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Revealing the dissimilar structure of microbial communities in different WWTPs that treat fish-canning wastewater with different NaCl content

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ABSTRACT

Studies that characterize the microbial communities in wastewater treatment plants (WWTPs) are numerous, yet similar studies in industrial WWTPs treating fish-canning effluents are limited. The microbial communities in samples of 4 fish-canning WWTPs that operated under different NaCl concentrations were investigated by qPCR and partial 16S rRNA gene Illumina sequencing. The absolute abundances of key microbial populations (Total *Bacteria*, *Archaea* and *Fungi*, ammonium oxidizing bacteria (AOB), Mycolata, *Candidatus* Microthrix, *Ca.* Accumulibacter and *Ca.* Competibacter) presented statistical differences among the WWTPs. The NaCl concentration negatively affected the absolute abundance of *Bacteria* and *Fungi*, filamentous, and phosphate (PAO) and glycogen (GAO) accumulating bacteria, while AOB and *Ca.* Microthrix populations were statistically higher in the WWTP with higher NaCl contents. On the other hand, the main bacterial operational taxonomic units (OTUs) were classified as members of *Kouleothrix* (*Chloroflexia*, *Chloroflexi*) and *Tetrasphaera* (*Actinomycetia*, *Actinobacteria*), family *Beijerinckiaceae* (*Alphaproteobacteria*, *Proteobacteria*), order *Betaproteobacteriales* (*Gammaproteobacteria*, *Proteobacteria*), *Sphingobacteriales* (*Sphingobacteriia*, *Bacteroidetes*) and *Frankiales* (*Actinobacteria*, *Actinobacteria*), class *Anaerolineae* (*Chloroflexi*), phylum *Chloroflexi* and Bacteria_unclassified. The structure of the bacterial community was highly dissimilar among the 4 WWTPs, as the identities of the dominant OTUs differed significantly among them. Therefore, the individual characteristics of the different WWTPs, mainly NaCl concentration, were responsible for the narrow assemblage of the bacterial communities. Different OTUs belonging to the phyla *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes* and *Proteobacteria* were revealed as salt-tolerant. Taking into account these results, NaCl content was an important driver of the abundance of microbial populations and the bacterial community structure in the analysed industrial facilities.

1. Introduction

In the last 30 years, the total *per capita* consumption of fish and seafood has increased globally by 50% [1]. This is due to the consumer's perception that fish is a healthy alternative to other protein sources [2]. To meet this demand, processed fish production has been increased with a rapid growth of fish-preserving and fish-canning industries across the world [3,4].

Biological wastewater (WW) treatment depends mainly on the structure and function of the microbial communities that carry out the transformation of contaminants and removal of nutrients [5]. Effective WW treatment is necessary to avoid environmental damage after the discharge of effluents into natural water streams. The abundance and diversity of the activated sludge (AS) bacterial biomass determines the metabolic pathways that occur in the WW treatment plant (WWTP) that finally dictate the quality of the treated effluent. Different operational and environmental parameters influence the abundance of key microbial populations and regulate the assembly of the complex bacterial communities of the AS [6,7].

Fish canning industries consume large quantities of water that are utilized in different stages of fish processing, generating high amounts of WW [8,9]. In general, treatment of industrial WW is often much more difficult than that of urban wastewater [10]. Particularly, fish-canning WWs are characterized by a high salt content and high loads of

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organic matter, nitrogen, phosphorus and fat [8]. High salt concentration in WWTPs results in osmotic stress, causing disintegration of cells by reducing cell turgor and dehydrating cytoplasm [11]. Consequently, a NaCl increase can negatively impact on the treatment processes of these WW, as biological activity can decrease if salinity raises. In addition, high chemical oxygen demand (COD) and particulate matter concentration usually hinders fish-canning WW treatment. Due to these inconveniences, these effluents can cause drastic effects on the environment if not properly managed and disposed of. To cope with these problems, different types of WW treatment systems have been tested [12,13]; however, a large knowledge gap still exists. It is unclear whether microbial populations abundance and community assemblages are affected by the salinity and strength of fish canning WW. Furthermore, the understanding of the relationships between the microbial communities and the operational/environmental parameters of full-scale industrial WWTPs is limited. Therefore, answers to these questions represent a challenge in the field of environmental engineering [6,14].

This study investigated microbial communities' structure in the AS of 4 different WWTPs that receive influents from fish-canning industries differing in their NaCl content, in order to address the following questions: (1) Were there significant differences in the abundances of key microbial populations and the bacterial community structures among facilities?, (2) Is there a common bacterial core among the different WWTPs?, (3) What were the relationships between microbial populations' abundances and operational variables?, (4) Was the NaCl content an important driver of the shaping of bacterial communities' structure? and (5) Which were the halophilic/halophobic bacteria of the AS? Therefore, the information collected in this study will contribute to the characterization and understanding of the microbial community structure and ecology in fish-canning WWTPs. In addition, due to the limited scientific literature addressing the effect of NaCl on the bacterial diversity in this particular type of industrial facilities, the assignation of the potential roles of the dominant OTUs found in the current work will help to understand the biological treatment of other highly saline effluents.

2. Material and methods

2.1. Sampling

WW and AS from four different fish-canning WWTPs (F1, F2, F3 and F4) in Galicia (Northwest of Spain) were analysed. Full details were previously described by Correa-Galeote et al. [15]. Briefly, F1 and F2 were conventional activated sludge (CAS) systems treating WW with low and moderate salinity, respectively. F3 and F4 were systems composed by an anaerobic digestor for organic matter removal and valorisation, and a nutrient removal reactor (NRR) with intermittent aeration, aimed to enhance the elimination of nitrogen. F3 treated WW of moderate salinity and was composed by an anaerobic reactor (UASB), plus an anoxic/aerobic bioreactor comprising 2 intermittently-aerated and 1 fully-aerated compartments (the NRRs). F4 treated high salinity WW, most of which was pumped directly into an anaerobic reactor for organic matter valorization into biogas. The remaining WW was bypassed to a NRR, where it was mixed with the anaerobic digestor effluent. This last unit (the NRR) was a nitrifying/denitrifying sequencing batch reactor (SBR) operated in cycles (feeding, aeration, settling and withdrawal), where the organic matter present in the WW supported the denitrification of the oxidized ammonia into gas nitrogen. The AS samples were taken from the biological reactors in F1 and F2, from the fully-aerated compartment in F3, and from the SBR in F4 during aeration.

2.2. Analytical methods

pH was measured with a glass electrode (Crisson GLP22). The APHA Standard Methods [16] were followed to measure the Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) in bulk samples, and to characterize the dissolved matter (Total Organic Carbon (TOC), Volatile Fatty Acids (VFA), ammonium, Na^+ and Cl^- ions) after filtering the bulk liquid through a 0.45 μm-pore size cellulose-ester filter (Advantec, Japan). TOC was measured by catalytic combustion in a TOC-L CNS analyzer with the TNM-1 module (Shimadzu, Japan). VFAs were determined in a gas chromatograph (Hewlett Packard 5890A, USA) equipped with a flame ionization detector (FID) and an automatic injector (Hewlett Packard 7673A, USA). Ammonium was quantified by the Bower/Holm Hansen method, and ions (Na^+ and Cl^-) by ion chromatography (861 Advanced Compact IC, Metrohm, Switzerland).

2.3. DNA extraction and purification

Biomass from AS was sampled as described by Correa-Galeote et al. [15]. Two independent biological replicates were used from each fish-canning WWTP. DNA was isolated using the FastDNA-2 mL SPIN Kit for Soil and the FastPrep24 apparatus (MP-BIO, CA, USA) according to the manufacturer's instructions. The quality and concentration of DNAs were determined by electrophoresis on 1% agarose and fluorometry using an Invitrogen Qubit 4 Fluorometer (Waltham, Massachusetts, USA). DNAs were stored at −20 °C until further use.

