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Tracing sewage water by ^{15}N in a mangrove ecosystem to test its bioremediation ability[†]

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Mangrove forests could be a simple and effective alternative to conventional sewage treatment, particularly for island communities given its low cost and low maintenance. Due to their high adaptation capacity, these plants are able to tolerate and bioremediate the high levels of nutrients and pollutants found in sewage water. This solution could be applied to small tropical islands with high population density such as Mayotte in the Indian Ocean. This paper reports on a trial by stable isotopic ^{15}N tracing of such a bioremediation process on pre-treated wastewater near the village of Malamani, in the middle of the large coastal mangrove in the bay near Chirongui. The first results show a boost in the mangrove growth, but a longer period of observation is needed to confirm the beneficial effects, and also to clarify the role of the local crab population, whose engineering activities play an important part in the ecosystem. The exact denitrification process is not yet understood, and the mass balance equation also reveals loss of nitrogen-containing compounds, which needs to be analyzed more closely.

A mangrove is a sea-land interface ecosystem comprising 60 to 75% of subtropical and tropical intertidal coastline, equal to an area of 150000 km^2.[1,2] Mangroves are roughly restricted to latitudes between 30° north and 30° south.[3] They play an important ecological and socio-economic role, as they are important nursery grounds and breeding sites for birds, mammals, fish, crustaceans and reptiles, and they also provide protection against wave action, shoreline erosion, hurricanes and tsunamis.[1,3,4] They are traditionally used for wood, food, fuel, medicine and habitats, especially in developing countries.[5] Mangrove vegetation is able to develop high adaptation capacity for extreme environmental conditions: unstable substratum, alternating aerobic and anaerobic conditions, desiccation (low tide) and waterlogging (high tide) conditions, and salinity fluctuations.[6] They act as a filter for fluxes from adjacent lands which carry suspended matter, nutrients and contaminants.[7]

This high adaptation capacity suggests that these plants should be able to tolerate the pollutants contained in sewage.[8] Agricultural, industrial, and domestic wastewaters are one of the biggest causes of nitrogen and phosphorus release into the aquatic ecosystem. Excess nitrogen loads are largely responsible for coastal eutrophication leading to widespread hypoxia and anoxia, habitat degradation, alteration of food-web structure, loss of biodiversity, and increased frequency, spatial extent and duration of harmful algal blooms.[9] Coastal eutrophication constitutes a major environmental problem on a worldwide scale.[9]

Natural and constructed wetlands such as mangroves could be a simple and effective alternative to conventional sewage treatment, particularly for island communities given the low cost and low maintenance.[10–15]

Nedwell suggested the use of a mangrove-like wastewater secondary treatment system.[16,17] He hypothesized that these wetlands may be more resistant to nutrient enrichment and eutrophication than neighboring lagoon and reef ecosystems. Mangroves may be able to purify wastewater containing high nitrogen concentrations through a complex set of biological, physical and chemical processes, such as absorption by plants or microorganisms, or adsorption into sediments. Denitrification seems to be the major process of atmospheric nitrogen loss into intertidal sediments.[18–21] No change in a mangrove plant community was observed after wastewater discharge,[8] and many studies have shown positive growth response following nitrogenous fertilization such as enrichment experiments,[22–24] the addition of livestock wastewater[25] and domestic sewage,[8] or natural enrichment by guano.[26]

Mayotte, a small French island in the Indian Ocean, has one of the highest population densities in the world and rapid demographic increase (500 inhabitants km^{-2}, INSEE – French National Institute for Statistics and Economic Studies, Paris, France). As a result, Mayotte Island faces difficulties with

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its domestic wastewater, particularly as it has only one treatment plant and some vegetative filter strip systems. The issue of wastewater treatment is especially important in Mayotte, because it has an exceptional ecological heritage: the mangrove forest is the major buffer area where groundwater and river water are purified before coming out in the lagoon. A double coral reef surrounds this closed lagoon, with the clear water being filtered by the mangrove formation. The French International Union for Conservation of Nature (IUCN 2003) has identified more than 70 areas of major ecological interest on the island. For this reason, since 2006 the water and wastewater syndicate of Mayotte (SIEAM), in partnership with the CNRS (Toulouse, France), has been conducting a wastewater treatment trial using the bioremediation ability of mangroves near the village of Malamani.

