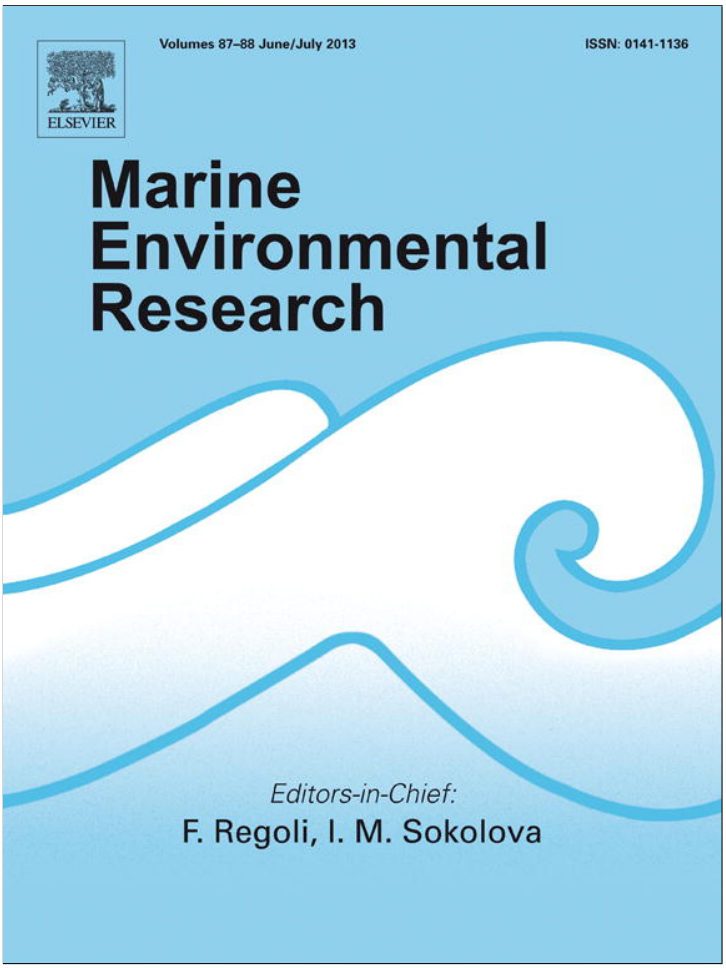

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Dynamics of wood fall colonization in relation to sulfide concentration in a mangrove swamp

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ABSTRACT

Wood debris are an important component of mangrove marine environments. Current knowledge of the ecological role of wood falls is limited by the absence of information on metazoan colonization processes over time. The aim of this study was to provide insights to their temporal dynamics of wood eukaryotic colonization from a shallow water experiment in a mangrove swamp. Combined *in situ* chemical monitoring and biological surveys revealed that the succession of colonizers in the mangrove swamp relates with the rapid evolution of sulfide concentration on the wood surface. Sulfide-tolerant species are among the first colonizers and dominate over several weeks when the sulfide content is at its maximum, followed by less tolerant opportunistic species when sulfide decreases. This study supports the idea that woody debris can sustain chemosynthetic symbioses over short time-scale in tropical shallow waters.

1. Introduction

Distributed at various depths on the seafloor, sunken woods have retained scientist interest for long time. Since they were first reported from more than 1500 m depth during the Challenger expedition in the 19th century, so-called 'sunken wood' colonizer assemblages have been described from a variety of sources including:

(i) natural sunken wood collected from dredge materials (Wolff, 1979; Pailleret et al., 2007; Samadi et al., 2007; Nishimoto et al., 2009)
(ii) experimentally immersed wood substrates (Turner, 1977; Maddocks and Steineck, 1987; Lorion et al., 2009; Bernardino et al., 2010; Gaudron et al., 2010; Bienhold et al., 2013)
(iii) analyses of fossil sunken wood communities (Kiel and Goedert, 2006)

These studies particularly emphasized the abundance and diversity of wood associated fauna in tropical areas where vegetable debris are naturally abundant on the seafloor.

According to Turner (1977), wood ingesters and microbe grazers would constitute the basis of the food web completed by detritivores, with predators and scavengers feeding on these groups. Recent isotopic studies confirmed wood as a carbon source for both direct and indirect users (Nishimoto et al., 2009). Focusing on the surrounding sediment, first experimental studies of macrofaunal succession have confirmed the relatively slow decomposition of wood compared to other vegetable remains and the mediating role of wood borers in the utilization of woody material in the deep sea (Bernardino et al., 2010; Bienhold et al., 2013). Fossil wood-fall

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communities could have displayed the same structure as modern wood falls, although different wood ingesters might have lead to different temporal patterns in the colonization and degradation of wood (Kiel and Goedert, 2006; Kiel et al., 2008).

More unexpected was the observation that sunken woods in the deep-sea share numerous colonizers with so-called chemosynthetic habitats. The reliance of wood invertebrate species to chemosynthetic lineages was first hypothesized by Distel et al. (2000). The observation of bacterial ectosymbionts on the gills of a deep-sea wood mussels (Gros and Gaill, 2007; Gros et al., 2007), that were later confirmed to be sulfide-oxidizers (Duperron et al., 2008) provided further evidence to this idea. Wood falls host a number of higher taxonomic levels known from hydrothermal vents and cold seeps (Kiel and Goedert, 2006; Samadi et al., 2007; Kiel et al., 2008; Bernardino et al., 2010; Gaudron et al., 2010).

Sulfide can be produced from wood in the marine environment from synergetic microbial activities involving cellulolytic bacteria and sulfate reducing bacteria (SRB) with or without the mediation of wood borers, at shallow to great depth (Fors et al., 2008; Palacios et al., 2006, 2009; Bienhold et al., 2013). Owing to the difficulty of accessing deep-sea environments, previous studies have provided only snapshots in the succession of species on wood, preventing to address the relationship between wood habitat conditions and colonization dynamics. In particular, the temporal variation of sulfide, a major factor in metazoan colonization, is still poorly constrained, although a recent aquarium study simulating deep-sea conditions have revealed a relatively rapid temporal dynamics (Yücel et al., 2013).

Even if diversity is likely to be represented by different groups, mangrove swamps offer an opportunity to further investigate the relationships between sulfide enrichment and colonizers diversity, and their temporal dynamics, on degrading wood in tropical environments. In a mangrove swamp, sulfide enrichment was confirmed on natural sunken wood surface (Laurent et al., 2009; Muller et al., 2010), combined with the occurrence of a strict chemoautotrophic symbiosis between a ciliate *Zoothamnium niveum* and sulfide-oxidizing bacterial ectosymbionts (Rinke et al., 2006). The dynamics of such sulfide emission is however poorly constrained to date.

The questions we addressed in this study were twofold: 1 – What are the main eukaryotes colonizing immersed woods over timescales of days to weeks? 2 – Do these groups relate to sulfide concentration on wood surface? Answers to these questions provide insights to the role of wood fall in tropical mangrove ecosystem, where they are naturally abundant. These substrates remain an overlooked component of the mangrove ecology, despite vegetable debris are recognized as a primary source of energy for the ecosystem (e.g. leaves, roots) (Nagelkerken et al., 2008). The results also offer additional cues to the general mechanisms driving sunken wood community dynamics in marine environments, providing interesting comparative information to address differences or common features with deep-sea wood falls.

