Received: 21 April 2020 | Revised: 18 August 2020 | Accepted: 21 August 2020

DOI: 10.1111/are.14872

ORIGINAL ARTICLE

Aquaculture Research WILEY

A case study of biofilter activation and microbial nitrification in a marine recirculation aquaculture system for rearing Atlantic salmon (*Salmo salar* L.)

Irene Roalkvam[1] | Karine Drønen[1] | Håkon Dahle[1,2] | Heidrun I. Wergeland[1]

[1]Department of Biological Sciences, University of Bergen, Bergen, Norway

[2]K.G. Jebsen Centre for Deep Sea Research, University of Bergen, Bergen, Norway

Correspondence
Irene Roalkvam, Department of Biological Sciences, University of Bergen, Bergen, Norway. Email: irene.roalkvam@uib.no

Funding information
Norwegian Seafood Research Fund (FHF), Grant/Award Number: Project number 901470

Abstract

Marine recirculation aquaculture system (RAS) is a prominent technology within fish farming. However, the nitrifying bacteria in the biofilter have low growth rates, which can make the biofilter activation a long and delicate process with periods of low nitrification rates and variations in water quality. More knowledge on the microbial development in biofilters is therefore needed in order to understand the rearing conditions that favour optimal activation of the biofilters. In this case study, we investigated the activation of two biofilters in a marine RAS for Atlantic salmon post-smolt associated with either high or low stocking densities of fish by monitoring the microbial communities and chemical composition. The results showed that the microbial communities in both biofilters were similar during the first rearing cycle, despite variations in the water quality. Nitrifying bacteria were established in both biofilters; however, the biofilter associated with low stocking density had the highest relative abundance of ammonia-oxidizing *Nitrosococcus* (1.0%) and nitrite-oxidizing *Nitrospira* (2.1%) at the end of the first rearing cycle, while the relative abundance of ammonia-oxidizing *Nitrosomonas* (2.3%–2.9%) was similar in both biofilters. Our study showed that low fish stocking density during the first rearing cycle provided low and steady concentrations of ammonium, nitrite and organic load, which can stimulate rapid development of a nitrifying population in new marine RAS biofilters.

KEYWORDS

16S rRNA gene, biofilter, microbial community, nitrifying bacteria, recirculating aquaculture system

1 | INTRODUCTION

Development of new technology within aquaculture the industry is important in order to expand seafood production or pursue cost-effective operations within fish farming. Today, recirculating aquaculture systems (RASs) are used for rearing salmon in countries such as Canada, Chile, China, Faroe Island, France, Iceland, Norway, Poland, Scotland, Tasmania and United States (Dalsgaard et al., 2013; De Guzman, 2018; Furuset, 2018; Summerfelt, 2015), but investments are also made in other countries worldwide. In Norway, the great expansion in marine RAS for post-smolt Atlantic salmon and Rainbow trout is mainly a measure against sea lice infections. With the newest

RAS technology, the hourly degree of water recirculation can be kept above 99%, which makes it possible to establish fish farms in areas or countries with limited water resources.

Due to the high degree of water retention, the RAS has several steps of water quality improvement. One of these steps involves biological removal of ammonia and dissolved organic matter by microorganisms that colonize the medium inside the biofilter, referred to as biofilm carriers hereafter. These biofilms form complex microbial communities over time, comprising different bacteria with various metabolic and physical properties. The nitrifying bacteria in the biofilter convert the toxic ammonia secreted by the fish to non-toxic nitrate in a two-step process. The oxidation of ammonia to nitrite is usually performed by species within *Nitrosomonas* in marine RAS, while the oxidation of nitrite to nitrate is often performed by species within *Nitrospira* (Blancheton, Attramadal, Michaud, d'Orbcastel, & Vadstein, 2013; Foesel et al., 2008; Keuter, Beth, Quantz, Schulz, & Spieck, 2017; Ruan, Guo, Ye, Liu, & Zhu, 2015; Schreier, Mirzoyan, & Saito, 2010; Tal, Watts, Schreier, Sowers, & Schreier, 2003). Cultured representatives of these nitrifying bacteria are known to have low growth rates (Koops & Pommerening-Röser, 2015a; Spieck & Lipski, 2011), which can make activation of new RAS biofilters a time-consuming process. The slow development of nitrifying bacteria makes the biofilter less efficient during the activation period, causing low water quality due to increased concentrations of ammonia and nitrite, which can be harmful for the fish or inhibit further microbial biofilter development. It is important to obtain further knowledge on the development of nitrifying bacteria in RAS biofilters and reveal factors that can promote growth of the nitrifying population in order to reduce the activation period. This is of great economic interest for the aquaculture industry and could also improve the welfare of farmed fish.

As laboratory experiments are not necessarily a good model for microbial colonization of a large-scale industrial RAS, we performed a case study by surveying the microbial succession of two biofilters in a marine RAS for post-smolt Atlantic salmon during the first 17 weeks of the biofilter activation period. The RAS had two parallel biofilters where the fish tanks connected to each biofilter received either low or high stocking density. The aim of the study was to compare the establishment of the nitrifying population in each biofilter and reveal how the development of microbial communities was affected by stocking density and variations in physiochemical water parameters.

2 | MATERIALS AND METHODS

2.1 | The rearing facility

The RAS surveyed in this study was located on the west coast of Norway. This fish farm comprises two RAS units for rearing post-smolt Atlantic salmon, prior to on-growing in seawater cages. The two RAS units were engineered and installed in 2015 and 2017, respectively, of which the newest unit was explored in this study. Samples for microbial analyses and physiochemical water parameters were obtained throughout the first rearing cycle in the new RAS unit, which started in December 2017 and ended in March 2018.

The RAS unit comprised four fish tanks with a volume of 1,150 m^3 each and with a dilution rate of 150 m^3/hr/tank. There were 2 biofilters in the RAS unit, where one biofilter was connected to 2 fish tanks. Each biofilter contained 300 m^3 of BioWater^{TM} Biofilm carriers (Biowater Technology) made from high-density polyethylene (HDPE) with a surface area of 828 m^2/m^3. Maximum fish stocking capacity of the RAS unit was a total of 500,000 post-smolt individuals, which grew from approximately 100 g to approximately 500 g during a rearing cycle of 4 months. The feed conversion ratio was 0.8, but feeding was adjusted according to fish appetite. Fish in tanks connected to Biofilter 1 received feed supplied by Alltech (Emerald, 3–4 mm), whereas fish in tanks connected to Biofilter 2 received feed supplied by BioMar (Orbit, 3–4 mm).

The biofilters in the RAS unit were inoculated with microorganisms from the biofilter in the older RAS unit, as this biofilter was matured and fully operational at the time of biofilter activation in the newest RAS unit. Approximately 200 L of biofilm carriers and water from the old RAS biofilter was transferred to each of the new biofilters, which gave an inoculum size of approximately 0.07%. An oxidative agent (Loz supplied by Loz AS) was added periodically for removal of mainly nitrite and organic compounds, in order to compensate for the low performance of the immature biofilter. The Loz might also oxidize ammonia to some degree. Fish tanks associated with Biofilter 1 received up to 9.6 L/hr Loz on a daily basis in weeks 5–17, while fish tanks associated with Biofilter 2 received up to 0.2 L/hr Loz sporadically in weeks 11 and 13.

