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Microbial communities in full-scale woodchip bioreactors treating aquaculture effluents

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ABSTRACT

Woodchip bioreactors are being successfully applied to remove nitrate from commercial land-based recirculating aquaculture system (RAS) effluents. In order to understand and optimize the overall function of these bioreactors, knowledge on the microbial communities, especially on the microbes with potential for production or mitigation of harmful substances (e.g. hydrogen sulfide; H_2S) is needed. In this study, we quantified and characterized bacterial and fungal communities, including potential H_2S producers and consumers, using qPCR and high throughput sequencing of 16S rRNA gene. We took water samples from bioreactors and their inlet and outlet, and sampled biofilms growing on woodchips and on the outlet of the three full-scale woodchip bioreactors treating effluents of three individual RAS. We found that bioreactors hosted a high biomass of both bacteria and fungi. Although the composition of microbial communities of the inlet varied between the bioreactors, the conditions in the bioreactors selected for the same core microbial taxa. The H_2S producing sulfate reducing bacteria (SRB) were mainly found in the nitrate-limited outlets of the bioreactors, the main groups being deltaproteobacterial *Desulfobulbus* and *Desulfovibrio*. The abundance of H_2S consuming sulfate oxidizing bacteria (SOB) was 5–10 times higher than that of SRB, and SOB communities were dominated by *Arcobacter* and other genera from phylum Epsilonbacteraeota, which are also capable of autotrophic denitrification. Indeed, the relative abundance of potential autotrophic denitrifiers of all denitrifier sequences was even 54% in outlet water samples and 56% in the outlet biofilm samples. Altogether, our results show that the highly abundant bacterial and fungal communities in woodchip bioreactors are shaped through the conditions prevailing within the bioreactor, indicating that the bioreactors with similar design and operational settings should provide similar function even when conditions in the preceding RAS would differ. Furthermore, autotrophic denitrifiers can have a significant role in woodchip biofilters, consuming potentially produced H_2S and removing nitrate, lengthening the operational age and thus further improving the overall environmental benefit of these bioreactors.

1. Introduction

Land-based recirculating aquaculture systems (RAS) are a current state-of-art technology for environmentally sustainable aquaculture production. Being based on high water recycling, they discharge nutrient-rich effluents, which require efficient discharge treatment to meet the tightening environmental regulations. Woodchip denitrification reactors have been successfully applied to remove nitrate from e.g. agricultural tile drainage water, ground water, and aquaculture effluents (Christianson et al., 2021), being now commercially applied to treat RAS discharges (von Ahnen et al., 2018). The common understanding is that in these bioreactors, nitrate removal is based on an active microbial community growing on woodchips, that conducts denitrification i.e. reduces water nitrate into N_2 gas using woodchip carbon as an electron donor (Lopez-Ponnada et al., 2017).

In the woodchip bioreactors, the volumetric nitrate removal rates are considered to depend on influent water characteristics (N loading rates, temperature) (Lepine et al., 2020), and the previously measured removal rates vary from 17 to 39 g NO_3–N m^{-3} d^{-1} achieved in the pilot-scale systems (Lepine et al, 2016, 2020) to 5 and 11 g NO_3–N m^{-3}

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d^{-1} measured in the full-scale reactors treating commercial RAS discharges located outside under low environmental temperature conditions (<10°C) (von Ahnen et al., 2018). In addition to nitrate removal rates, removal efficiency (as %) is an important variable for estimating the operation of woodchip reactors. Removal efficiency has been found to increase with hydraulic retention time (Hoover et al., 2016), explained with the increased contact time between denitrifying microbial biofilm and water nitrate. However, overly long retention times in relation to incoming nitrate concentration can lead to nitrate limitation in the outlet part of the reactors, decreasing the overall nitrate removal rate of the reactor (von Ahnen et al., 2018). Indeed, we recently demonstrated that nitrate limitation and high carbon to nitrate ratio can promote nitrate reduction to biologically reactive ammonium (Aalto et al., 2020), which might be transferred further to nature with bioreactor discharge. Furthermore, nitrate limitation can also cause additional problems, as odorous and toxic hydrogen sulfide (H_2S) has been found to be produced when nitrate concentration falls below 3 mg/L or ORP is lower than −100mV (Malá et al., 2020).

