



PEATLANDS AFFECTED BY BIOGEOCHEMICAL STRESSORS

CONSEQUENCES OF INCREASED SALINITY AND NITRATE LEVELS
FOR BIOGEOCHEMICAL CYCLING, MICROBIOLOGICAL PATHWAYS,
ECOHYDROLOGY AND BIODIVERSITY

GIJS VAN DIJK

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Gijs van Dijk
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“Alles ist Wechselwirkung”
(everything is interaction)

Alexander Von Humboldt (1803)

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[Brunssummerheide, by G. van Dijk]

1.

Introduction

1.1 GENERAL INTRODUCTION TO PEATLANDS

As all studies included in my thesis have been carried out in peatland ecosystems, I will start with a short introduction to these peculiar ecosystem types. Peatlands are areas within landscapes where soil organic material accumulates over time. Under wet conditions, often with an average annual precipitation surplus and/or seepage influence, (partly) anaerobic soil conditions prevail, and organic material produced only partly decomposes and therefore accumulates over time (Succow & Joosten, 2001). The specific environmental conditions affect the species assemblages within different peatland types, and thereby also the organic matter composition of the peat soil. Peatlands are generally defined as ecosystems with organic soil layers comprising at least 30% organic matter and having a thickness of at least 30 cm (Joosten & Clarke, 2002).

Although they can be found worldwide from the tropics to the arctic, the majority of peatlands are found in the temperate, boreal and subarctic areas of the Northern hemisphere (Fig. 1.1). Worldwide peatlands cover around 4 million km² ($\approx$ 2.6 % of the total land surface) and represent a major long-term store of soil carbon. About 30% of all global soil carbon is stored in peat soils (Joosten & Clarke 2002). As peatlands can vary in type and characteristics, they can be very heterogeneous on a landscape scale. This heterogeneity provides a strong spatial variety of habitat conditions, resulting in a high diversity of species communities. Some peatlands are among the most productive ecosystems with a wide range of high productive species, whereas others are low in productivity can harbor a wide range of less productive but specific set of species (Wheeler & Proctor 2000). Relative to their small cover of the land surface, peatlands provide a disproportionately large number of natural resources and services. Next to supporting a high biodiversity, peatlands provide several key functions such as acting as a large freshwater reservoir, hydrological regulation, carbon sequestration and filtering of nutrient and pollutants. Peatlands have received growing attention from both scientists and policy makers due to global change, the importance of ecosystem services they provide, and their high vulnerability to anthropogenic disturbance.

Peatlands cover a large variety of spatial scales, from small paludified ponds up to entire raised bog landscapes with more than ten meter of peat. Peatland formation can take place under a variety of environmental conditions and by different vegetation communities including both bryophytes and vascular plants such as sedges and shrubs. Peatland formation can start from open water (paludification), or at locations in a landscape where water stagnated due to the presence of less permeable sediment layers or continuous groundwater seepage or surface water influence. Next to freshwater peatlands, there are also peatlands that have been formed under brackish conditions. They show a large variety in age; in the Northern hemisphere most larger peatlands started to develop after the last glaciation (about 7,500 – 10,000 years ago)

but there are also younger peatlands (Rydin & Jeglum, 2013). Under the right conditions for peat formation, organic material can accumulate over time, which will lead to decreasing influence of groundwater and/or surface water and increasing influence of precipitation. This succession leads to a change in environmental conditions (driven by local biogeochemical and (eco)hydrological conditions) and succession of species assemblages through time. Simultaneously new peat is formed accumulating in the top layer, while deeper in the profile decomposition processes still take place. This often leads to heterogeneous conditions in vertical direction. In general, with increasing depth, mineral richness increases, and peat layers become more anaerobic and compacted, while the hydraulic conductivity decreases and the degree of decomposition increases. During succession the rhizosphere becomes more and more isolated from groundwater and surface water, decreasing the availability of minerals and nutrients. In later successional stages peat mosses (*Sphagnum* spp.) strongly increase in cover. Being ecosystem engineers, they strongly modify their surroundings to their own benefit, especially because of their high water holding capacity and ability to acidify their environment. This means that biogeochemical conditions influence the presence and abundance of species and peat formation, and vice versa.

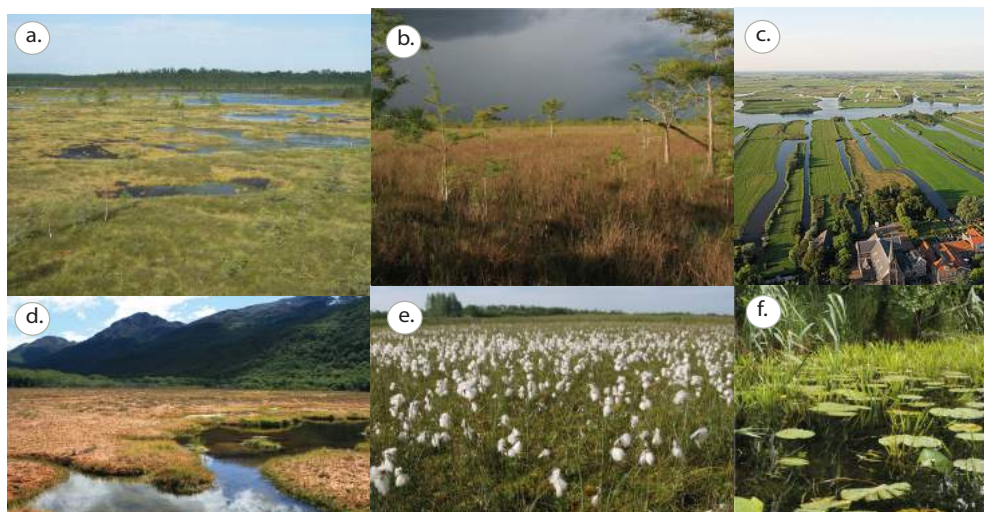


Fig. 1.1. Overview with some pictures of a variety of different peatlands, a. Nigula bog in Estonia, b. The Everglades in Florida USA, c. The IJperveld, North Holland the Netherlands, d. Andorra bog in Tierra del Fuego Argentina, e. an terrestrialized peat pond in Westbroek, Utrecht, the Netherlands, f. peat formation from open water in the Naardermeer, Noord-Holland, The Netherlands. [c. by P. Boerman, all other pictures by G. van Dijk].

Anthropogenic influence can have a major impact on biogeochemical and other environmental conditions, changing not only species abundance and diversity and thereby also peat formation, but also changing the balance between peat decomposition and formation by altered decomposition rates.

1.2 ANTHROPOGENIC INFLUENCE ON PEATLANDS

Like most other ecosystem types, peatlands suffer from anthropogenic forcing including habitat destruction and fragmentation, water, air and soil pollution, eutrophication, and heavily altered hydrology for instance leading to water shortage. This has detrimental effects on peatland functioning and services, e.g. leading to reduced hydrological regulation, carbon storage and biodiversity (Lamers et al. 2002). Peatlands that often needed hundreds to thousands of years to be formed, can be destroyed within years. At a global scale, 20% of all peatlands have been lost entirely or have been degraded to a large extent. For densely populated areas (such as Europe) this percentage even goes up to 50% (Joosten & Clarke 2002, Lamers et al. 2014). The remaining more or less intact peatlands are increasingly under pressure. The combination of an increasing human population, agricultural intensification, drainage, eutrophication and human-induced climate change will inevitably affect the future functioning of the remaining peatlands to a large extent. This will also have large negative consequences for the services that they provide. Scientific knowledge, including practical and directly applicable, of how anthropogenic forcing influences peatland functioning and how this can be restored, is therefore highly valuable to policy makers, managers and scientists. Only when based on evidence-based studies, good decisions can be made on how to manage peatlands and their surrounding landscapes in a sustainable way under ever-increasing anthropogenic pressure.

1.3 THESIS SCOPE

Although peatlands received growing attention from scientists and policy makers in recent decades, there are still many uncertainties with respect to the exact anthropogenic influences, their mechanistic understanding, and optimal mitigation options. Given their complex functioning and heterogeneity, it can be very challenging to make predictions about effects and mitigation for peatlands. My thesis will focus on two themes that received much less attention than others (Fig. 1.2);

- **Theme 1 Groundwater nitrate enrichment and peatland functioning**
- **Theme 2 Surface water salinization and peatland functioning**

Although groundwater nitrate enrichment and surface water salinization may also occur under natural conditions, these conditions are often caused by human impact. Both themes are studied here using a range of research methods and covering different disciplines such as (landscape) ecology, (eco-)hydrology, microbiology and environmental science, with a strong focus on biogeochemical processes.

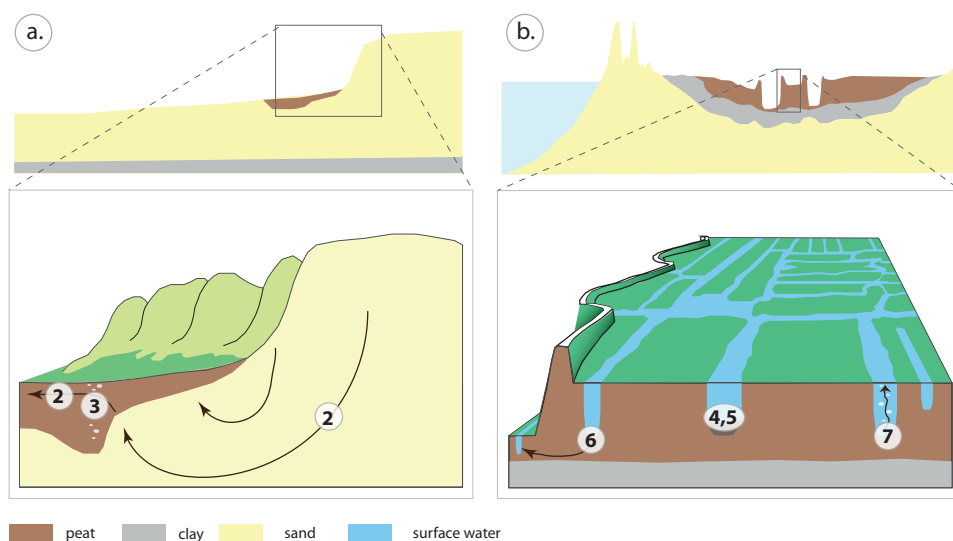


Fig. 1.2. Schematic overview of the landscape types in which the research presented in this thesis was carried out. On the left the sand hill landscape with percolation mires (province of Limburg, the Netherlands) where the effects of groundwater nitrate enrichment were studied. On the right the Dutch polder landscape (province of North Holland, the Netherlands) where the effects on enhanced surface salinity were studied. The different chapters within this thesis are indicated with their number.

1.3.1. Groundwater nitrate enrichment (Theme 1)

The effects of enhanced nitrogen availability on peatland functioning received a lot of attention during the last decades (e.g. Aerts et al. 1995, Bobbink et al. 1998, Lamers et al. 2000; Fritz et al. 2014; Van den Elzen et al. 2017). The majority of these studies, however, focused on the effects of enhanced atmospheric nitrogen deposition. Although for groundwater-fed peatlands, groundwater nutrient concentrations can be expected to be equally or even more important, the influence of groundwater nitrogen input received much less attention. Since the 1970s, increased groundwater nitrate concentrations have become a significant and increasing environmental issue worldwide (Rivett et al. 2008, Burow et al. 2010, Thornburn et al. 2003, Howden et al. 2011), due to global agricultural intensification leading to enhanced nitrate leaching to the groundwater (Di and Cameron 2002, Gundersen et al. 2006). Groundwater nitrate enrichment often originates from a combination of sources: increased nitrogen deposition (particularly

in forests due to strongly enhanced throughfall) originating from agricultural and industrial sources and leaching to the groundwater, and direct nitrate leaching from agricultural fields (Kirchmann et al. 2002, Di & Cameron 2002, Beaudoin et al. 2005). Nitrate enrichment mainly plays a role in aquifers poor in organic material, where nitrate is not removed due to the absence of reactive (labile) organic material and consequential low denitrification rates.

Ecological effects of groundwater nitrate enrichment

Groundwater nitrate enrichment can lead to a direct increase of nitrogen availability in the rhizosphere of plants and phyllosphere of bryophytes in groundwater-fed peatlands, but can also have a strong influence on microbial redox processes and pathways as nitrate is the most favorable alternative electron acceptor under anaerobic conditions. Peat moss (*Sphagnum* sp.) dominated peatlands are characterized by a low availability of nutrients, but can to a certain extent buffer increased nutrient loads due to the high nutrient sequestration capacity of peat mosses (e.g. Lamers et al. 2000, Fritz et al. 2014). A prolonged increase of the nitrogen loading will however lead to nutrient saturation of mosses (Lamers et al. 2000), alterations in moss physiology and nutrient leakage resulting in increased nitrogen availability and an increase of more eutrophic species such as graminoids, rushes and trees like *Betula* and *Salix* (Limpens et al. 2003, Tomassen et al. 2003, Limpens et al. 2004, Fritz et al. 2012). Indirectly, increased groundwater nitrate concentrations can also lead to internal eutrophication in groundwater fed mires due to increased decomposition of organic matter (i.e. Rivett et al. 2008, Smolders et al. 2010).

Effects on microbial redox processes

In peatlands oxygen is generally scarce in the soil due to water saturation, and an increased loading of the system with a strong alternative terminal electron acceptor such as nitrate can therefore have a strong impact on biogeochemical functioning and microbiological pathways. Via these processes groundwater nitrate enrichment may not only influence the nitrogen cycle but also other elemental cycles like those of carbon, sulfur, iron and phosphorus cycles, with consequences for nutrient availability, greenhouse gas emissions and species abundance (Lucassen et al. 2014, Smolders et al. 2010). One of the important greenhouse gasses emitted from peatlands to the atmosphere is methane (CH_4), which is produced by anaerobic decomposition of the peat soil. It is however estimated that about 50% of the methane produced in wetlands is consumed before it reaches the atmosphere (Cicerone & Oremland 1988, Bodelier 2011). This microbial methane sink is usually assumed to be fueled by oxygen. The influence of enhanced nitrate presence on soil methane production and potential soil methane oxidation is however not well understood (Gärdenäs et al. 2011, Bodelier 2011). Studies in other freshwater ecosystems point out that the presence of alternative electron acceptors such as sulfate (SO_4^{2-}), iron (Fe(III)) and nitrate (NO_3^-) do influence both the production of methane (methanogenesis) and the consumption of methane (methanotrophy) via anaerobic methane oxidation (Lovely & Klug 1983, Segers 1998, Smolders et al. 2002).

Another indirect effect of groundwater nitrate enrichment is nitrate reduction via organic electron donors in deposits in the aquifer such as sedimentary organic matter (i.e. Hartog et al. 2002), ferrous iron bearing carbonates (Straub et al. 1996, Claudhuri et al. 2001) and ferrous iron bearing sulfides (Postma et al. 1991, Zhang et al. 2009, Haaijer et al. 2007, Smolders et al. 2010). These processes can lead to mobilization of sulfate and bicarbonate and an immobilization of dissolved ferrous iron (within the aquifer and/or within the peatlands), which can lead to internal eutrophication of the peatland (Roelofs 1991, Lamers et al. 1998, Smolders et al. 2006; 2010). Especially the influx of sulfate in combination with a decreased input of iron may cause phosphate release due to the fact that sulfide, produced by microbial sulfate reduction, interferes with the iron-phosphorus cycle. Sulfide may react with dissolved reduced iron and particulate iron (hydr)oxides. The products are under anoxic conditions insoluble iron sulfide minerals, while phosphate adsorbed to the iron (hydr)oxides or present as iron phosphates becomes mobilized (Sperber 1958, Caraco et al. 1989, Smolders & Roelofs 1993). Groundwater nitrate enrichment can lead to sulfate mobilization by anaerobic oxidation of sulfide deposits (Haaijer et al. 2007) and increased availability of phosphorus by increased decomposition, which may have a large effect on the biodiversity in peatlands (Lamers et al. 2012, Wassen et al. 2005, Lamers et al. 2014). However if nitrate concentrations are high and nitrate reaches the peat surface, sulfate and iron reduction are impaired and mobilization of phosphate from iron-phosphate complexes is prevented (Lucassen et al., 2004).

Main conclusions theme 1

Groundwater nitrate concentrations are still increasing on a global scale. Therefore there is an strong urge for scientific studies on the effects of raised groundwater nitrate concentrations on biogeochemical processes in groundwater-fed ecosystems (including peatlands), and the consequences for their functioning and services, species diversity and management. In my thesis, the effects of groundwater nitrate enrichment on the functioning of groundwater-fed peatlands and their internal biogeochemical processes are studied in detail using a case study and additional laboratory experiments.

1.3.2 Surface water salinization (Theme 2)

As mentioned in paragraph 1.1., peatlands can be formed under a broad range of environmental conditions, including a fresh to brackish water range. In general it can be stated however that if the salinity level of organic sediments increases decomposition processes become faster than the accumulation of new organic matter which will lead to little peat accumulation over time in combination with the inability of peat forming species to grow under saline conditions. Therefore the majority of peatlands have developed under predominantly freshwater conditions. Coastal marshes and salt marshes can however also form peat. And some coastal peatlands are mainly freshwater peatlands which are only influenced by salinity along the sea side or even partly float during storm tide, avoiding flooding sea water. Some freshwater peatlands however became influenced by brackish or saline water at a later stage in their succession. Anthropogenic manipulation by water management that severely changed hydrological processes, in combination with land use change, sea level rise, land subsidence and natural changes in hydrology and seasonal precipitation intensity have led to an increase in the intrusion of saltwater into coastal freshwater wetlands worldwide (e.g. Church et al. 2013, Herbert et al. 2015) and increased saline groundwater intrusion into coastal zones in general (e.g. Rengasamy 2006, Oude Essink et al. 2010, Velstra et al. 2011). Increased surface water and groundwater salinity in general can affect agricultural land use (Katerji et al. 2003, Rengasamy 2010), threaten groundwater resources used for drinking water abstraction, and influence the ecological functioning of natural freshwater wetlands due to the strong biogeochemical and ecological consequences of salinization (e.g. Pitman & Lauchli 2002, Lamers et al. 2002a, Oude Essink & Kooi 2011, Bonte & Zwolsman 2010, Herbert et al. 2015).

Box. 1. Sea water composition and salinity levels

The salinity level in water can differ from low concentrations (fresh water) up to high concentrations (marine water) (Fig. 1.3) or even extreme saline water (briny water). For salinity several scales and units are used, both in practice as in science different terms and units are used. Within the present thesis two units are used, both the chloride concentration (in mM L^{-1}) and in PSU (Practical Salinity Unit). The majority of the experiments carried out in this thesis are in the range of 0.3 – 3.5 or 0.9 to 9.0 PSU (shown with the shaded rectangle in Fig. 1.3). Enhanced water salinity does result in increased concentrations of a combination of elements. To understand the biogeochemical and ecological effects of enhanced salinity it is essential to realize that not only chloride and sodium concentrations do increase but also concentrations of calcium, magnesium, sulfate, potassium and a series of other elements in lower levels. In Fig 1.3b the average precentral composition of sea water is presented on a mass base.

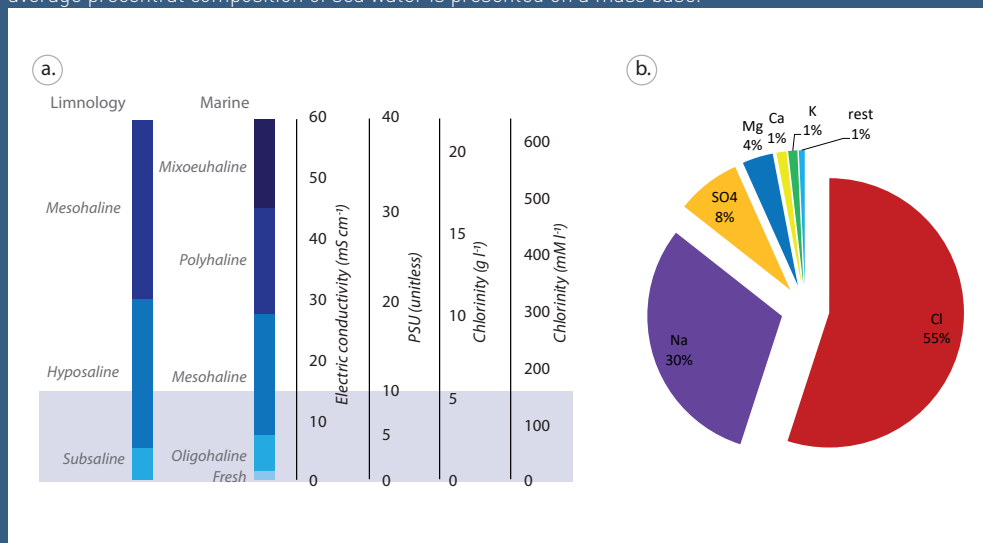


Fig. 1.3a. Salinity in different units [after Herbert et al. 2015], **b.** the average precentral composition of sea water is presented on a mass base.

Salinization in a Dutch context

The Netherlands, being a delta with many wetlands and historic gradients from fresh to saline water, already show a long history of managing surface water salinity levels (Van de Ven (eds.) 2003, Raats 2005; Van Diggelen 2015). The western part of the Netherlands is an example of a former raised bog landscape with secondary brackish water influence since the middle ages, showing salinization by the combination of flooding and land subsidence (Van de Ven (eds.) 2013). Because of prolonged drainage some regions have experienced more than four meters of subsidence during the last 400 years, large parts of which are now situated below mean seawater level (Schothorst, 1977, Nieuwenhuis & Schokking 1997). These lowlands are well protected against flooding by the construction of dikes and the implementation of

complex water management systems. The creation of this “polder-landscape” in combination with still ongoing land subsidence, however, has resulted in a strongly altered hydrology. This has increased the risk of salinization of the surface water by enhanced influence of seepage of brackish to saline groundwater. On top of that, climate change has increased the risk of salinization due to sea level rise (Oude Essink, 2001)) and decreased freshwater discharge in rivers during summer, resulting from a decreased precipitation and increased temperature (Zwolsman & van Bokhoven 2007, Witte et al. 2012). Salinization will not only enhance the ionic concentration, which has direct effects on plant and animal communities (i.e. Remane & Schlieper 1958, Nielsen et al. 2003) including agricultural crops and cattle (Pitman & Lächli, 2002, Rengasamy 2010), but will also influence chemical equilibria and microbial pathways (Herbert et al. 2015).

Box 2. Brackish water influence in Dutch former raised bog landscapes (part 2)

Currently the majority of these former wetlands are managed as intensively used agricultural peat meadows (Dutch: ‘veenweiden’), which demands ongoing drainage to warrant sufficiently low groundwater levels (Hoeksema 2007). A small percentage of the former wetlands is managed by nature management authorities and can be seen as semi-natural wetlands. These wetlands are, however, still partly managed for extensive agricultural use and affected by the intensive agricultural practices in their surroundings. Historic brackish water influence is still noticeable in deeper soil layers, the aquatic sediment and surface water. It will take centuries before peat sediments will be fresh again. Although the present surface water composition is less saline in these areas, the occurring species still have to deal with enhanced ion (including sulfate) concentrations in the sediment. On top of this, the areas have also suffered from a combination of other anthropogenic influences including increased nutrient input by intensive agricultural use and a strictly managed surface water level, both having contributed to the loss of characteristic biodiversity (Van ’t Veer 2009, Van ’t Veer et al. 2012). This led to the present situation where both species adapted to freshwater conditions as well as species adapted to brackish water conditions face severe problems. The present low abundance, or even absence, of aquatic vegetation in these coastal wetlands strongly calls for studies on the effects of increased salinity as a potential measure to restore the former brackish conditions, in order to increase the biodiversity of these wetlands. These will not only generate mechanistic knowledge about the processes occurring, but also about practical options to use this knowledge in water management and policymaking.

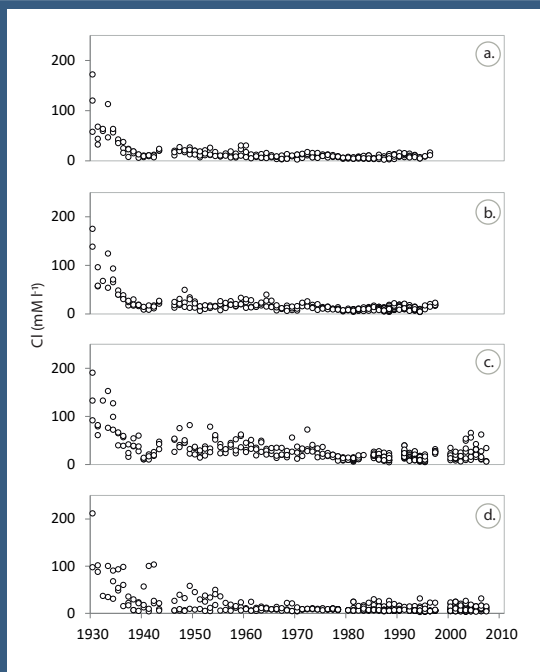


Fig. 1.4 Surface water chloride concentrations at four locations in the Province of North-Holland (Netherlands) during the period of 1930 -2010. A. gemaal aan het NoorHollandsch kanaal B. Gouwsloot kruising met Noordhollandsch kanaal C. Gemaal kadoelen D. Ringvaart Wijde Wormer brug Neck (open source data from waterboard HHNK, [www.12tux.nl]).

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Consequences of increased surface water salinity

Salinization not only leads to increasing levels of chloride and sodium, but also of potassium, magnesium and sulfate, with potential direct and indirect effects on biogeochemical processes influencing (among others) nutrient availability and carbon fluxes. The strong increase in cation concentrations leads to potential changes in physicochemical processes and chemical equilibria, aggregation and sedimentation of suspended matter and fast displacement of cations (e.g. calcium and ammonium) bound to the cation adsorption complex in the sediment (Rysgaard et al. 1999, Weston et al. 2006, 2010). By influencing sediment biogeochemistry, salinization directly influences nutrient availability (both phosphorus and ammonium) (Rysgaard et al. 1999; Baldwin et al. 2006; Weston et al. 2006; Weston et al. 2011). Although this topic received increasing attention during the last decades there is still no consensus on how enhanced surface water salinity will influence nutrient availability. Due to the interacting (and sometimes opposite) effects of salinization on chemical processes, microbial functioning and physicochemical effects, it appears to be very difficult to predict the effects of salinization on nutrient cycles in the sediment. Geological and historical differences and varying intensities of former influence of saline water, resulting in gradients of ion concentrations (for example sulfate concentrations) and sediment composition in peat sediments makes predictions about the effects of enhanced salinity even more complex, and often, to a certain extent, location specific. Previous studies have shown that salinization can have a direct effect on nitrogen cycling by affecting nitrification (Rysgaard et al. 1999, Magalhães et al. 2005, Noe et al. 2013) and denitrification (Giblin et al. 2010, Marks et al. 2016), while the availability of phosphorus in the sediment was shown to either increase (i.e. Portnoy and Giblin 1997, Lamers et al. 2002b, Weston et al. 2006) or decrease (Baldwin et al. 2006, Van Diggelen et al. 2014) as a consequence of enhanced salinity. Another major biogeochemical driver behind the effects of salinization is the enhanced availability of sulfate, which can stimulate microbial sulfate reduction, precipitation of sulfide with iron and, consequently, mobilization of phosphorus in wetlands (Smolders and Roelofs 1995; Lamers et al. 1998; Lamers et al. 2002b; Smolders et al. 2006).

Previous studies also show contrasting results about the influence of salinity on decomposition of organic matter and carbon emissions to the atmosphere. Several studies show increased sulfate concentrations, originating from salinization, to enhance organic matter breakdown and increase carbon mineralization and CO₂ emissions (Weston et al. 2006, Craft et al. 2009, Chambers et al. 2011, Weston et al. 2011, Marton et al. 2012). Studies comparing systems along a salinity gradient did however find that the more saline the system was, the less carbon they so emitted (Bartlett et al. 1987, Poffenbarger et al. 2011, Weston et al. 2014, Vizza et al. 2017). Increased sulfate reduction, which will often happen after salinization of anaerobic organic rich sediment (such as wetland sediments), is known to lead to decreased methanogenesis (Weston et al. 2006) because (1) the process of sulfate reduction is thermodynamically more

energy efficient (Segers, 1998) and methanogens are outcompeted for organic compounds (Lamers et al. 1998, Smolders et al. 2002) and (2) increased salinity and the production of toxic sulfide can lead to physiological stress for methanogenic bacteria (i.e. Chambers et al. 2011; Lamers et al. 2013).

Next to biogeochemical processes, physicochemical processes are also able to influence ecological functioning (species diversity and interactions between species) and ecohydrological processes (e.g. via the influence on soil hydraulic conductivity), especially in peatland soils. To predict the effects of enhanced salinity one also has to take into account the interacting effects of processes on different spatial scales in the landscape. For example, increased sulfate concentrations (as a consequence of increased salinity) are known to enhance sulfate reduction in anaerobic sediments and suppress methanogenesis (Segers 1998, Lamers et al. 2002b, Baldwin et al. 2006, Weston et al. 2006). Reduced methanogenesis and methane ebullition may, however, also influence hydraulic conductivity in peatland soils (Baird et al. 2003, Strack et al. 2005). It is therefore plausible that enhanced salinity, via its effects on biogeochemical (including microbiological) processes, could also affect hydrological processes. Although there is quite some evidence about the various separate processes and speculation about the interactions among the different processes on different scales, there is still little experimental proof for the exact regulation and net outcome of interacting effects.

Main conclusions theme 2

The chance of salinization is increasing on a global scale due to increasing anthropogenic influences including climate change. This stresses the urgency of scientific studies on the effects of salinization on ecosystems including peatlands. Especially studies on the effects of enhanced salinity on the interactions among biogeochemical (including microbial), hydrological and ecological processes in peatlands are needed. Such knowledge will help scientists, managers and policymakers to make decisions for the conservation and restoration of the biodiversity, natural resources and services peatlands provide. In my thesis the effects of enhanced salinity on the functioning of peatlands are studied in detail using a combination of a series of controlled laboratory experiments, field experiments and other field studies.

1.4 THESIS AIM AND OUTLINE

1.4.1. Aims of this thesis

As shown in the sections above, the effects of biogeochemical stressors such as enhanced nitrate concentrations and salinity levels can have a major influence on peatland functioning. However, based on current knowledge it is still very challenging to make predictions about how these stressors may impact peatland functioning. Therefore there is an strong urge for additional scientific and practically applicable knowledge. My thesis aims to produce new insights into how biogeochemical stressors (i.e. nitrate and salinity) influence biogeochemical processes, microbiological pathways and (eco-)hydrological processes in peatlands, and to unravel which consequences this might have for their ecological functioning, biodiversity and management. The results and discussion presented are based on a combination of fundamental and evidence-based, applied interdisciplinary research.

1.4.2. Outline of thesis

Theme one – Groundwater nitrate enrichment and peatland functioning

Chapter 2 describes a detailed, explorative field study of a groundwater-fed mire in a sand hill landscape in which the effects of groundwater nitrate enrichment are studied. The chapter includes a landscape ecological and ecohydrological description of how the study area functions, followed by a detailed analysis of how groundwater nitrate enrichment affects this area via direct and indirect processes.

In **Chapter 3** the effects of groundwater nitrate enrichment on microbiological pathways in a groundwater-fed mire are described. The chapter focuses on the effects of enhanced nitrate concentrations on anaerobic methane oxidation and the bacteria involved in a field study combined with laboratory incubations.

Theme two – Surface water salinization and peatland functioning

Chapter 4 Describes a controlled laboratory experiment in which the effects of enhanced surface water salinity on biogeochemical processes in the sediment were studied. The effects of both constant freshwater, constant brackish water and fluctuating salinity levels were studied on two sediment types, a peat and a clay sediment.

In **Chapter 5** the results of a long-term field experiment with enclosures are described in which the effects of surface water salinization on sediment biogeochemical processes were studied. This chapter focuses on the effects of enhanced surface water salinity on sediment nutrient availability.

Chapter 6 discusses the results of an interdisciplinary study on the combined sediment biogeochemical and physiochemical effects of surface water salinization on the hydraulic conductivity of the aquatic sediment in a long-term field experiment. The chapter shows how enhanced surface water salinity can cause increased hydraulic conductivity of the hyporheic zone of peatlands.

In **Chapter 7** the effects of surface water salinization on sediment decomposition and carbon fluxes to the atmosphere are studied in a long-term field experiment in a peatland. The effects of enhanced surface water salinity were studied using a combination of biogeochemical sediment and pore water analyses, microbiological analyses and carbon flux (diffusive & ebullative) measurements.

Chapter 8 discusses an explorative field study of a former freshwater peatland at the seaside of a dike, which is still influenced by seawater during spring tide. This study shows gradients found in this unique peatland that is the last remaining example of former coastal freshwater peatlands with seawater influence at the seaside. The chapter also discusses the special feature of this peat remnant: the ability of parts of the peat remnant to float during spring tide.

Based on all findings described in Chapter 2 to 8, a synthesis is presented in chapter 9 about the consequences of groundwater nitrate enrichment and enhanced surface water salinization on the functioning of peatlands. This synthesis also discusses the role of this knowledge for decision-making including dilemmas for peatland management, and includes recommendations for future research.

Table. 1.1 Overview of research questions, associated hypotheses, and chapters, see also fig. 1.2.

Research question	Hypothesis	Chapter
Theme one – Groundwater nitrate enrichment and peatland functioning		
How does groundwater nitrate enrichment influence the functioning of a groundwater-fed mire?	Groundwater nitrate enrichment has large biogeochemical consequences (sulfate mobilization) in the aquifer and (internal eutrophication) in the mire itself. These direct and indirect effects will lead to changes in species composition.	2
How does groundwater nitrate enrichment influence methane oxidation in a groundwater-fed mire?	Steep vertical gradients in carbon and nitrogen biogeochemistry occur, caused by groundwater nitrate enrichment. In the absence of oxygen, nitrate enrichment leads to methane oxidation and reduces methane emissions to the atmosphere.	3
Theme two – Surface water salinization and peatland functioning		
How do increased or fluctuating surface water salinity levels affect biogeochemical sediment processes in peat and clay sediments?	The effects of surface water salinity differ between clay and peat sediments with respect to nutrient mobilization. Increased salinity is expected to enhance sediment nutrient fluxes to the water layer. Fluctuating salinity levels are expected to have less effect compared to constantly increased salinity.	4
How does surface water salinization affect sediment biogeochemical processes in a peatland sediment?	Enhanced surface water salinity will lead to enhanced nutrient availability in the short term, but to decreased nutrient availability in the longer term.	5
How does surface water salinization influence sediment hydrological properties in the hyporheic zone of peatlands?	Surface water salinization will influence both biogeochemical and physiochemical processes in the sediment, and this combination affects the hydraulic conductivity and groundwater flow in the hyporheic zone of peatlands.	6
To what extent will surface water salinization influence sediment decomposition, microbiological processes involved, and carbon fluxes to the atmosphere?	Enhanced surface water salinity will affect microbiological processes mainly by increasing sulfate reduction rates and decreasing methanogenesis, leading to reduced methane emissions and increased carbon dioxide emissions to the atmosphere.	7
How were former freshwater coastal peatlands, that have become very rare, influenced by salinity and what can be learned from this for current and future salinization of coastal wetlands?	Steep vertical and horizontal biogeochemical gradients existed in these coastal freshwater peatlands, and salinity only influenced a small border zone of these wetlands.	8

Box. 3. B-WARE Research Centre

The studies presented in the present thesis have been realized by, and conducted at B-WARE Research Centre (Biogeochemical Water Management & Applied Research on Ecosystems). The Centre is a spin-off company of the Department of Aquatic Ecology and Environmental Biology of the Institute for Water and Wetlands Research (IWWR) at the Radboud University (for more info see page 255). The studies presented were often carried out in collaboration with fellow researchers from the Radboud University and from other institutes. At the end of this thesis a list of co-authors and their affiliations is presented.



Radboud University





(Brunssummerheide, by G. van Dijk)

THEME ONE

**Groundwater nitrate
enrichment and peatland
functioning**



[Brunssummerheide, by G. van Dijk]

2

Effects of groundwater nitrate and sulfate enrichment on groundwater fed mires, a case study

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ABSTRACT

Mires and peatlands are heavily influenced by anthropogenic stressors like acidification, eutrophication, desiccation and fragmentation. Groundwater fed mires, which are often well protected against desiccation due to constant groundwater discharge, can however be influenced by groundwater pollution such as nitrate enrichment, a threat which has received minor attention in literature. This paper demonstrates how groundwater nitrate enrichment can affect groundwater fed mires through direct nitrate influence and indirect nitrate-induced sulphate mobilization from geological deposits. Biogeochemical and ecohydrological analyses suggest that the study area in the South of the Netherlands is influenced by different water sources (rainwater, local groundwater and regional groundwater) with differing chemical composition, leading to horizontal and vertical biogeochemical gradients. Groundwater nitrate enrichment led to shifts in species assemblages and plant nutrient content in zones influenced by nitrate enriched groundwater. Nitrate-mediated sulphate mobilization in the aquifer led to elemental sulphur deposition, and in some places sulphide toxicity. Significant internal eutrophication was not observed, probably due to relatively low phosphorus concentrations in the soil. This study documents and explains the effects of increased groundwater nitrogen and indirect sulphur input on biogeochemical functioning of a groundwater fed mire and describes possible future consequences for conservation and biodiversity. Future management of groundwater fed mires in nitrate-polluted aquifers should include the reduction of nitrate leaching to the aquifer at the recharge areas, this is the only sustainable solution to protect the biodiversity in these mires.

INTRODUCTION

Mires under threat from anthropogenic influences

The functioning and biodiversity of mires are under threat by desiccation (due to drainage and conversion to agriculture), eutrophication, acidification and fragmentation (Joosten & Clarke, 2002, Lamers et al. 2002, Van Diggelen et al. 2006, Lamers et al. 2014). Especially when several of the afore mentioned processes co-occur, they result in increased peat decomposition and enhanced (internal) eutrophication leading to decreased biodiversity (Grootjans et al 2006, Smolders et al. 2006, Houtier et al. 2009, Lamers et al. 2014). The functioning of mires and peatlands is to a large extent driven by the combination of hydrological and biogeochemical processes (Succow & Joosten, 2001, Lamers et al. 2014). For groundwater fed mires in particular, the factors groundwater discharge and the chemical composition of the groundwater are major drivers for mire functioning and biodiversity. Whereas rainwater fed mires often suffer from desiccation during periods with less precipitation or as a consequence of anthropogenic drainage, intact groundwater fed mires, are protected from desiccation due to constant groundwater discharge, which also generates a constant supply of minerals and nutrients. Changes in groundwater pressure and or changes in groundwater chemical composition can therefore directly affect the functioning of these systems. Several studies already demonstrated how hydrological and chemical alterations can affect the functioning of mires, for example by changing redox conditions (i.e. increased oxygen, sulphate and nitrate concentrations) (De Mars & Wassen 1999, Reddy et al. 2000, Lamers et al. 2001, Lamers et al. 2002, Smolders et al. 2010, Cirkel et al. 2013).

Until now, the effects of groundwater nitrate enrichment on groundwater fed systems has received relatively little attention in literature in contrast to other forms of nitrogen pollution. Since the 1970s, however, elevated groundwater nitrate concentrations have become an increasingly important global environmental problem in agricultural areas (e.g. Rivett et al. 2008, Burow et al. 2010, Thornburn et al. 2003, Howden et al. 2011). Groundwater nitrate pollution mainly occurs in aquifers poor in labile organic material, such as sandy or loess soils, where nitrate remains present on its flow path to the aquifer, due to the absence of organic material and consequentially low denitrification rates. Nitrate often originates directly from agricultural fields (Kirchmann et al. 2002, Di and Cameron 2002, Beaudoin et al. 2005) but can also leach from forests, which intercept aerial reactive nitrogen compounds originating from agricultural and industrial sources (Rothe and Mellert 2004, Borken and Matzner 2004, Gundersen et al. 2006). The influence of these processes is only expected to increase in the future due to worldwide agricultural intensification (e.g., Di and Cameron 2002, Smolders et al. 2006, Shibata et al. 2017).

Influence of groundwater nitrate enrichment on mires

Although many studies have focussed on the effects of enhanced nitrogen availability in mires and other oligotrophic (semi)aquatic systems (i.e. Aerts et al. 1992, Bobbink et al. 1998, Aerts et al. 1999, Lamers et al. 2000, Fritz et al. 2014, Van den Elzen et al. 2018), the consequences of an increased nitrogen loading through nitrate-enriched groundwater have received less attention. In groundwater fed mires, increased groundwater nitrate concentrations can lead to a direct increase of the nitrogen availability for bryophytes and higher plants. Mires are partly buffered against increased nutrient loads (Lamers et al. 2000, Fritz et al. 2014) but a prolonged high nitrogen input may result in an increase of more eutrophic species such as graminoids, rushes and trees like *Betula* and *Salix* (Verhoeven et al. 1996, Limpens et al. 2003, Tomassen et al. 2003, Limpens et al. 2004, Fritz et al. 2012, Lamers et al. 2014, Cusell et al. 2014).

Nitrate as a favourable electron acceptor may increase anoxic decomposition rates of organic matter (Rivett et al. 2008) (i.e. Hartog et al. 2002) and the oxidation rates of ferrous iron bearing carbonates (Straub et al. 1996, Chaudhuri et al. 2001) and ferrous iron bearing sulphides (Postma et al. 1991, Schwientek et al. 2008, Zhang et al. 2009, Haaijer et al. 2007, Korom et al. 2012). These processes can lead to sulphate mobilization from both sulphur-rich geological deposits in the aquifer and from sulphur in the peat material of the affected mire (Lucassen et al. 2004, Zhang et al. 2009). Consequential sulphate reduction in mires may lead to internal eutrophication (Lamers et al. 1998, Lucassen et al. 2004, Smolders et al. 2006; Smolders et al. 2010), due to the fact that the produced sulphide interferes with the iron-phosphorus cycle, causing the mobilization of phosphate. Especially in combination with increased nitrogen, this may have an even more pronounced negative impact on the biodiversity in mires (Wassen et al. 2005, Lamer et al. 2012, Fritz et al. 2012, Lamers et al. 2014).

Groundwater nitrate concentrations are still increasing on a global scale. This leads to an urge to study the effects of groundwater nitrate enrichment on the functioning of groundwater fed ecosystems, and to define measures that could be taken to conserve and/or restore mires under the influence of nitrate-rich groundwaters. For example, a recent study by De Mars et al. (2017) confirmed that a large number of base-rich groundwater fed systems in North Western Europe receive groundwater that is heavily enriched with nitrate. They showed that groundwater fed systems in the Netherlands contained the highest groundwater nitrate concentrations compared to other NW European countries. Studies on the national scale do also report high groundwater nitrate levels, well above the limit of 50 mg l⁻¹ formulated in the European Nitrates Directive in 1991 (Council Directive 91/676/EEC, 1991) (Fraters et al. 2006, Baumann et al. 2012). In the present paper, we test in what way enhanced groundwater nitrate concentrations and nitrate mediated sulphate mobilization affect mire functioning and vegetation. We describe a case study of a groundwater fed mire in the Netherlands, in which groundwater movement and biogeochemical parameters are studied to unravel the functioning

of the mire and the potential consequences of groundwater nitrate enrichment. Within this ecohydrological and biogeochemical landscape analyses special attention is paid to the source, effect and fate of groundwater nitrate enrichment. We hypothesize that groundwater nitrate - and, the potentially related, sulphate enrichment affects vegetation development and leads to internal eutrophication.

MATERIALS AND METHODS

Study area

The study site is a groundwater fed mire with a surface area of 15 hectares, in the protected nature area the Brunssummerheide (50°55'39.63" N, 5°59'50.73" S) located in the south-east of the Netherlands (Fig. 2.1). It can be described as a base poor percolating mire with some aspects of bog vegetation.

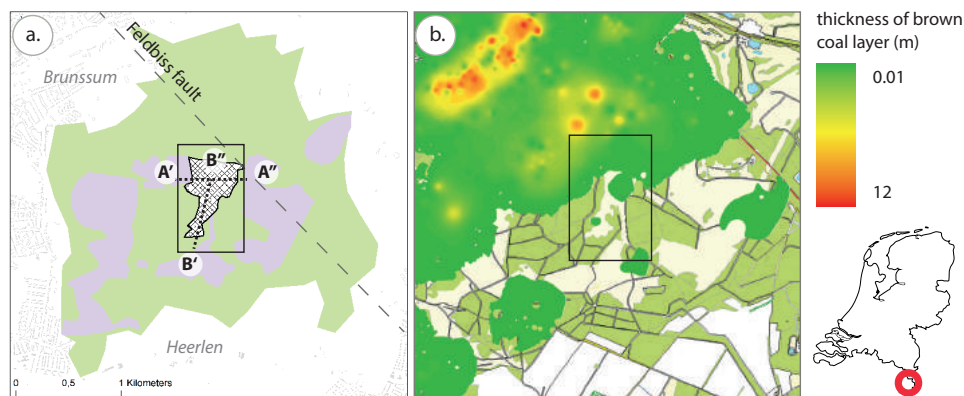


Fig. 2.1 a.) Map of the Brunssummerheide, with the study area shown in the centre (rectangular in a. & b. and shaded zone in a.), at the right the location of the area is shown on a map of the Netherlands (red circle). Forest is shown in green and heathland shown in purple. The geological fault is shown as a broken line. In a. two fine broken lines do represent the two cross section transects of Fig. 2 (A'-A'' & B'-B''). b.) The presence and thickness of lignite and marcasite containing brown coal layers in the underground is shown for the study area (data from TNO, 2013 (online DINoloket database)).

The mire is imbedded in small erosion valley incised in a Miocene fine sandy soil layer (grain size < 300 μM) (Heksenberg formation) with a thickness of up to 150 m deep, which is underlain by a thick impermeable clay layer (Formation of Rupel). The peat layer of the mire varies in thickness from several decimetres up to three metres. Both the mineral base and the surface of the mire are sloping northwards (from South to North, 85 to 77 meters above Amsterdam Ordnance Datum (AOD) ($\approx 1.15^\circ$) at the mire base and 85 to 80 meters above AOD for the surface ($\approx 0.72^\circ$)). The surrounding sandy flanks are covered with pine forest (*Pinus*) and some small

patches with deciduous trees (*Betula*). On a larger scale, the area surrounding the mire is covered with heather and pine forest and is surrounded by urban areas. Two fault lines are present in the region, the Heerlerheide fault (about 2.5 km west of the mire) and the Feldbiss fault (about 0.5 km east of the mire). The presence of the Feldbiss fault in particular, influences the local hydrology of the area due to its low permeability (caused by smearing of clay layers) (Borsje and Poppema 1997, Swierstra 2008). The flow pattern of the regional groundwater is directed towards the Northwest parallel of these fault lines. However, the presence of the draining headwaters of the Roode beek including the mire locally results in a northern flow direction. The impermeable Feldbiss fault provokes upwelling of groundwater to the surface (Swierstra 2008). Within the Miocene sand layer two brown coal deposits are present (Frimmersdorf and Morken, which were partly mined between 1917 and 1949), of which the Frimmersdorf is situated north (downstream) of the study area. The Morken layer is thicker and is mainly present north of the study area, but fragments also occur within the catchment area of the mire itself. The exact occurrence of smaller fragments of the Morken layer within the catchment has not been studied in detail, but geological drill cores collected within the area clearly reveal the presence of these fragmented marcasite containing lignite and deposits (Dubelaar and Menkovic 1998, coring information database TNO, 2013).

The combination of relatively large differences in elevation and presence of the impermeable Feldbiss fault close by and the incision of the erosion valley, led to the development of a discharge area with permanently wet conditions and peat formation. As the mire is fed by groundwater originating from a sandy aquifer poor in minerals, a weakly buffered percolation mire has developed. The percolation mire and its vegetation can be called a poor fen (Gorham 1955, Succow & Joosten 2001, Van Dijk et al., 2012). The surplus of water from the mire is approximately 20 l/s and is drained by the Roode beek (Swierstra 2008). The peat layer itself mainly consists of remains of rushes (*Juncus*) and peat mosses (*Sphagnum*). Within the peat layer at different depths several sand bands are present (varying in thickness and gravel content) indicating its spring like origin as well as the former wind- and/or water-mediated deposition of eroded sand on top of already existing peat layers. The actual vegetation consists of a combination of oligotrophic and minerotrophic plant species including several *Sphagnum* species, *Narthecium ossifragum*, *Eriophorum angustifolium*, *Andromeda polifolia*, *Molinia caerulea*, *Juncus acutiflorus* and some juvenile Willow and Alder trees. In the Northern part *Sphagnum*-dominated peat is present with *Narthecium ossifragum* (community *Erico-Sphagnetum magellanicum*; plant communities according to Schaminée (et al. 1995)), while further south *Molinia caerulea* becomes more dominant (community *RG Molinia caerulea* [*Oxycocco Sphagnetum*] and *RG Molinia caerulea* [*Scheuchzerieta Sphagnetum*] (the distribution of a selection of species is presented in Results S2).

Sampling

Surface water, pore water and groundwater

Surface water samples were collected in June and July of 2008 on 107 locations where open water was present using polyethylene bottles. Anaerobic soil pore water samples were sampled with ceramic cups (5 cm, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) connected via Teflon tubes to vacuum 60ml syringes. On eleven locations in the mire ceramic cups were placed in depth gradients (in total 34 ceramic cups, varying in depth from 5 cm to a maximum of 270 cm). Soil pore water samples were collected on five moments over a period of ten months. In 2012 and 2013 additional groundwater samples were collected simultaneously with plant samples, for groundwater $\delta^{15}\text{N}-\text{NO}_3^-$ and plant tissue $\delta^{15}\text{N}$ analyses respectively. All water samples were transported to the laboratory under cool and dark conditions and stored at 4 °C before analysis. Sulphide concentrations were determined directly after sampling by fixing 10.5 ml pore water with 10.5 ml Sulphide Anti Oxidant Buffer, and using an Orion sulphide electrode and a Consort Ion meter (type C830) for analysis. In August 2008 along four transects, electric conductivity measurements indicating total dissolved ion content, were carried out in depth profiles (to a maximum depth of 200 cm), with a WTW-Relch conductivity meter.

Hydrology

The groundwater levels were measured at eight locations with piezometers with 1 m filters installed at 1.5 m (n=5) and at 5 m (n=3) below the surface (see Fig. 2.2). Groundwater levels were automatically recorded by divers (TD Divers, Van Essen Instruments) (recordings were taken twice a day and corrected for local air pressure, from 2008-2014). The piezometers are part of an ecohydrological monitoring network of the Sigrano company and the Province of Limburg. Data are available from the DINO database (TNO, www.dinoloket.nl).

Vegetation

Samples of a selection of species [*Sphagnum fallax* (n=5), *Molinia caerulea* (n=5), *Calluna vulgaris* (n=3), *Betula pendula* (n=6)] and *Pinus sylvestris* (n=3)] were collected from seven locations (when present) differing in groundwater influence to analyse tissue N and $\delta^{15}\text{N}$ content, on 27 May 2013. For *Sphagnum fallax* the upper 5 cm of the bryophyte (including *capitulum*) was collected while for the other four plant species only the one year old leaves were collected.

Precipitation and nitrogen deposition

To estimate the local aerial nitrogen deposition, precipitation collectors were placed for a period of ten months (Aug-May in 2008-2009) and collected and sampled regularly. Precipitation samples were preserved by the addition of 1 ml of 1M HgCl_2 prior to installation in the field. Precipitation collectors consisted of funnels attached to dark bottles which were located at

nine locations (six randomly spread in the forest (within the mire and on the surrounding flanks) and three on randomly assigned locations on the open mire itself. In the funnel a mesh was placed to prevent leaves, needles or insects to enter. Precipitation samples were analysed as described below.

Chemical analysis

Surface water, pore water, ground water and precipitation samples

The pH was measured using a combined pH electrode with an Ag/AgCl internal reference (Orion Research, Beverly, CA, USA) and a TIM800 pH meter. Alkalinity was measured by titration to pH 4.2 with 0.01 M HCl using an ABU901 Autoburette (Radiometer, Copenhagen, Denmark). 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 with 0.1 ml HNO_3^- 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, ammonium, ortho-phosphate, 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). For details on chemical analyses see Van Dijk et al. (2015). The $\delta^{15}\text{N}-\text{NO}_3^-$ signature of groundwater samples was analysed at the NIOZ, Yerseke the Netherlands, using an adaptation of the ammonia diffusion method described by Sigman et al. (1997).