The inoculation of the biofilters was termed week 1 of the activation period. The fish tanks initially received maximum fish stocking density shortly after inoculation and activation of the biofilters: The first half of the fish batch was stocked in week 3, distributed into 2 of the tanks where each tank was associated with 1 of the biofilters. Then, the second stocking occurred in week 6 in the remaining 2 tanks. However, technical issues caused high mortalities in the fish tanks connected to Biofilter 2 in week 6, only a few days after the fish stocking. This resulted in two different biofilter activation strategies, where Biofilter 1 supported fish tanks with 100% of maximum fish stocking density (~125,000 fish per tank), referred to as high stocking density hereafter, while Biofilter 2 supported fish tanks with approximately 8% of maximum fish stocking density (~10,000 fish per tank), referred to as low stocking density hereafter.

2.2 | Quantification of water quality parameters

Nitrogen species in the water was analysed using a Odeon fitted with a photopod (Ponsel Measure) and with suitable tube tests for ammonia (0.08–1.6 mg/L-N), nitrite (0.01–0.6 mg/L-N) and nitrate (0.06–1.8 mg/L-N) (Orchidis). The concentrations of ammonia and nitrite were measured daily, while the concentration of nitrate was measured at irregular intervals in order to confirm nitrate formation.

Conductivity, pH and temperature were measured continuously using probes (Schneider probes and Unitronics computer software); however, a single representative reading per day is included in this data set. The salinity data were calculated from measured conductivity and temperature, using a standardized formula (Fofonoff & Millard, 1983).

2.3 | Biological sample material and water chemistry data

Samples for microbial community analyses were retrieved weekly and the water quality was monitored regularly from week 3 onwards, when the fish tanks were stocked. The biological sample material comprised microorganisms from water in the fish tanks and biofilm from biofilter carriers. From one of the fish tanks connected to each biofilter, 240 ml of water was collected and filtered using 0.22 μm Sterivex filter units (Merck). From each biofilter, 2 biofilm carriers were collected in a falcon tube. The samples were kept cold during transportation to the University of Bergen and stored at −20°C until further analyses.

2.4 | DNA extraction and amplicon library analysis

Microbial community analyses were based on 16S rRNA gene amplicon libraries that were sequenced using the Ion Torrent technology. From each biofilter, 2 biofilm carriers were sampled for DNA extraction using DNeasy Power Biofilm kit (Qiagen). Biofilm carriers were incubated in Falcon tubes with lysis buffers from the kit at 65°C, as suggested by the kit protocol, with occasional vigorous shaking. The supernatants were then transferred to bead tubes supplied by the kit, and the DNA extraction was completed following the protocol supplied with the kit. The plastic casing of filters containing microorganisms from fish tank water were cracked open using a pair of tongs, before the filter material was removed from the plastic core using a sterile scalpel. Then, the DNeasy Power Water kit (Qiagen) was applied on the filters, as described by the manufacturer.

An Amplicon library was constructed for the samples using the primers 519f and 806r in a two-step PCR, as described in Roalkvam, Drønen, Dahle, and Wergeland (2019b). The bioinformatics pipeline included filtering, clustering into operational taxonomic units (OTUs), trimming, chimera removal and taxonomic classification; see Roalkvam et al. (2019b) for details.

2.5 | Statistical analyses

The principal coordinate analysis (PCoA) was performed using the 'Vegan' package in R version 3.5 (Oksanen et al., 2018; R Core Team, 2018), using the 'cmdscale' function with Bray–Curtis as dissimilarity index. The analysis was performed on data at genus level with values given as relative abundance.

2.6 | Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this study did not include animal specimens.

3 | RESULTS

The marine RAS in this study contains 2 biofilters that operate 2 fish tanks each. The biofilter associated with a high fish stocking density (100% of maximum stocking density) is referred to as Biofilter 1 hereafter, while the biofilter associated with a low fish stocking density (8% of maximum stocking density) is referred to as Biofilter 2 hereafter.

3.1 | Water chemistry and fish mortality during the first rearing cycle

In water from Biofilter 1, the temperature was 9.3–17.7°C during the rearing cycle, except for a period between weeks 10 and 16 when the intake water had a higher portion of colder fresh water (Figure 1a). This corresponded to a period with wounds on the fish, where lower salinity was used in order to improve recovery of the fish. The average rearing salinity was 22.3ppt NaCl, except for the wound treatment period, when the salinity was below 20ppt NaCl. The pH was above 7.5 during the first 4 weeks after start-up, probably due to carbonates from concrete residues after construction work, but later decreased to an average pH of 7.0 for the remaining rearing cycle (Figure 1a). In Biofilter 2, the water quality was more stable compared to Biofilter 1, with no periods of increased freshwater addition. The temperature was 8.9–14.9°C, and the average salinity was 27.4ppt NaCl (Figure 1b). The pH was above 7.5 for a longer period compared to water in Biofilter 1, and did not decrease until 12 weeks after start-up (Figure 1b).

In Biofilter 1, the concentration of ammonium increased considerably the first 10 weeks of the rearing cycle (Figure 1c), reaching a maximum concentration of 17.6 mg/L-N. The concentration of nitrite increased considerably in weeks 10–14, with measured concentration above 100 mg/L-N nitrite (Figure 1c). The irregular quantification of nitrate concentrations confirmed that nitrate was produced and showed high concentrations (<220 mg/L-N) at some sample points (Figure 1c). Due to the low stocking density in the fish tanks connected to Biofilter 2, the concentration of ammonium remained below 8.3 mg/L-N throughout the cycle (Figure 1d). Loz was only added sporadically and in low concentrations in order to support nitrate formation from nitrite. During the rearing cycle, the concentration of nitrite remained below 50 mg/L-N, whereas the concentration of nitrate remained below 15.6mg/L-N (Figure 1d).

For all fish tanks, mortalities were recorded during the rearing cycle. The number of dead individuals from the batch of fish in each

