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## Impact of cigarette butts on microbial diversity and dissolved trace metals in coastal marine sediment

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Malika Belhassen

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3 1 **Impact of cigarette butts on microbial diversity and dissolved trace metals in coastal**  
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5 2 **marine sediment**  
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62 19 **Abstract**  
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21 Cigarette butts are the most common plastic form of litter found in the marine coast, threatening  
22 the quality of the seawater and marine life. However, the impact of cigarette butts known to  
23 contain toxic chemicals has been investigated to date in very few marine species. This study  
24 aimed to evaluate the effects of cigarette filters (smoked or unsmoked) on the microbial  
25 diversity inhabiting coastal sediments by high-throughput sequencing of the 16S rRNA genes.  
26 Both bacterial structure and metals distribution were impacted by cigarette filter addition in  
27 laboratory sediment experiments, compared to control sediment incubations without filter. Both  
28 smoked and unsmoked cigarette filters decreased pH and dissolved Cd, Mo and V  
29 concentrations in marine sediment incubations, while they increased dissolved Fe, Mn, Zn  
30 levels in the surrounding environment. Smoked filters dramatically decreased the relative  
31 abundance of the phyla *Bacteroidetes* and *Cyanobacteria*, while the members of the phyla  
32 *Gammaproteobacteria*, *Firmicutes* and *Thermotogae* were enriched by smoked filters in marine  
33 sediments. Bacterial taxa associated with deep marine environments or hydrothermal seep fields  
34 were selected by smoked cigarette filters. This study demonstrated for the first time the  
35 microbial community changes and impacts from toxic cigarette filters in coastal marine  
36 sediments.

103 38 **Keywords:** cigarette butt, bacteria, diversity, marine sediments, trace metals, Mediterranean  
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121 **1. Introduction**  
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125 43 Cigarette butts (CB) are one of the most common plastic forms of litter found in the  
126 44 environment (Kadir and Sarani, 2015). From 5 to 6 trillion cigarettes were smoked worldwide  
127  
128 44 environment (Kadir and Sarani, 2015). From 5 to 6 trillion cigarettes were smoked worldwide  
129  
130 45 every year by one billion smokers living in large majority in low- and middle-income countries  
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132 46 (Dropp et al., 2018; Kostova et al., 2014; WHO, 2017; Zafeiridou et al., 2018). Most of them  
133  
134 47 are discarded in the environment, and are transported by wind, rain, river, and marine currents  
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136 48 to coastal areas. Nowadays, CB are the most collected item during the cleaning of beaches  
137  
138 49 (Araujo and Costa, 2019; Addamo et al., 2017; Novotny et al., 2009). They can account for up  
139  
140 50 to 40% of marine litter collected on beaches in some Mediterranean areas (Munari et al., 2016;  
141  
142 51 Vlachogianni, 2019). CB are mainly composed of cellulose acetate, a kind of plastic, which  
143  
144 52 slowly biodegrade for several years depending on environmental conditions (Benavente et al.,  
145  
146 53 2019; Bonanomi et al., 2015). Moreover, CB are classified as hazardous waste according to  
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148 54 European regulation (Rebinschung et al., 2018), mainly due to the toxic chemicals they contain,  
149  
150 55 such as nicotine, metals (e.g. cadmium, arsenic) and others organic compounds derived from  
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152 56 tobacco combustion (Shevchenko, 2012; Moriwaki et al., 2009; Moerman and Potts, 2011).  
153  
154 57 Furthermore, it was estimated that a single CB could contaminate 1000 L of water (Green et  
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156 58 al., 2014). Due to their toxicity and slow degradability, CB in marine ecosystems pose a  
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158 59 potential human health risk through their transfer, fragmentation, accumulation in the food  
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160 60 chain and subsequent consumption.  
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166 62 To date, the ecological risk due to CB pollution in marine ecosystems is largely underestimated  
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168 63 (Kadir and Sarani, 2015). A recent review on CB pollution in coastal ecosystems has reported  
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170 64 only a few studies involving the quantification of CB in coastal ecosystems, which are largely  
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172 65 concentrated to American and European coasts (Araujo and Costa, 2019). Moreover, few  
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180 66 ecotoxicological studies have investigated the exposure and effects of CB on aquatic biota,  
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182 67 despite the wide diversity of marine organisms. The few studies available reported that CB  
183  
184 68 leachates were toxic to the marine bacterium *Aliivibrio fischeri* (formerly *Vibrio fischeri*) and  
185  
186 69 the cladoceran *Ceriodaphnia cf. dubia* (Micevska et al., 2006), the marine fish *Atherinops*  
187  
188 70 *affinis* (Slaughter et al., 2011), the polychaete worm *Hediste diversicolor* (Wright et al., 2015)  
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190 71 and three intertidal snail species (Booth et al., 2015). It has been shown that CB were toxic to  
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192 72 *A. fischeri* at 0.48 mg butts/L, and that smoked filters were more toxic than unsmoked filters  
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194 73 (Micevska et al., 2006). However, the CB toxicity studied from a model microbial organism,  
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196 74 such *A. fischeri*, could not predict the CB toxicity to the other microbial species found in the  
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198 75 environment, because each species is involved in complex interactions and the marine  
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200 76 environment presents a very diverse metabolically and phylogenetically microbial community  
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202 77 (Micevska et al., 2006). Thus, studies using environmental samples with indigenous  
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204 78 microorganisms are required to evaluate the effects of CB on marine ecosystems.  
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210 80 Microbial communities play a critical role in coastal and marine ecosystems and pollutant  
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212 81 transfer. The toxic chemicals entering marine ecosystems can seriously modify microbial  
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214 82 diversity and their ecological functions (Zouch et al., 2018; Johnston et al., 2009; Gillan et al.,  
215  
216 83 2005). Microbial diversity can also be used as a bioindicator of contaminant stress and  
217  
218 84 ecological status of coastal ecosystems, because microbial communities are very sensitive to  
219  
220 85 slight changes in their surrounding environment (Aylagas et al., 2017; Sun et al., 2012). Thus,  
221  
222 86 microbial richness and activity of coastal sediment could be potentially affected by CB  
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224 87 accumulation in their environments. Despite the abundant CB accumulation in the coastal  
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226 88 ecosystems (due to their slow degradation rate), no studies have yet evaluated their potential  
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228 89 impact on microbial diversity of marine sediments. In addition, sediments may act as an  
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230 90 important sink for CB and associated trace metals (e.g. cadmium, lead) trapped in the cigarette  
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91 filter before being released into the environment. The composition and content of metals can  
92 vary depending on CB (Chiba and Masironi, 1992), but only one study has evaluated the  
93 distribution of associated trace metals released by CB in the coastal environment to date  
94 (Dobaradaran et al., 2018).

95  
96 This study aimed to evaluate for the first time the microbial community composition and  
97 diversity as a function of CB exposure and specifically the dissolved trace metals apparently  
98 leached into CB-contaminated sediments in an urban and highly frequented coastal  
99 environment. Here, the effects of both smoked and unsmoked cigarette filters were evaluated  
100 on microbial community structure, metal distribution and nutrient concentrations in laboratory  
101 sediment incubations. Microbial diversity was evaluated by high-throughput 16S rRNA gene  
102 sequencing analyses at the end of the 4-day experiments and compared to controls (without  
103 cigarette filter) as well as initial community.