Plant tissue analysis

Vegetation samples were oven dried at 70°C for at least 48 hours. Dried vegetation samples were ground using a mortar with liquid nitrogen and/or a MM 301 mixer mill (Retsch). Vegetation samples were rinsed with deionised water prior to drying to wash away nitrogen originating from atmospheric deposition that may have accumulated on leaves. Total element concentrations were determined by digesting 200 mg of dried (24 h, 70°C) and homogenized sample in 4 ml concentrated HNO_3 (65%) and 1 ml H_2O_2 (30%) using an Ethos D microwave (Milestone microwave MLS 1200 Mega). Samples were analysed by ICP, as described above. Total nitrogen and carbon concentrations were measured with a CNS analyser (Model NA 1500; Carlo Erba Instruments, Milan, Italy). Vegetation $\delta^{15}\text{N}$ signature was measured with a DeltaPlus isotope ratio mass spectrometer (IRMS) (Thermo Finnigan) that was coupled, through a ConFlo III interface (Thermo Finnigan), to a Flash EA 1110 Elemental Analyzer (Thermo Finnigan).

Statistics

Significant differences among groups were tested using one-way ANOVA tests. Data was tested for normal distribution (Shapiro-Wilk tests and visual inspection of Q-Q plots). Not normally distributed data were tested for significant differences using Kruskal-Wallis tests. Normally distributed data were checked for equality of error variances using Levene's tests. We tested for normality and homogeneity of variances using the Shapiro-Wilk test and Levene's Test. Correlation relationships in pore water concentrations were defined using Pearson correlation. All tests were carried out using SPSS Statistics for Windows (Version 24. IBM Corp. Armonk, NY, 2016).

RESULTS

Ecohydrological functioning

The studied mire was found to be influenced year round by weakly buffered groundwater (alkalinity usually $< 0.5 \text{ meq l}^{-1}$) originating from the sandy aquifer and can be classified as a percolation mire. Along the edges of the mire usually little evidence was found for seepage, however closer to the runnels and in the centre of the mire an average hydraulic head above the peat surface was recorded ($> 2\text{-}10 \text{ cm}$ above peat surface) indicating year round seepage, see Fig. 2.2. The high hydrological resistance of the peat layer in combination with large variations in elevation the hydraulic head does create large differences in hydraulic head and groundwater flow. In the surrounding sandy hills the hydraulic head is higher than the hydraulic head under the mire indicating an inflow of groundwater of both regional and local origin (Fig. 2.2).

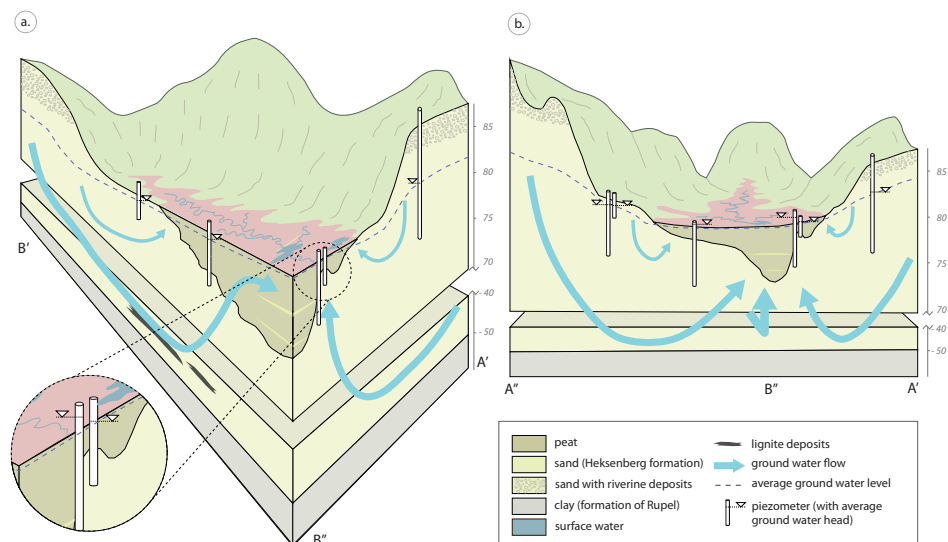


Fig. 2.2. Schematic overview and cross section of the study area with supposed flow of groundwater, within a south-north A'-A'' (a.) and an east-west cross section B'-B'' (b.). Elevation, groundwater head and soil profiles are based on field measurements and interpolations. Piezometers are shown with average groundwater head. In Fig. 1. the location of the cross sections is shown. Dotted lines represent the average groundwater level. Scale in meters to Amsterdam Ordnance Datum.

Groundwater flow patterns were studied using the hydraulic heads in piezometers, depth profiles of electrical conductivity (EC) in the peat profile along transects and a surface covering survey of EC measurements. Due to the relatively high ion concentrations in regional groundwater compared to rainwater and groundwater of more local origin, patterns of differing water composition could be observed; ranging from predominantly rainwater fed to predominantly regional groundwater fed and all stages in between. Groundwater flow patterns were studied using EC measurements along a series of cross sections and a surface water survey (transects in Fig. 2.3 and Fig. 2.4). The edges of the mire, in the far South side (transect IV) and in the north-eastern part of the study area (east of transect I), were mainly influenced by rainwater ($EC < 200 \mu S cm^{-1}$). More to the centre of the mire and along several runnels the influence of local origin and/or a mix of regional groundwater and rainwater was established ($EC 200-750 \mu S cm^{-1}$). The influence of regional groundwater ($EC 700-1000 \mu S cm^{-1}$) was observed at a depth of one to two meters in the peat profile in the southern (upstream) part of the mire (transect III). In the centre of the mire (at location C, middle of transect II) upwelling (spring) of regional groundwater was observed ($EC 700 - > 1000 \mu S cm^{-1}$) with water rising from the underlying sand aquifer up to the rhizosphere. At this location a runnel starts which runs North (downstream) partly over and partly percolating through the upper part of the peat. Further to the North at the western side of transect I, this resulted in increased EC levels (up to $700-1000 \mu S cm^{-1}$) in the upper section of the peat profile.

Biogeochemical functioning – groundwater influence

Biogeochemical analyses of pore water and surface water samples showed similar patterns as found with EC measurements and groundwater level data (Figs. 2.3 & 2.4). On locations influenced by regional groundwater highest Ca^{2+} and Cl^- concentrations were found ($\text{Ca}^{2+} > 200 \mu\text{mol}\cdot\text{l}^{-1}$ and $\text{Cl}^- > 500 \mu\text{mol}\cdot\text{l}^{-1}$). In contrast, locations that were mainly influenced by groundwater of local origin and/or a mix of regional groundwater and rainwater showed Cl^- concentrations of $200\text{--}500 \mu\text{mol}\cdot\text{l}^{-1}$ and Ca concentrations $100\text{--}200 \mu\text{mol}\cdot\text{l}^{-1}$, while Cl^- and Ca^{2+} concentrations were $< 200 \mu\text{M}$ and $< 100 \mu\text{mol}\cdot\text{l}^{-1}$ respectively for locations mainly influence by rainwater. Other parameters, including Na^+ , K^+ and pH followed a similar pattern (see Results S1) and showed a significant positive correlation (R^2 0.6 to 0.88 $P < 0.001$) with concentrations of Ca^{2+} and Mg^{2+} indicating the common origin (groundwater) of these ions.

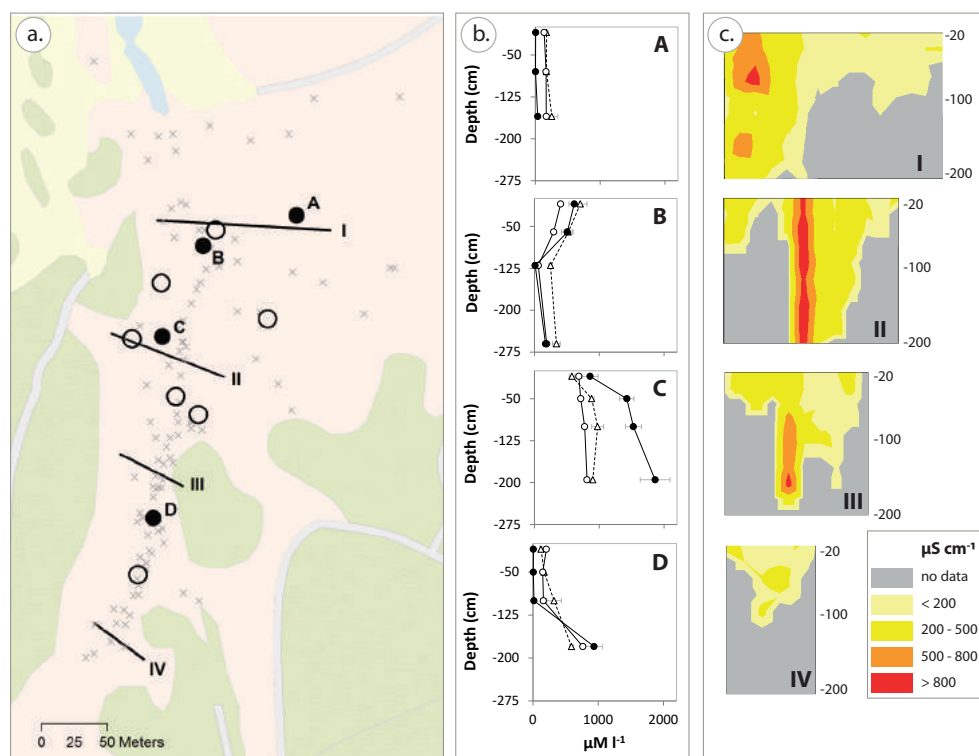


Fig. 2.3 a.) Location of the pore water depth profiles (open and filled circle, filled circles shown in b.), EC transect shown as lines (transect I to IV, shown in c.), surface water sampling locations shown with grey crosses. b.) average pore water concentrations (n=5) of sulphate (open circles), nitrate (filled circles) and chloride (open triangles) in depth profiles on 4 locations (A-D) in the mire. c.) Depth transects of EC ($\mu\text{S}\cdot\text{cm}^{-1}$) measurements in depth profiles along transects, interpolated within transects.

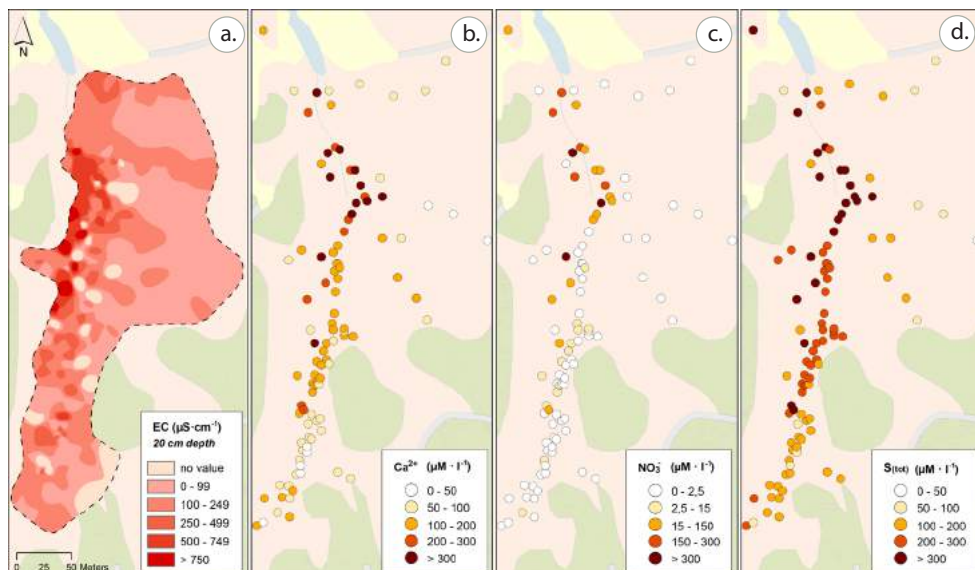


Fig. 2.4. a. Surface covering interpolated image of EC ($\mu\text{S cm}^{-1}$) at 20cm of depth in the soils. b. Surface water calcium concentrations. c. Surface water nitrate concentrations. d. Surface water sulphate concentrations.

Biogeochemical variation and nutrient availability

Elements associated with groundwater (i.e. $\text{Ca}^{2+}+\text{Mg}^{2+}$ and Cl^{-}) showed a significant positive correlation with nitrate concentrations (R^2 0.88, $P < 0.001$) and sulphate concentrations (R^2 0.78, $P < 0.001$) in both the pore water and surface water samples (Fig. 2.5), indicating the groundwater to be enriched by these ions. The mire is relatively poor in phosphorus with average values of 1.4 (max. 9) $\mu\text{M l}^{-1}$ in surface water and values ranging from below the detection limit to 2.5 $\mu\text{mol l}^{-1}$ in the pore water samples. Nitrate was the dominant form of nitrogen in both surface water and pore water (see Results S1). The majority (>75%) of the surface water samples contained low nitrate concentrations ($< 5 \mu\text{mol l}^{-1} \text{NO}_3^{-}$; Fig. 2.5b).

In the centre of the mire, especially in the northern part, remarkably high nitrate concentrations were observed in the surface water (up to $> 600 \mu\text{mol l}^{-1} \text{NO}_3^{-}$) (Fig. 2.3 & 2.5b). On the mire flanks and in the southern upstream part of the mire low pore water nitrate concentrations were observed in the top of the peat profile (i.e. depth profiles A and D in Fig. 2.3). Pore water nitrate concentrations were much higher on locations where regional groundwater influence was found (900-1800 $\mu\text{mol l}^{-1}$) in deeper layers of the soil (D in Fig. 2.3) or in the entire peat profile (from the bottom up to the rhizosphere) (C in Fig. 2.3). Pore water and surface water sulphate concentrations showed a comparable pattern, although sulphate concentrations also seemed to be higher on locations influenced by groundwater of local origin and/or a mix of regional groundwater and rainwater, low in nitrate. For $\text{Ca}^{2+}+\text{Mg}^{2+}$ concentration below 500

$\mu\text{mol l}^{-1}$ nitrate concentrations were (very) low and steeply increased above this threshold (Fig. 2.5). On some locations that were influenced by sulphate rich and nitrate poor groundwater sulphate reduction and free sulphide were found in the pore water (from $25 \mu\text{M l}^{-1} \text{H}_2\text{S}$ in the rhizosphere up to $300 \mu\text{M l}^{-1} \text{H}_2\text{S}$ at a depth $> 0.5 \text{ m}$). In some streams yellowish elemental sulphur deposits were also found on dead organic matter (see Fig. 2.6).

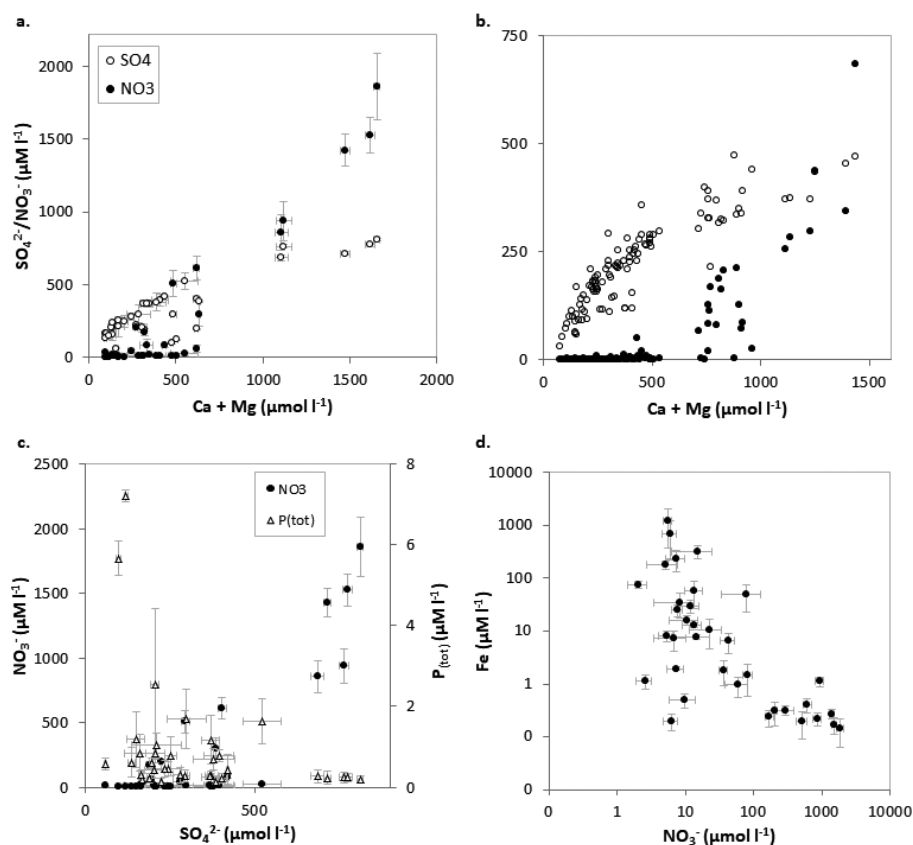


Fig. 2.5. Sulfate (open circles) and nitrate (closed circles) concentrations in the pore water (a.) and the surface water (b.) plotted against calcium + magnesium concentrations. Values are presented as means ($n=5$) $\pm$ SE for pore water samples, collected on 11 locations at several depths, while surface water samples are based on a single measurement on 107 locations. (c.) Pore water nitrate (filled circles) and total phosphorus (open triangles) concentrations plotted against sulphate concentrations. (d.) Pore water nitrate concentrations plotted against iron concentrations, values are presented as means ($n=5$) $\pm$ SE. Note different scales on the axes, and in (d) axes are on a logarithmic scale.

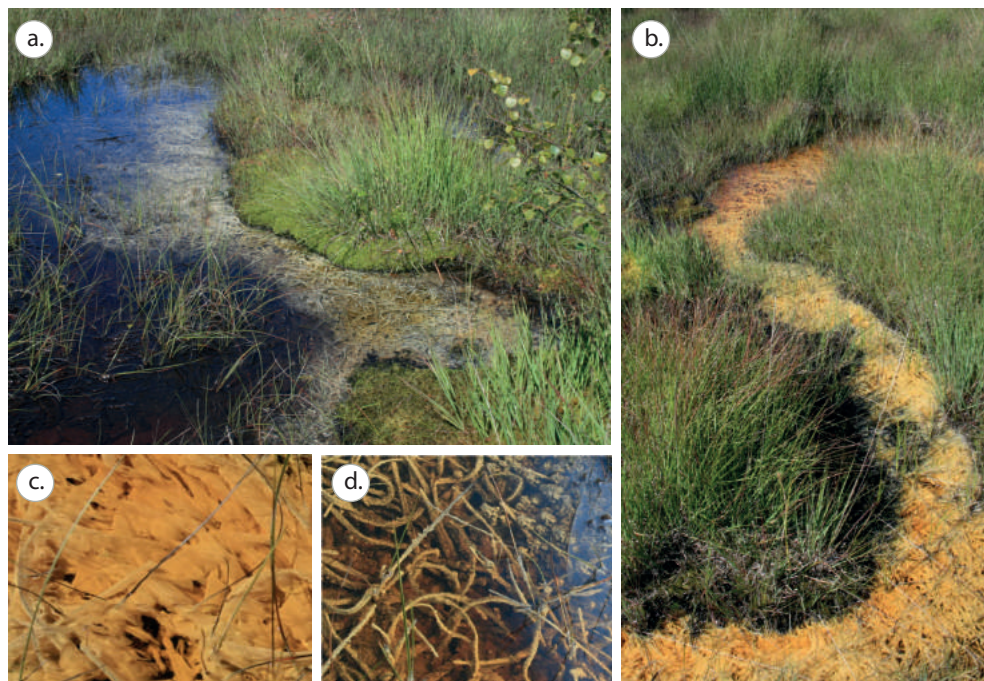


Fig. 2.6. Elemental sulphur deposition on dead plant material on spots where groundwater seepage occurs (A) or in entire spring streams (B). In C and D two close ups are presented. All photos by G. van Dijk.

Effects of nitrate and sulphate enrichment on species distribution

The distribution of plant species correlated with the influence of different sources of water, ranging from predominantly rainwater influenced to predominantly regional groundwater influenced. Zones with dominance of species like *Sphagnum magellanicum*, *S. papillosum*, *Erica tetralix* and *Eriophorum vaginatum* were predominantly rainwater influenced. Zones with dominance of species like *S. fallax*, *S. flexuosum* and *Narthecium ossifragum* were predominantly influenced by groundwater from local origin or a mixture of this local groundwater with rainwater. On spots where nitrate rich groundwater reaches the rhizosphere the dominant species is still *Sphagnum fallax* but other species like *Juncus acutiflorus*, *J. effusus* and juvenile *Salix* sp. and *Alnus* sp. trees have a higher cover than on locations where nitrate enriched groundwater does not reach the rhizosphere.

Groundwater versus aerial nutrient input

When water samples are divided in groups based on the dominant water source, rainwater, groundwater of local origin or groundwater of regional origin, a pattern (not significant, due to sample size) could be observed indicating that the $\delta^{15}\text{N-NO}_3$ signature of the regional

groundwater, which is often richer in nitrate, is higher compared to the signature of the rainwater and local groundwater (Fig 2.8).

Not only regional groundwater was a potential source of nitrogen and sulphur but also dry and wet atmospheric deposition. Atmospheric deposition measurements in the present study area showed a significantly higher total nitrogen and total sulfur deposition in the surrounding pine forest compared to the mire itself (Fig. 2.7a). Average total nitrogen deposition was $8.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (40% nitrate, 60% ammonium) and total sulphur deposition was $4.4 \text{ kg S ha}^{-1} \text{ yr}^{-1}$ on the mire itself ($n=3$), while the total average deposition of nitrogen and sulphur was $23.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (27% nitrate, 73% ammonium) and $8.9 \text{ kg S ha}^{-1} \text{ yr}^{-1}$ respectively in the surrounding pine forest ($n=6$). Average precipitation which reached the soil surface was significantly lower (> 40%) in the forest than in the mire due to interception of rainwater by trees (Fig. 2.7).

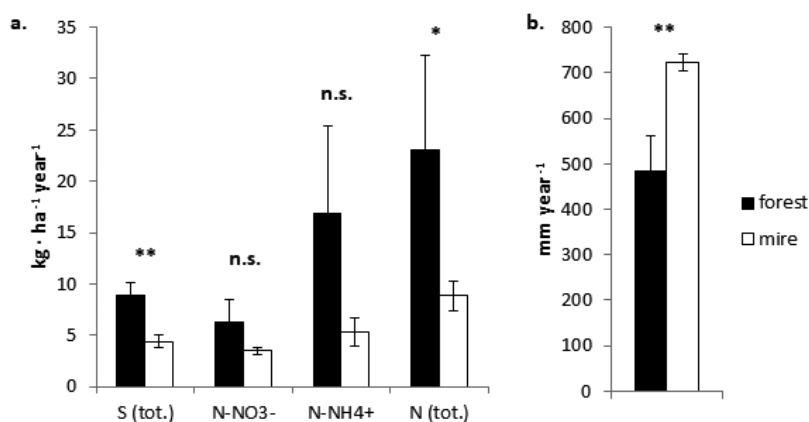


Fig. 2.7 (a). Total sulphur and total nitrogen (both nitrate and ammonium) deposition and precipitation (b) on the mire itself (white bars, $n=3$) and in the surrounding forest (black bars, $n=6$). Values are presented as means $\pm$ SE. Significance of differences between groups is indicated with single ($P < 0.05$) and double asterisks ($P < 0.01$).

$\delta^{15}\text{N}$ signature analyses of plant tissue of a series of species occurring in zones with different water sources indicated that individuals grown on locations with groundwater enriched in nitrate displayed higher $\delta^{15}\text{N}$ values (Fig. 2.8). $\delta^{15}\text{N}$ signatures seemed to differ between groups receiving water from different sources. Dividing the plant tissue analyses in three classes based on the water source by which they are influenced (RW: rainwater, LG: local groundwater and RG: regional groundwater) there was a trend of increased $\delta^{15}\text{N}$ signatures for species on locations with the influence of nitrate enriched regional groundwater, comparable with the trend observed in the groundwater itself (Fig 2.8).

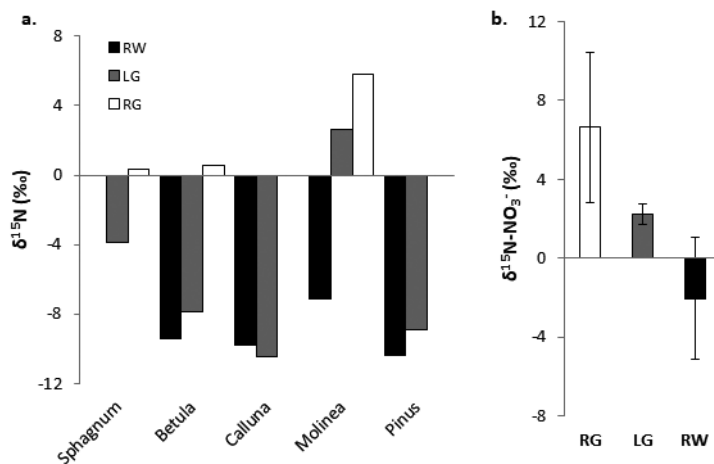


Fig. 2.8. a. Plant tissue $\delta^{15}\text{N}$ signatures for the different measured plant species. $\delta^{15}\text{N}$ signature of all species divided in different groups based on their location on the mire; no error bars are shown because some bars are based on only one measurement. b. $\delta^{15}\text{N-NO}_3^-$ signatures analyses of seven groundwater samples in the study area divided in three groups based on major water source (RW sites with dominated rainwater influence (black bars), LG: sites with local groundwater influence (grey bars), RG: sites with locations with regional groundwater (white bars)).

DISCUSSION

Functioning of the groundwater fed mire

In the centre of the mire, ground water heads were permanently above the surface, fluctuations were small, and the concentrations of nitrate and other dissolved ions were the highest. From this, it can be concluded that the mire is fed by a permanent discharge of groundwater, which most likely originates from a regional hydrological system. The chemical composition of the ground and surface water points to the occurrence of various other groundwater flows, so different parts of the mire were influenced by different groundwater flows, which created contrasting biogeochemical conditions and vegetation assemblages in the mire.

Groundwater nitrate and sulphate enrichment – potential sources

An important driver of change in the functioning of the studied mire is the nitrate and sulphate enrichment of the regional groundwater. Based on modelling results of Swierstra (2008) the size of the catchment area is estimated to be around 275-300 ha which includes forest (approx. 50 %), heathlands (approx. 25 %) and agricultural area (approx. 25 %). Possible sources for groundwater nitrate enrichment could therefore be nitrate leaching from agricultural areas (originating from both inorganic fertilisers and manure) and nitrate leaching from forests (indirect nitrate enrichment via atmospheric nitrogen deposition) (Dise and Wright 1995, Bobbink and Hettelingh 2011). Sources for sulphate enrichment can be similar although additional nitrate mediated sulphate mobilization from sulphur rich geological deposits in the aquifer could play a role here (Smolders et al. 2010), given the fact that lignite and marcasite deposits are present in the area. Based on the hydrological and chemical data collected in the present study, the proportional contribution of different sources for nitrate and sulphate cannot be established. For nitrate, we investigated $\delta^{15}\text{N}$ -signatures of plants and groundwater nitrate to gain more insight in the origin of nitrate.

The results indicate that the groundwater is more enriched in ^{15}N in zones where regional groundwater reaches the surface, and that the $\delta^{15}\text{N}\text{-NO}_3^-$ signature is lower in zones dominated by groundwater of local origin and lowest in groundwater at locations that are mainly rainwater fed. The higher isotope signature of regional ground water can be explained by the fact that most biological processes preferentially use ^{14}N resulting in increased residual ^{15}N in the remaining nitrogen. For instance, nitrate in groundwater is subject to denitrification processes. Denitrifying microorganisms performing this reaction preferably use the lighter ^{14}N isotope which leads to ^{15}N becoming more abundant in the remaining nitrate (e.g. Böttcher et al. 1990, Lehmann et al. 2003, Madigan et al. 2009, Zhang et al. 2009, Zhang et al. 2012). The regional groundwater also passes pyrite containing lignite deposits in the aquifer, where part of the nitrate in the groundwater can mediate denitrifying pyrite oxidation, which can in turn result in isotopic fractionation of nitrate. Indeed, the groundwater $\delta^{15}\text{N}\text{-NO}_3^-$ signature does correlate with the groundwater sulphate concentration (R^2 0.59, $P < 0.05$), indicating that this process might play a role.

Groundwater $\delta^{15}\text{N}\text{-NO}_3^-$ signature will of course also depend on the nitrate source (Nestler et al. 2011). According to Nestler et al. 2011 the $\delta^{15}\text{N}\text{-NO}_3^-$ signature of the groundwater measured in the present study falls within the range of artificial fertiliser, soil nitrogen and atmospheric deposition. This might exclude manure or sewage as the origin of the nitrate, but no clear distinction can be made between artificial fertiliser, soil nitrogen and atmospheric deposition, although atmospheric deposition is usually characterized by lower $\delta^{15}\text{N}\text{-NO}_3^-$ values than those measured in groundwater nitrate in our study. We found a higher nitrogen deposition in the forest ($23.0 \text{ kg N ha}^{-1} \text{ year}^{-1}$) in comparison to the mire ($8.8 \text{ kg N ha}^{-1} \text{ year}^{-1}$) (Fig. 2.7), which

can lead to nitrate leaching to the sandy aquifer due to the relatively thin forest top soil with little organic matter (Gundersen et al. 1998, Dise et al. 1998, Macdonald et al. 2002, Borken and Matzner 2004, Gundersen et al. 2006). Aerial nitrogen deposition measured on the mire itself could be relatively low due to the relatively small surface of the open mire, imbedded in dense pine forest which intercept aerial nitrogen deposition. Intensive agricultural land use is well known to lead to nitrate leaching to the groundwater (Goulding 2000, Di and Cameron 2002; Smolders et al., 2010). Nitrate and chloride concentrations of the regional groundwater are substantially higher compared to groundwater from local origin, which indicates that nitrogen originating from aerial nitrogen deposition is probably only an additional source (Apello and Postma 2007). Only the intensive use of fertilizers on the agricultural fields (25 % of the catchment area) at the southeast side of the catchment area near the village of Nieuwhagen can explain the severe nitrate pollution of the regional groundwater.

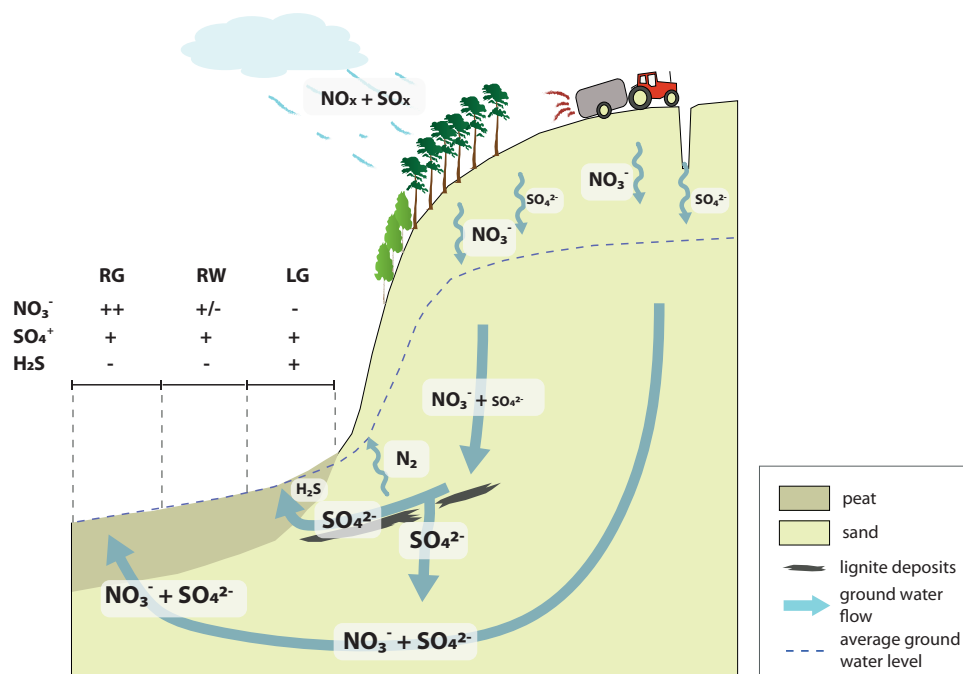


Fig. 2.9. Schematic overview of sources and processes influencing groundwater nitrate and sulphate enrichment in the present study area, (RG: zone dominated by regional groundwater, RW: zone dominated by rainwater, LG: zone dominated by local groundwater).

Aerial sulphur deposition was higher in the surrounding pine forest compared to the open mire itself (Fig. 2.7), indicating that sulphate leaching from forest soils in the catchment might be a possible source. Agricultural activities are also known to lead to sulphate leaching to

the groundwater due to the application of manure and different forms of sulphate containing fertilizers. An important additional source for groundwater sulphate enrichment can be the mobilization of sulphate from sulphur bearing deposits in the aquifer. The present study revealed the presence of marcasite rich lignite deposits in the aquifer. In combination with high groundwater nitrate concentrations. This ferrous iron bearing sulphide deposits can be oxidized via nitrate reduction, leading to the mobilization of sulphate (Postma et al. 1991, Lucassen et al. 2004, Schwientek et al. 2008, Zhang et al. 2009, Haaijer et al. 2007, Smolders et al. 2010, Korom et al. 2012). Sulphate mobilized from these geological deposits will end up in the groundwater, while nitrate is converted to nitrogen gas (Fig. 2.9).

Groundwater nitrate and sulphate enrichment – potential effects on ecosystem functioning and biodiversity

Nitrate and sulphate are electron acceptors that influence redox processes to a major extent, especially in wet soils that are rich in organic material such as peatlands (Lamers et al. 2012). As the process of nitrate reduction is thermodynamically more favourable than the process of sulphate reduction, sulphate reduction is only expected on locations with low nitrate concentrations, which is in line with our observations. Sulphide produced within the rhizosphere or in the soils of small ponds and streams in the mire, that were under the influence of nitrate poor and relatively sulphate rich groundwater (Fig. 2.6, 2.9), can be highly toxic for plants and animals (i.e. Lamers et al. 2013). Elemental sulphur deposition on dead plant material observed in this study very probably resulted from the oxidation of groundwater sulphide after it reaches the surface.

Little is known about the ecological effects of the presence of these sulphur deposits, but one can imagine that they might be toxic for aquatic invertebrates and will reduce food availability for these animals. Plants and bryophytes in the direct surrounding of these sulphur deposits seemed to be dead (pers. obs. G. van Dijk, see Fig. 2.6). However, it seems more likely that this is caused by the (temporal) presence of free sulphides (under the microbial mats (Fig. 2.6) free sulphide was observed on several locations). Apart from these direct effects of sulphide, the influx of sulphate is known to cause enhanced decomposition of organic matter, decreased methane production (e.g. Pester et al. 2012, Hausmann et al. 2016) and mobilization of internally bound phosphorus (internal eutrophication) (i.e. Lamers et al. 2001, Lamers et al. 2002, Smolders et al. 2006). No clear indications of internal eutrophication were found in the present study as pore water phosphorus concentration showed no correlation with pore water sulphate or nitrate concentrations (Fig. 2.5). A detailed study on microbiological and biogeochemical processes along a depth profile in the study area, however, showed that nitrate enrichment influences carbon and nitrogen cycling via stimulation of anaerobic methane oxidation by microorganisms within the peat profile (Zhu et al. 2012, Pester et al. 2012, Hausmann et al. 2016).

It can be concluded that internal eutrophication does not seem to play an important role. This might also be the case because almost no phosphorus in this ecosystem is bound to iron-complexes, that could easily be mobilised under enhanced sulphate reduction. Groundwater nitrate concentrations in the present study ($800\ \mu\text{M}$ - $2000\ \mu\text{M}\ \text{NO}_3^-$ [$50\text{-}125\ \text{mg}\ \text{l}^{-1}\ \text{NO}_3^-$]), exceed the maximum groundwater nitrate concentrations allowed by the EU-Nitrate directive ($800\ \mu\text{M}\ \text{NO}_3^-$ [$50\ \text{mg}\ \text{l}^{-1}\ \text{NO}_3^-$]). These high groundwater nitrate concentrations alone can already result in direct eutrophication and cause a change in vegetation composition. Vascular plants, bryophytes and tree species were found to have enhanced $\delta^{15}\text{N}$ signatures on locations influenced by nitrate enriched groundwater indicating uptake of anthropogenic N. The constant influence of nitrate enriched groundwater leads to a huge total nitrogen flux through the mire. High nitrogen loads have been shown to lead to an enhanced competitive advantage of graminoids and trees, at the expense of more oligotrophic species such as *Sphagnum* mosses (i.e. Limpens et al. 2003, Tomassen et al. 2004), which was also observed in the present study.

Sphagnum mosses can actively take up both ammonium and nitrate, with particularly ammonium being taken up very efficiently (Jauhiainen et al. 1998, Fritz et al. 2014). This ability prevents the dominance of vascular plants under ombrotrophic conditions (Van Breemen 1995, Lamers et al. 2000). However, when nitrogen loads exceed certain thresholds this will lead to nitrogen saturation of *Sphagnum* mosses, leaving available nitrogen for other, faster growing species (Lamers et al. 2000, Tomassen et al. 2004). Nitrate loading on locations with nitrate enriched groundwater clearly exceed the thresholds for nitrogen uptake in *Sphagnum* mosses. Based on maximum critical nitrogen loads (MCL), solely the aerial nitrogen deposition in the study area is at its maximum limit or exceeds the maximum critical loads for ombrotrophic bogs (MCL of $10\ \text{kg}\ \text{N}\ \text{ha}^{-1}\ \text{y}^{-1}$, $8.8\ \text{kg}\ \text{N}\ \text{ha}^{-1}\ \text{y}^{-1}$ in the study area) and coniferous forests (MCL of $20\ \text{kg}\ \text{N}\ \text{ha}^{-1}\ \text{y}^{-1}$, $23\ \text{kg}\ \text{N}\ \text{ha}^{-1}\ \text{y}^{-1}$ in the study area) (Bobbink & Hettelingh 2011). Additional nitrogen loading via nitrate-enriched groundwater (roughly estimated at $350\text{-}880\ \text{kg}\ \text{N}\ \text{y}^{-1}$ (based on concentrations and discharges mentioned in the present paper)) will therefore definitely exceed maximum critical loads for these habitats on locations with (direct) influence of nitrate enriched groundwater.

Although relatively little is known on the (toxic) effects of nitrate, it cannot be excluded that these high concentrations have (toxic) effects on organisms, possibly via the denitrification intermediate nitrite. Reviews on the effects of nitrate concentrations on aquatic invertebrates in streams and springs mainly point to biogeochemical effects and effects on redox processes and little on direct toxicity (Ferreira et al. 2006, Camargo and Alonso 2006). Within our study area, different aquatic invertebrate communities were observed in several streams and pools, which were to a varying degree influenced by nitrate enriched groundwater, although other driving forces (e.g. pH, size and vegetation structure) will also have their effect on the community composition (Van Dijk et al. 2014).

From an ecosystem point of view, the study area has a high biodiversity due to the presence of ecohydrological and biogeochemical gradients on a small scale, ranging from oligotrophic acidic conditions to mesotrophic, slightly buffered conditions (present study and Van Dijk et al. 2014) which generates a large heterogeneity of habitats. Especially the combination of different habitats and the gradients between them can result in suitable habitats for a large number of species and for rare species adapted to these gradients (Wheeler and Proctor, 2000, Verberk et al. 2006, Van Kleef et al. 2012). To some extent biogeochemical stressors like nitrate and sulphate might even enhance heterogeneity and biodiversity on an ecosystem scale, but only as long as their influence remains locally confined. As spring mires (such as the present study area) are often small in size, these systems are probably not very resilient after facing a large scale impact of these biogeochemical stressors for a longer time.

Lessons for the future – a management dilemma between groundwater quantity and groundwater quality

The present paper stresses, as others did before (Bedford et al. 1996, Grootjans et al. 2006, Lamers et al. 2014) the importance of interactions between biogeochemical processes and (eco-)hydrological processes for the functioning of ecosystems, especially for groundwater fed mires. Understanding the functioning of systems is important when aiming at restoration of these systems and mitigation of anthropogenic influences. The present paper stresses the potential direct and indirect biogeochemical effects and ecological consequences of groundwater nitrate enrichment on base-poor groundwater percolating mires. Mitigating these effects is not an easy task, however. One faces a management dilemma as direct reduction of regional groundwater influence enriched with nitrate would result in reduced water availability, leading to desiccation, enhanced decomposition and internal eutrophication.

One option could be to restore and to increase the influence of local groundwater, poorer in nitrate, by removing forests in the catchment area. Old topographical maps and aerial pictures reveal that forest cover has steeply increased during the 20th century (Swierstra 2008). Transformation of the current pine forests in the catchment areas into the heathland vegetation of more than a century ago will lead to a reduced interception of precipitation by at least 20% (Fig 2.7) and also reduces aerial nitrogen deposition. This will increase the influence of local nitrate poor groundwater over the base poor regional groundwater. This case study clearly shows that the interaction between percolating mires and nitrate enriched groundwater can be complex and does to a large extent depend on the local geological and ecohydrological setting. Therefore one has to be careful when extrapolating and generalizing results. Nevertheless it can be concluded that nitrate and sulphate polluted groundwater can raise serious management issues for groundwater fed mires and groundwater fed ecosystems in general.

Reducing nitrate leaching in the groundwater recharge areas is the best long-term solution, as this would lead to a strong reduction of the nitrate load and a reduced aquifer sulphate mobilization. Measures to reduce the leaching of nitrate from agricultural soils might include the reduction of nitrogen gifts to soils to levels slightly below those expected to give optimum yield, or enhance the N use efficiency and a range of counter measures (such as catch crops) (see Kirchmann et al. 2002, Shibata et al. 2017). However, reducing nitrate leaching from agricultural lands is not easily realised and also faces political and practical difficulties. Despite this, and even though it may take up to a few decades before the results of a decreased nitrate leakage to the regional groundwater are noticeable in the discharge area, this is the only sustainable solution to protect the biodiversity in this mire.

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APPENDIX

Results S1: Table 2.1. Average pore water chemical composition (n= 5 in time) On depth profiles (locations A-D of depth profiles is shown in Fig. 2.3), alkalinity is in presented in meq l⁻¹ all other elements are presented in μmol l⁻¹.

		pH	alk.	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	Cl ⁻	Fe ²⁺	SO ₄ ²⁻	P _(tot.)	NO ₃ ⁻	NH ₄ ⁺
	depth	meq l ⁻¹		μmol l ⁻¹									
A	-10	4.3	0.2	73	19	24	142	172	8	136	0.6	5	1
	-80	4.4	0.1	84	21	22	156	172	1	165	< 1	3	2
	-160	4.2	0.3	72	21	31	244	254	2	169	< 1	36	2
B	-10	5.0	0.2	390	227	75	631	707	0	404	< 1	614	< 1
	-60	5.7	0.2	340	146	62	558	522	0	296	< 1	506	1
	-120	5.9	0.9	99	58	48	295	248	16	60	0.6	11	73
	-260	5.9	0.4	248	73	53	433	339	< 1	190	< 1	171	1
C	-10	5.0	0.3	678	428	88	423	579	< 1	685	< 1	858	< 1
	-50	5.2	0.2	1032	443	112	627	884	< 1	714	< 1	1425	< 1
	-100	5.4	0.2	1180	436	130	657	974	< 1	774	< 1	1526	< 1
	-195	5.3	0.3	1110	545	121	649	900	< 1	811	< 1	1861	< 1
D	-10	5.3	0.3	81	56	42	122	124	711	205	2.5	6	5
	-50	5.7	0.5	57	55	41	113	172	1223	150	1.2	5	14
	-90	5.5	0.5	67	71	45	260	324	324	160	0.8	15	20
	-190	4.8	0.1	642	479	99	410	592	1	764	< 1	937	< 1

Results S2:

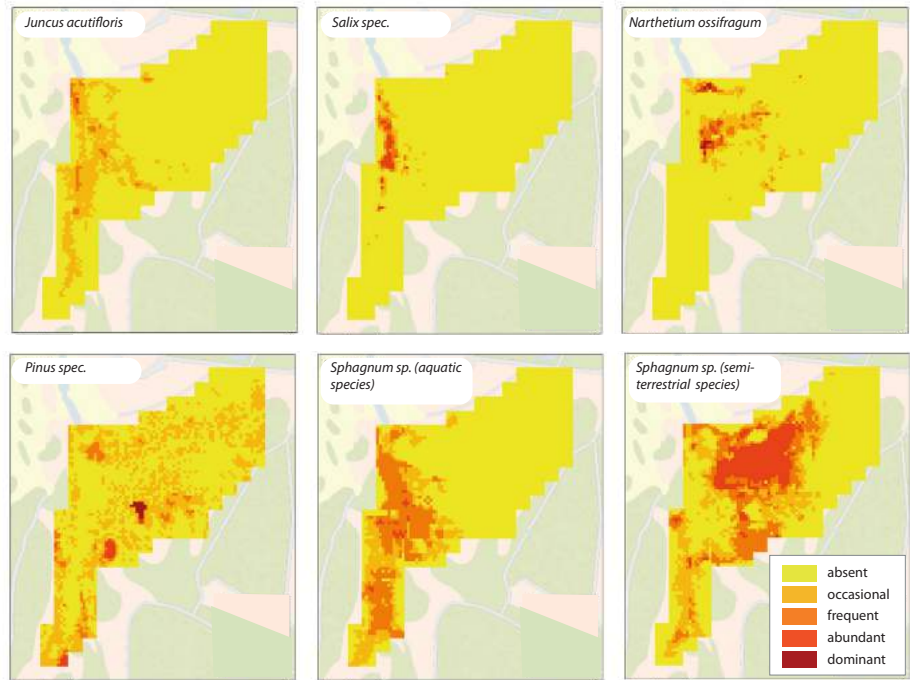


Fig. S 2.1. Maps of the study area with the distribution and abundance of different species or genera in 5x5 m plot on a tansley scale.



[Brunssummerheide with K. Ettwig and B. Zhu, by G. van Dijk]

3

Anaerobic Oxidization of Methane in a Minerotrophic Peatland: Enrichment of Nitrite-Dependent Methane-Oxidizing Bacteria

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ABSTRACT

The importance of anaerobic oxidation of methane (AOM) as a methane sink in freshwater systems is largely unexplored, particularly in peat ecosystems. Nitrite-dependent anaerobic methane oxidation (n-damo) was recently discovered and reported to be catalyzed by the bacterium "*Candidatus Methyloirabilis oxyfera*," which is affiliated with the NC10 phylum. So far, several "*Ca. Methyloirabilis oxyfera*" enrichment cultures have been obtained using a limited number of freshwater sediments or wastewater treatment sludge as the inoculum. In this study, using stable isotope measurements and porewater profiles, we investigated the potential of n-damo in a minerotrophic peatland in the south of the Netherlands that is infiltrated by nitrate-rich ground water. Methane and nitrate profiles suggested that all methane produced was oxidized before reaching the oxic layer, and NC10 bacteria could be active in the transition zone where countergradients of methane and nitrate occur. Quantitative PCR showed high NC10 bacterial cell numbers at this methane-nitrate transition zone. This soil section was used to enrich the prevalent NC10 bacteria in a continuous culture supplied with methane and nitrite at an in situ pH of 6.2. An enrichment of nitritereducing methanotrophic NC10 bacteria was successfully obtained. Phylogenetic analysis of retrieved 16S rRNA and *pmoA* genes showed that the enriched bacteria were very similar to the ones found in situ and constituted a new branch of NC10 bacteria with an identity of less than 96 and 90% to the 16S rRNA and *pmoA* genes of "*Ca. Methyloirabilis oxyfera*," respectively. The results of this study expand our knowledge of the diversity and distribution of NC10 bacteria in the environment and highlight their potential contribution to nitrogen and methane cycles.

INTRODUCTION

Wetlands are the largest single source of methane with estimated emissions of 103 Tg per year, which account for about 20% to 40% of the global annual atmospheric methane flux (Bastviken et al. 2011, Denman et al. 2007, Hanson & Hanson 1996). It is estimated that about 50% of the methane produced in wetlands is consumed before it reaches the atmosphere; this significant microbial methane sink is usually considered to consist exclusively of aerobic methanotrophic bacteria, which degrade methane using oxygen as electron acceptor (Bodelier 2011, Cicerone & Oremland 1988, Hanson & Hanson 1996, Reeburgh 2007). In ecosystems where oxygen is depleted but sufficient alternative electron acceptors, e.g. sulfate or nitrate are present, methane can also be converted anaerobically (Knittel & Boetius. 2009, Raghoebarsing et al. 2006). Anaerobic oxidation of methane (AOM) coupled to sulfate reduction is performed by a consortium of anaerobic methanotrophic archaea (ANME) and sulfate reducing bacteria (SRB) (Knittel & Boetius 2009, Strous & Jetten 2004). Its significance is well established for marine ecosystems, where it may consume more than 90% of the produced methane (Reeburgh 2007). In freshwater wetlands, and especially peatlands, electron acceptors are scarcer, with concentrations typically in the low μM range (Pester 2012). Due to this reason, redox processes are mostly limited by electron acceptor supply, very dynamic and highly susceptible to alterations e.g. by influx of polluted groundwater and atmospheric deposition of nitrogen and sulfur species (Gauci 2004, Stevens et al. 2011). The influence of nitrogen pollution on methane oxidation is complex, and not all feedback loops are well understood (Bodelier 2011, Bodelier & Laanbroek. 2004, Gärdenäs et al. 2011, Liu & Greaver. 2009). In principal the role of the alternative electron acceptors nitrate and sulfate for diverting carbon fluxes away from methane production is better established, given that sulfate and nitrate reduction are thermodynamically more favorable than methanogenesis (Gauci 2002, Lovley & Klug 1983, Lovley & Phillips. 1987, Vile et al. 2003). However, these alternative electron acceptors can in principle also enable methane oxidation (Strous & Jetten. 2004, Zhu et al. 2010), but this topic has received only little attention with respect to methane cycling in peatlands (Smemo & Yavitt 2011).

In the meantime, for other freshwater ecosystems, more and more evidence about the occurrence of AOM coupled to sulfate (Eller et al. 2005, Schubert et al. 2011), iron(III) (Sivan et al. 2011) and nitrate reduction (Deutzmann & Schink. 2011, Raghoebarsing et al. 2006, Smith et al. 1991, Van Breukelen & Griffioen 2004) has become available. Whereas nothing is known about the microorganisms mediating ferric iron reduction with methane, for sulfate reduction a very similar consortium of methanotrophic Archaea and SRB as in marine ecosystems is hypothesized to be responsible (Eller et al. 2005, Schubert et al. 2011). Nitrate- or nitrite-dependent AOM (n-damo), when linked to organisms, was so far always found to be performed by one bacterial species affiliated to the NC10 phylum (Deutzmann & Schink 2011, Ettwig et

al. 2008). Genome sequencing, expression studies and physiological experiments indicated that this bacterium, then named *Candidatus Methylopirabilis oxyfera*, is an “intra-aerobic” methanotroph that produces its own oxygen from the dismutation of nitric oxide into dinitrogen gas and oxygen. The produced oxygen is then used for canonical aerobic methane oxidation starting with the methane monooxygenase enzyme complex (Ettwig et al. 2010). Although 16S rRNA sequences similar to *M. oxyfera*’s were found in various environments (Ettwig et al. 2009), so far n-damo enrichment cultures have only been obtained from two types of ecosystems: eutrophic freshwater sediments and wastewater treatment sludge. The dominant bacteria in all described cultures were closely related ($\geq 97\%$ identity of the 16S rRNA gene sequence) to *M. oxyfera* (Ettwig et al. 2009, Ettwig et al. 2010, Hu et al. 2009, Luesken et al. 2011). Currently it is unclear, however, if *M. oxyfera*-related species are the only nitrite-dependent methane oxidizing bacteria; if methane oxidation is a general feature of NC10 phylum bacteria or limited to (close relatives of) *M. oxyfera*, and how important these bacteria are for methane cycling in various ecosystems.

In this paper, we studied a minerotrophic peatland infiltrated by nitrate-containing groundwater. At the sampling site, no methane emission was detectable. Porewater profiling revealed a nitrate-methane transition zone below the oxic layer that could provide an ecological niche for n-damo microorganisms. NC10 bacteria abundance in soil cores was analyzed using quantitative PCR, and the section with highest cell numbers of *M. oxyfera*, coinciding with the methane-nitrate transition zone, was used as inoculum for the enrichment of n-damo bacteria. Mimicking field conditions as much as possible by using nitrite-amended peatland water in continuous cultivation, a new cluster of *M. oxyfera*-like bacteria was enriched.

