Seasonal Pattern of the Biogeochemical Properties of Mangrove Sediments receiving Shrimp Farm Effluents (New Caledonia)

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Abstract

Coastal tropical shrimp farming may impact the adjacent ecosystems through the release of large quantities of effluents rich in nutrients. In New Caledonia, mangroves are considered as a natural biofilter to reduce impacts on the surrounding World Heritage listed lagoon. Our main objective was to understand the influence of effluent discharge on the biogeochemistry of mangrove sediments. A monitoring of the physico-chemical parameters of mangrove sediments was carried out during a whole year, including active and non active periods of the farm. The parameters studied were: i) benthic primary production (Chl-a concentrations), ii) physico-chemical parameters of sediments (redox potential, pH, salinity, TOC, TN, TS, $\delta^{13}C$ and $\delta^{15}N$), iii) concentrations of dissolved nitrogen, iron and phosphorus. A mangrove developing in the same physiographic conditions, presenting the same zonation, and free of anthropogenic input was used as reference. The concentration of benthic Chl-a measured at sediment surface in the effluent receiving mangrove was twice to three times that measured in the control zone whatever the season. We thus suggest that nutrients inputs significantly increased the phytobenthic production in the effluent receiving mangrove during the whole year, even after the cessation of discharges and because of natural seasonal dynamic of phytobenthos. Although the flow of surface OM was increased, the OM content at depth was not higher than in the control mangrove. However, the contribution of mangrove detritus to the sedimentary organic pool was higher probably as a result of higher density and much greater individual size of the mangrove trees. Unlike the control mangrove sediment, the effluent receiving mangrove sediment was not stratified, redox potential values were high and presence of Fe^{3+} was detected down to 50 cm depth, probably as a result of a larger root system, allowing a better sediment oxygenation and accentuated OM decomposition processes, and thus limiting ecosystem saturation.

Keywords: Shrimp farming; Effluents; Mangrove; Sediment biogeochemistry; New Caledonia

Introduction

Mangroves are tropical and subtropical ecosystems developing at the edge between land and sea. They extend over 140 000 km² of the coastal zone and over a latitudinal range of 30°N to 38°S [1,2]. Mangroves are crucial both ecologically and economically, supporting a wide variety of ecosystem services [3-5]. For instance, mangroves are very efficient to trap suspended materials because of the density of their aerial roots, and also because it is a low energy environment [6]. Thus, they may act as a filter, retaining particles from watershed, and avoiding over-deposition in coastal waters. Because it is a highly active biogeochemical environment, several countries used them as natural biofilters to treat urban or aquaculture sewages. The annual economic values of mangroves, estimated by the cost of the products and services they provide, have been estimated to be USD 200,000–900,000 ha^{-1} [7]. Income related to fisheries undertaken by the presence of mangroves is estimated at 10,000 $ per hectare per year, with a high variability between areas and market value [8]. In addition, the value of biofilter functions ranges between US $ 1193 ha^{-1} year^{-1} and US$ 5820 ha^{-1} year^{-1} [8]. Once mangroves covered more than 200,000 km² [9]. Unfortunately, mangrove forests are among the most threatened global ecosystems [10-13]. The destruction of the ecosystem takes place all-around the world, especially in emerging countries, where 90% of mangroves are located. Aquaculture accounts for 52% of mangrove loss globally, with shrimp farming alone accounting for 38% of mangrove deforestation [12,14]. World-wide, shrimp farming has increased almost exponentially since the mid-1970's due to short production cycles and their high product values, reaching a total annual production of over 2.3 billion tons in 2008 [15]. In tropical and sub-tropical zones, particularly in South America, Indonesia and Thailand, shrimp farms have been developed at the expense of mangrove forests, which are destroyed for the establishment of the rearing ponds [16]. In addition to the direct loss of mangroves during construction, shrimp farms also impact the adjacent mangroves through the release of large quantities of effluents rich in particulate and dissolved organic and inorganic nutrients [17-19]. During a production cycle, it has been estimated that only 29 and 16%, respectively, of total nitrogen and phosphorus added to the ponds as food and fertilizers inputs, is actually assimilated by shrimps [20]. In New Caledonia, shrimp farming is still a relatively small industry consisting of 19 farms with ponds covering 680 ha, with an annual production of 2,000 tons in 2006 [21]. In contrast to other parts of the World, there are no direct losses of mangroves due to shrimp farming, as local regulations prevent construction of farms within the mangroves themselves. Therefore, farms are built upstream of the mangrove forests within the salt-flats. However, ponds effluents are discharged into the adjacent mangroves, which are considered as a "natural biofilter", to

reduce or eliminate impacts on the surrounding World Heritage listed lagoon and coral reef [19]. Ponds are continuously irrigated in order to maintain water column oxygenation, with water that is pumped directly from the lagoon through a canal, and introduced by gravity at a rate increasing from 5 to 25% of the pond volume over the course of the rearing cycle. Excess water overflows the ponds and is discharged into the adjacent mangroves. Following the shrimp harvest, the ponds are drained and left dry for ~4 months, the "assec" period, until the subsequent production cycle [21]. Several studies have investigated the impacts of shrimp farm effluents on mangrove ecosystems, especially in mangrove creeks. Effluents have been shown to significantly increase water column chlorophyll *a*, dissolved inorganic nitrogen (NH_4^+ and NO_x) and total nitrogen and phosphorus concentrations [19-22]. In addition, they enhance primary and bacterial production [23,24] and induce significant increases in biological oxygen demand, promoting water column hypoxia or anoxia [19,25]. However, these effects are generally localized to areas close to effluent outlets, and water quality parameters and phytoplankton biomass return to natural levels within a few months after the cessation of the discharge [19,25]. In contrast, much less is known concerning the effects of shrimp farm effluents on processes within the benthic compartment [26,27]. Within this context, our main objective was to understand the influence of the effluents release on the biogeochemistry of mangrove sediments. Due to the cyclical nature of the farming practiced in New Caledonia, a monitoring of the physico-chemical parameters of the mangrove sediment was carried out during a whole year, including the complete cycle of farming, and the "assec" period, to see if the sediment can regenerate during this period. In New Caledonia, farms are built on salt-flats, and the first mangrove strata receiving effluents is generally the *Avicennia* stand. A densification of this stand following implantation of

a shrimp farm was observed [28]. The parameters followed during this study were: i) the benthic primary production (chlorophyll biomass), ii) the physico-chemical parameters of sediments (redox potential, pH, salinity, total organic carbon (TOC), total nitrogen (TN), total sulfur (TS), $\delta^{13}C$ and $\delta^{15}N$), iii) and the concentrations of dissolved nitrogen, iron and phosphorus. A mangrove in the same bay and developing in the same physiographic conditions but free of any anthropogenic input was used as reference.

