinity, ORP, DO) were performed between 11:30 AM and 2:00 PM on the first, second and third week, while environmental parameters (temperature and light intensity) were constantly measured every 8 minutes for the three weeks. Over the duration of the experiment, pH did vary among the two areas ($p < 0.02$), fluctuating between 8.51 and 8.58 at RSA, and 8.98 and 9.12 at RSB. Salinity was similar at both sites ($p > 0.07$), ranging from 38.22 to 42.19 psu at RSA and from 38.07 to 40.25 psu at RSB. Despite the very different values in the third week, ORP did not result to be associated with the areas ($p > 0.4$). ORP levels decreased sevenfold at RSA, from -22.7 mV to -170.8 mV, suggesting a strong reducing activity, while slightly increased at RSB, from -21.5 mV to -13.7 mV. DO varied significantly ($p < 0.03$) fluctuating between 24.3% and 86.3% at RSA and between 30% and 93.5% at RSB. Over the three weeks, the mean air temperature was 29.5 °C with a minimum of 25.2 °C during the night and a maximum of 37.8 °C during the day. Mean light intensity greatly fluctuated, with peaks up to 61600.91 lux and nadirs down at 5516.19 lux.

3.2 Hydrogen sulfide (H_2S) concentrations

A distinct and remarkable distribution pattern of the sulfide concentrations occurred at the study site (Fig. 5), showing a significant difference ($p < 0.05$) in the final sulfide concentrations of the two areas. Pore-water H_2S concentrations increased throughout the experiment independently of the area and the mangrove species (see Supplementary Table S2). Specifically, at the end of the third week, the sulfide concentrations almost doubled at RSA beneath *R. mangle* roots (R1-R5), from 10.21 ± 7.21 to 20.16 ± 2.86 mM (n=5), and nearly tripled for *A. germinans* (B1-B5), from 8.76 ± 6.56 to 23.55 ± 2.89 Mm (n=5). In contrast, at RSB, remarkably lower sulfide concentrations were observed for both species, from 0.05 ± 0.03 to 7.27 ± 4.05 mM (n = 5) for *R. mangle*, 0.57±1 and 9.62 ± 2.06 mM (n = 5) for *A. germinans.* Overall, beneath their roots, *R. mangle* seedlings showed 63.9% higher sulfide concentrations at RSA compared to RSB, while for *A. germinans* seedlings, the concentrations were 59.2% higher at RSA than at RSB. In the bare sediments (S1-S5), about 20 cm far from the plants, the H_2S levels increased from 15.50 to 21.60 mM (n = 5) at RSA and from 0.62 to 5.46 mM (n = 5) at RSB. A paired t-test showed a significant ($p < 0.03$) effect of *A. germinans* on the bare sediments at RSB at the end of the experiment, suggesting that black mangroves can increase the

sulfide concentrations in the sediment when the initial levels are low. In contrast, the seedlings of *R. mangle* showed no significant effect on bare sediments ($p > 0.1$) at RSB. No significance was detected at RSA for both species, indicating a negligible effect of the two mangrove species on the sulfide concentrations on bare sediments.

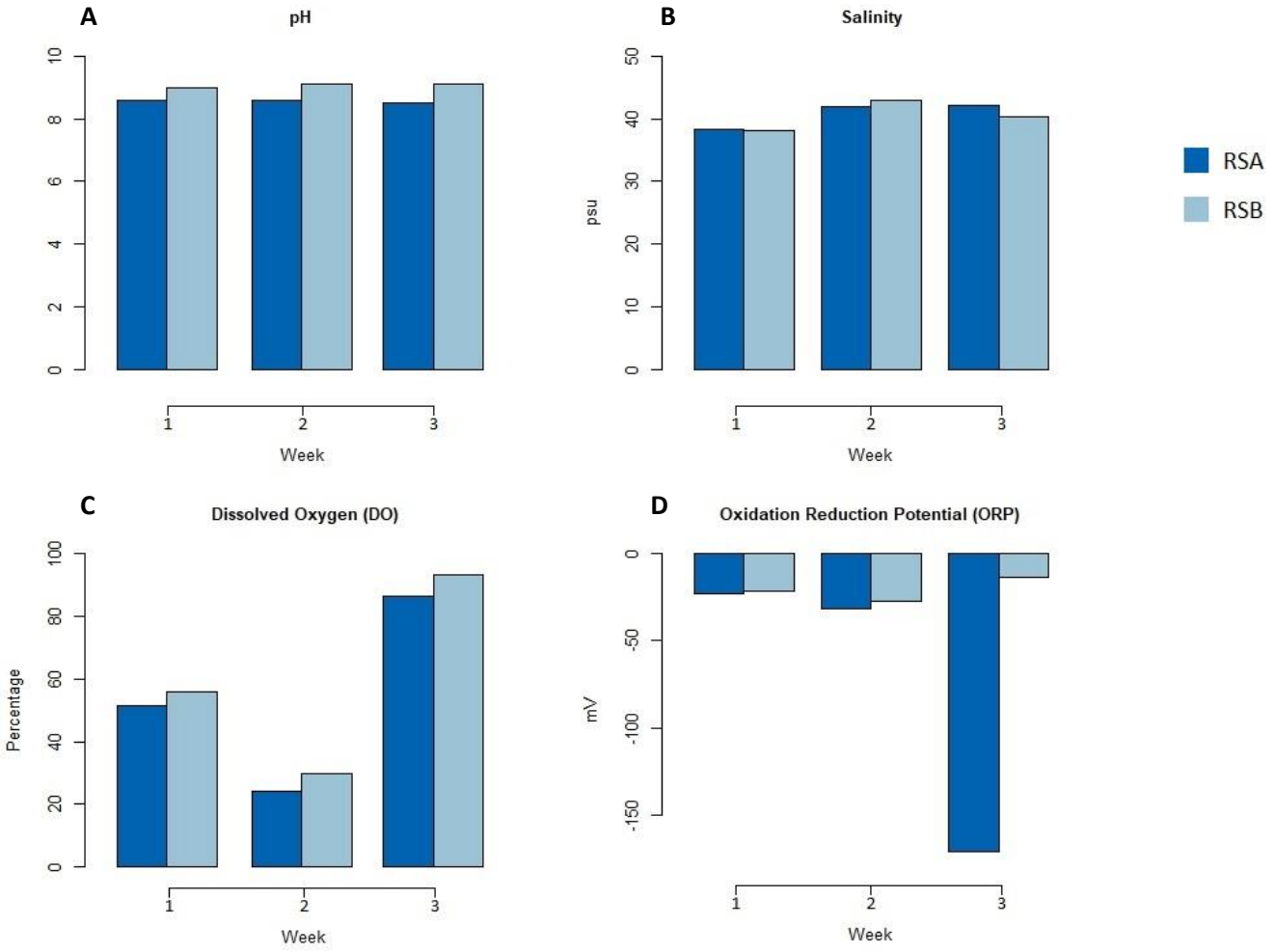


Figure 4. Barplots showing the environmental parameters measured in the two areas. Dark blue represent RSA while light blue RSB. (A) pH. (B) Salinity. (C) DO percentages. (D) Oxidation reduction potential (ORP).

