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# Dynamics of microbial communities across the three domains of life over an annual cycle with emphasis on marine mucilage in the Southern Bay of Biscay resolved by microbial fingerprinting

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## Abstract

Marine mucilage has been described as worldwide phenomena occurring sporadically, or frequently in certain coastal areas. They are transitory phenomena that can remain in the photic zone for several days or weeks. Their occurrence has been more frequent and persistent, and their magnitude has increased during the last decades. Their formation typically reflects an imbalance in the marine microbial communities, and has frequently be linked to global changes. Recurrent marine mucilage events have been observed in recent years in the Southern Bay of Biscay near the French coast, mainly



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south of the Adour estuary. In this study, we used a fingerprinting method (T-RFLP) targeting 16S and 18S rRNA genes to investigate marine microbial community composition and dynamics over an annual cycle across the three domains of life in coastal water at three depths, in the Adour River upstream of the mouth and in the marine mucilage. In line with studies conducted in marine environments we highlighted that the dynamics of marine microbial communities in the Bay of Biscay coincided with two environmental contexts: winter and spring on one hand and summer and autumn on the other hand. More interestingly, this dynamics affects also the marine pelagic mucilage that was observed during the sampling campaigns. Thus, the composition of the marine mucilage that appears in winter/spring context diverges from the composition of the marine mucilage that occurred during the summer/autumn context. We highlighted also that marine microbial communities were partly different from marine mucilage communities with an enrichment of some microorganisms suggesting that marine mucilage behaves as a microhabitat in seawater and possesses distinct microbial assemblages from the surrounding communities.

## Keywords

Mucilage; littoral; coastal seawater; T-RFLP fingerprinting; microbial community structure; river

# Introduction

The marine realm is a vast ecosystem that covers more than 70% of the earth surface. It is composed of multiple habitats that represent a large range of environmental conditions as contrasted as polar and tropical waters, sub-seafloor and sunlit surface, open and coastal waters. All marine habitats host microorganisms encompassing the three domains of life: Bacteria, Archaea and Eukarya (Protists and Fungi) as well as biological entities such as viruses and viroids (Bolhuis and Cretoiu, 2016; Massana and Logares, 2013). Hence it has been calculated the bacterial and archaeal marine biomass was similar to that of the soil biomass (Overmann and Lepleux, 2016). Important advances have been done to document the diversity of the marine microbiome during the recent years (e.g. Rusch et al., 2007; Sunagawa et al., 2015), however knowledge is still lacking to better understand the role of microorganisms in marine ecosystem functioning. Particularly, information on how global changes affect the marine microbiome is dramatically scarce. Only recently, studies on microbial marine ecosystems have been extended beyond bacterial communities with increasing interest on archaeal and viral communities (e.g. Beman et al., 2011; Culley et al., 2010; Gustavsen et al., 2014; Hugoni et al., 2013; Sheik et al., 2014; Sintes et al., 2015; Suttle, 2007; Yilmaz et al., 2016; Zhang et al., 2015). Few studies have targeted the eukaryotic component in spite of its contribution to microbial diversity, its role in biogeochemical processes and its incidence on bacterial community dynamics (Caron et al., 2008). Integrative approaches considering microorganisms across the three domains of life are even scarcer. Also since microorganisms interact with each other and are therefore intimately linked, it is important to study all domains simultaneously (Bacteria, Archaea and Eukarya) in order to understand global changes in marine ecosystem networks (Fuhrman et al., 2015). Hence, the role of the coastal marine microbial communities in ecosystems functioning and services deserves further investigation, especially since coastal areas are impacted by human activities and submitted to important environmental variations (freshwater runoff, river and wastewater treatment plant discharges, etc).

65 A consequence of these disturbances are phytoplankton blooms and marine mucilage events (Cloern,
66 1996; Godrijan et al., 2013; Hoppenrath, 2004; Winder and Cloern, 2010). These consist in gelatinous
67 evolving stages caused by non-settling early marine snow. Their formation typically reflects an
68 imbalance in the marine microbial communities leading to formation of large aggregates in seawater.
69 Marine mucilage are transitory phenomenon that can remain in the photic zone for several days or
70 weeks. Their size ranges from 1 cm to several kilometers (De Lazzari et al., 2008; Precali et al., 2005).
71 They are a worldwide phenomena that occur sporadically or recurrently in certain coastal areas. Their
72 occurrence has been described in the Mediterranean Sea (Danovaro et al., 2009), in the Ariake Sound
73 in West Japan (Fukao et al., 2009), in the Tasman Bay (MacKenzie et al., 2002), near the USA Pacific
74 Coast (Alldredge and Crocker, 1995), in the Gulf of Mexico (Green and Dagg, 1997) and in the North
75 Sea (Lancelot, 1995). The phenomenon was first reported in the North Adriatic Sea, in the scientific
76 literature in 1729 (in Fonda Umani et al., 1989). Since then their occurrence has been more frequent
77 and persistent, and their magnitude has progressed, while a causal link to global changes was
78 suggested (Danovaro et al., 2009). Studies worldwide suggest that the trigger for marine mucilage
79 onset is multifactorial. Abiotic factors include climatic and anthropogenic pressure and the associated
80 changes in nutrient concentration in seawater (De Lazzari et al., 2008; Umani et al., 2006), whereas
81 biotic factors are the consequence of the microbial response to these changing conditions (Cozzi et al.,
82 2004; Flander-Putrle and Malej, 2008). Although increased scientific interest has been paid to mucilage
83 events, the causes of appearance, the dynamics, and the respective role of microorganisms in such
84 systems have yet to be elucidated.

85 In this study, we focused on recurrent marine mucilage events that have been observed in recent years
86 in the South Biscay Bay near the French coast, mainly south of the Adour estuary. Preliminary studies
87 indicated that these events are becoming more abundant and invasive in this area (Susperregui N.,
88 personal communication); they occur in spring and in autumn. Whereas most studies investigating
89 marine mucilage have focused on eukaryotic microbial communities, we target the microbial
90 component across the three domains of life, namely Bacteria, Archaea and Eukarya, in order to

91 describe the community dynamics in the context of mucilage formation, and compare the community
92 composition with that of the surrounding environmental compartments. We used molecular
93 fingerprinting (T-RFLP), easy and fast approach, to characterize the microbial community dynamics in
94 the mucilage and in the surrounding seawater during one year. In order to evaluate possible links with
95 continental inputs, the analysis was also performed in the Adour River water, a few kilometers
96 upstream of the mouth. T-RFLP was based on 16S and 18S rRNA gene sequences in order to obtain a
97 global picture of the microbial community structure. Physical-chemical and climatic parameters were
98 also recorded to explore the relationship between these parameters and the composition of the
99 microbial communities.

100 year

101

# 102 Material and Methods

103 *Study sites and sample collection*

104 The study took place between May 2013 and May 2014, with 39 time points. Five distinct
105 environmental compartments were investigated: river water, sea water (two stations) from three
106 different depths, and marine mucilage when present.

107 The Biarritz sea site, known to be impacted by mucilage, was routinely sampled monthly, and
108 intensively sampled (every 3 or 4 days, during 4 weeks) during episodes of marine mucilage, until
109 mucilage receded. The northern, the Tarnos sea site was sampled monthly. This location is less prone
110 to mucilage events, however this particular year we noted the presence of mucilage concurrently to
111 the Biarritz site.

112 A third site was located in the River Adour, four kilometers upstream of the mouth (figure 1 and table
113 S1); samples were collected one or two days prior to the monthly or intensive sampling sessions.

114 Sampling at sea was performed at high tide to avoid freshwater influence, conversely river water was
115 collected at low tide to avoid marine influence (salinity ≤ 0.74 $g.kg^{-1}$). Due to bad conditions at sea, no
116 sampling took place in January nor February 2014.

