

# Salinization of coastal freshwater wetlands; effects of constant versus fluctuating salinity on sediment biogeochemistry

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**Abstract** Globally, coastal lowlands are becoming more saline by the combined effects of sea level rise, land subsidence and altered hydrological and climatic conditions. Although salinization is known to have a great influence on biogeochemical processes, literature shows contrasting effects that challenge the prediction of future effects. In addition, the effects of fluctuating salinity levels, a more realistic scenario than constant levels, on nutrient cycling in coastal wetland sediments have hardly been examined. A better understanding is therefore crucial for the prediction of future effects and the definition of effective management. To test the effects of constantly brackish water (50 mmol Cl l<sup>-1</sup>, 3.2 psu) or

fluctuating salinity (5–50 mmol Cl l<sup>-1</sup>), versus constantly low salinity (5 mmol Cl l<sup>-1</sup>, 0.32 psu) on nutrient biogeochemistry, we conducted a controlled laboratory experiment with either peat or clay sediments from coastal wetlands. Increased salinity showed to have a fast and large effect. Sediment cation exchange appeared to be the key process explaining both a decrease in phosphorus availability (through calcium mobilization) and an increase in nitrogen availability, their extent being strongly dependent on sediment type. Supply of brackish water decreased surface water turbidity and inhibited sediment methane production but did not affect CO<sub>2</sub> production. Constant and fluctuating salinity levels showed similar longer term effects on nutrient and carbon cycling. The contrasting effects of salinization found for nitrogen and phosphorus, and its effects on water turbidity indicate major ecological consequences for coastal wetlands and have important implications for water management and nature restoration.

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## Introduction

Large-scale land use change in coastal zones, sea level rise, land subsidence, and changes in hydrology and

seasonal precipitation intensity have greatly increased the risks of salinization worldwide (Church and White 2006; Pachauri and Reisinger 2008; Oude Essink et al. 2010). As a result, there is a strong need for information about the effects of salinization on both agricultural and natural areas in coastal wetlands. Most coastal wetlands are subjected to strong hydrological alteration (e.g. Turner and Lewis 1997; Lamers et al. 2002a; Carreño et al. 2008) and the resulting, ongoing land subsidence, especially in peatland areas, exacerbates the potential future effects of sea level rise.

Salinization is generally seen as a threat to the management of coastal freshwater wetlands. However, for coastal wetland areas that used to be brackish in the past, but have been turned into freshwater agricultural lands by large-scale hydrological modifications, increased salinity is essential for the rehabilitation of brackish communities. Often, however, these coastal wetlands have not only been modified by decreased salinity levels, but additionally received increased nutrient inputs by intensive agricultural use, leading to large scale eutrophication and loss of their characteristic biodiversity that is seriously endangered in Europe (Van't Veer 2009, Van't Veer et al. 2012). The presently low abundance or even absence of aquatic vegetation in these coastal wetlands strongly calls for studying the effects of increased salinity on the potential to restore these wetlands and their biodiversity.

For both situations, knowledge about the potential effects of salinity levels on nutrient biogeochemistry is crucial. It is known that increasing levels of chloride, sodium, potassium, magnesium and sulfate as a result of increasing salinity can have major effects on biogeochemical processes (Weston et al. 2006; Setia et al. 2010) and on the dynamics of plant, animal and microbial communities (Nielsen et al. 2003; Munns and Tester 2008). By changing sediment chemistry, salinization directly influences both sediment pore water and surface water concentrations of phosphorus and ammonium (Rysgaard et al. 1999; Baldwin et al. 2006; Weston et al. 2006, 2011). Although some studies (e.g. Baldwin et al. 2006; Weston et al. 2006; Van Diggelen et al. 2014) report a decrease of the phosphorous concentrations during salinization, others (e.g. Beltman et al. 2000) show an increase in bio-available phosphate possibly

due to desorption by chloride. Furthermore the increase in sulfate due to salinization may also stimulate microbial sulfate reduction, precipitation of sulfide with iron and, consequently, mobilization of phosphorus in wetlands (Smolders and Roelofs 1995; Lamers et al. 1998, 2002b; Smolders et al. 2006a, b).

Literature shows contrasting results about the influence of salinity increase on organic matter decomposition and carbon emissions. Some studies report increased carbon mineralization and increased C emissions (Weston et al. 2006; Chambers et al. 2011; Weston et al. 2011; Morrissey et al. 2014). Other studies, however, do not show a significant effect of salinization on C emissions (Hemminga et al. 1991; Setia et al. 2012), or even report decreasing C emissions (Loeb et al. 2008; Setia et al. 2010). For methanogenesis and methane (CH<sub>4</sub>) emissions, however, most studies consistently report a decrease due to salinization (Baldwin et al. 2006; Weston et al. 2006; Chambers et al. 2011). The study of Weston et al. (2011) is a notable exception, reporting increased CH<sub>4</sub> fluxes under increased salinity.

Most studies on the effects of salinization have focused on constant, rather than fluctuating salinity levels. Brackish water environments are, however, not only characterized by an intermediate salt concentration between freshwater and seawater, but also by fluctuating salinity levels. Both natural and anthropogenic salinization in the field will often result in salinity fluctuation over time (also called poikilohaline waters, as opposed to homoiohaline), the effects of which have hardly been tested under controlled conditions in the past. The biogeochemical effects of fluctuating salinity levels are, therefore, still poorly understood.

To disentangle the effects of salinization on the different biogeochemical sediment processes involved, we studied the effects of constant and fluctuating salinity increase on two characteristic coastal wetland sediment types (peat and clay) in a controlled, flow-through aquarium experiment. We expected salinization to cause major changes in biogeochemical processes, affecting both nutrient availability and carbon fluxes. Furthermore we hypothesized that fluctuating salinity levels might cause less pronounced effects on biogeochemical processes than constant salinity levels.