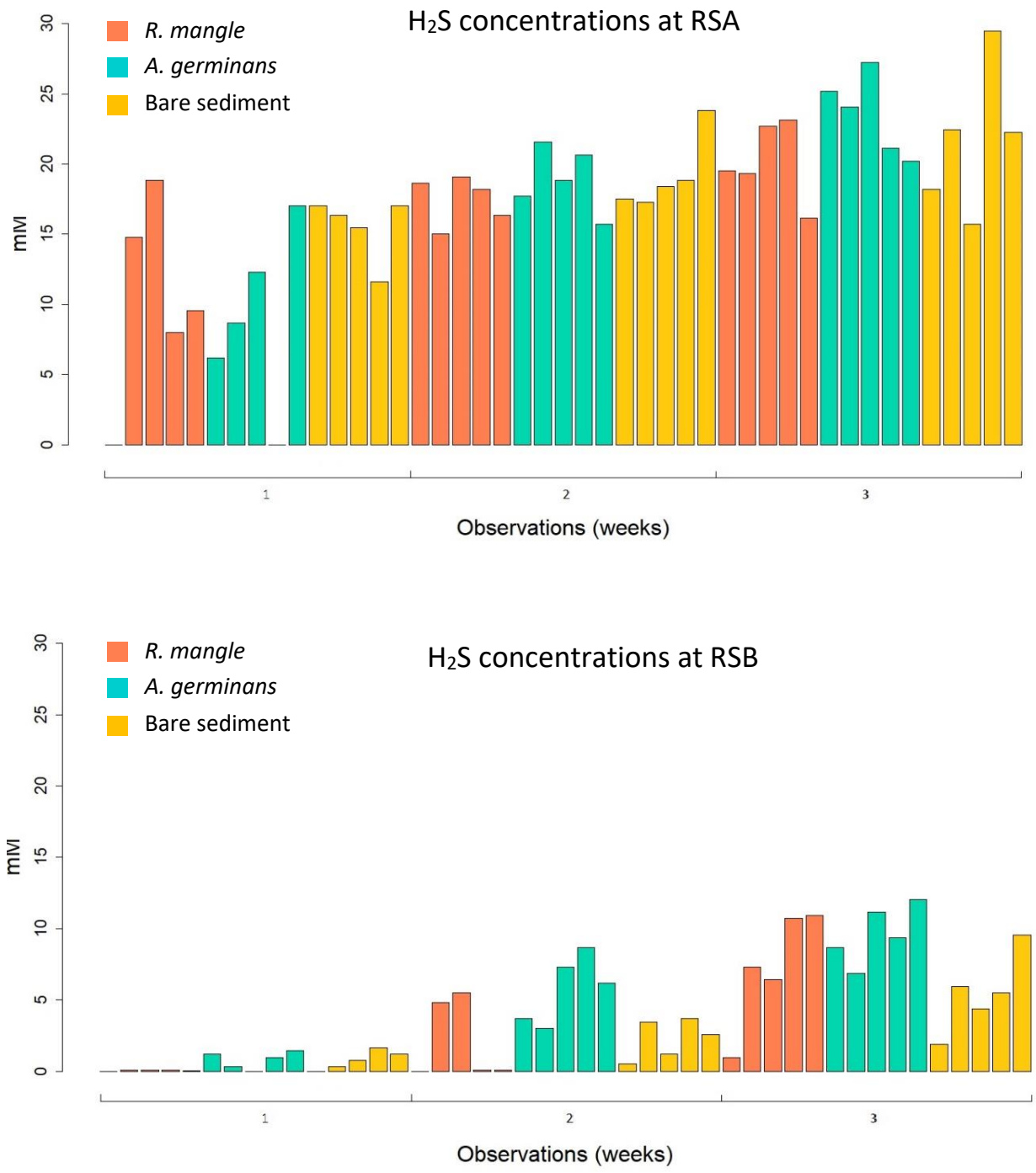


Figure 5. H_2S concentration in mM (bars) at the two study areas: RSA (above) and RSB (below). Negative values have been removed from the visualization and are more likely attributable to errors in detecting sulfide levels using the blue methylene method.

3.3 Physiological response to H_2S

To investigate the effects of H_2S on the growth of the two mangrove species, I examined the physiological condition and visible responses of the seedlings in terms of leaf loss rate. For clarity, previous observations from Mangrove Maniacs (Johnson, personal observation) have reported that it is erroneous to consider a seedling without leaves as definitely dead. There were few reports of seedlings that had lost all their leaves but managed to survive and sprout again during previous restoration attempts. Hence, it is still possible that after the end of the experiment, some seedlings

may have sprouted again, but it is very unlikely if conditions have not changed. At RSA, the two mangrove species showed remarkably different outcomes. At the end of the third week, *R. mangle* at RSA reported the worst result, with two seedlings that have lost all their leaves and three which have lost some leaves. *A. germinans* reported a lesser stress response, with only one seedling which has lost one leaf. On the contrary, at RSB, only two *R. mangle* seedlings and one *A. germinans* seedling reported a minor leaf loss. A Kaplan-Meier curve is used here to show the probability of not losing leaves in the three weeks (Fig. 6). Additionally, one seedling per species per area is shown in Fig. 7 for a better and more clear visual comparison of the effects of the pore-water H_2S. To evaluate the hypothesis of whether the leaf loss rate of both mangrove species (and their consequent decline) was a consequence of the different sulfide concentrations, logistic regression and a two-way ANOVA test were performed, showing a statistical significance ($p < 0.05$) between the mangrove species, leaf loss rate, sulfide levels and the two different areas. Because of the unvegetated nature of the pool and the absence of relevant organic matter inputs, the raising and different sulfide levels can be attributable to a more anoxic condition in the sediments or a strong microbial reducing activity (discussed below).

3.4 Microbial community structure in the rhizosphere and in bare sediment cores

A total of 147.600 sequences were obtained from the twelve rhizosphere samples that were collected on the third week of the experiment. The number of filtered reads per sample ranged from 8.085 for sample S1A to 13.392 for sample R1A. A one-way ANOVA was performed to test the significant difference ($p < 0.05$) in the microbial community between the two areas. Analysis of the relative frequency showed that the *Proteobacteria* was the most dominant phylum across the samples (Fig 8; see also Supplementary Figure 1 for a different visualization), accounting for the 30.7% of the total reads obtained and represented by the class of the *Alphaproteobacteria* (54.5%) and the *Gammaproteobacteria* (45.5%). *Cyanobacteria* was the second major phylum (25.3%), followed by *Desulfobacteria* (formerly belonging to the Deltaproteobacteria) (16.6%), *Bacteroidetes* (10.3%), *Planctomycetes* (3.7%), *Spirochetes* (2.9%), *NB1-j* (2.4%), *Actinobacteria* (1.9%), *Firmicutes* (1.5%), *Acidobacteria* (1.2%) and *Gemmatinomadetes* (1%). The microbial communities supported by the two mangrove species and within the two areas are notably distinct. The results from PCA were confirmed by one-way ANOVA, showing similarities ($p > 0.05$) between the microbial communities in the rhizosphere of *R. mangle* and in the bare sediment, while different communities ($p < 0.05$) between *A. germinans*. Sediment cores from *R. mangle* showed that *Desulfobacteria* were dominating at RSA (62%), while only a minor presence (7%) was found at RSB, whereas *Cyanobacteria* prevailed (47% vs 13% at RSA). *Proteobacteria* were also significantly higher at RSB compared to RSA (27% vs 8%). *Bacteroidetes* were equal in both areas (12%). Finally, *Planctomycetes* and *Spirochetes* were the least abundant phyla, accounting only for a minor percentage, respectively 2% and 3% at RSA and 3% and 4% at RSB. Rhizosphere cores retrieved from *A. germinans* revealed a distribution pattern of the major phyla similar between the two areas and which was dominated by *Proteobacteria* in both areas (47% at RSA and 41% at RSB, respectively). *Planctomycetes* were the second most abundant group at RSA (15% vs 10% at RSB) while *Desulfobacteria* were the second most abundant at RSB (21% vs 9% at RSA). *Cyanobacteria* (8%) and *Firmicutes* (4%) were only observed at RSB while NB1-j (9%) and *Acidobacteria* (5%) were only found at RSA. *Gemmatimomadota* (4% at RSA and 3% at RSB, respectively) and *Actinobacteria* (8% and 3%) were also found. Interestingly, *Gemmatimomadetes* were only observed in the cores of *A. germinans* and nowhere else. Microbial communities in bare sediments were not

significantly different in both areas ($p > 0.05$), and they were dominated by *Cyanobacteria* (41% at RSA and 40% at RSB respectively), followed by *Proteobacteria* (28% and 22%), *Bacteroidetes* (14% and 18%), *Desulfobacteria* (8% and 6%), *Spirochetes* (6% and 5%), *Planctomycetes* (3% and 5%). At RSB only, a minor percentage of *Acidobacteria* (3%) and *Firmicutes* (2%) was also found.