Two types of wood substrates were experimentally immersed and monitored over 3 months: *Rhizophora mangle* (Rhizophorae), the local mangrove tree, and, for comparison, *Cocos nucifera* (Arecaceae), which is a major component of natural sunken wood collections from tropical areas (Wolff, 1979) (Fig. S1). Temporal changes in the sulfide concentration on the wood surface and in the abundance and diversity of main eukaryotic groups were both monitored. The biogeochemical mechanisms sustaining sulfide production during wood degradation in those particular conditions lie outside the scope of the present paper. The settlement dynamics of different faunal groups were analyzed in the light of the sulfide concentration recorded on the wood surface over the experiment duration. The reliance on sulfide as an energy source was further considered by exploring the occurrence of chemoautotrophic symbioses among the colonizers. Similarly to the succession model proposed for whale and wood falls by previous authors, a model is proposed to explain the temporal colonization pattern on wood falls in the mangrove swamp, and its consistency with the observations performed at greater depth is further discussed.

2. Methods

2.1. Experimental strategy and colonizer surveys

Parcels composed of a metallic structure covered by a plastic mesh (1 × 0.5 × 0.5 m) were filled either with freshly cut fractions of branches (10 pieces about 30 cm long with various thicknesses) of the dicotyledon mangrove tree *R. mangle*, or 10 palm branches (~50 cm each) of the monocotyledon palm tree *C. nucifera* depending of the experiment (Fig. 1). One piece in each parcel was

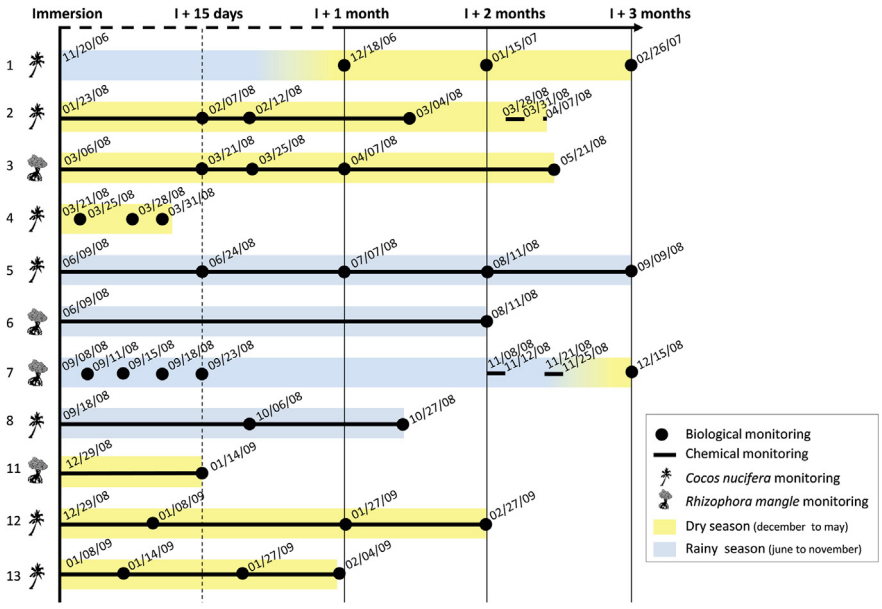

Fig. 1. Summary of the experiments performed at different periods of the year (dry and wet seasons), with the two type of wood (*Rhizophora mangle* and *Cocos nucifera*).

equipped with a sulfide measuring system as described in Section 2.2 (Fig. S1C and D).

The parcels were immersed in the mangrove swamp of Guadeloupe (16°N 61.5W) West Indies, at about 2-m depth. These parcels (0.25 m^3) reflected a substantial amount of accumulated wood debris, although smaller than the large wood deployments made in previous deep-sea experiments (Bernardino et al., 2010; Bienhold et al., 2013). In order to avoid contact with the organic-rich sulfidic sediment, the devices were maintained about 50 cm above the bottom using a metallic holder, which was not in contact with the wood. Thirteen experiments of various durations were realized independently, at different periods of the year (dry and wet season), as summarized in Fig. 2. Between November 2006 and January 2009, 7 and 4 replicate experiments involving the temporal surveys of major colonizer groups and their qualitative abundance were performed, respectively, for *C. nucifera* and *R. mangle*. Biological colonization was monitored regularly by *in situ* visual inspection of the parcels (presence of (i) biofilm covering the woods, (ii) giant colonial ciliates already described as sulfur-oxidizing bearing animals (Bauer-Nebelsick et al., 1996; Rinke et al., 2007) that can be easily observed with naked eyes underwater). Every two weeks, one piece of each type of wood was collected from the parcel, delicately placed underwater in a container, minimizing distance from the parcel in order to avoid the loss of small animals from the surface of the woods. The sample was then brought to the laboratory and processed within 4 h after collection in order to collect and identify the associated fauna.

In order to qualitatively define the abundance of the organisms colonizing the wood, we have defined three stages of abundance according to concentration of each type of animal colonizing the piece of wood removed from the parcel. Typical abundances for the three stages are illustrated in Fig. S3. Considering assays as replicates, biological observations were pooled together according to the type of wood and the results summarized in Table 3. When symbiotic associations were suspected among the associated fauna, the samples were specially analyzed using dedicated techniques (see Section 2.3).

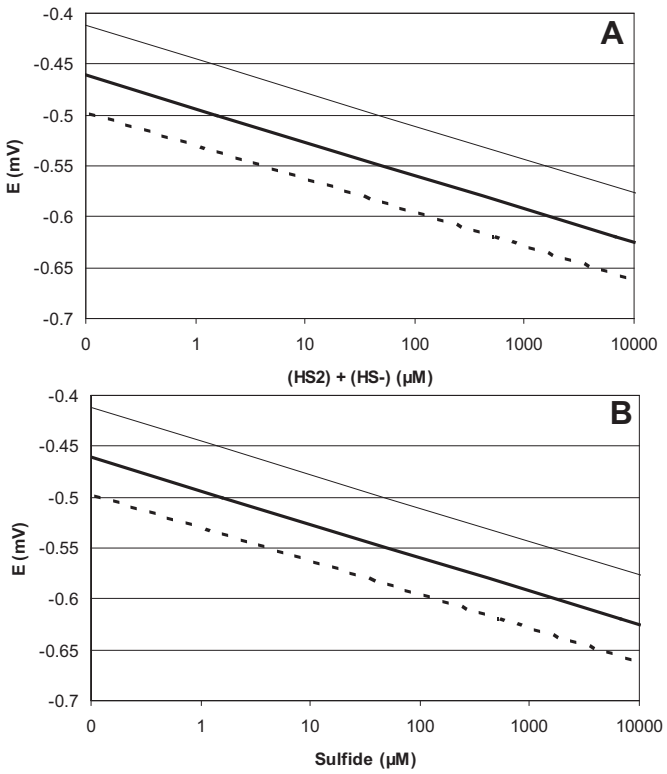

Fig. 2. Electrode potential as function of the concentration of sulfide (e.g. H_2S and HS^-) concentration calculated from calibration coefficients at pH 6 (dotted line), pH 7 (bold line), and pH 8 (thin line) for the two electrodes used in this study. (A) Electrode S5, (B) Electrode S10.