Several studies have suggested optimal and cost-efficient design parameters for construction and operation of woodchip bioreactors treating RAS effluents (e.g. hydraulic retention time, manifold design, woodchip replacement rate; Lepine et al., 2020, 2018, 2016; von Ahnen et al., 2016). At the same time, the comprehensive understanding on the microbiology of these bioreactors is still thin, being mainly based on laboratory-scale bioreactors, which are operated under strictly controlled conditions (e.g. Kiani et al., 2020; von Ahnen et al., 2019), or the focus has been on measuring the abundance and activity of denitrifying microbes (Aalto et al., 2020). However, in addition to the denitrifying microbes, these systems are expected to host a diverse community of microbes, including groups degrading complex wood-derived organic matter into biodegradable form to be available for denitrifying microbes. In addition to bacteria, fungi can play an important role in offering a low but steady degradation of cellulose and lignin in woodchips (Fatehi-Pouladi et al., 2019; Porter et al., 2015) and have shown to have potential to remove nitrate in woodchip bioreactors (Aldossari and Ishii, 2020; Yao et al., 2020), but their prevalence in woodchip bioreactors is currently unexplored. Furthermore, the occurrence of H_2S under nitrate limited conditions indicates the presence of sulfate reducing bacteria (SRB), which use a variety of simple organic matter compounds (e.g. organic acids, amino acids) as electron donors to produce hydrogen sulfide from sulfate (Muyzer and Stams, 2008). Indeed, SRB have been found in woodchip bioreactors treating synthetic inorganic wastewater, colonizing both deep and surface layers of woodchips (Yamashita et al., 2011), indicating potential for H_2S production also in woodchip bioreactors treating RAS effluents, which is currently unquantified. However, there is also a group of sulfur oxidizing bacteria (SOB) that can consume the hydrogen sulfide produced, which could thrive in woodchip bioreactors with long retention times and H_2S production.

Woodchip bioreactors are currently applied for nitrate removal in commercial RAS, but in order to optimize their performance in both fresh and marine water, and to avoid potential unwanted microbial processes, it is important to gain knowledge on the overall microbial community, including currently fully neglected fungi, as well as on the microbial groups that can produce or consume H_2S. In this study, we examined the microbial communities in three full-scale woodchip reactors treating the effluents of three commercial RAS, where the rates and microbiology of nitrate removal has already been examined (Aalto et al., 2020). In order to understand the interactions between bacterial and fungal communities, we measured the copy numbers of universal bacterial and archaeal (16S rRNA) and fungal (ITS) marker genes using qPCR. In addition, we examined the distribution and abundance of sulfate reducing bacteria carrying *dsr*A-gene in order to reveal the genetic potential for the harmful hydrogen sulfide production in these systems. Finally, we compared the bacterial community diversity and structure using next generation amplicon sequencing targeted on 16S rRNA gene, focusing especially on the microbial groups with known functions of sulfate reduction and sulfide oxidation as well as on the relative importance of heterotrophic and autotrophic denitrifiers.

2. Materials and methods

2.1. Study sites

The three woodchip bioreactors (Suppl. Fig. 1a) treated effluents from commercial freshwater RAS rearing rainbow trout (*Oncorhynchus mykiss*), each bioreactor located to a different RAS (RAS 1–3), with a distance of 50–100 km between RASs. The bioreactor 1 was constructed with horizontal flow and bioreactors 2 and 3 with a vertical flow from the surface to bottom (Suppl. Fig. 2). The bioreactors contained a blend of woodchips with volumes of 250, 650, and 1250 m^3 of woodchips, respectively, and were operated with hydraulic retention times (HRT) of 10, 11, and 18 h, respectively. A detailed description of the study sites is provided in Suppl. Fig. 2.

2.2. Sampling

Water samples for microbiological analysis were collected using syringe filters (0.22 μm Millipore Express® PLUS PES membrane) from inlet, outlet, and in 1–3 sampling points (SP1-3) within the woodchip bioreactors in October 2017 and March 2018 (see Suppl. Fig. 2). Per sampling point, one water sample was taken in October 2017, and two replicate water samples in March 2018. The water was filtered until filter was fully saturated, the volume of filtered water varying between 6 and 189 mL. In addition, woodchip biofilm samples from the subsurface layer in the middle of the bioreactor and outlet biofilm samples from the biomass growing near the outlet pipe were collected manually in October 2017. Samples were frozen immediately after the sampling to −20 °C. Analysis of water chemistry (nitrate-nitrogen, nitrite-nitrogen, total ammonial nitrogen, sulfate-sulfur) was done from the same sampling points, using ion chromatograph (Thermo Scientific Dionex Ion Chromatography, Mettler Toledo) and spectrophotometric method (for TAN; see Aalto et al. (2020)) (Suppl. Table 1).