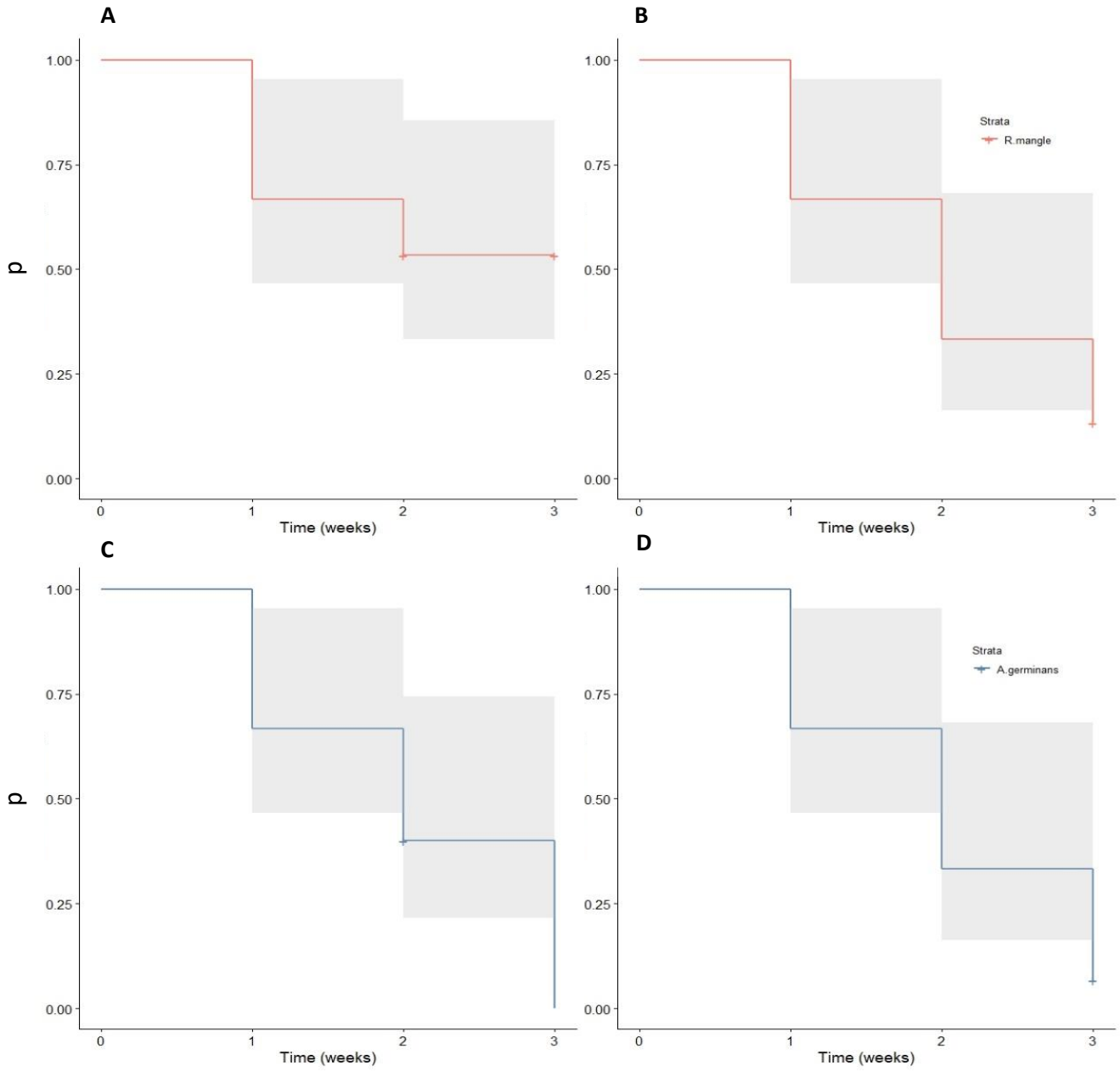


Figure 6. Kaplan-Meyer curves show the seedlings' probability (p) of not losing leaves in three weeks. At p = 1, all the plants have the same probability of losing leaves in the first week. p = 0 is the most desirable probability, indicating that plants with p close to 0 are the plants that most likely are not going to lose leaves. Shadowed areas are confidence intervals. (A) stands for *R. mangle* seedlings at RSA, and the data shows that after 3 weeks, plants have more than a 50% possibility of not losing leaves. In contrast, R. mangle at RSB (B) has less than a 25% probability of not losing leaves after the third week. For *A. germinans*, at RSA (C) the probability of not losing leaves after three weeks is close to 5% and about 10% at RSB (D)

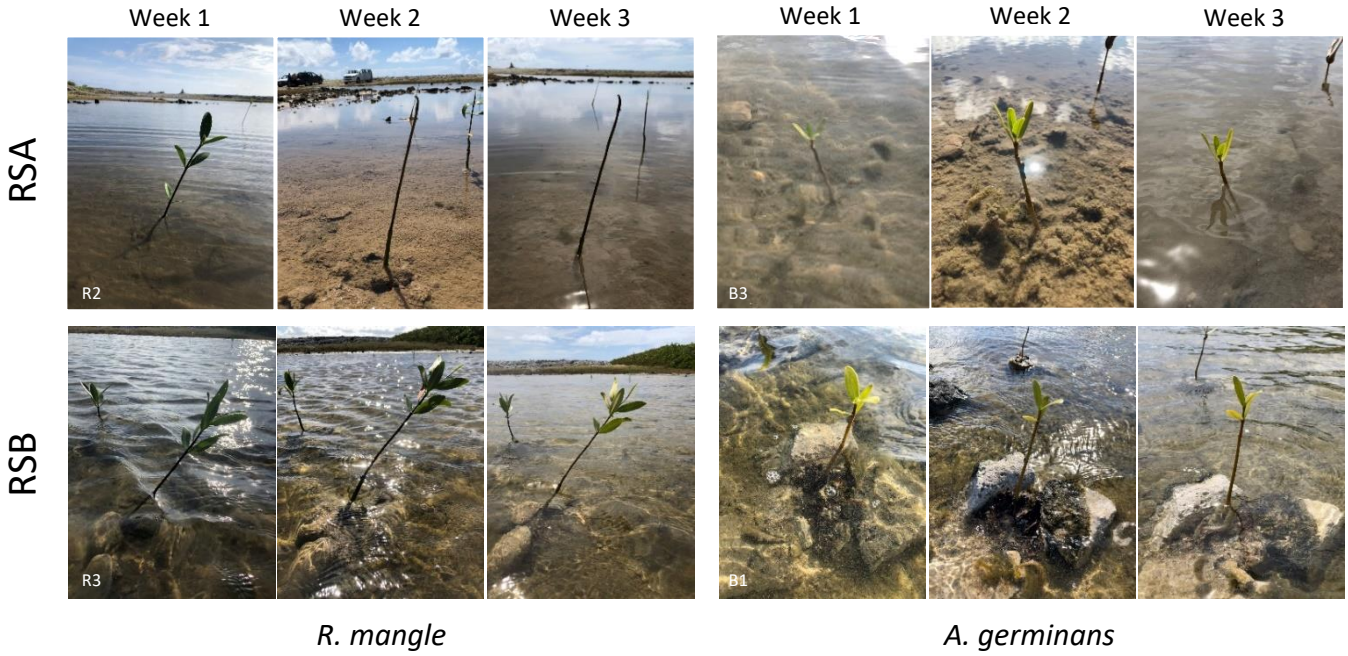


Figure 7. **A collage of photos of the seedlings of the two species taken in the first, second and third week in the two areas**. On the left side, *R. mangle* seedlings (R2 at RSA and R3 at RSB) and, on the right side, *A. germinans* seedlings (B3 at RSA and B1 at RSB).

