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Temporal dynamics of active prokaryotic nitrifiers and archaeal communities from river to sea

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25 # Abstract

To test if different niches for potential nitrifiers exist in estuarine systems, we assessed by pyrosequencing the diversity of archaeal gene transcript markers for taxonomy (16S rRNA) during an entire year along a salinity gradient in surface waters of the Charente estuary (Atlantic coast, France). We further investigated the potential for estuarine prokaryotes to
30 oxidize ammonia and hydrolyze urea by quantifying thaumarchaeal *amoA* and *ureC*, and bacterial *amoA* transcripts. Our results showed a succession of different nitrifiers from river to sea with bacterial *amoA* transcripts dominating in the freshwater station while archaeal transcripts were predominant in the marine station. The 16S rRNA sequence analysis revealed that *Thaumarchaeota* Marine Group I (MGI) were the most abundant overall but other
35 archaeal groups like *Methanosaeta* were also potentially active in winter (December-March) and *Euryarchaeota* Marine Group II (MGII) were dominant in seawater in summer (April-August). Each station also contained different *Thaumarchaeota* MGI phylogenetic clusters, and the clusters' microdiversity was associated to specific environmental conditions suggesting the presence of ecotypes adapted to distinct ecological niches. The *amoA* and *ureC*
40 transcript dynamics further indicated that some of the *Thaumarchaeota* MGI subclusters were involved in ammonia oxidation through the hydrolysis of urea. Our findings show that ammonia oxidizing *Archaea* and *Bacteria* were adapted to contrasted conditions and that the *Thaumarchaeota* MGI diversity probably corresponds to distinct metabolisms or life strategies.

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Keywords: Ammonia oxidation / *amoA* / *Archaea* / gradient / diversity

50 # Introduction

During the last decades, studies on microbial ecology have provided compelling evidence of the ubiquity and abundance of *Archaea* in a wide variety of aquatic habitats [1,2]. Further, the discovery of genes encoding enzymes related to nitrification in archaeal metagenomes from soil and marine waters [3-5] and the isolation of the first autotrophic archaeal nitrifier,
55 *Nitrosopumilus maritimus* [6] have led to a dramatic shift in the classical view that *Bacteria* were the main responsible for nitrification.

The wide distribution of ammonia oxidizing *Archaea* (AOA), affiliated with *Thaumarchaeota*, across a variety of aquatic environments is now well established through reports on the abundance of the gene encoding archaeal ammonia monooxygenase α-subunit
60 (*amoA*) in oceanic waters [7,8] and freshwater ecosystems [9,10]. Nevertheless, the relative contribution of AOA *versus* ammonia oxidizing *Bacteria* (AOB) remains unclear and factors that regulate ammonia oxidizing microorganisms' activity and diversity in aquatic ecosystems have not yet been fully elucidated. While in marine ecosystems AOA often outnumber AOB [8,11], the ecology of nitrifiers appears more complex along salinity gradients. Indeed, some
65 studies reported that AOB dominate under saline estuarine conditions [12-14], while others showed that AOA always dominate in estuarine systems [15]. In lakes, contrasting results have been reported as AOB were absent from an oligotrophic high-altitude lake [16] but were predominant in nutrient-rich compared to oligotrophic waters [9]. Additionally the discovery that some *Thaumarchaeota* may degrade urea to use nitrogen for their metabolism [17,18],
70 and the absence of the *ureC* gene (encoding the alpha subunit of a putative archaeal urease) in the representative marine isolate, *Nitrosopumilus maritimus* [19], raises new questions about the existence of different archaeal nitrifier ecotypes able to cope with different environmental conditions or potential competitors [17,18,20]. However, little is known about their possible niche differentiation associated with their various metabolisms [17,21] and their ability to

75 assimilate inorganic carbon [22]. In particular, their potential activity in relation to seasonal changes, salinity and chemical gradients, remains poorly understood.

Microbial communities in estuaries and some coastal margins vary greatly in space and time because of sharp gradients in salinity and nutrients [23,24]. The mixing of fresh- and saltwater creates steep physico-chemical gradients that are coupled to shifts in the resident 80 microbial communities, and particularly ammonia oxidizers [15]. Community transitions from marine to freshwater are explained by salinity, which is the main factor driving community structure globally [25,26]. Temperature, nitrite and ammonia concentrations, and net primary productivity have, however, also been shown to produce major effects on the nitrifiers community structure. Transitions between bacterial and archaeal ammonia oxidizers 85 communities have been frequently detected [13,27,28].

Estuarine *Archaea* usually originate from both marine and freshwater environments but also from soils and sediments [15,29]. In riverine ecosystems, *Euryarchaeota* and *Thaumarchaeota* are both present but their proportion can vary according to the studied location. Indeed, *Thaumarchaeota* dominated in the Rhine river, while *Euryarchaeota* were 90 the most abundant in the arctic Mackenzie river [30,31]. Even though the diversity of riverine *Archaea* starts to be described, their ecology and seasonal dynamics remain poorly understood because of the lack of temporal surveys. In addition, the potential activity (at the 16S rRNA level) of riverine archaeal communities is not known as aquatic microorganisms have traditionally been studied at the DNA level [32,33]. However, the recent use of both 16S 95 rRNA genes and 16S rRNA has shown the importance of differentiating the potentially active communities from the total communities for a better understanding of the ecology of aquatic microorganisms [34,35].

Here we studied an estuary to test at a domain level whether *Bacteria* and *Archaea* had different niches for nitrification. We then looked more specifically at *Archaea* to test if

100 different thaumarchaeal ecotypes had different habitats and metabolisms. We quantified thaumarchaeal *amoA* and *ureC* transcripts in comparison to bacterial *amoA* over one year along a salinity gradient in the surface water of the Charente Estuary (west coast of France), and we described the community structure of potentially active *Archaea* by pyrosequencing cDNA from the V3-V5 region of 16S rRNA gene.

105

Materials and Methods

Study sites, sampling and chemical analyses

The Charente is a 350 km coastal river draining a 10,000 km² basin and emerging in the bay of Marennes Oleron. The sampling area (Figure 1) started at St-Savinien, upstream of the

110 Charente (freshwater station, 45°52'37''N, 00°41'10''W) and ended in the Charente estuary (marine station, 45°59'54'', 01°09'56''W), with one intermediary station (mesohaline station, 45°58'11''N, 01°00'50''W). Each station was characterized by a specific salinity class ranging from 0 to 35 PSU (Supplementary Table 1). Surface water (0.5 m depth) was collected monthly in each station from April 2011 to March 2012, except May 2011 in the marine

115 station, by using a 10-L Niskin Bottle. Water temperature, salinity and pH were determined with a multiparameter probe (YSI GRANT 3800). Phosphate (PO_4^{3-}) and ammonia (NH_4^+) contents were analyzed using Merck colorimetric kits (Millipore) according to standard American Public Health Association (1992) methods. Chlorophyll *a* (Chl*a*) concentration was determined by spectrophotometry [36,37].

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RNA extraction and pyrosequencing

A sub-sample (300 mL) added with an equal volume of RNA Later (ammonia sulfate 7.93 M, sodium citrate 0.025 M, EDTA 0.02 M, pH 5.2) was pre-filtered through 5-µm pore-size polycarbonate filters (Millipore) and collected on 0.2-µm pore-size (pressure <10 kPa)

125 polycarbonate filters (Millipore) and stored at –80°C until nucleic acid extraction. The RNA extraction method was modified from Hugoni *et al.* [35] using a combination of mechanic and enzymatic cell lysis, followed by extraction using the AllPrep DNA/RNA kit (Qiagen, Valencia, CA). The RNA samples were tested for the presence of contaminating genomic DNA by PCR and then reverse transcribed with random primers using the SuperScript® VILO

130 (Invitrogen). The amplification of the V3-V5 region of the 16S rRNA genes was performed with universal archaeal primers Arch349F and Arch806R (Table 1, [38]), followed by pyrosequencing using a Roche 454 GS-FLX system with titanium chemistry by a commercial laboratory (MR.DNA, Shallowater, TX, USA).

