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Impact of seawater and canopy cover on the phyllosphere bacterial community of *Rhizophora mucronata* leaves

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***Abstract*—** *The plant-microorganism interaction is a well-studied topic in the world of science due to the sustainable management of the ecosystems. The phyllosphere remains the habitat of some microorganisms where several interactions take place. In order to assess whether the mangrove leaves can harbor a bacterial population and analyze the abundance in these leaves microbiotas, leaf samples of mangroves species (Rhizophora mucronata) were collected in the mangroves of Ouroveni in East-Mbandjini, Grande-Comoros. Through the 16S rRNA genes sequencing, the results showed that in the different experimental group, 105303, 110873, 124703, 146954 and 112225 OTUs were identified respectively, where the canopy was open (C1), semi-open (C2), completely closed (C3), and where the plants are submerged (S) and non-submerged (NS) in seawater. The identified OTUs was positively correlated with leaves-wax ($p < 0.05$, $r^2 = 0.91$), nitrogen ($r^2 = 0.72$), phosphorus content ($r^2 = 0.62$) and the factor "seawater" ($r^2 = 0.93$). It was however highly and negatively correlated with the canopy cover ($r^2 = 0.93$). Considering the factor "seawater", the relative abundance of bacteria in the submerged leaves was significantly higher compared to that from the non-submerged plants. By taking into account the factor "canopy cover", it was revealed that more the canopy cover was open, the less was the relative abundance of bacteria. Thus, the finding of this present study affirm that the leaves of mangroves can be a major habitat to host a large population of bacteria that can be influenced by local abiotic factor.*

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I. INTRODUCTION

The symbiotic relationship between plants and microorganisms is an interesting studied subject in the world of science. They can cohabit together in such a way that each of these two hetero-specific organisms benefit from this association. Until these several years, the research were mainly focused on microorganisms and their relationships with their host plants (Fatima and Senthil-Kumar 2015; Fester et al. 2014). However, several reports showed that different parts zone of plant host can harbor microorganisms which can be used for different scientific need. We distinguished therefore, phyllosphere, the endosphere and the rhizosphere which are considered as a habitat for microorganisms. The phyllosphere is the aerial part of plants mainly the leaf surface, which is an environment largely inhabited by microorganisms (Koskella 2020), while the rhizosphere is the part of the soil penetrated by plant roots and associated microorganisms (Liu et al. 2020). Studies by the rhizosphere are much more advanced compared to that of the phyllosphere. However, quite a large number of the phyllosphere reports are reported recently due to the massive production of data resulting from the use of omics and related technique. This enhanced a significant advance in the understanding of microbial dynamics in the aerial organs of plants, mainly in the leaves.

The community of microorganisms living both on the surfaces of plant organs (phylloplane) or inside plant tissues (endosphere), is composed by bacteria, viruses, fungi, algae, archaea and rarely by protozoa and nematodes (Vacher et al. 2016). The phyllosphere designates the community of microorganisms that live in a symbiotic relationship with plants, in particular on leaves, stems, buds and flowers. It is a complex and relatively unknown world of microbes interacting with each other and with host plants, especially with aerial organs. Nowadays, scientific studies are looking at this new world for a better understanding of this new subject (Lindow and Brandl 2003) and for other interests such as phylloremediation (Wei et al. 2017), pest control (Tripathi et al. 2020), invasion of pathogenic microorganisms on plants in general and leaves in particular (Wang et al. 2019), services for agriculture (Zhang et al. 2019), forestry, etc.

The microbiota of the phyllosphere can be translated to the overall microbial habitat potentially influencing the fitness and functions of their host; which would have an impact on plant biogeography and ecosystem functioning (Yuan et al. 2018). Following this consensus, the microbiota phyllosphere of several plant species, including economically important crop plants, has been explored for their agro alimentary functions. It is now well documented that phyllosphere microbial consortia regulate many plants that have a vital role in plant health as well as plant production (Yuan et al. 2018). Due to their agricultural potential, the phyllosphere microbiota serves as an imperative alternative to chemical fertilizers, which not only facilitate crops to thrive in poor-resource and stressful environments, but also provide resistance to combat dangerous pathogens without disrupt the essential ecosystem balance (Weyens et al. 2015).

Recent advanced development in molecular tools, high-throughput screening procedures and fusion of omics techniques has greatly improved the understanding of bacterial communities associated with phyllosphere including their structural, functional and ecological properties. Among the phyllosphere microorganisms living on the leaf surface, bacteria is far outnumber other epiphyte groups, both in cell numbers and in diversity of taxonomic groups (Zada et al. 2021). After the soil, the phyllosphere ranks second as the habitat containing the greatest concentration of microorganisms on earth. Indeed, the leaf area of terrestrial plants is estimated at more than 6.4 *108 Km^2 (Izuno et al. 2016). Given that the bacterial density on the leaf surface reaches 10^6-10^7 cells per cm^2 (Zhang et al. 2019), the phyllosphere remains an indisputable habitat for different types of microorganisms.

