Assessment of oil weathering and impact in mangrove ecosystem: PRISME Experiment.

Ronan Jézéquel*[1], Karine Duboscq[1], Léa Sylvi[2], Emma Michaud[3], Lise Millera Ferriz[2], Enora Roic[3], Robert Duran[4], Cristiana Cravo-laureau[4], Valérie Michotey[2], Patricia Bonin[2], Franck Gilbert[5], Marc Tedetti[2], Cécile Militon[2], Philippe Cuny[2]

[1] *CEDRE,* 715 rue Alain Colas – CS 41836, 29218 Brest cedex 2, France

[2] Aix Marseille Université, CNRS/INSU, Université de Toulon, IRD, Mediterranean Institute of Oceanography (MIO) UM 110, 13288, Marseille, France

[3] Laboratoire des Sciences de l'Environnement Marin (LEMAR), UMR 6539 (CNRS/UBO/IRD/Ifremer), Institut Universitaire Européen de la Mer, Technopôle Brest-Iroise, Rue Dumont d'Urville, 29280 Plouzané, France.

[4] Université de Pau et des Pays de l'Adour, UMR CNRS 5254 IPREM – EEM. 64013 Pau, FRANCE.

[5] Université Paul Sabatier, ECOLAB UMR 5245 CNRS-UPS-INPT. 31062 Toulouse. France.

Abstract 2017-410

Mangroves are among the most sensitive marine ecosystems to oil pollution due both to the sensitivity of mangroves species and to the high persistence of hydrocarbons in these environments. Despite their ecological and socio-economic value, the potential effects of an oil spill on French Guiana mangroves remain so far unknown. Yet, there is an increasing transboundary risk of oil spill due to Brazilian offshore oil exploitation (in mid-April 2013, there were 122 offshore drilling rigs in Brazil, including 29 under construction – Brazilian Amapá region located in the French Guiana border area is thought to become an important world oil production area in the coming years).

The aim of the PRISME project was to assess the natural degradation of oil in mangrove sediment as well as its impact on benthic communities (micro, meio and macrobenthos): a one-month in situ experiment was conducted in the young French Guianese mangrove (around 3 years old) at the mouth of the Sinnamary estuary. The experimental units consisted in eight plastic cores (Ø : 10 cm ; height: 30 cm) manually introduced within sediments. A thin layer of oiled sediment (2 cm, 20 000 ppm) was applied on four cores while the remaining four cores were considered as control (no oil addition). Three cores were additionally sampled at the beginning of the experiment as initial control sediments. After one month in situ, the eight cores were collected and sliced on site into different sedimentary layers aliquots for later analyses (hydrocarbons, bacterial, meio, macrofauna fauna diversity, bioturbation, biogeochemical parameters). Samples were sent to the different laboratories involved in this multidisciplinary project.

Results and knowledge gained from this experimental work were used to develop an approach for assessing coastal vulnerability for oil spills preparedness in mangroves.

INTRODUCTION

Mangrove forests, which grow at the interface between land and sea in the intertropical areas, play a fundamental role in the functioning of coastal ecosystems and provide ecosystem services (nurseries, coastal stabilization, natural attenuation, biogeochemical cycling) and socio - economic values (fishing, tourism, logging, landscape) estimated at 200 000 USD/ha per year (Costanza et al. 1997). Mangroves, considered "outstanding communities" by the French National Alliance for Environmental Research (AllEnvi), cover an area of about 698 square kilometers in French Guiana (FG) representing 70% of French mangroves (4th place in South

America). Mangroves of FG have, moreover, a specific dynamic compared to most other mangroves in the world. The Guiana coast is indeed a unique environment, characterized by a particularly active morpho- sedimentary dynamics which is under the direct influence of the massive suspended-sediment discharge of the Amazon river (754 106 tons yr− 1 ± 9%) (Anthony et al., 2010). This dynamic is accompanied by a heterogeneous remodeling of coastline characterized by marked deposition (formation of mud banks) but also of erosion phases affecting the coastline. It induces high and variable turbidity, also controlled by coastal and tidal currents. The advance of the Amazon freshwater plume and the freshwater inputs of Guiana rivers, coupled with coastal hydrodynamics and seasonal regimes also induce high variability of salinity of coastal waters. Because they can cope with the varying salinities and because of their fast-colonizing and fast-growing capacities, mangrove forests constitute the only adapted natural vegetal system suited to this unstable environment. Annually around ~300 million m^3 of sediment from the Amazon are transported by ocean currents forming mud banks that migrate along the coast from east to west toward the Orinoco River. Mangrove dynamics follow the one of mud banks. Mangroves quickly colonize the banks as soon as mud is sufficiently consolidated (Gratiot et al., 2007). Five successive steps in the development and maturation of the mangroves can be distinguished: mud bank above water level, mangrove pioneer (propagules settlement), young, mature, and senescent forest (Fromard et al., 1998).