## Materials and methods

### Site description

Two aquatic sediment types, peat and clay, were used in the experiment. The peat sediment was collected in the formerly brackish wetland “Ilperveld” (52°26′15.90–4°56′19.06). In this area, drainage of former peatlands led to strong land subsidence (Nieuwenhuis and Schokking 1997) of several meters since the early middle ages, while sea water levels increased with almost a meter since then. As a result, large areas became increasingly influenced by brackish to saline water from the inland sea Zuiderzee through flooding events and groundwater discharge. Since 1930, when this sea became a large freshwater lake after the construction of a major dam (‘De Afsluitdijk’), saline influence decreased, resulting in an almost complete collapse of brackish biodiversity (Van Vierssen and Breukelaar 1993; Van’t Veer et al. 2012; Van Dam 2009). Both sediment layers, the upper partly decomposed sludge layer and the deeper intact peat, were collected from a canal. The second sediment was collected in a lake (53°16′28.50–6°59′43.02) characterized by marine clay deposition near the Eems Dollart Estuary with a stronger historical salinity influence.

Characteristics of both sediment types used in the experiment are summarized in Table 1. The peat sediment was rich in organic matter, (especially the lower layer, 91 %), while the clay sediment contained much less organic matter (10 %). The peat sediment was richer in sodium and sulfur because of stronger former influence of brackish water. The peat sludge sediment and the clay sediment were both rich in iron and phosphorus (Table 1).

### Experimental set-up

#### *Aquarium experiment*

Forty glass aquaria were filled with a 12 cm layer of carefully mixed sediment (20 with clay, 20 with peat). The peat sediment consisted of 6 cm of intact peat at the bottom covered with 6 cm of peat sludge, mimicking the field situation. Next, water was carefully added to the aquaria to a depth of 12 cm. The aquaria were kept at 15 °C in a climate controlled chamber in the dark, to prevent the growth of vegetation and algae. Two pore water samplers (Rhizon SMS 5 cm; Eijkelkamp Agrisearch Equipment, Netherlands) were placed diagonally in the sediment of each aquarium. After 3 weeks, artificial surface water was pumped through the aquaria for 6 months, at a rate of 1.11 l day<sup>-1</sup> (retention time of 3.4 days). Three surface water treatments were used: constant freshwater (Fw, 5 mmol Cl l<sup>-1</sup>, 0.32 psu, constant brackish water (Bw, 50 mmol Cl l<sup>-1</sup>, 3.2 psu) and a fluctuating freshwater—brackish water treatment (Fl, 5–50 mmol Cl l<sup>-1</sup>), with 4 replicates per treatment (Table 2; Fig. 3). Salinity levels were chosen based on historic surface water salinity levels in Dutch coastal wetland systems (Van’t Veer et al. 2012). The fluctuating regime consisted of an alternation of 4 weeks of freshwater and 2 weeks of brackish water, which the exception of the third brackish water period which lasted 3 weeks. Sea salt (Tropic Marin, Wartenberg, Germany) including sulfate was used instead of NaCl to provide a more realistic representation of increased salinity effects, and to be able to extrapolate them to field conditions.

In addition to the effects of the different salinity treatments, the effect of introduction of micro-

**Table 1** Organic matter content and total element concentrations of the sediments used in the experiment, average concentrations are given with S.E.M. between brackets

Type [depth (cm)]	Organic matter content (%)	Bulk density (kg DW/l FW)	Na (μmol g <sup>-1</sup> DW)	Ca	K	Fe	P	S
Peat								
Sludge (0–6)	48 (0.01)	0.21 (<0.01)	113 (5)	398 (10)	39 (2)	227 (12)	27 (1)	654 (22)
Intact peat (6–12)	88 (0.02)	0.12 (0.01)	195 (10)	276 (11)	14 (2)	33 (12)	9 (1)	370 (19)
Clay								
(0–12)	10 (0.03)	1.07 (0.01)	10 (1)	123 (4)	45 (1)	264 (6)	18 (0.4)	46 (1)

**Table 2** Chemical composition of the surface water of the three treatments used (mmol per liter)

Duration	Fw	Fl		Bw
		Constant	1 month	
Cl <sup>-</sup>	5	5	50	50
Na <sup>+</sup>	7.5	7.5	45	45
Ca <sup>2+</sup>	1	1	2.5	2.5
HCO <sub>3</sub> <sup>-</sup>	3	3	3	3
SO <sub>4</sub> <sup>2-</sup>	1	1	5	5
Mg <sup>2+</sup>	0.5	0.5	4	4
K <sup>+</sup>	0.1	0.1	0.8	0.8
pH	8	8	8	8

Fw Fresh water, Fl fluctuating salinity, Bw brackish water

organisms adapted to a brackish environment was studied. For this, aquaria were filled with the similar two sediment types, but additionally enriched with brackish sediment (5 % addition based on fresh weight volume) originating from two brackish ditches (51°33'35.87, 4°08'49.26 and 51°31'56.05, 4°06'31.25) in four replicates per sediment type and treated similarly. As the addition of brackish sediment did not significantly alter nutrient concentrations and carbon emissions, the results will not be shown in the present paper.

Sediment pore water and surface water samples were taken every 2 weeks. Pore water samples were taken by connecting 100 ml vacuum bottles to the pore water samplers. Samples of both pore water samplers were pooled for each aquarium. Samples for pore water gas concentration were collected by connecting vacuum 12 ml glass exetainers (Labco exetainer<sup>®</sup>, High Wycimbe, UK) to the pore water samplers by which 6 ml of pore water was collected, and gas concentrations were measured in the headspace.

