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Abstract:

The sensitivity of denitrifying community to salinity fluctuations was studied in microcosms filled with marine coastal sediments subjected to different salinity disturbances over time (sediment under frequent salinity changes vs sediment with "stable" salinity pattern). Upon short-term salinity shift, denitrification rate and denitrifiers abundance showed high resistance whatever the sediment origin is. Denitrifying community adapted to frequent salinity changes showed high resistance when salinity increases, with a dynamic nosZ relative expression level. Marine sediment denitrifying community, characterized by more stable pattern, was less resistant when salinity decreases. However, after two successive variations of salinity, it shifted toward the characteristic community of fluctuating conditions, with larger proportion of *Pseudomonas*-nosZ, exhibiting an increase of nosZ relative expression level. The impact of long-term salinity variation upon bacterial community was confirmed at ribosomal level with a higher percentage of *Pseudomonas* and lower proportion of nosZII clade genera.
Keywords: salinity fluctuations, microcosm, denitrification, resistance, community shift, *Pseudomonas*

1. Introduction:

Coastal lagoons are productive ecosystems with high economic importance, frequently threatened by eutrophication and oxygen depletion due to anthropogenic nitrogen and/or phosphorus enrichment. In aquatic ecosystems, nitrogen cycle regulates the amount of nitrogen available for phytoplankton. The largest source of nitrogen (N_2) is located in Earth's atmosphere, however, it has limited availability for biological use. In contrast, ammonium (NH_4^+), nitrate (NO_3^-) or nitrite (NO_2^-) that are easily absorbed and metabolised, are in much lower concentrations. The scarcity of usable nitrogen molecules and their availability can affect some key processes, such as, the productivity, dynamics and the eutrophication level of many types of environments (Kjerfve, 1985; Kjerfve et al., 1996; Ogilvie et al., 1997; Bonin et al., 1998; Rysgaard et al., 1999; Bonin, 2000; Gardner et al., 2006). Nitrogen cycle is a network of processes by which nitrogen is converted into multiple forms including organic nitrogen, ammonium, nitrite, nitrate, nitrous oxide (N_2O), nitric oxide (NO) or dinitrogen (N_2) gases. In shallow zones, biogeochemistry of the sediments and the overlying water are tightly coupled (Howarth et al., 2011). As a result, the relative percentages of the different forms of nitrogen ex-fluxing from the sediment into the water column have important implications for the status of coastal zones such as lagoons (Eyre and Fergusson, 2002), suggesting the major role of sediment microorganisms in achieving these processes. Among them, denitrifiers, that are able to reduce stepwise nitrate or nitrite through few intermediate gaseous nitrogen compounds to dinitrogen (Zumft, 1997), have a pivotal role in removing nitrogen from ecosystems and in counterbalancing possible excesses. Denitrification is catalysed by diverse facultative anaerobic microorganisms composed almost exclusively of Bacteria and Archaea (Rusch, 2013). Bacterial denitrifiers are affiliated to more than 80 genera, spread over different phyla including *Proteaobacteria* (Alpha-, Betat-, Gamma-, Deltat-classes), *Bacteriodetes*, *Firmicutes*, *Verromicrobia*, *Gemmatimonadetes*, *Spirocheates*, *Deferribacters*. In contrast, Archaea denitrifiers are affiliated to a lower number of genera (26) that belong mainly to *Euryarchaeoa* phylum (24 genera) dominated by *Halobacteria* class (23 genera) over *Archaeglobi* class (1 genus, *Ferroglobus*); and to a lower extend of *Crenarchaeota* phylum (1 genus, *Thermoprotei* class) and *Thaumarchaeota* phylum (1 genus, *Nitrososphaeria* class). The nosZ gene, encoding for nitrous oxide reductase enzyme and catalysing the last step of denitrification, is one of the genes used to follow the denitrifying community (Scala and Kerkhof, 1998; Rosch et al., 2002; Rontani et al., 2010). Recently, phylogenetic studies of nosZ genes in soil revealed two distinct clades; nosZI and nosZII (Jones et al., 2013) of which organism would react differently toward environmental parameters such as pH, nitrogen, calcium concentration and cropping system (Domeignoz-Horta et al., 2015).

Microbial community of lagoons and coastal ecosystems are subject to continuous environmental changes, such as those involving salinity or nutrient concentrations. To maintain their ability to function they must tolerate rapid and repeated fluctuations (Castel et al. 1996, Baho et al. 2012). Variations in salinity impose considerable osmotic and matric stress on microorganisms and can cause decreases or increases in cytoplasmic volume; damage to membranes, proteins and nucleic acids; and cellular lysis. In literature, contradictory tendencies have been reported on the effects of salinity changes on denitrification. Salinity has no effect on denitrification for several coastal or estuarine areas (Nielsen et al., 2001; Fear et al., 2005; Magalhaes et al., 2005), whereas other studies have shown an inverse relationship between denitrification rates and salinity (Rysgaard et al., 1999; Giblin et al., 2010). However, it is difficult to assess the direct impact of salinity changes in field studies because many environmental and chemical parameters are correlated or interact at sampling sites (Jackson and Vallaire, 2009). Only a few experimental studies (e.g., (Nowicki, 1994; Rysgaard et al., 1999; Magalhaes et al., 2005; Laverman et al., 2007) address the resistance and resilience of microbial sediment activity in the face of salinity perturbations but without taking into account microbial community.

In the present study, we used a microcosm approach to examine specifically the effects of short-term salinity variations on denitrifying activity and community structure, taking into account the disturbance history of the sediment. The sediments selected for this study originated from the brackish semi-enclosed Berre lagoon subjected to eutrophication (SE France, Fig.S1) that contained extremely low macrofauna, that is a parameter that could impact proportion of nosZ-harboring bacteria (Bonin et al., 2015). An indirect effect of salinity fluctuation on macrofauna was in consequence excluded in this study.

Different scenarios of salinity variations were performed in this study in order to test the resistance of the communities from both stations that experience different patterns of long-term salinity stress. Several questions were addressed in this study (i) whether the long-term salinity history has an effect on the denitrification rates and community (including nosZI/nosZII taxon repartition), (ii) whether short-term salinity variation has an impact on denitrification rates and community (nosZI/nosZII taxon repartition), (iii) what are the main factors affecting community resistance and which taxa take advantage of fluctuating conditions?

2. Materials and methods:

2.1 Sampling sites:

The sediments selected for this study originated from the brackish semi-enclosed Berre lagoon subjected to eutrophication, which offers a particular historical record of salinity. The northern part is subjected to frequent salinity changes due to Mediterranean river flows sharp fluctuation (Delpy et al., 2012) and to a hydroelectric power plant that introduces freshwater inputs in a fluctuating mode. The southern part of the lagoon is in front of a channel leading to the Mediterranean Sea. It is, consequently, under marine influence and experiences only few salinity fluctuations of lower intensity. The two selected stations showed, from an annual survey, similar parameters in porous water such as nitrate, nitrite, ammonium concentrations and % of Lost-On-Ignition (LOI , 11 ± 2 % and 13 ± 2 % for S1 and S3) (Zaghmouri et al., 2013). The two sampling stations (Fig. S1) were distinguished by their contrasting salinity patterns. The first station (S1, ~ 6 m deep) is located in the northern part of the lagoon in front of the power plant and the Arc and Touloubre River mouths and is subject to freshwater loads. Its annual salinity ranges between 15 and 30. The second station (S3, ~ 9 m deep) is located in the southern part of the lagoon beyond the Caronte channel, which connects the lagoon to the Mediterranean Sea. Station S3 is under marine influence; its annual salinity ranges from 20 to 35.

2.2 Sample collection:

The sediments used in the experiment were collected in December 2010 at station S3 (with an *in situ* salinity of 33 and an *in situ* temperature of 15.9°C) and in March 2011 at station S1 (with an *in situ* salinity of 24 and an *in situ* temperature of 14.6°C) (Fig. S1). Both sediments were rich in mud and thus characterized by a low permeability. An annual survey has shown no difference in concentration of O_2 in bottom water, or concentration of nitrate, nitrite and ammonium in the porous water nor in organic matter content between both stations (Zaghmouri et al., 2013). For each station, a set of 5 Plexiglas cores (25 cm length, 10 cm inner diameter) was collected. The bottom half of the cores was filled with sediment (12 cm length). The top half was filled with *in situ* bottom water and aerated to maintain sediment saturation and to prevent the establishment of anoxic conditions during transportation.

2.3 Description of microcosms:

The Plexiglas cores were used for microcosms incubations. They consisted of cylinders of 10 cm in diameter and 25 cm in height with a total volume of approximately 2 L (Fig. S2). The microcosms were filled with undisturbed sediment from the cores to a height of 12 cm and were fully re-filled with synthetic seawater (SSW) (Baumann et al., 1971) and NaCl to adjust the desired salinity, then hermetically closed to the atmosphere so that no gaseous headspace remained. In each reservoir, salinity was controlled by refractometry. The salinity of the SSW was adjusted by adding NaCl. The $Na^{15}NO_3^-$ and $^{14}NH_4Cl$ concentrations in the reservoirs were adjusted to 250 µM and ~5 µM, respectively. Each microcosm was equipped with an inflow device (0.3 cm inner diameter), extending into the water column and ending 1 cm above the surface sediment, through which the flowing medium entered the system. The outflow device was located on the opposite side and extended 1 cm above the surface sediment. Cores were connected to a 5L-SSW reservoir providing the experimental treatment. The reservoirs were open and provided with a stream of air bubbles to prevent N_2 accumulation and to maintain air saturation in the water phase. Water was pumped via a peristaltic pump from the 5L SSW reservoir to the individual microcosms at a flow rate of 0.09 L.h^{-1}, creating a residence time of approximately 10.5 h in each microcosm. During the incubation, the water column in each microcosm was gently mixed using a magnetic stirrer driven by an external magnet (30 r.p.m.). Mixing tests prior to the experiment showed complete mixing within the water portion of the microcosms. The microcosms were incubated at the *in situ* temperature. The microcosms were stabilised at *in situ* seawater salinity for 72 h to reach a steady state (i.e., constant N-NO$_3^-$ and N-NH$_4^+$ uptake rates) before beginning the experiment.