117 Water was collected with a Niskin bottle one meter below the surface in the Adour River, and at three
118 different depths from the two sea sampling stations (see Figure 1 inset, for details). Triplicates of river
119 and sea water were filtered individually through 0.2 µm pore-size cellulose nitrate filters (Sartorius
120 Stedim Biotech GmbH). Filters were stored at -80°C. Marine mucilage samples were collected with a
121 plankton net at the intermediate depth and filtered through 60 µm pore-size nylon membranes
122 (NITEX). The mucilage was collected in triplicates from the surface of the filters, mixed with a sterile
123 spatula, and stored at -80°C.

124 *Environmental data collection*

125 Seawater temperature and salinity were measured *in situ* with a CTD sensor (Seabird). Suspended
126 particulate matter was determined gravimetrically. Filters for particulate organic carbon (POC) and
127 particulate organic nitrogen (PON) were decarbonated using HCl vapors (Lorrain et al., 2003) and
128 analyzed using a Flash Elemental Analyzer Series 1112 (Thermo Finnigan). Nutrients were analyzed
129 according to Aminot and Kérouel (2007), using an AutoAnalyzer 3 (Bran+Luebbe). Transparent
130 exopolymer particles (TEP) were determined with the dye-binding assay, as previously described
131 (Passow and Alldredge, 1995). TEP was measured in the particulate fraction (filtration 60 µm).
132 Extracellular polymeric substance (EPS) were determined according to the protocol of Dubois et al.
133 (1956). In addition, hydrological, oceanological and climatic data for river flow, sea wave height,
134 average air temperature, wind force, pluviometry and daily sunshine hours, were obtained from the
135 public databases HYDRO (http://www.hydro.eaufrance.fr), CANDHIS
136 (http://candhis.cetmef.developpement-durable.gouv.fr/) and Météo France.

137 *T-RFLP analysis*

138 Genomic DNA was extracted in triplicate from marine mucilage aggregates, and from filtered

139 freshwater and seawater using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA)
140 according to the manufacturer's recommendation. Filters were previously cut in small parts with a
141 sterile surgical blade. All genomic DNA extracts were stored at -20 °C until further analysis.

142 Microbial communities profiling was assessed by terminal restriction fragment length polymorphism
143 (T-RFLP) targeting 16S and 18S rRNA genes. Target genes were amplified by Polymerase Chain Reaction
144 (PCR), using fluorescently labeled primers FAM-357F (5'-CCTACGGGAGGCAGCAG-3') (Lane, 1991) and
145 HEX-926R (5'-hexa-chloro-fluorescein-phosphoramidite-CCGTCAATTCMTTTRAGT-3') (Muyzer and
146 Ramsing, 1995) for bacteria, FAM-Arch349F (5'-GYGCASCAGKCGMGAAW-3') and HEX-Arch806R (5'-
147 hexa-chloro-fluorescein-phosphoramidite-GGACTACVSGGGTATCTAAT-3') (Takai and Horikoshi, 2000)
148 for archaea, and HEX Euk-1A-F (5'-hexa-chloro-fluorescein-phosphoramidite-CTGGTTGATCCTGCCAG-
149 3') (Sogin and Gunderson, 1987) and Euk-516-GC-R (5'-ACC AGA CTT GCC CTC C-3') (Amann et al., 1990)
150 for eukaryotes. Each replicate DNA extraction was amplified independently. The reaction mixture
151 contained 1 µl of DNA template (10 ng), 1 µl of both primers (10 µM), and 12.5 µL of PCR Master Mix
152 Ampli Taq Gold 360 (Applied Biosystems, Foster City, CA). Sterile distilled water was added to obtain a
153 final volume of 25 µl. Cycling conditions were as follow: for the bacterial 16S rRNA gene, 95 °C for 10
154 min, 35 cycles at 95 °C for 45 s, 55°C for 45 s, and 72°C for 1 min, followed by 10 min at 72 °C; for the
155 archaeal 16S rRNA gene, 95 °C for 10 min, 40 cycles at 95 °C for 45 s, 61°C for 4 5s, and 72°C for 1 min,
156 followed by 10 min at 72 °C; and for the eukaryotic 18S rRNA gene, 94 °C for 10 min, 35 cycles at 94 °C
157 for 30 s, 56°C for 45 s, and 72°C for 1 min, followed by 10 min at 72 °C.

158 PCR products were purified with Illustra GFX$^{TM}$ 96 PCR and the GFX$^{TM}$ Gel Band Purification Kits (GE
159 Healthcare, Munich, Germany). PCR products concentrations were determined by comparison with
160 molecular markers (Smartlader, Eurogentec) after migration on a 1% agarose gel. Approximately 100
161 ng of purified amplicon was digested in 10 µl reaction with 20 U of enzyme *Msp*I (New England Biolabs
162 Inc., Ips- wich, MA) for 3 h at 37 °C. Terminal restriction fragments (T-RF) profiles were obtained from
163 the digested amplicons by suspending 1 µl aliquots in a mix of 8.75 µl formamide with 0.25 µl of

164 Genescan ROX 500 size standard (Applied Biosystems), followed by capillary electrophoresis on an ABI
165 PRISM 3130xl Genetic Analyser (Applied Biosystems).

166 *Pretreatment of T-RFLP data*

167 Raw data were first collected and analyzed using Genemapper software (version 1.4, Applied
168 Biosystem). All T-RFLP electrophoregrams were visually inspected to check the quality of the
169 electrophoresis. T-RF between 50 and 450 bp, labeled by HEX and above a threshold of 35 fluorescence
170 units, were included in the analysis, so as to avoid background noise. The resulting data were imported
171 into T-REX software (Culman et al., 2009) for further processing. Data were subjected to quality control
172 procedures including T-RF alignment (clustering threshold=1 bp) and noise filtering (peak area). For
173 statistical analyses, T-RF fluorescence intensity was standardized to the total fluorescence of each
174 electrophoregram. The average of three replicates was taken to obtain overall diversity per sample.
175 Finally, T-RF representing more than 1% of the cumulative peak height for the sample were included
176 in the analysis. Two samples were removed after filtering due to an excess of background noise.

177 *Statistical analyses*

178 Primer-E software (PRIMER-E Ltd., Ivybridge, UK) with Permanova + extension (Anderson et al., 2008)
179 was used to perform multivariate analysis on the environmental data and T-RFLP profiles. Differences
180 in environmental characteristics and diversity between Biarritz and Tarnos samples were evaluated by
181 paired t-test with R-software v.3.1.2. Principal Components Analysis (PCA) was used to ordinate
182 samples according to environmental variables after Log(x+1)-transformation and normalization.
183 Further agglomerative cluster analysis (AHC) based on Gower distance and SIMPROF permutation tests
184 (1000 permutation) were performed to check for significant grouping among sampling conditions.
185 Correlations among environmental variables were calculated using Spearman coefficient.

186 Non-parametric Shannon alpha diversity estimates, were calculated with R software with the vegan R-
187 package (Oksanen et al., 2013) for each sample. Mann-Whitney/Wilcoxon rank-sum test was used
188 using R-software v.3.1.2 to identify differences between groups based on Shannon diversity indices.

189 Biological Bray-Curtis indexes were used to obtain similarity distance matrix among samples from T-RF
190 data. PCoA (Principal Coordinate Analysis) was performed to highlight distances between the
191 communities. PERmutational Multivariate ANalysis Of VAriance (PERMANOVA) was performed to test
192 for significant differences between groups using the Bray-curtis matrix with 1000 permutations.
193 SIMiliraty PERcentage (SIMPER) analysis was performed with Primer-E software to identify T-RF
194 contribution to each compartment. T-RF distribution was assessed by heat map analysis using *ggplot2*
195 R-package (Wickham, 2009).