2.3. Quantification of bacteria, archaea, and fungi, and sulfate reducing bacteria

DNA extractions were done with DNeasy PowerLyzer PowerSoil DNA isolation kit (Qiagen) from freeze-dried biofilm and water filter samples. DNA concentrations were quantified with Qubit® dsDNA HS Assay Kit and Qubit 2.0. fluorometer (Thermo Fischer Scientific). The abundance of bacteria and archaea (16S rRNA gene: 515F–Y; Parada et al., 2016, 806R; Caporaso et al., 2011), fungi (ITS gene: ITS1F; Gardes and Bruns, 1993, ITS2; White et al., 1990), and sulfate reducing bacteria (*dsr*A gene: RH1-dsr-F/RH3-dsr-R; Ben-Dov et al., 2007) were quantified by qPCR amplification with Bio-Rad CFX96 Real-Time System (Bio-Rad Laboratories). The qPCR reactions of 25 μl consisted of 1x Maxima SYBR Green/Fluorescein qPCR Master Mix (Thermo Fisher Scientific), reverse and forward primers (0.4 μM for 16S rRNA and ITS, 0.2 μM for *dsr*A) and 6 ng of template DNA. The thermal conditions for 16S rRNA and ITS gene amplification were: initial denaturation at 95 °C for 10 min followed by 35 cycles at 95 °C for 30 s, 50 °C (16S rRNA)/52 °C (ITS) for 30 s and 72 °C for 60 s. For *dsr*A gene, the thermal conditions consisted of initial denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, 60 °C for 1 min and 72 °C for 30 s. Amplification efficiencies were 94–99%.

2.4. Microbial community composition

Microbial community composition was studied using next generation sequencing targeting V4 region of the 16S rRNA gene with primers 515F–Y (Parada et al., 2016) and 806R (Caporaso et al., 2011) using the

same samples than in qPCR. The sequence analysis was done using mothur (version 1.39.5; Schloss et al., 2009) as in von Ahnen et al. (2019). The total amount of sequences obtained was 1,527,588, and after subsampling for calculating alpha and beta diversities, each sample contained 13,552 sequences. Sequences have been submitted to NCBI Sequence Read Archive under BioProject PRJNA731005.

The previously published taxonomic information was used to pick the potentially sulfate reducing bacteria (Müller et al., 2015; Muyzer and Stams, 2008) and potential sulfur oxidizers/autotrophic denitrifying bacteria (Lu et al., 2018; Roalkvam et al., 2015) from the total microbial community data. The heterotrophic denitrification bacteria were picked based on the previous literature (Grießmeier et al, 2017, 2019, 2017; Saarenheimo et al., 2015). The percentage of autotrophic denitrification bacteria of all denitrifiers was calculated by dividing the amount of sequences assigned to autotrophic denitrifiers by the sum of the amount of sequences assigned to autotrophic and heterotrophic denitrifiers.

2.5. Statistical analysis

All statistical analyses were conducted using R version 3.6.3 (R Core Team, 2020). The processing of the sequencing data was done using package "phyloseq" (McMurdie and Holmes, 2013) and figures were produced with the package "ggplot2" (Wickham, 2016). The differences in the abundance of bacterial and fungal gene copies between the sampling points and years were examined with Two-way ANOVA, as the assumptions of the normality and variance homogeneity of the residuals were met.