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298 105 **2. Materials and Methods**  
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302  
303 107 **2.1. Studied area**

304 108 Sfax (with around 600,000 inhabitants) is the second largest city in Tunisia located on the  
305 109 southern coast of the Mediterranean Sea. Sfax is also located in the northern part of the Gulf of  
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309 110 Gabès having the highest tides in the Mediterranean Sea (up to 2.3 m, Sammari et al., 2006),  
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311 111 due to its large continental shelf with a very low slope. The southern coast of Sfax city,  
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313 112 extending from the solar saltworks to the commercial harbor, is impacted by numerous polluting  
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315 113 industrial sites discharging contaminants in coastal environments (Chifflet et al., 2019a; Zouch  
316  
317 114 et al., 2017). The north coast, stretching for more than 10 km to the small village of Sidi  
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319 115 Mansour, is more residential with the presence of beaches and small fishing ports, but it is  
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321 116 affected by increasing urbanization in recent years due to rise in population. The studied area  
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323 117 is in the northern coast of Sfax and corresponds to an urban beach intertidal area highly  
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325 118 frequented by local people. This coastal area had a lot of waste including CB and other litter  
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327 119 items (e.g. plastic bags and bottles, clothing, packaging) discarding by people or deposited by  
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329 120 coastal currents and tides, wind and rains, as observed in most urban coastal areas frequented  
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331 121 by people in Tunisia and other Mediterranean countries.  
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336 123 **2.2. Sample collection**

337  
338 124 Surficial sediments (0–5-cm layer) were sampled in October 2018 from one location  
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340 125 (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  
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342 126 and distributed in sterile plastic bags (~1 kg). Seawater was collected using a polyethylene pre-  
343  
344 127 cleaned bottle with HCl 10% v/v (analytical grade) then thoroughly rinsed with ultrapure water  
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346 128 (R = 18.2 MΩ.cm<sup>-1</sup>). Seawater and sediment samples were kept at 4°C until use (<3h after the  
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357 129 field sampling). The temperature and pH were measured *in situ* using the multiparameter Odeon  
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359 130 probe (Poncel, France).

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361 131 **Twelve** smoked cigarette filters (SF) were immediately collected after burning and the remnant  
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363 132 tobacco was removed from filters using sterile gloves. **Twelve** unsmoked cigarette filters (USF)  
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365 133 were also separated from tobacco according to this protocol. The **smoked and unsmoked**  
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367 134 **cigarette filters** were separately stored in metal-free **polypropylene tubes (VWR)** at room  
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369 135 temperature until used.

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### 373 374 137 **2.3. Microcosm experiments**

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376 138 Three experimental conditions were run in parallel and in triplicate: sediment incubations  
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378 139 without filter (NF; NF1, NF2, NF3), sediment incubations with addition of one unsmoked filter  
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381 140 (USF; USF1, USF2, USF3) and sediment incubations with addition of one smoked filter (SF;  
382  
383 141 SF1, SF2, SF3). For experiments, 4 g of sediment were mixed with 40 mL of 0.22- $\mu$ m filtered  
384  
385 142 seawater through a sterile cellulose acetate **filter** (Minisart, Sartorius) locked on plastic sterile  
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387 143 syringes (i.e. solid/liquid ratio of 10% w/w). Samples were introduced into 50 mL metal-free  
388  
389 144 polypropylene tubes (VWR) and gently **shaken** before incubating for 96 hours (i.e. 4 days, **a**  
390  
391 145 **duration defined from our previous results on sediment incubations described by Zouch et al.,**  
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393 146 **2017; Zouch et al., 2018; Chifflet et al., 2019b)** and under outdoor conditions (i.e. exposed to  
394  
395 147 **ambient light and temperature). All experiments were performed in triplicates. At the beginning**  
396  
397 148 **of the experiments (T0), the seawater supernatant was filtered (0.22- $\mu$ m) and stored in a metal-**  
398  
399 149 **free polypropylene tubes at  $-20^{\circ}\text{C}$  until chemical analyses (i.e. nutrients and metals). At the**  
400  
401 150 **end of the experiments (Tf), the cigarette filters (smoked or unsmoked) were removed from the**  
402  
403 151 **tubes and the seawater supernatant was filtered (0.22- $\mu$ m) and stored in a metal-free**  
404  
405 152 **polypropylene tubes at  $-20^{\circ}\text{C}$  until chemical analyses (i.e. nutrients and metals).** The pH of  
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407 153 experiments was measured using a pH-meter (inoLab 7110, WTW) calibrated with three  
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416 154 standard buffers (pH 4.0, 7.0 and 10.0 at 20 °C). Subsamples of sediment (T0 and Tf) were also  
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418 155 kept at -20°C for DNA extraction.  
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#### 422 157 **2.4. Chemical analyses**

425 158 Dissolved trace metals (Al, As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sb, V, Zn) concentrations  
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427 159 were measured by Argon Gas Dilution - Inductively Coupled Plasma - Mass Spectrometry  
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429 160 (AGD-ICP-MS, iCAP-Q, Thermo Scientific) after samples acidification (ultra-pure HNO<sub>3</sub> 1%  
430  
431 161 v/v). Because high dissolved solid contents induce ionization suppression, the AGD technique  
432  
433 162 is useful for reducing sample matrix content to about 0.2% of dissolved solids before entering  
434  
435 163 the plasma (Field et al., 1999). In AGD-ICP-MS analyses, the argon (Ar) gas flow through the  
436  
437 164 nebulizer is reduced while the total Ar gas flow to the plasma is maintained by the addition of  
438  
439 165 a make-up Ar gas flow to the aerosol leaving the spray chamber. The sample aerosol is thereby  
440  
441 166 diluted with Ar gas inside the ICP-MS sample introduction system. Analytical detection limits  
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443 167 were below the analysed samples. Accuracy of ICP-MS measurements were controlled using  
444  
445 168 certified reference nearshore seawater (CASS-6). Trace metals recoveries were between 98 and  
446  
447 169 108% except for Mn and Mo (78% and 122%, respectively; data not shown).  
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449 170 Inorganic nutrient concentrations were determined with a BRAN and LUEBBE Type 3 auto-  
450  
451 171 analyzer according to standard methods (Tréguer and LeCorre, 1975). Dissolved Inorganic  
452  
453 172 Nitrogen (DIN) is the sum of NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> values. Dissolved Inorganic Phosphorus  
454  
455 173 (DIP) corresponds to PO<sub>4</sub><sup>3-</sup> values. Dissolved Organic Nitrogen (DON) and Dissolved Organic  
456  
457 174 Phosphorus (DOP) were measured after mineralization processes at high temperature (120°C).  
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459 175 The oxidizing agents were sodium hydroxide and potassium persulfate for nitrogen and sulfuric  
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461 176 acid and potassium persulfate for phosphorus (Aminot and Kérouel, 2007).  
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#### 467 178 **2.5. DNA extraction, PCR and sequencing of 16S rRNA gene fragments**

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475 179 DNA extraction from triplicated initial sediment samples (without filter, NF-T0) and final  
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477 180 sediment samples (Tf) of the three experimental conditions (NF, USF, SF), performed in  
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479 181 biological triplicate (i.e., NF1, NF2, NF3; USF1, USF2, USF3; SF1, SF2, SF3), was carried out  
480  
481 182 using the UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Inc., CA), as previously  
482  
483 183 described by Quéméneur et al. (2016). Bacterial and archaeal 16S rRNA gene V4 variable  
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485 184 regions were amplified by PCR using the Pro341F/Pro805R prokaryotic universal primer set  
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487 185 (Takahashi et al., 2014), with barcode on the forward primer, as previously described by Dowd  
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489 186 et al. (2008), and were sequenced by the MiSeq Illumina (paired-end 2 x 300 bp) platform of  
490  
491 187 the Molecular Research Laboratory (Texas, USA). Sequence data were processed using MR  
492  
493 188 DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, sequences were  
494  
495 189 joined, depleted of barcodes then sequences <150bp removed, sequences with ambiguous base  
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497 190 calls removed. Sequences were denoised, then Operational taxonomic units (OTUs) generated  
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499 191 and chimeras removed. OTUs were defined by clustering at 3% divergence (97% similarity).  
500  
501 192 Final OTUs were taxonomically classified using BLASTn against NCBI non-redundant (NR)  
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503 193 reference database. The 16S rRNA gene sequences of OTU have been deposited in the Genbank  
504  
505 194 database under the accession numbers MN463061-MN463096.  
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## 511 196 **2.6. Statistical analyses**