196

# 197 Results and discussion

## 198 Physical-chemical characterization of samples at the intermediate depth revealed two contexts 199 during the year

200 Water of the Adour River collected 22 kilometers upstream the estuary was included in the survey in
201 order to understand freshwater and terrestrial nutriments inputs into the coastal seawater. The Adour
202 River has a pluvio-nival regime, in consequence its flow rate was higher during the snow melt and high
203 pluviometry of late winter and spring than during summer and autumn (Fig. 2a). During the sampling
204 period of this study the highest flow was observed in January 2014 (25/01/2014, 3643 $m^3$/day).
205 Environmental data collected in the Adour River and in the sea station showed that Particulate Organic
206 Carbon and Nitrogen (POC and PON, respectively) and nutrient salts (phosphate and silicate)
207 concentration in the Adour River were higher than in the seawater (Figure S1). However, the lag time
208 observed in the sea for these parameters showed clearly that the Adour discharge influences the
209 concentration of these elements at the two marine stations (Supplementary Table S1 and Figure S2).
210 Marine mucilage appeared in the southern Bay of Biscay after Adour River flow peaks of late winter
211 and spring and during the low-flow period of summer and autumn. Since climatic and physical-
212 chemical parameters might act synergistically to trigger marine mucilage formation, we investigated

213 their dynamics. Due to their proximity, climatic parameters at Biarritz and Tarnos sampling stations
214 were identical throughout the year, and environmental conditions similar (paired t-test, P=0.8213 for
215 monthly sampling). Thus, the exhaustive data set of Biarritz was used in the PCA analyses (Fig. 2b). All
216 together, the first two principal components (PC) of the PCA accounted for 53.5% of the variation in
217 the environmental data set. Seawater temperature, Adour River flow, TEP (Transparent Exopolymer
218 Particules) and silicate concentrations were essentially represented by PC1. This axis clearly separated
219 samples collected during the late summer and autumn period (hereafter called SA) from samples
220 collected the rest of the year namely winter and spring period (hereafter called WS). Indeed high
221 seawater temperatures mostly characterized SA samples whereas in contrast WS samples were mostly
222 positively correlated to Adour River flow, $SiOH_4$ and TEP concentrations. Interestingly the scattering of
223 the WS samples along PC1 and PC2 highlighted that these samples were collected during more
224 changing conditions whereas conditions during summer and autumn were more stable and
225 homogeneous, according to environmental parameters collected. Thus in addition to the above
226 mentioned descriptors, WS samples were mostly influenced by high concentrations of PON, POC,
227 suspended particulate material and $PO_4^{3-}$, elevated wind force and waves height. Clustering of all SA
228 samples (apart BM.16.07.13) was supported by permutation tests (80.33 % similarity, SIMPROF
229 p=0.076).

230 As outlined by Charles et al. (2012), these two periods show different dynamics in the Bay of Biscay.
231 Winters and springs are generally very rainy, high waves mix the sea, and the high Adour River outflows
232 are due to snow melt in the Pyrenees Mountains. In contrast, summers and autumns are sunny, the
233 sea is calm and Adour River outflows are low. Our observations highlighted that mucilage occurred
234 during these two different contexts in the Southern Bay of Biscay.

235 Over the period of our study, marine mucilage was regularly present during WS, which corresponds to
236 a period of repeated freshwater pulses. The frequency of the pulses did not allowed to link the two
237 events but as suggested by Degobbis et al. (1995) for the Adriatic Sea it is possible that the water pulses

238 we observed have favored mucilage formation under the cover of reduced water dynamics. However,
239 during SA period the onset of mucilage occurred once, in a contrasting situation. Indeed the Adour
240 river flow was low with no freshwater pulse, sea conditions were calm and the water temperature was
241 elevated. This observation indicates that freshwater pulse is not a necessary condition to trigger
242 mucilage formation which can depend of more complex phenomena. Hence several additional studies
243 in the Northern Adriatic Sea, including long term studies led over 10 years, revealed that in addition to
244 air and water temperature increase, the lack of mixing in low flow periods may have enhanced water
245 column stratification and favor phytoplankton blooms, whereas nutrient limitation may have increased
246 plankton EPS excretion (Cozzi et al., 2004; Degobbis et al., 1995; Degobbis et al., 2005; Deserti et al.,
247 2005; Russo et al., 2005; Umani et al., 2006). The onset of mucilage in the SA was observed in such
248 climatic context. Also in the Adriatic Sea, it has been suggested that a summer gyre has favored
249 mucilage occurrence further north (Cozzi et al., 2004). It is not known yet whether such currents have
250 an impact on marine mucilage formation in the southern Bay of Biscay, but but the influence of eddies
251 reported in this area (Ferrer et al., 2009; Koutsikopoulos and Le Cann, 1996)could be investigated in
252 the future.

## 253 Patterns of microbial diversity in marine seawater, and marine mucilage communities within the 254 year coincides with two environmental contexts

255 In the Northern Adriatic Sea marine mucilage tends to appear after phytoplankton blooms (Totti et al.,
256 2005). In the Southern Bay of Biscay, marine mucilage also appeared after periods known for their
257 dinoflagellates and diatoms blooms (Garcia-Soto and Pingree, 2009; Varela, 1996). In order to
258 investigate the respective dynamics of microbial communities during and outside marine mucilage
259 episodes in the Southern Bay of Biscay, we analyzed the microbial diversity in the three domains of life
260 during an annual cycle.

261 Seawater and mucilage showed contrasting diversity patterns of microbial alpha diversity

262 T-RFLP profiles revealed overall similar global richness for bacteria and eukaryotes with a total of 141
263 and 145 T-RF, respectively. Richness was much lower for Archaea with only 79 T-RFs (Fig. S1).
264 Noteworthy, archaeal 16S rRNA genes were not detected in marine mucilage despite the use of three
265 different primer couples and different PCR conditions. Although methodological bias cannot be
266 excluded, it has to be noted that 16S Bacteria and 18S Eukarya rRNA gene sequences were successfully
267 amplified with the same DNA extracts. To our knowledge, microorganisms belonging to archaea
268 domain had never been investigated and described in marine mucilage. Data from other studies are
269 thus clearly lacking to support our observation. We found however that this result echoes a study
270 conducted by DeLong et al. (1993) that failed to detect Archaea in marine aggregates. Since then, it
271 has been described that Archaea of the MGII group were associated to POM and aggregates (Delong,
272 2007; Orsi et al., 2015). In addition, Vojvoda et al. (2014) reported Archaea in both the marine snow
273 and the surrounding waters across seasons although at low density. However, they observed that
274 archaeal diversity and abundance increases with depth while presenting generally a higher diversity in
275 the smallest size-fractions of the particulate matter (Mestre et al., 2017). Furthermore, Mestre and
276 coworkers' study (2017) highlighted that Archaea compared to Bacteria were present at low
277 abundance in particulate matter fraction (representing around 8% of Prokaryotes sequences). As
278 discussed below, we showed also in this study that Archaea harbored the lowest diversity regardless
279 of the environment. In consequence, low abundance and low diversity may explain the difficulties to
280 amplify archaeal 16S rRNA gene sequences in the mucilage samples. Indeed, it is likely that mucilage
281 does not represent an adequate niche for Archaea because they cannot outcompete with R-strategist
282 Bacteria that colonize the mucilage, a transient nutrient-rich environment (Dang and Lovell, 2016;
283 Ghuneim et al., 2018). So, in order to determine whether Archaea colonize marine mucilage, further
284 efforts avoiding PCR bias, such as deep shotgun sequencing, will be required.

285 In the seawater column, the average Shannon diversity indices ($H_{mean}$, Table 1) indicated that archaeal
286 communities exhibited the lowest diversity compared to Bacteria and Eukarya. This result is consistent

287 with the conclusion of Massana et al. (2000) showing that surface waters belonging to different
288 provinces were dominated only by one or two OTUs in temperate areas. In addition, low diversity
289 would be linked to low archaeal cell densities in the first 100 m deep in the water column (DeLong et
290 al., 1999). Here, we also showed that the overall alpha diversity in the sea did not differ from that of
291 the Adour River (MWW, P=0.059). This result although different from a previous report showing that
292 archaeal diversity in freshwater was higher than in seawater (Hugoni et al., 2015) is however at the
293 edge of the statistical significance.