Our present study joins recent efforts to highlight the beneficial plant-microbe interaction in nature with particular reference to phyllosphere microbiota which can be used in the agricultural, or ecotoxicological sector to respectively boost global food security in conjunction with maintaining environmental sustainability. However, most studies on the microbe-plant relationship focus on terrestrial plants and little research is carried out on the marine domain and more particularly on mangroves. Given their particular ecology and the variable environmental conditions faced by these plants of the intertidal zone, it is obvious that these plants could constitute an exceptional habitat for phyllosphere microorganisms and bacteria in particular. This study aims to (i) highlight that the leaves of mangroves (*Rhizophora mucronata*) can host a large population of bacteria, (ii) analyze the abundance of bacteria in the leaves of *Rhizophora mucronata* taking into account different factors such as canopy cover and the seawater and (iii) express a correlation between leaf nutrients and the relative abundance of the bacterial population present on the leaves of *R. mucronate.*

II. MATERIALS AND METHOD

1- Design and collection of samples

The leaves of the mangrove species (*Rizhophora mucronata*) were collected in the intertidal zone of Ouroveni in East-Mbandjini, Grande-Comoros (longitude: 11°54'45 S, latitude: 43°41'08 E and altitude: 0 m). Leaves samples were collected by considering the canopy cover state and seawater as separate factors. Considering the canopy factor, three sampling zones were established: zone 1 corresponding to the canopy fully open (0-10%) and denoted C1; zone 2 corresponding to the semi open/close of the canopy (50-70%) and denoted C2 and zone 3 corresponding to the canopy fully close (100%) denoted C3. The percentage of the canopy was estimated by using a densiometer at a fixed point and rotating through the four cardinal points. The canopy percentage was then calculated according to occupied small square, as was described in (Elyamine 2012). In each branch where leaves were collected, we considered three levels which were basal denoted Ci-1, medium (Ci-2) and apical denoted Ci-3 where i can be 1, 2 or 3 accordingly. In addition to the canopy factor, plants submerged and not submerged in seawater were also considered. Leaves were collected with sterilized scissors with 70% ethanol on site. Twenty seven healthy green and mature leaves were collected for each mangrove zone at 1.5-2 m height. They were then sealed in a sterile 500 mL PVC bags and brought to the laboratory. After collect, leaves samples were divided into two groups; the first one was used for bacterial experimental purposes and the second one for the determination of leaves characteristics. An empty bag without leaves was considered as control denoted CR.

2- Determination of leaves characteristics

A party of mangroves species leaves were used to determine leaves surface area. Graph paper was used to draw the outer shape of leaf and calculate the surface area in square meters as was reported in (Pandey and Singh 2011). Ohers characteristics were determined in the laboratory of environmental microbiology at Shantou University, Guangdong, China. Leaves water and wax contents were expressed as the percentage of fresh weight and determined as was described in (Waight et al. 2007). Briefly, to determine leaf water content, the leaves samples were weighed (4 g) and dried for 24 h at 105°C in an oven. Thereafter, the dried sample was cooled in a desiccator and weighed. The percentage of leaf water content was calculated by using the following equation (1). The same weight of sample (4 g) was weighted and used to extract wax content with 20 mL hexane in a microwave extractor. The GF/C filter was used to filter the extract into a round bottom drying flask. The total was pre-weighed before drying by rotary evaporator. After drying, the round-bottomed flask was reweighed and the percentage of wax was calculated by using the following equation (2). Nitrogen (N) and phosphorus (P) contents were analyzed by using respectively, Kjeldahl method and double digestion with H_2SO_4 and perchloric acid method.

$$\text{Leaf water content (\%)} = \frac{\text{fresh weight-dried weight}}{\text{sample weight}} * 100 \quad \text{eq (1)}$$

$$\text{Leaf} - \text{wax (\%)} = \frac{\text{reweight flask} - \text{preweight flash}}{\text{sample weight}} * 100 \quad \text{eq (2)}$$

3- Leaves phyllosphere bacteria extraction

In laboratory, the samples were used to extract phyllosphere bacteria in the leaves surfaces. Leaves were transferred in sterile 500 mL Erlenmeyer where was already added autoclaved water, to suspend the leaves phyllosphere bacteria extract. The sample was alternately manually shaken, four times in total. The leaves were then removed and the solution was used as the phyllosphere bacteria extract.

4- DNA Extraction and amplification

Total genomic DNA of the different sample was extracted using an Ultra-Clean Microbial DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA). Polymerase Chain Reaction (PCR) amplification of the 16S rRNA genes from the V3-V4 region of each sample was conducted by using the universal primers, 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') as was described in (Huang et al. 2014). The extracted DNA was sent to Sangon Biotec Institute (SBI) platform at Shanghai, China, to be sequenced. DNA concentrations and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

5- Computational analysis

The de-duplication and filter-qualification of the raw fastq files, sequences classification, annotation and beta diversity distance calculation were performed by using Quantitative Insights Into Microbial Ecology (QIIME Version 1.9). UPARSE software (version 7.0.1001) was used to group the filtered sequences OTUs clustered with a 97% similarity cutoff. At 97% of confidence threshold, the taxonomy of each 16S rRNA gene sequence was analyzed using 16S rRNA database and the RDP Classifier (version 2.11). Different functional genes composition of bacterial community was determined by using PICRUST.

6- Statistical Analysis

Data were subjected to statistical analysis of variance (ANOVA) in SPSS (20) software. Differences between

means and multiples stepwise were performed using the appropriate post-hoc with a 95% confidence level. ANOSIM was used to evaluate similarities among different experimental group. The Shannon index was calculated to describe α diversity and the richness of microbiota. Different graphs were performed by using SigmaPlot and Origin pro.

III. RESULTS

1- Leaves characteristics

Leaf area, water content, leaf wax content and nutrients such as nitrogen and phosphorus were determined in leaves of *R. mucronata* species and plotted on the Table 1. Statistical results of leaf area in different collection areas show no significant difference. However, although no difference was observed, the leaves collected from the plants submerged in seawater (S1, S2 and S3) had a slightly reduced surface area.