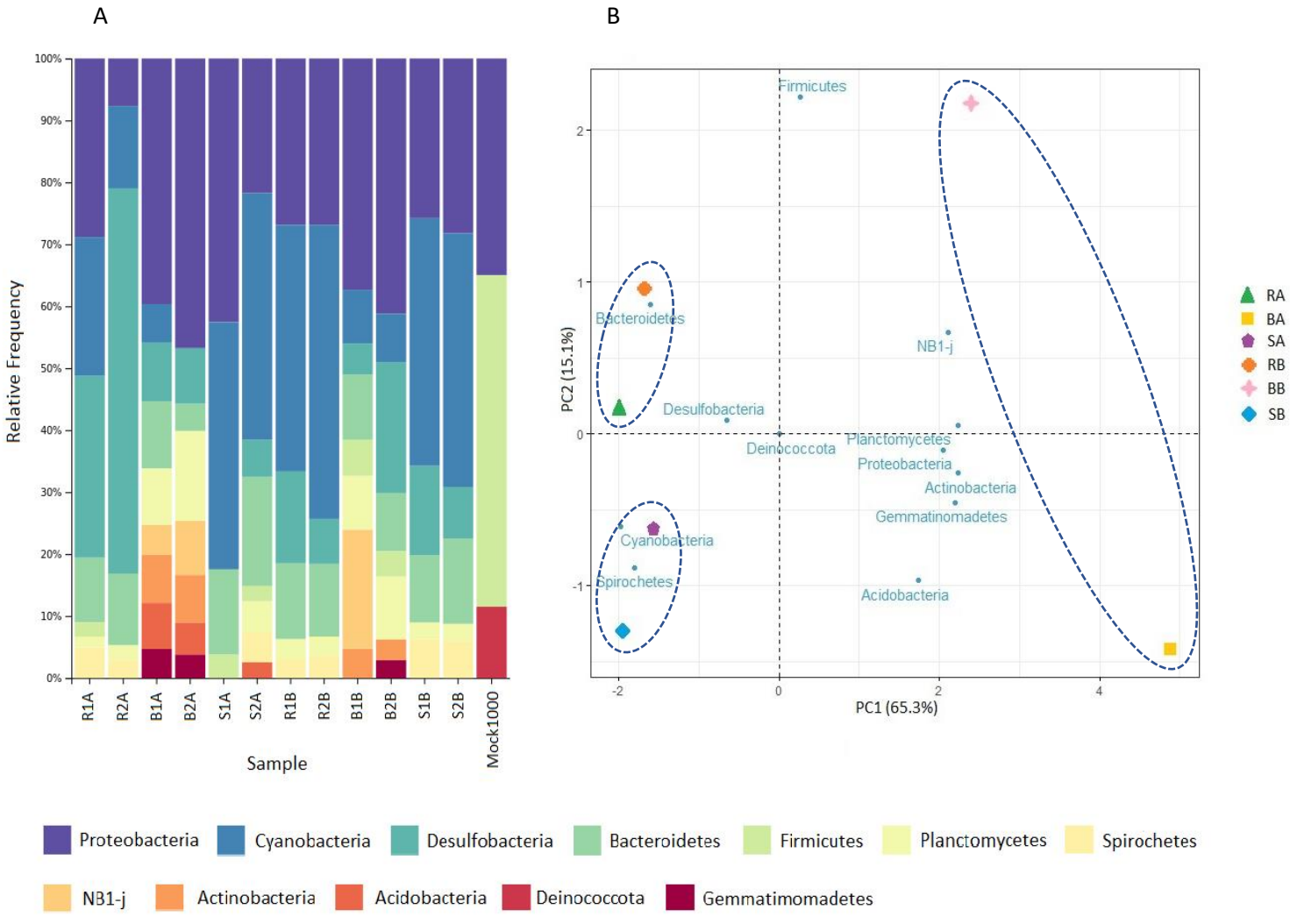


Figure 8. Continued

Figure 8. Continued

(A) Microbial community composition of the rhizosphere cores representing the relative abundance of the dominant microbial phyla. R1A, etc. Mock 1000 is a mock community consisting of 10 known microbial species in equal abundance (MSA-??, ATCC, USA). **(B) Principal component analysis (PCA)** of the microbial communities from the seedlings' rhizosphere (RA, BA, RB, BB) and bare sediments (SA, SB,). "R" stands for *R. mangle*, "B" for *A. germinans* and "S" for sediment; "A" for RSA (high sulfides) and "B" for RSB (low sulfides). The percentages of community variance in each axis are indicated in parentheses.

4. Discussion

In the present study, the effect of high, pore-water sulfide concentrations on two mangrove species was examined. Additionally, the microbial communities were investigated by extracting and isolating microbial genomic DNA retrieved from the rhizosphere cores of the two species. Unfortunately, despite being one of the three most common Caribbean species, data on the microbial consortia of the rhizosphere of *A. germinans* is lacking.

4.1 Sulfide tolerance

According to the literature, pore-water sulfide has disparate effects on plants. From promoting plant growth and root development to providing resistance against biotic and abiotic stress, sulfides are actively involved in many different physiological processes (Liu et al., 2021). Defining a tolerance threshold common for each response would probably be too ambitious, and most studies agree that the optimal concentrations that sustain mangroves are around 1 mM at the surface, while levels up to 5 mM have been demonstrated to have a detrimental effect (Lyimo et al., 2002; Li et al., 2021). In mangrove forests – areas that produce vast amounts of leaf litter – remarkably higher sulfide concentrations can be found, raising to 25 mM (Lyimo et al., 2002). What is still unclear is the full effect of sulfides on mangrove zonation, which has always been a matter of debate. According to Nickerson and Thibodeau (1985), *R. mangle* occurs in areas characterized by low sulfide concentrations (0.8 – 1 mM) or surrounded by bare sediments with similar sulfide concentrations. In contrast, underneath the pneumatophores of *A. germinans* trees, sixfold lower sulfide concentrations can be found (0.4 – 0.5 mM) compared to adjacent soils without pneumatophores (2.7 mM). The same investigators also demonstrated that roots of *A. germinans* have a more significant oxidizing effect on the rhizosphere than *R. mangle* (Thibodeau and Nickerson, 1986). They hence concluded that *R. mangle* was confined to areas of "pre-existing low sulfide concentrations". In contrast, McKee et al., (1988), demonstrated that sulfide concentrations beneath *R. mangle* roots might be significantly lower (0.3 mM) compared to adjacent bare sediment (1.6 mM), suggesting that both mangrove species can equally oxidize the soil and hence occur at comparable soil sulfide concentrations as long as their root systems are functional. In other words, the distribution pattern of the two mangrove species was certainly connected to sulfide concentration, but the definitive influence of pore-water sulfides on these patterns is not fully clear.

