Potential Nitrate Reduction Rates in Marine Mangrove Sediments in the Lesser Antilles

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Abstract

Mangrove sediments are generally nitrogen limited, with nitrate reduction to ammonium instead of denitrification in these sediments, resulting in nitrogen retention rather than nitrogen elimination. The goal of this work was to investigate the potential for nitrate reduction in marine mangrove sediments along a canal impacted by anthropogenic activity (Guadeloupe, West Indies) as a function of increased nitrogen load and how this would change nitrate transformation rates. In addition to that, the impact of the organic carbon load and the hydraulic retention time was assessed as factors affecting nitrate reduction rates. Potential nitrate reduction rates in the sediments along the canal, in the presence of indigenous organic carbon, ranged from 126 to 379 nmol cm$^{-3}$ h$^{-1}$ generally increasing upon increasing supplied nitrate. The potential for nitrate reduction increased significantly with the addition of mangrove leaves, whereas the addition of simple, easily degradable carbon (acetate), resulted in an almost five-fold increase in nitrate reduction rates. The hydraulic retention time also had an impact on the nitrate reducing capacity due to an increased contact time between nitrate and the benthic microbial community. Marine mangrove sediments have a high potential to mitigate nitrogen pollution, mainly governed by the presence of large amounts of degradable carbon in the form of litter. The hydraulic retention time as tested experimentally that can be extrapolated to the time of inundation of the mangrove sediments may increase the potential for nitrate reduction. Whereas the sediments are daily exposed to a small tidal effect, increased water retention could increase the nitrogen elimination potential in these mangrove sediments.

Introduction

Mangroves are coastal wetlands that are circumtropical distributed with an estimated global coverage between 10 to 24 million hectares (Kathiresan and Bingham, 2001). They protect coastlines, support coastal fisheries, enrich coastal waters and thrive under high saline conditions, tides, strong winds and elevated temperature. These ecosystems are highly productive and contribute significantly to the global carbon cycle (Bouillon et al., 2008). Litter fall represents a significant share of the carbon production in these systems (Alongi et al., 2005). Whereas part of this primary produced litter will be degraded in the water column, another part will settle on the sediment thus increasing the amount of sediment organic carbon. Part of this organic matter is degraded, mainly by microbial activity (Alongi, 1988); the non-degraded fraction will thus be buried. Aerobic heterotrophic microorganisms will degrade this freshly deposited organic matter at the sediment surface. After depletion of oxygen, anaerobic microbes will be capable of using alternative electron acceptors. In marine mangrove sediments sulfate is one of the major alternative electron acceptors, and has been shown to be responsible for an important part of the anaerobic mineralization (Kristensen et al., 2008).

The two main pathways of anoxic nitrate reduction in mangrove sediments are denitrification and dissimilatory nitrate reduction to ammonium (DNRA), whereas anammox has also been shown to occur (Amano et al., 2011; Balk et al., 2015; Brunet and GarciaGil, 1996; Burgin and Hamilton, 2007; Cao et al., 2016). For example, while Chiu et al (2004) indicated that in mangroves 55–76% of nitrogen loss is
through denitrification, Fernandes et al. (2012) estimated 99% DNRA in sediments. Cao et al. (Cao et al., 2016) also showed that DNRA was the dominant pathway for nitrate reduction in N limited mangroves in South east China. Ammonium is either produced via DNRA or organic matter degradation resulting in nitrogen retention (Enchrich-Prast et al., 2016). During denitrification and DNRA nitrite can be produced as an intermediate, whereas nitrous oxide (N\textsubscript{2}O) a strong greenhouse gas can be an intermediate during denitrification. Nitrate, nitrite, nitrous oxide and ammonium are reaction products and intermediates whose presence and proportion in the environment depend on the capacity of the microbial communities present to reduce nitrate. The assessment of the quantities of nitrogen likely to be eliminated and the identification of the metabolic pathways of reduction vary from one study to another. The ability to remove nitrate is influenced by various factors such as nitrate levels, the nature and availability of organic matter and local environmental conditions. Anaerobic nitrate removal processes require an organic carbon source as an energy source. The efficiency of nitrate removal depends on carbon availability and quality, i.e. the chemical composition (Chen et al., 2017; Hume et al., 2002; Pulou et al., 2012; Shiau et al., 2016).

Mangrove sediments, usually rich in organic matter and largely anoxic, have been sites for wastewater disposal (Kathiresan and Bingham, 2001). Interest in the nitrate reduction capacity of mangrove sediments has been growing and has been the subject of numerous studies conducted mainly on terrestrial mangroves (Fernandes et al., 2012a; Fernandes et al., 2016). Due to their hydromorphism and the accumulation of organic matter, mangrove sediments and soils are known to be potentially active reducing zones for nitrogen removal (Fernandes et al., 2016) used to treat nitrogen rich wastewater (Corredor and Morell, 1994; Shiau et al., 2016).

In the current study, we investigate the potential for nitrate reduction in mangrove sediments in an area downstream of a domestic wastewater treatment plant (WWTP) and along a mangrove density gradient in the “Canal des Rotours” Guadeloupe, West Indies. The nitrate-reducing capacity of the sediments, located in the same pedoclimatic context, along this canal was determined. The sediment and the benthic nitrate reduction rates along the canal are subject to a gradient of intrusion of marine waters into fresh water and human activities. The impact of key variables on potential nitrate reduction rates in mangrove sediments was determined using flow through reactors. The effect of nitrate and carbon concentrations as well as the flow rate, supplying nitrate to the sediment was determined. To do this, in addition to the physico-chemical characterization of the different sediments along the profile, laboratory experiments were conducted to test the impact of environmental and anthropogenic parameters on sediment reactivity. The effects of flow rate (retention time), nitrate concentrations (electron acceptor) and labile carbon availability (electron donor) were studied on potential nitrate reduction rates. For this purpose, the kinetics of nitrate reduction and the potential rates of nitrate reduction and nitrite and ammonium production under controlled conditions were determined using the continuous flow reactor method for surface sediments (0-1 cm depth) of marine mangrove sediments. Characterization of the organic matter present in the mangrove sediments were related the nitrate reduction potential.
We hypothesize that the sediments are nitrate limited and that the hydraulic retention time of nitrate containing water will increase the nitrate removal capacity. Taking into account the high organic carbon content of mangrove sediments, it is hypothesized that the quantity of organic carbon is sufficient and nitrate reduction is not limited by organic carbon. The mangrove sediments might thus exhibit a purifying filter for insular land-based inputs before discharging into lagoon waters, particularly in the case of the marine mangroves of Guadeloupe because of the low tidal range that governs them.