2.4 Microcosm experiment:

To assess the impact of salinity variations on the nitrogen cycle, three set up were developed to simulate salinity fluctuations mimicking those recorded in the lagoon with each type of sediment (S1, S3) (Fig. 1)

The first scenario aimed to maintain the salinity close to the *in situ* values throughout the experiment (Experiment S1M1 and S3M1). The second one was designed to follow the effect of one salinity shift: one increase from 20 to 35 for S1 (Experiment S1M2) or two decrease levels for S3: from 35 to 25 (Experiment S3M2A) or from 35 to 15 (Experiment S3M2B). The third one intended to follow the shift back to the initial *in situ* salinities after the previous disturbance: S1M2 to 20 (Experiment S1M3) and S3M2 A and B to 35 (Experiment S3M3A, S3M3B). The SSW in each microcosm reservoir was renewed at the beginning of each salinity fluctuation phase.

Before starting the experiments, during the 1st phase (3 days), five replicates of each station microcosms were maintained at *in situ* salinity in order to stabilise the system (salinity of S1 =20, S3=35). During the 2nd phase (~0.5 days of transition plus 4.5 days of stability) M1 microcosms were kept at *in situ* salinity, duplicates of microcosms were subjected to salinity-shift perturbation: M2 microcosms were subjected to the most extreme salinity values recorded in the lagoon during the last 20 years (35 for S1M2/M3 and 15 for S3M2B/M3B, (GIPREB, 2010)); S3 M2A and M3A microcosms were subjected to an intermediate salinity decrease (S=25). During the 3rd phase (~0.5 days of transition plus 4.5 days of stability), S1M2, S3M2A and S3M2B microcosms were maintained at the same salinity as in phase 2 (S=35; then down to 25 and after, up to 35 respectively), whereas 3 microcosms were shifted back to the initial *in situ* salinities after disturbance (S1M3 to 20, S3M3A and S3M3B to 35).

Whatever the sediment and the scenario, the conditions were maintained during several days with the same salinity in the microcosms and more than 6 individual measurements were determined independently in each microcosms for each phase of the scenario.
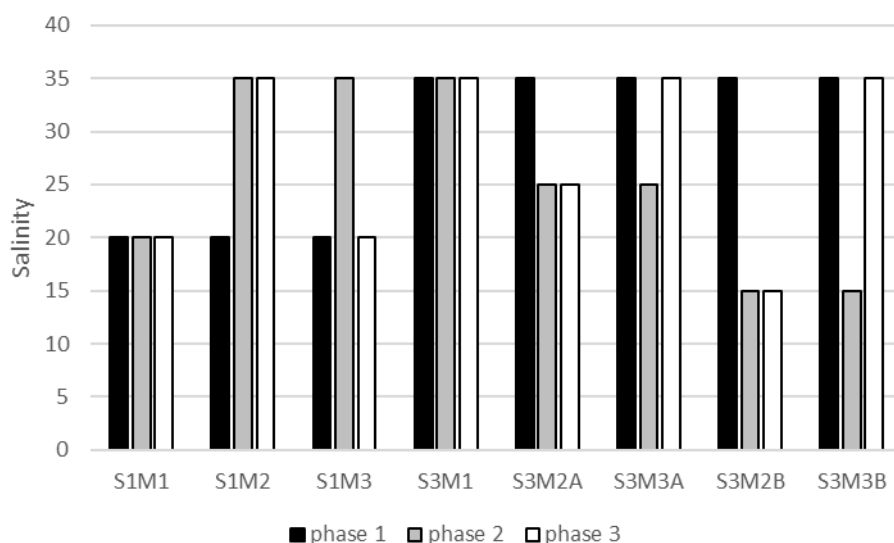
Fig. 1. Summary scenarios of salinity fluctuations in S1 and S3 sediment. Phase 1 (*in situ* salinity , black); Phase 2 (salinity-shift perturbation, grey) except for M1 microcosms; Phase 3 (maintained disturbance or back to *in situ* salinity, white) except for M1 microcosms . Phase 1, 2 and 3 lasted 3, 5 and 5 days respectively. Sx correspond to sediment type (Station S1 or Station S3), Mx correspond to the name of the microcosms.

2.5 Microbial activities:

To quantify denitrification, Anammox and dissimilative nitrate reduction into ammonium (DNRA) for each microcosm and for a given phase, the rates of substrate consumption or product accumulation were measured. For each microcosm during a given phase and for each activity, rates were calculated as the

mean of at least 6 individual measurements, determined independently each day within 10h interval during the steady state of each phase. Water was collected in vacuum Venoject® (Venoject Terumo, Japan) headspaces twice a day (10h interval) at the inlet and outlet of each microcosm. Concentration of nutrients (NH_4^+, NO_3^-, NO_2^-), dissolved gases (N_2) and their isotopic composition were measured in each sample.

Denitrification and Anammox rates:
For the measurement of denitrification and Anammox, Venoject® (Venoject Terumo, Japan) headspaces from each microcosm were injected into a mass spectrometer to determine N_2 concentrations (Anagaz 100 Quadruple mass spectrometer, MKS, England). The concentrations of $^{29}N_2$ and $^{30}N_2$ were measured in addition to $^{28}N_2$, Ar and O_2 (Minjeaud et al., 2008). At each sampling time, the denitrification and Anammox rates were calculated with the comprehensive method outlined by Spott & Stange (2007). This approach allows the calculation of the contribution of denitrification and Anammox to an N_2 mixture and includes considerations of possible contamination by atmospheric N_2. The following calculations (Eqs. 1-3) were used to determine the portion of atmosphere (A), denitrification (B) and Anammox (C) contributing to the N_2 mixture:

$$A = \frac{2b(\alpha_{30}-cd)+(c+d)(b^2-\alpha_{30})-(b^2-cd)(\alpha_{29}+2\alpha_{30})}{(a-b)[2(ab+cd)-(a+b)(c+d)]} \quad \text{Eq. 1}$$

$$B = \frac{2a(cd-\alpha_{30})+(c+d)(\alpha_{30}-\alpha^2)+(\alpha^2-cd)(\alpha_{29}+2\alpha_{30})}{(a-b)[2(ab+cd)-(a+b)(c+d)]} \quad \text{Eq. 2}$$

$$C = \frac{2ab-\alpha_{29}(a+b)+2\alpha_{30}(1-b-a)}{2(ab+cd)-(a+b)(c+d)} \quad \text{Eq.3}$$

where α_{28}, α_{29} and α_{30} are the molar fractions of masses 28, 29, and 30, respectively, within the N_2 mixture and a, b, c and d are the ^{15}N atom fractions of N_2 (a), NO_3^- (b), NO_2^- (c) and NH_4^+ (d), respectively. Due to relative high concentration of nitrate (250µM) in the overlaying water compared to *in situ* water (ranging from 0.3 to 36 µM for S1 and 0.2 to 7.4 µM for S3 (Zaghmouri et al., 2013), denitrifying and Anammox activities were considered as potential rates.

DNRA (Dissimilative Nitrate Reduction to Ammonium)
Nitrate concentration in the overlying water column were analysed with a Technicon-Auto-Analyser according to Treguer and Le Corre (Treguer and Le Corre, 1975). Ammonium concentrations were determined using the fluorometric method (Kérouel and Aminot, 1997). The isotopic composition of $N-NO_3^-+NO_2^-$ and $N-NH_4^+$ was determined according to Bonin et al. (Bonin et al., 1998). Ammonium was extracted from each sample by microdiffusion at 60°C after treatment with a mid-alkali (MgO) to convert NH_4^+ to NH_3 (Brooks et al., 1989). Ammonia was trapped on acidified (25 µl, 0.5 N H_2SO_4), precombusted Whatman GF/C filters, and the ^{15}N content was analysed by mass spectrometry (Tracer Mass, European Scientific) (Bonin, 1996). The rates of DNRA were calculated as previously described (Gilbert et al., 1997).

$$DNRA = \frac{(AT\% \ NH_4^+)([NH_4^+])}{(AT\% \ enrichment \ NO_3^-)(Time)} \quad \text{Eq. 4}$$

where AT% NH_4^+ and AT% NO_3^- is the molar fraction of $^{15}N-NH_4^+$ and $^{15}NO_3^-$ respectivelly. Due to relative high concentration of nitrate (250µM) in the overlaying water, DNRA activity was considered as potential.

Nitrate reduction rates:
Nitrate reduction rates (NaR) were calculated by combining denitrification and DNRA rates (NaR= Dtot + DNRA) (Dong et al., 2009; Jantti et al., 2011), Dtot corresponds to total denitrification rate.

2.6 Prokaryotic enumeration and diversity
Immediately after sampling, triplicate of the first 2cm of sediment were collected from microcosm sub-cores and were frozen (-80°C) and stored about 6 months at this temperature until processing. DNA was extracted from 0.5g (dry weight) of the first 2cm of the microcosm sediment using MoBio UltracleanTM Soil DNA isolation kits (Mo Bio Laboratories, California). RNA was extracted from 1g of sediment using RNA Power Soil : Total RNA Isolation Kit (Mobio) according to manufacturer instruction. Reverse transcriptions were performed on 20ng of total RNA using SuperScript III First Strand Synthesis System for RT-PCR (in vitrogen) with random hexamer (Promega) (details in supplement material). Absolute gene or cDNA quantifications (Bacterial 16S rDNA, archaeal 16S rDNA genes and

nosZ), were performed by qPCR according to the procedure described in detail in supplement material. Serial dilution of plasmids harbouring the gene of interest was used for standard construction.

