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Spatio-temporal variations in the composition of organic matter in surface sediments of a mangrove receiving shrimp farm effluents (New Caledonia)

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HIGHLIGHTS

- Fatty acid 18:1ω9 is a relevant marker to monitor effluent pathway in the mangrove.
- OM nature and distribution at sediment surface varied in relation to farm activity.
- Enhancement of litter-decomposer biomass and activity stimulates litter degradation.
- Diatoms dominate the microalgae community under effluent runoff conditions.
- Chl-*a* concentrations suggest permanent effect of effluent on primary production.

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ABSTRACT

In order to investigate spatio-temporal variations in the composition and origin of the benthic organic matter (OM) at the sediment surface in mangrove receiving shrimp farm effluents, fatty acid (FA) biomarkers, natural stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), C:N ratios and chlorophyll-*a* (chl-*a*) concentrations were determined during the active and the non-active period of the farm. Fatty acid compositions in surface sediments within the mangrove forest indicated that organic matter inputs varied along the year as a result of farm activity. Effluents were the source of fresh particulate organic matter for the mangrove, as evidenced by the unsaturated fatty acid (UFA) distribution. The anthropogenic MUFA 18:1ω9 was not only accumulated at the sediment surface in some parts of the mangrove, but was also exported to the seafront. Direct release of bacteria and enhanced in situ production of fungi, as revealed by specific FAs, stimulated mangrove litter decomposition under effluent runoff condition. Also, microalgae released from ponds contributed to maintain high benthic chl-*a* concentrations in mangrove sediments in winter and to a shift in microphytobenthic community assemblage. Primary production was high whether the farm released effluent or not which questioned the temporary effect of shrimp farm effluent on benthic microalgae dynamic. This study outlined that mangrove benthic organic matter was qualitatively and quantitatively affected by shrimp farm effluent release and that responses to environmental condition changes likely depended on mangrove stand characteristics.

1. Introduction

Mangroves develop along more than 70% of tropical and subtropical coastlines (Spalding, 1997; Giri et al., 2010). These ecosystems are among the most productive ecosystems on earth (Alongi and de Carvalho, 2008; Bouillon et al., 2008; Alongi, 2011) but are increasingly threatened by the development of aquaculture and especially shrimp farming (Alongi, 2002; Duke et al., 2007; Polidoro et al., 2010). In South America and South East Asia, shrimp farms have been developed at the expense of mangrove forests, which are destroyed for the establishment of the rearing ponds (Menasveta, 1997). In addition to the direct loss of mangroves during construction, shrimp farms also impact the adjacent mangroves through the release of large quantities of effluents rich in particulate and dissolved organic and inorganic nutrients (Paez-Osuna, 2001; Jackson et al., 2003). In New Caledonia shrimp farm ponds are built on the unvegetated upper part of the intertidal

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zone (i.e. salt-flat areas). Ponds are continuously irrigated in order to maintain water column oxygenation, with water that is pumped directly from the lagoon at a rate increasing from 5 to 30% of the pond volume over the course of the rearing cycle. Excess water, enriched in nutrients and in particulate and dissolved inorganic and organic matter, overflows the ponds and is discharged into the adjacent mangrove forests (Thomas et al., 2010; Molnar et al., 2013). Mangroves, more specifically *Rhizophora* sp. stands, have demonstrated high nutrient assimilation capacity (Robertson and Phillips, 1995). Mangroves are thereby used as so-called "natural biofilter" in order to avoid impacts on the surrounding World Heritage listed lagoon (Lemonnier and Faninoz, 2006; Thomas et al., 2010). In this context, most studies have investigated to date the effect of untreated shrimp farm effluents on water column processes. Effluents temporarily affect water quality (Trott and Alongi, 2000; Thomas et al., 2010) by increasing chl-*a* and nutrient concentrations (Costanzo et al., 2004; Thomas et al., 2010) and by enhancing primary and bacterial productions (McKinnon et al., 2002a; Burford et al., 2003). Also, the trophic fate of effluents has been investigated (McKinnon et al., 2002b). In contrast, much less is known concerning the effects of shrimp farm effluents on the mangrove benthic compartment. A recent study (Molnar et al., 2013) demonstrated direct impacts of shrimp farm effluents on benthic metabolism and N-cycling processes in sediments from the same shrimp farm as the one studied herein. Increase in nutrient regeneration rates recorded in aquaculture-impacted mangroves (Christensen et al., 2003; Nizzoli et al., 2006; Molnar et al., 2013) is hypothesized to be associated to stimulated bacterial and phytoplankton biomass productions (Molnar et al., 2013); but the development of these microbial communities, and their contribution to the OM pool remain to be identify in such polluted environments.

Fatty acid biomarkers successfully detail the organic matter composition (Parrish et al., 2000) in that they allow to distinguish bacteria from fungi biomass (Frostegard and Baath, 1996), allochthonous from autochthonous particulate OM (e.g. Xu and Jaffe, 2007); they are also a good indicator of microalgae biomass (Meziane et al., 1997, 2006; Hu et al., 2006). In complement to fatty acids, stable isotopes enable to confirm relative contributions of multiple organic matter sources to the bulk OM pool of surface sediments (Dunn et al., 2008; Volkman et al., 2008). Such indicators, together with chlorophyll-*a* were used in order to test the following hypothesis: (1) particulate organic material originated from shrimp farm effluents is redistributed over the mangrove area under effluent runoff and tidal influence, (2) OM composition of mangrove surface sediments exhibits quantitative and qualitative temporal variations, i.e. effluents are expected to induce an enrichment in labile organic material, and notably in bacterial and micro-algal biomass, in mangrove surface sediments, (3) OM changes are expected to be dependent on mangrove stand characteristics.

The main objectives of this study were to i) understand how releasing shrimp farm effluents in a mangrove are related to OM composition modifications at mangrove sediment surface, ii) to identify the spatio-temporal evolution of OM at sediment surface regarding mangrove stands, season, or farm activity.

2. Material and methods

2.1. Study site

The study was conducted in a mangrove located on the west coast of New Caledonia (21°56′S 166°04′E, in Saint Vincent Bay; Fig. 1), which covers 28.9 ha and receives shrimp farm effluents from the "Ferme Aquacole de la Ouenghi". The west coast of the main island of New Caledonia is characterized by a semi-arid tropical climate. The smallest thermal amplitude and the highest temperatures occur from December to February (i.e. in summer); and the largest thermal amplitude and the lowest temperatures occur from July to September (i.e. in winter). Tropical depressions can occur during summer, which is thus also the wet season. From the lower to the upper tidal zone, vegetation of the studied mangrove forest is mainly composed of a succession of *Rhizophora stylosa* (ca. 80% of the mangrove area), *Avicennia marina*, and a salt-flat occupied sporadically by *Sarcocornia quinqueflora* (Fig. 1), which is the typical mangrove zonation in New Caledonia (Marchand et al., 2011a). The adjacent semi-intensive farm is composed of two 1 m deep rearing ponds (L and K, Fig. 1), which are stocked with the blue shrimp *Litopenaeus stylirostris*. Shrimps in ponds are fed with locally produced feed pellets (35–40% protein, SICA©, NC) daily added throughout the rearing period, with inputs increasing (from ~0.25 to ~3.5 kg ha^{-1} d^{-1}; Farm manager, pers. comm) as shrimps grow. Shrimp farm activity is launched in January for ~8 months after which farm proceeds a ~4 month break (August–November) to drain and dry the ponds (Della Patrona and Brun, 2009). Consequently, the rearing starts during the middle of the summer, and finishes during the middle of the winter. During the active period of the shrimp farm daily effluent water discharges can reach up to 30% of pond volumes (Della Patrona and Brun, 2009) i.e. up to 54,000 $m^3 \cdot d^{-1}$ can be released into the mangrove. Part of the effluent waters is diverted around the salt-flat area in little sandy-made channels and released towards the natural mangrove channel ('D' outlet; Fig. 1); whereas another part is directly released onto the sediment of the *A. marina* stand ('E' and 'W' outlets; Fig. 1) and flowed undiluted across upper tidal flats. During high tides seawater from the adjacent lagoon reaches the upper part of the *A. marina* stand.

