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Benthic nitrate removal capacity in marine mangroves of Guadeloupe, Lesser Antilles

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Abbreviations: DNRA, dissimilatory nitrate reduction to ammonium; anammox, anaerobic ammonium oxidation; WWTP, wastewater treatment plant; C_{org}, organic carbon, C:N ratio, carbon to nitrogen ratio; EA-IRMS, elemental analyzer coupled to isotope ratio mass spectrometry; CR1, sampling station 1; CR2, sampling station 2; CR3, sampling station 3; GL, green leaves; YL, yellow leaves; NO_3^-, nitrate; NO_2^-, nitrite; NH_4^+ ammonium; C, Carbon; NRR, nitrate reduction rates; NiPR, nitrite production rates; APR, ammonium production rate, Kruskal-Wallis; KW

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Abstract

Mangrove sediments are known to be potentially active reducing zones for nitrogen removal. 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, Lesser Antilles). To this end, the effect of nitrate concentration, organic carbon load and the hydraulic retention time was assessed as factors affecting these nitrate reduction rates. Nitrate reduction potential was determined using flow-through reactors in marine mangrove sediments collected along 'The Canal des Rotours' in Guadeloupe. Potential nitrate reduction rates, in the presence of indigenous organic carbon, generally increased upon increasing nitrate supply from around 120 nmol cm^{-3} h^{-1} (low nitrate) up to 378 nmol cm^{-3} h^{-1} (high 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 (up to 748 nmol cm^{-3} h^{-1}). 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 mangrove sediments from this Caribbean island, currently exposed to a small tidal effect could increase its nitrate elimination capacities due to prolonged water retention via engineering.

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1. INTRODUCTION

Mangroves are coastal wetlands that have a circumtropical distribution with an estimated global coverage between 10 to 24 million hectares (Kathiresan & Bingham, 2001). These ecosystems are highly productive and contribute significantly to the global carbon (C) cycle (Bouillon et al., 2008). Litter fall represents a significant share of the C 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 is 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. The major alternative electron acceptor in marine mangrove sediments is sulfate (Kristensen et al., 2008), whereas nitrate (NO_3^-) may also contribute to anaerobic mineralization (Fernandes, Bonin, et al., 2012; Fernandes et al., 2016).

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; Cao et al., 2016). Ammonium (NH_4^+) is either produced via DNRA or organic matter degradation resulting in nitrogen (N) retention (Enchrich-Prast et al., 2016). During denitrification and DNRA, nitrite (NO_2^-) can be produced as an intermediate whereas nitrous oxide (N_2O) represents an intermediate concerning only the denitrification process. NO_2^-, N_2O and NH_4^+ are reaction products and intermediates whose presence and proportion in the environment depend on the capacity of the microbial communities present to reduce NO_3^-. The efficiency of NO_3^- removal depends on C availability and quality, i.e. the chemical composition (Chen et al., 2017; Pulou et al., 2012; Shiau et al., 2016).

Interest in the NO_3^- reduction capacity of mangrove sediments, usually rich in organic matter and largely anoxic, is increasing this last decade and has been the subject of numerous studies conducted mainly on terrestrial mangroves (Fernandes, Bonin, et al., 2012; 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 N removal (Fernandes et al., 2016) used to treat nitrogen rich wastewater (Corredor & Morell, 1994; Shiau et al., 2016). Taking into account the high C_{org} content of mangrove sediments, it can be hypothesized that the quantity of C_{org} is sufficient, with NO_3^- reduction not being limited by C_{org} but limited by NO_3^-. Furthermore, the contact time of NO_3^- containing water with the sediments in estuarine mangroves may affect NO_3^- removal capacity; it can be hypothesized that an increased hydraulic retention time (HRT) will increase the benthic NO_3^- removal capacity.

