Salinity affects nitrate removal and microbial composition of denitrifying woodchip bioreactors treating recirculating aquaculture system effluents

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Abstract

This study investigated the effect of salinity on microbial composition and denitrification capacity of woodchip bioreactors treating recirculating aquaculture system (RAS) effluents. Twelve laboratory-scale woodchip bioreactors were run in triplicates at 0, 15, 25, and 35 ppt salinities, and water chemistry was monitored every third day during the first 39 days of operation. Microbial communities of the woodchips bioreactors were analyzed at the start, after one week, and at the end of the trial.

Woodchip bioreactors removed nitrate at all salinities tested. The highest NO_3-N removal rate of 22.0 ± 6.9 g NO_3-N/m^3/d was obtained at 0 ppt, while 15.3 ± 4.9, 12.5 ± 5.4 and 11.8 ± 4.0 g NO_3-N/m^3/d were obtained at salinities of 15, 25 and 35 ppt, respectively. Nitrate removal rates thus decreased with salinity, being 54-69% lower than at 0 ppt. Leaching of total ammonia nitrogen (TAN) and orthophosphate (PO_4-P) from woodchips was initially higher at saline treatments compared to 0 ppt, while initial leaching of BOD_5 appeared to be similar across all treatments. Production of alkalinity per g NO_3-N removed was higher at 0 (3.6 ± 0.5 g$CaCO_3$/gNO_3-N) and 15 ppt (3.5 ± 0.8) than at the more saline treatments (25 ppt: 2.0 ± 0.9, 35 ppt: 1.12 ± 0.5 g$CaCO_3$/gNO_3-N), indicating that heterotrophic denitrification was the dominant nitrate removing process at 0 and 15ppt, while autotrophic denitrification processes probably interfered with the alkalinity balance at 25 and 35 ppt. In the woodchip reactors, Gammaproteobacteria was the most abundant taxa. However, salinity shaped the woodchip microbiome, resulting in an increase in the

abundance of sulfide oxidizing autotrophic denitrifiers, but decrease in the overall abundance of denitrifying microbes at higher salinities, which presumably explained the reduced nitrate removal rates at elevated salinities.

This study demonstrates that woodchip bioreactors can be applied to remove nitrate from saline RAS effluents albeit at lower nitrate removal rates compared to freshwater installations.

1. Introduction

Practical and effective methods for removing nitrate from recirculating aquaculture system (RAS) effluents are needed, to ensure a reduced environmental impact and a sustainable growth of the aquaculture industry (van Rijn et al. 2016). One approach to remove nitrates from waste waters are denitrifying woodchip bioreactors, which are typically trenches filled with woody substrate, that have been successfully applied to treat tile drainage for more than twenty years (Schipper et al., 2010; Christianson et al., 2012, Addy et al., 2016). Laboratory and pilot-scale studies have demonstrated that woodchip bioreactors may be applied to treat effluents from freshwater RAS (Lepine et al., 2016; von Ahnen et al., 2016 a,b; Christianson et al., 2016; Christianson et al., 2018). These previous studies have focused on examining factors regulating woodchip bioreactor operation (e.g. hydraulic retention time Lepine et al., 2016), the possibilities to increase N removal rates (von Ahnen et al., 2016a), start-up phase at pilot-scale (von Ahnen et al., 2016b), as well as potential operational risks (e.g. bioreactor clogging Christianson et al., 2016), or mitigation of potential phosphorus leaching after bioreactor start-up (Sharrer et al., 2016; Christianson et al., 2017). Recently, woodchip bioreactors have been successfully implemented in full-scale at three commercial recirculated freshwater trout farms in Denmark (von Ahnen et al., 2018).

However, since the production of marine fish species including smolt and even full grow-out of Atlantic Salmon (Salmo salar) in saline waters is being increasingly moved on land (Dalsgaard et al., 2013), knowledge on the capability of woodchip bioreactors to treat saline RAS effluents is now requested by the aquaculture industry. So far, only one study in the international scientific literature (He et al., 2018) has looked at woodchip bioreactors treating saline waters, by investigating denitrification performance of three woodchips types; eastern white pine (Pinus strobus), red oak (Quercus rubra), and eucalyptus (Eucalyptus camaldulensis) as well as other organic carbon sources (methanol, fish waste, elemental sulfur pellets with oyster shells, mixtures of sulfur and woodchips) when treating synthetic saline RAS water (15 ppt, 97.2 ± 1.8 mg NO_3-N/l). In their microcosm study, bottles were incubated under static conditions at 23.5 ± 2.1˚C and achieved volumetric removal rates for the three wood species which ranged from 1.2 ± 1.0 (eucalyptus) to 16.5 ± 1.1 (pine) $gN/m^3/d$.

The microbial communities of woodchip reactors are still rather poorly known. Recent laboratory experiments proved that Alpha-, Beta- and Gammaproteobacteria were dominating in woodchip bioreactors and high microbial denitrification and anammox activities was found on the woodchips (Grießmeier et al. 2017; Grießmeier & Gescher 2018). However, the effect of salinity on woodchip

68 microbiome is currently unknown. In a previous study by Bakke et al. (2017), the microbial communities
69 within RAS have been found to differ between three systems operated at different salinities (12, 22, and
70 32 ppt), suggesting that significant changes in microbial communities could be expected also in woodchip
71 reactors. Furthermore, since salinity decreases nitrate removal rates in natural aquatic systems (e.g.
72 Rysgaard et al. 1999), changes in denitrification rate might also be expected.

73 In this study, we examined the effect of salinity on the performance and microbial composition of
74 laboratory-scale woodchip bioreactors treating RAS effluent waters under start-up conditions using
75 combined measurements of water chemistry and next generation sequencing technique.

76 ## 2. Materials and Methods

77 ### 2.1 Experimental set-up

78 The experimental set-up comprised 12 horizontal-flow woodchip bioreactors (described in von Ahnen et
79 al., 2016a) operated at four different salinities, n=3 per salinity level. Each woodchip bioreactor contained
80 poplar (Populus sp.) woodchips with an average length of 0.7 ± 0.4 cm, width of 0.6 ± 0.3 cm and thickness
81 of 0.3 ± 0.2 cm (n=20) with a submerged woodchip volume of 3l and was operated at an empty bed contact
82 time (EBCT) of 12 hours. Total woodchip porosity was measured according to Christianson et al. (2010)
83 and was 70 ± 1 % (n=3).