512  
513 197 **Depending on the results of a Shapiro-Wilk normality test, experimental data were analyzed**  
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515 198 **using the Analysis of Variance (ANOVA) followed by Tukey's post-hoc test or the non-**  
516  
517 199 **parametric Kruskal-Wallis test followed by Dunn's test with Bonferroni correction to assess**  
518  
519 200 **the effect of treatments on the nutrients, pH and trace metals, and the relative abundance of**  
520  
521 201 **microbial taxa (classes/phyla and dominant phylotypes). *P* values of <0.05 were considered as**  
522  
523 202 **statistically significant differences. A Spearman rank correlation test was chosen to investigate**  
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525 203 **the relations among bacterial taxa and chemical parameters (showing significant differences**  
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534 204 between treatments), and we accepted correlation coefficients ( $r_s$ ) with  $P$  values of  $<0.05$  as  
535  
536 205 significant associations. Linear regression analyses were run to assess relationships between  
537  
538 206 log-transformed response variables (bacterial classes/phyla showing significant differences  
539  
540 207 between treatments) and the most discriminant explanatory variables (metals and pH). The data  
541  
542 208 obtained at the beginning and at the end of the experiments were also analyzed by heatmap,  
543  
544 209 Hierarchical Clustering Analysis (HCA) and Principal Component Analysis (PCA). The most  
545  
546 210 discriminant variables (among metals, microbial taxa and pH) showing significant difference  
547  
548 211 between the experimental conditions (i.e.  $P$  values of  $<0.05$ ) and explaining the sample  
549  
550 212 distribution on the PCA ordination were selected and represented on the biplot. All statistical  
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552 213 analyses were performed using XLSTAT 2019.1.2 (Microsoft Excel add-in program;  
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554 214 Addinsoft, Paris, France).

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### 216 3. Results and discussion

#### 218 3.1. Characteristics of initial microbial ecosystem

219 The initial coastal seawater used in laboratory experiments had a temperature of 20.9°C, a pH  
220 value of  $8.3 \pm 0.3$ , and variable levels of trace elements (Table 1). Nutrients were abundant with  
221 dissolved organic nitrogen (DON) and phosphorus (DOP) concentrations around  $14.52 \pm 1.72$   
222  $\mu\text{M}$  and  $8.54 \pm 2.81 \mu\text{M}$ , respectively. The initial microbial diversity of the sandy surficial  
223 sediment was dominated by five bacterial phyla: *Proteobacteria* ( $50.4 \pm 3.3\%$ ), *Bacteroidetes*  
224 ( $19.0 \pm 0.9\%$ ), *Firmicutes* ( $7.5 \pm 0.4\%$ ), *Cyanobacteria* ( $4.7 \pm 2.8\%$ ), *Actinobacteria* ( $4.5 \pm$   
225  $1.3\%$ ; Figure 1). This bacterial community was diverse with only 5 abundant OTUs (>1%)  
226 belonging to classes *Cyanophyceae*, *Gammaproteobacteria* and *Flavobacteriia* (making up less  
227 than 10% of all reads). *Archaea* accounted for  $7.5 \pm 1.3\%$  of the microbial community and was  
228 mainly represented by the family *Halobacteriaceae* (phylum *Euryarchaeota*; Figure 1).

#### 230 3.2. Variations of physicochemical parameters in CB experiments

231 The evolution of nutrients, pH and trace metals in seawater between the beginning (T0) and the  
232 end (Tf) of the 96h experiments are given in Table 1. No significant nutrient difference was  
233 observed between the studied conditions ( $p > 0.05$ ). Significant pH decreases were observed in  
234 both USF (unsmoked filter) and SF (smoked filter) conditions from  $8.3 \pm 0.3$  (NF, T0) to  $7.5 \pm$   
235  $0.3$  ( $p=0.007$ ; USF, Tf) and  $7.4 \pm 0.1$  ( $p=0.005$ ; SF, Tf). On the contrary, no significant pH  
236 difference was observed between NF-T0 and NF-Tf controls (no filter; from  $8.3 \pm 0.3$  at T0 to  
237  $8.0 \pm 0.3$  at Tf;  $p > 0.05$ ), indicating that CB addition decreased the pH of seawater. The variation  
238 in environmental conditions, such as pH, alters the trace metals mobility between seawater and  
239 sediments by inducing changes in dissolved trace metals concentrations (Hamzeh et al., 2014)  
240 and variations in microbial responses (Zouch et al., 2018). CB are among the most numerous

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651  
652 241 littered items in the environment and are potential sources of environmental pollution, including  
653  
654 242 trace metals (Araújo and Costa, 2019; Dobaradaran et al., 2018; Novotny et al., 2011). In our  
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656 243 experiments, different models of trace metals distribution were observed depending on cigarette  
657  
658 244 filter addition (Table 1). Overall, dissolved trace metals in SF conditions were higher than USF  
659  
660 245 conditions showing a higher release of trace metals from smoked than unsmoked CB. However  
661  
662 246 due to specific physicochemical conditions (pH, salinity) of coastal environment and chemical  
663  
664 247 behavior of trace metals, some elements (Al, As, Co, Cu, Ni, Pb and Sb) presented no significant  
665  
666 248 difference between NF, USF and SF conditions at Tf. These results showed a limited impact of  
667  
668 249 cigarette filters (smoked or unsmoked) on natural distribution of trace metals in this intertidal  
669  
670 250 environment. Conversely, both Fe and Mn levels significantly increased in seawater at Tf in  
671  
672 251 USF ( $277.13 \pm 55.91 \mu\text{g-Fe/L}$ ,  $p=0.01$ ;  $37.43 \pm 4.14 \mu\text{g-Mn/L}$ ,  $p=0.04$ ) and SF conditions  
673  
674 252 ( $376.87 \pm 176.04 \mu\text{g-Fe/L}$ ,  $p=0.04$ ;  $37.46 \pm 1.36 \mu\text{g-Mn/L}$ ,  $p=0.01$ ), compared to controls ( $4.53$   
675  
676 253  $\pm 2.81 \mu\text{g-Fe/L}$  and  $8.84 \pm 5.65 \mu\text{g-Mn/L}$ , respectively). To a lesser extent, a similar pattern  
677  
678 254 was also observed for dissolved Zn. The increase in dissolved trace metals concentrations of  
679  
680 255 some elements (Fe, Mn and Zn) in both USF and SF conditions compared to NF conditions at  
681  
682 256 Tf, highlighted a trace metals inputs from cigarette filters in coastal environment. These results  
683  
684 257 agreed with Moerman and Potts (2011) which showed that Fe, Mn and Zn in CB were highly  
685  
686 258 leached (10%, 23% and 19% respectively) in water. On the contrary, significant loss of  
687  
688 259 dissolved Cd was observed at Tf in both USF ( $0.010 \pm 0.002 \mu\text{g/L}$ ) and SF conditions ( $0.018 \pm$   
689  
690 260  $0.005 \mu\text{g/L}$ ) compared to NF controls ( $0.300 \pm 0.103 \mu\text{g/L}$ ;  $p<0.02$ ). Similarly, high decrease  
691  
692 261 in dissolved V were measured at Tf in both USF ( $1.80 \pm 0.15 \mu\text{g/L}$ ) and SF conditions ( $2.02 \pm$   
693  
694 262  $0.23 \mu\text{g/L}$ ) compared to controls ( $6.31 \pm 0.35 \mu\text{g/L}$ ;  $p<0.02$ ). To a lesser extent, a similar pattern  
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696 263 was also observed for dissolved Mo. The decrease in dissolved trace metals (Cd, Mo and V)  
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698 264 concentrations may be due to adsorption processes on CB. Indeed, cigarette filters (made of  
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700 265 cellulose acetate) are a synthetic polymer which may behave like other plastics exporting trace  
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711 266 metals in the marine environment (Ashton et al., 2010; Holmes et al., 2012; Holmes et al.,  
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713 267 2014).