3. Results & discussion

Bacteria and fungi were abundant in all the collected samples (Fig. 1). No archaeal sequences were observed in the libraries constructed using the broad-range primers, which should cover also archaeal sequences. The relationship of bacterial and fungal abundances varied between sampling points within reactors and between years (Two-way ANOVA, 2017: Sampling point × Gene: $F_{4,95} = 15.1$, $P < 0.001$, 2018: no interaction, Sampling point: $F_{2,54} = 94.7$, $P < 0.001$, Gene: $F_{1,54} = 67.7$, $P < 0.001$; Suppl. Table 2). In 2017, the abundances of bacteria and fungi were similar in the water and biofilm samples collected from the reactors (Tukey post-hoc analysis, $P > 0.05$, Suppl. Table 2), while bacteria were more abundant than fungi in the inlet and outlet water samples ($P < 0.001$), and in the outlet biofilm samples ($P < 0.001$). In 2018, bacteria were in general more abundant than fungi ($P < 0.001$). When comparing the abundance between sampling points, the abundances of both bacteria and fungi were higher in the water samples collected within the reactors than in the inlet or outlet water samples ($P < 0.001$). The equally high abundance of fungi and bacterial in woodchip biofilm and water samples collected within the bioreactors indicates that fungi are also important members of the woodchip microbiome, although being neglected in the previous studies. Recently, fungi inhabiting woodchip bioreactors have found to possess denitrification capacity (Aldossari and Ishii, 2020), but in our recent paper, we found no evidence for that in these bioreactors (Aalto et al., 2020). This suggests that the role of fungi is probably more on degrading lignocellulose or complex organic substances into more bioavailable forms, as has been recently suggested to happen during the woodchip composting process, where fungi were abundant on the woodchip surface (Kouanda and Hua, 2021). By degrading lignin, fungi facilitate the function of bacteria carrying out enzymatic hydrolysis under anaerobic conditions, which produce simple carbon compounds supporting the denitrifying microbial community. The specific roles of the fungi and bacteria support high abundance of both groups within the reactors. Furthermore, our results suggest that the high bacterial abundance in the bioreactors is supported by bacteria entering with the incoming water, since the bacterial abundance is high in inlet water samples, while fungi probably