2.2. Chemical measurement

Sulfide was monitored during all experiments, both at the dry and wet seasons, on each type of wood. Punctual measurements were also performed. This was done using an Ag/Ag_2S electrode sensitive to S^{2-} (diameter: 0.8 mm) combined with miniaturized glass electrode sensitive to pH (diameter: 1.5 mm) as described elsewhere (Le Bris et al., 2001, 2008; 2012). Each electrode was connected to an underwater potentiometric autonomous data logger (NKE, France), and the two loggers were attached to one of the wood pieces inside each parcel, while the tip of the pH and sulfide electrodes were tightly attached and carefully positioned at the surface of the vegetal substrate (Fig. S2). The measurement rate was set to 1 measure every 30 min, ensuring the autonomy of the device over more than two months.

Calibrations were performed in the laboratory, before and after recovery to check the stability of the electrode response over the experiments (Contreira Pereira, 2012). The reproducibility of calibration curves confirmed that surface potential records were suitable for a semi-quantitative assessment of sulfide concentration over time (Contreira Pereira, 2012). For this purpose, calibration coefficients for each silver sulfide electrode used were calculated for the whole period of experiment. The relationship between the free sulfide concentration (i.e. $[H_2S] + [HS^-]$) and the potential recorded at the electrode was defined using these coefficients, the pH measured by the glass electrode and the first acidity constant of H_2S defined in Rickard and Luther III (2007) (Fig. 4). According to the sensitivity of the Ag/Ag_2S to S^{2-}, the pH has a large influence on this relationship. Although outside the scope of the study, the wide variations in pH that where monitored are presented as supplementary material and accounted to estimate sulfide ranges. The relationship was defined for 3 pH values (6, 7 and 8) for which the used electrodes were confirmed to display a Nernstian response. No quantitative estimate was done below pH 6, we considered that measurement accuracy could not be sufficiently constrained.

2.3. Ultrastructural analyses of wood colonizers

Samples collected were observed and photographed with a stereomicroscope before preparation for Scanning Electronic Microscope (SEM), Transmission Electronic Microscope (TEM) observations or *in situ* hybridization techniques in order to detect possible symbioses (Table 1). Samples for Scanning Electron Microscope observations were fixed 2 h at 4 °C in glutaraldehyde 2% in caccodylate buffer (900 mOsm, pH 7.2). They were dehydrated in graded concentrations of acetone, critical point dried in CO_2 and sputter-coated with gold before viewed with a Hitachi S 2500 at 20 kV. For Transmission Electron Microscope observations samples were prefixed for one hour at 4 °C in 2.5% glutaraldehyde in 0.1 M pH 7.2 caccodylate buffer adjusted to 900 mOsm. After a brief rinse, they were fixed for 45 min at room temperature in 1% osmium tetroxide in the same buffer, then rinsed in distilled water and post-fixed with 2% aqueous uranyl acetate for one more hour before embedding and observation in a Leo 912 TEM at 120 kv. Samples for CARD-FISH (fluorescence *in situ* hybridization and catalyzed reporter deposition) experiments were first fixed for 1–3 h in 4% paraformaldehyde in seawater, washed three times in seawater and dehydrated in graded concentrations of ethanol to

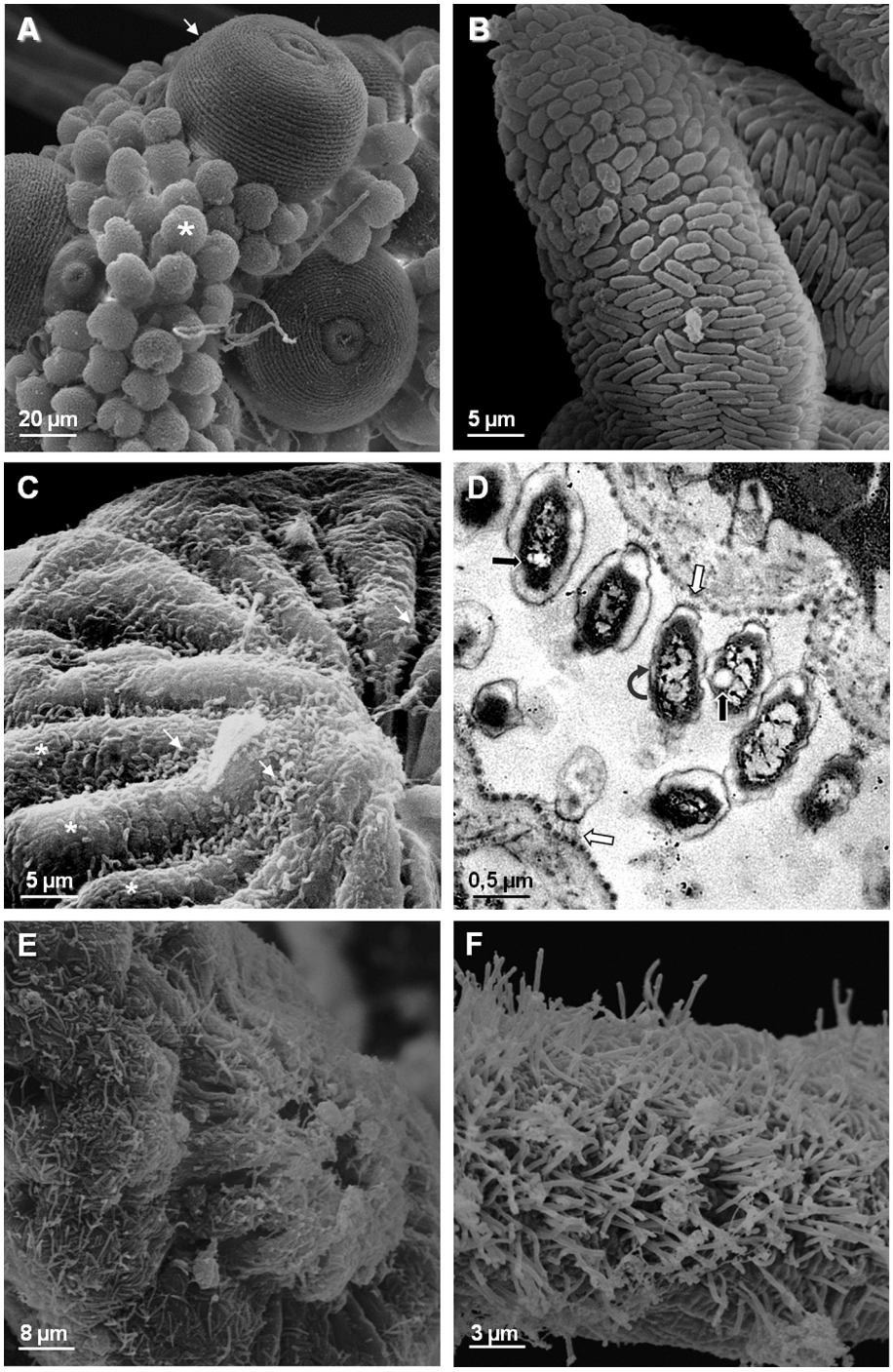

Fig. 3. SEM observations of *Zoothamnium niveum* showing its bacterial coat. (A) Microzooids (asterisk) and macrozooids (arrow) are both covered by a bacterial coat. (B) The modification of bacterial shape from the oral to aboral part of a *Z. niveum* microzooid, characterizing this symbiotic association. (C) SEM observations of the whole body of the loxosomatid entoproct, including the retracted tentacles (asterisks) covered by bacteria (arrows). (D) TEM observations of the bacteria at the surface of the loxosomatid entoproct confirming the ectosymbiotic nature of the association. Their double membrane (curved arrow) and the presence of electron lucent granules (black straight arrows) in their cytoplasm are also shown, together with a particular attachment system (white straight arrows). Lophophore (E) and stalk (F) of the unidentified entoproct densely covered by rod bacteria.