My results are only partially comparable to the cited studies because, having used only the seedlings of the two species, their root system was not fully developed, and roots represent an important site for oxygen diffusion from their tissue (Sherman et al., 1998). Thus, the data should not be confounded with the sulfide patterns of a proper mangrove forest, but they are fully representative of the tolerance experienced by transplanted mangrove seedlings. To support this, the experiment was designed to isolate only the sulfides and exclude other environmental parameters, because these were considered standardized in the two study areas. As a result, the short-term, physiological stress-response of losing

leaves is explicative of the different sulfide tolerance experienced by *R. mangle* and *A. germinans*. In this regard, it is then determined that a high sulfide concentration is a major contributor to the decline in the health of *R. mangle*. A t-test showed that only at RSB the difference between sulfide concentrations at the beginning and at the end of the experiment was significant ($p < 0.05$). At RSA, concentrations were chronically higher, reaching a level whereby they were almost comparable to concentrations at 30 – 40 cm in a natural mangrove forest in Tanzania (25 mM, Lyimo et al., 2002), highlighting a very anoxic condition. Overall, the presence of replanted *R. mangle* seedlings played a contrasting role in sulfide distributions in the two areas. *R. mangle* decreased sulfide concentrations by 14% at RSA while increasing them by 18% at RSB when compared to the bare sediment. Instead, *A. germinans* seedlings positively influenced sulfide levels by 20% at RSA and 41% at RSB. However, the only significant result ($p < 0.05$) was the latter. This does not necessarily mean that their influence is irrelevant, rather, since most of the current studies have investigated adult mangroves, more research focused on the contribution of young mangroves to bare sediments is necessary. Additionally, from a distinct perspective, this means that, if on the one hand *A. germinans* increases H2S levels in the pore water, on the other it can also tolerate higher concentrations compared to *R. mangle* at the seedling stage. The undeniably visible response is the significant difference between the leaf loss rate of the two species, clearly indicating the stress that both species are facing and responding to differently. At the end of the experiment, at RSA, *R. mangle* seedlings showed a severely stressed response with a leaf loss rate of 71.9%, compared to only 6.2% for *A. germinans*. At RSB instead, the leaf loss rate was 8.3% for *R. mangle* and 7.1% for *A. germinans*. Surprisingly, *A. germinans* seedlings were able to tolerate concentrations ten times higher than previously observed, reaching a maximum of 29.4 mM compared to the 2.7 mM observed by Nickerson and Thibodeau (1985) and the 1.7 mM of McKee et al., (1988), showing only small stress signs. This outcome is in contrast with a previous study in Singapore (Lee et al., 1996) where red mangroves seedlings have succeeded in highly sulfidic areas while black mangroves degenerated. It was then suspected that, because of the aerial roots, red mangroves could have been more adapted to soils characterized by elevated sulfide levels. These results, combined with the observations from the previously cited studies, confirm that the occurrence of both mangrove species is linked to pore-water H_2S concentration and reveals the better tolerance of *A. germinans* seedlings.

4.2 Microbial community structure of rhizospheres and bare sediment

The wide range of settings in which mangroves grow exposes them to diverse chemical-physical characteristics. Because of that, mangroves support various microbial communities in the surrounding sediment, in the water column and inside the plant's tissues (Feller et al., 2010; Lee et al., 2015). These communities, which are influenced by both abiotic and biotic factors (Constancias et al., 2015; Shang et al., 2017), are fundamental for the occurrence of mangroves (Holguin, 2001; Andreote et al., 2012). They play a significant role in sediment biogeochemistry and nutrient cycling, as well as providing defense against some pathogens and promoting cell death upon senescence (Holguin et al., 2001; do Carmo et al., 2011). In exchange, mangroves release root exudates which are being used by the microbial community as a nutrient source (Holguin et al., 2001). As demonstrated by culture techniques, numerous studies have already confirmed that mangrove sediments host typical microbial communities especially associated with the metabolisms of sulfur and iron (Nedwell et al., 1994; Sherman et al., 1998; Young et al., 2006). The solid relationship between microbial communities and mangroves could contribute to the success of a species at the expense of another. In this section, I

will concentrate mostly on the microbial phyla and classes that most participate in the reduction of sulfate and oxidation of sulfide and are consequently influenced by the presence or absence of oxygen. However, as for the previously discussed sulfide tolerances, these results represent the microbial communities of nursery-raised, transplanted seedlings and of the bare sediment at 'Red Slave' and not a mature mangrove forest.

The occurrence of the phylum *Proteobacteria* has been widely observed in mangrove sediments across different studies (Andreote et al., 2012; Wu et al., 2016; Santana et al., 2019; Nobrega et al., 2021). *Proteobacteria* are highly diverse and extensively distributed in marine environments, and three of the five classes were observed: *Alphaproteobacteria*, *Gammaproteobacteria* and *Desulfobacteria* (also known as *Deltaproteobacteria* according to the NCBI taxonomy). The class of *Alphaproteobacteria* includes several plant symbionts, and their unifying feature is that they are oligotrophs, adapted to live in low-nutrient environments. On the other hand, *Gammaproteobacteria* includes communities capable of decomposing organic matter and taking part in nutrient cycling in marine environments. Within *Gammaproteobacteria*, the genus *Thiohalocapsa* is part of the so-called "purple sulfur bacteria (PSB)" and was found in the bare sediments of both areas (RSA and RSB) and the rhizosphere of *R. mangle* at RSB. PSB are phototrophic bacteria capable of photosynthesis, but instead of using water as a reducing agent, they use H_2S, which is oxidized to elemental sulfur (Brenner et al., 2007). *Desulfobacteria* was the third most abundant group within the rhizosphere of the seedlings and in the bare sediment. This class belongs to the phylum *Desulfobacterota* that has largely been described as a crucial component of sulfate-reducing bacteria (SRB) in many marine sediments. SRBs are anaerobes (Kjeldsen et al., 2004) that perform anaerobic respiration using sulfate (SO^{2-}_4) as the final electron acceptor in the electron transport chain, reducing it to H_2S. In these anoxic sediments, sulfate reduction accounts for up to 50% of the mineralization of the organic matter present (Kristensen et al., 2008). The activity of SRBs was evident from the presence of sulfide peaks up to 29 mM in the bare sediments and was further supported by the low redox balance of -170.8 mV at RSA during the third week when rhizosphere cores were collected. The dominance (62%) of *Desulfobacteria* in the rhizosphere from *R. mangle* at RSA and the notably smaller component (8%) in the bare sediment and in *A. germinans* could be the reason for the severe stress response experienced by the red mangroves. This could be explained by the release of exudates from roots during the initial growth phase of the plant which stimulates the activity of SRB (Hines et al., 1989, Lynch and Whipps, 1990, Alongi et al., 1993; Nedwell et al., 1994). Several phyla and classes (*Gemmatimomadota*, *Desulfuromonadia*, *Phycisphaerae*, *Bacilli*) were only detected in the seedling's rhizosphere, suggesting that microbial communities established during nursery growth have been maintained after replantation. *Gemmatimonadota* is widely distributed in many natural habitats, from grasslands, lake sediments and alpine soils. This microbial class is predominantly aerobic and their occurrence only in the rhizosphere of *A. germinans* could suggest better soil aeration from its roots even at the seedling stage. If this is also the case for adult black mangroves, the original observations from Nickerson and Thibodeau (1985) suggesting that A. germinans has higher oxidative potential could then be confirmed. Roots and their exudates can facilitate or indirectly select the establishment of microbial communities (Holguin et al., 2001). As a consequence, it is further demonstrated here that they can potentially tip the balance for the success of a restoration plan. To date, data on the rhizomicrobiome diversity are severely lacking. Therefore, identifying the different microbial communities that consistently inhabit the rhizosphere of *R. mangle* and *A. germinans* is

essential to understanding the complex interactions among microorganisms-sediments-plants in mangroves. Additionally, it will be relevant to investigate how and to what extent mangroves can influence the microbial communities of degraded areas and to determine the role they play during the initial growth and the ecological importance of these communities in mangrove ecosystems.