In order to study the impact of NO_3^-, C_{org} and HRT on NO_3^- reduction rates, we investigated the potential for NO_3^- reduction in mangrove sediments in an area downstream of a domestic wastewater treatment plant (WWTP) along the “Canal des Rotours” Guadeloupe, Lesser Antilles. The effect of NO_3^- and C concentrations as well as the flow rate were investigated in controlled laboratory experiments. For this purpose, the potential benthic NO_3^- reduction as well as NO_2^-

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and NH_4^+ production rates were determined using the continuous flow reactor method (Laverman et al., 2006). In addition to benthic NO_3^- reduction rates, water quality and a physico-chemical characterization of the different sediments along the Canal de Rotours were determined.

2. MATERIAL AND METHODS

2.1 Study site and sampling

Potential NO_3^- reduction rates were determined along 'The Canal des Rotours', near the town Morne à l'eau located in Guadeloupe, French Lesser Antilles (Figure 1). The canal bed has a width that varies between 18 and 30 meters and a maximum depth of 2.9 m. This shallow canal of 6 km length consists of a straight section that crosses Morne à l'eau, a second part passes an agricultural area and then meandering through a mangrove forest until it reaches the coast (Figure 1). The mangrove forest along the canal is primarily covered by red mangrove trees, *Rhizophora mangle,* becoming denser 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. Morne à l'eau discharges its wastewater treatment plant (WWTP) effluent directly into the canal (Figure 1).

To evaluate the benthic NO_3^- 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. The sediments for the experiments were collected at the shore corresponding to salinities varying in an inverse fashion to mangrove density, with the pore water salinity ranging from 23‰ at CR1, 29‰ at CR2 and 34‰ at CR3.

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 first centimeter of the muddy immersed sediments was sampled in November and December 2016. The sediments were transported to the laboratory and flow-through reactor experiments were started within 2h upon sampling. A subsample of the sediment (30 grams) was dried at 37°C during one week and crushed in a ball mill for sediment analysis.

2.2 Nitrogen transformation rate determination

Effect of NO_3^- concentrations on NRR (experiment 1)

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Nitrate reduction rates were determined in surface sediments (0-1 cm, duplicates) incubated in flow through reactors (see Supplemental Figure S1) (Laverman et al., 2006). The volume of the sediment contained in the reactors was 13.85 cm^3. The sediment section of the reactors was 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-rings 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. The inflow solutions consisted of milliQ water containing 33 g l^{-1} NaCl and different concentrations of KNO_3.

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 ± 0.1ml 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 reactors were wrapped with aluminum foil to perform the experiment in dark to inhibit light-sensitive processes like oxygen production by photosynthesis. The experiments were done 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 potential rates in order to determine and compare the impact of key environmental variables (NO_3^-, C_{org}, HRT) on nitrate reduction in surficial mangrove sediments.

Sediments from all three locations CR1, CR2 and C3, were used to determine the impact of NO_3^- concentrations on the NRR. Four concentrations of NO_3^- were used (0.6-0.7 mM, 1.4-1.9 mM, 3.0 - 3.1 mM, and 6.0 mM).

The effect of C source on NRR (experiment 2)

Sediments from CR2 were used to assess the influence of the nature and availability of C on the potential NRR. Three different sources of C were provided to the sediment of CR2. Additions were made either by mixing the sediment with mangrove leaves or via the input solution (2mM acetate); all reactors were supplied with a 6.2 mM NO_3^- solution at a flow rate of 2.7 ml h^{-1}. Sediment that was used for C additions was mixed with green leaves or yellow leaves (weight leaves: weight sediment 2.5:1). Green leaves were collected freshly from *Rhizophora mangle* trees, the yellow leaves correspond to senescent leaves.

Effect of flow rate on NRR (experiment 3)

Similar reactor experiments were used to test the impact of flow rate on NRR at five different flow rates (0.5, 1.2, 2.7, 6.2, 9.8 ml h^{-1}) to sediments from CR2 provided with NO_3^- concentration of 6.1 mM.

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2.3 Determination of rates and kinetic parameters

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

$$R = \frac{\Delta C * Q}{V}$$

equation 1

Where ΔC 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} as applied in experiments 1 and 3, and a reactor volume of 13.85 cm^3 this largely exceeds the replacement of initial pore water and a (semi) steady state situation. Nitrogen reduction rates were determined over a longer period in the experiments with C additions (24h to 72h).