#### *Emission and production of CO<sub>2</sub> and CH<sub>4</sub>*

In situ CO<sub>2</sub> and CH<sub>4</sub> emissions were analyzed by carefully placing funnels (11 cm in diameter, volume of 0.25 dm<sup>3</sup>) on the water surface for 6 h at the end of the experiment. Gas samples were analyzed in the funnel headspace at t = 0, 3 and 6 h. At the end of the experiment sediment of all treatments was collected

for anaerobic incubation. For this incubation 6 ml of anaerobic aquatic sediment and 2 ml dinitrogen (N<sub>2</sub>) flushed water were mixed in 15 ml airtight bottles. The headspace of the bottles was flushed for 5 min with N<sub>2</sub> directly after sealing, and the bottles were kept in the dark at 15 °C. The increase of the CO<sub>2</sub> and CH<sub>4</sub> concentrations in the headspace was measured after 1, 3, 6, 24 and 168 h, after sampling (0.1 ml for CH<sub>4</sub> and 0.5 ml for CO<sub>2</sub>), an equal amount of N<sub>2</sub> was added to prevent pressure differences. Emission and production rates of CO<sub>2</sub> and CH<sub>4</sub> were determined using linear regression.

#### *Surface and pore water samples*

The pH values of surface water and pore water samples were measured using a combined pH electrode with a Ag/AgCl internal reference (Orion Research, Beverly, CA, USA) and a TIM800 pH meter. Turbidity was measured using a Turb 550 turbidity meter (WTW GmbH, Weilheim). Total dissolved inorganic carbon concentrations were measured using infrared gas analysis (IRGA, ABB Advance Optima, Zürich, Switzerland). Prior to elemental analyses, 10 ml of each sample was stored at 4 °C until analyses with 0.1 ml (65 %) HNO<sub>3</sub> to prevent metal precipitation. For the analyses of P, Ca, Mg, Fe, S, K and Al inductively coupled plasma spectrophotometry (ICP-Optical Emission Spectrometer, Thermo Scientific iCAP 6000 Series ICP) was used. To determine nitrate (Kamphake et al. 1967), ammonium (Grasshoff and Johanssen 1972), orthophosphate (Henriksen 1965), sodium and chloride concentrations, 20 ml of each sample was stored at -20 °C and analyzed colorimetrically with an Auto Analyzer 3 system (Bran and Luebbe). Sodium and potassium were determined with a Technicon Flame Photometer IV Control (Technicon Corporation).

#### *Gas samples*

CO<sub>2</sub> concentrations were measured using an infrared gas analyzer (IRGA, ABB Advance Optima, Zürich, Switzerland). CH<sub>4</sub> concentrations were measured with a Hewlett-Packard 5890 gas chromatograph (Avondale, California) equipped with a flame-ionization detector and a Porapak Q column (80/100 mesh) operated at 120 °C with N<sub>2</sub> as carrier gas.

### Sediment samples

Wet and dry weights of the sediments were measured by drying a known volume of sediment before and after heating at 70 °C until constant weight. The organic matter content was determined by loss-on-ignition for 4 h at 550 °C. Total concentrations of Fe, S and P in the sediment samples were determined by digesting 200 mg of dried (24 h, 70 °C) and homogenized sample in 4 ml concentrated HNO<sub>3</sub> and 1 ml 30 % H<sub>2</sub>O<sub>2</sub> (Milestone microwave MLS 1200 Mega). Sample extracts were analyzed after dilution by ICP, as described above. Exchangeable cation concentrations of the sediments (Na, Mg, Mn, Ca, K and NH<sub>4</sub>) were determined at the end of the experiment by shaking (105 r.p.m.) 17.5 g of fresh sediment for 60 min with a 0.2 M SrCl<sub>2</sub> solution. The supernatant was analyzed using ICP (see details above).

### Statistics

All treatment effects were tested with SPSS Statistics for Windows (Version 21.0. IBM Corp. Armonk, NY; Released 2012). Effects for surface water and sediment pore water were tested for the period in which the pumps were active ( $t = 33$ –201 days). To test for significance of treatment effects in the repeated design used, data were tested using a generalized linear mixed model, using treatment as fixed factor and time as repeated measures; the co-variance type used was AR(1): heterogeneous. Both sediment types were analyzed separately. A Bonferroni post hoc test was used to test for statistical differences between treatments. Treatment effects for the sediment analyses and decomposition analyses (both single measurements) were tested using a One-Way ANOVA and a Post-Hoc least significant difference test. Differences between treatments with P values below 0.05 were referred to as significant differences.

## Results

### Sediment pore water

In both sediment types pore water concentrations of chloride (Cl), sodium (Na), sulfur (S) and potassium

(K) increased significantly in the constant brackish water treatment (Bw) compared to the constant freshwater treatment (Fw), showing saltwater intrusion into the sediment (Figs. 1, 2). Moreover, pore water concentrations of calcium (Ca), magnesium (Mg), ammonium (NH<sub>4</sub><sup>+</sup>), and for the clay sediment also iron (Fe), were much higher for Bw and fluctuating freshwater—brackish water treatment (Fl), and exceeded freshwater treatment concentrations. In addition, pore water pH decreased for these treatments (Fig. 2). At the end of the experiment pore water ion concentrations were about 25 % lower for Fl than for Bw (Peat sediment: Cl 27 %, Na 29 %, Ca 15 %, NH<sub>4</sub> 5 %, Clay sediment: Cl 34 %, Na 32 %, Ca 29 %, NH<sub>4</sub> 13 %). Due to diffusion to the water layer, anion and cation concentrations in the sediment pore water of both sediments decreased over time for Fw.