The leaves water content of this mangrove species was also measured. It was observed that the water content in the leaves of submerged plants (S1, S2 and S3) was significantly higher compared to that in the leaves of non-submerged plants (NS1, NS2 and NS3).

The leaves of the plants collected from the different plants showed a significant difference in wax content. Leaf-wax content in non-submerged plants (NS1, NS2, and NS3) was higher compared to that in leaves of plants from submerged ones. Therefore, the order of leaf-wax content was arranged as follows: Ci<S<NS.

The nitrogen content in the green leaves of different mangrove plants was also determined. The leaf N content of non-submerged plants (NS1, NS2, and NS3) was significantly higher than that of submerged plants (S1, S2, and S3). Additionally, considering the canopy cover, the leaves collected in the zone where the canopy was totally closed (C3), the N content was more considerable compared to that in the leaves in the other two zones (C1 and C2).

The phosphorus content of the leaves of the plants collected in the different zones was also measured. The P content in leaves of non-submerged mangrove plants (NS1, NS2, and NS3) was slightly higher than that of submerged plants. The order of P content in the leaves of different mangrove plants was as follows: Ci<S<NS.

Table.1: leaves characteristics including the surface, water, wax, nitrogen and phosphorus content

Group experimental	Leaves Surface (cm^2)	Water content (%)	Wax contents (%)	Nitrogen (mg/Kg)	Phosphorus (mg/Kg)
C1	24.76 ± 9.5	26.85 ± 3.8	17.36 ± 4.3	0.95 ± 0.3	0.08 ± 0.2
C2	28.04 ± 7.6	29.67 ± 2.4	14.75 ± 7.2	0.99 ± 1.2	0.07 ± 06
C3	29.54 ± 5.3	28.68 ± 2.7	16.45 ± 6.6	1.05 ± 1.2	0.05 ± 1.4
S1	27.66 ± 4.5	39.65 ± 4.2	17.56 ± 4.4	1.86 ± 0.4	0.11 ± 1.1
S2	25.25 ± 8.2	41.85 ± 7.2	15.65 ± 7.2	1.67 ± 2.6	0.10 ± 0.3
S3	24.77 ± 6.5	43.12 ± 7.4	16.46 ± 4.1	1.98 ± 1.5	0.13 ± 1.7
NS1	28.98 ± 6.4	27.76 ± 4.1	22.87 ± 5.6	2.98 ± 0.3	0.29 ± 0.6
NS2	27.46 ± 8.9	27.78 ± 8.5	22.98 ± 4.3	2.65 ± 0.3	0.19 ± 0.7
NS3	27.23 ± 8.7	26.75 ± 4.4	21.98 ± 4.5	2.34 ± 2.2	0.29 ± 0.7

Data are the mean of three replicate ± SD and were compared by Duncan's multiple range tests at $p < 0.05$.

2- Bacterial community in the leaves of the species *R. mucronata*

After sequencing the 16S rRNA genes, the number of OTUs identified in the different leaves of mangrove plants was significantly higher compared to those identified in the control (CR1 and CR2). In the different experimental groups, 105303, 110873, 124703, 146954 and 112225 OTUs were identified respectively in the leaves where the canopy was fully open (C1), semi-open/closed (C2), totally closed (C3), where the plants were submerged in seawater (S) and where the plants were out of the water (NS) (Table 2). The OTUs identified were different in the three different areas, taking into account the canopy cover. The results show that the more the canopy is closed, the more the number of OTU increases. On the other hand, considering seawater as a factor, the number of OTUs identified on the leaves of submerged plants was significantly higher than that identified in non-submerged plants. These results suggest that the number of OTUs in

the phyllosphere depends not only on water availability, but also on canopy cover. The richness estimated by the Shannon and Chao indices showed a slight difference in favor of the presence of seawater (S) and also at canopy closure (C3). However, no significant difference was noted when comparing the results from the leaves of submerged and non-submerged plants.

Table 2: Different bacterial OTUs and estimated bacterial abundance and diversity alpha indexes in different mangroves species leaves.

Experimental Group	Code bar	Seq_Num	Num- OTUs	Shannon index	Chao index
CR1	TCCGAC	45372	68563	2.06 ± 0.23	356.28 ± 36.33
CR2	AGCTAG	42387	59564	2.02 ± 0.24	346.14 ± 41.33
C1	CTGACG	61408	105303	2.18 ± 0.23	376.18 ± 46.33
C2	CACGAT	78803	110873	3.39 ± 0.67	411.14 ± 30.87
C3	CGCATA	89494	124703	3.48 ± 0.22	445.11 ± 28.42
S1	CGCCAT	91882	148373	4.39 ± 0.24	478.17 ± 32.86
S2	TCTATT	91075	147330	4.34 ± 0.26	467.16 ± 30.59
S3	AGGCGG	91930	145160	4.53 ± 0.45	483.15 ± 34.61
NS1	ATTGTG	73480	111830	3.13 ± 0.47	444.15 ± 37.38
NS2	TATCGA	74961	112982	3.62 ± 0.55	456.13 ± 41.18
NS3	GCCGCT	73712	111863	3.35 ± 0.74	444.15 ± 31.18

Data are the mean of three replicate ± SD and were compared by Duncan's multiple range tests at $p < 0.05$. Seq_Num is the quality number of samples reads and Num-OTUs is the 16S rRNA sequences OTUs obtained by sample clustering and normalized.