2.4 Analytical methods

Nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) were determined colorimetrically in the input and outflow solutions using a Nutrient Autoanalyzer, Quatro (Thermo fisher). 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 a 80-µm mesh for leaves. Sediment samples were decarbonated by hydrochloric acid (Harris et al., 2001). The C_{org} and organic N (N_{org}) contents were determined using an Elemental analyzer (Vario PYRO cube, Elementar). Isotope abundance ratios of N_{org} and C_{org} of the solid samples were determined by isotope ratio mass spectrometry coupled to an elemental analyzer (EA-IRMS; Elementar-Isoprime). Results are reported in the internationally accepted delta notation expressed in ‰. Analytical precision is about 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

2.5 Statistical analysis

Significant variability of measured rates along the duration of the experiment were tested for the different experiments in R (version 4.2.2, 2022-10-31). Prior to further analysis, data were tested for normality (Shapiro-Wilk normality test) and equal variance (Bartlett equal variance test). As the data lacked normality and equal variance, the non-parametric Kruskal-Wallis (KW) One Way Analysis of Variance on ranks was conducted. This way, the impact on NRR upon carbon addition (experiment 2) or variable flowrate (experiment 3) were tested. The lack of normality and equal variance of the data of experiment 1 (nitrate and site) omitted the use of a two-way anova testing the combined effect of nitrate concentration and site. Therefore, the effect of nitrate concentration and site on NRR were tested separately using the KW One Way Analysis of Variance on ranks. These tests were followed by a Pairwise Multiple Comparison Procedure using Dunn's method or a Tukey test in order to discriminate differences between treatments.

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3. RESULTS AND DISCUSSION

3.1 Nitrogen and carbon dynamics along the Canal des Rotours

Maximum NH_4^+ concentrations were measured immediately downstream of the WWTP (19.4 µM) decreasing to 2.3 µM at the mouth of the estuary (Table 1). The maximum concentrations of NO_2^- and NO_3^- were measured immediately downstream of the WWTP discharge and a clear decrease was observed along the canal (Table 1). Whereas maximum nutrient concentrations close to the WWTP are likely due to discharge from the station, other sources of inorganic N (i.e urban and agricultural activity) also decrease from upstream to downstream. The decrease in inorganic nitrogen concentrations along the canal are most likely due to nutrient dilution with oligotrophic marine waters. Biological activity, such as nitrification or denitrification may also lower the inorganic N concentrations in the water column along the canal (Lee et al., 2008). Overall, the measured inorganic N 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 (Enrich-Prast et al., 2016; Fernandes et al., 2016).

The amount of sedimentary C_{org} increased from upstream to downstream (26.3% to 34.7%, Table 1). These values are similar to tidal-influenced mangrove sediments, with a similar vegetation, *Aviciennia germinans* and *Rhizophora mangle*, encountered in Florida with C_{org} content up to 26% (Balk et al., 2015). The values of $\delta^{13}C$ vary little along the canal with no particular trend, ranging from -28.5 to -27.4 ‰ (Table 1). The $\delta^{13}C$ values associated with C:N ratios in the sediments confirm that the organic matter at all three sites originate from terrestrial C3 plants (Kristensen et al., 2008; Lamb et al., 2006) and are consistent with the isotopic signature of other mangroves (Cremiere et al., 2017; McKee et al., 2002). The sedimentary N content remains stable along the canal (1.3-1.5%) and consequently C:N ratios increasing from upstream to downstream (22.3 to 27.5, Table 1). Similar to the gradient in inorganic N in the water column, sedimentary $\delta^{15}N$ decreases from 9 ‰ close to the WWTP outlet to 1.2 ‰ at the most downstream, marine site. Wastewater with a relative high $\delta^{15}N$-NH_4^+ (Kendall, 1998; Sebilo et al., 2006) may enrich and increase the sediment $\delta^{15}N$ close to the WWTP outlet. The increase in the density of mangroves, with lower $\delta^{15}N$ signatures, is most likely responsible for the decrease of the $\delta^{15}N$ of the sediments from upstream to downstream.