**Materials And Methods**

**Study site and sampling**

Potential nitrate reduction rates were determined along ‘The Canal des Rotours’, near the town Morne à l’eau located in Guadeloupe, French West Indies. The Canal des Rotours was created in the 19th century during the slavery period in order to improve river transport on the island with a length of 6 km (Figure 1). The canal bed has a width that varies between 18 and 30 meters and a maximum depth of 2.9 m. The shallow canal consists of a straight section that crosses the community Morne à l’eau. A second part passes an agricultural area and then meandering through a mangrove forest until it reaches the coast. The mangrove forest is primarily covered along the canal by red mangrove trees, *Rhizophora mangle*, becoming more and more dense downstream. The downstream part is flooded and subject to tide resulting in a limited horizontal as well as a vertical salinity stratification, with a tidal range not exceeding 40 cm.

The village Morne à l’eau discharges its wastewater treatment plant (WWTP) effluent directly into the canal. The plant treats urban and industrial wastewater and receives external inputs in the form of waste and grease as well as sludge from three other smaller stations. The WWTP releases, on a daily basis, 30 mg l\(^{-1}\) suspended solids, 15 mg N l\(^{-1}\) (Kjehdahl nitrogen) and 2 mg P l\(^{-1}\) (Total phosphorus), no specific treatment reducing N is applied in the WWTP (“Régie Eau Nord Caraïbes de la Communauté d’Agglomération du Nord Grande Terre”, Franck Zadigue, pers. comm.).

To evaluate the nitrate removal capacities along the canal, three stations (CR1, CR2 and CR3) were selected from the release point to the estuary depending on the density of the mangrove forest. The wastewater plant and CR1 are located in an inhabited area while the two other sites CR2 and CR3 are composed of mangrove, becoming denser towards the estuary. The sediments were sampled from the canal or mangrove riverbank.

Water samples (triplicate) were collected upstream, downstream and near the outlet of the wastewater treatment plant to analyze nutrient concentrations at a depth of 50 cm. For each site, the two first centimeters of the muddy immersed sediments were sampled in November and December 2016. The sediments were transported to the laboratory and flow-through reactor experiments were started within 2h upon sampling. For each site, eight reactors were run (2 per treatment). A subsample of the sediment (30 grams) was dried at 37\(^\circ\)C during one week and crushed in a ball mill.
In addition to nutrient, carbon and nitrate removal capacities, a bathymetric profile of the canal at the three sampling was conducted. The floor profiles at each sampling site were carried out using a handheld sounder (Plastimo Echotest II) over the entire width of the stations, with a distance between two measurements set at one meter. The salinity profiles of the water column in the canal were determined by measuring salinity at different depths with a multi-parameter probe (Multiline P4) at 10 cm of depth intervals.

**Nitrogen transformation rate determination**

To determine the effect of environmental variables on nitrate reduction rates, surface sediments (0-1 cm) from the three sampling stations were incubated in flow through reactors (Laverman et al., 2006). In order to assess the reactivity of the sediments along the canal with respect to nitrate reduction, the sediments underwent three types of treatments as presented in Table 1. For some treatments, only the sediments from CR2 were incubated. This site was chosen because of its location closest to the canal, i.e. a tide-influenced marine mangrove area with little anthropogenic influence.

The reactors used in this study consist of a sediment section sealed by a Plexiglas® ring of 1 cm height with 0.2 µm pore size nitrocellulose filters and glass fiber filters (1.2 mm thick, 47 mm diameter) at each end and O-ring to prevent leakage. To ensure a radially homogeneous flow, input and output canals are present at the center of the caps, in direct contact with the filters.

| experiment | date      | station | Treatment | \( \text{Q}_{\text{avg}} \) (ml h\(^{-1}\)) | \([\text{NO}_3^-]\) (mM) | Carbon |
|------------|-----------|---------|-----------|-----------------|---------------------|--------|
| [\text{NO}_3^-] | Sept 2016 | all     | 2.8       |                 | 0.6 - 6             | -      |
| Carbon     | Oct 2017  | CR2     | 2.7       | 6.1             | YL, GL, Ac          |        |
| Flow rates | Aug 2017  | CR2     | 0.5, 1.2, 2.7, 6.2, 9.8, 6.1 | 6.1 | - |

The inflow solutions consisted of milliQ water containing 33 g l\(^{-1}\) NaCl and different concentrations of KNO\(_3\). To determine the maximal nitrate reduction rates and at which concentration the reduction is limited by the nitrate supply, four concentrations of nitrate were chosen (0.6-0.7 mM, 1.4-1.9 mM, 3.0 -3.1 mM, and 6.0 mM). In order to assess the influence of the nature and availability of carbon on the potential nitrate reduction rates, three different sources of carbon were provided to the sediment. Additions were made either by mixing the sediment with mangrove leaves or via the input solution (2mM acetate); all reactors were supplied with a 6 mM nitrate solution at a flow rate of 2.7 ml h\(^{-1}\) (see Table 1).
The elevated nitrate concentration was chosen based on the results of the nitrate addition experiments, making sure nitrate was not limiting in order to test the effect of carbon. Sediment that was used for carbon additions was mixed with green leaves or yellow leaves (weight leaves: weight sediment 2.5:1). Green leaves from *Rhizophora mangle* trees, the yellow leaves correspond to senescent leaves. The two types of leaves differ in total carbon content as well as C:N ratio and should result in an enrichment of the sediment with organic carbon. The reactor experiments that tested the impact of flow rate on nitrate reduction rates applied five different flow rates to sediments of CR2 with nitrate concentration of 6.1 mM.

Nitrate was the only external electron acceptor supplied to the sediment by the inflow solution. A peristaltic pump with a continuous flow rate of 2.8 ± 0.1 ml h\(^{-1}\) was used to supply the inflow solution to the reactor (ISMATEC Reglo digital, model ISM834). To ensure anoxic conditions, the inflow solutions were vigorously purged with argon gas during 10 minutes. The oxygen concentration of the purged inflow solution was measured in the input bottle with a fiber optic oxygen transmitter (fibox3, PreSens Precision Sensing GmbH, Germany) and was below 6 µM O\(_2\) (maximum value 5.9 ± 0.03% µM). The reactors were wrapped with aluminum foil to perform the experiment in dark to inhibit light-sensitive processes like oxygen production by photosynthesis. The results shown here where obtained at room temperature (27±0.4°C). After the incubation of the sediment for 12h in order to develop steady state conditions, outflow was collected continuously and sampled every 4 h during 2 days. Collection tubes were changed at indicated fixed time intervals and stored at -20°C prior to chemical analyses. Note that all rates are optimal, potential rates in order to determine and compare the impact of key environmental variables (nitrate, carbon, retention time) on nitrate reduction in surficial mangrove sediments.