3- Correlation between different identified OTUs and different factors

Correlation test was performed to assess the possible relationship between different leaves characteristics (leaves surface area, leaves water content, leaf-wax, nitrogen and phosphorus content) and local abiotic environmental factor (canopy cover and seawater) with the abundance of bacteria in the different mangroves leaves (Figure 1). It was revealed that bacterial abundance moderately correlated to plant leaves surface area (Fig 1A, $r^2 = 0.053$). However it showed no correlation with plant leaves water content (Fig 1B, $r^2 = 0.1795$). The identified OTUs in all different mangroves leaves was found to positively correlated with leaf-wax, nitrogen and phospgorus content and the factor seawater (Fig 1C, $r^2 = 0.904$, Fig 1D, $r^2 = 0.72$, Fig 1E, $r^2 = 0.62$ and Fig 1F, $r^2 = 0.93$) respectively. On the other hand, the abundance of identified OTUs was negatively correleted with the factor canopy cover (Fig 3G, $r^2 = 0.93$).

IV. BACTERIAL COMPOSITION ON THE *R. MUCRONATA* LEAVES

4.1 Based on class level

The bacterial relative abundance of *R. mucronata* leaves was assessed at the class level (Figure 2). It was shown that in the three experimental groups (Ci, Si and NSi), Gammaprotebacteria was the most dominated class with more than 50% on average of the total bacteria identified. Betaprotebacteria and Bacilli are the next with 23% and 19% respectively. The relative abundance of Proteobacteria in general including Gamma, Alpha and Betaproteobacteria is far the highest with more than 78%. Compared to the control, apart from individuals belonging to the class of Gammaproteobacteria, most of the bacteria identified presented less than 1%.

4.2 Based on family level

The relative abundance of bacteria in *R. mucronata* leaves was further assessed at the family level (Figure 3). Enterobacteraceae were the most dominant bacterial family in the leaves of all the collected mangrove plants, whether they were in where the canopy was open (C1), semi-open (C2) or fully closed (C3), or where plants are submerged or not (Si or NSi). Rhodocyclaceae is the

second family identified on the leaves of the species *R. mucronata* with 21%.

4.3 Based on genus level

The relative abundance of bacteria in the leaves of *R. mucronata* was finally assessed at the genus level (Figure 4). The genus *Pantoa* was the most abundant in C3-3, S-1, S-3, N-S1, and NS-3 with more than 60% on average and less abundant or even almost absent in CR1, C1-1, C1-2 and C3-1. On the other hand, the genus *Metthyloversalitis* was rather more dominant in CR2, C1-1, C2-2, C3-1 and C3-2. In the experimental groups C1-3, C2-1 and C2-3, more than half of the bacteria belonging to the Enterobacteriaceae family are unclassified.

