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Abstract

Cigarette butts are the most common plastic form of litter found in the marine coast, threatening the quality of the seawater and marine life. However, the impact of cigarette butts known to contain toxic chemicals has been investigated to date in very few marine species. This study aimed to evaluate the effects of cigarette filters (smoked or unsmoked) on the microbial diversity inhabiting coastal sediments by high-throughput sequencing of the 16S rRNA genes. Both bacterial structure and metals distribution were impacted by cigarette filter addition in laboratory sediment experiments, compared to control sediment incubations without filter. Both smoked and unsmoked cigarette filters decreased pH and dissolved Cd, Mo and V concentrations in marine sediment incubations, while they increased dissolved Fe, Mn, Zn levels in the surrounding environment. Smoked filters dramatically decreased the relative abundance of the phyla *Bacteroidetes* and *Cyanobacteria*, while the members of the phyla *Gammaproteobacteria*, *Firmicutes* and *Thermotogae* were enriched by smoked filters in marine sediments. Bacterial taxa associated with deep marine environments or hydrothermal seep fields were selected by smoked cigarette filters. This study demonstrated for the first time the microbial community changes and impacts from toxic cigarette filters in coastal marine sediments.

Keywords: cigarette butt, bacteria, diversity, marine sediments, trace metals, Mediterranean Sea

1. Introduction

Cigarette butts (CB) are one of the most common plastic forms of litter found in the environment (Kadir and Sarani, 2015). From 5 to 6 trillion cigarettes were smoked worldwide every year by one billion smokers living in large majority in low- and middle-income countries (Dropp et al., 2018; Kostova et al., 2014; WHO, 2017; Zafeiridou et al., 2018). Most of them are discarded in the environment, and are transported by wind, rain, river, and marine currents to coastal areas. Nowadays, CB are the most collected item during the cleaning of beaches (Araujo and Costa, 2019; Addamo et al., 2017; Novotny et al., 2009). They can account for up to 40% of marine litter collected on beaches in some Mediterranean areas (Munari et al., 2016; Vlachogianni, 2019). CB are mainly composed of cellulose acetate, a kind of plastic, which slowly biodegrade for several years depending on environmental conditions (Benavente et al., 2019; Bonanomi et al., 2015). Moreover, CB are classified as hazardous waste according to European regulation (Rebinschung et al., 2018), mainly due to the toxic chemicals they contain, such as nicotine, metals (e.g. cadmium, arsenic) and others organic compounds derived from tobacco combustion (Shevchenko, 2012; Moriwaki et al., 2009; Moerman and Potts, 2011). Furthermore, it was estimated that a single CB could contaminate 1000 L of water (Green et al., 2014). Due to their toxicity and slow degradability, CB in marine ecosystems pose a potential human health risk through their transfer, fragmentation, accumulation in the food chain and subsequent consumption.

To date, the ecological risk due to CB pollution in marine ecosystems is largely underestimated (Kadir and Sarani, 2015). A recent review on CB pollution in coastal ecosystems has reported only a few studies involving the quantification of CB in coastal ecosystems, which are largely concentrated to American and European coasts (Araujo and Costa, 2019). Moreover, few

ecotoxicological studies have investigated the exposure and effects of CB on aquatic biota, despite the wide diversity of marine organisms. The few studies available reported that CB leachates were toxic to the marine bacterium *Aliivibrio fischeri* (formerly *Vibrio fischeri*) and the cladoceran *Ceriodaphnia* cf. *dubia* (Micevska et al., 2006), the marine fish *Atherinops affinis* (Slaughter et al., 2011), the polychaete worm *Hediste diversicolor* (Wright et al., 2015) and three intertidal snail species (Booth et al., 2015). It has been shown that CB were toxic to *A. fischeri* at 0.48 mg butts/L, and that smoked filters were more toxic than unsmoked filters (Micevska et al., 2006). However, the CB toxicity studied from a model microbial organism, such *A. fischeri*, could not predict the CB toxicity to the other microbial species found in the environment, because each species is involved in complex interactions and the marine environment presents a very diverse metabolically and phylogenetically microbial community (Micevska et al., 2006). Thus, studies using environmental samples with indigenous microorganisms are required to evaluate the effects of CB on marine ecosystems.

Microbial communities play a critical role in coastal and marine ecosystems and pollutant transfer. The toxic chemicals entering marine ecosystems can seriously modify microbial diversity and their ecological functions (Zouch et al., 2018; Johnston et al., 2009; Gillan et al., 2005). Microbial diversity can also be used as a bioindicator of contaminant stress and ecological status of coastal ecosystems, because microbial communities are very sensitive to slight changes in their surrounding environment (Aylagas et al., 2017; Sun et al., 2012). Thus, microbial richness and activity of coastal sediment could be potentially affected by CB accumulation in their environments. Despite the abundant CB accumulation in the coastal ecosystems (due to their slow degradation rate), no studies have yet evaluated their potential impact on microbial diversity of marine sediments. In addition, sediments may act as an important sink for CB and associated trace metals (e.g. cadmium, lead) trapped in the cigarette

filter before being released into the environment. The composition and content of metals can vary depending on CB (Chiba and Masironi, 1992), but only one study has evaluated the distribution of associated trace metals released by CB in the coastal environment to date (Dobaradaran et al., 2018).

This study aimed to evaluate for the first time the microbial community composition and diversity as a function of CB exposure and specifically the dissolved trace metals apparently leached into CB-contaminated sediments in an urban and highly frequented coastal environment. Here, the effects of both smoked and unsmoked cigarette filters were evaluated on microbial community structure, metal distribution and nutrient concentrations in laboratory sediment incubations. Microbial diversity was evaluated by high-throughput 16S rRNA gene sequencing analyses at the end of the 4-day experiments and compared to controls (without cigarette filter) as well as initial community.

2. Materials and Methods

2.1. Studied area

Sfax (with around 600,000 inhabitants) is the second largest city in Tunisia located on the southern coast of the Mediterranean Sea. Sfax is also located in the northern part of the Gulf of Gabès having the highest tides in the Mediterranean Sea (up to 2.3 m, Sammari et al., 2006), due to its large continental shelf with a very low slope. The southern coast of Sfax city, extending from the solar saltworks to the commercial harbor, is impacted by numerous polluting industrial sites discharging contaminants in coastal environments (Chifflet et al., 2019a; Zouch et al., 2017). The north coast, stretching for more than 10 km to the small village of Sidi Mansour, is more residential with the presence of beaches and small fishing ports, but it is affected by increasing urbanization in recent years due to rise in population. The studied area is in the northern coast of Sfax and corresponds to an urban beach intertidal area highly frequented by local people. This coastal area had a lot of waste including CB and other litter items (e.g. plastic bags and bottles, clothing, packaging) discarding by people or deposited by coastal currents and tides, wind and rains, as observed in most urban coastal areas frequented by people in Tunisia and other Mediterranean countries.

2.2. Sample collection

Surficial sediments (0–5-cm layer) were sampled in October 2018 from one location (34°46′05.2″N–10°48′49.9″E) at high tide and at 0.5 m from the shore with a plastic spatula and distributed in sterile plastic bags (~1 kg). Seawater was collected using a polyethylene pre-cleaned bottle with HCl 10% v/v (analytical grade) then thoroughly rinsed with ultrapure water (R = 18.2 MΩ.cm^{-1}). Seawater and sediment samples were kept at 4°C until use (<3h after the

field sampling). The temperature and pH were measured *in situ* using the multiparameter Odeon probe (Poncel, France).

Twelve smoked cigarette filters (SF) were immediately collected after burning and the remnant tobacco was removed from filters using sterile gloves. Twelve unsmoked cigarette filters (USF) were also separated from tobacco according to this protocol. The smoked and unsmoked cigarette filters were separately stored in metal-free polypropylene tubes (VWR) at room temperature until used.

2.3. Microcosm experiments

Three experimental conditions were run in parallel and in triplicate: sediment incubations without filter (NF; NF1, NF2, NF3), sediment incubations with addition of one unsmoked filter (USF; USF1, USF2, USF3) and sediment incubations with addition of one smoked filter (SF; SF1, SF2, SF3). For experiments, 4 g of sediment were mixed with 40 mL of 0.22-µm filtered seawater through a sterile cellulose acetate filter (Minisart, Sartorius) locked on plastic sterile syringes (i.e. solid/liquid ratio of 10% w/w). Samples were introduced into 50 mL metal-free polypropylene tubes (VWR) and gently shaken before incubating for 96 hours (i.e. 4 days, a duration defined from our previous results on sediment incubations described by Zouch et al., 2017; Zouch et al., 2018; Chifflet et al., 2019b) and under outdoor conditions (i.e. exposed to ambient light and temperature). All experiments were performed in triplicates. At the beginning of the experiments (T0), the seawater supernatant was filtered (0.22-µm) and stored in a metal-free polypropylene tubes at −20°C until chemical analyses (i.e. nutrients and metals). At the end of the experiments (Tf), the cigarette filters (smoked or unsmoked) were removed from the tubes and the seawater supernatant was filtered (0.22-µm) and stored in a metal-free polypropylene tubes at −20°C until chemical analyses (i.e. nutrients and metals). The pH of experiments was measured using a pH-meter (inoLab 7110, WTW) calibrated with three

standard buffers (pH 4.0, 7.0 and 10.0 at 20 °C). Subsamples of sediment (T0 and Tf) were also kept at −20°C for DNA extraction.