MATERIAL AND METHODS

Site description

The Brunssummerheide peatland (50°55′39.63″N/5°59′50.73″E) is a small (15ha) spring fen located in an oligotrophic sandy valley fed by locally upwelling, weakly buffered nitrate-polluted groundwater. The peat layer is relatively thin (maximum 2.5 m) and vegetation is dominated by *Sphagnum* spec., *Narthecium ossifragum* and *Molinia caerulea*. At the sampling site, nitrate-enriched groundwater overflows the peatland surface and infiltrates into the peat layer.

Porewater profile determination and soil sampling

Nitrate and methane profiles were determined by measuring the concentrations in porewater samples collected using 5 cm ceramic cups (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) connected to Teflon tubes. Porewater samples were obtained at least in duplicate from the depth of 20 cm to 220 cm at 5 or 10 cm intervals in December 2009 and

June 2010. Porewater for methane analyses was collected in vacuumed anaerobic glass bottles (40 ml) prefilled with 5 g sodium chloride and sealed with butyl rubber stoppers. For chemical analyses, porewater was collected in 60 ml syringes. Samples were transported to the laboratory within two hours in a cooling box, and stored at 4 °C for maximum 14 days before analysis. Methane in the bottle headspace was measured after pressure equilibration with argon using gas chromatography as described previously (Ettwig et al. 2009). Nitrate was analyzed colorimetrically on a Traacs 800+ auto-analyzer as described previously (Tomassen et al. 2003). Redox potential measurements were performed by gently pushing platinum electrodes into pre-drilled holes and allowing them to equilibrate. Stable readings were obtained after 30 min (Fritz et al. 2011). Soil samples were obtained from 50 cm to 130 cm depth with a Russian peat corer, sliced into 5-20 cm intervals in the field, immediately put into self-sealing plastic bags, and stored in air-tight bins with oxygen scavenger (Anaerogen, Oxoid, USA), then transported to the laboratory and stored anaerobically at 4 °C until further analysis.

Incubation

Initially, 200 ml soil slurry of the depth layers of 80-100 cm, 100-120 cm and 120-135 cm (sampled in July 2009) were incubated in separate bottles (500 ml). Surface water from the peatland was collected and used for medium preparation after removal of particles by filtering through a hemo-filter (Hemoflow HF80S, Fresenius Medical Care, USA). The medium contained: 2 mM KHCO_3 , 0.2 mM $\text{Na}^{15}\text{NO}_2$ (99.6% ^{15}N ; Isotec, USA) and 0.5 mM NaNO_3 . The bottles were made anaerobic by 6 cycles of vacuuming and gassing with Ar/CO_2 (75:25), followed by 5 min of flushing with Ar/CO_2 . Then 10 ml $^{13}\text{CH}_4$ was injected into the headspace (final concentration ca. 20%). The pH in the bottles was around 6.0 and the bottles were incubated at 25 °C, with gentle shaking at 100 rpm. The production of $^{13}\text{CO}_2$ was measured by GC-MS in the headspace (see below).

After three months incubation, the bottle with strongest $^{13}\text{CO}_2$ production was used as inoculum for continuous culturing in a 3 liter glass bioreactor (working volume 1.5 l; Applikon, Schiedam, The Netherlands) that was operated in sequencing batch mode to prevent biomass loss. One cycle constituted of 23 h of continuous supply of medium, 0.5 h settling, finally followed by 0.5 h discharging with a level-controlled pump. To keep the culture anaerobic, the reactor was continuously flushed with 20 ml min^{-1} Ar/CO_2 (95:5) and 5 ml min^{-1} methane. The temperature was controlled at 25 °C and the pH at 6.0 to 6.2. Dissolved oxygen, temperature and pH in the reactor were monitored by respective electrodes. Medium was prepared as described above, except using unlabeled nitrite. The nitrite concentration in the reactor was estimated daily with Merckoquant test strips (0-80 mg l^{-1} ; Merck, Germany), and the concentration in the medium was slowly increased from 0.2 mM to 2.5 mM dependent on the activity of the continuous culture. Nitrite concentrations in the reactor were kept below 20 mg l^{-1} (0.44 mM). The medium loading to the reactor was between 200 to 500 ml per day.

Activity analysis

Methane oxidizing activity in bottles was measured by determining the amount of $^{13}\text{CO}_2$ produced from $^{13}\text{CH}_4$ oxidation with GC-MS (Agilent 5975C inert MSD; Agilent, United States) as previously described (Ettwig et al. 2009). Activity in the reactor was tested in batch experiments with the whole culture. First medium supply was stopped and unlabeled nitrite was allowed to be depleted. The reactor was flushed with Ar-CO_2 (95:5) for 1 h while stirring, and checked for residual methane in the headspace. When undetectable, 0.2 mM $^{15}\text{NO}_2^-$ and 50 ml $^{13}\text{CH}_4$ were added. 20 μl gas samples were taken every hour for $^{13}\text{CO}_2$, $^{15,15}\text{N}_2$, $^{15,14}\text{N}_2$ analysis. At the same time, 1 ml culture liquid was taken and centrifuged; the supernatant was kept at 4 °C for nitrite analysis. Nitrite concentrations were determined with colorimetric methods as described elsewhere (Kartal et al. 2006). The influence of pH on activity was determined in batch incubations of 10 ml biomass in 40 ml serum bottles, buffered with MES (2-(N-morpholino) ethanesulfonate, 20 mM) to pH values between 5.9 and 6.7, and with MOPS (3-(N-morpholino) propanesulfonate, 20 mM) to pH values between 6.75 and 7.4 (measured at the end of incubation).

DNA isolation

Total DNA from soil samples was isolated with the PowerSoil[®] DNA isolation kit (MO BIO Laboratories Inc., USA) according to the manufacturer's manual. Approximately 0.3 g homogenized soil was used for DNA isolation, and two independent isolations were carried out for each depth interval. DNA was eluted three times with pre-warmed Milli-Q water from the column to ensure that the entire DNA had been collected. DNA in the third elution was undetectable by agarose gel electrophoresis ($<0.2 \text{ ng } \mu\text{l}^{-1}$). DNA obtained from the same depth interval was pooled for qPCR analysis to minimize the influences from soil inhomogeneities. DNA from enrichment cultures was isolated with a method based on bead-beating and SDS lysis, as described previously (Ettwig et al. 2009). DNA quality was checked on agarose gel, and concentrations were measured in triplicate with NanoDrop (ND-1000, ISOGEN Life Science, The Netherlands).

Quantitative PCR

In order to quantify n-damo bacteria and all bacteria in different depths of the soil cores, quantitative PCR (qPCR) targeting the 16S rRNA gene was performed. To account for imperfect primer matching and known variability of results (Ettwig et al. 2009), two different primer pairs were used for each group. For NC10 phylum bacteria, primer pairs p1F & p1R and p2F & p2R, and for all bacteria, primer pairs 1100F & 1492R and 533F & 805R (Table 1) were applied. All q-PCR assays were performed according to the MIQE guidelines (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) (4). qPCR experiments were carried out with the Bio-Rad IQTM 5 cycler real-time detection system

using IQTM SYBR green Supermix (Bio-Rad, United States) in 25 μ l reaction volume as previously described (Ettwig et al. 2009), except using 65 °C for n-damo specific primer pairs and 58 °C for universal primer pairs as annealing temperature, which had been determined as most suitable for the present samples by gradient PCR. The qPCR reactions were carried out in 96 well plastic plates (Bio-Rad, United States) sealed with Opti-Seal Optical disposable adhesive (BIOplastics, The Netherlands). Fluorescence signals were obtained at 72 °C at the end of the elongation step of each cycle. PCR products obtained with n-damo specific and universal bacteria primer pairs were cloned and sequenced using the vector pGEM-T Easy (Promega, United states). The sequences retrieved were of the correct length (201 bp for p1F & p1R; 292 bp for p2F & p2R; 291 bp for 515F & 805R and 410 bp for 1100F & 1492R), and the obtained n-damo sequences were similar (>97.2% identity) to the sequence of *M. oxyfera* (accession no. FP565575). Standard curves for n-damo bacteria and general bacteria were constructed with plasmids containing corresponding inserts, taking into account the molecular mass of the plasmid including the insert, and the plasmid concentration. Plasmids copy numbers used as standard were between 30.7 to 3.07 $\times 10^8$ μ l⁻¹ for NC10 bacteria, 86.9 to 8.69 $\times 10^8$ μ l⁻¹ for all bacteria. Two soil cores with partial overlap were analyzed. Both cores were sliced in sections between 5 and 10 cm in the field (see soil sampling and DNA isolation). In Fig. 1, each depth interval is represented by its average depth. DNA isolated from soil of 85-90 cm depth was used to test dilution effect; 10 times and 100 times had a maximum difference of 8.7%, compared with non-diluted ones. For NC10 bacteria non-diluted DNA was used as templates; but for primers targeting all bacteria, 100 times diluted DNA was used. PCR efficiencies calculated based on standards were between 90.6% and 99.2%. Both standards and samples were run in triplicates. The copy numbers in samples were calculated based on comparison with the threshold cycle values of the standard curve, taking into account the dilution and the amount of total DNA obtained per gram soil.

Phylogenetic analysis

PCR was performed with DNA isolated from the soil layer used as an inoculum (80-100 cm depth), the enrichment culture after 3 months of incubation in bottles, and the continuous culture after 1 and 17 months of enrichment in the reactor. 16S rRNA sequences of n-damo bacteria were obtained with universal bacteria primer 8F or n-damo specific primer 193F in combination with n-damo specific primer 1043R (Table 1). PCR products of the correct size were ligated into the pGEM-T Easy cloning vector (Promega, United States) and amplified in *Escherichia coli* DH5 α . Plasmids were isolated from 10 to 15 randomly selected white colonies per library using the GeneJet miniprep kit (Fermentas, Lithuania), and were sequenced at the DNA Diagnostics Center of Nijmegen University Medical Center. The sequences were aligned to reference sequences with the MUSCLE algorithm. Phylogenetic trees were constructed with MEGA5 using the neighbor-joining method and the robustness of tree topology was tested by bootstrap analysis (1000 replicates).

With the same DNA samples also functional gene (particulate methane monooxygenase subunit A, *pmoA*) clone libraries were constructed. The particulate methane monooxygenase catalyzes the first step of methane oxidation and is well conserved in methane oxidizing bacteria, therefore *pmoA* is widely accepted as a marker gene for assessing diversity of aerobic and *M. oxyfera*-like anaerobic methanotrophs in the environment (Luesken et al. 2011, McDonald et al. 2008). Two different forward primers targeting either most methanotrophs (A189b) or only close relatives of *M. oxyfera* (cmo182) were combined with a specific reverse primer (cmo682) (Table 3.1). A *pmoA* phylogenetic tree based on nucleotide sequences was constructed as described above.

Table 3.1. Primer Pairs used for qPCR analysis and clone library construction

Designation* and purpose	Sequence (5'–3')	Reference	Annealing temp (°C)	Target
qPCR analysis				
p1F	GGGCTTGACATCCCACGAACCTG	Ettwig et al. 2009	65	NC10 bacterial 16S rRNA
p1R	CGCCTTCCTCCAGCTTGACGC	Ettwig et al. 2009		
p2F	GGGGAAGTCCAGCGTCAAG	Ettwig et al. 2009	65	NC10 bacterial 16S rRNA
p2R	CTCAGCGACTTCGAGTACAG	Ettwig et al. 2009		
533F	GTGCCAGCMGCCGCGGTAA	Turner et al. 1999	58	All bacterial 16S rRNA
805R	GACTACCAGGGTATCTAATC	Leser et al. 2002		
1100F	YAACGAGCGCAACCC	Downes et al. 2009	58	All bacterial 16S rRNA
1492R	GGTTACCTTGTTACGACTT	Weisburg et al. 1991		
Clone library construction				
8F	AGAGTTTGATYMTGGCTCAG	Juretschko et al. 1998	55-65	NC10 bacterial 16S rRNA
193F	GACCAAAGGGGGCGAGCG	Ettwig et al. 2009		
1043R	TCTCCACGCTCCCTTGCG	Ettwig et al. 2009		
A189bF	GGNGACTGGGACTTYTGG	Luesken et al. 2011	55-65	NC10 bacterial pmoA
cmo182F	TCACGTTGACGCCGATCC	Luesken et al. 2011		
cmo682R	AAAYCCGGCRAAGAACGA	Luesken et al. 2011		

* F, forward; R, reverse.

Fluorescence in situ hybridization

On a monthly basis, 1.5 ml biomass was harvested from the reactor and forced through a 0.5 mm needle to break big cell aggregates. Then the sample was centrifuged and the pellet was washed twice with 1 ml 1×PBS, and fixed with paraformaldehyde on ice for three hours. Fluorescence in situ hybridization (FISH) was performed as previously described (Ettwig et al. 2010), using 40% formamide concentration. The following oligonucleotide probes were used: S⁻*-DBACT-0193-a-A-18 and S⁻*-DBACT-1027-a-A-18 specific for *n-damo* bacteria (Raghoebarsing et al. 2006) and a mixture of EUB I-III and V for most Bacteria (Denman et al. 2007). Images were acquired with a Zeiss Axioplan 2 epifluorescence microscope equipped with a CCD camera, together with the Axiovision software package (Zeiss, Germany).

Nucleotide sequences accession numbers

Representative 16S rRNA and *pmoA* gene sequences were deposited at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>) with the accession numbers JX262153- JX262155 (*pmoA*) and JX262156-JX262161 (16S rRNA).

RESULTS

Porewater profiles

Porewater depth profiles of the Brunssummerheide sampling location were determined on five occasions between July 2009 and May 2011, with an overall very similar pattern. Representative winter (December 2009) and summer (June 2010) profiles are shown in Fig.3.1. Nitrate concentration decreased with depth, and became undetectable below 100 cm. No methane was detected in the upper 80 cm, but methane gradually increased below the depth of 80 cm and reached the maximum concentration at around 120 centimeters (Fig. 3.1 A, B). Redox data indicated that the soil was completely anoxic below 50 cm depth, and living roots of vascular plants were not found below 60 cm depth. The maximum concentration of nitrate (0.6 mM) in June 2010 (Fig. 3.1 B) was about 0.2 mM higher than that in December 2009 (Fig. 3.1. A), possibly due to relatively stronger evaporation of surface water and higher groundwater influx in summer. The maximum concentration of methane remained similar in both seasons, as well as the overall pattern: an opposing gradient at around 80-100 cm depth.

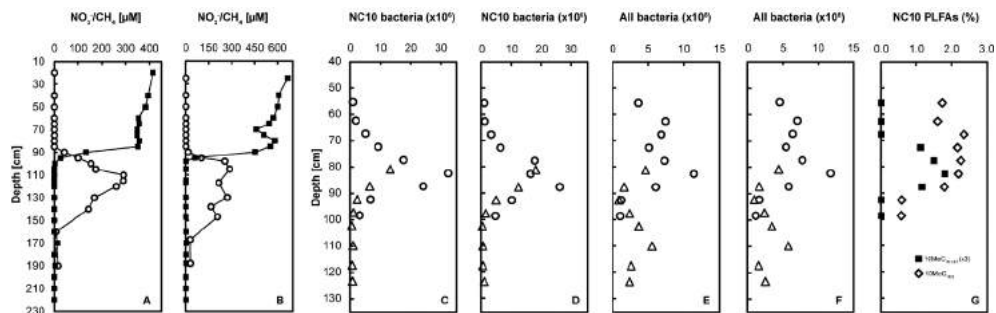


Fig. 3.1 Depth profiles of the Brunssummerheide peatland. (A, B), Nitrate (filled square) and methane (open circle) concentrations in porewater sampled in December 2009 (A) and in June 2010 (B). [C-F] Bacterial cell numbers (cells g^{-1} wet soil) as assessed by qPCR on DNA extracted from two overlapping soil cores from 51 to 102 cm (open circles), and from 77 to 127 cm (open triangles). NC10 bacteria abundance was determined with primer pairs p1F & p1R (C), and p2F & p2R (D). Total bacterial abundance was determined with primer pair 535F & 805R (E), and 1100F & 1492R (F). (G) Relative abundance of the phospho-lipid fatty acids 10-methyl-hexadecanoic acid (10MeC16:0, open diamonds) and 10-methyl-hexadecanoic acid (10MeC16:1 $\Delta 7$, multiplied by 3, closed squares) diagnostic of NC10 bacteria [data from Kool et al., [Kool et al. 2012]].

Quantifying abundance of NC10 bacteria in different soil depths

Total bacterial and NC10 phylum abundance in different soil depths was determined in two overlapping cores by qPCR using primers targeting the 16S rRNA genes. The highest cell numbers ($1.3\text{--}3.2 \times 10^7 \text{ g}^{-1}$ wet soil) of NC10 bacteria were found at 80–85 cm depth (Fig. 3.1 C, D), coinciding with the concomitant decrease of methane and nitrate (Fig. 3.1 A, B), and a peak in abundance of NC10 phylum-characteristic phospho-lipid fatty acids (Fig. 3.1 G; Kool et al. 2012). In contrast, total bacteria cell numbers, ranging from 0.9 to 11.8×10^8 cells g^{-1} wet soil, did not show a depth-related pattern (Fig. 3.1 E, F).

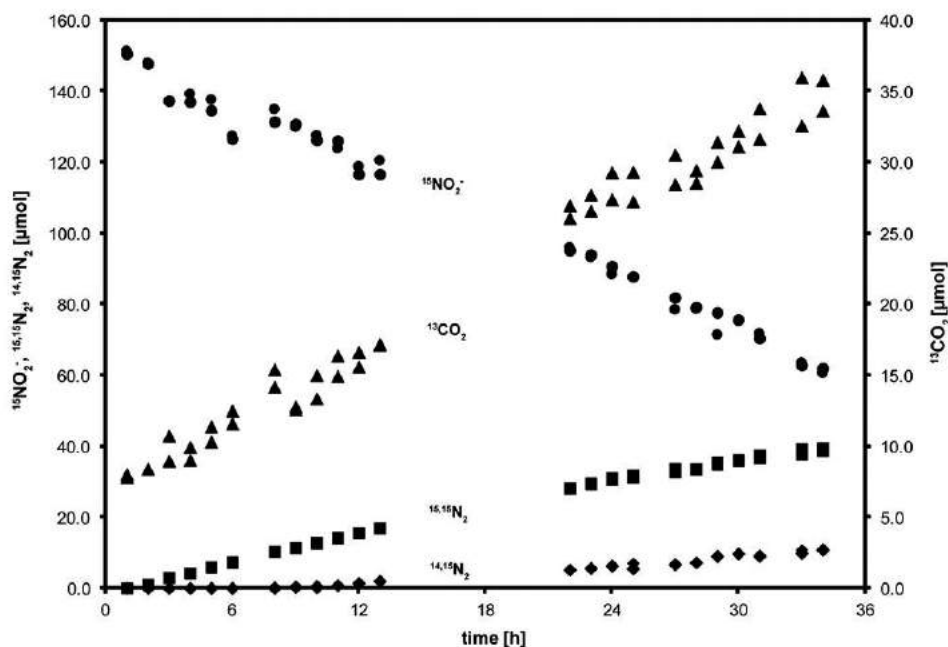


Fig. 3.2 Activity test of the enrichment culture at month 10 with $^{15}\text{NO}_2^-$ and $^{13}\text{CH}_4$. Nitrite (filled circle) was consumed, $^{15,15}\text{N}_2$ (filled square), $^{14,15}\text{N}_2$ and $^{13}\text{CO}_2$ (filled triangle) were produced. The $^{13}\text{CO}_2$ production rate was $20.2 \mu\text{mol d}^{-1}$, and the rate of $^{15,15}\text{N}_2$ production was $29.0 \mu\text{mol d}^{-1}$.

Enrichment and activity

Nitrite dependent methane oxidizing activity was initially determined by measuring the fraction of $^{13}\text{CO}_2$ in total CO_2 after supply of $^{13}\text{CH}_4$ and nitrite to three soil sections (80-100; 100-120; 120-135 cm). Despite the addition (and permanent presence) of nitrate (0.5 mM), all soil cores produced some methane in the first two weeks of incubation, but no methane oxidation could be detected (detection limit approximately $0.5 \text{ nmol d}^{-1} \text{ g}^{-1} \text{ soil}$). After about 3 month's incubation, the 80 - 100 cm section showed methane oxidation activity ($9.0 \text{ nmol d}^{-1} \text{ g}^{-1} \text{ soil}$, assessed as CO_2 production), and an increase in this rate indicated microbial growth. This incubation (80-100 cm) was used as inoculum to start a sequencing batch reactor for the enrichment of the responsible microorganism. Over the first 9 months of enrichment activity remained low with a nitrite reduction rate of about $50 \mu\text{mol d}^{-1} \text{ L}^{-1}$, and then started to increase to about $1.0 \text{ mmol } (\text{NO}_2^-) \text{ d}^{-1} \text{ L}^{-1}$ in month 15. Batch tests and experience with previous NC10 bacteria enrichment cultures had indicated that nitrite was preferred over nitrate; consequentially the medium, prepared with *in situ* water was not only amended with nitrate, but also nitrite. To test the coupling of nitrite reduction to methane oxidations, both activities were tested in batch experiments after 10 months with ^{15}N - and ^{13}C -labelled substrates during the enrichment period (Fig. 3.2). Nitrite-N was completely recovered as nitrogen gas, concomitantly methane

was fully oxidized to CO_2 . The ratio of $^{13}\text{CO}_2$ and $^{15,15}\text{N}_2$ production was 3:4.3, similar to the theoretical stoichiometry of 3:4 [38]. An activity test at different pH values demonstrated that the culture preferred circumneutral conditions, but was active down to the lowest tested value of 5.9 (Fig. 3.3).

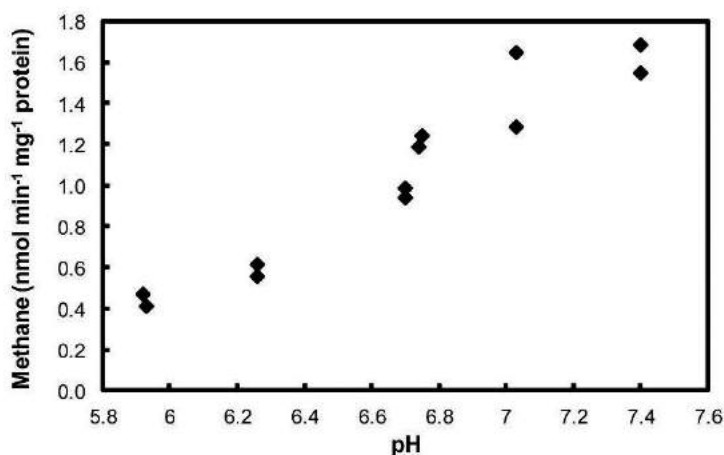


Fig. 3.3 Methane-oxidizing activity of the n-damo enrichment culture incubated at different pH values.

FISH analysis of the enriched bacteria

FISH was performed on biomass of the enrichment culture fixed every month, but no clear hybridization with NC10 specific probes was observed until after 8 months of medium supply. Even though small numbers of NC10 bacteria must have been present, they remained undetectable at first due to strong autofluorescent background and hybridization inhibition, presumably caused by peat material. Starting at month 9, NC10 cells could be detected (Fig. 3.4A). With the progression of incubation, both total cell numbers visualized by DAPI stain, and the percentage belonging to the NC10 phylum gradually increased (Fig. 4 B, C) and coincided with an increased activity of the culture. At month 14 about 50%, and at month 19 more than 80% of the population did hybridize with the NC10 specific probes (Fig. 3.4).

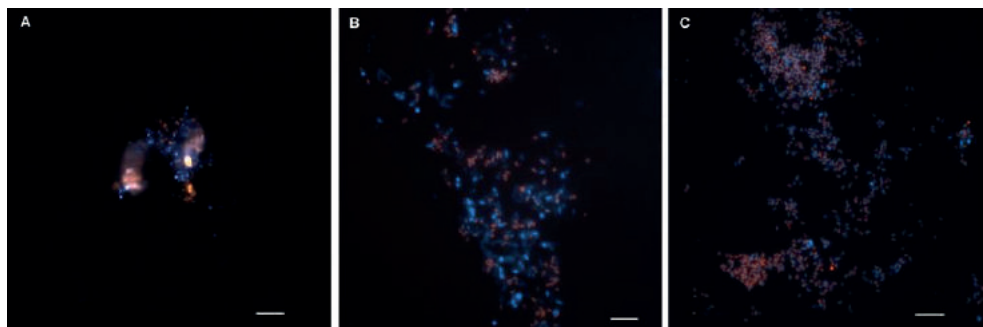
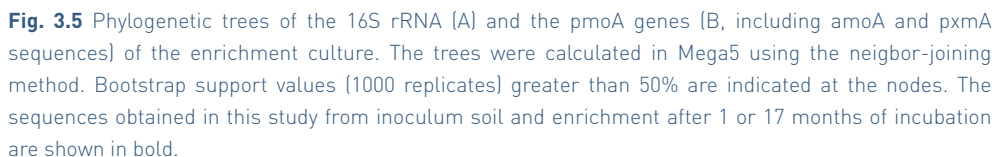


Fig. 3.4 Fluorescence in situ hybridization of the enrichment culture at different times of incubation. A: month 9; B: month 14; C: month 19. NC10 bacteria appear in pink, due to co-hybridization of NC10 bacteria specific probes 193-Cy3 and 1027-Cy3 (red) and a mixture of probes EUBI-III, IV-Cy5 (light blue) for most eubacteria and DAPI (dark blue). (Scale bars: 5μm).

16S rRNA and *pmoA* gene phylogenetic analysis

M. oxyfera-related 16S rRNA and *pmoA* genes were successfully obtained from both inoculum soil and the enrichment after 1 or 17 months of incubation. Long (>1000 bp) 16S rRNA sequences obtained with primer 8F (universal) and 1043R (NC10 specific) were used for phylogenetic analysis. Results showed that the 16S rRNA sequences belong to the group A of NC10 bacteria (14), forming a cluster (differences between 0.1% to 2.7%) with sequences retrieved from coal-tar contaminated aquifer (AF351214, AF351217, FJ810544) and lake Constance sediment (HQ906524, HQ906538) [9]. These sequences share only 94.9% to 95.5% identity with *M. oxyfera* (Fig. 3.5 A).

The phylogenetic analysis of the *pmoA* gene showed similar results. *pmoA* sequences from both soil and enrichment culture again cluster together with *pmoA* sequences retrieved from Lake Constance sediment (HQ906571, HQ906568, HQ906566) [9]. These *pmoA* sequences had an identity with those of *M. oxyfera* of 86.2-90.9% on nucleotide level, but the 95.8-97.9% on amino acid level indicated functional conservation (Fig. 3.5 B). No significant difference could be observed between the inoculum and the 17 months old enrichment culture, indicating that no population shift within the NC10 phylum had occurred. Both the 16S rRNA and *pmoA* genes phylogenetic results suggested that a new cluster of NC bacteria had been enriched.



DISCUSSION

The Brunssummerheide peatland is a spring fen in an oligotrophic sand valley fed by nitrate-polluted groundwater, and therefore contains nitrate concentrations in the upper peat layer which are uncommonly high for pristine peatlands (Waughman 1980). Also in contrast to many other peatlands (Clymo & Bryant. 2008, Kip et al 2010, Lai 2009, Le Mer & Roger. 2001), methane was not detected in the upper 70-80 cm of the depth profile at 5 sampling occasions in different seasons from 2009 to 2011, even though methane was produced in the deep anoxic zone (below 100 cm, Fig. 3.1A, B). As roots of vascular plants do not reach that deep in the Brunssummerheide (maximum 60 cm), this suggested the existence of an anoxic methane sink in the peat, independent from oxygen and aerenchymal transport by roots, for which oxidized nitrogen species could serve as electron acceptor. The counter gradient of methane and nitrate at the depth of 80 cm may provide an ideal niche for, and may be at least partly created by the recently characterized n-damo bacteria. Targeting their 16S rRNA gene in DNA extracts from different depths confirmed this: Highest n-damo cell numbers (up to 3.2×10^7 cells g^{-1} soil) and ratios (3 to 8% of total bacterial community) were observed at the depth of 80-90 cm (Fig. 3.1 C, D), coinciding with the methane-nitrate transition zone (Fig. 3.1 A, B). At this depth, also a peak in abundance of phospho-lipid fatty acids diagnostic for NC10 phylum bacteria was detected (Fig. 3.1 G; Kool et al. 2012). The n-damo cell number and lipid profiles also agreed with the finding that among soil samples from 80-100, 100-120 and 120-135 cm depth only the 80-100 cm sample showed anaerobic methane-oxidizing activity upon incubation. Despite the relatively high numbers of n-damo bacteria detected at a depth 80-90 cm, it took several months to obtain an enrichment culture with measurable activity. Also detection by fluorescence *in situ* hybridization using NC10 phylum-specific probes, hampered by a strongly auto-fluorescent background from the organic-rich inoculum, was only possible after 9 months of continuous cultivation with constant supply of nitrite and methane. This may be due to the "dilution" of the naturally NC10 phylum-enriched soil layer with less active deeper layers (90-100 cm) in the inoculum, and a very low growth rate at the prevailing conditions, especially the pH (6.0 – 6.2). The pH optimum test showed that the NC10 phylum bacteria enriched in the continuous culture were only acidotolerant to a certain extent, not acidiphilic. They were active down to a pH below 6, but their physiological optimum was clearly higher, above 7 (Fig. 3.3). This is a prime example for the discrepancy between physiological and ecological optimum. In contrast to previous *M. oxyfera* enrichment cultures from neutral, eutrophic sediments (Ettwig et al. 2009), which had a similar optimum (around 7.5), but were not active at a pH below 7 (assessed under similar conditions, O. Rasigraf, MSc thesis, 2011, unpublished), a different ecotype was dominant in the more acidic and low nutrient environment. According to the species delineation of 97% identity of the 16S rRNA gene for bacteria in general and 93% of the *pmoA* gene diagnostic for methanotrophic bacteria (Lücke & Frenzel 2011), the NC10 phylum bacterium dominating the Brunssummerheide enrichment culture even constitutes a new species within the genus *Methyloirabilis*.

Like other NC10 enrichment cultures (Ettwig et al 2009, Hu et al. 2009, Luesken et al. 2011), the enrichment period was characterized by a long phase without measureable activity, followed by a period of slow, but exponential increase in nitrite consumption rate. In the present case, nitrite-reducing activity remained low for the first 9 months, and then started to increase to about $1.0 \text{ mmol } (\text{NO}_2^-) \text{ d}^{-1} \text{ L}^{-1}$ in month 15. After this increase it was not possible to stimulate the growth of the culture further and a sort of stationary phase was reached similar to other enrichments of NC10 bacteria (Ettwig et al. 2009, Hu et al. 2009, Kampman et al. 2012). The doubling time of the Brunssummerheide *Methyloirabilis* strain was estimated to be about two months, which is 4 to 8 fold lower than the values reported before (Ettwig et al. 2009). It is difficult to predict whether this reflects the growth rate under field conditions. On one hand some factors like a higher temperature (25 °C), the optimum temperature of methanotrophs in most peat soils (Hanson & Hanson 1996) in contrast to 10-15 °C *in situ* and constant substrate supply may be beneficial, but other factors like stirring, use of surface- instead of porewater or a decrease in microbial partner communities may also be disadvantageous for growth in the laboratory.

However, once established, the methanotrophic community does not need to grow fast to constitute a relevant methane sink in the environment. According to previous estimations, *Methyloirabilis* cells in an enrichment culture have an activity of 0.1 to 0.4 fmol $\text{CH}_4 \text{ cell}^{-1} \text{ d}^{-1}$ (Ettwig et al. 2009), indicating that the Brunssummerheide soil of 80 - 85 cm depth with about $1.3 \text{ to } 3.2 \times 10^7 \text{ cells g}^{-1} \text{ soil}$ may convert between 1.3 and 12.8 nmol $\text{CH}_4 \text{ d}^{-1} \text{ g}^{-1} \text{ soil}$. This range is at the lower end of methane oxidation rates reported for aerobic methanotrophs (Segers 1998) in wetlands, but apparently high enough to balance the methane diffusing upwards from deeper, methanogenic soil layers.

Nitrite is clearly the preferred electron acceptor of previously reported *M. oxyfera* enrichments (Ettwig et al. 2008, Ettwig et al. 2009, Hu et al 2009, Raghoebarsing et al. 2006). When nitrite was depleted in the present *Methyloirabilis* enrichment culture, methane oxidizing activity in the presence of nitrate (1 mM) ceased; upon addition of fresh nitrite, methane consumption started again (data not shown), demonstrating that the methane-oxidizing activity of Brunssummerheide enrichment is also nitrite dependent. Although nitrite was also detected in the depth profile, its concentrations were much lower (max. 4.2 μM , mostly around the detection limit of the colorimetric method) than those of nitrate. There was no depth-related pattern, and values were not constant over time. The nitrite needed by n-damo bacteria active in the soil might be supplied by other microorganisms (e.g. denitrifying bacteria) or *Methyloirabilis* itself converting nitrate to nitrite using organic carbon compounds other than methane. This would explain why nitrate is sufficient as an electron acceptor for methane oxidation *in situ* and in the initial batch incubations, whereas after enrichment, concomitant with a relative loss of other

bacteria and a degradation of labile organic carbon, this supply path is insufficient and nitrite addition becomes mandatory for methane oxidation.

The present study shows an additional, so far hardly investigated pathway linking the biogeochemical cycling of nitrogen and methane in peatlands. Given the world-wide increasing groundwater nitrate and atmospheric nitrogen loads (Lucassen et al. 2004, Smolders et al. 2010), this methane sink may become more relevant for mitigating the mobilization of carbon in the form of methane from wetlands in the future.

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THEME TWO

**Surface water salinization
and peatland functioning**



(Laboratory experiment, G. van Dijk)

4

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

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Leon P.M. Lamers,

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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⁻¹, 3.2 psu) or fluctuating salinity (5-50 mmol Cl·l⁻¹), versus constantly low salinity (5 mmol Cl·l⁻¹, 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 CO₂ 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.

Keywords: Eutrophication, Nutrient, Carbon, Poikilohaline, Brackish, Sea Level Rise

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 & White 2006; Pachauri & 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 et al. 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; Weston et al. 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; Lamers et al. 2002b; Smolders et al. 2006).

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 (CH₄) 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 CH₄ 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.

MATERIAL 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 *et al.* 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 4.1. 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 4.1).

Table 4.1. Organic matter content and total element concentrations of the sediments used in the experiment, average concentrations are given with S.E.M. between brackets

		Organic matter content	Bulk density	Na	Ca	K	Fe	P	S
	type (depth (cm))	(%)	kg DW/l FW	(μmol · g ⁻¹ DW)					
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)

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 5cm; Eijkelkamp Agrisearch Equipment, Netherlands) were placed diagonally in the sediment of each aquarium. After three weeks, artificial surface water was pumped through the aquaria for six months, at a rate of 1.11 l·day⁻¹ (retention time of 3.4 days). Three surface water treatments were used: constant freshwater (Fw, 5 mmol Cl·l⁻¹, 0.32 psu, constant brackish water (Bw, 50 mmol Cl·l⁻¹, 3.2 psu) and a fluctuating freshwater - brackish water treatment (Fl, 5-50 mmol Cl·l⁻¹), with 4 replicates per treatment (Table 4.2 and Fig. 4.2). 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 four weeks of freshwater and two weeks of brackish water, with the exception of the third brackish water period which lasted three 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.

Table 4.2 Chemical composition of the surface water of the three treatments used (mmol l⁻¹)

	Fw	Fl	Bw	
		Fw	Bw	
Duration	Constant	1 mth	2 wks	Constant
Cl ⁻	5	5	50	50
Na ⁺	7.5	7.5	45	45
Ca ²⁺	1	1	2.5	2.5
HCO ₃ ⁻	3	3	3	3
SO ₄ ²⁻	1	1	5	5
Mg ²⁺	0.5	0.5	4	4
K ⁺	0.1	0.1	0.8	0.8
pH	8	8	8	8

In addition to the effects of the different salinity treatments, the effect of introduction of micro-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 two 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®, 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 CO₂ and CH₄

In situ CO₂ and CH₄ emissions were analyzed by carefully placing funnels (11 cm in diameter, volume of 0.25 dm³) on the water surface for six hours at the end of the experiment. Gas samples were analyzed in the funnel headspace at t=0, 3 and 6 hours. 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 (N₂) flushed water were mixed in 15 ml airtight bottles. The headspace of the bottles was flushed for five minutes with N₂ directly after sealing, and the bottles were kept in the dark at 15°C. The increase of the CO₂ and CH₄ concentrations in the headspace was measured after 1, 3, 6, 24 and 168 hours, after sampling (0.1 ml for CH₄ and 0.5 ml for CO₂), an equal amount of N₂ was added to prevent pressure differences. Emission and production rates of CO₂ and CH₄ 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_3 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 & Johanssen, 1972), ortho-phosphate (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_2 concentrations were measured using an infrared gas analyzer (IRGA, ABB Advance Optima, Zürich, Switzerland). CH_4 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_2 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 hours 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_3 and 1 ml 30% H_2O_2 (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_4) 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_2 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 (Fig. 4.1a & b). Moreover, pore water concentrations of calcium (Ca), magnesium (Mg), ammonium (NH_4^+), 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. 4.1b). 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_4 5%, Clay sediment: Cl 34%, Na 32%, Ca 29%, NH_4 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 $55 \mu\text{mol P}\cdot\text{l}^{-1}$ to $15 \mu\text{mol P}\cdot\text{l}^{-1}$) as well as for Fl) (from $55 \mu\text{mol P}\cdot\text{l}^{-1}$ to $30 \mu\text{mol P}\cdot\text{l}^{-1} \approx 50\%$ decrease at the end) compared to the freshwater (Fw) treatment (Fig. 4.1a). Peat sediment showed a small (8.5%) decrease under Bw treatment, but not for the Fl treatment.

Pore water NH_4^+ concentrations in the clay sediment increased significantly in both the Bw (55%) and the Fl treatment (45%), although slower for the latter (Fig. 4.1a). In the peat sediment, in contrast, NH_4^+ concentrations decreased in all treatments over time, but remained slightly (but significantly) higher for Bw (Fig. 4.1a). Pore water NO_3^- concentrations remained below the detection limit ($1 \mu\text{mol}\cdot\text{l}^{-1}$) for both sediment types in all treatments (data not shown).

Table 4.3. P values of statistical analysis of treatment effects (Fw = freshwater treatment, Bw = brackish water treatment, Fl = fluctuating treatment) in pore water and surface water for both sediment types. Significant effects are presented in bold.

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

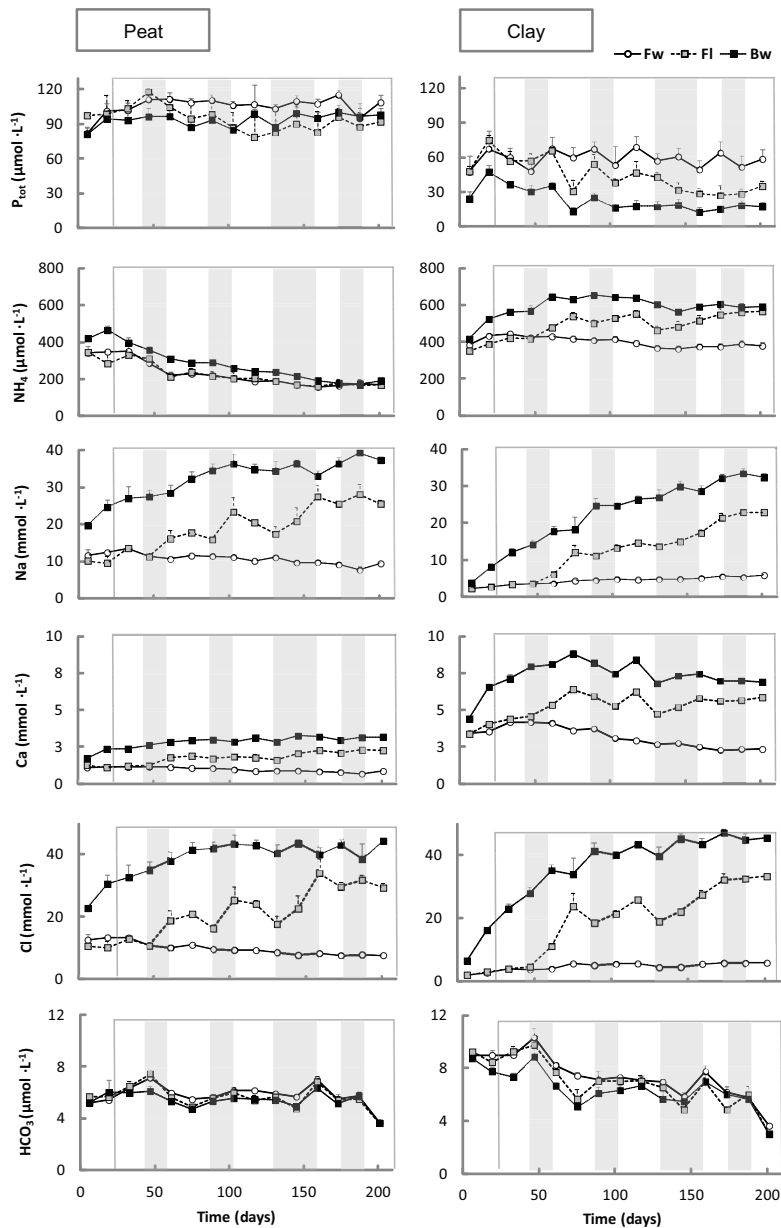


Fig. 4.1a Sediment pore water concentrations for peat (left) and clay (right) sediments over time (+ S.E.M., $n = 4$). The square indicates the period during which the surface water was refreshed. Treatments: freshwater (Fw, white circles with black line), fluctuating salinity (FI, grey squares with dotted line) and brackish water (Bw, black squares with grey line). The shadings indicate the increased salinity episodes for the fluctuating salinity treatment

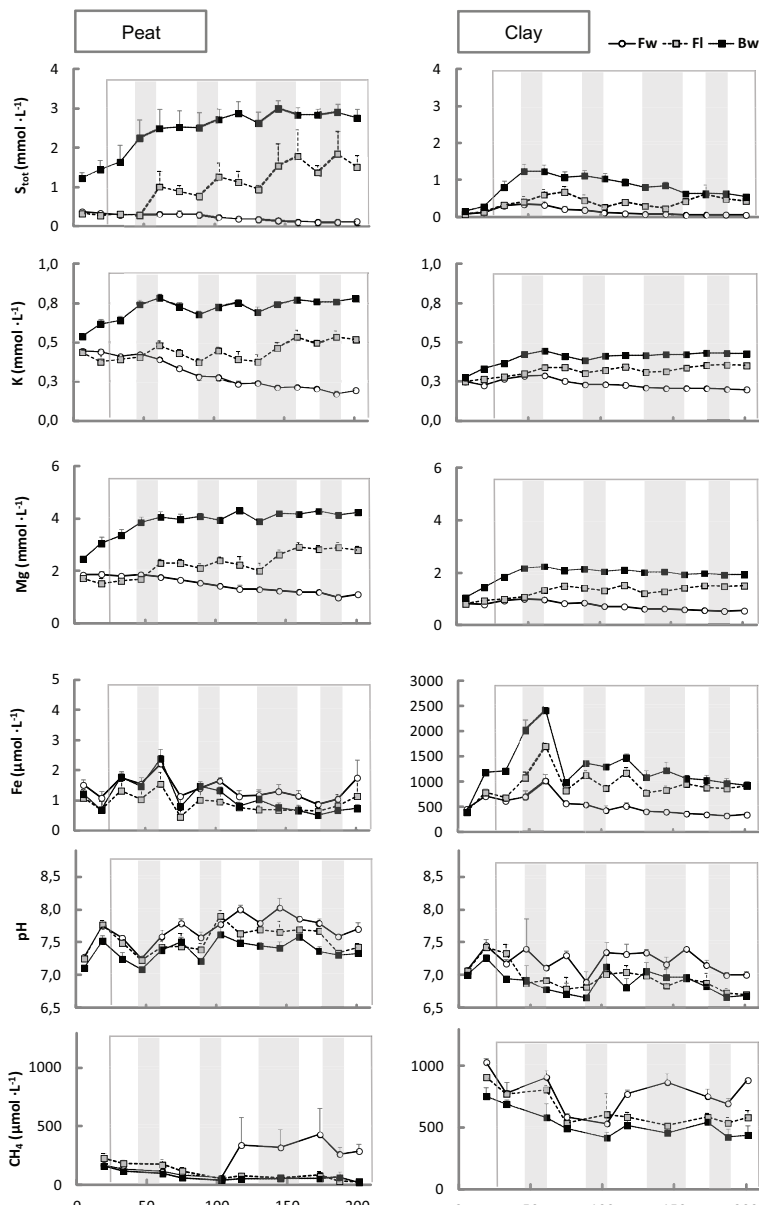


Fig. 4.1b Sediment pore water concentrations for peat (left) and clay (right) sediments over time (? S.E.M., $n = 4$). The square indicates the period during which the surface water was refreshed. Treatments: freshwater (Fw, white circles with black line), fluctuating salinity (Fl, grey squares with dotted line) and brackish water (Bw, black squares with grey line). The shadings indicate the increased salinity episodes for the fluctuating salinity treatment. Note the different scales for iron.

Sediment cation adsorption

Compared to the Fw treatment, SrCl_2 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 4.4). The Bw treatment reduced SrCl_2 extractable Ca and NH_4^+ concentrations in the clay sediment. Peat sediment showed a similar, but not significant trend.

Table 4.4. SrCl_2 extractable cation concentrations in both sediment types. 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.

	Peat			Clay		
	Fw	Fl	Bw	Fw	Fl	Bw
<i>SrCl₂ extractable concentration (mmol · kg DW⁻¹)</i>						
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)a	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
NH₄	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

Inorganic carbon and methane

For both sediment types pore water CH_4 concentrations were significantly lower in both Bw and Fl (Fig. 4.1b). CO_2 and total inorganic carbon (including bicarbonate) in the sediment pore water did not differ significantly (data not shown). CO_2 emissions to the atmosphere were around $80\text{--}160 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ for all treatments, without treatment or sediment type effect. CH_4 emissions were below the detection limit of $0.0001 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$; data not shown. Anaerobic incubation of the sediments at the end of the experiment showed no treatment effects for CO_2 production. CO_2 production was, however, significantly higher for the peat sediment versus the clay sediment (Fig. 4.3). CH_4 production was, however, significantly lower for peat sediments treated with Bw or Fl compared to the Fw treatment (Fig. 4.3). For the clay sediment, CH_4 production rates were very low, without any treatment effects (Fig. 4.3).

Surface water

Surface water chemistry followed the chemical composition of the treatment water (chloride concentrations in Fig. 4.2, other elements not shown). For FL, 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 FL 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 FL treatment (Fig. 4.2 and Table 4.3).

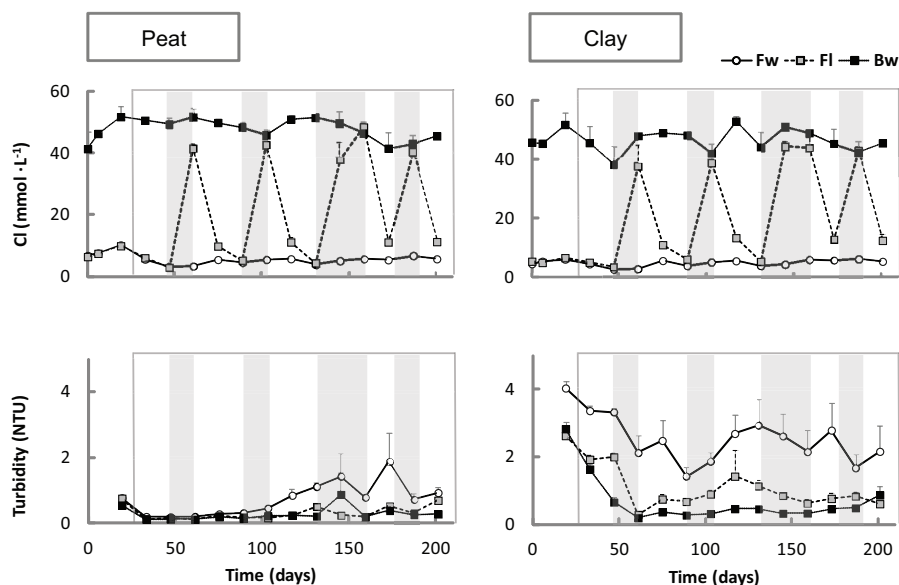


Fig. 4.2 Chloride concentrations and turbidity in the surface water above the peat sediments (left) and clay sediments (right) over time (+ S.E.M., $n = 4$). The square indicates the period in which the surface water was refreshed. Treatments: freshwater (Fw, white circles), fluctuating salinity (FL, grey squares) and brackish water (Bw, black squares). The shadings indicate the increased salinity episodes for the fluctuating salinity treatment

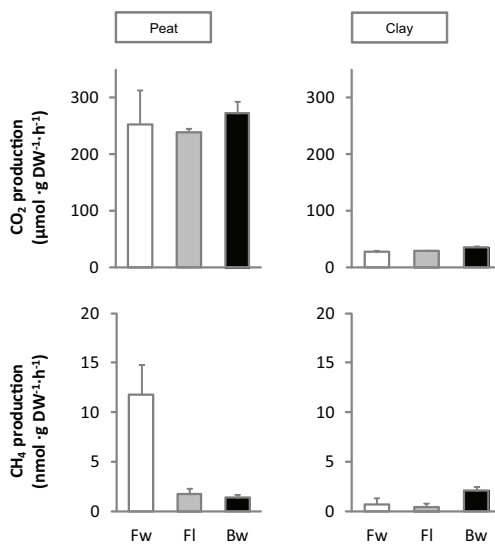


Fig. 4.3. Production rates of CO₂ (top) and CH₄ (bottom) during incubation of peat (left) and clay sediments (right) (+/- S.E.M., n = 4). Treatments: freshwater treatment (Fw, white bars), fluctuating (FI, grey bars), brackish water (Bw, black bars)

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, H⁺ and NH₄⁺ from the cation adsorption complex in both sediment types, as well as against Fe in the clay sediment. Although SrCl₂ extractable Ca and NH₄⁺ concentrations were similar or even higher on a mass base for peat sediment, the increase of NH₄⁺ 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 et al. 1977; Loeb et al. 2008; Baldwin et al. 2006; Weston et al. 2006). The absence 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. 2006; 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 & 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 et al. 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., 2006)). Under these conditions, P mobilization rates to the water layer will also largely depend on porewater P concentrations, regardless of 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; Lamers et al. 2002b). In the peat sediment, S was present in such excess relative to Fe (Table 4.1 and 4.4) 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 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_x precipitation did not affect the adsorption of phosphate within the course of the experiment (Zak et al. 2006; Loeb 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). 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 & 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 & 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 (Fig. 4.1b & 4.3), 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, CH_4 concentrations in the sediment pore water were 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 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 CO_2 production and emission reported in literature largely differ, varying from no effect, as found in the present paper, to decreased or increased CO_2 emissions. Results of the present study indicate no significant short-term difference between constant or pulsed salinity on 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 CO₂ production, but decreased CH₄ 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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(Enclosure experiment in Ilperveld, R. Kuiperij and G. van Dijk, by E. Hoftijser)

5

Salinization lowers nutrient availability in former brackish freshwater wetlands; unexpected results from a long-term field experiment

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ABSTRACT

Worldwide, coastal freshwater wetlands are facing salinization at an increasing rate due to large-scale land use change, freshwater extraction, climate-driven sea level rise and droughts, and land subsidence. Although it is known that increased surface water salinity may seriously influence wetland functioning, effects shown in previous studies are contradicting and evidence from controlled, long-term field experiments is scarce. We therefore tested the effects of 4 levels of increased surface water salinity, from oligohaline to mesohaline conditions (0.9, 2.25, 4.5, 9 PSU), on physicochemical and biogeochemical processes in the sediment of a formerly brackish freshwater wetland. For this, we used 16 enclosures in a controlled, 5-year field experiment. In contrast to other studies, salinization unexpectedly led to a decreased availability of nitrogen and phosphorus in the sediment, both in the short and in the long term, even though sulfate reduction rates had increased. Salinization resulted in the mobilization of calcium, manganese and ammonium, but not of iron. Decreased phosphorus availability was probably caused by co-precipitation with calcium. In addition, enhanced vertical water flow as a result of increased hydraulic conductivity may have contributed to a lower nutrient availability. Increased salinity shifted the dominant mineralization process from methanogenesis to sulfate reduction. We show here that surface water salinization has major consequences for the ecological and biogeochemical functioning of coastal freshwater wetlands, and discuss the implications for the prediction of salinization effects and for future water management and restoration of brackish wetlands.