Bacterial nosZ diversity analysis was performed by DGGE after amplification of 686 pb fragment using nosZ 1211F and nosZ 1897R primers (Rosch et al., 2002) as previously described by (Rontani et al., 2010). About 500ng of nosZ-PCR products were loaded onto 1mm thick, 8% (wt/vol) polyacrylamide gel with a denaturation gradient from 20% to 80% (Rontani et al., 2010). After running and DNA staining, DGGE bands comparison between samples were realized with Gel Compar II software (Applied Maths) and relative band intensity within samples were also measured. Major bands were excised from the gel for S1 and S3 samples and sequenced with Sanger method (GATC company). After sequence alignment (ClustalW, (Thompson et al., 1994) phylogenetic trees were constructed using the Neighbor-Joining method (Saitou and Nei, 1987) and plotted using the Njplot Mega 7 program (Tamura et al., 2007). Sequences accession numbers: KY996804-KY996809, bioproject PRJNA384371.

For ribosomal prokaryotic diversity analysis, the V4 region of the bacterial and archaeal 16S rDNA genes (16S) of DNA samples were amplified using universal primer set, 515F (5′-GTGCCAGCMGCCGCGGTAAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) (Caporaso et al., 2012), using 1U Pfu DNA polymerase (Promega). The 16S amplicons were sequenced by the MiSeq Illumina (paired end 2* 250) platform Get of Genotoul. The paired-end raw reads were firstly overlapped and merged by the FLASH assembly software (Magoc and Salzberg, 2011). The analysis of the 16S-assembled data was relied on the use of QIIME package and its recommendations (Kuczynski et al., 2012). The removal of low quality bases and chimera sequences were performed by QIIME script and UCHIME (Edgar et al., 2011), respectively. The high quality sequences were then clustered into Operational Taxonomic Unit (OTU) using a 97% of sequence identity threshold and the UCLUST algorithm (Edgar, 2010). The OTU table was filtered for low abundance OTUs (Bokulich et al., 2013) followed by a subsampling-based normalization implemented in QIIME (Kuczynski et al., 2012). The full taxonomic hierarchy classification was performed by UCLUST taxonomy. Finally, the alpha, beta diversity and community structure was realized by the core_diversity_analyses.py script (Kuczynski et al., 2012).

2.7 Statistical analysis:

Student's *t* test was used to examine the influence of salinity changes on nitrogen processes. The significance level for all tests was set at p<0.05. All tests were performed with the XLSTAT® (XLSTAT software® 2008, Addinsoft, Paris). Repeated Measures-Hierarchical analyses of variance analysis (rm-ANOVA) was used to examine the variance in the nitrogen processes within microcosms along a time series. In fact, as there are five different salinity treatments, each of which has a specific sequence of salinity shifts in time, and as the experiment is a time-series with three dependant phases, the use of rm-ANOVA is the most appropriate in this study. The significance level for all tests was set at p<0.05. All tests were performed with XLSTAT® (XLSTAT software® 2008, Addinsoft, Paris). Past software was used for PCA analysis, and test of the K mean clustering.

Results:
3.1 *In situ* biodiversity

The microbial ribosomal diversity (Fig. 2) of the top 2cm of S1 sediments, located in the less saline part and S3 in the marine part of the lagoon (Fig. S1), was characterized.

This layer was chosen as the microbial exposition to salinity fluctuation should be maximal in this part. For each 16S rRNA sample data, the Good's coverage estimator showed that the sequencing depths were sufficient to cover 98.9% of the microbial diversity for both sampling sediments. The number of OTUs was 1557 and 1544 for S1 and S3, respectively. The microbial richness measured by the Shannon diversity index (9.19 and 9.29) indicated that S1 and S3 sampling sites have similar microbial diversity. Simpson's diversity index, corresponding to a measurement of diversity taking into account the number of species present, as well as the relative abundance of each species, was close to 1 in this study, suggesting very high diversity level in these sediments.
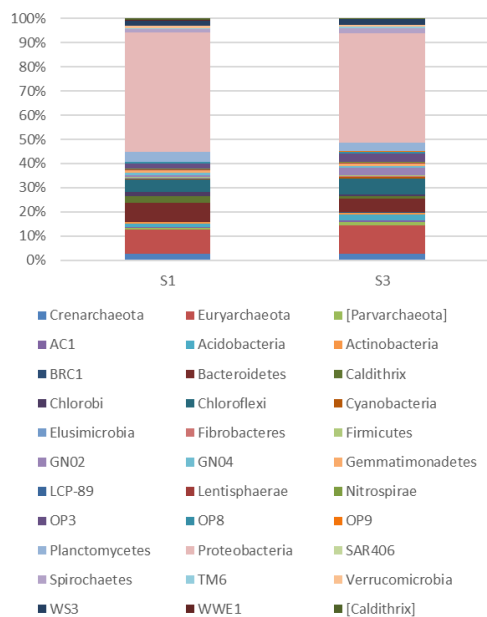
Fig. 2. Relative abundance of phylums present in S1 and S3 microcosms at the beginning of the incubation

Archaea accounted for 12.7 and 14.3 % of the sequences for S1 and S3 respectively, with about 10% belonging to *Euryarchaeota* phylum, 2% to *Crenarchaeota*, and 1% to *Parvarchaeota* (Fig. 1). The sequences of these phyla were not affiliated to classes harbouring denitrifiers, even for S3 that contained *Archaeglobi* sequences in very low percentage (0.002% data not shown) as their deeper affiliations (*Archaeoglobus* genus) did not confirm a potential denitrification property. Among bacteria, Proteobacteria was the major dominant phylum (46.4 and 40.3% of the sequences for S1 and S3 respectively) of which 21% correspond to Delta-proteobacteria for both stations. The percentage of Gamma-proteobacteria was higher in S1 (20.3%) than in S3 (13.3%). Unlike Archaea, numerous bacterial genera (17) known to harbour denitrifiers were identified in ribosomal analysis, with a cumulative percentage reaching 0.89 and 1.33% of the sequences for S1 and S3 respectively (Fig.2). All these data suggest a large predominance of Bacteria over Archaea among denitrifiers in both sediments. Although ribosomal diversity may overestimate denitrifying community due to discrepancy between ribosomal information and presence of denitrification genes, strong differences could be observed between bacterial denitrifying genera present at S1 station, subjected to frequent salinity fluctuations, and S3 station under marine influence. Genera *Pseudomonas*, *Acinetobacter*, *Janthinobacterium* and *Maribacter* were more abundant in the fluctuating station (S1), whereas *Pseudoalteromonas*, *Thiobacillus*, *Shewanella*, *Arcobacter*, *Opitutus*, or *Sulfurimonas* were more abundant under marine influence (S3). In addition, *Marinobacter*, *Pseudoruegeria* or *Dehalobaterium* genera were found with similar percentage in both conditions. Both sediments contained about 0.22% of genera that have been shown to possess nosZI clade gene whereas proportion of genera harbouring nosZII clade was 2 fold higher in S3 compared to S1 (0.77 vs 0.35) (Fig. 3).
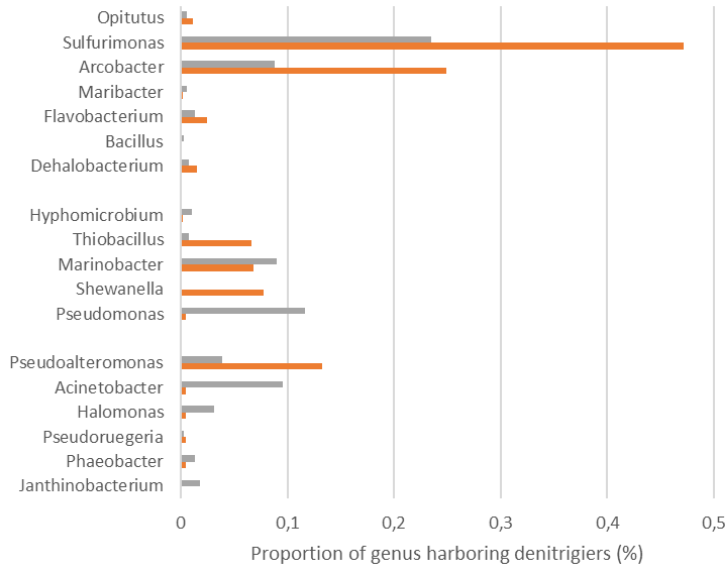
Fig. 3. Relative abundance (%) of genera containing denitrifiers in S1 and S3 microcosms at the begining of the incubation from ribosomal information. NosZ clades were indicated according to Jones et al 2013. ND: not determined

3.2 Effect of salinity fluctuations on denitrifying activities in microcosms

In order to test the effect of salinity fluctuations on the processes of nitrogen cycle, 10 microcosms containing S1 or S3 sediments were settled. The incubations were separated in 3 phases (Fig.3). During the first phase of the incubation (Phase 1), for the both sampling sites, five microcosms were incubated at their *in situ* salinity (20 and 35 for S1 and S3, respectively). Activities of the entire core associated to the nitrogen cycle (potential denitrification, nitrate reduction, Anammox, DNRA) were measured in Phase 1 (Table 1, Fig.S3).