The objective of this study was to perform isotopic tracing with ^{15}N of Malamani's pre-treated wastewater to assess the fate of nitrogenous compounds in areas which could be affected by discharges such as sediments, groundwater and vegetation. This approach is intended to provide a first estimation of the retention of N in the mangrove and to allow rough calculations of the capacity of this system to retain nutrients, especially N and P.

EXPERIMENTAL

Study area

Sampling was carried out in March and April 2009 on 'Grande Terre' Mayotte Island. The experimental pilot site is located in the Chirongui Bay, downstream from Malamani village (see Fig. 1(a)). Malamani domestic wastewaters (400 inhabitants) are collected and sent to a decanter and storage tank thus providing wastewater pre-treatment by screening, grit removal and sedimentation. Delivery and discharge of domestic, pre-treated wastewater into the mangrove are conducted through a web of pipelines which acts as secondary wastewater treatment and is supposed to limit nutrient transfer into the lagoon. Since April 2008, the discharges have been controlled by a remote terminal unit (SOFREL, Lacroix system, Vern-sur-seiche, France). They are carried out 1 h before low tide for an outflow of up to 10 m^3.day/plot (e.g. one discharge every second low tide). Four plots (45 m × 15 m) were delineated in 2007 (see Fig. 1(b)) in two representative mangrove strata dominated by either *Ceriops tagal* or *Rhizophora mucronata*: two control plots and two impacted domestic wastewater plots. Similarly, a 25 piezometric network (170 cm deep) was set up to study the physical and chemical characteristics of the mangrove groundwater.

Some of the mangrove soil characteristics from the Ceriops and Rhizophora strata are given in Table 1. In both strata the soil texture is dominated by the silt fraction with higher silt values for the Rhizophora strata, situated closer to the lagoon. The soil pH is neutral – slightly acidic (6.6 < pH <6.8). The C concentration in the soil varies from 6 to 7%, which is similar to that of other mangroves in the same geographical area. The bulk soil N concentration of 0.3–0.5% is low, resulting in high C/N ratios. The N concentration in the Ceriops and Rhizophora leaves is rather low, suggesting that N is a limiting factor.

Nitrogen enrichment, sampling and analytical techniques

For the ^{15}N pulse labeling experiment, two independent subplots (3 m × 3 m) were delineated in both strata; each subplot contained a piezometer. For each subplot, a 100 L tank was filled with wastewater, and 1.5 g of highly labeled $^{15}NH_4NO_3$ (98 atom%) was added. This approach leads to slight differences in the initial N concentration and ^{15}N enrichment of the wastewater, but it is impossible to use a single tank due to the harsh environment. After being mixed, the labeled wastewater was homogeneously poured over the 9 m^2 subplot at low tide. In each case, blanks were sampled beforehand to determine the natural abundance of ^{15}N in all studied compartments (soil, soil-extractable N-NH_4^+, ground water and leaves).

Groundwater

In each subplot, the infiltrated labeled water was sampled in the piezometer over 30 h (n=22). A volume of 400 mL was filtered and kept for the colorimetric analysis, and 10 mL for the isotope measurement.

Soil water

In each subplot, five surface soil samples (100 g between 0 and 5 cm deep) were taken over 30 h (n=112): 60 g for the 1 M KCl extraction followed by colorimetric analysis of both mineral N forms, and 20 g were dried at 65 °C prior to determination of bulk soil [15]N. The soil water content was measured on a 20 g subsample after drying at 105°C.

Leaves

In each subplot two sets of 15 leaves (5 leaves from three trees) were taken (every day in the first week, and every two days in the second week) from trees whose roots were within the subplot limits (n=168). Each group of leaves was weighed before and after drying (60 °C, 48 h). The dry leaves were pulverized before isotopic analysis.