Oil spills may represent a major threat to the mangrove communities with a sensitivity to oil pollution that may vary according to the development stage of mangrove. Previous studies carried out on different mangroves around the world have shown that the effects and persistence of oil contamination on these ecosystems is extremely variable. Depending on the oil characteristics but also on the physico-chemical characteristics of the coastal area and of the

mangrove communities, spilled oiled may be rapidly degraded (e.g. Burns et al., 2000; Farias et al., 2008) or, on the contrary, impact the mangrove communities for several years (e.g. Burns et al., 1993; Kingston, 2002, Baca et al., 2014). Whatever the case, due to the peculiarities of FG mangrove, from what we know so far, it is extremely difficult to predict the fate and effects that would occur following a major oil spill on the FG mangroves.

In the case of an oil spill, the young mangrove stage of development would be the first one to be impacted by oils slick coming ashore. The present paper describes the protocols used during a field experiment conducted in young mangroves of Sinnamary estuary (French Guiana). After one month in natural environment, oil-contaminated and non-contaminated samples of sediment were collected and sent to laboratories for analyses. The objectives were to:

- Assess the natural degradation of oil through chemical analyses;

- Study the response of microbial communities to oil pollution (diversity and abundance);

- Assess the effects of oil on macrofaunal biodiversity .

MATERIAL AND METHODS

Site location

A young mangroves forest dominated by *Avicennia germinans* located in the Sinnamary estuary and previously characterized by Aschenbroich et al. (2016), was selected for PRISME (Devenir d'une contamination PétRolIère dans les Sédiments de la Mangrove guyanaisE et son impact sur les communautés benthiques) experiment during the dry season. In case of an oil spill, this successional stage of mangrove colonization is supposed to be one of the first to be impacted by oil slick coming ashore.

Experimental Protocol

Experimental units used for the experiment consisted in eight plastic cores made of 30 cm length and 10 cm diameter PVC tubing. These PVC cores, left open at each end, were inserted in October 2015 into the natural sediment at two locations (four cores in each) separated by 10 meters and located under the shadow of mangrove trees (Figure 1): one for the incubation of the contaminated cores (HC+) and the other one for the incubation of control, uncontaminated cores (HC-). To simulate the oil spill, surface sediments were artificially contaminated. Two liters of surface sediment (0-2 cm depth) were collected close to the inserted cores in both locations. These sediments were manually mixed during 20 minutes with 70 mL of a light crude oil (API = 43) (20 000 ppm), previously topped at 200°C in order to simulate 12 hours of natural weathering. A volume of 12 mL of the contaminated mixture was transferred at the surface sediment of each of the four HC+ cores in the corresponding incubation location (Figure 2B). To quantify the macrofaunal reworking activity, 1 mL of a microsphere solution (diameter = 9,8 μm ± 0,553; Fluoresbrite[®] Fluorescent Microspheres, Polysciences Inc.) was added in each core over the 1.5 cm thick layer of the deposited mixture (Figure 2C). Finally, in order to stabilize the microspheres, a volume of 5 mL (corresponding to a sediment deposit layer of 0.5 cm thick) of the contaminated mixture was added at the surface of each HC+ core (Figure 2D). The uncontaminated, control cores (HC-), were prepared similarly but using an uncontaminated sediment mixture instead of the contaminated one. The PVC cores protruded about 2 cm above the sediment/water interface but did not induced observable trapping of detritus within the cores after one month of in situ incubation.

Figure 1. Photography of the control incubation area. The experimental site was located in a sheltered area colonized by a young mangrove forest of *A. germinans* trees of about 2 to 4 meters height.
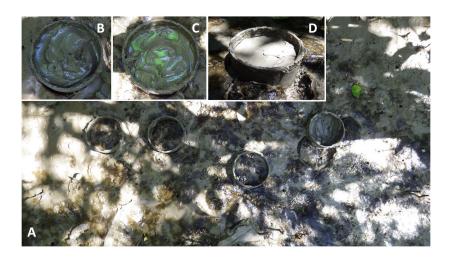

Figure 2. (A) Top view of the inserted cores in contaminated incubation area. Top view of a core after the sequential steps of addition of: (B) the contaminated mixture, (C) the microspheres and, (D) the microspheres' stabilization sediment layer.