Table 1. Denitrification, nitrate reduction, dissimilative nitrate reduction into ammonium and anammox rates, quantification and proportion of denitrifiers in stations S1 and S3 in control microcosms

Phase		S1	S3
P1	Denitrification ($\mu mol.cm^{-2}.h^{-1}$)	0.88 ± 0.16	8.25 ± 1.23
	Nitrate reduction ($\mu mol.cm^{-2}.h^{-1}$)	0.88 ± 0.16	8.26 ± 1.32
	DNRA ($\mu mol.cm^{-2}.h^{-1}$)	$6. \ 10^{-3} \pm 0.1 \ 10^{-3}$	$9.25 \ 10^{-5} \pm 4.4 10^{-5}$
	Anammox ($\mu mol.cm^{-2}.h^{-1}$)	$7.2 \ 10^{-3} \pm 0.6.10^{-3}$	$8.6 \ 10^{-5} \pm 0.3 \ 10^{-5}$
	nosZ/g wet sediment	$1.01 \ 10^{7} \pm 0.12.10^{7}$	$0.52 \ 10^{7} \pm 0.07 \ 10^{7}$
	% of denitrifier /bacterial genome equivalent *	1.4	2.66
P3	% of denitrifier/ bacterial genomes equivalent*	2.89	1.04
	cDNA nosZ / cDNA 16S rRNA (%)	$3.62 \ 10^{-5}$	$20.98 \ 10^{-5}$

Rates of phase 1 (n=30) were measured on 5 microcosms at the beginning of the incubation (phase 1) from 5 microcosms filled with sediment of station S1 or S3. NosZ/16S and cDNA nosZ/16S rRNA % were measured at the end of phase 3 in control microcosms (no salinity changed). *4.19x nosZ /16S rDNA , 4.19 being the average number of ribosomal gene per bacterial genomes

For both stations, whatever the activity, there was no difference in the initial activity among the microcosms of the same set (rm-ANOVA, p>0.05, data not shown). Moreover, control microcosms (S1M1 and S3M1) presented no significant change of potential denitrifying rates all along the experiment (P1, P2, P3; Fig. S3 (*t* test, n=18, p>0.05)) allowing to validate the stability and the homogeneity of the microcosms. Potential denitrification and nitrate reduction rates were in micromolar range whereas DNRA and Anammox were in nanomolar range or less (Table 1). Due to extreme low potential DNRA and Anammox rates and to the importance of potential denitrification found in S1 and S3 microcosms, DNRA and Anammox were not presented as they were negligible in this study. Nitrate reduction mirrored denitrification all along this study and thus was not shown. Therefore, denitrification appeared to be the major process removing nitrogen in both sediments.

Significant differences of magnitude in potential denitrification were observed between both microcosm series (S1 or S3) with a potential activity almost 10 fold higher for S3 sediment compared to S1. Denitrification rates reached 8.25 ± 1.23 $\mu mol.cm^{-2}.h^{-1}$ in S3 vs. 0.86 ± 0.15 $\mu mol.cm^{-2}.h^{-1}$ in S1 (*t* test, n=12, p<0.05 Table 1).

To test whether the difference of activity was due to a direct effect of salinity on the fitness of denitrifiers or change in the community, the effect of salinity fluctuation was investigated on microcosms of both stations. We hypothesised that if the difference of activity was due to modification of activity level of denitrifiers, an increase of salinity for S1 would lead to an increase of activity and the opposite for S3. Different scenarios of salinity variations were performed (Fig.1) and comprised (i) no fluctuation (control, S1M1 and S3M1), (ii) one fluctuation mimicking realistic situations (increase of salinity for S1M2 and two levels of salinity decrease for S3M2A and S3M2B) or (iii) two fluctuations (the previous shift then a return back to the initial salinity) (Fig. 1). Activities were recorded each day during the incubation period and were averaged over the phases (Fig. S3). Whether for an increase of salinity for S1 or a decrease of salinity for S3, or one fluctuation, no significant variation of denitrification rate was detected between the phases (P1, P2, P3, Fig S2) (*t* test, n=12, p>0.05). However, microcosms subjected to two shifts of salinity presented higher variances in denitrification rates within P3 phase. In comparison with control microcosms (S1M1 and S3M1) (Fig 4A), no significant difference in the potential denitrification rates could be observed between 10 days shift of salinity, and a shorter shift (5 days) followed by a return to *in situ* salinity (S1M2 vs S1M3, S3M2A vs S3M3A or S3M2B vs S3M3B). Whatever the scenario, denitrifying activities of microcosms containing S3 sediment were still significantly higher than those of S1 (Fig. S3). The long-term salinity history seems to have an effect on the denitrification rates in contrast to short-term variations.
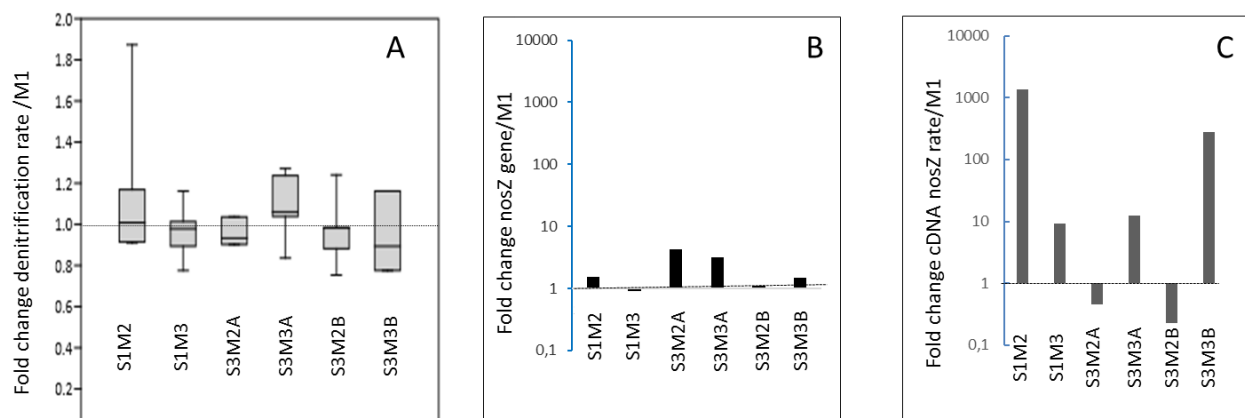
Fig. 4. Potential denitrification rate (A) , abundance of denitrifiers (nosZ gene)(B), nosZ induction rate (mRNA nosZ) (C) at the end of the phase 3 expressed as fold change compared to control microcosms. (A) Results are presented as box-plots: the center vertical line marks the median, the edges of the box mark first and third quartiles and the vertical lines indicate the range of values that fall within 1.5 (midrange) of the hinges, (B) ratio of DNA- nosZ/DNA-16S rRNA normalized with control ratio, (C) Induction rate correspond to ratio of cDNA- nosZ/cDNA-16S rRNA normalized with control ratio. Ratio of 1 was marked with an horizontal hatched bar.

3.3 Denitrifiers in microcosms: abundance and diversity

In a second time, the impact of long or short term variation of salinity on denitrifying community was analysed. The ribosomal information does not allow identifying with certainty denitrifiers since not all the organisms of a genus present this property. In consequence, a gene coding for an enzyme of this process, *nosZ,* was used to follow this community. From ribosomal analysis, no archaeal classes or genus harbouring denitrifiers were identified in the initial sediment. In consequence, this study focused only on bacterial denitrifiers in the top 2cm of the sediments through *nosZ* quantification (absolute and relative to 16S rDNA), expression and diversity analysis. This approach was performed in the initial sediment and in the 8 microcosms at the end of the incubation in order to record the effect of the entire scenarios.

A 10-fold difference in denitrifying activity was observed between S1 and S3, and the relative *nosZ*/bacterial genome equivalent reflected partly this variation since it was about 2 fold higher in S3 than in S1. In contrast, the *nosZ*-absolute abundance presented an opposite tendency (Table 1). This observation could be due to higher activity of the present bacteria or to our technical approach since our DNA extraction method and qPCR quantification were unable to distinguish between DNA originating from active bacteria and extracellular DNA (eDNA) originating from dead and lysed cells. As preservation of eDNA in sediment could lead to overestimation of denitrifying bacteria abundance and shaded difference of alive denitrifying bacteria between both stations, transcriptomic approach has been also performed. The *nosZ* expression (evaluated by nosZ-cDNA/16S r-cDNA) was 5.7 fold higher for S3 than for S1 at the end of the incubation of control microcosms (Table 1). The higher denitrification activity recorded in S3 could be the result of higher activity level of denitrifiers in this sediment.