2.2. Field sampling

Surface sediments of the Ouenghi mangrove were collected at the same sites during a non-active period (i.e. drying of ponds in December 2009) and during an active period (July 2010) of the adjacent shrimp farm. At the active-period sampling time, due to a shortage of shrimp larvae, only the smaller pond (7.5 ha; pond K) was in production. Mangrove forest was randomly sub-divided into 51 sampling sites (Fig. 1) using a hand-held GPS receiver (Colorado 300, Garmin) for geographical coordinate's registrations. Because of the high density of trees and aerial roots, several sites were not accessible and the use of a systematic sampling approach (Caeiro et al., 2003) was therefore not possible. For fatty acid and stable isotope analysis, surface sediments were sampled in triplicates during the active period; during the non-active period, due to logistical problems, only one replicate was gathered; one replicate corresponded to the pooling of five sediment cores of 1 cm depth × 2 cm Ø. For chl-*a* analysis, four replicates were collected at each sampling period; each replicate corresponds to the pooling of four 1 cm-depth × 2 cm-Ø sediment cores. Additionally, in order to qualify the particulate organic matter composition of effluents, water was collected in sampling bottles and filtered on Glass-Fiber Filters (GF/F) within 10 min that followed. Food pellets used for shrimp nutrition were grounded to assess their fatty acid composition. All samples were swiftly transported to the laboratory, freeze-dried and stored at −20 °C until analyses.

2.3. Chlorophyll-a analysis

Chl-*a* was extracted from freeze-dried sediment by using a methanol 93% solution and its concentrations determined according to Yentsch and Menzel (1963) using a Turner Designs TD700 fluorimeter equipped with an optical kit no. 7000-961 including an excitation filter of 340–500 nm wavelength, and an emission filter up to 665 nm wavelength. Chl-*a* in methanol was then excited at 450 nm and fluorescence emission was measured at 664 nm.

2.4. Fatty acid analysis

Fatty acids were extracted following the method of Bligh and Dyer (1959) slightly modified as in Meziane et al. (2007). FAs were then separated and quantified by way of gas chromatography (GC; Varian CP-3800 equipped with flame ionization detector). Separation was

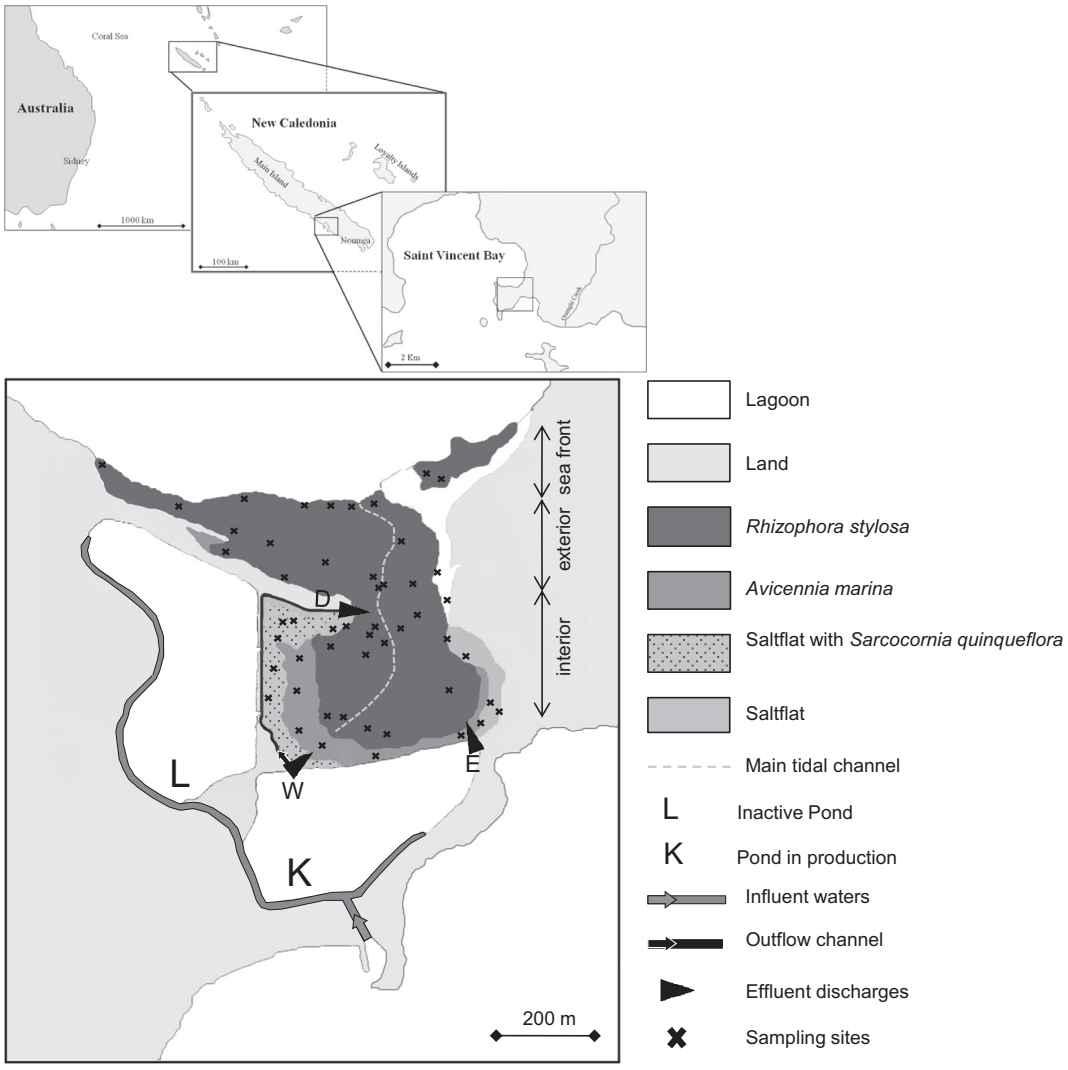

Fig. 1. Map showing the location of the Ouenghi mangrove in Saint Vincent Bay on the west coast of New Caledonia; showing the effluent outlets: at the west (W) and east (E) side of the K pond, and wastewaters flowing from the dyke outlet (D); and mangrove stand succession: salt-flat, *Avicennia marina* stand, *Rhizophora stylosa* stands. The locations of sampling sites are symbolized by the crosses.

performed using a Supelco OMEGAWAX 320 column (30 m × 0.32 mm i.d., 0.25 μm film thickness) with H_2 as carrier gas. After injection of 1 μl of sample at 60 °C, the temperature was raised to 150 °C at 40 °C min^{-1}, then to 240 °C (held 14 min) at 3 °C min^{-1}. Most FA peaks were identified by comparing their retention times with those of authentic standards (Supelco Inc., Bellefonte, PA, USA). For some samples, peaks of FAs were confirmed with GC–Mass Spectrometry (GC–MS; Varian 220). FAs are designated as *X*:*YωZ*, where *X* is the number of carbons, *Y* is the number of double bonds and *Z* is the position of the ultimate double bond from the terminal methyl. The concentration of each FA (C_{FA} $mg_{of\ FA}/g_{of\ dry\ weight}$) was calculated according to Schomburg (1987):

$$C_{FA} = A_S/A_{IS} \times C_{IS}/W_S$$

where A_S is the peak area of the FA, A_{IS} is the peak area of the internal standard (FA 23:0), C_{IS} is the concentration of the internal standard (mg) and W_S is the dry weight of sample (g).

2.5. Stable isotope analysis

Sediment for isotopic ratio analysis ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) were dried, grounded and pre-treated by adding a weak HCL solution (1 N) in order to remove carbonate from samples (Schink et al., 1979) while minimizing effects on isotopic composition (Kennedy et al., 2005). The analysis was performed on samples from 32 selected stations at the UC Davis Stable Isotope Facility (Department of Plant Sciences, University of California at Davis, Davis, California) using a Europe Hydra 20/20 mass spectrometer equipped with a continuous flow IRM device and are reported in standard delta notation ($\delta^{13}C$ or $\delta^{15}N$), defined as parts per thousand (‰) deviation from a standard (Vienna Peedee belemnite for C and atmospheric N_2 for N): $\delta^{13}C$ or $\delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1000$ (Peterson and Fry, 1987). The analytical precision (standard deviation for repeated measurements of the internal standards) for the measurement was 0.06‰ and 0.13‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.6. Statistical treatments

2.6.1. Multivariate analysis

Homogeneous groups of station and their temporal variations were displayed by n-MDS analysis (PRIMER ® 6; Clarke, 1993) applied to a similarity matrix based on Bray–Curtis dissimilarity coefficient (Bray and Curtis, 1957). Differences in FA composition among factors were tested using one-way analysis of similarity (ANOSIM) and the statistic test was computed after 5000 permutations. No transformation was applied to the data and factors used for analysis were the sampling period