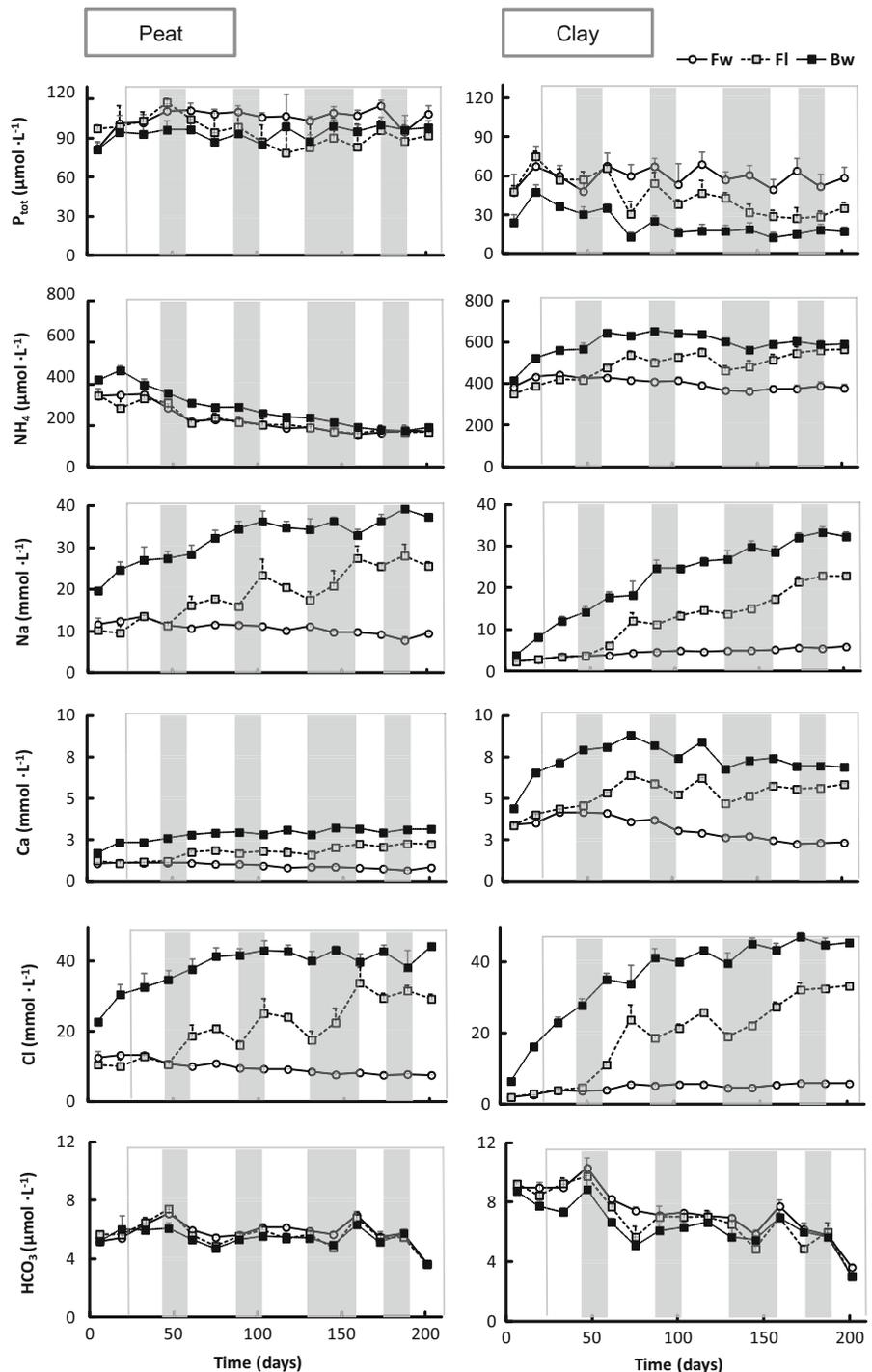
Brackish water (both Bw and Fl) led to a strong immobilization of phosphorus in clay sediments. Total phosphorus (P) concentrations in the sediment pore water were high but decreased 70 % in clay sediment as a result of both Bw (from 55 to 15  $\mu\text{mol P l}^{-1}$ ) as well as for Fl (from 55 to 30  $\mu\text{mol P l}^{-1} \approx 50$  % decrease at the end) compared to the freshwater (Fw) treatment (Fig. 1). Peat sediment showed a small (8.5 %) decrease under Bw treatment, but not for the Fl treatment.

Pore water NH<sub>4</sub><sup>+</sup> concentrations in the clay sediment increased significantly in both the Bw (55 %) and the Fl treatment (45 %), although slower for the latter (Fig. 1). In the peat sediment, in contrast, NH<sub>4</sub><sup>+</sup> concentrations decreased in all treatments over time, but remained slightly (but significantly) higher for Bw (Fig. 1). Pore water NO<sub>3</sub><sup>-</sup> concentrations remained below the detection limit (1  $\mu\text{mol l}^{-1}$ ) for both sediment types in all treatments (data not shown).

### Sediment cation adsorption

Compared to the Fw treatment, SrCl<sub>2</sub> extractable concentrations of Na and K increased significantly in the Bw and Fl treatments for both sediment types, and for Mg in the clay sediment (Table 4). The Bw treatment reduced SrCl<sub>2</sub> extractable Ca and NH<sub>4</sub><sup>+</sup> concentrations in the clay sediment. Peat sediment showed a similar, but not significant trend.

**Fig. 1** Sediment pore water concentrations for peat (*left*) and clay (*right*) sediments over time (+ S.E.M.,  $n = 4$ ). The *square* indicates the period during which the surface water was refreshed. Treatments: freshwater (Fw, *white circles with black line*), fluctuating salinity (Fl, *grey squares with dotted line*) and brackish water (Bw, *black squares with grey line*). The shadings indicate the increased salinity episodes for the fluctuating salinity treatment