2.4. Chemical analyses

Dissolved trace metals (Al, As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sb, V, Zn) concentrations were measured by Argon Gas Dilution - Inductively Coupled Plasma - Mass Spectrometry (AGD-ICP-MS, iCAP-Q, Thermo Scientific) after samples acidification (ultra-pure HNO_3 1% v/v). Because high dissolved solid contents induce ionization suppression, the AGD technique is useful for reducing sample matrix content to about 0.2% of dissolved solids before entering the plasma (Field et al., 1999). In AGD-ICP-MS analyses, the argon (Ar) gas flow through the nebulizer is reduced while the total Ar gas flow to the plasma is maintained by the addition of a make-up Ar gas flow to the aerosol leaving the spray chamber. The sample aerosol is thereby diluted with Ar gas inside the ICP-MS sample introduction system. Analytical detection limits were below the analysed samples. Accuracy of ICP-MS measurements were controlled using certified reference nearshore seawater (CASS-6). Trace metals recoveries were between 98 and 108% except for Mn and Mo (78% and 122%, respectively; data not shown).

Inorganic nutrient concentrations were determined with a BRAN and LUEBBE Type 3 auto-analyzer according to standard methods (Tréguer and LeCorre, 1975). Dissolved Inorganic Nitrogen (DIN) is the sum of NO_2^-, NO_3^- and NH_4^+ values. Dissolved Inorganic Phosphorus (DIP) corresponds to PO_4^3 values. Dissolved Organic Nitrogen (DON) and Dissolved Organic Phosphorus (DOP) were measured after mineralization processes at high temperature (120°C). The oxidizing agents were sodium hydroxide and potassium persulfate for nitrogen and sulfuric acid and potassium persulfate for phosphorus (Aminot and Kérouel, 2007).

2.5. DNA extraction, PCR and sequencing of 16S rRNA gene fragments

DNA extraction from triplicated initial sediment samples (without filter, NF-T0) and final sediment samples (Tf) of the three experimental conditions (NF, USF, SF), performed in biological triplicate (i.e., NF1, NF2, NF3; USF1, USF2, USF3; SF1, SF2, SF3), was carried out using the UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Inc., CA), as previously described by Quéméneur et al. (2016). Bacterial and archaeal 16S rRNA gene V4 variable regions were amplified by PCR using the Pro341F/Pro805R prokaryotic universal primer set (Takahashi et al., 2014), with barcode on the forward primer, as previously described by Dowd et al. (2008), and were sequenced by the MiSeq Illumina (paired-end 2 x 300 bp) platform of the Molecular Research Laboratory (Texas, USA). Sequence data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, sequences were joined, depleted of barcodes then sequences <150bp removed, sequences with ambiguous base calls removed. Sequences were denoised, then Operational taxonomic units (OTUs) generated and chimeras removed. OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against NCBI non-redundant (NR) reference database. The 16S rRNA gene sequences of OTU have been deposited in the Genbank database under the accession numbers MN463061-MN463096.

2.6. Statistical analyses

Depending on the results of a Shapiro-Wilk normality test, experimental data were analyzed using the Analysis of Variance (ANOVA) followed by Tukey's post-hoc test or the non-parametric Kruskal-Wallis test followed by Dunn's test with Bonferroni correction to assess the effect of treatments on the nutrients, pH and trace metals, and the relative abundance of microbial taxa (classes/phyla and dominant phylotypes). *P* values of <0.05 were considered as statistically significant differences. A Spearman rank correlation test was chosen to investigate the relations among bacterial taxa and chemical parameters (showing significant differences

between treatments), and we accepted correlation coefficients (r_S) with *P* values of <0.05 as significant associations. Linear regression analyses were run to assess relationships between log-transformed response variables (bacterial classes/phyla showing significant differences between treatments) and the most discriminant explanatory variables (metals and pH). The data obtained at the beginning and at the end of the experiments were also analyzed by heatmap, Hierarchical Clustering Analysis (HCA) and Principal Component Analysis (PCA). The most discriminant variables (among metals, microbial taxa and pH) showing significant difference between the experimental conditions (i.e. *P* values of <0.05) and explaining the sample distribution on the PCA ordination were selected and represented on the biplot. All statistical analyses were performed using XLSTAT 2019.1.2 (Microsoft Excel add-in program; Addinsoft, Paris, France).

3. Results and discussion

3.1. Characteristics of initial microbial ecosystem

The initial coastal seawater used in laboratory experiments had a temperature of 20.9°C, a pH value of 8.3 ± 0.3, and variable levels of trace elements (Table 1). Nutrients were abundant with dissolved organic nitrogen (DON) and phosphorus (DOP) concentrations around 14.52 ± 1.72 µM and 8.54 ± 2.81 µM, respectively. The initial microbial diversity of the sandy surficial sediment was dominated by five bacterial phyla: *Proteobacteria* (50.4 ± 3.3%), *Bacteroidetes* (19.0 ± 0.9%), *Firmicutes* (7.5 ± 0.4%), *Cyanobacteria* (4.7 ± 2.8%), *Actinobacteria* (4.5 ± 1.3%; Figure 1). This bacterial community was diverse with only 5 abundant OTUs (>1%) belonging to classes *Cyanophyceae*, *Gammaproteobacteria* and *Flavobacteriia* (making up less than 10% of all reads). *Archaea* accounted for 7.5 ± 1.3% of the microbial community and was mainly represented by the family *Halobacteriaceae* (phylum *Euryarchaeota*; Figure 1).

3.2. Variations of physicochemical parameters in CB experiments

The evolution of nutrients, pH and trace metals in seawater between the beginning (T0) and the end (Tf) of the 96h experiments are given in Table 1. No significant nutrient difference was observed between the studied conditions ($p>0.05$). Significant pH decreases were observed in both USF (unsmoked filter) and SF (smoked filter) conditions from 8.3 ± 0.3 (NF, T0) to 7.5 ± 0.3 ($p=0.007$; USF, Tf) and 7.4 ± 0.1 ($p=0.005$; SF, Tf). On the contrary, no significant pH difference was observed between NF-T0 and NF-Tf controls (no filter; from 8.3 ± 0.3 at T0 to 8.0 ± 0.3 at Tf; $p>0.05$), indicating that CB addition decreased the pH of seawater. The variation in environmental conditions, such as pH, alters the trace metals mobility between seawater and sediments by inducing changes in dissolved trace metals concentrations (Hamzeh et al., 2014) and variations in microbial responses (Zouch et al., 2018). CB are among the most numerous

littered items in the environment and are potential sources of environmental pollution, including trace metals (Araújo and Costa, 2019; Dobaradaran et al., 2018; Novotny et al., 2011). In our experiments, different models of trace metals distribution were observed depending on cigarette filter addition (Table 1). Overall, dissolved trace metals in SF conditions were higher than USF conditions showing a higher release of trace metals from smoked than unsmoked CB. However due to specific physicochemical conditions (pH, salinity) of coastal environment and chemical behavior of trace metals, some elements (Al, As, Co, Cu, Ni, Pb and Sb) presented no significant difference between NF, USF and SF conditions at Tf. These results showed a limited impact of cigarette filters (smoked or unsmoked) on natural distribution of trace metals in this intertidal environment. Conversely, both Fe and Mn levels significantly increased in seawater at Tf in USF (277.13 ± 55.91 µg-Fe/L, $p=0.01$; 37.43 ± 4.14 µg-Mn/L, $p=0.04$) and SF conditions (376.87 ± 176.04 µg-Fe/L, $p=0.04$; 37.46 ± 1.36 µg-Mn/L, $p=0.01$), compared to controls (4.53 ± 2.81 µg-Fe/L and 8.84 ± 5.65 µg-Mn/L, respectively). To a lesser extent, a similar pattern was also observed for dissolved Zn. The increase in dissolved trace metals concentrations of some elements (Fe, Mn and Zn) in both USF and SF conditions compared to NF conditions at Tf, highlighted a trace metals inputs from cigarette filters in coastal environment. These results agreed with Moerman and Potts (2011) which showed that Fe, Mn and Zn in CB were highly leached (10%, 23% and 19% respectively) in water. On the contrary, significant loss of dissolved Cd was observed at Tf in both USF (0.010 ± 0.002 µg/L) and SF conditions (0.018 ± 0.005 µg/L) compared to NF controls (0.300 ± 0.103 µg/L; $p<0.02$). Similarly, high decrease in dissolved V were measured at Tf in both USF (1.80 ± 0.15 µg/L) and SF conditions (2.02 ± 0.23 µg/L) compared to controls (6.31 ± 0.35 µg/L; $p<0.02$). To a lesser extent, a similar pattern was also observed for dissolved Mo. The decrease in dissolved trace metals (Cd, Mo and V) concentrations may be due to adsorption processes on CB. Indeed, cigarette filters (made of cellulose acetate) are a synthetic polymer which may behave like other plastics exporting trace

metals in the marine environment (Ashton et al., 2010; Holmes et al., 2012; Holmes et al., 2014).

3.3. Microbial diversity in CB experiments

The microbial diversity in sediment according to USF conditions (unsmoked filter), SF conditions (smoked filter) and NF controls (no filter) were evaluated at Tf (96h) and compared to the initial microbial diversity in sediment (NF controls at T0). Changes in overall microbial structure were observed at the phylum/class level depending on experimental conditions (Figure 1). Significant differences between treatments ($p<0.05$) were detected in the relative abundance of six bacterial taxa: *Alphaproteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Gammaproteobacteria*, *Firmicutes* and *Thermotogae* (Figure 2). A shift in relative abundance of microbial families was also observed depending on experimental conditions (Figure 3).