[15]N isotopic analysis

The resulting powders were sent to Iso-analytical Laboratory (Crewe, UK) where they were analyzed using a Roboprep-CN elemental analyser coupled with a GEO 20–20 isotope ratio mass spectrometer (both from Europa Scientific, Crewe, UK). The calibration was carried out using working laboratory (IA=Iso-Analytical) and international (IAEA) standards: wheat flour (IA-R001), and ammonium sulfate (IAEA-305-A and IAEA-311, or IA-R045 and IA-R046). The results are expressed in per mil relative to $\delta^{15}N_{Air}$:

$$\delta^{15}N_{Air}(‰) = \left(\left(^{15}N/^{14}N_{sample}\right) / \left(^{15}N/^{14}N_{standard}\right) - 1 \right) * 1000$$

[15]N Atom % is given by the relationship:

$$(R \text{ enriched sample}/R \text{ un} - \text{enriched sample} - 1) \times 100,$$

where R=$^{15}N/^{14}N$=0.0036765.

Statistical tests

The Mann-Whitney U-test was performed to test differences between mean values in the Ceriops and Rhizophora data.
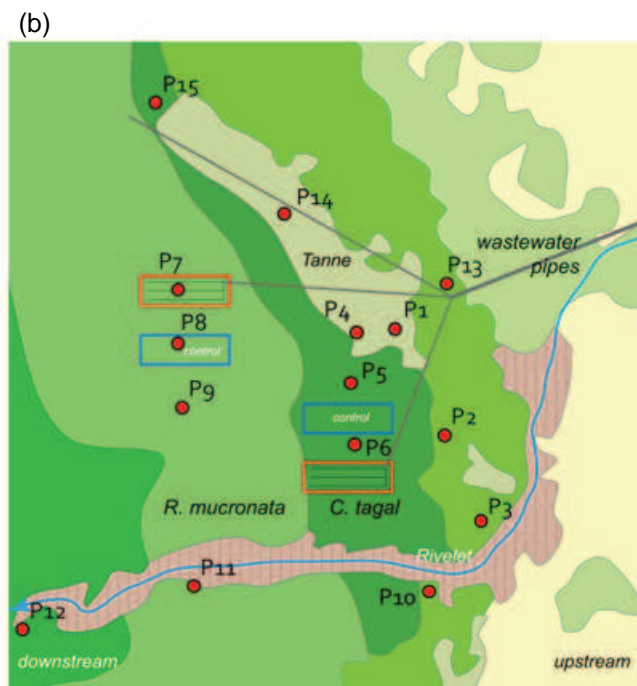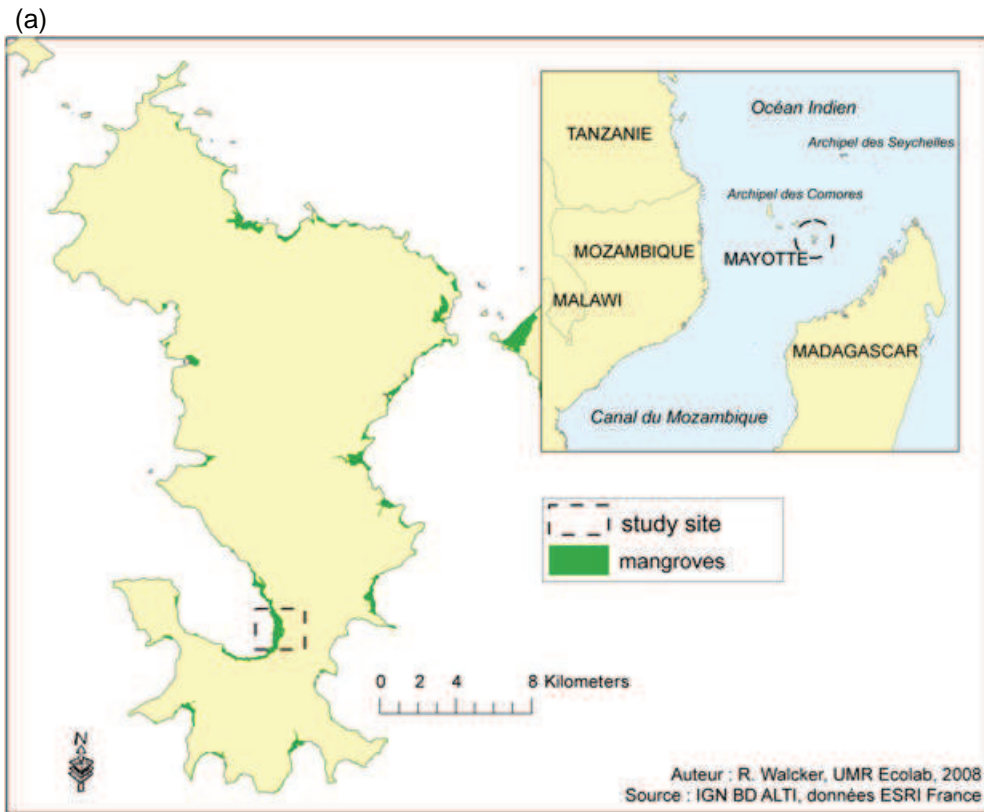
Figure 1. (a) Maps of Mayotte, Malamani, and distribution of the Mangrove. (b) Location of the plots and the piezometers in Malamani mangrove.