The responses of denitrifying community to short term salinity fluctuations were also analysed (nosZ abundance and expression). Relative abundances of nosZ-genes were little impacted by these disturbances compared to controls (Fig. 4B): little or no differences were observed between the 4 microcosms (S1M2, S1M3, S3M2B, S3M3B), and an increase of 4 fold was observed for S3M2B and S3M3B (which experienced a decrease of salinity from 35 to 25) (Fig. 4B). In contrast, the salinity scenarios seem to have a stronger impact at nosZ transcript level (Fig. 4C). For the sediment of marine origin (S3), subjected to single decrease of salinity of 10 days (S3M2A and S3M2B), a decrease of nosZ relative expression level was observed. When two successive fluctuations of salinity occurred (i.e a decrease of 5 days followed by a return to *in situ* salinity for 5 days), an increase of nosZ relative expression level (12.5 and 276 fold for S3M3A and S3M3B) was observed compared to control. For sediment that had experienced long-term fluctuation (S1), the response seems different and more dynamic. A single increase of salinity (S1M2) or a fluctuation (S1M3, on which a short shift followed by a return to *in situ* salinity was applied) induced an increase of 1370 and 9 fold of nosZ cDNA relative expression compared to control (Fig. 4C). As a result, an effect of short-term salinity variation was visible at nosZ transcript level and could lead to changes in the major member of the denitrifying community.
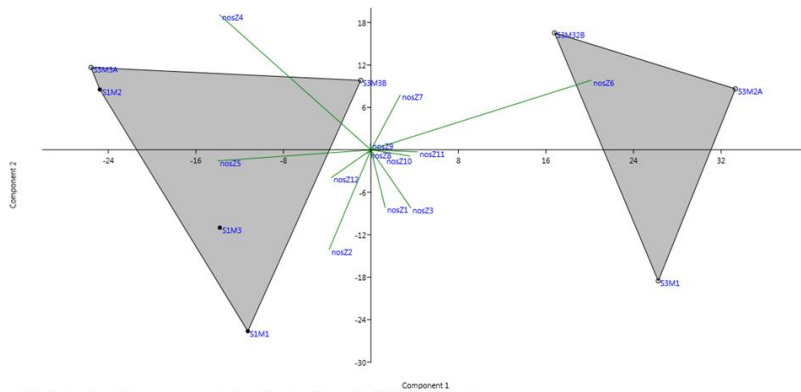
Fig. 5. Principal Component Analysis of nosZ OTU-intensity.
Groups were determined with K mean. Black symbol: sediment subjected to long term saline variation,
white symbol: sediment of marine environment, with long term saline stability

Denitrifiers diversity was analyse by PCR-DGGE on nosZ to allow the detection of the major nosZ-OTUs. Major bands were further identified by sequencing. In comparison to the total intensity of DGGE bands within a sample, the cumulative intensity of organisms identified by sequencing varied from 74 to 92% (Fig S4). Analysis in principal component of nosZ –OTU band intensity from DGGE gel originating from the 8 microcosms has identified two clusters (Fig. 5). One grouped microcosms with marine sediments that have experienced none or a single shift of salinity (S3M1, S3M2A and B). The nosZ6-OTU that present about 98% similarity at amino acid level with nosZ of alpha proteobacteria – *Sulfitobacterium mediterraneum* (Fig. S5), showed high intensity in these microcosms.

The other group on Fig. 5 contained marine sediment microcosms that have undergone two fluctuations of salinity (S3M3A and B) and S1 sediment counterpart that have experienced long-term salinity shifts, whatever the recent scenario is (S1M1, S1M2, S1M3). A group of major OTUs were particularly intense and corresponded to nosZ2, 4 and 5. They presented more than 90% similarity with nosZ sequences of different strains of *Pseudomonas* with nosZ4 being 99% similar to nosZ of *Pseudomonas stutzeri CCUG*. Few OTUs including nosZ3 (97% similarity with uncultured bacteria from marine sediment) and nosZ1 (clustering among Rhodobacteracea nosZ sequences with 95% similarity with *Labrenzia aggregate*) present few variation of intensity whatever the treatment or the sediment origin (Fig. 5, S4).

3. Discussion:

Denitrification was the major process responsible of nitrogen cycle in the microcosms used in this study, as it has been shown from an annual survey in this area (Zaghmouri et al., 2013). We assessed the sensitivity of denitrification to long-term and short-term salinity changes in two sampling sites from Berre Lagoon exhibiting similar physical and chemical parameters. Variation of salinity could modify (i) the number of denitrifier cells, (ii) percentage of active denitrifying cells, (iii) the activity of the enzyme or (iv) the structure of the community. Denitrification rate in S3 was 10 fold higher than in S1 in agreement with a 5.7 higher relative *nosZ* expression.

Short term-salinity variation unaffected the number of *nosZ* genes and had no significant impact on denitrification potential rate in the entire core, whatever are the scenario or the historical salinity pattern of the sediment. The stability of a net ecosystem denitrification in spite of salinity stress has been shown to be strongly influenced by the initial evenness of the community (Wittebolle et al., 2009). The high diversity of both sediments used during this study is an element favouring their resistance. Our results highlighted also the absence of direct relationship between denitrification rates of the entire core collected from the microcosms and the normalized abundance of nosZ gene or their transcripts measured in the top 2cm. No evident relationship has been seen between denitrification rates and the normalized abundance of other gene of denitrification in another microcosm study (Bowen et al., 2014) or in global analysis (Graham et al., 2016). It was attributed to the fact that denitrification is performed by facultative micro-organisms with different phenotypic plasticity, of which some part could be inactive. However in our study, since activity was performed on the entire sediment column of which a large part of sediment

was not affected by salinity fluctuation, the apparent resistance of denitrifying community could be also due to aggregation of activity from these different depths as reported for nitrate reduction (Papaspyrou et al., 2014).

In literature, denitrifier communities in continental shelf sands appear to be dominated by members of the Alphaproteobacteria based on analysis of the nosZ gene (Hunter et al., 2006; Mills et al., 2008). Presence of nosZ Alphaproteobacteria-like sequences was also detected in our study. Few OTUs including nosZ3 (97% similarity with uncultured bacteria from marine sediment) and nosZ1 (clustering among Rhodobacteracea nosZ sequences with 95 % similarity with *Labrenzia* aggregate) presented few variations of intensity whatever the treatments or the sediments origin and were probably resistant to salinity changes without taking advantage of them. In contrast, nosZ6 affiliated to *Sulfitobacter mediterraneus* was less resistant as its intensity discriminated the group of microcosms of marine sediment subjected to none or one shift of salinity to the other PCA group of other microcosms.

Phylotype nosZ2, nosZ4 and nosZ5, affiliated with *Pseudomonas,* dominated or increased in microcosms subjected to frequent variations in salinity. For example, the abundance of nosZ4, lower in S3M1, increases in the S3-microcosms after the salt stresses. Promotion of *Pseudomonas* genus in short term fluctuation is in agreement with the impact of long-term salinity fluctuation observed from ribosomal diversity analysis of the same sediment. Members of *Pseudomonas stutzeri* have been isolated world-wide from various habitats including aquatic and terrestrial ecosystems (Sikorski et al., 2002). From culture experiment, it has been shown that many *Pseudomonas* species are capable of rapid growth and often outcompete numerically abundant microbes from the original system, leading them to be categorized as optimal exploiters of nutrient pulses (van Niel, 1955; Gibson, 1957) and are able to rapidly switch from aerobic respiration to denitrification (Lycus et al., 2017). This is also in agreement with the reactive reaction of nosZ mRNA synthesis of community subjected to frequent change.

In marine microcosms subjected to two fluctuations of salinity, the relative stability of activity, the modification of present denitrifiers observed with PCA data and the high relative nosZ transcript level suggested that the induction of transcript resulted from a modification of the structure of the denitrifying community. This is consistent with apparition of nosZ-OTU characteristic of fluctuating environment. The community shift could be an explanation of the higher variances of activity observed for S3M2A and S3M3B microcosms.

Ribosomal diversity was also used to investigate the proportion of nosZI and nosZII-clade harbouring genera. Case studies on the ecology of nitrous oxide reducers have shown the niche partitioning between the two communities, nosZII clade seemed to be more sensitive to environmental changing conditions than nosZI clade (Domeignoz-Horta et al., 2015). Sharp difference have been identified herein in genera that harbour nosZII clade that were more numerous in station with more stable salinity conditions (S3), whereas no difference was observed for nosZI clade. Calcium, or nitrogen concentration and pH, has been shown to affect nosZ clades partitioning (Domeignoz-Horta et al., 2015) and our results extend this finding to salinity fluctuation.

4. Conclusion

If a disturbance occurs, an ecosystem can respond differently: (1) If the disturbance is low, then the system may resist the change. Resistance is the intensity at which a system can be disturbed from its reference state without the change being essentially irreversible (Levin, 2001; Levin and Lubchenco, 2008). (2) If the disturbance is greater and limited in time, the system can show resilience, i.e., once the disturbance has stopped with a certain rate it can return to its initial state in term of functioning. (3) If the disturbance becomes more frequent or more severe, the ecosystem can partially recover (Palumbi et al., 2008) or can undergo profound changes, shifting to an alternative state (regime shift) (Folke et al., 2004; Palumbi et al., 2008)

In this study, we used a microcosm approach to examine the impacts of salinity fluctuations on denitrification using sediments from the Berre lagoon. We showed , in both sediments, the resistance of the functional response allowing the maintenance of the initial level of denitrification and thus the elimination of nitrate in excess whatever their different historical salinity pattern. Our results revealed that nosZ-harbouring community of marine sediment resisted to a single fluctuation, even of large range. In contrast, for two variations of salinity, a shift toward community close to that of sediment subjected to long-term salinity oscillations was observed, presenting large part of *Pseudomonas* denitrifiers. This shift of community was accompanied with high nosZ relative expression level. The number of salinity

changes had little impact on denitrifiers assemblage present in sediments subjected to long-term salinity fluctuations. However, salinity changes increased the relative level of nosZ expression indicating dynamic reaction of this community. From ribosomal information, genera harbouring nosZII clade were more abundant in saline stable condition.

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Supplement material

Salinity shifts in marine sediment: importance of number of fluctuation rather than their intensities on bacterial denitrifying community

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Material and methods

qPCR quantification:
Templates for the standard curve were made by 10-fold serial dilution of recombinant plasmid. DNA standards were created by PCR either from strains or sediment extracted genomic DNA. The corresponding fragment was cloned after purification into pGEMT vector (Promega, WI, USA). After purification, using the Wizard® Plus SV Minipreps Start-Up Kit (Promega), the concentration of plasmids was determined using NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, DE, U.S.A). The target abundance for standards were calculated using the following formula: gene abundance $=6.023 \; 10^{23}$ (copies.mol^{-1}) x standards concentration (g.mL^{-1})/molecular mass (g.mol^{-1}), assuming that double stranded DNA has a molecular mass of 660 Da. Standards for Bacterial and Archaeal ribosomal gene corresponded to pGEMT plasmids (Promega), harboring one copy of Gammaproteobacterial or an MG-II Euryarchaeaotal SSU rRNA gene fragment, respectively. Data were analyzed by comparative CT method. The regression equation was calculated on the graph, plotted between the CT value and the copy number of the standard ranging from 4.5 x 10^6 to 4.5 x 10^2 and from 7.48 x 10^6 to 7.48 x 10^3 copies in the reaction for Archaea and Bacteria, respectively. At the end of the PCR reactions, the specificity of the amplification was checked from the first derivative of their melting curves and also analyzed by an agarose gel electrophoresis.
For nosZ gene quantification, primers nosZ 2F and nosZ 1897R were used. Templates for standard curve were made by 10-fold serial dilution of recombinant plasmid harboring one nosZ copy of Marinobacter sp BC38 fragment (KM816604) in order to reach 10^2 to 10^5 genes/mL.