Both USF and SF conditions were dominated by *Proteobacteria* (40.2 ± 1.4% and 53.4 ± 2.7% of total reads) and *Firmicutes* (21.5 ± 1.2% and 28.6 ± 2.3%; Figure 1). Among *Proteobacteria*, *Gammaproteobacteria* were abundant in both USF and SF conditions, but largely predominant in SF conditions (43.7 ± 2.6% of total reads), especially *Alteromonadaceae* and *Vibrionaceae* families, indicating they were enriched by CB addition in marine sediment. Interestingly, the proportions of *Thermotogae* (represented by *Petrotogaceae* family) significantly increased with smoked filter addition (8.6 ± 1.9% in SF vs. <0.5% of total reads in controls, $p=0.007$; Figures 2 and 3). Furthermore, the relative abundance of *Firmicutes* (represented by *Clostridiaceae* and *Bacillaceae* families) was 2.3 times higher in SF conditions than in controls ($p=0.01$). On the contrary, the relative abundance of *Bacteroidetes* (*Flavobacteriaceae, Sphingobacteriaceae* and *Saprospiraceae*) dramatically decreased in SF conditions (2.6 ± 1.1%), compared to USF conditions (15.4 ± 3.4%, $p=0.045$) and controls (21.2 ± 8.2%, $p=0.017$). Similarly, the relative abundance of *Alphaproteobacteria* (*Rhodobacteraceae*) was significantly lower in SF

conditions (5.7 ± 2.6%) than in USF conditions (14.0 ± 1.3%, p=0.007) and controls (15.0 ± 3.3%, p=0.027). The relative abundance of *Cyanobacteria* (*Chroococcales* and *Oscillatoriales*) were also 10 to 60 times lower in USF and SF conditions (0.74 ± 0.03 and 0.12 ± 0.03%, respectively) than in controls (7.3 ± 2.0%; p<0.02). These results indicated that growth of *Alphaproteobacteria*, *Bacteroidetes* and cyanobacterial species may be inhibited by CB, while growth of some species belonging to *Gammaproteobacteria*, *Firmicutes* and *Thermotogae* may be enhanced by cigarette filter addition. No significant change in relative abundance of *Archaea* (ranging from 3.3 to 10.9% of total reads and mainly represented by *Halobacteriaceae*) was observed in sediment depending of CB addition (p>0.05).

A Principal Component Analysis (PCA) was performed to identify the factors that affect the microbial community at the beginning and at the end of the experiment (Figure 4). The first two principal components explained 71.2% of the variability in the data. Two groups (with or without cigarette filter) were identified by Hierarchical Clustering Analysis (HCA). Correlations of the key bacterial taxa (classes/phyla) with environmental variables showed some dependencies on metal distribution and pH (Table S1). Significant and positive correlations were observed between the *Firmicutes* or *Thermotogae* proportions and dissolved Fe and Mn (r_s>0.72, p<0.05). In contrast, the proportions of *Bacteroidetes* and *Cyanobacteria* were significantly and negatively correlated with Fe and Mn (p<0.05). Their relative abundances were also significantly correlated with pH values (negatively for *Firmicutes* and *Thermotogae* and positively for *Bacteroidetes* and *Cyanobacteria*; p<0.05). However, such discriminant chemicals parameters (Fe, Mn and pH) were not identified as significant determinants of bacterial diversity by our multiple regression models (except Mn for *Cyanobacteria*, Table S2), suggesting that other unmeasured environmental parameters (e.g. organic compounds) could play a key role in shaping bacterial communities.

3.4. Dominant bacterial species in CB experiments

The number of abundant species (dominant OTU >1% of total reads) in sediment increased over time in 96h incubations, but it was significantly lower in SF conditions (accounting for 77.0 ± 3.6%) than in NF controls (making up 42.0 ± 8.3%) at TF ($p<0.05$).

In USF conditions (unsmoked filter), dominant OTUs at TF were mainly affiliated to *Firmicutes* (15.5 ± 8.7%) and *Fusobacteria* (12.7 ± 8.9%; Table S1). The majority of *Firmicutes* OTU showed significant difference between treatments ($p<0.05$; Table S3). They were grouped in two families (*Bacillaceae* and *Clostridiaceae*) and related to fermentative *Exiguobacterium*, *Alkaliphilus* and *Vallitalea* species isolated from marine and/or hydrothermal ecosystems (Ben Aissa et al., 2014; Ben Aissa et al., 2015; Kim et al., 2005). The dominant fusobacterial OTU2 was affiliated to the thermophilic and fermentative *Hypnocyclicus thermotrophus* isolated from a microbial mat sampled near a hydrothermal vent in the Greenland Sea (Roalkvam et al., 2015). Others dominant species were affiliated to *Alphaproteobacteria* (e.g. *Roseibacterium* genus) and *Gammaproteobacteria* (e.g. *Marichromatium* genus), whose relative abundance increased significantly with the addition of unsmoked filter ($p<0.05$). Unlike controls, *Cyanobacteria* (particularly the families *Chroococcales* and *Oscillatoriales*) were not represented among dominant species in USF conditions.

In SF conditions (smoked filter), abundant OTUs were mainly assigned to *Gammaproteobacteria*, followed by *Firmicutes*, *Alphaproteobacteria*, *Epsilonproteobacteria*, *Halobacteria* and *Thermotogeae* (Table S4). As detected in controls (without cigarette filter), *Gammaproteobacteria* were dominated by *Marinobacter* and *Marinobacterium* spp., as well as *Idiomarina* and *Oceanimonas* spp., but *Vibrio* spp. (accounting for 13.5 ± 2.3% of SF reads) were only predominant in presence of CB. The abundant OTU11 was closely related to *Vibrio diabolicus* (99.0% 16S rRNA sequence similarity), a mesophilic and polysaccharide-secreting bacterium isolated from a deep-sea hydrothermal field in the East Pacific Rise (Raguénès et al.,

1997). The other abundant and gammaproteobacterial phylotypes enriched by CB were closely affiliated with *Vibrio owensii* (100% similarity with OTU647 and OTU1430), isolated from diseased cultured crustaceans (Cano-Gomez et al., 2010), and *Vibrio harveyi* (98.9% similarity with OTU4626), a model marine and bioluminescent microorganism known as pathogen of aquatic fauna (Wang et al., 2015). Among *Firmicutes*, three OTU developed well in presence of CB and were affiliated to mesophilic and fermentative bacterial species belonging to genera *Exiguobacterium* (99.5% similarity), *Alkaliphilus* (92.7% similarity) and *Serpentinicella* (98.4% similarity). These species were isolated from deep South Africa gold mine and serpentinite-hosted Prony hydrothermal field in New Caledonia (Takai et al., 2001; Ben Aissa et al., 2015; Mei et al., 2016). Two others dominant phylotypes were only retrieved in presence of CB and were affiliated to the classes *Epsilonproteobacteria* (*Arcobacter halophilus*, 99% similarity) and *Thermotogeae* (family *Petrotogaceae* accounting for 8.6 ± 1.8% of SF reads represented by *Geotoga subterranea*, 99.0% similarity with OTU5). The relative abundance of this OTU5 increased significantly with the addition of smoked filter ($p<0.05$). The moderately thermophilic and fermentative *Geotoga subterranea*, was isolated from brines collected from oil fields in USA (Davey et al., 1993), while *Arcobacter halophilus* was isolated from hypersaline lagoon water in the Hawaiian Islands (Donachie et al., 2005) and also found in industrially multi-contaminated coastal sediment (Zouch et al., 2017).

No *Cyanobacteria* and *Bacteroidetes*, which are important in photosynthetic activities and organic matter degradation, respectively, were represented among dominant species in sediment incubations with CB (SF conditions, Table S2). The absence of such key microbial groups among dominant bacteria in our experiments with smoked filters could be explained by the release of toxic chemical compounds inhibiting their respective cell growth into surrounding medium. However, *Bacteroidetes* species, particularly those belonging to the class *Flavobacteria*, constitute a major component of the bacterial community in metal- and oil-

contaminated marine sediment (Zouch et al., 2018; Kappell et al., 2014). In marine ecosystems, *Bacteroidetes* were also known to growth attached to particles, surfaces or algal cells (Fernandez-Gomez et al., 2013). Moreover, *Cyanobacteria* were also overrepresented on plastic debris compared to the surrounding free-living and organic particle-attached fractions in 'plastisphere' studies (Jacquin et al., 2019). Thus, the depletion of *Cyanobacteria* and *Bacteroidetes* from sediment fraction might be also explained by: (i) the potential death of sensitive algal biomass and *Cyanobacteria*, causing hypoxia and providing competitive advantage for *Firmicutes* against *Bacteroidetes* in sediment, and/or (ii) a potential colonization of CB by *Cyanobacteria* and *Bacteroidetes*. These latter assumptions remain to be verified through future experiments analyzing both sediment and CB fractions, as previously investigated in microbial colonization studies of plastic and microplastic (Harrison et al., 2014; Dussud et al., 2018; Jacquin et al., 2019).

4. Conclusions

This study showed that CB changed the microbial diversity of coastal marine sediment, and the physicochemical parameters of its surrounding environment (e.g. pH, trace metals). Cigarette filter addition decreased the pH of seawater and the concentrations of dissolved Cd, Mo and V, whereas they increased dissolved Fe, Mn and Zn concentrations in coastal ecosystem. We also found that smoked filter addition to coastal sediment led to a depletion in *Cyanobacteria* and heterotrophic *Bacteroidetes* (especially *Flavobacteriaceae* and *Saprospiraceae*), with a concomitant enrichment in heterotrophic *Gammaproteobacteria* (*Alteromonadaceae* and *Vibrionaceae*), *Firmicutes* (*Bacillaceae and Clostridiaceae*), and *Thermotogae* (*Petrotogaceae*) related to deep and/or hydrothermal ecosystems and adapted to extreme conditions (e.g. high temperature and metals concentrations). The changes in bacterial

community diversity, and selection of specific groups by cigarette filter addition, support the need to evaluate the effect of potential toxic substances on global bacterial diversity (e.g. 16S rRNA PCR tests), in addition to test model marine bacterial species (e.g. *A. fischeri* bioluminescence inhibition test) in future development of ecotoxicology studies.

Acknowledgments

This project was financially supported by IRD (French National Research Institute for Sustainable Development) in the framework of the French-Tunisian International Joint Laboratory "LMI COSYS-Med". The project leading to this publication has received funding from European FEDER Fund under project 1166-39417. We thank L. Causse from the AETE-ISO platform (OSU/OREME, Université de Montpellier) for performing trace metals analysis in seawater samples.