**Determination of rates and kinetic parameters**

Potential nitrate reduction rates (NRR) nitrite production rate (NiPR) and ammonium production rate (APR) were calculated using equations 1:

\[
R = \frac{\Delta C \cdot Q}{V}
\]

Where \(\Delta C\) is the concentration difference of the compound between the input and output solution, (in µmol L\(^{-1}\)), Q the volumetric flow rate of the solution through the reactor in ml h\(^{-1}\) and V is the volume of the sediment contained in the reactors which was 13.85 cm\(^3\). The rates of consumption or production (in nmol.cm\(^{-3}\).h\(^{-1}\)) were obtained between 24h and 36 to 48 hours. With a volumetric flow rate of 2.8 ml h\(^{-1}\) and a reactor volume of 13.85 cm\(^3\) this largely exceeds the replacement of initial pore water and a (semi) steady state situation. The rates were determined over a longer period in the experiments with carbon additions (24h to 72h).

In order to determine kinetic parameters and maximum potential nitrate reduction rates for all stations, nitrate reduction rates are presented as a function of the nitrate concentrations of the input solution according to equation 2.
As the nitrate concentration increases, so do the reduction rates. This increase is described by Michaelis-Menten kinetics (see (Laverman et al., 2006), which allows the maximum potential reduction rates \( R_{\text{max}} \) and the half-saturation concentrations \( K_m \) to be determined from the nitrate reduction rates measured at steady state during the experiments.

### Analytical methods

Dissolved inorganic nitrogen compounds were determined colorimetrically in the input solution and the outflow solutions. Nitrate (\( \text{NO}_3^- \)) and nitrite (\( \text{NO}_2^- \)) using a Nutrient Autoanalyzer, Quatro 5Thermo fisher) and the ammonium (\( \text{NH}_4^+ \)) was determined according to the indophenol blue coloration method (Koroleff 1969).

Sediments, green and yellow (senescent) leaves were dried at 37°C for one week, ground and sieved through a 1-mm mesh for sediments and an 80-µm mesh for leaves. Sediments samples were decarbonated by hydrochloric acid (Harris et al., 2001). The C and \( N_{\text{org}} \) contents were determined using an Elemental analyzer (Vario PYRO cube, Elementar). Isotope abundance ratios of organic nitrogen and organic carbon of the solid samples were determined by continuous-flow isotope ratio mass spectrometry coupled to an elemental analyzer (EA-CF-IRMS; Elementar-Isoprime). Results are reported in the internationally accepted delta notation expressed in ‰. The international for C and N is Pee Dee Belemnite and atmospheric nitrogen respectively. Different reference materials were used for carbon and nitrogen: tyrosine (\( \delta^{13}\text{C} = -23.2\%\text{oo}, \delta^{15}\text{N} = 9.98\%\text{oo}, \text{laboryatory standard} \)), IAEA-N1 (\( \delta^{15}\text{N} = 0.3\%\text{oo} \)) IAEAN2 (\( \delta^{15}\text{N} = 20.3\%\text{oo} \)) and IAEA-N3 (\( \delta^{13}\text{C} = -23.2\%\text{oo} \) and \( \delta^{15}\text{N} = 4.5\%\text{oo} \)). Analytical precision is about 0.1‰ for \( \delta^{13}\text{C} \) and 0.2‰ for \( \delta^{15}\text{N} \).

### Statistical analysis

For the different tested conditions, significant variability of measured rates along the duration of the experiment were tested by one-way repeated measures analysis of variance (ANOVA) using Sigma-Plot software. Data were tested for normality (Shapiro-Wilk normality test) and equal variance (Bartlett equal variance tests), in the no-parametric cases, Dunn's ANOVA on ranks were performed. In both case, ANOVA were followed by a Tukey test in order to discriminate differences.

### Results

Water column and sediment characteristics along the canal (Canal des Rotours)

The bathymetric profile at the different locations shows a strong stratification of salinity of the water column (Figure 2). Near the wastewater plant the surface layer (first 60 cm) is freshwater and the
underlying layer is seawater, this layer decreases until the mouth of the mangrove. Differences in temperature and salinity between island and ocean waters result in a stratification observed at all sampling stations. The sediment is in contact over the entire profile with marine waters with salinity over 30. Sediments for the experiments were collected at the shore corresponding to salinities in the pore water ranging from 23 at CR1, 29 at CR2 and 34 at CR3.

Ammonium, nitrate and nitrite concentrations were measured in October 2017 in the water column for all stations (Table 2). Maximum ammonium concentrations were measured immediately downstream of the WWTP (19.4 µM). The concentrations decreased from upstream to downstream. At the mouth, the concentrations measured were 2.3 µM. As for ammonium, the maximum concentrations of nitrite and nitrate were measured immediately downstream of the WWTP discharge and a clear decrease was observed along the canal. Nitrite concentrations decreased from 2.1 to 0.7 µM, nitrate decreased from 18.3 to 3.0 µM.

| #  | Location | distance (km) | [NO₃] (µM) | [NO₂⁻] (µM) | [NH₄⁺] (µM) | C (%) | N (%) | C:N | δ¹³C (‰) | δ¹⁵N (‰) |
|----|----------|--------------|------------|------------|-------------|------|------|-----|-------|--------|
| CR1| WWTP     | 0.0          | 18.3       | 2.1        | 19.4        | 26.3 | 1.4  | 22.3| -27.4| 9.0    |
| CR2| intermediate | 2.4       | 2.9        | 0.4        | 11.5        | 30.2 | 1.3  | 26.7| -28.5| 2.5    |
| CR3| downstream | 3.7         | 3.0        | 0.7        | 2.3         | 34.7 | 1.5  | 27.5| -27.8| 1.2    |

Sediment characteristics, total C, N, C:N ratio as well as δ¹³C and δ¹⁵N, from the same locations as the water samples, are listed in Table 2. The amount of organic carbon increased from upstream to downstream (from 26.3–34.7%). The amounts of sediment organic nitrogen were relatively stable from one station to another varying between 1.3 to 1.4%. The C:N ratios for the three sites ranged from 22.3 to 27.5 increasing from upstream to downstream. The δ¹⁵N of sediments decreased along the canal from 9‰ at the WWTP to 1.2‰ at station CR3. The values of δ¹³C vary little along the canal with no particular trend, ranging from -28.5 to -27.4‰ (Table 2).

The effect of nitrate concentrations on nitrogen transformation rates

Potential nitrate reduction rates

The sediments from the three stations were incubated with four different input solutions and at a flow rate of 2.8 ml h⁻¹ (Table 1). Two examples of the input supply over time (constant) and the concentrations of nitrate and nitrite over time are shown in SI Figure 1. In order to assess the nitrate removal capacity of mangrove marine sediments along the canal, the measured equilibrium concentrations at the reactor outlet were used to calculate the nitrate reduction rates (NRR), nitrite (NIPR) and ammonium (APR) and production rates (equation 1).
Figure 3 shows the nitrate reduction rates for all sampled stations as a function of the nitrate concentrations provided by the input solution. The potential rates of nitrate reduction vary depending on the nitrate concentrations provided by the input solution. For the lowest nitrate input concentration (0.6 and 0.7 mM), potential nitrate reduction rates are 126 and 138 nmol.cm\(^{-3}\)h\(^{-1}\) respectively for CR1 and CR2, these rates increase till 374 and 378 nmol.cm\(^{-3}\)h\(^{-1}\) at 6 mM (see SI Table 1). For these two sampling areas, the higher the concentration of nitrate in the nutrient solution, the higher the potential reduction rates. For the site at the mouth of the canal, CR3, this increase is smaller, with average potential reduction rates increasing from 119 to 219 nmol.cm\(^{-3}\)h\(^{-1}\) between the lowest and highest nitrate concentration provided by the input solution.