Keywords: phosphorus, ammonium, methanogenesis, sulfate reduction, brackish, cation exchange

INTRODUCTION

Anthropogenic forcing with respect to land use, water management and hydrological processes in combination with sea level rise and climate change (Church et al. 2013) has led to a worldwide increase in the intrusion of saltwater into coastal freshwater wetlands (Herbert et al 2015). The resulting surface water salinization has large biogeochemical and physiological consequences, and threatens groundwater and surface water resources for drinking water abstraction, agricultural practices and the ecological functioning of freshwater wetlands (e.g. Pitman & Lauchli 2002, Lamers et al. 2002a, Katerji et al. 2003, Rengasamy 2010, Bonte & Zwolsman 2010, Oude Essink & Kooi 2011, Brouns et al. 2014, Herbert et al. 2015). Coastal wetlands can be under the direct or indirect influence of increased salinity from various sources and via several pathways, e.g. decreased freshwater inflow due to summer droughts (Milly et al. 2005), anthropogenic land and water use change in the coastal zone, and via salinization of groundwater aquifers (Barlow & Reichard 2010, Ferguson & Gleeson 2012, Tayler et al. 2013).

Salinization not only alters ionic concentrations, but also chemical equilibria and microbiological pathways. Due to the myriad of effects of salinization on biogeochemical processes, as linked to microbiological functioning and chemical processes, it is challenging to predict the net effects of salinization on nutrient cycling in the sediment, and literature shows contrasting results. From previous studies it is known that salinization can influence sediment nutrient cycles via several direct and indirect pathways. Increased ionic concentrations in surface water or sediment porewater change physicochemical processes and chemical equilibria, facilitate aggregation and sedimentation of suspended matter, and induce fast displacement of cations (incl. calcium and ammonium) bound to the cation adsorption complex in the sediment, (Rysgaard et al. 1999, Weston et al. 2006, 2010, Van Dijk et al. 2015). Salinization can also directly influence the nitrogen (N) cycle including nitrification (Rysgaard et al. 1999, Magalhães et al. 2005, Noe et al. 2013) and denitrification rates (Giblin et al. 2010, Marks et al. 2016), and the phosphorus (P) cycle by increasing (i.e. Portnoy and Giblin 1997, Lamers et al. 2002b, Weston et al. 2006) or decreasing (Baldwin et al. 2006, Van Diggelen et al. 2014, Van Dijk et al. 2015) P availability in the sediment.

Apart from these direct effects on nutrient availability, more indirect effects may occur by affecting the sulfur (S) and iron (Fe) cycle (Smolders et al. 2006). As salinization leads to an increase in the concentration of sulfate (SO_4^{2-}), which is reduced as an alternative terminal electron acceptor in anaerobic sediments rich in organic material, this may lead to internal eutrophication (Lamers et al. 2002b, Smolders et al. 2006; Geurts et al., 2010). In organic aquatic sediments, which are common in coastal wetlands, SO_4^{2-} reduction and consequential sulfide (H_2S) production is closely coupled to the Fe and P cycle. Sulfide may mobilize P by reducing Fe(III)[O]OH complexes to which phosphorus is adsorbed, while the precipitation

of iron(II) sulfides (FeS_x) strongly lowers dissolved Fe concentrations (Lamers et al., 2002b; Smolders et al. 2006, Reddy and DeLaune 2008). Increased SO_4^{2-} reduction rates generally decrease the rates of methanogenesis (Weston et al. 2006, Loeb et al. 2007) because (1) SO_4^{2-} reduction is thermodynamically more favorable (Segers, 1998) and methanogens are outcompeted for organic compounds such as acetate (Lamers et al. 1998, Smolders et al. 2002) and (2) increased salinity and the production of toxic sulfide can lead to physiological stress in methanogens [i.e. Chambers et al. 2011; Lamers et al. 2013]. Increased SO_4^{2-} concentrations can enhance net organic matter breakdown (C mineralization) and increase CO_2 emissions (Chambers et al. 2011, Weston et al. 2011, Marton et al. 2012; Weston et al. 2006, Craft et al. 2009). However, a comparison of different systems along a salinity gradient shows that the more saline systems often emit less net carbon (C) to the atmosphere (Bartlett et al. 1987, Poffenbarger et al. 2011, Weston et al. 2014, Vizza et al. 2017).

The biogeochemical and ecological effects of salinization seem to be influenced by a range of biogeochemical factors and are probably location specific, based on local differences in biogeochemical and physical sediment characteristics (Herbert et al. 2015 and references therein). The fact that coastal wetlands often show differences in the historical influence of brackish or saline water, resulting in increased salinity and increased S concentrations in their aquatic sediment, makes the prediction of the effects of enhanced salinity even more complex. In recent years more knowledge is gained about the effects of salinization. Often, short-term laboratory experiments have been used, while long-term manipulations in the field are scarce. To study long-term effects of salinization on biogeochemical processes and on nitrogen, phosphorus and carbon availability in organic sediments, we carried out a five-year experimental field study. The effects of four different surface water salinity levels were tested in a Dutch coastal freshwater wetland that was formerly influenced by saline water (see Materials and Methods). We expected salinization to cause major changes in biogeochemical processes, increasing porewater nitrogen- and phosphorus concentrations and decreasing porewater methane concentrations.

MATERIAL AND METHODS

Site description

The experiment was carried out in a former brackish freshwater wetland (Ilperveld, 1300 hectares), located to the north of Amsterdam (the Netherlands). The coastal wetland harbors a large surface area of open water in connection with canals including the Noordholland Canal. Between 2500 and 1500 years before present a raised bog landscape was formed here, dominated by *Sphagnum* mosses (Bakker & Smeerdijk 1982, Witte & van Geel 1985, Willemsen et al., 1996) Since the Middle Ages, however, the area has suffered from large-scale

anthropogenic disturbances (Vos 2015), including drainage, burning, increased agricultural activity, construction of ditches and canals, creation of deep polders, and peat extraction. This led to a strong land subsidence and to an increased influence of brackish water due to several floods with brackish and saline water from the Zuiderzee, a former bay of the North Sea (Raats 2005, Van 't Veer 2009). Since the 1930s the influence of brackish water decreased and salinity levels dropped due to the construction of a large dam (Afsluitdijk) converting the brackish Zuiderzee into a freshwater lake (Lake IJsselmeer) and eliminating seawater influence from the study area (De Beaufort, 1954). Since that moment, surface water chloride concentrations have decreased from approximately 13 PSU (200 mmol Cl L⁻¹) to approximately 1 PSU (15-20 mmol Cl L⁻¹). Although the IJperveld is classified as a freshwater wetland under the present conditions, the historic brackish water influence (i.e. increased chloride, sodium and sulfate levels) is still noticeable in the aquatic sediment and groundwater, see table 5.1 for the average chemical sediment composition at the experimental location at different depths. The high sulphur versus low iron concentrations and high sodium concentrations are indications for the former influence of brackish water.

Table 5.1. Average organic matter content (OM in %) and total element concentrations of (Na, Ca, S, Fe, P and N) (mmol kg DW⁻¹) at three depth sections at the experimental location [S.E.M. in superscript, (n=4)].

Sediment depth	OM	Na	Ca	S	Fe	P	N
	%	mmol kg DW ⁻¹					
0-15 cm	68,8 ^{1,1}	249,2 ^{5,2}	485,6 ^{5,9}	737,2 ^{29,9}	176,7 ^{14,4}	25,7 ^{1,6}	1105,1 ^{29,6}
15-30 cm	81,4 ^{3,4}	243,2 ^{9,6}	393,6 ^{26,8}	620,0 ^{56,0}	98,1 ^{29,2}	14,8 ^{3,4}	821,1 ^{110,7}
30-45 cm	91,6 ^{0,3}	270,3 ^{25,4}	307,0 ^{13,4}	451,6 ^{26,9}	18,8 ^{5,5}	5,9 ^{0,2}	560,5 ^{32,4}

The experiment was carried out in a dead-end canal (52° 27.225N - 4° 55.885E), approximately 250 meters long and 10-15 meters wide. In this wetland, a strict surface water level management is maintained only allowing water level fluctuations of a few centimeters. The canal is surrounded by reedlands and peat meadows in agricultural use. The underwater sediment at the study site consists of a top layer (14 cm) of peat sludge on top of a several meters thick sequential stratification of structured peat and clay layers (Westland formation), based on a sand layer (Formation of Twente) (National Dino Database, TNO) (see Fig. 5.1).

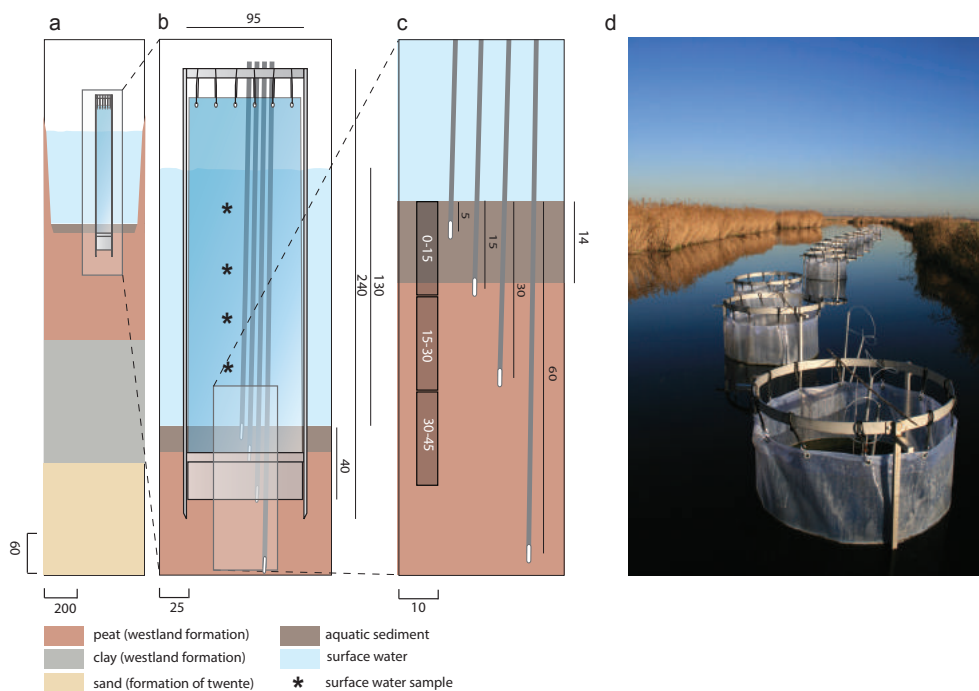


Fig. 5.1 a) Enclosure placed in the surface water of a canal with peat soil on top of clay and sand layers; b) schematic representation of an enclosure showing steel frame, flexible PVC foil cylinder, and ceramic cups; c) detailed overview showing sampling method including ceramic cups for porewater sampling at different depths and in shaded rectangles sediment sample depths (0-15, 15-30 and 30-45 cm below the water – sediment border). Scales in cm.; d) experimental set-up in the field (photo G. van Dijk).

Experimental design

To test the effects of increased surface water salinity, 16 enclosures (95 cm diameter) were placed in the middle of the canal to avoid side effects (Fig. 5.1). We randomly assigned four different salinity levels treatments ($n=4$) to the enclosures. Additional measurements were carried out at fixed locations ($n=4$) in the surrounding canal, as a control for enclosure effects. The enclosures consisted of stainless steel frames in which a cylinder of flexible PVC foil (≈ 1 mm thick, 130 cm long) was placed in June 2010. The PVC enclosures were open at the top and bottom and connected to the steel frame by rubber bands, keeping the upper part 30 cm above the water surface (Fig. 5.1). At the bottom the foil was fastened to a steel ring (30 cm length) placed 40 cm below the sediment-water interface. In this way miniature ponds with flexible volumes were created, in which interactions between the atmosphere (precipitation and evaporation) and the enclosed surface water remained intact.

Table 5.2. Overview of the chemical composition of the surface water of all treatments at the start of the experiment.

element	Unit	fresh (outside control)	fresh (inside control)	oligohaline	oligo-mesohaline	mesohaline
Salinity	PSU	0.9	0.9	2.25	4.5	9
Cl ⁻	g l ⁻¹	0.5	0.5	1.25	2.5	5
Cl ⁻	mmol l ⁻¹	14	14	35	70	140
Na ⁺	mmol l ⁻¹	12	12	32	64	128
S _(tot)	mmol l ⁻¹	1.3	1.3	2.5	5	10
Ca ²⁺	mmol l ⁻¹	2	2	2.5	3	5
HCO ₃ ⁻	mmol l ⁻¹	3	3	3	3	3

The different salinity levels (in a range from fresh to mesohaline) were established by adding concentrated solutions of artificial sea salt (Tropic Marin®, which is similar to the element composition of the North Sea) to the surface water in the enclosures (table 5.2). After six months, surface water salinity at a depth of 10 cm had decreased due to constant precipitation influence (salinity level did decrease the most in higher salinity treatments, up to ≈ 60%, see Fig. 5.2). To avoid precipitation from affecting the treatment, dissolved sea salt was added twice a year after measurements, to keep surface water ionic concentrations stable.

Sampling

The experiment lasted five years (2010-2015). During the first 1.5 years, the experiment was sampled monthly, followed by 3.5 years with less intensive sampling (1 to 4 times per year). Surface water and sediment porewater were collected from all replicates. During the first 1,5 years additional mixing of the surface water was applied in the enclosures every two weeks by carefully mixing by hand with a low rate for 2 minutes, to prevent stratification of the surface water layer (the remaining 3.5 years of the experimental period no additional mixing was applied). Due to stratification of the surface water layer over time, surface water results are only presented for the first months of the experimental period. Sediment porewater samples are presented for the complete five year duration of the experiment.

Surface water samples were taken from the upper 15 cm of the surface water layer. Sediment pore water samples were taken anaerobically at a depth of 15 cm in the aquatic sediment with a vacuumed 50 mL syringe connected to a ceramic cup by a Teflon tube. Porewater gas was collected by connecting a vacuumed 12 mL glass exetainer (Labco exetainer®, High Wycimbe, UK) to the ceramic cup, and methane measurements were subsequently measured in the headspace. Additional porewater for depth profiles (at 5, 15, 30 and 60 cm of depth) were collected in the control (0.9 PSU) and highest salinity (9.0 PSU) treatment after 2.5 years. At this time, sediment cores (4 subsamples pooled per enclosure) were also collected in all treatments using a sediment suction corer (diameter 4 cm; ref) and divided into three depth sections 0-15cm, 15-30 cm and 30-45 cm. All samples were cooled during transportation and stored under dark and cool conditions (4 °C) until analyses.

Chemical analyses

The pH values of surface water and porewater 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), after which CO₂ and HCO₃⁻ were calculated based on the pH equilibrium. CH₄ 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 N₂ as carrier gas. Sulfide concentrations were determined directly after sampling by fixing 10.5 mL of pore water with 10.5 mL Sulfide Anti Oxidant Buffer, and using an Orion sulfide electrode and a Consort Ion meter (type C830) for analyses (Van Gernerden, 1984). Prior to elemental analyses, 10 mL of each sample was stored at 4°C until analyses after adding 0.1 mL (65%) HNO₃ 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 & Johanssen, 1972), ortho-phosphate (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).

Dry weights and bulk densities of the sediment samples were measured by drying a known volume of fresh sediment at 70°C until constant weight. The organic matter content was determined by loss-on-ignition for 4 hours 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 HNO₃ and 1 mL 30% H₂O₂ (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_4) 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_2 solution. The supernatant was analyzed using ICP (see above). Concentrations of P for different fractions (labile, Ca, Fe and Al bound) were determined according to the sequential extraction method of Golterman (1996), the remaining residue was digested with HNO_3 (as described above) to determine the concentration of organically bound P (refractory non-labile organic P). In November and December 2012, extra sediment samples were collected with an Eckman sediment sampler (three subsamples of 180 cm^2 and 8 cm depth per enclosure) for benthic macro-invertebrate analyses. Samples were taken to the laboratory and stored at 4 °C until analysis within a few days. The three subsamples were carefully mixed and a part of the total fraction (for which was corrected later) was washed over three sieves with different mesh sizes (2, 1, & 0.4 mm) and sorted out to taxonomic genus level.

Data analyses

Treatment effects were tested with SPSS 21 (IBM SPSS Statistics) on $\ln(x+1)$ transformed data to achieve a closer fit to a natural distribution, except for pH. Treatment effects for surface water and sediment porewater were tested for the period of June 2010 to September 2011 (16 months, 16 measurement points in time) and for surface water from June 2010 to September 2010 (4 months, 5 measurement points in time). Data was tested using Linear Mixed Models, using replicas as subject factor, treatment as factor and time as co-factor (AR(1): Heterogeneous was used as co-variance type, based on AICC values). Differences between treatments within the Linear Mixed Models were further analyzed using LSD post-hoc tests. Treatment effects for sediments and porewater in depth profiles were tested among depth sections using One-Way ANOVA and LSD post-hoc tests.

RESULTS

Salinity effects on surface water biogeochemistry

The increase in surface water salinity had major consequences for surface water chemistry. However, due to reduced water movement and the exclusion of the influence of surrounding surface water, precipitation caused stratification of the surface water layer in the enclosures already after three months (Fig. 5.2, a. at the start, b. the stratified situation after six months.). After this period, we reduced the stratification effect by periodical stirring of the surface water layer. On the longer term stirring was stopped and extra sea salt had to be added regularly to maintain surface water salt concentrations at the original treatment levels.

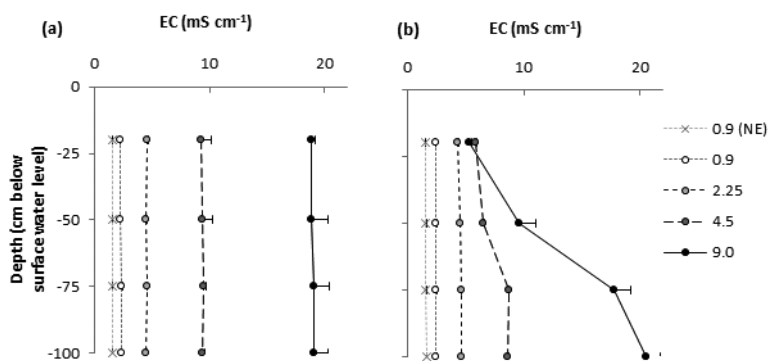


Fig. 5.2 Depth profiles of the electric conductivity (EC, in mS cm^{-1} average + S.E.M., [n=4].) in the surface water of all treatments (in PSU; NE= No Enclosure), under (a) non-stratified (at start), and (b) stratified conditions (after six months).

Despite the periodical stratification near the surface and the regular addition of salt, salinity concentrations did remain at treatment levels one meter below the water surface (about 30 cm above the aquatic sediment). As the chemical composition of the top layer of the surface water was affected to such an extent by the above-mentioned processes, we will not present surface water results in detail. Some clear effects were however visible during the first months. A significant ($p < 0.01$) decrease of the surface water phosphorus concentration at an increased salinity was observed (78% decrease between control and the two highest salinity treatments (4.5 and 9.0 PSU) after four months). Furthermore, after reaching the targeted treatment concentrations in July 2010, (see Fig. 5.3) the calcium concentration decreased faster in the following months compared to the chloride concentration and the total inorganic carbon concentration (TIC) did decrease faster in increased salinity treatments (Fig. 5.3). For the period presented in Fig. 5.3 calcium concentrations in the two highest salinity treatments and Cl concentrations of all salinity treatments differed significantly from the control treatment ($p < 0.001$). On the short term no significant differences were found between the inside and outside control treatments, indicating that the placement of enclosures didn't have major short term effects.

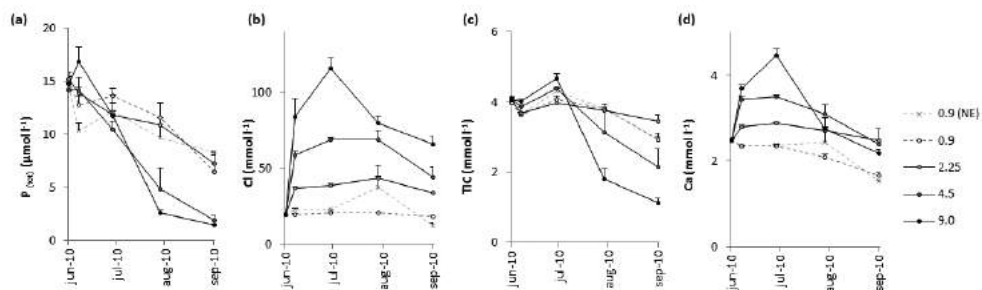


Fig. 5.3 Surface water phosphorus concentration (a), chloride concentration (b) the total inorganic carbon concentration (TIC) and the calcium concentration (d) in all treatments during the first four months of the experiment (given in PSU, (NE= No Enclosure)), (+ S.E.M., [n=4]).

Salinity effects on porewater nutrient concentrations

Porewater Cl and Na levels increased significantly in all salinity treatments ($p < 0.001$) up to 60 cm of depth ($p < 0.05$) (see Fig. 5.6). Pore water pH was not influenced by salinity treatment. Sediment porewater chloride and sodium concentrations at a depth of 15 cm, showed a very fast response (already within a month) to increased surface water salinity and did increase in all three salinity treatments (see Fig. 5.4). Within months porewater P concentrations decreased significantly ($p < 0.01$) in the highest salinity treatment (9 PSU), leading to a 40% decrease after five months compared to the control treatment. During the following months porewater P concentrations further decreased ($p < 0.05$) in the two highest salinity treatments. After 1 to 1.5 years, P concentrations were 33% lower in the 2.25 PSU, 54% lower in the 4.5 PSU and 69% lower in the 9 PSU treatment compared to the control treatment (see Fig. 5.4). Although surface water salinization initially led to a short term increase of porewater ammonium (NH_4^+) concentrations, NH_4^+ concentrations decreased in the longer term. After 1.5 years, a 30% decrease was reached in the 4.5 PSU treatment (n.s.), and a 60% ($p < 0.01$) decrease in the 9 PSU treatment, as compared to the control. In all salinity treatments, both P and NH_4^+ concentrations stabilized after a gradual decrease during the first 10 months (see Fig. 5.4). Porewater depth profiles showed significantly ($p < 0.01$) lower nutrient levels at both 15 and 30 cm depth for the 9 PSU treatment compared to the control (Fig. 5.6).

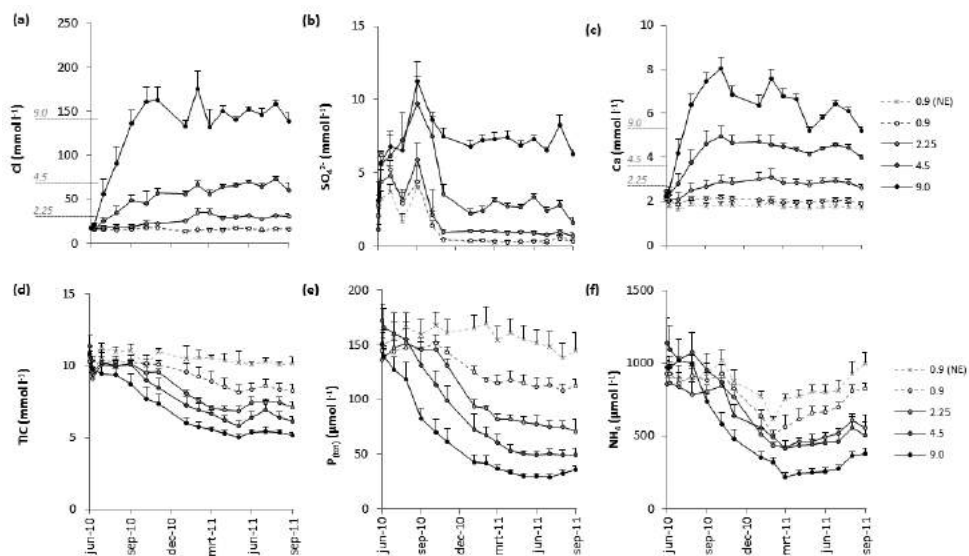


Fig. 5.4. Porewater concentrations of (a) chloride, (b) calcium, (c) sulfate, (d) total inorganic carbon (TIC), (e) total phosphorus, and (f) ammonium through time at 15 cm of depth in the sediment for all treatments (given in PSU; NE= No Enclosure). Averages + S.E.M. [n=4]. Note that concentrations are given in $\mu\text{mol l}^{-1}$ for P and NH_4^+ , and in mmol l^{-1} for other substances. In (a) and (c) treatment concentrations (indicated in PSU) are given in grey with dotted lines on the y-axes.

Salinity effects on the sediment cation exchange complex

High cation concentrations (mainly sodium) as a result of salinization caused the mobilization of cations from the cation exchange complex of the sediment. Sediment analyses showed decreased exchangeable ammonium concentrations in the brackish treatments, which was significant ($p < 0.05$) for the deeper sediment layer of the 9.0 PSU treatment (see Fig. 5.5). Total sediment P and N concentrations did, however, not differ. No significant treatment effects were found on different concentrations and precentral distribution of P fractions in the sediment. Most P (about 70%) was present as organic-P, about 15% was bound to calcium carbonates and about 10 % to iron (Fe) or aluminum (Al). Mobilization of NH_4^+ was seen on the short term, but we also found mobilization of Ca, and Mn (data not shown). For these elements, porewater concentrations were significantly increased in the two highest salinity treatments ($p < 0.05$ for 4.5 PSU; $p < 0.01$ for 9.0 PSU) see Fig. 5.4). However, no Fe mobilization was seen (data not shown). Although Ca was also added via the addition of sea salt, pore water concentrations increased to values higher than surface water concentration, indicating calcium mobilization in the sediment. After this mobilization pulse, porewater Ca concentrations showed a slight decrease and stabilized at higher levels in the highest salinity treatments (see Fig. 5.4). Sediment analyses (SrCl_2 -extractions) showed lower extractable Ca (figure 5.5e) and Mn

concentrations (data not shown) in the sediments treated with 9 PSU water ($p < 0.05$). Both total Na and exchangeable Na concentrations increased (significantly) in the two highest salinity treatments ($p < 0.01$ for both 4.5 and 9 PSU) (Fig. 5.5).

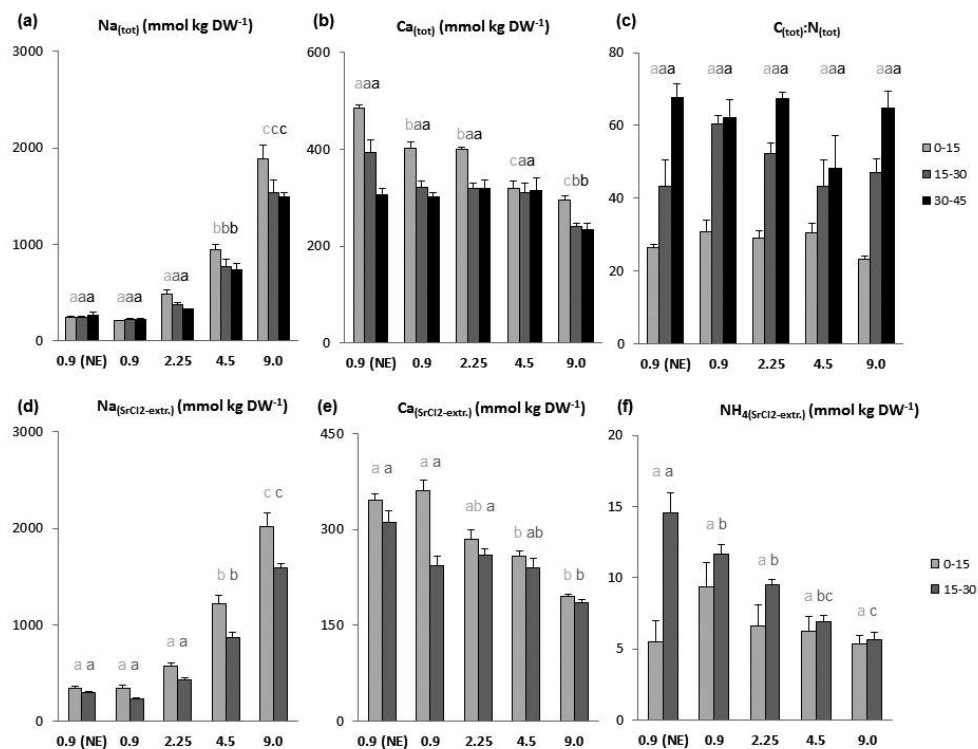


Fig. 5.5. Sediment characteristics for three depth sections (0-15, 15-30 and 30-45 cm of depth) in all treatments (given in PSU; NE= No Enclosure); (a) total Na concentration, (b) total Ca concentration, (c) total C:N ratio (mol/mol), (d) SrCl_2 extractable Na concentration, (e) SrCl_2 extractable Ca concentration, (f) SrCl_2 extractable NH_4^+ concentration (average + S.E.M., $n=4$). Different letters indicate statistical differences between treatments ($P < 0.05$).

Salinity effects on methanogenesis and sulfate reduction

Increased surface water salinity strongly decreased porewater methane (CH_4) concentrations (see Fig. 6) from around 0.8 mmol/l to 0.05 mmol/l down to a depth 30 cm ($p < 0.05$). In the longer term (after 1.5 years) concentrations were lowered by 94% in the 9.0 PSU treatment, 82% in the 4.5 PSU treatment and 42% in the 2.25 PSU treatment respectively. Porewater sulfate concentrations increased in all salinity treatments ($p < 0.05$). Porewater sulfide concentrations strongly increased from around 0.13 mmol/l to 4.9 mmol/l in the 9.0 PSU treatment (40 times increase; $p < 0.05$) in the sediment top layer (Fig. 5.6), but decreased with depth.

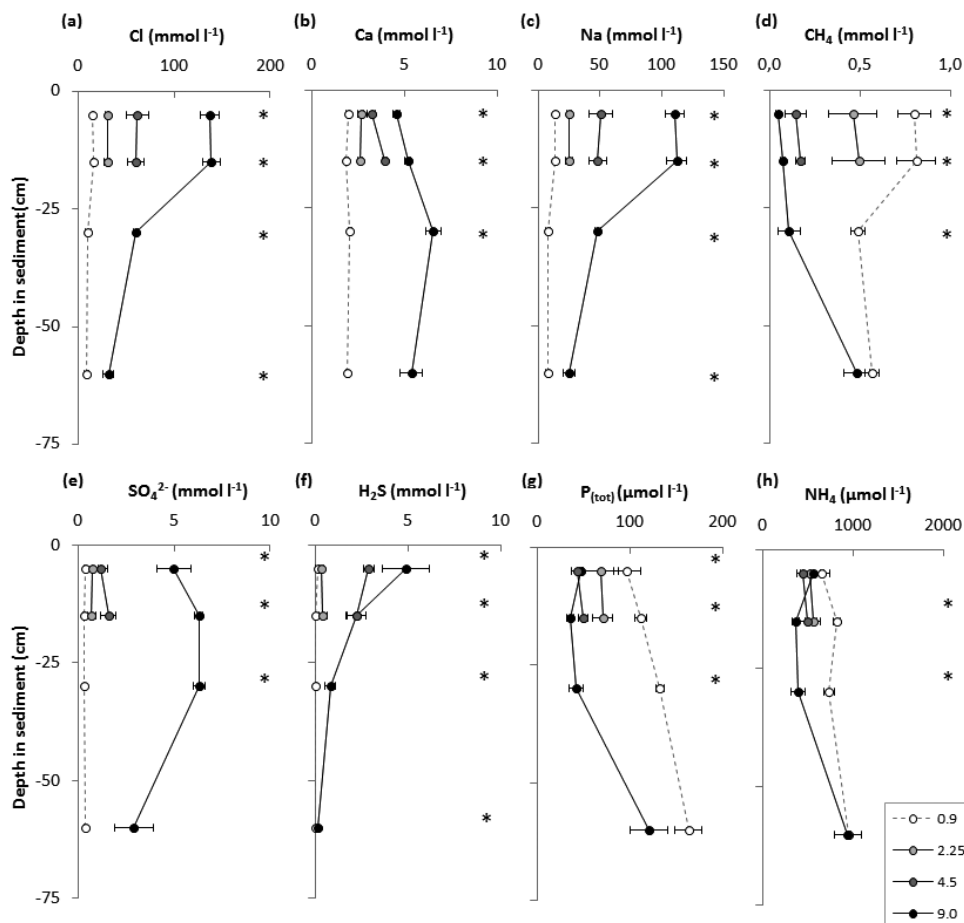


Fig. 5.6. Porewater concentrations for (a) chloride, (b) calcium, (c) sodium, (d) methane, (e) sulfate, (f) sulfide, (g) total P, (h) NH_4^+ in depth profiles [0.9 and 9 PSU at 4 depths, 2.25 and 4.5 PSU at 2 depths], 2.5 years after the start of the experiment for all treatments (PSU), average $\pm$ S.E.M. [$n=4$]; note different scales on the x-axes. Statistical differences were tested between 0.9 and 9 PSU treatment, * $p < 0.05$.

Salinity effects on benthic invertebrate community

Benthic invertebrates sampled between 0 and 8 cm depth at the end of the second year showed a strong decrease in numbers of individuals, from 2000–3000 m^{-2} sediment in the control and 2.5 PSU treatment, to almost absence (< 50 individuals m^{-2}) in the 4.5 and 9.0 PSU treatments ($p < 0.01$ for all invertebrates, Oligochaeta ($p < 0.01$), Gammaridae ($p < 0.05$) and Chironomidae ($p < 0.01$). Community composition also changed: the control community was dominated by worms (*Oligochaeta*), gammarids (*Gammaridae*) and chironomid larvae (*Chironomidae*), with a total spp. density of 1800 m^{-2} , whereas the highest salinity treatments only a small selection of

species of chironomid larvae and one gammarid species remained (total spp. density of 50 m⁻²). During the experimental period, no aquatic vegetation was present in the enclosures.

DISCUSSION

Long-term salinization lowers sediment phosphorus availability

In contrast to what was hypothesized increased salinity (> PSU) significantly lowered porewater total P concentrations. Although some other studies also showed this effect (e.g. Baldwin et al. 2006; Weston et al. 2006, Van Dijk et al. 2015) other studies showed the opposite (Sundareshwar and Morris 1999; Beltman et al. 2000, Jun et al. 2013). Our study does indicate that lowered porewater P concentrations may well be caused by co-precipitation of P with Ca or with Ca carbonate (Bale and Morris 1981; Morris et al. 1981; House 1999). This mechanism seems to be very likely given the combination of enhanced Ca concentrations due to the sea salt addition and increased Ca mobilization from sediment cation adsorption sites at increased salinities. Subsequent precipitation of Ca in the surface water is indicated by the faster decrease of Ca over time compared to chloride. The observed decrease of the TIC concentrations of the sediment porewater also indicate a potential precipitation of CaCO₃ following Ca mobilization in the sediment. During the first five months, surface water P concentrations also decreased at a higher rate due to salinization than Cl (from 9 µmol P l⁻¹ month⁻¹ for 4.5 PSU to almost 15 µmol l⁻¹ month⁻¹ for 9.0 PSU).

As was shown by Geurts et al. (2010), porewater P concentration and P release from the aquatic sediment to the overlying water layer are often linearly correlated under aerobic conditions for sediments showing low (< 1 mol mol⁻¹) porewater Fe:P ratios, as in our study. This implies that lower porewater P concentrations due to salinization will also result in decreased P release to the overlying water layer. An important issue related to P availability in wetlands is the potential role of increased S concentrations on sediment P mobilization. Several studies showed S-induced P mobilization and enhanced decomposition (i.e. Roden and Edmonds 1997; Lamers et al. 1998, 2002b, Zak et al. 2006) leading to internal eutrophication on the longer term (Smolders et al. 2006). Interestingly, in our study the opposite was found as lower porewater P concentrations correlated with enhanced SO₄²⁻ concentrations as a consequence of salinization. Low porewater Fe concentrations in combination with high SO₄²⁻ reduction rates, as indicated by high sulfide levels, indicate that adsorption of P onto ironhydroxides, or precipitation of P and Fe, is not a key mechanism of importance in the present study. This may be explained by the fact that our study site was rich in calcium and also showed historically increased sediment total S concentrations (see Materials and Methods), that were that high that most Fe had precipitated with sulfide, resulting in a minor effect of adsorption and desorption of P onto iron compounds. As a result phosphorus immobilization caused by precipitation of phosphorus with calcium is higher than potential phosphorus mobilization by enhanced sulphur concentrations

and sulphate reduction. Sulphur poor sediments (such as many wetlands without historic salinity influence) will most probably react differently and show phosphorus mobilization after salinization. Unequivocal proof of increased Ca bound phosphorus P in sediments affected by increased salinity cannot be provided, as P-fractionation analyses did not show differences. The latter may be explained by the fact that background total sediment P concentrations were too high to measure relative differences caused by sorption.

Long-term salinization lowers sediment nitrogen availability

Although long-term effects will be influenced by the enclosure effect, differences between treatments within enclosures can still provide relevant information on sediment processes. Up to more than five years after the start of the experiment chloride concentrations did remain stable (Fig. 5.7). Pore water nutrient concentrations did remain stable at lower levels in all salinity treatments. In the longer term salinity treatment effects on cation concentrations became smaller (Fig. 5.7) but after 5 years cation concentrations were still higher in the 9.0 PSU salinity treatment. (in Fig. 5.7), calcium is shown as an example).

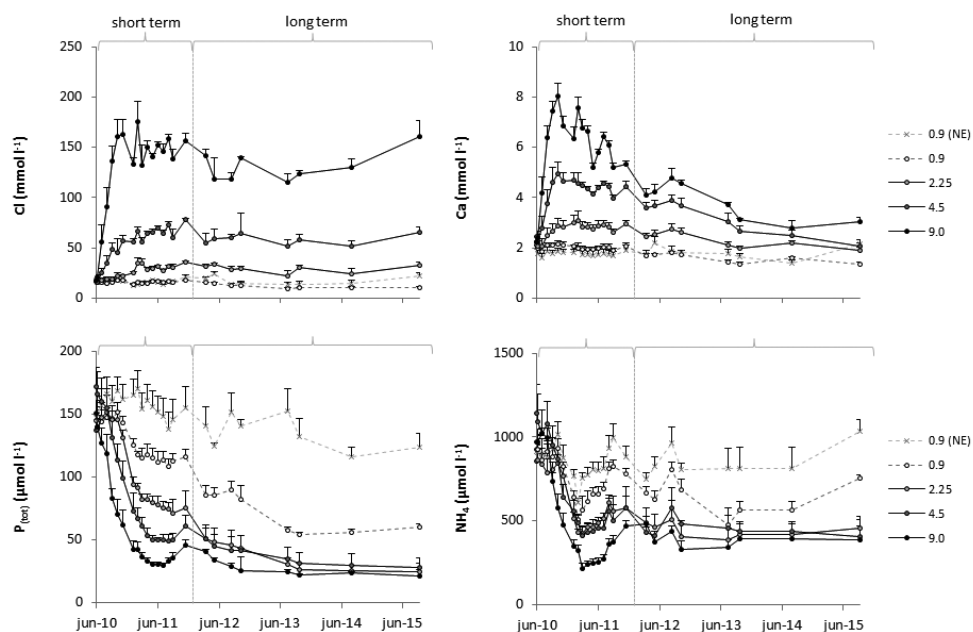


Fig. 5.7. Pore water concentrations (a. chloride, b. calcium, c. total phosphorus, d. ammonium) through time at 15 cm of depth in all treatments (given in PSU, (NE= No Enclosure)), (+ S.E.M., [n=4]). Note that scales are presented in μmol l⁻¹ and in mmol l⁻¹.

The observed salinity-induced decrease in NH_4^+ availability is in contrast to our hypothesis and to the results of many other studies reporting an increase due to displacement of NH_4^+ from sediment cation exchange sites by increased cation concentrations (Seitzinger et al. 1991; Weston et al. 2006, 2010, Van Dijk et al. 2015). Rapid NH_4^+ mobilization can already take place within hours after salinization (Weston et al. 2010), but long-term increased or fluctuating salinity may well deplete the amount of NH_4^+ bound to cation exchange sites (Weston et al. 2010, Noe et al. 2013, Van Dijk et al. 2015). In the present study, NH_4^+ mobilization was indeed found during the first weeks. Our analyses indicated NH_4^+ mobilization from cation exchange sites, which supports our hypothesis of short-term mobilization and long-term depletion. Sediment porewater analyses showed that concentrations decreased over time at higher salinity (> 2.25 PSU). The observed decrease is most probably caused by fast nitrification in the water layer and subsequent denitrification in the sediment of the NH_4^+ mobilized (Strauss et al. 2002; Burgin and Hamilton 2007). In sulfide-rich ecosystems sulfur-driven nitrate reduction by autotrophic denitrifying bacteria may also play an important role (Hatakeyama et al. 2013). In literature, there is no consensus on the effects of salinization on nitrification and denitrification processes (Herbert et al. 2015). There are studies that report that both processes are solely affected due to increased ionic strength (e.g. Laverman et al. 2007) or due to increased sulfide concentrations (i.e. Brunet and Garcia-Gil 1996), but a mechanistic explanation is challenging as alternative processes of N loss to the atmosphere as anammox could also play a role.

Increased hydraulic conductivity

An additional factor which might enhance decreased porewater P and NH_4^+ concentrations could be an increased vertical infiltration of P due to salinity-induced increase in hydraulic conductivity. Van Dijk et al. (2016) showed that enhanced salinity does not only influence physicochemical and biogeochemical processes but can also influence hydrological processes by increasing the hydraulic conductivity of sediments rich in organic matter due to the combined effect of pore dilation and reduced abundance of methane bubbles in the sediment pores.

Salinization effects on decomposition - sulfate reduction versus methanogenesis

There is no consensus in literature on how increased salinity affects sediment carbon cycling (Herbert et al. 2015). In general, salinization will lead to increased concentrations of the alternative terminal electron acceptor sulfate, which may enhance anaerobic microbial mineralization of organic matter in coastal wetlands (Lamers et al. 1998, Weston et al. 2006, Chambers et al. 2011, Weston et al. 2011, Marton et al. 2012). Although C mineralization rates and microbial activity were not determined in the present study, porewater concentrations of the end products of methanogenesis (CH_4) and of sulfate reduction (H_2S) can be used as proxies to study how these processes are affected by salinity. Our study shows a clear shift from methanogenesis dominance under control conditions to a sulfate reduction dominance under increased salinity (> 2.25 PSU). This effect has also been shown in several other studies

(e.g. Barlett et al. 1987, Weston et al. 2006, Chambers et al. 2011, Vizza et al. 2017) as sulfate is a thermodynamically more favorable process than methanogenesis. As a result, methanogens are outcompeted by sulfate reducers, or even directly inhibited by an increase in ionic strength (Weston et al. 2006, Chambers et al. 2011). Apart from effects on sulfate reduction and methanogenesis Weston et al. (2006) also showed a temporary enhancement of iron reduction rates after salinity increase. In our study, this probably did not occur due to the low availability of Fe^{3+} (as discussed above). Even after five years of increased salinity, the methanogenic bacteria community did not seem to have adapted to increased salinity as methane concentrations were still low in the 4.5 and 9.0 PSU treatments (data not shown). This means that increased surface water salinity will decrease CH_4 emissions from former brackish coastal freshwater wetlands. Based on the results from the present study, however, it is difficult to speculate on the effects of salinization on the carbon emissions to the atmosphere.

Consequences of surface water salinization for the benthic community

In addition to the effects of increased salinity on biogeochemical cycling, some important consequences were observed for the benthic fauna community. It is well known that increased salinity results in a loss of biodiversity in aquatic ecosystems (i.e. Remane 1934, Hart et al. 1991). Although in the present study nutrient levels did decrease, they are probably not limiting so we expect ionic stress and sulphide toxicity to overrule the effects of decreased nutrient availability on benthic fauna. Free sulphide is known to increase mortality and toxicity effects of macroinvertebrates and to delay population recovery after recolonization (Lamers et al. 2013, Kanaya et al. 2015). Characteristic brackish benthic fauna and aquatic plants, are most probably absent due to dispersal limitation which was inherent to our experimental set up.

CONCLUSION

The present paper does show a strong, dose-dependent impact of a long-term (5 years) salinization on nutrient dynamics in coastal freshwater wetland sediments that partly contradicts short-term effects shown in other studies. This shows that care should be taken to extrapolate the outcome of short-term studies to longer-term effects. Although many studies report an increase of the nutrient (N, P) availability as a result of increased salinity, our study clearly shows that combined physicochemical and biogeochemical effects may well lead to a reduction of the nutrient availability in formerly brackish freshwater wetlands, both in the short and in the longer term (5 years). Very probably increased cation exchange is one of the key processes involved, resulting in NH_4^+ and Ca mobilization, Ca-P precipitation and nitrogen losses due to coupled nitrification/denitrification. Furthermore we demonstrated a shift from methanogenesis to sulfate reduction as the most dominant C mineralization process. This

means that salinization results in decreased nutrient availability and methane emissions, but in increased levels of sulfide, toxic to both plants and animals.

As worldwide salinization of coastal (and inland) systems is occurring at an alarming rate and scale (Herbert et al. 2015), results from long-term experiments such as the one presented here can be used to better predict the future effects of increasing surface water salinity on biogeochemical processes in the sediment. Our study shows that these effects do strongly depend on sediment characteristics, as in formerly brackish systems nutrient availability may still decrease even when sulfate reduction rates increase. This means that profound knowledge about the ecological and biogeochemical functioning of the systems involved, and on how increasing salinity levels influence these systems through time, is needed for decision support for managers and policy makers involved in wetland management and restoration.

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[Enclosure experiment in Ilperveld with J. Nijp, by G. van Dijk]

6

Salinity-induced increase of the hydraulic conductivity in the hyporheic zone of coastal wetlands

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ABSTRACT

In coastal zones globally, salinization of surface water and groundwater is rapidly taking place due to the combined effects of sea level rise, land use change, land subsidence, altered hydrology and climate change. Although increased salinity levels are known to have a great impact on both biogeochemical and hydrological processes in aquatic sediments, only few studies have included both types of processes and their potential interactions. In the present paper, we used a controlled three years experimental mesocosm approach in the surface water of a Dutch coastal wetland to test these interactions as a result of salinization, and to discuss mechanisms explaining the observed hydrological changes. In enclosures (1000 l), surface water salinity was experimentally increased from 14 mmol to 140 mmol Cl l⁻¹ (0.9 and 9 PSU) by adding sea salt. This not only strongly increased pore water salinity, but also increased sulphate reduction rates, leading to higher sulphide and lower methane concentrations. By analysing slug test data with three different slug test analysis methods, we were able to show that hydraulic conductivity of the hyporheic zone increased 2.8 times by salinization. This shows that increased salinity can strongly change the hydrological characteristics of the hyporheic zone in coastal wetlands. Based on our hydrological and biogeochemical measurements, we conclude that the combination of pore dilation and decreased methane production rates were major controls on the observed increase in hydraulic conductivity. The slug test analysis method comparison allowed to conclude that the adjusted Bouwer & Rice method results in the most reliable estimate of the hydraulic conductivity for hyporheic zones. Our work shows that both physical and biogeochemical processes are vital to explain and predict hydrological changes related to the salinization of hyporheic zones in coastal wetlands and provides a robust methodological approach for doing so.

Keywords: downward seepage, hydraulic conductivity, methane, methanogenesis, pore dilation, salinization

INTRODUCTION

Climate change, sea level rise and extensive land use change have resulted in an increasing occurrence of salinization of surface waters (Abacra & Post 2010, Herbert *et al.* 2015) and increased saline groundwater intrusion in coastal zones (i.e. Rengasamy 2006, Werner & Simmons 2009, Oude Essink *et al.* 2010, Velstra *et al.* 2011). This not only affects agricultural land use (Katerji *et al.* 2003, Rengasamy 2010), but also threatens groundwater resources used for drinking water abstraction, and the ecological functioning of natural freshwater wetlands due to large biogeochemical consequences of salinization (e.g. Pitman & Lauchli 2002, Lamers *et al.* 2002a, Oude Essink & Kooi 2011, Bonte & Zwolsman 2010, Herbert *et al.* 2015). In addition, anthropogenically lowered surface water levels and groundwater levels have resulted in increased land subsidence rates, especially in areas with peaty soils such as densely populated deltaic areas. The western part of the Netherlands is an example of a coastal wetland prone to both salinization and land subsidence, with regions showing more than four meters of subsidence during the last millennium, large parts of which are now situated below mean sea level (Schothorst, 1977, Nieuwenhuis & Schokking 1997). Although these low areas are generally protected against flooding by the construction of dikes and the implementation of complex water management systems, protection against salinization due to upwelling of brackish or saline groundwater or a shortage of freshwater supply, especially during dry summers also needs to be implemented (Oude Essink *et al.* 2010, De Louw *et al.* 2011, Velstra *et al.* 2011).

Water management in these areas needs to consider that increased salinity has a strong effect on soil- and water biogeochemistry as a result of the increase in ions such as chloride, sodium, magnesium and calcium, but also sulphate. Surface water salinization will primarily affect hydrological and biogeochemical processes in the hyporheic zone (here defined as the aquatic sediment, the interface between surface water and groundwater conform; Orghidan, 1959). The hyporheic zone is of special interest because it is a hotspot for biogeochemical processes and often harbours a large microbiological diversity and steep redox gradient (Boulton *et al.* 1998, Storey *et al.* 1999). Increased salinity in the sediment can have important biogeochemical and eco(hydro)logical consequences including direct effects on plant and animal communities (Remane & Schlieper 1958, Nielsen *et al.* 2003) including agricultural crops and cattle (Pitman & Lächli, 2002), but also changes in microbial communities and related biogeochemical cycling (e.g. Baldwin *et al.* 2006, Weston *et al.* 2006, Van Dijk *et al.* 2015).

Indirectly, the increased water density due to increased surface water salinity may also have physical and hydrological consequences. However, only few studies focus on the effects of surface water salinization on hydrological and biogeochemical processes and their interactions in the hyporheic zone of coastal wetlands, especially long-term effects have yet to

be analysed. Laboratory studies showed that salinity may influence the hydraulic conductivity of soils (Mehnert & Jennings 1985) modifying the infiltration rate of saline water into soils, and altering groundwater flow patterns. Most studies, however, deal with mineral soils, and little knowledge exists about the effects of salinity on the interaction between biogeochemical processes and the hydraulic conductivity of peat soils. Water flow through wetland sediments is affected by the hydraulic conductivity, which can vary greatly among different types of peat with varying degrees of decomposition [e.g. Boelter, 1969, Chason & Siegel, 1986, Stofberg *et al.* 2016]. Laboratory experiments and models [e.g. Ours *et al.* 1997, Comas & Slater 2004, Kettridge & Binley 2010] indicate that increased salinity can increase flocculation of particles such as humic acids in peat soils, causing pore dilation which potentially influences the hydraulic conductivity of peat soils. As a consequence of increased salinity, increased sulphate concentrations can enhance sulphate reduction in anaerobic sediments and suppress methanogenesis (Segers 1998, Lamers *et al.* 2002b, Baldwin *et al.* 2006, Weston *et al.* 2006) which is known to influence hydraulic conductivity in peatlands (Baird *et al.* 2003). Although these studies indicate large effects of salinity on hydrology or biogeochemistry, the combined measurement of biogeochemical and hydrological processes in long term field studies is to our knowledge currently missing. In the present paper, the effects of increased salinity (140 mmol $\text{Cl}^- \text{L}^{-1}$ (9 PSU); brackish conditions) on biogeochemical processes and the hydraulic conductivity in the hyporheic zone were studied in a controlled field experiment in a Dutch coastal peatland. We hypothesized that interacting biogeochemical and hydrological processes increases the infiltration rate of saline water into the sediment under increased surface water salinity.