135 **Bioinformatic analysis**

The 16S rRNA pyrosequencing dataset represented 175,637 raw sequences. Cleaning procedures consisted in the elimination of sequences presenting ambiguous bases "N", a quality score < 25, length less than 200bp and with a mismatch in the forward primer. The remaining reads were clustered at 97% similarity threshold [39] and representative sequence

140 for each OTU were inserted in phylogenetic trees for taxonomic annotation. The process was automated by PANAM that also computed richness and diversity indexes, Chao1 and Shannon respectively (http://code.google.com/p/panam-phylogenetic-annotation/downloads/list; [40]). Chimeras were detected using Uchime [41] and represented 1% of the cleaned sequences. After the removal of sequences affiliated with *Bacteria*, the dataset

145 contained a total of 27,803 archaeal sequences distributed into 1825 OTUs (Supplementary Table 1). Several sequences were affiliated with *Bacteria* belonging to the *Verrucomicrobia* phylum suggesting that the chosen *Archaea* primers were not as specific as thought, and that they may not have amplified all archaeal sequences in the estuary. For the analysis of the

seasonal dynamics, the 16S rRNA samples were randomly resampled down to 212 sequences using PANAM.

A phylogenetic tree including OTUs retrieved in the 3 sampling stations was constructed by aligning both OTUs and reference sequences from the literature using Muscle [42] and neighbor joining phylogenies were built using Mega5 [43] and a bootstrap iteration of 500. This allowed us to delineate major *Thaumarchaeota* MGI clusters and evaluate the proportion of OTUs belonging to each cluster retrieved in each station.

RT quantitative PCR analysis

The qPCR protocol modified from Hugoni *et al.* [9] targeted the cDNA transcribed and included the primers described in the Table 1. Briefly, transcript numbers of thaumarchaeal *amoA* and *ureC* and bacterial *amoA* were determined in triplicate. The reaction mixture (25 µL) contained MESA GREEN qPCR MasterMix Plus for SYBR Assay® (1X, Eurogentec) added with 0.8 µg of BSA, 0.7 µM of primers and ultra-pure sterile water. One µL of cDNA was added to 24 µL of mix in each well. qPCR reactions consisted of an initial denaturing step at 94°C (for 15min for thaumarchaeal *amoA*, and 5min for thaumarchaeal *ureC* and bacterial *amoA* transcripts) followed by 40 cycles (thaumarchaeal *amoA*: 94°C 15sec, 52°C 30sec, 72°C 30sec; thaumarchaeal *ureC*: 94°C 1min, 55°C 1min, 72°C 2min and bacterial *amoA*: 95°C 30sec, 56°C 40sec, 72°C 2min). Standard curves were generated from a mix of clone representatives from the environments studied (sequences were obtained using the Arch AmoR and Arch AmoF primers [44] and have been archived in GenBank under accession numbers: KF432403 and KF432404 for the thaumarchaeal *ureC* gene, JN089917 and JN089905 for the archaeal *amoA* gene, JX003650 and JX003657 for the bacterial *amoA* gene). All reactions were performed with standard curves spanning from 10^1 to 10^8 copies per µL. Mean PCR efficiencies and correlation coefficients for standard curves were as follows:

for the thaumarchaeal *ureC* assay, 98 %, $r^2 = 0.98$, for the thaumarchaeal *amo*A assay, 108 %,

175 $r^2 = 1.00$, and for the bacterial *amoA* assay, 107 %, $r^2 = 1.00$.

Statistical analysis

Canonical correspondence analysis (CCA) was performed to assess the relationships between active archaeal taxonomic groups and environmental parameters. CCA was performed on 6

180 environmental factors (temperature, salinity, Chl*a* content, pH, phosphate, and ammonium concentrations) and the taxonomic groups abundance matrix (inferred from 16S rRNA reads number).

To explain the variation of archaeal *amoA* and *ureC* transcripts abundance, a redundancy analysis (RDA) was used after a forward selection [45] of the 10 thaumarchaeal

185 OTUs susceptible to explain a significant part of changes in archaeal *amoA* and *ureC* transcripts abundance (inferred from the qPCR assays).

The statistical analyses were conducted using R associated to the package VEGAN (http://cran.r-project.org/web/packages/vegan/index.html).

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Results

Environmental characteristics of the Charente estuary

Three stations of the Charente estuary (Figure 1) were sampled monthly during one year along a salinity gradient (Supplementary Table 1). The freshwater, mesohaline and marine stations were characterized by a mean salinity of 0, 14.9 and 33.2 PSU and a mean Chl*a*

195 concentration of 17.6, 4.34 and 2.74 µg L^{-1}, respectively (Supplementary Table 1). Ammonia concentrations were on average lower in the marine station (0.019 mg L^{-1}, SD=0.034) than in the freshwater station (0.081 mg L^{-1}, SD=0.035). Conversely phosphate concentrations were

higher in the marine station (0.096 mg L^{-1}, SD=0.056) than in the freshwater station (0.038 mg L^{-1}, SD=0.034).

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Bacterial and archaeal *amoA* and archaeal *ureC* transcript dynamics

Archaeal and bacterial *amoA* transcript numbers were quantified by RT-qPCR during one year (Figure 2.A and B). Few archaeal *amoA* transcripts were detected overall in the freshwater station. Nevertheless, three distinct periods of higher transcript numbers were

205 observed: one from May to June, then a second during September and October and finally from December to January (Figure 2.A). The mesohaline station was characterized by higher archaeal *amoA* transcript numbers from May to September with a peak in June. Conversely, the marine archaeal *amoA* transcript numbers showed an increase in abundance from October to January, with a peak in January.

210 In the freshwater station, bacterial *amoA* transcript numbers increased first in June and then peaked in September, reaching up to 225 times more transcripts than *Archaea* at the same period (Figure 2.B). In the mesohaline station, bacterial *amoA* transcripts showed similar dynamics to that of *Archaea* with three peaks, although their magnitude was overall lower. The marine station had the lowest number of bacterial *amoA* transcripts, with an

215 increase from August to October, earlier in the year compared to archaeal *amoA*.

Thaumarchaeal *ureC* transcript abundance was also determined using RT-qPCR during a one-year period to assess the genetic potential of estuarine *Archaea* for ureolytic nitrogen metabolism (Figure 2.C). Transcript numbers were overall very low but were still within the detection range according to our standard curve. The freshwater station presented

220 the highest transcripts abundance that corresponded precisely to the periods of highest archaeal *amoA* abundance in this station. In both the mesohaline and marine stations, lower

numbers of *ureC* transcripts were detected all year, except in May and June at the mesohaline station when numbers were slightly higher.

225 **Active *Archaea* community structure and dynamics**

The changes in the community structure of active archaeal populations were evaluated over time from river to sea by pyrosequencing cDNA from the 16S rRNA gene (Supplementary Table 1). The community diversity was evaluated through the Shannon index on normalized datasets, and was significantly higher in the freshwater than in the marine station (t-test, p
230 =0.008, Supplementary Figure 1). Moreover, the number of OTUs was also different between stations with 550 OTUs observed in the freshwater station against 923 and 352 in the mesohaline and marine stations respectively. We did not manage to amplify archaeal cDNA from October to November at the marine station.

