WATER LEVEL FLUCTUATIONS IN RICH FENS

AN ASSESSMENT OF ECOLOGICAL BENEFITS AND DRAWBACKS

Ivan S. Mettrop

Water level fluctuations in rich fens
An assessment of ecological benefits and drawbacks

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This research project was financially supported by the National Program Kennisnetwerk Ontwikkeling en Beheer Natuurkwaliteit (O+BN) of the Ministry of Economic Affairs, Agriculture and Innovation.

ISBN 978-94-91407-23-9

Cover: Short-term summer inundation with base-rich and nutrient-poor surface water is considered beneficial in the management of non-floating rich fens, as elucidated in Chapter 5. Martin Chytrý is acknowledged for permission to use his high quality images of *Hamatocaulis vernicosus* in this photomontage.

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Academisch Proefschrift

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. D.C. van den Boom
ten overstaan van een door het College voor Promoties ingestelde
commissie, in het openbaar te verdedigen in de Agnietenkapel
op woensdag 21 oktober 2015, te 14.00 uur

door

Ivan Sebastiaan Mettrop

geboren te Amsterdam

Promotiecommissie

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Faculteit der Natuurwetenschappen, Wiskunde en Informatica

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Chapter 1

General introduction

1.1. Rich fens

A rich fen is a mire type that is characterized by base-rich and nutrient-poor (mesotrophic) conditions (Sjörs, 1950; Van Wirdum, 1991; Kooijman, 1993; Wheeler and Proctor, 2000). The term 'rich' not only refers to the high concentrations of minerals, but also to the high floristic diversity (Wassen et al., 2005; van Diggelen et al., 2006). The vegetation composition in rich fens (*Scorpidio-Caricetum diandrae*) strongly depends on sufficient supply in the topsoil of mineral-rich surface water and/or groundwater, which has been in contact with base-rich substrates (Gore, 1983; Van Wirdum, 1993; Wheeler and Proctor, 2000). As a consequence, rich fens harbor a large number of threatened minerotrophic vascular plant species and brown mosses, which depend on relatively high acid neutralizing capacity (ANC), high pH, and low nutrient availability.

Rich fens have become very rare in densely populated and heavily exploited landscapes, and are therefore protected as EU priority habitat *H7140A – Transition mires and quaking bogs (Quaking fens)*, which is one of the different peatland habitat types as differentiated within Natura 2000 legislation. The distinction between many Natura 2000 wetland habitat types is based on different successional stages in the encroachment of open water by vegetation (terrestrialization), which is to a great extent determined by successive biogeochemical conditions and processes (Tallis, 1983). With respect to the conservation and management of rich fens, it is therefore important to focus on the processes that are involved in this terrestrialization process.

The earliest stage of this succession is characterized by aquatic vegetation such as *Chara* spp. in small open water bodies (habitat type H3140; Figure 1.1). In this phase, only small amounts of organic matter have accumulated yet and mineralization rates are low (Koerselman and Verhoeven, 1992). *Stratiotes aloides* L., with its sturdy leaves, may facilitate further encroachment by providing physical support and is considered an important constituent of the next step in succession (Sarneel et al., 2011; Harpenslager et al., 2015), leading to habitat type H3150. As a result of further hydrosere succession, floating root-mats are formed, which stay in direct contact with the minerotrophic surface water. Since microbial decomposition is still slowed down under these waterlogged, anaerobic conditions, dead remains of constituent plants gradually accumulate, and formation of peat is initiated (Tallis, 1983). This relatively thin, base-rich but nutrient-poor peat layer provides the

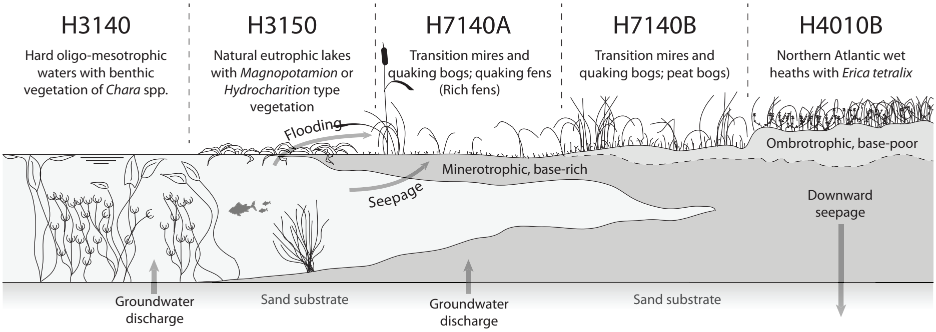

Figure 1.1. Schematic overview of the terrestrialization process and the different stages/Natura 2000 habitat types in order of hydrosere succession from open water towards (semi-)terrestrial peatland.

required conditions for development of rich fens (Habitat type H7140A) and their characteristic bryophytes such as *Scorpidium scorpioides* (Hedw.) Limpr., *Scorpidium cossonii* (Schimp.) Hedenäs, and *Hamatocaulis vernicosus* (Mitt.) Hedenäs, also referred to as brown mosses. For preservation of rich fens, it is essential that the surface layer stays in direct contact with minerotrophic, base-rich water. Once the peat growth exceeds the minerotrophic water layer and peat deposits become isolated, the influence of poorly buffered rainwater becomes bigger (ombrotrophic conditions), which leads to a decreased ANC and formation of peat bog vegetation (H7140B) (Van Wirdum, 1991; Koerselman and Verhoeven, 1992; Van Diggelen et al., 1996). In addition, aerobic conditions and hence oxidation processes, in which oxygen is used as a terminal electron acceptor, lead to acidification (Stumm and Morgan, 1996), which in turn results in reduced ANC. Development of habitat type H7140B is a relatively rapid process that is accompanied by the invasion of *Sphagnum* species (Bellamy and Rieley, 1967; Tallis, 1983; Kuhry et al., 1993; Laine et al., 2011). Since *Sphagnum* species release protons in exchange for other cations (Clymo, 1963; Kooijman and Bakker, 1994), acidification of the rich fen bryophyte layer is intensified (Van Wirdum et al., 1992). The increased gradual loss of contact with minerotrophic water, increased peat accumulation by *Sphagnum*, and further succession leads to development of wet heaths, defined as habitat type H4010B.

In the Netherlands, where both fens and bogs were exploited for fuel since medieval times, but particularly during the 18th and 19th centuries, these different wetland habitat types successfully developed in residual peat excavation turbaries (Van Wirdum et al., 1992; Vermeer and Joosten, 1992). However, during the past 50 years, the hydrosere succession by vegetation in open water is inhibited (Lamers et al., 2002), resulting in absence of rich fen rejuvenation. In addition, transition from rich fens to bogs is enhanced, resulting in deterioration of present rich fen habitats.

Consequently, rich fens dominated by *S. scorpioides*, *S. cossonii* and *H. vernicosus* have become very rare in the Netherlands (Kooijman, 1992; Paulissen et al., 2013; Cusell, 2014a). Also other countries in Western Europe show serious deterioration of brown moss-dominated rich fens over the past decades (JNCC, 2007).

1.2. Environmental constraints

The major constraints on the conservation and restoration of rich fens in agricultural areas in Europe are considered to be acidification, eutrophication and toxicity, next to direct drought effects on communities (e.g. Lamers et al., 2015). All of these environmental constraints are induced by anthropogenic disturbance.

Acidification

The cause of acidification lies in several different processes. Hydrological isolation from base-rich groundwater and surface water, caused by natural succession and/or anthropogenic intervention, has led to reduced ANC in fen peatland regions with intensive agriculture (e.g. van Wirdum, 1991; Van Diggelen, 1996). Presumably, increased atmospheric N-deposition as a result of fossil fuel combustion and intensive cattle farming has exacerbated the acidification of fens due to direct influx of nitric acid and sulfuric acid, and indirectly by additional ammonium oxidation (nitrification) and sulfide oxidation during periods of drought (Gorham et al., 1987; Lamers et al., 2015). Finally, the shift from base-rich bryophytes to *Sphagnum* spp. may lead to further acidification, since *Sphagnum* spp. can release protons in exchange for other cations (Clymo, 1963; Kooijman and Bakker, 1994).

Eutrophication

The term eutrophic refers to relatively high availability of primary nutrients, and eutrophication refers to the increased availability of elements limiting primary production in an ecosystem. In general, eutrophication causes the disappearance of characteristic slow-growing plant species and bryophytes, because they are outcompeted by strongly competitive, faster growing and generally more common species, leading to biodiversity loss (Wheeler and Shaw, 1991). In the case of rich fens, rapid succession towards poor fens or bogs (habitat type H7140B; Figure 1.1) is enhanced. So, to conserve the present brown moss-dominated rich fens, site conditions should be characterized by a relatively low nutrient availability (Kooijman, 1993). The nutrients phosphorus (P) and nitrogen (N) are the primary nutrient-limiting elements for rich fens (Verhoeven and Schmitz, 1991; Koerselman and Meuleman, 1996; Boeye et al., 1997; Wassen et al., 2005; Cusell et al., 2014b). Since nutrient availability is determined both by the balance of nutrient in- and outputs and by the internal cycle within the ecosystem, generally a distinction is made between external and internal eutrophication.

External eutrophication is defined as the increase of nutrient availability as a result of input from outside of the fen system. Influx of nutrient-rich surface water and groundwater, together with the major input of mainly N via atmospheric deposition, has caused severe deterioration of fens in the Netherlands over the past decades (Koerselman et al., 1990; Koerselman and Verhoeven, 1992; Lamers et al., 2002; 2015).

The increase of nutrient availability as a result of enhanced mobilization from the soil itself is called internal eutrophication (Roelofs, 1991; Smolders et al., 2006). Microbial mineralization of organic N and P is a major source of nutrients (Chapin, 1980; Verhoeven, 1986; Verhoeven et al., 1988). Particularly upon increased oxygen availability, and hence stimulation of decomposition rates, large amounts of nutrients can be released by means of mineralization of peat soils (Williams and Wheatley, 1988; Bridgham et al., 1998; Updegraff et al., 1995; Olde Venterink et al., 2002). In addition, P-availability may increase under anaerobic conditions as a result of net P-mobilization due to Fe reduction (Patrick and Khalid, 1974). Especially in Fe-rich soils with high P-contents, this anaerobic P-mobilization can be severe (Loeb et al., 2008; Zak et al., 2010; Cusell et al., 2013a). These ways of nutrient legacy from the topsoil can pose a major constraint on the restoration of fens, especially in (former) agricultural areas (Lamers et al., 2002; Zak et al., 2010).

Toxicity

Especially in agricultural areas, toxicity may be an additional problem for the rehabilitation of rich fens. As a result of fertilization and reduction of nitrate, but also as a result of atmospheric N-deposition (Verhoeven et al., 2011), ammonium concentrations may strongly increase, potentially reaching toxic concentrations to brown mosses such as *S. scorpioides* (Paulissen et al., 2004). In addition, sulfide and Fe(II) are considered potential toxins to rich fen vegetation (Lamers et al., 2015).

1.3. Restoration

Restoration objectives

Rich fen management aims at both the rejuvenation of rich fens via hydrosere succession from aquatic vegetation in open water, and the inhibition or resetting of the transition from present rich fens to poor fens or bogs.

The absence of hydrosere succession, and hence the absence of newly formed rich fens is generally attributed to P-eutrophication of surface water and banks of turbaries (Lamers et al., 2002), toxicity of sulfide and/or ammonium in underwater soil pore water (Roelofs, 1991; Smolders and Roelofs, 1993; Lamers et al., 2013), and the absence of species facilitating terrestrialization by habitat and/or dispersal constraints (Lamers et al., 2015). Both via external P-inputs and internal P-mobilization, growth of highly productive phytoplankton is stimulated, resulting in turbid surface waters and decline of macrophytes (Scheffer et al., 1993). Although the water quality in Dutch wetlands has slightly improved over the past 15-25 years, new development of rich fens with *S. scorpioides* is yet absent.

Moreover, active management of rich fens is focused on preventing transition to *Sphagnum*-dominated poor fens or bogs (Van Diggelen et al., 1996; Lamers et al., 2002). Without active management, only acid fens and bogs/woodlands will remain. So, succession in rich fens needs to be slowed down or even inhibited, especially given the fact that new formation is hardly taking place. Annual mowing is necessary, as highly competitive, fast-growing plant species easily become dominant at the expense of lower, slow-growing species, especially under more eutrophic conditions. Further, nutrients can be removed from the system by harvesting, leading to increased species-richness (Vermeer and Berendse, 1983). Moreover, it is essential that the top of the peat layer stays in direct contact with minerotrophic, base-rich and nutrient-poor ground or surface water to keep favorable conditions for minerotrophic plant species and brown mosses, and to prevent favorable conditions for *Sphagnum* spp.

Water table fluctuations as a restoration measure?

During the past decades, water levels in European rich fen areas have often become constricted within narrow limits as a result of adjacent agricultural water management. In pristine wetlands, however, water levels vary with the meteoric and groundwater balances in and around these wetlands (Baker et al., 2009). From a management perspective, the re-establishment of fluctuating water levels is considered for non-pristine fens in order to optimize the generic ecological quality (Vermeer and Joosten, 1992; Lamers et al., 2002; Cusell et al., 2013b). Since fluctuation of the water level is a major factor determining biogeochemical and ecohydrological processes and functioning of wetlands, potential benefits and disadvantages of re-establishment of fluctuating water tables have been considered in previous studies (e.g. Mettrop et al., 2012; Cusell et al., 2013b; 2014a).

During periods with lower water levels, aerobic oxidation processes prevail due to oxygen intrusion into the soil, potentially decreasing the ANC and pH (Stumm and Morgan, 1996), and increasing nutrient-mineralization (Olde Venterink et al., 2002). These effects could hamper the development of protected brown moss vegetation in rich fens, especially during summer (Cusell et al., 2013a). However, temporary drought may be beneficial to some extent, since Fe-oxidation can lead to rapid binding of phosphate in the soil (Richardson, 1985), which may temporarily reduce P-availability in porewater that may be important to conserve P-limited vegetation types. Moreover, the impact of drought may strongly differ among fens with different biogeochemical characteristics and vegetation. In fens with high iron (Fe) and/or sulfur (S) contents, the effects of drought-induced oxidation and acidification may be stronger than in calcium (Ca) rich fens, because Ca and $CaCO_3$ are not redox sensitive and changes in pH can be buffered (Stumm and Morgan, 1996). The response of P-availability to drought may also differ among fen types, since the P-binding capacity of the soil under oxic conditions is expected to strongly depend on the soil Ca and/or Fe contents. In addition, a high drought incidence can have direct effects via drought stress in vascular plants and bryophytes. As a result, typical wetland plant communities may be replaced by vegetation favored by drier conditions (Lamers et al., 2015). All these combined effects of a higher drought incidence may lead to favorable conditions for *Sphagnum* spp. at the expense of protected rich fen brown mosses.

During periods with increased water levels, inundation may occur. In the case of water rich in Ca and HCO_3, inundation can potentially increase soil ANC via infiltration, and also by internal alkalinity generation as a result of reduction processes (Stumm and Morgan, 1996). At the same time, however, P-availability may increase as a result of net P-mobilization (internal eutrophication) due to Fe reduction (Patrick and Khalid, 1974). Especially in Fe-rich soils with high P-contents, this anaerobic P-mobilization can be severe (Loeb et al., 2008; Zak et al., 2010; Cusell et al., 2013a). Moreover, high sulfate reduction rates and formation of iron sulfides may result in reduced soil P-binding to Fe, and hence additional P-mobilization in S-rich soils (Caraco et al., 1989; Smolders and Roelofs, 1993; Lamers et al., 1998). In addition, anaerobic conditions may lead to the formation of potential phytotoxins such as ammonium, sulfide, and Fe(II) (Lamers et al., 2015). Increased surface water influence, as a result of inundation, can also lead to higher nutrient inputs (external eutrophication; e.g. Wassen et al., 1996). In relatively nutrient-poor (mesotrophic) fens adjacent to agricultural areas, external P-input can be highly detrimental (Koerselman and Verhoeven, 1992; Lamers et al., 2015). This effect may also strongly depend on biogeochemical characteristics of the peat soil.

1.4. Main objectives and thesis outline

The main question raised in this thesis is: what are the ecological benefits and drawbacks of re-establishment of water level fluctuations as a management tool in rich fens, and what can be concluded after weighing these benefits and drawbacks in terms of nature and water management? The sequence of chapters is based on an increasing scale in experimental setup. In Chapter 2, the effects of different gradations of drought on acidification and mineralization rates are studied in a long-term incubation experiment, involving peat soil samples from brown moss-dominated rich fens and *Sphagnum*-fens. To gain more detailed insight into the influence of vegetation development during water level manipulations with different water qualities, and the importance of chemical soil characteristics, Chapter 3 describes a mesocosm experiment in which subsequent periods of drought and inundation were simulated with P-poor and P-rich supply-water. Chapter 4 and 5 report on large-scale field experiments, assessing the biogeochemical impacts of 2 weeks of inundation both in summer and winter, and 2 weeks of drought in summer. Both floating and non-floating fens are included with different fen vegetation types. In Chapter 6, the relative importance of Ca and Fe for nutrient availability, plant productivity and species composition in brown moss-dominated rich fens is discussed, based on extensive analyses of soil samples from the Netherlands (strong anthropogenic forcing) and central Sweden (weak anthropogenic forcing). Finally, Chapter 7 provides a synthesis in which results and conclusions from the preceding chapters are discussed and integrated in a comparative overview of potential ecological benefits and drawbacks from a management perspective.

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CHAPTER 2

Nutrient and carbon dynamics in peat from rich fens and *Sphagnum*-fens during different gradations of drought

Ivan S. Mettrop, Casper Cusell, Annemieke M. Kooijman, Leon P.M. Lamers

Soil Biology & Biochemistry 68 (2014), 317-328.

Abstract

Drought has major impacts on microbial decomposition and net N- and P-release in peat. The separate effects of aeration (oxygen intrusion) during moderate drought and desiccation (oxygen intrusion plus water deficiency) during severe drought are, however, poorly understood. This information is vital to understand the biogeochemical and ecological effects of different gradations of drought in peatlands. In addition, effects may differ between rich fen peat and *Sphagnum*-dominated poor fen peat. We therefore conducted a controlled incubation experiment involving both soil types to quantify the rates of decomposition, net N-mineralization, net P-release, denitrification, and the partitioning of C, N and P in soils and microbial biomass under three different incubation conditions. Soils were incubated under (1) anaerobic, waterlogged conditions, (2) aerobic, moist conditions, characteristic for moderate drought in which oxygen intrusion takes place, and (3) aerobic, desiccated conditions to simulate severe drought.

Our results show that under anaerobic, waterlogged conditions, net N-mineralization rates per mass dry peat soil and per microbial C mass were much higher (on average 10 times) in the *Sphagnum*-peat than in peat from rich fens, probably caused by higher microbial N-demand and N-immobilization in rich fens. The response upon aeration differed greatly between rich fen peat and *Sphagnum*-peat. Whereas aeration led to increased respiration and net N-mineralization rates in the rich fen peat, these rates did not change for *Sphagnum*-peat. The absence of aeration effects in *Sphagnum*-dominated fens suggests that decomposition rates are more strongly determined by litter quality than by oxygen intrusion. Upon further desiccation, both net P-release and DOC production, which remained unchanged upon aeration, increased significantly for both fen types. This may be due to microbial die-off and/or a change in microbial composition. The low anaerobic net N-mineralization rates and the strong response to aeration in rich fens compared to *Sphagnum*-fens, as well as the strong increase in P-availability upon further desiccation in both fen types, have important implications for peatland management in relation to drought.

2.1. Introduction

Acidification and eutrophication are considered a threat to nitrogen- and phosphorus-limited, minerotrophic base-rich fens, which are generally called ‘rich fens’ (Kooijman, 1992; Van Wirdum, 1993; Paulissen et al., 2004; Kooijman, 2012). These rich fens belong to the EU priority habitat H7140; Transition mires and quaking bogs. For the conservation of rich fens, it is important to keep these habitats base-rich, and nutrient-poor. As the water level is a key factor determining the biogeochemical processes and functioning of wetlands (Reddy and Patrick, 1974; Loeb et al., 2008) and wetland hydrology in densely populated regions across the world has strongly been affected by anthropogenic influence (Lamers et al., 2002; Limpens et al., 2008), it is important to gain insight into the biogeochemical processes resulting from water level drawdown with regard to net mobilization of nutrients in these fens.

As undisturbed wetlands are generally characterized by high water levels, the decomposition of organic matter is mainly carried out by microorganisms that require electron acceptors other than O_2 (McLatchey and Reddy, 1998). This leads to the sequential reduction of nitrate, iron and sulfate, and finally methanogenesis (Mitsch and Gosselink, 1993; Stumm and Morgan, 1996), which are relatively slow processes compared to aerobic decomposition. However, as many wetlands are affected by water level drawdown, the redox potential in the soil increases (see Appendix A. Supplementary data), and aerobic oxidation processes may prevail. This may lead to acidification as a result of the use of oxygen (Stumm and Morgan, 1996) and, if more severe, to limitations as a result of water shortage. These radical biogeochemical changes are expected to affect the availability of nutrients, especially in peatlands where microbial mineralization of organic N and P is the main source of nutrients (Verhoeven, 1986; Verhoeven et al., 1988). Although it has been generally assumed that lowering of the water level in fens results in increased microbial decomposition and thus increased mineralization of nutrients (Williams and Wheatley, 1988; Bridgham et al., 1998; Updegraff et al., 1995; Olde Venterink et al., 2002; Holden et al., 2004), the relationships between aeration and desiccation of peat soils and the actual net release of N and P are poorly understood (Olde Venterink et al., 2002).

Decomposition and mineralization may also be affected by the acid neutralizing capacity (ANC) of a peatland (Verhoeven et al., 1988; 1990; Kooijman and Hedenäs, 2009). It has been generally assumed that in mineral-rich wetlands the conditions for litter decay and nutrient turnover are more favorable than in mineral-poor wetlands, leading to higher net N-mineralization rates and increased nutrient availability for plants in rich fens as compared to ombrotrophic *Sphagnum*-fens (Bayley et al., 2005). However, high decomposition rates do not by definition lead to high net N- and P-mineralization rates (Kooijman et al., 2008; Kooijman and Hedenäs, 2009). In addition, net N- and P-mineralization do not necessarily increase with pH, and often increase from rich fens to poor fens (Verhoeven et al., 1988; 1990; Bridgham et al., 1998; Scheffer et al., 2001; Kooijman and Hedenäs, 2009). Additional experimental research is therefore needed to assess whether the ANC in fens also affects the changes induced by aeration and desiccation. Although oxygen deficiency is considered a major factor limiting microbial decomposition rates, these rates may also be strongly limited by litter quality and enzyme activity (Freeman et al., 2004) in poor, *Sphagnum*-dominated fens, which may interact with drought effects.

The main objective of this study was to gain insight into the effects of aeration (increased oxygen intrusion) and desiccation (oxygen intrusion plus water shortage) on decomposition rates and net release rates of nutrients upon water level drawdown in fens, and to investigate whether these responses are affected by ANC of the peat. Therefore, we conducted a laboratory incubation experiment involving soils from both rich fens and *Sphagnum*-dominated fens. Microbial processes were studied under (1) anaerobic, moist conditions, (2) aerobic, moist conditions, which are characteristic for moderate drought in which oxygen intrusion takes place, and (3) aerobic, desiccated conditions, characteristic for severe drought. We expected lowering of the pH and an increase of microbial decomposition rates and net nutrient mineralization rates upon drought. We also hypothesized that the net release rates of nutrients differ between rich fens and *Sphagnum*-fens due to differences in microbial immobilization characteristics. The following responses are discussed in this paper: (1) acidification as a result of oxygen intrusion, (2) changes in carbon (C) mineralization, (3) changes in net N-mineralization, and (4) changes in net P-release. In addition, implications for the hydrological management of both rich fens and *Sphagnum*-dominated fens are discussed.

2.2. Material and methods

Sampling

Peat soil samples were collected from three locations in the Netherlands (Figure 2.1): Stobbenribben (ST), Kiersche Wiede (KW) and Oostelijke Binnenpolder Tienhoven (BPT). Stobbenribben and Kiersche Wiede are situated in the northwestern part of the province of Overijssel and are part of the extensive Ramsar fen area Wieden-Weerribben, in which most of the peat soils remain relatively base-rich due to the supply of lithotrophic surface water (Van Wirdum, 1991). Binnenpolder Tienhoven is part of the Vechtplassen area, which is characterized by the discharge of base-rich groundwater in the river plain of the river Vecht (Schot, 1991). All locations also show sub-locations with lower ANC, characterized by *Sphagnum*-dominance.

Peat samples were collected in November 2011 and kept at field moisture content. From each of the three locations, five samples were collected from a mineral-

Figure 2.1 The three different research locations in The Netherlands: Stobbenribben (N 52°47'5.5'', E 5°59'1''), Kiersche Wiede (N 52°41'47.8'', E 6°7'57'') and Oostelijke Binnenpolder Tienhoven (N 52°10'30.7'', E 5°6'0.4'').

rich, brown moss-dominated site, and five from an ombrotrophic, *Sphagnum*-dominated site (*n*=30). Rich fen sites were characterized by the bryophytes *Scorpidium scorpioides* (Hedw.) Limpr. and *Hamatocaulis vernicosus* (Mitt.) Hedenäs. Bryophytes are good indicators of environmental conditions in the top layer, because they have no roots and remain in direct contact with the surrounding water through one cell layer thick leaves without cuticula (Proctor, 1982). *Sphagnum palustre* (L.), unable to survive in calcareous water (Clymo and Hayward, 1982), indicates relatively ombrotrophic conditions.

In Stobbenribben and Binnenpolder Tienhoven, rich fen samples were collected from sites dominated by *S. scorpioides*, and in the Kiersche Wiede from *H. vernicosus*-dominated sites. All *Sphagnum*-dominated samples were collected from sites dominated by *S. palustre*, which were situated within 25 meter from the rich fen sites. Samples were collected from the upper 10 centimeters of the peat soil, just below the living moss layer. Samples for bulk density were collected by using a steel corer with an exact volume of 100 ml. All samples were collected in plastic bags to avoid oxygen exposure, and stored at 4°C.

Experimental setup and chemical analyses

Three different conditions were simulated during incubation: (1) anaerobic (moist) incubation for 69 days, (2) aerobic (moist) incubation for 62 days and (3) aerobic (dry) incubation for 90 days. For logistical reasons, incubation periods differed, but the results have been corrected for these differences in incubation time. For all treatments, fresh samples were homogenized by hand, placed into petri dishes with a diameter of 15 cm, and stored in the dark at 20°C. Rich fen and *Sphagnum*-samples were incubated under field-moist conditions with a gravimetric moisture content of respectively 15 and 25 g water per g dry peat soil. To simulate permanently wet and anaerobic conditions, fresh soil samples were placed in a glove box (Plas-Labs Inc., 855 Series), filled with inert argon gas 5.0. For aerobic incubation, samples were placed under ambient air conditions. All anaerobic and moist aerobic samples were kept at field moisture by weekly adding demineralized water, based on the initial weight of the samples. For the dry, aerobic situation, samples were dried out gradually to air-dry conditions.

Before starting the incubation, initial soil characteristics of the soil samples were measured. Total C and N contents of dry peat soil were measured using a CHNS analyzer (Elementar, Vario EL Cube). Furthermore, portions of 250 mg dry peat soil were digested for 50 minutes in a microwave (Perkin-Elmer, Multiwave) with 4.0 ml HNO_3 (65%) and 1.0 ml HCl (37%), after which total P, Fe, Ca, Mg and S contents were measured by ICP (Perkin-Elmer, Optima 3000XL) (Bettinelli et al., 1989; Westerman, 1990).

Rates of CO_2 production (soil respiration), CH_4 uptake/production and N_2 emission were measured at the beginning and at the end of the incubation period in 100 ml serum bottles containing 7-10 g of peat soil. For the anaerobic samples, these serum bottles were filled inside the glove box to maintain anaerobic conditions. Rates of N_2 emission were only measured for anaerobic incubation, and rates of CH_4 emission or consumption (of ambient CH_4) were only measured for anaerobic and moist aerobic incubation. Over a period of two days, four measurements were carried out for each sample. Concentrations were measured by chromatography using Varian 3600 GC for CO_2 and CH_4, and Shimadzu GC-8A for N_2, with helium as carrier gas. Concentrations were determined by calibration relative to standard gas, and production rates were calculated from the differences in headspace concentrations in the serum bottles over time. Initial headspace concentrations were similar to ambient concentrations. Total denitrification rates may have been underestimated since only fluxes of N_2 were measured, and fluxes of N_2O were not taken into account.

Before and after incubation pH values of the soil samples were determined in water extracts. After 2 h of shaking, pH was measured with a Consort C831 pH meter, using a solid(g):liquid(g) ratio of 1:10. Also gravimetric moisture content, expressed as a percentage of the sample's dry weight, was determined for all fresh samples before incubation and for the samples that were incubated under dry aerobic conditions, by drying the soil samples for 24 hours at 105°C.

Concentrations of extractable inorganic N (NH_4 and NO_3), orthophosphate (o-PO_4), and dissolved organic carbon (DOC) in both fresh and incubated samples were determined via extraction with 50 ml 0.05M K_2SO_4 solution (Westerman, 1990). A solid(g):liquid(g) ratio of 1:50 was used for the rich fen samples and 1:80 for the *Sphagnum*-fens, because the *Sphagnum*-peat absorbs much solution. After 1 h of shaking in 100 ml bottles, extraction solutions were collected by using Rhizon SMS soil moisture samplers (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment, the Netherlands), which were connected to vacuum serum bottles. Concentrations were measured by using an Auto Analyzer (Skalar, San++ System, fitted with Skalar, SA1074). Rates of net N-mineralization and net P-release were calculated as the difference in total extractable inorganic N (NH_4 and NO_3) and P (o-PO_4) concentrations between initial samples and incubated samples.

Microbial C and N were determined by chloroform fumigation extraction (Jenkinson and Powlson, 1976; Brookes et al., 1985; Vance et al., 1987). Before and after incubation, samples were flushed with chloroform for 24 hours. Microbial C and N were determined by measuring total extractable DON (dissolved organic nitrogen), DOC and inorganic N (NH_4 and NO_3) concentrations in 0.05M K_2SO_4 extractions, as described in the previous paragraph. The differences between fumigated and non-fumigated samples were used to calculate the microbial C and N content, assuming an extractability of 0.45 (Jenkinson and Ladd, 1981; Wu et al., 1990).

Calculations of gross N-mineralization and microbial N-immobilization

In order to explain differences in net N-mineralization between treatments, several aspects of microbial growth and nutrient efficiency were calculated (Table 2.1). The equations were adapted after Kooijman et al. (2008), in which C and N dynamics were described based on existing theoretical models (Berendse et al., 1989; Tietema and Wessel, 1992). Measured values for the CO_2 emission (Q), net N-mineralization rates (NM), denitrification rates (D), N:C ratios of the peat substrate (NC_s), and averaged microbial N:C ratios during the incubation period (NC_m) were used to estimate the microbial growth efficiency (eC), which is the fraction of gross C-release that is used for microbial assimilation. In addition, gross N-release rates (GN), N-immobilization rates (I) and the microbial N-immobilization efficiencies (eN) were estimated. We, however, emphasize that this is only a clarifying approach to get insight into the microbial processes that are important, and by no means a complete model. The model was not applied to explain microbial characteristics concerning P, since the net P-release is not only associated with microbial net P-mineralization, but also to a high extent dependent on redox-sensitive chemical binding of P.

Statistical analysis

All statistical analyses were performed using SPSS 20.0 for Windows (IBM Inc., 2011). Significance was accepted at a confidence level of $P<0.05$. Initial differences in soil characteristics between rich fens and *Sphagnum*-fens were tested by applying

Table 2.1 List of symbols and used equations (derived and reformulated from Kooijman et al., 2008).

Measured variables		Unit
NM	Net N-mineralization	μmol N kg^{-1} d^{-1}
Q	Respiration (CO_2 emission)	μmol C kg^{-1} d^{-1}
NC_m	N:C-ratio in microbial biomass	mol N mol C^{-1}
NC_s	N:C-ratio in peat substrate	mol N mol C^{-1}
D	Denitrification	μmol N kg^{-1} d^{-1}
Calculated variables		**Unit**
eC	Microbial growth efficiency	mol C mol C^{-1}
GN	Gross N-release	μmol N kg^{-1} d^{-1}
I	N-immobilization	μmol N kg^{-1} d^{-1}
eN	Microbial N-immobilization efficiency	mol N mol C^{-1}
Equations used		
1	$NM = GN-I-D$	
2	$NM = ((NC_s*Q)/(1-eC))-((eC*NC_m*Q)/(1-eC))-D$	
3	$eC = ((NC_s*Q)-(NM+D))/((NC_m*Q)-(NM+D))$	
4	$GN = (1/(1-eC))*NC_s*Q$	
5	$I = (eC/(1-eC))*NC_m*Q$	
6	$eN = eC*(NC_m/NC_s)$	

a two-way ANOVA, using fen type and location as two independent variables (i.e. fixed factors). We distinguished between fens with minerotrophic species (*S. scorpioides* and *H. vernicosus*) and fens with ombrotrophic species (*S. palustre*). Potential differences resulting from treatment conditions were tested by three-way ANOVA with LSD (least significant difference) post hoc analyses, using fen type, treatment, and location as three independent variables (i.e. fixed factors). *P*-values in the text are indicated as follows: $*P<0.05$, $**P<0.01$, $***P<0.001$.

2.3. Results

Initial soil- and microbial characteristics

The initial soil characteristics before incubation clearly differed between both fen types for many variables (Table 2.2). As expected, pH values were considerably higher in rich fens than in *Sphagnum*-fens ($F_{1,24}=2893.0$***). The effect of location on pH was the strongest for rich fens, considering a significant interaction of location*fen type ($F_{2,24}=217.3$***). In the KW rich fen, initial pH was lower than in the other rich fens. Total N and P concentrations in rich fen peat were, on average, 1.8 times as high as in *Sphagnum*-fens, resulting in lower C:N ratios ($F_{1,12}$=10764.0***) and C:P ratios ($F_{1,12}$=504.1***) in rich fen peat. Total P concentrations were the lowest in the ST, resulting in significantly higher C:P ratios ($F_{2,12}$=314.5***) and N:P ratios ($F_{2,12}$=315.3***). The rich fen soils were also characterized by higher total concentrations of Ca ($F_{1,12}$=886.3***), although this was largely due to the ST site where Ca concentrations were 10 times higher for rich fen than for poor fen, as indicated by a significant interaction of location*fen type ($F_{2,12}$=595.2***). Fe concentrations were also higher in rich fen peat ($F_{1,12}$=655.3***), which was mainly due to the BPT rich fen where total Fe concentrations were about 10 times higher than in the other rich fens, as indicated by a significant interaction of location*fen type ($F_{2,12}$=1536.9***). The effect of location on concentrations of extractable NH_4 was significant ($F_{2,24}$=148.2***), and this effect was the strongest for rich fens, considering a significant interaction of location*fen type ($F_{2,24}$=26.3***). Also extractable NO_3 concentrations differed between locations ($F_{2,24}$=117.8***) and the effect of location was the strongest for rich fens, considering a significant interaction of location*fen type ($F_{2,24}$=93.1***). Both extractable NH_4 and NO_3 concentrations were higher in the ST rich fen than in the other rich fens. Extractable o-PO_4 concentrations did not significantly differ between locations ($F_{2,24}$=2.5^{NS}). In addition, bulk density was 2-3 times higher in rich fens ($F_{1,11}$=175.1***), while gravimetric soil moisture content was twice as high in *Sphagnum*-dominated fens ($F_{1,24}$=128.2***).

Anaerobic CO_2 production per kg dry peat soil at T=0 did not differ significantly between both fen types ($F_{1,24}$=3.0^{NS}) or between locations ($F_{2,24}$=0.2^{NS}) (Table 2.2). However, when expressed per volume fresh peat soil, anaerobic CO_2 production rates at T=0 in rich fens were significantly higher (factor 2.0 on average) than in *Sphagnum*-dominated fens ($F_{1,24}$=89.4***), due to the lower bulk density of *Sphagnum*-peat. When expressed per mass of microbial C, respiration was higher (factor 1.7 on average) in the *Sphagnum*-fens ($F_{1,24}$=64.0***), as the total concentration of microbial C was higher in rich fen peat ($F_{1,23}$=42.3***). Anaerobic CH_4 fluxes per kg dry peat soil at T=0 were negative for all samples, indicating microbial oxidation of CH_4 . The anaerobic oxidation of CH_4 was, on average, two times higher in *Sphagnum*-fens than in rich fens ($F_{1,24}$=86.1***). In addition, the overall concentration of microbial C in the KW location was significantly higher than in the other locations.