84 The effluent to be treated in the experimental woodchip bioreactors originated from a 8.5 m^3
85 experimental, freshwater RAS stocked with rainbow trout (Oncorhynchus mykiss) as described in von
86 Ahnen et al. (2016a). The effluent was pre-filtered through a 60 µm mesh, and kept in the aerated holding
87 tank (700 l). Every third day, the holding tank water was used to replace water in four small reservoirs (60
88 l each), which each acted as inlet water source to 3 experimental woodchip bioreactors (three bioreactors
89 of same salinity receiving water from one small reservoir). After water replacement, synthetic sea salt
90 (Blue Treasure SPS Sea Salt) was added to three of the small reservoirs to generate inlet waters with
91 salinities of 0, 15, 25 and 35 ppt, respectively. The woodchip bioreactors were operated continuously in
92 this fixed set-up during the entire study period.

93 ### 2.2 Water sampling and analyses

94 Water sampling was conducted every third day for 39 days during bioreactor start-up. Water from the 4
95 inlet-reservoirs was collected as grab samples, while outlet samples from all woodchip bioreactors were
96 collected as 24 hour pooled samples by collecting the entire bioreactor effluent in a container cooled by
97 ice over 24 hours. Immediately before water samplings, point measurements of oxygen, pH and

temperature were performed in the inlet reservoirs, and at the outlet side of the woodchip bioreactors using a portable meter (Hach Lange HQ40 multimeters, Düsseldorf, Germany).

After sampling, total BOD_5 was analyzed from unfiltered water samples ($BOD_{5\text{-total}}$; ISO 5815-2, 2003) and subsamples of water (1000 ml) were filtered through 0.45 µm mixed cellulose ester filters (Whatman, GE Healthcare, UK) and analyzed for dissolved BOD_5 following standard procedure ($BOD_{5\text{-diss}}$; ISO 5815-2, 2003). Measurements of BOD_5 were carried out on every fourth sampling event only.

Other subsamples were filtered through 0.2 µm syringe filters (Filtropur S 0.2 µm, Sarstedt, Germany) prior to analyses of total ammonia nitrogen (TAN; DS224, 1975) and measurements of the ions nitrite-N, nitrate-N, orthophosphate (PO_4-P), and sulfate (SO_4-S) using an ion chromatograph (Thermo Scientific™ Dionex™ Ion Chromatography, Mettler Toledo). Filtered subsamples were kept cold (3°C) after collection for subsequent analysis.

2.3 Sampling and analyses of the woodchip-associated microbial community

For microbiome analysis and qPCR, approximately 20 mL of woodchips were collected with sterilized tweezers from the center of the outlet side of the woodchip bioreactors after 1, 7 and 39 days (end of trial) of bioreactor operation, and stored at -20 °C until further analyses. To detach the microbes from woodchips, 20 ml of water was added to woodchips and sonication was performed in two periods of two minutes by an ultrasonic bath (Branson 1510). Woodchips were discarded, samples were freeze-dried (Alpha 1-4 LD plus, Christ) and DNA extraction done with PowerLyzer PowerSoil DNA extraction kit (MoBio Laboratories, Inc.). The Quant-IT PicoGreen® dsDNA Assay Kit (Invitrogen) was used to quantify DNA . Changes in the community composition, richness, and diversity of organisms harbouring the 16S rRNA gene were studied with next generation sequencing. Universal prokaryotic primers 515F-Y (GTGYCAGCMGCCGCGGTAA; Parada et al. 2016) and 806R (GGACTACHVGGGTWTCTAAT; Caporaso et al., 2011) were used to amplify the V4 region of the prokaryotic 16S rRNA gene. The abundance of nirK (nirK876/1040; Henry et al. 2004) and nirS (nirSC3aF/R3cd; Kandeler et al. 2006) genes in samples were quantified by qPCR amplification with Bio-Rad CFX96 Real-Time System (Bio-Rad Laboratories).

The qPCR reactions of 25 µl consisted of Maxima SYBR Green/Fluorescein qPCR Master Mix (Thermo Fisher Scientific), 5 ng of template DNA, and 0.4 µM of each primer (Sigma Aldrich). Thermal cycling consisted of 10 min initial denaturation at 95 °C, followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 60 s, followed by final elongation at 72 °C for 5 min.). For nirK and nirS genes, the annealing temperature was 60 °C and 55°C, respectively. For microbial community analysis, one µl of the PCR

product was used as a template in PCR where Ion Torrent PGM sequencing adapters and barcodes were added to the ends of PCR product. Linker and fusion primers (0.04 µM of M13_515F-Y, 0.4 µM of IonA_IonXpressBarcode_M13 and P1_806R) were used in 10 additional cycles with conditions otherwise identical to the first amplification (Mäki et al. 2016). Products were purified with Agencourt AMPure XP purification system (Beckman Coulter Life Sciences, Indianapolis, IN, USA), quantified, and pooled in equimolar quantities for sequencing on Ion Torrent PGM using Ion PGM Hi-Q View OT2 Kit for emulsion PCR, PGM Hi-Q View Sequencing Kit for the sequencing reaction and Ion 316 Chip v2 (all Life Sciences, Thermo Fisher Scientific).

Analysis of gene sequences was done using Mothur (version 1.39.5; Schloss et al., 2009). Sequences shorter than 200 bp, low-quality sequences with more than one mismatch in barcode/primer sequences, or with homopolymers longer than eight nucleotides, as well as barcodes, and primers were removed. Sequences were aligned using Silva reference alignment (Release 132). Chimeric sequences, denoted using Mothur's implementation of Uchime (Edgar et al., 2011), were removed from each library. Sequences were divided into operational taxonomic units (OTUs) at 97% similarity level, and singleton OTUs (OTUs with only one sequence in the entire dataset) were removed. Sequences were assigned taxonomies with a naïve Bayesian classifier (bootstrap value cut off = 80 %) (Wang et al. 2007) using Silva database (Release 132) and sequences classified as chloroplast, mitochondria and eukaryote were removed. Finally, the data was normalized by subsampling to 12,887 sequences. Sequences have been submitted to NCBI Sequence Read Archive under Sequences have been submitted to NCBI Sequence Read Archive under BioProject PRJNA509197.