Legends of Figures

Figure 1. Composition of microbial communities at the phylum or class level in the sediment at the beginning (T0) and (Tf) of experiments without filter (NF, control), with unsmoked filter (USF) and smoked filter (SF).

Figure 2. Comparison of the relative abundance of selected bacterial taxa (showing significant difference between treatments) in the sediment between the beginning (T0) and (Tf) of incubations without filter (NF, control), with unsmoked filter (USF) and smoked filter (SF). Values are means of abundance data from biological triplicates ± standard deviation.

Figure 3. Heat map showing the relative abundance of the families in the sediment at the beginning (T0) and (Tf) of experiments without filter (NF, control), with unsmoked filter (USF) and smoked filter (SF). Each column represents an experimental condition and each row represents a family. The colour intensity for each panel corresponds to the family abundance, red indicates high level of relative abundance, while yellow indicates low relative abundance. The dendrogram was constructed from the family abundance table using Euclidean distance. The scale represents 10% dissimilarity.

Figure 4. Principal Component Analysis (PCA) biplot showing the variation among the data (showing significant difference, i.e. with P values < 0.05) obtained from the beginning (T0) and (Tf) of experiments without filter (NF, control), with unsmoked filter (USF) and smoked filter (SF). White and black circles represent NF experiments at T0 and Tf, black square represent USF experiments and black triangles represent SF experiments. Ellipses represent the clusters

identified with Hierarchical Clustering Analysis (HCA). Arrows indicate the direction of maximum increase and strength (through the length) of each variable to the overall distribution. The blue arrows indicate metals and pH, and the red arrows represent the microbial taxa.

References

Addamo, A.M., Laroche, P., Hanke, G., 2017. Top Marine Beach Litter Items in Europe: a review and synthesis based on beach litter data, EUR 29249 EN, Publications Office of the European Union, Luwembourg, ISBN 978-92-79-87711-7, doi:10.2760/496717, JRC108181.

Aminot, A., & Kérouel, R., 2007. Dosage automatique des nutriments dans les eaux marines : méthodes en flux continu. Editions Quae.

Araújo, M. C. B., & Costa, M. F., 2019. A critical review of the issue of cigarette butt pollution in coastal environments. *Environ. Res*., 172:137-149.

Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. *Mar. Pollut. Bull.,* 60:2050–5.

Aylagas, E., Borja, Á., Tangherlini, M., Dell'Anno, A., Corinaldesi, C., Michell, C. T., Irigoien, X., Danovaro, R., Rodríguez-Ezpeleta, N., 2017. A bacterial community-based index to assess the ecological status of estuarine and coastal environments. *Mar. Pollut. Bull*., *114*(2), 679-688.

Ben Aissa, F. B., Postec, A., Erauso, G., Payri, C., Pelletier, B., Hamdi, M., Ollivier, B., Fardeau, M.-L., 2014. *Vallitalea pronyensis* sp. nov., isolated from a marine alkaline hydrothermal chimney. *Int. J. Syst. Evol. Micr.*, *64*(4), 1160-1165.

Ben Aissa, F. B., Postec, A., Erauso, G., Payri, C., Pelletier, B., Hamdi, M., Fardeau, M. L., Ollivier, B., 2015. Characterization of *Alkaliphilus hydrothermalis* sp. nov., a novel alkaliphilic anaerobic bacterium, isolated from a carbonaceous chimney of the Prony hydrothermal field, New Caledonia. *Extremophiles*, *19*(1), 183-188.

Booth, D. J., Gribben, P., Parkinson, K., 2015. Impact of cigarette butt leachate on tidepool snails. *Mar. Pollut. Bull.*, *95*(1), 362-364.

Benavente, M. J., Caballero, M. J. A., Silvero, G., López-Coca, I., Escobar, V. G., 2019. Cellulose Acetate Recovery from Cigarette Butts. In *Multidisciplinary Digital Publishing Institute Proceedings* (Vol. 2, No. 20, p. 1447).

Cano-Gomez, A., Goulden, E. F., Owens, L., Høj, L., 2010. *Vibrio owensii* sp. nov., isolated from cultured crustaceans in Australia. *FEMS Microbiol. Lett.*, *302*(2), 175-181.

Chiba M., & Masironi R., 1992. Toxic and trace elements in tobacco and tobacco smoke. *Bull. World Health Org.*, 70(2), 269-275.

Chifflet, S., Tedetti, M., Zouch, H., Fourati, R., Zaghden, H., Elleuch, B., Quéméneur, M., Karray, F., Sayadi, S. (2019a) Dynamics of trace metals in a shallow coastal ecosystem: insights from the Gulf of Gabès (southern Mediterranean Sea). AIMS Environ. Sci., 6(4): 277–297.

Chifflet, S., Quéméneur, M., Barani, A., Angeletti, B., Didry, M., Grégori, G., Pradel, N. (2019b). Impact of sterilization methods on dissolved trace metals concentrations in complex natural samples: optimization of UV irradiation. *MethodsX*, *6*, 1133-1146.

Davey, M. E., Wood, W. A., Key, R., Nakamura, K., Stahl, D. A., 1993. Isolation of three species of *Geotoga* and *Petrotoga*: two new genera, representing a new lineage in the bacterial line of descent distantly related to the "*Thermotogales*". *Syst. Appl. Microbiol.*, *16*(2), 191-200.

Dobaradaran, S., Schmidt, T. C., Nabipour, I., Ostovar, A., Raeisi, A., Saeedi, R., Khorsand, M., Khajeahmadi, N., Keshtkar, M., 2018. Cigarette butts abundance and association of mercury and lead along the Persian Gulf beach: an initial investigation. *Environ. Sci. Pollut. R.*, *25*(6), 5465-5473.

Donachie, S. P., Bowman, J. P., On, S. L., Alam, M., 2005. *Arcobacter halophilus* sp. nov., the first obligate halophile in the genus *Arcobacter*. *Int. J. Syst. Evol. Micr.*, *55*(3), 1271-1277.

Dowd, S. E., Callaway, T. R., Wolcott, R. D., Sun, Y., McKeehan, T., Hagevoort, R. G., Edrington, T. S., 2008. Evaluation of the bacterial diversity in the feces of cattle using 16S

rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiol.*, *8*(1), 125.

Drope J, Schluger N, Cahn Z, Drope J, Hamill S, Islami F, Liber A, Nargis N, Stoklosa M., 2018. The Tobacco Atlas. Atlanta: American Cancer Society and Vital Strategies

Dussud, C., Hudec, C., George, M., Fabre, P., Higgs, P., Bruzaud, S., et al., 2018. Colonization of non-biodegradable and biodegradable plastics by marine microorganisms. *Front. Microbiol.*, *9*, 1571.

Fernández-Gomez, B., Richter, M., Schüler, M., Pinhassi, J., Acinas, S. G., González, J. M., Pedros-Alio, C., 2013. Ecology of marine *Bacteroidetes*: a comparative genomics approach. *ISME J.*, *7*(5), 1026.

Gillan, D. C., Danis, B., Pernet, P., Joly, G., Dubois, P., 2005. Structure of sediment-associated microbial communities along a heavy-metal contamination gradient in the marine environment. *Appl. Environ. Microbiol.*, *71*(2), 679-690.

Green, A. L. R., Putschew, A., Nehls, T., 2014. Littered cigarette butts as a source of nicotine in urban waters. *J. Hydrol.*, *519*, 3466-3474.

Field M.P., Cullen J.T., Sherrell R.M., 1999. Direct determination of 10 trace metals in 50 μL samples of coastal seawater using desolvating micronebulization sector field ICP-MS. *J. Anal. Atom. Spectrom.*, 14, 1425-1431.

Hamzeh M., Ouddane B., Daye M., Halwani J., 2014. Trace metal mobilization from surficial sediments of the Seine river estuary. *Water Air Soil Poll.*, 225, 1878-1893.

Harrison, J. P., Schratzberger, M., Sapp, M., Osborn, A. M., 2014. Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. *BMC Microbiol.*, *14*(1), 232.

Holmes, L. A., Turner, A., Thompson, R. C., 2014. Interactions between trace metals and plastic production pellets under estuarine conditions. *Mar. Chem.*, 167:25–32.

Holmes, L. A., Turner, A., Thompson, R.C., 2012. Adsorption of trace metals to plastic resin pellets in the marine environment. *Environ. Pollut.*, 160:42–8.

Jacquin, J., Cheng, J., Odobel, C., Pandin, C., Conan, P., Pujo-pay, M., Barbe, V., Meisterzheim, A.-L., Ghiglione, J.-F., 2019. Microbial ecotoxicology of marine plastic debris: a review on colonization and biodegradation by the 'plastisphere'. *Front. Microbiol.*, *10*, 865.

Johnston, E. L., & Roberts, D. A., 2009. Contaminants reduce the richness and evenness of marine communities: a review and meta-analysis. *Environ. Pollut.*, *157*(6), 1745-1752.

Kadir, A. A., & Sarani, N. A., 2015. Cigarette butts pollution and environmental impact–A review. In *Applied Mechanics and Materials* (Vol. 773, pp. 1106-10). Trans Tech Publications.

Kappell, A. D., Wei, Y., Newton, R. J., Van Nostrand, J. D., Zhou, J., McLellan, S. L., Hristova, K. R., 2014. The polycyclic aromatic hydrocarbon degradation potential of Gulf of Mexico native coastal microbial communities after the Deepwater Horizon oil spill. *Front. Microbiol.*, *5*, 205.

Kim, I. G., Lee, M. H., Jung, S. Y., Song, J. J., Oh, T. K., Yoon, J. H., 2005. *Exiguobacterium aestuarii* sp. nov. and *Exiguobacterium marinum* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int. J. Syst. Evol. Micr.*, *55*(2), 885-889.

Mei, N., Postec, A., Erauso, G., Joseph, M., Pelletier, B., Payri, C., Ollivier, B., Quéméneur, M., 2016. *Serpentinicella alkaliphila* gen. nov., sp. nov., a novel alkaliphilic anaerobic bacterium isolated from the serpentinite-hosted Prony hydrothermal field, New Caledonia. *Int. J. Syst. Evol. Micr.,* 66: 4464-4470.