The \(R_{\text{max}}\) values deduced from the rates obtained at different nitrate input concentrations from the flow reactor experiments correspond to the maximum nitrate reducing activity that microbial populations in the sediment can eliminate given the availability and abundance of the electron donor (Laverman et al., 2006). In this study, therefore, the levels correspond to the nitrate reduction activity when the amounts of nitrate are non-limiting and when the latter is the only available electron acceptor. As shown in Table 3, there is no clear trend for the two kinetic parameters studied along the canal. The maximum rates (\(R_{\text{max}}\)) differ from one site to another and vary from 234 to 444 nmol.cm\(^{-3}\)h\(^{-1}\) with the highest \(R_{\text{max}}\) for the station CR2. The affinity for nitrate is very low, with very high \(K_m\) values in sediments close to the WWTP (CR1) and at CR2 with respectively values of 1355 and 1218 µM. The \(K_m\) is still high at CR3 with 700 µM but lower than at the two upstream sites.

**Table 3**

| #    | Location | \(R_{\text{max}}\) | \(K_m\) | \([\text{NO}_3^-]\) | C   | C:N |
|------|----------|--------------------|---------|-----------------|-----|-----|
|      |          | (nmol.cm\(^{-3}\)h\(^{-1}\)) | (µM)   | (µM) | (%) |      |
| CR1  | WWTP     | 433                | 1355    | 18.3 | 26.29 | 22.30 |
| CR2  | intermediate | 444            | 1218    | 2.9  | 30.22 | 26.65 |
| CR3  | downstream | 234               | 700     | 3.0  | 34.68 | 27.52 |

*Nitrite and ammonium production rates*

The decrease in nitrate concentrations was also accompanied by an increase in nitrite and ammonium concentrations, resulting in corresponding nitrite and ammonium production rates (SI Table 1). Nitrite production rates ranged from 2.8 to 200 nmol.cm\(^{-3}\)h\(^{-1}\). The maximum rate was reached at the highest concentration of nitrate in the input solution at the station closest to the WWTP (CR1). Levels varied from one site to another, the higher the nitrate concentration in the input solution, the higher the nitrite production rates. The nitrite production rates increased with increasing nitrate supply and thus the nitrate
reduction rates, note that the relative contribution (% in Table SI-1) of intermediate nitrite production increases as a function of increasing nitrate supply.

Ammonium production rates ranged from 27.4 to 101 nmol.cm$^{-3}$h$^{-1}$. Highest ammonium production rates were determined at CR2 from 59 to 101 nmol.cm$^{-3}$h$^{-1}$. The ammonium production (or release) rates per site show no significant differences as a function of supplied nitrate concentrations. However, the amount of ammonium produced as a function of nitrate reduced is elevated at low nitrate concentrations (25 – 43%) and decreases with increasing nitrate reduction rates (9-19%).

Effect of carbon addition: mangrove leaves and dissolved organic carbon

Green mangrove leaves contained 47.9% of carbon, whereas yellow leaves had a carbon content of 46.6% (SI Table 2). The nitrogen content was lower in yellow mangrove leaves (0.6%) compared to the green leaves (1.2%). The addition of the crushed leaves to the sediment increased the carbon content of the reactors from 37.2% in the non-amended sediment to 39.9% in the sediment with yellow leaves and 43.8% in the sediment with green leaves.

Figure 4 shows the nitrate reduction rates as a function of the carbon additions. Overall, the sediments with carbon additions showed higher nitrate reduction rates compared to the non-amended control with average nitrate reduction rates ranging from 165 to 748 nmol.cm$^{-3}$h$^{-1}$. The addition of acetate to the input solution significantly increased sediment nitrate reduction rates from 165 to 748 nmol.cm$^{-3}$h$^{-1}$. The nitrate reduction rates upon addition of green leaves (351 nmol.cm$^{-3}$h$^{-1}$) is significantly higher than that the non-amended sediment (165 nmol.cm$^{-3}$h$^{-1}$), whereas the addition of yellow leaves (260 nmol.cm$^{-3}$h$^{-1}$) is neither significantly different from un-amended nor green leave amended sediments. Even if the differences in rates between the two treatments (green and yellow leaves) are not statistically significant, the results show that the degree of degradation of mangrove leaves changes slightly nitrate reduction rates (260 versus 351 nmol.cm$^{-3}$h$^{-1}$).

Nitrite production rates show the same trend as nitrate reduction rates, with highest nitrite production, 302 nmol.cm$^{-3}$h$^{-1}$ (42% of the NRR) in the presence of acetate (SI Table 1). In the non-amended sediment and the sediment with leave addition, the nitrite production rates were between 15 to 25% of the nitrate reduced. Nitrite production rates upon the addition of acetate are significantly different from the production rates of sediments alone and those with the addition of mangrove leaves. In fact, the average nitrite production rates obtained in sediments with acetate addition is 7 times higher than in sediments where no acetate was added. At the same time, the rate of nitrate reduction associated with this nitrite production is 4.5 times higher in the presence of acetate than the rate obtained in sediments that have not undergone carbon addition.

Regardless the source of carbon supplied to the sediment, all the reactor outlets present a production of ammonium in variable quantities. The ammonium production rates show no significant differences
between treatments with average values ranging from 15 to 36 nmol.cm\(^{-3}\)h\(^{-1}\). The ammonium production rates (SI Table 1) show no significant differences between the treatments.

Effect of Flow Rate on Potential Nitrate Reduction Rates

The impact of different flow rates on nitrate reduction rates are shown in Figure 5. Lowest nitrate reduction rates are determined at 0.5 ml h\(^{-1}\) with an average of 148 nmol.cm\(^{-3}\)h\(^{-1}\). Rates increase with increasing flow rate reaching on average 259 nmol cm\(^{-3}\)h\(^{-1}\) at a flow rate of 2.6 ml h\(^{-1}\). Rates were slightly lower at a flow rate of 6.2 ml h\(^{-1}\) and increased again at 9.8 ml h\(^{-1}\). The nitrite production increased with an increasing flow rate, from 17 to 106 nmol.cm\(^{-3}\)h\(^{-1}\) at respectively 0.5 and 9.8 ml h\(^{-1}\). The percentage of nitrite production increased as well with increasing flow rates and reached up to 57% at a flow rate of 6.2 ml h\(^{-1}\). The same trend was observed for the production rates of ammonium; for the lowest flow rate, these are 22 nmol.cm\(^{-3}\)h\(^{-1}\) and reach 45 nmol.cm\(^{-3}\)h\(^{-1}\) at a flow rate of 9.8 ml h\(^{-1}\) (SI Table 1).