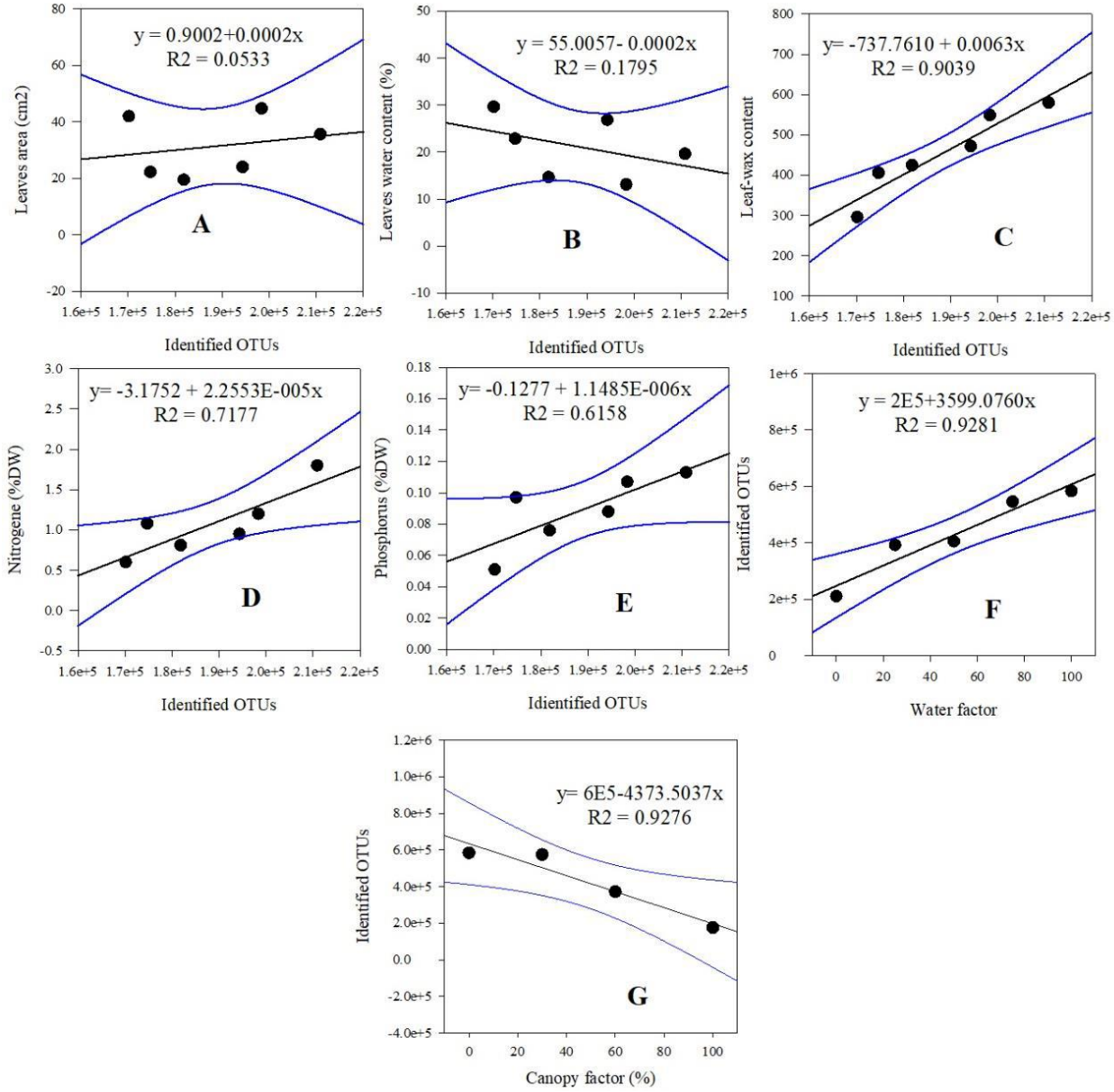

Fig.1 : Correlation between leaves areas (A), leaves water content (B), leaves wax (C), leaves nitrogen (D), phosphorus (E) and local abiotic factors (F and G) with bacterial identified OTUs. Bacterial identified OTUs moderately correlated to plant leaves surface area (r^2 = 0.053), showed no correlation with plant leaves water content (r^2 = 0.1795), positively correlated with leaf-wax, nitrogen and phospgorus content and the factor seawater (r^2 = 0.904, r^2 = 0.72, r^2 = 0.62 and r^2 = 0.93) respectively and negatively ($p < 0.05$, r2 = 0.93) with the canopy cover factor.

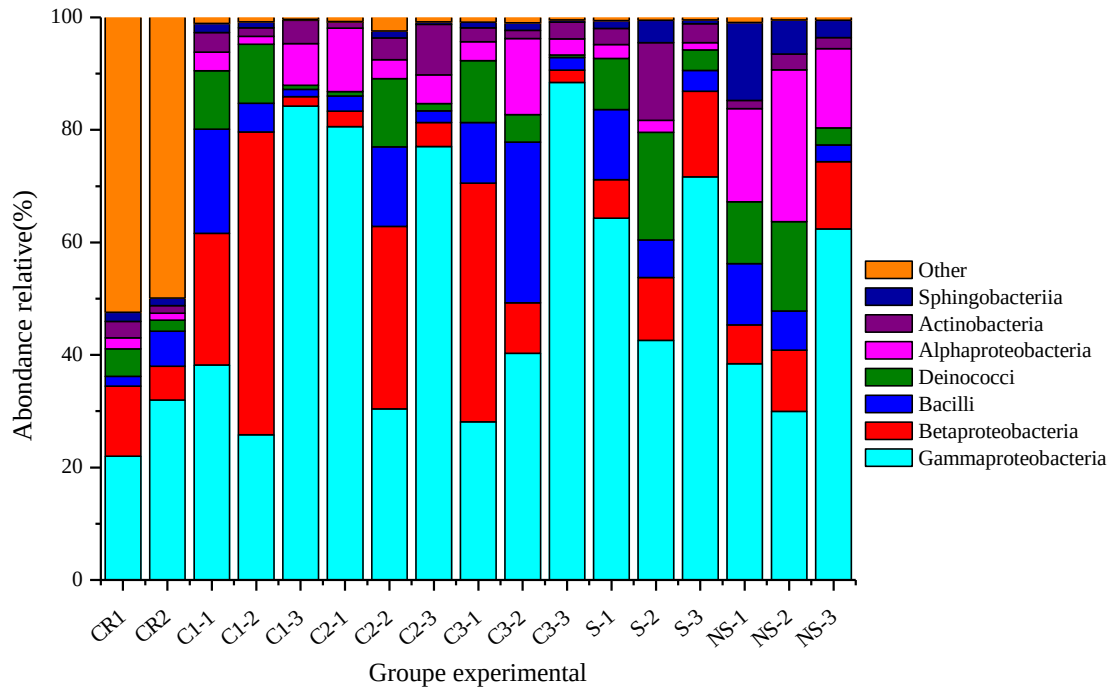

Fig.2 : Bacterial relative abundance at the class level. The horizontal and vertical axis represent respectively the name of each sample and the abundance ratio in three replications. Each color corresponds to the name of the class and at the same time indicates the abundance of the different classes