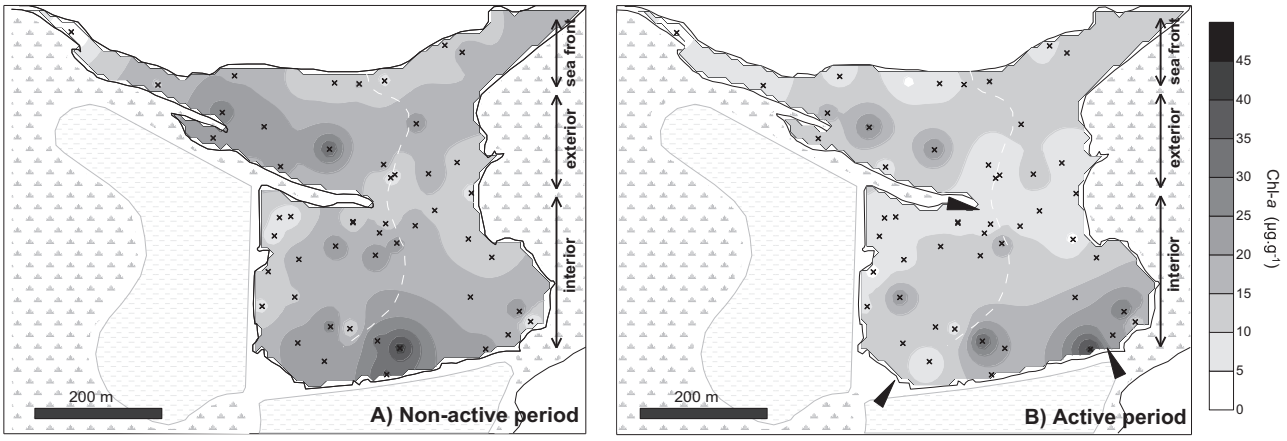

Fig. 2. Concentrations of chlorophyll-*a* (chl-*a*; $\mu g \cdot g^{-1}$) in surface sediments recorded during the non-active (A) and the active period (B) of the adjacent shrimp farm. Arrows represent effluent inputs from ponds (polygon filled with dotted lines). Dotted line represents the main tidal channel.

(i.e. non-active period vs. active period of the farm) and the vegetation stands according to their tidal position (i.e. the salt-flat, *Avicennia* and *Avicennia–Rhizophora* mixed stands as well as the interior, exterior and seafront *Rhizophora* stands). When differences in FA composition were detected between factors, similarity of percentage tests (SIMPER) were used to highlight which FAs conducted the observed differences.

2.6.2. Univariate analysis

Differences in concentrations of selected FAs and chl-*a* between sampling times and vegetation stands were tested using analysis of variance (R® software). Prior to ANOVA, chl-*a* data were log $(x + 1)$ transformed and all data were tested for homoscedasticity (Bartlett test) and normal distribution (Shapiro–Wilk). Tukey's HSD post-hoc tests were then used to determine differences between groups. C/N, $\delta^{13}C$ and $\delta^{15}N$ data were tested using a non-parametric test (Kruskal–Wallis test) and multiple comparisons between groups were assessed by pairwise Wilcoxon tests. For all tests the type I error (risk α) was set at 0.05.

2.7. Contour map representation

Surface maps were used to illustrate spatial variation of chl-*a*, selected or summed FAs, C/N ratio, $\delta^{13}C$ and $\delta^{15}N$, using Surfer (Golden Software Inc. 2002, version 8). The inverse distance to a power algorithm was employed as the interpolation method with a second degree power.

3. Results

3.1. Chlorophyll-a

Chl-*a* concentrations in mangrove surface sediments ranged from 1.72 ± 0.2 $\mu g \cdot g^{-1}$ dw to 42.25 ± 9.3 $\mu g \cdot g^{-1}$ dw during the non-active period, and from 2.92 ± 1.62 $\mu g \cdot g^{-1}$ dw to 41.56 ± 9.71 $\mu g \cdot g^{-1}$ dw during AP (Fig. 2). Concentrations slightly decreased from the non-active (16.15 ± 9.15 $\mu g \cdot g^{-1}$ dw av.) to the active period (12.06 ± 9.5 $\mu g \cdot g^{-1}$ dw av.) of the farm (one-way ANOVA; $F = 32.25$, $p < 0.001$). However, spatial pattern remained comparable between periods. Chl-*a* exhibited higher concentrations at the interior of the bay than at the exterior; the highest values were recorded close to the K pond whereas the lowest were recorded in the center of the bay within the *Rhizophora* stand.

3.2. Fatty acids

Identified FAs included saturated fatty acids (SAFAs; ≥11:0–30:0) and unsaturated fatty acids (UFAs). SAFAs were composed of short-chain fatty

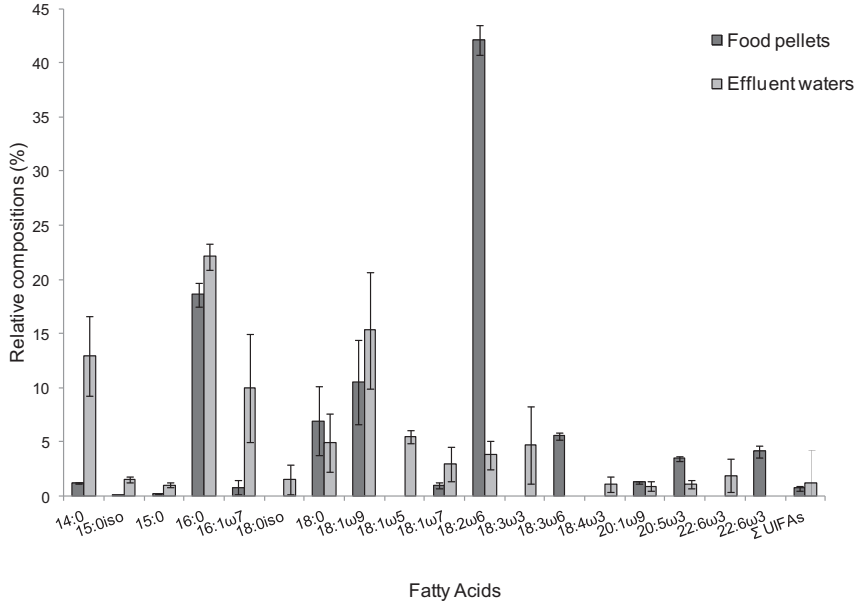

Fig. 3. Fatty acid proportion (% of total fatty acids) of food pellets used for shrimps in pond, and in particulate organic matter of water effluents. Σ UIFAs: sum of unidentified fatty acids.

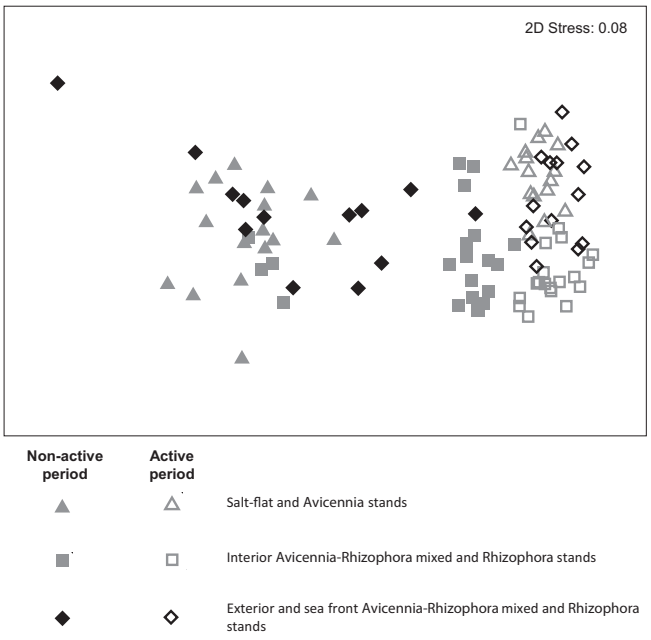

Fig. 4. None-metric multi-dimensional scaling (n-MDS) of FA compositions in surface sediments, during non-active farm period (filled symbols) and active period (open symbols).

acids, long-chain fatty acids (LCFAs; ≥24:0) and branched fatty acids (BFAs; e.g. *iso*15:0, *anteiso*15:0). UFAs included mono-unsaturated fatty acids (MUFAs; e.g. 16:1ω7 and 18:1ω9) and polyunsaturated fatty acids (PUFAs; e.g. 18:2ω6 and 20:5ω3).

3.2.1. Effluent and food-pellet fatty acid composition

FAs identified in food pellets distributed in ponds were mainly composed of the PUFA 18:2ω6 (42.08 ± 1.3%), MUFA 18:1ω9 (10.50 ± 4.0%) and SAFA 16:0 (18.62 ± 1.0%). Effluents were mostly made up of the common SAFA 16:0 (22.1 ± 1.2%), of the MUFAs 18:1ω9 (15.3 ± 5.4%) and 16:1ω7 (10.0 ± 5.0%), and notably of the PUFA 18:2ω6 (3.8 ± 1.3%) and of the MUFA 18:1ω7 (2.9 ± 1.6%; Fig. 3).