We evaluated active archaeal community composition, based on 16S rRNA
235 transcripts, and focused on the three main periods of high archaeal *amoA* transcripts abundance. In the freshwater station, different patterns of community composition were visible with time (Figure 3). The 16S rRNA *Thaumarchaeota* MGI sequences were more abundant from April to November representing about 83% of the archaeal sequences. These *Thaumarchaeota* were dominated by 11 different OTUs with best Blast match to sequences
240 recovered from lacustrine freshwaters, groundwater, rivers and mangrove sediments. In contrast, the number of sequences affiliated with methanogenic groups (i.e. *Methanosaeta, Methanoregula, Methanoculleus, Methanomicrobiales, Methanospirillum*) increased from December to March reaching up to 60% of the sequences. Five methanogen OTUs all belonging to the *Methanosaeta* genus dominated. They were closely related to sequences
245 found in salt marsh and lacustrine sediments, but also retrieved in groundwater and wetlands.

Among *Euryarchaeota* we detected few members of the Rice Cluster-V (RC-V) and Deep-sea Hydrothermal Vent Euryarchaeotic Group – 6 (DHVEG-6).

In the mesohaline station, the potentially active archaeal community was clearly dominated by *Thaumarchaeota* MGI all year round (from 86% to 93% of the sequences,
250 Figure 3), but by different subgroups at different times (Figure 4). Within *Euryarchaeota*, few methanogenic lineages were retrieved in the mesohaline station and, *Euryarchaeota* MGII represented about 7% of the sequences between December and March.

In the marine station, we could also distinguish changes with seasons. From April to August, *Thaumarchaeota* MGI represented about 17% of the 16S rRNA transcripts, the
255 remaining being almost exclusively *Euryarchaeota* MGII sequences (around 81%). Conversely, from December to March, *Thaumarchaeota* MGI represented about 70% of the sequences while *Euryarchaeota* MGII accounted for 27% of the sequences. Six abundant *Thaumarchaeota* OTUs were related to marine waters sequences but also to lacustrine freshwater, groundwater, rivers and mangrove sediment. On the other hand, the fifteen
260 abundant *Euryarchaeota* OTUs were related to marine sequences.

The canonical correspondence analysis showed that *Euryarchaeota* MGII was mainly related to salinity and consequently to the marine station. On the other hand, the potentially active *Thaumarchaeota* MGI were linked to the mesohaline station, while the majority of euryarchaeal groups (i.e. methanogenic lineages, RC-V, Lake Dagow Sediment cluster
265 (LDS), Miscellaneous Euryarchaeotic Group (MEG), Miscellaneous Crenarchaeotic Group (MCG)) were related to ammonia and to the freshwater station (Supplementary Figure 2).

We used all *Thaumarchaeota* MGI OTUs retrieved in all the stations to build a phylogenetic tree. The potentially active OTUs fell into six major subclusters (Figure 4). Among them, the clusters Marine A and B were initially recovered in marine ecosystems
270 [35,46] and the clusters Freshwater A and B, in freshwaters [9,44]. Interestingly, we also

found OTUs belonging to a subcluster related to sediments. OTUs present in the freshwater station belonged mainly to the Freshwater A and Sediment associated groups, while those in the mesohaline and the marine stations clustered mainly with sequences belonging to the Marine A subcluster. OTUs common to both the freshwater and the mesohaline stations fell
275 into the Sediment subcluster while those that were specific of the mesohaline and marine station mostly fell into the Marine A subcluster.

We further analyzed *Thaumarchaeota* MGI OTUs present in the mesohaline station and identified 3 monophyletic subclusters within the *Thaumarchaeota* MGI_Marine A cluster (Figure 4). Each of these subclusters had a specific seasonal dynamics (Supplementary Table
280 2 and 3). The first subcluster named MGI.A.b was potentially active from April to August, the second named MGI.A.a from September to November and the last one called MGI.A.c from December to March (Figure 4).

To gain insight into possible associations between 16S rRNA OTUs and functional genes, we used a forward redundancy analysis (RDA) of *amoA* and *ureC* transcript
285 abundances against the most abundant thaumarchaeal OTUs retrieved in all sampling stations (Figure 5). The ten most informative OTUs (i.e. accounting for 77% of the total variation) were selected to build a RDA. It showed that the potential ureolytic metabolism was correlated to three OTUs (R16_HUJB0N002GU20W, R24_HUJB0N002JCR91 and R10_HUJB0N002JLORV), affiliated with the Sediment and Freshwater A subclusters (Figure
290 4). On the other hand, the *amoA* potential activity was correlated with six OTUs among which five were affiliated with the Marine A subcluster while the last one was affiliated with the Freshwater B subcluster.

Discussion

295 The quantification of *amoA* transcript abundance in the surface waters of the Charente estuary over a 1-year period showed a transition in active ammonia-oxidizing populations from AOB in freshwaters to AOA in marine waters. The higher AOB transcript numbers in the freshwater station is in agreement with previous studies suggesting that AOB potential nitrification rates were inhibited in high salinity environment (around 30 PSU [27]).
300 Nevertheless, even if salinity appears to be an important factor in determining AOB distribution, Bernhard *et al.* [27] showed that AOB exhibited a broad range of salinity tolerance, suggesting that other environmental parameters needed to be considered to understand their activity dynamics. In contrast, AOA potential activity was very low in freshwater, while intermediary estuarine conditions (mesohaline station) favored higher
305 transcript abundance through the whole year. In contrast, previous studies based on DNA, have shown that archaeal *amoA* genes were more abundant in freshwater [13,14,47]. However, these studies and our data showed that the highest thaumarchaeal transcript abundance was retrieved when ammonia concentrations were the lowest, probably because of the high apparent affinity of *Thaumarchaeota* for reduced nitrogen [48]. In the marine station,
310 thaumarchaeal *amoA* transcript numbers were more important during the winter period, consistent with previous results showing highest archaeal *amoA* gene abundance in winter in the Mediterranean Sea [7]. Winter may be a period during which *Thaumarchaeota* MGI are more active and win the competition for ammonia against *Bacteria* [49]. In our study, AOA activity evaluated through 16S rRNA could not be statistically linked to any of the
315 environmental parameters measured, suggesting that other factors (i.e. other physico-chemical parameters or bottom up controls) shape AOA activity in this ecosystem.

We detected possible *Thaumarchaeota* MGI ecotypes presenting different seasonal dynamics and associated with varying levels of potential ammonia oxidation activity. In particular, the changes observed among Marine A subclusters suggest that the phylogenetic

320 diversity was associated with contrasted seasonal conditions. The variable potential activity levels of the different *Thaumarchaeota* MGI subclusters could suggest an adaptation to different niches and may indicate an ecological specialization, supporting the notion that different *Thaumarchaeota* MGI could have different metabolisms [21]. Although we could not precisely define ecological niches in the present study, our work confirms the idea that

325 *Thaumarchaeota* MGI is composed of different ecotypes, as previously proposed for the coastal Mediterranean Sea [35,50] and lakes [16,51,52]. The use of ureolysis to supply ammonia when AOB activity dominates is a possible example of the specific adaptations of some *Thaumarchaeota* MGI ecotypes to particular niches. This was illustrated in our study through the finding that the *Thaumarchaeota ureC* transcripts were detected in the freshwater

330 station, when AOB *amoA* transcripts outnumbered AOA. *Thaumarchaeota* MGI could use urea to fuel nitrification and thus adopt an alternative metabolic pathway when the availability of ammonia is limited and/or when competitors are present [18]. In our study, the presence of *ureC* transcripts was associated to the presence of specific *Thaumarchaeota* MGI OTUs affiliated with the Freshwater A and Sediment subclusters. Not all *Thaumarchaeota* MGI

335 OTUs might be able to use urea for nitrification, as illustrated by the weak *ureC* transcripts number found in the marine station despite the high abundance of *Thaumarchaeota* and as suggested by the absence of the *ureC* gene in the *N. maritimus* genome [19]. Thus we hypothesize that only some specific clusters have the ureolytic potential, and that this metabolism is preferentially present in *Thaumarchaeota* from freshwater systems. However,

340 caution is needed when interpreting such results as the detection of genes and transcripts does not warranty metabolic activities of *Archaea*. It was for instance recently suggested that *Thaumarchaeota* expressing *amoA* are not obligate ammonia oxidizers [53], and thus *amoA*-carrying *Archaea* are not necessarily ammonia oxidizers [54]. More work is needed to understand if and what AOA are involved in urea utilization and under which conditions.