.Table S1: Technical information about sample storage and DNA and RNA extraction and reverse transcription

Sample	Frozen at -80 °C upon collection	
DNA extraction	DNA Power Soil Total DNA Isolation Kit (Mobio)	
RNA extraction	RNA Power Soil : Total RNA Isolation Kit(Mobio)	
Reverse transcription reaction	kit Invitrogen " SuperScript III First Strand Synthesis System for RT-PCR (in vitrogen) with random hexamer (Promega)	Template RNA(20 ng) random hexamer 2.5 µM, RNAaseOUT RNA inhibitor 2U/µL, DTT 5mM, dNTP 0.5 mM each, SSIV buffer x1, DEPC-treated water qsp 20µl

Table S2: Technical information about qPCR

	bacterial 16S rRNA gene	Archaeal 16S rRNA gene	Nitrous oxide reductase gene
Gene symbol	rrs	rrs	nosZ
Amplicon length	172bp	169 bp	280bp

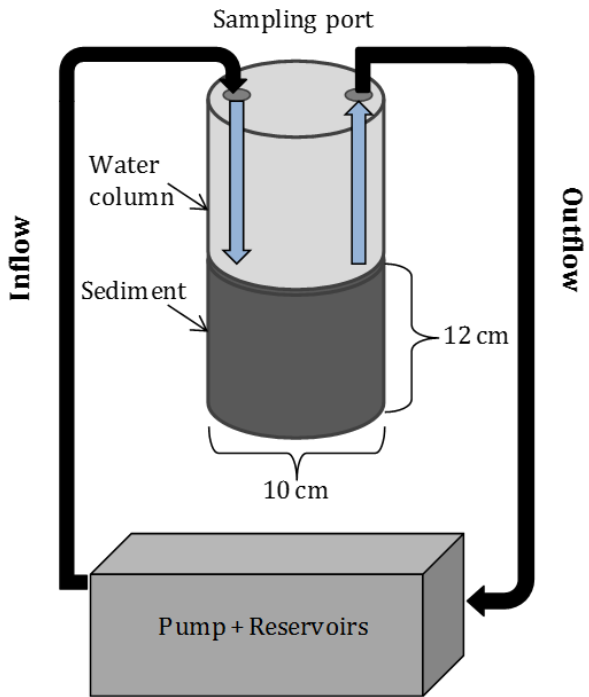|			
Primer sequence	DGGE300F : GCCTACGGGAGGCAGC AG (Michotey et al., 2012) univ516 : GTDTTACCGCGGCKGCT GRCA (Takai and Horikoshi, 2000)	Arc 931F : AGGAATTGGCGGGGG AGCA m1100R : BTGGGTCTCGCTCGTT RCC (Einen et al., 2008)	nosZ 2F : CGCRACG GCAASAA GGTSMSS GT (Henry et al., 2006)) nosZ1897R : CATGTGCAGNGC RTGGCA (Rosch et al., 2002Rosch et al., 2002)
Reaction volume and amount of DNA	20 μl	20 μl	20 μl
Primer concentration	0.25μM	0.25μM	0.25μM
Mg^{2+} concentration	According to SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSoAdvanced SYBR Green supermix (Bio-rad)
dNTP concentration	According to SSoAdvanced SYBR Green supermix(Bio-rad)	According to SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSoAdvanced SYBR Green supermix (Bio-rad)
Polymerase identity and concentration	According to SSo7d-fusion polymerase SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSo7d-fusion polymerase SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSo7d-fusion polymerase SSoAdvanced SYBR Green supermix (Bio-rad)
Additive	SYBR Green	SYBR Green	SYBR Green
Complete thermocycling parameters	Initial denaturation 98 °C for 2 min 40 cycle of : 98 °C for 5 s 55 °C for 10 s 72 °C for 12 s	Initial denaturation 98 °C for 3 min 40 cycle of : 98 °C for 10 s 62 °C for 10 s 72 °C for 20 s	Initial denaturation 95 °C for 5 min 40 cycle of : 98 °C for 15 s 55 °C for 15 s 72 °C for 40 s
Reaction set up	manual	manual	manual
Manufacturer instrument	CFX96 Real Time System (C1000 Thermal Cycler, Bio-Rad Laboratories)	CFX96 Real Time System (C1000 Thermal Cycler, Bio-Rad Laboratories)	CFX96 Real Time System (C1000 Thermal Cycler, Bio-Rad Laboratories)
qPCR validation			
specificity	Melting curve and gel electrophoresis	Melting curve and gel electrophoresis	Melting curve and gel electrophoresis
Cq of the NTC	30.8	35	>40
Calibration curves with slopes and y intercept	Slope=3.307 Y=40.472	Slope=3.254 Y=37.181	Slope=3.558 Y=44.682
PCR efficiency calculated from the slope	100%	102%	91 %
R^2 of calibration curve	0.99	0.99	0.997

Linear dynamic rang	$7.48\ 10^7$-$7.48.10^3$	$4.5\ 10^7$-$4.5.10^2$	$1.36\ 10^7$-$1.36.10^2$
Cq at the LOD	27.76	28	36.89
Data anaysis			
qPCR analysis program	CFX Manager™ Software	CFX Manager™ Software	CFX Manager™ Software
Method of cq determination	Single threshold	Single threshold	Single threshold
Number of technical replicate	2	2	2
Repeatability (intra-assay variation)	Expressed as SD error in figures and table	Expressed as SD error in figures and table	Expressed as SD error in figures and table
Reproducibility; (Inter-assay variation)	4%	4%	3%

Fig. S1: Location of Berre lagoon and sampling stations

Fig. S2: Schematic of a microcosm chamber setup. Each microcosm was connected to a peristaltic pump and to a SSW reservoir

Fig. S3: Variations in denitrification rates (D tot) (μmol.cm^{-2}.h^{-1}) during the 3 incubation phases in microcosms from S1 and S3 sampling sites. Results were as box-plots: the center vertical line marks the median, the edges of the box mark first and third quartiles and the vertical lines indicate the range of values that fall within 1.5 (midrange) of the hinges, the cross marks the mean value. Numbers in bold depict the number of microcosms replicates used for every salinity treatment in each phase. Numbers between brackets represent the number of rate measurements, determined independently twice per day during the steady state of each phase; all these values were used to build one box plot. The salinities maintained all along the incubation phase were notified (i.e.: S=20 For S1, phase 1).

Fig. S4: Relative abundance of major nosZ_OTU in microcosms containing S1 or S3 sediment at the end of the incubation

nosZI

nosZII

Fig. S5 genus nosZ maximum likelihood tree.

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The percentage of trees above c. 50 % in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 88 positions in the final dataset.

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Supplement material

Salinity shifts in marine sediment: importance of number of fluctuation rather than their intensities on bacterial denitrifying community

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Material and methods

qPCR quantification:
Templates for the standard curve were made by 10-fold serial dilution of recombinant plasmid. DNA standards were created by PCR either from strains or sediment extracted genomic DNA. The corresponding fragment was cloned after purification into pGEMT vector (Promega, WI, USA). After purification, using the Wizard® Plus SV Minipreps Start-Up Kit (Promega), the concentration of plasmids was determined using NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, DE, U.S.A). The target abundance for standards were calculated using the following formula: gene abundance $= 6.023 \ 10^{23}$ (copies.mol^{-1}) x standards concentration (g.mL^{-1})/molecular mass (g.mol^{-1}), assuming that double stranded DNA has a molecular mass of 660 Da. Standards for Bacterial and Archaeal ribosomal gene corresponded to pGEMT plasmids (Promega), harboring one copy of Gammaproteobacterial or an MG-II Euryarchaeaotal SSU rRNA gene fragment, respectively. Data were analyzed by comparative CT method. The regression equation was calculated on the graph, plotted between the CT value and the copy number of the standard ranging from 4.5 x 10^6 to 4.5 x 10^2 and from 7.48 x 10^6 to 7.48 x 10^3 copies in the reaction for Archaea and Bacteria, respectively. At the end of the PCR reactions, the specificity of the amplification was checked from the first derivative of their melting curves and also analyzed by an agarose gel electrophoresis.
For nosZ gene quantification, primers nosZ 2F and nosZ 1897R were used. Templates for standard curve were made by 10-fold serial dilution of recombinant plasmid harboring one nosZ copy of Marinobacter sp BC38 fragment (KM816604) in order to reach 10^2 to 10^5 genes/mL.
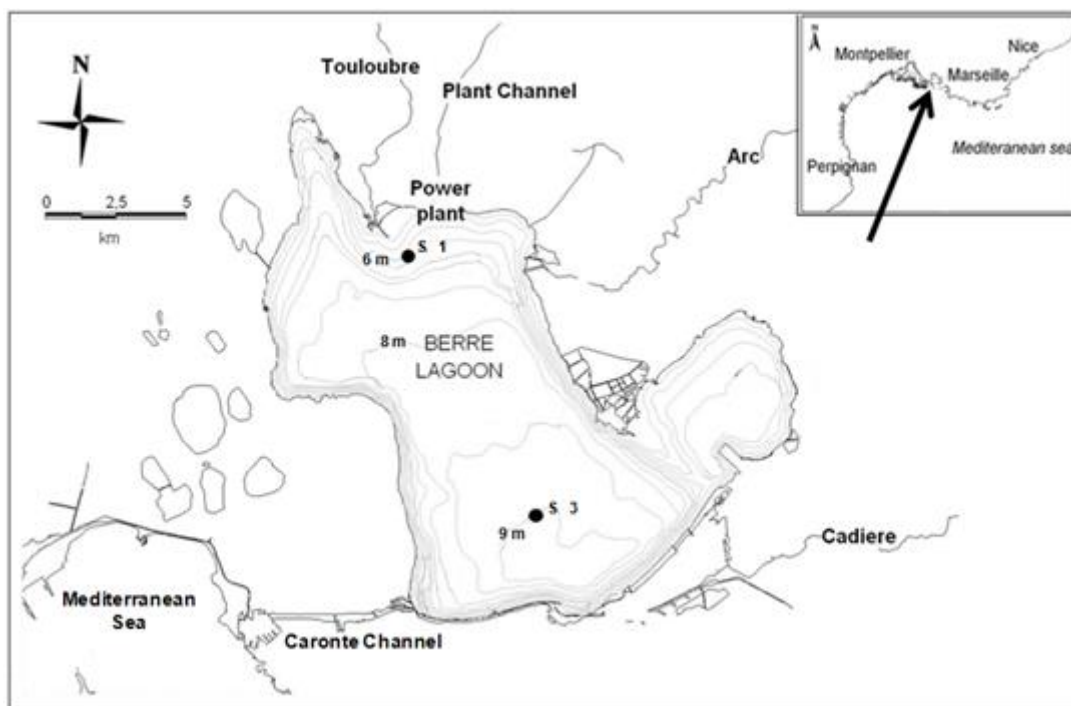
.Table S1: Technical information about sample storage and DNA and RNA extraction and reverse transcription