Treatment effects

Acidification

All outcomes of statistical analyses of the incubation results are shown in Table 2.3. Treatments had a significant effect on [H^+]. Both aeration and desiccation led to a net increase of [H^+], hence to significant lowering of the pH (Figure 2.2). Overall, the net increase of [H^+] during incubation was greater in *Sphagnum*-fens than in

Table 2.2 Initial characteristics of the peat soil and microbial biomass at T=0 at the different research sites. Data shown represent mean values and their standard deviations (n = 5). * = significant difference ($P < 0.05$) between rich fen peat and *Sphagnum*-peat, † = significant difference ($P < 0.05$) between locations. ST = Stobbenribben, KW = Kiersche Wiede, BPT = Binnenpolder Tienhoven, d.w.: dry weight of peat soil. Positive fluxes indicate release.

Fen type	Rich fen			*Sphagnum*-dominated fen		
Location	ST	KW	BPT	ST	KW	BPT
Dominant moss species	*S. scorpioides*	*H. vernicosus*	*S. scorpioides*	*S. palustre*	*S. palustre*	*S. palustre*
pH-H_2O *†	6.9 (0.1)	5.7 (0.2)	6.3 (0.2)	3.8 (0.0)	4.4 (0.0)	4.4 (0.1)
C_{total} (g kg^{-1} d.w.) †	464.0 (1.6)	481.7 (0.7)	331.3 (6.0)	469.9 (4.0)	454.1 (2.1)	460.9 (1.7)
N_{total} (g kg^{-1} d.w.) *†	17.5 (0.3)	22.5 (0.3)	16.4 (0.2)	11.4 (0.2)	10.6 (0.1)	10.3 (0.1)
P_{total} (g kg^{-1} d.w.) *†	0.6 (0.0)	1.0 (0.0)	1.1 (0.1)	0.3 (0.0)	0.7 (0.0)	0.5 (0.1)
Ca_{total} (g kg^{-1} d.w.) *†	22.6 (0.7)	9.1 (0.1)	11.4 (0.5)	2.2 (0.1)	7.5 (0.3)	10.3 (1.0)
Fe_{total} (g kg^{-1} d.w.) *†	1.3 (0.0)	2.0 (0.0)	17.0 (0.7)	1.1 (0.0)	6.0 (0.2)	1.9 (0.2)
Total Ca:Fe (mol mol^{-1}) *†	23.9 (0.4)	6.2 (0.0)	0.9 (0.0)	2.9 (0.1)	1.7 (0.1)	7.4 (0.1)
Substrate C:N ratio (g g^{-1}) *	26.5 (0.3)	21.4 (0.3)	20.2 (0.1)	41.3 (0.5)	42.7 (0.5)	44.9 (0.5)
Substrate C:P ratio (g g^{-1}) *†	823.4 (21.7)	480.3 (13.7)	294.7 (21.4)	1451 (42.9)	651.9 (14.6)	902.3 (7.2)
Substrate N:P ratio (g g^{-1}) †	31.1 (0.8)	22.4 (0.7)	14.6 (1.0)	35.1 (1.1)	15.3 (0.5)	20.1 (2.2)
ext. NH_4 (mg kg^{-1} d.w.) *†	117.4 (23.2)	18.3 (5.2)	3.8 (0.9)	53.6 (13.7)	15.6 (3.9)	5.6 (1.0)
ext. NO_3 (mg kg^{-1} d.w.) *†	23.0 (4.5)	1.2 (0.4)	1.0 (0.6)	2.2 (1.3)	1.3 (0.4)	0.7 (0.4)
ext. o-PO_4 (mg kg^{-1} d.w.)	12.1 (3.1)	18.8 (5.4)	7.8 (1.3)	18.6 (2.3)	11.2 (2.2)	16.9 (3.9)
Dry bulk density (mg cm^{-3}) *†	64.9 (6.1)	49.3(5.0)	81.2 (10.4)	25.4 (6.9)	26.3 (2.7)	25.3 (0.7)
Gravim. moisture content (%) *	982 (58)	1421 (75)	889 (156)	2404 (263)	1747 (438)	1910 (63)
Microbial C (mg g^{-1} d.w.) *†	6.6 (0.7)	9.9 (1.8)	3.8 (0.5)	3.4 (0.3)	5.1 (0.5)	3.8 (1.1)
Anaerobic CO_2 flux T=0 (mgC kg^{-1} d^{-1})	316.1 (77.1)	344.3 (97.3)	359.1 (34.8)	424.3 (59.5)	370.4 (30.9)	350.4 (74.8)
Anaerobic CO_2 flux T=0 (gC dm^{-3} d^{-1}) *	20.5 (5.0)	17.0 (4.8)	27.5 (2.9)	11.3 (2.2)	10.2 (1.0)	9.1 (2.2)
Anaerobic CO_2 flux T=0 (mgC gC_m^{-1} d^{-1}) *	48.6 (12.4)	36.3 (14.6)	104.1 (39.1)	131.6 (22.2)	76.8 (14.2)	103.4 (43.4)
Anaerobic CH_4 flux T=0 (mgC kg^{-1} d^{-1}) *†	-0.9 (0.2)	-1.3 (0.2)	-0.2 (0.2)	-2.2 (0.1)	-1.7 (0.2)	-0.3 (0.2)

rich fens. However, the effect of aeration and desiccation on lowering of the pH was stronger in rich fens, as indicated by a significant interaction of fen type*treatment. In the ST location the effect of aeration and desiccation on pH was less strong than in the other locations.

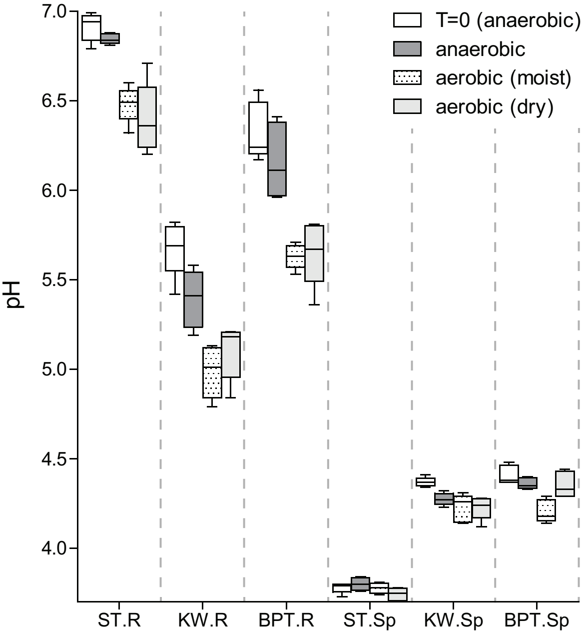

Figure 2.2 Box plots showing soil pH-H_2O values of samples from the six different study sites for the different treatments ($n = 5$). ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-fen, KW.Sp = Kiersche Wiede *Sphagnum*-fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-fen. Upper and lower quartiles are indicated, as well as whiskers showing minimum and maximum values. Significant effects of fen type and treatment are indicated in Table 2.3.

Carbon cycling

During incubation, the overall effects of fen type and treatment on the CO_2 emission per kg dry peat soil were significant and considering a significant interaction of fen type*treatment, the effect of treatment was stronger for rich fens (Table 2.3). Both aeration and desiccation led to increased CO_2 emission when expressed per kg dry peat, but only in rich fens and not in *Sphagnum*-fens (Figure 2.3). As the overall concentration of microbial C mass per kg dry peat was on average two times lower in *Sphagnum*-fens, overall CO_2 emission per mass unit microbial C was on average 1.5 times higher in *Sphagnum*-fens than in rich fens. Overall CO_2 emission expressed per volume peat soil was on average 3.0 times higher in rich fens than in *Sphagnum*-fens, due to the higher bulk density of rich fen peat. Also, DOC production per kg dry peat soil was significantly affected by treatment. DOC production showed a slight but significant decrease upon aeration, while desiccation resulted in a considerable increase of DOC concentrations. CH_4 fluxes expressed per kg dry peat became clearly positive under moist anaerobic conditions only in two rich fens (KW and BPT), while in all *Sphagnum*-fens CH_4 fluxes remained negative. CH_4 fluxes were negative for all fens upon aeration, and aeration seemed to have a leveling effect for both fen types.

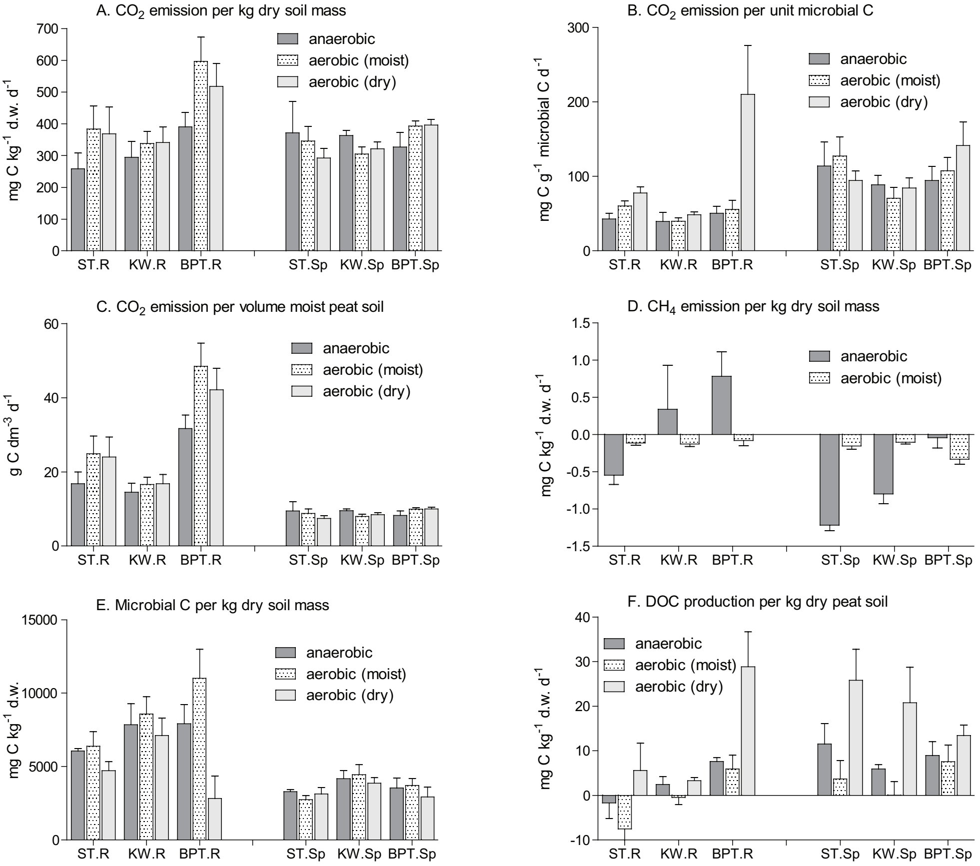

Figure 2.3 Average fluxes of CO_2 (A, B, C), fluxes of CH_4 (D), microbial C (E) and DOC production (F) under anaerobic, moist aerobic and dry aerobic conditions for samples from the six different study sites ($n = 5$). Positive fluxes indicate release. ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-fen, KW.Sp = Kiersche Wiede *Sphagnum*-fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-fen. Standard deviations are indicated. Significant effects of fen type and treatment are indicated in Table 2.3.

Nitrogen cycling

Especially under anaerobic conditions, net N-mineralization rates per kg dry peat soil and per microbial C mass were much higher (on average 10 times) in the *Sphagnum*-fens than in the rich fens (Figure 2.4, Table 2.3). Due to the high bulk density of rich fen peat compared to *Sphagnum*-peat, the differences in net N-mineralization when expressed per volume peat soil were smaller, but on average still 4 times higher in *Sphagnum*-peat than in rich fen peat. Anaerobic denitrification rates per kg dry

Table 2.3 Outcomes of statistical analyses of the effects of fen type, treatment, location and their interaction effects, as tested by three-way ANOVA with LSD post hoc analyses. *F*-ratios are shown with their level of significance: *$P < 0.05$, **$P < 0.01$. D.f. denominator = 72, except for CH_4 flux per kg d.w. (d.f. denominator = 48) and N_2 flux per kg d.w. (d.f. denominator = 24). Different letters indicate significant differences ($P < 0.05$) between treatments, n.s.: not significant, d.w.: dry weight of peat soil.

Dependent variable	Fen type	Treatment	Location	Fen type x treatment	Fen type x location	Treatment x location	Anaerobic	Aerobic	Aerobic
	(d.f. =1)	(d.f. = 2)	(d.f. = 2)	(d.f. = 2)	(d.f. = 2)	(d.f. = 4)	(moist)	(moist)	(dry)
Net d$[H^+]$	22.0**	7.9**	10.3**	3.2*	2.1	3.1*	a	b	b
d(pH)	136.4**	25.1**	8.7**	14.6**	0.5	1.0	a	b	b
CO_2 flux (per kg d.w.)	13.7**	9.7**	39.1**	13.2**	15.5**	5.1*	a	b	b
CO_2 flux (per $C_{microbial}$)	49.1**	25.0**	34.5**	16.6**	7.2**	20.9**	a	a	b
CO_2 flux (per dm^2)	697.0**	15.9**	138.5**	17.8**	121.0**	5.3**	a	b	b
Microbial C (per kg d.w.)	282.9**	35.6**	21.4**	24.3**	3.4*	12.5**	b	c	a
CH_4 flux (per kg d.w.)	79.8**	3.1	40.0**	53.1**	1.4	50.1**	n.s.	n.s.	-
DOC production (per kg d.w.)	44.2**	96.0**	22.1**	0.9	38.3**	1.9	b	a	c
DOC production (per dm^2)	5.5*	82.1**	64.7**	13.7**	77.0**	8.6**	b	a	c
Net N-mineralization (per kg d.w.)	149.1**	33.4**	173.5**	62.9**	14.3**	21.4**	a	b	a
Net N-mineralization (per $C_{microbial}$)	267.7**	7.5**	126.6**	23.0**	42.4**	18.7**	a	b	a
Net N-mineralization (per dm^2)	13.2	45.1**	119.2**	65.2**	6.9**	20.8**	a	c	b
N_2 flux (per kg d.w.)	65.1**	-	105.8**	-	27.8**	-	-	-	-
Gross N-mineralization (per kg d.w.)	546.5**	13.7**	102.7**	13.2**	86.9**	6.4**	a	c	b
Gross N-mineralization (per $C_{microbial}$)	58.6**	33.5**	51.7**	26.4**	36.9**	23.4**	a	a	b
N-immobilization (per kg d.w.)	633.1**	8.6**	164.2**	3.1	70.3**	10.9**	a	b	b
N-immobilization (per $C_{microbial}$)	167.1**	44.5**	92.6**	16.9**	30.1**	19.9**	a	a	b
Net P-release (per kg d.w.)	0.7	351.4**	8.7**	0.8	14.3**	13.6**	b	a	c
Net P-release (per $C_{microbial}$)	3.4	341.7**	1.6	20.4**	10.2**	8.1**	b	a	c
Net P-release (per dm^2)	18.7**	255.9**	13.9**	44.8**	16.7**	19.2**	a	a	b

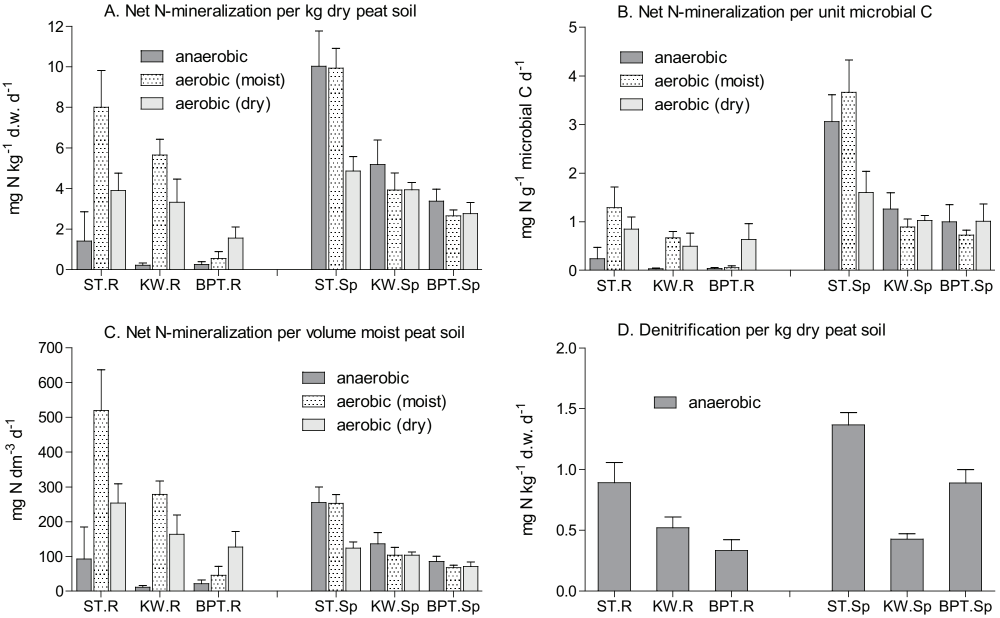

Figure 2.4 Rates of net N-mineralization (A, B, C) under anaerobic, moist aerobic and dry aerobic conditions, and rates of anaerobic denitrification (D) for samples from the six different study sites (*n* = 5). ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-fen, KW.Sp = Kiersche Wiede *Sphagnum*-fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-fen. Standard deviations are indicated. Significant effects of fen type and treatment are indicated in Table 2.3.

peat soil were relatively high in the rich fens compared to the net N-mineralization rates (on average 91%) and relatively low in the *Sphagnum*-fens (on average 14%), and in absolute terms anaerobic denitrification rates were lower in rich fen peat than in *Sphagnum*-peat. In contrast to net N-mineralization, estimated gross N-mineralization was overall higher in rich fens than in *Sphagnum*-fens, both expressed per kg dry peat soil mass, and per microbial C mass (Figure 2.5, Table 2.3). Estimated microbial N-immobilization was considerably higher in rich fens than in *Sphagnum*-fens per kg dry peat soil and per microbial C mass. The microbial N-immobilization rates in rich fens could even be up to 82-98 % of the gross N-mineralization.

Treatment had a significant effect on net N-mineralization when expressed per kg dry peat soil, per microbial C mass, and per volume peat soil (Figure 2.4, Table 2.3). According to a significant interaction of fen type*treatment, the two different fen types respond differently to treatment. Upon aeration, net N-mineralization in rich fens was on average 9.7 times higher than under anaerobic conditions when expressed per kg dry peat and on average 3.8 times higher when expressed per

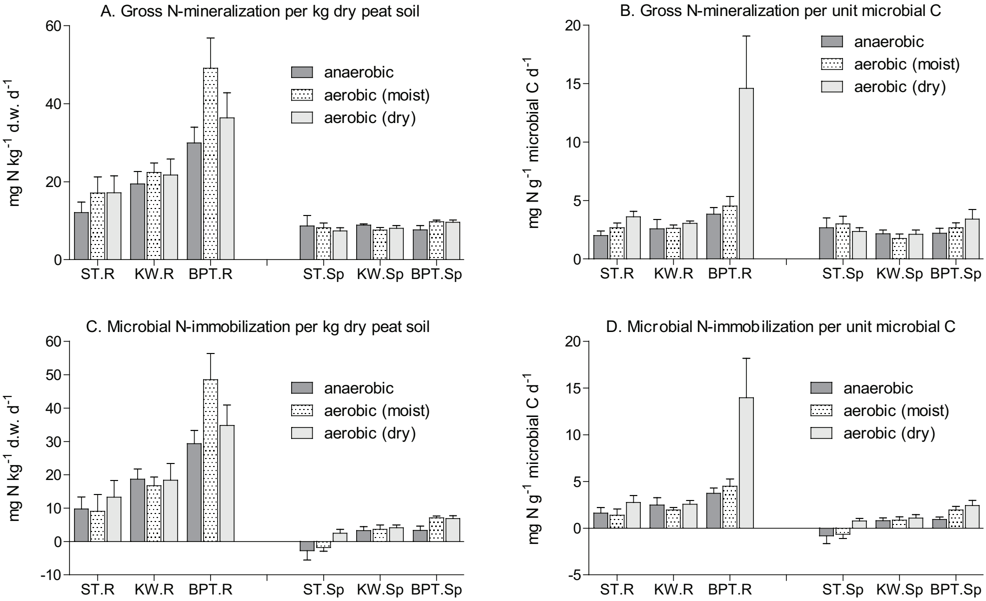

Figure 2.5 Rates of gross N-mineralization (A, B) and N-immobilization (C, D) for samples from the six different study sites under different incubation conditions (*n* = 5). ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-fen, KW.Sp = Kiersche Wiede *Sphagnum*-fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-fen. Standard deviations are indicated. Significant effects of fen type and treatment are indicated in Table 2.3.

volume peat soil. In *Sphagnum*-fens, treatments did not significantly affect the net N-mineralization rate. Also estimated gross N-mineralization per kg dry peat soil was significantly affected by treatments, and given a significant interaction of fen type*treatment, the effect of aeration and desiccation on gross mineralization was again related to rich fen peat rather than to *Sphagnum*-peat. However, no significant interaction of fen type*treatment on microbial N-immobilization per kg dry peat soil was observed, which means that the effect of treatments on N-immobilization did not differ between rich fen peat and *Sphagnum*-peat.

The three rich fen locations responded differently with respect to their net N-mineralization rates upon treatments (Figure 2.4). The microbial biomass showed a relatively high increase in the BPT rich fen, but not in the ST and KW rich fens, where the increase of net N-mineralization per kg peat soil upon aeration was due to increased microbial activity rather than increase of microbial biomass. In all three rich fens, gross N-mineralization increased upon aeration, but microbial immobilization increased only in the BPT rich fen. Upon desiccation, gross N-mineralization per microbial biomass C increased considerably especially at the BPT rich

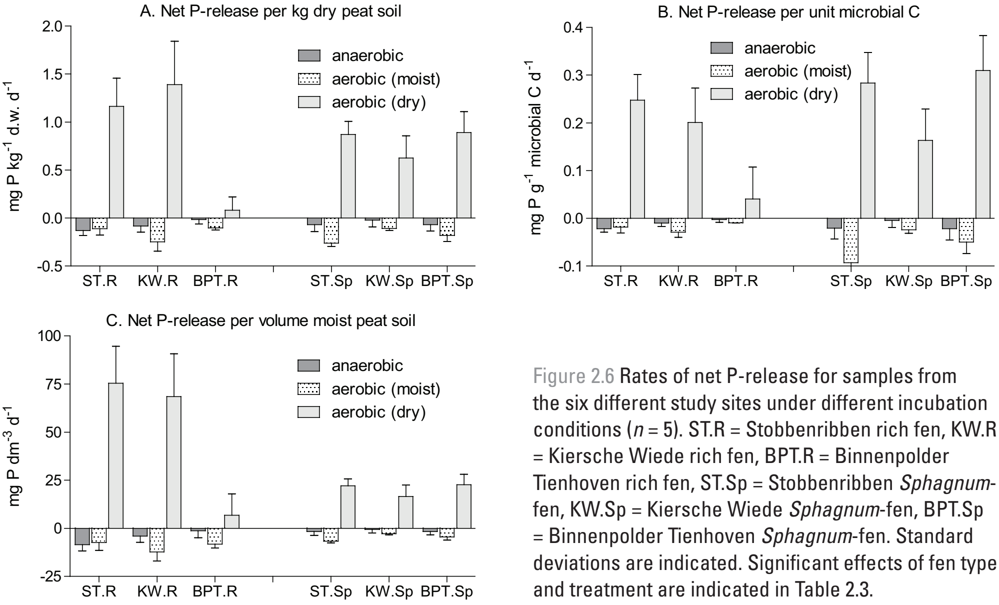

Figure 2.6 Rates of net P-release for samples from the six different study sites under different incubation conditions ($n = 5$). ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-fen, KW.Sp = Kiersche Wiede *Sphagnum*-fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-fen. Standard deviations are indicated. Significant effects of fen type and treatment are indicated in Table 2.3.

fen. However, due to a concomitant increase of the microbial N-immobilization per unit microbial C mass, the increase of net N-mineralization per kg dry soil and per volume peat soil was relatively limited.

Phosphorus cycling

The overall effect of treatment on net P-release was significant (Figure 2.6, Table 2.3). The net P-release was negative under moist anaerobic and moist aerobic incubation, which means that in all of the fens there was net P-immobilization. However, after the soil samples dried out completely, net P-release increased considerably per kg dry peat soil, per microbial C mass and per volume peat soil. When expressed per kg dry peat soil, the effect of desiccation was similar for both rich fens and *Sphagnum*-fens, as indicated by a non-significant interaction of fen type*treatment. However, when expressed per microbial C mass the net P-release upon desiccation was higher in *Sphagnum*-peat. When expressed per volume peat soil, the net P-release was higher in the rich fens due to the higher bulk density, especially in the ST and KW rich fen. There seemed to be a shift in composition of the microbial population upon desiccation, because both the increase of DOC and o-PO_4 concentrations were relatively higher than the increase of inorganic N-concentrations upon desiccation in comparison to the other treatments.

2.4. Discussion

Does aeration lead to severe acidification?

One of the main questions with regard to water level drawdown is whether stimulation of aerobic oxidation processes leads to severe acidification and subsequently to vegetation changes. Lowering of pH as a result of water level drawdown is assumed to be temporary. When the water level is increased again, most of the protons produced will most likely be consumed due to the anaerobic reduction of alternative electron acceptors (Loeb et al., 2008). However, a drop in pH may temporarily lead to favorable conditions for establishment of *Sphagnum* spp., which is a threat to typical rich fen vegetation (Kooijman, 2012). In the ST rich fen, which is the most Ca-rich location, pH values did not drop below 6.0, which seems to be a critical value for rich fens dominated by *S. scorpioides* (Kooijman, 2012). However, in the KW and BPT rich fens, which have lower Ca-concentrations, pH values dropped to respectively 5.0 and 5.6, indicating that *Sphagnum* may get a competitive advantage.

Anaerobic respiration in rich fens versus *Sphagnum-f*ens

Anaerobic respiration rates per kg dry peat soil did not differ between rich fens and *Sphagnum*-dominated fens, which is not consistent with previous work, in which decomposition rates per mass peat soil were generally lower in *Sphagnum*-dominated fens than in rich fens under moist anaerobic conditions (e.g. Farrish and Grigal, 1988). In these studies, the low decomposition rate of *Sphagnum*-peat has been attributed to several *Sphagnum*-specific characteristics, such as acidification (Verhoeven et al., 1990) and chemical composition (Belyea, 1996; Aerts et al., 1999). Cell walls of *Sphagnum*-litter contain phenolic compounds that would inhibit the activity of microorganisms involved in decomposition processes because of their recalcitrant nature and antibiotic properties (Van Breemen, 1995; Aerts et al., 2001). As the activity of phenol oxidase is limited under anaerobic conditions (Freeman et al., 2004), phenolics may accumulate and inhibit more general degradative enzymes such as glucosidases, phosphatases and sulphatases (Freeman et al., 2001), leading to reduced breakdown of organic matter (Fenner and Freeman, 2011). However, in view of the fact that anaerobic respiration rates per kg d.w. between fen types did not differ in this study, one may wonder whether these *Sphagnum*-specific characteristics really are determining the respiration rates under anaerobic conditions. When expressed per volume peat soil however, respiration rates were indeed higher in rich fens, but this was due to the higher bulk density in rich fens than in *Sphagnum*-fens.

The respiration rate per microbial biomass C in rich fens was lower than in *Sphagnum*-fens, which may be pH-related. Enwall et al. (2007) found a negative correlation between soil pH and the microbial metabolic quotient or also expressed as $q\mathrm{CO}_2$ (respiration-to-biomass ratio), indicating a decreased efficiency of heterotrophic microorganisms to convert organic carbon into microbial biomass in rather acidic soils. This would explain the high respiration rate per microbial biomass C in *Sphagnum*-fens. Moreover, this lower respiration rate per microbial biomass C in rich fens seems to be compensated for by a larger number of microorganisms in rich fens than in *Sphagnum*-fens.

The negative CH_4 fluxes for all soils under initial anaerobic conditions indicate net uptake/consumption of CH_4 within the system, which has also been observed in previous research (Yavitt et al., 1990; Danevçiç et al., 2010). Low or even negative emission rates may reflect methane oxidation by microbial communities associated with living and dead *Sphagnum* and other moss species (Raghoebarsing et al., 2005; Liebner et al., 2011), and may also reflect suppression of methanogenesis by other electron acceptors, because reduction of e.g. nitrogen is yielding a higher amount of Gibbs free energy (Stumm and Morgan, 1996). High soil N-concentrations have frequently been linked to decreases in methanogenesis (Bridgham and Richardson, 1992; Bender and Conrad, 1994). This may to some extent explain the persistent negative methane emission rates for both rich fen and *Sphagnum*-fen in the N-rich ST location. The high potential for microbial communities associated with *Sphagnum*-peat to oxidize CH_4 and reduce the emission of this greenhouse gas to the atmosphere, especially under anaerobic conditions, (Raghoebarsing et al., 2005) is reflected in this study.

Different response of respiration to aeration and desiccation between fen types

Carbon respiration generally increases upon aeration of peat soils (e.g. Moore and Knowles, 1989; Freeman et al., 1993; Oechel et al., 1998; Blodau and Moore, 2003; Danevçiç., 2010; Fenner and Freeman, 2011). In this study, aeration indeed led to increased respiration, but only in rich fens. The ratio of microbial soil respiration to microbial biomass (qCO_2) can be used as a measure of changes in microbial biomass in response to disturbance, in which the index supposedly declines during succession/ecosystem development, and increases during disturbance (Wardle and Ghani, 1995). Upon desiccation, the microbial soil respiration per microbial biomass C increased in the rich fens, indicating reduced microbial efficiency. In the *Sphagnum*-fens however this was not the case. Decomposition and respiration can be limited by oxygen deficiency, but also by litter quality and enzyme activity (Freeman et al., 2001). In *Sphagnum*-fens, decomposition is probably not only limited by oxygen deficiency, but also by the high concentrations of phenolic compounds in *Sphagnum*-litter (Van Breemen, 1995; Aerts et al., 2001). In spite of the fact that in all fens aeration presumably stimulated the activity of phenol oxidase, the enzyme responsible for the breakdown of phenolic compounds (Fenner and Freeman, 2011), aeration did not lead to increased respiration rates in *Sphagnum*-fens, probably because *Sphagnum*-litter contained too many phenolic compounds. Rich fens presumably contain lower concentrations of phenolic compounds. Also, the low pH in *Sphagnum*-fens may have been relatively unfavorable for the degradation of phenolic material (Pind et al., 1994). In rich fens, stimulation of phenol oxidase by aeration may therefore have led to phenol-concentrations that are low enough for other degradative enzymes in rich fens to be active, such as glucosidase and phosphatase (Freeman et al., 2004), resulting in increased respiration upon aeration in rich fens. These mechanisms should be further investigated.

The response of DOC production upon aeration and desiccation

Rates of DOC production did not follow the clear pattern as observed for CO_2 production, probably because DOC can be both sink and source of carbon and therefore can be affected by many factors. The slight decrease of DOC concentrations as a result of aeration seems in accordance with Glatzel et al. (2003), who showed that DOC concentrations are often higher under anaerobic conditions than under aerobic conditions, because of accumulation of intermediate metabolic products instead of formation of CO_2. However, DOC production may also decrease upon aeration due to increased biological activity and increased consumption of DOC as a substrate for respiration (Pastor et al., 2003).

Upon total desiccation however, DOC production increased considerably. This may suggest an increase in overall decomposition, as documented in previous work (Mitchell and McDonald, 1992; Olde Venterink et al., 2002), but in our study respiration did not increase. The increased DOC production may instead be related to die-off of microbes, as a result of water shortage, by which cellular constituents are released. This idea was supported by a decreasing microbial C mass upon total desiccation.

Anaerobic N-mineralization and N-immobilization in rich fens versus *Sphagnum*-fens

For the connection to plant production in relation to nutrient availability for roots, nutrient mineralization rates per unit volume are most important. This study clearly showed higher net N-mineralization rates per unit volume under anaerobic conditions in *Sphagnum*-fens than in rich fens. Also, when expressed per kg dry peat, anaerobic net N-mineralization rates were significantly higher in *Sphagnum*-peat, which is in conformity with results from previous studies (Verhoeven and Arts, 1987; Verhoeven et al., 1988, 1990; Updegraff et al., 1995), but does not correspond with the general idea that conditions for litter decay and mineralization are generally more favorable in mineral-rich than in mineral-poor wetlands (Bayley et al., 2005). Also, the finding that net N-mineralization rates per microbial C mass were higher in *Sphagnum*-dominated fens than in rich fens corresponds well with previous experiments carried out by Kooijman and Hedenäs (2009).

These differences in anaerobic net N-mineralization rates between both fen types cannot be related to denitrification, because anaerobic denitrification rates in all fens were relatively low compared to the high net N-mineralization rates as measured in *Sphagnum*-peat. Other N-removing pathways, such as anaerobic ammonium oxidation (anammox) (Burgin and Hamilton, 2007), are not likely to be seriously affecting the measured net N-mineralization either, since the quantitative contribution of these pathways in semi-terrestrial fens are assumed to be relatively small compared to denitrification (White and Reddy, 2009).

The higher anaerobic net N-mineralization per kg dry peat, per microbial C mass en per volume peat soil in *Sphagnum*-fens cannot be explained by differences in gross N-mineralization either, as estimated gross N-mineralization was higher in rich fens than in *Sphagnum*-fens. Since the net N-mineralization rate is a net result of gross N-mineralization and microbial N-immobilization, microbial immobilization characteristics are often determinative for the N-availability for plants (Robertson and Groffman, 2007). In this study, estimated microbial N-immobilization rates were significantly higher in rich fens than in *Sphagnum*-fens, which probably explains the differences in net N-mineralization between fen types. Microbial decomposition of organic matter is regulated by a variety of heterotrophic bacteria and fungi (Coulson and Butterfield, 1978), and changes in microbial N-demand may be associated with shifts in bacterial and fungal composition occurring over pH gradients (Kooijman et al., 2008; Kooijman and Hedenäs, 2009). In rich fens, bacteria are generally more abundant and active in anaerobic decomposition, while in acidic peatlands the bacterial population and its activity are generally limited and fungal activity becomes more dominant (Winsborough and Basiliko, 2010). Bacteria generally have a lower C:N biomass ratio and a higher N-demand than fungi (Hassink et al., 1993; Robertson and Groffman, 2007), which is possibly caused by the fact that bacteria use amino acids rather than carbohydrates for osmoregulation (Kuehn et al., 1998). Considering these ideas, it is rather likely that the higher N-immobilization rates per microbial C mass as estimated in our study may provide an explanation for the lower net N-mineralization rates in rich fen peat compared to *Sphagnum*-peat under anaerobic conditions.

Different response of net N-mineralization to aeration and desiccation between fen types

Aeration in wetlands due to drought is generally assumed to result in an increase of net N-mineralization rates per kg peat (Grootjans et al., 1985; 1986; Williams and Wheatley, 1988; Bridgham et al., 1998; Updegraff et al., 1995; Olde Venterink et al., 2002; Holden et al., 2004). In this study, aeration and desiccation only resulted in increased net N-mineralization per kg peat and per volume peat for the rich fens, not only because of increased microbial biomass, but also because of increased net N-mineralization per unit microbial C. Interestingly, net N-mineralization rates in *Sphagnum*-fens were not affected by increased availability of oxygen. This striking difference in response between both fen types is probably caused by differences in concentrations of phenolic compounds and degradative enzymes, just as in the case of the respiration results. It may be that only in rich fens the concentrations of phenolic compounds are low enough to allow other enzymes to be active, resulting in increased net N-mineralization upon aeration and desiccation. These mechanisms should be further investigated.

The effect of aeration and desiccation on net P-release

The impact of aeration and desiccation on processes concerning net P-release is rather complicated, because apart from mineralization processes, there are redox-sensitive processes of chemical P-binding. For P it has been shown by Olde Venterink et al. (2002) that aeration and increased decomposition rates do not necessarily lead to an increased net P-release. We confirm these findings, as we did not find any significant differences in net P-release upon aeration, even though respiration rates increased in rich fens. This may be caused by the fact that released o-PO_4 can be bound immediately after mineralization, for example as Fe-phosphates (Patrick and Khalid, 1974; Richardson, 1985) or Ca-phosphates (Boyer and Wheeler, 1989; Reddy et al., 1993). Since the mobilization and immobilization of Fe-phosphates is redox-sensitive (Patrick and Khalid, 1974; Lijklema, 1980; Boström et al., 1982; Richardson, 1985), oxidation processes under aerobic conditions presumably led to the formation of Fe(III) oxides and hydroxides, which may have reduced net P-mobilization.

In contrast to aeration, full desiccation led to an enormous increase of the net P-release. These results are in contrast with a potential decrease due to redox sensitive Fe-P complexation. Microbial die-off resulting from drought, as supported by the reduced microbial biomass C upon desiccation, may have resulted in a net increase of extractable o-PO_4 concentrations. It seems though that also a change in microbial population took place upon desiccation, because both the increase of DOC and o-PO_4 concentrations were relatively higher than the increase of inorganic N-concentrations upon desiccation in comparison to the other treatments. Additional research is required, focusing on the balance between biogeochemical P-binding and microbial P-mobilization as a result of drought.