MATERIALS AND METHODS

Site description

The field experiment was carried out in a large permanent ditch (more details below) located in a freshwater coastal wetland (Ilperveld) located north of Amsterdam (the Netherlands). This semi-natural wetland is a nature reserve including large areas of open water connected via sluices and pumping stations to surrounding canals (e.g. Noordhollands Kanaal) with a strictly regulated surface water level of about 1.5 m below mean sea level. The wetland has been influenced by anthropogenic use since the Middle Ages. We selected the Ilperveld because it was affected by the infiltration of brackish surface water and several flooding events during the last centuries as a result of anthropogenic land subsidence. Since the 1930s, the influence of brackish water decreased and salinity levels dropped due to the construction of a large dam (Afsluitdijk) eliminating seawater influence (De Beaufort, 1954). The chemical sediment composition, however, is still indicative of the former brackish influence (increased chloride, sodium and sulphur concentrations). The soil surface level of the area varies between 1.3 and 1.5 m below mean sea level (-1.3 to -1.5 m). Directly adjacent to the study area there are

a number of deeper polders (agricultural lands that need active pumping to avoid flooding; surface level at -4 to -5 m).

The IJperveld subsoil is characterized by a sequential stratification of peat and clay layers (Westland formation (Figure 6.1)). The toplayer consists of a 2 m thick peat deposit, situated on top of a 3 m thick layer of sequential stratification of peat and clay layers. These layers are underlain by a 30 m sandy aquifer (Formation of Twente) based on a sand layer rich in loam and silt (Formation of Drenthe) (National Dino Database, TNO). The area is part of a large former *Sphagnum* bog that was formed between 2500 and 1500 years before present (Bakker & Smeerdijk 1982, Witte & van Geel 1985, Willemsen *et al.*, 1996). The area has been strongly affected by anthropogenic influence (i.e. drainage, construction of ditches and canals, peat extraction, and the creation of deep polders), which had a major impact on its hydrological functioning. The current average phreatic groundwater level fluctuates around the average surface water level (-1.53 m NAP (below mean sea level)). The hydraulic head of the phreatic aquifer is at about -2.5 m NAP in the southern and -3 m NAP in the northern part (i.e. 0.97 m and 1.47 m below surface water level, respectively). Despite strong land subsidence, the groundwater head of the phreatic layer is higher compared to the regional hydraulic head, indicating downward seepage of approximately $0.1 \text{ mm} \cdot \text{d}^{-1}$ based on hydrological modelling (Nelen & Schuurmans, 2005).

The experiment was carried out in a dead-end canal (52° 27.225N - 4° 55.885E) of about 250 meters long and 10-15 meters wide, with a water depth of 1.3 m, surrounded by reedlands and agricultural peat meadows. The aquatic sediment was unvegetated and consisted of a 15 cm decomposed organic sludge layer on top of a 1.5 m thick layer of *Sphagnum* peat on clay (Figure 6.1). Both the sludge and the peat layer are rich in organic matter (75% and 90%). The bulk density of the peat (80 g DW l^{-1} ; 48 h at 70°C) is higher compared to that of the sludge layer (60 g DW l^{-1}).

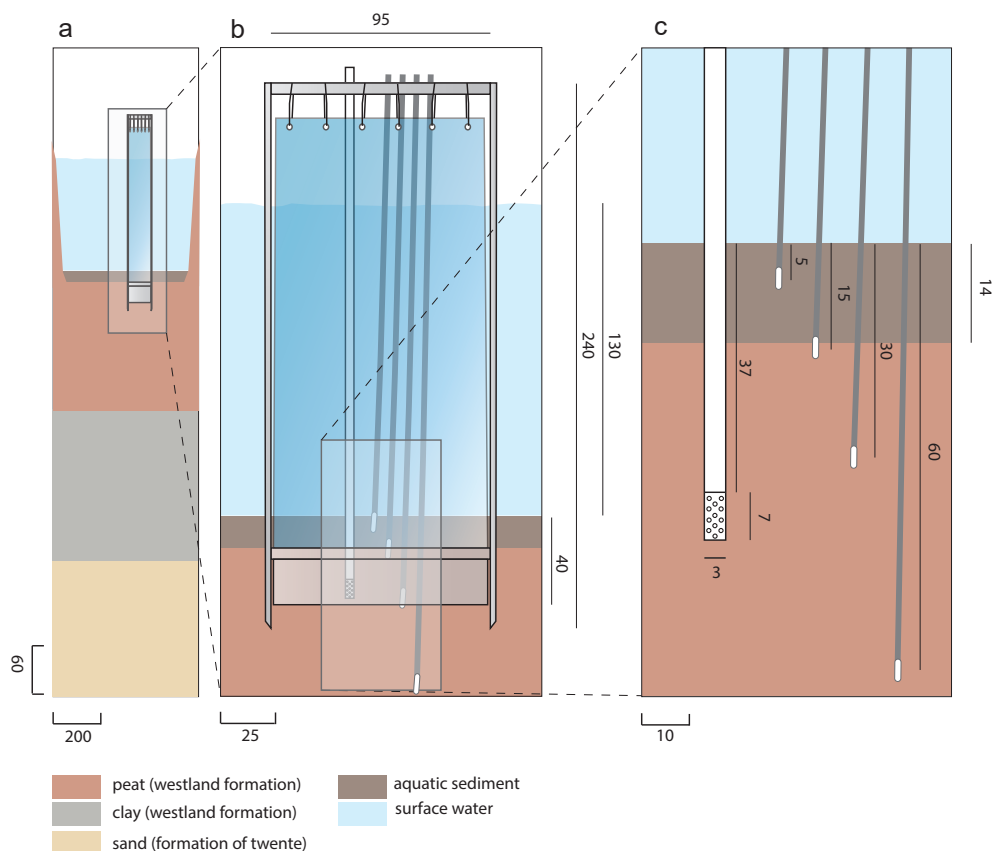


Fig. 6.1 (a) Enclosure placed in the water column of a canal with peat soil on top of clay and sand. (b) Enclosure overview showing steel frame, flexible polyvinylchloride enclosure, and piezometer and ceramic cups. (c) Detailed overview of sampling methods, on the left (in white) the piezometer with filter at the bottom, on the right ceramic cups for pore water sampling at different depths. Scales in cm.

EXPERIMENTAL SET-UP

Enclosure experiment

To test the effect of salinity on hydraulic conductivity of the hyporheic zone, we placed 16 enclosures (diameter 95cm) in an unvegetated canal. To avoid canal-edge effects we placed the enclosures in the middle of the canal, and randomly assigned an increased salinity ($n = 4$) and a control ($n = 4$) treatment (eight extra enclosures, with two intermediate salinity treatments were placed in which only chemical parameters and the enclosure volume were determined). The enclosures had stainless steel frames and flexible PVC (polyvinylchloride) foil (≈ 1 mm thick). Flexible PVC foil was used to reduce the effect of the enclosure itself and to assure the

effect of waves, wind and mixing were not fully excluded, which would have been the case with fixed sides. The top of the open PVC enclosures was connected to the steel frame by rubber bands, keeping the upper part 30 cm above the water surface (Figure 6.1). At the bottom the enclosures were fastened to a steel ring (30 cm length), placed at 40 cm below the sediment-water interface. In this way miniature ponds with flexible volumes were created, in which interactions between the atmosphere (precipitation and evaporation) and the enclosed surface water remained intact. Vertical exchange of water between the surface water, the hyporheic zone, underlying sediments and groundwater also remained intact (Figure 6.1). Lateral flow of the surface water was eliminated, while lateral pore water flow (up to the installation depth of 40 cm beneath the sediment-water interface) was strongly reduced inside the enclosure. During the experimental period enclosures of both the control and the increased salinity treatment must nevertheless have lost water via the aquatic sediment underneath the steel ring due to the average precipitation surplus of about 300 mm year⁻¹ (30 yr average, Sluijter *et al.* 2011, recent years KNMI, 2015) at the study location and an equal surface water level inside and outside the enclosures (visual observations).

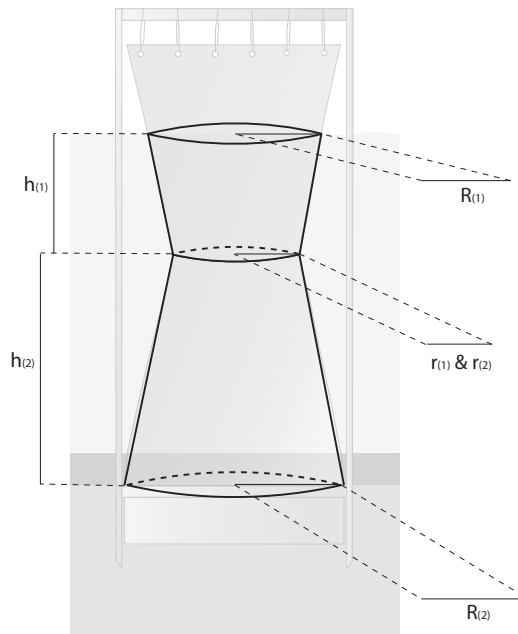


Fig. 6.2 A schematic illustration of how the enclosure volume was calculated, for calculating the content of different parts of two cones

Salinity Treatment

In four enclosures the surface water salinity was increased from the ambient canal condition of 14 mmol l⁻¹ Cl (0.9 PSU, low salinity, control treatment), to 140 mmol l⁻¹ Cl, (9 PSU increased salinity treatment), by adding artificial sea salt (Tropic Marin®). The 140 mmol l⁻¹ treatment was selected as a realistic salinity level for future salinization in Dutch coastal wetlands. To keep salinity as constant as possible, and to avoid dilution by rainwater, sea salt was added periodically during the experimental period when surface water salinity levels dropped to values that were 10% lower than the target values; pore water salinity however remained stable.

Hydrological measurements

Surface water volumes of the flexible enclosures were measured at the start and end of the experiment (12th August 2010 to 30th July 2013, total duration of 35 months). The volume of the enclosures was calculated based on measurements of enclosure diameter at the surface water level (R1 in Figure 6.2), based on the volume of a two- truncated cone (Equation 1) and assuming that the middle of the flexible bag had the smallest diameter. Based on the difference between enclosure diameter (at the surface water level) and the diameter of the steel frame at the top, the angle of the cones was calculated. The volume of the enclosures was calculated by summing the volume of two truncated cones, using Equation 1 and Figure 2.

$$V = \frac{1}{3} \pi h (R^2 + Rr + r^2) \quad (1)$$

To measure the in-situ effect of salinity on the saturated horizontal hydraulic conductivity (K_s), slug tests were used, after placement of piezometers, to quantify K_s in the hyporheic zone (Ong & Zlotnik, 2011).

The bottom-end of the piezometers was closed water-tight with a cap, and the perforated section was covered with nylon cloth to prevent clogging of the filter. Time series of water pressure (frequency 2 s⁻¹) were obtained using divers (TD Divers DI240, Van Essen Instruments), which were placed in the piezometers. The piezometers consisted of cylindrical polyvinylchloride tubes (outer diameter 31 mm), with the filter (70 mm) located in the denser *Sphagnum*-dominated peat layer at a depth of 35 – 42 cm below the surface water-aquatic sediment boundary (Figure 6.1). The filter was constructed by drilling holes (diameter 0.7 mm, 84% perforation of total filter surface) in the piezometer (Figure 1).

In slug tests, a known volume of water is rapidly poured into a piezometer, which induces a stepwise increase in water pressure which then decreases over time from which the saturated hydraulic conductivity was calculated. Piezometers were installed in both the control and the salinity treatment enclosures (one per enclosure). Typically, piezometers or wells are

'developed', a procedure where drilling debris is removed from the piezometer intake (Baird et al., 2004; Kill 1990; Buttler 1998). Because of the fragile nature of hyporheic zone and peat sediments, we decided to not develop the piezometers, as it involves inducing strong flows, which may alter the pore architecture. After the water level in the piezometers stabilized, a slug of 100 ml water of a salinity similar to the local surface water was added. To verify the quality and reproducibility of the slug tests, three replicate tests were taken per piezometer. Due to technical errors in two of the divers the hydraulic conductivity could only be calculated using 3 replicates, whereas enclosure volume and chemical depth profiles were calculated using 4 replicates for each treatment.

Comparison of slug test analyses

Various approaches have been described in literature to calculate K_s (saturated hydraulic conductivity) using slug test data (e.g. Bouwer & Rice, 1976; Cooper et al., 1967; Hvorslev, 1951; Hyder et al., 1994; van Beers, 1983), all assuming a homogeneous aquifer with uniform thickness. The hydrostatic time lag method developed by Hvorslev (1951) has frequently been employed in wetland studies (Baird et al., 2008; Baird et al., 2004; Surridge et al., 2005; Whittington & Price, 2006). Three additional assumptions for this method are that: 1) the filter vertically stretches the whole aquifer (i.e. fully penetrating piezometer), 2) the aquifer is confined (it remains saturated), and 3) water storage change is negligible (steady state flow).

The approach developed by Bouwer & Rice (1976) (BR) is suitable for both confined and phreatic unconfined aquifers, and for partially penetrating piezometers (Bouwer, 1989). Briefly, the BR method is based on the Thiem equation of steady state flow to/from a piezometer (Bouwer & Rice, 1976; Thiem, 1906) (Equation 2):

$$Q(t) = 2\pi K_s L \frac{h(t)}{F} \quad (2)$$

where $Q(t)$ is flow rate, L is the filter length, $h(t)$ is the increase in water level in the piezometer relative to the initial groundwater level, and F is a shape factor correcting for the piezometer geometry. Bouwer and Rice (1976) empirically developed equations to derive F from the piezometer radius, position of the filter below the groundwater table and estimated aquifer thickness. The performance of these empirical equations for many realistic field applications (requiring relatively small filter length as compared to filter diameter) is, however, limited (Brown et al., 1995; Hyder & Butler, 1995; Zlotnik et al., 2010). More recently, (Zlotnik et al., 2010) established a general, analytical closed-form expression of the BR shape factor (F_{zgd}) to account for piezometer geometry (Equation 3).

$$F_{ZGD} = \left(\sum_{i=1}^{\infty} \left\{ \left(\cos \left(\beta_i \frac{H}{D} \right) - \cos \left(\beta_i \frac{H-L}{D} \right) \right)^2 \times \frac{K_0 \left(\frac{\beta_i r_w}{D} \right)}{\beta_i^3 K_1 \left(\frac{\beta_i r_w}{D} \right)} \right\} \right) / \frac{L r_w}{2 D^2} \quad (3)$$

Here, K_0 and K_1 are the modified Bessel functions of third kind and 0th or 1st order and $\beta_i = \pi (i-0.5)$. r_w^* represents the piezometer radius scaled by the anisotropy coefficient (Equation 4).

$$r_w^* = \frac{r_w}{\sqrt{K_h/K_v}} \quad (4)$$

The coefficients K_h and K_v are the horizontal (h) and vertical (v) saturated hydraulic conductivity. We assumed the peat sediment had an anisotropy ratio of 1. See Zlotnik *et al.* (2010) for details on the derivation and Nijp (2015) for the calculation of F_{ZGD} using the R software (R Core Team, 2014). The only remaining assumption is that the storativity is negligible. Following recommendations by Butler (1998) to minimize the effects of compression on the calculation of K_s , we selected a normalized head range of 0.2 – 0.3. The quality of the K_s estimates was assessed by comparing observed and simulated water pressures using Willmott's modified index of agreement (Legates *et al.*, 2011; Willmott *et al.*, 1985).

Chemical analyses

Surface water samples and sediment pore water samples were taken in the period of June to August 2013. Sediment pore water samples were collected at 5, 15, 30 and 60 cm depth in the sediment with a vacuumed syringe connected to a ceramic cup via a Teflon tube. Pore water depth profiles were collected in the highest and lowest salinity treatment (the treatments where the slug tests took place), in the two intermediate salinity treatments pore water samples were only collected at 10 cm depth. Extra sediment pore water samples were collected for pore water methane analyses by connecting vacuumed 12 ml glass exetainers (Labco exetainer®, High Wycombe, UK) in which concentrations were measured in the headspace and recalculated for the water volume using Henry's constant.

For the analyses of sulphur, calcium, and magnesium inductively coupled plasma spectrophotometry (ICP-Optical Emission Spectrometer, Thermo Scientific iCAP 6000 Series ICP) was used. Prior to elemental analyses, 10 ml of each sample was stored at 4°C until analyses with 0.1 ml (65%) HNO_3^- to prevent metal precipitation. To determine sodium and chloride concentrations, 20 ml of each sample was stored at -20°C and analysed colorimetrically with an Auto Analyzer 3 system (Bran and Luebbe). Sodium and potassium were determined with a Technicon Flame Photometer IV Control (Technicon Corporation). CH_4 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 N₂ as carrier gas. Sulphide concentrations were determined directly after sampling by fixing 10.5 ml pore water with 10.5 ml Sulphide Anti Oxidant Buffer (SAOB), and using an Orion sulphide-electrode and a Consort Ion meter (type C830) for analyses (Van Gernerden, 1984).

Data analysis

The differences in enclosure volume between the control and salinity treatment was tested with a one-way ANOVA. We tested for normality and homogeneity of variances using the Shapiro-Wilk test and Levene's Test, no transformations were applied to the data. Differences in pore water element concentrations over depth were assessed using a generalized linear mixed model, using salinity treatment and depth as fixed factor and time as repeated factor; the covariance type used was AR(1) Heterogeneous. A Least Significant Difference post-hoc test was used to test for statistically significant differences. Differences in hydraulic conductivity (K_s) between salinity treatments were assessed with a two-way ANOVA with salinity treatment and slug test analysis method as fixed factors, and enclosure as within-subjects factor. The three replicated slug tests per enclosure yielded very similar K_s estimates (median deviation from mean per enclosure was 3%), indicating that the reproducibility of slug tests is high and no well development was required. This was supported by a Repeated Measures ANOVA with slug test analysis method and salinity treatment as fixed factors and repetition as within-subjects factor, showing that K_s did not significantly differ at different points in time ($P > 0.2$). We therefore removed the factor time from our analysis. Data were normally distributed (Shapiro-Wilk Test; $P > 0.07$), but the assumption of homogeneous variances among treatment-method groups was not met ($P = 0.001$). To account for this violation, we calculated robust standard errors using a robust parameter estimate covariance matrix in the Generalized Linear Model procedure (White 1980). All tests were carried out using SPSS Statistics for Windows (Version 21.0. IBM Corp. Armonk, NY, 2012).

RESULTS

Salinity effects on hydrology

During the start of the experiment the water volume of the flexible enclosures was around 1050 l and did not differ between both salinity treatments ($P = 0.55$) (Figure 6.3a). After 3 years however, the water volume in the enclosures with increased salinity had decreased by 61% ($P < 0.001$) (Figure 6.3a). The enclosure volume measured after two experimental years decreases logarithmically as function of salinity ($R^2 = 0.99$; Fig. 6.3b). An opposite relation was found between enclosure volume and pore water methane concentrations ($R^2 = 0.99$; Fig. 6.3c). Two intermediate salinity treatments are presented in Figure 6.3, which are not further discussed

in the present paper as other hydrological measurements (slug tests) were only done in the 14 and 140 mmol Cl l⁻¹ treatments.

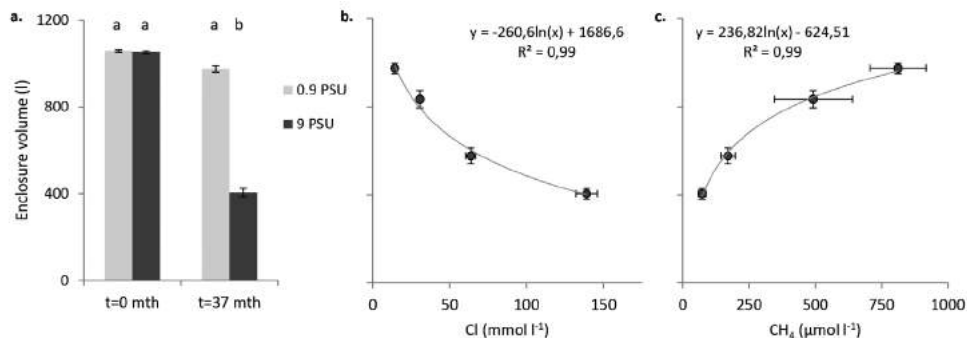


Fig. 6.3 (a) The average water volume at the start of the experiment ($t = 0$ months) and after 3 years ($t = 37$ months) for the control treatment (0.9 PSU, 14 mmol/L Cl) and increased salinity treatment (9 PSU, 140 mmol/L Cl), ($\pm$ S.E.M., [$n = 4$]). (b) and (c) the average final enclosure volume (error bars represent $\pm$ S.E.M., [$n = 4$]) for both salinity treatments and for two additional intermediate salinity treatments (31 and 64 mmol/L Cl) plotted against the pore water chloride concentration and the pore water methane concentration respectively.

The average hydraulic conductivity (K_s) of the aquatic peat sediment was on average 2.8 times higher ($P < 0.01$) in enclosures with increased surface water salinity (Figure 6.4). This increase appeared not to be affected by the method used to calculate K_s , all methods fitted the data well (in 95% of all K_s estimates, the Willmott index of agreement between observed and modelled hydraulic head exceeded 0.94), but showed a significant effect of salinity. Compared to the BR method with Zlotnik shape factor (FZGD), however, the Hvorslev method yielded conductivity estimates which were 24% larger K_s ($P = 0.25$); and the BR method (without FZGD) yielded a 28% lower K_s ($P = 0.08$) (see Figure 6.4).

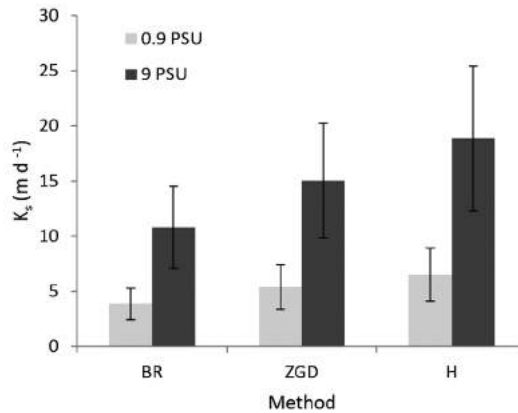


Fig. 6.4. Effect of salinity treatment (colors) in slug test on average saturated hydraulic conductivity K_s for three analysis methods; H = Hvorslev (Hvorslev, 1951), BR = Bouwer–Rice (Bouwer & Rice, 1976), ZGD = Bouwer–Rice with shape factor conform Zlotnik et al. (2010), (error bars represent $\pm$ S.E.M., [n = 3])

Effects of surface water salinization on sediment biogeochemistry

Increased surface water salinity had a significant impact on chemical composition and biogeochemical processes in the aquatic sediment. Chloride (Cl) concentrations not only increased significantly ($P < 0.005$) up to the treatment level in the top layer but also deeper in the sediment, up to 30 mmol l^{-1} Cl, 20 % of the treatment level (Figure 6.5). After 2.5 to 3 years of surface water salinization the pore water Cl concentration in the salinity treatment was increased to the treatment level up to a depth of 15 cm, and was still significantly increased ($P = 0.004$) at a depth of 60 cm. Similar patterns were found for cations (Sodium, Magnesium, Calcium, Potassium; data not shown). Pore water Cl concentrations in the control treatments remained around 12 mmol l^{-1} in the depth profile and even showed a minor decrease in the upper 5 and 15 cm. In the high salinity treatment both pore water total sulphur (S) concentration as well as sulphide concentration were increased (at a depth of 5 to 10 cm from about 0.4 to 5.0 mmol l^{-1} ($P = 0.003$) for total S and from 0.13 to 4.9 mmol l^{-1} ($P = 0.01$) for sulphide (Figure 6.5). In contrast, pore water methane (CH_4) concentrations were strongly decreased (from 0.8 to 0.047 mmol CH_4 l^{-1} , > 90 % decrease in the top layer) up to a depth of at least 30 cm in the salinity treatment ($P < 0.005$) (Figure 6.5). Pore water depth profiles were not collected in the two intermediate salinity treatments; pore water samples at 10 cm depth were analysed and showed intermediate effects, and are therefore not discussed in more detail.

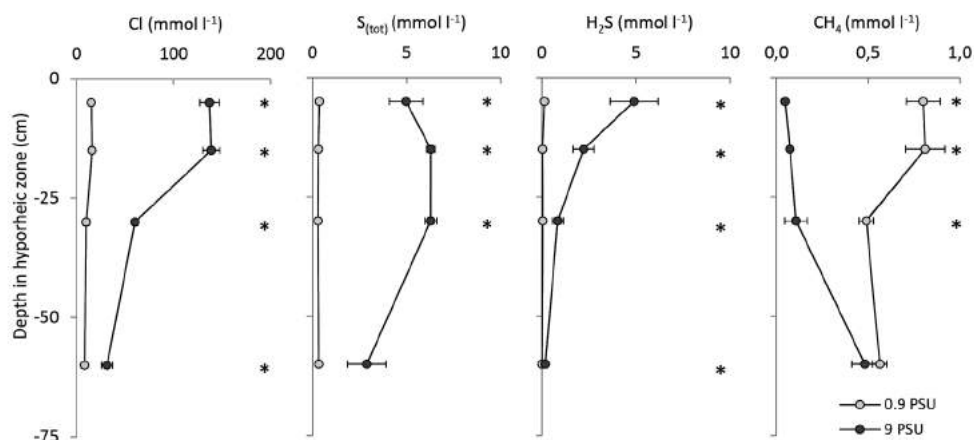


Fig. 6.5. Depth profiles for pore water concentrations in the aquatic sediment (a) chloride, (b) total sulphur, (c) sulfide, and (d) methane in mmol/L, for control treatment (○) and 140 mmol Cl per L salinity treatments (●) 37 months after the start of the experiment. * $p < 0.05$; note different scales for the x-axes, [error bars represent $\pm$ S.E.M., [n = 4]]

DISCUSSION

Possible mechanisms explaining increased hydraulic conductivity due to salinization

The striking negative correlation between salinity and change of enclosure volume through time presented in this study was unexpected. The decreased volume of the flexible enclosure as a result of increased surface water salinization can be caused by several processes.

First, increased salinity may increase water density and might thereby enhance vertical (downward) water flow (Holzbecher 1998). In the present study the water density increased from 1000.646 kg/m³ in the control treatment to 1007.735 kg·m⁻³ in the highest salinity treatment. Based on Darcy's Law this would lead to a 0.7 % increase in vertical water flux (assuming similar viscosity, temperature of 20°C and permeability, but different water density for the control and increased salinity treatment). As we found that the increased salinity treatment induced an 83% increase in hydraulic conductivity (Figure 6.4), the contribution of increased water density to the observed enhanced vertical water flow can only be marginal.

Second, increased surface water salinity may influence the evaporative water losses from the surface water. Increased salinity would, however, have decreased evaporation rates due to increased attraction of solvent molecules in the water (Salhotra *et al.* 1985).

Thirdly, a physical explanation of increased hydraulic conductivity in peat soils with increased salinity could be the process of pore dilation. Dilation of macro pore spaces is caused by the flocculation of the organic acids located on the surfaces of peat fibers as a result of an increased salinity. By modifying the pore water chemistry, increased salinity alters the physical properties of the sediment and can influence the hydraulic conductivity in peat soils (Ours *et al.* 1997, Hoag & Price 1997). The effect of pore dilation on K_s was further studied by Comas & Slater (2004), who established a quantitative relation between the electric conductivity (EC) and K_s for peat soils ($K_s \propto EC^n$, with n varying from 0.25 – 0.30). Based on Comas & Slater (2004), we estimated the maximum contribution of pore dilation to the observed salinity induced changes in K_s using Equation 5.

$$K_s = K_0 [EC]^{0.3} \quad (5)$$

Here, K_0 represents the K_s at an EC of 1 S m^{-1} , which was estimated from the average K_s and EC in the control treatment. Following this formulation, the contribution of pore dilation on the K_s due to increased EC (in the present study an increase in EC from 0.19 S m^{-1} to 1.84 S m^{-1}) was estimated to cause a 97.6 % increase in the high salinity treatment relative to the control treatment (factor 1.98). The relative increase in K_s found in the present study in the high salinity treatment relative to the control treatment is however 179.2 % (a factor 2.97). This indicates that, even though pore dilation alone can explain a substantial part of the K_s increase, 82 % of the increase in K_s found in the present study remains unexplained. This large unexplained part of the increase in K_s suggests that other mechanisms could also play an important role.

Fourthly, biogeochemical processes may have influenced the hydraulic conductivity (K_s) in the hyporheic zone. Surface water salinization was shown to have a large impact on sediment biogeochemistry in the present study (Figure 5), not only leading to an increase of chloride and sodium, but also to an increase of sulphur concentrations (Weston *et al.* 2006, Van Dijk *et al.* 2015, Herbert *et al.* 2015). These increased sulphur concentrations increase sulphate reduction rates, as shown by the strong increase in pore water sulphide concentrations (Figure 6.5). As demonstrated in several previous studies (Barlett *et al.* 1987, Denier van der Gon & Neue 1995, Lamers *et al.* 2002b, Baldwin *et al.* 2006), increased sulphate concentrations in anaerobic, organic matter rich conditions result in increased sulphate reduction and increased sulphide concentrations. Compared to chloride (which can be used as an inert tracer), the total sulphur concentration shows a less strong increase in the top of the sediment of the increased salinity treatment. The missing part of the total sulphur concentration in the pore water must have been transformed to sulphide by the sulphate reducing microorganisms, indicated by increased sulphide concentrations in the top layer (Figure 6.5). The sulphide produced is released to the water layer or atmosphere or bound to iron as FeS_x in the sediment. Increased salinity significantly decreased pore water methane concentrations (94-78% decrease in the top

30 cm of the aquatic sediment), also indicated in the correlation in Figure 6.3 and 6.5, showing a negative correlation between pore water chloride and methane concentrations ($R^2 = 0.93$). This can be explained by the direct effect of increased sulphate availability on methanogenesis. Thermodynamically, sulphate reduction is a more favourable process than methanogenesis (Segers 1998). Sulphate-reducing bacteria therefore outcompete methanogens for access to organic substrates, which suppresses methanogenic activity when sufficient sulphate is present (Lamers *et al.* 1999, Smolders *et al.* 2002). Additionally, methanogenic activity may also be hampered due to physiological (osmotic) salt stress (only on the short term; Chambers *et al.* 2011) or sulphide toxicity (Cappenberg, 1975), reducing the methane production even further.

Decreased methanogenesis and consequential lower pore water methane (CH_4) concentrations decrease the formation of CH_4 gas bubbles. Due to the very low solubility of methane in sediment pore water, accumulation and resulting oversaturation of CH_4 likely resulted in the formation of CH_4 gas bubbles in the pores of anaerobic peat soils including aquatic sediments. Methane gas bubbles are known to produce an upward pressure in the pore water of peat soils, which in combination with air pressure variations may lead to CH_4 ebullition (Kellner *et al.* 2004, Kellner *et al.* 2006, Kettridge *et al.* 2012). Upward moving CH_4 gas bubbles can get stuck in the dense structure of peat soils (i.e. Kellner *et al.* 2004), such as *Sphagnum* peat in our case, and thereby reduce water movement. Increased occurrence of CH_4 gas bubbles and CH_4 ebullition can therefore decrease the hydraulic conductivity of organic rich sediments such as the hyporheic zone in the control treatment of the present study (Reynolds *et al.* 1992, Baird *et al.* 1995, Baird *et al.* 2003, Strack *et al.* 2005). Literature reports large decreases in the hydraulic conductivity of peat soils as a consequence of increased gas bubble content; Baird *et al.* (2003) report a decrease by a factor two, while Reynolds *et al.* (1992) found a decrease by a factor four. Although the hydraulic conductivity in peat soils can vary greatly due to large variations in peat quality, bulk density and rate of decomposition (e.g. Boelter, 1969, Chason & Siegel, 1986, Stofberg *et al.* 2016), gas bubbles may affect the hydraulic conductivity in a wide range of peat soils.

Apart from methane, other gas bubbles (sulphide, nitrogen gas, entrapped air) can also influence the hydraulic conductivity modifying groundwater flow (Beckwith & Baird 2001, Marinas *et al.* 2013). Increased nitrogen gas content is, however, not expected in the present study. As sulphide is more soluble than methane gas (solubility of sulphide: 0.46 grams of gas dissolved in 100 g of water, solubility of methane: 0.0026 grams of gas per 100 g of water, both at 15 °C when the total pressure above the solution is 1 atm. (Gevantman 1999)), the effect of the increased sulphide concentration is expected to be lower. Field observations (Gijs van Dijk, 2016) indicate that almost no ebullition occurs in the high salinity treatments whereas much ebullition was observed in the control enclosures, which underlines that the increase in sulphide does not lead to increased ebullition.

In the increased salinity treatments the strong decrease (by 78-94%) of pore water methane concentrations will therefore very likely have enhanced hydraulic conductivity. Additionally, at the start of the experiment, the increase in salinity may also have decreased the solubility of CH_4 in the pore water (Yamamoto *et al.* 1976), thereby enhancing the formation of CH_4 gas bubbles and stimulating CH_4 ebullition. In combination with the formation of larger pores (pore dilation) and the reduced production of methane this may have led to an accelerated loss of CH_4 enhancing hydraulic conductivity, also indicated by the strong relation between pore water methane concentration and the enclosure volume ($R^2 = 0.99$, Figure 6.3c).

Effect of slug test analysis methods on the hydraulic conductivity estimates

Repeated measurements per location yielded very reproducible estimates of the hydraulic conductivity (median deviation among three replicates per location was 3%). The hydraulic conductivity estimates in this study are in the range of other K_s values for moderately decomposed *Sphagnum* peat, generally between about 0.01 and 50 m d^{-1} (Surridge *et al.*, 2005, Whittington & Price, 2006, Baird *et al.*, 2008, Nijp, 2015). The increased salinity treatment values are in the higher end of the hydraulic conductivity range observed for comparable peats. However, different methods used to analyse slug test data generated different hydraulic conductivity estimates.

Typically, the interest of eco(hydro)logists lies in hyporheic zone or in the unconfined (i.e. rainwaterfed) aquifer, as this is the zone with most ecological and biogeochemical activity. A filter fully penetrating an aquifer or placed in the centre of an aquifer, an assumption for the Hvorslev (1951) method, is often less relevant and less practical. In addition, the performance of the Bouwer & Rice (1976) approach may be limited for many realistic field applications, as the empirically developed equations for the shape factor are relatively inaccurate for piezometers with a small filter length relative to the filter diameter (Brown *et al.*, 1995; Hyder & Butler, 1995; Zlotnik *et al.*, 2010). More recently, Zlotnik *et al.* (2010) developed an analytical closed-form expression for the shape factor in the Bouwer-Rice method. This general shape factor enables the analysis of slug tests with the full range of possible and more eco(hydro)logically relevant piezometer geometries and positioning within aquifers. In this study, not accounting for the specific positioning and partial penetration of piezometer filters by employing the Hvorslev method resulted in an overestimation of saturated hydraulic conductivity estimates of the hyporheic zone (in this study of about 23 %) as compared to the Bouwer-Rice approach with the Zlotnik shape factor, which matches well with results by Hyder *et al.* (1994) and Nijp (2015). The application of the Bouwer-Rice (BR) slug test analysis, in which the positioning and partial penetration of piezometers is accounted for with empirical equations (Bouwer & Rice, 1976), resulted in an underestimation of K_s of about 28.2% (as compared to the Bouwer-Rice approach with the Zlotnik shape factor). Although differences among slug test analysis methods were not significantly different, likely due to small samples size ($n = 3$), we recommend to use the BR

approach in combination with the general shape factor presented by Zlotnik *et al.* (2010) to estimate the hydraulic conductivity in the hyporheic zone.

Consequences of salinization on the hydrology of coastal wetlands

In this study we show that increased salinity significantly increased the hydraulic conductivity in the hyporheic zone of a coastal wetland in a long term controlled field setup. It is important to include this strong salinity-induced increase in hydraulic conductivity, into account in hydrological model simulations related to salinization effects. Especially coastal wetlands and other anaerobic sediments rich in organic material with potential methanogenic activity, susceptible to future salinity changes, are sensitive to such changes.

Salinization of deeper aquifers and surrounding areas can have major biogeochemical, agricultural and ecological consequences, influencing the nutrient, carbon and sulphur cycle (Van Dijk *et al.* 2015, Herbert *et al.* 2015). In peat aquifers with net infiltration and high methanogenic activity, increased salinity in the hyporheic zone might on the long term result in a nonlinear positive feedback where water infiltration becomes increasingly enhanced by salinity-induced increased hydraulic conductivity. This might result in an increased net infiltration from coastal wetlands to underlying aquifers and surrounding areas or losses through preferential flow paths and saline hotspots similar to those observed by De Louw *et al.* (2013) and De Louw *et al.* (2010) for other soil types, although, at present it is unknown if results found in the present study are also applicable on a situation with saline water intrusion via the aquifer. For the present study area these processes will probably be small due to the presence of thick clay layers. Changes of the hydraulic conductivity in the hyporheic zone, being a hotspot for biogeochemical and microbiological processes, can have a major impact on nutrient, sulphur and carbon cycles and the organisms living in this zone.

CONCLUSION

Although an increase in salinity is known to increase the hydraulic conductivity of mineral soils (Frenkel *et al.* 1977, McNeal 1967, Mandal *et al.* 2008, Singh *et al.* 2011, Zhu *et al.* 2013), the strong effect of salinity on the hydraulic conductivity in organic soils has to our knowledge not been shown in earlier studies. This effect is probably due to the combination of physicochemical effects (pore dilation) and biogeochemical effects (depressed methanogenesis).

An extrapolation of the findings in the present paper, based on a long term field experiment, on a landscape scale is difficult to make. Our study, however, shows that increased salinity not only influences physical - and biogeochemical processes but as a result also affects hydrological processes in the phreatic layer. Our study also provides a robust methodological approach for measuring influences of physical - and biogeochemical processes on the hydraulic conductivity.

Future models and predictions of effects of climate change and increased salinization induced by sea level rise on water resources would therefore benefit from incorporating both physicochemical and biogeochemical processes. This would improve our understanding of the effects of increased salinity on hydrological processes. Our findings stress the need for an interdisciplinary approach to understand processes in terms of multiphase (liquid-gas) flow with shifting chemical equilibria in the hyporheic zone of wetlands threatened by salinization.

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(Enclosure experiment Ilperveld with J. Stelzer, by S. Kosten)

7

Salinization decreases sediment carbon emissions: short- versus long-term effects

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ABSTRACT

Aquatic sediments and wetland sediments in particular contribute to a large extent to the natural methane emission to the atmosphere. Driving factors such as temperature, sediment quality and precipitation patterns have received ample attention. Much less research has been dedicated to the influence of salinity on carbon cycling in wetland sediments, although methanogenesis may be profoundly impacted by changes in salinity. This is an important research gap as salinization of (coastal) wetlands continues to increase on a global scale.

In the present study we show surface water salinization to have a substantial significant decreasing effect on net CH_4 diffusive and ebullitive CH_4 fluxes (up to > 95 % above 2 – 2.5 PSU) in sulfate rich wetlands using a combination of biogeochemical sediment pore water analyses and flux measurements in a long term field experiment and a short term laboratory experiment. The effects of salinization on net CO_2 fluxes to the atmosphere remain unclear and was partly influenced by primary production in the water column in the present study.

Salinity induced enhanced ionic concentrations, sulfate reduction and physiochemical effects showed to be driving factors behind decreased methane fluxes to the atmosphere. The present paper indicates that short term salinity might increase CH_4 fluxes on the short term but does lead to decreased CH_4 fluxes on the long term. Currently the influence of (climate change driven) enhanced salinity on carbon emissions from wetlands is underestimated. Our data suggest the factor salinity to be very influential on wetland CH_4 fluxes. On an aerial basis the effect of salinization can be in the same order of magnitude as other climate change driven factors like temperature.

Key Words: salinity, methanogeneses, sulfate reduction, greenhouse gas emissions, methane ebullition

INTRODUCTION

Aquatic sediments and wetlands emit 30–60% of the total natural methane (CH_4) emission to the atmosphere (Birdgham et al. 2013, Kirschke et al. 2013, Turetsky et al. 2014). Wetland greenhouse gas emissions (GHG) however are very variable as they are the result of the interaction of several driving factors, which leads to uncertainties in total budgets of GHG emissions to the atmosphere (e.g. Kirschke et al. 2013). Driving factors such as temperature (e.g. Gudas et al. 2010, Yvon-Durocher et al. 2014, Marotta et al. 2014), sediment quality (e.g. Gudas et al. 2010) and precipitation patterns (e.g. Kirschke et al. 2013, Gallo et al. 2014) have received ample attention. But changes in salinity may also profound impact the carbon cycling in wetland sediments (Herbert et al. 2015). Although methanogenesis may be profoundly impacted by changes in salinity (e.g. Weston et al. 2006, Herbert et al. 2015) the quantitative effect of increased salinization on methane emissions (diffusive and ebullitive) is however poorly constraint. CH_4 is the second most potent greenhouse gas in the atmosphere after carbon dioxide (CO_2) and has 34 times the global-warming potential of CO_2 over a 100-year horizon (IPCC, 2013).

Effectd of salinization on the carbon cycle and GHG emissions is an important research gap as salinization of (coastal) wetlands continues to increase due to sea level rise, intensified anthropogenic land use (Abacra & Post 2010, Herbert et al. 2015, Cañedo-Argüelles et al. 2016) and increased saline groundwater intrusion in coastal zones (e.g. Werner & Simmons 2009, Oude Essink et al. 2010, Velstra et al. 2011). Increased salinity is known to affect biogeochemical sulfur-, carbon- and nutrient cycles (Herbert et al. 2015). Although salinization has received increasing attention during the last decades the debate on how enhanced surface water salinization affects (1) sediment microbial communities and processes, (2) sediment carbon cycling and (3) carbon fluxes to the atmosphere is still continuing. As wetlands in particular contribute most to global methane emissions, and salinity is known to influence methanogenesis, evidence based knowledge of the effects of enhanced salinity on net carbon emissions is of great importance.

Salinization not only leads to increased ionic concentrations but also to increased concentrations of terminal electron acceptors, in particular sulfate, and altered chemical equilibria which can have several direct and indirect effects on biogeochemical and microbiological cycles in wetlands (Herbert et al. 2015). Effects of enhanced salinity on the carbon cycle reported in literature do however differ (Table 7.1), some studies report enhanced sediment carbon respiration (e.g. Weston et al. 2011, Chambers et al. 2011, Marton et al. 2012) while others report decreased sediment carbon respiration (i.e. Neubauer et al. 2013, Weston et al. 2014). The same holds for carbon fluxes as some studies found no significant effect of salinity on carbon fluxes (e.g. Wilson et al. 2015, Vizza et al. 2017, Welte et al. 2017) or reported increased carbon fluxes (Weston et al. 2011) while others showed the opposite (e.g. Neubauer et al. 2013, Weston et al. 2014).

Table 7.1. Overview of studies reporting the effect of enhanced salinity on carbon fluxes to the atmosphere in freshwater wetlands and coastal wetlands. Studies that report effects on pore water carbon concentrations but lack emission fluxes and studies in non wetland like systems are not included. N.D. = no data, N.S.E. = no significant effect of salinity. In the columns CO₂ and CH₄ a + does indicate an increasing flux as a result of salinization and a – a decreasing flux respectively.

Ecosystem	Effect of enhanced salinity on carbon flux		Method	Reference	
	CO ₂	CH ₄	Laboratory (L) or field (F) conditions	Experimental approach (E _s experimental period < 0.6 months & E _i experimental period > 6 months) versus comparison of systems differing in salinity (S)	
coastal freshwater wetland	+	-	L	E _s	Chambers et al. 2011
freshwater wetland - salt marsh	+	-	L	E _s	Chambers et al. 2013
tidal forest	+	-	L	E _s	Marton et al. 2012
tidal freshwater marsh	+	+	L	E _i	Weston et al. 2011
coastal marshes	+/-	N.S.E.	F	S	Wilson et al. 2015
freshwater coastal wetlands	N.S.E.	-	L	E _i	Van Dijk et al. 2015
tidal freshwater marsh	-	-	F	E _i	Neubauer 2013
tidal freshwater marsh	-	-	L	E _s	Neubauer et al. 2013
tidal marshes	-	-	F	S	Weston et al. 2014
salt marshes	N.D.	-	F	S	Bartlett et al. 1987
fresh to salt marshes	N.D.	-	F & L	E _s & S	DeLaune et al. 1983
tidal marshes	N.D.	-	F	S	Poffenbarger et al. 2011
tidal freshwater to brackish marshes	N.D.	-	F	S	Holm et al. 2016
coastal wetland	N.D.	-	L	S	Vizza et al. 2017
coastal wetland	N.D.	N.S.E.	L	E _s	Vizza et al. 2017
marine mangrove forest to freshwater wetland	N.D.	N.S.E.	F	S	Welti et al. 2017

When the carbon emission between systems are compared along a salinity gradient, the general pattern which is that more saline systems emit less carbon, especially less methane (Bartlett et al. 1987, Poffenbarger et al. 2011, Weston et al. 2014, Holm et al. 2016, Vizza et al. 2017). Although enhanced ionic concentration can affect carbon fluxes (Chambers et al. 2011) it is generally accepted that one of the most important effects of increased salinity for

the carbon cycle is the increase in sulfate availability leading to increased sulfate reduction in anaerobic carbon rich sediments which (1) will affect the iron-sulfur cycle and can influence nutrient availability (e.g. Lamers et al. 2002, Smolders et al. 2006), (2) can lead to increased concentrations of the toxic sulfide (Lamers et al. 2013) and (3) can lead to reduced methanogenesis (Smolders et al. 2002, Weston et al. 2006).

Next to suppressed methanogenesis, it can be expected that microbial mineralization of organic matter, CO₂ production and CO₂ emission will be enhanced as a consequence of salinization due to increased sulfate concentrations and possible other electron acceptors (Chambers et al. 2011, Weston et al. 2011, Marton et al. 2012) such as iron and manganese (which can be mobilized in the sediment due to salinity induces cation mobilization (Van Dijk et al. 2015)).

Most estimations on sediment carbon fluxes reported in literature are based on short term laboratory experiments (days to months) (e.g. Chamber et al. 2011, Chambers et al. 2013, Marton et al. 2012, Neubauer et al. 2013, Vizza et al. 2017) and do often not take long term treatment effects into account; while only very few studies are carried out under field conditions. Additionally, to our knowledge there are no studies reporting on the effects of enhanced salinity on ebullitive fluxes while ebullitive methane fluxes are frequently the dominant emission pathway of methane from peatlands and sediments to the atmosphere (Glaser et al. 2004, Coulthard et al. 2009).

In the present paper we report on effects of increased surface water salinity (short and long term) on sediment carbon fluxes (diffusive and ebullitive) using a combination of a long term (6 year) controlled field experiment with a short term (months) laboratory experiment. We hypothesize that increased surface water salinity will increase CO₂ fluxes and decrease CH₄ fluxes leading to a net decrease of GHG emissions to the atmosphere, at the short and long term. Next, we hypothesize that sediment mineralization processes will switch from methanogenesis dominated to sulfate reduction dominated as a consequence of increased sulfate availability under enhanced surface water salinity.

MATERIALS AND METHODS

Site description

The field experiment was carried out in a large permanent dead-end canal located in a freshwater coastal wetland ([52° 27.225N - 4° 55.885E, Ilperveld, north of Amsterdam (the Netherlands)]. The sediments for the laboratory experiment were collected in the same canal. This wetland is a nature reserve situated in an agricultural landscape with multiple canals connecting both types of land use. The area was originally a freshwater raised bog with

brackish water influence during the last centuries (via brackish surface water and several flooding events) but has been heavily influenced by anthropogenic use since the Middle Ages (Van 't Veer et al. 2009). Although the surface water at the study site is currently almost fresh (0.9 PSU [$14 \text{ mmol l}^{-1} \text{ Cl}$]), the chemical sediment composition and biogeochemical functioning is still influenced by former inundations by brackish water (as reflected by the elevated pore water concentrations of chloride, sodium and sulfur, Van Dijk et al. in prep [Chapter 5]). The canal in which the field experiment was carried out was 10-15 meters wide, with a water depth of 1.3 m with mainly unvegetated aquatic sediment. The aquatic sediment consists of a 14 cm decomposed organic sludge layer on top of 1.5 to 2 m thick layer peat layer (*Sphagnum* moss dominated), this peat layer is based on sequential stratification of peat and clay layers (Westland formation).

Experiment set-up field experiment

To test the effect of increased surface water salinity on sediment carbon fluxes and microbiological processes we placed 16 enclosures (diameter 95cm) and installed 4 control locations outside of the enclosures in June 2010. We created 4 salinity levels (each in $n=4$, but during the experimental period one enclosure was lost, resulting in $n=3$ for the control treatment) ranging from the ambient canal condition of 0.9 PSU [$14 \text{ mmol l}^{-1} \text{ Cl}$] to 9.0 PSU [$140 \text{ mmol l}^{-1} \text{ Cl}$] (see section "salinity treatment" for details). Salinity levels were maintained by regular addition of sea salt to compensate for dilution by rainwater over time to avoid loss by infiltration. To avoid canal-edge effects we placed the enclosures in a row in the middle of the canal and randomly assigned salinity treatments. The enclosures consisted out of stainless steel frames with flexible PVC (polyvinylchloride) foil ($\approx 1 \text{ mm}$ thick) (see Fig. 7.1). The top of the open PVC enclosures was connected to a steel frame via rubber bands, keeping the top of the PVC foil 30 cm above the water surface (Fig. 7.1). The enclosures were pushed 40 cm into the sediment, the bottom was open to keep the interaction between surface water, the atmosphere (precipitation and evaporation) and the deeper sediment intact.

Samples for the present study were taken from spring to autumn in 2016, six years after the start of the experiment. We focused on the warmest months of the year as previous studies have shown that these months have highest emissions, ebullition for instance largely stops below a temperature of 10°C (Aben et al., in press). Difference in spring to autumn emissions between treatments will therefore likely reflect differences in year-round cumulative fluxes. The placement of enclosures might influence sediment processes, especially in long term field experiments as the present study (Van Dijk et al. in prep [Chapter 5])). To obtain insight in the effect of the enclosures on diffusive and ebullitive fluxes we also measured outside the enclosures, these fluxes were found not to differ significantly from fluxes in the control treatment (data not shown).

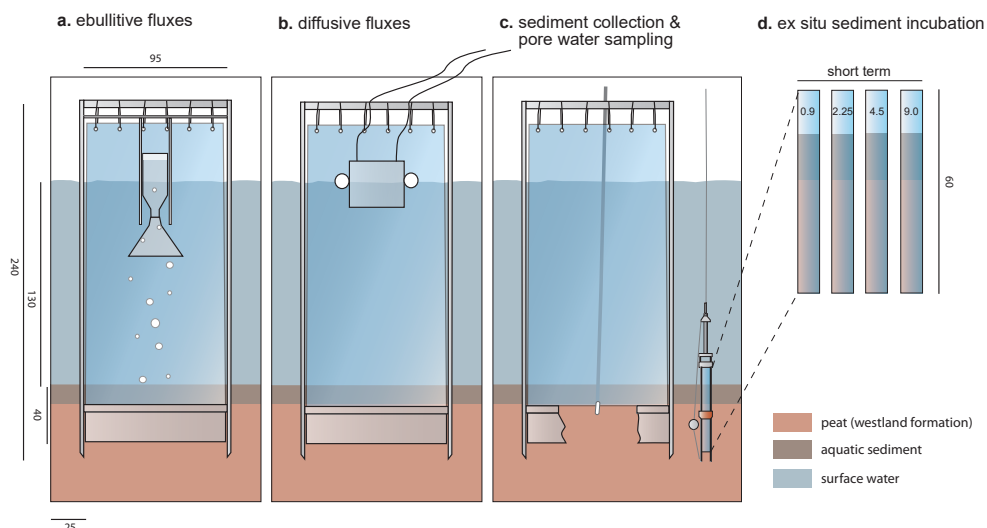


Fig. 7.1. A schematic view of the field experiment with enclosures (a-c) and the laboratory experiment (d). (a) measurements of ebullitive gas fluxes, (b) measurements of diffusive fluxes, (c) collection of sediment with corer outside the enclosures and pore water sampling with ceramic cup inside the enclosure, (d) ex-situ laboratory experiment with sediment in cores, numbers denote salinity treatment (in PSU). Scales in cm.