Table 1. Soil chemistry characteristics in two adjacent stands of Rhizophora and Ceriops mangroves situated at Malamani, Mayotte. Leaf C and N of both species

		Rhizophora	Ceriops
Soil	Texture (%) (sand/silt/clay)	21/76/3	40/57/3
	pH (H_2O)	6.80	6.62
	Total C (%)	7.32	5.25
	Total N (%)	0.26	0.15
	C/N	28	35
	P total (%)	0.36	0.44
Leaf	Total C (%)	48.5	47.6
	Total N (%)	1.48	1.22

The following symbols are used on the figures for the significance level: $0.05 < $p-value$ < 0.01$:(*); $0.01 < $p-value$ < 0.001$: (**); p-value$ < 0.001$: (***). These statistical tests were carried out using the program R version 2.9 and SigmaPlot 9.0.

RESULTS

$N-NH_4^+$ accounted for 95–100% of the mineral N in the sewage water before dispersion into the mangroves, due to the reduction conditions in the buffer tank. After the addition of the labeled ^{15}N, the $N-NH_4^+$ concentration was 20.4 mg $N-NH_4^+$ L^{-1} in the wastewater applied to the Rhizophora plot and 23.0 mg $N-NH_4^+$ L^{-1} in the wastewater applied to the Ceriops plot (Table 2). About 215 and 255 mg $N-NH_4^+$ were applied per m^2 in the Rhizophora and Ceriops plots, respectively, which corresponds to approximately 0.04% of bulk soil organic N (0–30 cm). The addition of $^{15}N-NH_4^+$ to the sewage increased the ^{15}N label to 8.66 and 8.06 atom % ^{15}N. These values are lower than the theoretical atom % ^{15}N, probably due to loss of $N-NH_4^+$ and $^{15}N-NH_4^+$ and through volatilization. We observed a decrease in both the $N-NH^{4+}$ concentration and the ^{15}N label in the 100 L storage tank that was left open for a few hours. In fact, only 70% of the ^{15}N was detected in the sewage solution, which indicates a loss of N by volatilization. Table 3.

The soil water content was higher in the Rhizophora plot than in the Ceriops plot, reflecting the spatial distribution of the two species (Fig. 2(a)): the Rhizophora plot is closer to the lagoon than the Ceriops plot. Over the whole 30 h period, the soil water content remained reasonably constant. In both species the addition of wastewater did not change the amount of total soil organic N, which varied slightly over the 30 h (Fig. 2(b)). The N concentration was higher in the Rhizophora soil (2.7 g N kg soil^{-1}) than in the Ceriops soil (1.6 g N kg soil^{-1}). As a consequence of adding similar amounts of ^{15}N to both plots, the resulting enrichment

was higher in the Ceriops soil than in the Rhizophora soil (Fig. 2(c)). The ^{15}N label increased strongly after ^{15}N-labeled wastewater application, reaching the highest values after 2–4 h in Rhizophora and 8 h in Ceriops, followed by a continuous decrease of the atom % ^{15}N during the following hours. Thirty hours after the start of labeling, the bulk soil atom % ^{15}N was still higher than before the labeling because of immobilization/retention of applied wastewater N.

The dynamics and concentration of soil-extractable $N-NH_4^+$ are quite similar in both species. Wastewater addition resulted in a steep increase in the $N-NH_4^+$ concentration – almost all the applied $N-NH_4^+$ was recovered in this fraction 2 h after application. Later, the $N-NH_4^+$ concentration remained stable or tended to decrease. Nevertheless, the $N-NH_4^+$ concentrations were highly variable, a sign of heterogeneous distribution or retention of the applied $N-NH_4^+$. The atom % ^{15}N of soil-extractable $N-NH_4^+$ increased dramatically immediately after the application of labeled wastewater, showing that most soil-extractable $N-NH_4^+$ originates from the wastewater. After this peak, the atom % decreased from 8 atom % ^{15}N to 4–5 atom % ^{15}N in both species. In the hours that followed, the atom % ^{15}N remained almost constant in the Rhizophora, while a steep decrease was observed in the Ceriops.