Micevska, T., Warne, M. S. J., Pablo, F., Patra, R., 2006. Variation in, and causes of, toxicity of cigarette butts to a cladoceran and microtox. *Arch. Environ. Con. Tox.*, *50*(2), 205-212.

Moerman, J. W., & Potts, G. E., 2011. Analysis of metals leached from smoked cigarette litter. *Tob. Control*, *20*(Suppl 1), i30-i35.

Moriwaki, H., Kitajima, S., Katahira, K., 2009. Waste on the roadside, 'poi-sute'waste: its distribution and elution potential of pollutants into environment. *Waste manage.*, *29*(3), 1192-1197.

Novotny, T., Lum, K., Smith, E., Wang, V., Barnes, R., 2009. Cigarettes butts and the case for an environmental policy on hazardous cigarette waste. *Int. J. Environ. Res. Public health*, *6*(5), 1691-1705.

Novotny, T. E., Hardin, S. N., Hovda, L. R., Novotny, D. J., McLean, M. K., Khan, S., 2011. Tobacco and cigarette butt consumption in humans and animals. *Tob. Control*, *20*(Suppl 1), i17-i20.

Quéméneur, M., Garrido, F., Billard, P., Breeze, D., Leyval, C., Jauzein, M., Joulian, C., 2016. Bacterial community structure and functional *arrA* gene diversity associated with arsenic reduction and release in an industrially contaminated soil. *Geomicrobiol. J.* 33, 839–849.

Raguénès, G., Christen, R., Guezennec, J., Pignet, P., Barbier, G., 1997. *Vibrio diabolicus* sp. nov., a new polysaccharide-secreting organism isolated from a deep-sea hydrothermal vent polychaete annelid, *Alvinella pompejana*. *Int. J. Syst. Evol. Micr.*, *47*(4), 989-995.

Roalkvam, I., Bredy, F., Baumberger, T., Pedersen, R. B., Steen, I. H., 2015. *Hypnocyclicus thermotrophus* gen. nov., sp. nov. isolated from a microbial mat in a hydrothermal vent field. *Int. J. Syst. Evol. Micr.*, *65*(12), 4521-4525.

Rebischung, F., Chabot, L., Biaudet, H., Pandard, P., 2018. Cigarette butts: A small but hazardous waste, according to European regulation. *Waste manage.*, *82*, 9-14.

Sammari, C., Koutitonsky, V.G., Moussa, M., 2006. Sea level variability and tidal resonance in the Gulf of Gabes, Tunisia. *Cont. Shelf Res.* 26, 338–350.

Slaughter, E., Gersberg, R. M., Watanabe, K., Rudolph, J., Stransky, C., Novotny, T. E., 2011. Toxicity of cigarette butts, and their chemical components, to marine and freshwater fish. *Tob. Control*, *20*(Suppl 1), i25-i29.

Sun, M. Y., Dafforn, K. A., Brown, M. V., Johnston, E. L., 2012. Bacterial communities are sensitive indicators of contaminant stress. *Mar. Pollut. Bull.* 64, 1029–1038.

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., Nishijima, M., 2014. Development of a prokaryotic universal primer for simultaneous analysis of bacteria and archaea using next-generation sequencing. *PloS one*, *9*(8), e105592.

Takai, K., Moser, D. P., Onstott, T. C., Spoelstra, N., Pfiffner, S. M., Dohnalkova, A., Fredrickson, J. K., 2001. *Alkaliphilus transvaalensis* gen. nov., sp. nov., an extremely alkaliphilic bacterium isolated from a deep South African gold mine. *Int. J. Syst. Evol. Micr.*, *51*(4), 1245-1256.

Treguer, P., & LeCorre, P., 1975. Manuel D'Analyse des sels nutritifs dans l'eau de mer (Utilisation de l'Autoanalyser II Technicon). *Lab. d'Oceanologie Chim., Univ. de Bretagne Occidentale, Brest, France*.

Vlachogianni, Th., 2019. Marine Litter in Mediterranean coastal and marine protected areas – How bad is it. A snapshot assessment report on the amounts, composition and sources of marine litter found on beaches, Interreg Med ACT4LITTER & MIO-ECSDE.

Wang, Z., Hervey, W. J., Kim, S., Lin, B., Vora, G. J., 2015. Complete genome sequence of the bioluminescent marine bacterium *Vibrio harveyi* ATCC 33843 (392 [MAV]). *Genome Announc.*, *3*(1), e01493-14.

WHO Report on the Global Tobacco Epidemic, 2017: Monitoring tobacco use and prevention policies. Geneva: World Health Organization; 2017.

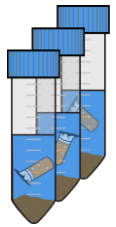
Wright, S. L., Rowe, D., Reid, M. J., Thomas, K. V., Galloway, T. S., 2015. Bioaccumulation and biological effects of cigarette litter in marine worms. *Sci. Rep.*, *5*, 14119.

Zafeiridou, M., Hopkinson, N.S., Voulvoulis, N., 2018. Cigarette smoking: an assessment of tobacco's global environmental footprint across its entire supply chain, and policy strategies to reduce it. Geneva: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO.

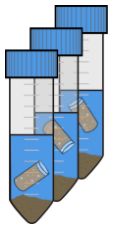
Zouch, H., Karray, F., Armougom, F., Chifflet, S., Hirschler-Réa, A., Kharrat, H., Kamoun, L., Ben Hania, W., Ollivier, B., Sayadi, S., Quéméneur, M., 2017. Microbial Diversity in Sulfate-Reducing Marine Sediment Enrichment Cultures Associated with Anaerobic Biotransformation of Coastal Stockpiled Phosphogypsum (Sfax, Tunisia). *Front. Microbiol.*, *8*, 1583.

Zouch, H., Cabrol, L., Chifflet, S., Tedetti, M., Karray, F., Zaghden, H., Sayadi, S. & Quéméneur, M., 2018. Effect of acidic industrial effluent release on microbial diversity and trace metal dynamics during resuspension of coastal sediment. *Front. Microbiol.*, *9*, 3103.

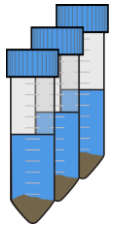
Figure 1



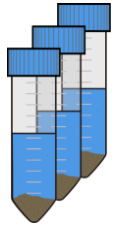
SF (Tf)
Final sediment with smoked filter



USF (Tf)
Final sediment with unsmoked filter



NF (Tf)
Final sediment without filter (control)



NF (T0)
Initial sediment without filter (control)