Sediment sampling

After one month of experiment, cores were collected and immediately sliced on site into 0.5 cm layers for the first 2 cm, and then into 1 cm layers until the bottom of the core

(Figure 3A). From each of these layers, 3 mL of sediment subsamples were collected for the analysis of the depth distribution of the microspheres. Several composite subsamples of sediments of the 0 – 2 cm, 2 – 4 cm, 4 – 6 cm and >6 cm core horizons were furthermore sampled for the different analyses to be done:

- chemical analyses, 5g immediately frozen on site in dry ice box and stored later at -20°C in glass bottles in the laboratory until analysis;

- microbial analyses, 1.5 mL immediately frozen in liquid nitrogen, stored on site in a dry ice box , and then at -80°C in Eppendorf® tubes in laboratory until analysis;

- meiofauna analyses, 4 mL (Figure 3B) immediately fixed with 4% buffered formaldehyde (Figure 3C) and stored later at room temperature (25°C);

- macrofauna analyses: sediments remaining after the subsampling steps were fixed with 4% buffered formaldehyde (Figure 3C), and stored later at ambient temperature.

Figure 3. (A) Sampling of the sediment for the microbial analyses. (B) Sampling of the sediment for the analysis of the meiofauna. (C) Fixation with 4% buffered formaldehyde of the sediment samples for the benthic macrofauna and meiofauna analyses.

Samples Analyses

At the time this paper was written, the analyses of meiofauna and macrofaunal reworking activity were not completed and therefore the results are not discussed here.

Analyses of oil weathering

The sediment samples were split into three aliquots for moisture analyses (50°C for 24 hours), oil analyses and storage for complementary analyses, if needed. Oil was extracted from sediment samples using an ASE 350 (Accelerated Solvent Extraction, Dionex) (Table 1). Organic extracts were dried over Na_2SO_4 (activated at 400°C for 4 hours) and concentrated to approximately 2 mL using a Syncore (Büchi, Germany).

Table 1 Settings of the ASE 350.

Weight of sample (g)	2
Solvent	Methylene chloride
Temperature (°C)	100
Pressure (psi)	1700
Cycles	2
Heating (min)	5
Static (min)	5
Flush volume (%)	75
Purge (s)	60

Prior to GC/MS analyses, extracts were fractionated using a SPE (Solid Phase Extraction) cartridge (silica/cyanopropyl (SiO2/C3-CN; 1.0/0.5 g, 6 mL; Interchim, France) (Alzaga et al., 2004). Saturate and aromatic fractions were eluted simultaneously with 8 mL of methylene chloride/pentane (80:20, v/v) and concentrated to 2 mL.

Alkanes and aromatics were quantified using gas chromatography coupled with mass spectrometry (GC/MS). The GC/MS was an HP 6890N equipped with a split/splitless injector (Splitless time: 1 min, flow 50 mL/min) coupled to an HP 5973 Mass Selective Detector (MSD)

8

(Electronic Impact: 70 eV, voltage: 1200 V). The injector temperature was maintained at 300°C.

The interface temperature was 300°C. The GC/MS temperature gradient was from 50°C (1 min)

to 300°C (20 min) at 5°C/min. The carrier gas was Helium at a constant flow of 1 ml/min. The

capillary column used was an HP-5 MS: 30 m×0.25 mm ID×0.25 µm film thickness. *n*-Alkanes

and PAH semi-quantifications were performed using Single Ion Monitoring (SIM) mode with the

most representative fragment (saturates) or the molecular ion (PAHs) of each compound at a

minimum of 1.4 cycles/s. 17α(H),21β(H)-hopane (m/z=191) was used as a conserved internal

biomarker during analysis (Prince et al., 1994, Venosa et al., 1997). Total Petroleum

Hydrocarbon quantification was determined with chromatograms obtained in Scan mode.

Different concentrations of the crude oil used for the experiment diluted with methylene chloride

were used to calibrate the method and then to quantify the TPH extracted from the sediment

samples.