294 It is worthy to note that the overall alpha diversity for the three domains was homogenous along the
295 water column in the seawater (Table 1, MWW >0.05 for all comparisons). Considering the overall level
296 of alpha diversity for eukaryotes, we observed that average Shannon diversity indexes were
297 significantly higher in the Adour River (2.34 ± 0.45) than in the marine mucilage and the seawater
298 (Table 1, MWW P<<0.001 for both comparisons). In addition, overall alpha diversity for Eukaryotes was
299 also higher in the seawater than in the marine mucilage indicating that the later showed the lowest
300 eukaryotic diversity (Table 1, MWW P<<0.001). In contrast, the global alpha diversity of Bacteria was
301 significantly lower in the Adour River (1.85 ± 0.29) and the marine mucilage (1.97± 0.58) than in the
302 seawater (minimum of 2.22 ± 0.31) (MWW, P≤0.01 for both comparisons) whereas Adour diversity
303 was equivalent to that of the marine mucilage (MWW, P=0.58). In contrast, Danovaro et al. (2009)
304 found that bacterial diversity was more elevated in marine mucilage than in the surrounding marine
305 waters in the Adriatic Sea. The discrepancies between our study and that of Danovaro et al. (2009) can
306 be explained by differences in terms of, biochemical composition of mucilage, development stages,
307 maturity and advance in recruitment and succession of microbial species on mucilage surfaces that
308 affect microbial composition of communities (Dang and Lovell, 2000; Del Negro et al., 2005). Indeed,
309 a closer examination of Shannon diversity dynamics during the year (Fig. 3) showed that the alpha
310 diversity, both Eukarya and Bacteria, in the marine mucilage exceeded the diversity recorded in the
311 seawater at some dates. The increase of diversity was generally sharp and followed by a decline of
312 diversity in the mucilage that was not observed in the seawater column. This observation suggested

313 that the microbial community dynamic in the marine mucilage might be controlled by endogenous
314 conditions that are becoming less favorable inside the mucilage with time. Indeed, several papers
315 describe the succession of microbial species observed during the different stage of mucilage events
316 (Bongiorni et al., 2007). Marine mucilage includes an important fraction of extracellular polymeric
317 substances (EPS), mainly produced by algae (Bongiorni et al., 2007; Svetlicic et al., 2005). These
318 molecules are recognized as a major nutrient source in the oceans (Bongiorni et al., 2007; Casillo et al.,
319 2018) in addition to energy resources. They also provide solid surfaces suitable for attachment and
320 colonization (Decho, 1990; Decho and Gutierrez, 2017). It has been shown that fresh mucilage of the
321 Adriatic was dominated by diatoms (Najdek et al., 2005), and then the microbial composition of
322 mucilage changes with age and size (Najdek et al., 2002). The main pioneer bacterial colonising the
323 mucilage has been identified belonging to the *Alteromonas* genus in the Adriatic Sea, before observing
324 the ecological succession of heterotrophic bacteria and phytoplankton (Blažina et al., 2011). The
325 abundance of the dominant phytoplankton species has been shown to increase with the age of the
326 mucilage (Totti et al., 2005). It is likely that mucilage is a dynamic habitat where the composition and
327 abundance of microorganisms change over time until its diversity is reduced, reflecting its collapse.

328 Overall bacterial, archaeal and eukaryotic diversities in the seawater followed the same dynamic
329 except for some dates. For instance, an intriguing dynamic during autumn 2013 was noticed with an
330 increase of bacterial diversity while in the same time archaeal diversity decreased in the seawater
331 column, particularly at the sub-surface. Opposite trends observed between diversity patterns of the
332 three domains of life suggested negative interactions between the different communities, which
333 include either top-down control or unfavorable environmental conditions at the time of the year for
334 some lineages. Finally, T-RFLP analysis did not revealed any specific trend in the variation of alpha
335 diversity (considering the three domain of life) around or during periods of marine mucilage
336 appearance in the seawater or river water. However, this observation did not exclude that the relative
337 abundance of some species could have been altered by some replacement processes, without affecting
338 the alpha diversity.

339 Microbial dynamics fit with two environmental contexts

340 The overall structure of the microbial communities during the year indicated that the Adour River
341 water exhibited more stable archaeal and bacterial communities than the marine seawater, while the
342 magnitude variations of the eukaryotic communities were similar in the river and the sea (Table 2).
343 Such high level of similarity between the Adour River samples was unexpected because estuarine
344 waters may contain a large range of microbial taxa originating from marine water and freshwater as
345 well as from soil and sediments that are expected to generate some dynamics in terms of microbial
346 diversity. The behavior of the archaeal community was particularly interesting because information on
347 seasonal dynamics of Archaea is dramatically lacking in estuarine and freshwater ecosystems (Hugoni
348 et al., 2015). The high similarity among samples revealed in our study suggested that planktonic
349 estuarine archaeal communities were poorly affected by seasonal variation in the Adour River. This
350 conclusion was clearly highlighted on the PCoA plot (Fig. 4) and well supported by PERMANOVA test
351 (P=0.571). Similarly, the bacterial communities were highly similar between samples (74.77 %)
352 suggesting that they were even more stable than Archaea during the same period. In the seawater,
353 similar stability was observed, but to a lesser extent. Noteworthy, the bacterial composition of the
354 marine mucilage varied during the year at the same range than of the seawater.

355 In contrast to the Adour River, the marine archaeal communities, as well as the marine eukaryote and
356 bacterial communities, clustered into two groups, which corresponded to the two marine
357 environmental contexts WS and SA (Fig. 4). This observation was confirmed by PERMANOVA tests
358 ($P \leq 0.003$). Fuhrman et al. (2015) underlined the importance of temporal dynamics at different time
359 scales in oceanic microbial communities. Although our data were collected only during one year, the
360 observed pattern suggested that the microbial communities in the Southern Bay of Biscay were also
361 influenced by seasonal factors as it has been highlighted previously in numerous marine environments
362 (Cram et al., 2015; Fuhrman et al., 2006; Gilbert et al., 2011; Giovannoni and Vergin, 2012; Pinhassi
363 and Hagstroem, 2000; Steele et al., 2011). For the archaeal communities, the first two PCO axes
364 explained 66.6 % of the variation in the data set (Fig. 4). PCO2 clearly separated the samples according

365 to these two periods. For eukaryotes and bacterial communities, the first two PCO axes explained 34.8
366 % and 34.3 % of the variation, respectively. The observed pattern was consistent for the first five axis
367 that represent around 60 % of the total variation in both cases. This result was consistent with Gilbert
368 et al. (2011) that identified the day length cycle as main environmental parameter explaining most of
369 the variability in the seasonal pattern of species diversity. Interestingly, although we did not considered
370 this parameter in our study it appears that our results are consistent with Gilbert's study. Also all the
371 parameters that co-varied with this cycle appeared thus far more decisive than trophic interactions in
372 marine ecosystems. In the Adour River such pattern was not observed, except for the bacterial
373 communities (P= 0.026). However this result did not mean that seasons do not influence the structure
374 of archaeal and eukaryotic communities but rather that the two contexts defined from data collected
375 from the sea do not apply to the riverine system. Moreover the power of our test was also reduced in
376 this compartment where collected data are much less numerous than in the marine environment.