3.2.2. Spatial variability in FA composition in both periods

According to the n-MDS analysis (Fig. 4), all sediment samples collected during the active period had comparable FA profiles one to another (at 80%, Bray–Curtis similarity threshold), whereas higher dissimilarity between sites was observed during the non-active period. Indeed, dissimilarity in FA composition between stands was lower during the active period (from 11% to 19%, SIMPER) than during the non-active period of the farm (>20%, SIMPER).

At this latter time significant differences between stand ($R = 0.660$, $p < 0.001$, ANOSIM) was driven by the original FA composition of the interior *Rhizophora* stand compared to the others. *Rhizophora* (interior) sediments exhibited higher concentrations of UFAs (Fig. 5A) such as the 18:1ω7, 18:2ω6, 16:1ω7, 20:5ω3 and 18:1ω9, as well as BFAs *iso*- and *anteiso*15:0 than other stands (Figs. 5 to 9, left panels) (Tukey's HSD; $p < 0.05$ for all). On the contrary during the active period of the farm FA concentrations were more homogeneously distributed between stands which led to no significant spatial discrimination of the above mentioned FAs (one-ANOVA, $p > 0.05$).

3.2.3. Temporal change in the FA pool in mangrove surface sediments

Shift in mangrove sediment FA compositions from the non-active to the active period of the farm was significant (ANOSIM, $R = 0.694$, $p < 0.01$). Dissimilarity between periods (30%, SIMPER) was mainly due to a greater contribution of UFAs 16:1ω7, 18:1ω7, 18:1ω9, 20:5ω3 and 18:2ω6, and a lower contribution of SAFAs 16:0, 15:0 and 24:0, 26:0, 28:0 to surface sediments during the active period than during the non-active period of the farm (Table 1). This resulted in higher UFA/SAFA ratio values during the active period than during the non-active period (one-way ANOVA; $F = 147.18$, $p < 0.001$, Fig. 5). Also, concentrations of UFAs such as the 18:1ω7 ($F = 65.05$), 18:2ω6 ($F = 71.32$), 16:1ω7 ($F = 70.88$), 20:5ω3 ($F = 53.90$), and 18:1ω9 ($F = 107.6$) increased significantly in mangrove surface sediments from the non-active to the active period of the farm as well as the concentration of the BFAs *iso*-15:0 ($F = 37.64$) and *anteiso*-15:0 ($F = 38.82$) (one-way ANOVA; $p < 0.001$ for all; Figs. 5 to 9). Also, LCFA concentrations slightly increased (not significant, Fig. 10) despite the drop of their contribution to the overall FA composition (Table 1). On the contrary, concentrations of some PUFAs such as 18:3ω6 ($F = 12.17$, $p < 0.001$) and 20:3ω6 ($F = 21.07$, $p < 0.001$) decreased twofold (Fig. 8C, D) from the non-active to the active period of the farm and contributed less to the FA pool (Table 1).

3.2.4. Site-dependent responses of environment condition changes

All stands exhibited higher contribution of the UFAs and BFAs mentioned above whereas lower contribution of the SAFAs were recorded during the active period than during the non-active period of the farm (SIMPER). However, change in FA concentrations was less important in the interior *Rhizophora* stand than elsewhere. More specifically, the PUFA 20:5ω3 did not increase significantly whereas the MUFA 16:1ω5 did, in the interior *Rhizophora* stand from one

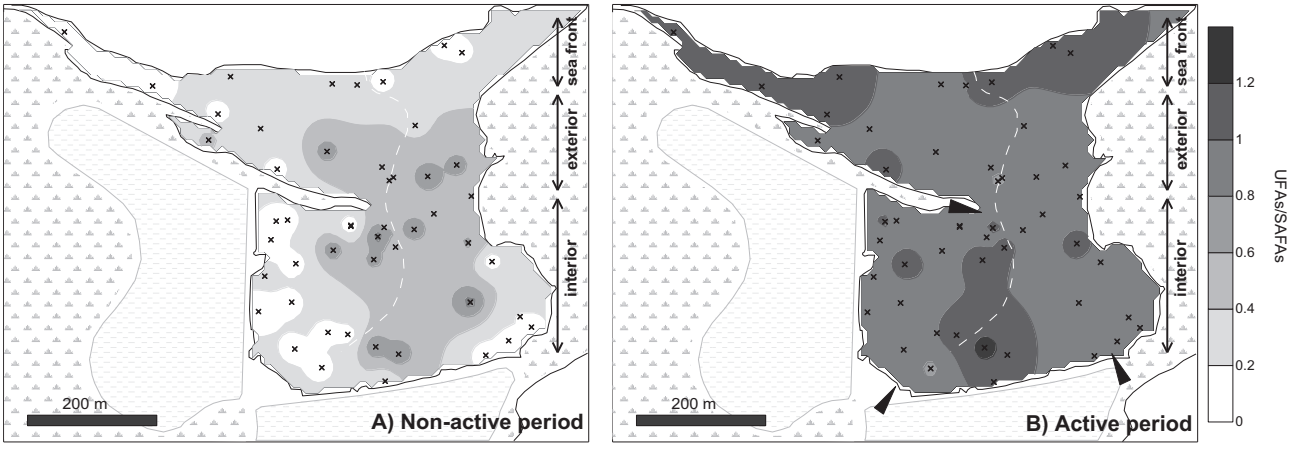

Fig. 5. Values of the UFA/SAFA ratio in mangrove surface sediments during the non-active (A) and the active period (B) of the adjacent shrimp farm. Arrows represent effluent inputs from ponds (polygon filled with dotted lines). Dotted line represents the main tidal channel.

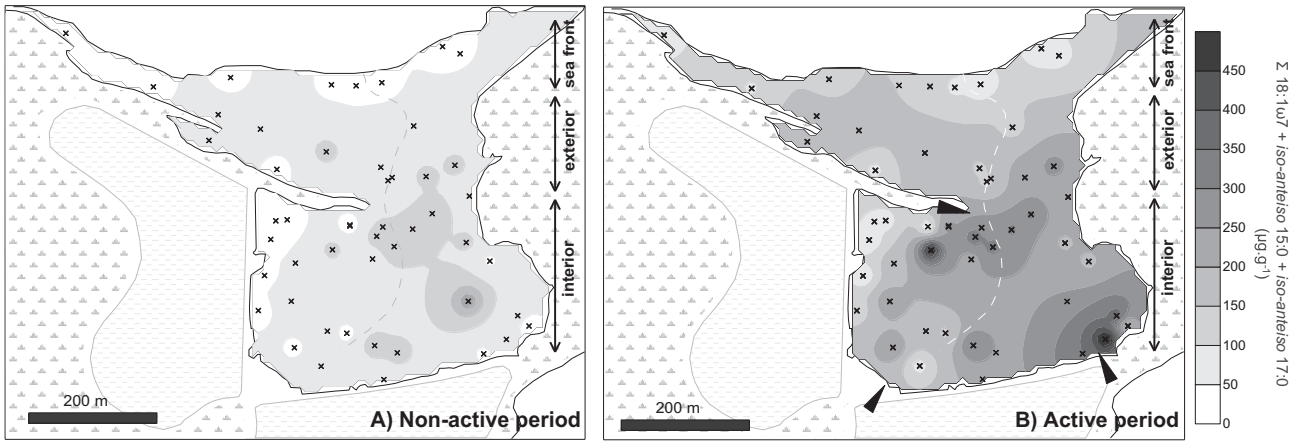

Fig. 6. Sum of bacterial marker concentrations (18:1ω7 + *iso–anteiso* 15:0 + *iso–anteiso* 17:0; $\mu g \cdot g^{-1}$) in surface sediments during the non-active (A) and the active period (B) of the adjacent shrimp farm. Arrows represent effluent inputs from ponds (polygon filled with dotted lines). Dotted line represents the main tidal channel.

period to another (one-way ANOVA; $p < 0.001$). In accordance, ANOSIM found that sediments of the interior *Rhizophora* stand displayed weak FA composition changes from the non-active to the active period of the farm compared to other stands, resulting in an average dissimilarity of 17.44% between both periods. The salt-flat sediments exhibited the greatest shift in FA composition between both periods (43.23%, ANOSIM).