2.4 Statistics and calculation of removal rates and correlation analyses

Volumetric removal rates ($g/m^3/d$) were calculated based on concentration differences between bioreactor inlet and outlet samples and the EBCT of the woodchip bioreactors. The EBCT was calculated from the volume of the submerged woodchips and the flow rates. Results are throughout presented as means ± SD.

Differences between removal rates were assessed by Kruskal–Wallis One-Way ANOVA on Ranks followed by Tukey Test of Multiple Comparisons using SigmaPlot version 13.0 (SystatSoftware Inc., CA, USA). The statistical analysis for microbiome were conducted using R version 3.4.3 (R Core Team, 2018). Non-metric multidimensional scaling (NMDS) conducted with metaMDS function in vegan package; (Oksanen et al., 2013) plots calculated based on Bray–Curtis distance matrix were used to visualize dynamics in the

158 microbial community structure (OTUs represented by at least two reads). Before NMDS, Wisconsin and
159 square- root-transformations were applied to OTU abundance data.

160 ## 3 Results and discussion

161 ### 3.1 Nitrogen removal

162 In all bioreactors, dissolved oxygen concentrations were ~1 mg O_2/l (Fig. 1a), and pH values ~7 (Fig. 1b.)
163 at the outlets, indicating that suitable conditions for denitrification prevailed throughout the experimental
164 period (Henze et al. 2002). Indeed, nitrate was removed in all bioreactors during the experimental period
165 of 39 days, albeit removal rates were significantly higher in freshwater bioreactors than in the bioreactors
166 with salinities of 15, 25 and 35 ppt, respectively (Tab. 1, Fig. 1c). In a previous study with high-salinity
167 synthetic wastewaters, salt concentrations larger than 1% (10 ppt) have been found to suppress
168 heterotrophic denitrification in a downflow packed column with plastic media fed with sugar solution
169 (Dincer and Kargi 1999). In agreement with this, we found reduced nitrate removal rates with increasing
170 salinity also in woodchip beds, although there was no significant difference between removal rates
171 obtained at 25 and 35ppt (Tab. 1).

172 In the freshwater treatment/bioreactors, the nitrate removal rates achieved (22.02 ± 6.87 g NO_3-N/m^3/d;
173 Tab. 1) fitted into the upper range of removal rates reported for drainage water treatment (2-22 gN/m^3/d;
174 Schipper et al., 2010; Christianson et al., 2012). However, removal rates in woodchip bioreactors may
175 decline over the first years of operation (Addy et al. 2016), suggesting that the removal rates might have
176 declined if this trial was continued further after the 39 days. Nitrate removal rates at three full-scale
177 woodchip bioreactors operated at commercial freshwater recirculated trout farms in Denmark ranged
178 from 4.5 – 7.8 gNO_3-N/m^3/d during the first year. The average temperature was 10.1 °C as compared to
179 the 14.4 °C in this study. At 15 ppt, the average nitrate removal rate in the bioreactors filled with poplar
180 woodchips was 15.30 ± 4.86 g NO_3-N/m^3/d, similar to findings by He et al. (2018), who found nitrate
181 removal rates of 11.6 - 16.5; 1.2 – 4.9; and 4.7 – 9.5 g NO_3-N/m^3/d for eastern white pine (Pinus strobus),
182 eucalyptus (Eucalyptus camaldulensis) and red oak (Quercus rubra), respectively, in a microcosm study
183 incubating woodchips with 800 ml of synthetic saline RAS water at 15 ppt, initial nitrate concentrations of
184 approximately 100 mg NO_3-N/l and at 23.5 ± 2.1°C. At 25 and 35 ppt, nitrate-N removal rates were 12.46
185 ± 5.41 and 11.82 ± 3.96 g NO_3-N/m^3/d, respectively.

186 The amount of alkalinity produced per g NO_3-N reduced in the woodchip bioreactors decreased as salinity
187 increased (0 ppt: 3.58 ± 0.47, 15 ppt: 3.52 ± 0.84, 25 ppt: 1.96 ± 0.86, and 35 ppt: 1.17 ± 0.53 g$CaCO_3$/gNO_3-

188 N removed, n=13). According to van Rijn et al. (2006), heterotrophic denitrification produces 3.57 mg
189 $CaCO_3$ for each mg NO_3-N reduced to N_2 gas, indicating that the alkalinity production at 0 and 15 ppt was
190 well explained by heterotrophic denitrification. At 25 and 35ppt, however, alkalinity production was lower
191 than would be expected on the basis of heterotrophic denitrification alone. At these salinities, sulfate was
192 net produced at rates of 1.38 ± 25.17 (25 ppt) and 6.38 ± 27.32 gSO_4-S/m^3/d (35 ppt). Since the reduction
193 of sulfate (SO_4^{2-}) to sulfide (S^{2-}) produces 100 mg $CaCO_3$ per mole sulfate reduced, while sulfate production
194 through sulfide-driven autotrophic denitrification consumes 20mg $CaCO_3$/mole H_2S (van Rijn et al., 2006),
195 the reduced alkalinity production at 25 and 35 ppt bioreactors could be explained by increased
196 autotrophic denitrification and/or by lower sulfate reduction. Minor average sulfate removals at 0 and 15
197 ppt (Tab. 1, Fig. 1d) may indicate that some nitrate limitation potentially occurred locally at microsites
198 within the woodchips. However, inlet concentrations of nitrate-nitrogen to all bioreactors increased
199 steadily from 55 to 86 mg NO_3-N/l during the study period (Fig. 1 c), and outlet nitrate concentrations
200 were above 40 mg NO_3-N/l at all times thus indicating that overall woodchip bioreactors were not limited
201 by nitrate availability during the experiment.