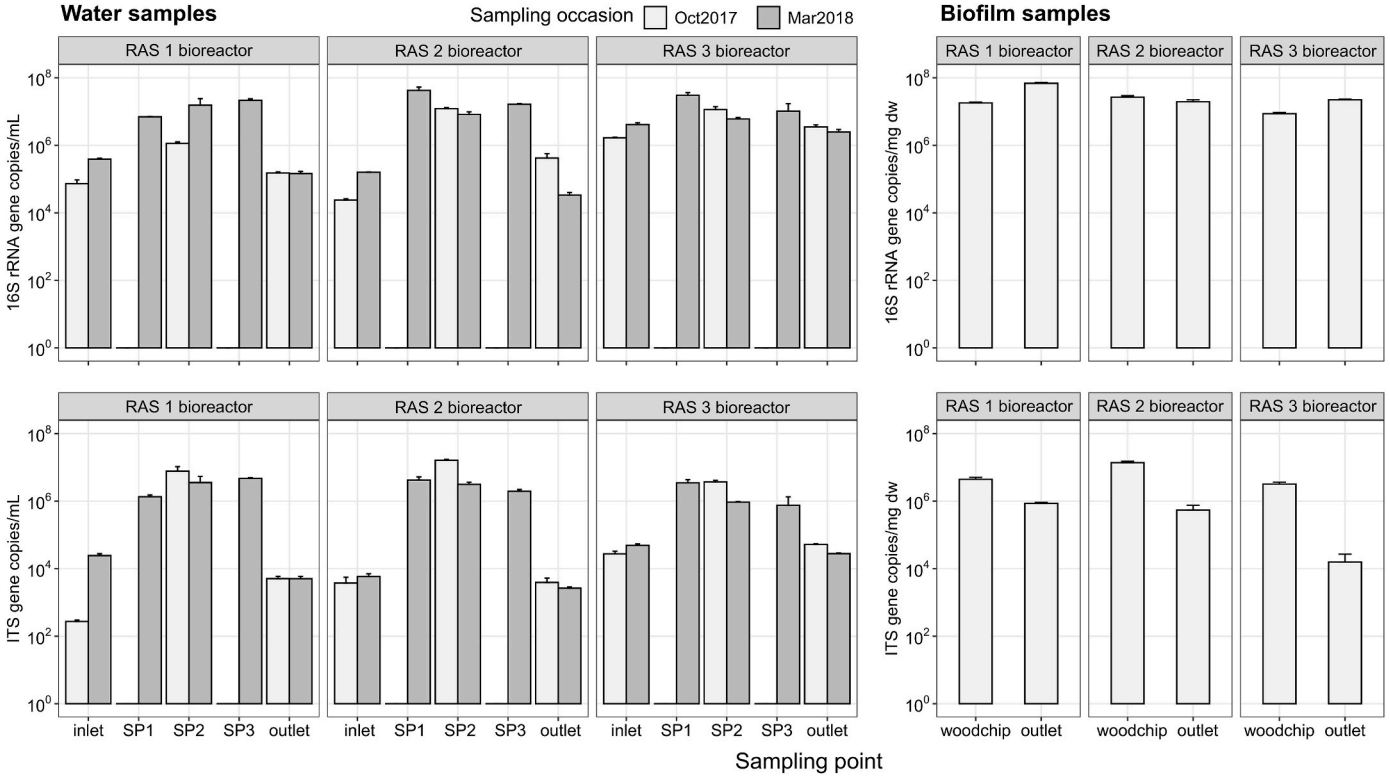

Fig. 1. The abundance (gene copies mL^{-1} or mg dw^{-1}) of bacteria (16S rRNA gene copies) and fungi (ITS gene copies) in the water samples collected in inlet, within the reactors (SP1-3), and outlet, and in biofilm on the woodchips and in the outlet at three bioreactors (each located to a different commercial RAS) on two sampling occasions (October 2017 and March 2018). No archaea were observed. Amount of replicates: water samples n = 2–3, woodchip biofilm n = 6, outlet biofilm n = 3.

enter with the woodchips and increase in abundance under the favourable conditions prevailing in the bioreactor, since their abundance was low in the incoming water. Fungi have shown to have capacity for degradation of pollutant and toxic materials (Singh et al., 2019), suggesting that woodchip bioreactors with an abundant fungal community can potentially remove potentially harmful substances (e.g. antibiotics, disinfection chemicals), but more studies are needed in future to verify this.

The abundance of the microbes carrying the typical sulfate reduction marker gene, *dsr*A, was quantified in the woodchip and outlet biofilm samples in October, and in bioreactor water samples in March (Fig. 2). In addition, the abundance was measured in the inlet and outlet samples in the bioreactor 1. The results revealed that sulfate reducing bacteria (SRB) were abundant in both the woodchip and outlet biofilm samples, although being also present in the water samples within the reactors. Furthermore, the SRB abundance increased gradually from SP1 to SP3 in the bioreactor 1, along the decreasing nitrate concentrations (Aalto et al., 2020). The abundance of SRB was low in inlet and outlet water samples, indicating that they disappear fast when conditions are not favourable i.e. when the amount of organic matter decreases or oxygen concentrations increase, since generally SRB only thrive under organic matter rich and anoxic conditions (Muyzer and Stams, 2008).

The Bray-Curtis and Jaccard similarities were used to quantify the differences in the relative abundance of OTUs and in the occurrence of OTUs between different sampling points, sampling occasions, and bioreactors (Suppl. Table 3). The Jaccard similarities were always higher than the Bray Curtis ones. Since Jaccard similarity index is only based on presence or absence of OTUs, but Bray Curtis also takes the abundance also into account, the result indicates that microbial community dissimilarities i.e. the differences in the communities were due to differences in the relative abundance of the same OTUs. When inspecting further the Bray-Curtis similarities, the microbial communities in the inlet water showed moderate similarities (≤70%), which can be due to the differences in the water chemistry (Suppl. Fig. 2, Suppl. Table 1), location and system setup of the farm, e.g. bioreactors 2 and 3 being preceded by wetlands, while bioreactor 1 receives water directly from the RAS. The community similarity measured from bioreactor water was higher between bioreactors (64 ± 7%) than within bioreactors (55 ± 12%; see Suppl. Fig. 3), indicating that the conditions in the bioreactors select for the same core microbial taxa, but that the conditions vary within the bioreactors, supporting an overall diverse microbial community and occurrence of different members in the different parts of the bioreactor.

In all samples, the most abundant phylum was Proteobacteria, with Gammaproteobacteria as the most abundant class (66 ± 14% of proteobacterial seqs, 30 ± 7% of all seqs; Fig. 3). Previously, the key denitrifying bacteria in these bioreactors have been assigned to Alpha and Betaproteobacteria (Aalto et al., 2020), the latter now being classified under Gammaproteobacteria, but Proteobacteria include a wide diversity of different physiological functions and has observed to be the dominant phylum also in freshwater RAS (Aalto et al. unpublished) The members of phylum Epsilonbacteraeota (former Epsilonproteobacteria) were abundant in the inlet samples in bioreactor 2 in March and in bioreactor 3 at both sampling occasions (47 ± 4% of all seqs), in outlet water samples (26 ± 17% of all seqs) and outlet biofilm samples (36 ± 17% of all seqs). This phylum has been characterized to play a key role in sulfur cycle, but also to include pathogens vertebrates (Murphy et al., 2008; Roalkvam et al., 2015), potentially including fish pathogens originating from RAS. The water and woodchip biofilm communities collected within the bioreactors were more diverse (Shannon diversity index; water: 6.1 ± 0.4, woodchip biofilm: 6.1 ± 0.4) than the communities in the inlet and outlet water or outlet biofilm samples (inlet:

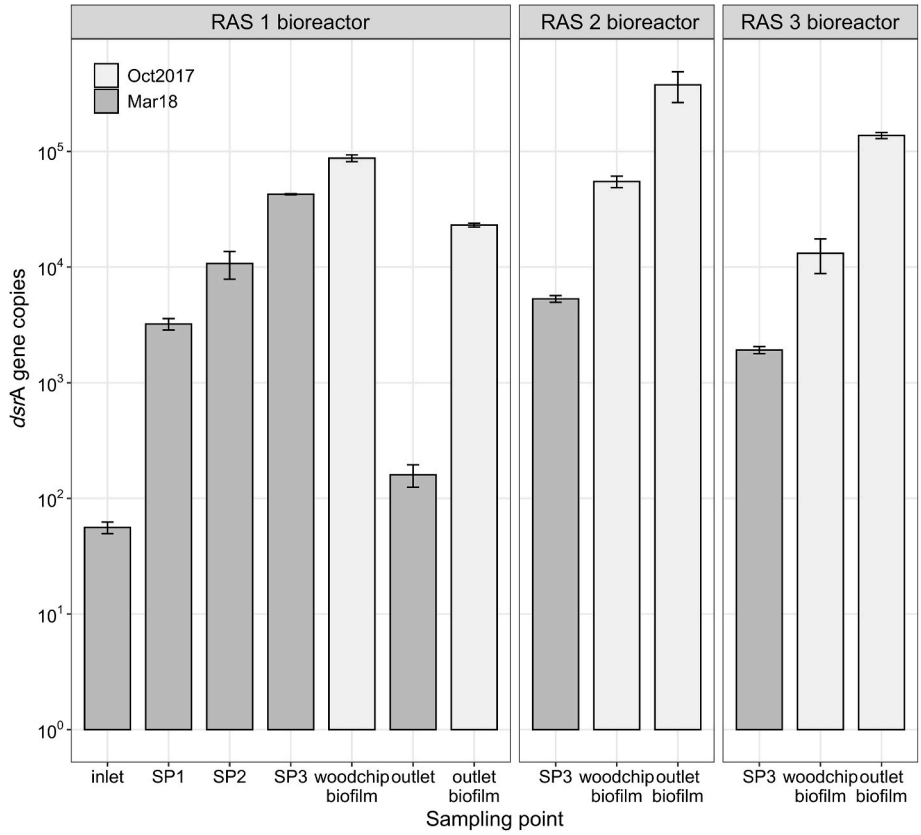

Fig. 2. The abundance of SRB (*dsr*A gene copies mL^{-1} or mg dw^{-1}) in the water samples collected in inlet, within the reactors (SP1-3), and outlet, and in biofilm on the woodchips and in the outlet at three bioreactors (each located to a different commercial RAS) on two sampling occasions (October 2017 and March 2018). Amount of replicates: water samples n = 2–3, woodchip biofilm n = 6, outlet biofilm n = 3.