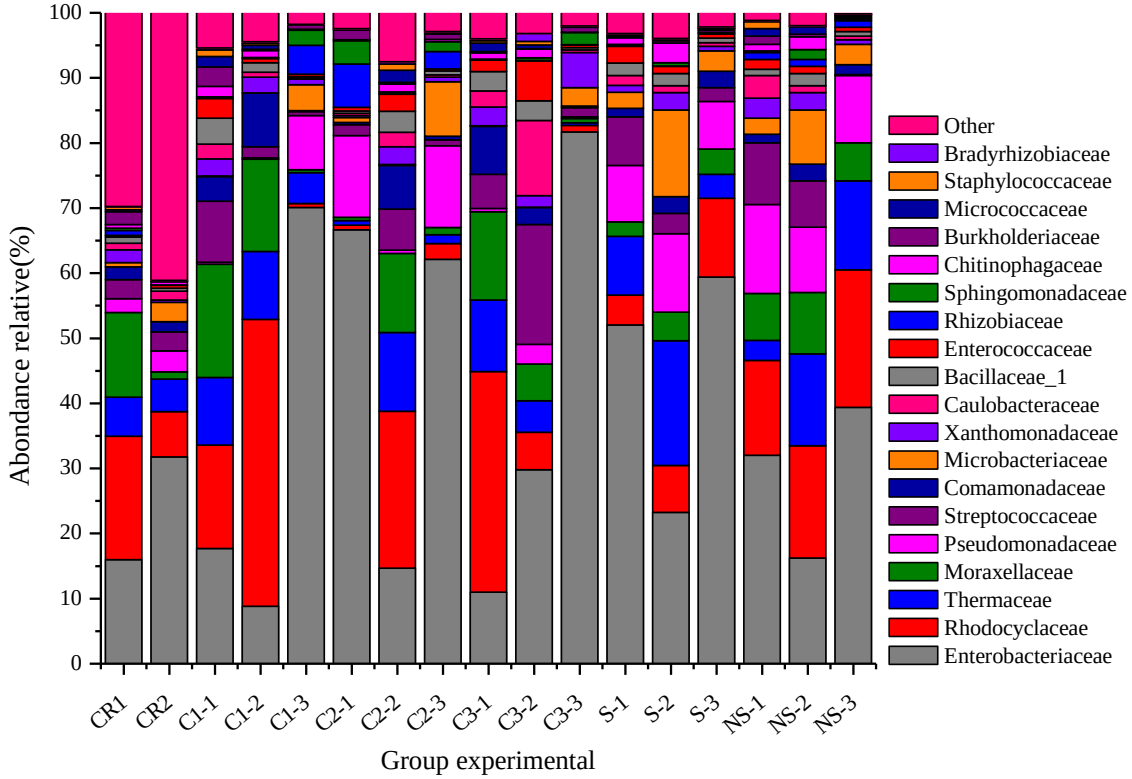

Fig.3: Bacterial relative abundance at the family level. The horizontal and vertical axis represent respectively the name of each sample and the abundance ratio in three replications. Each color corresponds to the name of the class and at the same time indicates the abundance of the different classes.

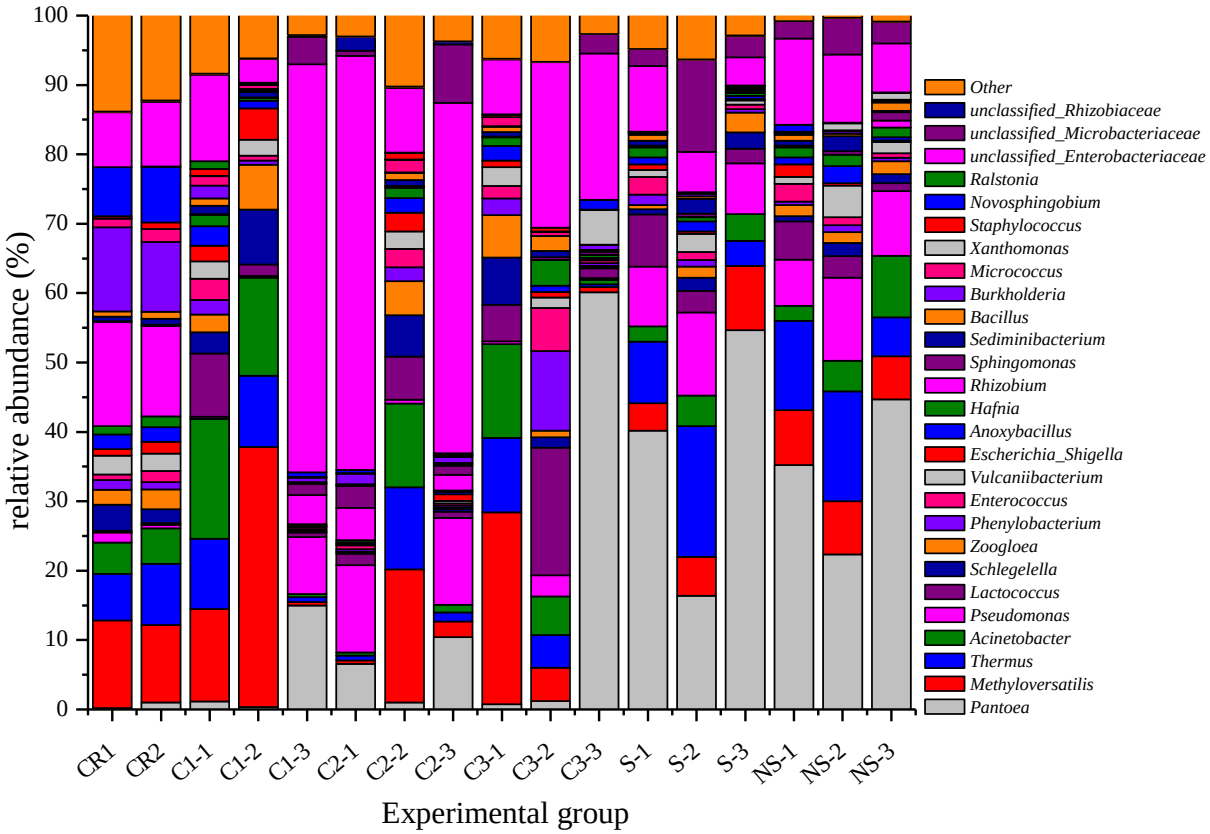

Fig.4 : Bacterial relative abundance at the genus level. The horizontal and vertical axis represent respectively the name of each sample and the abundance ratio in three replications. Each color corresponds to the name of the class and at the same time indicates the abundance of the different classes.