3.2 The effect of NO_3^- concentrations on nitrogen transformation rates

Potential NRR showed significant differences for both supplied nitrate concentration and between sites (KW analysis of variance $p<.001$). For the lowest NO_3^- input concentrations (0.6 and 0.7 mM), potential NO_3^- reduction rates are 126 and 138 nmol cm^{-3} h^{-1} respectively for CR1 and CR2, these rates increase to 374 and 378 nmol cm^{-3} h^{-1} at 6 mM (Table 2). For these two sampling areas, the higher the concentration of NO_3^- in the nutrient solution, the higher the NRR. For the site at the mouth of the canal, CR3, the increase in NRR is significantly smaller than for CR1 and CR2 (Dunn's

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pairwise comparison *p<.01,* Figure 2), with average NRR increasing from 119 to 219 nmol cm^{-3} h^{-1} between the lowest and highest NO_3^- concentration provided by the input solution.

The potential NRR 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 also in sediments collected from two mangroves in Saudi Arabia. The same type of mangrove trees colonizes the sediments in Florida as those found in the Canal des Rotours, i.e. *Rhizophora mangle* on the seafront and a mix of *Avicennia germinans* and *R. mangle* in the inner part of the mangrove forest. Environmental conditions such as different salinities, variations in annual temperatures at the sampling sites as well as differences in the C_{org} content of the sediments may explain the differences in NRR 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 N limited Indian mangrove sediments determined by the isotope pairing method (Fernandes, Michotey, et al., 2012). In these mangrove sediments, denitrification rates ranged between 70-200 nmol g^{-1} h^{-1} in sediments with total C_{org} contents 1-2 %. The amount and availability of C_{org} is a determining factor in NO_3^- removal (Herbert, 1999; Pulou et al., 2012), the large difference in total C_{org} between these mangrove sediments may explain some of the differences.

The differences in potential NRR along the canal are not related to the total amount of sediment C_{org} nor to the C:N ratios (Table 1). Based on the increasing C contents and C:N ratios along the canal, an increase in NRR would have been expected in the most downstream site. However, the highest NRR were determined in vicinity of the WWTP and in sediments halfway the canal, suggesting that these sediments are better adapted than those downstream. As the differences in rates at these sites are not correlated to the C quantity, the C quality or availability, as well as the microbial communities are likely responsible for the differences. The wastewater outlet may enrich and modify the microbial communities, i.e. favor the relative abundance of nitrate reducing bacteria, at the site close to the WWTP. Nitrate-reducing microbial populations in sediments adapt quickly to external 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 NO_3^- than those from less polluted sediments (Nedwell, 1975). The increase in total C_{org} 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 NO_3^- reducing capacities.

Nitrite and ammonium production from the sediments subject to different NO_3^- concentrations are listed in Table 2. Nitrite production rates ranged from 2.8 to 200 nmol cm^{-3} h^{-1}. The maximum rate was reached at the highest concentration of NO_3^- in the input solution at the station closest to the WWTP (CR1). The NiPR increased with increasing NO_3^- supply and thus the NRR.

Ammonium production rates ranged from 27.4 to 101 nmol cm^{-3} h^{-1}. Highest APR were determined at CR2 from 59 to 101 nmol cm^{-3} h^{-1}. The APR per site showed no significant differences as a function of supplied NO_3^- concentrations. However, the amount of NH_4^+ produced as a function of NO_3^- reduced is elevated at low NO_3^- concentrations (25-43%) and decreases with increasing NRR (9-19%).

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3.3 Effect of carbon addition: mangrove leaves and dissolved C_{org}

Carbon additions significantly increased NRR compared to the non-amended control (KW analysis of variance *p<.001*) with average NRR ranging from 165 to 748 nmol cm^{-3} h^{-1}. The NRR upon addition of green leaves (351 nmol cm^{-3} h^{-1}) was significantly higher than that the non-amended sediment (165 nmol cm^{-3} h^{-1}, Dunn's pairwise comparison *p<.05*), whereas the addition of yellow leaves (260 nmol cm^{-3} h^{-1}) was neither significantly different from un-amended (Dunn's pairwise comparison *p=0.07)* nor green leave amended sediments (Dunn's pairwise comparison *p=0.50*). The amount of C_{org} increased from 37.2 % C_{org} in the un-amended sediment to 43.8% in the sediment amended with green leaves and to 39.9% in the sediment amended with the yellow leaves. Even if the differences in rates between yellow leaves and green leaves or un-amended are not statistically significant (*p=0.50, respectively p=0.07*), the results show that the degree of degradation of mangrove leaves changes slightly NRR (260 for yellow leaves *versus* 351 and 165 nmol cm^{-3} h^{-1} for green leaves and unamended).