Experimental set-up laboratory experiment

To test differences between the effects of long term versus short term surface water salinization on sediment carbon fluxes sediment cores were collected in the same canal as where the enclosure experiment was carried out. Sediment cores were collected in 1.8 mm thick transparent PVC cores (diameter 6cm, height 60cm) with a UWITEC sediment corer (UWITEC, Mondsee, Austria) around the enclosures (Fig. 7.1 c & d). Sediment cores were filled with surface water from the study site, sealed off and transported to the laboratory within several hours and were placed under controlled conditions (dark and 10 °C). After stabilization (24 h) under controlled conditions the surface water on top of the sediment was removed (up to some millimeters above the sediment, to prevent oxygen entering the sediment), salinized to treatment level by the addition of artificial sea salt, and returned to the core.

Salinity treatment

In both the field experiment and the laboratory experiment local surface water was used which was salinized up to treatment level by adding artificial sea salt (Tropic Marin®). Four different salinity treatments were used, from 0.9, 2.25, 4.5 to 9.0 PSU (14, 35, 70 to 140 mmol l⁻¹ Cl). Two control treatments (0.9 PSU) were used, one inside the enclosures (n=4) and one outside the enclosures (n=4).

Diffusive carbon flux measurements

Diffusive carbon (CO_2 and CH_4) fluxes from surface water to the atmosphere in the field experiment were measured monthly from May to September (with the exception of August as there was no greenhouse gas (GHG) analyzer available) using an in situ floating transparent chamber connected in a closed loop to a GHG analyzer (model GGA-24EP, Los Gatos Research, Santa Clara, CA, USA) using Off-axis Integrated Cavity Output Spectroscopy (OA-ICOS) technology in September we used a Ultraportable Greenhouse Gas Analyser (Los Gatos Research, Inc. USA.). Both devices yielded similar results in a comparative test, ensuring consistency of measurements. The chamber (headspace volume about 8-10 L, diameter of 14.55 cm and height of 12 cm) was placed floating, half submerged, connected to the gas analyzer (as in Almeida et al. [2016]) on the surface water of each enclosure (see Fig. 7.1b). The gas diffusion measurements were carried out in duplicate (during daytime, 09:00 AM 6:00 PM) for about three to five minutes assuring that we obtained a linear slope during a minimal of 3 minutes. If ebullition occurred - which can be observed as a sudden increase in GHG concentrations interrupting the linear slope - the measurement was started again. Fluxes were calculated according to, using the following equation:

$$F = \frac{V}{A} * \text{slope} * \frac{P * F_1 * F_2}{R * T} \quad (1)$$

Where, F is gas flux ($\text{mg m}^{-2} \text{ d}^{-1}$), V is chamber volume (m^3), A is chamber surface area (m^2), slope is the change in GHG concentration over time (ppm/second); P is atmospheric pressure (atm); F_1 is the molecular weight for CO_2 (44.01) or CH_4 (16.04; g mole^{-1}); F_2 is the conversion factor of seconds to days; R is the ideal gas constant $0.082057 \text{ L atm K}^{-1} \text{ mol}^{-1}$; and T is gas temperature in Kelvin (K).

In the laboratory experiment diffusive carbon fluxes were measured using a similar method at $t = 1, 5, 9$ and 13 weeks. Before the measurements, surface water was carefully removed from the core to exclude processes in the water column (such as methane oxidation and dissolving of CO_2) to interfere with our assessment of sediment CO_2 and CH_4 release. Subsequently the core was closed with a lid with tubes connected to the gas analyzer. Gas diffusion measurements and flux calculations were done using equation 1. Diffusive CO_2 fluxes from the long term field experiment showed to be influenced by processes in the water column (e.g. growth of algae) to such an extent that these results are given less attention.

Ebullitive methane flux measurements

To estimate ebullitive methane fluxes in the field experiment one inverted funnel (base opening diameter of 23 cm) connected to water-filled 1L glass bottles was placed (from June -October) half submerged in each enclosure, see Fig. 7.1a). The bottles were replaced after

an average period of one month. Subsequently, the bottles were taken to the laboratory where the headspace volume was determined and the methane concentrations in the headspace was analyzed using 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 N₂ as carrier gas. The amount of gaseous CH₄ in each bottle was determined by multiplying the CH₄ concentration (C_{gas}) by the volume of gas (V_{gas}). The CH₄ in the bottles was assumed to be in equilibrium with the water phase. Hence, the amount of CH₄ dissolved in the water (C_{water} * V_{water}) was calculated using Henry's law and its solubility constant for CH₄, taking the respective water temperature into account. The total amount of CH₄ in each bottle was calculated by summing the aqueous and gaseous content and divided by funnel surface (A) and time (Δt) to calculate CH₄ ebullition per square meter (equation 2).

$$\frac{(C_{gas} * V_{gas}) + (C_{water} * V_{water})}{\Delta t * A} \quad (2)$$

Total CH₄ fluxes were calculated by taking the sum of the average ebullitive flux over the period before the diffusive flux and the diffusive flux. This total CH₄ flux was related to the element pore water concentrations sampled at the day of the diffusive flux measurement. To correlate CH₄ fluxes with temperature and air pressure meteorological data was obtained from the weather station of the KNMI at Schiphol (www.knmi.nl/nederland-nu/klimatologie). Mean monthly difference in air pressure was calculated by subtracting the mean minimal air pressure over five day periods of the mean maximal air pressure over five day periods during the different periods the bottles for the ebullitive flux sampling were in the field.

Sediment pore water sampling and analyses

Sediment pore water samples for chemical analysis were collected at a depth of 5 cm in the sediment with a 60ml vacuumed syringe connected to a ceramic cup via a Teflon tube (from May – September). For analyses of pore water total sulfur, calcium, potassium and total phosphorus inductively coupled plasma spectrophotometry (ICP-Optical Emission Spectrometer, Thermo Scientific iCAP 6000 Series ICP) was used. Prior to elemental analyses, 10 ml of each sample was stored at 4°C until analyses with 0.1 ml (65%) HNO₃ to prevent element precipitation. To determine pore water 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). Other sediment pore water samples were collected for pore water methane and sulfide analyses by connecting vacuumed 12 ml glass exetainers (Labco exetainer®, High Wycimbe, UK) with 0.5 ml of 1M HCl to the same ceramic cups. Concentrations of methane and sulfide were measured in the headspace (after removing the vacuum with N₂ gas) of the exetainers using gas chromatography and recalculated for the water volume using Henry's constant. Methane concentrations were measured as described in the previous paragraph. Sulfide concentrations analyzed using a 7890B gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped

with a Carboxpack BHT100 glass column (2 m, ID 2 mm), flame ionization (FID) and flame photometric detector (FPD).

Data analysis

Data was tested for normal distribution (Shapiro–Wilk tests and visual inspection of Q–Q plots). Not normally distributed data were tested for significant differences using Kruskal–Wallis tests. Normally distributed data were checked for equality of error variances using Levene’s tests. Significant differences among groups were assessed using one-way ANOVAs with LSD post hoc tests for equal variances or using Welch tests and GamesHowell post hoc tests for non-equal variances. Correlations between concentrations of different compounds in the pore water were defined using Pearson correlation. All tests were carried out using SPSS Statistics for Windows (Version 24 IBM Corp. Armonk, NY, 2016).

RESULTS

Long term salinization effects on carbon fluxes to the atmosphere

Net diffusive CO_2 and CH_4 fluxes from the sediment were found to vary in time (Fig. 7.2), and showed clear differences between the different salinity treatments. Diffusive CH_4 fluxes were significantly reduced in all increased salinity treatments ($P < 0.05$) in June, July and September. Diffusive CO_2 fluxes on the contrary reacted less strong to increased salinity and were significantly higher in increased salinity treatments ($P < 0.05$) in some months, (Fig. 7.2).

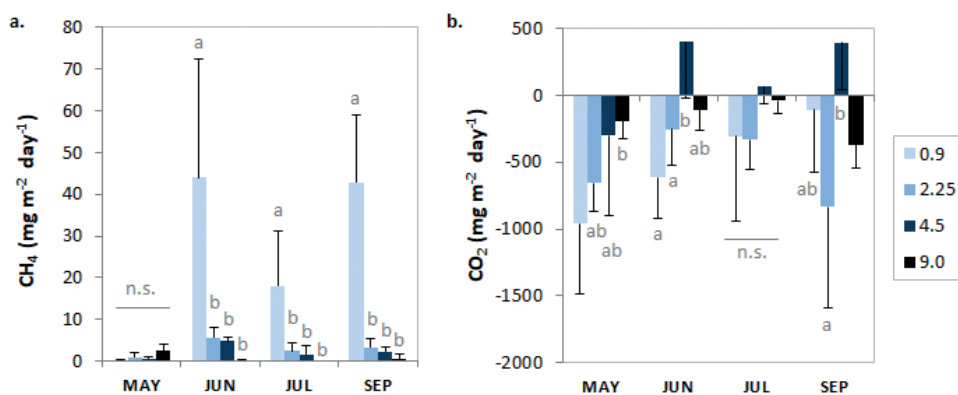


Fig. 7.2. Diffusive fluxes for (a) CH_4 and (b) CO_2 at four moments during the year for the four different salinity treatments (0.9 up to 9.0 PSU). Values are presented as means + St.dev. Significance of differences between groups is indicated per month with letters ($P < 0.05$), n.s. = not significantly different.

Enhanced surface water salinity significantly reduced ebullitive methane fluxes in all salinity treatments ($P < 0.05$) (Fig. 7.3a). Compared to the control treatment (0.9 PSU) the highest salinity treatment (9.0 PSU) had, on average, a 99.9% (from 7.36 to 0.007 $\text{mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$) reduced ebullitive methane flux. The volume of the ebullitive gas flux was also reduced under enhanced surface water salinity (see Fig. 7.3b).

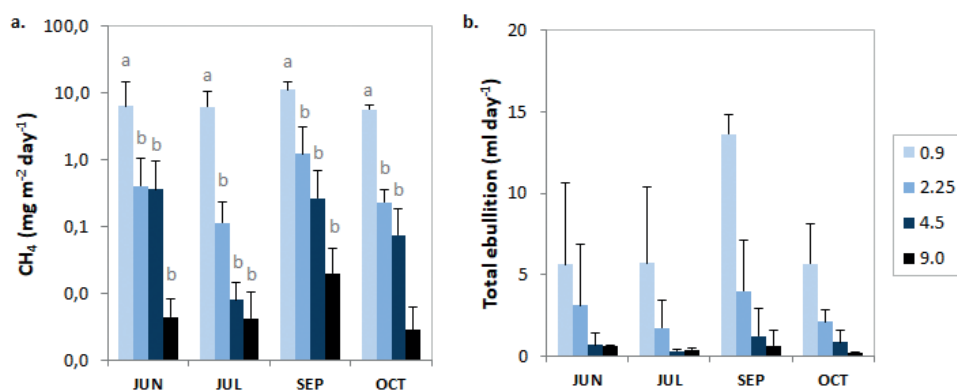


Fig. 7.3. a). Ebullitive CH_4 flux (a) presented on a log scale and (b) total ebullitive gas flux at four moments during the year for the four different salinity treatments (0.9 up to 9.0 PSU). Values are presented as means + St.dev. Significance of differences between groups is indicated per month with letters ($P < 0.05$) n.s. = not significantly different.

Both diffusive and ebullitive methane fluxes in the control treatment showed a positive correlation with average temperature (see Fig. S 7.1 Appendix). Average monthly fluctuations in air pressure did not correlate with ebullitive methane fluxes in the control treatment (see Fig. S 7.1 Appendix).

Carbon fluxes under long term salinization, the role of sediment biogeochemistry

Ebullitive methane fluxes correlated negatively with pore water chloride and sulfide concentrations (R^2 0.72, $P < 0.01$, R^2 0.69, $P < 0.01$ respectively) (Fig. 7.4 a & b). Pore water element concentration like phosphorus, ammonium and other ions were strongly linked to enhanced salinity and showed weaker or not significant correlations with ebullitive methane fluxes (data not shown). Pore water chloride, pore water sulfide concentrations and pore water sulfate concentrations (data not shown) however showed a significantly negative correlation with pore water methane concentrations (R^2 0.76, $P < 0.01$, R^2 0.75, $P < 0.01$ respectively) (Fig. 7.5 a & b). Average element pore water concentrations differed per treatment, but did not differ over time, (see table S. 7.1 with average pore water concentrations in supplementary material).

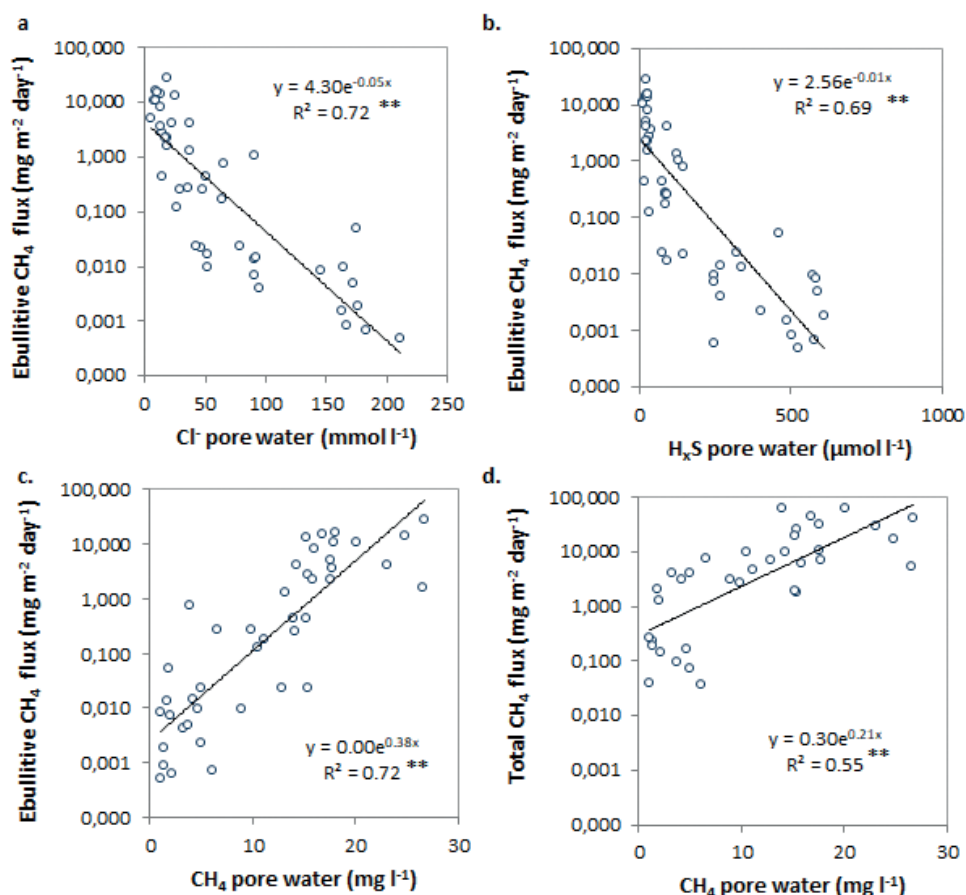


Fig. 7.4. (a) correlation of pore water CH_4 concentration with ebullitive CH_4 fluxes, (b) correlation of pore water CH_4 concentration with total CH_4 flux (diffusive + ebullitive), (c) correlation of pore water Cl^- concentration with ebullitive CH_4 fluxes, (d) correlation of pore water H_2S concentration with ebullitive CH_4 fluxes. Single and double asterisks indicate significance at the $P < 0.05$ and $P < 0.01$ level, respectively.

Ebullitive methane fluxes showed a strong positive correlation with sediment pore water methane concentrations (R^2 0.72, $P < 0.01$) Fig. 7.4c. The total methane flux (diffusive + ebullitive) also showed a correlation with pore water methane concentrations, this correlation is however less strong (R^2 0.55, $P < 0.01$) Fig. 7.4d).

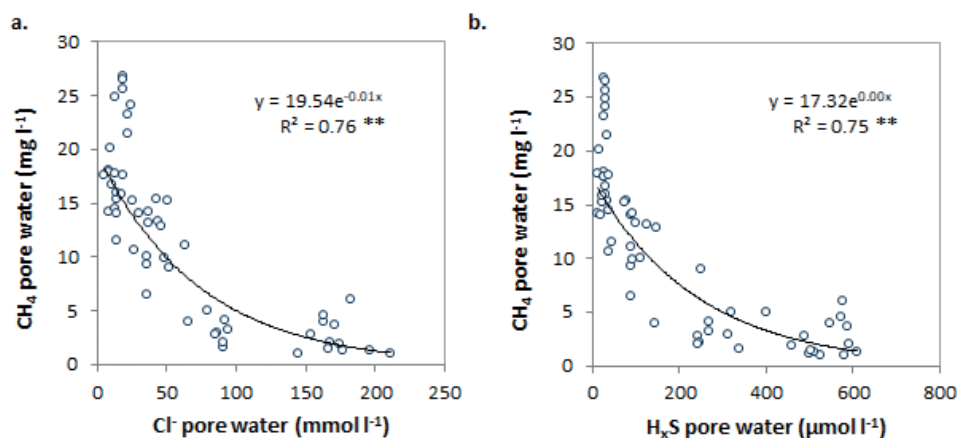


Fig. 7.5. correlation of pore water CH_4 concentration with pore water Cl^- concentration (a), and pore water H_2S concentration (b). Double asterisks indicate significance at the $P < 0.01$ level.

Short term effect of salinization on carbon fluxes

In contrast to CH_4 fluxes from the field experiment the short term laboratory experiment showed significantly higher average diffusive sediment CH_4 release in the highest salinity treatment after week 1 and week 5 (Fig. 7.6 a & c). However, diffusive sediment CH_4 release rapidly decreased in time in the increased salinity treatments and after 13 weeks the pattern observed for the diffusive flux was comparable to the pattern for the long term fluxes measured in the field. After 13 weeks the carbon dioxide emission became significantly lower for the two highest salinity treatments.

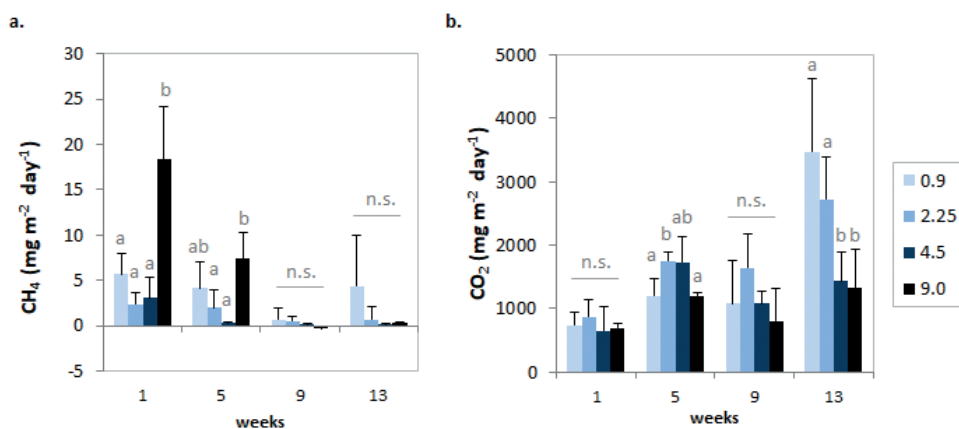


Fig. 7.6. Diffusive fluxes of CH₄ and CO₂ in sediments under short term salinization incubated in the laboratory for 13 weeks. The same salinity treatments were used as in the field experiment (0.9 up to 9.0 PSU). Values are presented as means + St.dev. Significance of differences between groups is indicated per month with letters ($P < 0.05$) n.s. = not significantly different.

DISCUSSION

Short term versus long term salinization

Our results reveal a large difference between short term and long term enhanced salinity on methane fluxes. Increased salinity within a range of 0.9 to 9.0 PSU resulted in a > 95 % decrease of methane fluxes in the long term whereas an opposite effect (threefold increase) was found shortly after sediments were exposed to high salinities. Interestingly a laboratory experiment of Weston et al. (2011) also showed enhanced methane flux as a result of enhanced salinity during the first months of their experiment. These contrasting findings likely explain the discrepancies found in literature regarding the effect of salinization on CH₄ fluxes as reviewed in table 1. These findings furthermore indicate that the GHG emission from large areas of wetlands around the world are prone to be severely altered due to ongoing sea-level rise, intensified anthropogenic land use and increased saline groundwater intrusion in coastal zones. The exact area of wetlands that are threatened by salinization is unknown but $1.5 \cdot 10^6$ km² of forest, wetlands or other protected areas are salt affected (Wicke et al. 2011) hinting the large extent to which wetland salinization may be occurring (Herbert et al. 2015), likely leading to an overall decrease in CH₄ emission in the long run.

An explanation for enhanced methane fluxes on the short term could be a temporarily enhanced methanogenic activity due to a salinity induced mobilization of dissolved organic matter (DOC). Although salinity dependent DOC mobilization is still poorly understood (Herbert et al.

2015), it could be caused by changes in the macromolecular structure of the organic matter with increased ionic strength (Tombacz & Meleg, 1990), or the die-off of microorganisms or (benthic) algae after sudden increase in osmotic stress. Both processes may result in short term enhanced availability of DOC (Nyang, 2003; Weston et al., 2006) which could temporarily stimulate methane production. Enhanced salinity might also stimulate methane fluxes on the short term by reducing the solubility of methane (Yamamoto et al. 1976, Wiesenburg & Guinasso 1979) which will result in enhanced bubble formation and ebullition. Ebullitive fluxes do only occur when the pore water reaches oversaturation of methane.

The present paper showed surface water salinization to significantly reduce both diffusive and ebullitive methane fluxes on the long term, above the threshold of pore water chloride concentrations of 40 mmol l⁻¹ (2.6 PSU), (on average 96.3 % reduction of diffusive CH₄ flux and 99.9% reduction of ebullitive flux in the highest salinity treatment versus the control treatment in the field experiment). This reduced methane flux to the atmosphere can very well be explained by salinity induced enhanced sulfate reduction as reported by several studies on the effect of enhanced salinity on carbon fluxes (e.g. Barlett et al 1987, Weston et al 2006). Decreased pore water methane concentrations, increased pore water sulfide concentrations and the significant negative correlations between pore water chloride, sulfate and sulfide, with pore water methane and (ebullitive) methane fluxes found in the present study are strong indications for a shift from a methanogenesis dominated sediment to a sulfate reduction dominated sediment under salinization. Sulfate reduction mediated depressed methanogenesis is caused by a combination of processes. First the process of sulfate reduction is thermodynamically more energy efficient (Kristjansson et al. 1982, Segers 1998) leading sulfate reducers to outcompete methanogens for organic substrates (e.g. acetate) which can lead to a complete inhibition of methanogenesis (Lovley et al. 1982, Lamers et al. 1998, Smolders et al. 2002). Second, the process of sulfate reduction can lead to physiological stress for methanogenic bacteria (Chambers et al. 2011) especially in the case of salinization where the combination of two physiological stressors are present; sulfide and increased ionic strength (Weston et al. 2006, Lamers et al. 2013).

As both methanogenic and methanotrophic bacteria occur over a wide salinity range (e.g. methanogenic activity was even found to occur in hypersaline environments (McGenity 2010)), one could expect the communities to adapt to enhanced salinity conditions over time. The present paper does however indicate the methanogenic bacteria in the present study still to be suppressed after six years of enhanced salinity.

The process of (an)aerobic methane oxidation within the sediment or the sediment-water boundary could also influence net methane fluxes (Valentine 2002). Enhanced salinity could potentially influence methane oxidation and especially iron- and sulfate-mediated anaerobic methane oxidation. Especially in marine ecosystems the consortium of anaerobic

methanotrophic archaea and sulfate reducing bacteria have shown to be potentially of great influence on anaerobic methane oxidation and net methane emissions (Reeburgh 2007). Both iron- and sulfate-mediated anaerobic methane oxidation were reported from a broad salinity range (Valentine & Reeburgh 2000, Le Mer & Roger 2001, Egger et al. 2014), indicating that these processes might have been of influence in the net methane flux in the present study. Sensitivity of methanotrophs for short term and long term enhanced salinity and salinity induced biogeochemical consequences is however still poorly understood. It is therefore difficult to assess the influence of (an)aerobic methane oxidation on net methane fluxes in the present study.

The short term laboratory experiment showed significant negative treatment effects on sediment diffusive CO₂ fluxes. Due to high interference in the water column the long term effects of enhanced salinity on CO₂ fluxes are not discussed in detail.

Salinity induced physiochemical effects

Salinization is known to influence a range of physiochemical and biogeochemical processes, which can have consequences for sediment pore sizes and movement of gasses and water within them. Controlling mechanisms of sediment gas bubble formation, pore water and gas movement and gas bubble emissions is however still poorly understood (e.g. Kellner et al. 2004, Kellner et al. 2006, Kettridge et al. 2012, Liu et al. 2016). A full understanding of the mechanisms regulating methane ebullition in particular from wetlands (which are known for heterogeneity in structure, pore size and hydraulic conductivity (e.g. Stofberg et al. 2016)) is therefore still incomplete (e.g. Kellner et al. 2006, Comas et al. 2008, Bon et al. 2013, Chen & Slater 2015). How enhanced salinity might influence these mechanisms and interactions is even more difficult to predict. One of the known physiochemical effects of enhanced salinity is flocculation of the organic acids located on the surface of peat fibers within the sediment resulting in sediment pore dilation, which can influence the hydraulic conductivity of peat soils (Ours et al. 1997, Hoag & Price 1997, Comas & Slater 2004, Van Dijk et al. 2017). Our previous work in a subset of the same enclosures (Van Dijk et al. (2017)) showed that an increase in salinity can significantly enhance sediment hydraulic conductivity. We related this increase in hydraulic conductivity in part to pore dilation but we speculated that it was to a large extent caused by a decrease in gas production in the sediment as gas bubbles can effectively block vertical water movement (Kellner et al., 2004). The present work confirms the negative relationship between gas production in the sediment and hydraulic conductivity (Fig. 7.7) study. This salinity induced reduced methanogenesis and increased hydraulic conductivity might result in enhanced infiltration and increased groundwater salinity in freshwater aquifers with net infiltration.

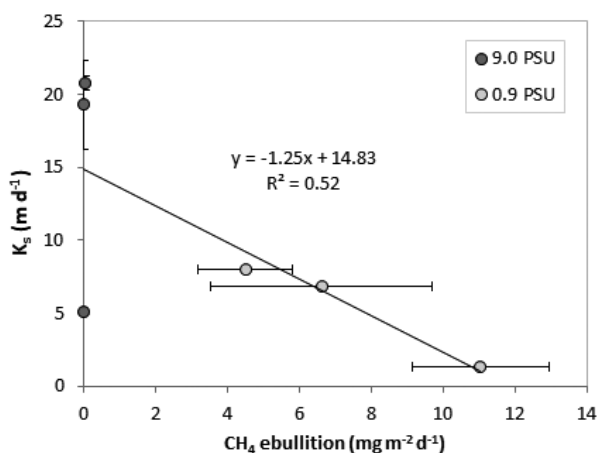


Fig. 7.7. The correlation ($P = 0.107$) between the ebullitive methane flux (average of $n=4$ points in time $\pm$ SEM) and the hydraulic conductivity (average of $n=3$ measurements $\pm$ SEM, data based on Van Dijk et al. (2017) in the same enclosures of the control ($n=3$) and highest salinity treatments ($n=3$).

Salinity induced pore dilation in combination with faster oversaturation of methane might stimulate ebullitive methane fluxes on the short term which will also force other bubbles and porewater to move and create preferential flow paths (Boudreau et al. 2005, Scandella et al. 2011) which might accelerate the short term ebullitive flux.

Salinization versus other environmental factors

Clearly environmental factors other than salinization influence diffusive and ebullitive CH₄ and CO₂ fluxes to the atmosphere. Factors like temperature (Gudas et al. 2010, Yvon-Durocher et al. 2014), available organic matter as substrate for microorganisms (Vizza et al. 2017, Welti et al. 2017) are known to influence diffusive CO₂ and CH₄ fluxes. Increases of 6–20% in methane ebullition per 1°C increase have been reported (Aben et al. in press). Within the present paper temperature was also positively correlated with diffusive and ebullitive fluxes (see Fig. S 7.1 Appendix). Air pressure fluctuations are known to influence the timing of ebullitive methane fluxes (e.g. Waddington et al. 2009), and was not shown to correlate with ebullitive fluxes in our study. Although the climate warming related predicted CH₄ emissions from wetlands may play a role in a larger area of wetlands, on an aerial basis the effect of salinization is considerably larger with an increase in salinity above 2 – 2.5 PSU leading to a possible reduction in CH₄ emissions up to > 95% as observed in our study. Clearly chemical sediment composition will influence the effect of salinization on a local scale.

Although we show the factor salinity did significantly decrease methane fluxes there might be an interaction with salinity and the availability of organic matter as substrate for methanogenic bacteria. The availability of organic matter was however not studied within the present paper, some studies have shown the availability of organic matter to overrule the effect of salinization (Vizza et al. 2017, Welti et al. 2017). Weston et al. (2011) for example, found an increase of both methanogenesis and sulfate reduction after an combined increase of salinity and available organic matter. Several studies also showed that sediment characteristics in general do influence the effects of enhanced salinity on methanogenic activity and net carbon fluxes (e.g. Neubauer et al. 2013, Wilson et al. 2015, Van Dijk et al. 2015). The driving factors influencing the effect of the interaction of salinization and sediment composition on the availability of organic matter and methane fluxes do warrant further study.

CONCLUSION

The present paper shows that enhanced surface water salinity can significantly reduce carbon emissions from wetlands to the atmosphere in the long term. In a six year field experiment and laboratory experiments both diffusive and ebullitive methane fluxes were reduced up to > 95 % above 2 – 2.5 PSU. A short term laboratory experiment, however showed that an increased salinity could result in an increased methane emission during the first weeks of the experiment. Suppressed methanogenesis due to the increase availability of sulfate stimulating thermodynamically more favorable sulfate reduction, very probably is key drivers behind the suppressed methane fluxes to the atmosphere. Nevertheless additional factors such as salinity induced physiochemical effects in the sediment might also play a role. The present paper indicates that short term salinity experiments do deliver insight in short term effects but might deliver a misleading outcome when compared to results obtained from long term field experiments.

Currently the influence of salinization on carbon emissions from wetlands are underestimated and might affect water management and ecosystem services of wetlands. The present paper showed that the factor salinity can have a strong effect on wetland methane fluxes, in the same order or magnitude of even higher as other climate change driven factors like temperature, which received proportionally more attention in literature.

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SUPPLEMENTARY MATERIAL

Table S 7.1 Average pore water concentrations a 15 cm of depth in the sediment in the four salinity treatments of the field experiment during the period carbon fluxes were measured (may-sep).

PSU	Cl	Na	Ca	K	S _(tot)	H _x S	CH ₄	P _(tot)	NH ₄
					mmol l ⁻¹				μmol l ⁻¹
0.9	11.9	18.3	1.2	0.2	0.3	25.6	1007.1	32.3	424.7
2.25	40.3	31.6	1.9	0.4	0.9	99.3	582.0	16.7	313.8
4.5	86.9	68.9	2.1	0.9	2.7	241.4	301.9	25.7	440.2
9.0	164.7	145.9	2.7	2.3	6.3	526.9	194.2	12.8	268.0

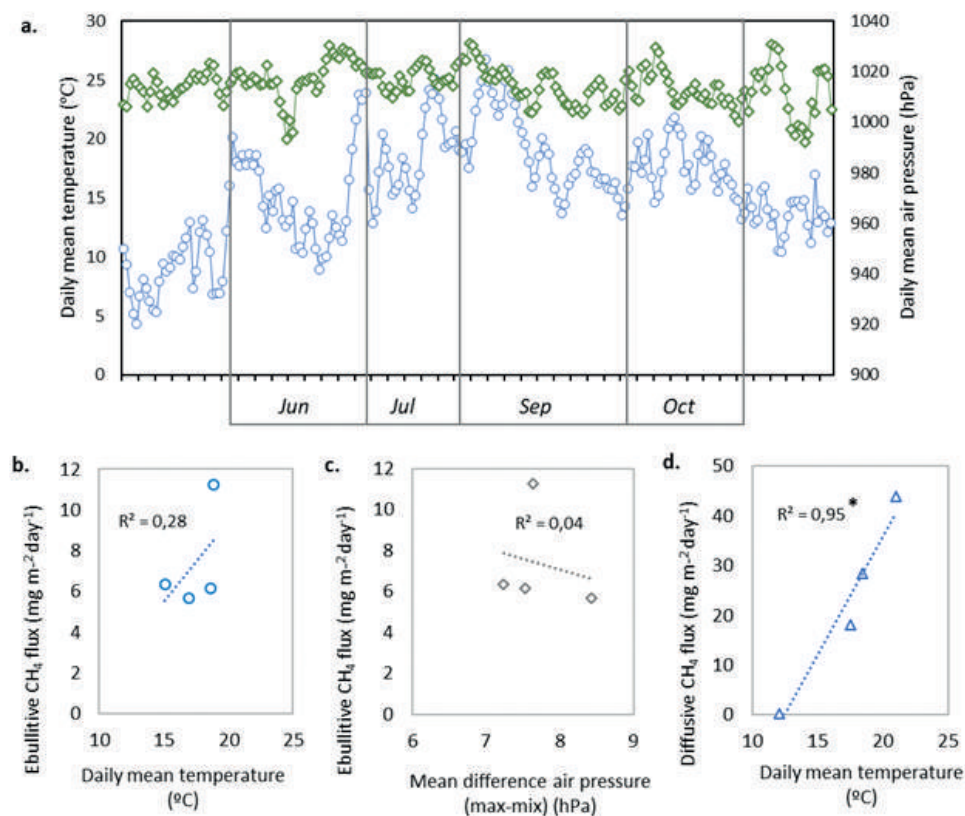


Fig. S 7.1. Daily mean temperature and mean air pressure near the study area [data from KNMI at meteorological station at Schiphol], the sampled periods of ebullitive fluxes are indicated with rectangles. b. correlation between daily mean temperature during the sampling period and the ebullitive CH₄ flux. c. correlation between the mean difference in air pressure during the sampling period and the ebullitive CH₄ flux. d. correlation between the mean daily temperature (5 days before sampling) and the diffusive CH₄ flux. All correlations presented are carbon fluxes from the control enclosures. A single asterisks does indicate significance at the $P < 0.05$ level.



[Sampling on the Sehestedter bog, by L. Lamers]

8

Biogeochemistry and vegetation of a rare, endangered floating coastal raised bog remnant in Europe, the Sehestedter bog

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submitted to Wetlands

ABSTRACT

The majority of coastal bogs (evolved as freshwater bogs, which in recent history got influenced by the sea) have either been eroded or suffered from anthropogenic forcing and lost their natural functioning and contact with the sea. Here, we report on a bog remnant still influenced by saline water which floats during storm floods. We investigated biogeochemical processes along gradients and discuss mechanisms that enhance buoyancy which is of vital importance for the conservation of the bog.

Saline influence does show a steep gradient and marine clay deposits within the peat provide the 'hinge' above which the peat is floating. Our results provide important insight into the functioning of former coastal bogs and do point out that buoyancy of the remnant is driven by a combination of various factors: the difference in densities between the bog and sea water, desiccation along the edges and methane production in the bog. If coastal bogs lose their ability to float the impact of erosion and the sum of several other processes (i.e., peat decomposition, salt stress, clay sedimentation, internal eutrophication and depressed methanogeneses) will cause a shift in environmental conditions and lead to loss of buoyancy and consequential loss of characteristic species.

Keywords: salinity, raised bog, buoyancy, Sehestedter Aussendeichsmoor, coastal wetland

INTRODUCTION

In former times, large raised bog complexes along the coast (evolved under freshwater dominated conditions) were often directly influenced by the sea along the edges on the seaside during later stages in their evolution; some only during storm floods, others more often. Already for centuries humans did had an increasing influence on coastal bogs. From roughly 1000 years AD onwards large parts of the coastal bogs in North-western Europe became disconnected from marine influence by dike construction, or were drained for agricultural use or turf extraction (Pons 1992). Former coastal peat layers influenced by seawater were also used for the exploitation of salt after burning (called 'selnering' which has been described for a series of locations along the Wadden Sea coast (Borger 1992, Van Geel & Borger 2002, Behre 2005, Leenders 2013)) which did probably increase the vulnerability of coastlines to flooding.

The remaining inland peatlands in coastal regions are often remnants of former large coastal bogs and include large parts of the North-western part of the Netherlands and Germany. Although these bogs often were close to the coast, they were formed in periods with high fresh groundwater influence (due to regression phases of the North Sea or the formation of elevated levees) and switched later in time to dominantly rainwater bogs. Due to the increased freshwater influence saltmarsh like condition did change into suitable conditions for swamps and which resulted in the formation of fen peat (from 8000-4000 BP). Plants like *Phragmites* sp., *Typha* sp., *Carex* sp. and *Sparganium* sp. did form fen peat and carr forests and as the influence of groundwater reduced large ombrotrophic (i.e. rainwater-fed) raised bogs were formed (Behre & Kucan 1999, Bakker & Van Smeerdijk 1982, Pons 1992, Vos 2015). Some of the coastal raised bogs became influenced by salinity in a later stage due to rising seawater levels, flooding events and anthropogenic influences. The majority of these coastal bogs lost their natural functioning for coast protection and were eroded, drained or exploited and often lost direct hydrological connection with their surroundings. The study area in the present paper is the last raised bog remnant still under direct marine influence, not eroded yet, present in Europe.

For the management and restoration of coastal peatlands, which often already have undergone significant anthropogenic change, information on the (historic) functioning of coastal peatlands is of major importance. As large parts of anthropogenic influenced coastal bogs were impacted by floods of salt water in the past and present coastal wetlands might suffer from increased salinity levels in future due to rising sea level or anthropogenic causes knowledge of the functioning of intact coastal bogs is important (Herbert et al., 2015). At present, however, reference sites for large European coastal peatlands are absent, except for the present study area. Almost all European coastal peatlands have either been lost or modified to such an extent that they can no longer serve as a reference. In North-western Germany, however, a remnant

of a former large bog along the coast is still present. The area (Sehestedter Aussendeichsmoor or Schwimmendes Moor) is a small raised bog remnant on the seaside of the dike. According to Behre (2005) this is the last remaining European raised bog remnant outside of a dike that is subjected to marine influence during storm tide and storm floods. During high storm floods the sea water level increases up to more than 1.6 meters above average high tide which can cause flooding of the bog remnant if it would not float. Due to the buoyancy of the bog, which is discussed in further detail later in the present paper, the bog remnant does however start floating during high storm tides. The ability to float reduces the impact of salt water on the bog, if the remnant would not float it will be flooded. This feature of floatation during storm floods is a phenomenon which occurred more often in several former coastal bogs, but has become an unique feature as nowadays the Sehestedt Bog is the last remaining place in Europe where this phenomenon still occurs. The bog only does float during storm flood when seawater levels reach 1.6 m or more above mean high tide. In recent years the bog floated several times; in winter 2006/2007 this occurred even three times (Behre 2007b). Although the history and deterioration of the bog remnant and the influence of storm flood have been described in detail (Künnemann 1941, Behre & Kucan 1999, Behre 2005, 2007b), so far little attention has been paid on the biogeochemical functioning of the bog remnant. More insight in the biogeochemical functioning of the bog can however help to understand which mechanisms do maintain the ability to float and how changes in salinity levels might influence the functioning of bogs. In the present paper we therefore report on the biogeochemical characteristics of the soil and pore water of the bog remnant and analyze its functioning related to salinity and the implications for future conservation. Special attention is paid on both horizontal and vertical salinity gradients in the bog and the impact of these gradients on ability to float and future consequences for present characteristic species composition.

MATERIAL AND METHODS

Site description

The Sehestedter Aussendeichsmoor or 'Schwimmendes moor' (German for swimming or floating peatland; further called Sehestedt Bog) is a raised bog remnant of a former extensive raised bog formed behind an elevated levee along the coast in the Jade Bay in North-western Germany (Figure 8.1). Peatland formation started with swamp and minerotrophic peat accumulation during the regression phase in the North Sea between 1500 and 1000 B.C., when a shift occurred from marine to brackish and freshwater conditions which resulted in the occurrence of swamps and fenpeat formation (Behre 2003, 2005, 2007b). During further succession minerotrophic fens and alder woods developed, which eventually shifted to a raised bog dominated by peat mosses (*Sphagnum* spp.) during the next regression of the North Sea by the end of the last century B.C. (Behre 2003, 2007a). At the beginning of the 13th century

storm surges broke through the elevated levee which caused large erosion of swamps behind the levee and started the formation of the Jade Bay (Behre 2005). During several centuries the swamps and parts of the coastal bogs eroded away and the Jade Bay extended far beyond its present size. Although several storm floods led to major erosion the coastal bog had a special resistance for flooding as during storm tides the seaward side bog starts floating which prevents the bog being flooded (Behre 2005).

During the last centuries however a large proportion of the bog was eroded during storm floods. In 1725, a dike was built which separated the bog in a part outside of the dike and an inland part of the remaining bog. The bog area outside covered an area of 165 ha in 1725, which was reduced due to erosion to 107 ha in 1820, to 21 ha in 1932 and to a present area of about 9.5 ha (Behre 2005). The bog remnant is boarded with a 8 m dike on south-eastern side and saltmarsh vegetation at the north-western side and is surrounded by a small band of reed (*Phragmites australis*) around the bog (see Figure 1). The remnant itself is covered a vegetation composition associated to raised bogs (*Sphagnum* spp., *Andromeda polifolia* and *Oxycoccus palustris*) as well as spreading bush vegetation of *Myrica gale* with elements of heather vegetation communities (*Calluna vulgaris* and *Erica tetralix*) and Birch wood (*Betula pubescens*).

Field sampling

In October 2012, vegetation surveys and electric conductivity (EC) depth profiles were made on eight locations along a transect between the salt marsh and the dike. (figure 8.1). At four locations (nr. 2, 4, 6 & 8 in figure 8.1) along the transect, soil, pore water and pore water gas samples were taken at different depths (from 0 to 4.5 m of depth below the surface). Three of the four sample locations were taken on the bog itself (nr. 2, 4 and 6) and one (nr. 8) on the salt marsh (Figures 8.1 and 8.2). Vegetation surveys were carried out for 2x2 m squares using the Braun Blanquet scale. EC depth profiles were carried out with WTW conductivity probe.



Fig. 8.1. Aerial picture of the northern part of the Sehestedt bog (©Google Earth), showing the study transect (yellow dots indicate depth profile sites). The sea is on the western side of the dike. At the right bottom a map of the North western region of Germany in white, in grey the north east of the Netherlands, the red dot indicates the study area.

Anaerobic soil pore water samples and gas samples were collected using 5 cm ceramic cups (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) connected via teflon tubes to vacuumed syringes. For the sampling of pore water gas concentrations (methane and sulphide), pore water was collected in 12 ml glass exetainers (Labco exetainer®, High Wycimbe, UK), 0.5 ml of 0.1 M HCl was added to conserve the sample. Uncompressed soil samples were obtained using a Russian peat corer (Eijkelkamp Agrisearch Equipment, Netherlands), immediately put into sealed plastic bags, transported at 4 °C to the laboratory and stored at 4 °C until further analysis.

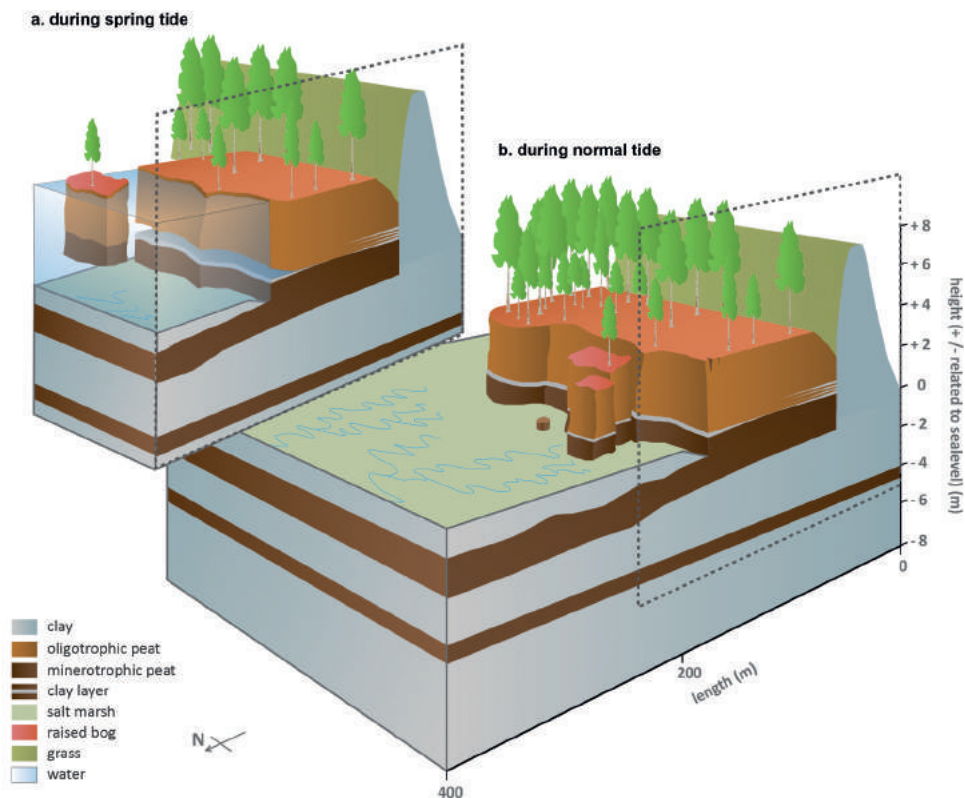


Fig. 8.2. Schematic overview of the area, based on data of the present paper and data from Behre (2005), scales in meters (length and high +/- sea level). a. is the area during a spring flood when a part of the bog is floating. b. is the area under normal conditions. figure by G. van Dijk.

Chemical analyses

Surface and pore water

The pH of surface water and pore water samples was measured using a combined pH electrode with an Ag/AgCl internal reference (Orion Research, Beverly, CA, USA) and a TIM800 pH meter. Alkalinity was measured by titration to pH 4.2 with 0.01 M HCl using an ABU901 Autoburette (Radiometer, Copenhagen, Denmark). Prior to elemental analyses, 10 ml of each sample was stored at 4°C with 0.1 ml HNO₃ 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, ammonium, orthophosphate, 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), for details concerning the analyses see (Van Dijk et al. 2015). Sodium and potassium were determined

with a Technicon Flame Photometer IV Control (Technicon Corporation). Methane and sulfide concentrations were analyzed using a gas spectrometer (HP 5890 gas chromatograph).

Soil analysis

Dry weights and bulk densities of soil samples were measured by drying a known volume at 70°C until constant weight, whereas organic matter content was determined by loss-on-ignition (4 h, 550°C). Total element concentrations (Mg, S, Fe, Ca, P) of soil samples were determined by digesting 200 mg of dried (24 h, 70°C) and homogenized sample in 4 ml concentrated HNO₃ and 1 ml 30% H₂O₂ (Milestone microwave MLS 1200 Mega). Samples were analyzed by ICP, as described above. Total nitrogen and carbon concentrations were measured with a CNS analyser (Model NA 1500; Carlo Erba Instruments, Milan, Italy).

RESULTS

Soil characteristics

The bog comprises a 3-4 m thick peat layer situated on marine clay (Figure 8.2), note the large differences in surface level between the three samples on the bog and the one on the salt marsh (Figures 8.1 and 8.2). The peat layer consists of about 1 m of minerotrophic (*Phragmites*, *Carex*) peat on top of the marine clay basis. On top of this minerotrophic layer a *Sphagnum* dominated ombrotrophic peat layer of about 2-3 m is present. Under the whole raised bog, especially on both the western (seaward) and the eastern (landward) side of the bog, small clay layers (varying in thickness) are intercalated at the transition between the minerotrophic and ombrotrophic peat. On the eastern location (location 2), five thinner bands (of 1-15 cm) of clay are present, while on the western side only one thicker clay layer (30 cm) is present. In the middle of the bog (location 4), clay bands between the two peat types are absent. On the saltmarsh, a top layer of more than a meter of marine clay is present with a peat layer underneath (Figure 8.2). This peat layer consists of about 1.5-2 meters of decomposed peat with a small clay band in the middle. Underneath this peat layer, marine clay is present again (Figure 8.2). Organic matter content of the soil samples clearly indicate the difference between the peat (O.M. > 95%) and clay (O.M. < 25%) layers (Figure 8.3).

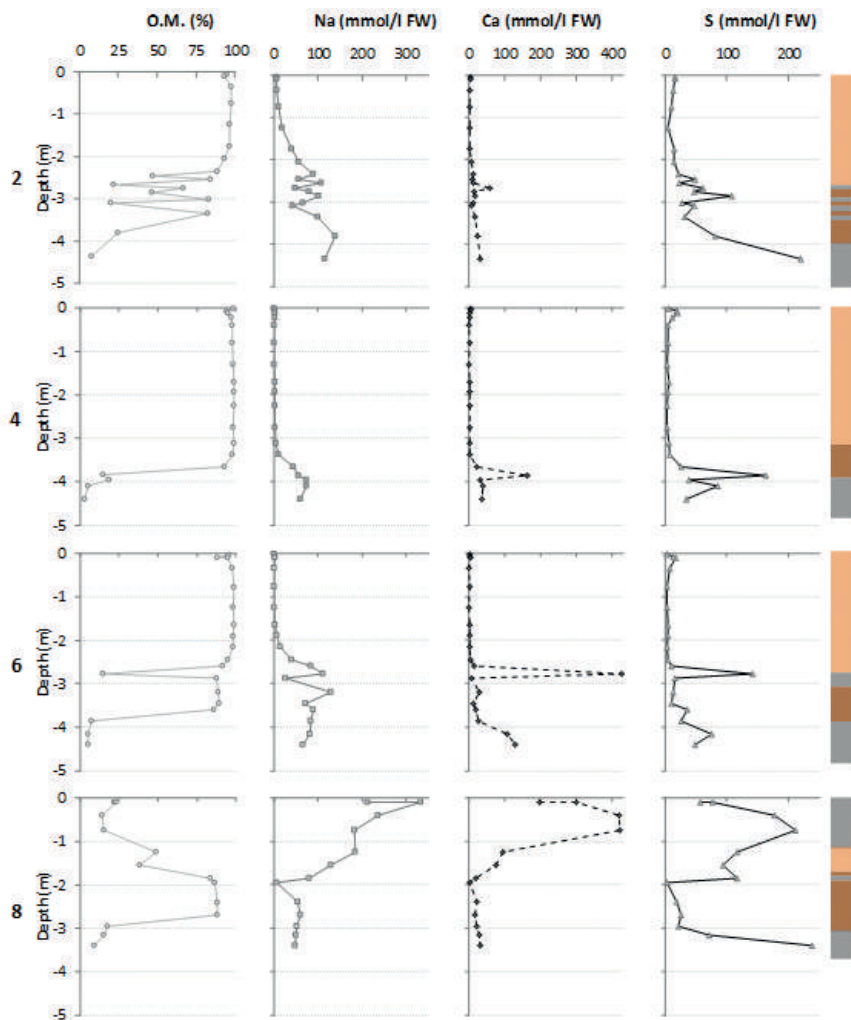


Fig. 8.3. Depth profiles of chemical soil characteristics of locations 2 (landward), 4, 6 and 8 (seaward), from the left to the right; organic matter content (%), and total sodium, calcium and sulfur concentration, all in mmol/l FW. On the right, soil types are shown: light brown = ombrotrophic peat, dark brown = minerotrophic peat, and grey = clay. Note that profiles are presented in meters below surface level, while surface levels differ per location, nr. 2, 4 and 6 are on the bog, 3-4 m above sea level, nr. 8 is at the salt marsh, 0-0.5 m above sea level.

In the upper pore water of the central part of the bog marine influence is almost absent (chloride concentrations < 10 mmol/l) and oligotrophic conditions are present. Total sodium (Na), calcium (Ca) and sulfur (S) concentrations in the soil profiles (Figure 8.3) show differences in marine influence within the peatland. In the minerotrophic peat layers and around clay bands marine influence is clearly indicated by increased Na, Ca and S concentrations (Figure 8.3). On both sides of the bog (locations 2 and 6) increased Na and to a lesser extent S and Ca concentrations are present higher in the soil profile, on location 6 up to 2 m and on location 2 even up to 1 m below surface level. Deeper in the marine clay layer Na concentrations decrease again.