Microbial Community Analyses

Total DNA was extracted from sediment samples (0.25-0.30 g) of sediment using the

PowerSoil DNA isolation kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's

recommendations. DNA was eluted in 100 µL water, quantified using a Shimadzu BioSpec-nano

UV-Vis Spectrophotometer and stored at −20 °C until use. For the analysis of the microbial

diversity, PCR amplification was carried out using the primer set 515F (5'-GTG CCA GCM

GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3') for the V4

region of 16S rRNA gene. The 25 µL PCR mixture contained 1U of Pfu DNA polymerase, 0.4

µM of each primer, 0,4 mM of dNTP, 1U of Pfu DNA Polymerase (Promega), 1X Buffer with

$MgSO_4$ (Promega), and 2 µL of template DNA (dilution 1/10). The reactions were performed in

an iCycler thermocycler (Bio-Rad) under the following thermocycling steps : initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 20 s, annealing at 50°C for 20 s, and elongation at 72°C for 25 s, with a final extension at 72°C for 10 min. PCR products were visualized and quantified by agarose gel electrophoresis. Equal amounts of the PCR products were polled together and sequenced on an Illumina MiSeq plateform (Genotoul, Toulouse, France). Raw data were processed using QIIME v1.8 (Caporaso et al., 2010) according to the following workflow: (1) quality filtering step removing reads according to Bokulich et al. (2013) recommendations, (2) chimera checking using usearch61, (3) de novo Operational Taxonomic Units (OTUs) picking step using –m uclust parameter (Edgar, 2010) and similarity threshold 0.97; (4) assignation taxonomy step based on the Greengenes taxonomy and Greengenes reference database v13.8 (MacDonald et al., 2012) and (5) alpha diversity index (Chao1 richness estimator, Shannon diversity index), rarefaction curves and beta diversity calculation step. The coverage percentage was estimated by Good's method (Good, 1953). Multivariate analysis were performed using the function ordinate from the phyloseq package in RStudio (version 0.99.491 ; http://www.rstudio.com/).

The bacterial and archaeal abundance were mesured by the determination of bacterial and archaeal 16S rRNA gene copy numbers, respectively. Q-PCR of the 16S genes was performed using the GoTaq® qPCR Master Mix (Promega) and a CFX96 Real Time System (C1000 Thermal Cycler, Bio-Rad Laboratories, CA, USA). For the determination of the bacterial 16S rRNA gene copy numbers, the primer set 300F (5'- GCC TAC GGG AGG CAG CAG-3') and Univ516R (5'- GTD TTA CCG CGG CKG CTG RCA-3') was used. The real-time PCR cycles consisted of an initial denaturation at 98°C for 2 min, followed by 30 cycles of denaturation at 98°C for 2 min, followed by 35 cycles of denaturation at 98°C for 5 s, hybridization at 55 °C for

10 s and elongation step at 72°C for 12 s. For the determination of the archaeal 16S rRNA gene

copy numbers, the primer set 931F (5'- AGGAATTGGCGGGGGAGCA-3') and 1100R (5'-

YGGGTCTCGCTCGTTRCC-3') was used. The real-time PCR cycles consisted of an initial

denaturation at 98°C for 3 min, followed by 35 cycles of denaturation at 98°C for 10 s,

hybridization at 62 °C for 10 s and elongation at 72°C for 20 s. To check the specificity of the

system, melt curve analysis was performed at the end of each PCR run. Standard curves were

constructed with 4,4.108 to 4,4.102 copies of a pGEM-T Easy plasmid containing a

Pseudomonas stuzeri 16S rRNA insert (bacterial 16S rRNA gene) and 3,2.108 to 3,2.101 copies

of a pGEM-T Easy plasmid containing an euryarchaeote insert (archaeal 16S rRNA gene). The

resulting conditions lead to a Q-PCR efficiency higher than 99% ($R2=0.99$). 16S rRNA genes

abundances were standardized by the mass of DNA recovered per g dry sediment and log 10

transformed before statistical analysis performed with R software. The analysis of variance

(ANOVA) was performed in order to investigate the effects of treatments on bacterial and

archaeal abundances.

Macrofauna Analyses

        The fixed macrofauna samples were both sieved with a 250 µm and 500 µm mesh to

retain macroorganisms which were further preserved in a 70 % ethanol solution with rose bengal

solution until sorting and identification. The macrofauna was identified at the lowest practical

taxonomic level to estimate the taxa richness (number of taxa) with stereoscopic and optic

microscopes using key reference (Aschenbroich et al., 2017).