70% ethanol (for hybridizations on whole organisms) or 100% ethanol (for LR White resin sections). Organisms dehydrated in 100% ethanol were then embedded in LR White resin (Gros and Maurin, 2008), 1 µm-thick sections were cut and collected on Superfrost slides while whole organisms were rehydrated in 50% then 30% ethanol and collected on Superfrost slides. Hybridizations on both types of samples were done according to Pernthaler et al. (2002) with the universal eubacterial probe EUB 338 (5′-GCTGCCTCCCGTAGGAGT-3′) [Sekar et al., 2004] labeled with carboxyfluorescein (FITC). 4′-6-Diamidino-2-phenylindole (DAPI) was mixed with Dako-cytomation before mounting the cover slides in order to detect the presence of DNA on the sections. The slides were then visualized under an epi 80i epifluorescence microscope (Nikon, France).

Table 1
Analyses realised on organisms collected from immersed vegetal substrates.

			SEM	TEM	Hybridizations
Annelida	Polychaeta errantia		x		x
	Polychaeta sedentaria		x		x
Arthropoda	Malacostraca	Amphipoda	x		
Crustacea		Décapoda	x		
		Isopoda			
	Maxillopoda	Cirripedia			
		Copepoda			
	Ostracoda				
Chordata	Ascidiacea		x		x
Tunicata					
Ciliophora	Oligohymenophora		x	x	x
	Heterotrichea		x		
Cnidaria	Anthozoa	Actinaria	x		
Ectoprocta	Gymnolaemata		x		
Entoprocta			x	x	x
Mollusca	Bivalvia				x
	Gastropoda		x		
Nematoda			x		
Platyhelminthes	Turbellaria		x		x

3. Results

3.1. Colonization patterns

Diverse faunal communities were described on both wood types (Table 2). During the 3 months of immersion, annelids, arthropods, tunicates, ciliates, cnidarians, ectoprocts, entoprocts, molluscs, nematodes, and platyhelminthes were collected at the surface of the experimental substrates. No organism was found inside the wood. The qualitative analysis of abundance reveals temporal patterns in the most represented groups (Table 3) which are detailed below.

After 4 days of immersion, few organisms were already observed at the wood surface. Some heterotrich ciliates were found on both substrates, as well as a single gastropod in one of the *R. mangle* experiments. After 10 days, wood colonizers considerably diversified. Sedentary polychaetes, crustaceans, other ciliates, and nematodes were present on both substrates. Moreover some ascidians colonized *R. mangle* and some ectoprocts were observed on *C. nucifera*.

Heterotrich ciliates belonging to the families Folliculinidae and Stentoridae are the first organisms colonizing the experimental substrates. Both families were present after 4 days of immersion on *R. mangle*. On *C. nucifera,* the Stentoridae joined the Folliculinidae

Table 2
List of identified wood colonizing organisms.

Annelida	Polychaeta	Errantia	Amphinomidae	
			Eunicidae	
			Nereididae	
			Syllidae	
		Sedentaria	Ampharetidae	
			Cirratulidae	
			Capitellidae	
			Sabellidae	
			Serpulidae	
			Terebellidae	
Arthropoda	Malacostraca	Amphipoda		
Crustacea		Decapoda	Alpheidae	
			Majidae	*Mithrax sculptus*
			Palamonidae	
			Portunidae	*Callinectes* sp.
		Isopoda		
	Maxillopoda	Cirripedia	Balanidae	
		Copepoda		
	Ostracoda			
Chordata	Ascidiacea	Aplousobranchia	Clavelinidae	*Clavelina* sp.
Tunicata		Phlebobranchia	Diazonidae	*Rhopalaea abdominalis*
			Perophoridae	*Ecteinascidia turbinata*
Ciliophora	Oligohymenophora (Peritrichia)	Sessilida	Vorticellidae	*Pseudovorticella* sp.
				Zoothamnium niveum
	Heterotrichea	Heterotrichia	Folliculinidae	
			Stentoridae	*Stentor* sp.
Cnidaria	Anthozoa	Actinaria	Actiniariae	*Viatrix globulifera*
Ectoprocta	Gymnolaemata	Cheilostomata	Bugulidae	*Bugula* sp.
		Ctenostoma	Vesiculariidae	*Zoobotryon* sp.
Entoprocta	Solitaria	Loxosomatida	Loxosomatidae	
	Coloniales	Stolonata	Barentsiidae	
			Pedicellinidae	
Mollusca	Bivalvia	Limoida	Limidae	
		Mytiloida	Mytilidae	*Brachydontes* sp.
		Ostreoida	Ostreidae	*Crassostrea rhizophorae*
		Veneroida	Tellinidae	
			Veneridae	*Anomalocardia* sp.
	Gastropoda	Basommatophora	Planorbidae	
		Cephalaspidea	Aplustridae	
		Neogastropoda	Muricidae	*Murex brevifrons*
		Neotaenioglossa	Caecidae	*Caecum* sp.
Nematoda	Adenophorea	Desmodorida	Stilbonematidae	*Eubostrichus dianae*
Platyhelminthes	Turbellaria	Polycladida	Pseudoceritidae	*Pseudoceros crozieri*
				Pseudoceros texarus

Table 3
Estimation of abundances of organisms observed at the surface of *Cocos nucifera* and *Rhizophora mangle* after different immersion time. +: rare individuals observed. ++: groups moderately represented on the substrate. +++: abundant groups, covering large areas of the substrate.

Organisms collected		Vegetal substrates and immersion times (days)															
		Cocos nucifera								*Rhizophora mangle*							
		4	7	10	15	20	30	60	90	4	7	10	15	20	30	60	90
Annelida	Polychaeta errantia				x	x	x	xx	xxx						x	xxx	xx
	Polychaeta sedentaria		x	x	xx	xxx	xxx	xxx	xxx			x	x	x	x	xx	xx
Arthropoda	Malacostraca		x	x	x	x	xx	xx	xx		x	x	x	x	x	xxx	xx
Crustacea	Maxillopoda			x	xxx	xxx	xxx	xxx	xxx		x	x	x	x	x	xxx	xx
	Ostracoda		x					x			x			x			
Chordata	Ascidiacea						xx	xx	xx			x					
Tunicata																	
Ciliophora	Oligohymenophora		x	xx	xxx	xxx	xx	xx	xx		xx	xxx	xxx	xxx	xxx	xx	xx
	Heterotrichia	x	x	xx	xx	xx	xx	x	x	xx	x	x	xx	x	x		
Cnidaria	Anthozoa							x								x	
Ectoprocta	Gymnolaemata		x	x		x	x	x	x				x	x		x	x
Entoprocta								xx	xx					xx			
Mollusca	Bivalvia							x	x								
	Gastropoda					x	x	x	x	x			x			x	
Nematoda				x	x			x	x		x	x	x	x			x
Platyhelminthes						x		xxx	xx						x		

after one week of immersion. These heterotrich ciliates maintained all over the 3 month-experiment on *C. nucifera,* while they were no more observed on *R. mangle* after one month of immersion.