Materials and Methods

Study site and sampling strategy

New Caledonia (NC) is a French archipelago located in the southwestern part of the Pacific Ocean, and is characterized by the world's largest lagoon surrounding the main island. The climate of the main island is strongly influenced by the inter-tropical convergence zone (ITCZ) with mean annual rainfall close to 1100 mm and average monthly air temperatures fluctuating between 20°C and 27°C (Meteo France data). The west coast of the main island, where the study sites are (Figure 1), is characterized by a semi-arid tropical climate. The smallest thermal amplitude and the highest temperatures occur from December to February (i.e. in summer); and the largest thermal amplitude and the lowest temperatures occur from July to September (i.e. in winter) (Figure 2). Tropical depressions can occur during summer, which is thus also the wet season. In New Caledonia, a total of 24 mangrove species occupy ~25 000 ha; 88% of mangroves are located on the west coast due to coastal morphology, but biodiversity is higher on the east coast because of less arid conditions. Along the west coast of the main island, which is characterized by a semi-arid climate, the same mangrove zonation can be observed. *Rhizophora* trees, representing
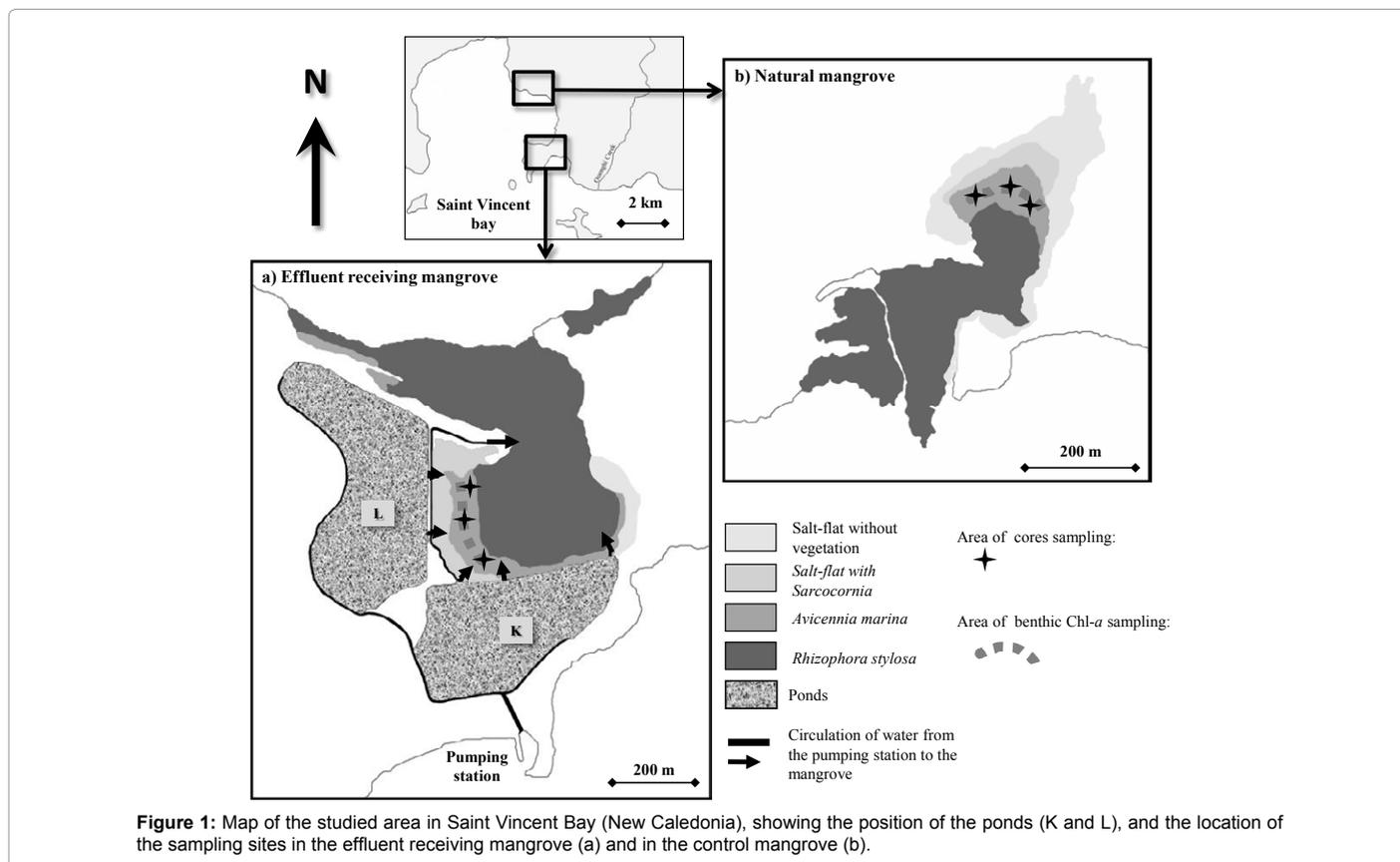

Figure 1: Map of the studied area in Saint Vincent Bay (New Caledonia), showing the position of the ponds (K and L), and the location of the sampling sites in the effluent receiving mangrove (a) and in the control mangrove (b).
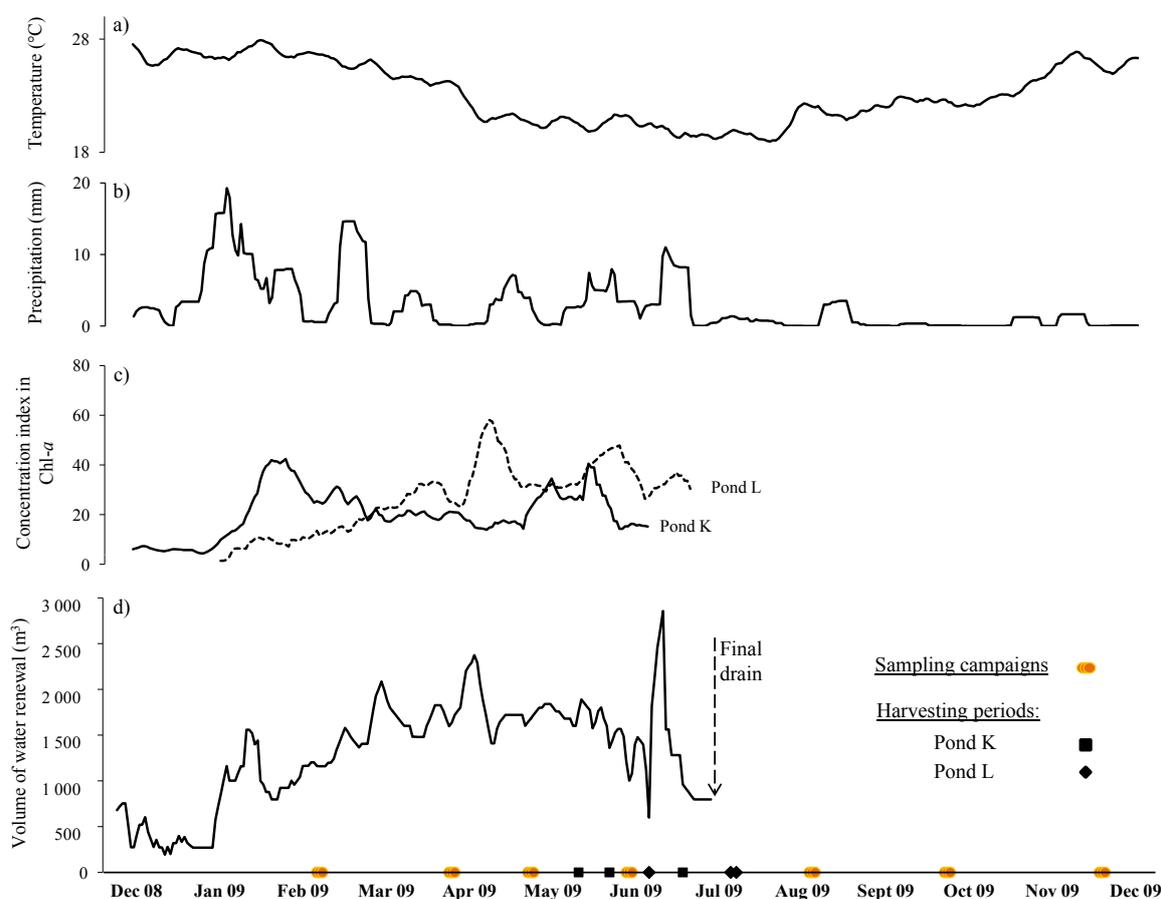
Figure 2: Temperature (a) and precipitation (b) measured at the closest Météo-France station (averaged over 10 days), concentration index in Chl-a measured in the ponds water (pond K and L) (c), and total volume of water renewal by day for the two ponds (L+K). Harvesting periods of the shrimp farm and sampling campaigns are specified.