345 The occurence of active methanogenic groups in the freshwater station, particularly abundant during the winter months (December-March), may be due to the presence of sediments or mud mixed with the water during rainfalls and/or storms. Our results also suggest that the types of methanogens present may thrive better in freshwater environments rather than in marine and brackish environments. The retrieval of sequences affiliated with 350 methanogens in the mesohaline station could suggest that they were slowly dispersed from their habitat (i.e. freshwater), resulting in decreasing activity with increasing salinity [55]. Some specific freshwater groups like LDS or RC-V clusters were also found (4.8 and 11.9 % of the reads respectively), indicating discharge from nearby rivers and from the catchment area. The RC-V and LDS cluster represent highly diverse groups of *Euryarchaeota* detected 355 in various ecosystems [56]. They have been identified in rivers [30,31] where they represent a large proportion of the archaeal cell counts [31]. In the marine station, there was a clear succession between *Thaumarchaeota* MGI and *Euryarchaeota* MGII. The predominance of potentially active *Thaumarchaeota* MGI occurred during the winter period, as previously shown in the North-Western Mediterranean Sea [35]. *Euryarchaeota* MGII dominated the 360 active archaeal assemblage during the summer period. The putative presence of genes encoding the proteorhodopsin, retrieved in members of marine *Euryarchaeota* MGII.a [57,58] could explain the ecological success of this group in the marine station. Our study also confirms the potential activity of some less abundant groups, like Marine Benthic Group B (MBG-B) and the recently proposed "*Bathyarchaeota*" formerly known as Miscellaneous 365 Crenarchaeotic Group [59,60] retrieved in the freshwater and mesohaline stations. This suggests that these groups considered as ubiquitous [61] and usually found in sediments [62] could be active in estuarine water column. A recent study using a single-cell genome approach reported that some members of the MCG are capable of protein remineralization in

anoxic sediments and it may be that these organisms are undertaking such a process in this

370 environment [63].

In summary, our study clearly showed that the potential activity of prokaryotic nitrifiers was influenced by the physico-chemical gradients retrieved through an estuarine ecosystem. At the domain level, *Archaea* and *Bacteria* occupied different ecological niches with AOB being more active in freshwater and AOA in marine waters. Our results also

375 suggest the presence of different thaumarchaeal ecotypes, which maybe able to degrade urea adding to the growing evidence that additional metabolisms may be present within *Thaumarchaeota*.

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Figures and tables legend

Figure 1. Location of sampling stations (median position over 12 sampling dates) along the Charente Estuary. The freshwater station was called A, the mesohaline station: C, and the marine station: E.

Figure 2. Abundances of thaumarchaeal (A) and bacterial (B) *amoA* transcripts and (C) thaumarchaeal *ureC* transcripts per mL of water during the one year survey.

Figure 3. Proportion of 16S rRNA genes transcripts for archaeal groups retrieved in the freshwater station, the mesohaline station, and the marine station during three distinct periods: from April to August, from September to November and from December to March.

Figure 4. 16S rRNA transcripts phylogenetic tree including potentially active *Thaumarchaeota* MGI OTUs retrieved in the three sampling stations of the Charente estuary. Bootstrap values >40 are shown. Histograms on the right represented the number of OTUs retrieved in each station, in the freshwater and mesohaline stations, in the mesohaline and marine stations and in the 3 stations. Seasonal changes in *Thaumarchaeota* OTUs from the Marine A cluster were illustrated for OTUs in the mesohaline station which were active from September to November (■), from April to August (●), and from December to March (▲).

Figure 5. RDA plot of *ureC* and *amoA* transcripts abundance (□) compared with active abundant *Thaumarchaeota* MGI OTUs (▲) according to the stations sampling points (●). The correspondant abbreviated names for OTUs were as follows: R24_HUJ (R24_HUJB0N002JCR91), R10_HUJ (R10_HUJB0N002JLORV), R16_HUJ (R16_HUJB0N002GU20W), R34_HUJ (R34_HUJB0N002JELSP), R8_HTR (R8_HTRM39R02IN9W4), R16_HUJ (R16_HUJB0N002ITPEH), R17_HTR (R17_HTRM39R02HMXG2), R22_HTR (R22_HTRM39R02HVYCX), D32_HUJ (D32_HUJB0N002F6TFA), R22_HTR (R22_HTRM39R02GQZTZ).

Table 1. Primers used for qPCR and pyrosequencing, and PCR annealing conditions used in

585 this study.

Supplementary Data

Supplementary Figure 1. Box plot of Shannon index from the three different sampling
590 stations. A significant difference in diversity between two stations is marked with a star (*, $p < 0.008$).

Supplementary Figure 2. Ordination diagram from CCA of major active archaeal groups compared with environmental data.

Supplementary Table 1. Quality checked (QC) and *Archaea* affiliated sequences obtained
595 for each sample from surface water collected monthly in the Charente estuary. Environmental parameters (temperature, salinity, pH and Chl*a*, ammonia and phosphates concentrations) associated to each point are presented. ND: not determined.

Supplementary Table 2. Mean number of 16S rRNA sequences associated with each abundant OTU retrieved in the freshwater, mesohaline and marine stations. ND: not
600 determined.

Supplementary Table 3. Monthly community structure at the subcluster level in *Thaumarchaeota* MGI. Number of OTUs were presented for each subgroup and number of sequences between brackets. ND: not determined.

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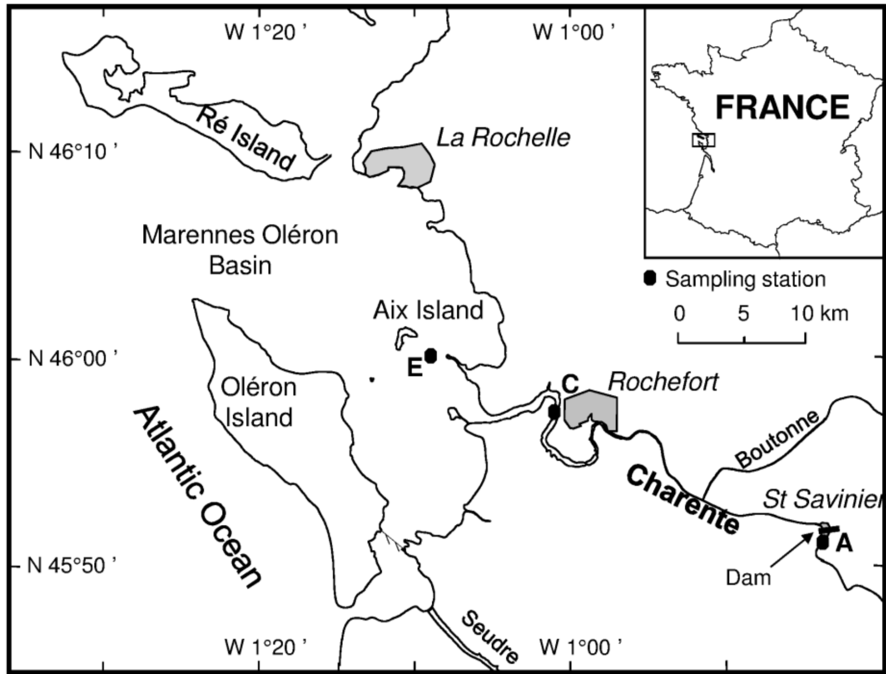