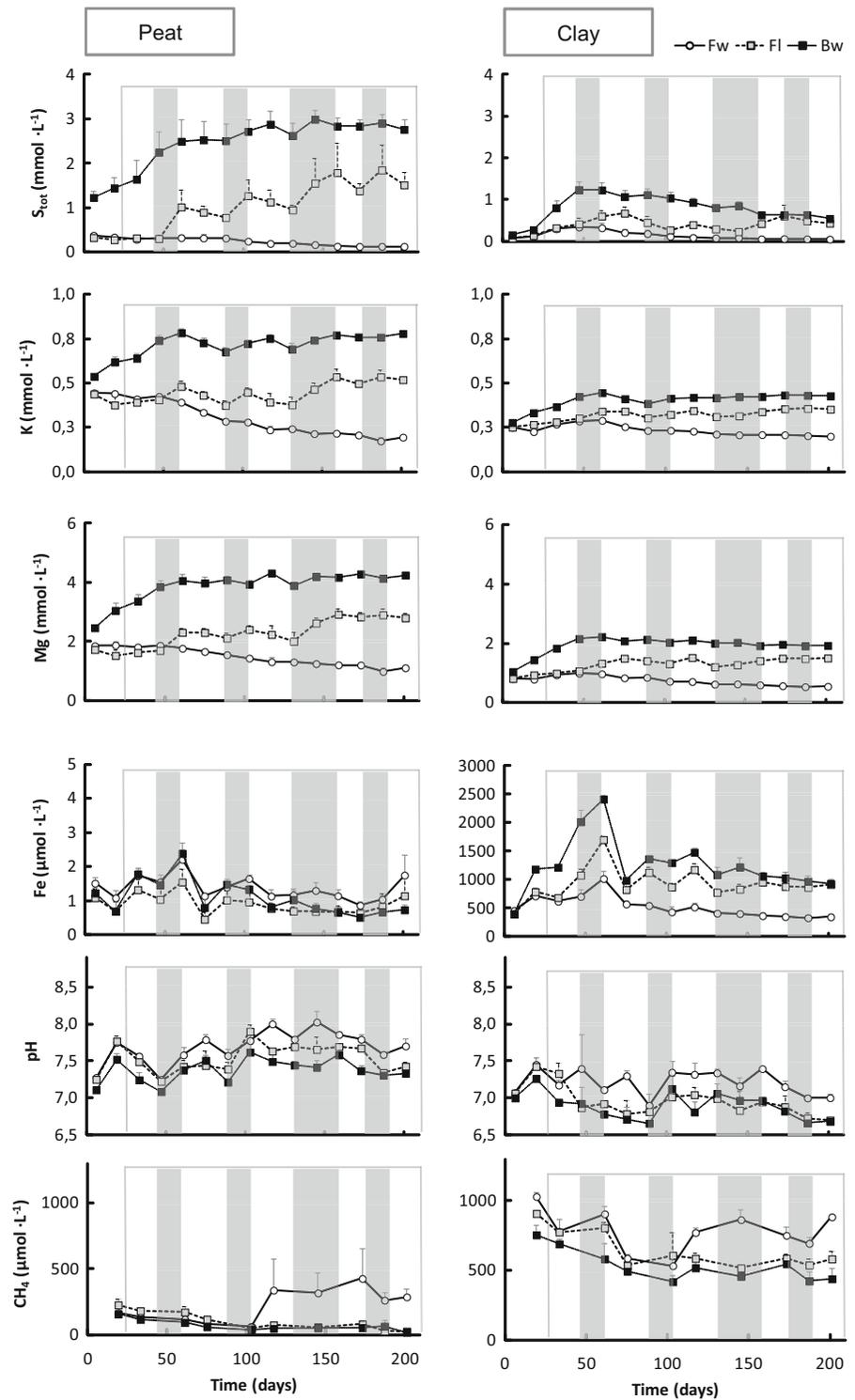


### Inorganic carbon and methane

For both sediment types pore water  $\text{CH}_4$  concentrations were significantly lower in both Bw and Fl

(Fig. 2).  $\text{CO}_2$  and total inorganic carbon (including bicarbonate) in the sediment pore water did not differ significantly (data not shown).  $\text{CO}_2$  emissions to the atmosphere were around  $80\text{--}160 \mu\text{mol m}^{-2} \text{h}^{-1}$  for

**Fig. 2** Sediment pore water concentrations for peat (*left*) and clay (*right*) sediments over time (+ S.E.M., n = 4). The *square* indicates the period during which the surface water was refreshed. Treatments: freshwater (Fw, *white circles with black line*), fluctuating salinity (FI, *grey squares with dotted line*) and brackish water (Bw, *black squares with grey line*). The shadings indicate the increased salinity episodes for the fluctuating salinity treatment. Note the different scales for iron



all treatments, without treatment or sediment type effect.  $\text{CH}_4$  emissions were below the detection limit of  $0.0001 \mu\text{mol m}^{-2} \text{h}^{-1}$ ; data not shown. Anaerobic incubation of the sediments at the end of the experiment showed no treatment effects for  $\text{CO}_2$  production.  $\text{CO}_2$  production was, however, significantly higher for the peat sediment versus the clay sediment (Fig. 4).  $\text{CH}_4$  production was, however, significantly lower for peat sediments treated with Bw or FI compared to the Fw treatment (Fig. 4). For the clay sediment,  $\text{CH}_4$  production rates were very low, without any treatment effects (Fig. 4).

### Surface water

Surface water chemistry followed the chemical composition of the treatment water (chloride concentrations in Fig. 3, other elements not shown). For FI, the chemical composition was similar to Bw at the end of each brackish period, and similar to Fw at the end of each freshwater period. The chemical composition for FI was similar to Bw, and did not differ significantly from the Fw treatment at the end of freshwater periods. Surface water nutrient concentrations were not affected by treatment, most probably because of the high refreshment rates (data not shown). Despite this