377 The overall composition of the marine mucilage regarding bacterial and eukaryotic communities was
378 similar between the two sampling stations, Biarritz and Tarnos, that are 8 km apart (Fig. 4,
379 PERMANOVA tests P=0.7 for both comparisons). Interestingly regarding the two environmental
380 contexts (SA and WS), our analysis revealed that the composition of the marine mucilage, both for
381 bacterial and eukaryotic communities, was different (PERMANOVA, P=0.002 for bacterial and P=0.026
382 for eukaryotic communities) at the Biarritz sampling station (not compared for Tarnos due to sample
383 size limitation). This seasonal pattern highlighted that marine mucilage that occurs in the Southern Bay
384 of Biscay involved different cohorts of microorganisms. This result was in accordance with previous
385 studies on marine mucilage from the Northern Adriatic Sea (Degobbis et al., 1995). Finally, our study
386 also suggested that the marine mucilage represent a microhabitat colonized by microbial communities
387 different to that observed in the seawater column, particularly at the intermediate depth where was
388 collected the marine mucilage. The bacterial communities of the mucilage showed only 58.5 % and
389 63.2 % similarity with bacterial communities of the seawater at SA and at WS respectively
390 (PERMANOVA tests, P= 0.001 for both tests). Similar observation was made for the eukaryotic

391 communities that showed 63.5 % of similarity with those of the seawater at SA (PERMANOVA tests, P=
392 0.001) and 64.5 % at WS (PERMANOVA tests, P=0.118).

## 393 The composition of bacterial and eukaryotic communities reveals links between marine
## 394 mucilage and seawater microbial communities

395 In order to describe the relationships of the mucilage microbial communities with those of the
396 seawater, the abundance of the most representative bacterial and eukaryotes T-RFs was compared by
397 heat map analysis (Fig. 5). Although nearly similar patterns were observed for both bacteria and
398 eukaryotes, some T-RFs were more abundant either at SA or WS. This observation indicated that some
399 T-RFs appeared according to the season. In contrast, some T-RFs were ubiquitous as for example T-RF
400 X276G.278G (Eukarya) and T-RF X364G.365G (Bacteria) that were present at similar abundance during
401 the year in both the mucilage and the seawater. Other T-RFs found in both mucilage and seawater
402 during the year became predominant at some dates in either the mucilage or the seawater, as the
403 eukaryotic T-RF X374G.378G for example. Also, seasonal patterns were observed, as for example the
404 T-RF X279G.280G (Eukarya) and T-RF X225G.226G (Bacteria) that were mainly recorded at SA.
405 Noteworthy, the T-RFs that were detected at low abundance in marine mucilage were specifically
406 detected in the seawater at the intermediate depth. In contrast, the bacterial T-RF X368G.369G and
407 the T-RF X296G.297G, which were among the most abundant T-RFs in the mucilage during the year,
408 were seasonally detected in the seawater intermediate depth. Finally, specific T-RFs were also
409 identified for either the mucilage (T-RF X85G) or the seawater (T-RF 380G.381G). Although we cannot
410 ascertain that identical T-RF found in different samples were strictly made of the same species, their
411 absence or their abundance can be more robustly considered. Hence the increase of some
412 representative T-RFs in marine mucilage is consistent with previous studies showing that marine
413 mucilage communities were built with eukaryotes that aggregated from the seawater (Godrijan et al.,
414 2013; Hoppenrath, 2004). Moreover, in the northern Adriatic Sea, in the area where marine mucilage
415 appears, most of the eukaryotic species observed in mucilage have also been detected in seawater in

416 this area, regardless of its presence. (Totti et al., 2005). Thereby, it is worthy to note that marine
417 mucilage would concentrate some marine species within its matrix. The differences we noticed
418 between marine mucilage and seawater bacterial communities were in accordance with the results of
419 Danovaro et al. (2009) highlighting composition differences between marine mucilage and surrounding
420 seawater. Our study highlighted that the mucilage owned different abundant T-RFs to that observed
421 in the seawater, observation that was consistent with Del Negro et al. (2005) reporting that the
422 microbial community dynamics of the mucilage was different to that of the seawater.

423

# 424 Conclusion

425 In this study, fingerprinting method (T-RFLP) was used to investigate marine microbial community
426 dynamics across the three domains of life in coastal water at three depths, in the river upstream of the
427 mouth of the Adour River and in the marine mucilage that occurred along the coast of the Southern
428 Bay of Biscay. We aimed to reveal potential relationships between these communities with a special
429 emphasis on marine mucilage composition. Sampling performed over an annual cycle allowed to
430 investigate these relationships. In line with numerous studies conducted in marine environments we
431 highlighted that the dynamics of coastal marine microbial communities in the Southern Bay of Biscay
432 coincided with two environmental contexts: winter and spring on the one hand and summer and
433 autumn on the other hand. More interestingly, this dynamics affected also the marine mucilage that
434 was observed during the sampling campaigns conducted during this study. Thus, the composition of
435 the marine mucilage in winter/spring diverged from the marine mucilage of summer/autumn. We
436 highlighted also that although marine microbial communities were partly different from marine
437 mucilage communities for all domains of life. Some microorganisms would however originated from
438 surrounding water, in particular micro-eukaryotes. Intriguingly Archaea were not detected in the
439 mucilage. Thus, marine mucilage acts as a microhabitat in sea water that owned distinct microbial

assemblages established, in part, by the recruitment of specific organisms from surrounding communities.

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# Figure captions

**Figure 1**: Map of the sampling stations in the Southern Bay of Biscay. Two marine sampling stations were located off the Basque coast: Biarritz station (43° 29’ 211 N, 1° 36’ 095 W) and Tarnos station (43° 33’ 445 N, 1° 32’ 428 W). The freshwater station (43° 30’ 076 N, 1° 17’ 522 W) was situated on the Adour, one of the main river of the Southern Bay of Biscay. Inset: details of sampling depths, and sample codes.

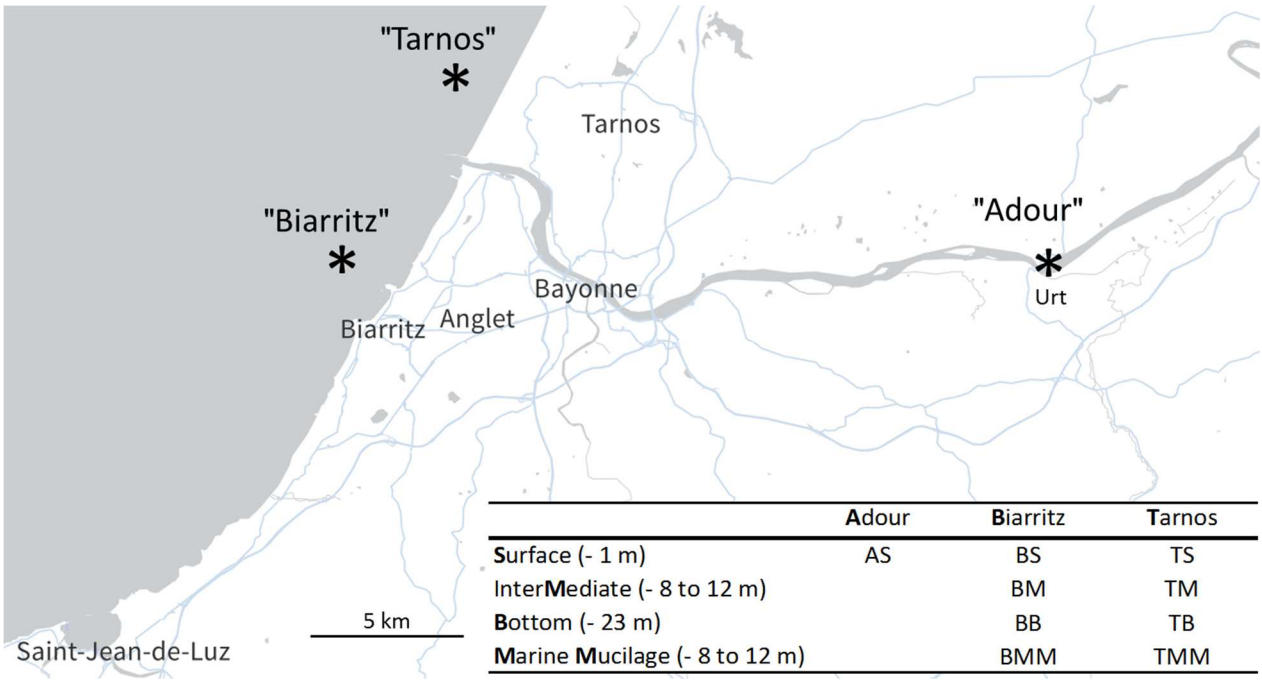
**Figure 2:** Adour River flow and characterization of sampling points recorded at Biarritz station at the intermediate depth according to environmental parameters during the one-year sampling campaign. a): Adour River flow. Black arrows shows Adour River samplings, dark grey arrows shows seawater samplings during days without marine mucilage, light grey arrows shows seawater and marine mucilage samplings. b): PCA on environmental parameters surveyed for all sampling points during the one-year sampling. Empty black squares represent the samples collected during summer and autumn (SA) and the grey triangles represent the samples collected during winter and spring (WS). Physical-chemicals parameters were measured in the seawater at the intermediate depth that is the depth where marine mucilage was also collected. Climatic parameters represent the day average. Seawater and air temperatures are in Celsius degrees, salinity is in g.kg-1, suspended particulate matter concentration is in mg.L-1, PON, POC, EPS and TEP concentration are in µg.L-1, SiOH4 and PO43- concentration is in µmol.L-1, Adour River flow is in m3.s-1, sea wave height is in meters, pluviometry is in millimeters per day, atmospheric pressure is in hPa and daily sunshine is in hours per day. Samples labelled with a cross represent samples collected outside a period of mucilage.