2.4. Quantitative polymerase chain reaction

Quantification of total *Bacteria*, *Archaea* and *Fungi* and functional marker genes was performed by quantitative PCR (qPCR) on a QuantStudio-3 Real-Time PCR system (Applied Biosystems). Gene quantification (2 independent experiments, 3 replicates per DNA sample) targeting the bacterial and archaeal 16S rRNA and fungal 18S rRNA genes were used as proxy for total *Bacteria*, *Archaea* and *Fungi*. Ammonia monooxygenase (*amoA*) genes were amplified to estimate population abundances of ammonia-oxidizing bacteria (AOB) and archaea (AOA). The *amoA* genes were used as proxy to determine nitrogen removal populations, as the oxidation of ammonia to nitrite has been proposed as the limiting step of biological nitrogen removal [17]. Quantification of 16S rRNA of the Mycolata group and genus *Candidatus* Microthrix (hereafter referred to as Microthrix) was used to assess the abundance of filamentous bacteria, and 16S rRNA of *Candidatus* Accumulibacter and *Candidatus* Competibacter (hereunder referred to as *Accumulibacter* and Competibacter) were used as proxy for polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs), respectively. Reactions were performed in 25 μL containing 2.5 μL of 10× DreamTaq buffer (Thermo Scientific, MA, USA), 0.5 μL of True Pure dNTPs (8 mM) (Canvax, Córdoba, Spain), 0.15 μL of each primer (10 μM), 0.125 μL of 5 U/μL DreamTaq Hot Start DNA Polymerase (Thermo Scientific, MA USA), 0.125 μL of 20× SYBR Green I (Thermo Scientific, MA, USA), 0.0625 μL of 20 mg/mL of bovine serum albumin solution (New England Biolabs, MA, USA), 19.38 μL of ultrapure water, and 2 μL of template DNA. Primers sequences and PCR conditions are provided in Table S1 in the Supplementary Material. As standards for the quantification of the respective target genes, dilution series of cloned linear fragments developed previously were used [18–20]. Amplicons of the 16S rRNA gene of Accumulibacter and Competibacter were generated from a mix of environmental DNA used in this work. The PCR products were cloned with the aid of the TOPO® TA cloning system (Invitrogen, CA, USA). Clones carrying plasmids showing 98% and 97% identity to the sequences of the 16S rRNA of Accumulibacter (GeneBank accession CP001715.1) and Competibacter (GeneBank accession AY962319.1) were used as standards.

2.5. Bacterial 16S rRNA gene amplicon sequencing

Amplification (two independent replicates per WWTP) of the V3-V4 region of the 16S rRNA gene was performed using the prokaryotic specific-primers Pro341F (5′-CCTACGGGNBGCASCAG-3′) and Pro805R

(5′-GACTACNVGGGTATCTAATCC-3′) modified by Takahashi et al. [21]. Raw data from Illumina MiSeq sequencing were processed using the MothurMiSeq guidelines in the software Mothur v1.44.1 [22]. The different first and reverse fastq read files were combined to generate the individual files. Quality control, primer trimming, and filtering were made according to the default settings. Improved sequences were merged into unique sequences that were aligned against the SILVA SEED v132 database. Aligned sequences were subjected to a de-noise analysis (three-difference mismatches) and putative chimeric sequences were detected and removed using the VSEARCH tool. Pairwise distances between aligned DNA sequences were conducted for the remaining unique sequences and operational taxonomic units (OTUs) were defined according to their phylogeny distance (97% similarity). OTUs with an abundance of only 1 sequence read in the whole set were considered PCR artifacts and were removed for diversity analysis. The consensus sequence of the different OTUs was obtained through the get.oturep command. Finally, each consensus sequence was taxonomically classified using the SILVA SEED v132 database that follows the whole-genome taxonomy recently proposed by Parks et al. [23]. Rarefaction curves for the OTUs abundance data were constructed using the Analytic Rarefaction software (University of Georgia, Athens, USA; https://strata.uga.edu/software). Simpson, Shannon and Sørensen biodiversity indices were calculated according to Hill et al. [24] and Chao et al. [25]. The nucleotide sequences have been deposited in GeneBank under the accession numbers MW082835-MW084339.

2.6. Multivariate statics analysis

The statistical differences of the different abiotic and biotic data sets among the samples were analysed using non-parametric tests ($p < 0.05$ significance level) in XLSTAT v2020 (Addinsoft, New York, USA). The PC-ORD software [26] was used for the multivariate statistical analyses. To determine the differences in the biotic variables, abundances of bacterial groups measured by PCR or relative abundance (RA) of the different OTUs were relativized by the maximum before the construction of non-parametric multidimensional scaling (NMS) analyses, according to the corresponding Sørensen dissimilarity matrices. The abiotic data sets (logarithmic transformed) were coupled into the NMS in order to obtain the correlations among the ordination of samples and the physicochemical parameters of the different WWTPs.

3. Results and discussion

3.1. Samples' characterization

WW and AS characterizations are shown in Table 1. In general, there was a high variability of the composition of the effluents from the 4 fish-canning factories. In this sense, the NaCl concentrations of the WWs were low in F1 and F3 (NaCl influent concentrations 0.78 and 0.83 g/L, respectively), moderate in F2 (NaCl concentration 1.66 g/L) and high in F4 (NaCl concentration 14.90 g/L). The TOC concentrations were moderate in F1, F2 and F3 (TOC influent concentrations 0.49, 0.26, and 0.36 g/L, respectively); however, F4 bore the highest organic load (TOC 1.97 g/L). Similarly, the N pollution was moderated in F1 and F4 (NH_4^+ influent concentrations ranging 13.36–37.36 mg N-NH_4^+/L) and higher for F2 and F3 (NH_4^+ influent concentrations ranging 150–175 mg N-NH_4^+/L). Despite these differences in the salt concentration, organic and N loads, the removal efficiencies fulfilled the EU discharge standards [27] in the four facilities.

3.2. Quantification of bacterial, archaeal and fungal populations

Quantitative qPCR was used to assess the abundance of *Bacteria*, *Archaea* and *Fungi*, and the results were summarized in Fig. 1. According to the Kruskal-Wallis and Conover-Iman tests, there were significant differences among the 4 WWTPs for all genes quantified.

The total bacterial populations were in the range of 6.59×10^{13}–4.81×10^{12} gene copies/L AS. The highest copy numbers were found in the F1 facility, intermediate values were measured in F2 and F3 (with no statistical differences between both facilities) and the lowest values in F4 (the facility with the highest NaCl concentration). The abundance of total bacterial populations is a general marker of the WW treatment process, in which a wide range of contaminant organic compounds are mineralized by bacteria to water and carbon dioxide [28]. Despite the statistical differences among WWTPs, the bacterial 16S rRNA gene copy numbers were up to three orders of magnitude higher than those previously described by Layton et al. [29] and Shu et al. [30] in an industrial WWTP treating different chemically-enriched WWs and a municipal WWTP, respectively. However, similar values were found by other authors in different sewage sludges [31,32]. Recently, Abzazou et al. [33] proposed that higher abundances of bacteria in the AS of WWTP results in better nutrient removal efficiencies.

Regardless of the sample, the absolute abundances of *Archaea* were lower than those of *Bacteria*, and shifted over 4 orders of magnitude (from 2.22×10^9 in F3 to 7.26×10^4 gene copies/L AS in F2). The main role of archaeal populations in WWTPs is to perform the final steps of the mineralization of the organic matter [34]. Abzazou et al. [33] found higher abundances of total *Archaea* than those here described when they analysed the structure of microbial communities in municipal WWTPs.

The abundance of total *Fungi* was lower compared to the two prokaryotic domains and was higher in the F1 samples ($4.36 \times 10^7 \pm 1.77 \times 10^7$ gene copies/L AS), with no differences among the other 3 facilities ($2.05 \times 10^6 \pm 1.75 \times 10^5$ gene copies/L AS). Fungal populations are involved in several processes in WWTPs, such as organic matter biodegradation, the establishment of sludge flocs, and detoxification [35]. The abundance of fungal markers fell within the same range previously reported by Maza-Márquez et al. [36] in different municipal WWTPs, but with a lower 18S rRNA/Bacterial 16S rRNA ratio than that described by Wei et al. [37]. However, it must be also taken into consideration that the quantification of Fungi may differ depending on the selected gene markers and primers [36].