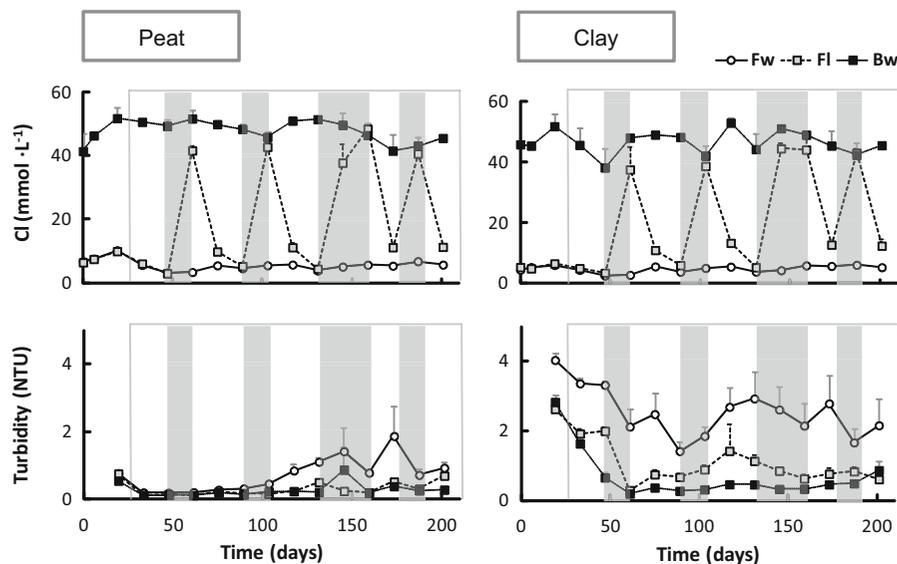
high rate, turbidity decreased significantly in both the Bw and the FI treatment (Fig. 3; Table 3).

## Discussion

### Salinity-induced cation exchange

For both constant and fluctuating salinity the input of brackish water resulted in a rapid exchange of Na, Mg and K supplied via the surface water against Ca,  $\text{H}^+$  and  $\text{NH}_4^+$  from the cation adsorption complex in both sediment types, as well as against Fe in the clay sediment. Although  $\text{SrCl}_2$  extractable Ca and  $\text{NH}_4^+$  concentrations were similar or even higher on a mass base for peat sediment, the increase of  $\text{NH}_4^+$  and Ca was much stronger in the clay sediment. This can be explained by the fact that, on a volume base, clay sediments have a larger cation exchange complex (CEC) than peat sediments.

As a result of cation exchange, pore water Ca concentrations strongly exceeded concentrations in the surface water. This salinization-induced Ca and Fe exchange has also been reported in other studies (Poonia and Talibudeen 1977; Loeb et al. 2008; Baldwin et al. 2006; Weston et al. 2006). The absence



**Fig. 3** Chloride concentrations and turbidity in the surface water above the peat sediments (*left*) and clay sediments (*right*) over time (+ S.E.M.,  $n = 4$ ). The square indicates the period in which the surface water was refreshed. Treatments: freshwater (Fw, white circles), fluctuating salinity (FI, grey squares) and brackish water (Bw, black squares). The shadings indicate the increased salinity episodes for the fluctuating salinity treatment

**Table 3** P values of statistical analysis of treatment effects (*F<sub>w</sub>* freshwater treatment, *B<sub>w</sub>* brackish water treatment, *F<sub>l</sub>* fluctuating treatment) in pore water and surface water for both sediment types

	Peat			Clay		
	Fw Bw	Fw Fl	Bw Fl	Fw Bw	Fw Fl	Bw Fl
Pore water						
P <sub>(tot)</sub>	<b>0.01</b>	0.06	0.91	<b>0.00</b>	<b>0.01</b>	<b>0.00</b>
NH <sub>4</sub>	<b>0.01</b>	0.06	1.00	<b>0.00</b>	<b>0.00</b>	0.69
Na	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
Cl	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
Ca	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
pH	<b>0.00</b>	<b>0.00</b>	<b>0.02</b>	<b>0.00</b>	<b>0.00</b>	0.20
HCO <sub>3</sub>	<b>0.00</b>	0.16	0.54	<b>0.05</b>	0.66	0.10
S <sub>(tot)</sub>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.02</b>
K	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
Mg	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
Fe	<b>0.01</b>	<b>0.05</b>	1.00	<b>0.00</b>	<b>0.01</b>	<b>0.03</b>
CH <sub>4</sub>	1.00	1.00	1.00	<b>0.00</b>	<b>0.00</b>	0.20
Surface water						
Cl	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
Turbidity	<b>0.03</b>	<b>0.01</b>	0.93	<b>0.00</b>	<b>0.01</b>	<b>0.05</b>
Ca	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
P <sub>(tot)</sub>	0.36	0.43	0.45	1.00	<b>0.00</b>	<b>0.00</b>
pH	<b>0.01</b>	<b>0.03</b>	1.00	<b>0.00</b>	<b>0.00</b>	1.00

Significant effects are presented in bold

of Fe exchange in the peat sediment can be explained by the brackish, sulfur (S) rich history of the location. The peat sediment is rich in reduced S, and most Fe in such sediments is bound as iron sulfides, while only a small fraction is bound to the cation adsorption complex (Smolders et al. 2006a, b; Geurts et al. 2010; Van Diggelen et al. 2014).

#### Effects on sediment nutrient dynamics

We found that salinization lowered sediment pore water P concentrations. Although some studies have shown a similar decrease of dissolved P in pore water during salinization (e.g. Baldwin et al. 2006; Weston et al. 2006), others have shown an increase by desorption (Sundareshwar and Morris 1999; Beltman et al. 2000). Our results do not exclude P desorption, but at least indicate that the immobilization of P was much stronger

than its mobilization, resulting in a net decrease of pore water P concentrations. The decrease in P can most likely be explained by co-precipitation with calcium or calcium carbonate (Bale and Morris 1981; Morris et al. 1981; House 1999) and possibly also by binding to newly formed iron (hydr)oxides in the clay sediment (Roden and Edmonds 1997; Sundareshwar and Morris 1999). This hypothesis is supported by the observation that P levels indeed decreased most strongly in the clay sediment, showing the highest exchange of both Ca and Fe upon salinization.