These results indicate that despite the high C_{org} contents, NRR were limited by available carbon. Most likely, the C rich mangrove sediments contain C_{org} that is little available and recalcitrant, possibly due to preceding microbial mineralization activities occurring in the sediment. The rapid increase in NRR upon the addition of acetate shows that the resident microbial community is able to utilize this easily degradable C source instantaneously. The strong significant effect of acetate (Dunn's pairwise comparison *p<.05)* in the sediments in the canal des Rotours indicates the presence of active NO_3^- 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 C content in the sediments from the canal de Rotours, in comparison to the Taiwanese soils with little C_{org} (0.7-3%), may explain the presence of a more diverse and active denitrifying community in our sediments.

Mangrove leaves are rich in carbohydrates, amino acids, lignin-derived phenols, tannins (Kristensen et al., 2008). *Rhizophora mangle* leaves contain between 25 to 36% of sugars of which glucose represents more than 50% (Marchand et al., 2005). The fact that yellow leaves are richer in lignin, tannin and cellulose than green leaves and contain less sugars and hemicellulose (Kristensen et al., 2008) most likely explains the slight difference in NRR between the green and yellow leave amended sediments.

Nitrite production rates show the same trend as NRR, with highest NiPR, 302 nmol cm^{-3} h^{-1} (42% of the NRR) in the presence of acetate (Table 2). In the non-amended sediment and the sediment with leave addition, the NiPR were between 15 to 25% of the NO_3^- 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. Regardless the source of carbon supplied to the sediment, all the reactor outlets present a production of NH_4^+ in variable quantities. The APR show no significant differences between treatments with average values ranging from 15 to 36 nmol cm^{-3} h^{-1}. The NiPR and APR obtained here suggest that some of the NO_3^- reduced is not totally transformed into N_2 and that incomplete denitrification and DNRA may occur simultaneously in these sediments. At low NO_3^- concentrations, the relative production of NH_4^+ is high compared to the

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amount of NO_3^{--} reduced. This points in the direction of DNRA, with a reduction of NO_3^-, via NO_2^- to $NH_4^{+\,+}$, as has also been shown in Indian mangrove sediments where NO_3^- reduction was dominated by DNRA (Fernandes, Bonin, et al., 2012). This is in good agreement with the assumption that the ratio between C and NO_3^- plays a role in the whether denitrification or DNRA prevails, i.e. at low NO_3^- concentrations DNRA is favored, whereas at high NO_3^{--} concentrations denitrification would be dominant (Tiedje, 1988; Yin et al., 2002). Our approach aimed to determine the NO_3^- removal capacity of mangrove sediments, the applied NO_3^- concentrations show that at low NO_3^- concentrations nitrogen is likely retained in the system.

3.4 Effect of flow rate on NRR

The different flow rates have a significant effect on NRR (KW *p<.001,* Figure 3). Lowest NO_3^- reduction rates are determined at 0.5 ml h^{-1} with an average of 154 nmol cm^{-3} h^{-1}. Rates increase with increasing flow rate reaching on average 290 nmol cm^{-3} h^{-1} at a flow rate of 2.6 ml h^{-1}, not significantly different from the rates at the lowest flow rate (Dunn's *p =0.40).* Rates increase with increasing flowrates with 318 nmol cm^{-3} h^{-1} at flow rate of 6.2 ml h^{-1} and 263 nmol cm^{-3} h^{-1} at 9.8 ml h^{-1} and significantly different from rates at the lowest flow rate of 0.5 ml h^{-1} (Dunn's *p<.05*). No significant differences between the rates 2.6, 6.1 and 9.3 were observed (Dunn's *p = 1.0*). The NiPR 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} (Table 2). The percentage of NiPR increased as well with increasing flowrates 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 NH_4^+; 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} (Table 2). At the lowest flow rate, a large part of the supplied NO_3^- (69.1%) is reduced (see Table 2), NO_3^- concentrations within the sediment may have become limiting resulting in relative lower NRR as previously observed by Pallud et al. (2007). At high flow rates, NO_3^- reduction may be limited by the rate of NO_3^- diffusion (Willems et al., 1997) which may also explain the slight decrease in reduction rates at the high flow rates. *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 NO_3^- reduction.