55% of the total mangrove areas in New Caledonia, develop mainly in zones swept daily by tides, while *Avicennia* trees, representing 14% of the total mangrove areas, develop at higher elevations, at the borders of the salt-flat, which occupies the higher area in the intertidal zone. The effluent receiving mangrove and the control mangrove are located on the west coast of New Caledonia in the northern part of the same bay, Bay of Saint Vincent, and present the same zonation (Figure 1). None of the studied mangroves are crossed by a river; they are thus mainly subject to the marine influence, without significant freshwater input except by runoff during rainfall. The control mangrove (21°54'S, 166°04'E) covered 20 ha (Figure 1a) and was free of significant anthropogenic influences. The effluent receiving mangrove (21°56'S, 166°04'E; total area of 28.9 ha (Figure 1b), located 2 km from the control mangrove) receives effluent discharges from the "Ferme Aquacole de la Ouenghi" shrimp farm (FAO). Visual density and size distributions of the mangrove trees differed between the two studied mangroves. In the control mangrove, the *Avicennia* trees were sparsely distributed, bush-like and never exceeded 1 m in height. In contrast, in the mangrove receiving shrimp farm effluents, there was a higher density of trees. Pictures of the effluent receiving mangrove before the farm constructions show that the density was the same than in the control one. The FAO shrimp farm exploitation started in 1989 and operates two 1 m deep rearing ponds of 10.5 (L) and 7.5 (K) ha, respectively, which are constructed within and have replaced the original salt-flat.

Like the majority of shrimp farms in New Caledonia, FAO operates a semi-intensive rearing system. Ponds are stocked with the blue shrimp, *Litopenaeus stylirostris,* at a density of ~17 individuals.m^{-2} in December/January, which are reared for ~8 months (Figure 2). The shrimp are fed with locally produced feed pellets (35-40% protein, SICA, New Caledonia), which are added daily throughout the rearing period, with inputs increasing from ~0.25 to ~3.5 kg.ha^{-1}.d^{-1} over the rearing cycle as the shrimps grow (Farm manager, Pers. Comm.). The volume of water discharged into the mangrove corresponds to the volume of the water renewed daily, and increases progressively with the growth of post-larvae and adult organisms. Effluent discharges are massive, especially during harvesting periods and during the final drain. However, not being renewed before the final drain, the volume of water released in the mangrove does not correspond to the curve of water renewal (Figure 2). The ponds were drained on 17 and 28 July 2009 respectively for ponds L and K, which corresponds to the last shrimp harvest and to the maximum water discharge to the mangrove. In both mangroves (control and effluent receiving), 8 sampling campaigns were conducted from February 2009 to February 2010 in the *Avicennia* stands. Sampling campaigns were conducted to cover the entire production cycle of the farm with four campaigns during the rearing period, and 4 campaigns during the "assec" period. Surface sediment samples were collected to determine their benthic Chl-a contents. Five subareas were defined for each *Avicennia* stand, and four replicates were collected

in each subarea. Each replicate corresponded to the pooling of five 1cm depth x 2cm Ø syringes contents. The samples were then frozen at -20°C before being lyophilized for analysis. In addition, sediment cores (50 cm deep) were collected in triplicate during low tide using an Eijkelkamp gouge auger in each mangrove stand. Core locations were chosen close to the vegetation but being carefully to avoid the presence of main roots in samples. After being collected, cores were wrapped in plastic film and aluminium foil, and were transported to the air-conditioned laboratory within 10 min. Samples were collected every 5 cm from 0 to 50 cm depth. Samples were taken and frozen until they were lyophilized for analysis of Total Organic Carbon (TOC), Total Sulphur (TS), Total Nitrogen (TN), as well as for nitrogen and carbon stable isotopes. Pore-waters were extracted from 5 cm long sediment section with soil moisture samplers Rhizon® [29,26]. All samples were filtered through 45 μm cellulose acetate syringe-filter. An aliquot of filtered pore-water was acidified with HNO_3 to prevent precipitation of reduced dissolved iron and store refrigerated for Fe analysis (Fe^{2+} and Fe^{3+}). A second aliquot was frozen at -25°C for nutrient analysis (NH_4^+, NO_x, DON, DIP, DOP).

Analytical methods

Chlorophyll a analysis: Chl-a was extracted from freeze-dried sediments by using a methanol 93% solution and their concentrations determined fluorometrically [30]. Fluorometer used was a Turner Designs TD700 equipped with an optical kit n°7000-961 including an excitation filter of 340-500 nm wavelength, and an emission filter up to 665 nm wavelength. Pigments in methanol were then excited in the fluorometer with a 450 nm wavelength beam of light and fluorescence emitted at 664 nm. Chl-a data are represented in a Boxplot (realized with R). They were, first, analyzed by a non-parametric Kruskall-Wallis test, and then by a Wilcoxon test to compare mean values for pairs (control mangrove vs. effluent receiving mangrove, between campaigns), a Benjamini and Hochberg correction were used.

Salinity, pH, redox potential and ΣH_2S measurements: Physico-chemical parameters were determined in an air-conditioned laboratory within 30 min after the core sampling. Salinity, pH, redox and ΣH_2S were measured like previously described [26]. pH was measured with a WTW pH meter electrode. Salinity was determined with an Atago refractometer. We used a WTW sulphide ion specific electrode allowing, with the appropriate calculations (taking into account pH and salinity), the determination of the total sulphide concentration (TS, S^{2-}, H_2S and HS^-) [31] based on the calibration of the electrode by titration, as previously described ($\log (S^{2-})=-0.024*E(V)-0.878$) [32]. The redox potential was measured with a combined Pt and Ag/Ag/Cl reference electrode connected to a WTW pH/mV/T meter. Redox data are reported relative to a standard hydrogen electrode after adding 202 mV to the original mV values obtained with an Ag/AgCl reference electrode at 25°C. The following redox scale was used:

- oxic > 400 mV, contain measurable dissolved oxygen,

- 100mV > Suboxic > 400 mV, lack measurable oxygen or sulphide but contain dissolved iron or manganese, and no reduction of sulfate.

- Anoxic < 100 mV, sulfato reduction.

Pore-water analysis: Dissolved iron (Fe^{2+} and Fe^{3+}) were measured in pore-waters by colorimetric procedures with a precision of ± 5% [33]. Sulphate was analysed according to a nephelometric method with a precision of about 5% [34]. NH_4^+ concentrations were determined by the fluorimetric method [35] using a TurnerDesigns TD700 fluorimeter. NO_x (nitrate+nitrite) concentrations were determined using an

Autoanalyzer III (Bran+Luebbe) [36]. Total Dissolved Nitrogen (TDN) concentrations were determined as NO_x following oxidation. Dissolved Organic Nitrogen (DON) concentrations were calculated by difference between the TDN and the measured inorganic forms (NH_4^+ and NO_x). Dissolved Inorganic Phosphorus (DIP) concentrations were determined using the autoanalyzer III (Bran+Luebbe) [37]. Dissolved Organic Phosphorus (DOP) was determined by difference of the TDP pool minus DIP pool.

Sediment solid phase analysis: *Sediment samples were analyzed only once at the beginning of the study to compare the sediment characteristic of the two Avicennia stands.*

Total organic carbon (TOC) was determined using a Rock-Eval 6 pyrolisis [38,39]. Total nitrogen (TN) and total sulphur (TS) concentrations were measured by combustion at 1100°C with a CNS-2000 LECO apparatus. Prior to analysis for the determination of $\delta^{13}C$ and $\delta^{15}N$, 20 ± 1 mg from each sample were freeze-dried and grounded. Surface sediments were treated with the addition of 1N HCl solution to remove carbonates. The isotopic ratio (R) values of dried samples ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) were determined at the UC Davis Stable Isotope Facility (Department of Plant Sciences, University of California at Davis, Davis, California) using a Europe Hydra 20/20 mass spectrometer equipped with a continuous flow IRM device and are reported in standard delta notation ($\delta^{13}C$ or $\delta^{15}N$), defined as parts per thousand (‰) deviation from a standard (Vienna Peedee belemnite for C and atmospheric N_2 for N). The analytical precision (standard deviation for repeated measurements of the internal standards) for the measurement was 0.06‰ and 0.13‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Results and Discussion