3.3. Quantification of key groups involved in WW treatment

The abundances of bacterial and archaeal ammonia oxidizers, Mycolata and Microthrix, Accumulibacter and Competibacter, were also

Table 1
Main parameters analysed in the wastewater (WW) and the activated sludge (AS) samples (n = 2) retrieved from fish-canning WWTP.

		NaCl (g/L)	VSS (g/L)	TOC (g/L)	VFA (mg COD/L)	NH_4^+ (mg N-NH_4^+/L)	pH
F1	WW	0.781 ± 0.006	1.08 ± 0.01	0.487 ± 0.003	N.D.	13.36 ± 0.34	6.38 ± 0.01
	AS	0.474 ± 0.007	3.69 ± 0.09				7.28 ± 0.01
F2	WW	1.658 ± 0.011	1.25 ± 0.05	0.265 ± 0.005	N.D.	150 ± 6.78	7.39 ± 0.01
	AS	1.717 ± 0.009	1.98 ± 0.19				7.00 ± 0.01
F3	WW	0.834 ± 0.014	1.09 ± 0.01	0.363 ± 0.008	761 ± 0.01	175 ± 5.14	6.73 ± 0.01
	AS	1.361 ± 0.009	3.76 ± 0.16				7.07 ± 0.01
F4	WW	14.902 ± 0.012	1.38 ± 0.01	1.968 ± 0.007	N.D.	37.36 ± 10.03	9.11 ± 0.01
	AS	12.757 ± 0.009	2.53 ± 0.15				7.64 ± 0.01

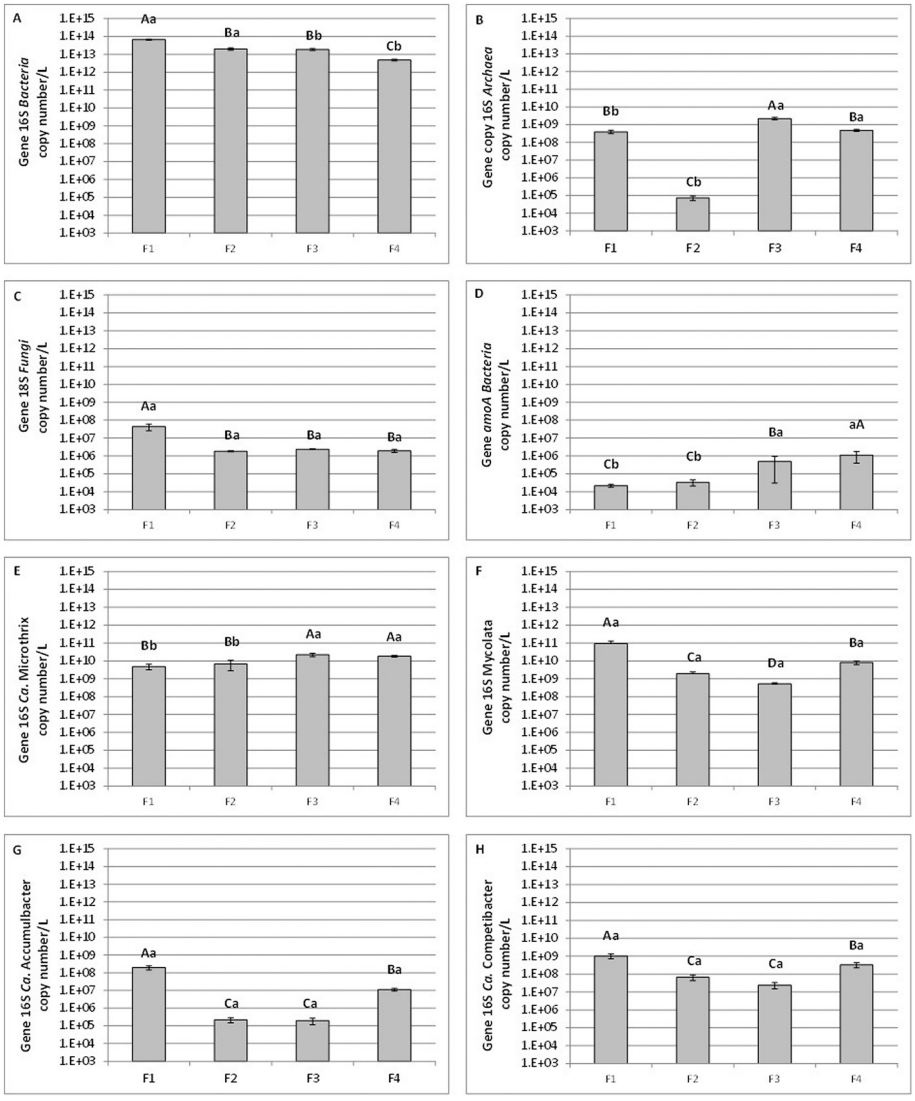

Fig. 1. Gene copy numbers (No. of copies/L activated sludge) for the bacterial (A) and archaeal (B) 16S rRNA gene, fungal 18S rRNA (C), bacterial *amoA* (D), 16S rRNA of genus *Candidatus* Microthrix (E), 16S rRNA of Mycolata group (F), 16S rRNA of *Candidatus* Accumulibacter (G) and 16S rRNA of *Candidatus* Competibacter (H) determined by quantitative PCR in activated sludge samples (n = 2) retrieved from fish-canning industrial WWTP. For a given population, capital letters indicate significant differences among the four WWTPs, according to the Kruskal-Wallis and Conover-Iman tests ($p < 0.05$), and lower-case letters indicate significant differences between the two WWTP configurations (F1 and F2: CAS systems and F3 and F4: NRR).

determined by qPCR (Fig. 1). The abundance of bacterial *amoA* genes greatly varied among samples of the different facilities, being lower in F1 ($2.21 \times 10^4 \pm 3.96 \times 10^3$ gene copies/L AS) and F2 ($3.29 \times 10^4 \pm 1.23 \times 10^4$ gene copies/L AS), intermediate in F3 ($4.92 \times 10^5 \pm 4.61\ 10^5$ gene copies/L AS), and higher in F4 ($1.10 \times 10^6 \pm 7.00 \times 10^5$ gene copies/L AS). The *amoA*-bearing bacteria are exploited to reduce the ammonia concentration of the effluents in WWTPs, in which they are primarily responsible for the initial step of nitrification converting ammonia to nitrite [29]. The AOB population abundances were reduced in these fish-canning WWTPs, compared to those found in other municipal WWTPs [38,39]. On the other hand, despite several attempts to amplify the AOA counterpart populations, they were more often below the detection limit, indicating that they would be hardly involved in nitrification in the analysed WWTPs. The lack of detection of AOA was in agreement with several previous works that have described a low AOA/AOB ratio in different WWTPs [39,40]. Recently, Gwak et al. [41] have proposed that the high level of complex mixtures of organic compounds that typically occurs in WW specifically inhibits the growth of AOA without affecting AOB. Subsequently, data of ammonia oxidizing *Archaea* were removed from analysis henceforth.

Microthrix (*Actinobacteria*) populations were less abundant in F1 and F2 samples (4.80×10^9 and 6.76×10^9, respectively) compared to those from F3 and F4 (2.21×10^{10} and 1.82×10^{10}, respectively). On the other hand, the 16S rRNA copy numbers of Mycolata, a group of filamentous *Actinobacteria* that contain mycolic acids in their cell wall (particularly *Gordonia*, *Millisia, Mycobacterium, Nocardia, Skermania* and *Tsukamurella* [42,43]), oscillated from 5.10×10^8 to 9.05×10^{10} gene copies/L AS, measured in the F3 and F1 facilities, respectively. Filamentous bacteria are essential for the treatment of WW, as they provide the structural inner backbone necessary to form flocs or granules in AS [44]. However, an excessive proliferation of filamentous bacterial groups is often related to severe bulking and/or foaming [45]. It is noteworthy, that, at the sampling time, the F1 WWTP experimented an isolated episode of bulking. Taking into account that the Microthrix/Mycolata ratio was <1 in the F1 facility, the dominance of Microthrix over other filamentous bacteria could be related to the observed bulking phenomenon. Nonetheless, the gene copy numbers of both Microthrix and Mycolata measured here were within the ranges previously found in different WWTPs [19,20,46,47].