RESULTS

Oil weathering

Figure 4 compared the initial concentration of oil in the sediment to the TPH concentrations of both HC+ and HC- conditions according to depth. In the HC- cores, the TPH concentrations (0.72 ± 0.34 mg/g) are related to the presence of biogenic hydrocarbons naturally abundant in this type of environment. There was no change in the biogenic hydrocarbon concentrations with the depth. In the HC+ cores, initial TPH concentration was measured at 23.3 ± 2.83 mg/g immediately after introduction of oiled sediment. This result is close to the 20 000 ppm targeted and therefore, validate the protocol used for introducing the oiled sediment for this study. After one month in environment, TPH concentrations decreased in the surface layer (0 – 2 cm). This change can be attributed (i) to a natural removal of 59 % of oil from the sediment to seawater and (ii) to an oil migration in the sediment column: TPH concentration increased significantly in the 2 – 4 cm sediment layer from 0.72 mg/g to 8.4 ± 2.33 mg/g.
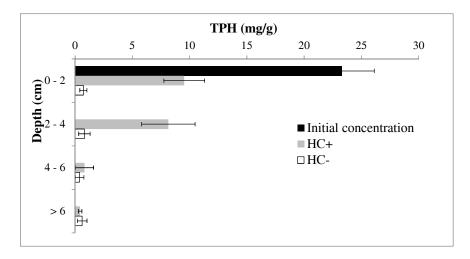

Figure 4. Means and standard deviation (*n*=3) of TPH concentrations (mg/g) in sediment column after one month of exposure for HC+ and HC – conditions according to depths (cm).

The degree of weathering was determined based on 17α(H),21β(H)-hopane (m/z=191) used a conservative internal marker within the oil. This compound has proven to be recalcitrant to natural degradation processes (evaporation, dissolution biodegradation or photo-oxidation) (Prince et al., 1994, Venosa et al., 1997)

Figures 5 and 6 show respectively the distributions of *n*-alkanes and PAHs relative to hopane (Hopane Unit) in the initial oil and in the oil extracted from samples of the first two layers of the sediment column (0-2 cm / 2-4 cm) after one month in natural environment. Both *n*-alkanes and PAHs distributions highlight that natural degradation occurred significantly during one month of exposure. n-C_{10} to n-C_{27} alkanes appeared highly degraded (90% after only 30 days) mainly by biodegradation as (i) the oil was topped at 200°C before sediment pollution and (ii) n-C_{17}/pristane and n-C_{18}/phytane ratios decreased significantly (Table 2).

PAHs distribution indicates a high degradation rate of 89% after (only) one month in natural environment. All the PAHs compounds appeared significantly degraded except for the heaviest ones (with more than 5 aromatic rings) well-known to be less sensitive to the biodegradation process.
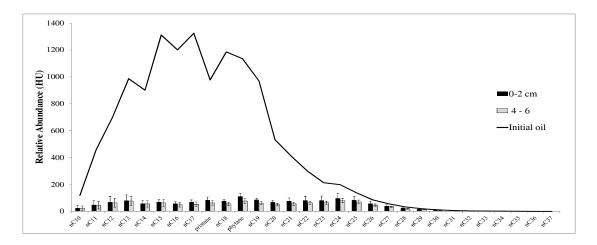
Figure 5. Means and standard deviation (*n*=3) of *n*-alkanes distributions (Hopane Unit) for the initial oil and oil extracts of sediment samples (0-2 cm and 2-4 cm depths) after one month of experiment.
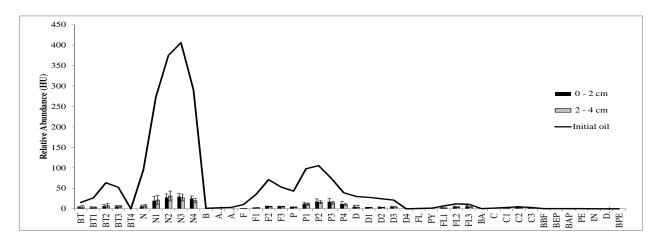

Figure 6. Means and standard deviation (*n*=3) of PAHs distributions (Hopane Unit) for the initial oil and oil extracts of sediment samples (0-2 cm and 2-4 cm depths) after one month of experiment.

Table 2. Results of n-C_{17}/pristane and n-C_{18}/phytane ratios for initial oil and oil extracts of sediment samples (0-2 cm and 2-4 cm depths) after one month of experiment.