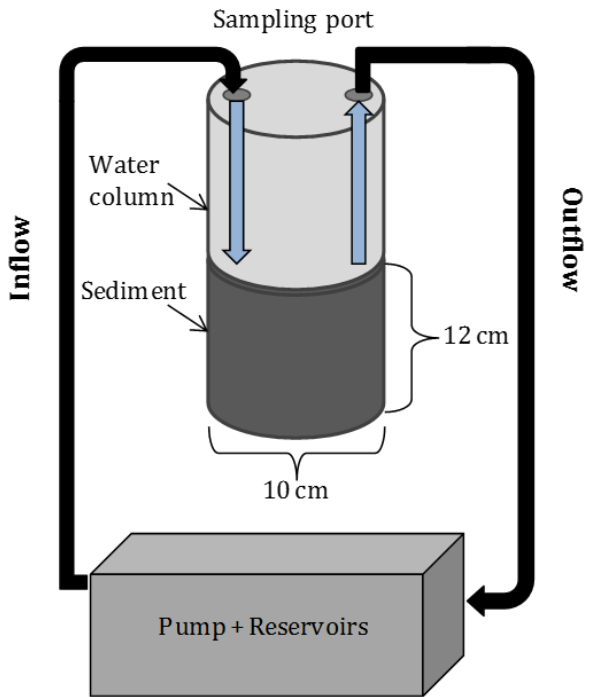| Sample	Frozen at -80 °C upon collection	
DNA extraction	DNA Power Soil Total DNA Isolation Kit (Mobio)	
RNA extraction	RNA Power Soil : Total RNA Isolation Kit(Mobio)	
Reverse transcription reaction	kit Invitrogen " SuperScript III First Strand Synthesis System for RT-PCR (in vitrogen) with random hexamer (Promega)	Template RNA(20 ng) random hexamer 2.5 µM, RNAaseOUT RNA inhibitor 2U/µL, DTT 5mM, dNTP 0.5 mM each, SSIV buffer x1, DEPC-treated water qsp 20µl

Table S2: Technical information about qPCR

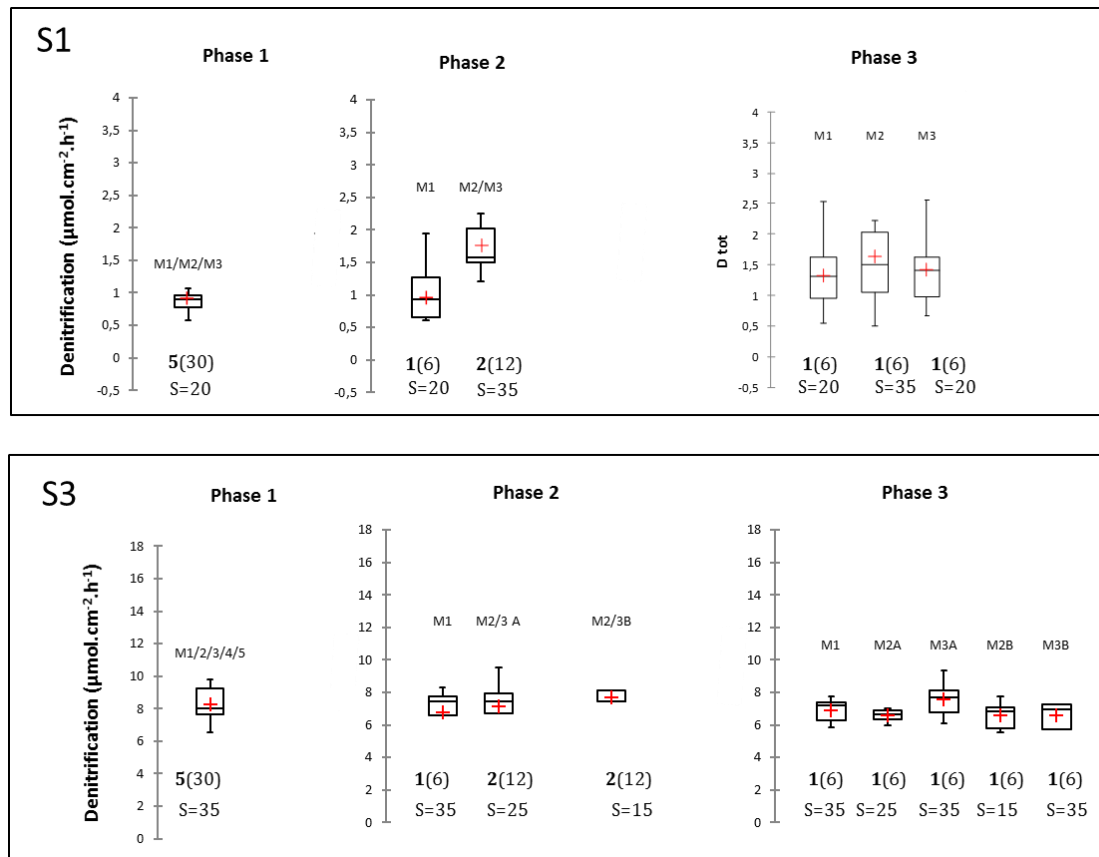|	bacterial 16S rRNA gene	Archaeal 16S rRNA gene	Nitrous oxide reductase gene
Gene symbol	rrs	rrs	nosZ
Amplicon length	172bp	169 bp	280bp
Primer sequence	DGGE300F : GCCTACGGGAGGCAGCAG (Michotey et al., 2012) univ516 : GTDTTACCGCGGCKGCTGRCA (Takai and Horikoshi, 2000)	Arc 931F : AGGAATTGGCGGGGGAGCA m1100R : BTGGGTCTCGCTCGTTRCC (Einen et al., 2008)	nosZ 2F : CGCRACGGCAASAAGGTSMSSGT (Henry et al., 2006)) nosZ1897R : CATGTGCAGNGCRTGGCA (Rosch et al., 2002)
Reaction volume and amount of DNA	20 µl	20 µl	20 µl
Primer concentration	0.25µM	0.25µM	0.25µM
Mg^{2+} concentration	According to SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSoAdvanced SYBR Green supermix (Bio-rad)
dNTP concentration	According to SSoAdvanced SYBR Green supermix(Bio-rad)	According to SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSoAdvanced SYBR Green supermix (Bio-rad)
Polymerase identity and concentration	According to SSo7d-fusion polymerase SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSo7d-fusion polymerase SSoAdvanced SYBR Green supermix (Bio-rad)	According to SSo7d-fusion polymerase SSoAdvanced SYBR Green supermix (Bio-rad)
Additive	SYBR Green	SYBR Green	SYBR Green
Complete thermocycling parameters	Initial denaturation 98 °C for 2 min 40 cycle of : 98 °C for 5 s 55 °C for 10 s 72 °C for 12 s	Initial denaturation 98 °C for 3 min 40 cycle of : 98 °C for 10 s 62 °C for 10 s 72 °C for 20 s	Initial denaturation 95 °C for 5 min 40 cycle of : 98 °C for 15 s 55 °C for 15 s 72 °C for 40 s
Reaction set up	manual	manual	manual
Manufacturer instrument	CFX96 Real Time System (C1000 Thermal Cycler, Bio-Rad Laboratories)	CFX96 Real Time System (C1000 Thermal Cycler, Bio-Rad Laboratories)	CFX96 Real Time System (C1000 Thermal Cycler, Bio-Rad Laboratories)
qPCR validation			
specificity	Melting curve and gel electrophoresis	Melting curve and gel electrophoresis	Melting curve and gel electrophoresis
Cq of the NTC	30.8	35	>40
Calibration curves with slopes and y intercept	Slope=3.307 Y=40.472	Slope=3.254 Y=37.181	Slope=3.558 Y=44.682
PCR efficiency calculated from the slope	100%	102%	91 %

