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Mangrove microbial diversity and the impact of trophic contamination

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ABSTRACT

Mangroves are threatened ecosystems that provide numerous ecosystem services, especially through their wide biodiversity, and their bioremediation capacity is a challenging question in tropical areas. In a mangrove in Mayotte, we studied the potential role of microbial biofilm communities in removing nutrient loads from pre-treated wastewater. Microbial community samples were collected from tree roots, sediments, water, and from a colonization device, and their structure and dynamics were compared in two areas: one exposed to sewage and the other not. The samples from the colonization devices accurately reflected the natural communities in terms of diversity. Communities in the zone exposed to sewage were characterized by more green algae and diatoms, higher bacteria densities, as well as different compositions. In the area exposed to sewage, the higher cell densities associated with specific diversity patterns highlighted adapted communities that may play a significant role in the fate of nutrients.

1. Introduction

Recent assessments suggest that about one-third of mangrove, sea grass and salt marsh areas around the world have already been lost over recent decades as a result of reclamation, deforestation, engineering and urbanization (Lewis et al., 2011; Peixoto et al., 2011; Penha-Lopes et al., 2011), as well as transformation to provide aquaculture ponds (Alongi, 2002). Many coastal lagoons in the tropics support dense mangrove forests, which are productive areas harboring a wide diversity of organisms. Adapted to intertidal zones, they are subjected to highly variable physicochemical conditions of salinity, flooding, light, and temperature, which give rise to the high diversity that characterizes mangrove ecosystems (Feller et al., 2010).

Mangroves are often established on nutrient-rich sediments, and are able to absorb excess nutrients without suffering any major structural or functional disturbance (Saenger, 2002). Nedwell (1975) was the first to show that pretreated wastewater discharge into a mangrove swamp in Fiji reduced eutrophication in adjacent coastal waters, and therefore suggested that mangroves might serve as the final stage in sewage treatment. Since then, most attempts to evaluate the potential role of mangrove to remove nutrients from sewage have been done in the form of experimental studies (Chu et al., 1998; Wu et al., 2008), or on constructed pilot sites (Yang et al., 2008; Tam et al., 2009). Yang et al. (2008) highlighted the need for complementary treatment to eliminate pathogenic bacteria. However, only a few studies have investigated natural mangrove sites in intertidal zones (Wang et al., 2010; Penha-Lopes et al., 2011). Wang et al. (2010) explored changes in water quality in a subtropical mangrove estuary (China), and highlighted the fact that large quantities of nutrients may be trapped by the mangrove during flood periods. In a comparison of non-impacted and peri-urban subtropical mangroves (Mozambique), Penha-Lopes et al. (2011) showed that both the structure and the fitness of a natural shrimp population (*Palaemon concinnus*) were impacted by nutrient levels. Tam showed as early as (1998) that adding wastewater to mangrove soils seems to stimulate the growth of microbial populations, probably because of the nutrients and carbon supplied in wastewater, but this compartment has not been thoroughly explored within the framework of domestic-sewage discharge in mangroves. Estuarine waters are dynamic environments in which sediments, and marine and fresh water mix, resulting in salinity and nutrient gradients. Shifts in physical, chemical, and microbiological properties between freshwater and adjacent coastal marine environments occur over short periods of time, driven by tides and freshwater flows, which create an intense abiotic pressure that influences the composition of bacterioplankton communities (Crump et al., 1999). Microorganisms have large

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population sizes and display long dispersal distances, high reproductive rates, and remarkable genetic diversity, suggesting that they can cross environmental boundaries, including salinity, more readily than multicellular organisms (Logares et al., 2009).

On solid surfaces, microorganisms organize themselves into microbial biofilms, mainly consisting of heterotrophic bacteria and autotrophic eukaryotes that are often known collectively as the "microphytobenthos", which is embedded in a microbial organic matrix (Decho, 2000). These biofilm communities are present in the upper layers of sediment (Holguin et al., 2001) as well as on tree roots (Toledo et al., 1995; Gomes et al., 2010), where they carry out a number of different functions, including nutrient transformation, sediment stabilization, plant-growth promotion, and even providing protected suitable areas for pathogens entering marine systems. The impacts of pollutants such as polycyclic aromatic hydrocarbons (PAH, for a review see Fernandez-Luqueno et al. (2011)) and phthalates have been studied in mangrove ecosystems. Conversely, changes in trophic conditions due to nutrient loading have received much less investigation in these tropical zones (Underwood, 2002; Ramanathan et al., 2008), unless they are threatened by urban sewage or aquaculture. Yet such changes, which have been extensively studied in freshwater ecosystems, are known to modify the functionality of biofilm communities, as well as their diversity (e.g. Pesce et al., 2008; Berthon et al., 2011).