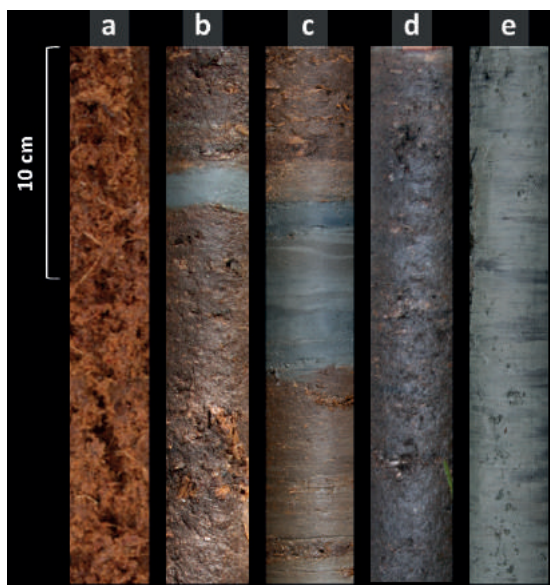


Fig. 8.4. Photos of different sediment types found in the soil cores, a.) ombrotrophic peat, b.) thin clay band of 'intercalated clay' in ombrotrophic peat, c.) thick clay band in minerotrophic peat, d.) minerotrophic peat, e.) marine clay. Photos by G. van Dijk.

Soil pore water chemistry

The elemental composition of the pore water showed similar depth gradients as the soil profile (see Table 8.1). On the raised bog remnant itself (location 2, 4, and 6), the upper 50 cm is dominated by rainwater intrusion (Table 8.1 and Figure 8.6). The top 50 cm showed low pore water pH (3.2 to 4) and alkalinity (<0.05 meq/l) levels. These low pH and alkalinity conditions were maintained to depths of around 2 meter. Only at greater depths (> 3m), especially in fen peat layers, pH increases to values around 6.5 and alkalinity reaches 15 meq/l. Pore water nutrient concentrations (total phosphorus, P_{tot} , and ammonium, NH_4^+ ; Table 1)) are relatively low ($\approx 10 \mu\text{mol } P_{\text{tot}}$ and $\approx 25 \mu\text{mol } \text{NH}_4^+$) close to the surface in the bog itself but increase with

depth. At greater depths ($> 2\text{m}$), pore water of both the bog and the salt marsh showed much higher nutrient concentrations were found, which were more than 5-20 times higher than in the top layer of the bog. Pore water nitrate (NO_3^-) concentrations were low at all locations at all depths. Pore water conditions differ between the edges and the center of the bog, and show an increased saline influence higher in the profile on both ends, especially at location 2 at the inland side.

At the center of the bog (loc. 4), conditions remain acidic and ombrotrophic ($\text{pH} < 4.5$, alkalinity 0.1 or lower and $< 10 \mu\text{mol Cl/l}$) to over 3 m below the surface. Along with this increased salinity influence (shown as pore water Cl concentration in Figure 8.5) total pore water P and NH_4^+ concentrations increase (see Figure 8.5a). Sites without salinity influence show much lower pH and pore water bicarbonate (HCO_3^-) concentrations (Figure 5b.). Higher pore water total S and sulfide (H_2S) concentrations are only present at locations with increased Cl-salinity (Figure 8.5c.). Methane (CH_4) concentrations reach $500 \mu\text{mol/l}$ to more than $3000 \mu\text{mol/l}$, and higher values are only reached at low H_2S concentrations (Figure 8.5d.).

Table 8.1. Chemical pore water composition at four depths for 4 locations along the transect. Note that concentrations are given in different units.

loc.	depth cm	pH	alk meq/l	EC mS/cm	CO ₂ mmol/l	HCO ₃ mmol/l	CH ₄	H ₂ S μmol/l	Fe	Ca	S mmol/l	Na mmol/l	Cl	P	NO ₃ μmol/l	NH ₄
2	-12	3.1	< 0.1	1.0	0.3	< 0.1	0.3	0.2	23.6	0.3	0.5	4.7	6.3	9.2	1.4	24.8
	-52	3.5	< 0.1	0.6	4.0	< 0.1	683.4	0.4	25.3	0.1	0.1	2.6	3.3	4.6	2.4	84.3
	-202	4.6	0.2	7.8	9.4	0.1	805.3	73.0	29.8	0.6	2.2	53.3	57.8	8.6	0.8	481.6
	-332	6.7	17.5	18.0	6.6	15.2	308.4	876.2	2.4	4.7	22.8	152.5	26.1	109.2	0.9	816.5
	-402	6.8	15.5	16.2	5.8	15.1	579.8	445.2	3.1	4.5	7.8	134.4	101.8	79.1	3.4	1013.6
4	-12	3.5	< 0.1	0.2	1.5	< 0.1	0.3	0.2	10.6	0.1	0.5	4.1	4.2	5.3	1.4	27.3
	-52	4.0	< 0.1	0.1	2.4	< 0.1	187.2	0.3	14.7	0.0	0.1	1.6	1.8	2.9	1.3	82.8
	-202	4.5	0.1	0.1	3.7	0.1	775.7	0.4	4.6	0.0	0.1	0.6	0.6	1.8	3.3	159.7
	-317	4.4	0.1	0.9	5.4	0.1	930.2	0.9	27.1	0.1	0.1	5.8	7.0	8.3	0.8	368.0
	-402	6.8	10.7	8.5	4.1	10.1	505.9	0.8	3.4	3.1	0.1	51.0	55.7	123.1	2.7	658.2
6	-12	3.6	< 0.1	0.2	1.0	< 0.1	0.3	0.3	12.2	0.0	0.2	2.1	1.9	12.6	1.7	20.4
	-52	3.9	< 0.1	0.1	2.2	< 0.1	83.9	0.3	6.8	0.0	0.1	0.7	0.8	2.6	1.5	27.1
	-202	4.1	< 0.1	14.9	5.5	< 0.1	3203.6	0.8	16.6	0.1	0.0	4.1	5.3	4.9	0.5	373.6
	-332	6.5	14.4	15.2	9.6	11.9	1848.8	0.6	300.4	4.1	0.0	83.5	99.6	278.2	1.1	1683.7
	-402	6.7	7.9	0.9	3.8	7.6	681.7	0.7	229.4	2.8	0.0	63.8	75.5	43.5	5.1	795.9
8	-12	7.0	25.7	38.3	4.7	18.0	97.4	86.7	14.6	10.0	10.2	255.7	328.4	189.0	4.8	196.4
	-52	7.1	25.0	32.1	5.5	28.7	8070.9	0.8	5.1	9.4	0.7	221.3	273.7	450.8	2.8	1667.5
	-152	6.2	7.5	26.3	19.7	13.5	602.5	1.4	2.6	9.2	0.1	177.9	227.2	275.5	1.2	2351.3
	-202	6.3	6.8	8.6	7.9	6.1	607.2	0.6	1372.6	4.7	0.1	48.6	63.0	4.9	3.9	1574.2
	-302	6.3	6.7	x	6.2	4.7	22.1	0.5	1476.6	5.2	0.1	51.4	72.2	25.2	1.1	1780.3

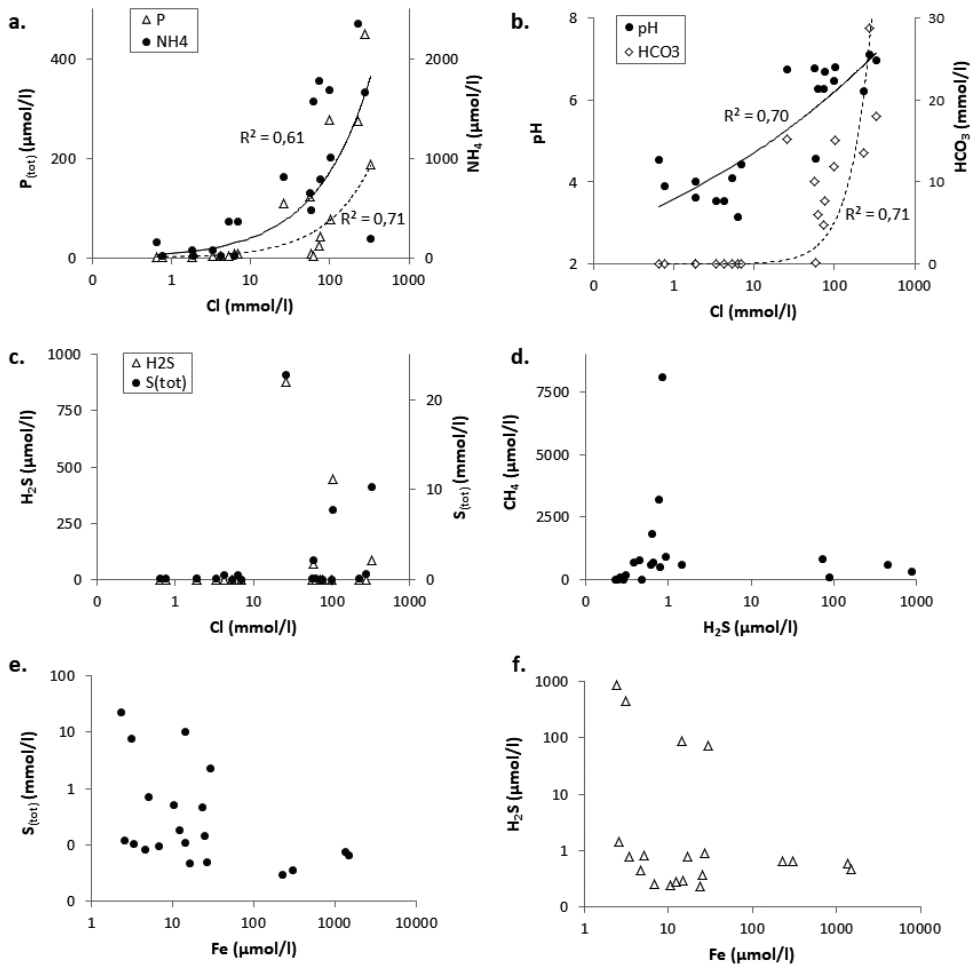
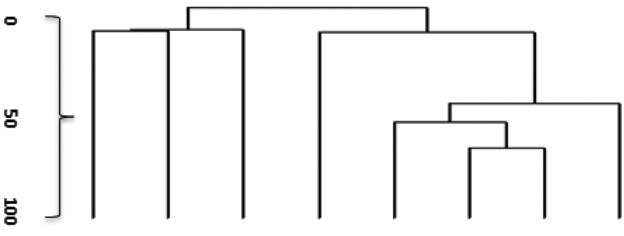


Fig. 8.5. Correlations between different pore water characteristics for all locations sampled. Note that the horizontal axis presents a log scale. A. Chloride to total phosphorus (in triangles) on the left axes and ammonium (filled circles) on the right axes. B. Chloride to pH (filled circles) on the left axes and bicarbonate (filled circles) on the right axes. C. Chloride to sulphide (triangles) on the left axes and total sulphur (filled circles) on the right axes. D. sulphide to methane on the left axes. E. iron to total Sulphur on the left axes. F. iron to sulphide on the left axes.

Vegetation

The Sehestedt bog harbors an interesting set of vascular plant and bryophyte species typical for communities of oligotrophic raised bogs, and saltmarshes (Behre 2005). On the raised bog remnant itself (locations 3–6) raised bog vegetation (Oxycocco-Ericion) is present with relatively high abundance of heather like species (*Calluna vulgaris*, *Erica tetralix* and *Oxycoccus palustris*) indicating desiccation. Less prominent species in these plots are *Eriophorum angustifolium*, *Molinia caerulea*, *Narthecium ossifragum* and some peat mosses *Sphagnum magellanicum*, *Sphagnum papillosum* and *Sphagnum rubellum*. Along the edges of the bog, plant species characteristic for raised bog are absent, and a more minerotrophic vegetation is present with dominance of *Phragmites australis* and species such as *Festuca rubra*, *Urtica dioica*, *Calamagrostis epigejos* and *Elytrigia atherica*. (Table 2). Location 8 is characterized by saltmarsh vegetation, including *Bolboschoenus maritimus*, *Triglochin maritimus*, *Aster tripolium*, *Atriplex portulacoides*, *Puccinellia maritima*, *Suaeda maritima*, and shows little similarity with other plots. Along all edges of the bog (including seaward) a small *Betula pubescens* forest is present. On the raised bog itself a large number of *Betula* seedlings are present and patches where *Myrica gale* is dominant.

Table 8.2. Vegetation surveys for all locations (1-8, see Fig. 8.1) along the transect, clustered in a dendrogram (above the table) based on similarity in species occurrence.

								
	1	7	8	2	3	4	5	6
Graminoids								
<i>Holcus lanatus</i>	r							
<i>Calamagrostis epigejos</i>		1						
<i>Elytrigia atherica</i>		1						
<i>Festuca rubra</i>		2m	2a					
<i>Phragmites australis</i>	3	4						
<i>Bolboschoenus maritimus</i>			2m					
<i>Triglochin maritimus</i>			r					
<i>Eriophorum vaginatum</i>				2b			x	
<i>Eriophorum angustifolium</i>					x	r	x	
<i>Molinia caerulea</i>					r			2b
Other vasucal plants								
<i>Chenopodium polyspermum</i>	x							

<i>Cirsium spec. (sdl.)</i>	1				
<i>Glechoma hederacea</i>	x				
<i>Hypericum tetrapterum</i>	r				
<i>Solanum dulcamara</i>	r				
<i>Urtica dioica</i>	3				
<i>Atriplex littoralis</i>		x			
<i>Linaria vulgaris</i>		x			
<i>Potentilla anserina</i>		x			
<i>Sonchus arvensis var. maritimus</i>		r			
<i>Aster tripolium</i>			2a		
<i>Atriplex portulacoides</i>			2b		
<i>Atriplex prostrata</i>			x		
<i>Limonium vulgare</i>			x		
<i>Puccinellia maritima</i>			2b		
<i>Salicornia europaea</i>			x		
<i>Spartina anglica</i>			x		
<i>Suaeda maritima</i>			2a		
<i>Andromeda polifolia</i>			x	1	1
<i>Calluna vulgaris</i>			4	4	x
<i>Dropteris carthusiana</i>			r		
<i>Erica tetralix</i>			3	1	1
<i>Vaccinium oxycoccus</i>			3	3	4
<i>Narthecium ossifragum</i>				x	x
Peat mosses					
<i>Sphagnum papillosum</i>			x		r
<i>Sphagnum rubellum</i>			x		
<i>Sphagnum magellanicum</i>			x		r
<i>Sphagnum fallax</i>			r		
<i>Sphagnum palustre</i>					r
Other mosses					
<i>Pleurozium schreberi</i>	x				
<i>Calliergonella cuspidata</i>	r		x		
<i>Polytrichum formosum</i>			x		
<i>Hypnum cupressiforme</i>				r	
<i>Calypogeia sp.</i>					r
<i>Aulacomnium palustre</i>				x	r
Trees and scrubs					
<i>Betula pubescens</i>			x	x	2b
<i>Frangula alnus</i>			r		x
<i>Myrica gale</i>					1
<i>Rubus cf. fruticosus</i>					r

DISCUSSION

Biogeochemical functioning of floating coastal bogs

The present study describes a rare atypical combination of an oligotrophic raised bog with marine influence in the direct surrounding, however surprisingly biogeochemical conditions in the bog remnant do still support raised bog vegetation to grow. As peat mosses, and bog vegetation in general, are very sensitive to high alkalinity and high ionic concentrations such as salinity (Clymo & Hayward 1982, Wilcox 1984, Wilcox & Abdrus 1987, Hájek 2006, Harpenslager et al. 2015), the ombrotrophic peat layers are clearly from older origin and have been formed under rainwater dominated condition and increased salinity levels found in *Sphagnum* peat are a result of saltwater intrusion from the sides during storm tides. Increased salinity influence from the sides also led to increased sulfur input in *Sphagnum* peat layers formed under sulphur poor conditions. This increased sulphur input, along with increased alkalinity, must have led to increased sulphate (SO_4^{2-}) reduction in anaerobic peat layers and enhanced peat mineralization (Lamers et al., 1998) and will have caused internal nutrient mobilization (Smolders et al. 2006), as is also indicated by the correlation between Cl and SO_4^{2-} and nutrient concentrations NH_4^+ and P (Figure 8.5). Additionally increased ionic concentrations, as a result of saline water intrusion, can enhance NH_4^+ availability due to cation exchange (Van Dijk et al. 2015). Although the upper two meters of the bog remnant do contain low salinity related ionic concentrations and low nutrient concentrations, a slight increase of Cl was found in the top 5 cm, probably caused by sea spray. In conclusion, the bog remnant does show strong horizontal and vertical biogeochemical gradients as a result of saltwater intrusion from the sides. As a result of the ability to float during storm tide, biogeochemical conditions in the rooting zone of plants in the center of the bog remnant did stay ombrotrophic (low alkalinity and low ion-concentrations) and relatively oligotrophic (low nutrient levels). For the functioning and conservation of raised bog like conditions and species composition the ability to float is of vital importance on the bog remnant.

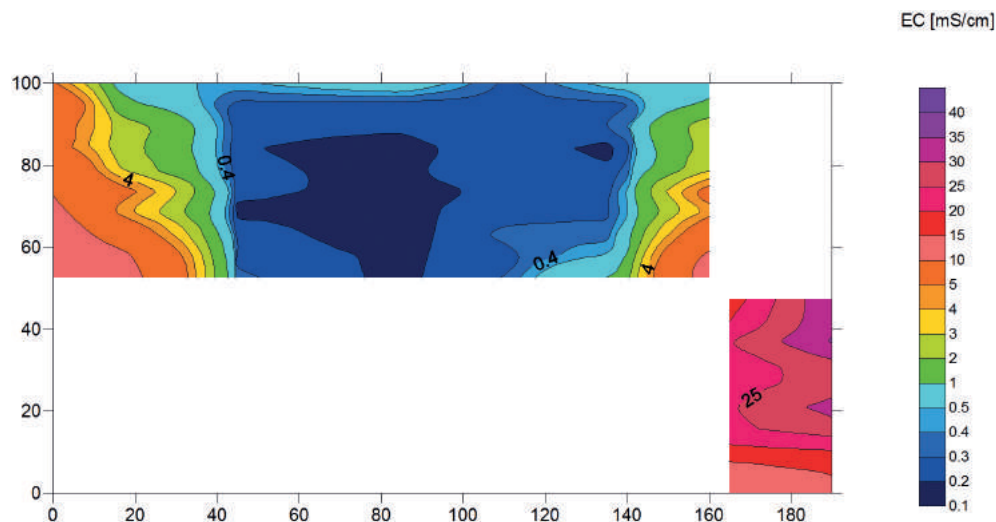


Fig. 8.6. Depth profile of interpolated electric conductivity measurements (in mS/cm) in the soil along transect from location 1 to 8, (see Fig 8.1).

To float or not to float

The ability of an entire bog remnant to float is a special phenomenon which did occur in more coastal peatlands in the past but is nowadays restricted to the Sehestedt bog. In literature, descriptions can be found of floating bigger or smaller bog segments, during high storm tide. During a large flood in 1509, for example, a large section of a bog floated 15 km eastward from the Dutch west side of the Dollart Estuary to German shore, carrying 12 cattle. In the 18th and 19th century observations were made of floating bogs including small wooden houses and trees (Behre 2005 and references within).

The bog remnant of the present study does only fully floats during high storm floods (in some winters several times, in 2006 there was a strong storm flood, splitting the bog remnant in two parts; Behre 2005 & Behre 2007b) when mean high water levels increase up to > 1.6 m above average seawater level (Künnemann 1941, Behre 2005).

What does however cause this ability to float and which mechanisms do influence its buoyancy? The buoyancy of the bog remnant is probably caused by a combination of mechanisms. First, one potential mechanisms could be the difference in densities between the freshwater in the bog peat and the seawater (1000 versus 1025 kg/m³), as in growing (sphagnum moss dominated) raised bog the water content is about 95-98 %, the density can be a mechanisms of influence. Increased growth of vascular plants (such as graminoids, heather like vegetation and bushes) do increase the density of the peat. Secondly the buoyancy of a peat layer is influenced by the weight of unsaturated peat on top (i.e. Fritz et al. 2008), desiccation along the edges of the bog

and drainage of the entire remnant will therefor enhance the buoyancy of the bog remnant. This mechanism will especially enhance the buoyancy of the bog remnant along the edges on the sea side. Thirdly the buoyancy of a peat soil is influenced by entrapped gas bubbles partly consisting of methane originating from in-situ methane production (methanogenesis) (Tokida et al. 2005), other gasses present in bubble in the pore water can be nitrogen gas, carbon dioxide or even sulphide. For methane for example oversaturation of gas (due to constant production over the years) and the formation of methane gas bubbles in the pores of the bog in combination with the capacity of the soil to keep gas bubbles trapped does create buoyancy (Lamers et al. 1999, Smolders et al. 2002, Strack et al. 2005). Pore water methane gas concentrations (0.5 to > 1 mmol CH₄/l) found in the present study do facilitate buoyancy (Smolders et al. 2002, Kellner et al. 2004). Increased temperature, decreased air pressure and increased salinity do decrease the solubility of methane and thereby enhance (over)saturation of methane in the soil (Strack et al. 2005, Tokida et al. 2007). Decreased air pressure during storm tide combined with flood water pressure from the sides might lead to temporary methane oversaturation, bubble formation and enhanced buoyancy during storm tide. Behre (2005) does also describe observations of escaping gasses from the floating peat layer during storm flood.

From literature it is known that enhanced salinity influence can reduce the buoyancy of peat body's, increasing salinity influence from the edges might therefor decrease the buoyancy of the remnant in future. Methane production (methanogenesis) is influenced by several biogeochemical and physical factors such as temperature, pH and salinity (especially the presence of oxidized sulfur components in saline water) (Williams and Crawford 1984, Dunfield et al. 1993, Smolders et al. 2002, Weston et al. 2006; Chambers et al. 2011). Enhanced salinity influence along the edges does increase the sulfate concentrations and does lead to enhanced sulfate reduction (in anaerobic soils rich in organic matter). The process of sulphate reduction is thermodynamically more efficient than methanogenesis and will suppress methanogenic activity under salinity influence (Segers 1998, Smolders et al., 2002), the toxic end product of sulphate reduction, sulphide, might even further reduce methanogenic activity. Increased salinity is also found to increase hydraulic conductivity in soils, especially in peat soils due to the combined effect of pore dilation and suppressed methanogenesis (Ours et al. 1997, Hoag & Price 1997, Kettridge & Binley 2010, Van Dijk et al. 2016), which might also influence the buoyancy of the bog remnant on the long term. Increased hydraulic conductivity can in theory however both stimulate and reduce buoyancy of the bog remnant. Increased hydraulic conductivity can decrease buoyancy due to enhanced outflow of gas bubbles and potential increase salinity influence during storm tides on the one hand, but on the other hand increase buoyancy due to enhanced desiccation along the edges as a consequence of increased outflow of (ground)water due to increase hydraulic conductivity.

Deposition of clay layers within the peat body

As a consequence of the buoyancy of the bog, several small layers of so-called 'turn-up clay' (or 'klappklei' or 'intercalated peat layer') were deposited during events when it did float in the past, which stabilized peat layer underneath. In former times this phenomenon occurred in several coastal peatlands. In several studies along the North Sea coast younger marine clay layers were found deposited within peat layers of older origin (i.e. Baeteman 1981, Behre 2005, Groenendijk & Vos 2013). The bog remnant discussed in the present study does not always float entirely during every high storm flood, since often only the edges do float (Behre 2005), which makes the edges function as a sort of natural levee. The peat cores in the present study underline these observations, as fewer and thinner clay layers were present in the center of the bog. These clay bands were deposited both within the upper *Sphagnum* moss dominated ombrotrophic peat but mainly at the transition from minerotrophic to ombrotrophic peat soil. The location of these clay bands indicate that only the ombrotrophic part of the bog floats and that the lower fen peat layer did and does not float. The peat profile does probably break at this transition point between the two peat layers due to differences in bulk density (BD of the fen peat is 0.13 kg DW/l FW versus the ombrotrophic peat 0.08 kg DW/l FW, in peat layers where clay bands are present the bulk density increases up to about 0.3 kg DW/l FW (as a reference, the density of the marine clay underneath the bog remnant is 0.6 to 0.9 kg DW/l FW, depending on the amount of organic matter)). According to Künnemann (1941) the southern part of the bog was covered with a layer of clay during a flooding event in 1914 and has not floated since then.

Where salt marshes and raised bog meet, not a bright future

The impact of the combined effect of salinity influence, severe erosion and desiccation along the edges do not predict a bright future for conservation of the bog remnant. The ability to float has shown to be vital for maintaining environmental conditions for raised bog associated species composition. In former times, under less anthropogenic influence, large coastal bog bodies did only float along the edges, functioning as a natural levee and protecting the bogs themselves and their hinterland. The present remnant is however small and influenced by erosion and saline water during storm tides from all sides and has floated entirely as turn up clay layers were found under almost the entire remnant. A logic conclusion is therefore that the erosion found during the last decades (Behre 2005) will continue in future. During every high storm tide event, erosion and the influence of saline water intrusion will increase. Over time this might reduce the buoyancy of the bog among others by increased sulfate reduction rates, increased peat decomposition and reduced methanogenic activity. Behre (2005) reported already that the bog does float less on the inland side, which corresponds well with the observed highest salinity influence, high pore water H_2S concentrations and large number of intercalated clay bands found on the inland side. As a result of reduced buoyancy, the influence of saline water on the bog will increase and smaller layers will float (which is also shown by several intercalated clay

layers higher in the profile at this side) or the buoyancy of the whole peat profile will decrease at not float at all. On the other hand increased drainage and desiccation of the bog remnant can lead to increased buoyancy during storm tides but will on the long term lead to enhanced peat decomposition (due to the presence of oxygen), internal eutrophication and enhanced erosion.

Apart from factors mentioned above other factors also threaten the conservation of the present vegetation. Increased nitrogen deposition and desiccation will lead to a shift to dryer conditions, richer in nutrients with increased abundance of heather species, grammanoids and trees or scrubs. The cover of trees (mainly *Betula pubescens*) has increased (in 1936 almost no trees were present on the bog remnant, Behre 2005), caused by the combination of factors like management and drainage (during the last decades increased nitrogen deposition might have had an influence [Tomassen et al., 2003]). An increasing number of trees might also enhance the deterioration of the bog by enhancing evapotranspiration and might also decrease the buoyancy due to increase of weight and erosion along the sides. Erosion along the sides will create cracks in the bog remnant which will enhance drainage and desiccation.

The presence of an coastal bog remnant still under direct marine influence during storm tides does deliver a unique combination and gradient between raised bog like conditions and salt marsh like conditions. This area can give a remarkable insight in historical functioning of coastal wetlands and function as a reference site. Future influence of the sum of several causes; drainage, desiccation, salinization influence and erosion during storm tides will lead to the erosion of the entire bog remnant in future. Predicted effects of climate change might even enlarge the effects on the bog remnant, as a consequence of expected future sea level rise, up to 0.2 to more than 1 m by the year 2100 (Vermeer & Rahmstorf 2009, Albrecht et al. 2011, Church et al. 2013) and potentially higher flood events (Rhein et al. 2013). For future management of coastal bogs and peatlands lessons can be learned on how future erosion and salinization might influence the functioning of these systems and can be used for their conservation. Nowadays former coastal peatlands are often drained and used for agriculture, better understanding of their genesis may improve our understanding of current biogeochemical and hydrological functioning of drained and rewetted peatlands.

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[Brunsummerheide, by G. van Dijk]

9

Synthesis

9.1 BIOGEOCHEMICAL STRESSORS IN PEATLANDS

Biogeochemical processes influence concentrations and forms of chemical substances in the environment, including those of nutrients and toxins, and thereby influence organism both directly and indirectly. Peatlands are very heterogeneous, from the micro-scale up to the landscape-scale, often showing strong biogeochemical gradients in both horizontal and vertical direction. Knowledge about how anthropogenic forcing can alter biogeochemical processes and thereby impact these ecosystems is of great importance, both from a scientific point of view and from a management and policy point of view. Due to the heterogeneous nature of peatlands and complex interactions between chemical, physical and biological processes, it is difficult to predict the consequences of changing biogeochemical processes for the functioning of peatland ecosystems and species. As prediction based on theory and models is often challenging, evidence-based interdisciplinary research is essential. To move beyond correlative research (data from field monitoring) and demonstrate causality, experimental work under controlled conditions in the laboratory or field situation is required. In my thesis, the effects of enhanced groundwater NO_3^- concentrations and enhanced surface water salinity levels were therefore studied using an approach that linked field observations to field and laboratory experiments. In this synthesis, the results from the various studies presented in my thesis will be discussed in relation to research reported in literature. I will conclude with recommendations for management and for future research, addressing both challenges and chances.

THEME 1: GROUNDWATER NITRATE ENRICHMENT AND PEATLAND FUNCTIONING

9.2 GROUNDWATER NITRATE ENRICHMENT

Although several other studies already focused on the effects of enhanced nitrogen (N) availability in mires and other aquatic and semi-aquatic systems (e.g. Aerts et al. 1995, Bobbink et al. 1998, Lamers et al. 2000), N enrichment via groundwater has received much less attention. Groundwater-fed mires in particular are not only influenced by enhanced airborne nitrogen deposition, but also by groundwater NO_3^- enrichment. Since the 1970s, increasing NO_3^- concentrations have globally become an increasing environmental problem in aquifers that are poor in labile organic material (e.g. Rivett et al. 2008, Burow et al. 2010, Thornburn et al. 2003, Howden et al. 2011). The study presented in this thesis (chapter 2) and other recent studies (e.g. De Mars et al. 2017) show that many aquifers and groundwater-fed systems in Northwestern Europe receive groundwater that is heavily polluted with NO_3^- . Although European

countries already agreed in 1991 that groundwater should not contain levels above 50 mg l⁻¹ NO₃⁻ (European Nitrates Directive; Council Directive 91/676/EEC, 1991), present groundwater concentrations in the Netherlands often exceed this value, sometimes even showing two or three times the maximum allowable concentration (Fraters et al. 2006, Bouman et al. 2012). The study area discussed in this thesis receives groundwater with concentrations around 800 - 2000 µmol l⁻¹ NO₃⁻ (50-125 mg l⁻¹ NO₃⁻).

Groundwater enrichment with NO₃⁻ is often caused by direct NO₃⁻ leaching from agricultural fields (Kirchmann et al. 2002, Di & Cameron 2002, Beaudoin et al. 2005, Fraters et al. 2006) or by interception in forests of aerial N deposition originating from agricultural and industrial sources (Rothe and Mellert 2004, Borken and Matzner 2004, Gundersen et al. 2006). Although a number of source-oriented measures have been taken and average NO₃⁻ groundwater levels have been reduced since the 1990s, average groundwater concentrations below Dutch sand and loess soils tend to stabilize above the established value of 50 mg l⁻¹ NO₃⁻ (Fraters et al. 2006). On a global scale NO₃⁻ enriched groundwater is also expected to become a serious issue in the near future, due to the worldwide agricultural intensification (e.g., Di and Cameron 2002).

9.2.1 Influence of groundwater nitrate enrichment on groundwater fed mires

Consequences for nitrogen availability and biodiversity

The NO₃⁻ enrichment of groundwater can lead to increased N availability in the rhizosphere in groundwater-fed ecosystems and can indirectly influence a series of biogeochemical processes (e.g. Smolders et al. 2010). The present thesis shows that NO₃⁻ rich groundwater leads to its increased availability in a formerly N poor groundwater-fed mire. Nutrient poor mires are to some extent buffered against increased nutrient loads, because of the high nutrient uptake capacity of peat mosses (*Sphagnum spp.*) (e.g. Lamers et al. 2000, Fritz et al. 2014) that often dominate these systems. However, Lamers et al. (2000) showed that in the long term, increased aerial N loads lead to nutrient saturation of mosses, alternations in moss physiology and leakage of nutrients, which will lead to an increased cover of more eutrophic species such as graminoids, rushes and trees (Limpens et al. 2003, Tomassen et al. 2003, Limpens et al. 2004, Fritz et al. 2012). Studies on the longer term ecological effects of N enrichment via groundwater are, however, still scarce. In the present thesis it is shown that more eutrophic vascular species such as graminoids (including grasses and rushes) and trees like *Betula spp.* and *Salix spp.* mainly occur in zones where NO₃⁻ enriched groundwater reaches the rhizosphere (chapter 2). Secondly, plant tissue δ¹⁵N signatures of a series of bryophytes and vascular plants also indicate enhanced uptake of anthropogenic N (chapter 2). On the long term, groundwater NO₃⁻ enrichment may therefore increase N availability and enhance the growth of eutrophic, highly competitive species at the expense of biodiversity and characteristic species in groundwater fed mires.

Consequences for (external and internal) eutrophication

Previous studies already showed that increased groundwater NO_3^- concentrations can indirectly lead to sulfate (SO_4^{2-}) mobilization and internal eutrophication in groundwater fed mires due to oxidation of reduced sulfur (S) and increased decomposition of organic matter (i.e. Rivett et al. 2008, Smolders et al. 2010). Additionally it was known that groundwater NO_3^- enrichment can lead to NO_3^- reduction via sediment deposits in the aquifer such as sedimentary organic matter (i.e. Hartog et al. 2002), ferrous iron bearing carbonates (Straub et al. 1996, Claudhuri et al. 2001) and ferrous iron containing sulfides (Postma et al. 1991, Schwientek et al. 2008, Zhang et al. 2009). Groundwater NO_3^- enrichment can therefore both lead to SO_4^{2-} mobilization from sulfur rich geological deposits in the aquifer as from S within the mire under influence itself (Lucassen et al. 2004, Zhang et al. 2009). The results from the present thesis are in line with these findings, as a strong indication for NO_3^- induced SO_4^{2-} mobilization was found (chapter 2). In contrast to results in other studies (Lamers et al. 1998, Lucassen et al. 2004, Smolders et al. 2006, Smolders et al. 2010) no internal eutrophication was found, most probably due to the fact that the sulfide (produced by SO_4^{2-} reducers) did not provoke phosphorus mobilization from iron-phosphorus complexes. Further downstream of the study area, however, indications for internal eutrophication were observed (pers. obs. G. van Dijk), due to the presence of soils richer in iron (Fe) and iron bound phosphorus (P). This underlines the potential dangers for internal eutrophication as an indirect consequence of groundwater NO_3^- enrichment. In combination with increased N availability, internal mobilization of P enhances eutrophication and can lead to a dominance of eutrophic species and concomitantly reduced biodiversity in mires (Wassen et al. 2005, Lamers et al. 2012, Fritz et al. 2012, Lamers et al. 2014). The present thesis shows that SO_4^{2-} mobilization indeed leads to enhanced SO_4^{2-} reduction resulting in enhanced concentrations of toxic sulfide and elemental S deposits (Fig. 2.6 in Chapter 2). This results in a complete die-back of all vegetation. The presence of sulfide and S deposits was only found on locations where SO_4^{2-} was present and NO_3^- was absent, due to NO_3^- reduction and NO_3^- mediated SO_4^{2-} mobilization. This indicates that, as long as NO_3^- concentrations remain sufficiently high, NO_3^- can prevent SO_4^{2-} reduction and therefore prevent sulfide production and SO_4^{2-} induced eutrophication. NO_3^- functions as a microbial redox buffer preventing the reduction of ferric iron and SO_4^{2-} and oxidizing ferrous Fe compounds when present (Lucassen et al. 2004, Smolders et al. 2010). Although the potential ecological effects of free sulfide are known rather well (Lamers et al. 2013), knowledge on the ecological effects sulfur deposits present in the aquatic environment is scarce. These S deposits may be produced by chemolithotrophic sulfide oxidizers or by photosynthetic purple sulfur bacteria. Instead of water, these groups use hydrogen sulfide as their reducing agent, which is oxidized to produce granules of elemental S (Madigan et al. 1997). All bryophytes and vascular plants near these deposits appeared to be dead (pers. obs. G. van Dijk and Fig. 2.6 in Chapter 2). Free sulfide, and maybe also S deposits, are toxic and reduce the availability of food for aquatic detritivores and herbivores, with negative consequences for the aquatic food chain. Waters in the present

study area with S deposits and free sulfide showed a poor abundance and species diversity of aquatic invertebrates compared to other waters in the mire (Van Dijk et al 2014). The ecological consequences of the accumulation of toxic sulfide and the concomitant deposition of elemental sulfur remains subject for future research. In the short term this will probably lead to stress and reduced growth of plants and animals, while in the long term this can also be expected to affect peat accumulation .

Consequences for carbon cycle

At first glance groundwater NO_3^- enrichment would not be expected to have large effects on peatland carbon (C) cycles and net C emissions to the atmosphere. Mires and peatlands in general however are potentially one of the largest natural sources of methane (CH_4) (Kirschske et al. 2013). Enhanced concentrations of electron acceptors such as NO_3^- , ferric iron and SO_4^{2-} are known to inhibit methanogenesis (Scholten & Stams 1995, Achtnich et al. 1995). Although the mechanisms behind NO_3^- driven inhibition of methanogenesis are not fully understood (Klüber & Conrad 1996), the inhibitory effect of NO_3^- has been demonstrated in various anaerobic systems (Scholten & Stams 1995, Achtnich et al. 1995). A rather large percentage of CH_4 produced in peatlands is however consumed by aerobic methanotrophic bacteria before it reaches the atmosphere (Valentine 2002). In anaerobic ecosystems (such as the soils of the major part of undrained peatlands) CH_4 can be oxidized anaerobically using alternative electron acceptors e.g. SO_4^{2-} or NO_3^- . In marine systems, for example, a large part of the CH_4 produced is oxidized by a consortium of anaerobic methanotrophic archaea (ANME) and SO_4^{2-} -reducing bacteria (SRB) (Valentine & Reeburgh 2000). In general, however, electron acceptors such as NO_3^- and SO_4^{2-} are scarce in freshwater peatlands. The results in the present thesis show that groundwater NO_3^- enrichment does end this limitation, resulting in NO_3^- dependent anaerobic CH_4 oxidation (chapter 3). This pathway linking the biogeochemical cycling of N and CH_4 has only recently been discovered in peatlands. In theory NO_3^- derived SO_4^{2-} mobilization might also stimulate SO_4^{2-} dependent anaerobic oxidation of CH_4 in other zones of mires (where NO_3^- is less dominant or absent). Enhanced groundwater NO_3^- concentrations (and indirect SO_4^{2-} mobilization) are expected to decrease methanogenesis and enhance the anaerobic oxidation of CH_4 produced in deeper layers (not influenced by NO_3^-). This may lead to a decrease in CH_4 emissions to the atmosphere from groundwater fed wetlands. In the short term NO_3^- enrichment can decrease CH_4 (and thus net greenhouse gas) emissions. In the longer term, however, enhanced availability of NO_3^- and SO_4^{2-} may well lead to enhanced mineralization, resulting in reduced peat accumulation and thereby also reduced net C sequestration.

Table. 9.1 Overview of research questions and answers for theme 1 of this thesis.

Research question	Answer	
Theme one – Groundwater nitrate enrichment and peatland functioning		
How does groundwater nitrate enrichment affect the functioning of a groundwater fed mire?	In groundwater fed mires, enhanced groundwater nitrate concentrations will lead to (1) sulfate mobilization from geological deposits resulting in sulfide production and elemental sulfur deposition, and potentially also in the mobilization of phosphate (internal eutrophication), and (2) in the long term to enhanced growth of eutrophic species such as graminoids and trees at the expense of biodiversity and characteristic species.	2
How does groundwater nitrate enrichment influence methane oxidation in a groundwater fed mire?	Groundwater nitrate enrichment enables nitrate dependent anaerobic oxidation of methane in freshwater peatlands, which might lead to reduced net methane emissions to the atmosphere.	3

9.2.2 Recommendations for management and policy

- On the national and European scale average groundwater NO_3^- levels are often well above the EU maximum value. On a global scale, groundwater NO_3^- is expected to increase in the future due to further agricultural intensification. Although source-oriented measures have been taken, they do not seem to be adequate to sufficiently reduce NO_3^- leaching to the groundwater. During the last 50 years, entire aquifers have become enriched with NO_3^- which means that it may still take many decades or centuries for the groundwater to reach groundwater fed systems. This also means that it may take a long time for polluted mires to become 'clean' again after NO_3^- leaching to the groundwater at the source has stopped. This shows the strong urge to study the effects of groundwater NO_3^- enrichment on the functioning of groundwater fed ecosystems, and to define measures and legislation that (1) reduce NO_3^- leaching at the source and (2) conserve or restore groundwater fed, NO_3^- polluted, ecosystems.
- The long-term direct and indirect effects of groundwater NO_3^- enrichment on the functioning of groundwater fed ecosystems and their organisms are not well understood. In sharp contrast to the number of studies on the effects of enhanced aerial N deposition, relatively few studies have focused on the effects of enhanced N loading via groundwater. It is even unknown to what extent NO_3^- levels below the EU NO_3^- directive value may already affect groundwater fed ecosystems in a negative way. Therefore, additional evidence-based research is urgently needed to support managers and policy makers in decision-making.
- For the study area presented in this thesis (chapter 2 & 3) the regional groundwater is more enriched with NO_3^- than the local groundwater. A

potential management measure would therefore be to clear cut the forest in the catchment area, reducing the impact of anthropogenic aerial N influence and enhancing the influence of precipitation and groundwater recharge close to the mire by the release of canopy interception and evapotranspiration. This approach can be used both as a conservation and restoration measure for groundwater fed ecosystems. The applicability of this measure will, of course, strongly depend on local ecological, geological and ecohydrological conditions.

- In current nature management and policy, much emphasis is placed on measures to reduce the effects of aerial N deposition in natural areas. N loading from groundwater, however, receives relatively little attention and is therefore underrepresented in planning. As groundwater derived N loads may exceed aerial N input in groundwater fed systems and effects can be profound (chapter 2), it is strongly recommended to include this N input in N reduction strategies for groundwater fed ecosystems.

Take home messages theme 1: groundwater nitrate enrichment

- Groundwater nitrate pollution is becoming an increasingly important worldwide problem, and can have large consequences for the functioning of groundwater fed ecosystems.
- It leads to enhanced nitrogen availability and eutrophication in formerly nutrient poor, groundwater fed mires.
- It may cause sulfate mobilization from geological sulfur bearing deposits, which may induce internal phosphate mobilization, enhanced sulfide toxicity and elemental sulfur deposition in groundwater fed mires.
- As nitrate is a strong electron acceptor, enhanced concentrations can decrease methane emission, by the inhibition of methanogenesis and stimulation of nitrate dependent methane oxidation. By stimulating the anaerobic oxidation of organic matter (decomposition), it may however also compromise the net sequestration of carbon.
- For the conservation and restoration of groundwater fed mires, more attention is needed for measures to reduce nitrate leaching to the groundwater.

THEME 2: SURFACE WATER SALINIZATION AND PEATLAND FUNCTIONING

9.3 SURFACE WATER SALINIZATION IN PEATLANDS

Worldwide, anthropogenic forcing has influenced land use, water management and hydrological processes. In combination with sea level rise and climate change, these changes increase the risk of salinization, especially in coastal wetlands (Herbert et al. 2015). In this thesis, several aspects and effects of increased surface water salinity in wetlands were studied, with a focus on biogeochemical processes in the sediment.

9.3.1 Sediment biogeochemistry and nutrient availability

The studies presented in this thesis show that surface water salinization not only alters ionic concentrations, but also strongly influences chemical equilibria and microbiological pathways in the sediment. From previous studies it is known that salinization can influence sediment nutrient cycles via a myriad of direct and indirect processes, but as literature shows contrasting results, it is challenging to predict the net effects of salinization on sediment nutrient cycling.

Phosphorus availability

Salinization induced cation mobilization appeared to be one of the major drivers influencing nutrient availability (chapter 4 & 5) (Fig. 9.1). Surface water salinization resulted in a rapid exchange of cations dominant in brackish or salt water (e.g. Na^+ , Mg^{2+} and K^+) with cations bound to the cation adsorption complex of the sediment (e.g. Ca^{2+} , H^+ , Fe^{2+} and NH_4^+) (Chapter 4 & 5), which corresponds well with results reported in literature (Poonia et al. 1977, Loeb et al. 2008, Baldwin et al. 2006, Weston et al. 2006). Cation mobilization due to enhanced surface water salinity results in sediment specific effects as the amount and type of cation species mobilized is location specific.

A second important driver influencing sediment nutrient mobility and the mobility of phosphorus in particular is the sediment sulfur content and enhanced SO_4^{2-} concentration as a consequence of salinization. In chapter 4 it was shown that peatlands with a historical or geological influence of brackish water have sediments that are rich in reduced sulfur compounds. In such sediments most Fe is bound as iron sulfides, which leads to relatively small amounts of extra P being mobilized from Fe-P complexes after S enrichment (Smolders et al. 2006, Geurts et al. 2010, Van Diggelen et al. 2014). In literature both increasing P levels (e.g. Baldwin et al. 2006, Weston et al. 2006, Hartzell & Jordan 2012) and decreasing P levels have been reported as a consequence of enhanced salinity (Sundareshwar and Morris 1999, Beltman et al. 2000, Jun et al. 2013). The majority of these studies did show S-induced P mobilization and enhanced decomposition after SO_4^{2-} enrichment (e.g. Roden and Edmonds 1997, Lamers et al. 1998, 2002, Zak et al. 2006). Enhanced SO_4^{2-} concentrations generally cause

internal eutrophication in the longer term (e.g. Smolders et al. 2006). In the areas studied in this thesis however, decreased pore water P concentrations were found as a consequence of salinization. This could be explained by the fact that mobilization of calcium resulted in (co-) precipitation of P with Ca or Ca carbonate (Bale and Morris 1981; Morris et al. 1981, House 1999) In combination with the fact that sulfate reduction only leads to a minor increase in P availability in these sulfur rich sediments, this resulted in a net decrease of the P availability.

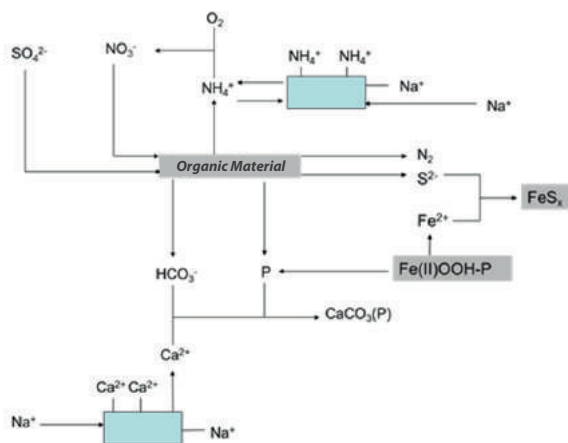


Fig. 9.1. Schematic representation of several biogeochemical processes related to nutrient availability affected by enhanced salinity.

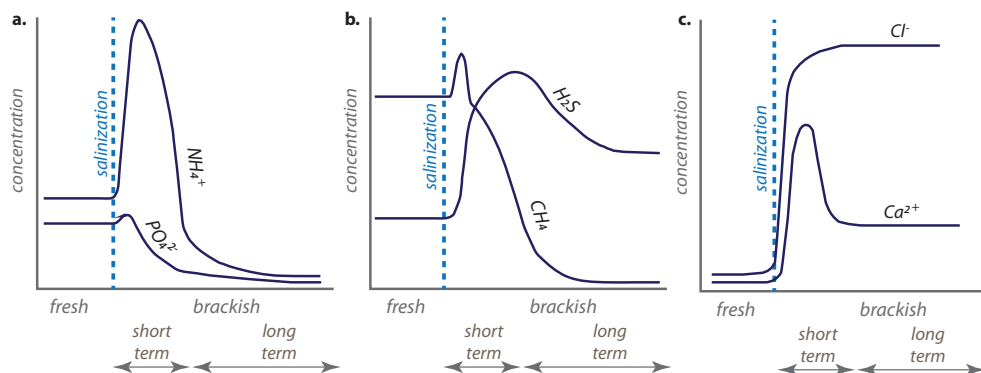


Fig. 9.2. Schematic representation of sediment pore water characteristics for a selection of ions over time during salinization: a. availability of ammonium and phosphate; b. production of hydrogen sulfide and methane; and c. concentrations of chloride and calcium.

Nitrogen availability

The results presented in chapter 4 and 5 show salinity induced ammonium (NH_4^+) mobilization due to displacement of NH_4^+ from the cation adsorption complex, which is in line other studies (e.g. Seitzinger et al. 1991, Weston et al. 2006, 2010). This process leads to increased NH_4^+ availability in the short term; rapid NH_4^+ mobilization can already take place within hours to days after salinization (Chapter 4; Weston et al. 2010). Prolonged salinity increase or fluctuating salinity may, however, deplete the amount of NH_4^+ bound to cation exchange sites in the long term. This will lead to decreased NH_4^+ availability or even NH_4^+ depletion (Chapter 4). Whether salinity induced NH_4^+ mobilization from the sediment does also lead to NH_4^+ accumulation in the surface water is, however, strongly determined by other steps in the N cycle (Strauss et al. 2002, Burgin & Hamilton 2007, Zhou et al. 2017). The observed decrease of pore water NH_4^+ concentrations in the studies here is most probably also caused by fast oxidation of mobilized NH_4^+ (nitrification) in the water layer and subsequent denitrification in the sediment (Strauss et al. 2002, Burgin & Hamilton 2007, Franklin et al. 2017). Studies by Marton et al. (2012) and Craft et al. (2009), for example, found N accumulation rates to decline with increased salinity, but also showed that these effects differ per sediment type and are therefore location specific. Some studies show that salinity is a significant factor in N mineralization (e.g. Noe et al. 2013, Brouns et al. 2014). Especially in sulfide rich ecosystems, such as S rich wetlands, sulfide-driven NO_3^- reduction by autotrophic denitrifying bacteria may also play an important role in the N cycle (Hatakeyama et al. 2013). In recent reviews and meta analyses it is shown that there is still no consensus about the explanation of the differential effect of salinization on net N cycling (Herbert et al. 2015, Zhou et al. 2017). There are studies that report that both nitrification and denitrification are solely affected by increased ionic strength (e.g. Laverman et al. 2007) or by increased sulfide concentrations (i.e. Brunet & Garcia-Gil 1996), but a mechanistic explanation is challenging as alternative processes for N loss to the atmosphere, such as anaerobic NH_4^+ oxidation (anammox) could also play a role. The net effect of salinization on sediment N availability is therefore sediment specific and the effects of enhanced salinity on N cycling warrant further research.

Consequences for phytoplankton growth

It is generally assumed that phytoplankton growth is limited by P in most freshwater systems and by N in saline systems (Redfield 1958, Hecky & Kilham 1988; although anthropogenic activity might have leveled off this difference between systems (Elser et al. 2007)), mainly because of adsorption of phosphates to iron minerals in freshwaters and low presence of these adsorption surfaces in saline systems due to higher SO_4^{2-} reduction rates and trapping of iron as FeS_x . Most of the studies in my thesis focus on biogeochemical processes in the sediment. Geurts et al. (2010) showed that pore water P concentrations and P release from the aquatic sediment to the overlying water layer is generally linearly correlated under aerobic conditions in the water layer, for sediments showing low ($< 1 \text{ mol mol}^{-1}$) pore water Fe:P ratios. This implies

that lower porewater P concentrations due to salinization will also result in a decrease in P release to the overlying water layer. In the short term, the effect of surface water salinization on the nutrient dynamics in the sediment, i.e. a combination of decreased P availability and temporally increased N availability, can be expected to intensify P limitation for phytoplankton (which is common in most freshwater wetlands [Redfield 1958, Hecky & Kilham 1988, Hartzell & Jordan 2012]). In the long term, however, one could expect lowered N availability due to reduced binding capacity of ammonium at cation exchange sites. In combination with increased S-induced P mobilization this could lead to N limitation, which is common in brackish waters [Howarth & Marino 2006].

9.3.2 Short versus long term and fluctuating versus constant

In this thesis the differences between short-term and long-term salinization (chapter 5 and 7), and between constantly enhanced salinity and fluctuating salinity levels (chapter 4) on sediment processes were studied. We show that in the longer term fluctuating surface water salinity, representative of many brackish systems due to tidal effects and fluctuating rainwater input, has similar effects as constantly enhanced surface water salinity. This can be explained by the fact that the sediment rapidly becomes saline (cation adsorption), while it takes more time to become fresh again (cation desorption) (Fig. 9.3). Effects of salinization on sediment characteristics also seem to be time-dependent and may differ between the short and long term. A large set of chemical and physical changes already occur within hours to days, where other chemical and especially microbiological processes occur within months to years. This indicates that effects found in the short term (often shown in literature) might be misleading as they differ greatly from long term effects (Fig. 9.2).