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### 717 269 **3.3. Microbial diversity in CB experiments**

720 270 The microbial diversity in sediment according to USF conditions (unsmoked filter), SF  
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722 271 conditions (smoked filter) and NF controls (no filter) were evaluated at Tf (96h) and compared  
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724 272 to the initial microbial diversity in sediment (NF controls at T0). Changes in overall microbial  
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726 273 structure were observed at the phylum/class level depending on experimental conditions (Figure  
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728 274 1). Significant differences between treatments ( $p < 0.05$ ) were detected in the relative abundance  
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730 275 of six bacterial taxa: *Alphaproteobacteria*, *Bacteroidetes*, *Cyanobacteria*,  
731  
732 276 *Gammaproteobacteria*, *Firmicutes* and *Thermotogae* (Figure 2). A shift in relative abundance  
733  
734 277 of microbial families was also observed depending on experimental conditions (Figure 3).

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736 278 Both USF and SF conditions were dominated by *Proteobacteria* ( $40.2 \pm 1.4\%$  and  $53.4 \pm 2.7\%$   
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738 279 of total reads) and *Firmicutes* ( $21.5 \pm 1.2\%$  and  $28.6 \pm 2.3\%$ ; Figure 1). Among *Proteobacteria*,  
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741 280 *Gammaproteobacteria* were abundant in both USF and SF conditions, but largely predominant  
742  
743 281 in SF conditions ( $43.7 \pm 2.6\%$  of total reads), especially *Alteromonadaceae* and *Vibrionaceae*  
744  
745 282 families, indicating they were enriched by CB addition in marine sediment. Interestingly, the  
746  
747 283 proportions of *Thermotogae* (represented by *Petrotogaceae* family) significantly increased with  
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749 284 smoked filter addition ( $8.6 \pm 1.9\%$  in SF vs.  $< 0.5\%$  of total reads in controls,  $p = 0.007$ ; Figures  
750  
751 285 2 and 3). Furthermore, the relative abundance of *Firmicutes* (represented by *Clostridiaceae* and  
752  
753 286 *Bacillaceae* families) was 2.3 times higher in SF conditions than in controls ( $p = 0.01$ ). On the  
754  
755 287 contrary, the relative abundance of *Bacteroidetes* (*Flavobacteriaceae*, *Sphingobacteriaceae*  
756  
757 288 and *Saprospiraceae*) dramatically decreased in SF conditions ( $2.6 \pm 1.1\%$ ), compared to USF  
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759 289 conditions ( $15.4 \pm 3.4\%$ ,  $p = 0.045$ ) and controls ( $21.2 \pm 8.2\%$ ,  $p = 0.017$ ). Similarly, the relative  
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761 290 abundance of *Alphaproteobacteria* (*Rhodobacteraceae*) was significantly lower in SF  
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770 291 conditions ( $5.7 \pm 2.6\%$ ) than in USF conditions ( $14.0 \pm 1.3\%$ ,  $p=0.007$ ) and controls ( $15.0 \pm$   
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772 292  $3.3\%$ ,  $p=0.027$ ). The relative abundance of *Cyanobacteria* (*Chroococcales* and *Oscillatoriales*)  
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774 293 were also 10 to 60 times lower in USF and SF conditions ( $0.74 \pm 0.03$  and  $0.12 \pm 0.03\%$ ,  
775  
776 294 respectively) than in controls ( $7.3 \pm 2.0\%$ ;  $p<0.02$ ). These results indicated that growth of  
777  
778 295 *Alphaproteobacteria*, *Bacteroidetes* and cyanobacterial species may be inhibited by CB, while  
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781 296 growth of some species belonging to *Gammaproteobacteria*, *Firmicutes* and *Thermotogae* may  
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783 297 be enhanced by cigarette filter addition. No significant change in relative abundance of *Archaea*  
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785 298 (ranging from 3.3 to 10.9% of total reads and mainly represented by *Halobacteriaceae*) was  
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787 299 observed in sediment depending of CB addition ( $p>0.05$ ).

789 300 A Principal Component Analysis (PCA) was performed to identify the factors that affect the  
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791 301 microbial community at the beginning and at the end of the experiment (Figure 4). The first two  
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793 302 principal components explained 71.2% of the variability in the data. Two groups (with or  
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795 303 without cigarette filter) were identified by Hierarchical Clustering Analysis (HCA).  
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797 304 Correlations of the key bacterial taxa (classes/phyla) with environmental variables showed  
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799 305 some dependencies on metal distribution and pH (Table S1). Significant and positive  
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801 306 correlations were observed between the *Firmicutes* or *Thermotogae* proportions and dissolved  
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803 307 Fe and Mn ( $r_s>0.72$ ,  $p<0.05$ ). In contrast, the proportions of *Bacteroidetes* and *Cyanobacteria*  
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805 308 were significantly and negatively correlated with Fe and Mn ( $p<0.05$ ). Their relative  
806  
807 309 abundances were also significantly correlated with pH values (negatively for *Firmicutes* and  
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809 310 *Thermotogae* and positively for *Bacteroidetes* and *Cyanobacteria*;  $p<0.05$ ). However, such  
810  
811 311 discriminant chemicals parameters (Fe, Mn and pH) were not identified as significant  
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813 312 determinants of bacterial diversity by our multiple regression models (except Mn for  
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815 313 *Cyanobacteria*, Table S2), suggesting that other unmeasured environmental parameters (e.g.  
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817 314 organic compounds) could play a key role in shaping bacterial communities.  
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### 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 *Hypnocyclus 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 *Thermotogae* (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.,



886  
887  
888 341 1997). The other abundant and gammaproteobacterial phylotypes enriched by CB were closely  
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890 342 affiliated with *Vibrio owensii* (100% similarity with OTU647 and OTU1430), isolated from  
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892 343 diseased cultured crustaceans (Cano-Gomez et al., 2010), and *Vibrio harveyi* (98.9% similarity  
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894 344 with OTU4626), a model marine and bioluminescent microorganism known as pathogen of  
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896 345 aquatic fauna (Wang et al., 2015). Among *Firmicutes*, three OTU developed well in presence  
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898 346 of CB and were affiliated to mesophilic and fermentative bacterial species belonging to genera  
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900 347 *Exiguobacterium* (99.5% similarity), *Alkaliphilus* (92.7% similarity) and *Serpentinicella*  
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902 348 (98.4% similarity). These species were isolated from deep South Africa gold mine and  
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904 349 serpentinite-hosted Prony hydrothermal field in New Caledonia (Takai et al., 2001; Ben Aissa  
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906 350 et al., 2015; Mei et al., 2016). Two others dominant phylotypes were only retrieved in presence  
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908 351 of CB and were affiliated to the classes *Epsilonproteobacteria* (*Arcobacter halophilus*, 99%  
909  
910 352 similarity) and *Thermotogae* (family *Petrotogaceae* accounting for  $8.6 \pm 1.8\%$  of SF reads  
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912 353 represented by *Geotoga subterranea*, 99.0% similarity with OTU5). The relative abundance of  
913  
914 354 this OTU5 increased significantly with the addition of smoked filter ( $p < 0.05$ ). The moderately  
915  
916 355 thermophilic and fermentative *Geotoga subterranea*, was isolated from brines collected from  
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918 356 oil fields in USA (Davey et al., 1993), while *Arcobacter halophilus* was isolated from  
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920 357 hypersaline lagoon water in the Hawaiian Islands (Donachie et al., 2005) and also found in  
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922 358 industrially multi-contaminated coastal sediment (Zouch et al., 2017).  
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924 359 No *Cyanobacteria* and *Bacteroidetes*, which are important in photosynthetic activities and  
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926 360 organic matter degradation, respectively, were represented among dominant species in  
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928 361 sediment incubations with CB (SF conditions, Table S2). The absence of such key microbial  
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930 362 groups among dominant bacteria in our experiments with smoked filters could be explained by  
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932 363 the release of toxic chemical compounds inhibiting their respective cell growth into surrounding  
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934 364 medium. However, *Bacteroidetes* species, particularly those belonging to the class  
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936 365 *Flavobacteria*, constitute a major component of the bacterial community in metal- and oil-  
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947 366 contaminated marine sediment (Zouch et al., 2018; Kappell et al., 2014). In marine ecosystems,  
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949 367 *Bacteroidetes* were also known to growth attached to particles, surfaces or algal cells  
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951 368 (Fernandez-Gomez et al., 2013). Moreover, *Cyanobacteria* were also overrepresented on  
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953 369 plastic debris compared to the surrounding free-living and organic particle-attached fractions  
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955 370 in ‘plastisphere’ studies (Jacquin et al., 2019). Thus, the depletion of *Cyanobacteria* and  
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957 371 *Bacteroidetes* from sediment fraction might be also explained by: (i) the potential death of  
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959 372 sensitive algal biomass and *Cyanobacteria*, causing hypoxia and providing competitive  
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961 373 advantage for *Firmicutes* against *Bacteroidetes* in sediment, and/or (ii) a potential colonization  
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963 374 of CB by *Cyanobacteria* and *Bacteroidetes*. These latter assumptions remain to be verified  
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965 375 through future experiments analyzing both sediment and CB fractions, as previously  
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967 376 investigated in microbial colonization studies of plastic and microplastic (Harrison et al., 2014;  
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969 377 Dussud et al., 2018; Jacquin et al., 2019).