	Initial oil	0-2 cm	2 - 4 cm
n-C_{17}/pristane	1.36 ± 0.06	0.83 ± 0.08	0.84 ± 0.09
n-C_{18}/phytane	1.05 ± 0.02	0.70 ± 0.05	0.72 ± 0.04

Oil effects on prokaryotic communities

In chronically oil-contaminated marine sediments, new inputs of oil generally induce a marked increase of the bacterial biomass (Head *et al.*, 2006; Miralles *et al.*, 2007; Stauffert *et al.*, 2013). In the sediments of the young mangrove studied, one month after the oil contamination, there is however no marked increase nor decrease of the total bacterial or archeal cell abundances. Indeed, Q-PCR estimation of the bacterial and archeal 16S rRNA gene copy numbers did not vary significantly between contaminated and uncontaminated sediments (p-value of 0.28 and 0.11, respectively). In the superficial sediment layer (0-2 cm) the 16S rRNA gene copy numbers per gram of wet sediment were about of 6.3×10^8 and 3.8×10^7 for bacteria and archaea, respectively. However, the addition of oil resulted in a microbial community with a different structure at the different sediment layers analyzed. In particular, as previously observed in Brazilian mangrove sediments contaminated with oil (Dos Santos *et al.*, 2011), the orders Oceanospirillales and Alteromonadales (class of the Gammaproteobacteria) increased. Interestingly, among these groups, there was an 8 to more than 25-fold increase of the number of sequences affiliated to the oil-degrading *Alcanivorax* and *Marinobacter* genera (Figure 7). Representatives of these genera are well-known actors of the biodegradation of oil hydrocarbons in marine contaminated sediments (Cuny *et al.*, 2011). They are probably involved in the efficient removal of oil hydrocarbons observed in this study.
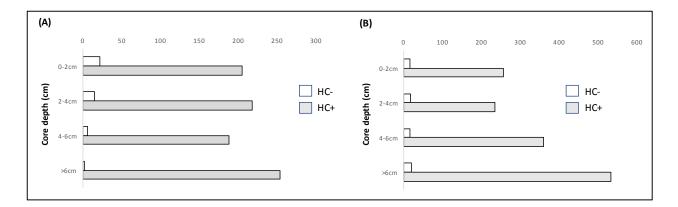
Figure 7. (A) Number of sequences affiliated to *Alcanivorax* genus. (B) Number of sequences affiliated to *Marinobacter* genus.

Interestingly, the highest numbers of sequences affiliated to these genera were observed at the deeper layers of the oil-contaminated sediments (4-6 cm and > 6 cm), where no oil hydrocarbons were detected. The development of the related hydrocarbonoclastic bacteria may be due to the vertical transfer of the more soluble fraction of oil, that most probably occurred at the beginning of the experimentation during the periodic tidal flushing of the sediment. These genera could thus be used as a sensitive proxy in monitoring oil contamination and biodegradation of mangrove sediments.

Macrofauna analyses

At the time this paper was written, the analyses of macrofaunal reworking activity weres still under progress and therefore not discussed here.

Hydrocarbon contamination had a major impact on the macrobenthic community of young mangrove sediments. Indeed, a mortality rate close to 90% was observed in the first two sedimentary horizons (0-2 and 2-4 cm) (Figure 8).
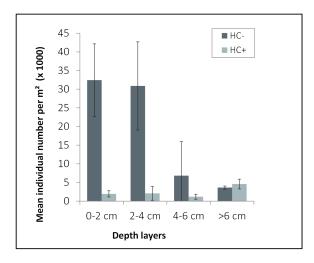
Figure 8. Mean individual density (Nb. m^{-2}) per depth layers (0-2 cm ; 2-4 cm ; 4-6 cm ; > 6 cm) for the uncontaminated (HC-) and oil-contaminated (HC+) treatments.

The phyla of annelids (capitellidae and oligochaete taxa) and nematodes appeared to be the most vulnerable relative to their abundance in the absence of hydrocarbons. On the other hand, other phyla increased their presence in the presence of petroleum (Foraminifera and an insect larva).