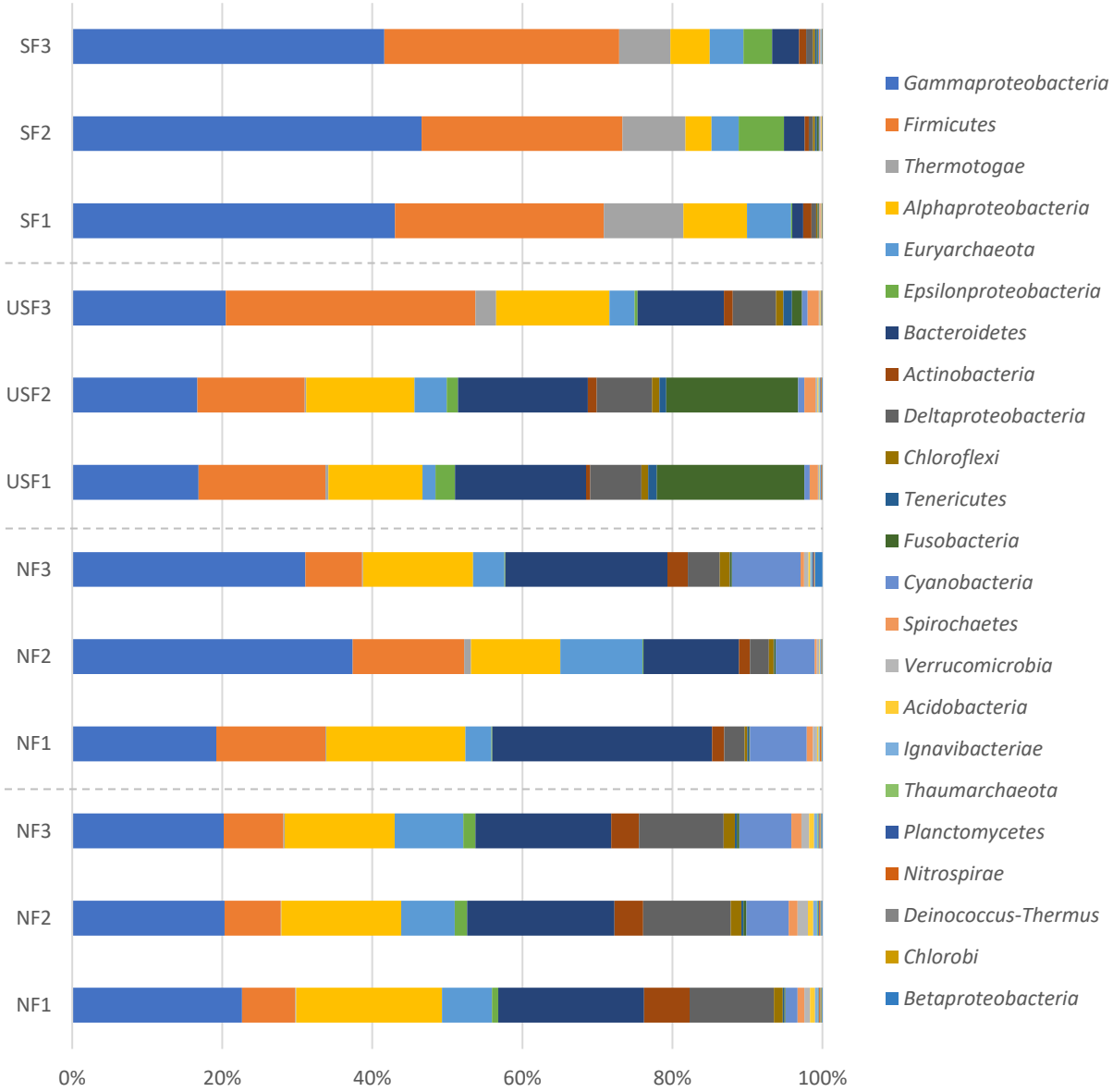


Figure 2

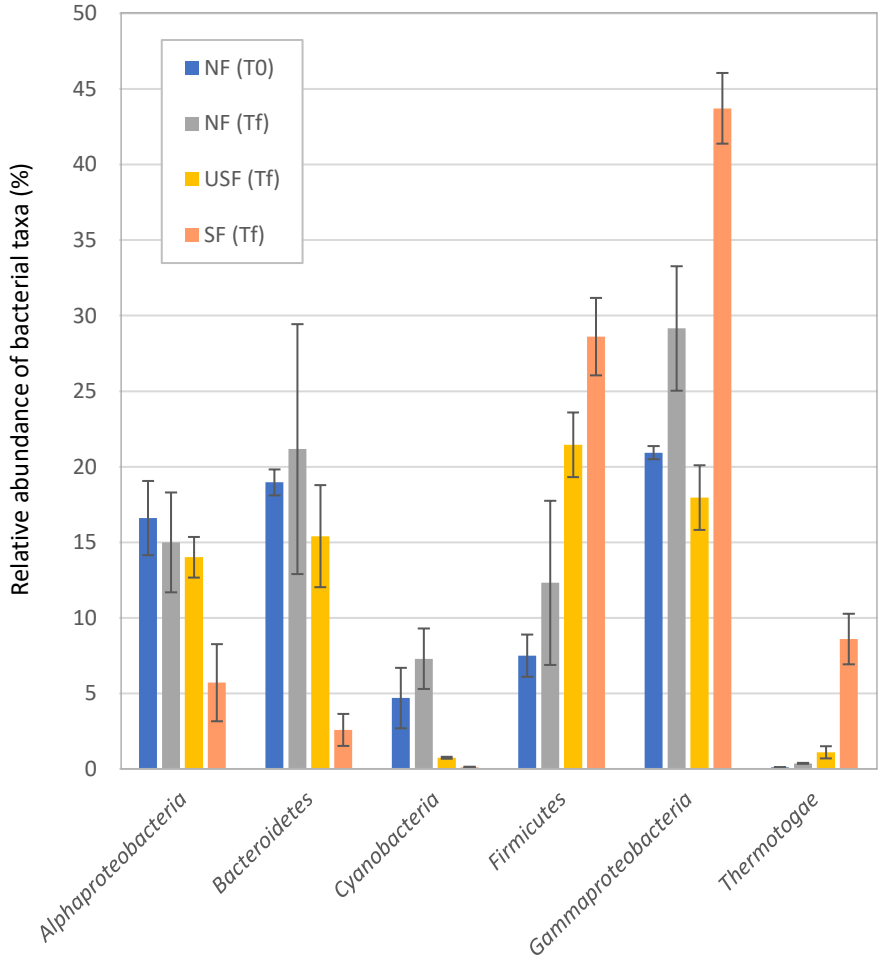

Relative abundance of bacterial taxa (%)
50
45
40
35
30
25
20
15
10
5
0
NF (T0)
NF (Tf)
USF (Tf)
SF (Tf)
Alphaproteobacteria
Bacteroidetes
Cyanobacteria
Firmicutes
Gammaproteobacteria
Thermotogae

Figure 3

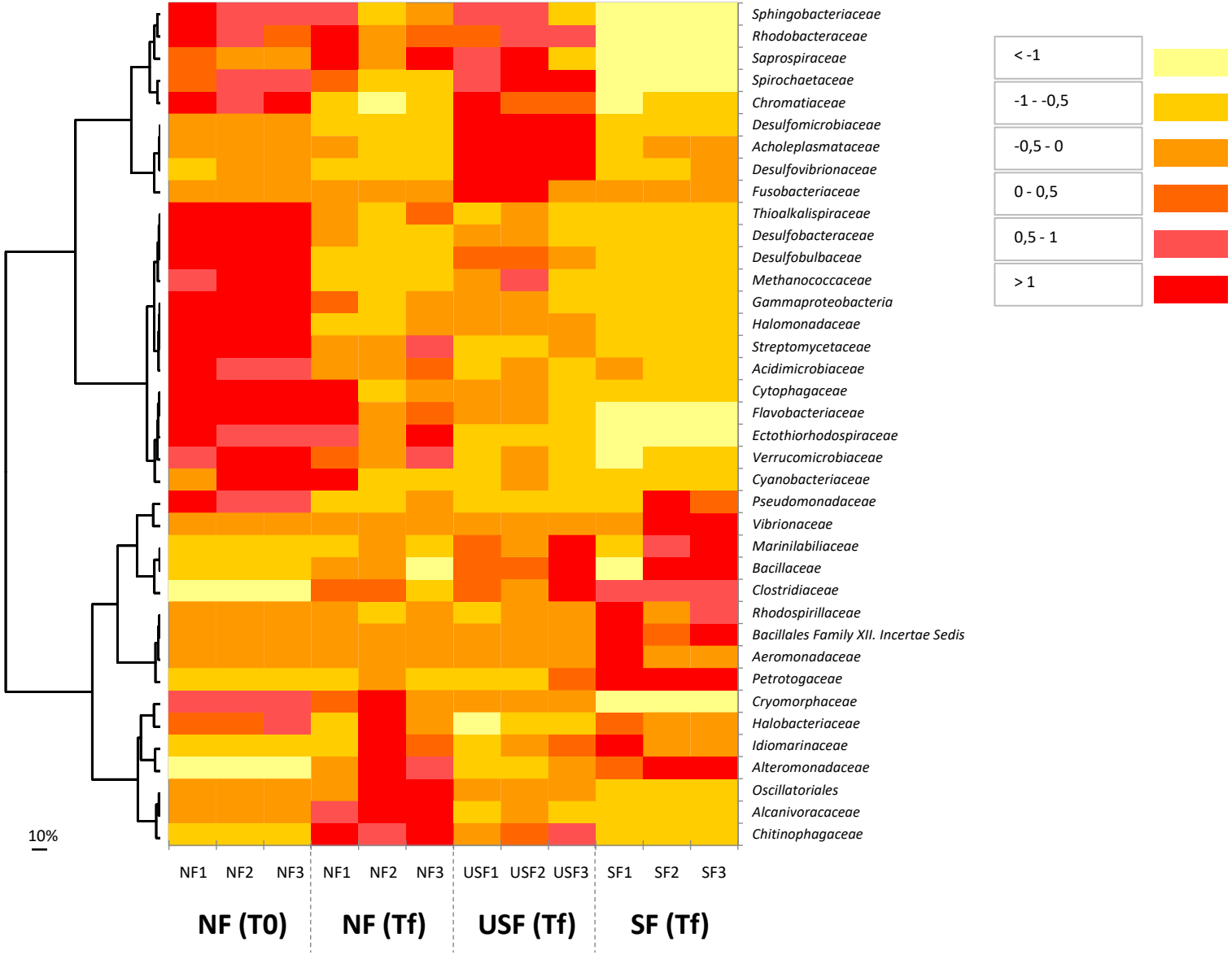

Sphingobacteriaceae
Rhodobacteraceae
Saprospiraceae
Spirochaetaceae
Chromatiaceae
Desulfomicrobiaceae
Acholeplasmataceae
Desulfovibrionaceae
Fusobacteriaceae
Thioalkalispiraceae
Desulfobacteraceae
Desulfobulbaceae
Methanococcaceae
Gammaproteobacteria
Halomonadaceae
Streptomycetaceae
Acidimicrobiaceae
Cytophagaceae
Flavobacteriaceae
Ectothiorhodospiraceae
Verrucomicrobiaceae
Cyanobacteriaceae
Pseudomonadaceae
Vibrionaceae
Marinilabiliaceae
Bacillaceae
Clostridiaceae
Rhodospirillaceae
Bacillales Family XII. Incertae Sedis
Aeromonadaceae
Petrotogaceae
Cryomorphaceae
Halobacteriaceae
Idiomarinaceae
Alteromonadaceae
Oscillatoriales
Alcanivoracaceae
Chitinophagaceae
< -1
-1 - -0,5
-0,5 - 0
0 - 0,5
0,5 - 1
> 1
10%
NF1 NF2 NF3 NF1 NF2 NF3 USF1 USF2 USF3 SF1 SF2 SF3
NF (T0)
NF (Tf)
USF (Tf)
SF (Tf)