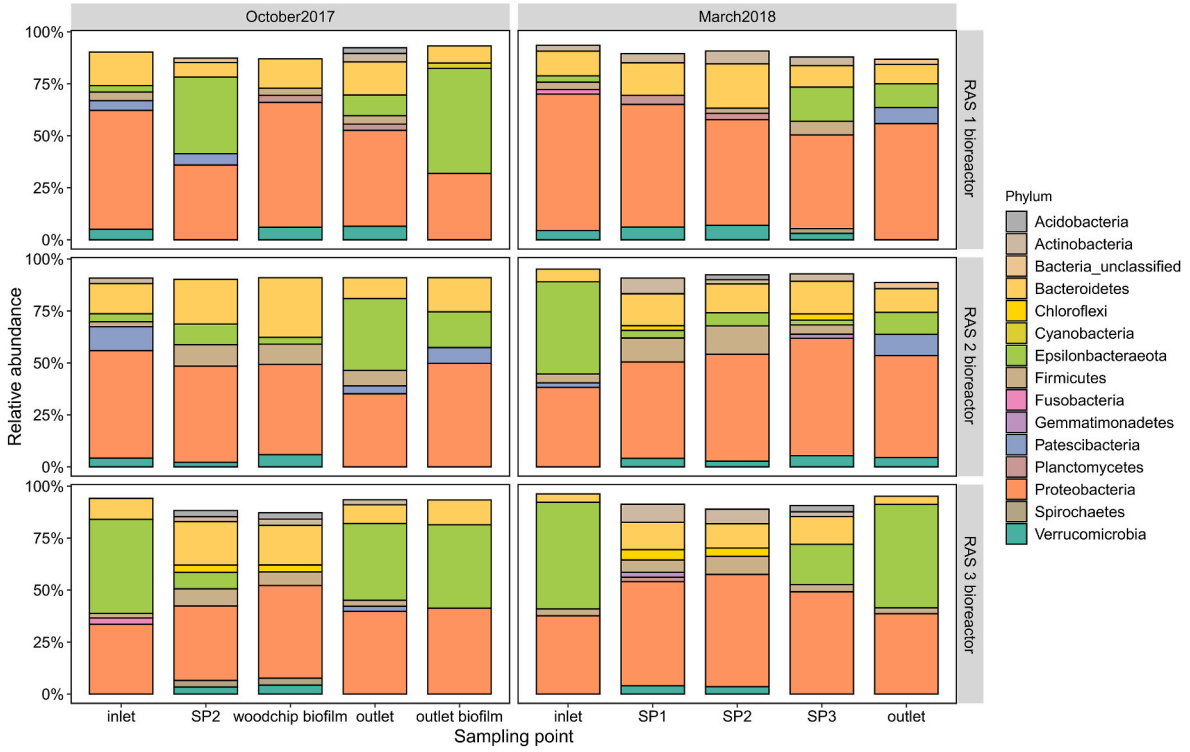

Fig. 3. The relative abundance of bacterial phyla in the water samples collected in inlet, within the reactors (SP1-3), and outlet, and in biofilm on the woodchips and in the outlet at three bioreactors (each located to a different commercial RAS) on two sampling occasions (October 2017 and March 2018).

4.4 ± 1.4, outlet: 5.0 ± 1.4, outlet biofilm: 4.2 ± 0.6, ANOVA $F_{4,42} = 8.1$, $P < 0.001$, Tukey post hoc test, $P < 0.05$, data not shown). This was partly explained by the relative abundance of the typical fermentative phyla Firmicutes and Bacteroidetes being higher in samples collected within the reactors ($7 \pm 3\%$, $16 \pm 5\%$, respectively) as compared to the other samples ($3 \pm 1\%$, $10 \pm 4\%$, respectively), indicating the presence of hydrolytic and fermentative anaerobic microbial processes degrading complex organic substances, facilitating denitrifying community.

When focusing on the sequences assigned with the microbial taxa related to sulfur cycle, the abundance of SRB was generally lower than that of sulfur oxidizers (SOBs) (Suppl. Fig. 4). Sulfate reducers were most abundant in water samples collected within the reactors and in the woodchip biofilm samples. Deltaproteobacterial *Desulfobulbus* was the most abundant SRB genus ($50 \pm 23\%$ of all SRB seqs, $1.2 \pm 1.5\%$ of all seqs), while the abundance of deltaproteobacterial *Desulfovibrio* was found to be high in the woodchip biofilm and outlet water samples from reactors 1 and 2 in October ($23 \pm 15\%$ of all SRB sequences, $0.6 \pm 0.5\%$ of all sequences; Suppl. Fig. 4). Previously, *Desulfobulbus* has been found to inhabit deep layers of woodchips and produce organic acids for heterotrophic denitrifying bacteria inhabiting the outer woodchip biofilm through incomplete oxidation of inorganic matter (Yamashita et al., 2011), while *Desulfovibrio* is commonly found in sediments and soils with high sulfate reducing activity. When combining these results with quantification of SRB genes, it seems that woodchip biofilms as well as biofilms growing on the bioreactor outlets are hotspots for SRB, as also the calculated sulfate removal rates indicate (Suppl. Fig. 2). However, the relative abundance of SRB remains low even at these sampling points as compared to other microbial groups (at highest 2.7% of all seqs). This indicates that H_2S production is not generally a significant issue in freshwater RAS woodchip bioreactors, but it can emerge under very specific conditions when the more favourable electron acceptor, nitrate, becomes limiting, as the H_2S producers are present. However, the human detection limit for H_2S is low (0.01–0.3 ppm; Guidotti, 2010), so even low amounts of H_2S gas produced with the typical unpleasant rotten egg smell can lead to conflicts between aquaculture facilities and surrounding municipalities. Thus, a correct design of the woodchip bioreactors to optimize the distribution of water within the bioreactor as well as to ensure a low enough HRT in relation to the incoming nitrate concentration is important to avoid conditions favouring H_2S production.