Oligohymenophoran ciliates were described on experimental substrates 7 days after immersion. Two species from this group were abundant: the colonial *Z. niveum* belonging to the family Zoothamniidae and the solitary *Pseudovorticella* sp. from the family Vorticellidae (Fig. S2). They developed in groups of several tens of colonies or individuals, forming white patches on the substrate. Their number increased regularly until the 20th day on *C. nucifera* and the 30th day on *R. mangle,* before exhibiting a slow decrease. Both *Z. niveum* and *Pseudovorticella* sp. respected the same pattern of abundances.

Annelids started to colonize the substrates after variable immersion times. Sedentary polychaetes are observed 7 days after immersion on *C. nucifera* and 10 days on *R. mangle*. They are

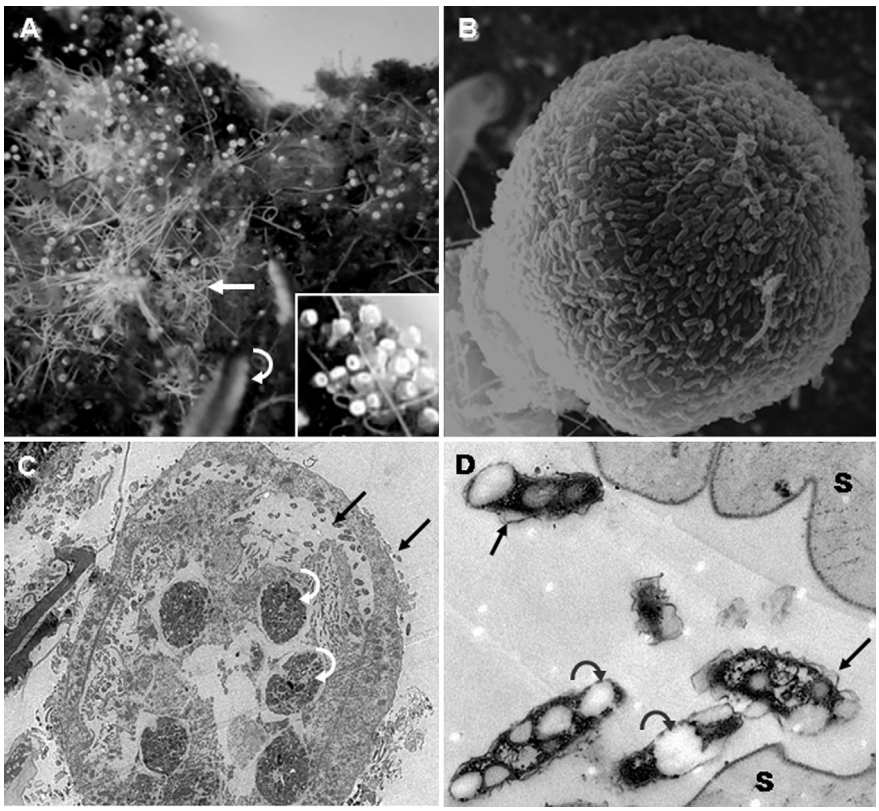

Fig. 4. Observation of *Pseudovorticella* sp. (A) On the substrate surface (insert), in the vicinity of *Z. niveum* (curved arrow) and white sulfur filamentous bacteria (straight arrow). (B) SEM view of bacteria covering the zooid of the vorticellid ciliate. (C) TEM view showing bacteria on the zooid surface (black straight arrows) and in the food vacuoles (white curved arrows). (D) TEM view of the bacteria at the surface of the stalk (S). Double membrane (straight arrows), characteristic of gram negative bacteria, and electron lucent granules (curved arrows) suspected to be sulfur granules are also shown.

followed by errant polychaetes (at 15 days for *C. nucifera* and 1 month for *R. mangle*). The number of these organisms increased on both substrates substantially after 2 months of immersion, principally for the families Sabellidae and Serpulidae. The third month revealed a large diversity of polychaetes. Numerous families were represented: Ampharetidae, Amphinomidae, Capitellidae, Cirratulidae, Eunicidae, Nereididae, Sabellidae, Serpulidae, Syllidae and Terebellidae. The crustaceans are shown to colonize *C. nucifera* faster than *R. mangle*. The most abundant representatives of this group are the Balanidae. Isopods are only detected on *C. nucifera* after 2 months of immersion.

Gastropods did not follow a regular colonization dynamics on *R. mangle*. They seemed to randomly pass on the substrate. After 4 days of *R. mangle* immersion a planorbid gastropod was observed, then *Caecum* sp. after 15 days and a nudibranch belonging to the family Aplustridae after 2 months. The tendency was different on *C. nucifera*. The planorbid gastropod was observed more regularly, as well as *Murex brevifrons* (and its egg-laying) during the first month of immersion. Few bivalves were also observed at the surface of *C. nucifera* after 2 and 3 months of immersion. These bivalves belonged to the families Limidae, Ostreidae, Tellinidae and Veneridae. During the 3-month experiment, no bivalve was observed on *R. mangle*. Similarly to gastropods, nematodes, ectoprocts, flatworms and ascidians did not seem to have a regular colonization process of woody plant remains.

3.2. Symbioses

Bacterial associations were detected on six species: the colonial ciliate *Z. niveum*, the solitary ciliate *Pseudovorticella* sp., the nematode *Eubostrichus dianae* and three entoprocts (a loxosomatid, a pedicellinid and an unidentified one). Bacteria associated with *Z. niveum* cover all zooids and branches except the adhesive disk (Fig. 2). They follow a regular arrangement with a transition from rod bacteria at the basal part of the zooids to a more coccoid shape at the oral part of the zooids as described by McLeod and Wing (2007) and Bauer-Nebelsick et al. (1996).

Pseudovorticella sp. was often observed in the vicinity of *Z. niveum* and filamentous mats of thiotrophic *Beggiatoa*-like bacteria (Muller, personal communication) (Fig. 3). According to SEM observations, the bacteria associated with *Pseudovorticella* sp. cover the whole body of the organism except the adhesive disk similarly to what is observed for *Z. niveum*. The arrangement is less organized than in *Z. niveum*, and only rod-shaped ectosymbionts were detected (Fig. 3). TEM observations confirmed the presence of the bacteria at the surface of both zooid and stalk but also inside food vacuoles (Fig. 3C). The ectosymbiotic bacteria have a double membrane characteristic of Gram negative bacteria. Some electron lucent granules contained in their cytoplasm might be interpreted as sulfur granules (Fig. 3D).

SEM observations of the loxosomatid entoproct (Fig. 2C) revealed that bacteria covered its whole body. TEM observations (Fig. 2D) confirmed that only extracellular bacteria are associated with this loxosomatid entoproct. These ectosymbionts are identified as Gram-negative bacteria owing to their double membrane. Like the ectosymbionts of *Pseudovorticella* sp. and *Z. niveum*, electron lucent granules were observed inside their cytoplasm, and might indicate their reliance on thiotrophy.

The pedicellinid entoproct was only analyzed using *in situ* hybridization experiments. Combination of DAPI coloration (Fig. 5A) and CARD-FISH experiments with the EUB 338 probe (Fig. 5B) confirmed that eubacteria cover the whole body of this entoproct. The unidentified entoproct that was detected with SEM observations also exhibited rod-shaped bacteria covering both the lophophore and the stalk of the organism. From the density of bacteria, it can be hypothesized that this association is truly symbiotic.