Although the species richness remains unchanged (10± 5 species), the composition of the community has undergone many changes (figure 9). This may have a strongly effect on the ecosystem functions. Thus, the disappearance of certain families of animals may be detrimental to the bioturbation activity, which is essential for sediment reworking as well as the mixing and transport of particles and solutes.
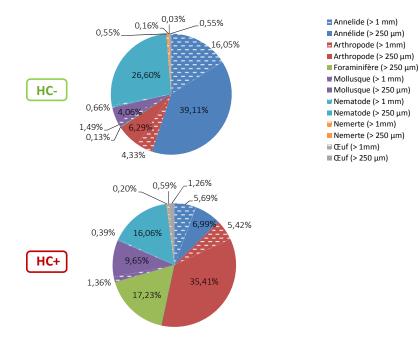
Figure 9. Relative abundance of phyla for the uncontaminated (HC-) and oil-contaminated (HC+) treatments

CONCLUSION

The PRISME project had two main objectives: first, conduct a field experiment in the French Guiana mangrove in order to assess the short term behavior and impact of oil in this sensitive ecosystem. The results of this field experiment highlighted a migration of oil in the sediment column which was confirmed by microbial strains analyses. Alkanes and PAHs appeared highly degraded after only one month of exposure. Microbial and chemical analyses confirmed respectively that hydrocarbonoclastic bacteria were present and active in this environment. Oil addition to the surface sediment (0-2 cm) had an impact on macrofauna community (density and diversity) in the first 6 cm of the sediment column.

The second main objective of the PRISME project was to gain knowledge and experience in order to prepare a major project which will include a larger-scale field

experiment. Among the perspectives discussed between the partners involved in PRISME project, the impact of oil on flora will be probably included in the final project: field site selected for PRISME project was characterized by the presence of a large number of young *A. germinans* trees and propagules which had the local characteristic of a very rapid growth (10-20 cm / month). This characteristic leads to a rapid change in the structure of this young mangrove forest and is one of the important lessons learned from the PRISME experiment. Special attention will therefore have to be paid to this specific characteristic of the French Guiana mangrove in order to set-up a successful long-term survey of the effects that oil contamination would have on this ecosystem. The behavior of oil within the mobile muds along the coast of this area certainly also deserves particular consideration for future experimental work.

ACKNOWLEDGEMENTS

REFERENCES

Alzaga, R., P. Montuori, L. Ortiz, J.M. Bayona, Albaiges, J. 2004. Fast Solid-phase Extraction-gas Chromatography-mass Spectrometry Procedure for Oil Fingerprinting. Application to the Prestige Oil Spill. Journal of Chromatography A, 1025 (1): 133-138.

Anthony, E.J., Gardel, A. Gratiot, N. Proisy, C. Allison, M.A. Dolique, F. Fromard, F., 2010.

The Amazon-influenced muddy coast of South America: A review of mud-bank–shoreline

interactions. Earth Science Reviews. 103, 99-121.

Aschenbroich, A., Michaud, E., Gilbert, F., Fromard, F., Alt, A., Le Garrec, V., Bihannic, I., De

Coninck, A., Thouzeau, G. 2017. Bioturbation functional roles associated with mangrove

development in French Guiana, South America. Hydrobiologia. doi 10.1007/s10750-017-3093-7.

Baca, B., E. Rosch, E.D. DeMicco, P.A. Schuler. 2014. TROPICS: 30-year Follow-up and

Analysis of Mangroves, Invertebrates, and Hydrocarbons. In: International Oil Spill Conference

Proceedings, Washington D.C. USA. 1, 1734-1748.

Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills, D. A. Caporaso,

J. G. 2012. Quality-filtering vastly improves diversity estimates from Illumina amplicon

sequencing. Nature Methods 10(1):57–9.

Burns, K.A., S. Codi, N.C. Duke. 2000. Gladstone, Australia Field Studies: Weathering and

Degradation of Hydrocarbons in Oiled Mangrove and Salt Marsh Sediments With and Without

the Application of an Experimental Bioremediation Protocol. Marine Pollution Bulletin 41:392-

402.

Burns, K.A., S.D. Garrity, S.C. Levings. 1993. How many years until mangrove ecosystems

recover from catastrophic oil spills?. Marine Pollution Bulletin 26:239–248.

Caporaso J.G., J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, et al.

2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods.

7(5):335–6. DOI: 10.1038/nmeth.f.303

Corsellis, Y., Krasovec, M., Sylvi, L., Cuny, P, Militon, C. 2016. Oil removal and effects of spilled oil on active microbial communities in close to salt-saturation brines. Extremophiles. 20(3):235–50.

Costanza, R., R. d'Arge, R. de Groot, S. Farberk, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R.V. O'Neill, J. Paruelo, R.G. Raskin, P. Suttonk, M. Van den Belt. 1997. The value of the world's ecosystem services and natural capital. Nature 387:253-260.