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#### 975 379 4. Conclusions

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977 381 This study showed that CB changed the microbial diversity of coastal marine sediment, and the  
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979 382 physicochemical parameters of its surrounding environment (e.g. pH, trace metals). **Cigarette**  
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981 383 **filter** addition decreased the pH of seawater and the concentrations of dissolved Cd, Mo and V,  
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983 384 whereas they increased dissolved Fe, Mn and Zn concentrations in coastal ecosystem. We also  
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985 385 found that **smoked filter** addition to coastal sediment led to a depletion in *Cyanobacteria* and  
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987 386 heterotrophic *Bacteroidetes* (especially *Flavobacteriaceae* and *Saprospiraceae*), with a  
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989 387 concomitant enrichment in heterotrophic *Gammaproteobacteria* (*Alteromonadaceae* and  
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991 388 *Vibrionaceae*), *Firmicutes* (*Bacillaceae* and *Clostridiaceae*), and *Thermotogae*  
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993 389 (*Petrotogaceae*) related to deep and/or hydrothermal ecosystems and adapted to extreme  
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995 390 conditions (e.g. high temperature and metals concentrations). The changes in bacterial  
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1006 391 community diversity, and selection of specific groups by **cigarette filter addition**, support the  
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1008 392 need to evaluate the effect of potential toxic substances on global bacterial diversity (e.g. 16S  
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1010 393 rRNA PCR tests), in addition to test model marine bacterial species (e.g. *A. fischeri*  
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1013 394 bioluminescence inhibition test) in future development of ecotoxicology studies.  
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1030 402 in seawater samples.  
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1067 404 **Legends of Figures**  
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1072 406 **Figure 1.** Composition of microbial communities at the phylum or class level in the sediment  
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1074 407 at the beginning (T0) and (Tf) of experiments without filter (NF, control), with unsmoked filter  
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1076 408 (USF) and smoked filter (SF).  
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1080 410 **Figure 2.** Comparison of the relative abundance of selected bacterial taxa (showing significant  
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1082 411 difference between treatments) in the sediment between the beginning (T0) and (Tf) of  
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1084 412 incubations without filter (NF, control), with unsmoked filter (USF) and smoked filter (SF).  
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1086 413 Values are means of abundance data from biological triplicates  $\pm$  standard deviation.  
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1089 415  
1090 416 **Figure 3.** Heat map showing the relative abundance of the families in the sediment at the  
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1092 417 beginning (T0) and (Tf) of experiments without filter (NF, control), with unsmoked filter (USF)  
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1094 418 and smoked filter (SF). Each column represents an experimental condition and each row  
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1096 419 represents a family. The colour intensity for each panel corresponds to the family abundance,  
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1098 420 red indicates high level of relative abundance, while yellow indicates low relative abundance.  
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1100 421 The dendrogram was constructed from the family abundance table using Euclidean distance.  
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1102 422 The scale represents 10% dissimilarity.  
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1107 426 **Figure 4.** Principal Component Analysis (PCA) biplot showing the variation among the data  
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1109 427 (showing significant difference, i.e. with P values  $< 0.05$ ) obtained from the beginning (T0) and  
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1111 428 (Tf) of experiments without filter (NF, control), with unsmoked filter (USF) and smoked filter  
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1113 429 (SF). White and black circles represent NF experiments at T0 and Tf, black square represent  
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1115 430 USF experiments and black triangles represent SF experiments. Ellipses represent the clusters  
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428 identified with Hierarchical Clustering Analysis (HCA). Arrows indicate the direction of  
429 maximum increase and strength (through the length) of each variable to the overall distribution.  
430 The blue arrows indicate metals and pH, and the red arrows represent the microbial taxa.

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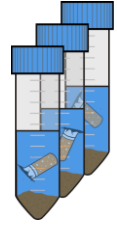
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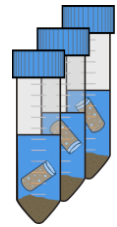
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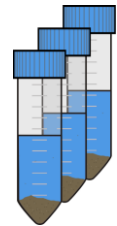
Figure 1



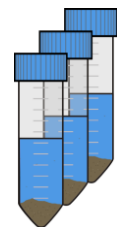
**SF (Tf)**  
Final sediment with  
smoked filter



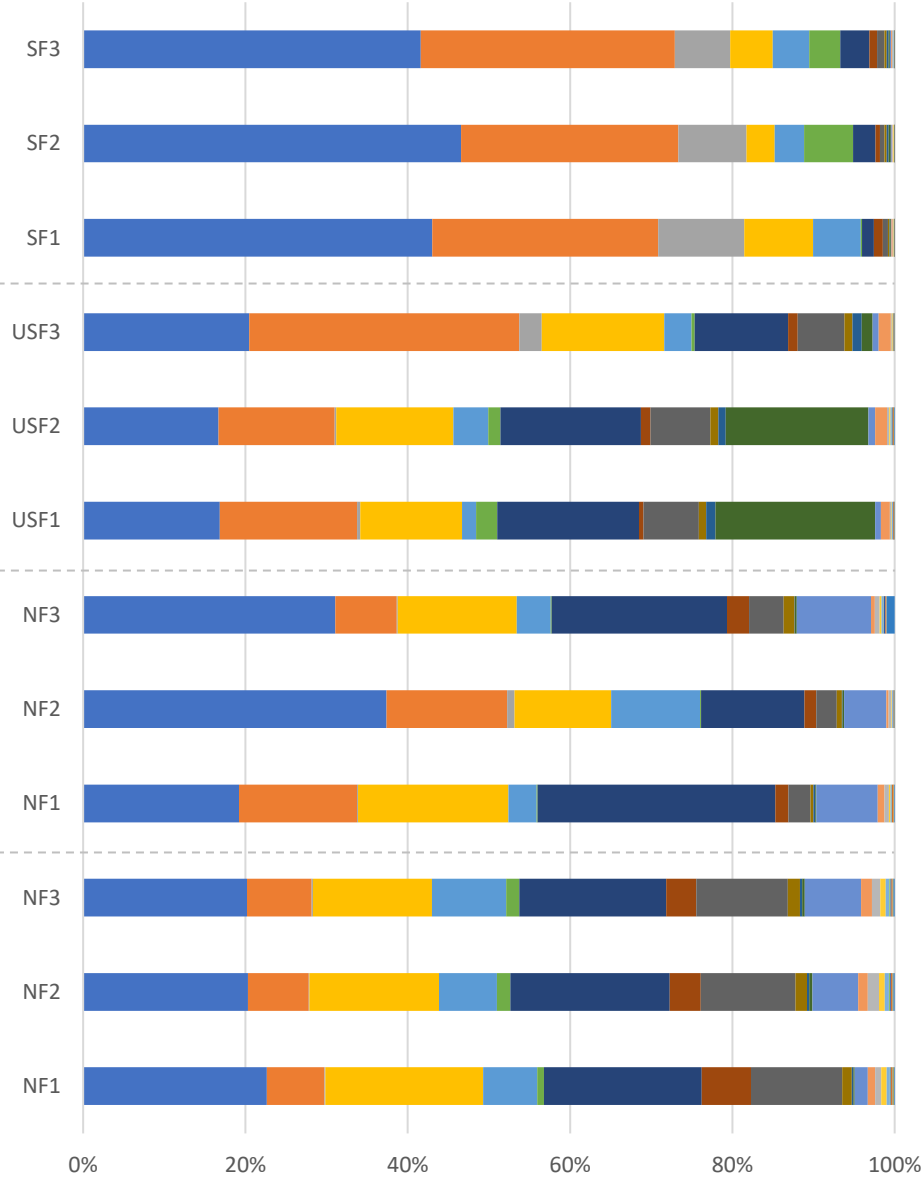
**USF (Tf)**  
Final sediment with  
unsmoked filter