In both species, no relevant amounts of tracer ^{15}N were detected in the soil solution collected by piezometer (data not shown). This detail supports the hypothesis that the applied NH_4^+ is retained/immobilized at the soil surface. Leaves are recognized as strong sinks for soil-borne N. In both species, the total leaf N remained fairly constant over a post-labeling period of 280 h (Fig. 3(a)). The uptake of applied ^{15}N by both tree species and further translocation to the leaves progressively increased their atom ^{15}N (Fig. 3(b)). The leaf ^{15}N dynamics showed a slow increase in ^{15}N (hour 0 to hour 120) followed by a second period (hour 120 to hour 290) where the ^{15}N enrichment remained stable (Rhizophora) or increased and decreased (Ceriops). Thus, even if the bulk soil ^{15}N enrichment of Rhizophora and Ceriops differed strongly, this was not reflected in the ^{15}N enrichment of the leaves. The retention of NH_4^+ by clay minerals and adsorption to soil organic matter allow a continuous increase in the atom % ^{15}N of the leaves. This is particularly interesting, because it demonstrates the uptake and immobilization of applied sewage through the vegetation.

The applied sewage ^{15}N is rapidly immobilized in the surface soil (40–60%, Fig. 4(b)) and taken up and immobilized through the leaf biomass (2–4%, Fig. 4(a)). The recovery of ^{15}N in the soil-plant system is 42–64%. Depending on the tide, losses are possible through exportation with the seawater into the lagoon, or through volatilization for losses such as ammonia. Although both are possible, the volatilization process is largely supported by the environmental conditions, namely high temperature and the high pH of the wastewater.

Table 2. $N-NH_4^+$ concentration in wastewater after tracer addition, amount of $^{15}N-NH_4^+$ added, ^{15}N enrichment of labeled waster water, applied amounts of N and ^{15}N per m^2

	$N-NH_4^+$	$^{15}N-NH_4^+$	atom% ^{15}N	applied N	applied ^{15}N
	----mg N(^{15}N) L^{-1}----			-----mg N (^{15}N) m^{-2}------	
Rhizophora	19.4	0.277	8.66	215	18.7
Ceriops	23.0	0.277	8.06	255	20.6

Table 3. Retention/immobilization of applied wastewater N in the mangrove biomass

	N applied[a]	N uptake (leaf)[b]	Leaf N[c]	N immobilized (biomass)[d]	N lost by litter fall
			(g N m^{-2} year^{-1})		
Rhizophora	220	8.8	10.2	6.6	5.1
Ceriops	220	4.4	6.5	4.3	3.3

[a]Amount of N imported by the wastewater (calculated according to the mean daily N-NH$_4^+$ concentration and a daily deposition of 10000 L).
[b]Amount of applied N taken up by the leaves (derived from the experiment 2% of applied N in Ceriops and 4% of applied N in Rhizophora).
[c]Total leaf N (derived from the reserves and from N uptake).
[d]Immobilization of N through translocation of N into new leaves, roots, wood biomass.

DISCUSSION

Our ^{15}N tracer experiment provided a first insight into the destination of wastewater N deposited into mangroves. We focused on the main compartments (soil, leaves) and established ^{15}N budgets for the soil plant system. The