202 3.2 Other water quality parameters

203 Elevated nitrite concentrations were observed at all salinites during the first 12 days of woodchip
204 bioreactor operation (Fig. 1e). This is in agreement with previous findings by von Ahnen et al. (2016b),
205 who found elevated nitrite concentrations in the pilot-scale woodchip bioreactor effluent during the first
206 11 days after bioreactor start-up. Lepine et al. (2016) also observed elevated nitrite concentrations during
207 the first days after start-up in denitrifying woodchip bioreactors operated at different hydraulic retention
208 times.

209 Leaching of TAN (Fig. 1f) and orthophosphate (Fig. 1g) was more pronounced during the first days after
210 bioreactor start-up at salinities 15, 25 and 35 ppt as compared to 0 ppt. This might be due to the higher
211 density of the saline water as compared to freshwater, increasing the diffusion rate of TAN and
212 orthophosphate. In contrast, leaching of organic matter, measured as total and dissolved BOD_5 was similar
213 across all salinities, and total BOD_5 concentrations measured in the outlet approached inlet
214 concentrations after 12 days of operation (Fig. 1h,i), in agreement with von Ahnen et al. (2016b), where
215 leaching of dissolved and total BOD_5 occurred during the first two weeks of operation. The relatively short-
216 lasting leaching observed in that study as compared to previous woodchip bioreactor studies (reviewed
217 by Schipper et al. 2010) was likely due to the short EBCT (5 hours) applied, while in the current study, the
218 short-lasting leaching was probably due to the relatively fine woodchips being used. Leaching of dissolved

organic matter was accompanied by a decreasing outlet pH during the first days after bioreactor start-up (Fig. 1 b), presumably due to the leaching of organic acids, typically observed during bioreactor start-up (Lepine et al., 2016; von Ahnen et al., 2016b). The pH decreased less during bioreactor start-up at higher salinities, which was likely due to the higher alkalinity values (Fig. 1j). After 3 weeks of operation, outlet TAN concentrations increased at 35ppt, presumably due to microbial processes rather than leaching.

3.3 Microbial community

In the beginning of the experiment (day 1), the microbial community compositions of woodchips were similar across all salinity levels (Fig. 2). However, after 7 days, the communities of the freshwater (0 ppt) and 15 ppt woodchip bioreactors were developed distinct from the more saline treatments (25 and 35 ppt), and this difference was even more notable at the end of the experiment (day 39). Across all microbial classes observed in the reactors, the relative abundances of Bacteroidia (belonging to Bacteroidetes), Alpha- and Deltaproteobacteria were lower in the more saline treatments, especially at the end of the experiment (Fig. 3). Similarly, the relative abundance of Actinobacteria and Bacilli (belonging to Firmicutes) was lower, while the abundance of Gammaproteobacteria was higher at day 39 in the saline treatments than in the freshwater reactors.

The number of sequences (reads) assigned to denitrifying microbial orders (Saarenheimo et al. 2015; Grießmeier et al. 2017; Grießmeier & Gescher 2018) decreased in saline bioreactors in the course of the experiment (Fig. 4), suggesting that the decreased nitrate removal activities were linked to the abundance of these denitrifying microbes. The abundance of Betaproteobacteriales, Rhizobiales and Xanthomonadales decreased with increasing salinity, suggesting that they could be the main contributors to heterotrophic denitrification, and thus were most abundant in the freshwater bioreactors. In contrast, the abundance of Campylobacteriales was promoted in all saline bioreactors. This group was dominated by Arcobacter (Suppl. Fig. 1), which has previously been found to oxidize sulfide into elemental sulfur (S^0) (De Gusseme et al. 2009), that can be further oxidized into sulfate, thus explaining the observed sulfate production in 25 and 35 ppt bioreactors. Furthermore, Arcobacter has also been found to be capable of reducing nitrate and oxidizing sulfide, suggesting them as possible autotrophic denitrifiers in saline bioreactors. However, the overall abundance of these groups remained low even in the saline treatments (Fig. 4), explaining why the nitrate removal rates were lower in the more saline bioreactors. Furthermore, the abundance of the common sulfate reducers, Deltaproteobacteria, decreased in the most saline bioreactors, suggesting that less sulfide was produced in saline treatments, which could partly explain the moderate abundance of autotrophic denitrifiers. However, further studies are needed to elucidate the

interactions between heterotrophic and autotrophic denitrifiers in woodchip reactors more thoroughly. The microbial community composition analysis could also explain other observed water chemistry than nitrate reduction: observed nitrite accumulation in the beginning of the experiment could be due to Enterobacteriales, which decreased throughout the experiment (Suppl. Fig. 2). Campylobacteriales have been suggested to be capable of dissimilatory nitrate reduction to ammonium (Grießmeier et al. 2017), which could explain the higher TAN production at 35 ppt.

The qPCR of nitrite reductase genes, nirS and nirK, showed that nirS gene was more abundant than nirK in all bioreactors (Fig. 5). At the beginning of the experiment, the abundance of nirK and nirS genes were similar for all salinities. The abundance of nirS gene systematically increased toward the end of experiment, although the abundance of genes in 25 ppt (9.2E+06 gene copies) and 35 ppt (3.4E+06) was notably lower than in 0 ppt (6.0E+07) and in 15 ppt (2.8E+07) (Fig. 5). The higher gene copy numbers in 0 ppt indicate higher amount of denitrifying prokaryotes present in the system, and thus higher genetic potential for denitrification. Similar pattern was observed in the abundances of nirK genes. Thus, denitrifying prokaryotes were less abundant in higher salinities than in freshwater environments and the genetic potential for denitrification was thus lower in higher salinity bioreactors. This finding is consistent with the reduced nitrate removal rates measured and the suppressed levels of denitrifying microbes in the more saline water.

Conclusions

Denitrifying woodchip bioreactors may be used to treat saline aquaculture effluents. However, denitrification rates in saline waters appear to be reduced compared to freshwater applications, which need to be considered in the planning and design of woodchip bioreactors for the saline RAS effluents. Analyses of the microbial community revealed that salinity altered the woodchip microbiome significantly, promoting autotrophic denitrifiers, but decreasing the overall denitrification potential.