3.5 Optimization of NRR in mangrove sediments

In order to understand the effect of NO_3^- concentrations, C input and water/sediment contact time on potential NO_3^- removal, NO_3^- removal percentages were calculated for the different treatments applied to the sediments (Table 2). The efficiency of NO_3^- removal decreases with increasing NO_3^- concentrations provided by the input solution. At the same flow rate, and thus same contact time between the microorganisms and NO_3^-, all NO_3^- is reduced at the lowest concentration, while the NRR range between 13 and 56% at the highest NO_3^- concentration (6 mM).

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The elimination of NO_3^- with the C_{org} present in the sediment was 13 %, whereas the addition of acetate significantly improved NO_3^- removal to 63% while for the yellow and green leaf additions the removal was 21% and 30% respectively (Table 2). The reduction efficiency strongly depends on the nature of the C 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 C_{org} supplied may influence the metabolic pathway for NO_3^- removal as was shown in wastewater sludge (Akunna et al., 1993) with complete denitrification, in the presence of lactic acid and acetic acid, compared to 50% DNRA in the presence of glucose and glycerol. In the context of the Canal des Rotours, the metabolic pathways of elimination were not identified. In order to optimize NO_3^- removal capabilities for this system, estimating different pathways and end products would need further, detailed investigations.

In addition to N and C load, HRT is a critical factor in the optimization of purification capacities (Fisher & Acreman, 2004) as it should be long enough to allow the biological processes to reduce NO_3^-. The decrease in retention times (flow rates) is accompanied by an increase in the potential efficiency of NO_3^- elimination from an average of 6% at the highest flowrate to 52% at the lowest flow rate (Table 2). A similar increase in NO_3^- removal associated with increasing substrate contact time was observed in other studies in restored, freshwater and mangrove wetlands (Jansson et al., 1994; Toet et al., 2005; Willems et al., 1997; Woltemade & Woodward, 2008; Wu et al., 2008). A study of the influence of retention time on the NO_3^- 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 NO_3^- removal increased from 21 to 86% (Toet et al., 2005). In order to make sure that mangrove sediments can assure the complete elimination of NO_3^- from a wastewater treatment, it will be necessary to assess discharge rates in relation to cumulative nitrogen loads to ensure effective removal and allow NO_3^- to diffuse to the sediment for reduction. Increasing the hydraulic retention time, optimal drainage, installation of retention sheets have been applied in river streams (reviewed by Craig et al., 2008). Many countries use mangroves as low-cost green alternatives for the treatment of wastewater and storm water from coastal areas (Chen et al., 2011; Corredor & Morell, 1994; 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). The identification of the initial natural state, the development of ecological engineering (adding C, 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 & Jones, 1998).

The results of the current study demonstrate that mangrove sediments along the Canal de Rotours may act as 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. The effectiveness of the potential reduction varies according to the amounts of NO_3^- provided, the nature of the C source available to the microorganisms, the hydraulic retention time and the sampling periods. A better knowledge of the environment will thus allow the

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implementation of adapted management practices by an optimization of the ecosystem functions, such as nitrogen elimination.

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AUTHOR CONTRIBUTIONS

Anniet M. Laverman: Conceptualization, Methodology, Supervision, Validation, Writing – original draft. **Mathieu Sebilo**: Writing – review & editing. **Jennifer Tocny**: Data curation, Formal analysis, Writing – review & editing. **Olivier Gros**: Funding acquisition, Project administration, Resources, Writing – review & editing.