We found high pore water P concentrations that decreased in time under both a constant and fluctuating salinity. Geurts et al. (2010) showed a linear correlation between pore water P concentration and P release from the aquatic sediment to the overlying water layer under aerobic surface water conditions and low Fe:P in the pore water, as was the case in the peat sediment here. At pore water Fe:P > 1, as for the clay sediment, P mobilization rates can be expected to be low as long as the water layer is aerobic. If, however, the water layer becomes anaerobic, as often occurs in eutrophic systems with high organic matter loads such as the one studied here, this type of Fe–P interaction at the sediment–water boundary layer no longer occurs due to lack of Fe oxidation (Smolders et al. 2006a, b). Under these conditions, P mobilization rates to the water layer will also largely depend on porewater P concentrations, regardless Fe:P ratios.

So one would expect a similar 50–70 % reduction in P release towards the surface water, which under more stagnant conditions would lead to a lower availability of P in the water layer. As they are often P limited, this would have a positive effect on the currently eutrophied aquatic ecosystems. Due to the high refreshment rate of surface water used in the present study, necessary to test the salinization effects in sediments under controlled, stable conditions, surface water nutrient concentrations remained below the detection limit. In most wetlands, the refreshment rates of the surface water will however be much lower compared to our experimental set-up.

The increase of sulfate did not cause P mobilization, a phenomenon often seen in freshwater wetlands (Roden and Edmonds 1997; Lamers et al. 1998, 2002b). In the peat sediment, S was present in such excess relative to Fe (Tables 1, 4) that it can be assumed that all Fe is bound to sulfide and that Fe does not play a major role in the phosphate adsorption under anaerobic

**Table 4** SrCl<sub>2</sub> extractable cation concentrations in both sediment types

	Peat			Clay		
	Fw	Fl	Bw	Fw	Fl	Bw
SrCl <sub>2</sub> extractable concentration (mmol kg DW <sup>-1</sup> )						
Ca	451.4 (37.9)a	428.3 (40.3)a	343.2 (22.7)a	116.9 (5.3)a	109.2 (2.6)ab	102.9 (2.1)b
K	8.1 (0.7)a	14.1 (1.8)b	18.5 (0.8)c	9.2 (0.5)a	9.9 (0.6)a	12.2 (0.3)b
Mg	216.6 (18.7) <sup>a</sup>	231.7 (19.8)a	208.5 (15)a	20.9 (1.3)a	22.0 (1.3)a	28.9 (1.2)b
Mn	4.5 (0.5)a	4.1 (0.4)a	3.1 (0.5)a	2.2 (0.1)a	2.0 (0.1)a	1.9 (0.1)a
Na	162.7 (5.7)a	331.2 (25.6)b	568.2 (23.7)c	16.9 (1.1)a	44.5 (1.2)b	79.1 (0.9)c
NH <sub>4</sub>	10.1 (0.78)a	8.5 (0.3)a	7.9 (1.1)a	10 (0.2)a	8.5 (0.6)b	7 (0.3)c

Average concentrations are given with S.E.M. between brackets, significant differences between treatments ( $P < 0.05$ ) per sediment type are indicated by different letters

circumstances. Hence, an increased sulfide production by the increased supply of sulfate did not mobilize phosphate in this sediment. In the clay sediment on the other hand, Fe was present in such excess relative to S as well as to P that sulfide formation and FeS<sub>x</sub> precipitation did not affect the adsorption of phosphate within the course of the experiment (Zak et al. 2006; Loeb et al. 2008). Only on the longer term, brackish conditions with constant supply of sulfate and increased sulfide formation may, but not necessarily do, result in P release in this type of sediment.

Ammonium mobilization as a result of salinization-induced cation exchange is known to be an important process in aquatic sediments (Seitzinger et al. 1991; Rysgaard et al. 1999; Weston et al. 2006, 2010). This can be expected to cause eutrophication in N limited aquatic ecosystems. Whether this also leads to ammonium accumulation in the water layer is, however, also strongly determined by nitrification and subsequent denitrification rates (Strauss et al. 2002; Burgin and Hamilton 2007). Marton et al. (2012) and Craft et al. (2009) found nitrogen accumulation to decline with increased salinity but found large differences between different sediment types, and further studies on this subject are therefore important.

The combination of the decrease in P availability and the increase in N availability can be expected to

intensify P limitation of phytoplankton (which is common in most freshwater wetlands) during the transition from freshwater into brackish water on the short term. On the long term, however, one would expect that less binding of ammonium onto the cation exchange sites of the sediment would result in a decrease of the sediment ammonium concentrations. On the longer term, lowered N availability and increased S-induced P availability could therefore lead to N limitation, which is common in brackish waters (Howarth and Marino 2006). Effects of salinization on sediment characteristics seem to be time-dependent and can differ between the short and long term. The large differences between biogeochemical effects for both sediment types tested in this paper warrant further study, in order to be able to predict effects of salinization based on sediment characteristics.