The abundance of Accumulibacter was higher in F1 (1.97×10^8 copy numbers/L of AS), followed by F4 (1.11×10^7 copies/L AS) and finally F2 and F3, with no statistical differences among them (1.94×10^5 and 2.18×10^5 copies/L AS, respectively). A similar trend was observed for Competibacter (1.02×10^9, 3.26×10^8, 6.54×10^7, and 6.54×10^7 copies/L AS, F1, F4, F2 and F3, respectively). Accumulibacter is one of the main microorganisms capable of polyphosphate accumulation to remove phosphorus (P) from wastewater, while GAOs are often viewed as PAO competitors as they consume carbon substrate without P

removal [48,49]. Although both candidate genera are generally associated to enhanced biological P removal (EBPR) systems [50], they were found worldwide in WWTPs that do not operate under EBPR conditions [51]. Interestingly, PAO and GAO populations followed the same tendencies in the 4 WWTPs, suggesting that they were modulated by the same factors. Compared to other works, the abundances of both populations were lower than previously reported in EBPR systems [52,53]; but similar to those observed in a SBR not configured for P removal [54]. The domination of GAO populations over PAO suggests that P removal due to Accumulibacter activity in these AS was minimal.

There were no statistical differences for the absolute abundances of total *Fungi*, Mycolata, PAO and GAO between the CAS and NRR systems according to the Mann-Whitney test (Fig. 1), suggesting that the type of configuration of the fish-canning facilities did not have an strong effect over these populations. The copy numbers of total *Bacteria* were higher in the CAS systems, while total *Archaea*, AOB and Microthrix populations were higher in the NRR systems, specifically designed for nitrogen removal (nitrification and denitrification). Besides, there is to note that the highest abundance of *Archaea* in the NRR compared to the CAS systems could be linked to the use of an anaerobic digester previous to the NRR, in which different archaeal groups perform key processes [55]. Therefore, the type of configuration was a factor that only modulated the abundance of some microbial groups. In agreement with that, Baek et al. [56] found slight divergences in the gene copy numbers of total *Bacteria* and of some functional populations in different industrial WWTPs.

Finally, to take into consideration the differences in VSS concentration among the different facilities, the absolute abundances of the targeted bacterial populations were also expressed as the number of gene copies/g VSS (Table S2). Generally considered, the values followed the same trends for the different populations and facilities than when the total abundances were standardized per liter of AS.

3.4. Bacterial community structure

The total number of high-quality sequences was 91,817 and the average number per library was 11,477 ± 1891 sequences. The number of sequences of each OTU and their classifications are shown in Table S3. The rarefaction curves based on the OTUs abundances (Fig. S1) forthcame to the saturation stage, indicating that the sequencing effort was adequate to obtain a good representation of the global bacterial community structure in the AS of the 4 WWTPs. The indices of alpha diversity are shown in Table S4. Overall, 1505 different OTUs were found; and the average number of OTUs per amplicon library was 367 ± 27. There were no statistical differences in the numbers of OTUs among the samples, although there were differences in both biodiversity indices. The highest values of the Shannon index and, reciprocally, the lowest of the Simpson index, were observed for the F2 samples suggesting that the bacterial community in this facility was more diverse and was distributed more evenly than those of the other WWTPs. According to Marzorati et al. [57], communities displaying high diversity and evenness have a low functional organization and respond better to changing environmental conditions maintaining their functionality. Neither the type of treatment nor the NaCl gradient produced significant changes in the values of the biodiversity indices, which fell within the same ranges previously reported in urban [58] and industrial (food processing) WWTPs [59].

Out of 1505 OTUs, a total of 1297 OTUs (69,211 sequences) belonged to 24 different bacterial phyla. Two hundred and six OTUs (25,559 sequences) which remained unclassified at the phylum level and 2 OTUs (20 sequences) which were classified as *Archaea*, were removed from subsequent analyses. Fig. 2 shows the differences in the bacterial community profiles at the phylum level among samples (complete data set available in Table S4). Overall, the bacterial community was mainly composed of *Proteobacteria* (average 28.11 ± 5.31%), *Chloroflexi* (25.86 ± 7.46%), *Actinobacteria* (11.87 ± 2.70%) and *Bacteroidetes* (8.15 ± 2.51%). The aforementioned phyla have been also often described dominant in municipal WWTPs [58,60–62] and in plants treating mixtures of 30% municipal and 70% industrial WW [63]. The fraction of the community which could not be classified at the phylum level (Bacteria_unclassified) was on average 16.61 ± 7.60%.

The phyla which were found here as major groups have been also previously reported dominant in AS of different types of WWTPs [60,64–68]. Regarding their functionality in the effective WW treatment, different genera within these phyla have been related to the removal of organic matter, N and P nutrients [58,64,65,69] and the degradation of complex organic substances [70]. In addition, their presence in AS is regarded relevant for an adequate formation of the flocs [71–73].

According to the Kruskal-Wallis and Conover-Iman combined tests, the RAs of the bacterial phyla significantly differed among the four facilities (Table S4). In this sense, Rodríguez-Sánchez et al. [74] found great divergences of the bacterial community structure in three membrane-based WWTPs operating under different salt levels. In order to go deeper into this characterization, a heatmap showing the RAs of the bacterial community populations grouped at the phylum level (Fig. S2a) was constructed. The dominance patterns indicated that, for the majority of the phyla, higher-than-average RAs were reached in a specific facility. As such, the community in F1 samples was dominated by *Bacteroidetes*, *Chloroflexi*, *Calditrichaeota* and *Fusobacteria*; *Actinobacteria*, *Chlamydiae*, *Cyanobacteria*, *Deinococcus-Thermus*, *Dependentiae*, *Hydrogenedentes*, *Patescibacteria*, *Proteobacteria*, *Verrucomicrobia* and WS2 were over-represented in F2 samples; F3 samples were characterized by the highest RAs of *Acidobacteria*, *Dadabacteria*, *Nitrospirae*, *Spirochaetes* and *Synergistetes*; and finally, F4 samples displayed higher than average values of *Armatimonadetes*, *Epsilonbacteraeota*, *Firmicutes* and *Gemmatimonadetes*, plus the biggest fraction of Bacteria_unclassified.

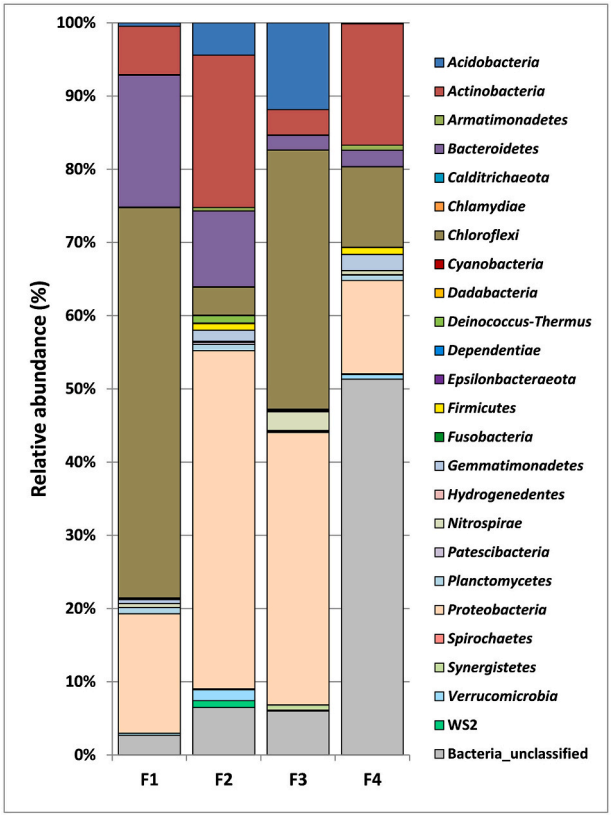

Fig. 2. Average relative abundance of bacterial phyla in activated sludge samples (n = 2) of WWTPs treating fish-canning wastewater, identified by high-throughput Illumina sequencing.