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Figure 1. Hugoni *et al.*

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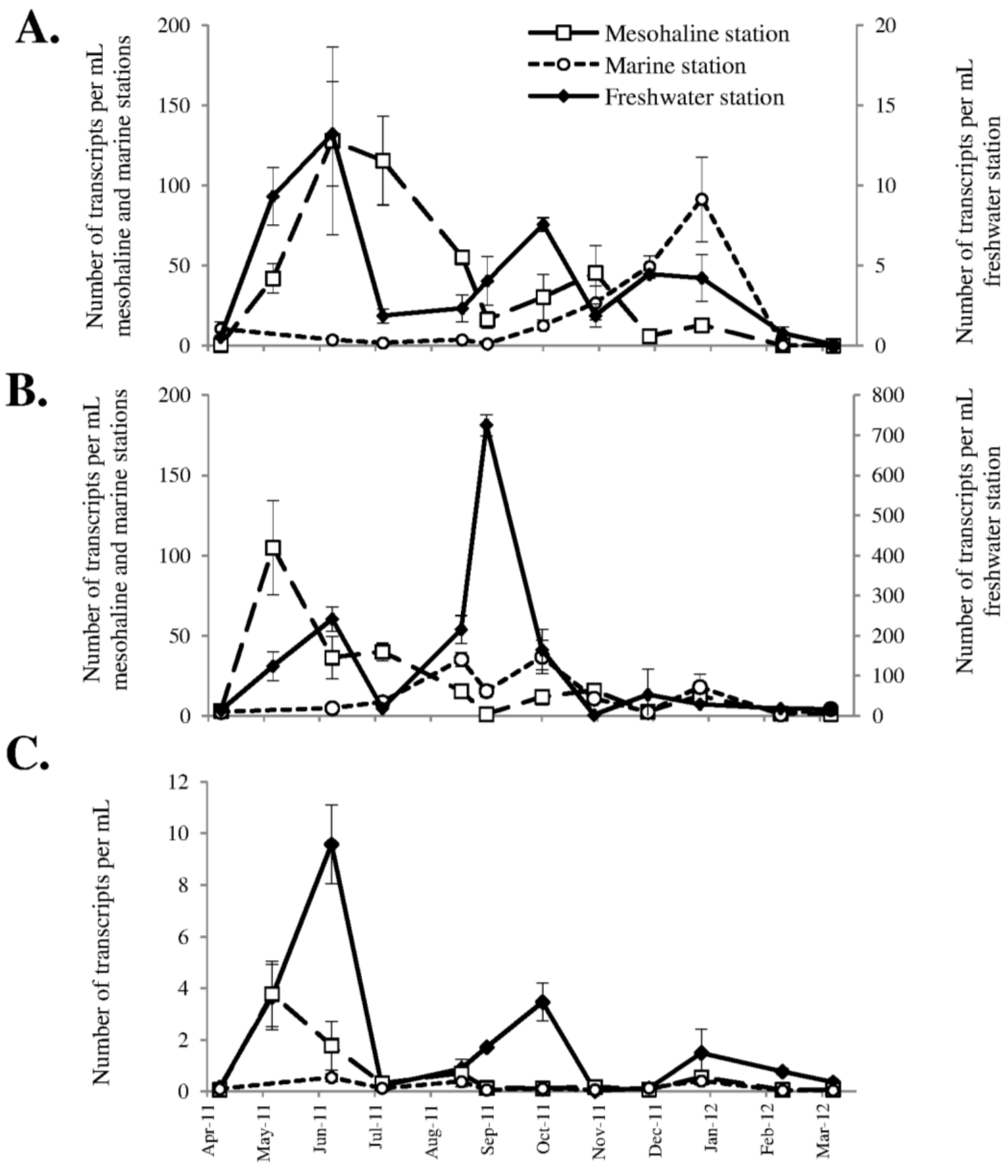

Figure 2. Hugoni *et al.*

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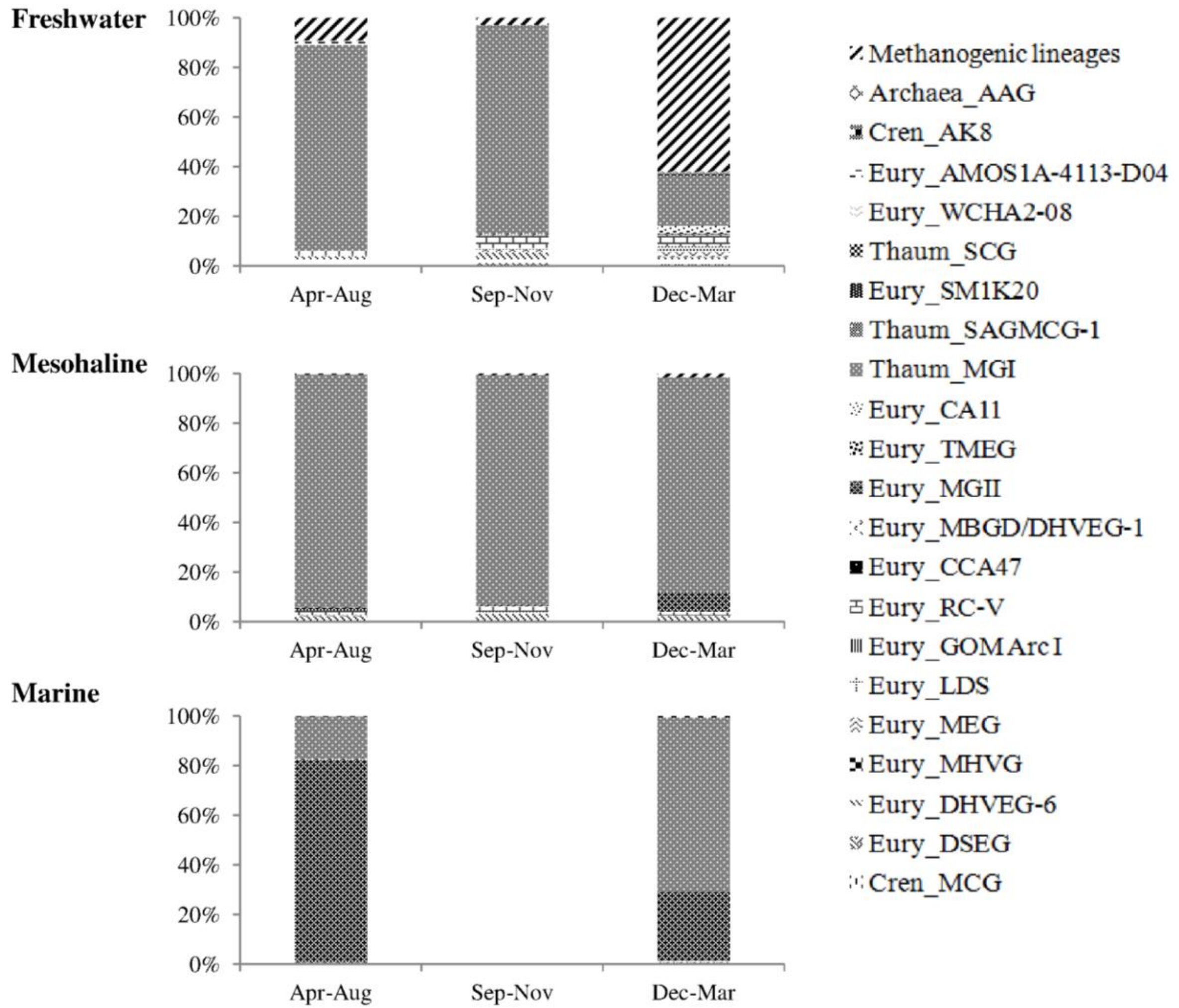