3.3. Spatial–temporal distributions of C and N stable isotope

During the non-active and the active periods, $\delta^{13}C$ ranged from −28.47‰ to −14.23‰ and from −28.48‰ to 22.05‰ respectively and $\delta^{15}N$ ranged from 1.73‰ to 5.97‰ and 2.07‰ to 4.02‰ respectively. $\delta^{13}C$ and $\delta^{15}N$ values in mangrove surface sediments significantly decreased from the non-active to the active period of the farm (Kruskal–Wallis, $p < 0.05$ and $p < 0.05$ respectively). As shown in Fig. 11 (C,D and E,F for $\delta^{13}C$ and $\delta^{15}N$ respectively) decreases were particularly marked for the salt-flat and the *Avicennia* stands (Wilcoxon; $p < 0.05$). C:N ratios ranged from 9.97 to 30.25 during the non-active period and from 8.78 to 22.69 during the active period. Ratios slightly decreased but not significantly from the non-active to the active period of the farm (Fig. 11A, B). Sediments of *Rhizophora* stands (i.e. interior, exterior and seafront) had higher C:N values than all other stands at both periods (Tukey's HSD; $p < 0.001$ and 0.05 for the non-active and the active period respectively).

4. Discussion

4.1. Distribution of shrimp pond effluent within the mangrove

Ubiquitous 14:0 and 16:0 FAs were identified in effluent waters (Fig. 3), as well as in mangrove surface sediments at both periods. However these FAs are present in most of the OM sources and therefore unreliable as effluent particulate OM tracers. The MUFA 18:1ω9 highly detected in effluent waters degrades quickly compared to SAFAs (e.g. 14:0, 16:0, LCFAs; Carrie et al., 1998); thus its detection in surface sediments corresponds to fresh organic matter inputs into the mangrove. The distribution of shrimp farm effluents at the sediment surface in the mangrove can therefore be determined by monitoring this specific MUFA. This MUFA was likely originated from microbial degradations of PUFA 18:2ω6 (Dewick, 2001), largely occurring in ponds (Avnimelech and Ritvo, 2003) and highly detected in food pellets (43% of total FA; Fig. 3). The latter was certainly not fully consumed by shrimps in the pond and was thus exported towards the mangrove as revealed by the detection of the 18:2ω6 in effluent waters (Fig. 3). Low tidal currents in relation with the high retention time of water within mangrove swamps favored the settlement of the anthropogenic particulate OM on the mangrove sediment surface. The distribution of the MUFA 18:1ω9 was characterized by hotspots close to the three effluent outlets: areas which are characterized by *Avicennia–Rhizophora* mixed vegetation known to act, because of their roots web complexity,

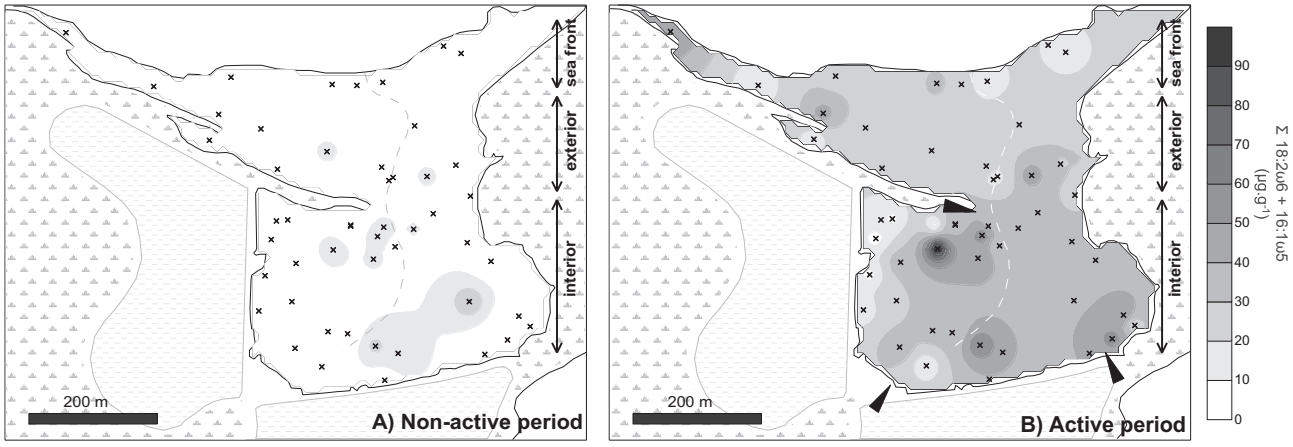

Fig. 7. Sum of fungal markers concentrations (18:2ω6 + 16:1ω5; $\mu g \cdot g^{-1}$) in surface sediments during the non-active (A) and the active period (B) of the adjacent shrimp farm. Arrows represent effluent inputs from ponds (polygon filled with dotted lines). Dotted line represents the main tidal channel.

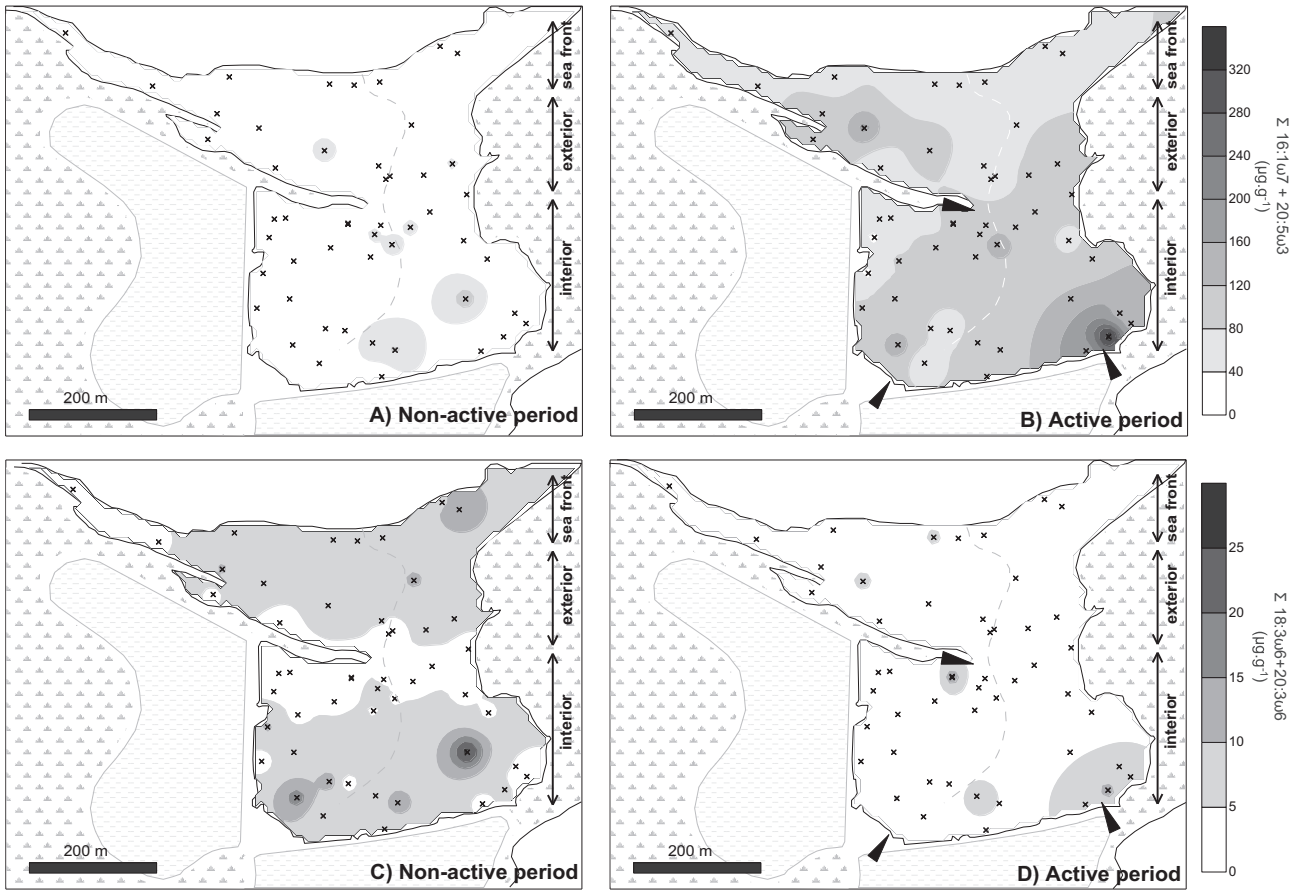

Fig. 8. Concentrations of microalgae markers in surface sediments: sum of the 16:1ω7 + 20:5ω3 ($\mu g \cdot g^{-1}$) during the non-active (A) and the active period (B) of the adjacent shrimp farm and of the 18:3ω6 + 20:3ω6 ($\mu g \cdot g^{-1}$) during the non-active (C) and the active period (D). Arrows represent effluent inputs from ponds (polygon filled with dotted lines). Dotted line represents the main tidal channel.

more efficiently as a suspended matter trap than any other stands (Kathiresan, 2003). However, the widespread identification of the anthropogenic MUFA (Fig. 9B) within the mangrove argues for effluent redistributions under tidal influence. The MUFA 18:1ω9 was also detected at the seafront. The latter result suggests that particulate OM originated from ponds was exported seawards, as observed for nutrients originated from effluents in the same mangrove (Molnar et al., 2013) and in Australian mangroves (Trott et al., 2004). Partial efficiency of mangrove forests in removing excess nutrients and the need of high surface ratio of *Rhizophora* sp.:pond (from 2:1 to 5.2:1 ha for nitrogen) for excess nutrients removal has been pointed out by Robertson and Phillips (1995) and Shimoda et al. (2007). In supplement our results showed that a *Rhizophora* sp.:pond ratio of ca. 3.1:1 ha does not enable the particulate organic matter originated from effluents to be completely filtered by the mangrove. Therefore, absolute effluent-filtering efficiency of mangroves would still have been to be reconsidered in relation to the effluent composition.