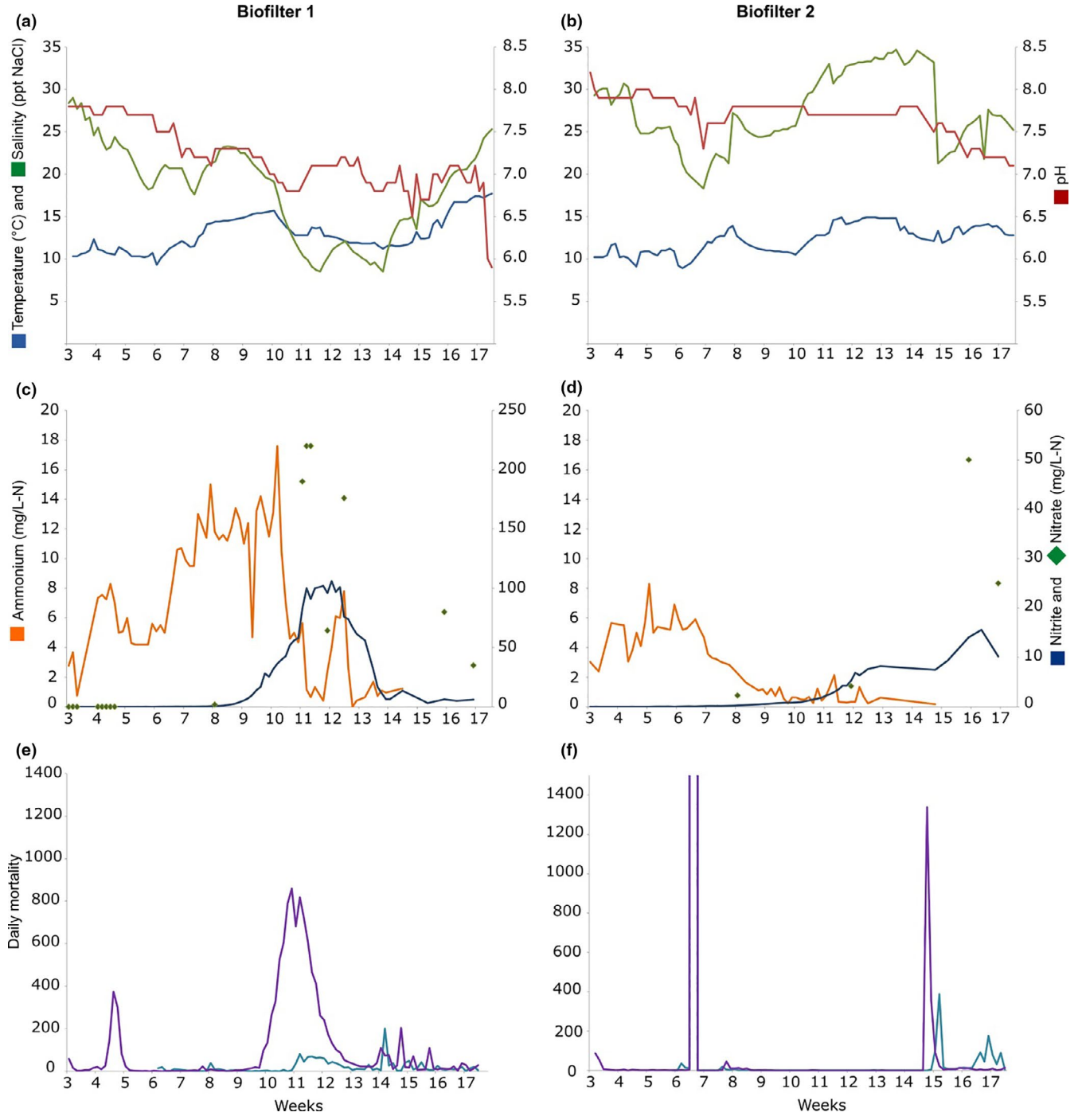

FIGURE 1 Water chemistry parameters and fish mortality were registered on a daily basis for Biofilter 1 and Biofilter 2. The parameters for pH, salinity and temperature are shown for Biofilter 1 (a) and Biofilter 2 (b). The concentrations of ammonium, nitrite and nitrate are also shown for Biofilter 1 (c) and biofilter 2 (d). Note the different scales on the second axes. Periods with relatively high daily mortality were detected in fish tanks connected to Biofilter 1 (e) and Biofilter 2 (f). The fish tanks connected to Biofilter 2 experienced technical issues in week 6 that killed ~92% of the stock (f). The daily mortality in parallel fish tanks associated with each biofilter is indicated

fish tank varied substantially. In fish tanks connected to Biofilter 1, the mortalities increased for a short period of time in weeks 4–5 (Figure 1e), possibly due to poor water quality and increased concentrations of ammonium. Furthermore, in the period between weeks 10 and 13, high mortalities were associated with poor water quality and increase in wound formations. Wounds occurred on the flank next to the caudal fin, and were probably caused by the fish rubbing against objects in the fish tanks (Figure S1). No fish pathogenic bacteria could be isolated from the wounds on plates with marine agar or blood agar with 2% NaCl. In fish tanks connected to Biofilter 2, the majority of the fish in both tanks perished in week 6 due to technical issues (Figure 1f). It was estimated that 10,000 fish remained in

each fish tank, which is 8% of the maximal stocking density. In week 15, a new batch of fish was added to the fish tanks connected to Biofilter 2 in order to reestablish the original stocking density. This increased the mortality transiently due to damages caused by transportation (Figure 1f).

3.2 | Microbial community structure

Variations in community structure between groups of samples and over time were analysed on genus level using PCoA. Microbial communities in fish tank water and biofilms from both biofilters were very similar during the first 4 weeks (Figure 2). During the following period (weeks 5–10), the microbial communities in fish tanks and the two biofilters gradually formed distinct clusters. Biofilms from both biofilters had highly similar communities at the end of the rearing cycle, as samples from weeks 10 to 17 clustered together. Contrarily, communities in water sampled from fish tanks connected to Biofilter 1 and Biofilter 2 were less similar, as these samples did not cluster together, and were also separated from biofilm sample (Figure 2).

On class level, biofilms from Biofilter 1 and Biofilter 2 seemed stable over time (Figure 3a,c). Several of the classes were abundant in biofilms from both biofilters, such as *Gammaproteobacteria* and *Flavobacteria* which comprise up to 97.2% and 50.0% in Biofilter 1 and up to 83.6% and 58.4% in Biofilter 2 respectively (see Figure S2 for microbial community structure on genus level). Some of the sampled biofilms in Biofilter 1 had considerably higher relative abundances of taxa within *Alphaproteobacteria*, *Cytophagia*, *Deltaproteobacteria* and *Betaproteobacteria*, compared to biofilms in Biofilter 2. Taxa found to be abundant in biofilms from Biofilter 2, but not Biofilter 1, included *Bacilli*, *Nitrospira*, *Acidimicrobiia* and uncultured members of BD1-5 clade, TM6 clade and *Crenarchaeota* group (Figure S2).

Throughout the rearing cycle, the variation in community structures of the fish tank water was higher than for the biofilms in the biofilters (Figure 3b,d). Similar to the biofilms, the water samples were dominated by *Gammaproteobacteria* and *Flavobacteria*, which comprised up to 97.6% and 58.4% in water connected to Biofilter 1 and up to 93.6% and 92.7% in water connected to Biofilter 2 respectively (see Figure S2 for microbial community structure on genus level). In addition, tank water connected to Biofilter 1 had high relative abundance of *Cytophagia*, which comprised up to 63.1% of the microbial communities in the water, and also higher relative abundance of *Alphaproteobacteria* compared to water from Biofilter 2 (Figure 3b). Furthermore, several taxa were present with a relative abundance above 2% in fish tank water connected to either Biofilter 1 or Biofilter 2. Among these, the taxa within BD1-5 clade and *Actinobacteria* were particularly abundant in fish tank water connected to Biofilter 2 (Figure 3d), where their relative abundances were 23.8% and 18.0% in some samples respectively.

3.3 | Development of the nitrifying population in the biofilters

Among known genera of nitrifying microorganisms, the most abundant in the biofilter samples were *Nitrosomonas* (*Betaproteobacteria*),