Mangrove zonation and solid phase characteristics of mangrove sediments

In New Caledonia, shrimp pounds are built upstream of the mangrove forests within the salt-flat areas. Water is pumped in daily from the nearby lagoon for a partial renewal of the pond water that maintains salinity at 32–39‰ and dissolved oxygen above 3.5 mg.L^{-1}. It flows through the intake canal while the excess pond water drains from the opposite end of the pond into the adjacent mangroves, mainly the *Avicennia* stand. Along the west coast of the main island, which is characterized by a semi-arid climate, the same mangrove zonation can be observed. *Rhizophora* trees, representing 55% of the total mangrove areas in New Caledonia [40], develop mainly in zones swept daily by tides, while *Avicennia* trees, representing 14% of the total mangrove areas, develop at higher elevations, at the borders of the salt-flat, which occupies the higher area in the intertidal zone. This topographic zonation induces differences in the length of immersion by tides, which is translated by the water content of the sediment. In a previous study [41], we suggested that surface ground elevation indirectly controlled zonation, by controlling the length of immersion, the amount of evaporation, and thus the salinity of pore-water; the latter being recognized as a key factor in mangrove zonation [42,43]. Within the *Avicennia* stand, the total effluent release by the shrimp farm over a rearing cycle was approximately 2.58 10^9 L (pers. com. farm manager), with total N and P loads being ~2.3 and ~0.5 tons, respectively [44]. These values were equivalent to loads of ~79 kg N ha^{-1} and ~19 kg P ha^{-1}, which are about the maximum quantities of N and P that can be processed and immobilized by a mangrove ecosystem [45]. Elevated nutrient concentrations were measured in an external marine creek of the effluent receiving mangrove, suggesting that mangrove was only a partial filter for the nutrient loads [44]. However,

higher density and much greater individual size of the mangrove trees in the effluent receiving mangrove compared to the adjacent control mangrove, suggest that a proportion of the nutrient loads had been assimilated into the mangrove biomass over the 20 years of operation of the studied shrimp farm. Concerning surface sediments, the quality and the quantity of the organic matter seemed to strongly differ between the two mangroves. POC and $\delta^{15}N$ were higher in the effluent receiving mangrove, while C/N values were lower, indicating a greater algae development at sediment surface compared to the control mangrove (Figure 3). In fact, the excess nutrients exported from shrimp ponds were predominantly in particulate organic forms [44], which may have induced a higher benthic primary productivity (see next chapter on Chl-a seasonal evolution). Surprisingly, within the sedimentary column, our results indicate that the effluent receiving mangrove was not enriched in organic matter compared to the control mangrove, with TOC values ranging between 2 and 5% in the upper 20 cm of the two mangroves (Figure 3). This organic enrichment can be considered as moderated compared to other mangrove swamps [46,47]. This result may be linked to the root system of *Avicennia* trees, which is mainly a radial cable root system that develops close to sediment surface and that is less developed than for other species like *Rhizophora* [32]. It can also result from intense decay processes of the Organic Matter (OM) developing in the effluent receiving mangrove (see next chapter on pore-water physico-chemical properties). At depth, a TOC increase, with values reaching more than 8% at 40 cm depth (Figure 3), resulted from an increased proportion of red tissues characteristics of *Rhizophora* wood, visually observed in the core. This observation was made in other mangroves developing on the west coast of New Caledonia [26,41], implying that the *Avicennia* forests develop on sediments that were previously colonized by *Rhizophora* trees. This TOC increase is correlated with an increase in C/N, from 15 in the upper cores to 50 at depth, and a decrease in $\delta^{13}C$ from -22 to -26‰ (Figure 3), confirming an increase proportion of higher plant debris in

the sedimentary organic pool. All the latter parameters presented the same depth trend in both mangroves. However, the upper sediment of the effluent receiving mangrove was characterized by significantly higher $\delta^{15}N$ values compared to the control mangrove (WMW Test, $p<0.05$) (Figure 3). $\delta^{15}N$ signatures of the different sources of OM were: $+4.0 \pm 1.7$‰ for *Avicennia* tissues, $+2.4 \pm 2$‰ for *Rhizophora* tissues, $+1.1 \pm 1.7$‰ for effluents, $+2.4 \pm 1.9$‰ for *cyanophycae*, $+1.3 \pm 1.6$‰ for diatoms. Since N benthic processes, e.g. nitrification and denitrification, usually leads to ^{15}N enrichment, our results are rather related to a different composition of the sedimentary organic pool. The sediment of the control mangrove presented a typical signature of a blend between microphytobenthos and higher plant debris, with $\delta^{15}N$ values ranging between $+2$ and $+3$‰, while the contribution *Avicennia* trees in the sedimentary organic pool seemed to be higher in the effluent receiving mangrove, with $\delta^{15}N$ values ranging between $+5$‰ at the top of the core and $+2$‰ at depth. Although elevated algae development at sediment surface, the increased proportion of *Avicennia* detritus in the sedimentary organic pool highlighted that a large quantity of effluents released in the mangrove had been assimilated into mangrove trees biomass.

Influence of shrimp farm effluents on the natural seasonal dynamic of microphytobenthos

Contribution of microphytobenthos to mangrove primary productivity is usually low due to light limitation induced by close canopy, low nutrient input, and growth inhibition resulting from the high tannin content of mangrove litter [48]. Consequently, Chl-a concentrations at sediment surface rarely exceed 5 $\mu g.g^{-1}$ [49]. Nevertheless, with an open canopy and high nutrient input, microphytobenthos can contribute to a high percentage of the sedimentary organic matter [50]. The Chl-a concentrations measured in the control mangrove ranged between 6.9 ± 2.0 and 12.7 ± 7.1 $\mu g.g^{-1}$ (Figure 4), which represent relatively high concentrations for mangrove

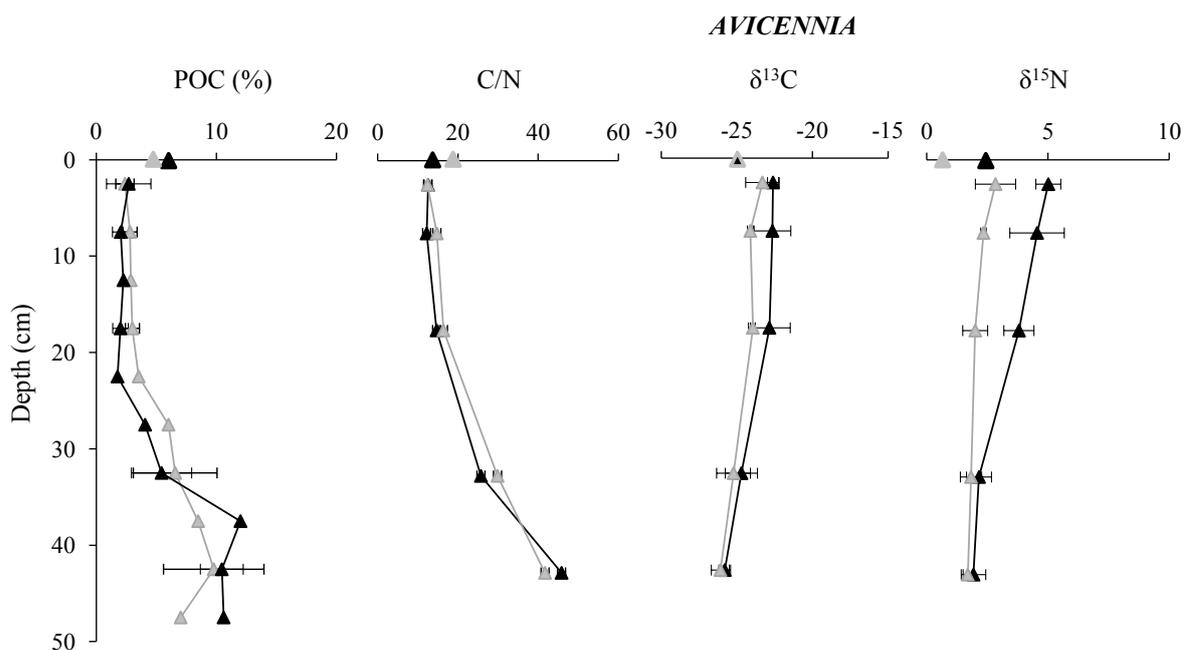**Figure 3:** POC content, C/N, $\delta^{13}C$ and $\delta^{15}N$ distribution with depth within the *Avicennia* sediment in the effluent receiving mangrove (black line) and in the control mangrove (gray line).