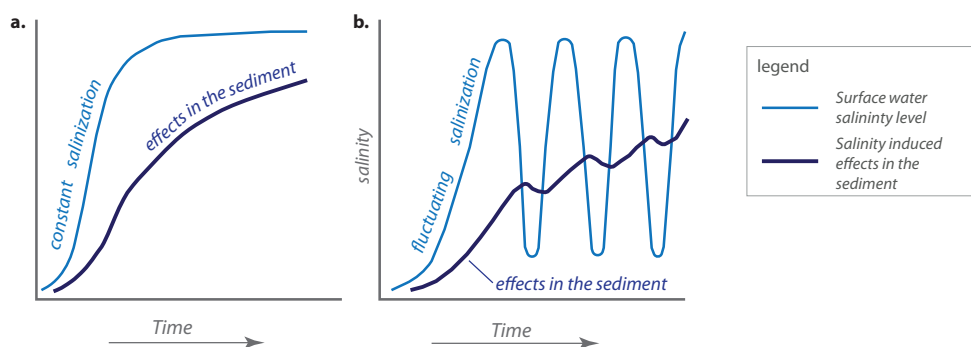


Fig. 9.3. Schematic representation of the biogeochemical effects in the sediment as a consequence of constant (a) versus fluctuating (b) salinity levels.

9.3.4 Carbon dynamics and microbial communities

Peatland soils show high concentrations of carbon and may therefore also emit high amounts of carbon to the atmosphere in the form of CO_2 and CH_4 . As wetland sediments are generally anaerobic, they contribute to a large extent to the natural emission of methane to the atmosphere (Bridgman et al. 2013, Kirschke et al. 2013, Turetsky et al. 2014). As enhanced surface water salinity (1) increases ionic concentrations, (2) increases concentrations of alternative terminal electron acceptors, and (3) affect geochemical processes and the composition of microbiological communities (Herbert et al. 2015), it can be expected that this will also influence C mineralization processes and the production and emission of greenhouse gasses. Several other factors driving C emission rates received much attention, including temperature (e.g. Gudas et al. 2010, Yvon-Durocher et al. 2014), sediment quality (e.g. Gudas et al. 2010) and precipitation patterns (e.g. Kirschke et al. 2013). Salinization, however, received proportionally less attention. Literature reporting on the effects of enhanced salinity on sediment C cycling and emission often shows contradictory results (Herbert et al. 2015). Some studies report enhanced C mineralization (Weston et al. 2011, Chambers et al. 2011, Craft et al. 2009, Neubauer 2013) while others show decreased mineralization (i.e. Roache et al. 2006). When comparing C emission along a salinity gradient, the general pattern that is often found is that saline systems net emit less C (Bartlett et al. 1987, Poffenbarger et al. 2011, Weston et al. 2014, Vizza et al. 2017). Here we show that experimentally enhanced surface water salinity only had a minor effect on sediment CO_2 production (Chapter 4) and on diffusive CO_2 fluxes (Chapter 7). Methanogenesis, however, was suppressed in all studies in this thesis. We found an up to > 80% decrease in pore water CH_4 concentrations (Chapter 4, 5, 6, 7 & 8), a > 90% decrease in diffusive methane fluxes (Chapter 7) and a > 90% reduction of ebullitive methane fluxes (Chapter 7) in the range from 0.9 to 9.0 PSU (14 - 140 mmol Cl l⁻¹). As mainly anaerobic peatland sediments were studied, which are rich in organic material, enhanced SO_4^{2-} concentrations were expected to result in a shift from a system dominated by methanogens to a system dominated by SO_4^{2-} reducers (e.g. Bartlett et al. 1987, Segers, 1998, Lamers et al. 1998, Smolders et al. 2002, Weston et al. 2006, Vizza et al. 2017). This was indeed confirmed in chapter 5, 6 and 7, where enhanced porewater sulfide concentrations were found, up to 40 times increase from 0.9 to 9.0 PSU (14 - 140 mmol Cl l⁻¹). Enhanced SO_4^{2-} reduction rates (1) affected the iron-sulfur and nutrient cycles, (2) increased concentrations of toxic sulfide and (3) suppressed methanogenesis. SO_4^{2-} reduction is thermodynamically more favorable than methanogenesis (Segers, 1998) causing SO_4^{2-} reducers to outcompete methanogens for organic compounds (e.g. acetate). This can even lead to a complete inhibition of methanogenesis (Lovley et al., 1982, Lamers et al. 1998, Smolders et al. 2002). Additionally, SO_4^{2-} reduction in combination with salinization may lead to physiological stress for methanogenic bacteria (Chambers et al. 2011) due to the combined stress of ionic strength and sulfide toxicity (Weston et al. 2006, Lamers et al. 2013). One might expect that methanogens might adapt to enhanced salinity conditions in the long term, as there is a wide range of methanogens which do occur in marine environments

(Segers 1998, Reeburgh 2007). Results in this thesis do, however, indicate that methanogenic activity is still suppressed in the sediments under enhanced salinity after six years (chapter 7). Although enhanced salinity led on the short term to enhanced diffusive methane fluxes (chapter 7), longer term (already after a few weeks) salinization showed to suppress methanogenesis. The short-term enhanced diffusive methane flux could be explained by a short-term enhanced productivity of methanogenic bacteria using the dissolved organic matter which was mobilized as a consequence of enhanced sediment salinity.

In my thesis, the main focus was on the effects of enhanced salinity on methanogenesis and SO_4^{2-} reduction. Effects on methane oxidation were not analyzed. The effects of enhanced salinity on aerobic and anaerobic methane oxidation in the sediment and at the sediment-water boundary may, however, also affect net methane emissions (Valentine 2002). Especially in marine ecosystems, a consortium of anaerobic methanotrophic archaea and SO_4^{2-} reducing bacteria have been shown to be responsible for high anaerobic methane oxidation rates, lowering net methane emission (Reeburgh 2007). Methane oxidation, and particularly iron- and SO_4^{2-} -mediated anaerobic methane oxidation, may well be influenced by enhanced salinity, and these processes have been shown to occur in a broad salinity range (Valentine & Reeburgh 2000, Le Mer & Roger 2001, Egger et al. 2014). Future research should therefore include the effects of enhanced salinity on methane oxidation in wetlands, to fully unravel its influence on net C emissions.

Interactions with the carbon cycle, physicochemical effects and hydrology

In the studies of this thesis we show that enhanced salinity suppresses methanogenesis. CH_4 emission to the atmosphere via ebullition often represents a large contribution to or even the dominant transport path of CH_4 from peatlands and sediments (Glaser et al. 2004, Coulthard et al. 2009, Aben et al. 2017); in our studies the ebullitive fluxes represented $\approx 25\%$ of the total CH_4 flux. A full understanding of the mechanisms regulating CH_4 production, and its ebullition in particular, in peatlands is however still incomplete (e.g. Kellner et al. 2006, Comas et al. 2008, Bon et al. 2013, Chen & Slater 2015). In chapter 6 it is shown that the combination of physicochemical effects (pore dilation) and biogeochemical effects (depressed methanogenesis) can even have hydrological consequences by increasing the hydraulic conductivity in sediments under enhanced salinity. The ebullitive methane fluxes presented in chapter 7 do indeed correlate with the hydraulic conductivity presented in chapter 6 in the same enclosures (Fig. 9.4).

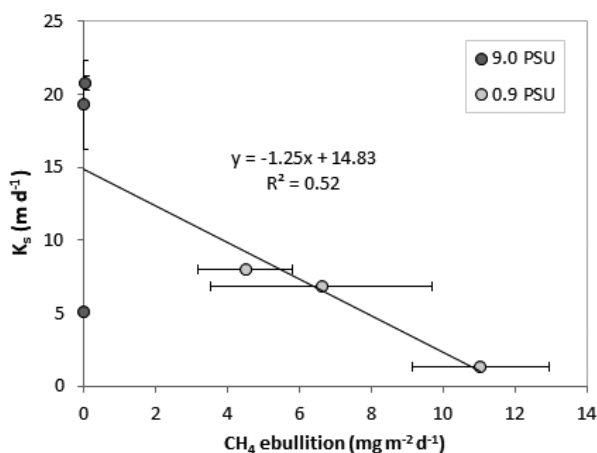


Fig. 9.4. Correlation ($P = 0.107$) between the ebullitive methane flux (average of 4 points in time $\pm$ SEM, Chapter 7) and the hydraulic conductivity (average of 3 measurements $\pm$ SEM, Chapter 6) in the same enclosure, for the control ($n=3$) and highest salinity treatment ($n=3$).

One can imagine that this has consequences for the mobility of pore water and its solutes in the sediment. The mechanisms controlling sediment gas bubble formation, movement and emission, and their consequences for water movement in sediment pores is, however, still poorly understood (e.g. Kellner et al. 2004, Kellner et al. 2006, Kettridge et al. 2012, Liu et al. 2016). How enhanced salinity might influence these mechanisms and their interactions is unknown, especially because it not only affects methanogenesis but may also influence the production and movement of other gasses (e.g. sulfide, nitrogen gas or even entrapped air) which could cause similar effects (Beckwith & Baird 2001, Marinas et al. 2013). Enhanced salinity is not expected to increase N_2 concentrations (Zhou et al. 2017). Sulfide gas concentrations on the other hand do increase after salinization (Chapter 5, 6 and 7). In comparison to methane, sulfide gas is expected to play a very minor role in bubble formation and bubble movement, due to the relatively high solubility of sulfide in contrast to methane (Gevantman 1999). In chapter 6 and 7 it is shown that, although net ebullitive fluxes of CH_4 decrease and hydraulic conductivity increases, porewater sulfide concentrations do increase. The exact contribution of sulfide bubbles to the atmosphere might however also be partly underestimated, during fieldwork a sulfide smell was observed indicating that sulfide could be a part of the ebullition (pers. Obs. G. van Dijk). Results in chapter 7 do also show lower ebullition rates under increased salinity (both lower methane ebullition and lower total gas ebullition) whereas pore water sulfide concentrations were higher under enhanced salinity, indicating that sulfide did not have a large contribution on total net ebullition.

CH_4 bubble formation and ebullition in general is influenced by physical and biogeochemical factors, such as the availability of easily decomposable organic matter and environmental

conditions (Chapter 5 and 7), atmospheric pressure (Waddington et al 2009, Chapter 8) and temperature (Gudas et al. 2010, Yvon-Durocher et al. 2014; Aben et al. 2017). Enhanced availability of organic matter to be mineralized, air and sediment pressure reduction and increased temperature will enhance methane production, leading to pore water oversaturation for methane and thereby stimulating gas bubble formation. Enhanced pore water salinity will also reduce the solubility of methane (Yamamoto et al. 1976, Wiesenburg & Guinasso 1979), which will lead to a minor enhancement of ebullition. When pore water methane oversaturation is reached and bubbles start to form, sediment buoyancy also increases causing the surrounding sediment to rise. If bubbles start to move, they can also force other bubbles to move, creating preferential flow paths (Boudreau et al. 2005, Scandella et al. 2011).

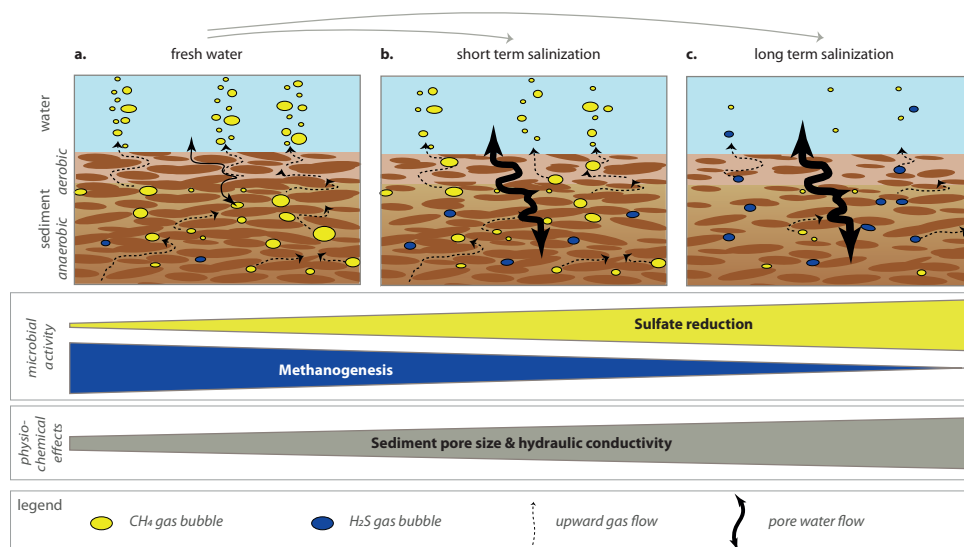


Fig. 9.5. Schematic representation of a peat sediment under a. freshwater conditions, b. short-term (weeks) salinization and c. long-term (years) salinization. Methane gas bubbles (yellow) and sulfide gas bubbles (blue) are shown, as well as their movement through the sediment (dashed lines). Below the figure the importance of methanogenesis (yellow) and sulfate reduction (blue) are indicated by the size of the triangle; the larger the triangle the greater the importance of the process. Note that the sediment pore size is increased in b. and c. Black arrows indicate pore water flow, increasing under enhanced salinity (b and c).

Another important consequence of salinization is the flocculation of suspended particles in the surface water (Chapter 4) and flocculation of organic acids on the surface of peat fibers within the sediment, resulting in sediment pore dilation (Chapter 6). Salinity-induced aggregation and flocculation of suspended matter in the water column is well known for estuaries for high salinity ranges (e.g. Sholkovitz 1976, Eisma 1986). Previous studies have shown that enhanced pore water ionic concentrations can influence sediment physical properties via pore dilation and thereby influence the hydraulic conductivity of peat soils (Ours *et al.* 1997, Hoag & Price 1997, Comas & Slater 2004). Due to the combination of all these processes enhanced salinity will increase ebullition in the short term and, due to SO_4^{2-} induced suppression of methanogenesis, lead to decreased methane production and emission, or even methane depletion, in the longer term. The results shown in this thesis indicate large effects of enhanced salinity on the carbon cycle and the hydraulic conductivity in the hyporheic zone (where most of the microbial activity takes place), affecting the movement of pore water and its solutes (Chapter 6 and 7, Fig. 9.4 and 9.5). It is, however, difficult to extrapolate these effects to peat soils or wetlands in general, as both biogeochemical processes (this thesis) and hydraulic conductivity (e.g. Stofberg *et al.* 2016) are known to be very heterogeneous in peat soils. These results warrant additional research on the interacting physiochemical and biogeochemical effects of enhanced salinity on gas and pore water movement.

9.3.3 Effects on aquatic and benthic organisms

Within the second theme of this thesis the main focus was on biogeochemical effects of enhanced surface water salinization. Effects on aquatic and benthic plants and animals organisms will therefore only be mentioned briefly here. Salinity will obviously also influence the abundance and distribution of aquatic and benthic organisms (e.g. Davis *et al.* 2003, James *et al.* 2003, Nielsen *et al.* 2003). Salt tolerance is species specific, but in general it can be stated that the majority of aquatic and benthic species are not able to live under brackish conditions (see Fig. 9.6), and even halophytes show an growth optimum under freshwater conditions compared to saline conditions (Geenway & Munns 1980).

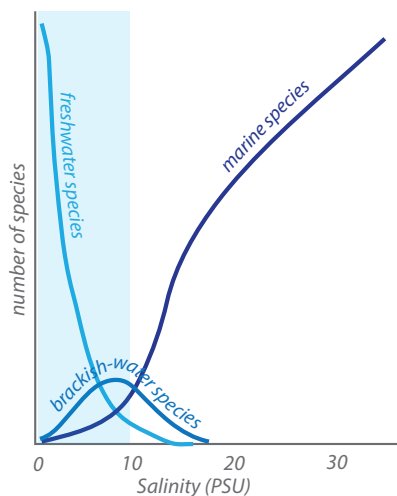


Fig. 9.6. Schematic representation of the number of species in relation to salinity (in PSU). The shaded rectangular represents the salinity range used in experiments in this thesis. After Remane (1958).

The most obvious adaptation that species in a brackish environment need is the active regulation of their internal osmotic pressure for fluctuating salinities. The two most common stressors for organisms under increased salinity are osmotic and ion-specific stress (Munns et al. 2008). Under higher salinities osmotic stress prevails over ion-specific stress (Munns et al., 2008; Rengasamy et al., 2010). Other potential stress factors include enhanced sulfide toxicity by enhanced sulfate reduction rates in the benthic zone (Lamers et al. 2013) or ammonia toxicity under high pH and ammonium rich conditions (Clarke & Baldwin 2002, Britto & Kronzucke 2002).

Table. 9.2. Overview of research questions and answers for theme 2.

Research question	Answer	Chapter
Theme two – Surface water salinization and peatland functioning		
How do (fluctuating) surface water salinity levels affect biogeochemical sediment processes in peat and clay sediments?	Sediment cation exchange appeared to be the key process explaining both decreased phosphorus availability and increased nitrogen availability, their extent being strongly dependent on sediment type. Constant and fluctuating salinity levels showed similar longer-term effects with respect to nutrient and carbon cycling.	4
How does surface water salinization affect sediment biogeochemical processes in a peatland sediment (with a focus on nutrient availability)?	Salinization unexpectedly led to decreased availability of nitrogen and phosphorus in the sediment, both in the short and in the long term, even though sulfate reduction rates had increased. Increased salinity shifted the dominant mineralization process from methanogenesis to sulfate reduction	5
How does surface water salinization influence sediment hydrological properties in the hyporheic zone of peatlands?	The combined effects of enhanced salinity on physiochemical and biogeochemical processes led to enhanced hydraulic conductivity of the hyporheic zone. This increase was mainly caused by the combination of pore dilation and methanogenesis suppression.	6
To what extent will surface water salinization influence sediment microbial decomposition processes and carbon fluxes to the atmosphere?	Salinization does enhanced CH ₄ fluxes on the short term but reduce methanogenic activity, pore water methane concentrations and diffusive and ebullitive fluxes to the atmosphere on the long term (mainly caused by a shift from methanogenesis to sulfate reduction). Sediment CO ₂ fluxes were less affected by salinity.	7
How did former freshwater coastal peatlands become influenced by high salinity and what can be learned for current and future salinization of coastal wetlands?	Due to the ability to float during spring tide, sharp salinity gradients are present along the borders of the bog remnant studied, with its center still functioning as a oligotrophic raised bog	8

9.3.4 Recommendations for management and policy

- As indicated in several sections of this chapter, there are still several processes for which there is no consensus in literature with respect to the effects of enhanced salinity. The studies presented in this thesis generated valuable additional knowledge about the effects of enhanced surface water salinity on biogeochemical processes, explaining the driving mechanisms. Although the studies carried out showed clear effects of enhanced salinity on physicochemical processes, methanogenesis, methane emission (diffusive and ebullitive fluxes), with important biogeochemical, ecological, and hydrological consequences, their regulation, interactions and implications for a larger scale warrant future interdisciplinary research.

- The new information presented in this thesis leads to a better understanding of the effects of enhanced salinity on aquatic and benthic organisms and communities, in combination with salinization studies with selected species in literature.
- A large part of the experiments were carried out in originally freshwater peatlands with historic brackish water influence. Although these systems are heavily influenced by eutrophication, land subsidence and strongly altered water management, they still form a special habitat (partly brackish peatlands) which is unique in Europe (Van 't Veer 2009, Van 't Veer et al. 2012). In contrast to what was expected, we showed that enhanced surface water salinity does not lead to enhanced nutrient availability, and may even reduce nutrient levels. It would therefore be a good option to test the effects of nutrient poor surface water with higher salinity on a larger scale, to be able to sustain and restore this unique Dutch landscape in the future.

Take home messages theme 2:

Surface water salinization

- Enhanced surface water salinity affects sediment phosphorus mobility to a great extent. As the effects are strongly linked to the characteristics of the sulfur, iron and calcium cycle, they are sediment specific. In S-rich wetlands salinization will lead to reduced P availability due to the formation of Ca-P complexes and minor effects of sulfide. In S-poor wetlands P availability can increase as a consequence of salinization.
- Enhanced salinity leads to the mobilization of ammonium from the cation adsorption complex in the short term and a potential reduction of N availability, or even N depletion, in the long term.
- Salinization promotes sediment cation (Ca, NH_4 , Mg, Fe etc.) mobilization in the short term. This cation exchange appeared to be the key process explaining several biogeochemical effects.
- Salinization causes a shift from methanogenesis to sulfate reduction in anaerobic wetlands sediments. This leads to enhanced sulfide concentrations and decreased diffusive and ebullitive methane fluxes (up to > 95% decrease above 2-2.5 PSU). CO_2 emissions are not influenced to a lesser extent.
- Surface water salinization leads to a combination of physicochemical and biogeochemical processes in the peat soil which have large consequences for gas production, gas movement and hydraulic conductivity.



[*Narthecium ossifragum* on the Brunssummerheide, by G. van Dijk]

Summary
Samenvatting
References
Curriculum vitae & publication list
Woord van dank

SUMMARY

Peatlands affected by biogeochemical stressors

This thesis covers two themes: (1) the effects of enhanced groundwater nitrate concentrations on groundwater fed mires, and (2) the effects of enhanced surface water salinity on peatland functioning. Mires (growing peatlands) are ecosystems in which dead organic material accumulates under wet and therefore (partly) anaerobic conditions. Peatland landscapes are often heterogeneous and rich in biogeochemical gradients. Relative to their small cover of the land surface, peatlands provide a disproportionately large number of services such as freshwater retention and storage, hydrological regulation, carbon sequestration, biodiversity and the filtering of nutrients and pollutants. In recent decades, they have received growing attention from both scientists and policy makers in relation to global change (including climate change), the importance of the ecosystem services they provide, and their great vulnerability to anthropogenic disturbance. Due to the complex functioning and heterogeneous nature of peatlands there are, however, still many uncertainties with respect to the nature and extent of anthropogenic impacts, their mechanistic understanding, and optimal mitigation options. One of the major causes for this complexity are the interactions among biogeochemical processes, physical processes and the species occurring. By combining different disciplines including landscape ecology, community ecology, microbiology and ecohydrology, with a main focus on biogeochemistry, this thesis addresses both themes using a combination of fundamental and evidence-based, applied research.

Theme 1: Groundwater nitrate enrichment and peatland functioning

Although groundwater nitrate concentrations are increasing on a global scale and may seriously affect groundwater fed ecosystems, relatively little is known about the consequences for ecosystem functioning. In the present thesis the effects of enhanced groundwater nitrate concentrations on the functioning of a groundwater fed mire are studied with respect to potential eutrophication and methane cycle, combining field and laboratory studies.

It is shown that groundwater nitrate enrichment can directly lead to **enhanced nitrogen availability and eutrophication effects** in nutrient poor, groundwater fed mires. It is shown to alter the vegetation composition and the nutrient levels in vascular plant and bryophyte tissue, increasing productivity and reducing biodiversity on the long term. Besides these direct effects, groundwater nitrate enrichment also has an indirect effect through the **mobilization of sulfate from geological sulfur deposits in the aquifer**. This can have a large impact on mire functioning as it may induce internal phosphate mobilization, sulfide toxicity and elemental sulfur precipitation in groundwater fed mires. **The combined enrichment with both electron acceptors (nitrate and sulfate) strongly affects chemical and microbial processes** with large consequences for nutrient and carbon cycling. This study also shows that the recently

discovered process of anaerobic nitrate dependent methane oxidation does also occur in mires, and can reduce methane emissions to the atmosphere in mires fed with nitrate rich groundwater. In the longer term, however, the enhanced availability of nitrate and sulfate may well lead to enhanced decomposition, slowing down peat accumulation and thereby also net carbon sequestration.

We therefore show clear effects of groundwater nitrate enrichment on biogeochemical cycling in groundwater fed ecosystems, with negative consequences for the conservation of these vulnerable systems and their biodiversity. Based on the outcome presented in this thesis and the predicted further increase of groundwater nitrate levels due to agricultural intensification, it is **urgently advised to rapidly take adequate measures to reduce nitrate leaching to the aquifer at the source and to mitigate its effects in nitrate sensitive ecosystems.**

Theme 2: Surface water salinization and peatland functioning

On a global scale the risk of salinization of coastal areas is increasing due to the combination of land use and climate change. This shows the urge for scientific studies on the effects of salinization on ecosystems, especially for systems providing important ecosystem services, such as peatlands. Current knowledge about the effects of enhanced salinity on biogeochemical, hydrological and ecological processes in peatlands, and about their interactions is, however, incomplete. This thesis shows the results of a number of studies on the effects of enhanced salinity on the functioning of peatlands, using a combination of controlled laboratory and field experiments.

Increased surface water salinity was shown to affect sediment nutrient mobility to a great extent, with important ecological consequences with respect to eutrophication. The effects on phosphorus mobility appeared to be strongly dependent on sediment sulfur, iron and calcium concentrations, and were therefore sediment specific. **In sulfur-rich wetlands salinization was shown to reduce phosphorus availability** due to the formation of calcium-phosphorus complexes, with minor effects of enhanced sulfate availability. In sulfur-poor wetlands the mobility of phosphorus could, however, increase as a consequence of salinization. **Sediment cation exchange appeared to be the key process explaining several salinity induced biogeochemical effects.** Surface water salinization results in sediment cation mobilization in the short term (e.g. calcium, magnesium and iron). In addition, increased ammonium mobilization from the cation adsorption complex enhances nitrogen availability in the short term, but potentially reduces nitrogen availability severely in the long term. Another important result is the fact that **surface water salinization causes a shift from methanogenesis to sulfate reduction** in anaerobic wetlands sediments, leading to **increased sulfide concentrations and reduced net greenhouse gas emissions.** Salinization leads to **reduced methanogenic activity** and **reduced diffusive and ebullitive methane fluxes** in the long term (up to > 95% decrease).

The study also shows that surface water salinization is able to **enhance the hydraulic conductivity of the sediment by a combination of physicochemical effects (pore dilation) and biogeochemical effects (decreased methanogenesis).**

The interdisciplinary studies presented in this thesis therefore illustrate that the major consequences of salinization for peatland functioning are caused by a complex interaction between physical, chemical, microbial and ecological processes. Although the complex effects warrant further study, the generated knowledge leads to a better understanding of the effects of enhanced salinity on peatlands and the contrasting effects reported in literature. In contrast to what was expected, we show that salinization does not necessarily increase nutrient availability in sulfate rich peatlands, and may even reduce surface water nutrient levels in the long term. This is valuable knowledge for a better understanding, prediction and mitigation of the effects of salinization on (coastal) peatlands and the ecosystem services they provide. In addition, however, a reduction in nutrient availability by salinization may also provide a good prospect for the restoration of the unique ecological characteristics of Dutch brackish peatlands in the future.



(Brunsummerheide, by G. van Dijk)

SAMENVATTING

Venen onder de invloed van biogeochemische stressoren

Dit proefschrift behandelt twee thema's over onderzoek in veengebieden. Het eerste thema richt zich op de effecten van nitraatvervuiling van grondwater op het functioneren van veengebieden en het tweede op verbrakking van oppervlaktewater op het functioneren van veensystemen. Veengebieden zijn ecosystemen waar organisch materiaal zich in de loop van de tijd ophoopt onder natte en (gedeeltelijk) anaerobe omstandigheden. Veengebieden zijn vaak heterogene landschappen, rijk aan biogeochemische gradiënten en bestaan voor minstens 30% uit organisch materiaal.

Veengebieden komen wereldwijd voor en de oppervlakte beslaat ongeveer 4 miljoen km² (2.6% van de totale landoppervlakte). Ondanks hun relatief geringe oppervlakte leveren veengebieden een verhoudingsgewijs grote bijdrage aan natuurlijke grondstoffen en zogenaamde ecosysteem-diensten, zoals zoetwater reservoir, regulering van de hydrologie, opslag van koolstof, biodiversiteit en een filterende functie van voedingsstoffen en vervuilende stoffen. Veenbodems vormen een belangrijke lange termijn opslag voor koolstof (naar schatting 30% wereldwijd). In verband hiermee en in relatie tot klimaatverandering en andere menselijke invloeden is de kwetsbare positie van veenecosystemen nadrukkelijker in de belangstelling gekomen.

In de afgelopen decennia zijn venen steeds meer onder de aandacht gekomen van de wetenschap en politiek door de toenemende antropogene invloeden op deze systemen en de potentiële negatieve consequenties hiervan op de natuurlijke grondstoffen en ecosysteem-diensten, die deze systemen leveren. Door de complexiteit van het functioneren en de heterogeniteit van veengebieden, bestaan er nog veel onzekerheden over hoe antropogene invloeden het functioneren van veengebieden beïnvloeden en welke consequenties die hebben. Een van de belangrijkste factoren die deze complexiteit veroorzaakt zijn biogeochemische processen en hun interacties met biotische en abiotische factoren, zoals voorkomende plantensoorten en fysische processen. Dit proefschrift richt zich, door een combinatie van fundamenteel empirisch en praktisch toegepast onderzoek vanuit verschillende vakgebieden, zoals (landschaps-)ecologie, microbiologie, (eco-)hydrologie met de nadruk op biogeochemie, op twee antropogene invloeden die tot op heden relatief weinig aandacht hebben gekregen in de wetenschappelijke literatuur: (1) de effecten van verhoogde nitraatconcentraties in grondwater op door grondwater gevoede hellingvenen en (2) de effecten van verhoogde oppervlaktewater zoutconcentraties op het functioneren van veengebieden.

Thema 1: Grondwater nitraat vervuiling en het functioneren van veengebieden

Ondanks dat nitraatconcentraties in het grondwater op wereldschaal stijgen en grondwater gevoede ecosystemen in toenemende mate hierdoor beïnvloed worden, is er relatief weinig bekend over de consequenties van deze antropogene invloed. In dit thema wordt onderzoek beschreven naar de effecten van verhoogde nitraatconcentraties in het grondwater op het functioneren van grondwater gevoede venen, met de focus op biogeochemische processen, potentiële (interne) eutrofiëring en de methaancyclus door de combinatie van veldstudie en laboratoriumonderzoek.

Het proefschrift laat zien dat nitraatvervuiling van grondwater direct kan leiden tot **verhoogde stikstof beschikbaarheid en eutrofiëring** in van oorsprong nutriënt arme grondwater gevoede venen. Verhoogde grondwater nitraatconcentraties blijkt te leiden tot een verandering in de vegetatiesamenstelling van vaatplanten en mossen en verandering van de chemische samenstelling in het veengebied. Op de langere termijn lijkt dit te kunnen leiden tot eutrofere ecosystemen met een geringere biodiversiteit. Naast deze directe effecten blijkt nitraatvervuiling van grondwater ook indirect te kunnen leiden tot **sulfaatmobilisatie vanuit zwavelhoudende bodemlagen in het watervoerende pakket**. Dit indirecte effect blijkt grote gevolgen te hebben op het functioneren van venen door verhoogde invloed van sulfaat (en sulfaatreductie), potentiële (interne) eutrofiëring, een verhoogde invloed van sulfide toxiciteit en neerslag van elementaire zwavel. **De combinatie van verhoogde concentraties van twee sterke reductoren (nitraat en sulfaat) blijkt chemische processen (o.a. redox processen) en microbiële processen** te beïnvloeden met grote consequenties voor nutriënt- en koolstof-cycli. Het proefschrift laat ook zien dat het recent ontdekte proces van nitraat afhankelijke methaanoxidatie in veengebieden optreedt en vermoedelijk gestimuleerd wordt door nitraatvervuiling, dat op de korte termijn kan leiden tot een gereduceerde methaanemissie naar de atmosfeer.

Zowel dit proefschrift als de recente literatuur indiceren duidelijke effecten van verhoogde nitraatconcentraties in het grondwater met potentieel grote consequenties voor biogeochemische cycli in grondwater gevoede systemen dat negatieve gevolgen kan hebben voor het behoud en de bescherming van deze kwetsbare systemen en hun biodiversiteit. Tot op heden zijn er relatief weinig maatregelen genomen om de nitraatvervuiling van het grondwater te verminderen. Op basis van de resultaten van dit onderzoek en de voorspelling van een in de toekomst zich verder intensiverende landbouw wordt **dringend geadviseerd om op korte termijn meer en adequatere maatregelen te nemen om de uitspoeling van nitraat naar het grondwater te voorkomen in combinatie met mitigerende maatregelen in kwetsbare ecosystemen die onder de invloed staan van met nitraat vervuild grondwater**.

Thema 2: Oppervlaktewater verbrakking en het functioneren van veengebieden

De combinatie van klimaatsverandering en toenemende antropogene invloeden op kustgebieden leidt op wereldschaal tot een steeds grotere kans van verhoogde zoutconcentraties in kustgebieden en het achterland. Dit verhoogt de behoefte van wetenschappelijk onderzoek aan de effecten van verhoogde zoutconcentraties op ecosystemen, met speciale aandacht voor systemen die veel natuurlijk grondstoffen en ecosysteem-diensten leveren, zoals veengebieden. De huidige kennis over de effecten van verhoogde zoutconcentraties op de (interactie tussen) biogeochemische, hydrologische en ecologische processen in veengebieden blijkt niet compleet. Dit proefschrift presenteert de resultaten van een serie onderzoeken aan de effecten van een verhoogde zoutconcentratie van het oppervlaktewater door een combinatie van gecontroleerde veld- en laboratoriumexperimenten.

Het laat zien dat verbrakking van het oppervlaktewater in hoge mate de mobiliteit van nutriënten in de waterbodem beïnvloedt, met belangrijke gevolgen met betrekking tot eutrofiëring. De effecten van verbrakking op de mobiliteit van fosfor blijkt sterk afhankelijk te zijn van de zwavel-, ijzer- en calciumconcentraties in de bodem en is daardoor afhankelijk van waterbodem eigenschappen. **In zwavelrijke veengebieden leidt verbrakking tot een verlaagde fosforbeschikbaarheid** door de formatie van calcium-fosfor complexen en kleine effecten van verhoogde sulfaatconcentraties. In zwavelarme veengebieden kan verbrakking echter juist leiden tot een stijging van de fosforbeschikbaarheid. **Uitwisseling van in de waterbodem gebonden kationen blijkt een van de sleutelprocessen te zijn die veel biogeochemische effecten van verbrakking kan verklaren.** Oppervlaktewater verbrakking blijkt op korte termijn te leiden tot kationmobilisatie in de waterbodem (o.a. Ca, NH₄, Mg, Fe). Verbrakking veroorzaakt mobilisatie van het kationadsorptiecomplex, dit blijkt op korte termijn de stikstofbeschikbaarheid te verhogen, maar kan leiden tot uitputting op langere termijn. Een ander belangrijk effect van oppervlaktewater verbrakking is de **verschuiving van activiteit van methanogene bacteriën naar sulfaatreducerende bacteriën** in anaerobe waterbodems in veengebieden. Deze verschuiving leidt tot **verhoogde sulfideconcentraties en gereduceerde koolstofemissie** naar de atmosfeer. Verbrakking leidt tot een **verlaagde activiteit van methanogene bacteriën** en **verlaagde diffuse en ebullatieve uitstoot van methaan naar de atmosfeer** op de langere termijn (tot > 95 % verlaging). Ook laat dit proefschrift zien dat verbrakking van oppervlaktewater kan leiden tot een **combinatie van fysisch-chemische (vergrootte bodemporiën) en biogeochemische effecten (o.a. gereduceerde methanogenese)** wat leidt tot een **verhoogde waterdoorlatendheid in de waterbodem**. De combinatie van een serie van interdisciplinair empirisch en toegepast onderzoek duidt op de grote consequenties van verbrakking op het functioneren van veengebieden en laat ook zien dat de interactie tussen fysische, chemische, microbiologische en ecologische processen als gevolg van verbrakking complex zijn. Ondanks de complexiteit van de effecten van verbrakking van oppervlaktewater, leidt het in dit proefschrift gepresenteerde onderzoek tot een beter begrip van de effecten

van verbrakking in veengebieden. Onverwacht blijkt een verhoging van de zoutconcentratie van het oppervlaktewater te leiden tot een verlaging van de beschikbaarheid van nutriënten op de lange termijn. Dit biedt toekomstperspectief voor bescherming en herstel van deze speciale, karakteristieke Nederlandse brakwater veengebieden in de toekomst. Tevens levert dit proefschrift waardevolle kennis waarmee effecten van toekomstige verbrakking van veengebieden en de door deze systemen geleverde ecosysteem-diensten, waaronder het filteren van nutriënten, koolstofopslag en biodiversiteit, beter kunnen worden voorspeld (en indien nodig gemitigeerd).

Veengebieden staan onder druk door menselijke activiteiten, zoals afgraven en versnippering, water- lucht- en bodemverontreiniging, eutrofiering en sterk aangetaste waterhuishouding die vaak tot water tekorten leiden. Dit heeft nadelige gevolgen voor het functioneren van de venen zelf, maar ook voor de natuurlijke grondstoffen en ecosysteem-diensten die venen leveren. Wereldwijd is ongeveer 20% van de veengebieden vrijwel verdwenen of sterk aangetast en menselijke invloed op bestaande gebieden neemt alleen maar toe. **Wetenschappelijke kennis, ook praktische en direct toepasbare, over hoe menselijke invloed het functioneren van veengebieden beïnvloedt en hoe dit kan worden herstelt, is daarvoor hoogst noodzakelijk om de juiste beslissingen te kunnen nemen over het duurzaam beheer van veengebieden en omliggende landschappen.**



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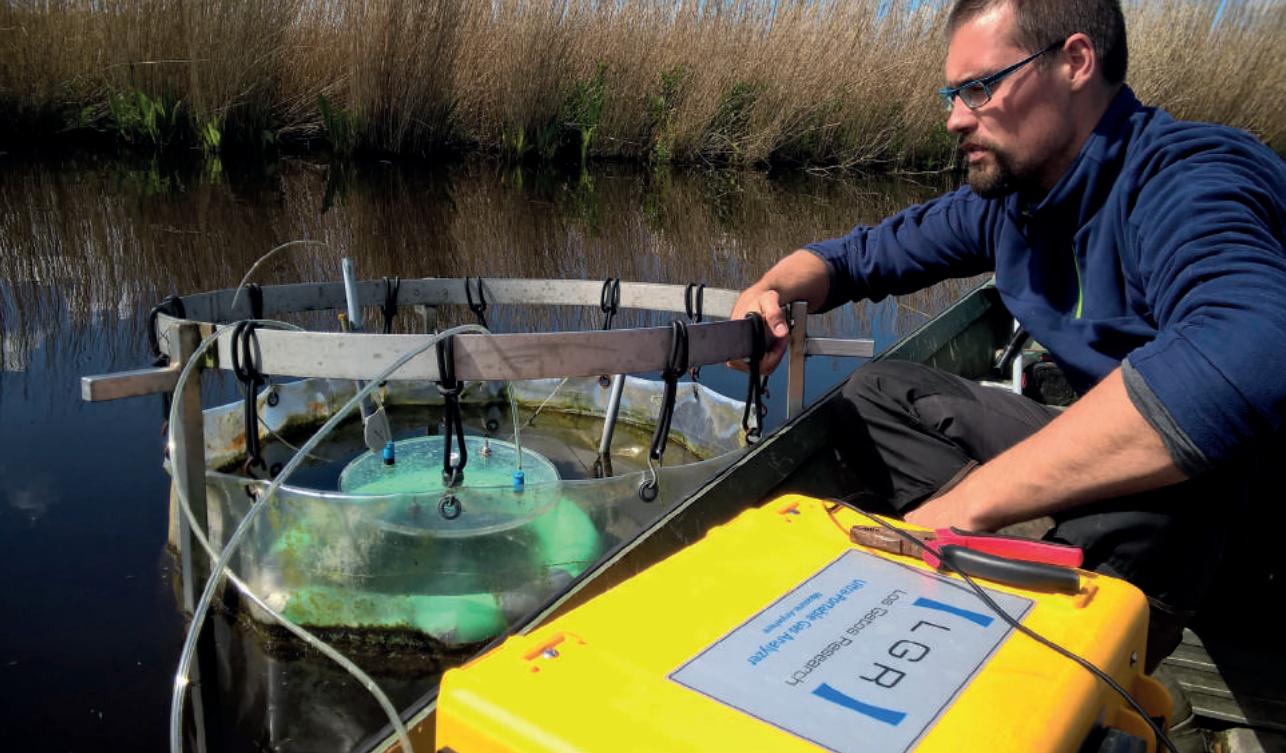
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(above G. van Dijk sampling in enclosure experiment, by S. Kosten.
below G. van Dijk and son S. van Dijk, by C. Bufel)

CURRICULUM VITEA - ABOUT

Gijs van Dijk was born on the 20th of November 1984 in the small town of Uffelte in the Province of Drenthe in a so called “nest of biologists”. With his mother being an ecologist and botanist working at the nature conservation organization Stichting Het Drentse Landschap and his father an ornithologist working at Sovon (Dutch Centre For Field Ornithology).

Gijs was raised both in the Province of Drenthe and in the Province of Gelderland (near the city of Nijmegen), did his primary school at the Jenaplan school Sterrendans in Nijmegen and his secondary school at the Stad and Esch college in Diever after which he started his studies on Environmental sciences at the Van Hall Larenstein in the city of Leeuwarden. During this study he was though a brought spectrum of subjects ranging from wastewater treatment to hydrology and aquatic ecology to soil management. Although his main interest was ecology this brought spectrum of subjects lay the basis for his later work by shaping a holistic view on ecosystems and enabled him to combine different fields of research. During this studies he did three internships, the first at Waterboard Groot Salland where his enthusiasms for the world of aquatic invertebrates was born. Hereafter he did an internship at Stichting Bargerveen/Radboud University (together with Sjoerd de Beer) on aquatic invertebrate communities in raised bogs in the Baltic States (under the supervision of Gert-Jan van Duinen), during this internship he got acquainted with ecological research which tasted for more. Gijs obtained his BSc degree on a study on the effects of winter flooding on plant communities and soil chemical parameters at the Rijks Universiteit Groningen (together with Jelmer Nijp) under the supervision of Ab Grootjans. Aside from his internships he rediscovered the extremely rare water beetle *Dytiscus latissimus* in the Netherlands which resulted in work for EIS-Nederland on the distribution of this species.

To gain more experience in the subjects of landscape ecology, aquatic ecology and practical applied research he decided to continue his studies and started a MSc in Biology at the Radboud University in Nijmegen. During this studies he did two internships, one at B-WARE Research Centre and one at the department of Aquatic ecology and environmental biology. The internship at B-WARE Research Centre was a partly self-organized landscape ecological study on a percolation mire at the Brunssummerheide (Province of Limburg, The Netherlands) and the effects of groundwater nitrate enrichment with a main focus on biogeochemical processes under the supervision off and together with Fons Smolders, Nicko Straathof, Christian Fitz, Gert-Jan van Duinen and Ab Grootjans. During this internship he started the second one (under the supervision of Christian Fritz and Fons Smolders) on the effects of addition of nutrients in nutrient poor *Sphagnum* moss dominated bogs in Tierra del Fuego (Argentina).

In the last phase of his studies he was asked by Fons Smolders and Leon Lamers to work at B-WARE Research Centre at a project on the effects of increased salinity in peatlands which in combination with his work on the percolation mire at the Brunssummerheide formed the basis of the present thesis. During his work at B-WARE Research Centre Gijs did work on a variety of projects and practical applied research. He worked at a brought range of ecosystems but specialized on peatlands and aquatic systems. A part of the studies he carried out is included in the present thesis. During his studies and later work at B-WARE Research Centre Gijs was able to publish over seven peer reviewed scientific articles, seventeen Dutch and German articles, two book chapters, several reports, and presented his work on national and international symposia.



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Department of Aquatic Ecology and Environmental Biology

W www.ru.nl/science/aquatic/

DONDERDAG 24 NOVEMBER 2005

Geelkever duikt weer op in Drenthe

De redactie wetenschap
ERDAM, 24 NOV. De groot-
terroofover van Europa, de
geelgerande waterroofo-
dyllus (latissimus), is eind ok-
tober in Drenthe herontdekt door
milieukunde Gijs van
Van Hall Instituut, Lelouwer-
in 1967 voor het laatst in Ne-
land gezien, bij Uffelte, Zuid-
Drenthe. Experts dachten
dat het insect was uitgestorven,
vermoeden nu dat het
tijd al die tijd over het
werd gezien. De precieze
plaats wordt geheim gehou-
om de kever tegen verza-
te beschermen.



Geelkever bacterie vree

Als bijvangst van een
natuuronderzoek in een
Limburgs veengebied
ontdekten microbiologen
een nieuwe bacterie. Deze
methaanvreter kan een
hulpje zijn bij
waterzuivering.



PETER DE JACKE

O p de Brunssummer-
Zuid-Limburgs be-
dersoekers van de
Universiteit Nijme-
ontdekt die een truce
bacterie onder de natu-
die hoort eerder in de natuur
waarschijnlijk. Zij bleek in de bodem
al waterstof te halen uit ni-
bet sterke broeikasgas methaan af
door pure zuinig van overbese-
traat, ademsig bit-
landbouwgronden.

vrijdag, 14 december 2012

ONDERZOEK Organisme Nieuwe bacterie Brunssummer

door onze verslaggever

BRUNSSUMMER - Onderzoekers van
Radboud Universiteit Nijmegen
onderzoekscenrum B-WARE
ben op de Brunssummerheide
nieuwe bacterie ontdekt. Het
om een bacterie die methaan (r-
gas) 'eet' met behulp van v
reinigende stikstofverbinding
De ontdekking werd ged
een hellingengebiedje van
verschillende plant- en d
ten. Terreinbeheerder Natu
menten riep de hulp in va
derzoekers om te bepalen hoe
kwetsbare gebiedje het beste
scherm zou kunnen worden. On-
derzoeker Gijs van Dijk van B-WA-
RE: „Diep in de veenbodem vond
ik hoge concentraties

De L
Limb

Terug van jaren weggeweest evervis en waterroofover

Van onze verslaggever
Karin Stals
GRONINGEN

Het zijn goede tijden voor zeld-
me dieren in Nederland. In Dren-
terroofover zich zien, terwijl le-
was. En op Texel speelde een ever-
vis aan. De kever was sinds 1967
nog nooit eerder op het Neder-
landse strand aangevoeld.
In 1970 dacht iemand die brude
geelgerande waterroofover te
niet bevestigd. Vorige maand
doek hij op in de neten van Gijs
van Dijk, milieukundestudent
megen. Maar waarschijnlijk is de
van verontreinigende bossen en land-
den, en elke veenbod

kever nooit weggeweest, legt Van
Dijk uit. Hij is moeilijk te spot-
bijn niet te zien, dan is hij
heel snel zwemt.
Waar hij de kever precies zag,
jagers. 'Ergens in Drenthe'. De
geen schatje. De larven hebben
'spitje, holle kaken waarmee ze
hun pool doorboren om de
vervolgens leg te zuigen', meldt
het persbericht. 'Ze lijken in alles
De kever - 3,6 tot 4,4 centime-
ter lang - is de grootste en zeld-
zaamste geelgerande waterroo-
schild zijn afgeplat, waardoor hij
dijkbaad kan zwemmen.
Ook op Texel wordt de vondst

van een bijzonder
zich is de evervis
maakt hij in Neder-
is uniek. Het platte
meter lang, lag op he-
De oogjes waren ni-
zakt, waaruit blijkt d
niet lang dood was.
was verdwaald, want
leen maar in diepte v
in de Atlantische Oce
den van Ierland en Eng
Het aangespoelde
was oranje, terwijl d
gesproken roodachtig
kreeftjes is gaan eten, el
kij niet op het manni
vis eet poliepen, en kw
maakt die zijn hier niet te
zegt bioloog Pierre Bou
Ecomare.

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Mind & Brain

Videos

Plants & Animals

Images

Earth & Climate

Space

CLIMATE MONITORING

software for simulating & analyzing climate

from universities, journals, and other

Science News

New Peatland Bacteria Feed On Greenhouse Gas and Ex

Dec. 14, 2012 — Researchers from Radboud University Nijmegen and B-WARE Research have discovered new methane-consuming bacteria in the soil beneath the Brunssummerheide in Limburg, the Netherlands. This may be the result of a new type of bacteria that they are now consuming

Wat wil dit zeldzame kevertje om te overleven?



DAGBLAD
NOORDEN
16 juli 2016, pag. 20

Kippenlevers eten met een numme

Wat heeft de ja-
renlang in Neder-
land uitgestorven
gewaande wa-
terroofover no-
dig om te overle-
ven? Bij Uffelte
zoeken ze het uit.



De brede geelgerande waterroofover was uitgestorven in Nederland.



PUBLICATION LIST

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Van Dijk, G., J.J. Nijp, K. Metselaar, L.P.M. Lamers, A.J.P. Smolders, 2017. Salinity-induced depression of methanogenesis increases hydraulic conductivity in the hyporheic zone of coastal wetlands, **Hydrological Processes**, 31, (4) 880-890

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Scholten I., H. van Kleef, **Van Dijk, G.**, Brouwer, J., Verberk, WCEP (*submitted to Insect Conservation and Diversity*). Larval development, metabolism and diet are possible key factors explaining the distribution decline of the specialized Broadest diver (*Dytiscus latissimus*)

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Cusell, C., B. de Haan, G. Kooijman, J. van Diggelen, **G. van Dijk** (*in prep.*) Vegetatieontwikkeling en de resultaten van decennia water- en natuurbeheer in De Wieden en De Weerribben

Van Dijk, G., A.J.P. Smolders, R. Loeb, C. Cusell, P.J. Westendorp, H. van Kleef, J.J. Nijp, S. Kosten, L.P.M. Lamers, (*in prep.*) Moet toekomstige verbrakking van laagveenpolders met een korreltje zout genomen worden?

*Some of the studies on which Gijs worked also got attention from the media such as his re-discovery of and later work on the waterbeetle *Dytiscus latissimus*, his work on the bog orchid *Hammarbya paludosa* and the discovery of anaerobic oxidating bacteria in a minerotrophic peatland (chapter 3). His research got attention in media like de Volkskrant, Trouw, het NRC, het Parool, VARA Vroege Vogels, Science Daily, Dagblad van het Noorden, De Limburger (see page 242).*



(Brunsummerheide, by G. van Dijk)

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Ooij, oktober 2017

Gijs

ONDERZOEKCENTRUM B-WARE

Onderzoekcentrum B-WARE is een spin-off bedrijf van de afdeling Aquatische Ecologie & Milieubiologie, welke deel uitmaakt van het onderzoeksinstituut IWWR (Institute for Water and Wetland Research) van de Radboud Universiteit Nijmegen.

Onderzoekcentrum B-WARE (Biogeochemical Water-management & Applied Research on Ecosystems) is een op duurzaamheid gerichte onderneming die inspeelt op de maatschappelijke behoefte aan nieuwe en toepasbare kennis op het gebied van water- en natuurkwaliteit. B-WARE is gespecialiseerd in het uitvoeren van toegepast en strategisch onderzoek naar de biogeochemische en ecologische processen die bepalend zijn voor de kwaliteit van natuur- en waterbeheer. In de praktijk richt zich het onderzoek voornamelijk op de sturende biogeochemische processen in hoogvenen, laagvenen, meren, plassen, vennen, beekdalen, polders en broekbossen. Daarbij wordt gebruik gemaakt van de nieuwste kennis en analysetechnieken. Naast de eigen analysefaciliteiten kan B-WARE gebruik maken van de analyseapparatuur van het Gemeenschappelijk Instrumentarium van de Radboud Universiteit Nijmegen.

De werkwijze omvat de combinatie van analyses in de veldsituatie en het opzetten en uitvoeren van veldexperimenten en experimenten onder gecontroleerde laboratoriumcondities, naast deskresearch. Deze werkwijze garandeert, zoals blijkt uit de praktijk, dat de door B-WARE verstrekte adviezen, bij uitvoering in de praktijk, een maximale kans van slagen hebben. De verkregen resultaten dragen direct bij aan het ontwikkelen van nieuwe inzichten in het natuur- en waterbeheer, zodat deze expertise kan worden toegepast op natuurherstel, natuurontwikkeling en waterberging.

Onderzoekcentrum B-WARE is er, sinds de oprichting in 2002, voor opdrachtgevers zoals ministeries, provincies, provinciale landschappen, gemeentes, particuliere terreinbeheerders en natuurbeschermingsorganisaties, waterschappen en adviesbureaus.

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Groundwater nitrate enrichment and peatland functioning

How does groundwater nitrate enrichment affect the functioning of groundwater fed mires and how does this influence methane oxidation in these systems?

Surface water salinization and peatland functioning

How do (fluctuating) and enhanced surface water salinity levels affect biogeochemical sediment processes in aquatic sediments? And how does this influence nutrient availability, hydrological properties and carbon fluxes to the atmosphere?



Radboud University