This study focuses on biofilm microbial communities in a tropical mangrove with two different trophic statuses, either exposed or not exposed to effluents from a domestic wastewater pre-treatment device. We tested an experimental approach using artificial substrates to collect natural biofilms, in order to avoid sampling biases due to environmental heterogeneity. Microbial communities were also sampled *in situ* to collect pelagic, benthic and root biofilms in order to compare their structures and diversities. This approach provides a very useful way of extending our knowledge about the microbial communities associated to mangrove ecosystems, and of evaluating the impact of urban sewage on these communities.

2. Materials and methods

2.1. Study sites

Two sites in a mangrove located in Chirongui bay, southwest of Mayotte Island in the Indian Ocean (12°55′S, 45°09′E, Fig. 1) were investigated in this study. This area was described in a previous study by Herteman (2010). Domestic wastewater collected from the contiguous village of Malamani (400-equivalent inhabitants) was subjected to primary treatment in a sedimentation tank which reduced the suspended matter concentration by 50%. Pre-treated wastewater was then discharged at low tide into a 45 m × 15 m mangrove zone dominated by *Rhizophora mucronata,* at the rate of 10 m^3 per 24 h. This site, designated the "impacted plot", was studied simultaneously with a "reference plot" located in the same mangrove zone, but not subjected to wastewater disposal. Our study was conducted in March 2009 at the end of the rainy season, during the spring tides phase, after this wastewater pre-treatment and discharge had been taking place for 1 year.

2.2. Sub-surface and porewater sampling

Salinity and pH were measured directly on the field in crab water holes (0 cm) and in deep piezometers (30 and 100 cm). Mean values were obtained from two-days measurements under similar hydrological conditions. Nutrient contents were measured 0.45-μm filtered water sampled at the same depth, and transported to the analytical laboratory (ARVAM, Reunion, France) in a cool-box at 4 °C. Nutrients concentrations were determined following French standard operating procedures (AFNOR). Interference with high NaCl concentrations was avoided using blank samples on a salinity gradient. In addition, the carbon source level was investigated through the chemical oxygen demand (COD) and biological oxygen demand (BOD) of the pre-treated wastewater that were measured three times during the first year of wastewater pre-treatment.

2.3. Collection of microbial communities

Six large frosted glass slides (47 cm^2 per slide) fixed in a perforated plastic box were used as artificial substrata allowing periphytic communities to colonize them (Morin et al., 2007). One box was placed in each plot (the impacted plot and the reference plot) at soil level, and maintained with ropes to arching stilt roots of *R. mucronata* for 8 days before sampling. This corresponded to a high tide period during which the sampling system remained under water for about 40% of the time. At the end of this period, the 6 slides from both of the sampling sites were collected, and transported to the field laboratory within 2 h in a cool-box. Colonized biofilms were then carefully removed from each replicate glass slide separately, using a razor blade, and suspended in

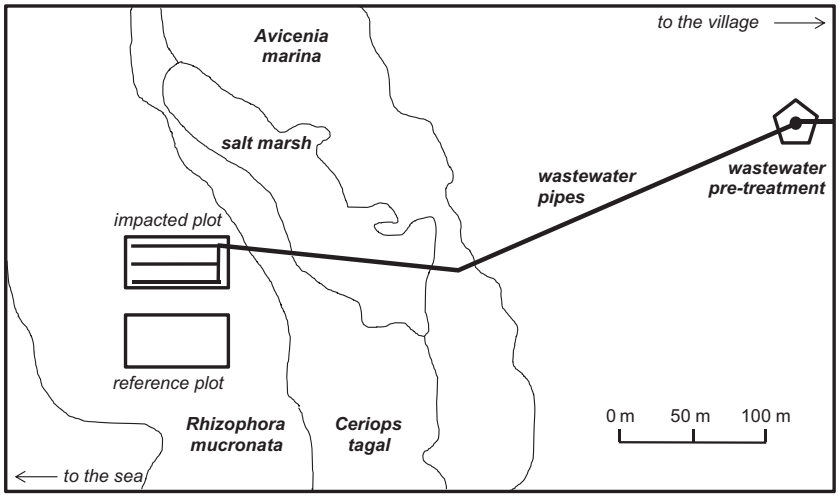

Fig. 1. Mangrove study area and land cover on Mayotte Island in the Indian Ocean.