sediments. Due to the semi arid conditions prevailing on the west coast of New Caledonia, and their position in the tidal range, *A. marina* trees are dwarf (~1m in height) and thus *Avicennia* stands are characterized by open canopy, meaning that solar radiation can reach sediment surface, which may explain the high chlorophyll-a content measured. These results are consistent with other studies concerning mangroves developing in the same climatic and physiographic conditions [51]. The Chl-a concentrations measured in the effluent receiving mangrove were significantly higher than in the control one (Wilcoxon Test, $p<0.01$), ranging from 17.7 ± 7.3 to 38.6 ± 27.5 µg.g^{-1} along the year (Figure 4). The impact of shrimp farm effluents on phytoplankton productivity in mangrove was already evidenced in creeks [19,23], but rarely on the microphytobenthos productivity [24]. Our results translate a high primary productivity at sediment surface as a result of nutrients input from the shrimp ponds. The high Chl-a concentrations measured may also result from the deposit of the phytoplankton-rich effluents. However, this algal mat was not homogenous as translated by the high standard deviations of the Chl-a measurements. The latter may be explained by the high heterogeneities of mangrove canopy closure and pneumatophore density compared to the control mangrove, as well as a higher crab density, which can induce different grazing and bioturbation [52]. Higher concentration in Pheo-*a*, representing the degradation products of Chl-a, were also measured in the effluent receiving mangrove (23.4 ± 3.8 to 28.8 ± 5.8 µg.g^{-1}) compare to the control one (5.2 ± 1.2 to 6.7 ± 1.9 µg.g^{-1}). This result may indicate efficient decomposition processes at sediment surface. From February to August, period that corresponds to a temperature decrease, Chl-a

concentrations were quite stable in the control mangrove (Wilcoxon Test, $p>0.05$), while they increased from October to February, when the temperature increased from $22.4 \pm 1.4°C$ to $27.8 \pm 1.1°C$ (Figure 2). Seasonal evolution of the microphytobenthos productivity was already evidenced in New Caledonia [53]. These authors observed biomass and productivity increases of micro and macro-algae with increase of temperature and irradiance during summer. The quantity of primary producers (chl-a biomass) at sediment surface increases with temperature, solar radiation, but also with soil water content, which depends both on the season and the position in the tidal zone [51]. In New Caledonia, the summer (from December to February) is characterized by high temperature but also intense rainfall, which can represent ideal conditions for the growth of microphytobenthos. The seasonal evolution of the Chl-a concentrations in the effluent receiving mangrove was similar to the one in the control mangrove, with higher values in summer. From February to June, when the farm was active, concentrations ranged between 17.7 ± 13.9 µg.g^{-1} and 25.7 ± 13.9 µg.g^{-1} (Figure 4), without any significant differences during the 4 sampling campaigns (Wilcoxon Test, $p>0.05$). In August after the final drain, the concentrations increased, reaching 30.0 ± 14.6 µg.g^{-1}. Thus the release of high quantity of enriched water in the mangrove induced a high microphytobenthos development, even during the winter period. During the non active period of the farm, from August to February, the Chl-a concentrations did not decrease, with a mean value of 27.8 ± 11.3 µg.g^{-1} (Figure 4). This result is opposite to what was observed for mangrove creeks, where phytoplankton biomass returned to natural levels within a few months after the cessation of the discharge [19,25].
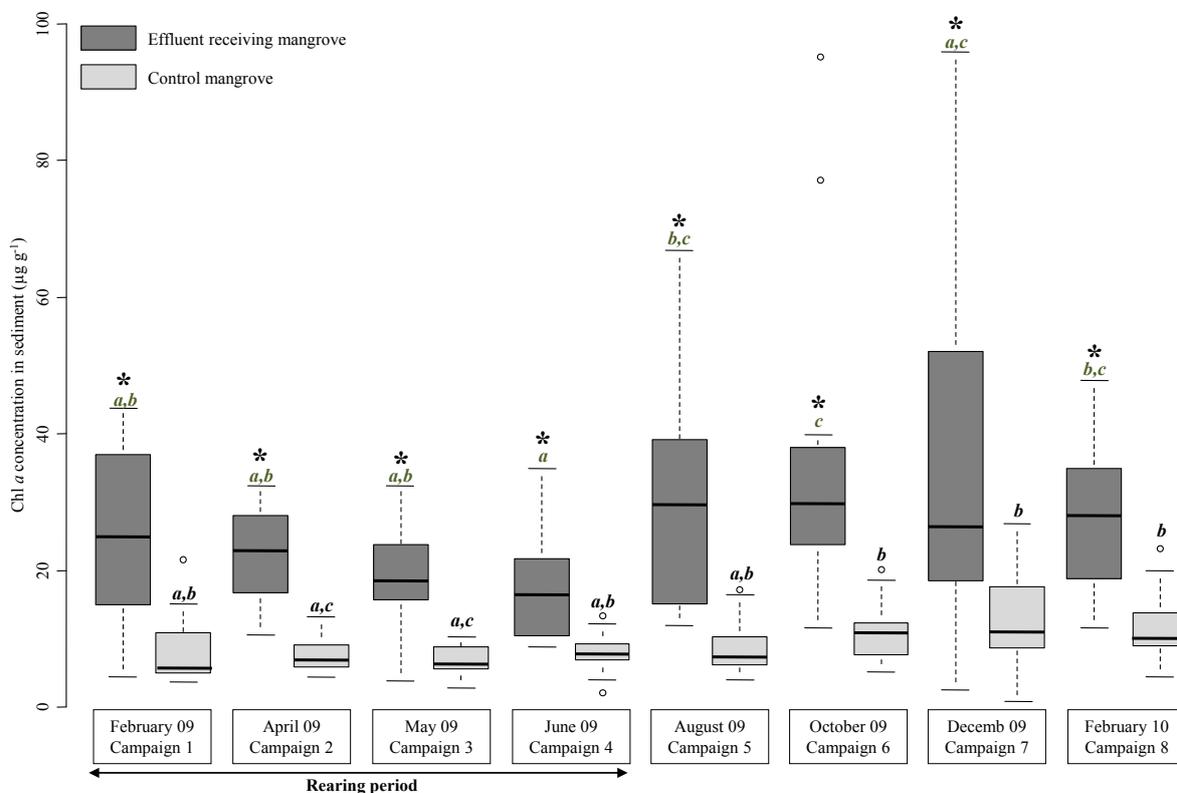

Figure 4: Boxplot representation of benthic Chl-a concentration (µg.g^{-1} of sediment) measured at sediment surface in the effluent receiving mangrove (dark grey) and in the control mangrove (light grey) during 8 campaigns between February 2009 and February 2010. Significant result of comparison of means test (WMW, $p<0.05$) between the two mangroves for each track is marked by a star. Significant differences test (WMW, $p<0.05$) between sampling periods for each mangroves are indicated by different letters.

Effluent release in the mangrove occurred during winter, when the metabolism of benthic organism are at their minimum [53,54], and the final drain occurred just before the seasonal temperature increase, period during which the microphytobenthos biomass increased in the control mangrove. Thus, in the same way that the effluents feed the trophic dynamic of the water column [24], they contribute to an increased development of micro-algae and bacteria, as already shown in mangrove sediments exposed to effluents from agricultural activities [55]. These results highlight that the Chl-a concentrations at mangrove sediment surface, and thus the microphytobenthos activity, were driven both by the release of effluents and the climatic seasonal evolution. The fact that the final drain occurred just before the temperature increase induced a boosted algal bloom, and the fact surface sediments never recovered its natural characteristics.