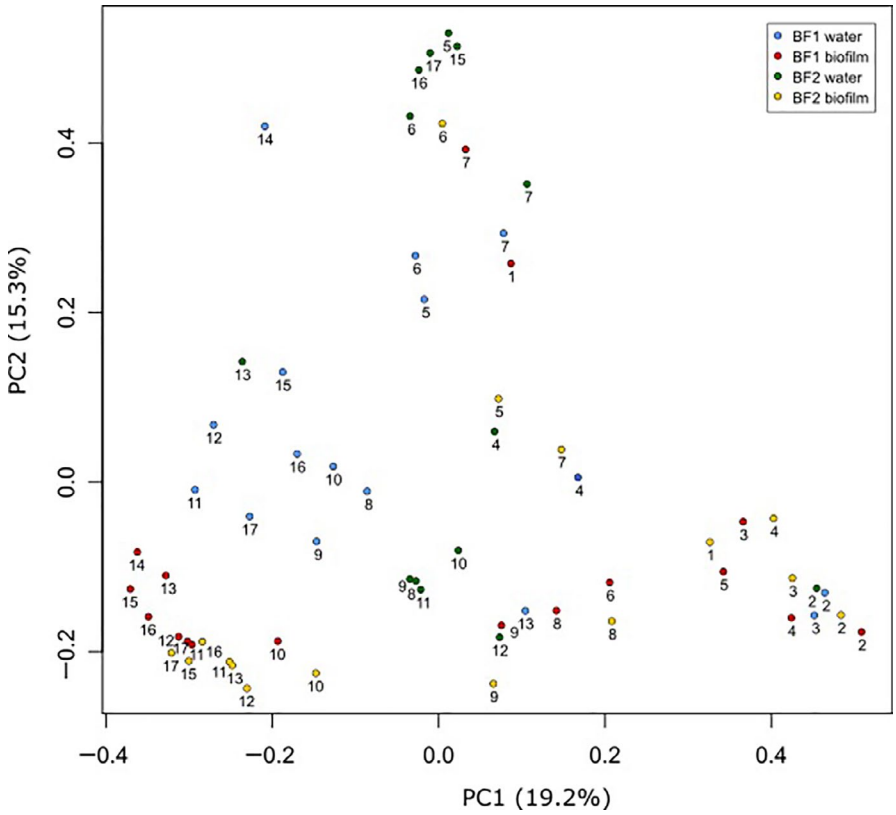

FIGURE 2 Principal coordinate analysis (PCoA) performed on all samples at genus level. Samples were compared using Bray–Curtis distance matrix, and the similarities were visualized in a PCoA plot. Numbers under the points refer to number of weeks into the sampling period

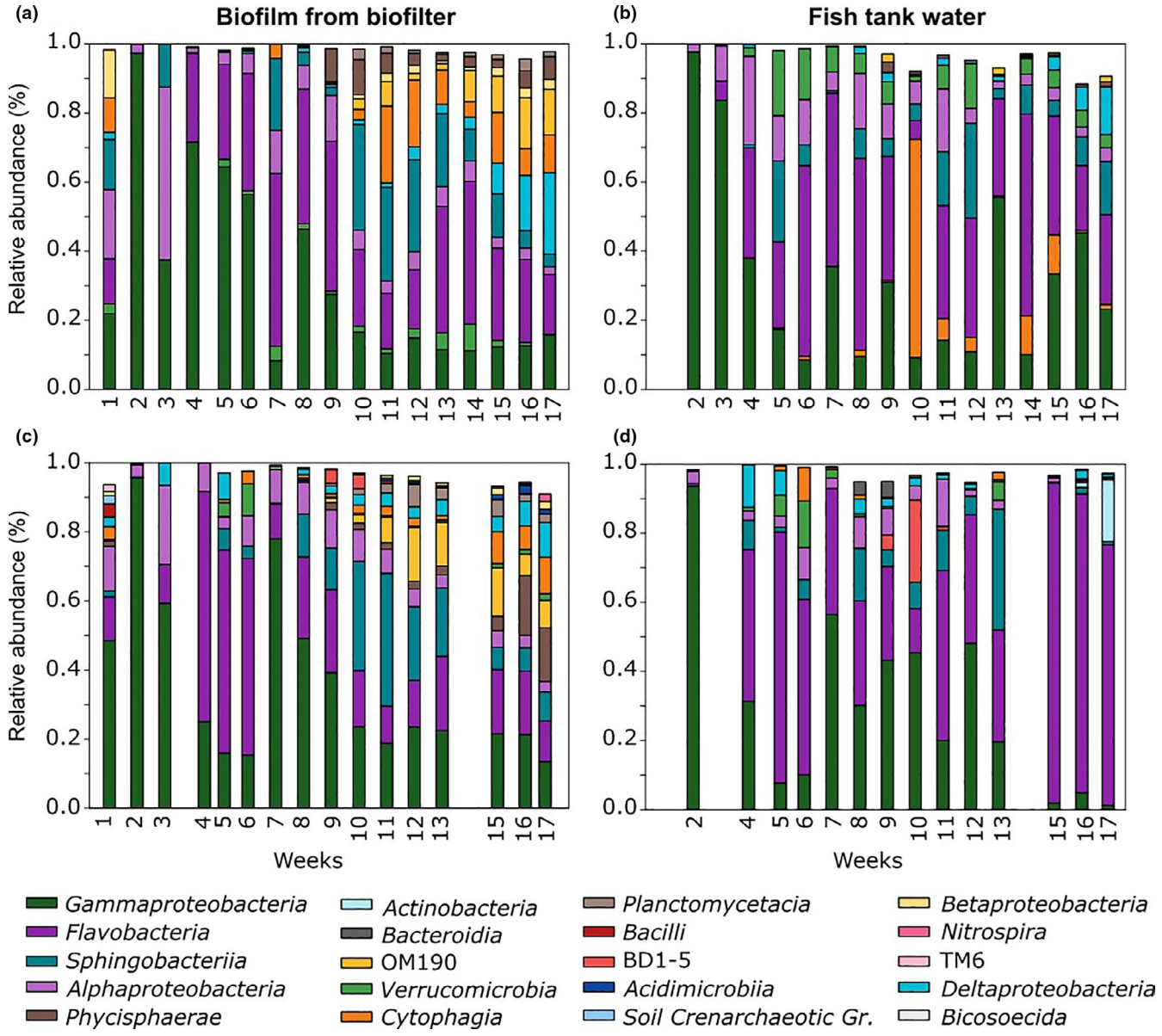

FIGURE 3 The microbial community structures were monitored using 16S rRNA gene sequencing. In a trend of increased diversity over time was observed in sample materials from both Biofilter 1 (a) and Biofilter 2 (c). The microbial communities in sampled fish tank water connected to Biofilter 1 (b) and Biofilter 2 (d) comprised many of the most abundant taxa also found in biofilms, but showed more variation during the rearing cycle. The microbial community structures are shown at class level; and taxa with a relative abundance of >2% in one or more samples are included. See Figure S2 for microbial community structures at genus level

Nitrosococcus (*Gammaproteobacteria*) and *Nitrospira* (*Nitrospira*) (Figure 4a,b). Cultured members of *Nitrosomonas* and *Nitrosococcus* are known to oxidize ammonia to nitrite (Koops & Pommerening-Röser, 2015a), while cultured members of *Nitrospira* are known to oxidize nitrite to nitrate (Spieck & Bock, 2015b). In Biofilter 1, the first nitrifying genus to be detected was *Nitrosomonas*, which occurred in week 9 (Figure 4a), and the decrease in ammonium concentration and subsequent increase in nitrite concentration during the following weeks was probably the first sign of microbial nitrification in the new RAS. In Biofilter 2, *Nitrosococcus* was the first nitrifying genus to be detected, which occurred in week 5 (Figure 4b). The relative abundances of both *Nitrosomonas* and *Nitrosococcus* increased notably in week 9, which corresponded well with the decrease in ammonium concentration starting in week 7. In Biofilter 1, the first step of nitrification seemed to be performed by *Nitrosomonas* only, which comprised a relative abundance of up to 2.9% at the end of the rearing cycle. The highest relative abundance of *Nitrosococcus* in Biofilter 1 was found to be 0.03% (Figure 4a). In Biofilter 2, *Nitrosococcus* seemed to coexist with *Nitrosomonas*, where the relative abundances of *Nitrosomonas* reached a maximum of 2.7%, whereas the relative abundances of *Nitrosococcus* reached a maximum of 3.3% (Figure 4b). Furthermore, the nitrite-oxidizing *Nitrospira* was detected in both biofilters, but seemed to develop more slowly than the ammonia oxidizers. Their