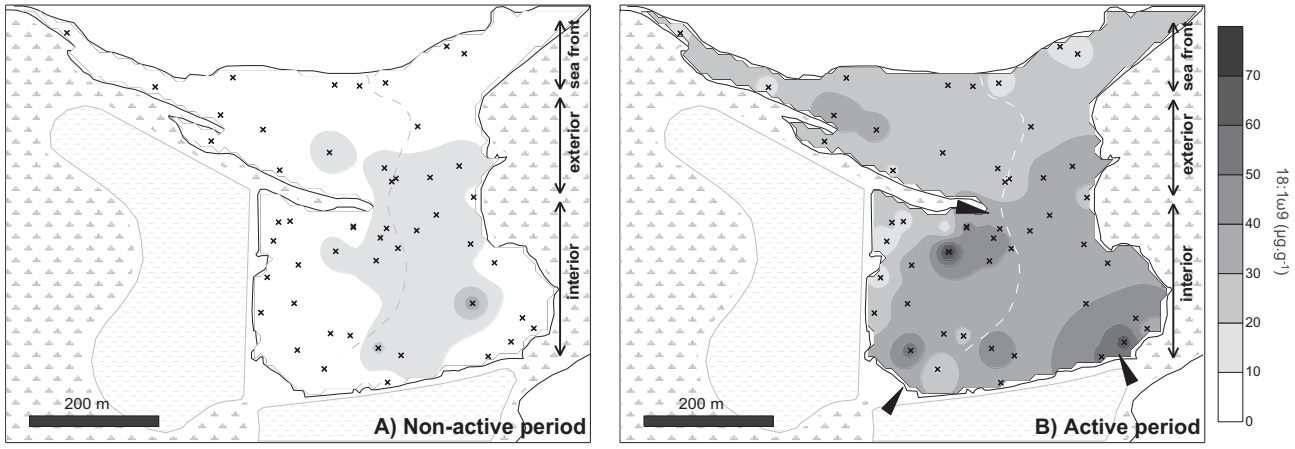

Fig. 9. Concentrations of 18:1ω9 ($\mu g \cdot g^{-1}$) in mangrove surface sediments during the non-active (A) and the active period (B) of the adjacent shrimp farm. Arrows represent effluent inputs from ponds (polygon filled with dotted lines). Dotted line represents the main tidal channel.

Table 1
Fatty acid contribution (%) to the overall sediment FA pool in mangrove surface sediments during the non-active and the active period of the adjacent shrimp farm for each vegetation stand.

	Non-active period						Active period					
	Salt-flat	*A. marina*	*A. marina–R. stylosa*	Interior *R. stylosa*	Exterior *R. stylosa*	*R. stylosa* seafront	Salt-flat	*A. marina*	*A. marina–R. stylosa*	Interior *R. stylosa*	Exterior *R. stylosa*	*R. stylosa* seafront
% SAFAs	58.8 ± 3.9	60.2 ± 4.0	50.9 ± 7.6	37.6 ± 5.8	53.3 ± 9.2	57.1 ± 13.0	39.7 ± 1.4	39.9 ± 1.8	35.5 ± 2.7	32.8 ± 1.8	35.6 ± 2.0	35.0 ± 2.7
% LCFAs	13.9 ± 3.8	13.2 ± 2.7	11.0 ± 3.6	13.2 ± 3.3	14.2 ± 3.0	14.7 ± 4.6	5.3 ± 1.9	5.4 ± 2.1	6.2 ± 2.5	7.7 ± 4.3	8.1 ± 2.4	5.8 ± 2.5
% UFAs	10.1 ± 3.1	10.1 ± 5.3	21 ± 14.5	30.7 ± 8.8	18 ± 11.4	12.4 ± 7.9	41.5 ± 2.6	40.2 ± 3	41.6 ± 5.7	38.7 ± 3.5	42.3 ± 2.9	43.7 ± 5.7
% Σ (16:1ω7 + 20:5ω3)	0.2 ± 0.1	1.4 ± 1.7	5.7 ± 5.6	7.8 ± 2.5	4.3 ± 4.1	1.8 ± 1.5	14.9 ± 2	13.2 ± 1.8	11.2 ± 3.1	9.7 ± 2.1	13.8 ± 2.6	12.3 ± 3.1
% Σ (18:3ω6 + 20:3ω6)	1.5 ± 2.4	3.2 ± 2.2	1.9 ± 0.9	1.2 ± 0.4	2.8 ± 0.9	3.7 ± 2.7	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.2
% 18:1ω7	0.6 ± 0.1	0.8 ± 0.7	2.6 ± 2.2	7.5 ± 2.0	2.6 ± 2.4	1.5 ± 1.6	5.5 ± 0.6	6.0 ± 1.4	7.9 ± 2.3	9.9 ± 1.0	6.8 ± 0.8	7.5 ± 1.8
% Σ odd-branched	17.1 ± 5.5	21.8 ± 3.0	18.6 ± 4.5	16.8 ± 2.2	20.4 ± 3.3	16.1 ± 3.9	15.8 ± 2.1	16.1 ± 1.4	16.8 ± 3.2	17.3 ± 1.3	14.9 ± 0.8	13.1 ± 1.9
% 18:1ω9	–	0.4 ± 0.6	1.6 ± 1.5	3.0 ± 0.9	1.4 ± 1.3	0.8 ± 0.8	4.7 ± 0.8	4.4 ± 0.7	4.1 ± 0.7	3.9 ± 0.5	3.8 ± 0.6	5.0 ± 0.7
% Σ (18:2ω6 + 16:1ω5)	–	–	1.6 ± 0.9	2.2 ± 0.4	0.9 ± 0.5	0.4 ± 0.3	2.3 ± 0.3	2.7 ± 0.4	3.8 ± 0.7	3.9 ± 0.8	3.6 ± 1.0	5.1 ± 1.1

4.2. Temporal changes in mangrove organic matter composition in surface sediments

Changes in effluent release conditions were concomitant with changes in the OM composition at the surface of mangrove sediments (Fig. 4). Indeed, more FAs were identified in mangrove sediments when the farm was active (Table 1) than during the non-active period. This diversification affected most of mangrove areas; but to a lesser extent the interior *Rhizophora* stand. Consequently, composition in OM was more similar between stands during the active period of the farm (Fig. 4). Effluent runoffs might play a role in the reduction of the inter-stand differences in OM sources as they, together with tide, redistribute organic matter over the whole mangrove area. Additionally, effluents runoff modify the length of immersion by continuously flooding mangrove sediments (Marchand et al., 2011b) which reduces the length of evaporation, and thus salinity of pore-water (Molnar et al., 2014). As salinity is a major factor controlling most of microbial community structure (e.g. Hendey, 1964; Alongi and Sasekumar, 1992; Ikenaga et al., 2010), artificial salinity decrease in sediment may lead to the change in the zonation of benthic microbial communities and in benthic OM composition at the sediment surface. Moreover effluent could organically enrich the high intertidal zones initially (i.e. during the non-active period) more depleted in fresh OM compared to the highly productive *Rhizophora* part of the mangrove. Indeed, the general increase of UFA/SAFA ratios from the non-active to the active period of the adjacent shrimp farm in the mangrove surface sediments states the presence of fresher OM during the rearing period, notably at the high intertidal zone (Fig. 5; Canuel and Martens, 1993; Carrie et al., 1998; Volkman et al., 2008). Record of high UFA proportion at this time, highly degradable (Carrie et al., 1998) suggests continuous fresh OM inputs from the ponds but can also results from in situ production, i.e. microbial development. The latter result is also translated by a drop of $\delta^{13}C$ and $\delta^{15}N$ values ($\delta^{13}C < -24‰$; $1.8 < \delta^{15}N < 3.8$), which reached typical values of sewage receiving environments during the active period (Rumolo et al., 2011). However, direct effect of effluent release on OM dynamics is not obvious, and therein proposed with caution, as the shrimp production cycle coincides with the natural seasonal cycle. It is thus difficult to tell if the huge increase in UFA/SAFA ratios observed here is simply a result of the seasonal evolution, a direct consequence of wastewater release or if both environmental forcing acted synergistically. Temporal change in each source of OM identified resulting in UFA increase was therefore compared with its natural seasonal evolution documented for comparable biotopes in the following sections.