**NF (Tf)**  
Final sediment without  
filter (control)



**NF (T0)**  
Initial sediment without  
filter (control)



- *Gamma*proteobacteria
- Firmicutes
- Thermotogae
- Alphaproteobacteria
- Euryarchaeota
- Epsilonproteobacteria
- Bacteroidetes
- Actinobacteria
- Deltaproteobacteria
- Chloroflexi
- Tenericutes
- Fusobacteria
- Cyanobacteria
- Spirochaetes
- Verrucomicrobia
- Acidobacteria
- Ignavibacteriae
- Thaumarchaeota
- Planctomycetes
- Nitrospirae
- Deinococcus-Thermus
- Chlorobi
- Betaproteobacteria

Relative abundance of prokaryotic taxa

Figure 2

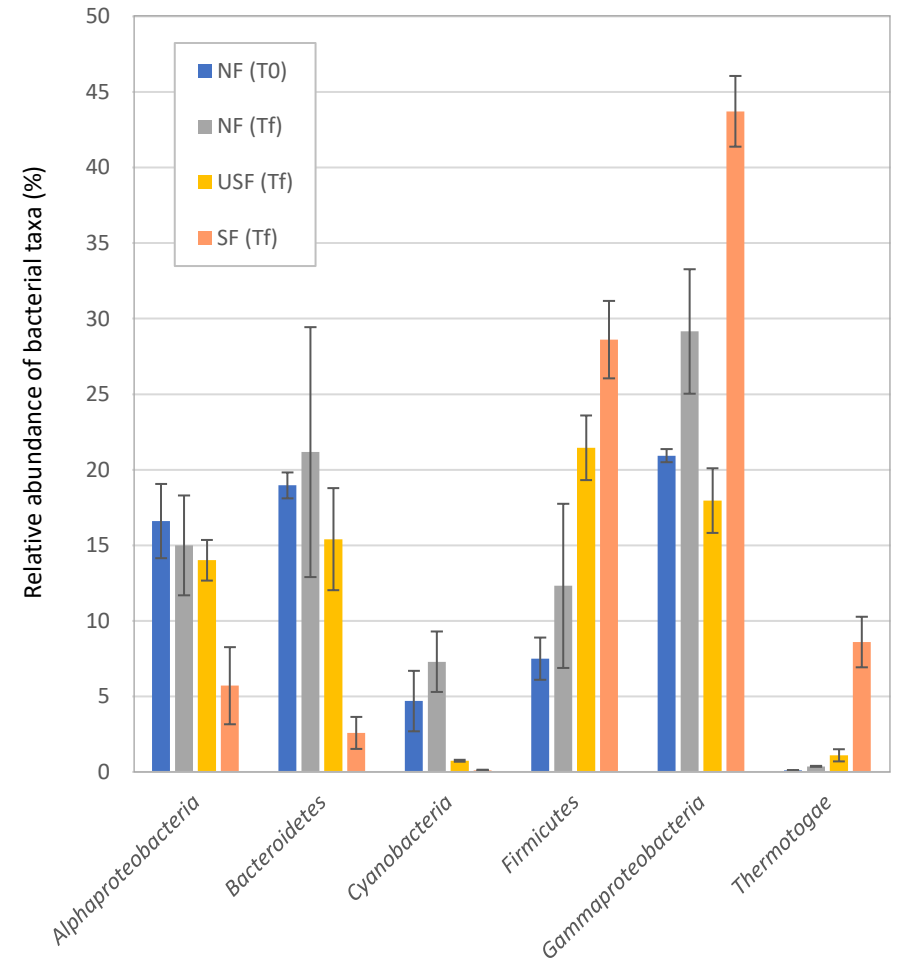


Figure 3

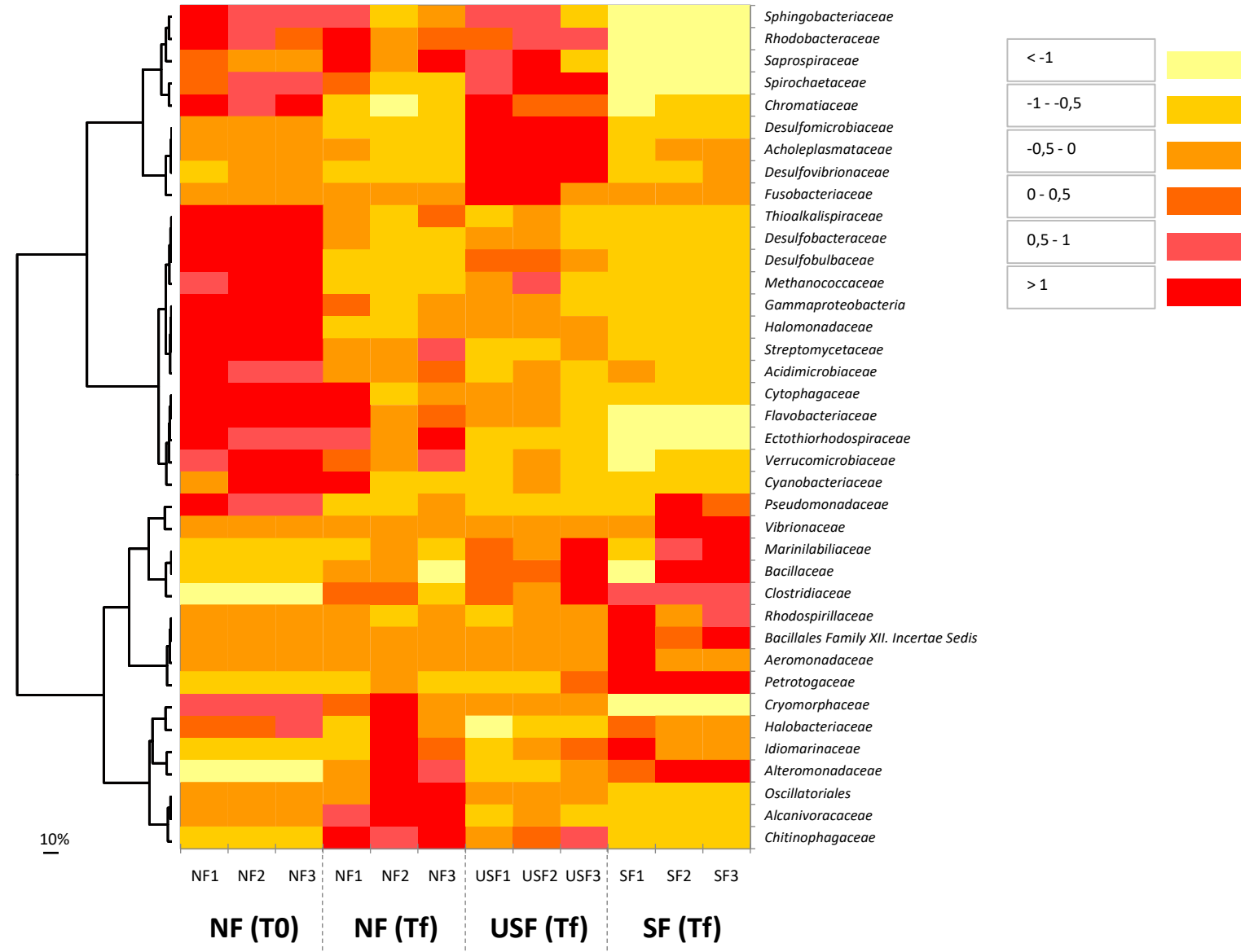
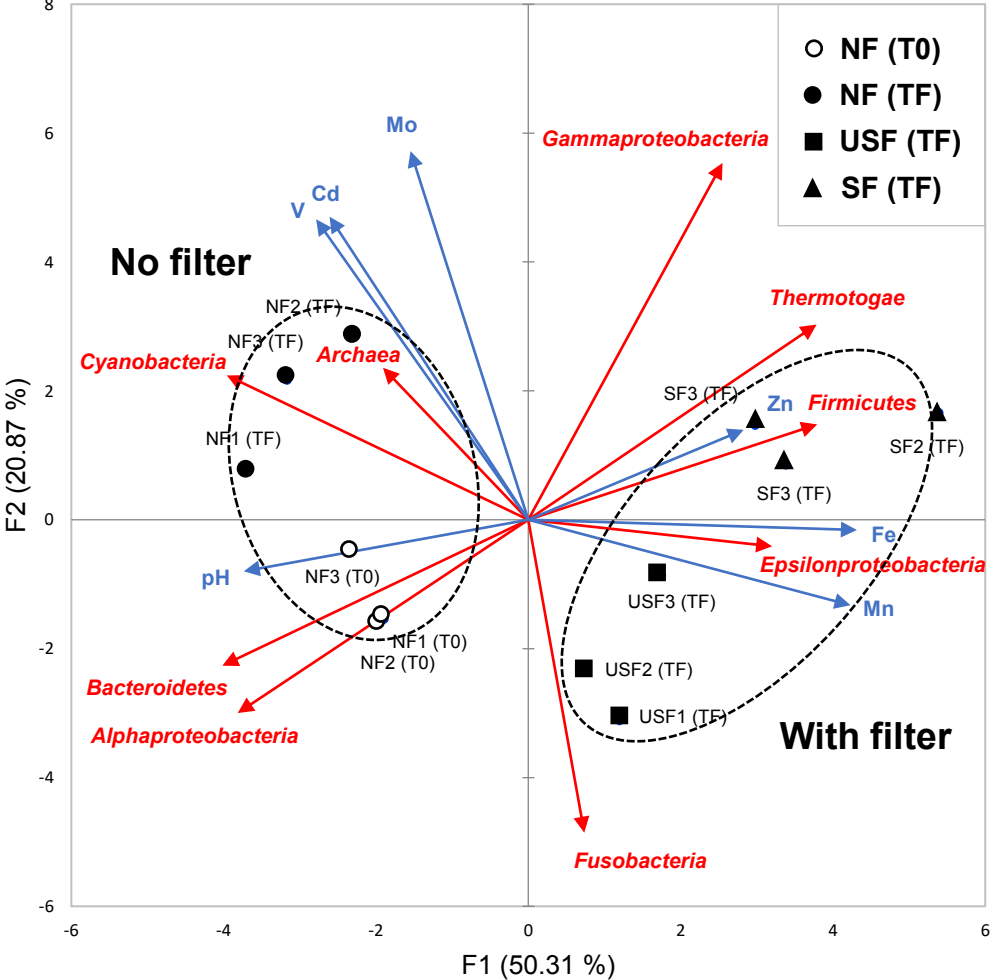


Figure 4





**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  $\pm$  standard deviation.**

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