Influence of shrimp farm effluents on mangrove pore-water properties

Natural seasonal evolution of mangrove pore-waters: Within the control mangrove, pore-water salinity varied along the year and with depth. In the upper cores, salinity increased from less than 40 in February to 65 in October, while below 35 cm depth, values were quite stable along the year, around 65 (Figure 5). Surface salinities may vary responding to physical factors (evaporation, intensity of rain, tidal flooding and position in the mangrove) and biological factors

(transpiration). In fact, salinity values increased from the hot and wet season to the cool season, which is characterized by low rainfall in New Caledonia. During this season, surface salinity was almost twice that of seawater, highlighting the influence of evaporation processes, as observed in Australia [56]. In addition, plant transpiration may increase pore-water salinity in mangrove sediments. In a previous study, an increase of pore-water salinity with *Avicennia* trees development was observed [32]. The high and stable basal salinities measured along the year cannot be explained by evapo-transpiration processes since *Avicennia* trees produce horizontal cable roots just below the sediment surface (in the upper 30 cm). Flow of underground water may explain this phenomenon, either by advection of salt water beneath fresh or less saline water, or vertical migration of water through crab holes [57], or via convection processes [32,58,59]. Redox conditions also varied along the year and with depth. During the whole year, the upper cores were characterized by Eh values ranging between 100 and 200 mV, slightly acidic pH (Figure 5), and the presence of Fe^{2+} and Fe^{3+} (Figure 6), indicating oxic to suboxic conditions. At depth, Eh values were lower than 0 mV, dissolved iron was under the detection limit, evidencing anoxic processes, as observed in other mangroves [60-62]. Within the upper sediment, suboxic to oxic conditions may be explained first by bioturbation. Crab burrows, or holes created by decomposing roots, may allow the renewal of electron acceptors during high tide [63]. Additionally, the root system of *Avicennia* trees has the capacity
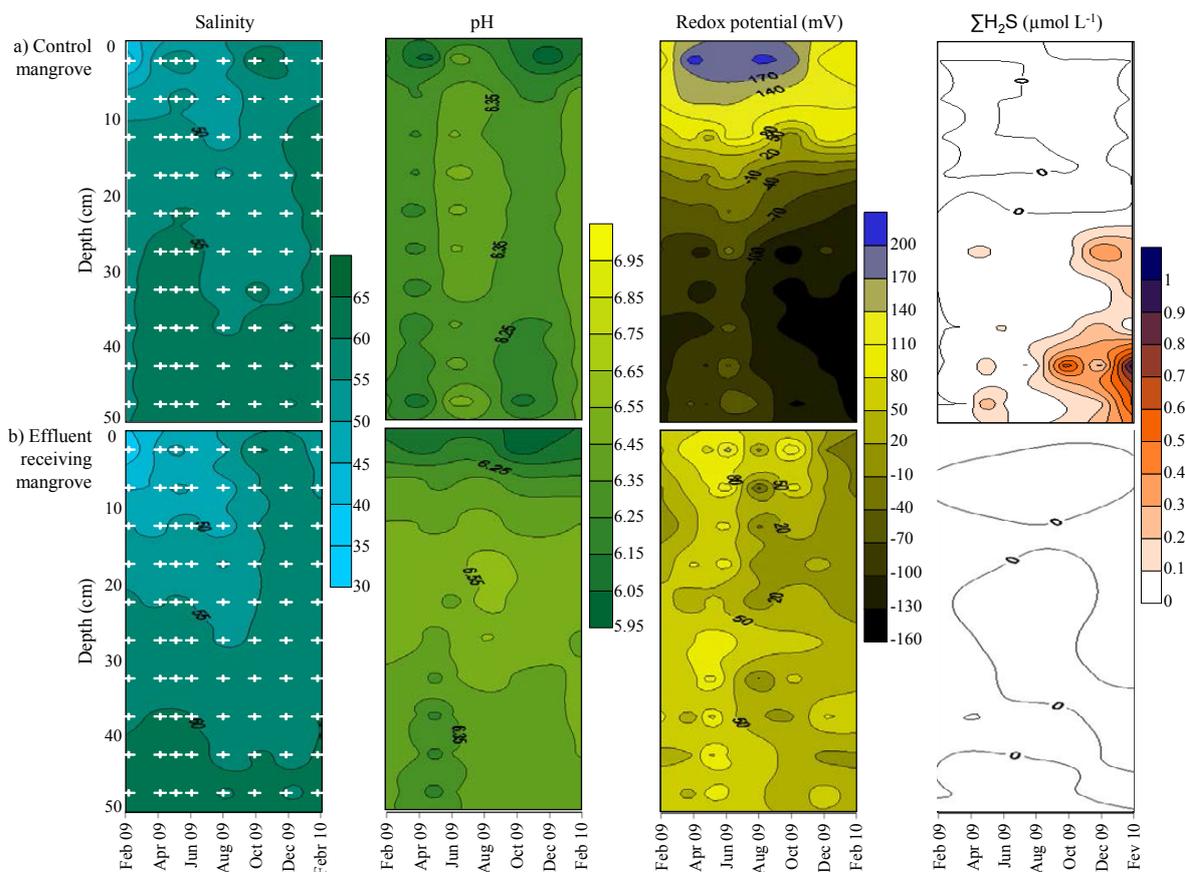

Figure 5: Temporal evolution of pore-water parameters: salinity, pH, redox potential, and total free dissolved sulfur ($\sum H_2S$) (means of tree replicates), in the control mangrove (a) and effluent receiving mangrove (b). Graphical representation was performed under Surfer 8, with Krigging interpolation method for extrapolating values between sampling periods. Measurement points are represented by crosses visible on the graphs of salinity (averaged on 3 replicates).

to diffuse oxygen into the sediment, and to create large oxidized rhizospheres [64-68]. In French Guiana, we demonstrated that the older the *Avicennia* forest, the higher the redox values, providing the sedimentary organic content was low, highlighting the key role of root growth on redox conditions [69]. Mangrove plant species distribution is correlated with soil redox potential and sulphide concentration, and reciprocal effects exist between soil characteristics and tree roots, with a negative correlation between pneumatophore density and sulphide concentrations [43]. Clark et al. [65] attributed the "lower oxidation zone" of their redox stratification model to the activity of the radial cable roots of *Avicennia*; while the "upper oxidation zone" was attributed to animal bioturbation. The two zones can merge in case of developed root system, resulting in suboxic to oxic conditions in the upper 30 cm of the sedimentary column. In this layer, increasing oxygen concentration is expected to favour the biodegradation of organic material and/or the oxidation of Fe sulfides, causing the observed pH decrease. At depth, the sedimentary organic content was higher as a result of the decomposing *Rhizophora* roots, explaining the anoxic conditions prevailing during the whole year. Sulphate reduction led to sulphide precipitation in this zone, explaining the total solid sulphur content reaching more than 6% (Figure 6). As soon as sulphate-reduction began, dissolved Fe concentrations decreased (Figure 6). reflecting the co-precipitation of Fe and S in the form of framboidal pyrite (optically observed in a previous study [61]). Fe(III)-bearing goethite and phyllosilicates were the major Fe hosts in the upward horizons of mangroves in Northern New Caledonia [70]. These mineral species progressively disappear with increasing depth where Fe(II)-bearing pyrite forms in the hydromorphic mangrove layers. Sediment was thus redox stratified whatever the season. Concerning the seasonal evolution of the redox conditions, Eh values were higher on the whole sedimentary column from April to August than from October to February, the hottest months (Wilcoxon Test, $p<0.05$) (Figure 5). In addition, the presence of dissolved sulphides (ΣH_2S) was evidenced at depth only from October to February (Figure 6). The temperature dependence of soil organic matter decomposition is still under debate, but may explained this trend [71]. In addition, the activity of decomposers, like fungi present even in anoxic and sulfidic sediments [72], may increase under these conditions. Eventually, summer was characterized by an increase of phytobenthos productivity as well as mangrove trees productivity [73], which may have induced a greater input of OM to