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Figure 3. Hugoni *et al.*

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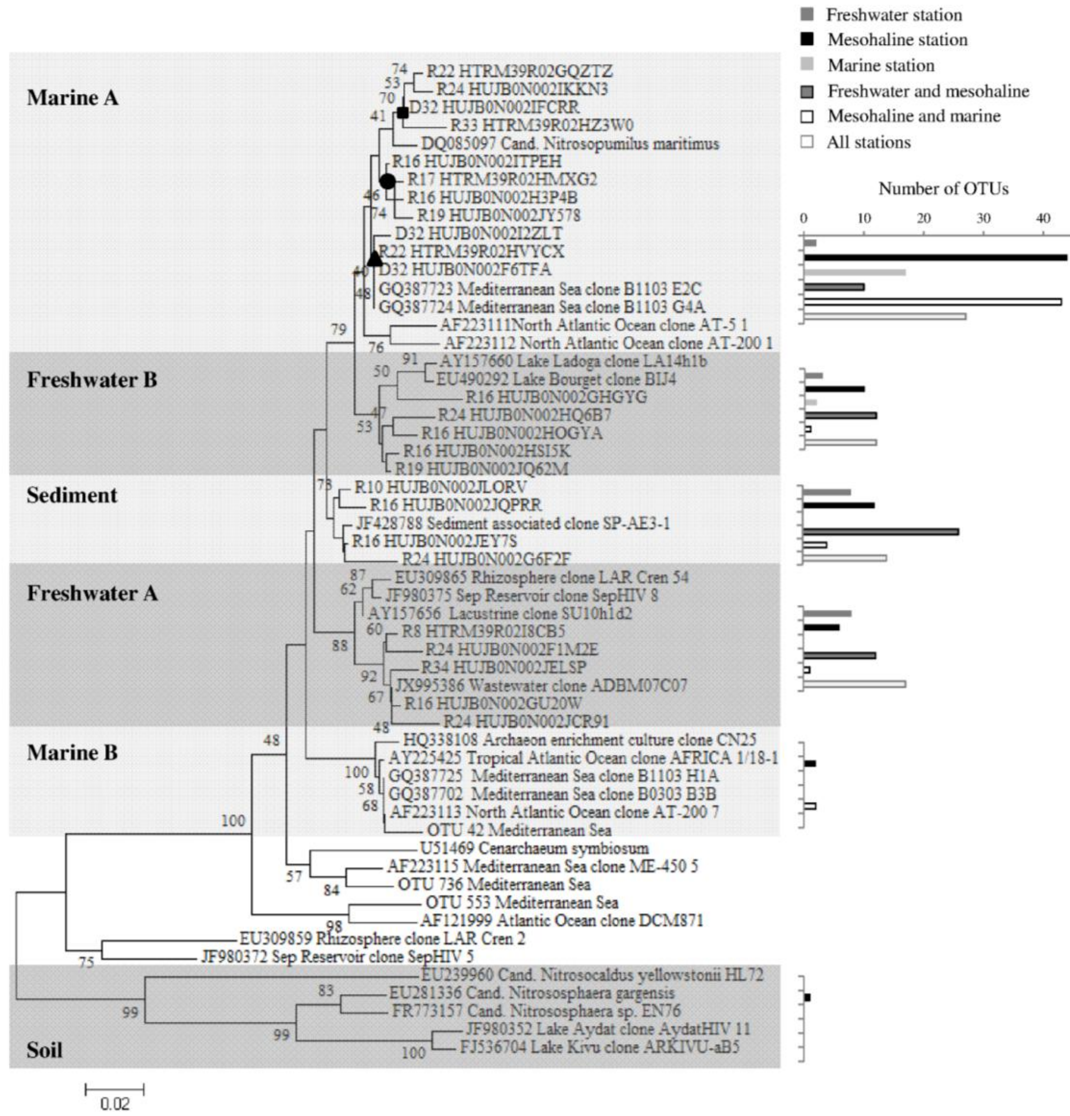

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Figure 4. Hugoni *et al.*

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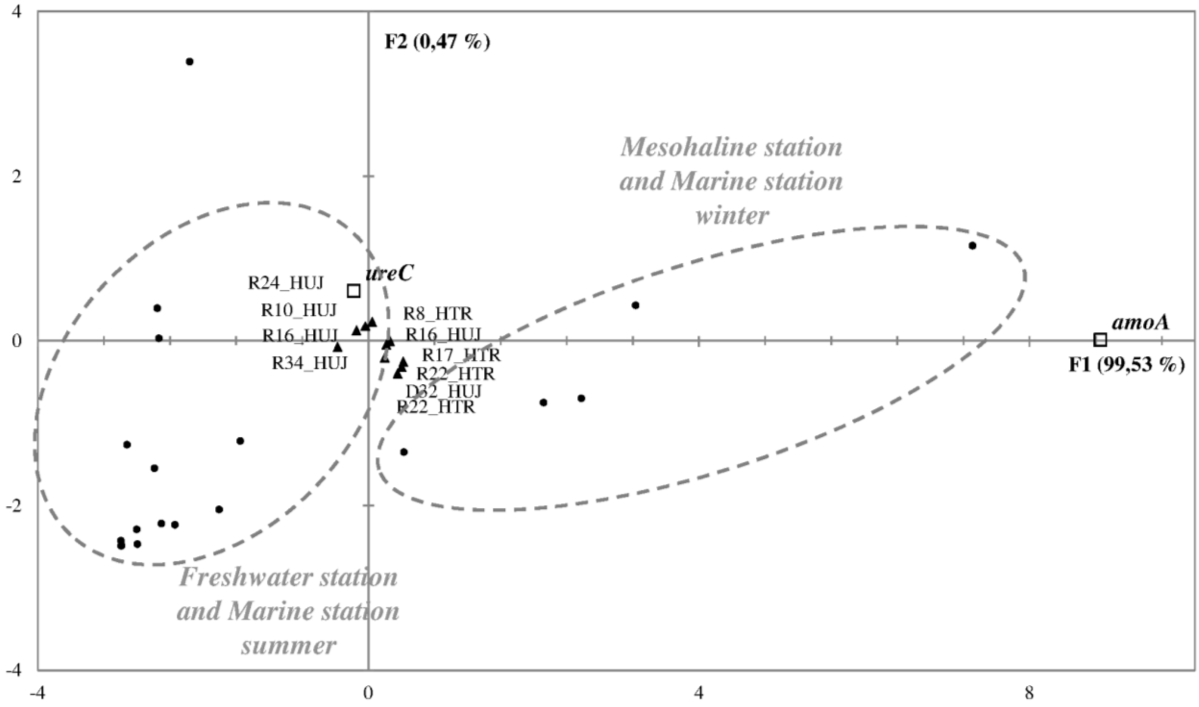