4.3 Potential for mangrove restoration

Worldwide, the extent of mangrove loss emphasizes, on the one hand, the degree of what we have lost, and, on the other hand, the many opportunities that exist to restore this important yet fragile ecosystem. From 2015 to 2019, Bonaire's government executed the "Ecological Restoration Project Lac and South Bonaire" whose main focus was the restoration of water circulation in Lac Bay. The volunteers from the Mangrove Maniacs continued to work inside and outside the bay, expanding the project to the southwest coast. In 2021 only, the group replanted more than one thousand *R. mangle* seedlings with the target of extending the sporadic cover of mangroves to create a more homogeneous cover and a natural barrier along the scarcely vegetated coast. This project cannot be considered part of a restoration plan, but certainly acts as a pilot experiment to test the efficiency of transplanting nursery-raised mangroves into new habitats, with the potential to restore the degraded areas around Lac Bay. The distribution of *R. mangle* in Lac Bay reflects its need to occur at the land-water interface, where the water regime is higher compared to more inland settings. Within the mangrove channels inside the bay, the water level is usually too high to support the aerial roots of *A. germinans*, while is great for the stilt roots of *R. mangle*. Previous replantation attempts showed that *R. mangle* seedlings collected and raised in Lac Bay could not survive in the degraded areas at its edges, where the flow rate has become insufficient. In contrast, these waterlogged, highly-sulfidic pools are naturally populated by *A. germinans*, reflecting the ability of the species to adapt and tolerate higher sulfide concentrations than *R. mangle*. Moreover, during the seasonal release of propagules, these areas are densely supplied by *A. germinans* propagules further suggesting that this species can withstand higher sulfide concentrations at every plant stage. And although data are missing on the detailed sulfide patterns in these degraded areas, it is clear that such high concentrations are tolerated only by *A. germinans* and not *R. mangle*. Consequently, including *A. germinans* in the restorative path of degraded areas could be one of the solutions to re-vegetate these otherwise unvegetated areas.

5. Conclusion

When restoring an area, a coral reef, or a forest, we should always remember that those ecosystems have been altered to "such an extent that it can no longer self-correct or self-renew" (Lewis, 2005). According to the literature, more than 160 restoration projects have been executed as of 2018 (Worthington and Spalding, 2018), but a universal "mangrove restoration toolkit" seems not feasible. However, over the last years, restoration techniques have been greatly refined and, if supported by scientific observations and properly applied, they rarely fail. This study showed that, when replanted in areas characterized by a high sulfide concentration, nursery-raised *R. mangle* seedlings did not survive. From a distinct perspective, it means that in a natural mangrove forest, a zonation pattern of the two species could develop in line with sediment sulfide concentrations, favoring *R. mangle* in less sulfidic areas and *A. germinans* where sulfide concentrations are higher. As a consequence, due to its better adaptability, replantation of *A. germinans* is advised in degraded, highly sulfidic pools. *A.*

germinans has been found to positively influence pore-water sulfide concentrations when low (RSB), increasing them almost twofold and, as a result, being able to withstand higher concentrations. In contrast, *R. mangle* showed no significant effects on sulfide concentrations. This result opposes the previous findings (e.g., Thibodeau and Nickerson (1986) and McKee (1988)) in which adult mangroves revealed to be able to oxidize sediments and consequently lower sulfide concentrations. However, this could be because this study reported the influence of mangrove seedlings, suggesting that mangroves may alter their influence at different life phases. Leaf loss rate, which is intended here as the most immediate short-term stress response possible, was influenced by pore-water sulfide concentration, and it was species-specific. Lastly, I also found a distinct bacterial composition between *A. germinans* and *R. mangle* that could be explained by i) different root exudates, ii) different terminal electron acceptors, iii) intrinsic different rhizomicrobiome and iv) sediment bio- and geo-chemical properties. Successful restoration has beneficial effects on local communities, can support countries to reach targets for carbon emissions and even aid in the pressing fight to moderate climate change. That is possible because, despite being unquestionably complex, the ecological restoration of mangrove ecosystems is achievable. However, these important findings leave us with new questions to be answered:

1- Is it possible to replant black mangroves as a natural mitigator of high sulfide concentrations in degraded areas as means of converting the sediment back to a condition where red mangroves can again thrive?
2 - What is the real potential of microbial communities in the rhizosphere for transplanted seedlings? To what extent they do change when transplanting a seedling from its natural habitat to a different area?
3 - Do microbial communities change intraspecifically between nursery-raised (within Lac Bay) and *in situ*-raised (within degraded areas) seedlings?
4 - To what extent are sulfate-reducing bacteria (SRBs) responsible for the steep leaf loss rate showed by *R. mangle* in the area with elevated sulfide concentrations? Although being suggested by this study, solid experimental evidence is required.
5 - Is it possible to inject plant-growth promoting bacteria to enhance the growth and the survival of dying seedlings? And consequently, is microbially-enhanced rhizosphere a valid path for conservation and restoration of mangrove ecosystems?
6 - Chronic high salinity is detrimental to mangroves (Kandasami and Bingham, 2001). How do the two effects (high salinity and sulfides) interact with each other? Which one is the major driver that inhibits mangrove growth?

Acknowledgments

First, I am extremely thankful to the Mangrove Maniacs group who shared with me all their profound knowledge of mangroves. I gratefully acknowledge Drs. Sabine Engel and Jessica Johnson (Mangrove Maniacs) for helping me design the experiment, Luuk Leemans for the field and technical support, Prof. Dr. Gerard Muijzer, Betsie Voetdijk, Anastasiia Barilo for assisting me in performing the analysis at the University of Amsterdam. Lastly, I thank Dr. Arie Vonk for his support in critically reading this manuscript and CRC® – Centro Ricerche Chimiche for the material needed to safely collect samples.

References

Alongi, D. M., and P. Christoffersen. "Benthic infauna and organism-sediment relations in a shallow, tropical coastal area: Influence of outwelled mangrove detritus and physical disturbance." Marine ecology progress series. Oldendorf 81.3 (1992): 229-245.

Alongi DM, Christoffersen P, Tirendi F (1993) The influence of forest type on microbial-nutrient relationships in tropical mangrove sediments. J Exp Mar Biol Ecol 171:201–223

Andreote FD, Jimenez DJ, Chaves D, Dias AC, Luvizotto DM, Dini-Andreote F, et al. The microbiome of Brazilian mangrove sediments as revealed by metagenomics. PLoS ONE. 2012; 7(6): e38600. doi:10.1371/journal.pone.0038600 PMID: 22737213

Ball, Marylyn C. "Patterns of secondary succession in a mangrove forest of southern Florida." Oecologia 44.2 (1980): 226-235.

Bayoumy, Mahmoud El, et al. "Removal of heavy metals and COD by SRB in UAFF reactor." Journal of Environmental Engineering 125.6 (1999): 532-539.

Brenner, Don J.; Krieg, Noel R.; Staley, James R. (2007). Bergey's Manual® of Systematic Bacteriology: Volume 2: The Proteobacteria, Part B: The Gammaproteobacteria. Springer Science & Business Media. ISBN 9780387280226.

Carlson, Paul R., et al. "Pore water chemistry of an overwash mangrove island." Florida Scientist (1983): 239-249

Chapman, Russell Leonard. "Algae: the world's most important "plants"—an introduction." Mitigation and Adaptation Strategies for Global Change 18.1 (2013): 5-12.

Chen J, Wu FH, Wang WH, Zheng CJ, Lin GH, Dong XJ, He JX, Pei ZM, Zheng HL (2011) Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. J Exp Bot 62:4481–4493.

Cheng, Hao, et al. "Differences in root aeration, iron plaque formation and waterlogging tolerance in six mangroves along a continues tidal gradient." Ecotoxicology 24.7 (2015): 1659-1667.

Constancias, Florentin, et al. "Contrasting spatial patterns and ecological attributes of soil bacterial and archaeal taxa across a landscape." Microbiologyopen 4.3 (2015): 518-531.

do Carmo FL, dos Santos HF, Martins EF, van Elsas JD, Rosado AS, Peixoto RS. 2011. Bacterial structure and characterization of plant growth promoting and oil degrading bacteria from the rhizospheres of mangrove plants. J Microbiol 49:535–543.