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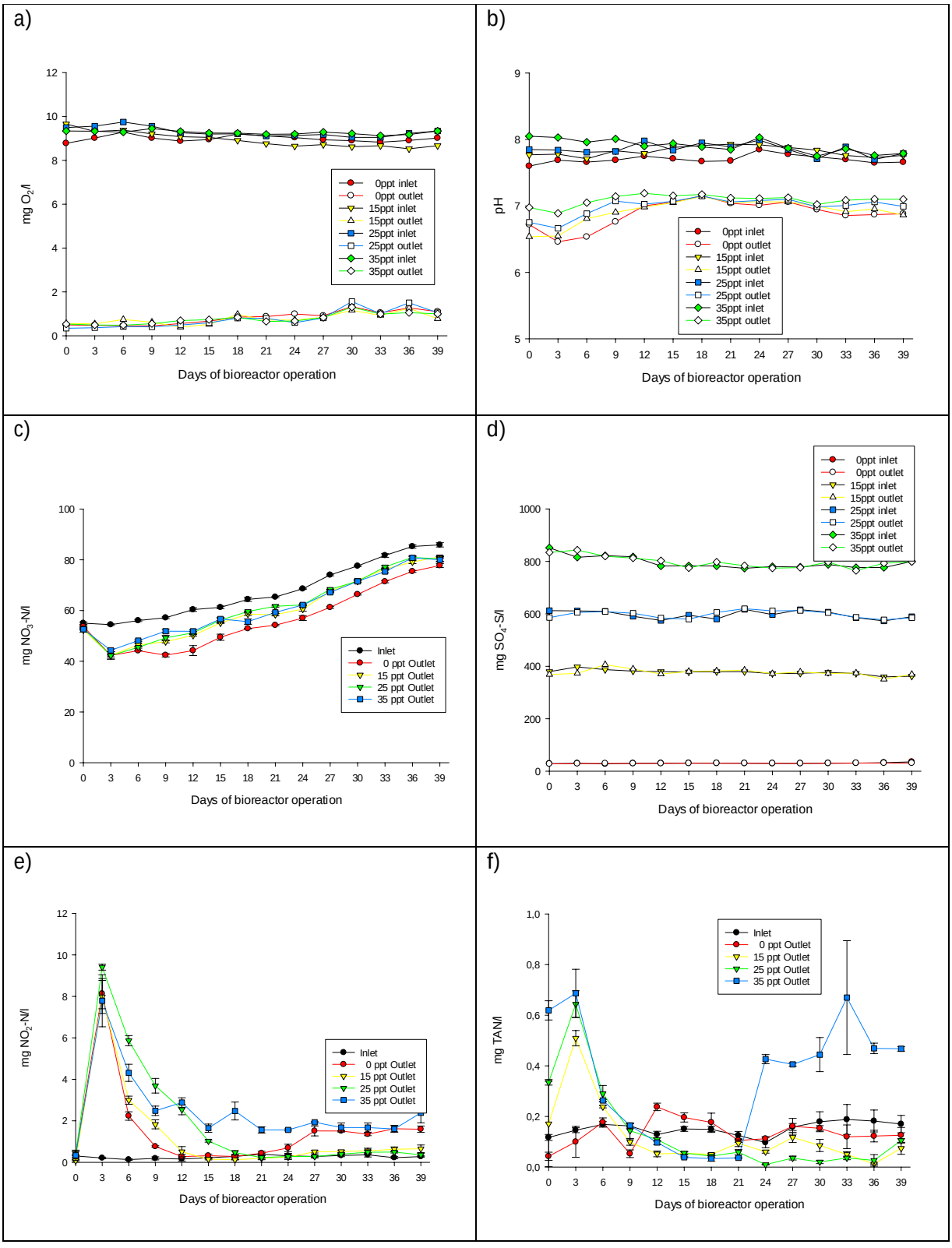
a)
mg O2/l
Days of bioreactor operation
0ppt inlet
0ppt outlet
15ppt inlet
15ppt outlet
25ppt inlet
25ppt outlet
35ppt inlet
35ppt outlet
b)
pH
Days of bioreactor operation
0ppt inlet
0ppt outlet
15ppt inlet
15ppt outlet
25ppt inlet
25ppt outlet
35ppt inlet
35ppt outlet
c)
mg NO3-N/l
Days of bioreactor operation
Inlet
0 ppt Outlet
15 ppt Outlet
25 ppt Outlet
35 ppt Outlet
d)
mg SO4-S/l
Days of bioreactor operation
0ppt inlet
0ppt outlet
15ppt inlet
15ppt outlet
25ppt inlet
25ppt outlet
35ppt inlet
35ppt outlet
e)
mg NO2-N/l
Days of bioreactor operation
Inlet
0 ppt Outlet
15 ppt Outlet
25 ppt Outlet
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f)
mg TAN/l
Days of bioreactor operation
Inlet
0 ppt Outlet
15 ppt Outlet
25 ppt Outlet
35 ppt Outlet