4.3. Bacterial and fungal growth

MUFA 18:1ω7 and BFAs *iso–anteiso*-15:0 and 17:0 are commonly used as bacterial biomarkers (Parkes, 1987; Meziane and Tsuchiya, 2002; Pinturier-Geiss et al., 2002; Dalsgaard et al., 2003; Dunn et al., 2008; Fig. 6). The rise of these specific FAs indicates that bacterial biomass in surface sediments strongly increased from the non-active period to the active period of the adjacent shrimp farm. These FAs may be originated from distinct bacterial communities (Edlund et al., 1986; Canuel, 2001; Pinturier-Geiss et al., 2002) but were most likely synthesized by anaerobic bacteria in phase of the bolstering of anaerobic conditions in the mangrove occurring during the active period as shown by the drop of the $\delta^{15}N$ values (Teranes and Bernasconi, 2000; Fig. 11E, F). Our results showed, that part of the bacterial biomass in mangrove sediments during the rearing period has been directly brought by effluents from shrimp ponds (Fig. 3) as found in other studies (McKinnon et al., 2002b; Burford et al., 2003). Direct effluent imports could account for the high increase in bacterial marker concentrations in the salt-flat and the *Avicennia* stand close to the outlets (Fig. 6B). Still, it cannot be excluded that part of the increase in bacterial biomass resulted from an in situ production that could be enhanced by nutrient-rich effluent releases (McKinnon et al., 2002a; Molnar et al., 2013).

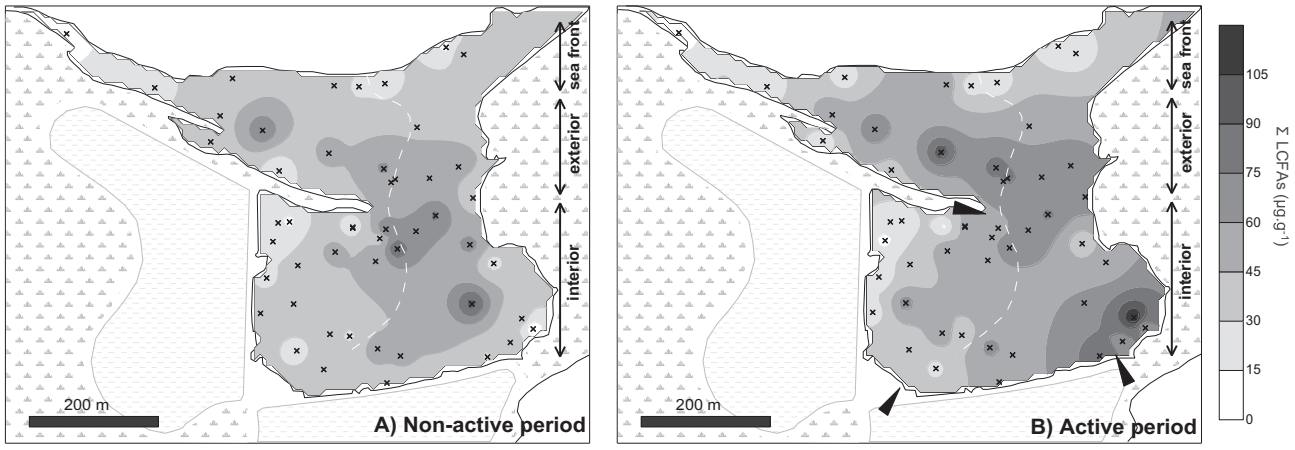

Fig. 10. Concentration of mangrove-derived organic matter markers in surface sediments: long chain fatty acids (LCFAs; $\mu g \cdot g^{-1}$) during the non-active (A) and the active period (B) of the adjacent shrimp farm. Arrows represent effluent inputs from ponds (polygon filled with dotted lines). Dotted line represents the main tidal channel.

However, due to wide geographical variation in bacterial seasonal dynamics (Alongi and Sasekumar, 1992; Mishra et al., 2012), global seasonal pattern could not be concluded from the literature. Therefore, whether the season or the effluent pollution contributed more to bacterial growth during the active period cannot be deduced in the present study.

During the rearing period, the growth of fungi associated to decaying mangrove leaves was revealed by the increased of the FAs 18:2ω6 and more specifically the development of *Arbuscular mycorrhiza* fungi was marked by the FA 16:1ω5 (Frostegard and Baath, 1996; Chen et al., 2001; Joergensen and Wichern, 2008; Calderon et al., 2012; Fig. 7). Increase in fungal biomass runs counter to the natural seasonal dynamic reported for such community. Indeed fungal growth in pristine mangroves is mainly driven by rainfall being stimulated during the rainy season (summer; Matondkar et al., 1980; Mishra et al., 2012). Thus, our results highlight the stimulating effect of effluent releases on benthic fungal development. In addition, the contribution of effluent releases to litter-decomposer growth was confirmed by the record of a highly degraded mangrove-litter during the active period, which is not expected during dry winter season (see below).

4.4. Litter-decomposer activity

LCFAs greatly contribute to mangrove leaf composition (Meziane et al., 2007), which makes their concentration a reliable proxy for mangrove leave derived OM in the environment (Volkman et al., 1980; Meziane et al., 2006). Concentrations of these markers in mangrove sediments exhibited a slight but unexpected increase from the non-active to the active period of the farm (Fig. 10). Indeed, in pristine mangroves LCFA contents are minimal during winter (Mfilinge et al., 2005). This variation does not result from higher mangrove-litter inputs as litter falls are reported to be minimal in winter (Mfilinge et al., 2005; Sharma et al., 2010) and seasonal mangrove litter production is not altered by wastewater release events (Tam et al., 1998). It could rather result from intense litter degradation processes of previously deposited material which led to the release and accumulation of LCFAs in surface sediments which remain for very long time in sediments (Mfilinge et al., 2003). In addition, C:N ratio values recorded in mangrove sediments indicate the presence of decaying litter in both periods. Indeed, C:N ratio >20 recorded within the *Rhizophora* stands confirmed the presence of mangrove-derived OM (Meyers, 1994). However, values were still relatively low (C:N <30; Fig. 11A–B) traducing the decomposition of the litter (as in Bouillon et al., 2003 and in Marchand et al., 2003, 2005) rather than a freshly deposited mangrove-litter which can results in a C:N value up to 280 (e.g. Meziane and Tsuchiya, 2000). The latter results indicate the presence of strongly degraded leaf material throughout the year and thus outline intense and unexpected degradation processes in winter. Indeed, maximum mangrove litter degradation rates usually occur during summer (Bosire et al., 2005; Mfilinge et al., 2005) as leaf degradation is enhanced with increasing temperature (Rezende et al., 2013). Therefore, litter degradation processes in winter are likely stimulated by effluent release through the boost of microbial litter-decomposer (such as bacteria and fungi) biomass and activity.

Enhancement of microbial activity in the mangrove during the active period of the adjacent shrimp farm was most probably responsible for the stimulated N-cycling processes and ammonium efflux measured in the studied mangrove (Molnar et al., 2013) in relation to organic matter degradation. Such activity could therefore act as a fertilizer for benthic primary producers. Thus, shrimp farm effluents could stimulate microalgae growth through nutrient load and bacterial inputs that stimulate nutrient processes.