Duke JA, 1983. Rhizophora mangle L. In: Handbook of energy crops. Lafayette, Indiana, USA: Center for New Crops and Plant Products, Purdue University. https://www.hort.purdue.edu/newcrop/duke_energy/Rhizophora_mangle

Fakhari S, Sharifi M, De Michele R, Ghanati F, Safaie N, Sadeghnezhad E (2019) Hydrogen sulfide directs metabolic flux towards the lignan biosynthesis in Linum album hairy roots. Plant Physiol Biochem 135:359–371.

FAO. 2007. The World's mangroves 1980-2005. 89pp.

Feller IC, Lovelock CE, Berger U, McKee KL, Joye SB, Ball MC. Biocomplexity in mangrove ecosystems.Ann Rev Mar Sci. 2010; 2: 395±417.

Fu, Yang, et al. "Central role of adenosine 5′-phosphosulfate reductase in the control of plant hydrogen sulfide metabolism." Frontiers in Plant Science (2018): 1404.

Hill K, 2001. Rhizophora mangle. Fort Pierce, Florida, USA: Smithsonian Marine Station at Fort Pierce. www.sms.si.edu/irLspec/Rhizop_mangle.htm

Hines, Mark E., Stephen L. Knollmeyer, and Joyce B. Tugel. "Sulfate reduction and other sedimentary biogeochemistry in a northern New England salt marsh." Limnology and Oceanography 34.3 (1989): 578-590.

Holguin G, Vazquez P, Bashan Y. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. Biol Fertil Soils. 2001; 33: 265±278. doi: 10.1007/s003740000319

Jin, Ren-Cun, et al. "The effect of sulfide inhibition on the ANAMMOX process." Water Research 47.3 (2013): 1459-1469.

Kjeldsen, Kasper U.; Joulian, Catherine; Ingvorsen, Kjeld (2004). Oxygen Tolerance of Sulfate-Reducing Bacteria in Activated Sludge. Environmental Science & Technology, 38(7), 2038–2043. doi:10.1021/es034777e

Kodikara, Kodikara Arachchilage Sunanda, et al. "Have mangrove restoration projects worked? An in-depth study in Sri Lanka." Restoration Ecology 25.5 (2017): 705-716.

Krauss, Ken W., et al. "Environmental drivers in mangrove establishment and early development: a review." Aquatic botany 89.2 (2008): 105-127.

Kristensen, Erik, et al. "Organic carbon dynamics in mangrove ecosystems: a review." Aquatic botany 89.2 (2008): 201-219.

Kryger, L., and S. K. Lee. "Effects of soil ageing on the accumulation of hydrogen sulphide and metallic sulphides in mangrove areas in Singapore." Environment international 21.1 (1995): 85-92.

Lee HJ, Jeong SE, Kim PJ, Madsen EL, Jeon CO. High resolution depth distribution of Bacteria, Archaea, methanotrophs, and methanogens in the bulk and rhizosphere soils of a flooded rice paddy. Front Microbiol. 2015; 6: e639. doi: 10.3389/fmicb.2015.00639 PMID: 26161079

Lee, S. K., W. H. Tan, and S. Havanond. "Regeneration and colonisation of mangrove on clay-filled reclaimed land in Singapore." Hydrobiologia 319.1 (1996): 23-35.

Lewis, R. R. (1990). "Creation and restoration of coastal plain wetlands in Florida." Wetland creation and restoration: Status

ofthe science. J. A. Kusler and M. E. Kentula, eds., Island Press, Washington, DC, 73-101.

Lewis III, Roy R. "Ecological engineering for successful management and restoration of mangrove forests." Ecological engineering 24.4 (2005): 403-418.

Li, Huan, et al. "Unraveling hydrogen sulfide-promoted lateral root development and growth in mangrove plant Kandelia obovata: Insight into regulatory mechanism by TMT-based quantitative proteomic approaches." Tree Physiology 41.9 (2021): 1749-1766.

Liu, Hai, et al. "Hydrogen sulfide (H2S) signaling in plant development and stress responses." Abiotech 2.1 (2021): 32-63.

Lugo, Ariel E., and Samuel C. Snedaker. "The ecology of mangroves." Annual review of ecology and systematics 5.1 (1974): 39-64.

Luo, Shilei, et al. "The role of hydrogen sulfide in plant alleviates heavy metal stress." Plant and Soil 449.1 (2020): 1-10.

Lyimo, Thomas J., Arjan Pol, and Huub JM Op den Camp. "Methane emission, sulphide concentration and redox potential profiles in Mtoni mangrove sediment, Tanzania." (2002).

Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129:1–10

MacNae, William. "A general account of the fauna and flora of mangrove swamps and forests in the Indo-West-Pacific region." Advances in marine biology. Vol. 6. Academic Press, 1969. 73-270.

McKee, Karen L., Irving A. Mendelssohn, and Mark W. Hester. "Reexamination of pore water sulfide concentrations and redox potentials near the aerial roots of Rhizophora mangle and Avicennia germinans." american Journal of Botany 75.9 (1988): 1352-1359.

Nedwell, D. B., T. H. Blackburn, and W. J. Wiebe. "Dynamic nature of the turnover of organic carbon, nitrogen and sulphur in the sediments of a Jamaican mangrove forest." Marine Ecology Progress Series (1994): 223-231.

Nickerson, N.H., Thibodeau, F.R., 1985. Associations between pore water sulfide concentrations and the distribution of mangroves. Biogeochemistry 1, 183–192.

M.S. Nóbrega, B.S. Silva, D.A. Tschoeke, et al., Mangrove microbiome reveals importance of sulfur metabolism in tropical coastal ..., Science of the Total Environment, http://dx.doi.org/10.1016/j.scitotenv.2021.151889

Odum, William E., and R. E. Johannes. "The response of mangroves to man-induced environmental stress." Elsevier Oceanography Series. Vol. 12. Elsevier, 1975. 52-62.

Rabinowitz, Deborah. "Dispersal properties of mangrove propagules." Biotropica (1978): 47-57.

Romañach, Stephanie S., et al. "Conservation and restoration of mangroves: Global status, perspectives, and prognosis." Ocean & Coastal Management 154 (2018): 72-82.

Santana, C. O., et al. "Microbial community structure and ecology in sediments of a pristine mangrove forest." bioRxiv (2019): 833814.

Shang, Yu, et al. "Inferring interactions in complex microbial communities from nucleotide sequence data and environmental parameters." PLoS One 12.3 (2017): e0173765.

Sherman RE, Fahey TJ, Howarth RW. Soil-plant interactions in a neotropical mangrove forest: iron, phosphorus and sulfur dynamics. Oecologia. 1998; 115: 553±563. doi: 10.1007/s004420050553

Smith III, Thomas J. "Seed predation in relation to tree dominance and distribution in mangrove forests." Ecology 68.2 (1987): 266-273.

Spalding, M., Kainuma, M. & Collins, L. World Atlas of Mangroves. A collaborative project of ITTO, ISME, FAO, UNEP-WCMC, UNESCO-MAB, UNU-INWEH and TNC. 319 (Earthscan, 2010)

Thibodeau, F.R., and N.H. Nickerson. 1986. Differential Oxidation of Mangrove Substrate by Avicennia Germinans and Rhizophora Mangle. Amer. J. Bot. 73(4):512-516.

Thom, Bruce G. "Mangrove ecology and deltaic geomorphology: Tabasco, Mexico." The Journal of Ecology (1967): 301-343.

Tomlinson, P. B. (1986) The Botany of Mangroves. Cambridge University Press, Cambridge, pp 62–115.

Twilley, Robert. (1995). Properties of mangrove ecosystems related to the energy signature of coastal environments. Maximum Power. 43-62.