Figure 4

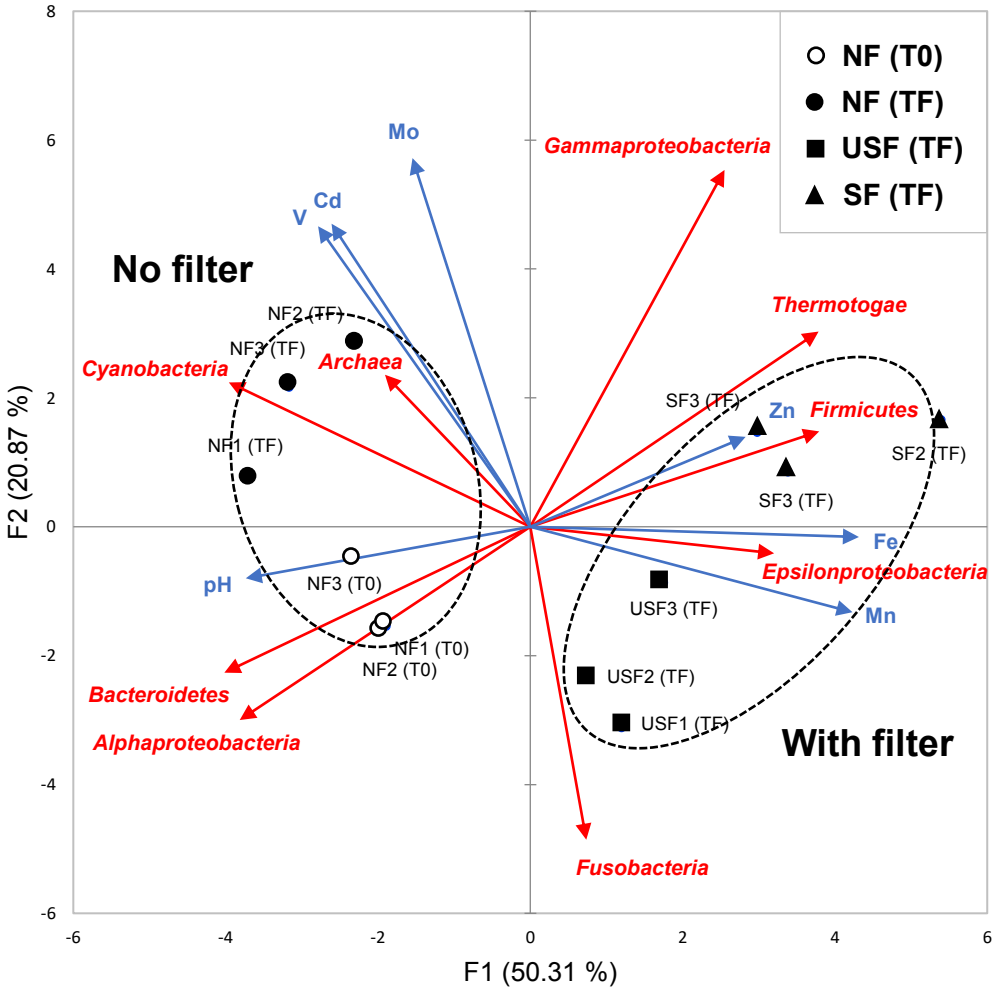

NF (T0)
NF (TF)
USF (TF)
SF (TF)
No filter
With filter
Mo
Cd
V
Gammaproteobacteria
Thermotogae
Cyanobacteria
Archaea
Zn
Firmicutes
Fe
Epsilonproteobacteria
Mn
pH
Bacteroidetes
Alphaproteobacteria
Fusobacteria
NF2 (TF)
NF3 (TF)
NF1 (TF)
SF3 (TF)
SF2 (TF)
SF3 (TF)
NF3 (T0)
NF1 (T0)
NF2 (T0)
USF3 (TF)
USF2 (TF)
USF1 (TF)
F2 (20.87 %)
F1 (50.31 %)
8
6
4
2
0
-2
-4
-6
-6
-4
-2
0
2
4
6

Table 1. Chemical properties (pH) and dissolved parameters (nutrients and trace metals) measured at the beginning (T0) and at the end (Tf) of experiments without filter (NF, control), with unsmoked filter (USF) and smoked filter (SF). Values are means of data from biological triplicates ± standard deviation.

Parameters	NF (T0)	NF (Tf)	USF (Tf)	SF (Tf)	Difference[1]
pH	8.3 ± 0.3	8.0 ± 0.3	7.5 ± 0.3	7.4 ± 0.1	NF > USF # SF
Nutrients (µM)					
DIN[2]	11.70 ± 0.70	9.89 ± 0.97	12.27 ± 1.54	11.51 ± 1.94	NF # USF # SF
DIP[3]	0.77 ± 0.30	0.62 ± 0.42	0.22 ± 0.18	0.47 ± 0.37	
DON	14.51 ± 1.72	14.75 ± 1.69	17.57 ± 1.86	17.10 ± 1.15	
DOP	8.54 ± 2.81	6.36 ± 3.64	2.95 ± 1.64	5.18 ± 3.47	
SiO_2	2.94 ± 0.50	2.26 ± 0.14	2.47 ± 0.51	2.88 ± 0.58	
Dissolved Metals (µg/L)					
Al	8.15 ± 2.61	8.14 ± 1.48	8.94 ± 0.35	9.93 ± 3.60	NF # USF # SF
As	3.33 ± 0.59	6.37 ± 1.50	6.43 ± 1.97	6.39 ± 1.25	
Co	0.10 ± 0.02	0.88 ± 0.27	0.55 ± 0.09	0.85 ± 0.19	
Cu	2.02 ± 2.96	6.14 ± 1.62	2.42 ± 2.46	4.98 ± 3.83	
Ni	1.02 ± 0.95	2.36 ± 0.67	2.25 ± 0.85	3.57 ± 1.59	
Pb	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.07 ± 0.05	
Sb	0.40 ± 0.06	1.86 ± 0.17	1.83 ± 0.34	2.01 ± 0.13	
Cd	0.01 ± 0.00	0.30 ± 0.10	0.01 ± 0.00	0.02 ± 0.00	NF > USF # SF
Mo	10.25 ± 1.57	14.45 ± 0.17	10.52 ± 0.96	11.69 ± 0.81	
V	1.85 ± 0.25	6.31 ± 0.35	1.80 ± 0.15	2.02 ± 0.23	
Fe	4.82 ± 0.43	4.53 ± 2.81	277.13 ± 55.91	376.87 ± 176.04	NF < USF # SF
Mn	9.77 ± 0.42	8.84 ± 5.65	37.43 ± 4.14	37.46 ± 1.36	
Zn	4.06 ± 2.66	4.12 ± 1.54	6.22 ± 3.00	4.66 ± 0.89[3]	

[1] Difference significantly higher (>) or lower (<) or no difference (#) between conditions (NF, USF and SF) evaluated using ANOVA or Kruskal-Wallis tests ($P<0.05$).
[2] Dissolved Inorganic Nitrogen (DIN) values correspond to the sum of NO_2^-, NO_3^- and NH_4^+ values.
[3] Dissolved Inorganic Phosphorous (DIP) values correspond to PO_4^{3} values.
[4] Values (mean ± standard deviation) obtained from two samples.

Declaration of interests

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author statement

MQ designed and performed experiments. FA, MQ and SC helped for data acquisition. AZ, MB, MQ and SC contributed to the analysis and interpretation of data. MQ wrote the manuscript in collaboration with SC. All authors read and commented on the draft manuscript. All authors agreed to the final version.

Table S1. Spearman's Rank correlation coefficients between the relative abundance of selected bacterial taxa and chemical parameters (showing significant difference between treatments) in sediment experiments. Values in bold are significant at $p \leq 0.05$.

Variables	Cd	Fe	Mn	Mo	V	Zn	pH
Alphaproteobacteria	0,014	**-0,713**	**-0,608**	-0,133	-0,056	-0,385	0,503
Bacteroidetes	0,245	**-0,881**	**-0,776**	0,000	0,091	-0,385	**0,685**
Cyanobacteria	0,42	**-0,916**	**-0,839**	0,266	0,385	-0,266	**0,650**
Gammaproteobacteria	0,476	0,287	0,133	0,294	0,189	0,224	-0,343
Firmicutes	-0,294	**0,72**	**0,783**	0,168	0,140	0,231	**-0,692**
Thermotogae	-0,189	**0,867**	**0,783**	0,196	0,077	0,224	**-0,664**

Table S2. Regression coefficients between the relative abundance of bacterial taxa (response variables) and selected chemical parameters (explanatory variables) in sediment experiments. Values in bold are significant at $p \leq 0.05$.

Response variables	Predictors	Coefficient	Standard error	t	Pr > |t|
Alphaproteobacteria	Constante	0,763	45,664	0,017	0,987
	Fe	-0,015	0,015	-0,997	0,352
	Mn	-0,014	0,203	-0,069	0,947
	pH	1,926	5,504	0,350	0,737
Bacteroidetes	Constante	-4,235	74,735	-0,057	0,956
	Fe	-0,014	0,024	-0,592	0,573
	Mn	-0,189	0,333	-0,568	0,588
	pH	3,313	9,007	0,368	0,724
Cyanobacteria	Constante	25,692	20,593	1,248	0,252
	Fe	-0,001	0,007	-0,119	0,909
	Mn	-0,236	0,092	-2,568	**0,037**
	pH	-2,175	2,482	-0,876	0,410
Gammaproteobacteria	Constante	113,393	130,556	0,869	0,414
	Fe	0,041	0,043	0,970	0,364
	Mn	-0,506	0,582	-0,870	0,413
	pH	-10,279	15,735	-0,653	0,534
Firmicutes	Constante	60,890	71,262	0,854	0,421
	Fe	-0,001	0,023	-0,023	0,982
	Mn	0,374	0,317	1,178	0,277
	pH	-6,656	8,589	-0,775	0,464
Thermotogae	Constante	11,623	34,674	0,335	0,747
	Fe	0,017	0,011	1,483	0,182
	Mn	-0,061	0,154	-0,392	0,707
	pH	-1,339	4,179	-0,320	0,758

Table S3. Blast analysis on the dominant microbial species (OTUs >1% of total sequences) in sediment at the end of the experiments (Tf) with unsmoked filter (USF, in bold), compared to conditions with smoked filter (SF, Tf) or no filter (NF, controls, T0 and Tf).
Values are means of data from biological triplicates ± standard deviations.