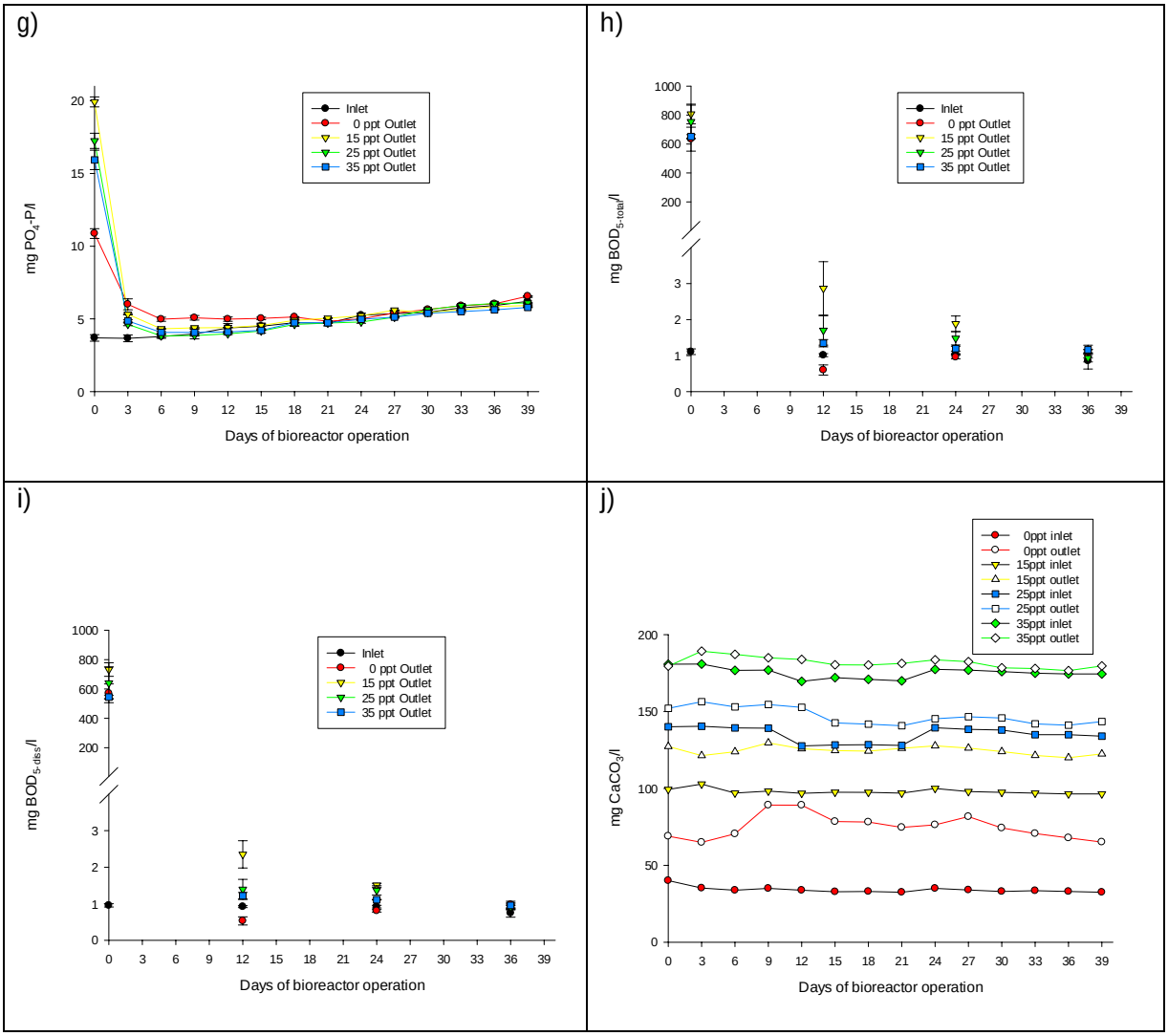

Figure 1. Inlet and outlet concentrations of NO_3-N (c), NO_2-N (e), TAN (f), PO_4-P (g), $BOD_{5\text{-total}}$ (h), $BOD_{5\text{-diss}}$ (i) SO_4-S (d), $CaCO_3$ (j) and dissolved oxygen a) as well as pH b) values for experimental woodchip bioreactors run at salinities of 0, 15, 25 and 35 ppt over 39 days.

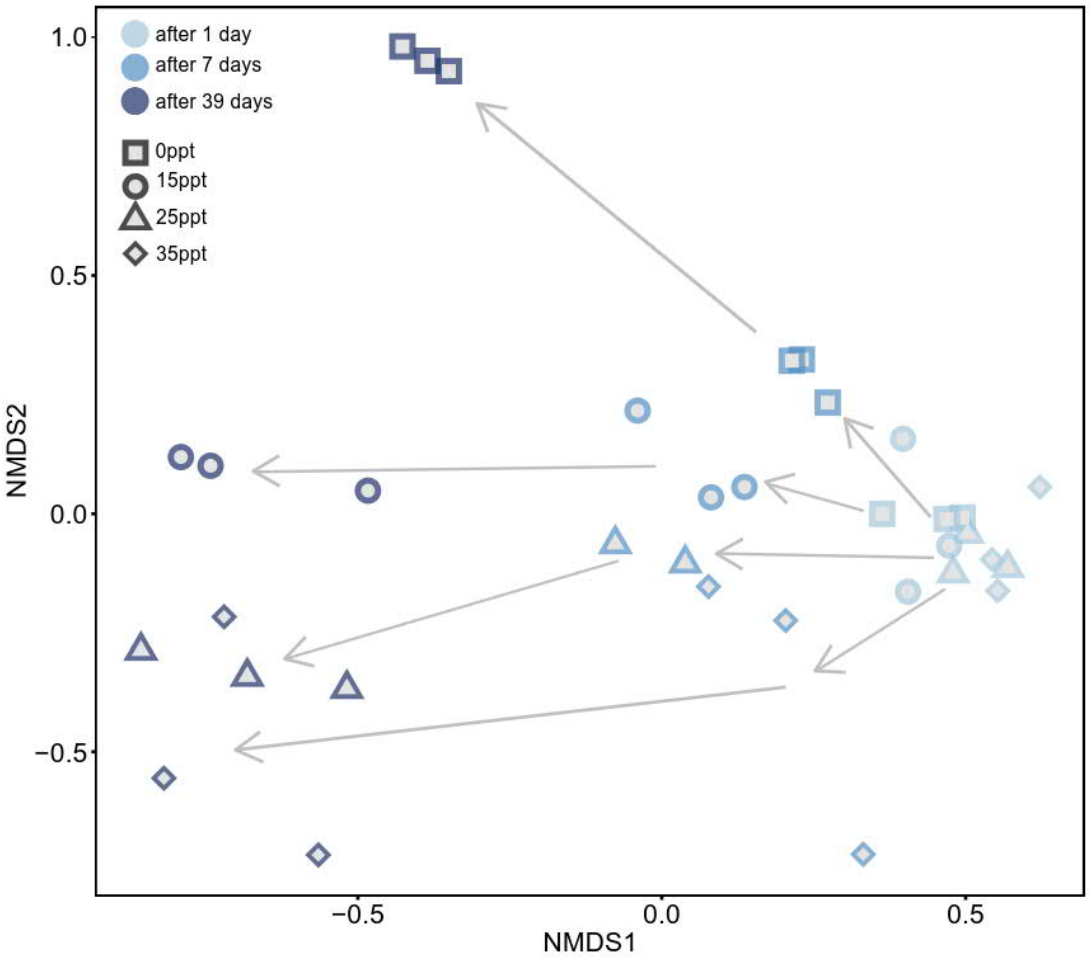

Figure 2. Nonmetric multidimensional scaling (NMDS) of woodchip microbiome at four different salinities and three sampling times. Arrows illustrate average change in community composition with time.