Mayotte mangroves, like mangroves in general, receive their mineral nutrients and organic matter through sediments imported by fresh water inflow, litter decomposition and its associated micro- and macro-organisms.[27–30] In general, however, the mangrove sediments remain poor in N- and P-containing compounds,[31] due to low upland inflow and
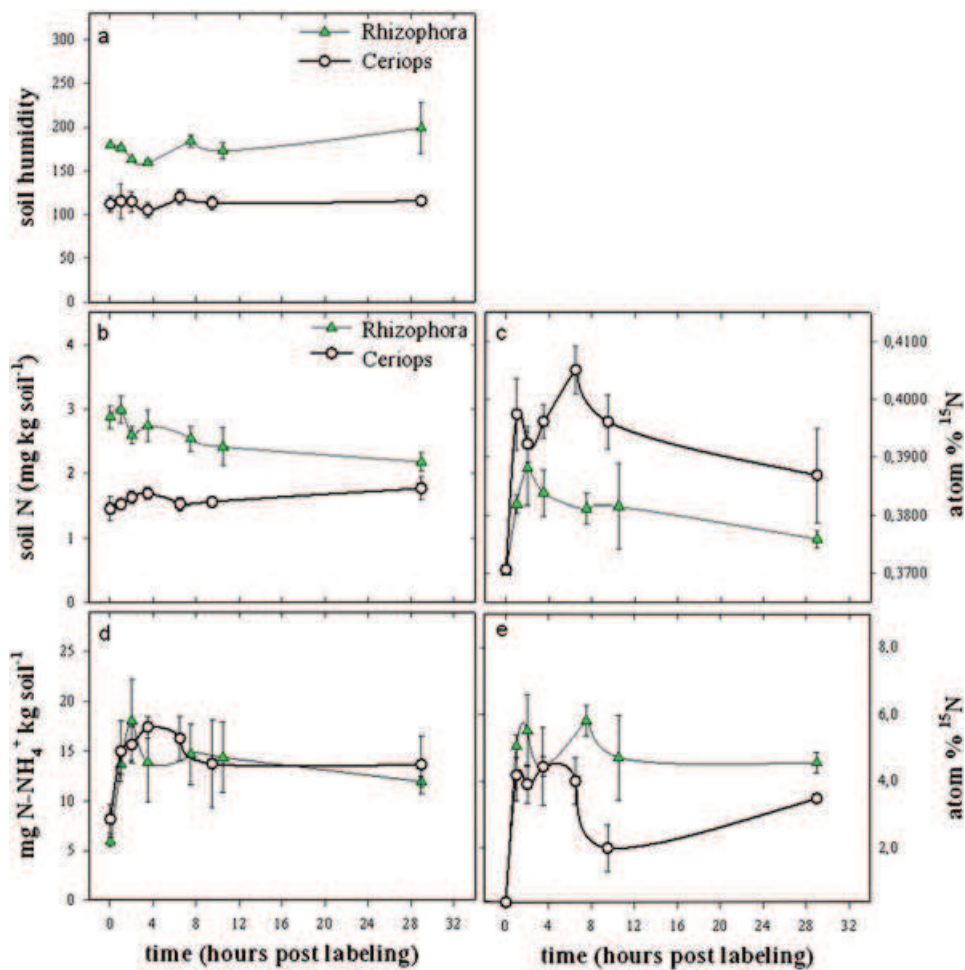

Figure 2. Variation of soil humidity (a), of bulk soil N (b), soil-extractable N-NH$_4^+$ (d) and their atom % ^{15}N (c and e) after application of ^{15}N-labeled wastewater to the soil surface in two adjacent plots of Rhizophora and Ceriops mangrove (n=5, mean value±standard deviation, number of *: Mann-Whitney test significance level for the difference).
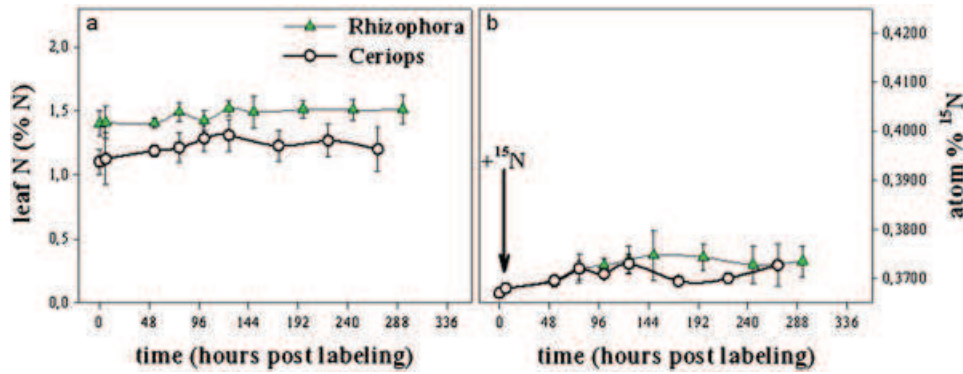
Figure 3. Development of leaf N concentration (a) and atom % ^{15}N (b) after application of ^{15}N-labeled wastewater to the soil surface in two adjacent plots of Rhizophora and Ceriops mangrove (n=5, mean value±standard deviation, number of *: Mann-Whitney test significance level for the difference).