4.5. Shift in microphytobenthic communities

The PUFA 20:5ω3 and the MUFA 16:1ω7 are largely used as diatom biomarker as they predominantly compose these organisms (Pond et al., 1997; Dalsgaard et al., 2003; Renaud et al., 1999; Napolitano et al., 1997). Increases of these marker concentrations at the sediment surface from the non-active to the active period exhibited high spatial heterogeneity (Fig. 8A, B). Indeed, differences in stand characteristics and distance from ponds led to spatially heterogeneous response of the benthic compartment to changes in environmental conditions (Fig. 4). Regarding the high increase of diatom biomass at the salt-flat and *Avicennia* stand, close to pond and effluent outlets (Fig. 8A, B) and in view of the large proportion of diatom markers recorded in effluent waters (Fig. 3) these microalgae were probably largely imported from ponds and accumulated on the high intertidal. In agreement, shrimp ponds have been previously reported as non-negligible sources of microalgae for adjacent biotopes (McKinnon et al., 2002b). The interior *Rhizophora* stand exhibited the lowest temporal modification in FA composition (Fig. 4) and in diatom marker concentration. High toxic tannin content of litter and light limitation induced by the dense canopy (Alongi, 1994) in this area probably inhibited diatom survival during the active period. In addition, microalgae can probably be in competition in term of nutrient uptake with *Rhizophora* sp. trees, which have high nutrient assimilative efficiency (Robertson and Phillips, 1995; Alongi et al., 2005).

The increase of diatoms in surface sediments was concomitant with the decreases of other micro-algal community markers such as 18:3ω6 and 20:3ω6 (Napolitano et al., 1990; Khozin et al., 1997; Fig. 8CD). Thus, a shift in microphytobenthic assemblages from the non-active period to the active period occurred and contributed to

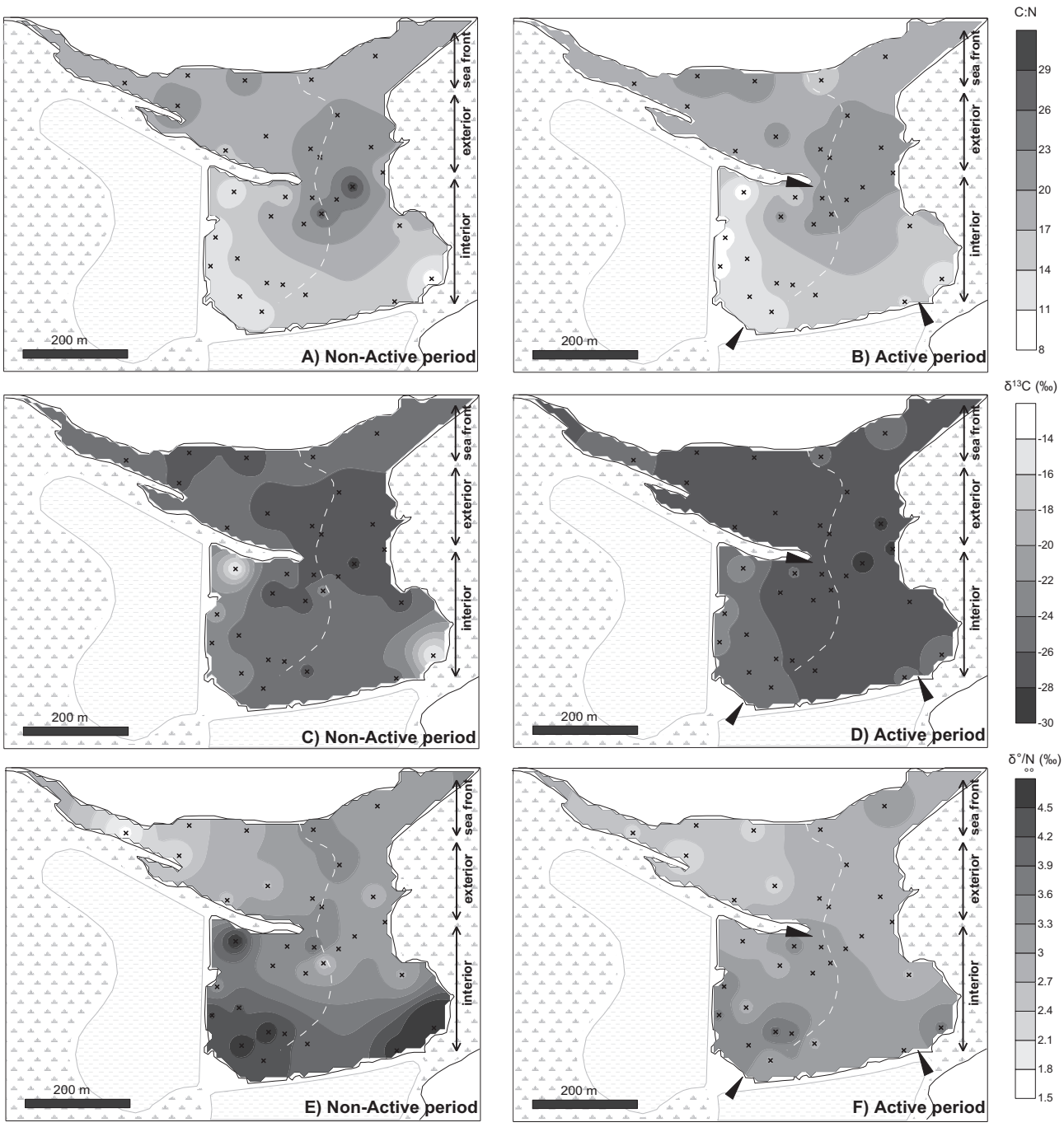

Fig. 11. Values of C:N ratio in surface sediments during the non-active (A) and the active period (B), of $\delta^{13}C$ (‰) during the non-active (C) and the active period (D) and $\delta^{15}N$ (‰) during the non-active (E) and the active period (F). Arrows represent effluent inputs from ponds (polygon filled with dotted lines). Dotted line represents the main tidal channel.

maintain high chl-*a* concentration over both seasons (Fig. 2). Therefore, the activity of the microphytobenthic community was not following the climatic seasonal pattern, which supposes net increase in primary producer biomass and productivity at the sediment surface during the wet summer in New Caledonia (Clavier and Garrigue, 1999). Additionally, diatom markers are supposed to exhibit low or no seasonal variation within unpolluted mangrove ecosystem (e.g. Meziane et al., 2006) contrary to what our results show. These results suggest that effluent releases led to diatom biomass enhancement that was responsible for high chl-*a* concentrations in winter. At both periods, chl-*a* concentrations were much higher than those measured in sediments of pristine intertidal mangrove (<5 $\mu g \cdot g^{-1}$ dw; review in Alongi and Sasekumar, 1992), but similar to those recently reported in mangrove sediments receiving wastewater (Burford et al., 2012). Besides the direct import of microalgae, shrimp farming through the release of high quantity of enriched water in the mangrove most probably stimulate the microphytobenthos development recorded during the winter period, and after the cessation of the release. In addition soil fertilization by bacterial and fungal activity occurring at this time may contributes to diatom growth. Effluent release in the mangrove occurred mainly during winter, when the metabolism of benthic organisms is at their minimum, and the final drain occurred just before the seasonal temperature increase, a period during which the microphytobenthos biomass should increase. The fact that the final drain occurred just before summer led to an increase in seasonal algal bloom, as observed by Molnar et al. (2014). Low isotopic values recorded during the non-active

period of the farm also suggest at least a mid-term effect of effluent on mangrove OM quality. Thus, microphytobenthic dynamic in the impacted mangrove is suggested to be driven by shrimp farm activity; and question of temporary effects (as suggested in Trott and Alongi, 2000) of shrimp farming on effluent receiving mangroves is proposed to be re-evaluated at least for the benthic compartment.

5. Conclusion

Particulate organic material originated from the shrimp farm pond was distributed at the sediment surface of the adjacent mangrove. Indeed, FA 18:1ω9, a good effluent tracer, was dispersed over all the mangrove area under tidal influence and exported towards the seafront which questioned the effluent-filtering efficiency of mangroves.

Qualitative and quantitative change in OM composition in mangrove surface sediments was related to farm activity. Mangrove sediments were enriched in fresh organic material; more specifically, bacterial, fungal and diatom biomasses were enhanced under effluent runoff conditions. Part of these microorganisms was continuously released from ponds whereas a portion resulted from in situ production. Stimulated activity of litter-decomposers led to highly degraded mangrove litter during the active-period of the farm. However, effluent releases did not involve net increase in total micro-algal biomass during the rearing period but rather a shift in microalgal communities. Nevertheless, high primary production recorded all over the year, whether the farm was active or not, suggests permanent effect of farm activity on mangrove benthic primary production.

However, quantitative changes in organic matter composition in response to runoff conditions depended on the distance from discharge points and mangrove stand characteristics. Consequently, benthic compartment of salt-flat and *Avicennia* stands were more affected by nutrient and organic matter inputs than *Rhizophora* stands. Our study emphasizes that the benthic compartment, in relation to stand characteristics, is a good proxy of the effect of effluent releases on mangrove ecosystems.

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