R2 of calibration curve	0.99	0.99	0.997
Linear dynamic rang	$7.48 \cdot 10^7 - 7.48 \cdot 10^3$	$4.5 \cdot 10^7 - 4.5 \cdot 10^2$	$1.36 \cdot 10^7 - 1.36 \cdot 10^2$
Cq at the LOD	27.76	28	36.89
Data anaysis			
qPCR analysis program	CFX Manager™ Software	CFX Manager™ Software	CFX Manager™ Software
Method of cq determination	Single threshold	Single threshold	Single threshold
Number of technical replicate	2	2	2
Repeatability (intra-assay variation)	Expressed as SD error in figures and table	Expressed as SD error in figures and table	Expressed as SD error in figures and table
Reproducibility; (Inter-assay variation)	4%	4%	3%

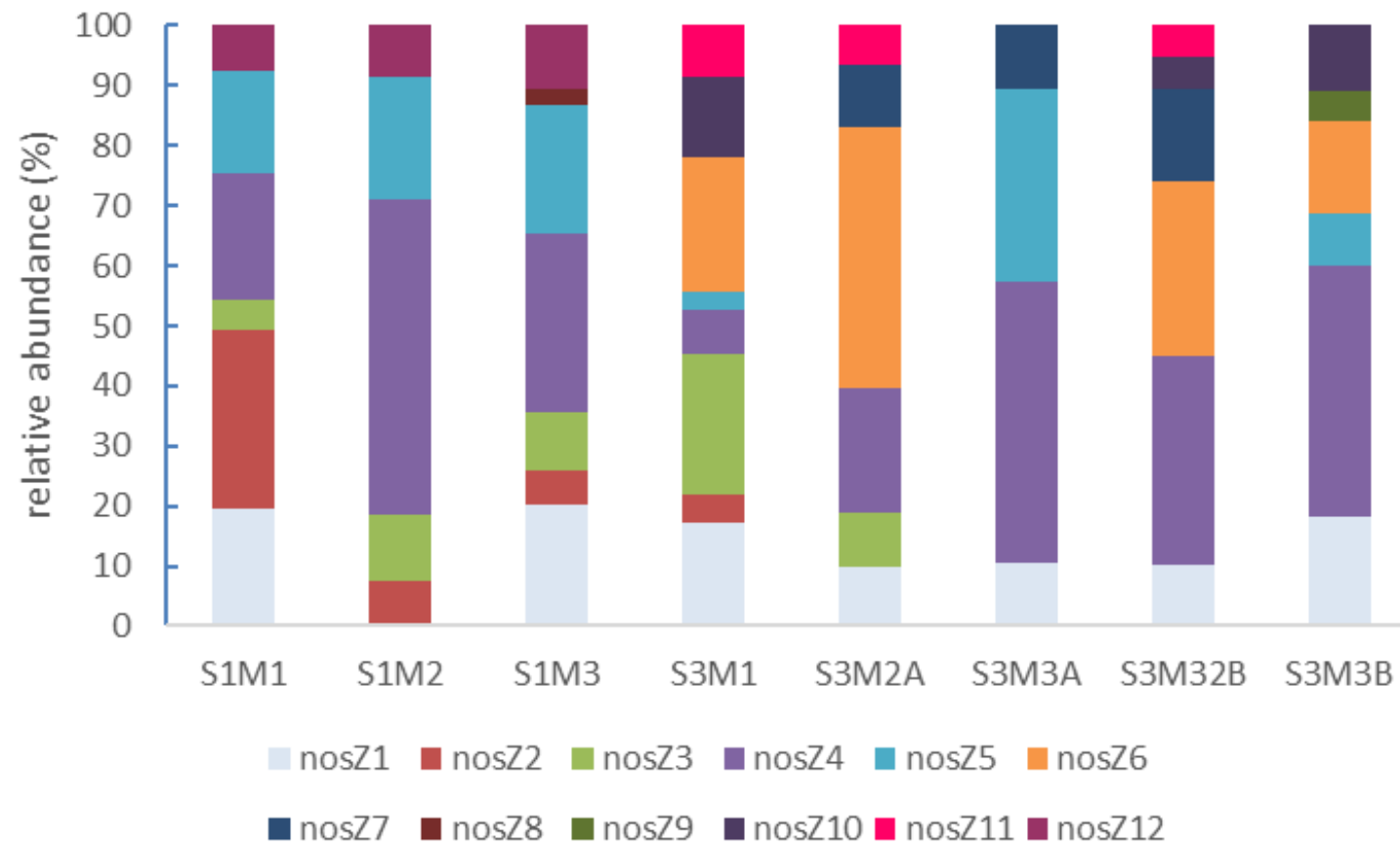**Fig. S1:** Location of Berre lagoon and sampling stations

Fig. S2: Schematic of a microcosm chamber setup. Each microcosm was connected to a peristaltic pump and to a SSW reservoir
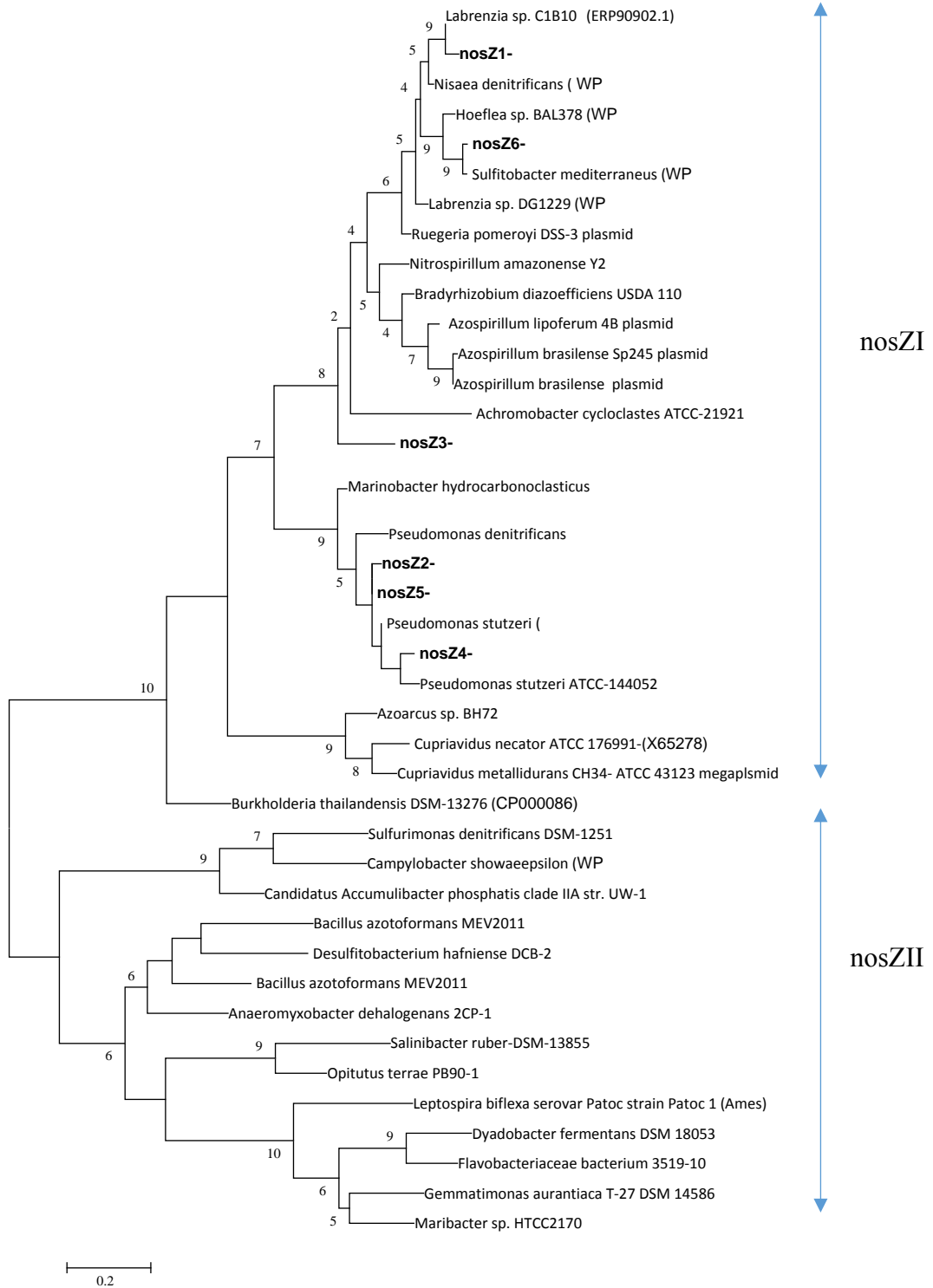
Fig. S3: Variations in denitrification rates (D tot) (μmol.cm^{-2}.h^{-1}) during the 3 incubation phases in microcosms from S1 and S3 sampling sites. Results were as box-plots: the center vertical line marks the median, the edges of the box mark first and third quartiles and the vertical lines indicate the range of values that fall within 1.5 (midrange) of the hinges, the cross marks the mean value. Numbers in bold depict the number of microcosms replicates used for every salinity treatment in each phase. Numbers between brackets represent the number of rate measurements, determined independently twice per day during the steady state of each phase; all these values were used to build one box plot. The salinities maintained all along the incubation phase were notified (i.e.: S=20 For S1, phase 1).

Fig. S4: Relative abundance of major nosZ_OTU in microcosms containing S1 or S3 sediment at the end of the incubation

Fig.S5: nosZ maximum likelihood tree
The percentage of trees above 50% in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 88 positions in the final dataset.

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