washing out by the ocean tide. In the case of Mayotte Island, the sediments imported into the mangrove by heavy rainfall and inundation are erosion products from old strongly weathered volcanic bedrock, namely lateritic soils with a high iron oxide content.[32] Such a nutrient-poor substrate could explain the relatively low growth and low productivity of the Mayotte mangrove. For instance, we found a annual litter productivity around 3.,8 t. h^{-1}.year^{-1} for the *Ceriops* strata, and 6.9 t. h^{-1}.year^{-1} for the *Rhizophora* strata. In other mangroves from the Indian-Pacific area, the litter productivity for both these species is 30 to 80% higher. In addition, nutrient import in the mangrove is event-based and not constant as it is in other mangroves situated close to big rivers. For this reason, the input of wastewater with higher concentrations of N and P could boost the mangrove growth. Our experiment showed that applied N as NH_4^+ is efficiently retained through adsorption to clay minerals or microbially immobilized in the soil substrate and taken up by the Rhizophora and Ceriops mangrove trees,. In both soils, the clay content is fairly low (3%) and there is strong competition between NH_4^+ and Na^+ for the cationic exchange sites. Thus, the ion exchange capacity to

retain NH_4^+ from added wastewater seems limited. The NH_4^+ cations are also, however, adsorbed on negative charges of soil organic matter.[33,34] The total C content in the Rhizophora and Ceriops is about 7%, suggesting that soil organic matter may adsorb a substantial part of NH_4^+. Mangroves are known and characterized by their capacity to accumulate and recycle organic matter efficiently.[6]

From our experiment, we assumed loss of N through volatilization. Volatilization of N-NH$_4$ is likely to occur in both the Rhizophora and the Ceriops plots. Other reasons, such as weight errors or non-homogeneous distribution of the label in the solution, are less likely. The key parameters which promote the volatilization of NH_4^+ such as surface-applied solution, high pH and high temperature (28–30 °C) are present in these mangroves. Under such conditions, the losses of NH_4^+ can reach 50 to 90% as is frequently observed in terrestrial ecosystems when liquid manure is applied.[35]

Another important mechanism of loss/export of ^{15}N from the mangrove is related to the tide. Depending on the amplitude of the tide, both species are flooded twice per day by saltwater. These tide events transform the mangrove
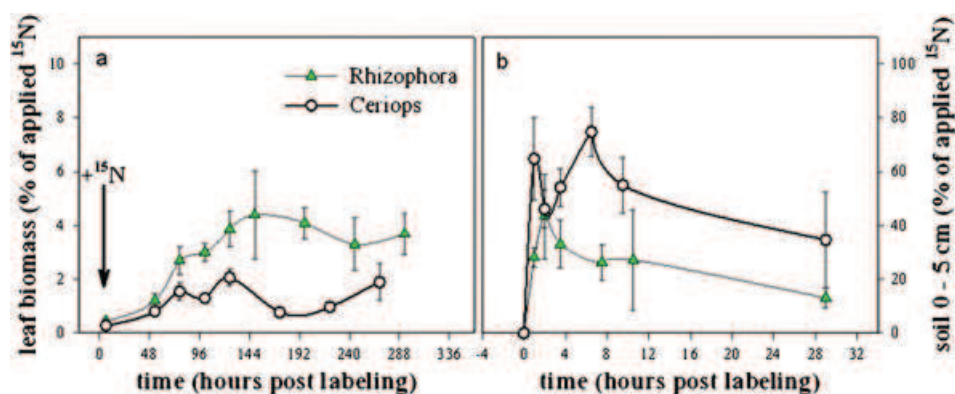

Figure 4. Temporal variation of the distribution of applied wastewater ^{15}N in the leaf biomass (a) and in the topsoil (0–5 cm) (b) (n=5, mean value±standard deviation, number of *: Mann-Whitney test significance level for the difference).

into an aquatic ecosystem, modifying essential physical and chemical soil parameters, such as dissolved oxygen, redox potential, etc.[3,6] Free, non-immobilized wastewater NH_4^+ situated on the soil surface is probably dissolved in the saltwater and transferred into the lagoon at low tide. According to the ^{15}N budgets for which we can account, 40–60% of the applied N is lost. Hence, we are not able to give exact numbers for the loss of NH_4^+ by the tidal movement.