Cuny, P., Cravo-Laureau, C., Grossi, V., Gilbert, F., Militon, C. 2011. Biodegradation of hydrocarbons in bioturbated marine sediments. In: Microbial bioremediation of non-metals: Current Research. 55–92.

Dos Santos H. F., Cury J. C., Do Carmo F. L., Dos Santos A. L., Tiedje J., Van Elsas J. D., et al. 2011. Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: bacterial proxies for oil pollution. PLoS ONE 6:e16943 10.1371/journal.pone.0016943, 2011.

Edgar, R.C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26(19), 2460-2461.

Farias, C.O., C. Hamacher, A.L.R. Wagener, A.L. Scofield. 2008. Origin and degradation of hydrocarbons in mangrove sediments (Rio de Janeiro, Brazil) contaminated by an oil spill. Organic chemistry. 39(3):289-307.

Ferrando, A., E. Gonzalez, M. Franco, M. Commendatore, M. Nievas, C. Militon, G. Stora, F. Gilbert, J.L. Esteves, P. Cuny. 2015. Oil spill effects on macrofaunal communities and bioturbation of pristine marine sediments (Caleta Valdés, Patagonia, Argentina): experimental evidence of low resistance capacities of benthic systems without history of pollution. Environmental Science and Pollution Research. 22: 15294-15306.

Fromard, F., H. Puig, E. Mougin, G. Marty, J.L. Betoulle, L. Cadamuro. 1998. Structure, above-ground biomass and dynamics of mangrove ecosystems: new data from French Guiana. Oecologia, 115: 39–53.

Gilbert, F., G. Stora, P. Cuny. 2015. Functional response of an adapted subtidal macrobenthic community to an oil spill: macrobenthic structure and bioturbation activity over time throughout an 18-month field experiment. Environmental Science and Pollution Research 22: 15285-15293.

Good I.J. 1953. The population frequencies of species and the estimation of population parameters. Biometrika. 40(3-4):237–64.

Gratiot, N., A. Gardel, E.J. Anthony. 2007. Trade-wind waves and mud dynamics on the French Guiana coast, South America: input from ERA-40 wave data and field investigations. Marine Geology. 236, 15-26.

Head, I. M., Jones, D. M., Röling, F. M. 2006. Marine microorganisms make a meal of oil. Nature Reviews Microbiology 4, 173-182.

Kingston, P.F. 2002. Long-term Environmental Impact of Oil Spills. Spill Science & Technology Bulletin 7: 53–61.

MacDonald C, D.K. Stringer, R.C Piper. 2012. Sna3 is an Rsp5 adaptor protein that relies on ubiquitination for its MVB sorting. Traffic. 13(4):586–98.

Miralles G., Nérini D., Manté C., Acquaviva M., Doumenq P., Michotey V., et al 2007. Effects of spilled oil on bacterial communities of Mediterranean coastal anoxic sediments chronically subjected to oil hydrocarbon contamination. Microb. Ecol. 54 646–661.

Prince, R.C., D.L., Elmendorf, J.R. Lute, C.S. Hsu, C.E. Haith, J.D. Senius, G.J. Dechert, G.S. Douglas and E.L. Butler. 1994. 17 α (H),21β (H)-hopane as a Conserved Internal Marker for

Estimating the Biodegradation of Crude Oil. Environmental Science and Technology. 28: 142-145.

Quintana C.O., M. Tang, E. Kristensen. 2007. Simultaneous study of particle reworking, irrigation transport and reaction rates in sediment bioturbated by the polychaetes Heteromastus and Marenzelleria. Journal of Experimental Marine Biology and Ecology 352:392-406.

Stauffert M, C. Cravo-Laureau, R. Jézéquel, S. Barantal, P. Cuny, F. Gilbert, C. Cagnon, C. Militon, D. Amouroux, F. Mahdaoui, B. Bouyssiere, G. Stora, F. Merlin, R. Duran. 2013. Impact of Oil on Bacterial Community Structure in Bioturbated Sediments. Gilbert JA, editor. PLoS ONE. 10;8(6).

Venosa, A.D., M.T. Suidan, D. King, and B. A. Wrenn. 1997. Use of hopane as a conservative biomarker for monitoring the bioremediation effectiveness of crude oil contaminating a sandy beach. Journal of Industrial Microbiology and Biotechnology. 18. 131-139.