<sup>1</sup> Difference significantly higher (>) or lower (<) or no difference (#) between conditions (NF, USF and SF) evaluated using ANOVA or Kruskal-Wallis tests (P<0.05).

<sup>2</sup> Dissolved Inorganic Nitrogen (DIN) values correspond to the sum of NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> values.

<sup>3</sup> Dissolved Inorganic Phosphorous (DIP) values correspond to PO<sub>4</sub><sup>3-</sup> values.

<sup>4</sup> Values (mean  $\pm$  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
<i>Alphaproteobacteria</i>	0,014	<b>-0,713</b>	<b>-0,608</b>	-0,133	-0,056	-0,385	0,503
<i>Bacteroidetes</i>	0,245	<b>-0,881</b>	<b>-0,776</b>	0,000	0,091	-0,385	<b>0,685</b>
<i>Cyanobacteria</i>	0,42	<b>-0,916</b>	<b>-0,839</b>	0,266	0,385	-0,266	<b>0,650</b>
<i>Gammaproteobacteria</i>	0,476	0,287	0,133	0,294	0,189	0,224	-0,343
<i>Firmicutes</i>	-0,294	<b>0,72</b>	<b>0,783</b>	0,168	0,140	0,231	<b>-0,692</b>
<i>Thermotogae</i>	-0,189	<b>0,867</b>	<b>0,783</b>	0,196	0,077	0,224	<b>-0,664</b>