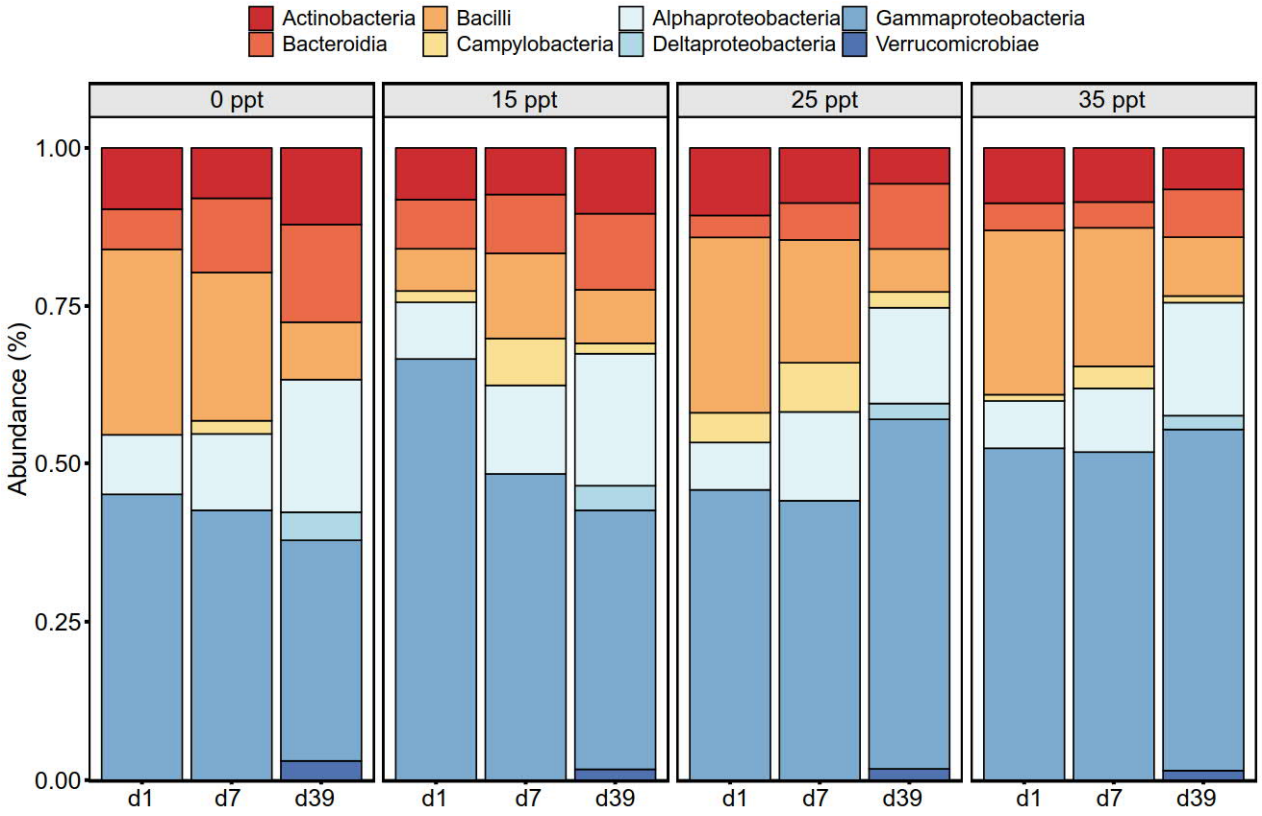

Figure 3. The relative abundances of microbial classes with relative abundance > 1% in different salinities and sampling occasions (d1= 1 day, d7= 7 days and d39= 39 days after the beginning of the experiment).

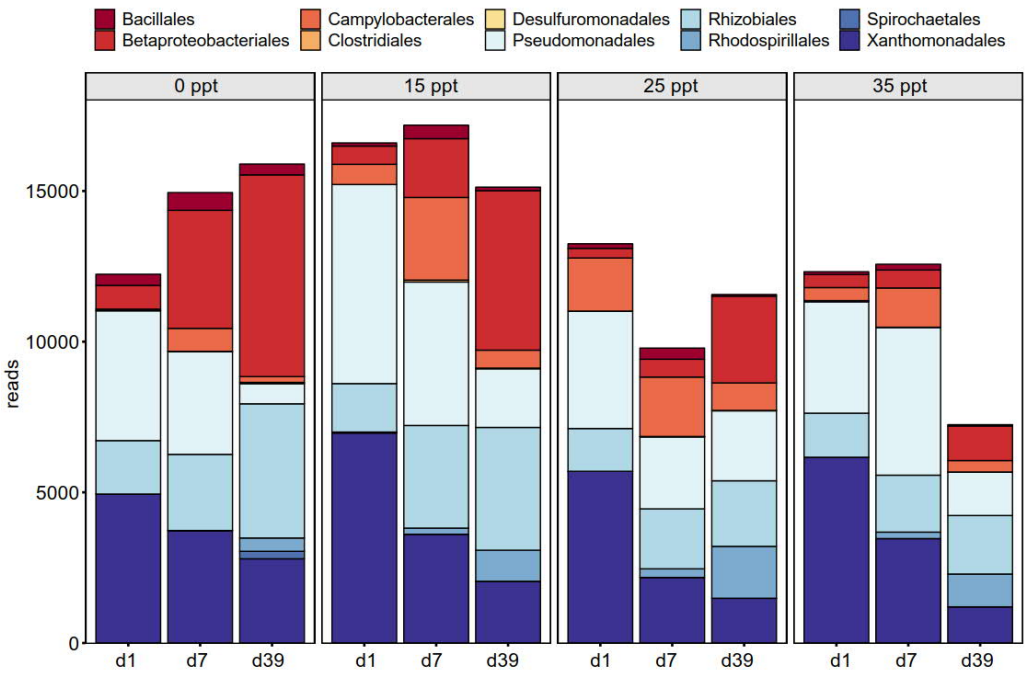

Figure 4. The amount of reads assigned to denitrifying microbial orders in different salinities and sampling occasions (d1= 1 day, d7= 7 days, and d39= 39 days after the beginning of the experiment).