Implications for the field situation

Water level drawdown initially leads to an increase in oxygen availability. Due to aerobic oxidation processes, pH values decreased after aeration and further desiccation. Although the pH will probably increase again during subsequent rising of the water level (Loeb et al., 2008), a temporary decrease in pH may lead to suitable conditions for dominance by *Sphagnum*-species that further acidify the habitat, which on its turn can lead to severe decline of the rich fen bryophyte vegetation. Moreover, aeration was shown to lead to increased N-availability per volume peat soil in the root zone of rich fens, which will possibly promote the degradation of rich fens because of increased encroachment of graminoid species at the expense of characteristic brown moss and slow-growing vascular species.

Severe desiccation should be avoided in any case from a management perspective, because this not only leads to increased net N-mineralization, but also to considerable net P-release and net DOC production per volume peat soil in the root zone. High concentrations of P are obviously a threat to nutrient-poor and predominantly P-limited rich fen habitats, and increased net DOC production can have negative chemical and ecological consequences, as it alters acid-base chemistry, and stimulates microbial decomposition rates (Wiegner and Seitzinger, 2004), hence lower carbon sequestration rates. Therefore, periods of low water levels are definitely undesirable with regard to the conservation of rich fens.

With regard to the conservation of *Sphagnum*-dominated fens, aeration as a result of water level drawdown did not seem to lead to increased respiration and net N-mineralization per volume peat soil in the root zone. Further desiccation, however, should also be avoided in *Sphagnum*-dominated fens, because of increased P-release and net production of DOC per volume peat soil.

However, it is important to emphasize that the laboratory conditions in this study are not completely identical to the field situation, since capillary action in peat often leads to relatively wet soils even if drought leads to lowering of the water table (Clymo and Hayward, 1982). The elasticity of a peat soil furthermore causes the surface to follow the water level when it moves down, a phenomenon called ‘moor-atmung’ or ‘mire-breathing’ (Ingram, 1983), which can also keep the surface of a peatland moist. The incubation results are nevertheless useful to assess the effects of drought in the field, since the oxygen availability can certainly increase significantly in periods of more than two months of relative drought. Redox profiles, that have been measured over time in peat soils from both a rich fen and a *Sphagnum*-fen in Stobbenribben, indicate that such periods of drought are common in the upper 10 cm of the soil (see Appendix A. Supplementary data).

Suggestions for further research

This experiment not only showed how net N- and P-mineralization rates are influenced by microbial immobilization, but also showed that these processes highly depend on the ANC of the peat. In order to get a more detailed understanding of microbial immobilization, stable isotope studies are suggested, which should focus on microbial growth and the C and N pathways in the microbial biomass of both rich fen soils and *Sphagnum*-dominated soils. In addition, more frequent measurements during incubation would provide more detailed information about the exact course of respiration, mineralization and immobilization rates over time. Due to the extensive experimental design, this was not possible in our study. Furthermore, additional measurements of litter quality and litter composition of *Sphagnum* and typical rich fen mosses, such as *S. scorpioides* and *H. vernicosus*, would reveal to what extent differences in chemical litter composition determine decomposition rates.

2.5. Conclusions

Our results show that under anaerobic conditions, net N-mineralization rates are lower in rich fens than in *Sphagnum*-fens, which seems to be due to significantly higher rates of microbial N-immobilization in rich fens. Although this phenomenon has been described in previous studies, it has never been demonstrated by estimating microbial N-immobilization. We suggest that these differences in net N-mineralization reflect differences in microbial N-demand and N-immobilization between the two fen types, associated with differences in bacterial and fungal dominance. The effects of aeration clearly differ between rich fens and *Sphagnum*-fens with respect to respiration and net N-mineralization. In rich fens, aeration seems to lead to increased respiration and net N-mineralization, while this was not the case in *Sphagnum*-peat. We therefore postulate that the biogeochemical effects of aeration as a result of moderate drought may be less severe in *Sphagnum*-fens, because the concentrations of phenolic compounds in *Sphagnum*-dominated peat are so high that activation of phenol oxidase by aeration only has a relatively limited effect. Furthermore, we showed that net P-release and DOC production increased significantly upon severe desiccation, which may be due to induced microbial die-off and/or a change in microbial composition. With respect to fen conservation management, the results of our study show that in particular in rich fens even moderate drought, during which oxygen availability increases but the peat is still moist, should be avoided because of acidification and increased N-availability, leading to dominance of graminoid species at the expense of characteristic brown moss and slow-growing vascular species. Above all, however, severe drought has a negative impact in both rich fens and *Sphagnum*-fens, not only because of direct adverse effects on wetland vegetation, but also because of severe increase of P-availability, leading to rapid succession, and possible loss of peat due to DOC-production.

Acknowledgements

The authors wish to thank IBED-colleagues Bert de Leeuw, Leen de Lange, Richard van Heck and Leo Hoitinga for their analytical assistance. This research was financially supported by the National Program Kennisnetwerk Ontwikkeling en Beheer Natuurkwaliteit (O+BN, Bosschap) of the Ministry of Economic Affairs, Agriculture and Innovation.

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Chapter 3

The ecological effects of water level fluctuation and phosphate enrichment in mesotrophic peatlands are strongly mediated by soil chemistry

Ivan S. Mettrop, Melchior D. Rutte, Annemieke M. Kooijman, Leon P.M. Lamers

Abstract

Since the re-establishment of a more natural water regime is considered by water management in wetlands with artificially stable water levels, the biogeochemical and ecological effects of water level fluctuation with different nutrient loads should be investigated. This is particularly important for biodiverse mesotrophic fens, sensitive to acidification and eutrophication. Mesocosm experiments were conducted to study the interactive effects of water level fluctuation and P-enrichment under controlled summer conditions, using peat cores including vegetation from three fens differing in biogeochemical characteristics.

The effects of fluctuating water levels on biogeochemistry and vegetation appeared to be highly dependent on peat chemistry, and more important than the effects of P-enrichment. Only when plant growth was stimulated by a favorable water level regime, P-enrichment led to increased P-consumption by plants. In rich fens with a high soil Ca-content, 7 weeks of lowered water table (-15 cm) did not lead to a drop in pH. However, soil subsidence, increased N-availability and decline of the rich fen bryophyte *Scorpidium scorpioides* give cause to concern. 7 weeks of inundation (+15 cm) offered possibilities for restoration in these fens, since alkalinity and Ca-concentrations increased, while soil P-mobilization did not occur. Even P-enrichment did not result in increased P-availability, presumably due to Ca-related precipitation of P. In rich fens with a high soil Fe-content, water table lowering should be avoided as well, because of soil subsidence, increased N-availability, decline of the rich fen bryophyte *Calliergon giganteum*, plus acidification due to Fe-oxidation. Shallow inundation, however, is also harmful, especially after mowing and with P-rich water, because plant growth was hampered, presumably by toxicity of NH_4^+ and/or Fe(II). In mineral-poor fens with a high soil P- and S-content, shallow inundation should be avoided, because of tremendous internal P-mobilization. Vitality of the dominant bryophyte *Sphagnum palustre*, however, was not affected. Low water tables affected neither vegetation, nor biogeochemistry, showing resistance to short-term drought in these fens.

Given the strong mediating effect of soil chemistry, risks and benefits of re-establishment of fluctuating water levels with clean or P-rich water need to be considered for different fen types separately in water and nature management.

3.1. Introduction

Mesotrophic fens, which are protected under the European Habitats Directive (*H7140 - Transition mires and quaking bogs*), are subject to serious deterioration in agricultural areas. Water shortage, acidification, eutrophication, and accumulation of toxins are considered to be major constraints on effective management and restoration of these fens (Lamers et al., 2015). Especially the combined effect of acidification and eutrophication is considered problematic, since species-rich vegetation communities may rapidly be transformed into species-poor *Sphagnum*-dominated communities (Kooijman, 1992). In agricultural areas, water level fluctuations are generally constricted within narrow limits by intensive hydrological management. In pristine wetlands, however, water levels vary with the meteoric and groundwater balances in and around these wetlands (Baker, Thompson and Simpson, 2009), affecting biogeochemical processes and plant succession. Therefore, water management authorities are considering re-establishment of fluctuating water levels in order to optimize the generic ecological quality in non-pristine fens (Cusell et al., 2013a). However, soil biogeochemical characteristics largely differ among different fen types, as influenced by Ca-rich or Fe-rich surface water and groundwater, or by historical flooding with sulfate-rich seawater. Also, with a higher incidence of water table fluctuation, water quality becomes an important factor, especially when fens are inundated from time to time. To support water management authorities in decision-making, therefore, a better understanding of the different biogeochemical and ecological effects of fluctuating water levels with different water qualities for various fen types is essential.

During periods of drought, aerobic oxidation processes prevail due to oxygen intrusion into the soil, potentially decreasing the acid neutralizing capacity (ANC) and pH (Stumm and Morgan, 1996), and increasing N- and P-mineralization (Olde Venterink et al., 2002; Chapter 2). These effects could hamper the development of protected brown moss vegetation in rich fens, especially during summer (Cusell et al., 2013b). However, temporary drought may be beneficial to some extent, since Fe-oxidation can lead to rapid binding of phosphate in the soil (Richardson, 1985), temporarily reducing P-availability in porewater that can be important to maintain P-limitation. Although the general effects are relatively well known, the actual impact of drought may strongly differ among fens with different biogeochemical characteristics. In Fe- and S-rich fens, the effects of drought-induced oxidation and acidification may be stronger than in Ca-rich fens, because Ca is not redox sensitive and changes in pH can be buffered (Stumm and Morgan, 1996). The response of P-availability to drought may also differ among fen types, since the P-binding capacity of the soil under oxic conditions is expected to strongly depend on the Ca and/or Fe contents.

During wet periods, the water table increases and inundation may occur. In the case of Ca-HCO_3-rich water, inundation and infiltration can increase soil ANC (Cusell et al., 2013a; Chapter 4). In addition, inundation leads to the sequential reduction of nitrate, iron and sulfate as alternative terminal electron acceptors. Since these microbial processes generate alkalinity, the ANC may further increase (Stumm and Morgan, 1996). At the same time, however, P-availability may increase as a result of net P-mobilization (internal eutrophication) due to Fe reduction (Patrick and Khalid, 1974). Especially in Fe-rich soils with high P-contents, this anaerobic P-mobilization can be severe (Zak et al., 2010; Cusell et al., 2013b). Moreover, high sulfate reduction rates and formation of iron sulfides (FeS_x) may result in additional P-mobilization in S-rich soils (Smolders and Roelofs, 1993; Caraco et al., 1998; Lamers et al., 1998b). In addition, anaerobic conditions may lead to the formation of potential phytotoxins such as NH_4^+, H_2S, and Fe(II) (Lamers et al., 2015).

Increased surface water influence, as a result of inundation, can also lead to higher nutrient inputs (external eutrophication) (e.g. Wassen et al., 1996). In relatively nutrient-poor (mesotrophic) fens adjacent to agricultural areas, external P-input can be highly detrimental (Lamers et al., 2015), and its effect strongly depends on biogeochemical characteristics of the peat soil.

The main objective of this study was to test the effects of water level fluctuation and water quality for fens differing in biogeochemical characteristics. To be able to study the interacting effects under controlled conditions, we carried out a mesocosm experiment involving two rich fens differing in soil Fe-content and a mineral-poor fen with a high soil P-content, typical for fen types in many parts of the world. Water level effects were not only studied separately, but also subsequently, to assess whether the effects of drought could be restored by inundation, and vice versa. Studying these different water level sequences over time is also important for the field situation because vegetation development varies greatly over the growing season. We measured soil surface height, ANC, nutrient dynamics and vegetation development. It was hypothesized that increased surface water P-loads would particularly promote vegetation growth. Further, we expected that drought would result in acidification, particularly in Ca-poor fens, because these are considered to be more sensitive than Ca-rich fens (Lucassen et al., 2002). Inundation was hypothesized to result in alkalinization, but also in internal P-mobilization, particularly in Fe-rich fens.

3.2. Material and methods

Three fen types

Peat cores were collected from three different locations with characteristic fen types, differing in chemical composition of peat and porewater.

The Stobbenribben rich fen (ST; N52°47’5.5”, E5°59’1”; dominated by *Scorpidium scorpioides* (Hedw.) Limpr.) is part of the Ramsar wetland area Weerribben-Wieden, and characterized by supply of lithotrophic base-rich surface water (Van Wirdum, 1991). As a result, relatively high pH and Ca-concentrations were detected in soil porewater (Table 3.1). The low soil P_{tot} content and high Ca_{tot} content of 247 mmol kg^{-1} d.w. resulted in a relatively high average soil molar Ca:P ratio of 27. Vegetation was dominated by Cyperaceae, predominantly *Carex elata* (All.), and to a lesser extent *Carex lasiocarpa* (Ehrh.), *Carex diandra* (Schrank) and *Carex rostrata* (Stokes).

The Oostelijke Binnenpolder Tienhoven rich fen (BPT; N52°10’30.7”, E5°6’0.4”; dominated by *Calliergon giganteum* (Schimp.) Kindb.) is part of the Vechtplassen area, and characterized by discharge of base-rich and Fe-rich groundwater in the former floodplain of the river Vecht. Although Ca-concentrations were relatively high, this site was especially rich in Fe, with porewater Fe-concentrations around 500 µmol L^{-1}. In addition, soil Fe_{tot} content was respectively 6-7 times higher than

Table 3.1 Initial soil characteristics of the three fen types. Means with standard deviations ($n = 24$) are shown, different letters indicate significant differences between fen types, and F-ratios are shown with their level of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. D.w.=dry weight of peat soil.

Variable	ST	BPT	ILP	$F_{2,69}$
Soil porewater				
pH	6.6 (0.2)^{b}	6.5 (0.2)^{b}	6.0 (0.2)^{a}	59.2**
Alkalinity (meq L^{-1})	3.1 (0.8)^{b}	7.1 (1.4)^{c}	1.7 (0.5)^{a}	191.8**
Ca (µmol L^{-1})	1503.5 (408.5)^{b}	3685.9 (953.1)^{c}	528.9 (129.5)^{a}	172.3***
Fe (µmol L^{-1})	6.6 (4.0)^{a}	477.3 (160.9)^{c}	97.5 (59.2)^{b}	126.5**
S (µmol L^{-1})	47.3 (17.9)^{b}	22.4 (4.6)^{a}	176.5 (50.4)^{c}	171.1**
o-PO_4 (µmol L^{-1})	0.5 (0.2)^{a}	0.1 (0.0)^{a}	37.0 (24.2)^{b}	55.1**
Soil				
Fe_{tot} (mmol kg^{-1} d.w.)	33.2 (15.3)^{a}	184.7 (24.0)^{b}	26.3 (6.0)^{a}	679.9***
Ca_{tot} (mmol kg^{-1} d.w.)	246.6 (18.2)^{c}	192.6 (22.9)^{b}	126.2 (14.8)^{a}	244.9***
S_{tot} (mmol kg^{-1} d.w.)	109.8 (29.1)^{b}	65.6 (9.2)^{a}	117.7 (14.6)^{c}	22.7***
P_{tot} (mmol kg^{-1} d.w.)	9.3 (1.4)^{a}	13.8 (1.3)^{b}	18.5 (3.6)^{c}	54.6***
Ca_{tot}:P_{tot} (mol mol^{-1})	27.0 (4.2)^{c}	14.0 (1.8)^{b}	8.4 (1.8)^{a}	266.9***
Fe_{tot}:P_{tot} (mol mol^{-1})	3.6 (1.7)^{b}	13.4 (1.7)^{c}	1.7 (0.4)^{a}	460.9***
Fe_{tot}:S_{tot} (mol mol^{-1})	0.3 (0.2)^{a}	2.9 (0.5)^{b}	0.3 (0.1)^{a}	578.0***

in the other two locations, resulting in a relatively high molar Fe:P ratio of around 13. Vegetation was dominated by *Menyanthes trifoliata* (L.) and Juncaceae, predominantly *Juncus subnodulosus* (Schrank), and *Juncus articulatus* (L.).

The mineral-poor Ilperveld fen (ILP; N52°26'35.7", E4°55'56.1"; dominated by *Sphagnum palustre* (L.)) was characterized by high porewater S_{tot} concentrations and a relatively high soil S_{tot} content, as a relic of flooding by the former Zuiderzee inland sea in the past. This fen type was further characterized by very high porewater o-PO_4 concentrations of around 40 µmol L^{-1}, respectively 75 and 370 times higher than for the ST and BPT rich fen types, while soil P_{tot} was only 1.3-2 times higher. Vegetation was dominated by *Phragmites australis* (Steud.) and *Carex riparia* (Curtis).

Experimental setup

In each fen type, 24 peat soil cores of the upper 30 cm, including mosses and vascular plants, were collected in December 2012 using PVC columns with a diameter of 16 cm and a length of 50 cm. Since sampling took place in winter, biomass was still low. The cores were subsequently used in a 14 week mesocosm-experiment.

The 24 soil cores per fen (n_{tot}=72) were treated with different water qualities and water level regimes. Within the factor water quality we distinguished between 'clean' or 'P-rich' supply-water. With regard to water level treatment three different situations were simulated: (1) a control treatment with water levels at the surface (0 cm) throughout the experiment, (2) a situation with initial drought, with water levels at -15 cm, followed by inundation, with water levels at +15 cm, and (3) the reverse regime: first inundation, then drought. Four cores were assigned per treatment combination (n=4). The experiment was conducted in a 18°C climate room to simulate summer conditions (relative air humidity of 50-60 % and 16 hours of light with a PAR intensity of 150 µmol m^{-2} s^{-1}). To be able to assess vegetation development during the water level sequences over time, all above-ground vegetation was clipped at soil surface level just before the start of the treatments.

To be able to compare the effect 'water quality' among fen types, uniform water qualities (based on surface water from ST) were applied for all fen types (Table 3.2).

Table 3.2 Chemical composition of the supplied water.

Chemical compound	Concentration (µmol L^{-1})
$CaCl_2.2H_2O$	1500
$MgCl_2.6H_2O$	300
KCl	100
$NaHCO_3$	2000
$NaH_2PO_4.H_2O$	15 (only P-rich treatments)

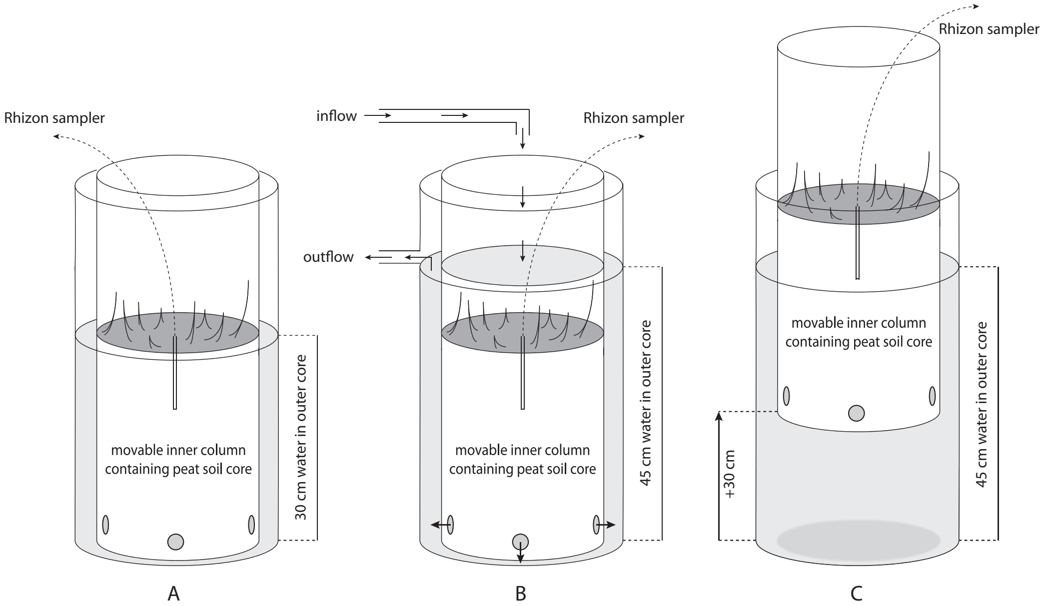

Figure 3.1 Experimental set-up. Via the principle of communicating vessels the water level in the soil cores was regulated. Reference cores with a water level at 0 cm (1.A) remained unchanged throughout the experiment. Inundation (+15 cm) was simulated by a pumping system (1.B), and drought (-15 cm) was simulated by raising the inner columns by +30 cm (1.C).

Water quality and level were regulated by placing the peat cores (with 4 pores of 5 mm diameter drilled at 1 cm above the bottom of their PVC columns) inside outer columns filled with either clean or P-rich water (Figure 3.1). In case of inundation, flow of water through the peat cores was simulated with a pumping system (Masterflex L/S), by which supply-water was added drop wise on top of the inner core and was discharged from the system via the outer core to simulate field conditions as well as possible. o-PO_4 concentrations in the P-rich supply-water amounted to 15 µmol L^{-1}, which is high but representative for surface waters in fens situated in or adjacent to agricultural areas in the Netherlands (e.g. Koerselman et al., 1990). This concentration was much higher than the initial porewater concentrations of the rich fens ST and BPT, but much lower than in the ILP fen. A flux of 56.6 L water per m^2 per day was applied via the pumping system, resulting in a P-supply of 9.6 g P per m^2 per year in the case of P-rich treatment.

The experiment was divided into ‘period 1’ and ‘period 2’ by a water level turning point halfway through the experiment (T=7 weeks), after which the cores with a -15 cm water level were subject to a +15 cm water level and vice versa. Period 1 represented the field situation shortly after winter, when plant biomass is still small. Period 2 represented the situation further in the season, when vegetation has already developed. Water level changes were regulated by raising or lowering the inner core 30 cm, while the water level remained unchanged in the outer core. In the cores subject to drought or control treatment, which were not part of the pumping system, water levels were adjusted with demineralized water three times a week to compensate for evapotranspiration.

Measurements

Peat soil characteristics of the upper 10 cm were determined by microwave destruction of 200 mg aliquots of dry, ground soil with 4.0 mL HNO3 (65 %) and 1.0 mL HCl (37%), and ICP analysis (Bettinelli et al., 1989). Porewater samples from the upper 10 cm of the peat soil were collected every week with permanently installed soil moisture samplers (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment, the Netherlands), connected to vacuumed serum bottles of 50 mL. pH-values were measured with a standard Ag/AgCl electrode and alkalinity was determined by titration down to pH 4.2 by using 0.01 mol L^{-1} HCl. Concentrations of o-PO_4, NO_3, NH_4^+ and dissolved organic matter (DOC) in porewater were measured using autoanalyzer (Skalar, San^{++} System, fitted with Skalar, SA1074). Total dissolved concentrations of Ca, Fe, and S were measured by an ICP Spectrometer (IRIS Intrepid II, Thermo Electron Corporation). In addition, water samples from the outflow of inundated cores were analyzed once during both periods. The height of the peat soil surface just under the living moss layer was measured relative to the inner core at a weekly base.

Plant community composition was recorded just before the turning point of the water level and at the end of the experiment. At these moments moss vitality was also assessed by measuring photosynthetic yields at the apex of five randomly selected individuals from each core after 30 minutes of dark adaption, using pulse-amplitude modulated (PAM) chlorophyll fluorometry in combination with saturating pulse analysis of fluorescence quenching (Junior-PAM fluorometer, Heinz Walz GmbH, Germany). Vitality was expressed as $(F_m\text{-}F_0)/F_m$, in which F_m stands for the maximum fluorescence upon intense light pulse and F_0 for the minimum of chlorophyll fluorescence at reduced light intensity, both measured regarding photosynthetic system II. At the end of the experiment above-ground plant biomass was harvested, dried at 70°C, separated into five groups of most common species (Cyperaceae, Juncaceae, Poaceae, Menyanthaceae and a ‘rest group’), and finally weighted per vegetation group. Total C and N contents in dried, ground plant biomass were measured with a CHNS analyzer (Elementar, Vario EL Cube, Hanau, Germany). Total P in dried plants was measured by total microwave digestion and ICP analysis, as described for soil analysis. Potential nutrient limitation for vegetation was assessed using vascular plant foliar N:P ratios (Koerselman and Meuleman, 1996).

Statistical analyses

Initial differences between the three fen types were tested by one-way ANOVA with least significant difference (LSD) post-hoc analyses, using 'fen type' as fixed factor.

Analysis of the treatment effects was conducted for each fen type separately, because of the large differences in chemical characteristics between the fen types. A linear mixed model was used to test the response to the two fixed factors 'water quality' and 'water level'. Since samples were taken several times consecutively from the same cores, the model was run with a residual repeated covariance structure ('AR(1): Heterogeneous') and time as repeated effect. In order to assess potential effects of the shift in water level halfway, the factor water level was categorized into six separate treatments: 0 cm in period 1, -15 cm in period 1, +15 cm in period 1, and 0 cm in period 2, -15 cm in period 2, +15 cm in period 2. Differences resulting from these water level treatments were further tested by LSD post-hoc analyses, and differences between the reference cores with water levels at 0 cm in period 1 versus period 2 were used as indicator for the effect over time.

Measurements on vegetation characteristics at the end of the experiment were tested for significant differences between fen types by applying a one-way ANOVA with LSD post-hoc analyses, using 'fen type' as fixed factor. Differences between treatments were tested separately per fen type by applying a two-way ANOVA with LSD post-hoc analyses, using water level and water quality as two main fixed factors.

All statistical analyses were performed using SPSS 20.0 for Windows (IBM Inc., 2011). P-values in the text are indicated as follows: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

3.3. Results

For reasons of clarity, responses to the treatment combinations are presented in the following order: (1) development of the above-ground vegetation as measured at the end of the experiment, (2) responses of soil and porewater characteristics during the experiment, and (3) moss vitality at the end of the experiment.

Above-ground vegetation development

Total above-ground biomass at the end of the experiment was clearly lower in ST than in BPT and ILP ($F_{2,69}=14.2^{***}$) (Figure 3.2A). Remarkably, supply of P-rich water did not lead to an overall increase in above-ground biomass in any fen type (ST:$F_{1,18}=0.0^{NS}$, BPT:$F_{1,18}=0.0^{NS}$, ILP:$F_{1,18}=1.0^{NS}$). Plant species composition was, however, affected by water quality. Cyperaceae declined with P-enrichment in the reference cores with water levels at 0 cm in all fen types.

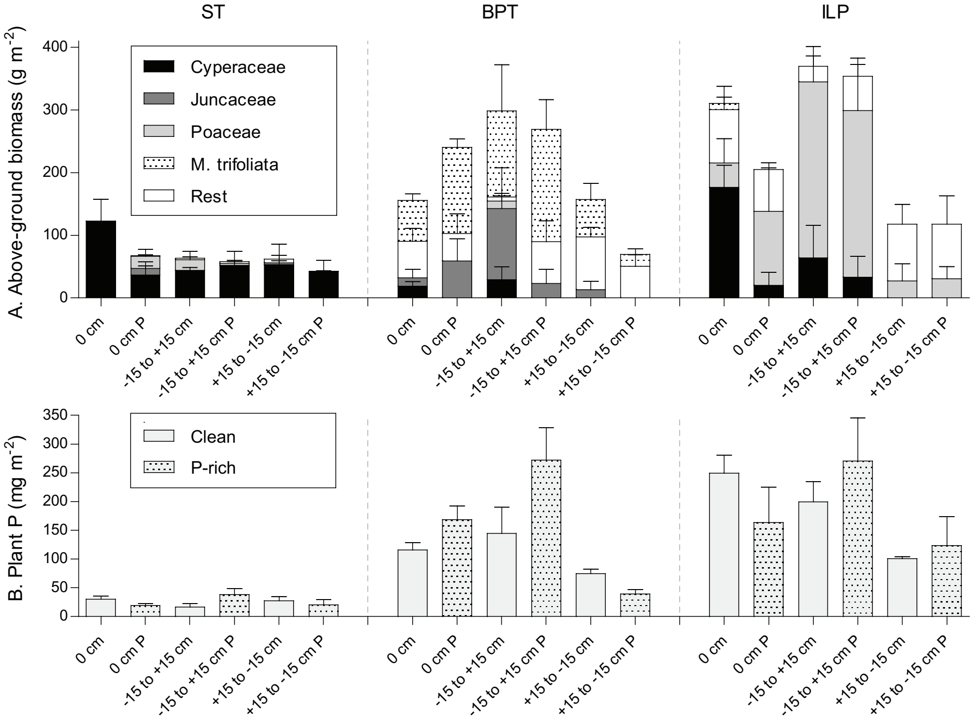

Figure 3.2 Above-ground biomass per m^2 divided into different vegetation groups (A) and P_{tot} in above-ground biomass per m^2 (B) at the end of the experiment. Different water level treatments are indicated, and P stands for P-addition. Means with S.E. are shown (*n* = 4).

Water level treatment did not affect total biomass in ST ($F_{2,18}=1.8^{NS}$), and biomass remained relatively low in all treatments. In BPT and ILP however, biomass was 3-4 times higher than in ST, and affected by water level treatment. Biomass was lower when the soil was first inundated, and then subjected to drought, than in the reference cores or the cores subjected to inundation after drought ($F_{2,18}=4.1^*$ and $F_{2,18}=3.5^*$). In addition, in BPT, an interaction between water level*water quality ($F_{2,18}=3.1^*$) suggested that the reduction in biomass when initially inundated was stronger with supply of P-rich water, although with clean water biomass production was also low. In ILP, particularly *P. australis* was stimulated by inundation after drought.

The three fen types clearly differed in plant tissue nutrient contents and type of nutrient limitation (Table 3.3). ST was characterized by the highest N:P ratio in vegetation of 40 on average ($F_{2,67}=49.1^{***}$), which was mainly due to the lower plant P content ($F_{2,68}=15.4^{***}$), suggesting P-limitation. In BPT and ILP, N:P

Table 3.3 Total N- and P-contents and N:P ratios of above-ground vascular plant tissue upon different treatments, as measured at the end of the experiment. Means with standard deviations are shown (*n* = 4).

Treatment	Plant P (g kg^{-1})	Plant N (g kg^{-1})	Plant N:P (g g^{-1})
Stobbenribben (ST)			
0 cm	0.27 (0.06)	12.3 (1.2)	46.9 (7.7)
0 cm P-rich	0.30 (0.08)	13.2 (2.1)	45.7 (10.8)
-15 cm to +15 cm	0.33 (0.12)	12.5 (3.0)	39.2 (5.4)
-15 cm to +15 cm P-rich	0.73 (0.18)	14.0 (2.4)	18.7 (2.9)
+15 cm to -15 cm	0.37 (0.20)	14.9 (3.8)	44.6 (11.1)
+15 cm to -15 cm P-rich	0.57 (0.29)	22.5 (5.9)	43.0 (10.6)
Binnenpolder Tienhoven (BPT)			
0 cm	0.82 (0.24)	21.1 (2.4)	26.7 (5.3)
0 cm P-rich	0.59 (0.13)	17.4 (2.5)	29.6 (2.0)
-15 cm to +15 cm	0.51 (0.08)	12.9 (1.7)	25.6 (5.9)
-15 cm to +15 cm P-rich	1.09 (0.27)	16.3 (4.3)	15.0 (1.7)
+15 cm to -15 cm	0.49 (0.08)	16.9 (0.9)	35.0 (4.6)
+15 cm to -15 cm P-rich	0.57 (0.05)	18.7 (2.0)	32.6 (1.9)
Ilperveld (ILP)			
0 cm	0.80 (0.09)	11.6 (2.3)	14.4 (1.3)
0 cm P-rich	0.91 (0.24)	11.9 (2.1)	13.5 (2.4)
-15 cm to +15 cm	0.56 (0.10)	11.7 (5.7)	20.2 (6.4)
-15 cm to +15 cm P-rich	0.78 (0.18)	11.4 (2.9)	14.7 (3.0)
+15 cm to -15 cm	0.91 (0.29)	13.5 (2.4)	15.4 (2.8)
+15 cm to -15 cm P-rich	0.85 (0.16)	15.7 (5.5)	18.5 (4.2)

ratios were on average 27 and 16 respectively, which suggest P-limitation for BPT and balanced availability of N and P for ILP. In ST and BPT, water quality did not affect plant N:P ratios in general ($F_{1,16}$=4.3^{NS} and $F_{1,18}$=4.2^{NS}). However, for both fens, water quality showed interactive effects with water level ($F_{2,18}$=4.8* and $F_{2,18}$=5.9*). In the inundation after drought treatment, in which vegetation growth was higher than in other treatments, tissue P-contents were higher with P-rich than with clean water, and N:P ratios 2 times lower. This suggests that the extra P was actually taken up by the vegetation, in contrast to the treatment with inundation first that strongly hampered biomass growth. In BPT, after drought, inundation with P-rich water even led to N:P ratios lower than 16, indicating balanced availability of N and P. In ILP, where P-availability was already relatively high, plant N:P ratios were neither influenced by P-enrichment ($F_{1,18}$=0.5^{NS}) nor by water level ($F_{2,18}$=2.0^{NS}).

Total P-uptake by above-ground phanerogams per m^2 was generally the highest when the soil was inundated with P-rich water after drought (Figure 3.2B), as indicated by interactions of water level*water quality (ST:$F_{2,17}$=3.0*; BPT: $F_{2,18}$=12.3***; ILP: $F_{2,18}$=3.9*). This peak coincided with higher tissue P-contents for all three fen types, and with a clear increase in biomass in BPT and ILP. Only when plant growth was stimulated by a favorable water level regime, P-enrichment led to increased P-consumption by vegetation. In contrast, in the control treatment and especially when the soils were inundated from the start of the experiment, total P-uptake per m^2 by the vegetation was low in all three fen types, even when P-rich water was supplied.

Soil and porewater characteristics

All statistics of treatment effects during the experiment are shown in Table 3.4.

Soil surface height

For all fen types, the reference cores with water levels at 0 cm showed no significant changes over time. As expected, soil surface height was influenced by water level treatment in all fen types, although this effect strongly depended on the sequence of the change (Figure 3.3 and Table 3.4). Inundation led to a slight increase of the soil surface of 1-2 cm in all fen types, both with and without prior drought. Drought during the first period did not show any effect. However, drought during period 2

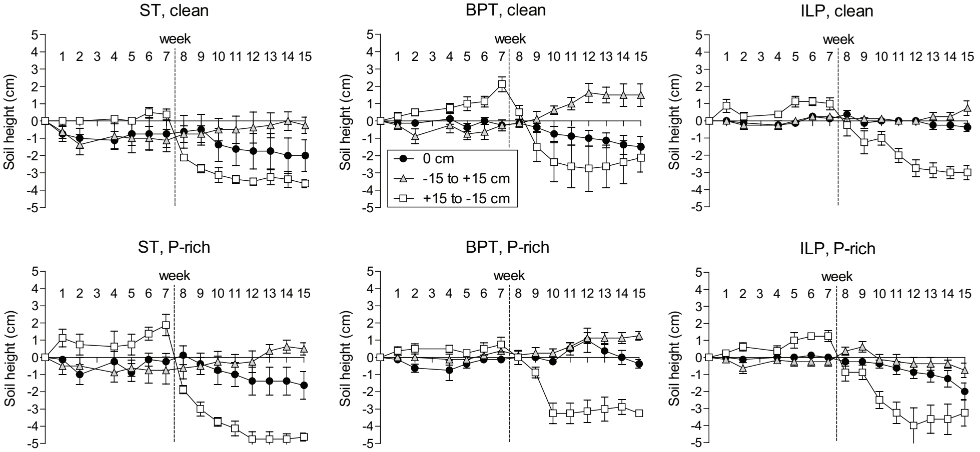

Figure 3.3 Height of the peat soil surface just below the living moss layer in relation to the inner core. The water level turning point in between period 1 and period 2 is indicated by the dashed line. Means with standard errors are shown (n = 4).

Table 3.4 Effects of water level, water quality and their interaction on porewater chemistry, as tested by a linear mixed model with LSD post hoc analyses for each location separately. *F*-ratios including denominator d.f. in parentheses are shown with their level of significance: $*P < 0.05$, $**P < 0.01$. Different letters indicate significant differences ($P < 0.05$) between water level treatments.