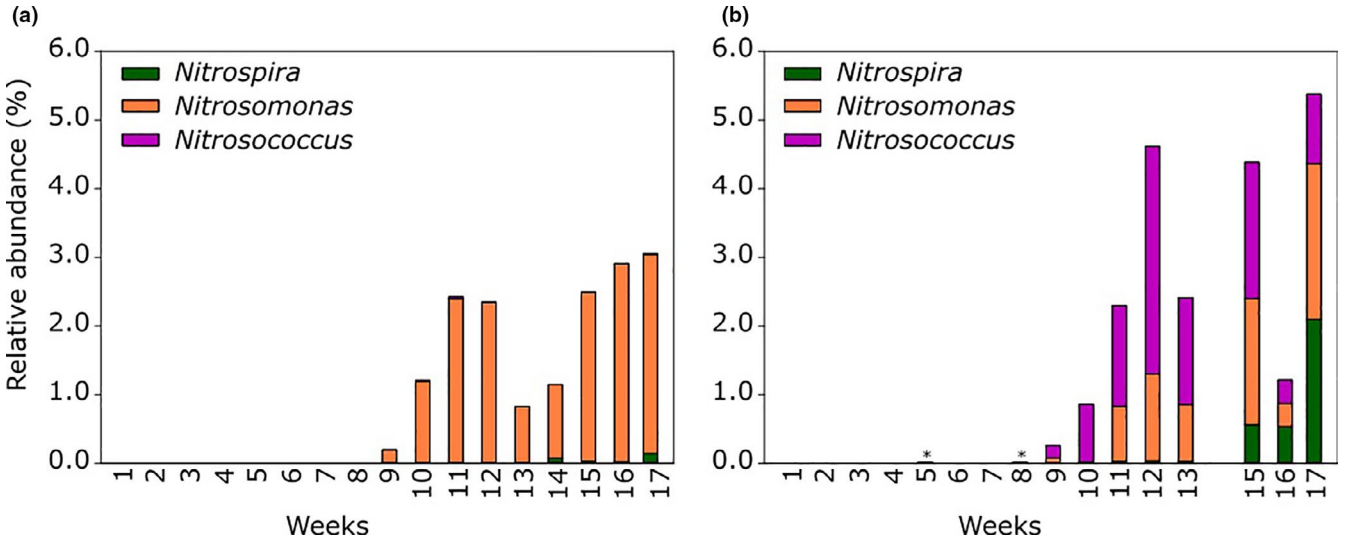

FIGURE 4 Relative abundances of the nitrifying populations in RAS biofilters. Nitrification in Biofilter 1 (a) and Biofilter 2 (b) was performed by representatives within *Nitrosomonas*, *Nitrosococcus* and *Nitrospira*. Low amounts of *Nitrosococcus* were detected in Biofilter 2 in weeks 5 and 8, which is marked with asterisks

relative abundances increased above 0.1% after 17 and 15 weeks in Biofilter 1 and Biofilter 2, respectively, meaning that the oxidizing agent (Loz) was needed in the early phase of biofilter activation in order to remove nitrite chemically. However, the development of the *Nitrospira* population in the two biofilters was very different. In Biofilter 1, the relative abundance of *Nitrospira* reached a maximum of 0.14%, whereas in Biofilter 2, the relative abundance of *Nitrospira* reached a maximum of 2.1%.

In addition, possible nitrifying *Thaumarchaeota* within Archaea was detected in biofilter biofilms. They comprised up to 0.08% of the microbial communities, except for the first week in Biofilter 2 when a relative abundance of 2.4% was detected (Figure 3c). The *Thaumarchaeota* was only detected in early stages of the biofilter activation (within first 4 weeks), and seemed to be outcompeted by the nitrifying bacteria once these established. All the genera of nitrifying bacteria were also detected in fish tank water, but with considerably lower abundances compared to the biofilms in the biofilters.

4 | DISCUSSION

In this case study, we have analysed the microbial colonization of biofilters and fish tank water in a marine RAS for Atlantic salmon post-smolt. Time series of both microbial community structure and physicochemical parameters provided detailed information about microbial development in relation to environmental changes during the first rearing cycle. Parallel laboratory experiments were not included in this study, as the natural development of nitrifying bacteria in an large-scale operational RAS with Atlantic salmon cannot easily be simulated in laboratories; however, some of the observations found in this study could generate interesting and important knowledge on nitrifying bacteria associated with RAS biofilters for future studies.

In general, a successful biofilter activation strategy involves rapid development of nitrifying bacteria that can oxidize ammonium to nitrite and further to nitrate, while keeping the nitrite concentrations below toxic levels. This has proved difficult to achieve due to the low growth rates of these microorganisms. Delong and Losordo (2012) suggested the 'cold start' method (natural activation of the biofilter by introducing a small stock of fish) or the 'seeding' method (activation of biofilter by inoculation with nitrifying bacteria) for biofilter activation in new RAS. In this study, seeding was attempted by inoculation with transferred biofilter material from an established biofilter; however the inoculum ratio was very small (<0.1%) compared to other studies where up to 15% inoculum ratios has been used (Bischoff-Lang, Koch, Thon, & Buck, 2015; Zhu et al., 2016). In addition, the incubation period between biofilter inoculation and fish stocking was very short. Given the slow growth of nitrifying bacteria, the inoculation of the biofilters probably had a very limited effect, as nitrifying bacteria can also be introduced to the biofilter via the fish in the RAS. Hence, the activation of biofilters in the current study was similar to the 'cold start' method analysed by DeLong and Losordo (2012). However, the differences in rearing conditions allowed us to compare 'cold start' with high stocking densities and low stocking densities.

Microbial succession occurred in biofilters and fish tank water in the new RAS, as would be expected. The microbial communities in the biofilms seemed stable over time compared to the free-living microorganisms in the water, which were probably more influenced by changing water parameters. The analysed biofilms in the two biofilters had many taxa in common at the end of the rearing cycle. The dominating taxa common for both biofilters included *Neptuniibacter*, *Glaciecola*, *Colwellia*, *Kordia*, *Flavobacterium*, *Algibacter*, *Planctomycetes* (OM190) and members within *Saprospiraceae* and *Alteromonadaceae*, which were closely related to cultured organoheterotrophic species isolated from seawater and marine habitats worldwide (Arahal

et al., 2007; Bernardet, 2015; Bowman & McMeekin, 2015; Deming & Junge, 2015; Kim, 2015; McIlroy & Nielsen, 2014; Nedashkovskaya & Kim, 2019; Xiao et al., 2019). However, few of the dominating taxa detected at genus level in this study were recognized among dominating taxa described in previous studies from aquaculture systems (Brailo et al., 2019; Rurangwa & Verdegem, 2015; Schreier et al., 2010). The differences in enriched taxa from RAS could be related to water quality and rearing conditions in the fish farms, the maturity of the biofilter at the sampling date, or choice of methods. Overall, the heterotrophic microorganisms represent the majority of the microbiome in RAS and are an important part of a healthy rearing environment for the fish. In addition, these bacteria could possibly prevent disease outbreak to a certain extent by outcompeting opportunistic pathogenic bacteria for resources, as previously suggested (Blancheton et al., 2013 and references therein).