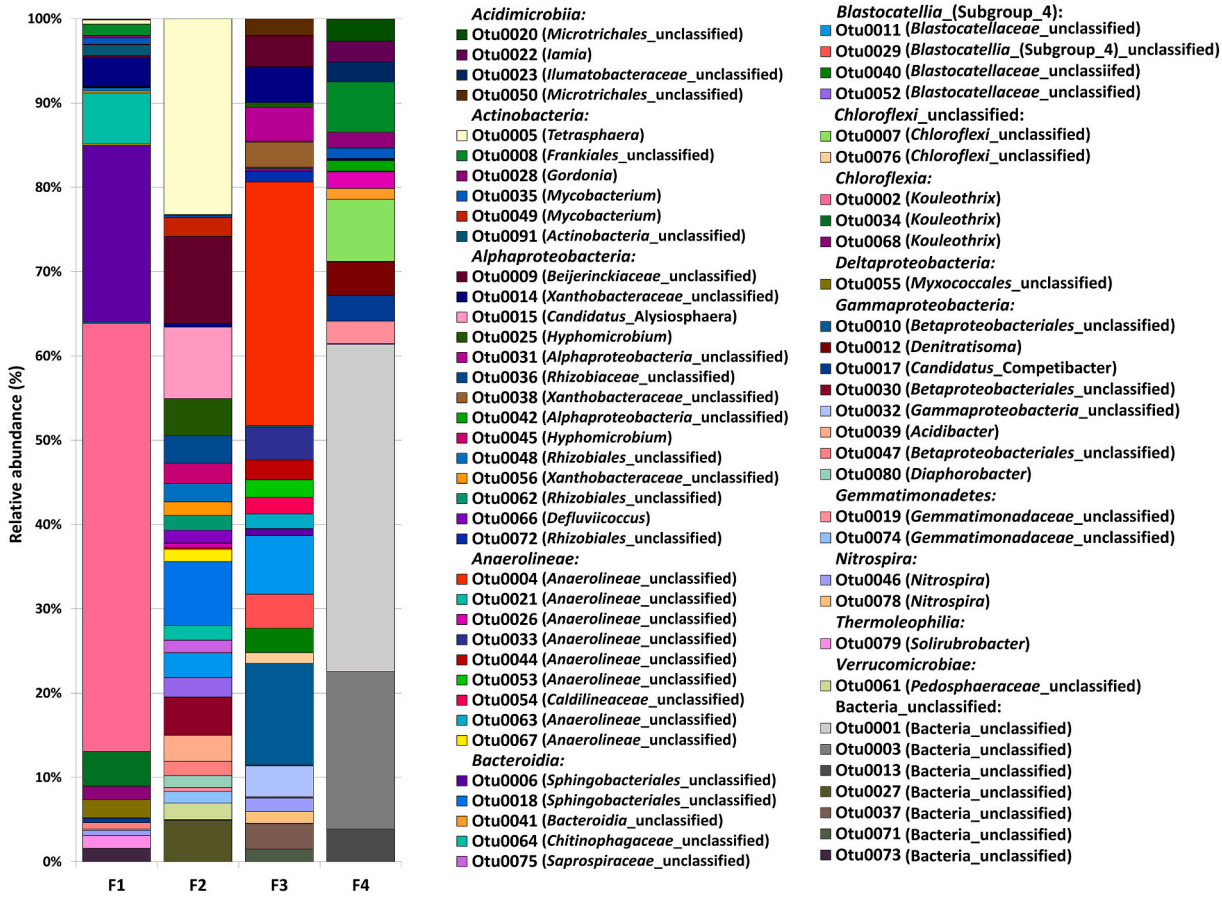

Fig. 3. Average relative abundance of the bacterial dominant OTUs (average RA > 1% in at least one WWTP) in activated sludge samples (n = 2) of WWTPs treating fish-canning wastewater, identified by high-throughput Illumina sequencing.

At the OTU level, there were 69 OTUs with mean relative abundances higher than 1% for a given WWTP, which were considered dominant OTUs (Fig. 3 and Table S6). The presence of these dominant OTUs is consistent with the analytical results of other works [58,63,75,76]. These 69 groups represented >81% of the total bacterial sequences. In general, according to the Kruskal-Wallis and Conover-Iman test (Table S6), the RAs of each dominant OTU were significantly different among the 4 WWTPs.

The top 10 more abundant OTUs (RA > 2%) accounted for 48% of the total bacterial community, and according to their RA (in decreasing order) were: Otu0001 (Bacteria_unclassified, 13.35%), Otu0002 (*Kouleothrix*, 7.42%), Otu0003 (Bacteria_unclassified, 6.43%), Otu0004 (*Anaerolineae*_unclassified, 4.97%), Otu0005 (*Tetrasphaera*, 3.49%), Otu0006 (*Sphingobacteriales*_unclassified, 3.14%), Otu0007 (*Chloroflexi*_unclassified, 2.55%), Otu0008 (*Frankiales*_unclassified, 2.26%), Otu0009 (*Beijerinckiaceae*_unclassified, 2.13%) and Otu0010 (*Betaproteobacteriales*_unclassified, 2.00%) (Table S6). Otu0002 (*Kouleothrix*) and Otu0006 (*Sphingobacteriales*_unclassified) were dominant in F1 WWTP. Otu0005 (*Tetrasphaera*) and Otu0009 (*Beijerinckiaceae*_unclassified) were the more abundant bacteria in the F2 system. Otu0004 (*Anaerolineae*_unclassified) and Otu0010 (*Betaproteobacteriales*_unclassified) were the dominant bacteria in F3 WWTP. Finally, Otu0001 and Otu0002 (both unclassified bacteria), Otu0007 (*Chloroflexi*_unclassified) and Otu0008 (*Frankiales*_unclassified) were the major OTUs in the F4 system. The two only dominant OTUs which could be classified at the genus level were well-known inhabitants of AS of WWTP. *Kouleothrix* (*Chloroflexi*) are filamentous bacteria that were firstly isolated in both domestic and industrial WWTPs. Despite their relation to bulking incidents [77], they are chemoorganoheterotrophs able to assimilate sugars and bacterial cell wall-debris *in situ*, and also play a main role in protein degradation [78,79]. Therefore, this genus could play important functions for an effective WW treatment. A high abundance of this genus was previously described in conventional WWTPs [80], being often reported more abundant in municipal than industrial WWTPs [67]. *Tetrasphaera* (*Actinobacteria*) are putative PAOs that can achieve a similar P-removal contribution compared to Accumulibacter. Besides, they can carry out diverse metabolic activities, as they are able to metabolize sugars and/or ferment glucose and various amino acids reducing the organic matter in WW [81,82]. The over-dominance of this genus in the AS of urban WWTPs not specifically designed for P-removal was previously reported by Świątczak et al. [83]; however, Mielczarek et al. [84] described that the relative abundance of this genus was <6% in either industrial or urban non-EBPR plants. The approximation to the functionality of the remaining OTUs was difficult, due to the versatility of the different members belonging to phyla *Actinobacteria, Bacteroidetes, Chloroflexi* or *Proteobacteria* [69,85–86]. *Actinobacteria* have been found dominant in municipal CAS [68] and advanced treatment systems [62,87]; however, the presence of *Frankiales* is poorly described in WWTPs. There is to note that the only valid genus within this order is *Frankia*, characterized for its ability to fix atmospheric nitrogen [88]. Unclassified *Sphingobacteriales* (*Bacteroidetes*) have been previously described as dominant in textile-dyeing industry WW [89]. The phylum *Chloroflexi*, and particularly the class *Anaerolineae*, are often found in WWTPs but most often in lower abundances than described here [71,90]. However, this phylum has been also regarded as dominant in different domestic and industrial WWTPs [58,68]. There is to note that *Chloroflexi* are commonly associated with extreme habitats, mainly hypersaline environments [90,91]; hence, their over-dominance in the 4 WWTPs analysed here is not surprising given the strength and high salinity of the influents. Generally