V. IMPACT OF LOCAL ABIOTIC FACTOR ON *R. MUCRONATA* LEAVES BACTERIA

5.1. Influence of canopy cover

The relative abundance of bacteria present on *R. mucronata* leaves was assessed at the family level, by considering the factor "canopy cover" (Figure 5). Among the different identified families, it was noted that the more the canopy cover was open, the lower was the relative abundance of bacteria. Enterobacteriaceae are the most dominant with more than 67% of all the identified bacteria in zone C. On the other hand, in comparison with the control, the relative abundance of bacteria in zone C1-1, C2-1 and C3-1 was lower. This supports the results that the relative abundance of bacteria is dependent on the state of the canopy cover.

5.2. Influence of seawater

The relative abundance of bacteria present on *R. mucronata* leaves was further evaluated at the family level by considering the factor "seawater" (Figure 6). In general, the results obtained in the different experimental groups (Si and NSi) are important compared to those found in the control. However, the variation in relative abundance of the different bacteria correlated with the factor seawater. The relative abundance of bacteria in leaves collected from submerged plants was significantly higher compared to that in the leaves collected from non-submerged plants. This again supports the fact that the relative abundance of bacteria was dependent on the water factor.

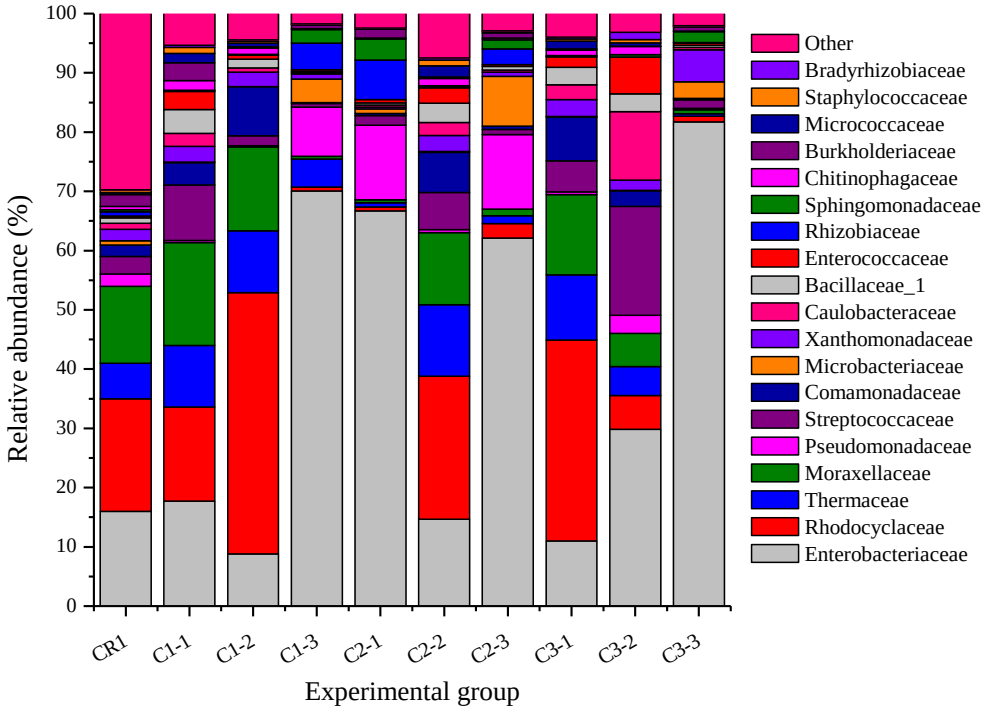

Fig.5: Relative bacterial abundance at the family level taking into account the factor canopy. The horizontal and vertical axis represent respectively the name of each sample and the abundance ratio in three repanditions. Each color corresponds to the name of the class and at the same time indicates the abundance of the different families.