Variable	Quality (d.f.=1)	Level (d.f.=5)	Level*Quality (d.f.=5)	Period 1			Period 2		
Stobbenribben (ST)				0 cm	-15 cm	+15 cm	0 cm	-15 cm	+15 cm
Soil height	0.07 (58.6)	53.98** (55.4)	4.78** (55.4)	b	b	c	b	c	a
pH	0.03 (76.8)	15.56** (76.8)	1.46 (76.8)	b	b	b	b	b	a
Alkalinity	0.61 (57.3)	30.88** (57.3)	0.71 (57.3)	d	b	c	d	e	a
Ca	0.27 (52.9)	25.41** (32.1)	1.47 (32.1)	b	a	bc	a	c	a
Fe	2.14 (67.9)	35.54** (55.5)	1.45 (55.5)	cd	b	d	c	e	a
S	0.47 (39.4)	43.82** (31.7)	0.79 (31.7)	b	c	b	b	a	d
o-PO_4	0.25 (75.1)	26.44** (46.0)	0.74 (46.0)	b	ab	b	b	c	a
NO_3	0.06 (30.7)	39.03** (18.9)	0.08 (18.9)	a	a	a	a	a	b
NH_4	0.03 (44.0)	25.39** (43.5)	2.11 (43.5)	c	b	cd	c	d	a
DOC	0.00 (94.1)	9.45** (70.7)	2.22 (70.7)	b	b	a	c	ab	b
Binnenpolder Tienhoven (BPT)				0 cm	-15 cm	+15 cm	0 cm	-15 cm	+15 cm
Soil height	0.41 (48.9)	28.16** (45.8)	1.42 (45.8)	b	b	c	b	c	a
pH	2.45 (77.7)	45.71** (73.3)	1.40 (73.3)	b	c	b	bc	a	d
Alkalinity	3.93 (73.3)	66.86** (71.2)	1.63 (71.2)	c	a	b	c	d	b
Ca	1.13 (50.0)	115.80** (33.9)	1.16 (33.9)	d	a	b	d	e	c
Fe	0.12 (63.9)	99.21** (44.6)	1.16 (44.6)	d	b	d	c	d	a
S	0.00 (59.2)	121.87** (42.1)	1.70 (42.1)	b	c	b	b	a	c
o-PO_4	0.32 (49.9)	46.37** (44.0)	3.11* (44.0)	c	b	d	b	c	a
NO_3	0.31 (37.2)	21.00** (34.2)	0.67 (34.2)	a	b	a	a	a	b
NH_4	0.99 (52.0)	54.52** (38.9)	21.17** (38.9)	c	b	d	c	bc	a
DOC	0.17 (41.7)	89.50** (37.6)	152.44** (37.6)	b	b	c	b	b	a
Ilperveld (ILP)				0 cm	-15 cm	+15 cm	0 cm	-15 cm	+15 cm
Soil height	7.04* (50.2)	75.01** (50.1)	2.66* (50.1)	c	c	d	b	bc	a
pH	0.00 (64.9)	23.84** (64.0)	7.14** (64.0)	bc	c	c	b	b	a
Alkalinity	0.08 (70.6)	60.27** (68.1)	4.13** (68.1)	c	b	d	cd	e	a
Ca	0.00 (76.9)	4.64** (61.4)	0.41 (61.4)	b	a	c	b	d	a
Fe	2.77 (63.6)	32.84** (41.8)	1.30 (41.8)	b	a	b	b	c	a
S	0.03 (74.9)	109.54** (48.8)	0.71 (48.8)	b	c	b	b	a	c
o-PO_4	0.75 (55.9)	45.14** (41.8)	1.20 (41.8)	c	b	e	c	d	a
NO_3	1.20 (59.8)	6.59** (44.1)	0.46 (44.1)	a	b	a	a	a	c
NH_4	0.51 (55.6)	12.12** (35.2)	1.21 (35.2)	b	a	c	b	b	a
DOC	0.23 (80.1)	3.42** (65.5)	2.50 (65.5)	ab	ab	b	b	a	b

led to significant subsidence of 3-5 centimeters in all fen types when preceded by inundation, when vegetation development was limited. In ST and ILP, this subsidence was stronger under P-rich conditions.

Porewater pH and ANC

Water quality (P addition) did not significantly influence Ca-concentrations, alkalinity or pH in any fen type (Figure 3.4A,B,C). Water level, however, did affect these three parameters (Table 3.4). Reference cores with water levels at 0 cm showed no change over time. Drought, however, decreased alkalinity for all fen types, accompanied by decreased Ca-concentrations especially for BPT. The decrease in alkalinity by drought was stronger when preceded by inundation in ST and ILP, resulting in lowered pH. Surprisingly, BPT showed a remarkable increase in pH with drought, leading to pH values of 7.0 on average, despite the strong decrease in alkalinity from 6.5 to 1.5 meq L^{-1} during period 1.

In ST and BPT, Ca-concentrations only increased upon inundation in period 2, when preceded by drought. Ca-concentrations increased to concentrations around 2000 µmol L^{-1} and 2500 µmol L^{-1} respectively, due to input of extra Ca from the supply-water. In ILP, Ca-concentrations gradually increased during both periods. Although alkalinity was also expected to increase due to supply of base-rich water and/or reduction processes, for ST and ILP this only happened upon inundation in period 2, when preceded by drought. In BPT, inundation led to different alkalinity responses than in ST and ILP. In BPT, which had higher alkalinity in porewater than the supply-water, a decrease in alkalinity was observed as a result of inundation during period 1, probably due to dilution.

Porewater Fe, S and DOC

Porewater Fe-, S- and DOC-concentrations were generally not affected by P-addition (Figure. 3.5A,B,C and Table 3.4). Also, the reference cores with water levels at 0 cm showed no significant changes in Fe-, S- and DOC-concentrations over time. However, in all fen types, the decrease of alkalinity upon drought was accompanied by a strong decrease in Fe- and increase in S-concentrations in porewater. Oxidation of Fe^{2+} resulted in decreased concentrations of dissolved iron, while oxidation of S^{2-}, partly enclosed in FeS_x, resulted in the formation of dissolved SO_4. Inundation showed the opposite response, with increased dissolved Fe-concentrations in all fen types, indicating reduction of Fe^{3+} to more soluble Fe^{2+}. Dissolved S-concentrations simultaneously decreased, indicating SO_4 reduction and subsequent FeS_x formation with part of the Fe^{2+} that became available.

In ST, where S-concentrations were moderately high, and especially in the S-rich ILP, these changes in soluble Fe and S-concentrations with drought were accelerated when preceded by inundation. This may point to increased formation of FeS_x during

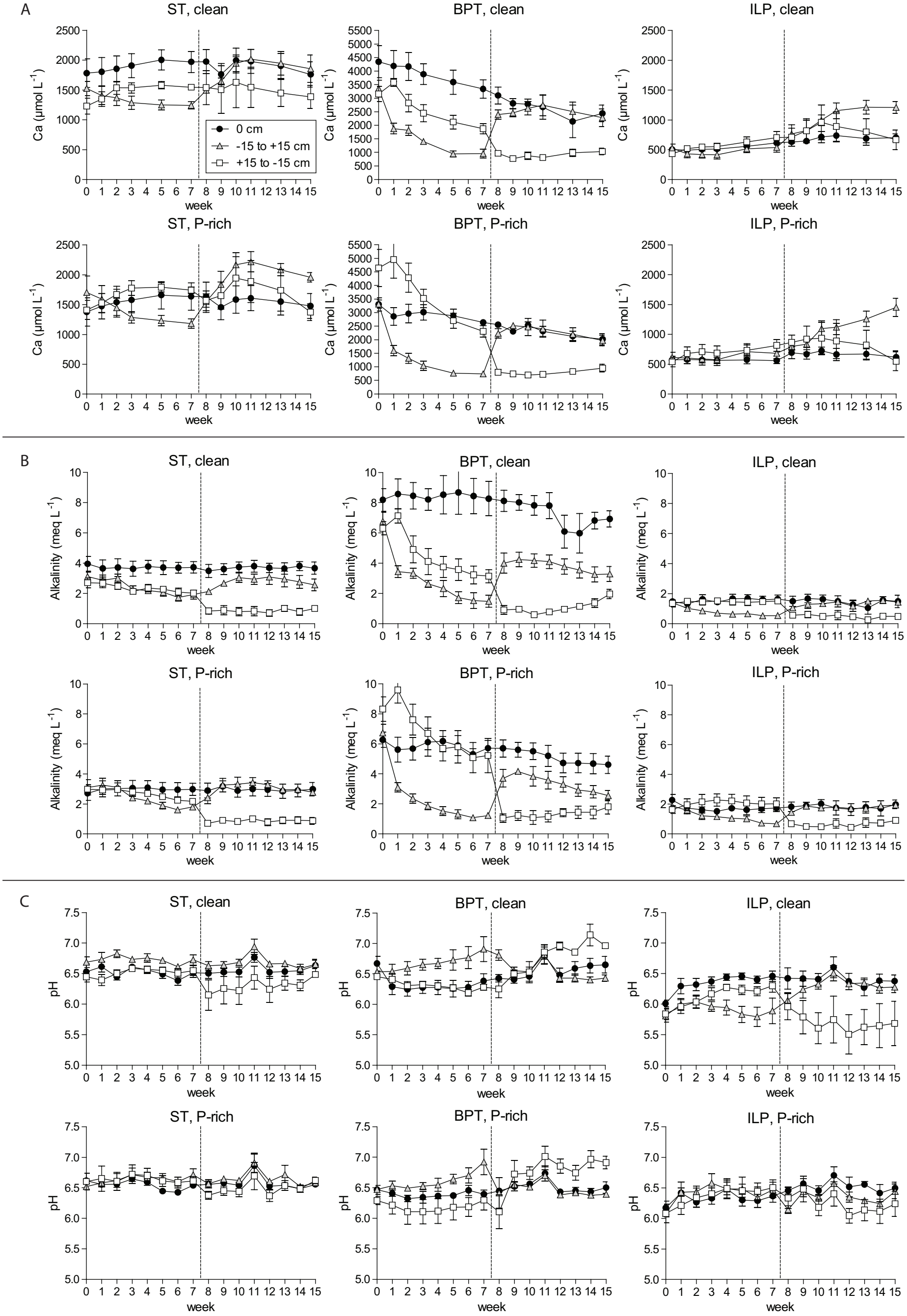

Figure 3.4 Porewater Ca-concentrations (4.A), alkalinity (4.B) and pH (4.C). The water level turning point in between period 1 and period 2 is indicated by the dashed line. Means with standard errors are shown ($n = 4$). Note that for Ca the scales on the y-axis differ between graphs.

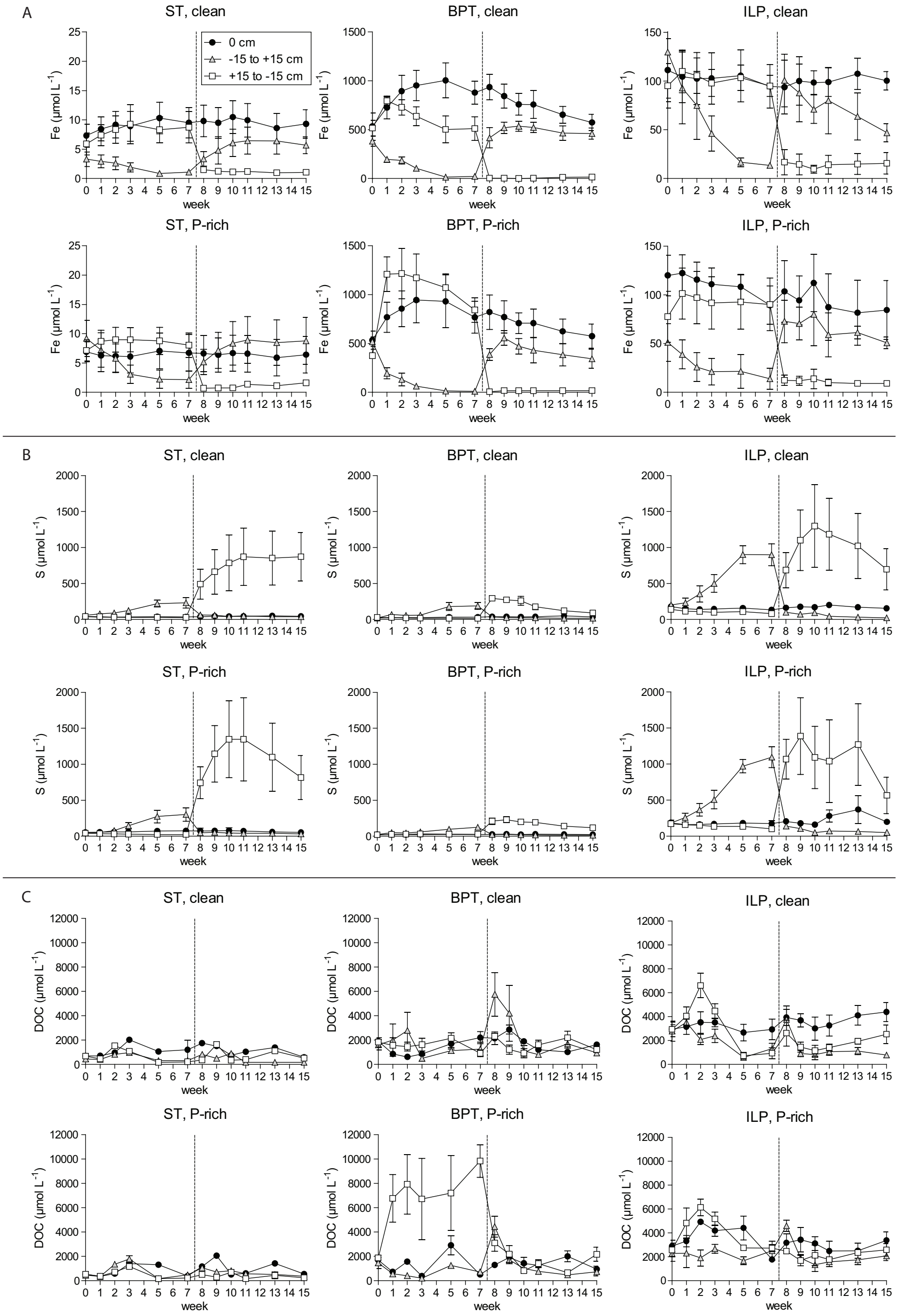

Figure 3.5 Fe (5.A), S (5.B), and DOC (5.C) concentrations in soil porewater. The water level turning point in between period 1 and period 2 is indicated by the dashed line. Means with standard errors are shown (*n* = 4). Note that for Fe the scales on the y-axis differ between graphs.

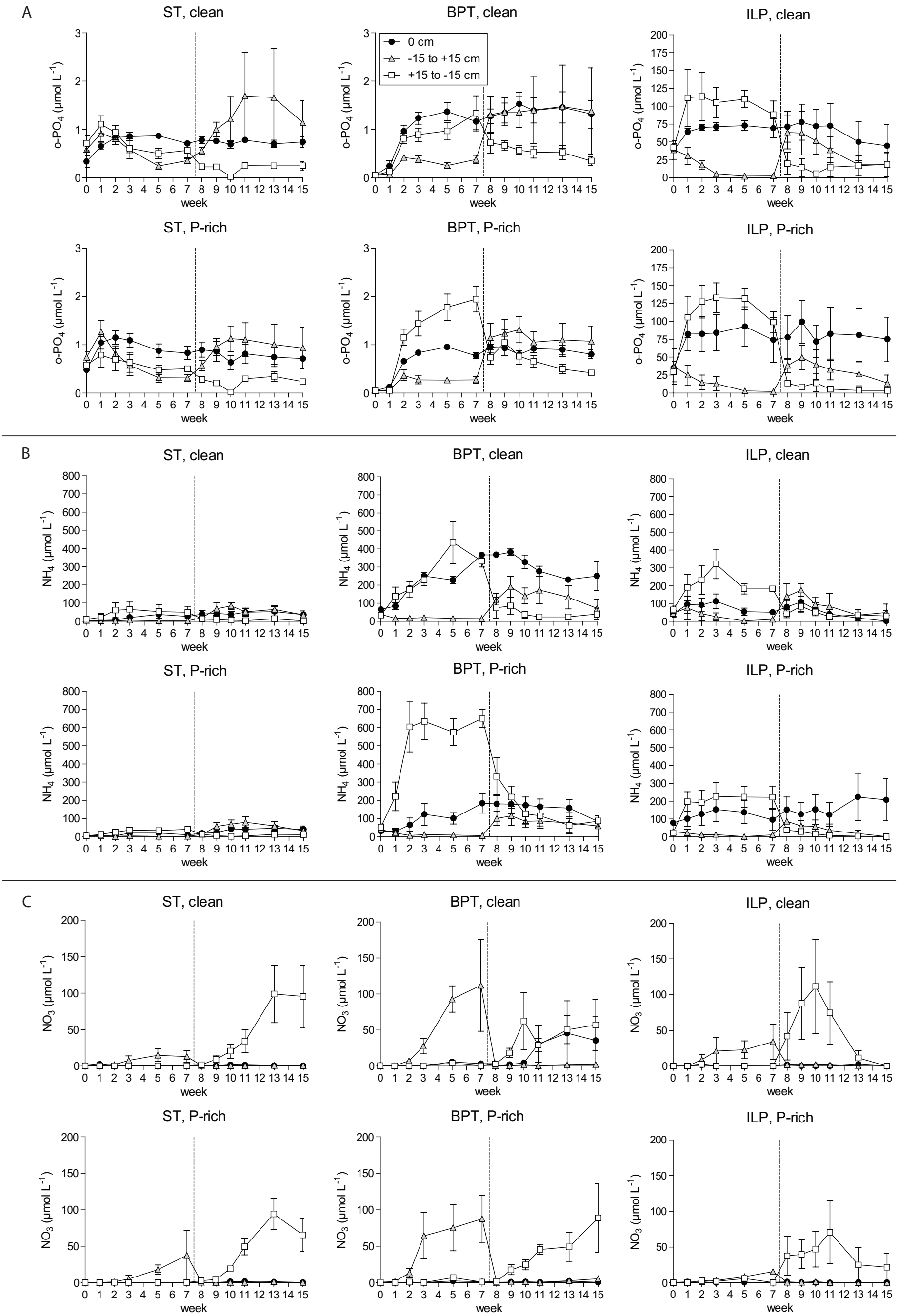

Figure 3.6 o-PO_4 (6.A), NH_4 (6.B) and NO_3 (6.C) concentrations in soil porewater. The water level turning point in between period 1 and period 2 is indicated by the dashed line. Means with standard errors are shown (n = 4). Note that for o-PO_4 the scales on the y-axis differ between graphs.

the preceding inundation period, which is oxidized during subsequent drought. In the Fe-rich BPT, oxidizable S-concentrations were low anyhow, in accordance with the low S_{tot} concentrations and high Fe:S ratio in the soil (Table 3.1).

Remarkable was the accelerated increase in Fe-concentrations in BPT upon inundation with P-rich water during period 1, accompanied by a considerable increase in DOC. This response was absent when clean water was supplied. In addition, this response was only observed in BPT. The increase in Fe-concentrations upon inundation with P-rich water in BPT was, however, not observed during period 2, when preceded by drought, and when the above-ground biomass had strongly increased.

Porewater nutrients

Despite differences in P-input via supply-water, porewater o-PO_4 concentrations were generally not affected by water quality in any fen type (Table 3.4). Upon P-enrichment, o-PO_4 concentrations were generally much lower than the 15 µmol L^{-1} of the supply-water in the rich fens ST and BPT, and values remained at the same low levels measured upon clean water treatment (Figure 3.6A). Also, o-PO_4 concentrations in the outflow of the outer columns were very low for all fen types (predominantly under the detection limit of 0.05 µmol L^{-1}). Because vegetation uptake only played a role in the inundation after drought treatment, when above-ground biomass was high, the absence of an increase in o-PO_4 concentrations upon P-enrichment in ST and BPT presumably points to chemical sorption of P in the soil. In ILP, however, porewater o-PO_4 concentrations were much higher than in the supply-water in both clean and P-rich treatment, and approximately 10 µmol L^{-1} higher with P-enrichment.

Contrary to P-addition, water level fluctuations significantly affected porewater o-PO_4 concentrations (Figure 3.6A and Table 3.4). The reference cores with water levels at 0 cm showed no significant changes over time, but differences between drought and inundation were highly significant. In all fen types, drought led to a decrease of porewater o-PO_4 concentrations, presumably because oxidized iron precipitated with P as Fe-P complexes. Inundation, on the other hand, increased o-PO_4 concentrations in all fen types as a result of Fe reduction and concomitant P-mobilization.

These water table effects on porewater o-PO_4 concentrations clearly differed among fen types. In the Ca-rich ST, o-PO_4 concentrations were relatively low and only slightly increased upon inundation after drought. In the Fe-rich BPT, however, o-PO_4 concentrations clearly increased upon inundation during both periods. Moreover, a significant water level*water quality interaction indicated that o-PO_4 concentrations increased especially upon inundation with P-rich water in the first period, when vegetation biomass was low. In ILP, o-PO_4 concentrations were already much higher than in ST and BPT, and increased considerably upon inunda-

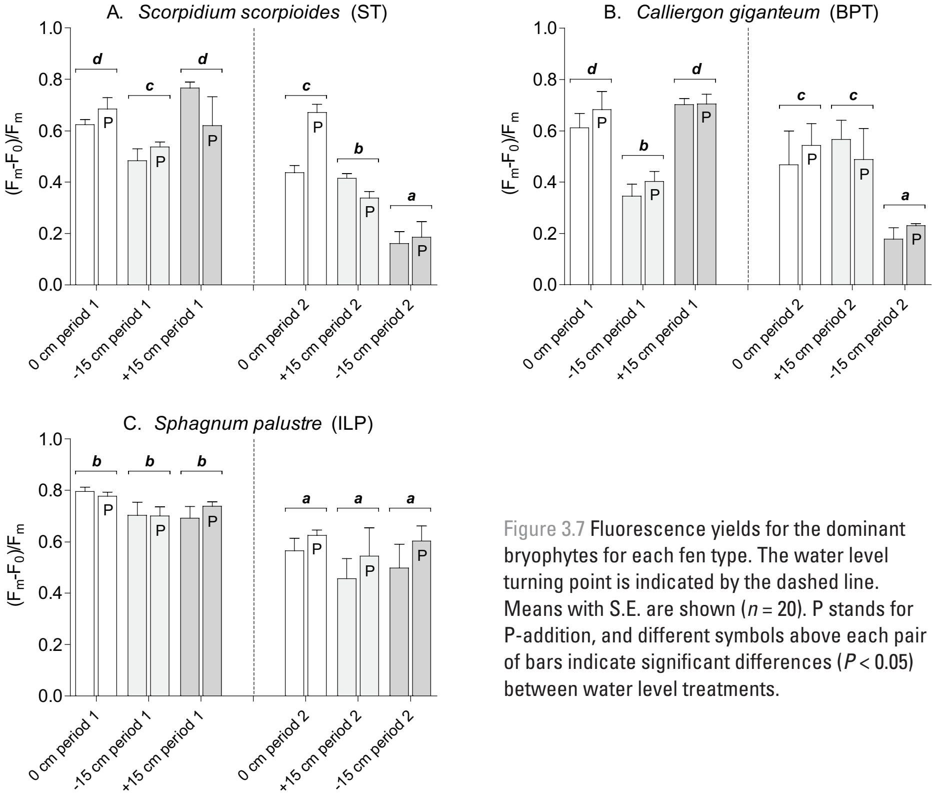

Figure 3.7 Fluorescence yields for the dominant bryophytes for each fen type. The water level turning point is indicated by the dashed line. Means with S.E. are shown (*n* = 20). P stands for P-addition, and different symbols above each pair of bars indicate significant differences ($P < 0.05$) between water level treatments.

tion. High values of around 130 µmol L^{-1} were reached with both clean and P-rich water. However, subsequent immobilization of P upon drought resulted in a decrease to, or even below, initial concentrations in all fen types.

Overall, there was no effect of water quality on NH_4 and NO_3 concentrations. However, in BPT, NH_4 concentrations increased upon inundation with P-rich water in the first period, when above-ground biomass was still low (Figure 3.6B,C). This was indicated by a water level*water quality interaction (Table 3.4). The effect of water level on NH_4 and NO_3 concentrations was again more important than that of water quality. While the reference cores with water levels at 0 cm showed no significant changes in NH_4 and NO_3 over time, the effects of drought and inundation were highly significant. Generally, drought led to decreased NH_4 and increased NO_3 concentrations due to ammonium oxidation (nitrification) by intruding O_2, while inundation led to increased NH_4 and decreased NO_3 concentrations as a result of decreased nitrification, increased denitrification, and dissimilatory nitrate reduction to ammonium. These effects were most obvious in BPT and ILP, where inundation with both clean and P-rich water during the first period led to a severe increase of NH_4. As already indicated, NH_4 concentrations further increased in BPT to values known to be phytotoxic of over 600 µmol L^{-1} when inundation was applied with P-rich water in the first period, accompanied by a strong increase in DOC (Figure 3.5C). Inundation with P-rich water during period 2, when vegetation biomass had already increased, did not have this strong effect. Furthermore, the increase of NO_3 concentrations by nitrification during drought in ST and ILP was much stronger when preceded by inundation, accompanied by a stronger decrease in alkalinity.

Moss vitality

Fluorescence yields in the reference situation with water levels at 0 cm were lower in all fen types after period 2 than after period 1, indicating that the experimental conditions in general were not optimal for the mosses. There was no general effect of water quality on the vitality of any of the mosses (BPT:$F_{1,228}=0.9^{NS}$, ST:$F_{1,228}=1.0^{NS}$, ILP:$F_{1,228}=3.2^{NS}$). However, there was a clear effect of water level for the two rich-fen species. Drought generally led to lower fluorescence yields of *S. scorpioides* in ST ($F_{5,228}=38.6^{***}$) and *C. giganteum* in BPT ($F_{5,228}=29.4^{***}$) (Figure 3.7). During subsequent inundation in period 2, vitality of *S. scorpioides* remained low, but *C. giganteum* showed a clear recovery. Drought after inundation, however, led to very low fluorescence yields for both rich fen moss spp. Vitality of *S. palustre* in ILP was, in contrast, not affected by drought or inundation ($F_{5,228}=0.1^{NS}$).

3.4. Discussion

The main objective of this study was to test the effects of water level fluctuation and water quality in fens differing in biogeochemical characteristics, under controlled conditions and for the combination of plant and soil (mesocosm). In all fens, effects of water level fluctuation were the most imminent, and general risks and benefits of drought and inundation could be observed, depending on the vegetation development.

Risks and benefits of higher drought incidence

Direct effects on plants, such as water shortage, and indirect effects such as acidification and N-eutrophication by increased mineralization are generally considered to be major potential constraints on vegetation development in relation to drought in mesotrophic fens (e.g. Lamers et al., 2015). However, temporary drought may also be beneficial for P-limited vegetation, since Fe-oxidation can lead to rapid binding of phosphate in the soil (Richardson, 1985), temporarily reducing P-availability in porewater. The potential risks and benefits need to be weighed up for different fen types separately.

Generally, vascular plant growth was not inhibited by drought, which we attribute to sufficiently deep rooting preventing water shortage. In BPT and ILP, vascular plant growth was even stimulated by drought directly from the start (similar to early spring conditions shortly after winter). This stimulation is, however, not necessarily favorable for mesotrophic peatlands, since high biomass production may lead to a less diverse species composition due to competition, and may offer less room and light for mosses.

While growth of vascular plants was not negatively affected by drought, vitality of rich fen bryophytes severely decreased. Although *C. giganteum* was able to recover during subsequent inundation, the decrease in vitality of *S. scorpioides* upon drought could not be restored within 7 weeks of subsequent inundation, due to reduced growth rates (Kooijman and Whilde, 1993). In contrast, vitality of *S. palustre* was not affected by drought at all, presumably due to the efficient capillary water transport and water storage of *Sphagnum* spp. (Clymo and Hayward, 1982), and to the fact that *Sphagnum* spp. are able to tolerate acidified conditions (e.g. Rochefort et al., 1990). These findings confirm the considerable competitive advantage of *Sphagnum* over rich fen bryophytes during drought, explaining drought-induced vegetation shifts from certain brown mosses to peat mosses.

Lowering of the water table led to subsidence of the peat soil surface in all fen types, but only when preceded by inundation. This suggests that subsidence is not solely due to reduced buoyancy by release of entrapped gas bubbles (Strack et al., 2006), or increased decomposition rates as a result of aeration (Chapter 2). Presumably, subsidence was further affected by the reduced vegetation development during prior inundation, which led to inhibited root growth and lower stability of the peat soil.

Drought generally led to decreased porewater Ca-concentrations, and especially decreased porewater alkalinity due to acidification. In the Fe-rich BPT, acidification seemed mainly be due to iron oxidation (Stumm and Morgan, 1996). In the Ca-rich ST, which contained slightly more S in the soil, and particularly in the S-rich ILP, the oxidation of sulfides may have been more important (Lamers et al., 1998a; Lucassen et al., 2002). Interestingly, prior inundation modified these drought effects. In all fen types, drought-induced acidification was accelerated when preceded by inundation, which may be due to increased concentrations of reduced components that could readily be oxidized during subsequent drought.

Despite the decrease in alkalinity upon drought, ANC remained sufficiently high to prevent a severe drop in pH in all fen types to values below 6.0, considered a critical value for rich fens dominated by brown mosses (Kooijman, 2012). Unexpectedly, pH values even increased to pH values of 7.0 upon drought in BPT, presumably due to degassing of CO_2-charged porewater, as demonstrated in previous studies in Fe-rich fens (Zak et al., 2004). A release of CO_2 to the atmosphere can lead to increased pH values ($pH = 6.4 + \log([HCO_3^-]/[CO_2])$) (Stumm and Morgan, 1996). The combined effect of decreased alkalinity and increased pH during drought in BPT may have induced Ca-mineral precipitation (Boyer and Wheeler, 1989), possibly explaining the decrease in porewater Ca-concentrations, despite a concentration effect by evapotranspiration.

In previous experimental studies, increased decomposition rates with drought are generally reported to result in increased N-mineralization in peat soils (Olde Venterink et al., 2002; Chapter 2). However, in terms of the actual porewater N-concentrations, increased plant uptake may compensate for this N-release. In all fen types, we found that drought at an early stage led to lower total porewater N-availability (NO_3 + NH_4) than early inundation, because vascular plant development was stimulated by this water level regime and thereby N-uptake by plants was enhanced.

In contrast to that of N, the availability of P in porewater decreased upon drought in all fen types. In the Ca-rich ST, we presume that mainly co-precipitation of P with calcite was involved (Boyer and Wheeler, 1989), which explains the relatively small response to oxic conditions. In the Fe-rich BPT, high rates of Fe-oxidation and subsequent Fe-P precipitation were involved (Patrick and Khalid, 1974; Richardson, 1985), explaining the more obvious decrease in P-availability. In the S-rich ILP, oxidation of FeS_x has presumably increased the reactive Fe^{3+} concentration, stimulating substantial P-binding in the topsoil (Roden and Edmonds, 1997). The binding of P to Fe temporarily reduces P-availability in porewater (e.g. Patrick and Khalid, 1974), but the question is whether this is really an advantage to P-limited vegetation. Fe-related P precipitation may be less relevant in terms of reducing P-availability to plants as generally assumed, an idea that was already reported by Pawlikowski et al. (2013). Many vascular plants are still capable of taking up P from accumulated Fe-phosphates in soils (Marschner, 1995).

All in all, the direct drought effects were not negative for vascular plants, but vitality of protected rich fen bryophytes severely decreased, giving *Sphagnum* a competitive advantage. Drought-induced acidification did not lead to considerable lowering of pH during 7 weeks, because of sufficient buffering in all fen types. In terms of nutrient-availability there were no considerable effects.

Risks and benefits of higher inundation incidence

Formation of potential toxins and increased P-mobilization are generally considered major constraints on vegetation development in relation to inundation in mesotrophic fens (e.g. Lamers et al., 2015). However, inundation may also be beneficial, since inundation with base-rich water in summer promotes buffering against acidification (Chapter 4). Also the potential risks and benefits of inundation need to be weighed up with a critical eye for different fen types separately.

Plant growth was hampered when inundated directly from the start (similar to early spring conditions shortly after winter), especially in BPT and ILP, which was probably due to anoxic conditions and formation of toxins in the first period. In BPT, inundation with P-rich water led to porewater Fe-concentrations over 1000 μmol L^{-1}, reported as toxic to *J. subnodulosus* (Snowden and Wheeler. 1993), which is confirmed by our results for development per group of species. In this fen type, NH_4 concentrations also considerably increased with inundation shortly after winter. Particularly with P-rich water, NH_4 concentrations increased well over 100 μmol L^{-1}, a level above which toxic effects can seriously damage bryophyte vegetation under summer conditions (Paulissen et al., 2004; Verhoeven et al., 2011), and toxic effects may be expected for plants (Lamers et al., 2015). When subjected to inundation after a period of drought (when plants already had the opportunity to grow), however, ammonium toxicity did not seem to be a severe problem anymore. Increased plant activity probably led to increased radial oxygen loss (ROL) from roots (Lamers et al., 2012), stimulating nitrification in the rhizosphere, and increased uptake of N. In the S-rich ILP, the decline in vegetation, especially of *P. australis*, upon inundation with both clean and P-rich water shortly after winter may very well be caused by sulfide toxicity (Armstrong et al., 1996). When subjected to inundation after drought however, when plants already had had the opportunity to grow, sulfide toxicity did not seem to be a problem anymore. Although sulfide concentrations in bulk soil still increased to toxic values, increased plant activity probably led to increased ROL, stimulating sulfide oxidation in the rhizosphere.

Otherwise, a wet period in spring, with reduced vegetation development, is not necessarily detrimental for mesotrophic peatlands. Competition by fast growing species may be limited this way, eventually resulting in increased biodiversity.

For rich-fen mosses, inundation, or at least water levels at the soil surface, turned out to be vital, not only to prevent water shortage, but partly also to restore direct effects of prior drought. *S. palustre* however, turned out to be well able to endure periods of inundation as well, regardless of the water quality. Even with base-rich inundation water, which was assumed to pose problems since *Sphagnum* spp. are generally associated with and adapted to acidic conditions, *S. palustre* thrived remarkably well.

Generally, inundation resulted in increased Ca-concentrations and alkalinity in porewater, but only when preceded by drought. This suggests that a prior period of drought promoted infiltration of base-rich water during inundation. In this way, inundation with base-rich water may contribute to a lasting increase in the ANC, as this is not only determined by the amount of bicarbonate in porewater, but also by the amount of Ca attached to the adsorption complex (Stumm and Morgan, 1996).

Moreover, the increase in porewater alkalinity during inundation after drought in the rich fens ST and BPT may point to additional alkalinity generation, resulting from anaerobic microbial reduction processes (Stumm and Morgan, 1996). An increased ANC by inundation, both by infiltration and by internal alkalinity generation, was previously demonstrated by field inundation experiments in similar fen types in summer (Chapter 4). In addition, anaerobic decomposition may have resulted in increased partial pressure of CO_2 in porewater (Estop-Aragonés et al., 2012), causing calcite to dissolve (Komor, 1994).

In ST, a period of 7 weeks of inundation seemed favorable to improve and/or conserve the porewater ANC, as desired from a management perspective. In BPT, the absence of an increase in porewater ANC in this experiment was primarily related to the dilution by supply-water with a lower alkalinity than the original porewater. This would, however, also be the case in the field situation, since alkalinity in surface water close to the sampled plots in BPT did not exceed 0.5 meq L^{-1} (unpublished data). Interestingly, in ILP, porewater alkalinity and Ca-concentrations remained lower than in the supply-water, which may indicate that buffer capacity was consumed. An important factor may be the exchange of Ca^{2+} for H^+ between supply-water and the H^+-rich adsorption complex of *Sphagnum*-mosses in the mineral-poor ILP (Clymo, 1963). In contrast, adsorption complexes of *S. scorpioides* in the rich fen ST and *C. giganteum* in the rich fen BPT may already have been saturated with Ca, as expected for minerotrophic moss species.

In the P-limited fens ST and BPT, P-availability remained relatively low. The high internal P-mobilization in ILP however, where the soil Fe:P ratio was low, is in accordance with previous findings for fen soils with high P-content (e.g. Zak et al., 2010). Furthermore, the high S-concentrations in ILP may have induced additional release of Fe-associated P during inundation. Since reduction of Fe and SO_4 leads to formation of FeS_x, the P-binding capacity of the peat sediment strongly decreases (Smolders and Roelofs, 1993; Caraco et al., 1998; Lamers et al., 1998b).

Net internal P-mobilization was lower upon inundation after drought than upon inundation directly from the start in BPT and ILP, which seemed to be related to P-consumption by plants. As mentioned, drought followed by inundation resulted in much higher plant biomass in these fen types. As reflected by the total amount of P in above-ground phanerogams per m^2 at the end of the experiment, the increase in biomass resulted in increased P-consumption, resulting in reduced net P-mobilization. In ST, the above-ground biomass did not differ between water level treatments, and net P-mobilization with inundation was relatively low. This can be explained by the fact that most P is bound to Ca, which is not sensitive to oxidation-reduction processes (Stumm and Morgan, 1996). Therefore, the link between net P-mobilization and P-consumption by plants seems to be less important in the Ca-rich ST.

All in all, the formation of toxins most likely results in significantly reduced vegetation development, especially with inundation in early spring. In addition, inundation increases the risk of internal P-mobilization, especially for fen soils with high P-content. On the other hand, inundation with base-rich water, especially after a period of drought, may contribute to an increased ANC.

Supply of P-rich water

In general, P-enrichment did not lead to increased above-ground biomass in any fen type, which was unexpected given the P-limitation of biomass production (as indicated by vegetation N:P ratios), and contrary to what we expected. Inundation with P-rich water only led to enhanced P-consumption by plants when preceded by a period of drought, when the vegetation had had the opportunity to develop, but this did not lead to higher production rates.