considered, *Betaproteobacteriales* is the most abundant taxon in WWTPs [92,93], playing a key role in nitrification, removal of phosphorus and of many organic pollutants. The dominance of unclassified *Betaproteobacteriales* was earlier reported in different AS from urban WWTPs [94] although in lower abundance (4.1%) than reported in this work (9.57%). Finally, a high percentage of unclassified bacteria was found in the samples analysed here, with 7 different bacterial unclassified OTUs as dominant populations. These relative abundance fell within the range reported by other authors in both the AS of municipal WWTPs [95] and anaerobic reactors treating the organic fraction of municipal solid wastes [66,70], except for the bacterial community found in F4 (50%), the facility with a higher level of salinity. A high relative abundance of unclassified bacteria, the so-called bacterial dark matter [96], is often found in extreme environments, where they could be playing important roles, and suggests many undiscovered microbial niches and interactions [97].

According to the Mann-Whitney test (Table S6), the RAs of Otu0005 (*Tetrasphaera*), Otu0015 (*Candidatus*_Alysiosphaera), Otu0047 (*Betaproteobacteriales*_unclassified), Otu0048 (*Rhizobiales*_unclassified), Otu0049 (*Mycobacterium*), Otu0056 (*Xanthobacteraceae*_unclassified), Otu0067 (*Anaerolineae*_unclassified) and Otu0074 (*Gemmatimonadaceae*_unclassified), were statistically more abundant in the CAS systems, while Otu0004 (*Anaerolineae*_unclassified) and Otu0012 (*Denitratisoma*) displayed a higher RA in the NRR systems (Table S6). Therefore, the type of configuration only selected a scarce number of OTUs. In contrast, Kruglova et al. [98] described a great divergence in the bacterial community structure in two WWTPs based on different technologies.

Overall, the RAs of AOB, Microthrix, Mycolata, and GAO populations calculated from the Illumina sequencing data were in agreement with the results observed for the qPCR data described above, according to the *p*-values of the Spearman correlation coefficients between the qPCR data and the corresponding Illumina data (Table S7). Therefore, this work highlights the complementarity between both techniques in the characterization of bacterial communities in environmental samples.

3.5. Beta diversity of bacterial communities in the fish-canning WWTPs

Only 7 of the 1297 OTUs identified in this study were common to the 4 WWTPs, whose relative abundances accounted for 5.72% of the total number of sequences. The shared OTUs were Otu0012 (*Denitratisoma*), Otu0014 (Xanthobacteraceae_unclassified), Otu0017 (Candidatus_Competibacter), Otu0022 (*Iamia*), Otu0036 (Rhizobiaceae_unclassified), Otu0056 (Xanthobacteraceae_unclassified), and Otu0072 (Rhizobiales_unclassified). This result suggested that there was a highly specific biodiversity at the OTU level for each WWTP. Similarly, Wu et al. [51], in a survey of 269 WWTPs around the world, reported that the common core accounted for 12% of the total number of sequences, and Meerbergen et al. [67] only found one common OTU (out of 1645) when they compared the diversity of *Bacteria* in 10 different industrial WWTPs. The heatmap of the RAs of the dominant OTUs (Fig. S2b) confirmed the divergences among WWTPs. In contrast, the shared eukaryotic taxa in these 4 WWTPs were 95% of total sequences [15]. Therefore, the structure of the bacterial communities was more dissimilar among WWTPs than observed for the eukaryotic communities. According to Zhang et al. [68], the 7 shared OTUs are the keystone of the bacterial community structure, playing a vital role in maintaining process efficiency.

The incidence-based Sørensen index was high for the pairwise comparisons without drastic changes between samples (0.61–0.73, Table S8). However, the beta diversity calculated in terms of abundance showed stronger differences among the number of individuals of the shared OTUs (Table S8), being higher for F1 and F2 (1.08), F2 and F4 (0.97) and F3 and F4 (0.98) than for the other pairwise comparisons (0.49–0.52). Therefore, a higher level of homology could not be linked either to the type of configuration or the NaCl gradient.

Finally, to visualize the global differences in the bacterial community composition among samples, a hierarchical cluster analysis based on the Bray-Curtis similarity matrix was performed (Fig. S3). This analysis found a greatest level of homology between samples F2 and F3, followed by F1 and, finally, by F4. Therefore, the cluster analysis confirmed that the specific characteristics of each industrial WW and the operational parameters were more important drivers of community shaping than the type of configuration. This result is in agreement with Ibarbalz et al. [99] and Isazadeh et al. [100], who have previously suggested that bacterial compositions in both urban and industrial WWTPs are more drastically affected by the individual intrinsic characteristics of WWTPs than by the type of configuration.

3.6. Correlation between operational parameters and microbial community structure in fish-canning WWTPs

Nonmetric Multidimensional Scaling (NMS) analyses, a kind of non-constrained ordination, were conducted taking into account the absolute abundance of key microbial populations (qPCR data) and the RA of the dominant OTUs derived from the Illumina sequencing analyses. The characteristics of the WW and AS and the operational parameters were linked to the corresponding NMS analyses, aiming to go deeper into the existing relationships between the microbial community structure and those abiotic variables.

As demonstrated in Fig. 4, the different microbial populations measured by qPCR were divided in two clusters, cluster 1 comprising total *Bacteria* and *Fungi*, GAO, PAO and Mycolata, and cluster 2 which grouped together total *Archaea*, AOB and Microthrix. The samples from the 4 WWTPs were distributed differently in the NMS space: F1 and F3 were strongly influenced by cluster 1 and cluster 2 populations' abundances, respectively, while F2 was not highly influenced by the microbial populations, and F4 located intermediately between F2 and F3. Therefore, the ordination of the samples was independent of the type of configuration. The vectors representing the trends of the operational parameters with a magnitude $r^2 > 0.5$ were overlapped on the NMS plots (Fig. 4). Pairwise Pearson product-moment (r) correlations with the trends of the microbial copy numbers and those of all the abiotic parameters were calculated (Table S9). Only strong correlations ($r \geq 0.7$, $r \leq -0.7$) will be discussed below.

According to Table S9, absolute abundances of both *Bacteria* and *Fungi* correlated negatively with the same parameters (WW pH, WW [NH_4^+], and AS [NaCl]), while the abundance of *Archaea* was linked to different factors (WW VFA and AS pH), suggesting an ecotype differentiation among *Archaea* and the other two broad groups of microbes. The abundance of gene markers of *Archaea* was not significantly related to salinity (Table S9), in agreement with the previous knowledge of microorganisms of this domain being able to endure wide salinity fluctuations and achieve stable community structures faster than *Bacteria* in nature [101].