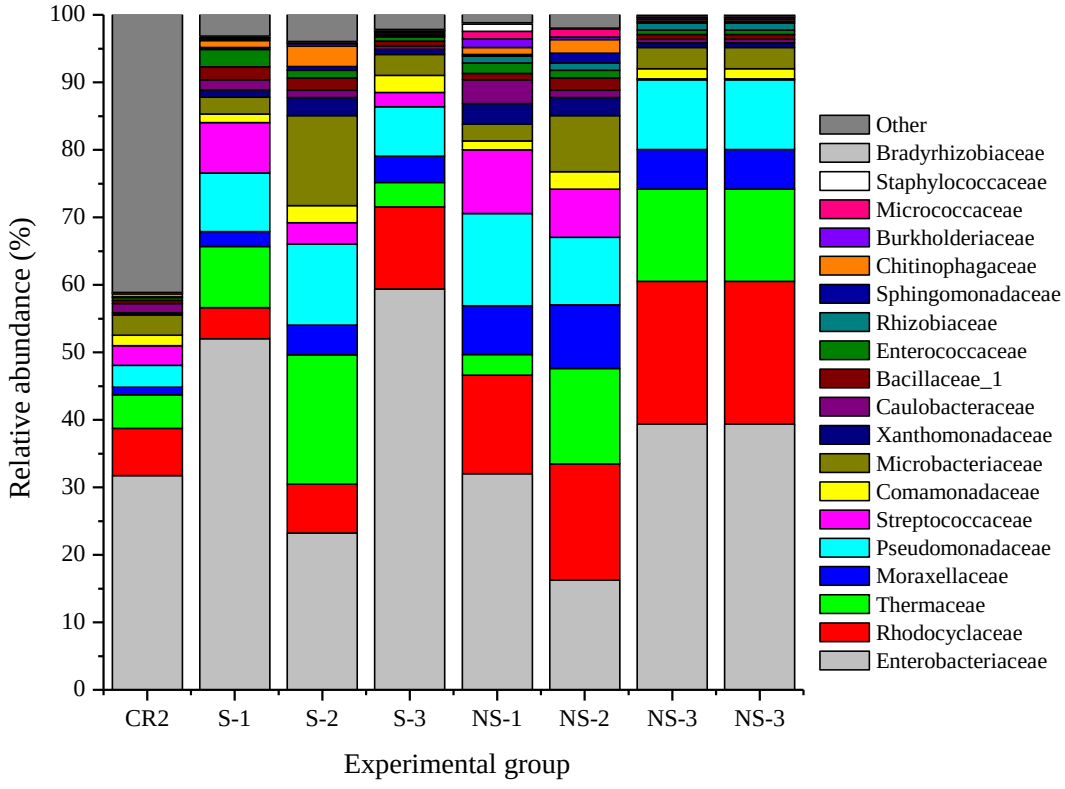

Fig.6: Bacterial relative abundance at the family level taking into account the seawater factor. The horizontal and vertical axis represent respectively the name of each sample and the abundance ratio in three repetitions. Each color corresponds to the name of the class and at the same time indicates the abundance of the different families.

VI. DISCUSSION

The wax content of the leaves has proven to be a critical parameter not only for the water conservation of the leaves, but also for the dynamism of the bacterial community phyllosphere of plant leaves. Studies have shown that, unlike the presence of trichomes (Reisberg et al. 2012), the composition of the cuticular wax influences the composition of bacterial communities in the phyllosphere (Reisberg et al. 2013; Bodenhausen et al. 2014). The leaf wax content in the different leaves of the species *R. mucronata* was different (Table 1). This result was consistent with that found by (Wang et al. 2008) which reported that the leaf wax content in *Rhizophora stylosa* was important. Leaf wax content was found to be positively correlated with OTUs identified. The leaves of plants, as organs is constantly exposed to pressures environmental, and exhibit several adaptive characteristics, such as the production of varieties of primary and secondary metabolites, among which are the constituents of the epicuticular wax that lines the leaf surface (Barthlott et al. 1998). These complex compounds consisting of long-chain aliphatic and cyclic components, including hydrocarbons, alcohols, aldehydes, flavonoids, etc., coat the outer surface of the epidermis of all the leaves of higher plants (Medina et al. 2006; Kunst and Samuels 2009) and plays an important role in the restriction of cuticular transpiration.

Water is an incontestable living factor for all organisms (animals, plants and microorganism). It absence could not only impact plant viability, but also affect the microbial community on plant leaves. Naturally, plants exposed to permanent water conditions exhibit low quantity of epicuticular wax than those in drought conditions (Oliveira et al. 2003; Cordeiro et al. 2011). This could explain the difference in the results observed among submerged and non-submerged mangrove species. The amount and distribution of water on the leaf is another highly dynamics of the foliar microclimate which greatly influences the development of micro organizations (Morris 2001). (Yadav et al. 2005) showed that leaf water content is the main factor in the abundance of phyllosphere bacteria in trees and shrubs Mediterranean, followed by leaf phosphorus content. The water content of the leaves of submerged mangroves was significantly higher than that of non-submerged mangroves (Table 1). Naturally, water in the leaves favors chemical reactions between the compounds dissolved in rain or dew water and those that escape from leaf. These reactions in turn have an effect on the microorganisms of the phyllosphere by altering water pH and nutrient availability (Morris 2001).

The results of the nitrogen and phosphorus content of the leaves were in agreement with that found by (Kembel and Mueller 2014) which reported that nutrients such as N and P influence bacterial community structure in tropical trees. Besides the nutrients on the mangroves leaves, it was found that the canopy cover was negatively correlated with the identified OTUs. Indeed, the greater was open the canopy cover, the lower the relative abundance of the identified bacteria was. Studies have shown that the environmental factors can alter the size and structure of communities in the phyllosphere in several ways, including environmental events such as rain (Vorholt 2012), the host plants and canopy cover (Khondoker et al. 2020). Studies by (Truchado et al. 2019; Aydogan et al. 2018) have shown as environmental factors such as temperature and solar radiation (prevent or promote canopy cover) have been implicated in the modification of the microbial community.

The correlation test revealed that the abundance of identified OTUs was not correlated with the surface of plant leaves. As a reminder, mangroves are halophilic plants i.e. that resist and thrive in saline conditions. The leaves of the mangroves are therefore known to secrete salt through salt glands located at their base. Studies on mangroves have shown a high accumulation of salt on their leaves, linked to the ability to the plant to resist salinity (Dias et al. 2012; Clough 1984). Although we know nothing about the effects of salt accumulation in the leaves on the microbial communities of the phyllosphere, due to salt exudation, it can be easily to imagine an impact negative on the whole microbial community. However, the non-correlation of the abundance of OTUs identified with leaf area suggested that the relationship between microorganisms and mangrove leaves could have another factor besides saline exudation and the adhesion surface which are considered to be contributors to the survival of the phyllosphere, as a means of dispersal (Grinberg et al. 2019).

VII. CONCLUSION

The present study showed that the leaves of the mangroves (*Rhizophora mucronata*) constitute a special environment capable of hosting a diverse bacterial community. The analysis of bacteria abundance and composition revealed that factors which influenced diversity and abundance of the different microbial taxa included the local abiotic environment to which the plant and its leaves are exposed, the nutrients and characteristics of the leaves. However, although much information has been obtained from individual studies on the plant microbiome, the present study suggest that meta-analyses controlling and others different methodologies are needed

to better understand the leaf-microbe associations of mangroves and whether they are suitable for particular beneficial effects.

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