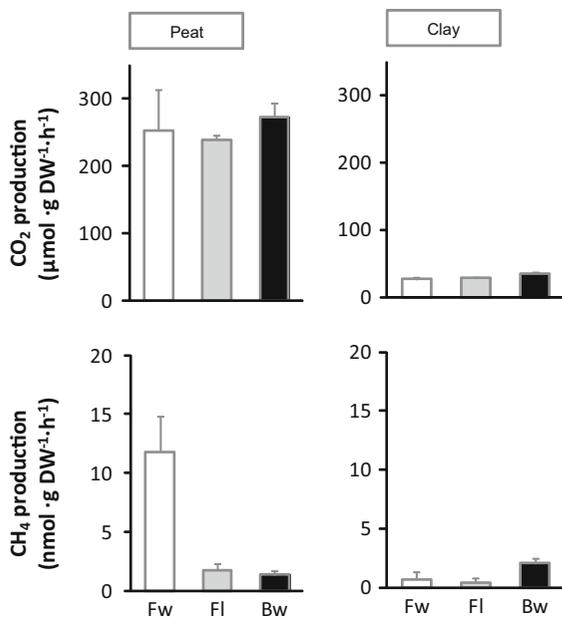
In the present study, effects of both constant and fluctuating salinity on biogeochemical processes in underwater sediments were tested. The experimental set-up, under controlled dark and plant free conditions, enabled us to gather information about direct effects of increased salinity on sediment biogeochemistry. This set-up, however, consequently led to some limitations in extrapolating the results to vegetated coastal wetlands. Although there were no aquatic plants present at the location where the peat sediment was collected, which has become characteristic of many coastal wetlands in the Netherlands, it is important to consider the potential effects of vegetation presence on the biogeochemical effects of salinization. Aquatic macrophytes will potentially have an effect by radial oxygen loss from their roots (Lamers et al. 2012). Although cation-exchange related effects of salinization will not be affected, rates of anaerobic redox processes such as sulfate reduction and methanogenesis might be reduced. In addition, aquatic vegetation might also reduce porewater nutrient concentrations due to uptake by roots. On the other hand, the input of fresh plant litter may also enhance anoxic conditions and sulfate reduction rates (Lamers et al. 2012). More in general, increased salinity levels and to a greater extent fluctuating salinity levels will not lead to high ionic concentrations, but also to the accumulation of potentially toxic substances such as sulfide and ammonium. These biogeochemical changes will therefore create a habitat that is only suitable for a selection of brackish species of flora and fauna.

## Turbidity changes

Salinity-induced aggregation and flocculation of suspended matter has been described earlier for estuaries for high salinity ranges (Sholkovitz 1976; Eisma 1986). The significant decrease in surface water turbidity under both fluctuating as well as constantly brackish water in the present paper indicates that these processes are also important within lower salinity ranges. The increase of the surface water turbidity in the peat sediment under the Fw treatment might have been caused by ebullition of methane, which was observed. Given the observed methane production rates and pore water methane concentrations in both sediments (Figs. 2, 4), more methane seems to escape from the peat sediment. Decreased turbidity of the surface water will stimulate the development of submerged macrophytes, which are often limited by light in eutrophic coastal wetlands as a result of high turbidity due to suspended particles and algal blooms.

## Effect on decomposition

Compared to the freshwater treatments,  $\text{CH}_4$  concentrations in the sediment pore water were



**Fig. 4** Production rates of  $\text{CO}_2$  (top) and  $\text{CH}_4$  (bottom) during incubation of peat (left) and clay sediments (right) (+ S.E.M.,  $n = 4$ ). Treatments: freshwater treatment (Fw, white bars), fluctuating (Fl, grey bars), brackish water (Bw, black bars)

significantly lower in the Bw and Fl treatments during the entire experimental period in the clay sediments and during the second half of the experiment in the peat sediments. Similar to several other studies (e.g. Mishra et al. 2003; Baldwin et al. 2006; Weston et al. 2006; Chambers et al. 2011) increased salinity significantly decreased  $\text{CH}_4$  production. Chambers et al. 2013 also found decreased methanogenesis with pulsed salinity in an intertidal wetland system. In sulfate rich environments methanogenic bacteria become outcompeted for organic substrates by sulfate reducing bacteria (Capone and Kiene 1988; Scholten and Stams 1995; Smolders et al. 2002, Lamers et al. 2013). Apart from reduced methanogenic activity caused by sulfate reduction, other studies also report osmotic stress leading to altered microbiological communities after salinization influencing biogeochemical cycles (Rysgaard et al. 1999; Pattnaik et al. 2000; Mishra et al. 2003; Baldwin et al. 2006; Chambers et al. 2011).

Salinity effects on  $\text{CO}_2$  production and emission reported in literature largely differ, varying from no effect, as found in the present paper, to decreased or increased  $\text{CO}_2$  emissions. Results of the present study indicate no significant short-term difference between constant or pulsed salinity on  $\text{CO}_2$  emissions.

## Fluctuating versus constant salinity

Effects of salinization are generally tested in laboratory experiments using a constant low versus a constant high salinity treatment. However, brackish environments are generally characterized by fluctuating salinity. In the present study the fluctuating salinity treatment showed to have intermediate biogeochemical effects (e.g. for nutrient and cation concentrations) in the short-term for both sediment types. In the longer term, however, the effects of fluctuating salinity became gradually more similar to those of constantly brackish water conditions. This also points out that repeated short periods of increased salinity in brackish systems can have large long-term effects on the biogeochemistry of aquatic sediments. Therefore, our hypothesis that fluctuating salinity levels cause less pronounced effects on biogeochemical processes than a constant increased salinity, can be rejected, considering the expected long-term effects similar to those of constant high salinity levels.

## Conclusion: salinization effects on aquatic ecosystems

Our results provide insights that are not only important to understand the impact of salinization on biogeochemical processes of freshwater aquatic sediments, but also for the restoration of brackish coastal wetlands. Increased salinity levels can have fast and major effects on biogeochemical processes in aquatic sediments of coastal wetlands. Sediment cation exchange appeared to be the key process explaining both the decreased phosphorus and increased nitrogen concentrations, potentially leading to a shift from N to P limitation in the surface water. On the long term both constant and fluctuating increased salinity levels may possibly lead to lower N availability as well, depending on the concentrations of ammonium bound to the sediment, and the effects of increased salinity on nitrification and denitrification rates. Increased salinity did not affect sediment CO<sub>2</sub> production, but decreased CH<sub>4</sub> production. No treatment effects on greenhouse gas emissions were found. The salinity-induced decrease in surface water turbidity can be expected to favor the growth of submerged aquatic macrophyte species adapted to brackish conditions. The present study showed a fluctuating salinity to have intermediate biogeochemical effects for both sediment types in the short term. In the longer term however, similar biogeochemical effects can be expected for both fluctuating and constant salinity levels under brackish water conditions.

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