3.3. Sulfide variability on the wood surface in relation to wood colonizers

Sulfide varied substantially throughout the experiment duration (Figs. 6 and 7). Both types of wood exhibit a rapid decrease in the electrode potential denoting the establishment of sulfidic conditions on their surface (4 days for *R. mangle* and 6 days for *C. nucifera*). Very acidic pH in the initial period (Fig. S3) prevents the maximum concentration of sulfide achieved on Stage II to be estimated, but substantial levels are expected owing to the low potential recorded. In comparison, punctual measurements performed throughout the experiments confirmed that sulfide remained undetectable in the water surrounding the immersed substrates.

Sulfide was detected on the surface of *R. mangle* after a non-sulfidic period lasting 2–4 days (Stage I) (Fig. 6). After a fast transient decrease (Stage II, from day 4 to day 6), the sulfide electrode

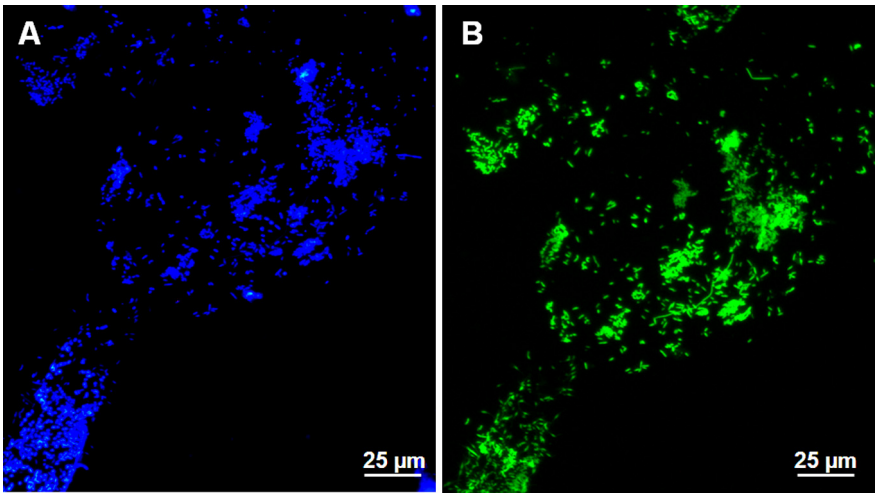

Fig. 5. Micrographs of the symbiotic pedicellinid entoproct showing its bacterial coat in epifluorescence. The blue coloration is obtained with DAPI (A) confirming the presence of microbial DNA. The green fluorescence obtained with the FITC labeled EUB 338 probe (B) matches with the blue coloration, and validates that eubacteria covered the whole body of the entoproct. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

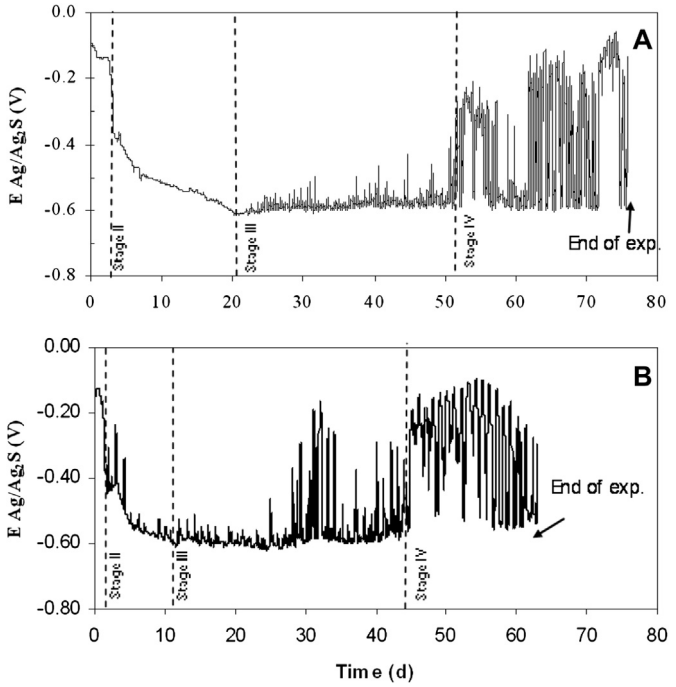

Fig. 6. Sulfide electrode potential as function of immersion time on the surface of *Rhizophora mangle* (A, B respectively for Experiment 3 and 6).

potential remained stable around −0.6 V, except for a short fluctuation period of few days in the second *R. mangle* experiment. After the pH has increased back above 6 and 7, sulfide concentration ranging from a few hundreds of μM to about 1 mM, respectively, are expected from calibration templates. These conditions are identified as Stage III and lasted about 30 days in each Rhizophora experiment (Fig. 6A and B). For the two *R. mangle* experiments, these highly sulfidic conditions lasted about 30 days. After this period, millimolar concentrations of sulfide alternating with non-sulfidic periods are displayed, according to the potential records. Stage IV lasted respectively 24 and 18 days on the first and second experiments, but was interrupted by the end of the experiments.

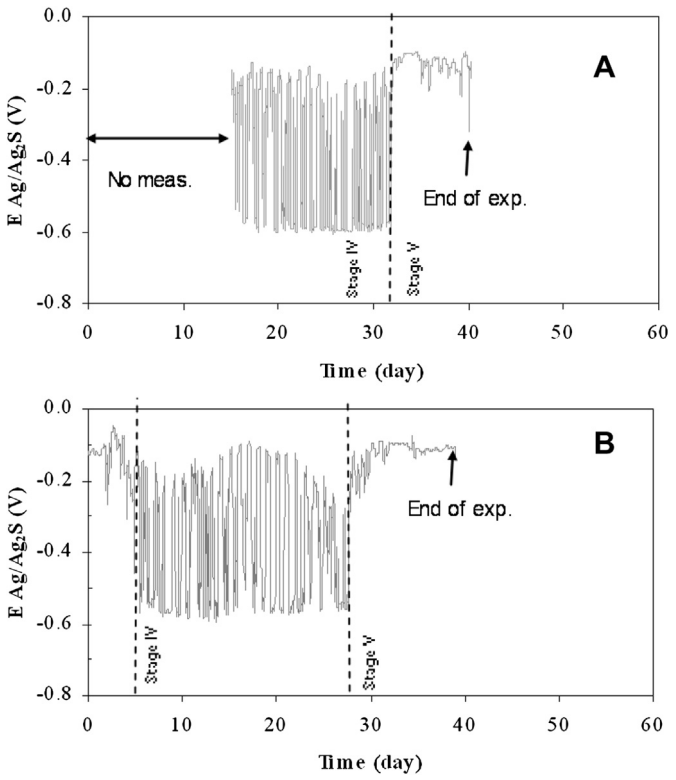

Fig. 7. Sulfide electrode potential as function of immersion time on the surface of *Cocos nucifera* (A, B respectively for Experiment 2 and 8). Arrows indicate the end of the experiments. The delayed start of chemical monitoring is also identified with an arrow in C.