In the Ca-rich ST and the Fe-rich BPT, P-enrichment did not result in increased porewater P-availability either. In ST, most of the added P seemed to be mainly immobilized within calcium phosphate in the soil (Boyer and Wheeler, 1989), while in BPT, most of the added P was presumably immobilized in soil Fe-P complexes (Patrick and Khalid, 1974; Richardson 1985). However, overall plant N:P ratios in the Fe-rich BPT were considerably lower than in the Ca-rich ST, which may imply that Fe-related P precipitation may be less relevant in terms of reducing P-availability than generally assumed, an idea that was already reported by Pawlikowski et al. (2013). Many vascular plants are still capable of taking up P from accumulated Fe-phosphates in soils (Marschner, 1995). In ILP, where P-binding elements such as Ca and Fe are sparse, P-enrichment seemed to primarily result in increased porewater P-concentrations, which is not relevant for plants as the P-availability was already high in this fen type. In addition, a small portion of the added P could be adsorbed by mosses in all fen types, but we assume that this way of P-immobilization is of minor importance.

Unexpectedly, P-rich inundation shortly after winter even had a negative effect on plant growth in BPT, in an indirect way. The strongly increased NH_4, DOC and Fe-concentrations upon inundation with P-rich water during period 1 indicate increased microbial activity with P-enrichment (Amador and Jones, 1995; White and Reddy 2000), which may have resulted in toxic concentrations of NH_4^+, Fe(II) and/or organic acids to plants. When preceded by drought however, inundation with P-rich water did not have these extreme effects in BPT, probably because in this case vegetation had the chance to develop. Increased plant activity probably led to increased ROL (Lamers et al., 2012), and in the case of P-rich water also to enhanced P-consumption. Enhanced plant development by a favorable water regime may thus have mitigated the stimulating effect of P-enrichment on anaerobic microbial activity later in the growing season.

Conclusions and implications for management

We here show that area-specific chemical properties of peat soils, as determined by the geohydrological setting in the landscape, strongly determine the responses to water level fluctuation and P-enrichment during flooding. In general, fluctuating water levels turn out to be much more important in terms of biogeochemical responses than P-enrichment, and the stage of vegetation development appears to be very important for its response.

In rich fens with Ca-rich soils due to groundwater and/or surface water supply, drought episodes up to 7 weeks will lead to a decline of characteristic rich fen bryophytes such as *S. scorpioides*. Vascular plant development however, is not expected to be considerably affected by changes in the water level. Further, drought in these fens does not lead to a considerable risk of lowering of pH due to their high ANC. Accelerated decomposition and N-mineralization, on the other hand, are serious reasons for concern. Increased N-availability may eventually promote the degradation of rich fens because of increased encroachment of graminoid species at the expense of characteristic brown moss and slow-growing vascular species (Verhoeven et al., 2011; Cusell et al., 2014). These adverse drought effects should therefore be prevented by inundation with surface water, especially late in the growing season after a period with high water levels. Moreover, periods of inundation with base-rich water in summer, especially when preceded by a period of drought, seem to be favorable in order to structurally improve the porewater ANC by supply of Ca and internal soil alkalinization. Short-term summer inundations as a management measure have been postulated previously to restore the ANC in the topsoil of Ca-rich fens that lack sufficient HCO_3^- and Ca-buffering to prevent acidification (Cusell et al., 2013a; Chapter 4), and our findings confirm this idea. Finally, inundation does not result in severe P-mobilization, and in case of supply of P-rich water, short-term inundation does not seem to be very harmful, presumably due to Ca-related precipitation of P.

In rich fens with Fe-rich soils (caused by current or former discharge of Fe-rich groundwater), short-term drought will also result in a decline of characteristic rich fen bryophytes such as *C. giganteum*. In contrast, a period of drought shortly after winter stimulates vascular plant development. In addition, drought results in an even higher degree of acidification than in Ca-rich fens due to Fe-oxidation, and increased decomposition and N-mineralization are considered detrimental in this fen type as well. Therefore, inundation with surface water is recommended. Inundation, however, should be prevented shortly after winter, when vegetation development, hence P-consumption by plants, is still limited. Especially inundation with P-rich water seems to stimulate microbial activity, despite Fe-related precipitation of P, resulting in NH_4^+ and/or Fe(II) toxicity. In agricultural areas, this may well generate a friction between preventing acidification and N-eutrophication during drought on the one hand, and preventing external eutrophication and accumulation of toxins during inundation on the other hand.

In mineral-poor fens with P- and S-rich soils, inundation in an early stage of the growing season leads to significantly reduced plant biomass. In this case, sulfide toxicity induced by inundation is presumed to limit plant growth, which may be beneficial in terms of preventing outcompetition by fast growing species, eventually resulting in increased biodiversity. On the other hand, inundation (even with relatively base-rich water) will not be favorable, given the strong internal P-mobilization. Since the *Sphagnum* mosses already predominate and there are no chances for base-rich bryophytes anyway, it is better to occasionally allow low water levels than to engender inundation in these S-rich fen types.

Given the outcomes of this study, the risks and benefits of the re-establishment of fluctuating water levels, with either clean or P-rich water, need to be considered for different fen types separately in water management and nature management plans before its implementation.

Acknowledgements

The authors wish to thank Peter Serné and Jelle Eygensteyn for their analytical work, and Peter Cruijsen for assistance with sampling in the field. This research was financially supported by Kennisnetwerk Ontwikkeling en Beheer Natuurkwaliteit (O+BN) of the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

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Chapter 4

Impacts of short-term droughts and inundations in species-rich fens during summer and winter: large-scale field manipulation experiments

Casper Cusell, Ivan S. Mettrop, E. Emiel van Loon, Leon P.M. Lamers,
Michel Vorenhout, Annemieke M. Kooijman
Ecological Engineering 77 (2015), 127-138.

Abstract

For the conservation and restoration of biodiverse rich fens, base-rich and nutrient-poor conditions are vital. In wetlands with artificially stable surface water levels, the re-introduction of temporary water level fluctuations has been postulated to restore the acid neutralizing capacity (ANC) of fens during inundation and to reduce surface water P-input during episodes with drought. This is the first study testing this hypothesis in large-scale field manipulation experiments in rich fens with threatened rich fen mosses, rich fens with *Calliergonella*, and poor fens with *Sphagnum*. Five different experiments were conducted: 2 weeks of raised levels (+10 cm) in a floating and a non-floating fen during winter, 2 weeks of high levels in a non-floating fen during summer, and 2 weeks of lowered levels (-15 cm) in a floating and a non-floating fen during summer.

For floating fens, both lowered and raised surface water levels in adjacent ditches did not show any effect on water tables, soil ANC or nutrient levels in fens. For non-floating fens, raised surface water levels led to inundation in all vegetation types, without affecting nutrient concentrations or vegetation. Although redox potentials decreased immediately in upper soils, ANC was generally not enhanced in winter due to limited infiltration into the waterlogged soils. In summer, in contrast, ANC increased because accelerated evapotranspiration led to enhanced infiltration of inundation water and higher temperatures resulted in microbial alkalinity generation. Short-term lowering of surface water levels in summer led to lower water tables in non-floating fens, but only when precipitation rates were low. Vegetation, ANC and nutrient concentrations were, however, not affected.

The effectiveness of short-term surface water level fluctuation to restore ANC strongly depends on peat buoyancy, water saturation of soils, season and weather conditions. This explains why short-term inundation in winter is often inadequate, while short-term inundation in summer does increase ANC. Short-term droughts

do not affect the ANC or nutrient availability. Our results are not only important for the hydrological management of fens, but also have implications for future management since short-term extreme weather events will occur more frequently due to climate change.

4.1. Introduction

Rich fens are well-buffered and nutrient-poor peatland habitats that occur at a pH of 5.5–7.5 (e.g. Sjörs, 1950; Wheeler and Proctor, 2000). These species-rich fens are protected under the European Habitats Directive (transition mires, type H7140) and harbor many threatened vascular plants and bryophytes. In recent decades many rich fens have been lost in Europe due to land use change (Kooijman, 1992; JNCC, 2007; Paulissen et al., 2013; Lamers et al., 2014). Part of this decline can be attributed to natural succession to *Sphagnum*-dominated fens (e.g. Clapham, 1940), but anthropogenic forcing, including high nitrogen deposition rates, have presumably accelerated this succession (Gorham et al., 1987; Gunnarsson et al., 2000). In addition, P-eutrophication has accelerated succession of P-limited rich fens to *Sphagnum*-dominated fens (Kooijman, 1993; Kooijman and Paulissen, 2006).

In wetlands with strongly regulated surface water levels as a result of adjacent agricultural water management, one of the proposed management tools to counteract acidification and P-eutrophication is the re-introduction of fluctuating surface water levels. Raised surface water levels may lead to increased alkalinity, pH, and/or Ca-concentrations in soil porewaters (Loeb et al., 2008a,b; Cusell et al., 2013a). Additionally, the acid neutralizing capacity (ANC) may be increased during inundation by microbial reduction of Fe(III), SO_4 and/or NO_3 (Gambrell and Patrick, 1978; Baker et al., 1986), or, more permanently, infiltration of base-rich surface water (Cusell et al., 2013a). Inundation may, however, also result in eutrophication due to higher P- and N-inputs (Wassen et al., 1996) or internal P-mobilization (Patrick and Khalid, 1974; Lamers et al., 1998a).

Allowing lowered water levels means a reduced input of external water, which will presumably result in a reduction of nutrient inputs since surface waters in Europe often contain high nutrient concentrations due to intensive agricultural land use around wetlands (Coops and Hosper, 2002; Lamers et al., 2014). At the same time, however, drought may lead to increased oxygen availability, increased microbial decomposition and thus increased mineralization of nutrients (Grootjans et al., 1986; Bridgham et al., 1998; Olde Venterink et al., 2002; Chapter 2). Furthermore, water level drawdown may result in acidification (Lamers et al., 1998a; Lucassen et al., 2002), as a consequence of aerobic oxidation processes by which protons are released (Stumm and Morgan, 1996).

Although all these water-level related processes have been studied intensively in mesocosm and incubation experiments, none of these studies examined their net effect in a field experiment. We present the first study in which the physical and biogeochemical responses to short-term (2 weeks) raising (during winter and summer), and draw-down (during summer) of the surface water level were tested for several years in large-scale field experiments in base-rich fens, and *Sphagnum*-dominated poor fens. The questions addressed in this study were: (1) what are the changes in water table and biogeochemical responses as a result of short-term (2 weeks) changes in surface water level, (2) do these responses differ between floating and non-floating fens, and (3) do the responses to raised surface water levels in non-floating fens differ between winter and summer conditions? Answers to these questions will not only be important for the hydrological management of fens, but are also likely to show future implications for the conservation of fens since short-term periods with intense precipitation or drought are predicted to occur more frequently due to climate change (e.g. Bronstert, 2003; Kundzewicz et al., 2006). Our expectation for (1) was that raised surface water levels lead to increased ANC, but also to P-eutrophication. In contrast, lowered surface water levels were expected to lead to acidification and eutrophication due to increased mineralization rates. For (2), we expected that the effects on biogeochemistry are larger in non-floating fens

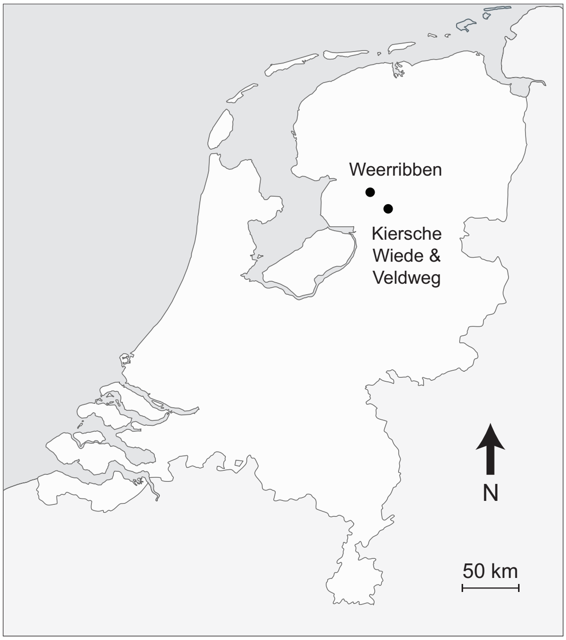

Figure 4.1 The three different experimental fen sites in The Netherlands: ‘De Weerribben’ (WEE; 52°47’52.2”N 5°55’19.9”E), and ‘De Kiersche Wiede’ (KW; 52°41’49.1”N 6°07’56.7”E) + ‘De Veldweg’ (VW; 52°41’30”N 6°06’45”E).

due to stronger water table fluctuations. For (3), we expected that the increase in ANC upon inundation is stronger in summer, because of higher infiltration and/or higher microbial alkalinity generation.

4.2. Materials and methods

Experimental design

Three fen sites in the Dutch Ramsar area and National Park "Weerribben-Wieden" were chosen for the experiments: a floating fen in "De Weerribben" (WEE) and two non-floating fens in "De Kiersche Wiede" (KW) and "De Veldweg" (VW) (Figure 4.1). All fen sites are annually mown with a brush cutter by the end of August to prevent the development of alder carr, and the hay is harvested.

The floating WEE had a buoyant 70 – 90 cm thick peat layer, floating above a sandy substrate 250 cm below soil surface. It comprised three vegetation types: (1) rich fen with *Calliergonella cuspidata* (Hedw.) Loeske dominating the moss layer, a porewater pH of 5.9 and an average water table at -8.8 cm ('Call'; *Caricion nigrae – Carex nigra-Agrostis canina* type), (2) poor fen type with *Sphagnum palustre* (L.) and *Sphagnum fallax* (H. Klinggr.) dominating the moss layer, a porewater pH of 5.0 and an average water table at -12.2 cm ('Sph'; *Caricion nigrae – Pallavicinio-Sphagnetum typicum* type), and (3) moor type dominated by ericaceous shrubs (*Erica tetralix* L.) with a *S. palustre* moss layer, a porewater pH of 4.8 and an average water table at -16.7 cm ('Moor'; *Oxycocco-Ericion – Sphagno palustris-Ericetum* type).

In contrast, the non-floating KW- and VW-fens were firmly connected to the sandy substrate located at a depth of 60 – 90 cm. These KW- and VW-fens included all three vegetation types that were mentioned for WEE, and additionally comprised rich fens with a porewater pH of 5.7 and an average water table at -3.0 cm ('Scor') with *Hamatocaulis vernicosus* (Mitt.) Hedenäs (*Caricion nigrae – Carex nigra-Agrostis canina* type) or *Scorpidium cossonii* (Schimp.) Hedenäs (*Caricion davallianae – Scorpidium-Carex diandra* type), respectively.

Table 4.1 Overview of the five experiments.

Experiment nr.	Fen site	Fen type	Season	Treatment of 2 weeks
Experiment 1	Weerribben (WEE)	Floating	November	Raised surface water level
Experiment 2	Kiersche Wiede (KW)	Non-floating	November	Raised surface water level
Experiment 3	Veldweg (VW)	Non-floating	July	Raised surface water level
Experiment 4	Weerribben (WEE)	Floating	July	Lowered surface water level
Experiment 5	Kiersche Wiede (KW)	Non-floating	July	Lowered surface water level

The present surface water level of the National Park fluctuates slightly between 0.73 and 0.83 m below mean sea level (BMSL) from March to November and is maintained at 0.83 m BMSL from December to February. The Park includes many historical, man-made ditches with a maximum depth of 1 m in order to be able to regulate the water table for reed harvesting and hay-making. At all fen sites, the vegetation types were located within a maximum distance of 50 m from adjacent ditches.

Five different experiments were conducted to evaluate the biogeochemical effects of surface water level changes on the four different vegetation types, in the three different fen sites mentioned (Table 4.1). In WEE and KW, raising and lowering of the water level was realized by the construction of dams around the areas (about 5 and 35 ha, respectively) and the use of pumps. Surface water levels were raised by 10 cm for 2 weeks in November to 0.63 m BMSL in the floating WEE-fen (experiment 1) and non-floating KW-fen (experiment 2). These raised levels were applied in 2009 and 2010 for WEE and between 2008 and 2011 for KW. In addition, the effects of high surface water levels in summer were examined in the non-floating VW-fen (experiment 3) during wet periods in July 2009 and 2010. During these periods, about 50 mm of rain in 2 weeks (3.5 – 4 mm/day) resulted in surface water levels of 0.73 m BMSL. In this case, therefore, surface water levels were not manipulated by pumps but equalled the levels in the entire National Park. Finally, surface water levels were lowered by 15 cm for 2 weeks in July 2009, 2010 and 2011 to about 0.98 m BMSL in the floating WEE-fen (experiment 4) and non-floating KW-fen (experiment 5).

Sampling

At all three fen sites, five homogenous plots of 2*2 m were selected for each of the vegetation types present. At each plot, water tables in the fen were manually recorded (a) 2 days before, (b) during and (c) 2 days after each experimental manipulation of the surface water level.

Before and after the treatments, soil porewater samples of the upper 10 cm were collected anaerobically with soil moisture samplers (Rhizons SMS 10 cm, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), connected to vacuumed plastic syringes (50 ml). The first 10 ml of each sample was discarded to exclude stagnant sampler water. Similar samples were collected in July 2008 to determine the initial biogeochemical conditions for all fen sites.

In experiment 2 (inundation of KW), we also collected inundation water above the vegetation in iodated polyethylene bottles of 100 ml after 1 week of inundation in 2009, 2010 and 2011, and in in five adjacent ditches that supplied the inundation water. Concentrations of Cl in inundation water and porewater were used as indicator of infiltration, because of its suitability as an inert tracer.

Chemical analyses of water samples

The pH-values of all water samples were measured, and alkalinities were determined by titration to pH 4.2, using 0.01M HCl. Surface water samples were filtered (GF/C glass-fibre filters, Ø= 1.2 µm; Whatmann, Brentford, UK). All samples were divided into two subsamples, and 1% HNO3 was added to one subsample to avoid metal precipitation. Total concentrations of soluble Ca, Fe and S were measured in the acidified subsamples by ICP-OES (Optima 3000 XL, PerkinElmer, Waltham, USA). In the non-acidified subsamples, concentrations of NH_4, NO_3, o-PO_4 and Cl were analyzed colorimetrically by continuous flow auto-analyzers (Skalar Analytical BV, Breda, the Netherlands).

Continuous redox measurements

Continuous measurements of the redox potential (E_h) were conducted in VW between September 2010 and July 2012. Two fiberglass probes with platinum sensor tips (PaleoTerra, Amsterdam, the Netherlands) were permanently installed in patches with Scor-, Sph- and Moor-vegetation, and these six probes were connected to a HYPNOS III data logger (MVH Consult, Leiden, the Netherlands; Vorenhout et al., 2011). Each probe contained seven sensor tips to record the E_m (measured potential) at -1, -3, -5, -10, -15, -20 and -50 cm below the soil surface every 15 min. E_m was measured as the potential between a sensor tip and a 3M Ag/AgCl reference probe. The E_h was calculated by adding a standard reference voltage and correcting for differences in pH, since pH indirectly modifies the Nernstian effect of the redox electrode:

$E_h = E_m + E_{ref} – 59*(7–pH)$, with E_{ref} being the potential of the reference probe.

Statistical analyses

Statistical analyses were performed in SPSS for Windows (SPSS 20.0.0, IBM, Armonk, USA). A two-way ANOVA with least significant difference (LSD) post-hoc test was used to determine significant differences in initial water tables (relative to the fen surface) and biogeochemical conditions between fen sites and vegetation types in July 2008. Since fen sites differed in terms of biogeochemistry and the ability to float, subsequent analyses were performed separately for the five different experiments. Since measurement plots were fixed, hence not independent over the years, a linear mixed model with year as repeated effect was used to determine the response to the fixed factors vegetation type and year (West et al., 2007). Within each year, the differences between measurements directly before and after the treatment were used as response variables. Significant differences between vegetation types and years were further examined by comparing their estimated marginal means in a LSD post-hoc test. P-values in the text are indicated as follows: $*P<0.05$, $**P<0.01$, $***P<0.001$.

In experiment 2, where raised surface water level led to inundations in KW, two additional linear mixed models, each with year as repeated effect and a single predictor variable, were used. The first model used vegetation type as fixed factor to evaluate if the inundation water had a homogenous composition or differed between the vegetation types. The second model used a categorized value for the start water table as fixed factor to evaluate the effect of this start water table on the increase of Cl-concentrations in soil porewaters during inundation.

4.3. Results

Initial conditions

Water tables were significantly higher in base-rich Scor- and Call-vegetation than in Sph- and Moor-vegetation, with mean depths of 5 – 10 cm and 15 – 23 cm below the surface in July ($F_{3,43}$=13.5***; Figure 4.2 and Appendix B.1). As expected, initial pH-values of 5.6 – 6.3 in soil porewaters for Scor- and Call-vegetation were also significantly higher than in Sph- and Moor-vegetation, where mean pH-values of about 4.7 were measured ($F_{3,42}$=29.4***). Scor- and Call-vegetation also showed significantly higher alkalinities, Ca- and Cl-concentrations than Sph- and Moor-vegetation, with initial alkalinities of about 1000 and 200 $\mu mol_c\ L^{-1}$ ($F_{3,40}$=21.5***), Ca-concentrations of around 500 and 200 $\mu mol\ L^{-1}$ ($F_{3,41}$=17.5***) and Cl-concentrations of around 900 and 500 $\mu mol\ L^{-1}$ ($F_{3,41}$=10.5***). It was remarkable that VW showed significantly higher pH, alkalinities and Ca-concentrations than the other two fen sites, especially in base-rich vegetation types as indicated by interaction effects of area and vegetation type (pH: $F_{5,42}$=2.6*, alkalinity: $F_{5,40}$=2.6*, Ca: $F_{5,41}$=4.3**). In contrast, concentrations of o-PO_4, NO_3 and NH_4 did not differ between vegetation types or fen sites, and were low in the soil porewaters of all vegetation types, with concentrations below 1, 3 and 10 $\mu mol\ L^{-1}$, respectively.

Experiment 1

Raised surface water levels in a floating fen during winter

Raising surface water levels by 10 cm had almost no effect on the water tables in the floating fens (Figure 4.3 and Appendix B.2). Along with this limited change in water tables, none of the biogeochemical variables was changed for any of the vegetation types, nor did vegetation suffer from flooding.

Experiment 2

Raised surface water levels in a non-floating fen during winter

Raising surface water levels by 10 cm in a non-floating fen in November led to inundation in all vegetation types during all 4 years through flooding and lateral flow

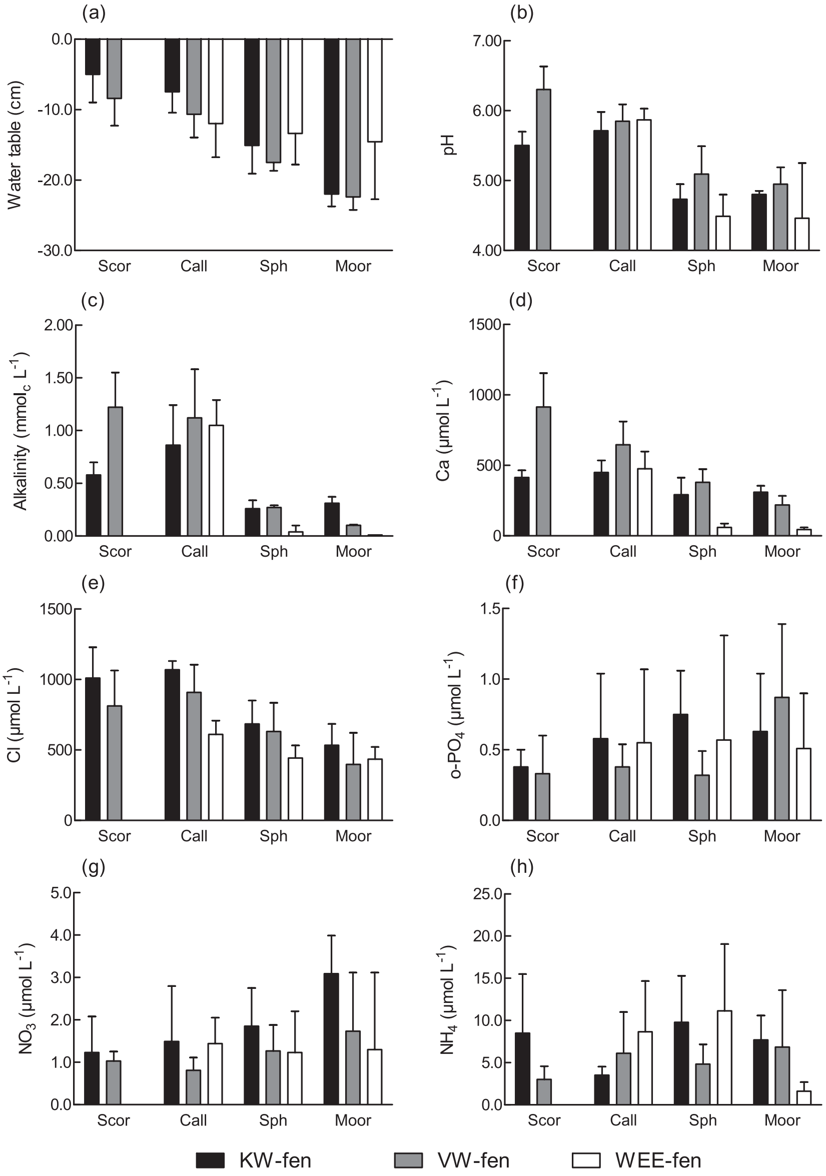

Figure 4.2 Water table (a), pH (b), alkalinity (c) and concentrations of Ca (d), Cl (e), o-PO$_4$ (f), NO$_3$ (g) and NH$_4$ (h) in soil porewater of four vegetation types (Scor = fen dominated by *Scorpidium cossonii* or *Hamatocaulis vernicosus*, Call = fen dominated by *Calliergonella cuspidata*, Sph = fen dominated by *Sphagnum palustre*, Moor with *Erica tetralix* and *Sphagnum palustre*) in three fens. Sample means are shown with their standard deviations (*n* = 5). KW = non-floating fen in Kiersche Wiede, VW = non-floating fen in Veldweg, WEE = floating fen in Weerribben. Statistical information is provided in Appendix B.

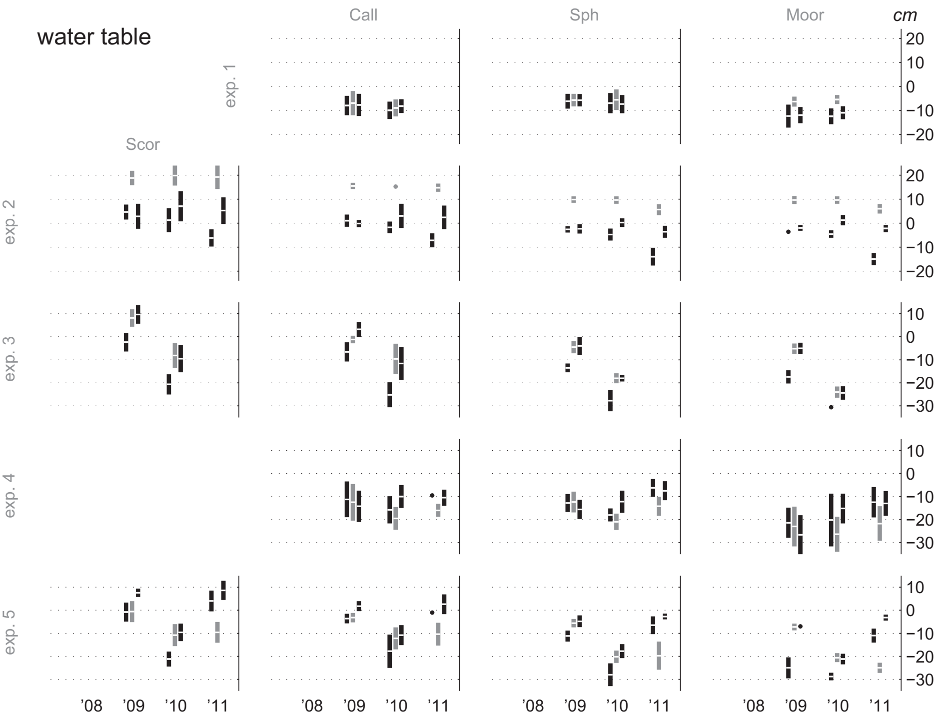

Figure 4.3 Effect of five surface water level treatments on the water table (cm above/below the fen surface) in four vegetation types, as measured 2 days before (black lines at the left of each triplet), during (grey lines) and 2 days after the treatments (black lines at the right of each triplet). Sample means (white centres of a line) are shown with their standard deviations (*n* = 5). See the caption of Figure 4.2 for a description of the vegetation abbreviations. Statistical information is provided in Appendix B.

from adjacent ditches (Figure 4.3; Appendix B.2), without visual effects of flooding on vegetation. Water table rises were largest in 2011 ($F_{2,14.6}$=116.0***) when initial water tables were lowest with 5 – 15 cm below the surface ($F_{2,15.5}$=157.4***), and smallest in 2009 when initial water tables were highest with levels around the soil surface. In 2009, most Scor- and Call-vegetation was already inundated at the start of the treatment. Furthermore, water tables rose more in Scor- and Call-vegetation than in Sph- and Moor-vegetation ($F_{3,15.1}$=8.3**).

In inundation waters, concentrations of Cl as an inert tracer did not differ between vegetation types during any of the monitored years, and were equal to those in the adjacent ditches that supplied the water (Figure 4.4). Concentrations of o-PO_4, NH_4 and NO_3 were also similar for all vegetation types and low with values of 0.05, 3 and 2 µmol L^{-1}, respectively. In contrast, alkalinities and Ca-concentrations of inundation water differed for Scor- and Call-vegetation versus Sph- and Moor-

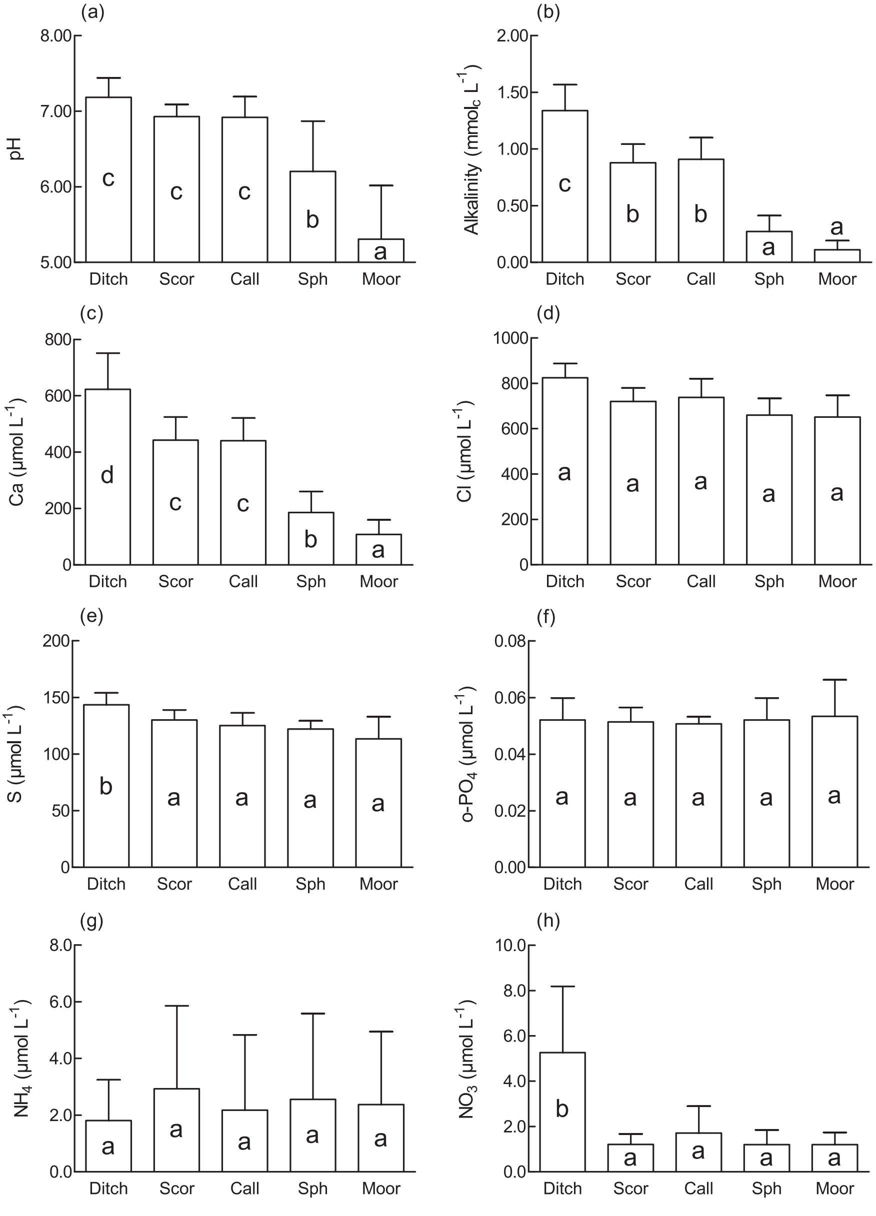

Figure 4.4 pH (a), alkalinity (b) and concentrations of Ca (c), Cl (d), S (e), o-PO_4 (f), NH_4 (g) and NO_3 (h) in the surface water of adjacent ditches and in the inundation water above four vegetation types in the KW-fen (experiment 2). See the caption of Figure 4.2 for abbreviations. Sample means for 2009, 2010 and 2011 are shown with their standard deviations ($n = 15$). Different letters indicate significant differences between vegetation types ($P < 0.05$).

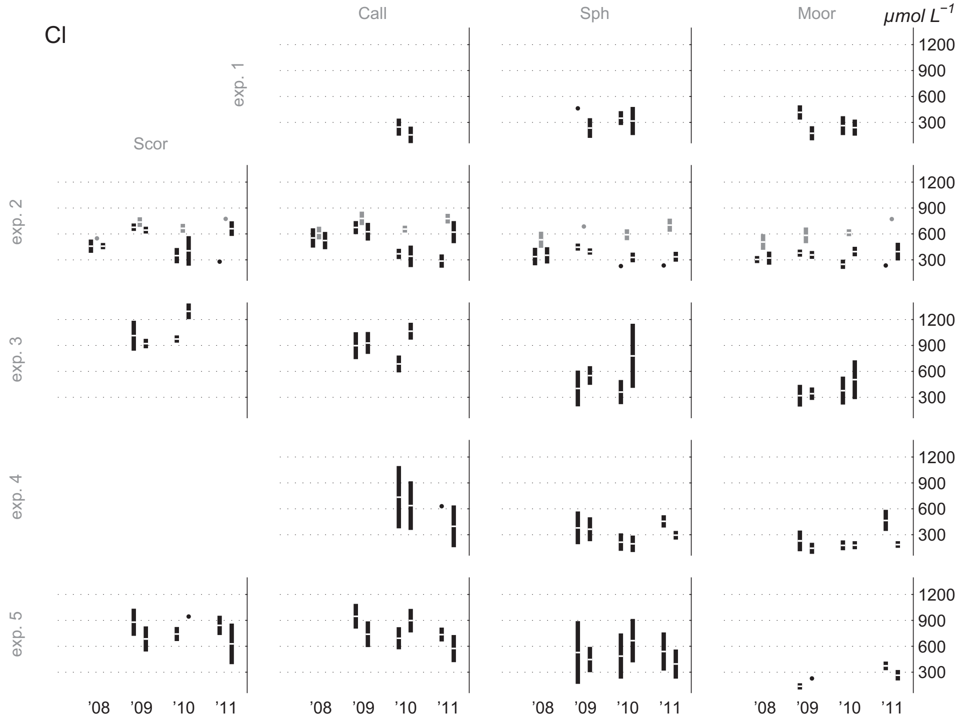

Figure 4.5 Effects of five surface water level treatments on the Cl-concentrations (μmol L-1) in four vegetation types, as measured 2 days before (black lines at the left of each triplet), during (grey lines; only in experiment 2) and 2 days after the treatments (black lines at the left of each triplet). See the caption of Figure 4.2 for abbreviations. Sample means (white centres of a line) are shown with their standard deviations (*n* = 5). Statistical information is provided in Apendix B.

vegetation, with alkalinities of around 900 versus 500 μmol$_c$ L^{-1} ($F_{4,17.3}$=80.6***) and Ca-concentrations of about 500 versus 200 μmol L^{-1} ($F_{4,20.1}$=68.0***). Also, pH decreased significantly from about 7.0 in ditches to 6.4 in the inundation water above Scor- and Call-, and to 5.4 above Moor-vegetation ($F_{4,19.4}$=36.0***).

In all years, Cl-concentrations in the inundation water were higher than the initial Cl-concentrations in the soil porewaters (Figure 4.5). However, inundation only led to increased porewater Cl-concentrations in Scor- and Call-vegetation in 2011 and in Sph- and Moor-vegetation in 2010 and 2011 (Appendix B.2). Additional analysis showed that porewater Cl-concentrations only increased when initial water tables were lower than 5 cm below the soil surface ($F_{4,17.1}$=9.1***; Table 4.2).