Discussion

Nitrogen and carbon dynamics along the Canal des Rotours

**Water column: dynamics of inorganic nitrogen**

The maximum nutrient concentrations close to the WWTP are likely due to discharge from the station. The decrease in inorganic nitrogen concentrations along the canal may be explained by nutrient dilution with marine waters usually exhibiting low inorganic nitrogen concentration. In addition to that, main sources of inorganic nitrogen, urban and agricultural activity, decrease from upstream to downstream. Microorganisms or vegetation may also rapidly consume nutrients resulting in a diminution along the canal (Kaiser et al., 2015; Lee et al., 2008). Decreasing nitrite and nitrate concentrations can also result from reduction processes such as denitrification along river corridors (Sebilo et al., 2003). The decrease in ammonium and nitrite, not accompanied by an increase in nitrate concentrations, suggests that the nitrification is not a major process in the water column, or that the rate is low compared to the discharge of the canal. In general, the measured inorganic nitrogen concentrations in the downstream, mangrove-dominated section of the canal are low and remain in the same order of magnitude as those generally observed in the water column of mangroves (e.g. (Enrich-Prast et al., 2016; Fernandes et al., 2016).

**Sediment: carbon quantity and quality**

The sedimentary organic carbon contents along the canal are high, slightly increasing from upstream to downstream (26-35%). These organic carbon contents are higher than those from mangrove sediments in the same geographical setting; carbon contents in the mangrove sediments in the “Manche a Eau” were in the range of 5 to 19% (Cremiere et al., 2017; Gontharet et al., 2017). In tidal-influenced mangrove sediments, with a similar vegetation, *Avicennia germinans* and *Rhizophora mangle*, in Florida, the organic carbon content reached maximum values of 26%, consistent with values found in the sediments
along the canal (Balk et al., 2015). The sedimentary N content remains stable along the canal (1.3-1.5%), in the same range as the N content of the *Rhizophora mangle* leaves. In general, mature green and photosynthetically active leaves have higher nitrogen concentrations than senescent leaves (Benner et al., 1990; Kathiresan, 2004), which is in agreement with the values for green (1.2%) and yellow *Rhizophora mangle* leaves (0.6%) at CR2.

The increase in total organic carbon along the canal is accompanied with increasing C:N ratios. Stable isotopes of carbon (δ\(^{13}\)C) and the ratio of organic carbon to total nitrogen (C:N) allow the identification of the origin of organic matter that accumulates in coastal areas. In particular, this method can differentiate between C3 and C4 plants and organic matter of marine and freshwater origin (Chmura and Aharon, 1995; Khan et al., 2015; Lamb et al., 2006). The elemental composition and isotopic signature of mangrove sediment can thus provide clues regarding the origin of the organic matter. The C:N ratio of terrestrial vegetation in C3 plants is typically higher than 12 as it is mainly composed of lignin and cellulose and poor in nitrogen. C4 grasses typically have C:N ratios higher than 30 (Lamb et al., 2006). The δ\(^{13}\)C values associated with C:N ratios in the sediments confirm that the organic matter at all three sites originates from terrestrial C3 plants (Kristensen et al., 2008; Lamb et al., 2006). These δ\(^{13}\)C values are consistent with the isotopic signature of the mangroves found in other studies that characterized *Rhizophora mangle* leaves with δ\(^{13}\)C between -28.3‰ and -25.3‰ (McKee et al., 2002; Wooller et al., 2003). These values are also similar to those obtained in the Manche-à-Eau mangroves with values between -26.4 and -24.4‰ (Cremiere et al., 2017; Gontharet et al., 2017).

The sedimentary δ\(^{15}\)N decreases from 9‰ close to the WWTP outlet to 1.2‰ at the most downstream, marine site. The value of δ\(^{15}\)N measured in the sediment immediately downstream of the WWTP discharge is relative high (9‰) and might be related to WWTP effluent. The mineralization and subsequent volatilization of organic nitrogen in wastewater leads to a significant increase of δ\(^{15}\)N values of the residual NH\(_4^+\) (Kendall, 1998; Sebilo et al., 2006). Wastewater with relative high δ\(^{15}\)N- NH\(_4^+\) may thus enrich and increase the sediment δ\(^{15}\)N as observed in the canal. The impact of the discharge is less important downstream due to dilution by marine waters and the contribution of the increase in vegetation cover on the upstream-downstream gradient that will decrease the δ\(^{15}\)N signal within the sediment. The δ\(^{15}\)N values obtained for leaves from *Rhizophora mangle* at site CR2 (2.6 and 6.5‰) are relatively high compared to those found in leaves of *Rhizophora mangle*, usually between -10‰ and 0‰ (McKee et al., 2002; Wooller et al., 2003). The increase in the density of mangroves, with low δ\(^{15}\)N signatures, and thus an increased input of these areas across the river corridor is most likely responsible for the decrease of the δ\(^{15}\)N of the sediments from upstream to downstream. The values of δ\(^{15}\)N decrease along the profile, whereas the C:N ratios increase (Table 2). These variations can be explained by mixing between an enriched organic matter pool immediately downstream of the WWTP discharges and a depleted pool at the level of the mangroves.

The water quality as well as the organic matter in the sediments along the canal is thus impacted in the upstream part by the WWTP, agricultural activity and possibly other human activities. The downstream
sites reflect an impact of the mangrove vegetation whose surface is increasingly dense along the canal.

Potential nitrate reduction rates

**Nitrate reduction rates in mangroves sediments**

The potential nitrate reduction rates achieved in sediments of the Canal des Rotours are comparable to those obtained by Balk et al. (2015) in mangrove sediments in Florida from coastal and forest mangroves and sediments collected from two mangroves located near a coral reef in Saudi Arabia. The same type of mangrove trees colonize the sediments in Florida as those found in the Canal des Rotours, i.e. *Rhizophora mangle* on the seafront and a mix of *R. mangle* and *Avicennia germinans* in the inner part of the mangrove forest. Vegetation found in the areas of sampling in Saudi Arabia consists solely of *Avicennia marina*. Environmental conditions such as different salinities, variations in annual temperatures at the sampling sites as well as differences in the organic carbon content of the sediments may explain the differences in nitrate reduction rates even if they are of the same order of magnitude. Nitrate reduction rates were in the same range, but slightly higher, than those estimated in nitrogen limited Indian mangrove sediments determined by the isotope pairing method (Fernandes et al., 2012b). In these mangrove sediments, denitrification rates ranged between 70 – 200 nmol g\(^{-1}\) h\(^{-1}\) in sediments with total organic carbon contents 1 – %. The amount and availability of organic carbon is a determining factor in nitrate removal (Herbert, 1999; Pulou et al., 2012), the large difference in total organic carbon between these mangrove sediments may explain some of the differences. The maximum nitrate reduction rates (\(R_{\text{max}}\)) in the canal des Rotours were also comparable to \(R_{\text{max}}\) of 172 nmol cm\(^{-3}\) h\(^{-1}\) determined by Corredor and Morell (1994) investigating nitrate elimination in mangrove sediments in Puerto Rico using the acetylene block method.