**Figure 3:** Diversity dynamics for the three domains of life (Archaea, Bacteria and Eukarya) measured through Shannon index over the year within five environmental compartments (Adour river, Seawater: surface, intermediate and botton, marine mucilage) at the Biarritz sampling station.

**Figure 4:** Changes in microbial community composition over the year across the three domains of life: Archaea, Bacteria and Eukarya. Each symbols in the PCoA plot represents a sample, with distance between samples calculated using Bray-Curtiss similarity measures. The legend indicated the color code for the plots, AS: Adour River, BS and TS: Biarritz and Tarnos surface seawater, BM and TM: Biarritz and Tarnos intermediate depth, BB and TB: Biarritz and Tarnos bottom seawaters, BMM and TMM: Biarritz and Tarnos marine mucilage, WS: winter and spring, SA: summer and autumn.

**Figure 5:** Heat map showing the T-RF distribution of the 20 most abundant T-RFs in marine mucilage and in the seawater at the intermediate depth over the year. Names of the T-RF are on the Y axis.

**Figure 1**

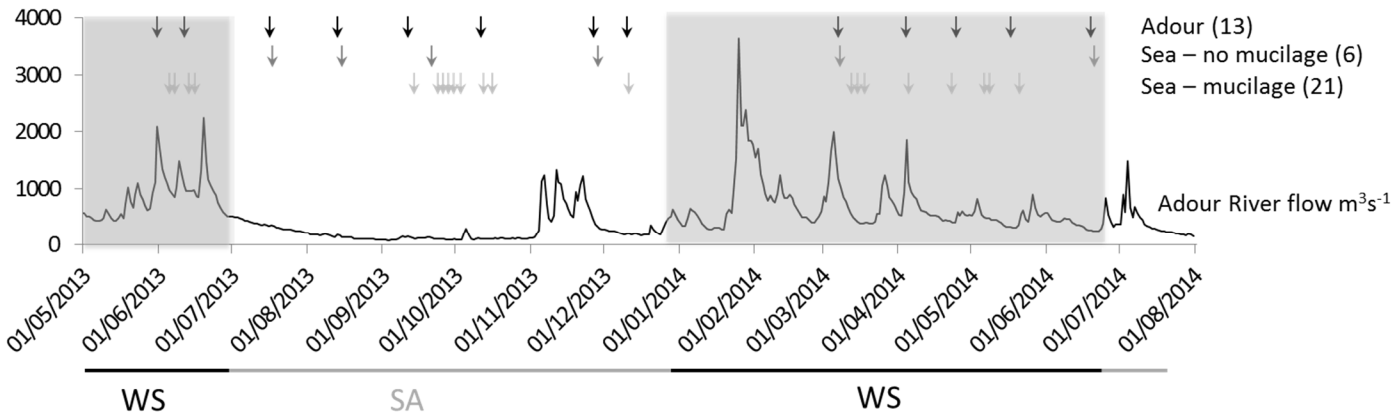



| | Adour | Biarritz | Tarnos |
|---|---|---|---|
| Surface (- 1 m) | AS | BS | TS |
| InterMediate (- 8 to 12 m) | | BM | TM |
| Bottom (- 23 m) | | BB | TB |
| Marine Mucilage (- 8 to 12 m) | | BMM | TMM |

**Figure 1**: Map of the sampling stations in the Southern Bay of Biscay. Two marine sampling stations were located off the Basque coast: Biarritz station (43° 29’ 211 N, 1° 36’ 095 W) and Tarnos station (43° 33’ 445 N, 1° 32’ 428 W). The freshwater station (43° 30’ 076 N, 1° 17’ 522 W) was situated on the Adour, one of the main river of the Southern Bay of Biscay. Inset: details of sampling depths, and sample codes.