Conflict of Interest

The authors declare no conflict of interest

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Figure captions

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, Morne à l'Eau upstream of the canal (A), the localization of the canal de Rotours in Guadeloupe (B) and the position of Guadeloupe in the Caribbean (C).

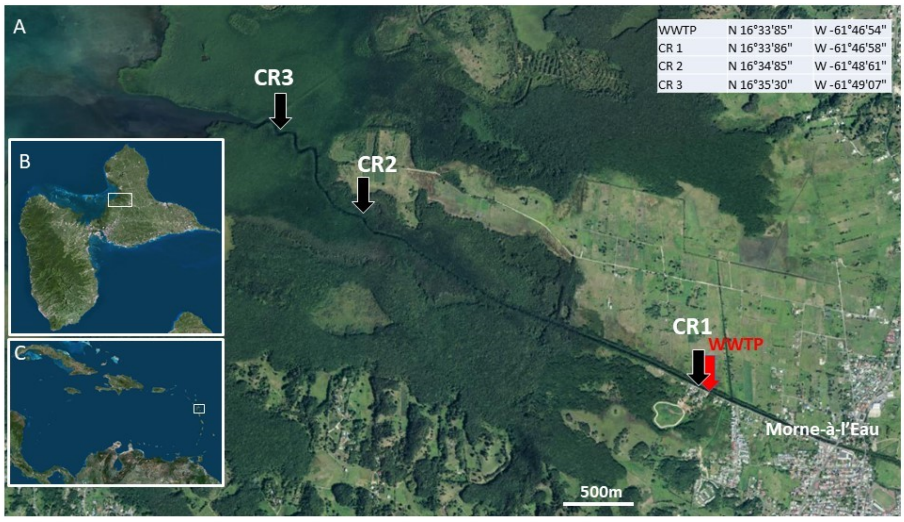

Figure 2. Box-plot of the NRR (n=14) in sediments of CR1, CR2 and CR3 as a function of different input concentrations (0.6 - 6 mM). Different letters indicate significant differences (Dunn's pairwise comparison *p<.05*) resulting from a one-way analysis of variance (KW). Note that the differences refer to differences within each site.

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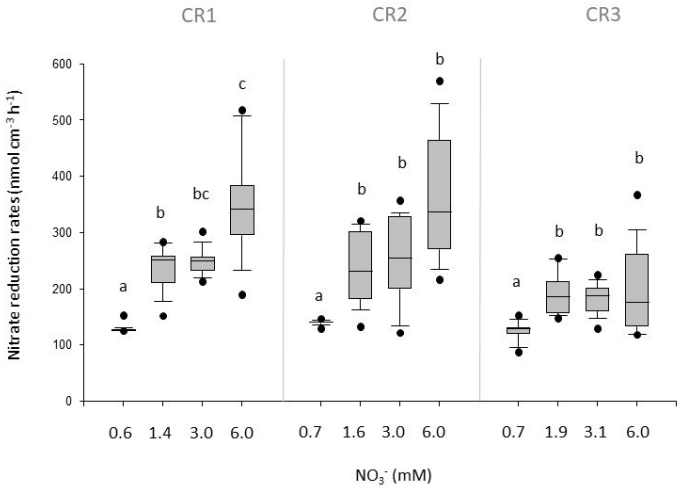
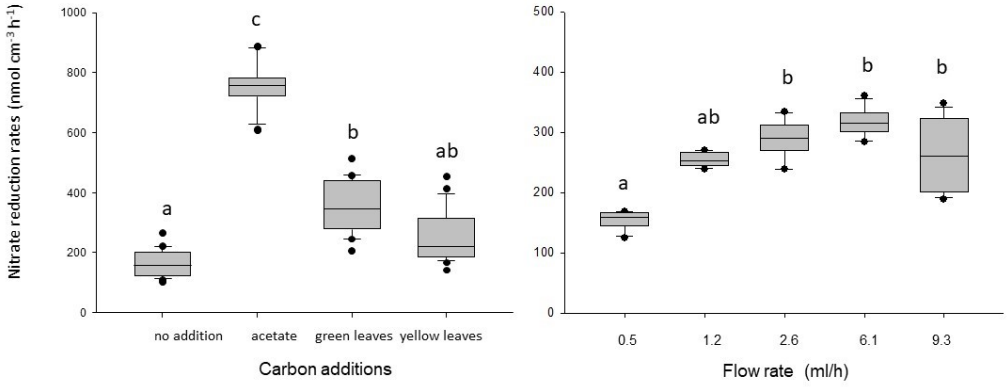