The absolute abundances of AOB were strongly correlated with AS [NaCl]. Accordingly, Pan et al. [102] previously stated that the numbers of copies of *amoA* genes of AOB in a municipal full-scale CAS were significantly higher at 2.5–10 g/L NaCl compared to salt-free conditions, while counts were lower at 15 g/L NaCl or higher. In contrast, no differences of the number of copies of AOB *amoA* genes were observed at either 10 or 30 g/L NaCl in a sequencing batch biofilm reactor treating saline industrial WW [103]. The negative effects of salinity on ammonia oxidation rates, at NaCl concentrations falling within the range of those in the facilities analysed here, have been reported in an aerobic-anoxic nitrifying sequencing batch reactor and a municipal full-scale CAS [102,103]. The tolerance of AOB to salinity widely varies depending on the species [104]; hence, NaCl concentration may influence nitrification performance by shifting the AOB community structure. In this sense, Cortés-Lorenzo et al. [105] observed no detrimental effects on nitrification in an aerated-anoxic submerged biofilter when it was fed with municipal WW at 0 or 3.7 g/L NaCl, while ammonia oxidation activity was significantly hampered at NaCl concentrations >24 g/L, and the

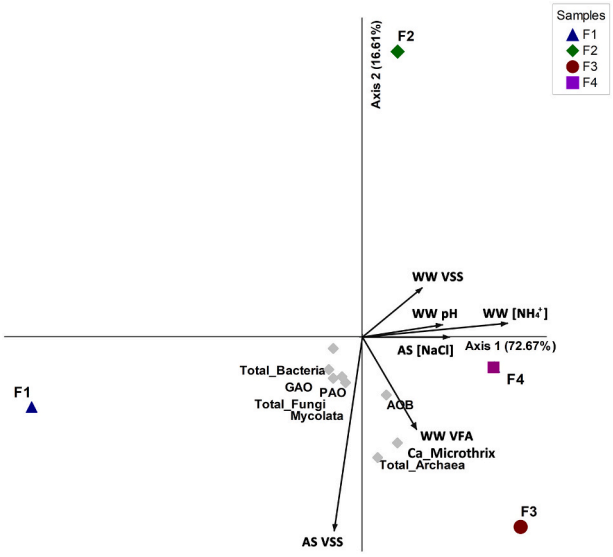

Fig. 4. Nonmetric Multidimensional Scaling (NMS) ordination of the activated sludge samples (n = 2) retrieved from the four fish-canning WWTPs, based on the gene copy numbers of the microbial groups Total Bacteria, Total Archaea, Total Fungi, AOB, Mycolata, *Ca.* Microthrix, *Ca.* Accumulibacter (PAO) and *Ca.* Competibacter (GAO), and their linking to the trends of the abiotic variables influencing the WWTPs (volatile suspended solids of activated sludge, VSS_AS; pH of wastewater, pH_WW; pH of activated sludge, pH_AS; NaCl concentration in wastewaters, NaCl_WW; NaCl concentration in activated sludges, NaCl_AS; volatile fatty acids in wastewater, VFA_WW; ammonium concentration in wastewater, NH4 + _WW).

change in efficiency was concomitant with population succession leading to a higher relative abundance of AOB phylotypes mostly described in saline environments.

PAO and GAO populations' abundance were both negatively correlated to the variables WW pH, WW [NH_4^+], and AS [NaCl], which followed very similar trends throughout the samples' ordination (Fig. 4). In this regard, Wang et al. [106] have previously stated that concentrations >10 g/L NaCl inhibited Accumulibacter's phosphate accumulation efficiency in batch tests, while Mielczarek et al. [107] failed to find significant correlations among WW [NH_4^+] and Accumulibacter or Competibacter abundance, in a survey conducted on 28 full-scale municipal EBPR plants in Denmark. Lopez-Vazquez et al. [108] highlighted that the differences in physicochemical properties (i.e., pH or temperature of the AS) have the same effect over PAO and GAO populations, indicating that they both respond in the same manner when exposed to similar environmental conditions. Also, it is important to note that the same niche selection makes the inhibition of the non-desired GAO over the beneficial PAO populations difficult.

The abundance of gene markers of Mycolata and Microthrix was driven by different variables, despite their belonging to the same phylum, highlighting a divergence in the colonization traits between both lineages of filamentous bacteria. Mycolata populations' abundance was strongly and negatively correlated with WW pH, WW [NH_4^+], and AS [NaCl], while in the case of Microthrix robust links were only detected with increasing values of WW VFA and AS [NaCl] and decreasing values of WW [NH_4^+] (Fig. 4, Table S9). Strong negative correlations were previously reported among the absolute abundances of Mycolata and WW pH, and those of Microthrix and AS [NH_4^+], in studies conducted in full-scale municipal WWTPs [19,20]. In contrast, Jiang et al. [109] failed to find robust correlations among WW [NH_4^+] and the abundances of Mycolata, Microthrix and other filamentous bacteria in a full-scale WWTP monitored during 5 years. Salinity is well known as a factor inhibiting the growth of filamentous bacteria in AS [110], and previous studies specifically reported a low tolerance of Microthrix to NaCl [103,111]; thus, the positive correlation observed here between the AS [NaCl] and the absolute abundance of Microthrix was unexpected (Table S9).

The detrimental effects of NaCl are well described in groups of bacterial populations evolutionarily and eco-physiologically different [112]. The results presented here suggest that both AOB and Microthrix were more halotolerant than the other bacterial groups targeted by qPCR in the four fish-canning WWT facilities (Table S9); however, contradictory reports in the literature exist, which highlight the need of further research to fully understand the effects of NaCl over the dominance patterns of Microthrix, AOB, PAO and GAO populations in WWTPs receiving saline influents.

Similarly to the trends observed in Fig. 4, the NMS based on the relative abundance of the dominant bacterial OTUs characterized by Illumina sequencing (Fig. 5) demonstrated that the samples were distributed across the whole NMS space without following any clear pattern of ordination. The substantial divergence in the structure of the bacterial communities is in disagreement with the previously described results for the eukaryotic communities in the same WWTPs samples, which were more narrowly distributed in their corresponding NMS [15]. Therefore, the particular characteristics of fish-canning effluents and operational parameters inflicted a narrower selection of the eukaryotic populations that participate in the WWTP functionality than that observed for the bacterial communities. This suggests a redundant functionality in the bacterial community since different bacterial taxa can carry out the same roles in the WWTPs. According to the statistical analyses here employed (NMS plot, Fig. 5, Table S10), WW [NaCl] was the main factor that influenced the distribution of the samples along axis 1, which explained 52% of the total variance, suggesting that this variable specifically modulated the structure of the dominant OTUs found in the 4 fish-canning WWTPs, despite the influence of other factors. This result is supported by previous literature, which highlighted the role of NaCl concentration as a major factor influencing AS community structure and WWTP performance [15,64,74]. In addition to that, other individual factors and, in lesser degree, the type of configuration, were also involved in the assembly of the microbial communities of each WWTP. In this sense, the trends of WW [NH_4^+] and AS VSS influenced in opposite ways the bacterial community structures of both F1 and F2 facilities. Similarly, the bacterial community structures of F3 and F4 facilities diverged mostly due to the differences of both WW [NaCl] and AS [NaCl], although WW TOC was also a very influential factor. In this sense, the capacity of these variables to modulate the structure of bacterial communities in WWTP has been previously and amply described in different scientific works [70,93,113–115].