UNEP (2014) The Importance of mangroves to People. In: van Bochove J, Sullivan E, Nakamura T (eds) A call to action. United Nations Environment Programme World Conservation Monitoring Centre, Cambridge. 128 pp.

Wang, Wenqing, et al. "Mangroves: obligate or facultative halophytes? A review." Trees 25.6 (2011): 953-963.

Worthington, Thomas, and Mark Spalding. "Mangrove restoration potential: A global map highlighting a critical opportunity." (2018).

Wu P, Xiong X, Xu Z, Lu C, Cheng H, Lyu X, et al. (2016) Bacterial Communities in the Rhizospheres of Three Mangrove Tree Species from Beilun Estuary, China. PLoS ONE 11(10): e0164082. doi:10.1371/journal.pone.0164082

Young, C.C., Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., 2006. Phosphate solubilizing bacteria from tropical soil and their tricalsium phosphate solubilizing bacteria abilities. Appl. Soil. Ecol. 34, 33–34.

Zhang, Yanying, et al. "Diversity and structure of diazotrophic communities in mangrove rhizosphere, revealed by high-throughput sequencing." Frontiers in microbiology 8 (2017): 2032.

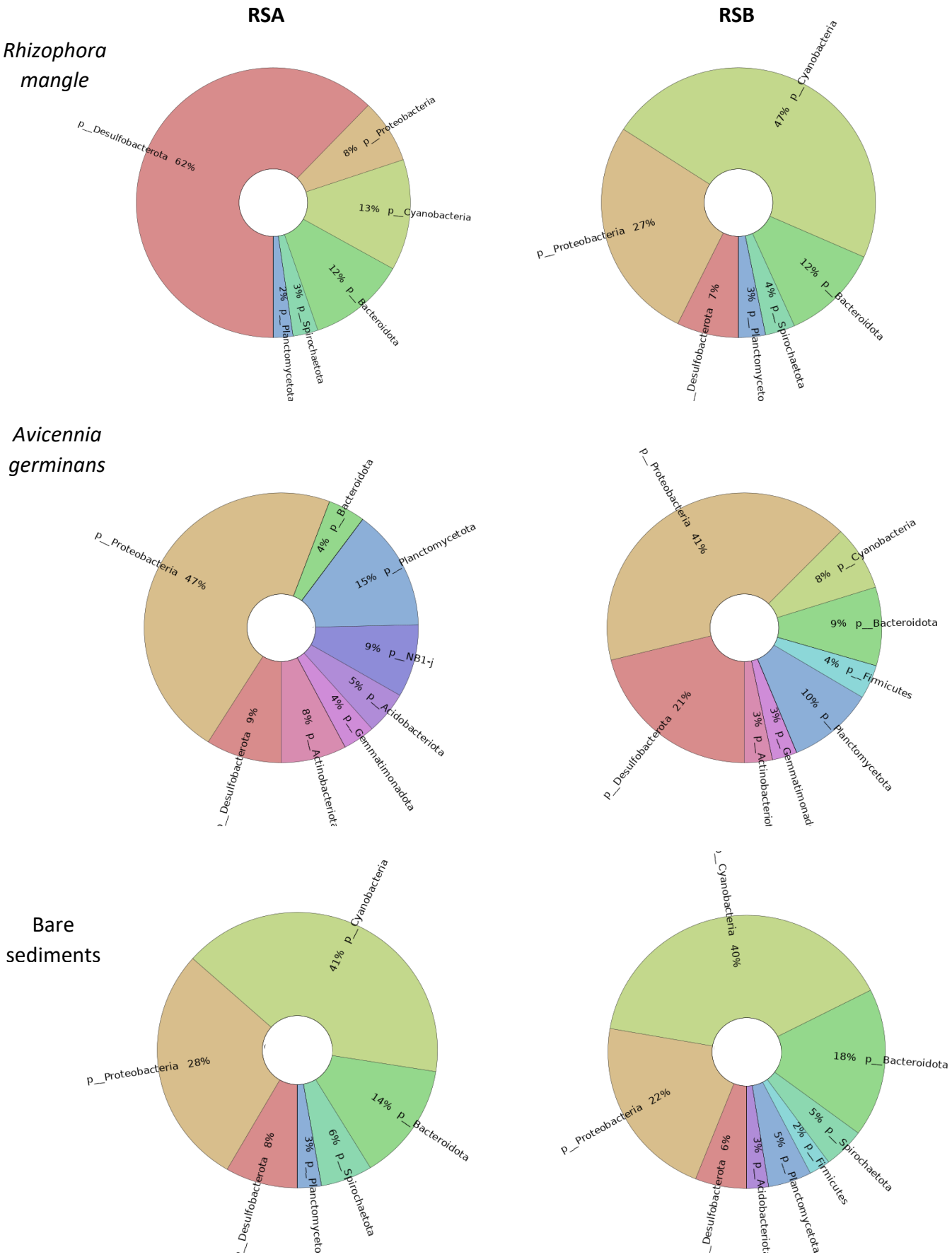
Supplementary material

Supplementary Table 1. List of the environmental parameters measured in the two areas during the three weeks.

	RSA			RSB		
Parameter	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
pH	8.58	8.57	8.51	8.98	9.1	9.12
Sal [psu]	38.22	41.95	42.19	38.07	42.85	40.25
ORP [mV]	-22.7	-31.8	-170.8	-21.5	-27.3	-13.7
D.O. [%]	51.7	24.3	86.3	56	30	93.5
Daily T [°C]	28.9	29.74	29.96	28.94	29.74	29.96
Daily light intensity [lux]	6639.57	36923.86	17942.07	6639.57	36923.86	17942.07

Supplementary Table 2. H_2S concentrations measured for each seedling (R = R. mangle, B = A. germinans) and bare sediment (S) during the three weeks. Sulfide concentrations were obtained from the absorbance read on the spectrophotometer after the analysis using the blue methylene method. Showed there are the values of each seedling and the mean of the five seedling per species per area.

	RSA			RSB		
	H2S (mM)			H2S (mM)		
ID	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
R1	-0.15	18.62	19.52	0	NA	0.98
R2	14.78	15	19.3	0.08	4.83	7.31
R3	18.85	19.07	22.69	0.08	5.5	6.41
R4	7.99	18.17	23.14	0.06	0.09	10.71
R5	9.57	16.36	16.13	0.05	0.06	10.93
Mean	**10.21** ± 7.21	**17.44** ± 1.71	**20.16** ± 2.86	**0.05** ± 0.03	**2.62** ± 2.95	**7.27** ± 4.05
B1	6.18	17.72	25.18	1.21	3.7	8.67
B2	8.67	21.56	24.05	0.3	3.02	6.86
B3	12.29	18.85	27.21	-1.05	7.31	11.16
B4	-0.37	20.65	21.11	0.98	8.67	9.35
B5	17.04	15.68	20.2	1.43	6.18	12.06
Mean	**8.76** ± 6.54	**18.89** ± 2.34	**23.55** ± 2.89	**0.57** ± 1	**5.78** ± 2.39	**9.62** ± 2.06
S1	17.04	17.49	18.17	-0.83	0.53	1.89
S2	16.36	17.26	22.46	0.3	3.47	5.96
S3	15.45	18.39	15.68	0.76	1.21	4.37
S4	11.61	18.85	29.47	1.66	3.7	5.5
S5	17.04	23.82	22.24	1.21	2.57	9.57
Mean	**15.5** ± 2.27	**19.16** ± 2.68	**21.6** ± 5.24	**0.62** ± 0.96	**2.3** ± 1.39	**5.46** ± 2.78



Supplementary Figure 1. Differential proportion of sequences in the microbial communities in the rhizosphere of *R. mangle* and *A. germinans* (first four plots) and in bare sediments (last two plots) in the two areas (RSA = left column; RSB = right column)