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Figure 5. Hugoni *et al.*

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Application	Primer	Primer sequence 5' – 3'	Annealing temperature	Targeted group	Reference
qPCR	amoA-1F	GGGGTTTCTACTGGTGGT	56°C	β-proteobacterial *amoA*	Rotthauwe *et al.*,1997
	AmoA-RNEW	CCCCTCBGSAAAVCCTTCTTC			
	CrenAmoAModF	TGGCTAAGACGMTGTA	52°C	Thaumarchaeal *amoA*	Mincer *et al.*, 2007
	CrenAmoAModR	AAGCGGCCATCCATCTGTA			
	Thaum-UreC F	ATGCAATYTGTAATGGAACWACWAC	55°C	Thaumarchaeal *ureC*	Alonso-Saez *et al.*, 2012
	Thaum-UreC R	AGTTGTYCCCCAATCTTCATGTAATTTTA			
Pyrosequencing	Arch349F	GYGCASCAGKCGMGAAW	55°C	Archaeal 16S rRNA	Takai *et al.*, 2000
	Arch 806R	GGACTACVSGGGTATCTAAT			
	amoA-1F	GGGGTTTCTACTGGTGGT	56°C	β-proteobacterial *amoA*	Rotthauwe *et al.*,1997
	Bacamo2R	CCCCTCKGSAAAGCCTTCTTC			
	Arch-amoAF	STAATGGTCTGGCTTAGACG	53°C	Thaumarchaeal *amoA*	Francis *et al.*,2005
	Arch-amoAR	GCGGCCATCCATCTGTATGT			

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Table 1. Hugoni *et al.*

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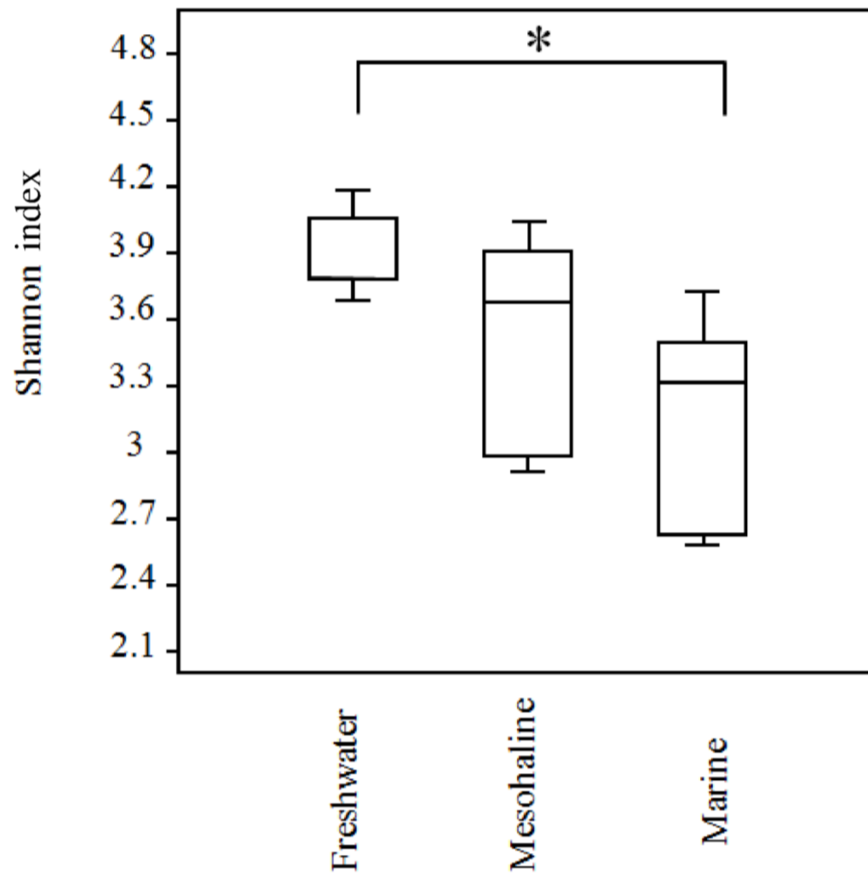

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Supplementary Figure 1. Hugoni *et al.*

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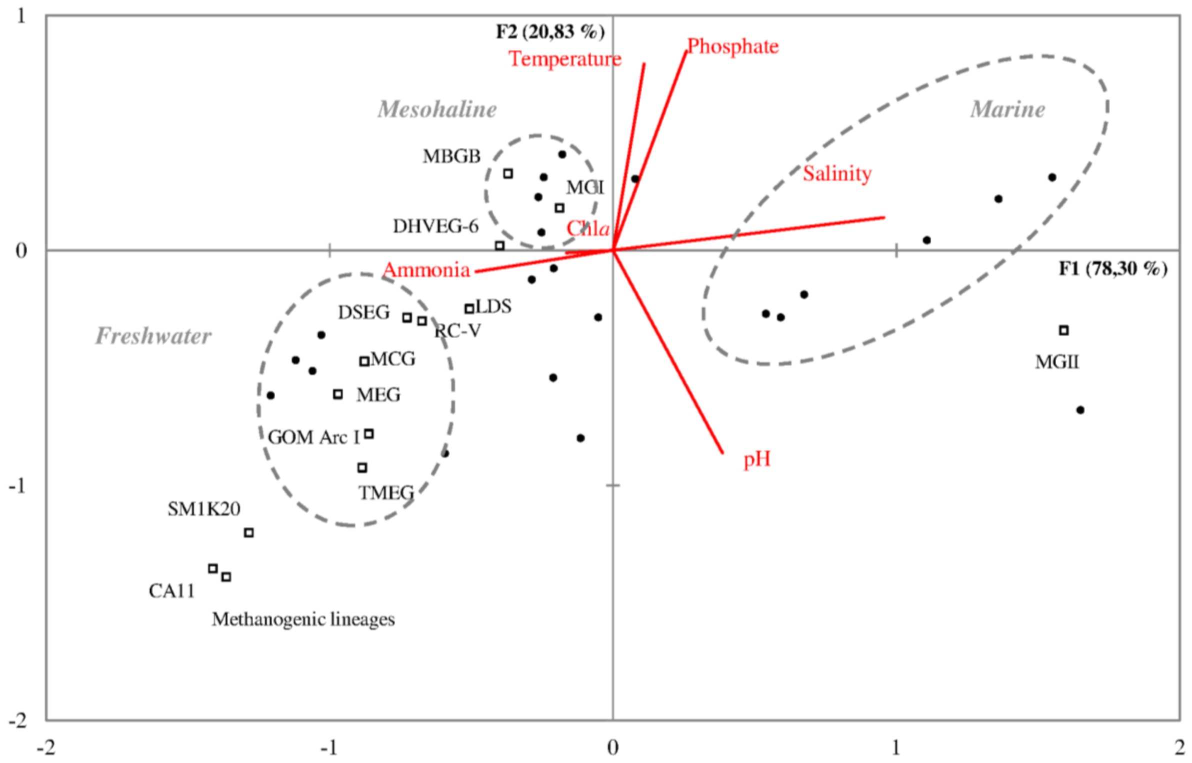