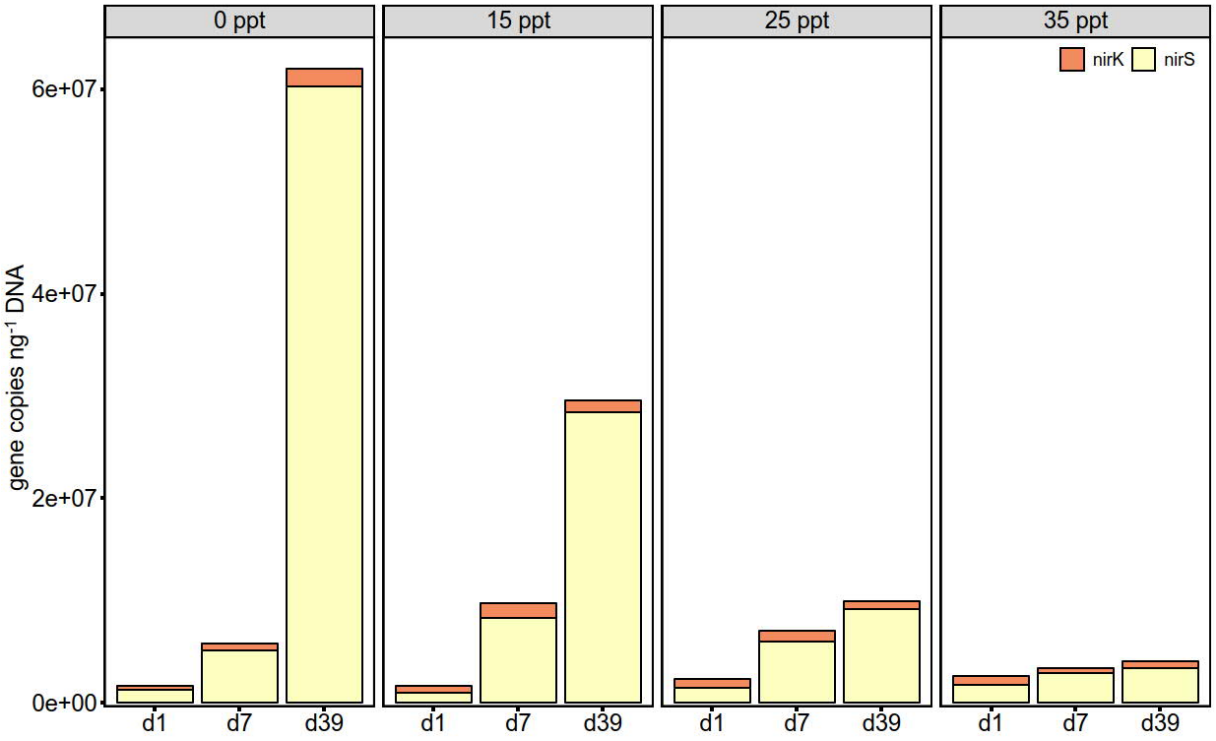

Figure 5. The total amount of nirK and nirS gene copy numbers in different salinities (d1= 1 day, d7= 7 days, and d39= 39 days after the beginning of the experiment).

Table 1. Average removal rates ($g/m^3/d$) as well as average inlet and outlet values for dissolved oxygen, pH and temperature (n=14) during the first 39 days of operation of experimental woodchip bioreactors run at salinities of 0, 15, 25 and 35 ppt. Mean values in rows not sharing a common superscript are significantly different from each other ($P<0.05$).

Removal Rate ($g/m^3/d$)	0 ppt	15 ppt	25 ppt	35 ppt
NO_3-N	22.02 ± 6.87^a	15.30 ± 4.86^b	12.46 ± 5.41^c	11.82 ± 3.96^c
NO_2-N	-2.52 ± 3.97^{ab}	-1.94 ± 4.11^{ab}	-3.15 ± 5.38^b	-4.33 ± 3.62^c
NH_4-N	-0.01 ± 0.12^a	0.07 ± 0.28^b	0.04 ± 0.35^b	-0.36 ± 0.48^{ac}
PO_4-P	-1.52 ± 3.60^a	-2.57 ± 8.27^a	-2.18 ± 7.09^a	-2.06 ± 6.39^a
SO_4-S	1.10 ± 2.76^a	1.03 ± 20.87^a	-1.38 ± 25.17^a	-6.38 ± 27.32^a
$CaCO_3$	-81.82 ± 17.21^a	-53.35 ± 6.34^b	-23.86 ± 10.41^c	-13.32 ± 8.45^c
$BOD_{5\text{-total}}$	-316 ± 554^{ac}	-405 ± 701^{bc}	-377 ± 662^c	-325 ± 563^c
$BOD_{5\text{-diss}}$	-284 ± 497^{ac}	-367 ± 635^{bc}	-320 ± 565^c	-273 ± 471^c
Inlet parameter				
Dissolved oxygen (mg O_2/l)	8.29 ± 0.14	8.95 ± 0.33	9.30 ± 0.21	9.27 ± 0.08
pH	7.70 ± 0.06	7.82 ± 0.07	7.85 ± 0.08	7.91 ± 0.10
Temperature (°C)	13.9 ± 1.0	14.0 ± 1.0	14.0 ± 0.9	14.0 ± 0.9
Outlet parameter				
Dissolved oxygen (mg O_2/l)	0.82 ± 0.31	0.76 ± 0.27	0.77 ± 0.40	0.77 ± 0.27
pH	6.84 ± 0.21	6.92 ± 0.18	6.99 ± 0.14	7.09 ± 0.08
Temperature (°C)	14.9 ± 0.9	14.8 ± 0.9	14.8 ± 0.8	14.8 ± 0.8