OTU name [NCBI Accession number]	Phylum/Class	Relative abundance (%)				Taxonomy closest cultivates [NCBI Accession number]	Identity (%)
		NF (T0)	NF (Tf)	**USF** (Tf)	SF (Tf)		
OTU_8 [MN463083]*	*Alphaproteobacteria*	0.34 ± 0.03	2.14 ± 0.63	**3.17 ± 0.79**	0.46 ± 0.33	*Roseibacterium beibuensis* [MG383386]	100
OTU_35 [MN463084]*	*Alphaproteobacteria*	0.03 ± 0.01	0.08 ± 0.05	**1.20 ± 0.20**	0.08 ± 0.07	*Rhodovulum marinum* [AM696692]	99.53
OTU_19 [MN463085]	*Bacteroidetes*	0.19 ± 0.04	0.98 ± 1.10	**1.42 ± 1.13**	0.01 ± 0.002	*Phaeodactylibacter xiamenensis* [NR_134132]	90.97
OTU_30 [MN463086]	*Bacteroidetes*	0.02 ± 0.004	0.11 ± 0.11	**0.69 ± 0.68**	0.59 ± 0.50	*Marinilabilia nitratireducens* [NR_132609]	96.84
OTU_16 [MN463087]	*Balneolaeota*	0.19 ± 0.03	1.76 ± 1.56	**1.01 ± 0.33**	0.05 ± 0.02	*Gracilimonas halophila* [NR_158001]	97.07
OTU_50 [MN463088]	*Deltaproteobacteria*	0.02 ± 0.01	0.02 ± 0.02	**0.63 ± 0.61**	0.07 ± 0.10	*Desulfovibrio psychrotolerans* [NR_042581]	92.05
OTU_60 [MN463089]	*Epsilonproteobacteria*	0.00 ± 0.01	0.01 ± 0.001	**0.64 ± 0.66**	0.01 ± 0.004	*Sulfurovum lithotrophicum* [CP011308]	88.24
OTU_1 [MN463064]*	*Firmicutes*	0.43 ± 0.06	4.38 ± 1.67	**4.33 ± 1.81**	6.17 ± 1.22	*Alkaliphilus transvaalensis* [NR_024748]	92.69
OTU_14 [MN463090]*	*Firmicutes*	0.04 ± 0.01	0.05 ± 0.01	**3.36 ± 2.30**	0.06 ± 0.01	*Vallitalea pronyensis* [NR_125677]	96.93
OTU_21 [MN463091]*	*Firmicutes*	0.38 ± 0.04	0.19 ± 0.22	**2.04 ± 0.29**	0.33 ± 0.30	*Pontibacillus litoralis* [NR_116372]	84.48
OTU_4 [MN463065]	*Firmicutes*	0.19 ± 0.01	0.16 ± 0.05	**4.25 ± 4.52**	5.60 ± 4.60	*Pontibacillus litoralis* [NR_116372]	84.00
OTU_31 [MN463092]*	*Firmicutes*	0.08 ± 0.02	0.18 ± 0.20	**0.82 ± 0.45**	0.09 ± 0.08	*Pontibacillus salicampi* [MH283830]	87.28
OTU_3 [MN463063]*	*Firmicutes*	0.10 ± 0.01	0.20 ± 0.15	**0.61 ± 0.89**	8.72 ± 6.31	*Exiguobacterium aestuarii* [MH881394]	99.55
OTU_2 [MN463093]	*Fusobacteria*	0.11 ± 0.01	0.12 ± 0.03	**12.68 ± 9.94**	0.15 ± 0.05	*Hypnocyclicus thermotrophus* [NR_145867]	92.96
OTU_22 [MN463094]*	*Gammaproteobacteria*	0.03 ± 0.01	0.04 ± 0.01	**10.88 ± 0.87**	0.31 ± 0.26	*Marichromatium gracile* [LT991979]	99.55
OTU_7 [MN463071]	*Gammaproteobacteria*	0.28 ± 0.04	3.18 ± 2.03	**2.11 ± 0.85**	3.54 ± 1.11	*Marinobacter hydrocarbonoclasticus* [MK131324]	100
OTU_28 [MN463095]	*Gammaproteobacteria*	0.10 ± 0.02	0.30 ± 0.03	**1.03 ± 0.78**	0.07 ± 0.07	*Alteromonas marina* [MH746022]	99.55
OTU_25 [MN463096]	*Gammaproteobacteria*	0.10 ± 0.02	2.06 ± 1.72	**1.02 ± 0.85**	0.09 ± 0.03	*Idiomarina taiwanensis* [KM407758]	99.55
OTU_5 [MN463085]*	*Thermotogae*	0.09 ± 0.02	0.31 ± 0.36	**0.98 ± 1.37**	8.55 ± 1.88	*Geotoga subterranea* [NR_029145]	99.00

* Significant differences between treatments using ANOVA or Kruskal-Wallis tests ($P<0.05$)

Table S4. Blast analysis on the dominant microbial species (OTUs >1% of total sequences) in sediment at the end of the experiments (Tf) with smoked filter (SF, in bold), compared to conditions with unsmoked filter (USF, Tf) or no filter (NF, controls, T0 and Tf). Values are means of data from biological triplicates ± standard deviations.

OTU name [NCBI Accession n°]	Phylum/Class	Relative abundance (%)				Taxonomy of closest cultivates [NCBI Accession n°]	Identity %
		NF (T0)	NF (Tf)	USF (Tf)	**SF** (Tf)		
OTU_12 [MN463061]	*Alphaproteobacteria*	0.06 ± 0.01	0.06 ± 0.01	0.16 ± 0.19	**3.10 ± 2.76**	*Thalassospira australica* [MH304396]	99.29
OTU_13 [MN463062]	*Epsilonproteobacteria*	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.03	**3.05 ± 2.85**	*Arcobacter halophilus* [MG195897]	99.53
OTU_3 [MN463063]*	*Firmicutes*	0.10 ± 0.01	0.20 ± 0.15	0.61 ± 0.89	**8.72 ± 6.31**	*Exiguobacterium aestuarii* [MH881394]	99.55
OTU_1 [MN463064]*	*Firmicutes*	0.43 ± 0.06	4.38 ± 1.67	4.33 ± 1.81	**6.17 ± 1.22**	*Alkaliphilus transvaalensis* [NR_024748]	92.69
OTU_4 [MN463065]	*Firmicutes*	0.19 ± 0.01	0.16 ± 0.05	4.25 ± 4.52	**5.60 ± 4.60**	*Pontibacillus litoralis* [NR_116372]	80.00
OTU_56 [MN463066]	*Firmicutes*	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	**0.61 ± 0.89**	*Serpentinicella alkaliphila* [NR_152685]	98.35
OTU_46 [MN463067]*	*Firmicutes*	0.01 ± 0.01	0.01 ± 0.00	0.05 ± 0.04	**1.54 ± 0.87**	*Halolactibacillus miurensis* [AB681280]	99.11
OTU_36 [MN463068]	*Firmicutes*	0.12 ± 0.01	0.11 ± 0.04	0.14 ± 0.08	**0.84 ± 0.68**	*Tissierella creatinini* [NR_117155]	95.04
OTU_9 [MN463069]	*Gammaproteobacteria*	0.09 ± 0.03	0.10 ± 0.03	0.35 ± 0.47	**5.02 ± 8.47**	*Oceanimonas doudoroffii* [NR_114185]	99.33
OTU_10 [MN463070]	*Gammaproteobacteria*	0.14 ± 0.03	1.68 ± 2.18	0.45 ± 0.42	**3.70 ± 2.55**	*Idiomarina seosinensis* [MG575737]	100
OTU_7 [MN463071]	*Gammaproteobacteria*	0.28 ± 0.04	3.18 ± 2.03	2.11 ± 0.85	**3.54 ± 1.11**	*Marinobacter hydrocarbonoclasticus* [MK131324]	100
OTU_6 [MN463072]*	*Gammaproteobacteria*	0.20 ± 0.01	1.44 ± 0.72	0.76 ± 0.20	**7.85 ± 3.27**	*Marinobacterium sediminicola* [NR_044529]	100
OTU_42 [MN463073]	*Gammaproteobacteria*	0.06 ± 0.01	0.06 ± 0.03	0.17 ± 0.10	**1.02 ± 1.39**	*Marinobacter vinifirmus* [KX418471]	99.55
OTU_11 [MN463074]	*Gammaproteobacteria*	0.16 ± 0.00	0.26 ± 0.10	0.25 ± 0.11	**5.35 ± 4.53**	*Vibrio diabolicus* [MK308588]	100
OTU_647 [MN463075]	*Gammaproteobacteria*	0.09 ± 0.01	0.12 ± 0.04	0.17 ± 0.08	**2.69 ± 2.27**	*Vibrio owensii* [CP033138]	100
OTU_3745 [MN463076]	*Gammaproteobacteria*	0.03 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	**1.37 ± 1.39**	*Marinobacterium georgiense* [MH044627]	98.88
OTU_24 [MN463077]	*Gammaproteobacteria*	0.03 ± 0.01	0.06 ± 0.02	0.04 ± 0.02	**1.34 ± 1.28**	*Marinobacterium georgiense* [NR_114163]	98.66
OTU_47 [MN463078]	*Gammaproteobacteria*	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	**1.11 ± 1.20**	*Pseudomonas aestusnigri* [MF155916]	99.78
OTU_1430 [MN463079]	*Gammaproteobacteria*	0.05 ± 0.03	0.05 ± 0.02	0.07 ± 0.03	**1.05 ± 0.89**	*Vibrio owensii* [CP033137]	100
OTU_4626 [MN463080]	*Gammaproteobacteria*	0.04 ± 0.01	0.05 ± 0.03	0.05 ± 0.02	**0.91 ± 0.77**	*Vibrio harveyi* [EU834007]	98.86
OTU_5 [MN463081]*	*Thermotogae*	0.09 ± 0.02	0.31 ± 0.36	0.98 ± 1.37	**8.55 ± 1.88**	*Geotoga subterranea* [NR_029145]	100
OTU_15 [MN463082]	*Archaea/Halobacteria*	0.75 ± 0.04	1.21 ± 0.83	0.59 ± 0.27	**1.11 ± 0.38**	*Halogranum amylolyticum* [NR_113451]	99.51

* Significant differences between treatments using ANOVA or Kruskal-Wallis tests ($P<0.05$)