In line with the absence of infiltration in 2008 and 2009, inundation had almost no biogeochemical effect in these years. However, during the inundation of 2011, when infiltration occurred in all vegetation types, biogeochemical effects were ob-

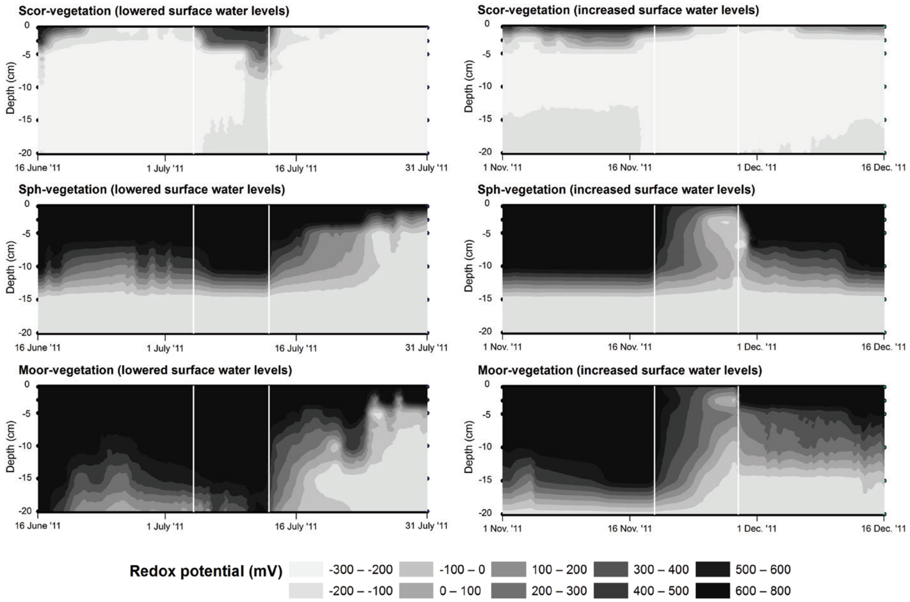

Figure 4.6 Redox potentials (E_h) in the upper 20 cm of the soil (vertical scale) in three vegetation types of the KW-fen (Scor: fen dominated by *Hamatocaulis vernicosus*, Sph: fen dominated by *Sphagnum palustre*, Moor with *Erica tetralix* and *Sphagnum palustre*) between June 16 and July 31 (2011; left), and November 1 and December 16 (2011; right). The vertical white lines indicate the initiation and end of the treatment period. For interpolation, ordinary kriging was applied in ArcGIS (ArcMap 10.0, ESRI, Redlands, USA).

served. Redox potentials (E_h) decreased almost immediately in Sph- and Moor-soils from about +600 to -100 mV in the upper 12 and 18 cm of the soils (Figure 4.6). On the other hand, E_h was only slightly affected in Scor-soils, because nearly the entire profile already showed anaerobic conditions before inundation, with E_h values of around -200 mV. In these soils, E_h only changed slowly from around 300 to -200 mV in the upper 2 cm of the soil. In contrast, porewater alkalinities and Ca-concentrations only increased significantly in Scor- and Call-vegetation, with 350 μmol$_c$ L^{-1} and 150 μmol L^{-1}, and remained equal in Sph- and Moor-vegetation, as indicated by the interaction vegetation type*year (alkalinity: $F_{9,8.4}$=7.8**, Ca:

Table 4.2 Effect of water table on Cl-infiltration into soil porewater of the KW-fen during inundations in 2009, 2010 and 2011 (experiment 4). Data shown are mean differences between the Cl-concentrations just after and before the inundations, and their standard deviations. Different letters indicate significant differences between water level categories ($P < 0.05$).

Initial water table:	Above the surface	0-2 cm below surface	3-5 cm below surface	6-9 cm below surface	> 9 cm below surface
Cl (μmol L^{-1})	64 (81)^A	53 (134)^A	46 (111)^A	282 (167)^B	185 (107)^B

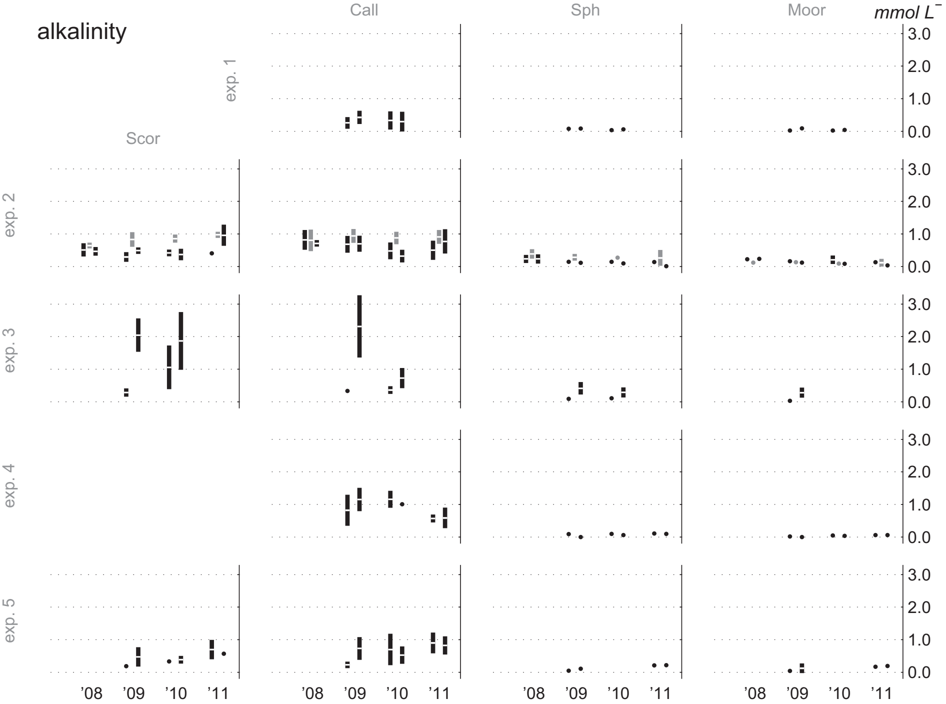

Figure 4.7 Effects of five surface water level treatments on the alkalinities (mmol$_c$ L^{-1}) in four vegetation types, as measured 2 days before (black lines at the left of each triplet), during (grey lines; only in experiment 2) and 2 days after the treatments (black lines at the left of each triplet). See the caption of Figure 4.2 for abbreviations. Sample means (white centres of a line) are shown with their standard deviations (*n* = 5). Statistical information is provided in Appendix B.

$F_{9,15.0}$=3.5*; Figs 4.7 and 4.8, and Appendix B.2). Finally, inundation in 2011 had no effect on Fe-, S-, o-PO_4, NH_4 and NO_3 concentrations in soil porewaters of any vegetation type (Appendix B.2 and C).

Experiment 3

Raised surface water levels in a non-floating fen during summer

Before the start of the treatment, water tables were significantly lower in July 2010 than July 2009 ($F_{2,16.0}$=290.0***; Figure 4.3 and Appendix B.2), with tables of 20 – 30 cm below the surface in 2010 (when the treatment was preceded by a very dry period) and tables of 3 – 20 cm below the surface in 2009. Rather heavy rainfall of 10 – 20 mm day-1 during the first treatment week of 2009 and 2010 led to a rise of water tables by 10 – 15 cm. In 2009, this rise resulted in inundation with surface water in Scor- and Call-vegetation, while lower initial water levels in 2010 prevented inundations.

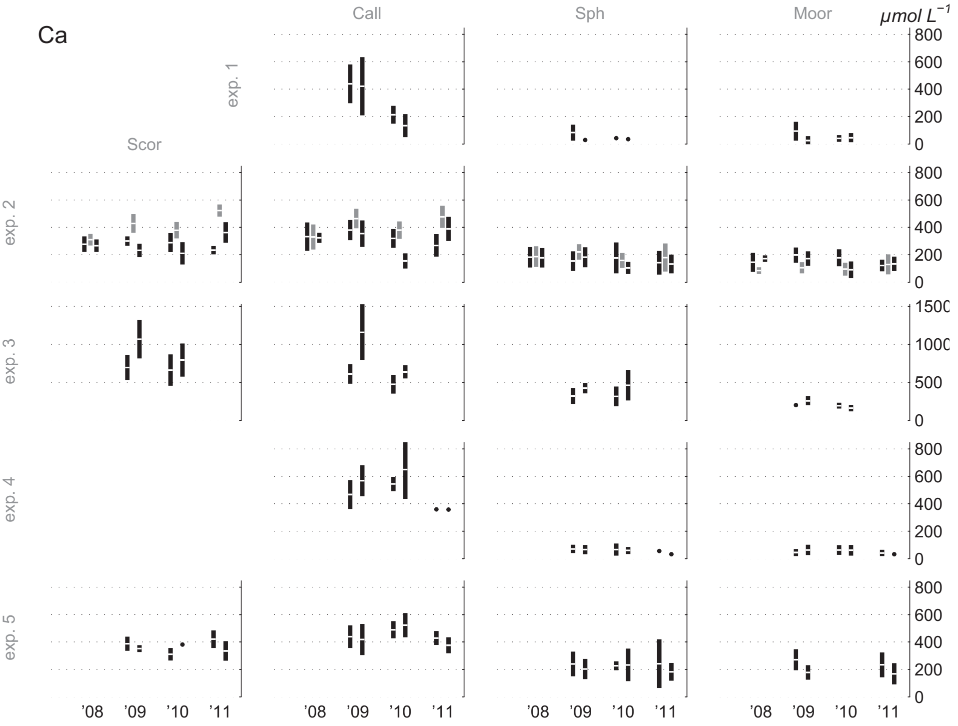

Figure 4.8 Effects of five surface water level treatments on the Ca-concentrations (μmol L^{-1}) in four vegetation types, as measured 2 days before (black lines at the left of each triplet), during (grey lines; only in experiment 2) and 2 days after the treatments (black lines at the left of each triplet). Sample means (white centres of a line) are shown with their standard deviations (*n* = 5). See the caption of Figure 4.2 for abbreviations. Statistical information is provided in Appendix B.

The raised surface water levels in 2009 and 2010 had no visual flooding effect on vegetation, and no effect on pH or o-PO_4, NH_4 and NO_3 concentrations in soil porewaters, but alkalinities and Ca-concentrations increased (Appendix B.2 and C). These effects were stronger in 2009 than in 2010 and differed between vegetation types, as indicated by the interaction vegetation type*year (alkalinity: $F_{3,10.9}$=4.0*, Ca: $F_{3,12.8}$=3.9*; Figs 4.7 and 4.8). In 2009, alkalinities and Ca-concentrations increased more (about 1900 μmol_c L^{-1} and 450 μmol L^{-1}) in the inundated Scor- and Call-vegetation than in the non-inundated Sph- and Moor-vegetation (about 300 μmol_c L^{-1} and 80 μmol L^{-1}). Non-inundated Scor- and Call-vegetation in 2010 showed significantly smaller increases in alkalinities (about 600 μmol_c L^{-1}) and Ca-concentrations (about 150 μmol L^{-1}) than in 2009, while Sph- and Moor-vegetation showed similar increases in alkalinities (about 250 μmol_c L^{-1}) and Ca-concentrations (about 80 µmol L^{-1}). Furthermore, Cl-concentrations increased by about 300 µmol L^{-1} in soil porewaters of all vegetation types during the non-inundated situation in 2010, but remained unaltered in 2009.

In 2009 and 2010, raised surface water levels led to decreased S-concentrations and increased Fe-concentrations in soil porewaters of all vegetation types (Appendix B.2 and C). In all vegetation types, S-concentrations decreased with 50 – 150 µmol L^{-1} in both years, while Fe-concentrations increased significantly more in 2009 than 2010 ($F_{1,13.7}$=25.6***), with 50 – 115 µmol L^{-1} and 15 – 25 µmol L^{-1}, respectively.

Experiment 4

Lowered surface water levels in a floating fen during summer

Two weeks of lowered surface water levels (-15 cm) had no clear effect on the water tables in the floating soils (Figure 4.3 and Appendix B.2), nor on vegetation. In July 2010 and July 2011 water tables were hardly affected, which may be attributed to a precipitation surplus of 1.0 – 1.5 mm day^{-1} at the end of the treatment period (weather station Marknesse: KNMI, 2014). In July 2009, when there was an evaporation surplus of about 2.5 mm day^{-1}, water tables lowered in all soils upon treatment ($F_{2,9.9}$=15.9**), but only 4 cm. In addition, there were no biogeochemical changes in any of the vegetation types (Appendix B.2 and C), except that porewater Cl- and S-concentrations decreased in 2011 (Cl: $F_{2,7.6}$=17.1**, S: $F_{2,7.5}$=5.2*), presumably due to dilution with rainwater.

Experiment 5

Lowered surface water levels in a non-floating fen during summer

Before the start of the treatments, water tables clearly differed among the 3 years ($F_{2,15.8}$=209.5***; Figure 4.3 and Appendix B.2), with lowest levels of 20 – 30 cm below the surface in 2010 (when the treatment was preceded by a very dry period) and significantly higher levels in 2009 (0 to -25 cm) and 2011 (+5 to -10 cm). In 2011, most Scor-vegetation was already inundated at the start of the treatment.

Lowering of surface water levels by 15 cm only led to lower field water tables in July 2011 ($F_{2,16.0}$=167.0***; Figure 4.3 and Appendix B.2), while water tables in the fens raised in July 2009 and 2010 due to heavy rainfall. These raised water tables in 2009 and 2010 had no effect on pH and Fe-, o-PO_4, NH_4 and NO_3 concentrations in soil porewaters (Appendix B.2 and C). The inundated locations with Scor- and Call-vegetation did, however, show significantly increased alkalinities (about 500 $µmol_c$ L^{-1}) in their soil porewaters in 2009, as indicated by the interaction effect vegetation type*year ($F_{5,14.4}$=3.1*; Figure 4.7), while Ca-concentrations did not change (Figure 4.8) and Cl-concentrations even decreased during this treatment (Figure 4.5). There were no visual effects of flooding on vegetation survival.

Although surface water levels were also raised by 4 – 6 cm after the treatment in July 2011 due to 2 days of rainfall (about 25 mm day^{-1}) after the end of the treatment, the lowered surface water levels did still lead to lower water tables during the treatment (Figure 4.3 and Appendix B.2). Water tables decreased by 10 – 15 cm in all vegetation types, leading to an increase of the redox potential (E_h) from around -200 to +500 mV in the upper 5 cm of Scor-soils (Figure 4.6). In contrast, E_h did not change in the upper 10 cm of Sph- and Moor-soils, since the initial E_h was already above +600 mV. However, none of the vegetation types showed significant changes in pH, alkalinity, Ca-concentrations or nutrient concentrations (Appendix B.2 and C). E_h decreased immediately in all vegetation types upon the 2 days of rainfall after the end of the treatment.

4.4. Discussion

Water table dynamics in floating fens hardly depend on surface water levels

As hypothesized, fluctuations in surface water levels had almost no effect on water tables in floating fens dominated by *Calliergonella* or *Sphagnum*, since the buoyant peat followed the surface water levels. This was not only the case during short-term experiments of 2 weeks, but also occurred during a similar surface water level rise of 3 months (field observation C. Cusell). As a result of the limited change in water tables, ANC and nutrient concentrations in soil porewaters did not change during the field experiments, not even after 3 months of lowered or raised surface water levels (Cusell et al., 2013b).

It has, however, been reported that lowered surface water levels can still lead to lower water tables in floating fens, especially when soil thickness increases (e.g. van Wirdum, 1993). Similarly, it has also been shown that raised surface water levels may lead to inundations in floating fens (O'Connell, 1981; Koerselman, 1989; van Wirdum, 1991), especially on rich fens with *Scorpidium* species (Cusell et al., 2013b). Such rich fens are usually located at or below, instead of clearly above the water table. Although there is still debate about the origin of this water above soil surface, which may be seepage of water from beneath the floating mat (van Wirdum, 1991) or flooding by surface water (Cusell et al., 2013b), it is clear that floating rich fens may get inundated when surface water levels get sufficiently high. The absence of inundation in the floating fens we studied may thus mainly be caused by the limited water level rise of only 10 cm and their high buoyancies, but may also reflect the absence of rich fens with *Scorpidium* species in the floating fens that were studied.

Short periods of lowered surface water levels do not lead to acidification or eutrophication

Non-floating fens did not respond uniformly to surface water level draw-downs of 2 weeks in summer, due to weather conditions. Water tables only dropped during an evapotranspiration surplus. Under these conditions, water tables dropped 10 – 15 cm in non-floating fens, while levels only dropped 4–6 cm in floating fens. The lowered water tables did not affect redox potentials (E_h) in the upper soil layers of non-floating fens with Sph- and Moor-vegetation, which were already above +600 mV. In contrast, a strong increase of E_h (from -200 to +500 mV) upon water level draw-down was found in the upper 5 cm of Scor-soils, indicating oxygen intrusion into these soils (e.g. Gambrell and Patrick, 1978). This short-term intrusion of oxygen did not lead to acidification or eutrophication, but it is well known that longer episodes of drought can stimulate net mineralization rates (Grootjans et al., 1986; Bridgham et al., 1998; Olde Venterink et al., 2002; Chapter 2) and acidification by aerobic oxidation processes (Lamers et al., 1998a; Lucassen et al., 2002; Chapter 2).

Inundation of non-floating fens

In non-floating fens, high water tables clearly led to inundation due to fixation of these fens to the sandy substrate, as is supported by diver data (Cusell et al., 2013b). High Cl-concentrations in inundation waters compared to soil porewaters showed that the inundation water originated from the adjacent ditches. Plots with highest water tables before the treatment, which were often dominated by *Scorpidium* species or *H. vernicosus*, showed the largest rise in water tables during inundation. This is presumably because they were situated in depressions that were 5 – 10 cm lower than the surface of *Sphagnum*-dominated vegetation, where water tables often bulge somewhat.

Effect of winter inundation on the ANC depends on infiltration rates

The absence of change in Cl-concentrations in soil porewaters during most winter inundations, despite higher Cl-concentrations in the inundation water, indicated that there was hardly any infiltration into the waterlogged soils. Infiltration only occurred where initial water tables were lower than 5 cm below soil surface. This is in accordance with Hooijer (1996) and Banach et al. (2009), who also found limited infiltration of inundation water in waterlogged riverine floodplain fens.

In the case in which two weeks of inundation during winter had no effect on the infiltration of HCO_3, Ca and Mg (ANC input), anaerobic reduction rates in the peat (internal ANC generation) did not occur either. However, longer-term inundations during winter can lead to both forms of ANC-increase in waterlogged rich fen soils, as demonstrated in a mesocosm experiment (Cusell et al., 2013a).

Two weeks of winter inundation in 2011 did lead to an increase in alkalinity and

Ca-concentration in soil porewater by 50–100%, but only in Scor- and Call-soils. Since alkalinity and Ca-concentration increased at a ratio of 2:1 in these plots, we attribute this increase only to infiltration of Ca- and HCO_3-rich inundation water, and not to anaerobic microbial reduction processes. Despite E_h values below -200 mV in Scor-vegetation, at which Fe(III)- and SO_4-reduction may lead to internal alkalinization (e.g. Ponnamperuma, 1984), unchanged Fe- and SO_4-concentrations in soil porewaters support the idea of limited alkalinity generation. This is most probably caused by the low temperatures and subsequent low microbial activity in winter (Loeb et al., 2008a,b).

For Sph- and Moor-vegetation, inundation led to an immediate decrease of E_h from +600mV to -100mV, but these anaerobic conditions did not result in internal alkalinity generation. Unlike in Scor-vegetation, infiltration of inundation water did not lead to an increase of alkalinities and Ca-concentrations in soil porewaters, which was related to lower alkalinities and Ca-concentrations in the water layer above Sph- and Moor-vegetation compared to Scor- and Call-vegetation. This striking difference in inundation water composition at a relatively short distance (10–20 m) can only be explained by the exchange of Ca^{2+} for H^+ between inundation water and the adsorption complex of living mosses and their peat. This exchange process has also been described for non-inundated conditions in *Sphagnum*-dominated fens (Clymo, 1963; Kooijman and Bakker, 1994). Acidification (alkalinity consumption) of inundation water may have mainly occurred at *Sphagnum*-dominated sites, because adsorption complexes of *Scorpidium* spp. and *C. cuspidata* were probably already saturated with Ca before inundation, while those of *Sphagnum* spp. often contain high concentrations of H^+.

Effect of inundation on the ANC depends on season

The increase of alkalinities and Ca-concentrations in soil porewaters upon 2 weeks of inundation of non-floating fens with Scor- and Call-vegetation was much stronger in summer than in winter. This clear seasonal difference may be explained by higher evapotranspiration in summer, which facilitates the infiltration of base-rich inundation water (Cusell et al., 2013a).

Since alkalinity and Ca-concentration increased at a ratio of 4:1 instead of 2:1, the increase in alkalinity in summer is presumably not only caused by the infiltration of base-rich inundation water, but also by other processes, i.e. evaporative concentration and microbial alkalinity generation. As long as water tables became sufficiently high (between 1 and 10 cm below the surface) in summer, raised surface water levels even led to increased alkalinities, Ca- and Cl-concentrations under non-inundated conditions. These increases cannot be caused by infiltration and are probably due to evaporative concentration. In addition, alkalinity production may have been higher during inundations in summer due to increased microbial alkalinity generation in the warmer anoxic peat soil. The simultaneous decrease in SO_4-concentrations (SO_4-reduction) and increase in Fe-concentrations (mobilization of Fe(II)) support this theory.

Short inundations with P-poor water do not lead to P-eutrophication

P-eutrophication did not occur in any of the experiments. It must, however, be noted that the inundation water contained very low o-PO_4 concentrations of 0.05 μmol L^{-1}. Other studies showed that inundation with P-rich surface water may well lead to P-eutrophication in fens (e.g. Wassen et al., 1996). Although P-uptake by vegetation may somewhat mask P-eutrophication, especially in summer, our field experiments also showed no evidence of increased internal P-mobilization in waterlogged soils upon 2 weeks of inundation. Several other experiments have, however, shown that prolonged inundation may well lead to internal P-mobilization in waterlogged soils (Patrick and Khalid, 1974; Loeb et al., 2008b), especially in P-rich fens (Cusell et al., 2013a), with SO_4-rich inundation water (Lamers et al., 1998b) and at higher temperatures (Cabezas et al., 2013). In the present study, the duration of two weeks seems to be sufficiently short and P-concentrations in flooding water seem to be sufficiently low to prevent P-eutrophication. However, P-eutrophication can certainly occur during flooding when soil quality and/or surface water quality are insufficient.

Implications for fen management

Rich fens, comprising many threatened vascular plants and bryophytes, can only persist under well-buffered and nutrient-poor conditions (e.g. Sjörs, 1950; Wheeler and Proctor, 2000; Kooijman and Paulissen, 2006). In wetlands with fixed surface water levels, the re-introduction of fluctuating levels has recently been proposed to restore the ANC and to reduce P-eutrophication, in order to conserve or restore fen biodiversity.

We did not see any direct effects of the experimental raising or lowering of the water table on vegetation. Our large-scale field experiments suggest that 2 weeks of raised surface water levels (+10 cm) may counteract acidification of base-rich fens by increasing the ANC, but only under specific conditions. A rise in surface water levels should lead to actual inundation, which was not the case for the floating *Sphagnum*-dominated fens studied. In contrast, in non-floating fens, 2 weeks of raised surface water levels did lead to inundation. The ANC, however, only increased when base-rich inundation water actually infiltrated into the soil. In winter, this only occurred when initial water tables were lower than 5 cm below the surface. In summer, infiltration was facilitated by higher evapotranspiration, as supported by the results of long-term mesocosm experiments (Cusell et al., 2013a). Furthermore, higher temperatures in summer led to internal alkalinity generation in non-floating fens as a result of Fe(III)- and SO_4-reduction, although this effect may be temporary since aerobic oxidation during subsequent droughts can lead to acidification (Lamers et al., 1998a; Loeb et al., 2008a).

Two weeks of lowered surface water levels (-10 cm) did not have severe acidifying or eutrophying effects. However, longer periods of drought can stimulate net mineralization and acidification, and are therefore not recommended.

Suggestions for further research

The experiments in this study were of relatively short duration. In order to get a better understanding of long-term effects, future experiments during multiple weeks are suggested, focusing both on drought and inundation. These experiments should preferably be conducted under different meteorological conditions. The analysis of such measured data can be useful toward determining an essential and fundamental set of peatland processes and feedbacks (Waddington et al., 2014).

In addition, additional field manipulation experiments during fall, summer and spring are needed to gain a better understanding of the impact during different seasons. Natural extreme events, such as droughts, are generally associated with increased temperatures, altering kinetics of biogeochemical reactions or decomposition rates as drivers of dissolved nitrogen turnover in fens (Cabezas et al., 2012). But more importantly from a management perspective, inundations with base-rich water may be much more effective in summer than in winter to restore the ANC. High temperatures are expected to result in accelerated evapotranspiration and enhanced infiltration of inundation water, but also in accelerated microbial alkalinity generation (Cusell et al., 2013a).

The absence of inundation in the floating fens we studied may be related to the limited water level rise of only 10 cm. Additional experiments with a larger rise in water level would reveal to what extent the buoyancy of floating fens is still limiting the influence of fluctuating water levels.

4.5. Conclusions

Short-term inundations can be profitable for rich fens as long as surface waters are nutrient-poor and infiltration does occur. This management tool is most suitable for non-floating fens and may best be applied in summer. Short-term periods with intense precipitation, which are very likely to occur more frequently in the future due to climate change, can thus have a positive effect on rich fens, especially in summer. Severe drought periods will, on the other hand, have negative direct and indirect (biogeochemical) effects on vegetation.

Acknowledgements

We wish to thank Anna Pommer, Filippo Fernandez, Richard Kanbier and Tanja Mook-Cusell for their help in the field, and Ton van Wijk, Piet Wartenbergh, Bert de Leeuw, Leen de Lange and Leo Hoitinga for analytical assistance. Natuurmonumenten, State Forestry (SBB) and Water Management Authority Reest & Wieden are acknowledged for allowing the experiments in their reserves and carrying out measures in the experimental areas. This study was funded by Water Management Authority Reest & Wieden, the Province of Overijssel and the Dutch Ministry of Economic Affairs, Agriculture and Innovation as a part of the Research Programme 'Ontwikkeling + Beheer Natuurkwaliteit' (OBN).

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CHAPTER 5

Short-term summer inundations as a measure to counteract acidification in rich fens

Ivan S. Mettrop, Casper Cusell, Annemieke M. Kooijman, Leon P.M. Lamers

Abstract

In regions with intensive agriculture, water level fluctuation in wetlands has generally become constricted within narrow limits. Water authorities are, however, considering the re-establishment of a more natural water level regime as a management tool in rich fens. This includes temporary inundation with surface water from canals and ditches, which may play an important role in counteracting acidification in order to conserve and restore biodiversity. Inundation may result in an increased acid neutralizing capacity (ANC) for two reasons: infiltration of base-rich inundation water into peat soils, and microbial alkalinity generation under anaerobic conditions. The main objectives of this study were to test whether short-term (2 weeks) summer inundation is more effective than short-term winter inundation to restore the ANC in the upper 10 cm of non-floating peat soils, and to explain potential differences. Large-scale field experiments were conducted for five years in base-rich fens and *Sphagnum*-dominated poor fens.

As demonstrated in Chapter 4, winter inundation did not result in an increase of porewater ANC. Infiltration of inundation water did not occur, because the peat was already waterlogged before inundation and evapotranspiration rates are relatively low. Also, low temperatures limited microbial alkalinity generation. In summer, however, when temperature and evapotranspiration rates were higher, inundation did result in increased porewater Ca- and HCO_3-concentrations, but only in areas with characteristic rich fen bryophytes. This increase was not only due to stronger infiltration into the soil, but also to higher microbial alkalinity generation under anaerobic conditions. In contrast, porewater ANC did not increase in plots dominated by *Sphagnum* spp. as a result of the ability of *Sphagnum* to actively acidify its environment. In both rich and poor fens, flooding-induced P-mobilization was not a reason for concern, since porewater o-PO_4 concentrations remained sufficiently low to safeguard P-limited vegetation. NO_3^- and NH_4^+ dynamics showed no considerable changes either.

In conclusion, short-term summer inundation with base-rich and nutrient-poor surface water is considered beneficial in the management of non-floating rich fens, and much more effective than winter inundation.

5.1. Introduction

Rich fens are minerotrophic peatland habitats that are characterised by base-rich and nutrient-poor conditions (Wheeler and Proctor, 2000). In Europe, these biodiverse rich fens have become very rare, mainly due to changes in land use, acidification and eutrophication (Lamers et al., 2015), and are therefore protected as EU priority habitat *H7140 - Transition mires and quaking bogs*. In terms of conservation and restoration of rich fens, conditions must be base-rich and nutrient-poor to prevent transformation of these species-rich communities to species-poor *Sphagnum*-dominated communities (Kooijman, 1992).

The cause of the problem of acidification lies in several processes. Hydrological isolation from base-rich groundwater and surface water, caused by natural succession and/or anthropogenic intervention, has led to reduced acid neutralizing capacity (ANC) in fen peatland regions with intensive agriculture (e.g. van Wirdum, 1991; Van Diggelen, 1996). Presumably, increased atmospheric N-deposition as a result of fossil fuel combustion and intensive cattle farming has exacerbated the acidification of fens due to direct influx of nitric acid, and indirectly by additional ammonium oxidation during periods of drought (Gorham et al., 1987; Kooijman, 2012). In addition, P-eutrophication may lead to rapid succession in rich fens, and hence a shift from minerotrophic bryophytes to *Sphagnum* spp. (Kooijman and Paulissen, 2006). Since *Sphagnum* spp. release protons in exchange for other cations (Clymo, 1963; Kooijman and Bakker, 1994), acidification of the bryophyte layer is intensified by P-eutrophication.

During the past decades, water levels in European rich fen areas have often been constricted within narrow limits as a result of adjacent agricultural water management. Water authorities are, however, considering the re-establishment of a more natural water regime in these areas, in which temporary inundation with surface water may play an important role in counteracting acidification. Short-term inundation with base-rich water has been postulated as a measure to restore the ANC in the top-soil of fens that lack sufficient HCO_3 and Ca buffering (Cusell et al., 2013a). Several studies have focused on potential benefits and disadvantages of raised water levels in fens. Winter inundation by raising surface water levels in the field did not result in enhanced ANC in non-floating rich fens (Chapter 4). However, during an unexpected inundation period in summer in these fens, alkalinization did increase, which suggested that inundation may be more effective in summer than in winter (Chapter 4). Two explanatory mechanisms were proposed (Cusell et al., 2013b). The primary explanation lies in infiltration of HCO_3- and Ca-rich inundation water into the peat soil. This is important, since especially Ca-input may contribute to a more permanent increase in ANC, as this is not only determined by the concentration of bicarbonate in porewater, but also by the amount of Ca attached to the adsorption complex (Stumm and Morgan, 1996). In winter, these infiltration rates may be limited in the already waterlogged soils. In summer, when temperatures are higher, infiltration of base-rich inundation water is presumably facilitated by high evapotranspiration rates (Chapter 4). In addition, porewater ANC may increase under anaerobic conditions by reduction of NO_3, Fe(III) and/or SO_4, which are processes that lead to microbial alkalinity generation (Baker et al., 1986; Stumm and Morgan, 1996). This microbial alkalinity production may be higher in summer as well, since increased temperatures generally promote microbial activity in anaerobic peat soils. In contrast to Ca supply via infiltration, the effect of internal alkalinization may be temporary, because subsequent aeration of the peat soil may lead to oxidation-induced acidification (Chapter 2; Chapter 3).

Besides the positive effects of inundation with base-rich water on the ANC of fen soils, also potential adverse effects have to be taken into account. Anaerobic conditions may result in net P-mobilization (internal eutrophication) due to Fe reduction (Patrick and Khalid, 1974), potentially further increased by SO_4 reduction (Lamers et al., 1998a), and hence increased P-availability (Zak et al., 2010; Cusell et al., 2013b). In addition, anaerobic conditions may lead to formation of potential phytotoxins such as NH_4^+, H_2S, Fe^{2+} and/or organic acids (Lamers et al., 2015). All of these potential adverse effects may be stimulated by inundation and need to be assessed as well.

The main objective of this study was to assess the effectiveness of short-term (2 weeks) summer inundation versus short-term winter inundation to restore ANC in the upper 10 cm of non-floating peat soils. Large-scale field experiments were conducted for several years in base-rich fens and *Sphagnum*-dominated poor fens. We expected short-term inundation with base-rich water to be much more effective in summer than in winter, primarily because relatively high summer temperatures may result in accelerated infiltration of base-rich inundation water, and additionally in accelerated microbial alkalinity generation. Presumably, improvement of the ANC is stronger in base-rich fens than in *Sphagnum*-dominated fens, due to the ability of *Sphagnum* to acidify its environment (Clymo, 1963; Kooijman and Bakker, 1994; Chapter 4). Furthermore, we expected limited internal P-mobilization and limited formation of toxins during short-term inundation of two weeks under summer conditions, based on results from earlier field studies in the same area (Chapter 4) and laboratory mesocosm experiments involving soils from these fen sites (Cusell et al., 2013b).

The results of this study are important in relation to the conservation and restoration of endangered rich fen habitats. Increased understanding of biogeochemical processes upon inundation is essential to support water and nature management authorities in environmental decision making, as it explains under which particular conditions restoration measures are expected to be successful. In addition, our results have important additional implications for future management in the face of climate change, since short-term extreme weather events, such as summer flooding, are predicted to occur more frequently (e.g. Bronstert, 2003; Kundzewicz et al., 2006).

5.2. Material and methods

Field site and experimental setup

The field experiments were conducted in two non-floating fens in the Dutch National Park Weerribben-Wieden: 'Kiersche Wiede' (KW; 52°41'49.1"N 6°07'56.7"E) and 'Veldweg' (VW; 52°41'30"N 6°06'45"E). Both fens comprised three vegetation types: (1) labelled 'Scor': rich fens with respectively *Hamatocaulis vernicosus* (Mitt.) Hedenäs (*Caricion nigrae – Carex nigra-Agrostis canina* type) in KW and *Scorpidium cossonii* (Schimp.) Hedenäs (*Caricion davallianae – Scorpidium-Carex diandra* type) in VW, (2) 'Call': rich fens with *Calliergonella cuspidata* (Hedw.) Loeske dominating the moss layer (*Caricion nigrae – Carex nigra-Agrostis canina* type), and (3) 'Sph': poor fens with *Sphagnum palustre* L. and *Sphagnum fallax* (H.) Klinggr. dominating the moss layer (*Caricion nigrae – Pallavicinio-Sphagnetum typicum* type).

An isolated part (9000 m^2) of the KW-fen was chosen as experimental site with raised surface water levels, while part of the VW-fen (9375 m^2) was chosen as a reference site in which the water level remained unchanged. Generally, surface water levels in the area are constricted within 0.73 and 0.83 m below mean sea level (BMSL). Short-term inundation of the fen surface in the KW-fen was achieved by raising the surface water level up to 0.63 BMSL during 14 days by using a pump. This meant a raise of 10 cm in November 2009, 2010 and 2011, and 15 cm in July-August 2013 and 2014. In both fens, the selected plots were located within a maximum distance of 50 m from adjacent ditches, and none of the fens was floating due to root attachment to the sand substrate at a depth of 60-90 cm.

Sampling and analyses

For each vegetation type (Scor, Call and Sph), five plots were selected in both KW and VW (n_{tot}=30). All measurements were carried out (a) 2 days before, (b) halfway during, and (c) 2 days after experimental manipulation of the surface water level. Water tables in the fen soils were manually recorded. Porewater samples from the upper 10 cm of the peat soils and from surface water in adjacent ditches were collected by using ceramic soil moisture samplers (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment, the Netherlands), connected to vacuumed plastic syringes of 50 mL. After 1 week of inundation, additional samples of the inundation water were collected, also by using these ceramic soil moisture samplers.

pH-values were measured with a standard Ag/AgCl electrode and alkalinity was determined by titration down to pH 4.2 using 0.01 mol L^{-1} HCl. Concentrations of dissolved o-PO_4, NO_3, NH_4, SO_4, Cl and dissolved organic matter (DOC) were measured by using auto-analyzer (Skalar, San^{++} System, fitted with Skalar, SA1074). Total concentrations of dissolved Ca, Fe, and S were measured in acidified subsamples by ICP (Perkin-Elmer, Optima 3000XL).

Ca and Cl-concentrations were used to calculate the ionic ratio (IR), which is equal to 2*[Ca]/(2*[Ca]+[Cl]. This IR index can be used as an indicator of the relative influence of groundwater and/or surface water versus rainwater in porewaters (van Wirdum, 1991). Further, porewater ratios of [alkalinity]/[Cl] and [Ca]/[Cl] were used as indicators of infiltration, because of the suitability of Cl as an inert tracer.