the sediment, greater oxygen consumption and thus lower Eh values in the upper core. Concerning nitrogen species, DON concentrations ranged from 30 to 150 $\mu mol.L^{-1}$ along the year (Figure 7). In a more developed mangrove forest in Malaysia, DON concentrations ranged from 79 to 315 $\mu mol.L^{-1}$ [60]. Mangrove ecosystems are characterized by a high net primary productivity (NPP), and high rate of organic accumulation in their soils [47,74], which may lead to high DON concentrations in pore-waters. The fact that the highest concentrations were measured at the root level, and during the hottest month, may evidence the role of the production of root exudates in the enrichment of mangrove soil in DON. The concentrations of NH_4^+ and NO_X were very low (Figure 7). NH_4^+ concentrations ranged between 1 and 4 $\mu mol.L^{-1}$ between 10 and 25 cm depth, the latter depth corresponding to the maximum densities of *Avicennia* roots [75]. NO_X concentrations mainly ranged between 1 and 2 $\mu mol.L^{-1}$ during winter, and were under the detection limit during the hottest months, December and February. High consumption of NH_4^+ by mangroves trees for their growth may explain these low concentrations [76,77]. In addition, ammonium is usually extremely rapidly recycled in pore waters (a few hours to two days) [77]. In presence of oxygen, nitrification can occur, producing nitrates that can be used by mangrove plants or subjected to denitrification processes. The fact that nitrates were under the detection limit during the hottest months may suggest that denitrification can be very efficient. In a previous study, we demonstrated that pristine mangrove sediments may be a net sink for inorganic nitrogen [44]. Dissolved organic phosphorus concentrations ranged between 1 and 5 $\mu mol.L^{-1}$ from February to August, and were lower than 1 $\mu mol.L^{-1}$ from October to February (Figure 8). In the upper 20 cm, the concentrations of DIP were almost negligible during the whole year. This superficial layer corresponds to the root zone of *Avicennia* and densest microalgae populations. DIP is a limiting nutrient and fuels the growth of mangroves. Moreover, as reduced iron was present in pore-waters of this layer, oxygen diffusion from *Avicennia* roots could induce the formation of a reactive oxide iron zone [70]. The presence of iron hydroxides and oxihydroxides, which have a strong adsorption capacity for P could constitute a huge trap of DIP [78]. They can rapidly stored DIP from the pore-waters and perhaps from the water column. Under the root layer, concentrations of DIP increased and ranged between 1 and 10 $\mu mol.L^{-1}$, especially during the hottest month. Anoxic conditions induced iron oxide dissolution, which release DIP into the

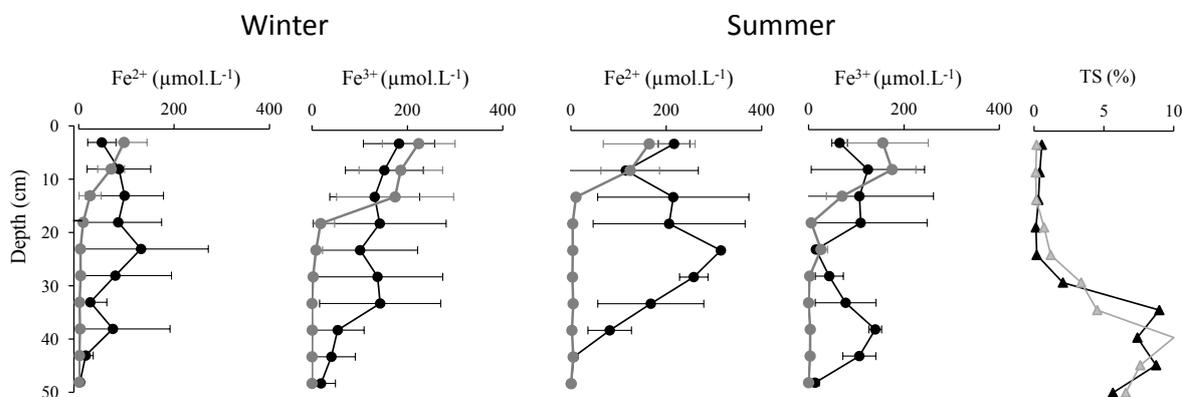**Figure 6:** Dissolved iron (Fe^{2+} and Fe^{3+}) and total solid sulphur (TS) in *Avicennia* sediments measured in June (winter) and in December (summer) 2009 in the effluent receiving mangrove (black line) and in the natural mangrove (gray line) (means of tree replicates ± σ). TS were only measured in June.
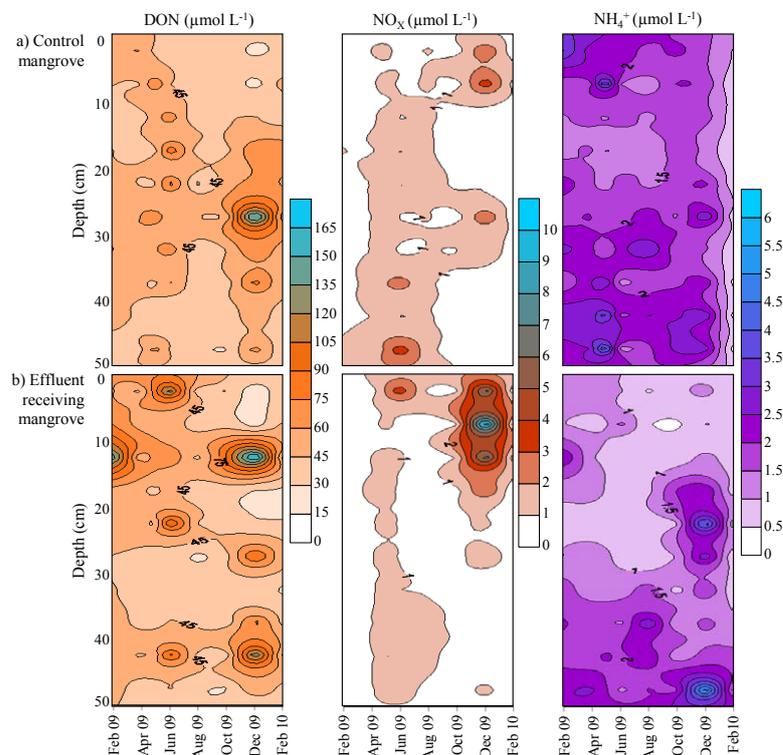
Figure 7: Temporal and depth evolution of the concentrations in DON, NO_X and NH_4^+ (means of tree replicates) in pore-waters in the control mangrove (a) and in the effluent receiving mangrove (b). Graphical representation was performed under Surfer 8, with Krigging interpolation method for extrapolating values between sampling periods.

Figure 8: Temporal and depth evolution of the concentrations in DOP and DIP (means of tree replicates) in pore-waters in the control mangrove (a) and in the effluent receiving mangrove (b). Graphical representation was performed under Surfer 8, with Krigging interpolation method for extrapolating values between sampling periods.
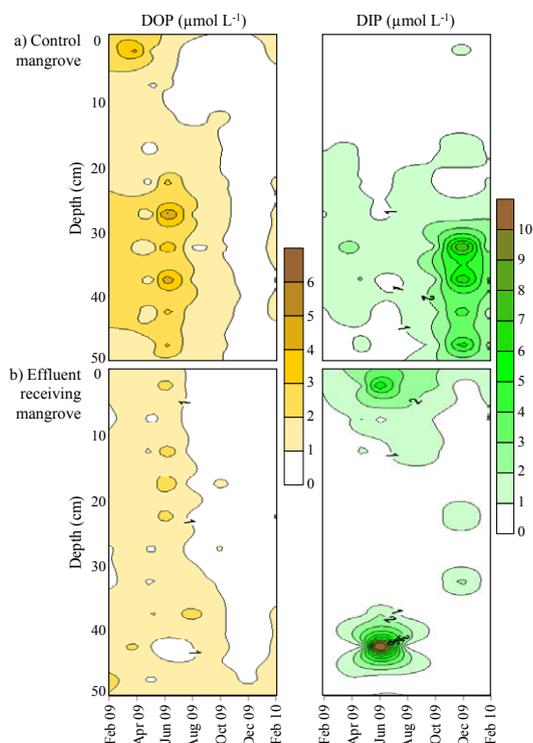
pore-water. The absence of root should contribute to the maintenance of concentration between 1 and 10 $\mu mol.L^{-1}$ in pore-waters at depth.