Figure 3. Box-plots of NRR with sediment from site CR2 as related to the different carbon sources (top panel, n=16) and at different flow rates (bottom, n=8). The different letters indicate significant differences between the carbon additions or flow rates (Dunn's p<.05).



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Tables

Table 1. Overview of the water and sediment characteristics at the three different sampling sites and the Rhizophora mangle leaves (GL = green leaves, YL = yellow leaves corresponding to senescent leaves ready to fall) used in the carbon addition experiments.

			Water column			Sediment				
#	Location	distance	$[NO_3^-]$	$[NO_2^-]$	$[NH_4^+]$	C	N	C:N	$\delta^{13}C$	$\delta^{15}N$
		(km)	(µM)			(%)			(‰)	
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
	GL					47.9	1.2	48.1	-28.7	2.6
	YL					46.6	0.6	90.6	-28.7	6.5

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Table 2. Average nitrate reduction (NRR), nitrite (NiPR) and ammonium (APR) production rates for the three stations (CR1, CR2, CR3). The different nitrate additions, flowrates and carbon additions are indicated with standard deviations in italic. The nitrate removal per station and treatment is indicated as a percentage between the input and nitrate in the output of the reactors.

Station	$[NO_3^-]_{input}$	Q	additions	NRR		NiPR		APR		NO_3^-
	mM	ml h^{-1}		Nmol cm^{-3} h^{-1}						removal
CR1	0.6	2.8	-	125.7	*1.0*	9.5	*3.7*	31.5	*12.6*	100.0%
	1.4		-	252.6	*22.7*	117.5	*20.4*	27.4	*12.6*	89.2%
	3.0		-	252.4	*28.3*	99.6	*14.5*	27.9	*22.4*	47.1%
	6.0		-	373.7	*61.2*	200.2	*33.6*	32.3	*17.4*	29.8%
CR2	0.7		-	137.8	*4.7*	6.5	*6.9*	58.9	*4.6*	100.0%
	1.6		-	300.4	*20.8*	76.3	*31.1*	62.4	*12.8*	93.6%
	3.0		-	277.7	*69.3*	90.8	*33.4*	100.7	*45.3*	59.4%
	6.0		-	378.4	*141.1*	109.7	*46.7*	70.7	*10.0*	30.8%
CR3	0.7		-	118.6	*16.9*	2.8	*1.5*	47.7	*5.5*	96.3%
	1.9		-	169.9	*23.9*	11.1	*8.2*	30.8	*4.5*	42.6%
	3.1		-	177.2	*21.2*	56.0	*9.7*	51.1	*12.3*	32.4%
	6.0		-	218.8	*104.2*	63.7	*7.4*	41.6	*7.6*	17.5%
CR2	6.1	0.49	-	154.1	*15.9*	16.9	*2.3*	22.1	*1.5*	69.1%
		1.22	-	254.8	*12.5*	57.9	*11.6*	33.7	*1.6*	46.1%
		2.55	-	289.7	*32.5*	63.3	*28.0*	38.3	*8.9*	23.5%
		6.12	-	317.7	*25.5*	74.9	*10.4*	36.0	*7.4*	11.4%
		9.25	-	263.3	*68.8*	106.4	*10.7*	44.7	*10.8*	6.3%
CR2	6.2	2.7	-	165.1	*44.6*	38.2	*8.0*	36.1	*14.5*	13.6%
			Ac	747.9	*75.1*	302.3	*119.7*	31.1	*32.4*	61.1%
			GL	351.4	*91.0*	51.6	*8.4*	14.8	*11.9*	30.9%
			YL	259.8	*91.0*	24.7	*24.9*	18.3	*16.9*	20.5%

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