Continuous redox measurements

In all vegetation types, the redox potential (E_h) in the upper 20 cm was measured during summer inundation in 2013 and 2014, to assess the extent to which oxygen availability was affected. The E_h was measured in Call-vegetation during summer inundation in 2013, and in Scor- and Sph-vegetation during summer inundation in 2014. Permanently installed fiberglass probes with platinum sensor tips at different heights (PaleoTerra, Amsterdam, the Netherlands), connected to a Hypnos data logger (MVH Consult, Leiden, the Netherlands; Vorenhout et al., 2011) were used to record E_m (measured potential) at -1 cm, -3 cm, -5 cm, -10 cm, -15 cm, and -20 cm below the soil surface every 15 minutes. Em was measured as the potential between a sensor tip and a 3M Ag/AgCl reference probe. The E_h was calculated by adding a standard reference voltage and correcting for differences in pH, since pH indirectly modifies the Nernstian effect of the redox electrode:

$E_h = E_m + E_{ref} – 59*(7–pH)$, with E_{ref} being the potential of the reference probe.

Statistical analysis

Initial differences in water tables and porewater chemistry were tested by a linear mixed model with least significant difference (LSD) post-hoc analyses, using location (KW vs. VW), vegetation type (Scor, Call and Sph) and season (winter vs. summer) as three fixed factors. Initial differences in surface water chemistry were tested with location and season as fixed factors. Since subreplicates were taken consecutively over the years from the same plots, the model was run with 'AR(1): Heterogeneous' as residual repeated covariance structure, with year as repeated effect. For all analyses yearly initial values, as measured 2 days before the experiment, were used.

Also for the treatment results a linear mixed model, with year as repeated effect, was used to test the response to three main fixed factors (1) water level treatment: inundation in KW vs. reference in VW, (2) season: winter vs. summer, and (3) vegetation type: Scor, Call and Sph. Differences between measurements before and after surface water level manipulation were used as response variables. In addition, differences in inundation water characteristics in KW were tested, using vegetation type and season as fixed factors. Differences in response between the three vegetation types were further tested by LSD post-hoc analyses.

All statistical analyses were performed using SPSS 20.0 for Windows (IBM Inc., 2011). *P*-values in the text are indicated as follows: $*P<0.05$, $**P<0.01$.

5.3. Results

Initial conditions

Surface water characteristics

The surface water quality in adjacent ditches differed between the experimental site KW and the reference site VW. Surface water in VW was, with an average Ca concentration of 1200 µmol L^{-1} and an alkalinity of around 2.5 $mmol_c$ L^{-1}, twice as base-rich as surface water in KW with average Ca concentrations of 630 µmol L^{-1} and an alkalinity of around 1.2 $mmol_c$ L^{-1} ($F_{1,7}=40.74**$ and $F_{1,6}=24.4**$). Neither Ca concentrations, nor alkalinities in surface water differed between seasons ($F_{1,7}=0.8^{NS}$ and $F_{1,9}=3.39^{NS}$). Surface water o-PO_4 concentrations did not differ between locations ($F_{1,36}=0.25^{NS}$) and were slightly increased in summer ($F_{1,38}=68.54**$), but still relatively low with values below 1.0 µmol L^{-1}. Surface water NO_3 concentrations were higher in summer than in winter ($F_{1,43}=44.73^{NS}$) and this summer-induced increase was stronger in VW than in KW, as indicated by a significant interaction of location*season ($F_{1,43}=18.24**$). However, NO_3 concentrations remained relatively low with average values under 10 µmol L^{-1}. Also NH_4 concentrations turned out to be higher in summer with average concentrations of 13.5 µmol L^{-1} versus 4.0 µmol L^{-1} in winter ($F_{1,44}=85.69**$), with no difference between the two locations ($F_{1,41}=0.47^{NS}$).

Soil porewater characteristics

Initial soil porewater Ca-concentrations, alkalinities and pH were generally lower in KW than in VW (Table 5.1). This differences are more obvious in the Scor- and Call-plots than in the Sph-plots, as indicated by significant interaction of location*vegetation type (Table 5.2). However, the ionic ratio (IR) did not differ significantly between the two fen sites, suggesting that the relative influence of base-rich surface water did not differ between KW and VW. Furthermore, water tables in the soil at $T=0$ did not differ between KW and VW, and overall nutrient concentrations were relatively low in both sites.

Table 5.1 Initial water tables and porewater characteristics for the different areas and vegetation types for combined seasons. Data shown represent mean values with S.E. (n = 25). Scor = fen dominated by *Scorpidium cossonii* or *Hamatocaulis vernicosus*, Call = fen dominated by *Calliergonella cuspidata*, Sph = fen dominated by *Sphagnum palustre*, Ditch = surface water in adjacent ditch. KW = Kiersche Wiede (experimental fen site), VW = Veldweg (reference fen site). IR (Ionic Ratio) = 2*[Ca]/(2*[Ca]+[Cl]).

	Scor		Call		Sph		Ditch	
Variable	KW	VW	KW	VW	KW	VW	KW	VW
Water table (cm)	-0.4 (1.0)	-1.1 (1.4)	-3.3 (0.8)	-5.1 (1.0)	-8.5 (1.0)	-10.9 (1.0)	-	-
Cl (μmol L^{-1})	480 (31)	752 (57)	427 (35)	617 (63)	276 (24)	487 (51)	664 (53)	895 (58)
IR (mol mol^{-1})	0.57 (0.01)	0.65 (0.02)	0.63 (0.02)	0.65 (0.02)	0.53 (0.03)	0.52 (0.02)	0.65 (53)	0.71 (0.01)
Ca (μmol L^{-1})	315 (15)	723 (63)	358 (20)	509 (35)	163 (15)	230 (16)	583 (45)	1123 (85)
Alkalinity (μmol_c L^{-1})	409 (33)	1280 (146)	567 (57)	789 (67)	115 (16)	235 (37)	1160 (99)	2248 (199)
pH	5.7 (0.1)	6.3 (0.1)	5.9 (0.1)	6.0 (0.1)	4.9 (0.1)	5.4 (0.1)	7.0 (0.1)	7.4 (0.1)
Fe (μmol L^{-1})	18.7 (4.0)	14.7 (3.4)	21.1 (7.4)	13.9 (2.5)	39.6 (10.5)	35.1 (6.3)	2.1 (0.3)	3.3 (1.0)
S (μmol L^{-1})	71.1 (15.5)	31.2 (3.7)	80.9 (19.8)	74.3 (11.2)	47.1 (9.3)	43.1 (3.8)	143.7 (7.7)	154.5 (12.3)
o-PO_4 (μmol L^{-1})	0.51 (0.13)	0.59 (0.11)	0.64 (0.15)	0.75 (0.22)	1.00 (0.20)	1.51 (0.38)	0.44 (0.12)	0.77 (0.32)
NH_4 (μmol L^{-1})	2.72 (0.40)	4.77 (0.92)	4.49 (1.45)	4.28 (0.59)	5.93 (1.61)	5.09 (0.78)	7.19 (1.99)	8.71 (2.41)
NO_3 (μmol L^{-1})	1.77 (0.46)	3.71 (0.84)	1.18 (0.17)	3.38 (0.68)	1.72 (0.24)	3.93 (0.93)	2.41 (0.70)	14.32 (6.10)

The initial water tables in the peat soil differed among vegetation types (Table 5.1 and 5.2). Scor-plots were characterized by water tables less than 1 cm beneath the soil surface, while Call-plots showed an average water table of -4 cm. In Sph-plots the water table was even lower with an average level at -10 cm. Furthermore, the influence of base-rich surface water was higher in Scor- and Call-plots than in Sph-plots, as indicated by a higher porewater IR. Consequently, porewater in Scor- and Call-plots showed significantly high alkalinities of about 0.8 and 0.7 $mmol_c$ L^{-1}, and Ca-concentrations of about 500 and 400 μmol L^{-1}, while in Sph-plots initial alkalinities were about 0.2 $mmol_c$ L^{-1} and average Ca-concentrations did not exceed 200 μmol L^{-1}. As expected, average initial porewater pH values of 6.0 in both Scor- and Call-plots were also significantly higher than in Sph-plots, where pH values of about 5.1 were measured. In contrast, both Fe- and o-PO_4 concentrations in porewater were two times higher in Sph-plots than in Scor- and Call-plots.

While the initial water tables in the peatsoil did not differ between winter and summer, initial Cl, Ca, S, o-PO_4, NH_4, NO_3 and DOC concentrations in porewater were generally higher in summer. Particularly in VW the initial NO_3 concentrations were increased in summer, as indicated by a significant interaction of location*season (Table 5.2).

Table 5.2 Effects of location, season, vegetation type and their interactions on the water table and porewater chemistry at $T = 0$ of each yearly experiment. For abbreviations see Table 5.1. F-ratios including denominator d.f. in parentheses are shown with their level of significance: $*P < 0.05$, $**P < 0.01$. Different letters indicate significant differences ($P < 0.05$) between vegetation types. ns = not significant.

	Location	Season	Veg	Location*Season	Location*Veg	Season*Veg	Scor	Call	Sph
Water table	0.01 (43.3)	0.89 (106.0)	55.24** (43.4)	3.16 (106.0)	0.95 (38.4)	1.19 (106.0)	c	b	a
Cl	52.59** (38.6)	19.86** (43.4)	27.54** (38.6)	5.37* (43.4)	1.57 (49.5)	7.18* (43.4)	b	b	a
IR	3.29 (47.7)	16.90** (65.7)	16.91** (47.6)	1.32 (65.7)	2.82 (49.5)	2.82 (65.7)	b	b	a
Ca	47.05** (38.1)	10.07** (99.7)	39.42** (38.1)	0.13 (99.7)	8.17** (40.0)	2.90 (99.6)	b	b	a
Alkalinity	27.38** (39.2)	0.01 (112.9)	23.45** (39.3)	0.08 (112.7)	7.99** (38.6)	0.86 (112.7)	b	b	a
pH	44.02** (38.6)	3.01 (76.7)	95.34** (38.7)	1.21 (76.5)	4.64* (37.8)	2.41 (76.6)	b	b	a
Fe	2.39 (60.9)	3.11 (68.8)	11.80** (60.9)	3.65 (68.6)	2.30 (39.7)	2.04 (68.6)	a	a	b
S	19.03** (30.8)	46.86** (30.3)	3.81* (30.8)	7.35* (30.4)	3.80* (35.1)	0.69 (30.4)	ab	b	a
$o\text{-}PO_4$	0.62 (31.8)	87.54** (33.9)	7.28** (31.8)	0.99 (33.9)	0.95 (42.4)	3.00 (33.9)	a	a	b
NH_4	0.67 (36.5)	98.14** (39.0)	3.33 (36.5)	0.23 (39.0)	0.14 (49.7)	2.01 (39.0)	ns	ns	ns
NO_3	25.62** (35.9)	40.59** (39.1)	0.58 (35.6)	15.91** (38.9)	1.54 (43.7)	0.15 (38.9)	ns	ns	ns
DOC	2.62 (42.1)	38.02** (72.5)	11.31** (42.3)	3.14 (71.8)	2.85 (45.2)	0.04 (72.1)	a	a	b

Winter inundation vs. summer inundation

Inundation and infiltration

Raising of the surface water level in adjacent ditches in KW clearly affected the height of the water tables in the peat soil in all vegetation types (Figure 5.1A). Both in winter and in summer, the soil surface in all KW-plots became inundated via lateral flow from the ditches, while water tables in the reference location VW did not change. The height of the inundation water level relative to the surface level generally differed among vegetation types in KW, even though in all plots the peat layer was attached to the underlying sand substrate. In Sph-plots the layer of inundation water was two times less thick than in Scor- and Call-plots ($F_{1,21}=34.95**$), due to the relatively higher position in the landscape of the soil surface in *Sphagnum*-plots.

In the reference site VW without inundations, neither in winter nor in summer there were changes in porewater Cl, which was used as an inert tracer for the rate of infiltration. In the experimental site KW, winter inundations in 2009 and 2010 with Cl-rich surface water from the adjacent ditches did not result in increased Cl-concentrations in porewaters either (Figure 5.1B). This indicates that, despite obvious inundation in all plots, infiltration in the peat soil did not occur. Only in the winter of 2011, when initial water tables in the peat soil were relatively low with levels around -10 cm, porewater Cl-concentrations increased, pointing to actual infiltration of Cl-rich inundation water.

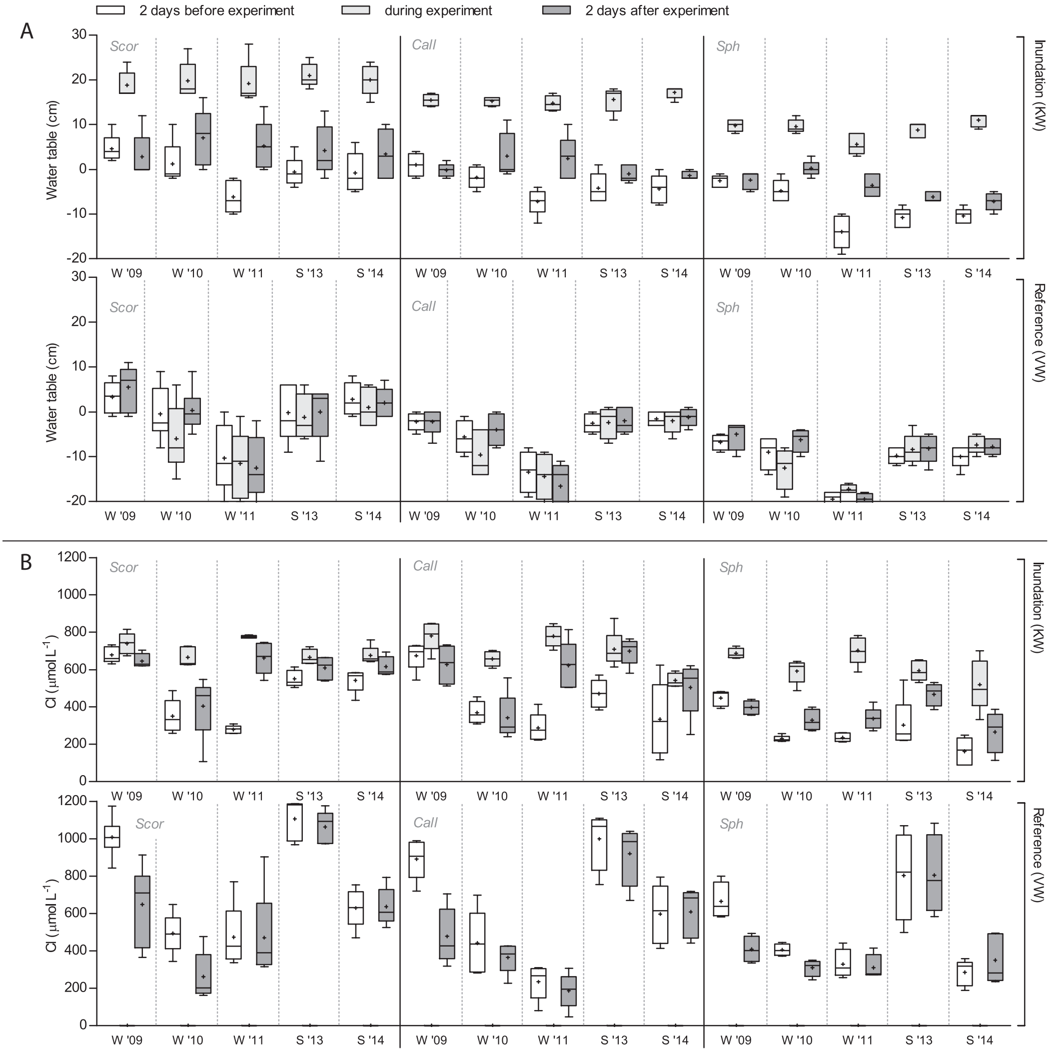

Figure 5.1 Water table (A) and Cl-concentrations (B) per vegetation type in porewater 2 days before the experiment, in inundation water during the experiment, and in porewater 2 days after the experiment. Sample means with standard deviations are indicated (*n* = 5). Statistical information is provided in Table 5.3. For abbreviations see Table 5.1.

In contrast, inundation during the summers of 2013 and 2014 resulted in a significant increase in both porewater Cl-concentrations and porewater IR, as indicated by an interaction of inundation*season (Table 5.3). Porewater Cl-concentrations approached 650 μmol L^{-1}, which was almost equal to the concentrations in the inundation water, and these changes did not differ among vegetation types. This implies

Table 5.3 Effects of inundation, season, vegetation type and their interactions on porewater chemistry during the experiments. For abbreviations see Table 5.1. *F*-ratios including denominator d.f. in parentheses are shown with their level of significance: *$P < 0.05$, **$P < 0.01$. Different letters indicate significant differences ($P < 0.05$) between vegetation types. ns = not significant.

	Location	Season	Veg	Scor	Call	Sph	Location*Season	Location*Veg	Season*Veg
Cl	99.07** (46.3)	42.70** (65.6)	2.10 (46.3)	ns	ns	ns	5.44* (65.7)	3.09 (41.1)	0.92 (65.6)
IR	10.30** (52.3)	0.70 (62.1)	1.01 (52.3)	ns	ns	ns	15.94** (62.1)	0.60 (55.3)	2.08 (62.1)
Ca	33.71** (44.3)	18.17** (93.6)	1.26 (44.3)	ns	ns	ns	3.65* (93.8)	3.53* (44.0)	2.42 (93.7)
Alkalinity	60.83** (45.9)	29.80** (83.5)	4.33* (46.0)	b	ab	a	4.05* (83.4)	16.89** (45.8)	3.30* (83.3)
pH	4.27 (46.8)	0.02 (57.1)	11.04** (46.8)	b	b	a	0.72 (57.0)	1.43 (41.3)	2.39 (57.1)
Fe	2.85 (39.3)	2.32 (43.6)	3.12 (39.2)	ns	ns	ns	0.01 (43.4)	0.84 (57.5)	1.30 (43.6)
S	1.69 (40.3)	0.17 (45.1)	0.83 (40.3)	ns	ns	ns	12.26** (45.0)	0.64 (39.0)	1.15 (45.0)
o-PO_4	7.68** (40.3)	4.03 (50.7)	0.11 (40.3)	ns	ns	ns	6.35* (50.7)	0.13 (46.0)	0.31 (50.7)
NH_4	3.46 (50.9)	1.40 (56.7)	0.13 (50.6)	ns	ns	ns	0.22 (56.5)	0.58 (44.0)	2.07 (56.4)
NO_3	84.19** (26.9)	78.36** (29.2)	3.86* (26.9)	b	a	a	73.3** (29.1)	1.75 (49.0)	3.33 (29.0)
DOC	3.09 (51.2)	23.82** (85.9)	10.92** (51.1)	a	a	b	0.36 (85.7)	5.51** (53.8)	4.52* (85.7)

that in all vegetation plots infiltration rates were higher during summer inundation than during winter inundation.

Changes in redox potential

Redox potentials (E_h) obviously decreased upon summer inundation (Supplementary data, Appendix D). Particularly in Scor-vegetation, the peat soil showed constant anaerobic conditions with E_h values below -200 mV during inundation. In Call-plots, anaerobic conditions were less prevalent, and the upper 5 cm was not characterized by anaerobic circumstances in the first week of inundation. Moreover, in Sph-plots, anaerobic conditions were eliminated after the first three days of inundation, and aerobic conditions with E_h values of 300-400 mV prevailed during the rest of the inundation period, even though the moss layer was submerged.

ANC and pH in porewater

In the reference site VW, there were no changes in porewater Ca-concentrations and alkalinity, neither in winter nor in summer. Winter inundation in KW did not result in increased Ca-concentration or alkalinity in porewater either (Figure 5.2A and B). Just like with Cl-concentrations, infiltration of base-rich surface water resulted in slightly increased porewater Ca-concentrations and alkalinity only in the winter of 2011, when initial water tables were relatively low.

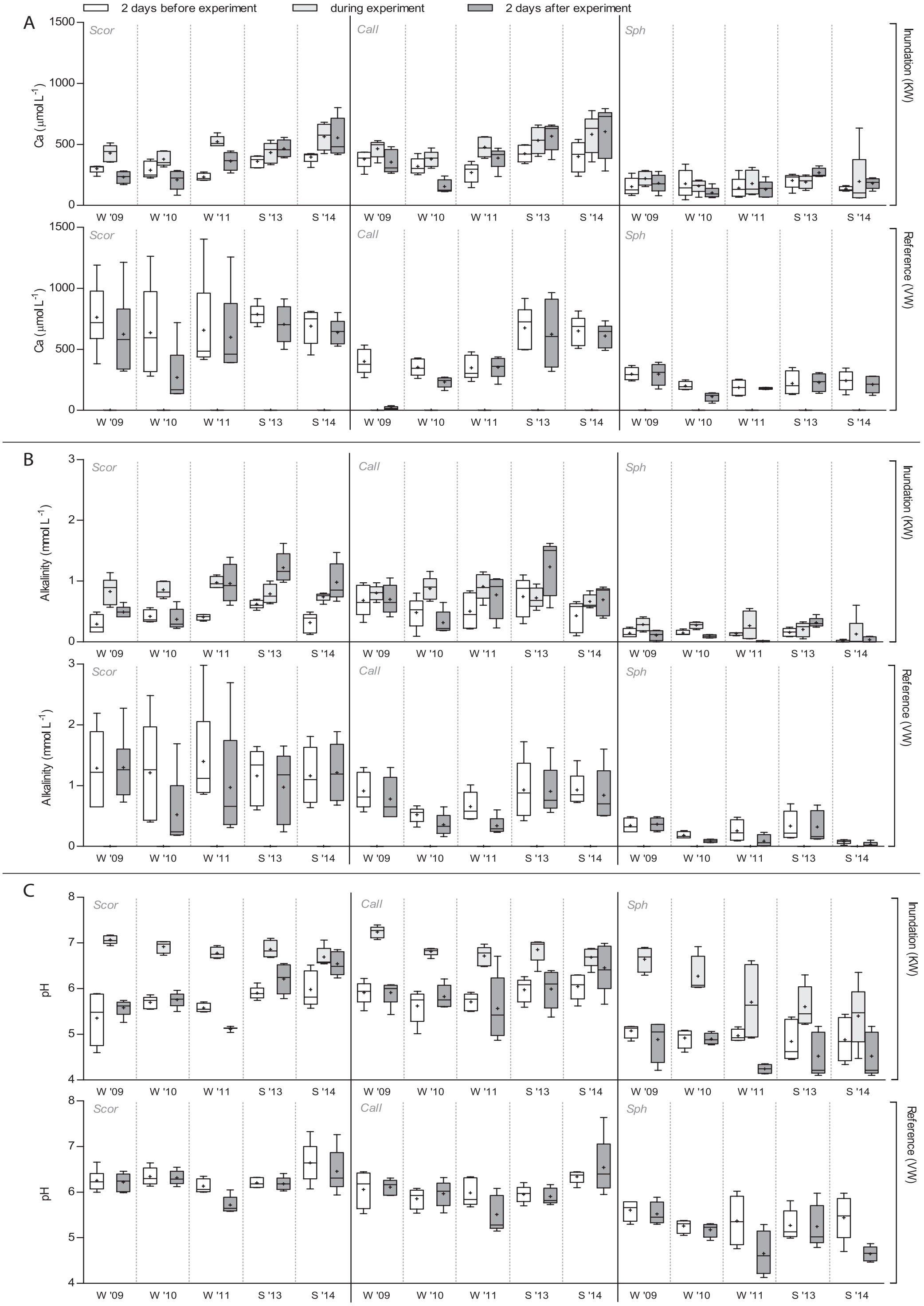

Figure 5.2 Ca-concentrations (A), alkalinity (B) and pH (C) per vegetation type in porewater 2 days before the experiment, in inundation water during the experiment, and in porewater 2 days after the experiment. Sample means with standard deviations are indicated ($n = 5$).

In contrast, summer inundations in KW in 2013 and 2014 resulted in a significant increase in both porewater Ca-concentrations and alkalinity, as indicated by an interaction of inundation*season (Table 5.3). Ca-concentrations increased with 120 µmol L^{-1} on average, and alkalinities showed an increase of about 0.4 $mmol_c$ L^{-1}. This increase in porewater ANC during summer inundation did, however, not result in a significant change in porewater pH.

The effect of inundation on the porewater ANC in KW did not only differ between winter and summer, but also among vegetation types. Both Ca-concentrations and alkalinity in porewater showed an increase in the Scor- and Call-plots, but this increase was absent in the Sph-plots, as indicated by an interaction of inundation*vegetation type (Table 5.3). Also, inundation water during both winter and summer experiments showed much lower Ca-concentrations and lower alkalinities in the Sph-plots ($F_{1,20}$=70.67** and $F_{1,21}$=84.14**). As a result, the pH values of inundation water at the Sph-plots were considerably lower than at the Scor- and Call-plots ($F_{1,21}$=52.53**), which was particularly true during summer as indicated by a significant interaction of vegetation type*season ($F_{1,29}$=6.98**).

Nutrients in porewater

Inundation in KW resulted in a small increase in porewater o-PO_4 concentrations of 0.3 µmol L^{-1} on average during summer inundation (Figure 5.3A and Table 5.3). This increase was only detected in 2013, and was presumably the result of internal P-mobilization, since the o-PO_4 concentrations in supplied surface water from the adjacent ditch were lower than these concentrations in porewater.

Porewater NO_3 concentrations were generally unaffected by inundation (Table 5.3) and remained very low in all vegetation types, regardless of the season (Figure 5.3B). NH_4 concentrations in porewater were, however, significantly affected by inundation and this effect differed between seasons (Figure 5.3C and Table 5.3). During summer inundation, an average increase of NH_4 concentrations of 13 µmol L^{-1} was measured, while during winter inundation NH_4 concentrations remained unaltered and very low.

5.4. Discussion

Conditions before inundation

Initial differences in pH and ANC between Sph-plots versus Scor- and Call-plots are due to the minor influence of base-rich water in Sph-plots, since *Sphagnum* spp. naturally occur further above the water table, are mainly fed by rain water acidify their habitat by releasing protons in exchange for other cations (Clymo, 1963). This reduces pH and ANC along the fen-bog gradient (Clapham, 1940; Segal, 1966; van

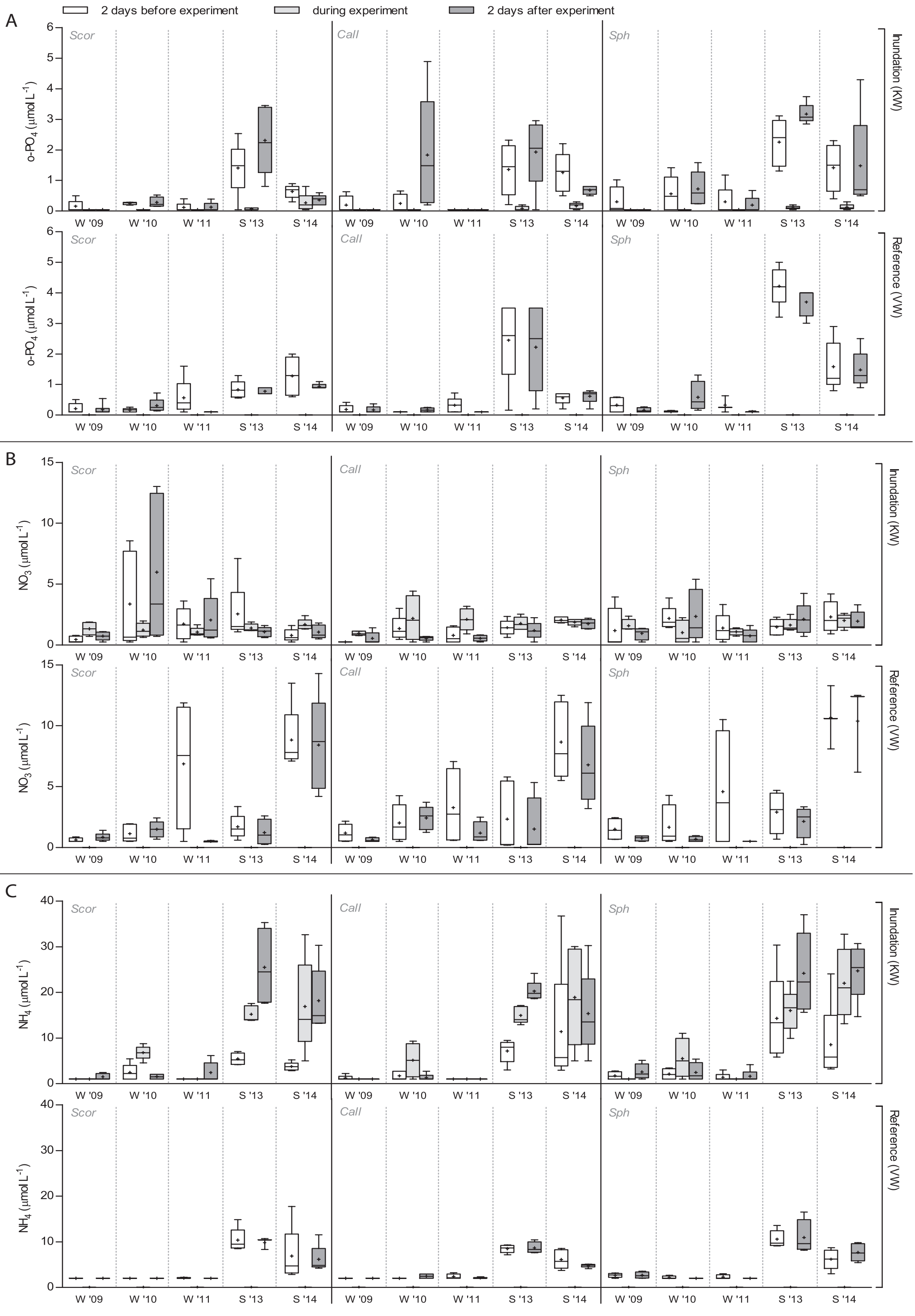

Figure 5.3 o-PO$_4$ (A), NO$_3$ (B) and NH$_4$ (C) concentrations per vegetation type in porewater 2 days before the experiment, in inundation water during the experiment, and in porewater 2 days after the experiment. Sample means with standard deviations are indicated (*n* = 5).

Wirdum, 1991). The lower pH in Sph-plots leads to increased solubility of iron and calcium phosphates, possibly explaining increased o-PO_4 concentrations in Sph-plots (Lindsay and Moreno, 1966).

The overall slightly increased initial porewater Ca- and Cl-concentrations in summer are presumably due to reduced dilution with rainwater due to increased evapotranspiration in summer (van Wirdum, 1991), as confirmed by an increased IR. While Ca- and Cl-concentrations were only 1.2 times higher in summer, o-PO_4 and NH_4 concentrations showed a stronger increase of 5-6 times, which we attribute not only to reduced dilution, but moreover to increased net mineralization rates as a result of the higher temperatures. Increased DOC-concentrations, as an indicator of increased decomposition, seem to confirm this idea.

Summer versus winter inundation

Generally, winter inundation did not result in infiltration of inundation water, because the peat soils were already waterlogged and evapotranspiration rates were relatively low (Chapter 4). Only when initial water tables were sufficiently low (about 10 cm below the soil surface), infiltration of base-rich surface water was facilitated in winter. In contrast, inundation in summer, when the peat is less water-saturated and evapotranspiration rates are higher, did result in enhanced infiltration of Ca- and HCO_3-rich inundation water.

Infiltration of base-rich surface water may, however, not have been the only process resulting in an increased porewater ANC upon inundation. To get insight into the relative contribution of internal, microbial alkalinity generation, porewater ratios of [alkalinity]/[Cl] and [Ca]/[Cl] were calculated (Table 5.4). In Scor-plots the

Table 5.4 Average porewater ratios of [alkalinity]/[Ca], and [Ca]/[Cl] in winter and summer, before and after inundation. For abbreviations see Table 5.1.

[Alkalinity]/[Cl]

Vegetation type		Scor	Call	Sph
Winter	Before inundation	1.0	1.3	0.4
	After inundation	1.0	1.0	0.2
Summer	Before inundation	0.8	1.5	0.4
	After inundation	1.8	1.6	0.5

[Ca]/[Cl]

Vegetation type		Scor	Call	Sph
Winter	Before inundation	0.8	0.7	1.1
	After inundation	0.6	0.5	0.9
Summer	Before inundation	0.8	0.8	1.8
	After inundation	1.0	0.7	1.5

ratio [alkalinity]/[Cl] was more than twice as high after summer inundation, while this was not the case after winter inundation. Further, the ratio [Ca]/[Cl] showed only a slight increase, which did not differ between winter and summer. This indicates a relatively higher production of alkalinity during summer inundation. Since inundation led to anaerobic conditions, as shown by redox-measurements, anaerobic microbially mediated redox processes occur which result in alkalinity generation (Baker et al., 1986; Stumm and Morgan, 1996). The response of redox potentials to summer inundation did not differ from the response to winter inundation (Chapter 4). We, however, suggest that especially during summer inundations, when temperatures are higher and microbial activity is enhanced, microbial alkalinity generation increases in the top-soil of Scor-plots. In the top-soil of Call- and Sph-plots, where anaerobic conditions during inundation were less prevalent than in Scor-plots, anaerobic microbial alkalinity generation may have been smaller, possibly explaining different response in porewater ANC among the vegetation types.

The decrease in E_h during summer inundation to values of <200 mV in the upper 10 cm in Scor-plots, reported to be representative for reduction of Fe(III) and SO_4 (Ponnamperuma, 1984), are in accordance with the idea of enhanced reduction processes. However, increased anaerobic decomposition and Fe(III) or SO_4 reduction was not reflected by changes in Fe-, S- or DOC-concentrations in porewater (Supplementary data, Appendix E). This may indicate that, despite of anaerobic circumstances, Fe(III) and SO_4 reduction rates were still limited due to the fact that peat soils in the National Park Weerribben/Wieden are relatively low in redox-sensitive Fe and S (Chapter 3). In addition, FeS_x precipitation may have reduced dissolved Fe and S levels.

The slight increase in internal P-mobilization during short-term summer inundation was not a cause for concern since porewater o-PO_4 concentrations remained sufficiently low and did not threaten P-limited vegetation. NO_3 concentrations showed no considerable change either. NH_4 concentrations, however, showed a clear increase during summer inundation, which we attribute to increased infiltration rates of inundation water with relatively high NH_4 concentrations originating from the ditches. Maximum concentrations of 20 µmol L^{-1} under summer conditions are, however, not considered toxic to bryophyte vegetation or plants (Paulissen et al., 2004; Verhoeven et al., 2011).

Effects for different vegetation types

Ca-concentrations, alkalinity and pH of inundation water in the Sph-plots were generally lower than in the Scor- and Call-plots. In addition, porewater ANC in the Sph-plots did not increase, even though infiltration occurred in all vegetation types and porewater ANC did increase in Scor- and Call-plots. The cation exchange capacity of the *Sphagnum*-comprising top layer of the Sph-peat soils has presumably hampered an increase in ANC via exchange of Ca^{2+} from inundation water for H^+ (Clymo and Hayward, 1992; Kooijman and Bakker, 1994; Paulissen et al., 2004; Chapter 4). Moreover, pH values in inundation water at Sph-plots turned out lower during summer inundation than during winter inundation, which may indicate that the acidifying effect of *Sphagnum* is enhanced in summer, possibly as a result of increased growth rates. This points at the significant role of *Sphagnum* as an ecosystem engineer in changing its habitat under similar conditions, once the moss has invaded the vegetation.

Conclusions and implications for management

In terms of counteracting acidification of rich fens, short-term summer inundation with base-rich surface water appears to be very efficient. In contrast to winter inundation, raising surface water levels in summer, when evapotranspiration rates are high, results in infiltration, and hence an increase of ANC. Secondly, internal alkalinity generation, as a result of anaerobic microbial redox processes, is enhanced by higher temperatures in summer. The latter effect will however be temporary, since aerobic oxidation during subsequent droughts can lead to re-acidification (Lamers et al., 1998b; Chapter 2). The first process of infiltration of Ca-rich water, however, may contribute to a lasting increase in the peat soil ANC, as the ANC not only determined by the amount of bicarbonate in porewater in the circum-neutral pH range, but also by the saturation of Ca and Mg at the adsorption complex (base saturation, buffering at slightly acidic conditions; Stumm and Morgan, 1996). The ability of rich fen soils to exchange H^+ for Ca^{2+} from the adsorption complex, and thereby buffer porewater pH, may be highly beneficial to counteract acidification during subsequent periods of drought in particular, when bicarbonate has been largely consumed and base cation exchange against H^+ initiates.

Only in Scor- and Call-plots porewater ANC was increased by summer inundation. In Sph-plots, the ANC remained relatively low, presumably due to exchange of Ca^{2+} from inundation water for H^+. Short-term summer inundation with base-rich water as a measure seems therefore only efficient at places where base-rich conditions still prevail. At the point when *Sphagnum* spp., which are able to acidify its environment, have already made their entry, the measure has no more effect. Therefore, short-term summer inundation is considered a preventive measure, in order to maintain and restore current rich fens.