The OTUs displaying the higher or lower values of the Pearson's correlation coefficient (r) with the concentration of NaCl ($r \geq 0.99$ or ≤ -0.99) are shown in Table 2. Those OTUs whose RAs were most positively correlated to NaCl concentration could be addressed as salt-tolerant and/or halophilic bacteria, which could perform the former functions of the halophobic bacteria in AS of WWTPs operated under high NaCl content. There is to note, that the OTUs here considered salt-tolerant/halophilic were distributed among different phyla (*Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes* and *Proteobacteria*) and the group of Bacteria_unclassified. Among the different mechanisms to cope with salt stress, the increase of osmolytes and the synthesis of antioxidant enzymes have been described in different members of *Actinobacteria* [116] and *Chloroflexi* [117], and the presence of an acidic proteome has been proposed as the main adaptation to NaCl stress in *Bacteroidetes* [118] and *Proteobacteria* [119]. Although some authors [120] have recently proposed *Gemmatimonadetes* as halophilic or halotolerant bacteria, the mechanisms involved in their NaCl adaptation are not yet established. On the other hand, some genera belonging to the phylum *Planctomycetes* have been described as well-adapted to high salinity conditions in some industrial WWTPs, such as those from textile industries [67]; however, in the current work, the RAs of OTUs taxonomically affiliated to phylum *Planctomycetes* within the total

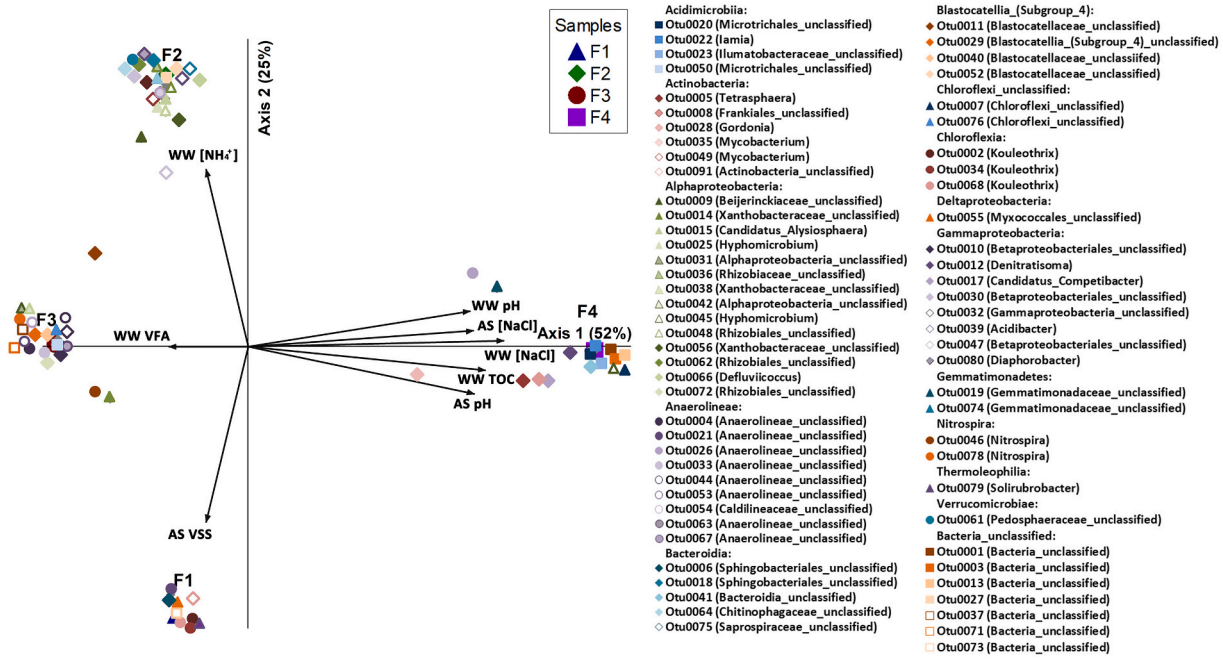

Fig. 5. Nonmetric Multidimensional Scaling (NMS) ordination of the activated sludge samples (n = 2) retrieved from the four fish-canning WWTPs, based on the RA of the dominant bacterial OTUs (Mean RA > 1% in at least one WWTP), and their linking to the abiotic variables influencing the WWTPs (volatile suspended solids of activated sludge, VSS_AS; pH of wastewater, pH_WW; pH of activated sludge, pH_AS; NaCl concentration in wastewaters, NaCl_WW; NaCl concentration in activated sludges, NaCl_AS; volatile fatty acids in wastewater, VFA_WW; ammonium concentration in wastewater, NH4 + _WW).

Table 2
Pearson product-moment correlation coefficients (r) between the NaCl concentration in WW and AS samples from different WWTPs and the relative abundance of bacterial OTUs (Mean RA > 1% in at least one WWTP) samples (n = 2) of WWTPs treating fish-canning wastewater according to Fig. 5. Only coefficients ≥0.99 or ≤ −0.99 are shown.

Class	OTU	NaCl_WW	NaCl_AS
Gemmatimonadetes	Otu0019 (Gemmatimonadaceae_unclassified)	1.00	1.00
Bacteria_unclassified	Otu0013 (Bacteria_unclassified)	0.99	0.96
Bacteria_unclassified	Otu0001 (Bacteria_unclassified)	0.99	0.96
Bacteria_unclassified	Otu0003 (Bacteria_unclassified)	0.99	0.96
Acidimicrobiia	Otu0020 (Microtrichales_unclassified)	0.99	0.96
Acidimicrobiia	Otu0023 (Ilumatobacteraceae_unclassified)	0.99	0.96
Alphaproteobacteria	Otu0042 (Alphaproteobacteria_unclassified)	0.99	0.96
Bacteroidia	Otu0041 (Bacteroidia_unclassified)	0.99	0.96
Chloroflexi_unclassified	Otu0007 (Chloroflexi_unclassified)	0.99	0.96
Gammaproteobacteria	Otu0012 (*Denitratisoma*)	0.99	0.96
Acidimicrobiia	Otu0022 (*Iamia*)	0.99	0.96
Alphaproteobacteria	Otu0072 (Rhizobiales_unclassified)	−0.99	−0.96
Blastocatellia_(Subgroup_4)	Otu0029 (Blastocatellia_(Subgroup_4)_unclassified)	−0.99	−0.96
Alphaproteobacteria	Otu0031 (Alphaproteobacteria_unclassified)	−0.99	−0.96
Anaerolineae	Otu0004 (Anaerolineae_unclassified)	−0.99	−0.96
Anaerolineae	Otu0033 (Anaerolineae_unclassified)	−0.99	−0.96
Anaerolineae	Otu0053 (Anaerolineae_unclassified)	−0.99	−0.96
Anaerolineae	Otu0054 (Caldilineaceae_unclassified)	−0.99	−0.96
Blastocatellia_(Subgroup_4)	Otu0040 (Blastocatellaceae_unclassified)	−0.99	−0.96
Chloroflexi_unclassified	Otu0076 (Chloroflexi_unclassified)	−0.99	−0.96
Gammaproteobacteria	Otu0010 (Betaproteobacteriales_unclassified)	−0.99	−0.96
Gammaproteobacteria	Otu0032 (Gammaproteobacteria_unclassified)	−0.99	−0.96
Nitrospira	Otu0078 (*Nitrospira*)	−0.99	−0.96
Bacteria_unclassified	Otu0071 (Bacteria_unclassified)	−0.99	−0.96
Nitrospira	Otu0046 (*Nitrospira*)	−0.99	−1.00
Alphaproteobacteria	Otu0038 (Xanthobacteraceae_unclassified)	−0.99	−0.96
Bacteria_unclassified	Otu0037 (Bacteria_unclassified)	−0.99	−0.96
Acidimicrobiia	Otu0050 (Microtrichales_unclassified)	−0.99	−0.98

community were very low, indicating that they most probably lacked important roles in these WWTPs.

Although this work was pioneer in the analysis of the NaCl effect on the bacterial community in fish-canning WWTPs, further research is needed to unravel the importance of NaCl for the shaping of microbial communities in saline influents of either fish-canning or other industrial WWTPs, in order to address their contribution to the adequate management of these saline effluents, according the data here showed. Future studies building on the results here presented could also aim at the identification of the key players in the microbial community necessary for an optimal treatment of the industrial fish-canning effluents.

4. Conclusion

The results presented here showed that high NaCl concentrations exerted a negative pressure over the absolute abundances of total *Bacteria* and *Fungi*, Mycolata, PAOs and GAOs in the AS of four full-scale fish-canning WWTPs. However, it was not a barrier for the proliferation of AOB populations. Higher NaCl concentrations correlated positively with an increased relative abundance of OTUs belonging to the phyla *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes* and *Proteobacteria*. The bacterial communities' structures were highly different among the 4 analysed WWTPs, indicating a strong modulating effect of the particular WW characteristics and the operational parameters of each facility, rather than the type of configuration. These findings provide novel insights into the structure of microbial communities in industrial WWTPs and the role of salinity as a key driver of their shaping.

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Declaration of competing interest

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

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