Another important factor to take into account is the burrowing activity of crabs. In both mangrove species, crabs are abundant and maintain a network of holes (around 10 to 20 burrows per m^2), restructure the soil, increase the aeration of the soil, build aggregates and feed on fresh organic matter. They are efficient ecosystem engineers. Depending on wastewater deposition, such holes are the preferential flow paths for applied sewage solution and they may reduce the amount of N remaining at the soil surface. Nonetheless, we detected no ^{15}N in soil water collected with piezometers. On the other hand, aggregates built by crabs may create or maintain more favorable conditions for microbial communities and thus increase the microbial immobilization of applied ^{15}N.

The tree uptake of applied ^{15}N indicated that applied wastewater ^{15}N remained in the soil even after several tides. Leaves are a good indicator for the uptake of soil-borne N, because of their high demand in order to maintain leaf photosynthesis. In our experiment, the two studied species showed similar uptakes of ^{15}N at a relatively low level. Based on the 280 h record, very little ^{15}N had been incorporated into the leaf biomass, but we cannot exclude a transfer of N out of the leaves into branches, flowers and seeds. The leaf N concentrations are low in both species, suggesting that N and other nutrients limit the growth and biomass production of the trees. Deposition of wastewater will improve this situation. Depending on the environmental conditions and a continuous nutrient supply through the wastewater deposition, a significant amount of N and other nutrients such as P are taken up by the trees.

The decrease in a few hours of the soil content after the ^{15}N labelling could be due to vegetation absorption, as is confirmed for the ammonium by the high ^{15}N signal in both the Ceriops and the Rhizophora trees. The second reason may be the microbial activity,[8] and the nitrification-denitrification process during the tide.[19] Our results, showing a decrease in the ^{15}N concentration of all the nitrogen compounds, confirm the rapid transformation or loss of these molecules. The colorimetric analyses of the soil and water samples reveal that part of the $^{15}NH_4$ is oxidized to nitrate in the surface sediment.

The nitrogen mass balance calculation on the soil and leaf pools shows that about 40–50% of the nitrogen compounds are found in the Rhizophora strata (mainly in their leaves), whereas only 10–30% are found in the Ceriops strata. The difference may be in the water compartment or it is possible that this missing nitrogen could be bioremediated and/or lost as gas (N_2O, N_2). The uncertainty resides in the amount of nitrogen compounds taken out by the tidal water. More data are needed to understand the effective yield of this bioremediation process and we plan a new isotope tracing study of the denitrification process to obtain a more complete mass balance table.

CONCLUSIONS

Before the installation of the wastewater pretreatment unit, the wastewater of Malamani village flowed into the rivelet and the local groundwater, and were certainly bioremediated by the vegetation of the back-mangrove forest (Erythrina mainly). Demographic pressure has, however, increased the volume of this wastewater, and the local inhabitants need more room for cultivation, which means that they have cut the trees in this back mangrove for agriculture purposes. This has created an urgent sanitary problem with eutrophication of the water pool and bacteriological contamination. By bringing the pretreated wastewater directly in the mangrove forest, the sanitary problem is solved downstream of the village, the mangrove growth and development is improved, and this previously unappreciated area has been put to good use. The difference in the bioremediation process is that previously the nitrogen compounds were mainly in nitrate form which was easy to transform into N_2O and N_2. Now the pre-treatment and the tank retention convert most of the nitrogen compound into ammonium, which is more easily absorbed by the vegetation but complicates the bioremediation process as it must first be reconverted into nitrate form. On the other hand, ammonium is more quickly absorbed than nitrates by the vegetation. These first results show that the mangroves can bioremediate the domestic pre-treated wastewater. A longer period of observation is needed to confirm the apparent beneficial effect on the vegetation, and also to see if the interdependence between the crabs and the mangrove trees is influenced. More work is also needed to clarify the final destination of the nitrogen compounds and to complete the mass balance.

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