The affinity for nitrate in these sediments was low, with values ranging from 700 – 1355 µM, largely exceeding nitrate concentrations in the water of the canal. The \(K_m\) values provide information on the bacterial community present in the sediment, defining the substrate concentration at which half of the enzymatic active sites are occupied. In other words, the higher the \(K_m\), the lower the affinity of the bacterial community for the substrate. The high \(K_m\) values in these sediments indicate that the bacterial community present is heterogeneous and includes organisms with both high and low affinity for nitrate (Pallud et al., 2007). Low \(K_m\) values correspond to a community of more specialized nitrate-reducing bacteria with a higher affinity for nitrate. All deduced \(K_m\) values are higher than the nitrate concentrations measured in the water column, indicating that the on-site nitrate reduction rates are nitrate limited. In the mangrove sediments from Porto Rico, using the acetylene block method, Corredor and Morell (1994) found a very low \(K_m\) of 3.3 µM, indicating a very high affinity for nitrate. Similar differences have been observed previously when comparing intact sediment incubation and homogenized incubation techniques (Laverman et al., 2006; Pallud et al., 2007).

**Nitrate rate reduction potential from upstream to downstream the “Canal des Rotours”**
The differences in potential nitrate reduction rates along the canal are not related to the amount of sediment organic carbon nor the C:N ratios. The highest nitrate reduction rates were determined in vicinity of the WWTP and in sediments halfway the canal, with lowest rates at the most downstream mangrove sediments. Based on the increasing carbon contents and C:N ratios along the canal an increase in nitrate reduction might have been expected. However, the differences in nitrate reduction rates along the canal suggest that sediments close to the WWTP and halfway the canal (CR1 and CR2) are better adapted and have higher nitrate reduction potential compared to sediments downstream. The highest nitrate reduction rates were found at site CR1 and CR2 at the highest nitrate concentrations, with deduced $R_{max}$ values of 433 and 444 nmol.cm$^{-3}$h$^{-1}$ respectively. The affinity for nitrate of the microbial community is low in these sediments and well below the nitrate concentrations in the overlying water (Table 3). As the differences in rates and affinity at these sites are not correlated to the carbon quantity, the carbon quality or availability, as well as the microbial communities are likely responsible for the differences.

It is possible to assume that different microbial communities developed based on the environmental characteristics of each zone. It may be expected that the effectiveness of nitrate reduction across sites may be dependent on the distribution, diversity and extent of activity of bacterial communities in sediments. It could be hypothesized that the microbial numbers and species involved in nitrate reduction are higher at CR 1 and CR2 compared to site CR3. The study of denitrifying gene (NosZ) abundance and denitrifying activity by Fernandes et al. (2012) on the sediments of two Indian mangroves indicates however that denitrification rates do not seem to be limited by the presence of denitrifying populations but by the availability of nitrate as a substrate. Whether this would be similar in the Canal des Rotours would have to be determined by enumeration of functional genes involved in nitrate reduction.

The most upstream sediment is impacted by the WWTP and anthropogenic influences (the village Morne à l'eau). In addition to the supply of inorganic and organic carbon and nitrogen, the wastewater may also enrich and modify the microbial communities at the site close to the WWTP. Nitrate-reducing microbial populations in sediments adapt quickly to externally imposed environmental changes, including substrate variations. This would be in line with work showing that bacterial populations in sediments subject to sewage discharges were kinetically more effective in reducing nitrate than those from less polluted sediments. Nedwell (Nedwell, 1975) showed that the $R_{max}$ of polluted sediment was higher (3.7 mg NO$_3^-$ N m$^{-2}$ h$^{-1}$) then in unpolluted sediments (1.1 mg NO$_3^-$ N m$^{-2}$ h$^{-1}$). This could explain the differences in $R_{max}$ and $K_m$ values of the nitrate reducing community close to the WWTP compared to those reached at the downstream sites since it is the latter that is directly subject to inorganic nitrogen discharges.

The sediment characteristics determined in the three mangrove sediments along the canal show no large differences, with organic matter with a terrestrial signature i.e. mangrove leaves. The most downstream (CR3), marine mangrove sediment shows the lowest nitrate reduction potential ($R_{max}$ 243 nmol.cm$^{-3}$h$^{-1}$) compared to the sediments halfway the canal (CR2, $R_{max}$ 444 nmol.cm$^{-3}$h$^{-1}$). Several environmental conditions can affect the quality and availability of the organic matter, i.e. the potential electron donors
for nitrate reduction as well as the microbial community and consequently influence the kinetic parameters associated with the sediments. As indicated by the salinity profiles, salinity remains significantly high at the water-sediment interface. For sampling sites CR 2 to 3, the mangrove area bordering the canal increases from upstream to downstream and thus plays an increasingly important role as a buffer strip, limiting lateral urban and agricultural input. The increase in total organic carbon as well as the C:N ratio increase from upstream to downstream, suggesting differences in organic matter quality that could play a role in the nitrate reducing capacities.

The nitrite and ammonium production rates suggest that some of the nitrate reduced is not totally transformed into N\textsubscript{2} and that incomplete denitrification and DNRA may occur simultaneously in these sediments. The intermediate production of nitrite increases with the increase of nitrate supplied to the sediment, the proportion of nitrite produced as a function of nitrate reduced increases as well. This suggests that the reduction of nitrate is incomplete and the first step in the nitrate reduction process is favored, with a minor contribution of the nitrite reduction to N\textsubscript{2} (complete denitrification) and/or ammonium (DNRA). At low concentrations of supplied nitrate, the relative production of ammonium is high compared to the amount of nitrate reduced. This points in the direction of DNRA, with a reduction of nitrate, via nitrite to ammonium. This is in good agreement with the work of Fernandes et al (Fernandes et al., 2012a) in Indian mangrove sediments showing that nitrate reduction is dominated by DNRA. High organic carbon content (as in the canal) and relative low nitrate concentrations are thus likely to favor DNRA. This is in good agreement with the assumption that the ratio between carbon and nitrate plays a role in the whether denitrification or DNRA prevails, i.e. at low nitrate concentrations DNRA is favored, whereas at high nitrate concentrations denitrification would be dominant (Tiedje, 1988; Yin et al., 2002). Our approach aimed to determine the nitrate removal capacity of mangrove sediments, the range of applied nitrate concentrations shows that at low nitrate concentrations nitrogen is likely retained in the system. Elevated nitrate levels, under the experimental conditions, are reduced to a large extent to nitrite. The conditions applied to the reactors do not allow the determination of the possible contribution of anammox in these sediments. In the case of anammox a consumption of nitrite and ammonium would be observed, the measurement of net production rates does not allow this differentiation between consumption and production.