**Figure 2**

**a)**




**b)**

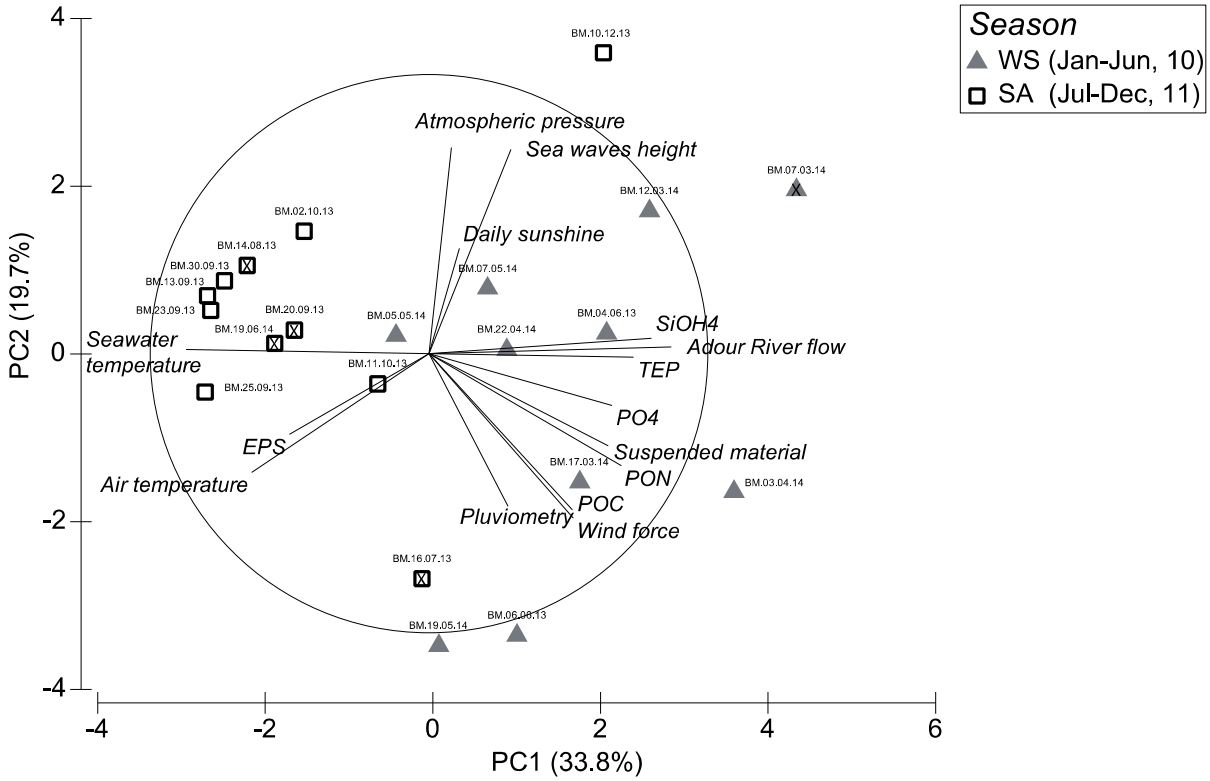
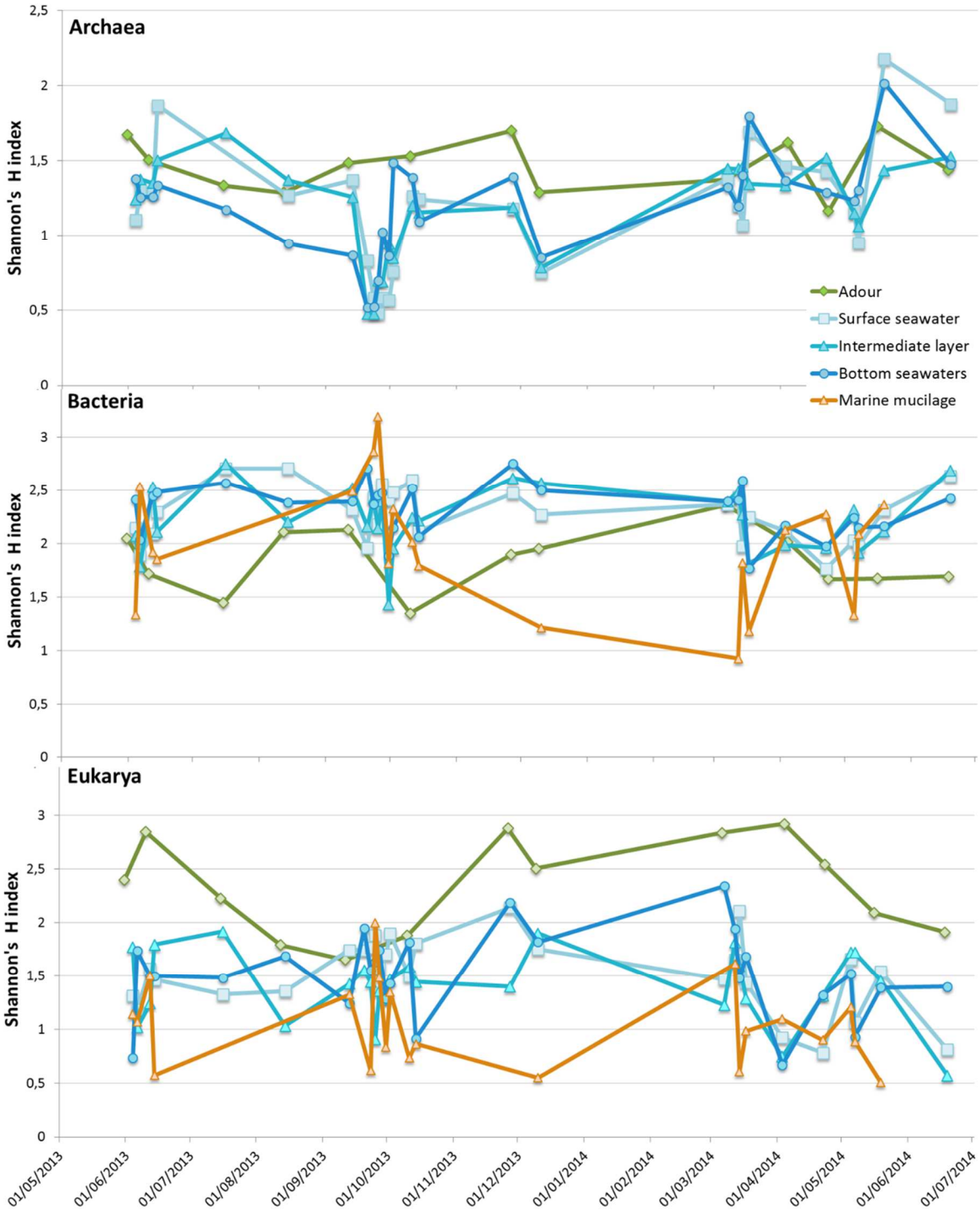



**Figure 2:** Adour River flow and characterization of sampling points recorded at Biarritz station at the intermediate depth according to environmental parameters during the one-year sampling campaign. **a**): Adour River flow. Black arrows shows Adour River samplings, dark grey arrows shows seawater samplings during days without marine mucilage, light grey arrows shows seawater and marine mucilage samplings. **b**): PCA on environmental parameters surveyed for all sampling points during the one-year sampling. Empty black squares represent the samples collected during summer and autumn (SA) and the grey triangles represent the samples collected during winter and spring (WS). Physical-chemicals

parameters were measured in the seawater at the intermediate depth that is the depth where marine mucilage was also collected. Climatic parameters represent the day average. Seawater and air temperatures are in Celsius degrees, salinity is in $g.kg^{-1}$, suspended particulate matter concentration is in $mg.L^{-1}$, PON, POC, EPS and TEP concentration are in $\mu g.L^{-1}$, $SiOH_4$ and $PO_4^{3-}$ concentration is in $\mu mol.L^{-1}$, Adour River flow is in $m^3.s^{-1}$, sea wave height is in meters, pluviometry is in millimeters per day, atmospheric pressure is in hPa and daily sunshine is in hours per day. Samples labelled with a cross represent samples collected outside a period of mucilage.

**Figure 3**

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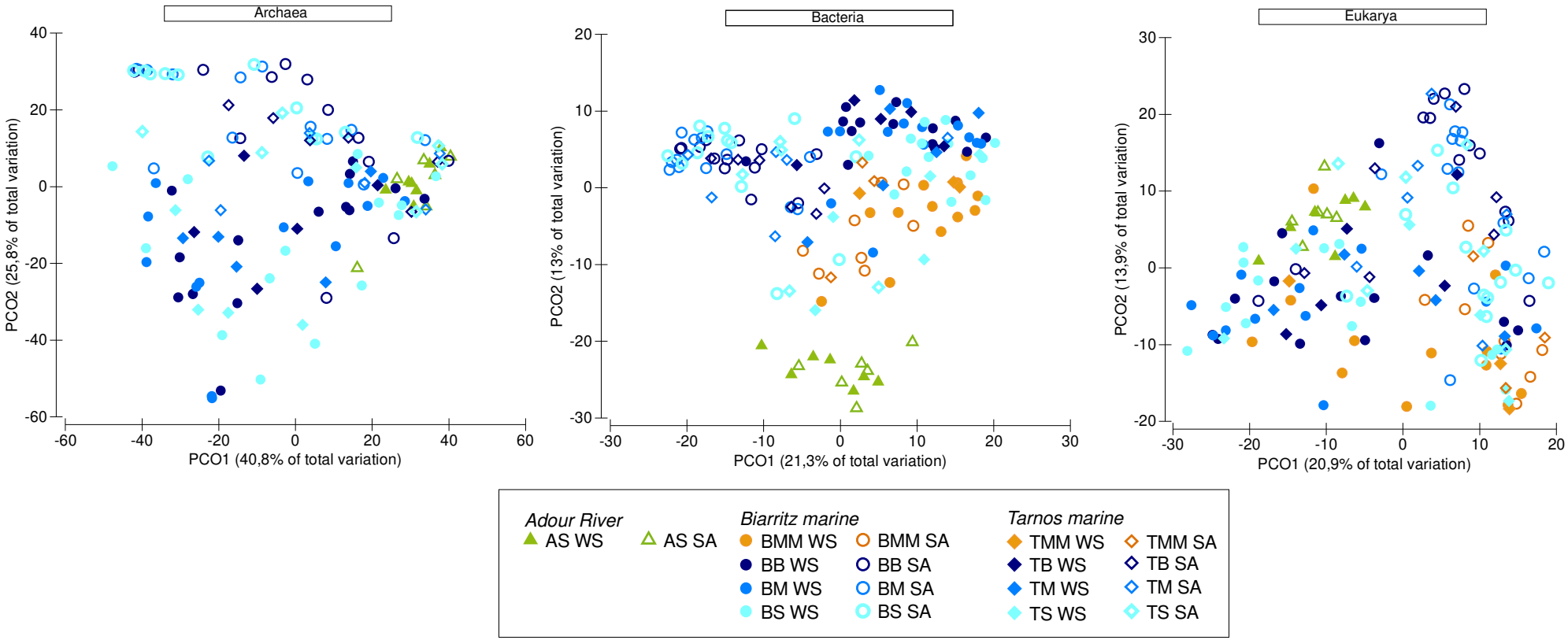
## Figure 4