Sulfur oxidizers were found at all sampling points, but the abundance was highest in the inlet and outlet samples. *Arcobacter* belonging to the phylum Epsilonbacteraeota was found to be the most abundant SOB genus ($57 \pm 32\%$ of all SOB seqs, $11 \pm 16\%$ of all seqs; Suppl. Fig. 4). *Arcobacter*, as well as the other SOB members of Epsilonbacteraeota, are capable of oxidizing H_2S, thiosulfate, and elemental sulfur (Roalkvam et al., 2015; Waite et al., 2017), producing sulfate. The number of sequences assigned to SOB was higher than that of SRB in all samples, indicating that the potentially produced H_2S can be quickly consumed in the woodchip bioreactors, avoiding the aforementioned risks. Furthermore, the SOB genera have been found to possess an autotrophic denitrification pathway or a nitrite ammonification pathway (Roalkvam et al., 2015), meaning that they can remove nitrate using sulfur compounds or other inorganic electron donors (Di Capua et al., 2019). This supports the previous suggestion on denitrification being of partly autotrophic origin in the woodchip bioreactors treating RAS effluents (von Ahnen et al., 2018). When comparing the amount of the sequences assigned to the potential autotrophic denitrifying SOB to the amount of sequences assigned to the heterotrophic denitrifiers (see von Ahnen et al., 2019), we found that heterotrophic denitrifiers were dominating in the water samples collected from the bioreactors and woodchip biofilm samples, but that the autotrophic SOB tended to have higher abundance or to even dominate in the outlet water and outlet biofilm samples (Table 1). This can be explained with heterotrophic denitrification being limited with the lower organic matter concentration in the outlet, while the higher potential availability of H_2S, other sulfur compounds or inorganic electron donors is expected to favour autotrophic denitrification. Our result means that a significant part of the N-removal in woodchip reactors can be provided by autotrophic denitrifiers, which are not dependent on the availability of organic carbon. This explains

Table 1
The percentage of autotrophic denitrifier sequences on total denitrification sequences (and the amount of total denitrifier sequences) in water samples in inlet, bioreactor, and outlet outlet water (average ± SD over sampling occasions and sampling point replicates) and biofilm on woodchips and in outlet in October 2017.

	RAS 1 bioreactor	RAS 2 bioreactor	RAS 3 bioreactor
Water samples			
Inlet	6 ± 1% (6183 ± 1058)	33 ± 33% (7710 ± 3821)	60 ± 1% (10753 ± 793)
Within the bioreactor	22 ± 23% (5024 ± 1058)	13 ± 6% (6221 ± 843)	16 ± 15% (6043 ± 993)
Outlet	27 ± 7% (5070 ± 286)	42 ± 15% (6632 ± 2354)	54 ± 6% (10522 ± 1261)
Biofilm samples			
Woodchip	4% (5447)	7% (4038)	5% (4494)
Outlet	56% (5594)	31% (5374)	42% (8652)

the previous findings on woodchip bioreactors providing stable N removal for years (e.g. Christianson et al., 2021; Lepine et al., 2020), although the operational age of the bioreactors can be also dependent on the nutrient or suspended solids concentrations in RAS effluent.

Altogether, our results show that woodchip bioreactors host a diverse community of bacteria and fungi. There is some genetic potential for H_2S production, mainly under nitrate-limited conditions that can be avoided with optimal bioreactor design and operation. Furthermore, the H_2S potentially produced can be rapidly consumed by sulfur oxidizing autotrophic denitrifiers, which utilize the hydrogen sulfide produced by sulfate reducing bacteria, but also other sulfur compounds or inorganic electron donors. This result corroborates the previous findings on the co-existence of autotrophic and heterotrophic nitrate removal in woodchip bioreactors, and the potential for simultaneous removal of nitrate and H_2S and efficient nitrate removal even under organic carbon limitation. This is highly beneficial for extending the operational age of the bioreactors, and in particular if woodchip bioreactors are applied as end-of-pipe N-removal treatment for marine RAS with sulfate rich effluents with a higher risk for H_2S production.

Credit author statement

Sanni L. Aalto: Conceptualization, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision. Suvi Suurnäkki: Formal analysis, Investigation. Mathis von Ahnen: Investigation, Writing - Review & Editing. Marja Tiirola: Conceptualization, Resources, Supervision, Funding acquisition. Per Bovbjerg Pedersen: Conceptualization, Resources, Writing - Review & Editing, Funding acquisition.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.113852.

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