A nitrifying consortium involving *Nitrosomonas* and *Nitrospira* is commonly found in marine RAS biofilters (Blancheton et al., 2013; Foesel et al., 2008; Keuter et al., 2017; Schreier et al., 2010; Tal et al., 2003), and is regarded characteristic for this type of environment. Previous analyses of the matured biofilter in the older RAS unit at the location in this study showed that the nitrifying population comprised *Nitrosomonas* (2.4% relative abundance), *Nitrosococcus* (0.5% relative abundance) and *Nitrospira* (12.3% relative abundance) (Roalkvam, Drønen, Dahle, & Wergeland, 2019). The current study therefore suggested that, among these genera, *Nitrosomonas* established rapidly with abundances similar to mature biofilters. In addition, *Nitrosococcus* bloomed in Biofilter 2, with relative abundances peaking at 3.3% in week 12. *Nitrosococcus* is less commonly found in aquaculture systems, but has previously been detected in such environments (Foesel et al., 2008; Schreier et al., 2010). A study of nitrifying bacteria from a wastewater treatment plant shows that *Nitrosomonas* and *Nitrosococcus* species can coexist in biofilms, possibly due to different oxygen requirements (Gieseke, Bjerrum, Wagner, & Amann, 2003). Furthermore, cultivated members within the *Nitrosococcus* genus have obligate salt requirements (Campbell et al., 2011; Koops & Pommerening-Röser, 2015b), which might explain the higher relative abundance of *Nitrosococcus* in Biofilter 2 compared to Biofilter 1, as Biofilter 2 had higher salinity during the entire rearing cycle. This suggests that different species of ammonia-oxidizing bacteria can coincide in RAS biofilters as a result of niche-specific lifestyles, but that the conditions are more suitable for *Nitrosomonas* in most cases.

Due to the slow development of nitrifying bacteria, periods with high concentrations of ammonium and nitrite can form in immature biofilters. Free ammonia can damage gills, internal organs and osmoregulation in fish, and the equilibrium between ammonia and ammonium in the water is mainly dependent on pH (Thorarensen & Farrell, 2011). It is not recommended to exceed 2 mg/L NH_4^+-N in rearing systems for salmonids (Norwegian Food Safety Authority, 2016), but cultured and well-characterized marine species of *Nitrosomonas* and *Nitrosococcus* can tolerate up to 500 mM (26.8 g/L) NH_4Cl and 1,000 mM (53.5 g/L) NH_4Cl as substrate for ammonia oxidation respectively (Koops & Pommerening-Röser, 2015b, 2015c). In this study, a *Nitrosomonas* population established in both biofilters despite periods of high ammonium concentrations (Figure 1a,b) suggesting that increasing concentrations of ammonium could impair the fish welfare rather than inhibiting the development of ammonia-oxidizing bacteria in the biofilters. Interestingly, the high salinity in combination with the water quality resulting from the low stocking density in Biofilter 2 could have promoted the development of ammonia-oxidizing *Nitrosococcus* 4 weeks earlier than the first detection of ammonia-oxidizing *Nitrosomonas* found in Biofilter 1, which could be of great importance for a rapid biofilter activation strategy.

The most significant difference between the two biofilters at the end of the rearing cycle was the relative abundances of nitrite-oxidizing bacteria. These microorganisms are crucial for converting toxic nitrite to non-toxic nitrate. The nitrite-oxidizing *Nitrospira* occurred earlier in Biofilter 2, compared to Biofilter 1, and also reached 14 times higher relative abundance of this genus by week 17. The nitrite-oxidizing bacteria are known to have low growth rates in general (Abeliovich, 2006; Spieck & Bock, 2015a), and the nitrite-oxidizing bacteria seem to be more sensitive to stress and changing growth conditions in marine RAS (Blancheton et al., 2013; Graham et al., 2007).

High concentrations of nitrite can cause hypoxia in fish, as it oxidizes haemoglobin to methaemoglobin that reduces the blood cells ability to bind oxygen (Jensen, 2003; Kroupova, Machova, & Svobodova, 2005). Nitrite concentrations below 0.5 mg/L-N are recommended for marine aquaculture systems (Norwegian Food Safety Authority, 2016); however, studies on harmful or lethal nitrite concentrations for fish in marine RAS are limited. Previous studies have shown methaemoglobin formation in sea bass exposed to 12.9 mg/L nitrite (Scarano, Saroglia, Gray, & Tibaldi, 1984) and chinook salmon exposed to 37.5 mg/L nitrite (Crawford & Allen, 1977). For Atlantic cod, nitrite concentrations as low as 1.0mg/L can impair growth (Siikavuopio & Saether, 2006). At the fish farm, the welfare of the fish was clearly compromised in the period with highest nitrite concentration in tanks connected to Biofilter 1, which resulted in low activity, wound formation and increased mortality rates. The fish also had reduced amount of mucus in this period, a condition possibly enhanced by the increased Loz concentration, which could impair the fish's natural protection against invading bacteria. The wounds on the flanks of the fish seemed to be caused by mechanical damage; however, it is difficult to confirm the cause of the wounds without further analyses. Contrarily, wound formation was not observed in tanks connected to Biofilter 2, where the nitrite concentrations were much lower.

The genus *Nitrospira* is very diverse, comprising several lineages with isolates from different environments (Daims, Lucker, & Wagner, 2016); however, there is limited knowledge on their physiology and metabolic capacity due to the low growth rates and challenging culture requirements. The low K_m (NO_2^-) shown in kinetic experiments and the chemical properties of the nitrite oxidoreductase used for nitrite oxidation suggest that most *Nitrospira* species are k-strategists that prefer low nitrite concentrations for growth (Daims et al., 2016; Nogueira & Melo, 2006; Spieck & Lipski, 2011).

This is also supported by the low nitrite tolerance in cultivated species, ranging from 1.5 mM (0.07 g/L) to 25 mM (1.15g/L; Off, Alawi, & Spieck, 2010). The highest nitrite concentrations measured in the water at the fish farm were within the maximum tolerance range for cultured *Nitrospira* species, but the possibility of growth inhibition of the uncultured *Nitrospira* species in this RAS due to high nitrite concentrations cannot be overlooked. Furthermore, cultured *Nitrospira* species are grown lithoautotrophically or mixotrophically, where a high concentration of organic matter is shown to inhibit growth (Ehrich, Behrens, Lebedeva, Ludwig, & Bock, 1995; Spieck & Bock, 2015b). Therefore, the high stocking density and resulting high organic load in the water connected to Biofilter 1 might be disadvantageous for the development of *Nitrospira* species during biofilter activation. Finally, growth experiments have shown that reduced partial pressure of oxygen (4 mg/L O_2) is recommended for cultivation of nitrite-oxidizing bacteria (Spieck & Lipski, 2011), and previous studies on nitrifying biofilms suggest that *Nitrospira* prefer the microaerophilic parts of biofilms (Okabe, Satoh, & Watanabe, 1999; Schramm, De Beer, Gieseke, & Amann, 2000). The oxidative agent Loz was added in periods during the first rearing cycle in order to compensate for the lack of microbial nitrite oxidation in the biofilters. Greater amounts of Loz were added to Biofilter 1 compared to Biofilter 2, due to the higher stocking density and nitrite concentrations. The highly reactive Loz might remove microaerophilic niches needed by the *Nitrospira* species, thereby preventing optimal growth conditions for these nitrite oxidizers in Biofilter 1. However, additional studies are needed to reveal optimum oxygen requirements for the uncultured nitrite-oxidizing bacteria found in marine RAS.

Overall, the relative abundance of *Nitrospira* did not exceed 0.14% in Biofilter 1 and 2.1% in Biofilter 2 during the first rearing cycle, which is far below relative abundance (<12.3%) observed in mature biofilters (Roalkvam, Drønen, Dahle, & Wergeland, 2019a). Although nitrifying bacteria established in the biofilters with 17 weeks, an activation period of 4 months did not seem to be enough time to ensure complete maturation of the biofilters or reaching maximum nitrification capacity. However, the community structures at genus level implied that activation of Biofilter 2 was more successful than Biofilter 1, due to its overall higher relative abundance of nitrifying bacteria. Moreover, the staff at the fish farm reported in the period after the first rearing cycle that the water in Biofilter 2 had a more stable chemical composition and lower ammonia and nitrite levels than water in Biofilter 1.