Seasonal evolution of pore-waters in the effluent receiving mangrove: Within the effluent receiving mangrove, pore-water salinity evolution with depth and along the year was similar to the one in the control mangrove (Figure 5). However, during the active period of the farm, a dilution characterized the upper sediment, with values close to 35, same that the lagoon water and 10 units lower than the pore-water of the control mangrove. In addition, the water content of the effluent receiving mangrove was slightly higher than for the control one (64% vs. 56%). The fact that releasing effluent in the mangrove led to a decrease of pore-water salinity may induce ecological modifications of the ecosystem. Mangrove is a zoned ecosystem, and each mangrove plant develops under specific conditions. In New Caledonia, pore-water salinity is a key driving factor of mangrove zonation [41]. Actually, *Avicennia* trees extend behind *Rhizophora* trees that develop on the seashore because they cannot cope with high pore-water salinities. Artificially decreasing salinity by releasing effluent may led to the development of favorable conditions for *Rhizophora* trees within the *Avicennia* stand, and the disappearance of the latter stand. This phenomenon was observed by many shrimp farmers downstream their ponds in New Caledonia. Nevertheless, during the non active period of the farm, salinity values returned to their normal values for this stand, and prevent the colonization by *Rhizophora* trees. In fact, salinity values were even higher than in the control one, probably as a result of the more develop root-system of *Avicennia* trees that led to higher transpiration processes. Surprisingly, mangrove sediments downstream the ponds were characterized by suboxic conditions almost during the whole year and on the whole depth profile, with Eh values higher than 50mV (Figure 5) and the presence of Fe^{2+} and Fe^{3+} down to 45 cm depth (Figure 6). In addition and opposite to the control mangrove, there was no stratification of the redox conditions with depth. Like for the salinity, the fact that trees were bigger downstream the ponds, and with a higher density, probably implied a more developed root system, and due to its physiological abilities, have induced a better oxygenation of the sediment. During the non active period of the farm, from August to February, Eh values were slightly lower (Figure 5). This period corresponds also to the beginning of the seasonal temperature increase. In the previous discussion concerning Chl-a seasonal evolution, this period was characterized by a bloom of microphytobenthos. Thus, the upper sediment was subjected to an increase input of organic matter that may have induced greater oxygen consumption for its decay. The most significant seasonal variation of the redox conditions was identified between 10 and 25 cm depth (WMW test, $p<0.05$), corresponding again to the root level. The fact that the Eh values at the root level were lower during summer may indicate that the production of labile OM from roots exudates stimulated bacterial processes. Consequently, the root system can influence the redox conditions in two ways: by releasing oxygen, leading to an increase of Eh values, and by the production of labile organic matter, leading to a decrease of Eh values. Downstream the biggest shrimp farm in New Caledonia (132 ha of ponds), previous results were contradictory, the sediment being anoxic for almost the whole depth profile during the period of effluent release, while oxidizing in the upper core during the period without effluent release [26]. In addition to the fact that the current study site was characterized by a smaller farm, with only two shrimp ponds, the farmer has decided to dig a channel to allow a better export of the effluents. This channel brings the effluents to the *Rhizophora* stand, which is closer to the sea. As a result, the effluents covered the *Avicennia* stand only when the channel overflows at high tide. The *Avicennia* stand is thus not always covered by a sheet of water

like downstream the bigger farm. *Rhizophora* stands are characterized by a higher productivity, a close canopy, elevated organic content in their sediments, and anoxic conditions with sulfate reduction processes [62]. In addition, they are submerged at each tide. Releasing the effluents in the *Rhizophora* stand will not modify the pore-water salinity of the stand. Additionally, the latter is already organic rich and subject to sulfate reduction process. Eventually, the close canopy will limit the light intensity reaching the soil and thus the development of microphytobenthos. We suggest that releasing effluent in the *Rhizophora* stand would be a better option, preventing any ecological modification neither of the *Avicennia* stand nor the *Rhizophora* stand. Since the shrimp farm released high quantity of total N and P, approximately 2.3 and 0.5 tons, respectively [44], we followed the concentrations of the different forms of dissolved P and N in the pore-waters in the effluent receiving mangrove. Three main differences with the control mangrove were evidenced: i) DON concentrations were higher during the whole year and on almost the whole depth profile, ii) NO_x concentrations in the upper sediment were higher during the hottest months, and iii) NH_4^+ concentrations were lower in the upper sediment whatever the season (Figure 7). Higher DON concentrations may be related to the more developed root system and the higher OM input into the sediment compare to the control mangrove. In French Guiana, a strong increase of DOC concentrations in pore-waters with plant development was observed [79]. We identified that N and P inputs were mainly as organics forms [44]. This excess nitrogen load can be eliminate by benthic nitrogen cycling as gaseous products (N_2, N_2O and NO) *via* denitrification and anaerobic ammonium oxidation (anammox) [80-82]. In a previous study, we observed that nitrification in the sediment at the outlet of a pond was the predominant source of nitrate fuelling nitrate reduction processes; nitrification rate was up to 210.6 ± 46.8 μmol m^{-2} h^{-1} [44]. The high NOx content characterizing the upper sediment during summer and the absence of ammonium may result from marked N benthic processes. As evidenced by the Chl-a seasonal distribution, the beginning of the summer was characterized by intense bacterial activity. Concerning dissolved phosphorus, the organic forms presented the same distribution than the control mangrove, but with lower values (Figure 8). DOP concentrations were lower than 2 $\mu mol.L^{-1}$ from February to August, and then under the detection limit. More intense decomposition processes resulting from the more developed root system may explain this trend. Concerning the inorganic form, we were not able to detect its presence in the upper sediment of the control mangrove probably as a result of its rapid utilization by mangrove trees and its possible precipitation with oxihydroxides. However in the effluent receiving mangrove, concentrations increased with farm activity, ranging between 1 and 5 $\mu mol.L^{-1}$ (Figure 8), evidencing the influence of effluents on this limiting nutrient, which may be favorable to mangrove growth.

Conclusion

This study highlighted an effective impact of the discharge of shrimp farm effluents on benthic primary productivity at mangrove sediment surface, as well as on the physico-chemical processes in the pore-waters, even several months after the cessation of the release. However, we did not evidence any sign of saturation, eutrophication or anoxia of the effluent receiving mangrove, probably as a result of, on the one hand, the physiological abilities of the root system to aerate the sediment, and on the other hand, the construction of a channel that conducts the effluents directly to the *Rhizophora* stand limiting the dispersing of the effluents within the *Avicennia* stand at the high tide periods. The main conclusions of this study can be summarized as followed.

- Quality and quantity of the organic matter at sediment surface strongly differed between the two mangroves. POC and Chl-a concentrations were higher at sediment surface in the effluent receiving mangrove, while C/N values were lower, indicating a greater algae development and a higher primary productivity as a result of nutrients input from the shrimp ponds.

- Chl-a concentrations increased during summer, which is the hot and wet season, reflecting a greater development of microphytobenthos. The activity of the latter at sediment surface in the *Avicennia* stand was driven both by the release of effluent and the climatic seasonal evolution. The fact that the final drain of the shrimp ponds occurred just before the seasonal temperature increase induced a boosted algal bloom, and the fact the sediment never recovered its natural characteristics.

- Organic enrichment of the effluent receiving mangrove sediment was moderated compared to other mangrove swamps, and there were not significant differences with the control mangrove. However, the higher density and much greater individual size of the mangrove trees were translated by a higher contribution of their debris in the sedimentary organic pool.

- Releasing effluents in the mangrove led to a decrease of pore-water salinity during the active period of the farm. However during the non active period of the farm, salinity values returned to their normal values for this stand, and prevent the colonization by *Rhizophora* trees, limiting ecological modification of the ecosystem.

- Surprisingly, mangrove sediments downstream the ponds were characterized by suboxic conditions almost during the whole year and on the whole depth profile. In addition and opposite to the control mangrove, there was no stratification of the redox conditions with depth up to 50 cm. The fact that trees were bigger downstream the ponds, with a higher density, probably implied a more developed root system, and due to its physiological ability, have induced a better oxygenation of the sediment.

- Concerning nitrogen cycling, higher DON concentrations were measured probably as a result of the higher mangrove trees development. In addition, the upper sediment of the effluent receiving mangrove was characterized by higher NO_x concentrations and lower NH_4^+ concentrations, evidencing more intense benthic N processes, notably nitrification as a result of the better oxygenation of the sediment.

Acknowledgement

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