Main differences between the two wood types were observed in the dynamics of sulfide enrichment on their surface. Sulfide was similarly detected on the surface of *C. nucifera* within about 6 days in the second *C. nucifera* experiment (since measurements were delayed in the first experiment, this was not observable) (Fig. 7). However, no permanent sulfidic stage (Stage III) was observed (Fig. 7A and B), conversely to what was observed on *R. mangle* (Fig. 6A and B). After a first non-sulfidic period (Stage I) lasting around 6 days, the potential strongly fluctuated denoting sulfide concentration ranging from low to non-sulfidic conditions (e.g. < 1 μM) to sulfide concentrations in the hundreds of micromolar to millimolar per liter range (corresponding to Stage IV on *R. mangle*) (Fig. 7A and B). The succession occurred within periods of hours. These fluctuating conditions lasted more than 3 weeks on the surface of *C. nucifera*, followed by a decrease of sulfide concentration below the detection limit (Stage V) beginning after 32 and 28 days of immersion in the two experiments (Fig. 5C and D)). The last stage (Stage V) corresponds to a final stage where sulfide has decreased below detection limit. This stage was only observed in the case of *C. nucifera* (Fig. 6A and B). Sulfide was still evidenced on *R. mangle* when we stopped *in situ* sulfide measurement after a much longer period (arrow in Fig. 5A and B).

The presence of most of dominant groups of wood colonizers revealed tight relationships with these temporal patterns. On *R. mangle*, the ciliate *Z. niveum* settled after the establishment of a sulfidic conditions on the substrate (i.e. after less than 4 days, beginning of Stage II) and reached its highest abundance between 10 and 60 days of immersion when the sulfide concentration has achieved its maximum (Stage III). This abundance slightly decreased at 60 days when the sulfide concentration decreased and became more fluctuating (Stage IV). On *C. nucifera*, *Z. niveum* reached its maximum in abundance (15–30 days) despite the absence of a permanent sulfidic stage (Fig. 7). The duration of the highest abundance period is reduced compared to *R. mangle*, with a decrease starting 30 days after immersion. The persistence of ciliates on *C. nucifera* in lower but still significant abundance up to 90 days whereas sulfide became undetectable after 30 days in both monitored experiments, either denotes that these sulfidic conditions are not obligatory for the colonies or that sulfidic condition still occur in some areas of the wood piece. Short-term measurements indeed confirmed that sulfide-rich patches remained locally available for thiotrophic organisms after 3 months.

Although less abundant than the previous groups, heterotrich ciliates are important wood colonizers in the mangrove swamp. These pioneer colonizers settled after only 4 days on both types of wood and they remained present throughout the experience duration. Their abundance less tightly related to sulfide levels, but similarly to the oligohymenophora ciliates, they are maximal during the period of sulfide enrichment at the surface of *C. nucifera*. However, unlike oligohymenophoran ciliates, they do not exhibit a marked preference for the highest sulfide conditions on *R. mangle*. (Stage III). This difference suggests that rather than being directly dependant on highly sulfidic conditions, they might found other advantages in the fluctuating habitat conditions encountered on *C. nucifera*.

Annelids and arthropods provide the most represented groups, after oligohymenophoran and heterotrich ciliates (Table 1). On *R. mangle*, they exhibit a distinct temporal pattern with regard to ciliates. These groups are abundant after 60 days when sulfide levels are fluctuating (Stage IV), and they are mostly excluded from the preceding high sulfide period (Stage III). This pattern suggests that they are unable to tolerate sulfide above a certain threshold or that they are constrained by oxygen availability. Oxygen-depleted conditions are indeed likely to associate with these high sulfide concentrations on the wood surface, due to microbial and chemical oxygen consumption linked with the oxidation of H_2S. Fluctuating conditions observed after 45–50 days on the surface of *R. mangle*, as well as on the surface of *C. nucifera*, revealed to be much more suitable for these organisms.

The dependence on sulfide was additionally questioned for three entoproct species potentially hosting bacterial symbioses. On *R. mangle*, these organisms are abundant around 3 weeks of immersion, and are then exposed to the highest sulfide levels (Stage III). However, the opposite is observed on *C. nucifera*, where entoprocts are only observed after 60 days when the sulfide concentration at the surface has decreased. Nematodes are occasionally present on the surface of wood and only one species, *E. dianae* (Ott et al., 2004; Himmel et al., 2009), was identified based on the aspect of its bacterial coat from the experimental substrates. In this study, the nematodes however do not exhibit a marked preference for sulfide-rich stages on wood.

4. Discussion

4.1. A succession model for wood colonizers in the mangrove swamp

Continuous monitoring evidenced successive stages, depending on the type of wood, with regard to sulfide exposure at the surface of immersed woods. Such stages can be defined as follow; initial non-sulfidic (I), transient increase in sulfide (II), permanently high sulfide conditions (III), intermittent sulfide exposure (IV) and the last stage where sulfide is no longer detected (Stage V). The most remarkable difference between woods was the absence of Stage III on *C. nucifera*.

Since the chemical characteristics of the environment vary over time, classical succession models (Connell and Slatyer, 1977) based on facilitation, tolerance and inhibition between species are not fully relevant to describe the colonization of wood. Instead widely and rapidly changing sulfide concentrations at the surface of wood appear as a primary factor influencing the diversity of dominant colonizers. The differences between *C. nucifera* and *R. mangle* colonization patterns are consistent with different sulfide enrichment dynamics, and particularly with the absence of a permanently high sulfide period on the surface of *C. nucifera*. Despite the temporal scale is considerably shorter in this study (days versus months or years) and may be confronted to the longer term degradation process, the dynamic colonization scheme proposed for organic falls in the deep-sea (e.g. Smith and Baco, 2003 for whale falls and Bienhold et al., 2013 for wood) provides an interesting comparison basis.

The whale fall model includes three successive ecological stages: the mobile scavenger stage, the opportunist stage and the sulfophilic stage. No marked mobile-scavenger stage has been identified on wood, although it is known as an important trophic group in the mangrove ecosystem (i.e. leaves consumers) (Torres-Pratts and Schizas, 2007). A strict sulfophilic stage, as defined from the predominance of organisms relying on chemoautotrophy, is observed on *R. mangle* alone. During the highly sulfidic stage, oligohymenophoran ciliates reach their maximal abundances and most other groups are poorly represented. These symbiotic organisms appeared on the wood surface only after sulfide is detected. The rapid settlement of *Z. niveum* is consistent with its strict dependence on sulfide (Ott and Bright, 2004; Rinke et al., 2007). A comparable colonization dynamics is described for the solitary vorticellid ciliate previously reported on natural sunken woods from the Guadeloupe mangrove swamp (Laurent et al., 2009) and for another vorticellid ciliate identified as *Vorticella* sp. observed on mangrove peat in Belize (Ott and Bright, 2004). Both were always associated with *Z. niveum* colonies confirming their preference for sulfidic habitat conditions. The ectosymbiotic associations described for the organisms could rely on chemoautotrophy using sulfide as an electron donor, similarly to *Z. niveum*. These ciliates are able to supply their chemoautotrophic symbionts with oxygen through a specific 'pumping' behavior (Vopel et al., 2001, 2002; 2005), and can thus sustain highly sulfidic oxygen-depleted conditions on the wood surface.

Among the other putative thiotrophic organisms, three entoprocts displayed abundances and arrangements of associated bacteria relevant to ectosymbioses, that were described for the first time for entoprocts. Their uneven relationship to sulfide however does not support the idea that these symbiotic entoprocts strictly depend on sulfide for growth. Similarly a few species of nematodes are known to form symbiosis with sulfide-oxidizing bacteria (Hentschel et al., 1999; Ott et al., 2004). The precise role of the symbionts is still undetermined, but they are generally supposed to play a detoxification role allowing them to colonize a sulfur-rich habitat toxic for most eukaryotes.