**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
<i>Alphaproteobacteria</i>	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
<i>Bacteroidetes</i>	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
<i>Cyanobacteria</i>	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	<b>0,037</b>
	pH	-2,175	2,482	-0,876	0,410
<i>Gammaproteobacteria</i>	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
<i>Firmicutes</i>	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
<i>Thermotogae</i>	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)	<b>USF (Tf)</b>	SF (Tf)		
OTU_8 [MN463083]*	<i>Alphaproteobacteria</i>	0.34 ± 0.03	2.14 ± 0.63	<b>3.17 ± 0.79</b>	0.46 ± 0.33	<i>Roseibacterium beibuensis</i> [MG383386]	100
OTU_35 [MN463084]*	<i>Alphaproteobacteria</i>	0.03 ± 0.01	0.08 ± 0.05	<b>1.20 ± 0.20</b>	0.08 ± 0.07	<i>Rhodovulum marinum</i> [AM696692]	99.53
OTU_19 [MN463085]	<i>Bacteroidetes</i>	0.19 ± 0.04	0.98 ± 1.10	<b>1.42 ± 1.13</b>	0.01 ± 0.002	<i>Phaeodactylibacter xiamenensis</i> [NR_134132]	90.97
OTU_30 [MN463086]	<i>Bacteroidetes</i>	0.02 ± 0.004	0.11 ± 0.11	<b>0.69 ± 0.68</b>	0.59 ± 0.50	<i>Marinilabilia nitratreducens</i> [NR_132609]	96.84
OTU_16 [MN463087]	<i>Balneolaeota</i>	0.19 ± 0.03	1.76 ± 1.56	<b>1.01 ± 0.33</b>	0.05 ± 0.02	<i>Gracilimonas halophila</i> [NR_158001]	97.07
OTU_50 [MN463088]	<i>Deltaproteobacteria</i>	0.02 ± 0.01	0.02 ± 0.02	<b>0.63 ± 0.61</b>	0.07 ± 0.10	<i>Desulfovibrio psychrotolerans</i> [NR_042581]	92.05
OTU_60 [MN463089]	<i>Epsilonproteobacteria</i>	0.00 ± 0.01	0.01 ± 0.001	<b>0.64 ± 0.66</b>	0.01 ± 0.004	<i>Sulfurovum lithotrophicum</i> [CP011308]	88.24
OTU_1 [MN463064]*	<i>Firmicutes</i>	0.43 ± 0.06	4.38 ± 1.67	<b>4.33 ± 1.81</b>	6.17 ± 1.22	<i>Alkaliphilus transvaalensis</i> [NR_024748]	92.69
OTU_14 [MN463090]*	<i>Firmicutes</i>	0.04 ± 0.01	0.05 ± 0.01	<b>3.36 ± 2.30</b>	0.06 ± 0.01	<i>Vallitalea pronyensis</i> [NR_125677]	96.93
OTU_21 [MN463091]*	<i>Firmicutes</i>	0.38 ± 0.04	0.19 ± 0.22	<b>2.04 ± 0.29</b>	0.33 ± 0.30	<i>Pontibacillus litoralis</i> [NR_116372]	84.48
OTU_4 [MN463065]	<i>Firmicutes</i>	0.19 ± 0.01	0.16 ± 0.05	<b>4.25 ± 4.52</b>	5.60 ± 4.60	<i>Pontibacillus litoralis</i> [NR_116372]	84.00
OTU_31 [MN463092]*	<i>Firmicutes</i>	0.08 ± 0.02	0.18 ± 0.20	<b>0.82 ± 0.45</b>	0.09 ± 0.08	<i>Pontibacillus salicampi</i> [MH283830]	87.28
OTU_3 [MN463063]*	<i>Firmicutes</i>	0.10 ± 0.01	0.20 ± 0.15	<b>0.61 ± 0.89</b>	8.72 ± 6.31	<i>Exiguobacterium aestuarii</i> [MH881394]	99.55
OTU_2 [MN463093]	<i>Fusobacteria</i>	0.11 ± 0.01	0.12 ± 0.03	<b>12.68 ± 9.94</b>	0.15 ± 0.05	<i>Hypnocyclicus thermotrophus</i> [NR_145867]	92.96
OTU_22 [MN463094]*	<i>Gammaproteobacteria</i>	0.03 ± 0.01	0.04 ± 0.01	<b>10.88 ± 0.87</b>	0.31 ± 0.26	<i>Marichromatium gracile</i> [LT991979]	99.55
OTU_7 [MN463071]	<i>Gammaproteobacteria</i>	0.28 ± 0.04	3.18 ± 2.03	<b>2.11 ± 0.85</b>	3.54 ± 1.11	<i>Marinobacter hydrocarbonoclasticus</i> [MK131324]	100
OTU_28 [MN463095]	<i>Gammaproteobacteria</i>	0.10 ± 0.02	0.30 ± 0.03	<b>1.03 ± 0.78</b>	0.07 ± 0.07	<i>Alteromonas marina</i> [MH746022]	99.55
OTU_25 [MN463096]	<i>Gammaproteobacteria</i>	0.10 ± 0.02	2.06 ± 1.72	<b>1.02 ± 0.85</b>	0.09 ± 0.03	<i>Idiomarina taiwanensis</i> [KM407758]	99.55
OTU_5 [MN463085]*	<i>Thermotogae</i>	0.09 ± 0.02	0.31 ± 0.36	<b>0.98 ± 1.37</b>	8.55 ± 1.88	<i>Geotoga subterranea</i> [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]	<i>Alphaproteobacteria</i>	0.06 ± 0.01	0.06 ± 0.01	0.16 ± 0.19	<b>3.10 ± 2.76</b>	<i>Thalassospira australica</i> [MH304396]	99.29
OTU_13 [MN463062]	<i>Epsilonproteobacteria</i>	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.03	<b>3.05 ± 2.85</b>	<i>Arcobacter halophilus</i> [MG195897]	99.53
OTU_3 [MN463063]*	<i>Firmicutes</i>	0.10 ± 0.01	0.20 ± 0.15	0.61 ± 0.89	<b>8.72 ± 6.31</b>	<i>Exiguobacterium aestuarii</i> [MH881394]	99.55
OTU_1 [MN463064]*	<i>Firmicutes</i>	0.43 ± 0.06	4.38 ± 1.67	4.33 ± 1.81	<b>6.17 ± 1.22</b>	<i>Alkaliphilus transvaalensis</i> [NR_024748]	92.69
OTU_4 [MN463065]	<i>Firmicutes</i>	0.19 ± 0.01	0.16 ± 0.05	4.25 ± 4.52	<b>5.60 ± 4.60</b>	<i>Pontibacillus litoralis</i> [NR_116372]	80.00
OTU_56 [MN463066]	<i>Firmicutes</i>	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	<b>0.61 ± 0.89</b>	<i>Serpentinicella alkaliphila</i> [NR_152685]	98.35
OTU_46 [MN463067]*	<i>Firmicutes</i>	0.01 ± 0.01	0.01 ± 0.00	0.05 ± 0.04	<b>1.54 ± 0.87</b>	<i>Halolactibacillus miurensis</i> [AB681280]	99.11
OTU_36 [MN463068]	<i>Firmicutes</i>	0.12 ± 0.01	0.11 ± 0.04	0.14 ± 0.08	<b>0.84 ± 0.68</b>	<i>Tissierella creatinini</i> [NR_117155]	95.04
OTU_9 [MN463069]	<i>Gammaproteobacteria</i>	0.09 ± 0.03	0.10 ± 0.03	0.35 ± 0.47	<b>5.02 ± 8.47</b>	<i>Oceanimonas doudoroffii</i> [NR_114185]	99.33
OTU_10 [MN463070]	<i>Gammaproteobacteria</i>	0.14 ± 0.03	1.68 ± 2.18	0.45 ± 0.42	<b>3.70 ± 2.55</b>	<i>Idiomarina seosinensis</i> [MG575737]	100
OTU_7 [MN463071]	<i>Gammaproteobacteria</i>	0.28 ± 0.04	3.18 ± 2.03	2.11 ± 0.85	<b>3.54 ± 1.11</b>	<i>Marinobacter hydrocarbonoclasticus</i> [MK131324]	100
OTU_6 [MN463072]*	<i>Gammaproteobacteria</i>	0.20 ± 0.01	1.44 ± 0.72	0.76 ± 0.20	<b>7.85 ± 3.27</b>	<i>Marinobacterium sediminicola</i> [NR_044529]	100
OTU_42 [MN463073]	<i>Gammaproteobacteria</i>	0.06 ± 0.01	0.06 ± 0.03	0.17 ± 0.10	<b>1.02 ± 1.39</b>	<i>Marinobacter vinifirmus</i> [KX418471]	99.55
OTU_11 [MN463074]	<i>Gammaproteobacteria</i>	0.16 ± 0.00	0.26 ± 0.10	0.25 ± 0.11	<b>5.35 ± 4.53</b>	<i>Vibrio diabolicus</i> [MK308588]	100
OTU_647 [MN463075]	<i>Gammaproteobacteria</i>	0.09 ± 0.01	0.12 ± 0.04	0.17 ± 0.08	<b>2.69 ± 2.27</b>	<i>Vibrio owensii</i> [CP033138]	100
OTU_3745 [MN463076]	<i>Gammaproteobacteria</i>	0.03 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	<b>1.37 ± 1.39</b>	<i>Marinobacterium georgiense</i> [MH044627]	98.88
OTU_24 [MN463077]	<i>Gammaproteobacteria</i>	0.03 ± 0.01	0.06 ± 0.02	0.04 ± 0.02	<b>1.34 ± 1.28</b>	<i>Marinobacterium georgiense</i> [NR_114163]	98.66
OTU_47 [MN463078]	<i>Gammaproteobacteria</i>	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	<b>1.11 ± 1.20</b>	<i>Pseudomonas aestusnigri</i> [MF155916]	99.78
OTU_1430 [MN463079]	<i>Gammaproteobacteria</i>	0.05 ± 0.03	0.05 ± 0.02	0.07 ± 0.03	<b>1.05 ± 0.89</b>	<i>Vibrio owensii</i> [CP033137]	100
OTU_4626 [MN463080]	<i>Gammaproteobacteria</i>	0.04 ± 0.01	0.05 ± 0.03	0.05 ± 0.02	<b>0.91 ± 0.77</b>	<i>Vibrio harveyi</i> [EU834007]	98.86
OTU_5 [MN463081]*	<i>Thermotogae</i>	0.09 ± 0.02	0.31 ± 0.36	0.98 ± 1.37	<b>8.55 ± 1.88</b>	<i>Geotoga subterranea</i> [NR_029145]	100
OTU_15 [MN463082]	<i>Archaea/Halobacteria</i>	0.75 ± 0.04	1.21 ± 0.83	0.59 ± 0.27	<b>1.11 ± 0.38</b>	<i>Halogramum amylolyticum</i> [NR_113451]	99.51

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