In conclusion, the results indicate that stocking density is not largely affecting the development of the class-level community structure in biofilters; however, it seems to greatly affect the water quality and thereby the establishment of nitrifying bacteria during the biofilter activation period. The biofilter associated with low stocking density provided rearing conditions with water quality that most likely encouraged a successful development of nitrite-oxidizing bacteria in the biofilter. This biofilter reached the highest overall relative abundance of nitrifying bacteria, where particularly the nitrite-oxidizing bacteria were significantly more abundant compared to the biofilter associated with high stocking density. Based on this study, a low stocking density during biofilter activation is recommended as this is shown to provide excellent growth conditions favouring a rapid development of a stable biofilter with an active nitrifying population. In addition, larger inoculum ratio and longer incubation of the biofilters before fish stocking are strongly suggested for future RAS biofilter activation strategies.

ACKNOWLEDGMENTS

This work was funded by the Norwegian Seafood Research Fund (FHF), through the project 'Microbial colonization in recirculation aquaculture systems (RAS)' (project number 901470). We declare that there are no conflicts of interest associated with this publication.

DATA AVAILABILITY STATEMENT

The 16S rRNA gene sequences are available at GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession number MN631385–MN632449.

ORCID

Irene Roalkvam https://orcid.org/0000-0002-6972-0001

REFERENCES

Abeliovich, A. (2006). The nitrite oxidizing bacteria. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The Prokaryotes: Volume 5: Proteobacteria: Alpha and Beta Subclasses* (pp. 861–872). New York, NY: Springer.

Arahal, D. R., Lekunberri, I., González, J. M., Pascual, J., Pujalte, M. J., Pedrós-Alió, C., & Pinhassi, J. (2007). *Neptuniibacter caesariensis* gen. nov., sp. nov., a novel marine genome-sequenced gammaproteobacterium. *International Journal of Systematic and Evolutionary Microbiology*, *57*(5), 1000–1006. https://doi.org/10.1099/ijs.0.64524-0

Bernardet, J. F. (2015). Flavobacteriaceae. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's manual of systematics of Archaea and bacteria* (pp. 853–871). https://doi.org/10.1002/9781118960608.fbm00069

Bischoff-Lang, A., Koch, L., Thon, M., & Buck, B. H. (2015). Biofilter inoculation in recirculating aquaculture systems. *Global Aquaculture Advocat*, 35–36.

Blancheton, J. P., Attramadal, K. J. K., Michaud, L., D'Orbcastel, E. R., & Vadstein, O. (2013). Insight into bacterial population in aquaculture systems and its implication. *Aquacultural Engineering*, *53*, 30–39. https://doi.org/10.1016/j.aquaeng.2012.11.009

Bowman, J. P., & McMeekin, T. A. (2015). Alteromonadales ord. nov. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's manual of systematics of Archaea and bacteria* (pp. 3057–3059). https://doi.org/10.1016/j.aquaculture.2018.12.078

Brailo, M., Schreier, H. J., McDonald, R., Maršić-Lučić, J., Gavrilović, A., Pećarević, M., & Jug-Dujaković, J. (2019). Bacterial community analysis of marine recirculating aquaculture system bioreactors for complete nitrogen removal established from a commercial inoculum. *Aquaculture*, *503*, 198–206. https://doi.org/10.1016/j.aquaculture.2018.12.078

Campbell, M. A., Chain, P. S. G., Dang, H., El Sheikh, A. F., Norton, J. M., Ward, N. L., … Klotz, M. G. (2011). *Nitrosococcus watsonii* sp. nov., a new species of marine obligate ammonia-oxidizing bacteria that is not omnipresent in the world's oceans: Calls to validate the names 'Nitrosococcus halophilus' and 'Nitrosomonas

mobilis'. *FEMS Microbiology Ecology*, *76*(1), 39–48. https://doi.org/10.1111/j.1574-6941.2010.01027.x

Crawford, R. E., & Allen, G. H. (1977). Seawater inhibition of nitrite toxicity to chinook salmon. *Transactions of the American Fisheries Society*, *106*(1), 105–109. https://doi.org/10.1577/1548-8659(1977)106<105:SIONTT>2.0.CO;2

Daims, H., Lucker, S., & Wagner, M. (2016). A new perspective on microbes formerly known as nitrite-oxidizing bacteria. *Trends in Microbiology*, *24*(9), 699–712. https://doi.org/10.1016/j.tim.2016.05.004

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., & Pedersen, P. B. (2013). Farming different species in RAS in Nordic countries: Current status and future perspectives. *Aquacultural Engineering*, *53*, 2–13. https://doi.org/10.1016/j.aquaeng.2012.11.008

De Guzman, M. L. (2018). New land-based salmon producer bares plans to build global RAS facilities. Retrieved from https://www.hatcheryinternational.com/recirc/new-land-based-salmon-production-company-bares-plans-to-build-global-ras-facilities-3307

DeLong, D. P., & Losordo, T. M. (2012). How to start a biofilter. Southern Regional Aquaculture Center Publication, no. 4502.

Deming, J. W. & Junge, K. (2015). Colwellia. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's manual of systematics of Archaea and bacteria* (pp. 1257–1369). https://doi.org/10.1002/9781118960608.gbm01090

Ehrich, S., Behrens, D., Lebedeva, E., Ludwig, W., & Bock, E. (1995). A new obligately chemolithoautotrophic, nitrite-oxidizing bacterium, *Nitrospira moscoviensis* sp. nov. and its phylogenetic relationship. *Archives of Microbiology*, *164*(1), 16–23. https://doi.org/10.1007/BF02568729

Foesel, B. Ã. U., Gieseke, A., Schwermer, C., Stief, P., Koch, L., Cytryn, E., … Schramm, A. (2008). *Nitrosomonas* Nm143-like ammonia oxidizers and *Nitrospira marina*-like nitrite oxidizers dominate the nitrifier community in a marine aquaculture biofilm. *FEMS Microbiology Ecology*, *63*(2), 192–204. https://doi.org/10.1111/j.1574-6941.2007.00418.x

Fofonoff, N. P., & Millard, R. C. (1983). Algorithms for computation of fundamental properties of seawater. *UNESCO Technical Papers in Marine Science*, *44*, 6–9. https://unesdoc.unesco.org/ark:/48223/pf0000059832

Furuset, A. (2018). Slik er trendene i RAS-markedet [Norwegian]. Tekfisk. Retrieved from https://fiskeribladet.no/tekfisk/nyheter/?artikkel=62642.