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## Figure 5

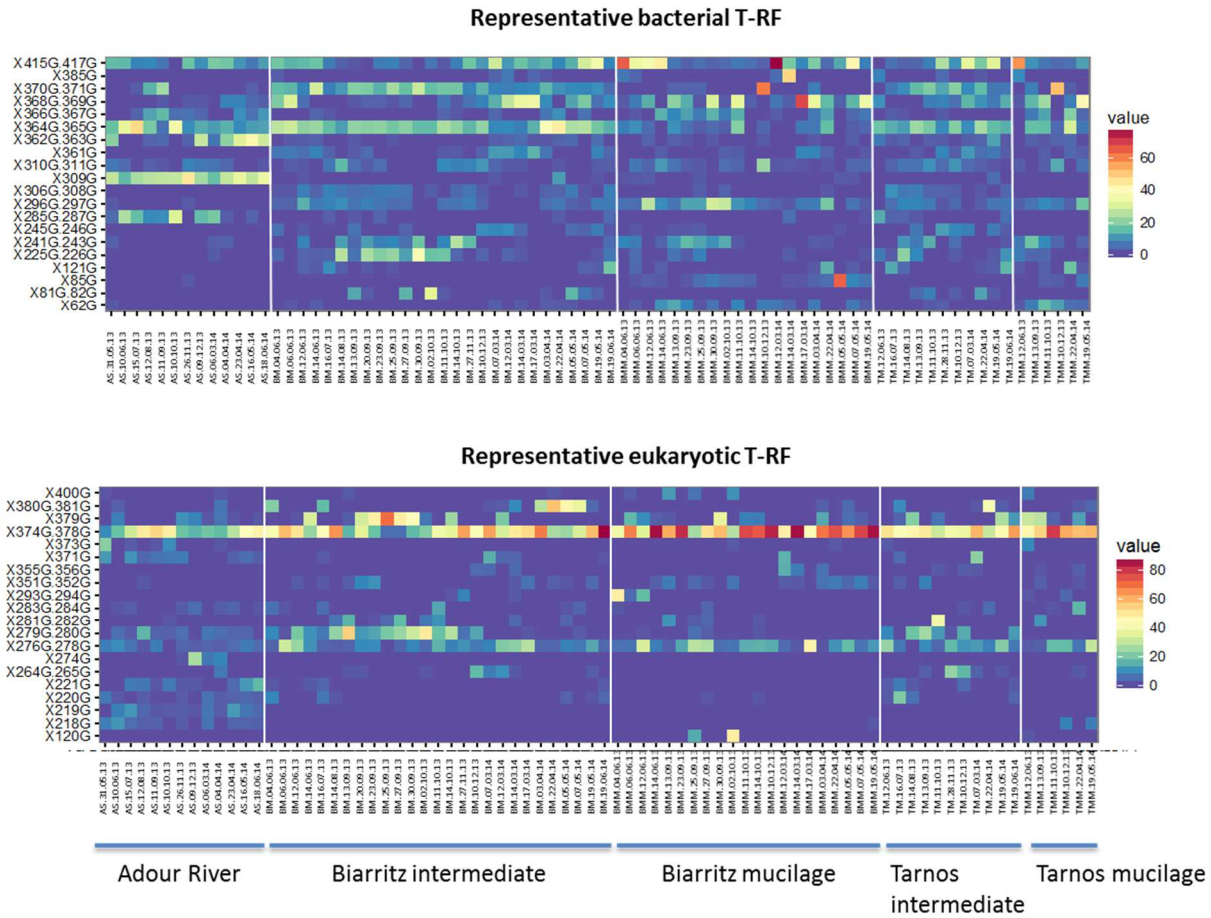


**Figure 5:** Heat map showing the T-RF distribution of the 20 most abundant T-RFs in marine mucilage and in the seawater at the intermediate depth over the year. Names of the T-RF are on the Y axis.

**Table 1:** Average Shannon diversity indices within the five environmental compartments (AS: Adour River surface, BS: Biarritz surface, BM: Biarritz in the intermediate depth, BB: Biarritz bottom, BMM: Biarritz marine mucilage, TS: Tarnos surface, TM: Tarnos in the intermediate depth, TB: Tarnos bottom, TMM: Tarnos marine mucilage) for the three domains of life.

| | **Environmental compartments** | | | | | |
|---|---|---|---|---|---|---|
| | Adour | Seawater | | | Marine | |
| **Domain** | river AS | surface S | intermediate M | bottom B | mucilage MM | Sampling site |
| Archaea | 1.54 ± 0.21 | 1.18 ± 0.43 | 1.18 ± 0.33 | 1.20 ± 0.34 | n. d. | B Biarritz |
| | | 1.26 ± 0.35 | 1.40 ± 0.38 | 1.27 ± 0.34 | n. d. | T Tarnos |
| Bacteria | 1.85 ± 0.29 | 2.28 ± 0.26 | 2.22 ± 0.31 | 2.33 ± 0.24 | 1.97 ± 0.58 | B Biarritz |
| | | 2.45 ± 0.31 | 2.45 ± 0.20 | 2.46 ± 0.19 | 1.98 ± 0.29 | T Tarnos |
| Eukarya | 2.34 ± 0.45 | 1.52 ± 0.35 | 1.40 ± 0.34 | 1.51 ± 0.40 | 1.04 ± 0.40 | B Biarritz |
| | | 1.43 ± 0.25 | 1.50 ± 0.24 | 1.69 ± 0.38 | 1.14 ± 0.29 | T Tarnos |

n.d.: not detected

**Table 2:** Average Bray-Curtiss similarity within the five environmental compartments (AS: Adour River surface, BS and TS: Biarritz and Tarnos surface seawater, BM and TM: Biarritz and Tarnos intermediate depth, BB and TB: Biarritz and Tarnos bottom seawaters, and BMM and TMM: marine mucilage) for the three domains of life.

| | **Environmental compartments** | | | | | |
|---|---|---|---|---|---|---|
| | Adour | Seawater | | | Marine | |
| **Domain** | River AS | surface S | intermediate M | bottom B | mucilage MM | Sampling site |
| Archaea | 63.38 | 45.22 | 48.84 | 47.18 | n.d. | B Biarritz |
| | | 47.4 | 55.3 | 55.76 | n.d. | T Tarnos |
| Bacteria | 74.77 | 68.43 | 68.21 | 68.67 | 63.02 | B Biarritz |
| | | 62.08 | 65.78 | 70.44 | 64.89 | T Tarnos |
| Eukarya | 62.63 | 63,46 | 62.79 | 61.55 | 67.7 | B Biarritz |
| | | 63.17 | 66.35 | 64.24 | 68.23 | T Tarnos |

n.d.: not detected