When more fluctuating sulfide conditions are established, opportunistic sulfide-tolerant groups join chemosynthetic ciliates on the substrate. Heterotrich ciliates are pioneers among these sulfide-tolerant opportunists. They do not require sulfide to settle as they are found on wood from the very first stage (Stage I), but their presence in the most sulfidic stage indicates that they can tolerate toxic sulfide and low oxygen conditions. Since ectosymbiotic associations have not been described from these groups, it can be hypothesized that these organisms feed on the wood surface where they can find organic material from decaying wood, bacteria, and protists. The higher abundance of these opportunists in the moderately sulfidic stage (Stage IV) on *C. nucifera* suggests that they could take advantage of the development of microbial mats. Other sulfide-tolerant opportunists are the sedentary polychaetes and copepods. Similarly, copepods can feed on decomposing mangrove leaves in the litter and associated bacteria (Kathiresan and Bingham, 2001; Torres-Pratts and Schizas, 2007). The co-occurrence of oligohymenophoran ciliates and these primary consumers species is conditioned by much less sulfidic conditions at the surface of this vegetal substrate.

The later colonization stage is characterized by an increased abundance of errantia polychaetes, Malacostaca, Platyhelminthes and, for *C. nucifera* alone, ascidians. These opportunists were occasionally present during the first weeks of the sulfide-rich period but their number increases after 30 or 60 days of immersion depending of the type of wood experimented. In *C. nucifera* (Fig. 7A and B) they are abundant from the 30th day of immersion while in *R. mangle* they are abundant few weeks later, corresponding to the fluctuating sulfide conditions (Fig. 6A and B). These data suggest that such invertebrates are not adapted to the habitat conditions characterized by the highest sulfide levels on wood. Many marine taxa developed biochemical or behavioral strategies to overcome sulfide toxic effects, but these adaptations may not be efficient above a certain threshold. Furthermore, oxygen depletion due to biotically or abiotically-driven sulfide oxidation in these conditions is likely and might also exert a significant constraint on these organisms. Conversely, they could tolerate intermittent exposure to

sulfide. Since they can move on the substrate, they may be able to sustain high sulfide concentrations for brief food supply avoiding a long exposure. Since the experiments were stopped before the natural end of the sulfide-rich period on *R. mangle*, the inventory of these sulfide-tolerant opportunists is however likely to be incomplete.

Interestingly, some groups, which commonly thrive in the mangrove sulfidic sediment, do not apparently find advantage from degrading wood. In particular, nematodes do not seem to be attracted by sulfide-rich habitats on wood, whereas they dominate the mangrove sediment meiofauna (Kathiresan and Bingham, 2001). This observation draws attention on the potential differences between succession models describing hard substrate epifauna and soft sediment infauna associated with organic falls, on which several previous succession studies in the deep-sea relied (Smith and Baco, 2003; Fujiwara et al., 2007; Bernardino et al., 2010).

4.2. Comparison with sunken wood colonization patterns in other marine environments

In the deep-sea, borers were thought to constitute the first level of wood communities, by converting the refractory components of the wood into available forms for microorganisms and are expected to colonize wood within months (Turner, 1977; Bienhold et al., 2013) Pholadid borers of the genus *Xylophaga* are able to colonize massive pieces of hard wood, as well as soft vegetal substrates like coconut shell or *Pandanus* fruits (Knudsen, 1961). Wood pieces immersed for about 100 days at great depth presented an advanced attack from *Xylophaga* and *Xyloredo* (Turner, 1973), and some species are able to colonize wood substrates in less than 2 weeks of immersion (Gaudron et al., 2010). Competitive advantages of various pholadids, resulting in the succession of different species on a same vegetal substrate, have been discussed in Voight (2007).

Surprisingly, not a single individual was observed on experimental substrates over 3-month. Additional observations indicated that teredinids where never found on *C. nucifera*, even after one year of immersion, suggesting that the structure of this wood might be inadequate for burrowing bivalves. Since abundant populations of teredinids are present in naturally sunken mangrove trees, this should not be the case for *R. mangle*. A plausible explanation could be the limited larvae pool in the mangrove swamp where these experiments where done. Indeed, natural sunken woods pierced by borers remain rare in this long-term experiment location (O.G. personal observations). The size of wood pieces could also have impeded larval settlement of wood borers (De Junqueira, 1991). Shallow-water species of teredinids thus appear less efficient in colonizing wood, at least in our deployment site.

These experiments additionally provide an interesting context to study the relation of shallow water sunken wood with chemosynthetic metazoans. In the deep-sea, experimental wood immersions revealed that chemosynthetic bivalves belonging to the families Thyasiridae and Vesicomyidae are able to colonize woody substrates within two weeks (Bernardino et al., 2010; Gaudron et al., 2010). Neither these groups, nor *Lucina pectinata* the only known thiotrophic bivalves in the Guadeloupe mangrove sediment (Frenkiel et al., 1996) were represented in this study. Instead, oligohymenophoran ciliates considered as representatives of "strict" chemosynthetic organisms were described. Their presence was not reported from deep-sea wood samples, but interestingly, *Z. niveum* ciliate was recently described on whale falls (Fujiwara et al., 2007). However, it should be noted that most deployment of woods in deep-waters impose strong experimental constraints and unicellular organisms are probably lost or destroyed during the recovering of samples from the seafloor. Thus, if ciliates and other unicellular can colonize wood falls in deep environment, they are unfortunately not yet described due to sampling limitations.

From this study an alternative succession model can be proposed for these two major sources of sunken wood in tropical shallow waters. The diverse communities of symbiotic organisms and microbe grazers that settle on wood within days to weeks in the mangrove swamp, while borers are absent, reflect a direct trophic relationship with degrading wood microbial communities. This includes the settlement of thiotrophic symbioses as well as sulfophilic organisms that may feed on microbial mats. Bacteria are known to colonize marine sunken woods and have been described from both inside the wood (Wirsen and Jannash, 1976; Fors et al., 2008) and on the surface (Bernardino et al., 2010; Yücel et al., 2013). The biofilm encountered on the surface of the wood was suggested to represent a carbon supply for some metazoans (Bernardino et al., 2010), but the dynamics of formation of this biofilm remains poorly known.

These sulfide-rich conditions that rapidly establish on the surface of wood can last as much as 40 days on the surface of *R. mangle* and 30 days on *C. nucifera*. Additional punctual measurements indicated that sulfide-rich patches could be sustained over at least 6 months (Laurent, unpublished data.). Further experiments could focus on the evolution of wood chemosynthetic communities during longer immersions (>6 months) on similar type of woods.

5. Conclusion

The most unexpected result in this study is the relatively rapid sulfide increase at the surface of wood in a tropical mangrove environment. In comparison sulfide was detected after only one month in aquarium conditions simulating Mediterranean deep-sea waters (13 °C) with much lower concentration at the surface of wood (Yücel et al., 2013). The pattern of wood colonization is consistent with this chemical evolution with strict autotrophic symbioses arriving first, dominating when sulfide levels are the highest, followed by less tolerant opportunistic species when sulfide levels decrease. This study shows that sunken woods in tropical shallow water follow a different colonizing scheme than proposed for deep-sea organic falls, even though common features are shared with the whale fall model of Smith and Baco (2003) such as the succession of sulfophilic organisms with opportunistic species.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2013.03.007.

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