**The effect of carbon additions on nitrate reduction rates**

The addition of organic carbon in the form of green leaves showed a significant increase in nitrate reduction compared to the non-amended control. The amount of organic carbon increased from 37.2% C\textsubscript{org} in the un-amended sediment to 43.8% in the sediment amended with green leaves. The addition of yellow leaves resulted in a smaller carbon increase (39.9%) compared to the non-amended control, resulting in a small, but non-significant increase of the nitrate reduction rates. The supply of acetate resulted in a significant increase of nitrate reduction rates as well as an elevated production of nitrite. These results indicate clearly that despite the high organic carbon contents, nitrate reduction rates are limited by easily available carbon (acetate). The significant increase in nitrate reduction in the presence of green leaves and a smaller impact of the yellow leaves confirms this increase is due to easily
degradable carbon. The rapid increase in nitrate reduction upon the addition of acetate shows that the resident microbial community is able to utilize this easily degradable carbon source instantaneously. Nitrate reducing bacteria use sugars and organic acids as external carbon sources, the addition of glucose showed for example an increase in denitrification rates in constructed wetlands (Lu et al., 2009). The effect of acetate in the sediments in the canal des Rotours indicates the presence of active nitrate reducing community, capable of instantaneously using acetate. This in contrast to observations in Taiwanese mangrove soils that showed little increase in denitrification rates upon addition of acetate (Shiau et al., 2016). The elevated carbon content in the sediments from the canal de Rotours, in comparison to the Taiwanese soils with little organic carbon (0.7-3%), may explain the presence of a more diverse and active denitrifying community in our sediments.

The increase in nitrate reduction rates upon acetate addition and the slight increase in the presence of mangrove leaf debris confirms that nitrate reduction is limited by the amount of organic carbon, particularly available carbon. Whereas acetate is an easy degradable carbon source, the leaves added as a carbon source are more complex. Mangrove leaves contain numerous compounds among which carbohydrates, amino acids, lignin-derived phenols, tannins (Kristensen et al., 2008). Marchand et al 2008 (Marchand et al., 2005) showed that the total organic carbon in Rhizophora mangle leaves is comprised between 25 to 36% of sugars of which glucose represents more than 50%. Yellow leaves are richer in lignin, tannin and cellulose than green leaves and contain less sugars and hemicellulose (Kristensen et al., 2008). The majority of the carbon compounds in green leaves is therefore more easily assimilated and/or degraded by micro-organisms than in yellow leaves, which could explain the slight difference in nitrate reduction rates between the two treatments (green and yellow leave addition). Furthermore, green leaves contribute thus in the supply of easily degradable carbon for denitrification in these systems.

Whereas the addition of acetate increases nitrate reduction rates, it also increases nitrite production rates. These rates are elevated and represent up to 40% of the nitrate reduced, indicating incomplete nitrate reduction, i.e. incomplete denitrification or DNRA. This intermediate nitrite production is moderate when more complex carbon in the form of plant debris is added. The form of and amount of carbon may thus modify the quantities of nitrite in the environment by influencing the processes involved. Nitrite accumulation with the addition of a carbon source has been observed in previous studies in wetland soils (Chen et al., 2017; Lu et al., 2009). Several factors have been shown to impact intermediate nitrite production among which C:N ratio and carbon source (Rocher et al., 2015). In general, nitrite accumulation is explained by a disequilibrium between the amounts of nitrate and carbon as well as between the enzymes producing and consuming nitrite. Microbial nitrate reduction requires a nitrate reductase, the membrane-bound Nar or the periplasmic nitrate reductase Nap, allowing nitrate to be reduced into nitrite with the concurrent oxidation of an electron donor, in general organic carbon (Jones et al., 2008; Kraft et al., 2011). The reduction of nitrite occurs via the nitrite reductase nirS or nirK into gaseous nitrogen (denitrification) or via the nitrite reductase Nrf (DNRA) (Kraft et al., 2011). Competition between theses reductases may occur when the carbon supply is insufficient and therefore resulting in nitrite accumulation (Chen et al., 2017). The supply of additional carbon and nitrate may also have led to an induction of nitrate reductase enzymes, whereas nitrite reduction enzymes will be induced upon a
production and presence of nitrite. Such a delay in the nitrite reduction step will thus result in a temporarily nitrite accumulation. It is therefore necessary to control these parameters in order to optimize the both nitrate and nitrite reduction in complete denitrification and elimination.

**Impact of hydraulic retention time, flow rates on nitrate reduction rates**

The flowrate at which nitrate is supplied to the sediment had an impact on nitrate reduction rates, with highest rates at 2.6 and 9.3 ml h\(^{-1}\), with slightly lower rates at 1.2 and 6.1 ml h\(^{-1}\) and lowest rates at the lowest flow rate of 0.5 ml h\(^{-1}\). At high flow rates, nitrate reduction may be limited by the rate of nitrate diffusion (Willems et al., 1997) which may explain the drop in production rates at the high flow rates. The increase in nitrate reduction by microbial populations present in wetland sediments associated with an increase in the contact time between these two elements has already been observed in other studies. In natural freshwater wetlands (Jansson et al., 1994; Willems et al., 1997) as well as in restored wetlands (Woltemade and Woodward, 2008) or in artificial freshwater and mangrove wetlands (Toet et al., 2005; Wu et al., 2008). Nitrate concentrations thus decrease with increasing residence time.

Hydrodynamic conditions, by modifying sediment characteristics, will spatially and temporally affect nutrient fluxes (Lin et al., 2016). *In situ*, the hydraulics of the system, such as tide, fresh water input from rivers, rainfall, influence of the groundwater table, will influence the functioning of wetlands by affecting, among others, the contact time between microorganisms and the substrate as well as salinity. Increasing the contact time between microorganisms in mangrove sediments and the substrate should increase the efficiency of nitrate reduction. Although reduction rates increase with flow rate, the percentage of nitrate removed is higher at low flow rates (Figure 6). At the lowest flow rates the nitrate removal is 53%, while at the fastest flow rate the nitrate removal percentage is 10 times lower at 5.6%. The decrease in flow rate leads to an increase in the residence time of the feed solution in the reactor, resulting in greater nitrate consumption.

Optimization of nitrate reduction rates in mangrove sediments

In order to understand the effect of nitrate concentrations, carbon input and water/sediment contact time on potential nitrate removal, nitrate removal percentages were calculated for the different treatments applied to the sediments at CR2 (Figure 6).