Supplementary Figure 2. Hugoni *et al.*

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	Date	QC seq.	*Archaea* affiliated sequences	*Thaumarchaeota* affiliated sequences	Temp. (°C)	Sal. (PSU)	pH	Chl *a* ($\mu g.L^{-1}$)	NH_4^+ ($mg.L^{-1}$)	PO_4^{3-} ($mg.L^{-1}$)
		16SrRNA								
Freshwater	14/04/2011	ND	ND	ND	16.9	0	8.65	3.618	0.113	0.008
	13/05/2011	ND	ND	ND	20.7	0	8.39	6.686	0.030	0.018
	15/06/2011	2966	177	147	21.7	0	8.28	28.48	0.081	0.008
	13/07/2011	4163	52	0	23.7	0	8.04	5.658	0.143	0.082
	26/08/2011	1613	20	0	23.5	0	8.18	63.665	0.036	0.111
	09/09/2011	3351	116	90	21.7	0	8.13	56.069	0.049	0.021
	10/10/2011	2057	212	158	18.3	0	8.22	33.94	0.065	0.056
	08/11/2011	3055	1616	1375	13.8	0	8.14	2.031	0.078	0.034
	08/12/2011	4909	394	229	11.5	0	8.21	0.691	0.122	0.014
	06/01/2012	5171	592	118	10.5	0	8.14	1.57	0.068	0.024
	20/02/2012	4501	946	41	7.2	0	8.1	7.089	0.081	0.011
	19/03/2012	306	35	0	12.1	0	ND	1.751	0.108	0.069
Mesohaline	14/04/2011	ND	ND	ND	15.4	14.3	8.48	5.264	0.0	0.011
	13/05/2011	ND	ND	ND	19.4	14.7	8.5	3.296	0.011	0.011
	15/06/2011	734	356	318	21	14.5	7.91	3.873	0.317	0.043
	13/07/2011	16263	7229	6833	22.1	15.4	7.78	5.054	0.043	0.114
	26/08/2011	1610	797	699	22.8	14.7	7.89	9.805	0.064	0.079
	09/09/2011	ND	ND	ND	21.1	14.3	7.58	4.276	0.0	0.069
	10/10/2011	4136	2798	2607	18	14.7	7.79	4.669	0.032	0.085
	08/11/2011	1169	724	645	13.7	14.3	7.9	2.736	0.033	0.056
	08/12/2011	1198	808	759	11.9	14.6	7.98	1.383	0.0	0.056
	06/01/2012	5042	3418	3041	10.2	15.7	8.12	0.661	0.05	0.03
	20/02/2012	ND	ND	ND	5.9	14.7	7.91	1.895	0.058	0.03
	19/03/2012	4784	1878	1478	10.4	15.2	7.87	9.28	0.020	0.056
Marine	14/04/2011	2313	1688	415	13.7	33.2	8.58	0.898	0.0	0.056
	15/06/2011	3298	1632	166	18.7	34.3	8.03	2.908	0.0	0.091
	13/07/2011	1909	586	97	21	35	8.03	5.078	0.0	0.088
	26/08/2011	ND	ND	ND	21.8	34.2	8.23	4.48	0.0	0.095
	09/09/2011	ND	ND	ND	20.7	35	8.07	1.946	0.0	0.062
	10/10/2011	ND	ND	ND	18.3	35.3	7.99	2.912	0.0	0.072
	08/11/2011	ND	ND	ND	14.5	34.8	8.03	1.312	0.0	0.261
	08/12/2011	429	337	310	12.6	33.6	8.02	0.803	0.0	0.072
	06/01/2012	1059	708	646	10.5	28.3	8.1	0.939	0.0599	0.075
	20/02/2012	914	334	219	6	32.8	7.95	1.419	0.071	0.082
	19/03/2012	1179	350	43	9.5	33.7	7.9	7.5	0.082	0.098

815 Supplementary Table 1. Hugoni *et al.*

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			Average number of sequences		
Station	**MGI Subluster**	**Representative OTU**	**Apr-Aug**	**Sep-Nov**	**Dec-Mar**
Freshwater station	**Marine A**	D32_HUJB0N002IFCRR	0	14	0
	Freshwater A	R34_HUJB0N002JELSP	17	60	24
		R16_HUJB0N002GU20W	16	43	10
		R24_HUJB0N002JCR91	5	39	8
		R8_HTRM39R02I8CB5	1	20	1
	Freshwater B	R24_HUJB0N002HQ6B7	10	20	3
		R16_HUJB0N002HSI5K	7	20	3
		R8_HTRM39R02IN9W4	0	15	2
	Sediment	R16_HUJB0N002JEY7S	19	44	9
		R10_HUJB0N002JLORV	6	20	5
		R7_HTRM39R02F36EW	5	20	3
Mesohaline station	**Marine A.c**	R22_HTRM39R02HVYCX	49	66	398
		D32_HUJB0N002F6TFA	5	19	303
		D32_HUJB0N002I2ZLT	1	6	84
	Marine A.b	R16_HUJB0N002H3P4B	323	101	47
		R19_HUJB0N002JY578	52	18	9
		R17_HTRM39R02HMXG2	59	16	3
		R16_HUJB0N002ITPEH	175	73	24
		R16_HUJB0N002ICU9P	87	34	8
	Marine A.a	D32_HUJB0N002IFCRR	129	131	105
		R17_HTRM39R02HNRQI	26	115	3
	Sediment	R16_HUJB0N002JEY7S	181	71	31
		R10_HUJB0N002JLORV	48	17	11
	Freshwater A	R24_HUJB0N002JCR91	48	24	18
		R16_HUJB0N002GU20W	115	43	50
		R34_HUJB0N002JELSP	199	84	78
	Freshwater B	R24_HUJB0N002HQ6B7	81	38	47
		R16_HUJB0N002HSI5K	47	19	29
Marine station	**Marine A**	D32_HUJB0N002F6TFA	34	ND	88
		R22_HTRM39R02HVYCX	47	ND	75
		D32_HUJB0N002I2ZLT	12	ND	18
		D32_HUJB0N002IFCRR	26	ND	12
		R16_HUJB0N002H3P4B	12	ND	7
		R25_HTRM39R02GJ5BA	10	ND	8

Supplementary Table 2. Hugoni *et al.*

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MGI Subluster	14-Apr	15-Jun	13-Jul	26-Aug	9-Sep	10-Oct	8-Nov	8-Dec	6-Jan	20-Feb	19-Mar
Marine A	ND	0	ND	ND	0	1 (1)	1 (42)	1 (1)	0	0	ND
Freshwater A	ND	4 (39)	ND	ND	4 (21)	4 (57)	4 (405)	4 (75)	4 (37)	3 (17)	ND
Freshwater B	ND	3 (17)	ND	ND	2 (15)	2 (9)	3 (140)	3 (16)	3 (8)	0	ND
Sediment	ND	3 (30)	ND	ND	3 (15)	3 (29)	3 (209)	3 (28)	3 (18)	2 (4)	ND
Marine A	ND	8 (140)	10 (2243)	10 (334)	ND	10 (894)	10 (258)	10 (516)	10 (2166)	ND	8 (269)
Freshwater A	ND	3 (35)	3 (977)	3 (75)	ND	3 (225)	3 (74)	3 (24)	3 (64)	ND	3 (349)
Freshwater B	ND	2(22)	2 (322)	2 (40)	ND	2 (95)	2 (18)	2 (3)	2 (8)	ND	2 (217)
Sediment	ND	2 (25)	2 (630)	2 (33)	ND	2 (135)	2 (39)	2 (19)	2 (37)	ND	2 (70)
Marine A	6 (307)	6 (78)	ND	ND	ND	ND	ND	6 (221)	6 (480)	6 (116)	1 (12)
Freshwater A	0	0	ND	ND	ND	ND	ND	0	0	0	0
Freshwater B	0	0	ND	ND	ND	ND	ND	0	0	0	0
Sediment	0	0	ND	ND	ND	ND	ND	0	0	0	0

845 Supplementary Table 3. Hugoni *et al.*