Gieseke, A., Bjerrum, L., Wagner, M., & Amann, R. (2003). Structure and activity of multiple nitrifying bacterial populations co-existing in a biofilm. *Environmental Microbiology*, *5*(5), 355–369. https://doi.org/10.1046/j.1462-2920.2003.00423.x

Graham, D. W., Knapp, C. W., Van Vleck, E. S., Bloor, K., Lane, T. B., & Graham, C. E. (2007). Experimental demonstration of chaotic instability in biological nitrification. *Isme Journal*, *1*(5), 385–393. https://doi.org/10.1038/ismej.2007.45

Jensen, F. B. (2003). Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *135*(1), 9–24. https://doi.org/10.1016/S1095-6433(02)00323-9

Keuter, S., Beth, S., Quantz, G., Schulz, C., & Spieck, E. (2017). Longterm monitoring of nitrification and nitrifying communities during biofilter activation of two marine recirculation aquaculture systems (RAS). *International Journal of Aquaculture and Fishery Sciences*, *3*(3), 51–61. https://doi.org/10.17352/2455-8400.000029

Kim, S. J. (2015). Kordia. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–4). https://doi.org/10.1002/9781118960608.gbm00319

Koops, H., & Pommerening-Röser, A. (2015a). The lithoautotrophic ammonia-oxidizing bacteria. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1821–1917): Wiley. https://doi.org/10.1002/9781118960608.bm00013

Koops, H., & Pommerening-Röser, A. (2015b). Nitrosococcus. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's manual of systematics of Archaea and bacteria* (pp. 1–3). https://doi.org/10.1002/9781118960608.gbm01111

Koops, H. (2015c). Nitrosomonas. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–6). https://doi.org/10.1002/9781118960608.gbm00991

Kroupova, H., Machova, J., & Svobodova, Z. (2005). Nitrite influence on fish: A review. *Veterinarni Medicina*, *50*(11), 461–471. https://10.17221/5650-Vetmed

McIlroy, S. J., & Nielsen, P. H. (2014). The family *Saprospiraceae*. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The prokaryotes: Other major lineages of bacteria and the Archaea* (pp. 863–889). Berlin, Heidelberg: Springer.

Nedashkovskaya, O. I., & Kim, S. G. (2019). Algibacter. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–18). https://doi.org/10.1002/9781118960608.gbm00294.pub2

Nogueira, R., & Melo, L. F. (2006). Competition between *Nitrospira* spp. and *Nitrobacter* spp. in nitrite-oxidizing bioreactors. *Biotechnology. Bioengineering*, *95*(1), 169–175. https://doi.org/10.1002/bit.21004

Norwegian Food Safety Authority. (2016). Merknad til § 21 Vannkvalitet i landbaserte akvakulturanlegg [Norwegian].

Off, S., Alawi, M., & Spieck, E. (2010). Enrichment and physiological characterization of a novel *Nitrospira*-like bacterium obtained from a marine sponge. *Applied and Environmental Microbiology*, *76*(14), 4640–4646. https://10.1128/AEM.00320-10

Okabe, S., Satoh, H., & Watanabe, Y. (1999). *In situ* analysis of nitrifying biofilms as determined by *in situ* hybridization and the use of microelectrodes. *Applied and Environmental Microbiology*, *65*(7), 3182–3191. https://doi.org/10.1128/AEM.65.7.3182-3191.1999

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Wagner, H. (2018). vegan: Community Ecology Package. Retrieved from http://CRAN.R-project.org/package=vegan

R Core Team (2018). *R: A language and environment for statistical computing (Version 3.5)*. Vienna, Austria: https://www.R-project.org/

Roalkvam, I., Drønen, K., Dahle, H., & Wergeland, H. I. (2019a). Comparison of active biofilm carriers and commercially available inoculum for activation of biofilters in recirculating aquaculture systems (RAS). *Aquaculture*, *514*, 734480. https://doi.org/10.1016/j.aquaculture.2019.734480

Roalkvam, I., Drønen, K., Dahle, H., & Wergeland, H. I. (2019b). Microbial communities in a flow-through fish farm for lumpfish (*Cyclopterus lumpus* L.) during healthy rearing conditions. *Frontiers in Microbiology*, *10*, 1594–1605. https://10.3389/fmicb.2019.01594

Ruan, Y.-J., Guo, X.-S., Ye, Z.-Y., Liu, Y., & Zhu, S.-M. (2015). Bacterial community analysis of different sections of a biofilter in a full-scale marine recirculating aquaculture system. *North American Journal of Aquaculture*, *77*(3), 318–326. https://doi.org/10.1080/15222055.2015.1017128

Rurangwa, E., & Verdegem, M. C. J. (2015). Microorganisms in recirculating aquaculture systems and their management. *Reviews in Aquaculture*, *7*(2), 117–130. https://doi.org/10.1111/raq.12057

Scarano, G., Saroglia, M. G., Gray, R. H., & Tibaldi, E. (1984). Hematological responses of sea bass *Dicentrarchus labrax* to sublethal nitrite exposures. *Transactions of the American Fisheries Society*, *113*(3), 360–364. https://doi.org/10.1577/1548-8659(1984)113<360:HROSBD>2.0.CO;2

Schramm, A., De Beer, D., Gieseke, A., & Amann, R. (2000). Microenvironments and distribution of nitrifying bacteria in a

membrane-bound biofilm. *Environmental Microbiology*, *2*(6), 680–686. https://doi.org/10.1046/j.1462-2920.2000.00150.x

Schreier, H. J., Mirzoyan, N., & Saito, K. (2010). Microbial diversity of biological filters in recirculating aquaculture systems. *Current Opinion in Biotechnology*, *21*(3), 318–325. https://doi.org/10.1016/j.copbio.2010.03.011

Siikavuopio, S. I., & Saether, B. S. (2006). Effects of chronic nitrite exposure on growth in juvenile Atlantic cod, Gadus morhua. *Aquaculture*, *255*(1–4), 351–356. https://doi.org/10.1016/j.aquaculture.2005.11.058

Spieck, E., & Bock, E. (2015a). The lithoautotrophic nitrite-oxidizing bacteria. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's manual of systematics of Archaea and bacteria* (pp. 259–610). Wiley. https://doi.org/10.1002/9781118960608.gbm007

Spieck, E., & Bock, E. (2015b). Nitrospira. In W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund, & S. Dedysh (Eds.), *Bergey's manual of systematics of Archaea and bacteria*.

Spieck, E., & Lipski, A. (2011). Cultivation, growth physiology, and chemotaxonomy of nitrite-oxidizing bacteria. *Methods in Enzymology*, *486*, 109–130. https://doi.org/10.1016/B978-0-12-381294-0.00005-5

Summerfelt, S. (2015). Update on land-based closed-containment systems for food fish. Paper presented at the Aquaculture Canada 2015: Blue Revolution 2.0, Nanaimo, BC, Canada. https://www.palomaquaculture.com/support-files/palom-aquaculture-steve-summerfelt-ras-update-june-5-2015.pdf

Tal, Y., Watts, J. E. M., Schreier, S. B., Sowers, K. R., & Schreier, H. J. (2003). Characterization of the microbial community and nitrogen transformation processes associated with moving bed bioreactors in a closed recirculated mariculture system. *Aquaculture*, *215*(1), 187–202. https://doi.org/10.1016/S0044-8486(02)00372-1

Thorarensen, H., & Farrell, A. P. (2011). The biological requirements for post-smolt Atlantic salmon in closed-containment systems. *Aquaculture*, *312*(1), 1–14. https://doi.org/10.1016/j.aquaculture.2010.11.043

Xiao, Y.-K., Yan, Z.-F., Kim, Y., Lee, H.-M., Trinh, H., Yang, J.-E., … Kook, M. (2019). *Glaciecola amylolytica* sp. nov., an amylase-producing bacterium isolated from seawater. *International Journal of Systematic and Evolutionary Microbiology*, *69*(4), 957–963. https://doi.org/10.1099/ijsem.0.003222

Zhu, S., Shen, J., Ruan, Y., Guo, X., Ye, Z., Deng, Y., & Shi, M. (2016). The effects of different seeding ratios on nitrification performance and biofilm formation in marine recirculating aquaculture system biofilter. *Environmental Science and Pollution Research International*, *23*(14), 14540–14548. https://doi.org/10.1002/9781118960608.gbm00780

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Roalkvam I, Drønen K, Dahle H, Wergeland HI. A case study of biofilter activation and microbial nitrification in a marine recirculation aquaculture system for rearing Atlantic salmon (*Salmo salar* L.). *Aquac Res*. 2020;00:1–11. https://doi.org/10.1111/are.14872