The efficiency of nitrate removal decreases with increasing amounts of nitrate provided by the input solution. Thus, for the same flow rate and the same contact time between the microorganisms in the sediment and nitrate, all nitrate is reduced at the lowest concentration, while the nitrate reduction ranges between 13 and 56% for the highest nitrate supplied (6 mM). The maximum reduction rates obtained in this study correspond to the maximum amounts of nitrate that can be eliminated by microbial activity. These rates should therefore be considered in the context that nitrate is the only electron acceptor available for the microorganisms and that this elimination is specific to the areas sampled.
Whereas the elimination of nitrate with organic carbon present in the sediment, resulted in a 13% elimination of the introduced nitrate, the addition of acetate significantly improved the removal of nitrate to 63% while for the yellow and green leaf additions the removal was 21% and 30% respectively. The reduction efficiency strongly depends on the nature of the carbon source supplied to the bacterial community present in the sediment, with acetate being the most efficient followed by green leaves and yellow leaves. The source and amount of organic carbon supplied may also influence the metabolic pathway for nitrate removal. The use of different carbon sources for the reduction of nitrate in wastewater sludge (Akunna et al., 1993) showed for example that 100% of the nitrate introduced was reduced by denitrification, in the presence of lactic acid and acetic acid, whereas in the presence of glucose and glycerol only 50% of the reduced nitrate was converted to ammonium via DNRA. The by-products of the reduction therefore depend on the nature of the carbon substrate present in the medium. In the context of the Canal des Rotours, the metabolic pathways of elimination were not identified. In order to optimize nitrate removal capabilities for this system, estimating different pathways and end products would need further, detailed investigation.

In addition to nitrogen carbon load, retention time is a critical factor in the optimization of purification capacities (Fisher and Acreman, 2004) as it should be long enough to allow the biological processes to reduce nitrate. At CR2, the decrease in retention times (flow rates) is accompanied by an increase in the potential efficiency of nitrate elimination from an average of 6% at the highest flowrate to 52% at the lowest flow rate. By influencing the flow rates and consequently the hydraulic retention times, it is possible to optimize nitrate removal. A study of the influence of retention time on the nitrate removal efficiency of a treatment wetland in the Netherlands showed that by increasing the retention time from 0.3 to 9.3 days, the percentage of nitrate removal increased from 21 to 86% (Toet et al., 2005). In order to make sure that mangrove sediments can assure the complete elimination of nitrate from a wastewater treatment, it will be necessary to assess discharge rates in relation to cumulative nitrogen loads to ensure effective removal and allow nitrate to diffuse to the sediment for reduction. In addition to that, in order to optimize nitrogen elimination, the mangrove area might require adjustment and modification regarding the hydrological retention time.

Mangroves, wetland buffer zones and streams are widely used to reduce nitrogen loads and will be even more in the future. Increasing the hydraulic retention time, optimal drainage, installation of retention sheets have been applied in river streams (reviewed by (Craig et al., 2008), and also in mangroves. Many countries already use mangroves as low-cost green alternatives for the treatment of wastewater and storm water from coastal areas (Chen et al., 2011; Corredor and Morell, 1994; Herteman et al., 2011; Lambs et al., 2011; Wong et al., 1997). However, changes in hydraulic retention time or other modification should be done in a controlled manner with long-term monitoring of the effects of these releases on the ecosystem as a whole. Monitoring and modeling will be necessary for decision-making regarding the framework of mangrove use in the elimination of inorganic nitrogen (Kelleway et al., 2017). Even though mangrove sediments have a potential for nitrate elimination, providing mangroves with nitrogen inputs in excess of the actual amounts that they are likely to eliminate can lead to negative effects such as eutrophication or greenhouse gas emissions (Chen et al., 2011). In addition to this, the long-term
response to the various other pollutants present in wastewater remains unknown (pesticide, metals, pharmaceutical compounds; e.g. (Kümmerer, 2010). The identification of the initial natural state, the development of ecological engineering (adding carbon, modifying the flow path, planting mangroves) and the evaluation of the success of the actions over the long term are essential for the implementation of restoration measures aimed at reducing perennial nitrogen quantities (Craig et al., 2008; Kaly and Jones, 1998).

**Conclusion**

Mangroves, which receive occasionally effluents from anthropic activities, are subject to nitrogenous inputs from various sources. The marine mangrove sediments in the study area have a high potentially capacity to remove nitrate. The addition of an external carbon source combined with a long hydraulic retention time would be an effective approach to achieve improved nitrate removal with low nitrite accumulation. Nitrate reduction rates depend on a range of environmental parameters and therefore may vary spatially and temporally. In this study, it was shown that the potential reduction of nitrate by mangrove marine sediments is limited by the availability of nitrate itself and by the amounts of organic carbon. The effectiveness of the potential reduction varies according to the amounts of nitrate provided, the nature of the carbon source available to the microorganisms, the hydraulic retention time and the sampling periods. Regardless the treatment applied to the sediment, the microorganisms present show great adaptability in their ability to reduce nitrate. Nevertheless, before the exploitation of this phenomenon can be sustained, it will be necessary to carry out further investigations on the functioning of this ecosystem and impact on the long term. A better knowledge of the environment will thus allow the implementation of adapted management practices by an optimization of the ecosystem functions, such as nitrogen elimination.

**Declarations**

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**Figures**

**Figure 1**

Localization of the sampling sites along the ‘canal des Rotours’ (source: www.geoportail.gouv.fr), including the coordinates, the position of the WWTP, the community Morne à l’Eau upstream of the canal and the “Grand Cul de sac marin” downstream of the canal.

**Figure 2**

Cross section of the canal at the three sampling sites with the depth of the canal and the salinity stratification due to the mixing of saline and freshwater. Depth of the canal was determined at 1 m width intervals, salinity at three locations along the width of the canal at different depth intervals. Measured salinity values at 10 cm depth intervals are shown (open circles) and salinity lines were drawn according. The distance in meters is from the left bank of the canal. Sampling site on the right shore used in the nitrate reduction experiments are indicated by an arrow.
Box-plot of the effect of nitrate addition on the nitrate reduction rates (n=14) determined by the flow through reactor method after incubation of the sediments from stations 1 to 3. The sediments were subjected to nutrient solutions with nitrate concentrations ranging from 0.6 till 6 mM (see detailed concentrations in the figure). The boxes encompassed the upper and lower quartiles. The dots indicate the extreme values while the horizontal lines correspond to the median values of the reduction rates. The letters identify the statistical groups, each letter being representative of a group whose differences are not significant for the parameter under study.
Figure 4

Box-plots of the effect of different carbon sources on the nitrate reduction rates (NRR, n=16) determined by flow through reactors with sediment of site CR3. The boxes include the upper and lower quartiles, the dots indicate the extreme values while the horizontal lines correspond to the median values of the reduction rates. The letters identify the statistical groups, each letter being representative of a group whose differences are not significant for the parameter under study.

Figure 5

Box-plot of the effect of flow variation on nitrate reduction rates (n=8). The boxes include the upper and lower quartiles, the dots indicate the extreme values while the horizontal lines correspond to the median values of the reduction rates.

Figure 6
Percentage nitrate removal as a function of nitrate concentrations (0.7 to 6 mM), carbon additions (A – acetate, GL - green leaves, YL - yellow leaves) and flow rates (0.5 to 9.3 ml h-1).

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