In addition to the importance of increased ANC to counteract acidification, raised water levels in summer may also be important to prevent other drought-induced problems that can be highly detrimental in rich fens. Increased oxygen availability during drought may lead to increased microbial decomposition, and hence increased mineralization of nutrients (Olde Venterink et al., 2002; Chapter 2), which can be highly detrimental for nutrient–limited rich fens. Furthermore, vegetation development and vitality of characteristic rich fen bryophytes are directly affected by drought in a negative way (Chapter 3). These adverse effects can be obviated as well by temporarily allowing raised water levels in summer.

Short-term summer inundation as a measure is, however, only considered beneficial under specific conditions. First, inundation has the most effect when the peat layer is attached to the underlying substrate via roots. In floating *Sphagnum*-dominated fens, raised surface water levels had almost no effect, because the buoyant peat follows changes in surface water levels and inundation does not occur, although this may be different in floating rich fens (Chapter 4). Further, surface water quality in adjacent ditches must not only be base-rich, but also nutrient-poor, as the adverse eutrophying effects of polluted inundation water on N- and P-limited vegetation are well-known (e.g. Lamers et al., 2015). In addition, porewater P-availability should not increase as a result of net P-mobilization (internal eutrophication) due to Fe(III) reduction in peat soils (Patrick and Khalid, 1974). Especially in Fe-rich soils with high P-contents, this anaerobic P-mobilization can be severe (Zak et al., 2010; Cusell et al., 2013b; Chapter 3). Moreover, sulphate reduction and formation of FeS_x may result in additional P-mobilization (Smolders and Roelofs, 1993; Caraco et al., 1998; Lamers et al., 1998a). Finally, anaerobic conditions should not lead to formation of potential phytotoxins such as NH_4^+, H_2S, Fe^{2+} and/or organic acids to plants, depending on soil chemistry (Lamers et al., 2015; Chapter 3). In the relatively Ca-rich fen sites of this study, both water and soil quality were suitable to obtain desired results from a management perspective. However, potential benefits and disadvantages of inundation need to be considered for different fen types with different water qualities separately in water management and nature management plans before implementation.

Acknowledgements

The authors wish to thank Leen de Lange, Peter Serné and Bert de Leeuw for analytical assistance. Natuurmonumenten and Water Management Authority Reest & Wieden are acknowledged for permission, financial support and cooperation in the experimental areas. This research was funded by Kennisnetwerk Ontwikkeling en Beheer Natuurkwaliteit (O+BN) of the Dutch Ministry of Economic Affairs, Agriculture and Innovation, the Water Management Authority Reest & Wieden and the province of Overijssel.

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CHAPTER 6

The relative importance of calcium and iron for nutrient availability, productivity and species composition in brown moss-dominated rich fens

Ivan S. Mettrop, Tessa Neijmeijer, Casper Cusell, Leon P.M. Lamers, Lars Hedenäs, Annemieke M. Kooijman

Abstract

Rich fens are characterized by minerotrophic conditions, in which calcium (Ca) and iron (Fe) concentrations show large variations. The relative importance of Ca and Fe, particularly in relation to the availability of phosphorus (P) for rich fen vegetation, is however largely unknown. To elucidate this, we examined the relation between vegetation characteristics and peat chemistry in 24 stands of rich fen vegetation: 12 in the Netherlands (strong anthropogenic forcing) and 12 in central Sweden (weak anthropogenic forcing). In addition, specific habitat preferences of three typical brown moss spp. were assessed.

Ca and Fe turned out to be important drivers of species composition in rich fens through their differential effects on plant P-availability. Fens dominated by *Scorpidium scorpioides* or *S. cossonii* were characterized by high porewater Ca-concentrations and total soil Ca-contents, but low P-availability. In these Ca-rich, but Fe-poor fens, foliar N:P ratios of vascular vegetation were above 20 g g^{-1}, indicating P-limitation due to Ca-P precipitation. In contrast, fens dominated by *Hamatocaulis vernicosus* were characterized by high porewater Fe-concentrations and total soil Fe-contents, but also relatively high P-availability. Total soil Fe-content showed a positive correlation with total soil P-content and P-concentration in plant tissue, and a negative correlation with foliar N:P ratios. N:P ratios in these fens were even below 13.5 g g^{-1}, indicating potential nitrogen (N)-limitation. The remarkable positive correlation between soil Fe-content and P-availability contrasts the idea that high Fe-contents automatically lead to low values of plant-available P. We instead propose that high groundwater Fe discharge leads to the accumulation of P that is still available to plants due to the relatively weak binding of P within abundant Fe-OM (Organic Matter) complexes. Furthermore, total biomass production was regulated by plant P-availability in Sweden. In the Netherlands, however, where above-ground biomass was 2.5 times higher, only the vegetation composition was regulated by plant P-availability. Finally, Dutch rich fens were more acidic than Swedish, which is probably related to the much higher atmospheric N-deposition.

We conclude that the relative roles of Ca and Fe strongly differ with respect to nutrient limitation and vegetation development in rich fens, and should therefore be included in studies relating vegetation development to geohydrological conditions.

6.1 Introduction

Mesotrophic and minerotrophic, species-rich fens are considered ecologically valuable because of their high floristic diversity including many red list species (Wassen et al., 2005; van Diggelen et al., 2006). These so-called ‘rich fens’ have become very rare in densely populated and heavily exploited landscapes, and are therefore protected as EU priority habitat *H7140 – Transition mires and quaking bogs*. Generally, the most important habitat characteristics explaining floristic diversity in fens are considered to be differences in water level, acid neutralizing capacity (ANC), nutrient-availability, and toxicity (e.g. Wheeler and Proctor, 2000; Hájek et al., 2006; Lamers et al., 2015).

Autogenic succession in fens, and/or anthropogenic intervention in areas with intensive agriculture, have resulted in hydrological isolation from base-rich groundwater and surface water, and hence reduced ANC (van Wirdum, 1991; Van Diggelen et al., 1996). Presumably, increased atmospheric deposition of nitrogen (N) has exacerbated the acidification of fens in industrialized countries (Gorham et al., 1987). In addition to sufficient ANC, phosphorus (P) limitation has been shown to be important to enable high biodiversity and the occurrence of rare and endangered bryophytes and plant species in rich fens (Boeye et al., 1997; Wassen et al., 2005; Cusell et al., 2014). In rather calcareous rich fens, Ca-related precipitation (co-precipitation with $CaCO_3$ and precipitation as Ca phosphates) reduces the bio-availability of P (Boyer and Wheeler, 1989; Wassen et al., 1990). In addition, the bio-availability of P has been reported to be reduced by Fe-related P-precipitation (to Fe oxides and hydroxides, and as organic Fe phosphates) in mires (e.g. Roden and Edmonds, 1997; Zak et al., 2004). For rich fens, however, the general assumption that Fe-rich conditions automatically imply a lower P-availability is called into question (Aggenbach et al., 2013; Pawlikowski et al., 2013; Cusell et al., 2014).

Therefore, and because it is important for the mechanistic understanding of the functioning and biodiversity of fens, the objective of this study was to reconsider the relative biogeochemical importance of Ca and Fe, particularly in relation to plant-available P in rich fens in regions with either high (the Netherlands) or low (central Sweden) anthropogenic pressure. In addition to soil porewater analyses, we therefore included additional soil extractions to assess different fractions of P in fen

Table 6.1 The different sampling locations in the Netherlands and central Sweden.

Species	The Netherlands	Coordinates	Central Sweden	Coordinates
Scorpidium scorpioides	Binnenpolder Tienhoven	52 10’31 N; 05 59’01 E	Gulåstjärnen	63 29’17 N; 14 53’48 E
	Stobbenribben	52 47’09 N; 05 59’03 E	Gulåstjärnen lakesite	63 29’17 N; 14 53’48 E
	Kikkerlanden	52 39’45 N; 06 02’27 E	Storflon	63 13’32 N; 16 00’45 E
	De Haeck	52 08’59 N; 04 50’36 E	Stormyran	63 13’15 N; 16 09’22 E
Scorpidium cossonii	Geleenbeekdal	50 55’34 N; 05 54’03 E	Flärkarna	63 04’01 N; 16 10’43 E
	Bennekomse Meent	52 00’22 N; 05 35’37 E	Gulåstjärnen	63 29’17 N; 14 53’48 E
	Veerslootlanden	52 37’09 N; 06 08’15 E	Gulåstjärnen lakesite	63 29’17 N; 14 53’48 E
	Veldweg	52 41’29 N; 06 06’45 E	Stormyran	63 13’15 N; 16 09’22 E
Hamatocaulis vernicosus	Blauwe Hel	52 00’48 N; 05 34’16 E	Flärkarna	63 04’01 N; 16 10’43 E
	Meppelerdieplanden	52 40’05 N; 06 07’37 E	Storflon	63 13’33 N; 16 00’45 E
	Kiersche Wiede	52 41’48 N; 06 07’57 E	Källmyren	63 24’10 N; 14 34’07 E
	Meppeler Diep	52 41’00 N; 06 08’51 E	Stormyran	63 13’15 N; 16 9’22 E

soils. Our main hypothesis was that the relative abundances of Ca and Fe are important drivers of rich fen functioning, diversity and species composition, through their differential effects on plant P-availability.

6.2. Materials and methods

Sampling

Samples were collected from 12 rich fens in the Netherlands and 12 rich fens in central Sweden (Table 6.1). Dutch samples were collected in August 2011, and Swedish samples from the province Jämtland in August/September 2012. We selected the sampling sites based on dominance of either *Scorpidium scorpioides* (Hedw.) Limpr., *Scorpidium cossonii* (Schimp.) Hedenäs, or *Hamatocaulis vernicosus* (Mitt.) Hedenäs. For each site, species composition and cover percentages of vascular plants and bryophytes were recorded in a 10 m^2 plot. In each plot, three subplots of 25 cm^2 with dominant coverage by one of the three bryophytes were randomly selected. At each subplot the height of the water level relative to the soil surface just beneath the living moss layer was measured, and above-ground biomass of the vascular vegetation was clipped at soil surface and harvested for further analysis. At each subplot, also porewater samples from the upper 10 cm of the soil were collected with Rhizon SMS soil moisture samplers (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), connected to vacuumed syringes of 50 mL. In addition, peat soil samples were collected from the upper 10 cm of the peat soil, just below the living moss layer. Furthermore, samples for bulk density were collected using a steel corer with an exact volume of 100 mL. All samples were collected in airtight plastic bags to avoid oxygen exposure, and stored at 4°C until further analysis.

Analytical techniques

Porewater pH-values were measured with a standard Ag/AgCl electrode and alkalinity was determined by titration down to pH 4.2 by using 0.01 mol L^{-1} HCl. Next, electrical conductivity (EC) of porewater samples was measured, and concentrations of o-PO_4, NO_3, NH_4, SO_4, Cl and dissolved organic matter (DOC) were measured by using an Auto Analyzer (Skalar, San++ System, fitted with Skalar, SA1074). Subsamples were acidified by adding 1% of concentrated HNO_3 to prevent metal precipitation, after which total concentrations of P, Ca, Fe, S, Mg, Al, Na, K, Zn and Mn were measured by Inductively Coupled Plasma (ICP) spectroscopy (Perkin Elmer, Optima 3000XL).

Dry weights and gravimetric moisture contents of the fresh peat soil samples, expressed as a percentage of the sample's dry weight, were determined by drying at 70°C until constant weight. Total organic matter (OM) contents were estimated by loss-on-ignition (550°C for 4 hours). Total C and N contents in dried peat soil samples were measured using a CHNS analyzer (Elementar, Vario EL Cube). In addition, 250 mg aliquots of dry soil were digested for 50 minutes in a microwave (Perkin Elmer, Multiwave) with 4.0 mL HNO_3 (65%) and 1.0 mL HCl (37%), after which total P, Ca, Fe, S, Mg, Al and K contents in diluted digestates were measured by ICP (Bettinelli et al., 1989; Westerman, 1990).

Other soil analyses were conducted on lyophilized peat soil samples in order to restrict redox sensitive reactions and to keep soil moisture contents equal. The total P-content (P_{tot}) (after heating at 500°C for 4 hours) and inorganic P-content (P_{inorg}) (not heated) were measured colorimetrically in 0.5M H_2SO_4 extracts (Murphy and Riley, 1962), after 16 h of shaking. P_{inorg} consists of directly available dissolved inorganic P (mostly orthophosphates), P bound to amorphous inorganic metal(hydr) oxides, and unavailable P incorporated in crystalline salts of Ca, Fe and Al-phosphates (Fixen and Grove, 1990). The amount of P incorporated in organic material (P_{org}) was calculated as the difference between P_{tot} and P_{inorg}. Concentrations of P bound to amorphous Fe and Al (P_{ox}; both organic and inorganic) were determined by 0.073M NH_4-oxalate/0.05M oxalic acid extraction at pH 3.0 after 4 h of shaking in the dark (Schwertmann, 1964) and subsequent element analysis by ICP. By considering the surplus of P_{ox} compared to P_{inorg}, a minimal estimate of P bound to Fe-OM complexes ($P_{Fe\text{-}OM}$) can be made, which is quite reliable particularly in Fe-rich soils with low Ca-contents (Kooijman et al., 2009).

Amorphous Fe- and Al-concentrations (Fe_{ox} and Al_{ox}) were also measured in the NH_4-oxalate/oxalic acid extracts. Fe_{ox} and Al_{ox} consist of non-crystalline inorganic

Fe- and Al(hydr)oxides and organic Fe and Al complexes (Fe_{pyr} and Al_{pyr}), of which the latter is incorporated in Fe/Al-OM complexes contributing highly to reversible P-adsorption. We additionally distinguished these Fe_{pyr} and Al_{pyr} fractions by determining organic Fe and Al complexes in 0.1 M $Na_4P_2O_7$ extracts (Wada, 1989), after one night of shaking and subsequent Auto Analyzer analysis.

Dried vascular plant biomass (standing stock) was used as a measure of productivity. Vegetation samples were weighed after drying at 70°C until constant weight, and ground. Total element concentrations in plants were determined by microwave destruction and CNHS analyzer in the same way as described for soil samples. Foliar N:P, N:K and K:P ratios ($g\ g^{-1}$) in vascular plants shoots were used as indicators of nutrient limitation, i.e. N-limitation if $N:P<14.5$ and $N:K<2.1$, P-limitation or co-limitation of NP if $K:P>3.4$ and $N:P>14.5$, and K-limitation or co-limitation of NK if $K:P<3.4$ and $N:K>2.1$ (Olde Venterink et al. 2003).

Statistical analyses

Differences between samples from the Netherlands and from Sweden were tested by applying a linear mixed model in SPSS 20.0 for Windows (IBM Inc., 2011), using 'country' as fixed factor. Since some pseudo replicates were taken from similar sites, we included a random factor 'locationcode' to correct by means of a covariance matrix (Variance Components). The dominant bryophyte species (*S. scorpioides*, *S. cossonii* or *H. vernicosus*) was not tested as a fixed factor in the mixed model, because the occurrence of these species depended on environmental variables, cover percentages within the different plots varied and, moreover, the species sometimes co-occurred. Unimodal relationships between the occurrence of the three different bryophyte spp. and the measured environmental variables were therefore tested with canonical correspondence analysis (CCA), by using CANOCO (Ter Braak, 1986). In the CCA, the bryophyte spp. occurrence, weighted according to their cover percentage, and all environmental variables (except pH) were logtransformed to improve their fit to a normal distribution (Williamson, 1972). Finally, strengths of linear relationships between separate variables were analyzed by using Pearson correlation coefficients (*r*). For all analyses, *P*-values in the text are indicated as follows: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

6.3. Results

Differences between the Netherlands and Sweden

Species composition

Species composition in the rich fens clearly differed between the Netherlands and central Sweden (Table 6.2). The communal vascular plant species comprised mainly

Table 6.2 List of communal species, and species that were most common in the Netherlands or in central Sweden.

Vascular plant spp.	Bryophyte spp.
Communal species:	
Carex lasiocarpa	*Bryum pseudotriquetrum*
Carex nigra	*Calliergon giganteum*
Carex panicea	*Campylium stellatum*
Carex rostrata	*Hamatocaulis vernicosus*
Drosera rotundifolia	*Scorpidium cossonii*
Epilobium palustre	*Scorpidium scorpioides*
Equisetum fluviatile	*Sphagnum contortum*
Equisetum palustre	*Sphagnum teres*
Menyanthes trifoliata	
Molinia caerulea	
Species most common in the Netherlands:	
Agrostis canina	*Calliergon cordifolium*
Alnus glutinosa	*Calliergonella cuspidata*
Calamagrostis canescens	*Fissidens adianthoides*
Cardamine pratensis	
Carex diandra	
Carex disticha	
Carex elata	
Cirsium palustre	
Galium palustre	
Hydrocotyle vulgaris	
Juncus articulatus	
Juncus subnodulosus	
Lysimachia vulgaris	
Lythrum salicaria	
Mentha aquatica	
Thelypteris palustris	
Utricularia minor	
Viola palustris	
Species most common in central Sweden:	
Betula nana	*Aneura pinguis*
Carex cordorrhiza	*Catascopium nigritum*
Carex dioica	*Cinclidium stygium*
Carex limosa	*Drepanocladus trifarius*
Dactylorhiza incarnata	*Helodium blandowii*
Eriophorum latifolium	*Loeskypnum badium*
Trichophorus alpina	*Paludella squarrosa*
Vaccinium uliginosum	*Sphagnum warnstorfii*
	Tomentypnum nitens

Cyperaceae such as *Carex lasiocarpa* (Ehrh.), *C. nigra* (L.) Reichard, *C. panicea* (L.) and *C. rostrata* (Stokes), and other common peatland species such as *Equisetum fluviatile* (L.) and *Menyanthes trifoliata* (L.). Communal bryophytes, apart from *S. scorpioides*, *S. cossonii* and *H. vernicosus*, were *Bryum pseudotriquetrum* (Hedw.) P. Gaertn., E. Mey and Scherb., *Calliergon giganteum* (Schimp.) Kindb., *Campylium stellatum* (Hedw.) C.E.O. Jensen, *Sphagnum contortum* (Schultz) Hüb. and *S. teres* (Schimp.) Ångström. Vascular plant species that were most common or even exclusively present in the Dutch fen sites comprised relatively eutrophic spp. such as *Agrostis canina* (L.) and *Calamagrostis canescens* (Weber ex F.H. Wigg.) Roth., *Cardamine pratensis* (L.), *Cirsium palustre* (L.), *Lysimachia vulgaris* (L.) and *Thelypteris palustris* (Salisb.) Schott., and bryophytes such as *Calliergon cordifolium* (Hedw.) Kindb. and *Calliergonella cuspidata* (Hedw.) Loeske. The vegetation in central Sweden comprised many northern species, which are absent or very rare in the Netherlands. This applies to vascular plant species such as *Carex dioica* (L.), *C. limosa* (L.), *Eriophorum latifolium* (Hoppe), and bryophytes such as *Catascopium nigritum* (Hedw.) Brid., *Cinclidium stygium* Sw., *Drepanocladus trifarius* (F. Weber and D. Mohr) Broth. ex Paris, *Helodium blandowii* (F. Weber and D. Mohr) Warnst., *Paludella squarrosa* (Hedw.) Brid. and *Tomentypnum nitens* (Hedw.) Loeske.

Despite the clear differences in species composition, the species numbers per location (about 25) did not differ between the Netherlands and central Sweden ($F_{1,22}=2.2^{NS}$). The number of different bryophyte species per site was, however, lower in the Netherlands than in Sweden ($F_{1,22}=6.0^*$), with respectively 21% versus 39% of the total species sum. The coverage of the bryophyte and vascular plant layers did not differ between countries ($F_{1,22}=2.3^{NS}$ and $F_{1,22}=2.4^{NS}$), and were on average 51and 64%, respectively.

Habitat characteristics

Although Ca-concentrations and alkalinities in porewater were similar, pH values were significantly lower in the Netherlands than in central Sweden (Table 6.3). Ca-concentrations of around 1.0 mmol L^{-1} and alkalinities of around 2.0 $mmol_c$ L^{-1} generally corresponded to pH values of around 6.3 in the Netherlands versus higher pH values of 6.7 in central Sweden. The porewater EC was, however, much higher in the Netherlands than in central Sweden, which we primarily attribute to increased concentrations of Na and Cl due to a closer location to the sea. In addition, porewater SO_4 concentrations were considerably higher in the Netherlands than in central Sweden, with values around 300 µmol L^{-1} versus 50 µmol L^{-1}. Overall, porewater DOC-concentrations in the Dutch sites were 6.7 times higher than in the Swedish sites with values around 3000 µmol L^{-1} versus 450 µmol L^{-1}. Porewater NH_4, NO_3 and o-PO_4 concentrations, however, did not significantly differ between the two countries (Table 6.3).

Table 6.3 Porewater characteristics for the three different vegetation types in the Netherlands and Sweden. Data shown represent mean values and standard errors ($n = 12$). F-values resulting from mixed model analysis of differences between countries are shown with their level of significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

	Netherlands			Sweden			Country
Variable	*S. scorpioides*	*S. cossonii*	*H. vernicosus*	*S. scorpioides*	*S. cossonii*	*H. vernicosus*	$F_{1,22}$
pH	6.3 (0.0)	6.4 (0.1)	6.1 (0.2)	6.7 (0.1)	6.7 (0.1)	6.8 (0.1)	6.3*
Alkalinity (mmol L^{-1})	2.2 (0.3)	2.1 (0.7)	1.4 (0.4)	2.1 (0.4)	2.6 (0.5)	1.7 (0.2)	0.6
EC (µS cm^{-1})	350 (24)	404 (64)	298 (36)	202 (37)	254 (41)	170 (21)	9.3**
Ca (µmol L^{-1})	989 (141)	1423 (267)	930 (134)	857 (175)	1104 (199)	676 (105)	1.4
Fe (µmol L^{-1})	62 (31)	17 (7)	158 (76)	62 (29)	45 (18)	85 (19)	0.4
Na (µmol L^{-1})	859 (74)	547 (87)	659 (93)	69 (8)	79 (12)	96 (6)	89.3***
Cl (µmol L^{-1})	1008 (75)	618 (83)	617 (96)	10 (3)	13 (4)	20 (4)	125.9***
SO_4 (µmol L^{-1})	140 (30)	228 (43)	562 (264)	51 (14)	45 (8)	73 (7)	7.6*
DOC (µmol L^{-1})	3107 (869)	2106 (502)	3761 (978)	676 (75)	392 (107)	321 (74)	17.6***
NH_4 (µmol L^{-1})	5.03 (1.94)	12.86 (5.40)	29.50 (9.50)	3.96 (0.68)	4.14 (0.82)	5.71 (0.70)	2.1
NO_3 (µmol L^{-1})	1.62 (0.38)	1.45 (0.47)	1.93 (0.30)	2.85 (0.70)	2.16 (0.90)	3.40 (0.88)	2.8
o-PO_4 (µmol L^{-1})	0.14 (0.04)	0.35 (0.13)	1.15 (0.38)	0.13 (0.05)	0.13 (0.04)	0.44 (0.14)	2.0

Table 6.4 Soil characteristics for the three different vegetation types in the Netherlands and Sweden. Data shown represent mean values and standard errors ($n = 12$). F-values resulting from mixed model analysis of differences between countries are shown with their level of significance: $*P < 0.05$.

	Netherlands			Sweden			Country
Variable	*S. scorpioides*	*S. cossonii*	*H. vernicosus*	*S. scorpioides*	*S. cossonii*	*H. vernicosus*	$F_{1,22}$
Water table (cm)	0.4 (0.6)	-2.5 (0.7)	-3.1 (1.5)	3.5 (0.3)	-0.2 (0.9)	0.3 (0.4)	3.7
Organic matter (%)	92 (1)	75 (5)	73 (6)	70 (9)	73 (8)	76 (4)	1.0
Ca_{tot} (mol m^{-2})	1.98 (0.17)	3.98 (0.27)	2.37 (0.19)	63.72 (32.38)	37.78 (21.33)	3.29 (0.26)	3.4
Fe_{tot} (mol m^{-2})	0.43 (0.14)	1.49 (0.36)	3.04 (0.99)	1.36 (0.62)	2.43 (0.97)	14.83 (4.42)	2.9
Fe_{ox} (mol m^{-2})	0.26 (0.10)	1.00 (0.24)	1.63 (0.49)	1.48 (0.55)	2.01 (0.85)	10.60 (2.68)	3.5
Fe_{pyr} (mol m^{-2})	0.16 (0.08)	0.65 (0.16)	1.18 (0.36)	0.43 (0.20)	0.73 (0.27)	2.39 (0.40)	0.4
Ca:Fe (mol mol^{-1})	12.5 (2.5)	4.3 (0.8)	5.7 (1.7)	879.7 (446.5)	275.2 (183.5)	0.4 (0.1)	0.1
Ca:(Ca+Fe) (mol mol^{-1})	0.60 (0.07)	0.33 (0.05)	0.34 (0.09)	0.54 (0.12)	0.46 (0.11)	0.06 (0.02)	0.9
S_{tot} (mol m^{-2})	1.74 (0.19)	1.82 (0.20)	1.68 (0.15)	1.91 (0.22)	2.01 (0.24)	1.43 (0.22)	0.1
N_{tot} (mol m^{-2})	7.28 (0.72)	14.78 (1.98)	14.79 (2.04)	13.68 (1.40)	15.66 (2.82)	7.73 (0.55)	0.1
P_{tot} (mol m^{-2})	0.12 (0.01)	0.26 (0.03)	0.55 (0.12)	0.17 (0.01)	0.25 (0.02)	0.35 (0.07)	0.1
P_{org} (mmol m^{-2})	80.1 (12.6)	204.9 (23.8)	454.5 (94.5)	132.4 (14.8)	187.4 (19.0)	176.8 (15.5)	0.1
P_{inorg} (mmol m^{-2})	10.8 (1.7)	28.5 (4.4)	41.1 (7.4)	20.1 (4.3)	33.6 (6.3)	107.3 (58.6)	1.3
P_{ox} (mmol m^{-2})	11.3 (1.9)	43.4 (6.1)	212.8 (57.9)	23.8 (1.8)	43.8 (8.3)	140.5 (61.0)	0.4
C:N (g g^{-1})	26.32 (1.7)	16.71 (0.4)	16.89 (1.4)	21.22 (1.6)	21.39 (2.8)	25.16 (0.8)	1.6
C:P (g g^{-1})	785.6 (75.1)	417.8 (29.9)	321.8 (82.7)	769.6 (65.8)	539.0 (57.7)	313.6 (39.8)	0.5
N:P (g g^{-1})	30.05 (2.1)	25.06 (1.8)	17.32 (3.7)	37.08 (2.8)	27.82 (3.8)	12.86 (1.7)	0.0
Ca:P (mol mol^{-1})	19.5 (2.9)	17.3 (1.9)	8.7 (2.7)	456.9 (229.0)	216.5 (140.4)	11.7 (1.6)	2.4
Fe:P (mol mol^{-1})	3.0 (0.8)	5.2 (0.7)	3.8 (0.9)	7.4 (3.3)	8.8 (3.0)	48.3 (17.9)	5.2*

Table 6.5 Vegetation characteristics for the three different vegetation types in the Netherlands and Sweden. Data shown represent mean values and standard errors ($n = 12$). F-values resulting from mixed model analysis of differences between countries are shown with their level of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

	Netherlands			Sweden			Country
Variable	*S. scorpioides*	*S. cossonii*	*H. vernicosus*	*S. scorpioides*	*S. cossonii*	*H. vernicosus*	$F_{1,22}$
Dried biomass (g m^{-2})	325 (64)	283 (56)	221 (42)	98 (16)	95 (12)	130 (18)	12.4**
N (g kg^{-1} d.p.)	10.84 (0.56)	11.84 (1.27)	16.33 (1.90)	10.03 (0.31)	12.75 (0.67)	14.11 (0.87)	0.0
N (g m^{-2})	3.20 (0.46)	2.89 (0.44)	3.26 (0.68)	0.99 (0.16)	1.20 (0.17)	1.77 (0.19)	18.0***
P (g kg^{-1} d.p.)	0.51 (0.03)	0.66 (0.10)	1.37 (0.19)	0.41 (0.03)	0.81 (0.13)	1.56 (0.21)	0.1
P (g m^{-2})	0.15 (0.02)	0.15 (0.02)	0.26 (0.05)	0.04 (0.01)	0.08 (0.02)	0.19 (0.03)	6.2*
K (g kg^{-1} d.p.)	7.29 (0.95)	9.48 2.18)	10.94 (2.84)	5.75 (0.49)	8.84 (1.15)	14.29 (0.67)	0.5
K (g m^{-2})	2.24 (0.41)	1.91 (0.27)	1.56 (0.25)	0.57 (0.10)	0.89 (0.18)	1.85 (0.25)	4.9*
Foliar N:P (g g^{-1})	21.6 (0.6)	19.4 (1.5)	12.4 (0.9)	25.0 (1.1)	18.0 (1.4)	10.1 (0.9)	0.3
Foliar N:K (g g^{-1})	1.98 (0.41)	1.75 (0.29)	2.24 (0.33)	1.87 (0.14)	1.77 (0.29)	1.00 (0.07)	1.1
Foliar K:P (g g^{-1})	15.09 (1.99)	13.47 (1.68)	7.16 (1.12)	13.95 (0.83)	11.76 (1.30)	10.27 (0.79)	0.4

Total soil Ca- and Fe-contents did not significantly differ between the Dutch and Swedish rich fens, but both variables showed greater variation and more extreme values in the Sweden (Table 6.4). Generally, Fe$_{ox}$ accounted for about 72% of the total soil Fe-content, and Fe$_{pyr}$ (the amount of Fe incorporated in organic matter (OM) complexes) accounted for 41% of this amorphous Fe-fraction. Neither Fe$_{ox}$, nor Fe$_{pyr}$ differed between the countries. Al$_{ox}$ was of minor importance, generally accounting for less than 10% of the sum of amorphous Fe and Al concentrations. Furthermore, the vast majority of P in all soils belonged to the organic fraction (P$_{org}$), ranging from 76 to 91 % of the total soil P-content. P$_{org}$, as well as the total organic matter content, did not differ between the Dutch and the Swedish fens.

While in the Swedish rich fens the average above-ground biomass was only 108 g m^{-2}, the biomass in the Dutch rich fens was 2.5 times higher with values around 277 g m^{-2} (Table 6.5). As a result, total N and P content in vegetation per m^2 soil were also higher in the Netherlands, while N and P content per mass vegetation did not differ. Furthermore, foliar nutrient ratios in vascular plants did not differ between the Netherlands and Sweden.

Differences among vegetation types

Species composition

The three characteristic brown moss spp. hardly co-occurred, but showed a slight overlap in some of the 10 m^2 plots, particularly for *S. scorpioides* and *S. cossonii*. With respect to other bryophyte spp., *C. stellatum*, *D. trifarius*, *Fissidens adianthoides* Hedw. and *S. contortum* clearly were most prevalent in the *S. scorpioides*- and

S. cossonii-dominated sites, while *C. cuspidata* was more common in the Dutch *H. vernicosus*-dominated sites.

Vascular plants composition did not show very obvious differences among the three characteristic brown mosses. Noteworthy is that *Liparis loeselii* (L.) Rich. only occurred in *S. scorpioides*-dominated fens, and *Caltha palustris* (L.) only occurred in *H. vernicosus*-dominated sites and not in *S. scorpioides*- or *S. cossonii*-dominated sites.

Habitat characteristics

Porewater o-PO_4 concentration and total soil P-content show the strongest positive correlation with axis 1 in the CCA (explaining about 51.6% of the total variation in the dataset; Table 6.6; Figure 6.1). Porewater o-PO_4 concentrations were considerably higher in *H. vernicosus* (on average 0.79 µmol L^{-1}) than in *S. scorpioides*-dominated sites (on average 0.14 µmol L^{-1}), while the *S. cossonii* type preferred an intermediate niche (on average 0.24 µmol L^{-1}; Table 6.3). The total soil P-content was more than 2 times higher in *H. vernicosus*-dominated sites than in sites with *S. scorpioides* or *S. cossonii* (Table 6.4). The differences in soil P were mainly reflected in the P_{ox} concentrations, with *H. vernicosus*-dominated sites showing average concentrations that were respectively 10 and 4 times higher than sites with *S. scorpioides* or *S. cossonii* (Figure 6.2), constituting 35% of the total soil P-content compared to 15 and 19% for *S. scorpioides* and *S. cossonii* respectively.

Porewater NH_4 concentrations showed a comparable pattern for the three dominant moss species. Sites dominated by *H. vernicosus* were characterized by the highest concentrations (on average 15.9 µmol L^{-1}). In sites dominated by *S. cossonii* intermediate values were detected (on average 7.9 µmol L^{-1}). In *S. scorpioides*-dominated sites the lowest NH_4 concentrations were detected (on average 4.5 µmol L^{-1}) (Table

Table 6.6 Canonical coefficients and intraset correlations of environmental variables with the first two axes of CCA. For variable specifications see Figure 6.1.

	Axis 1		Axis 2	
Variable	Coefficients	Correlations	Coefficients	Correlations
Water table	-0.054	-0.344	-0.672	-0.121
Foliar N:P ratio	-0.670	-0.826	0.160	-0.010
Alkalinity porewater	-0.004	-0.184	-0.861	0.058
Ca porewater	0.011	-0.062	0.871	0.292
Ca soil	-0.008	-0.194	0.754	0.286
Fe porewater	-0.204	0.288	0.402	-0.097
Fe soil	0.164	0.471	0.736	0.089
NH_4 porewater	0.179	0.449	0.392	0.125
o-PO_4 porewater	0.392	0.502	-0.355	-0.186
P soil	0.095	0.559	-0.927	0.018

6.3). Porewater NO_3 and total soil N-content was not important in the distribution of brown mosses (*t*-values<2.1).

In addition to the strong positive correlation of total soil P-content, porewater o-PO_4 and NH_4 concentrations to axis 1, the CCA clearly indicates that the foliar N:P ratio has the strongest negative correlation to axis 1 (Table 6.6; Figure 6.1).

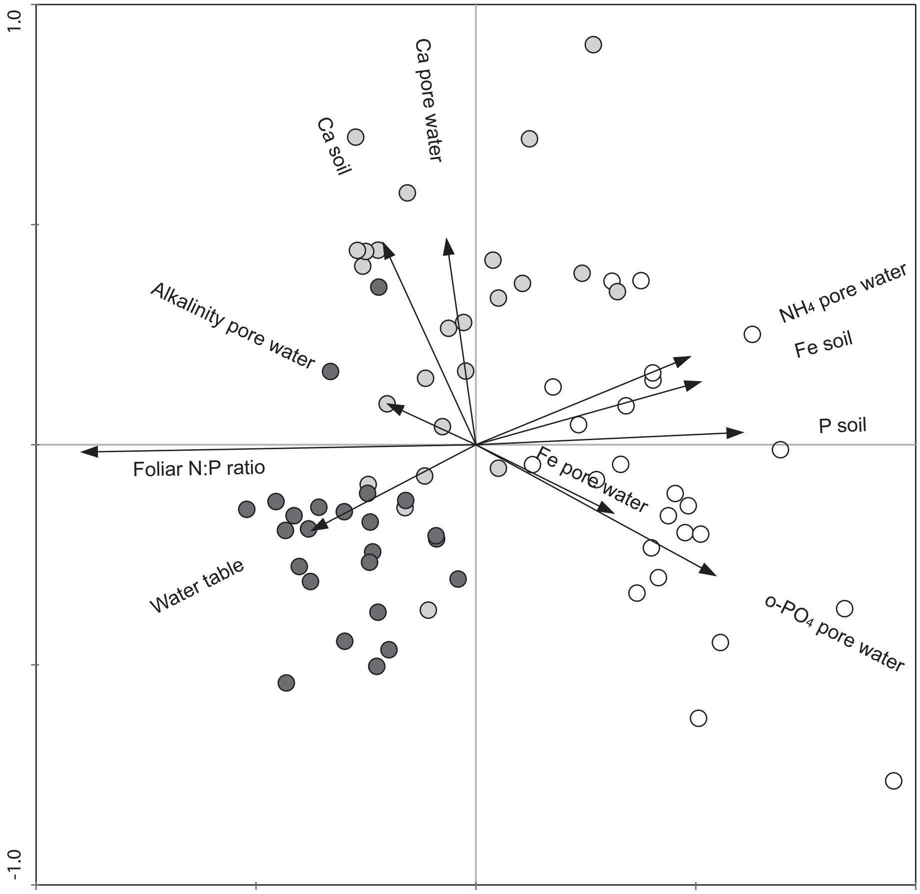

Figure 6.1 Canonical correspondence analysis (CCA) ordination diagram of all bryophyte relevés with environmental variables represented by arrows. The environmental variables are: water table (cm relative to soil surface); foliar N:P ratio in above-ground vascular plant biomass (g g^{-1}); alkalinity in porewater ($mmol_c$ L^{-1}); Ca, Fe, NH_4, o-PO_4 concentrations in porewater (μmol L^{-1}); and total Ca, Fe, P concentrations in the peat soil (mol m^{-2}). Vegetation types are based on dominant bryophyte spp. and indicated as follows: dark grey = *S. scorpioides*; light grey = *S. cossonii*; blank = *H. vernicosus*. The eigenvalues are 0.685 for axis 1 and 0.204 for axis 2.

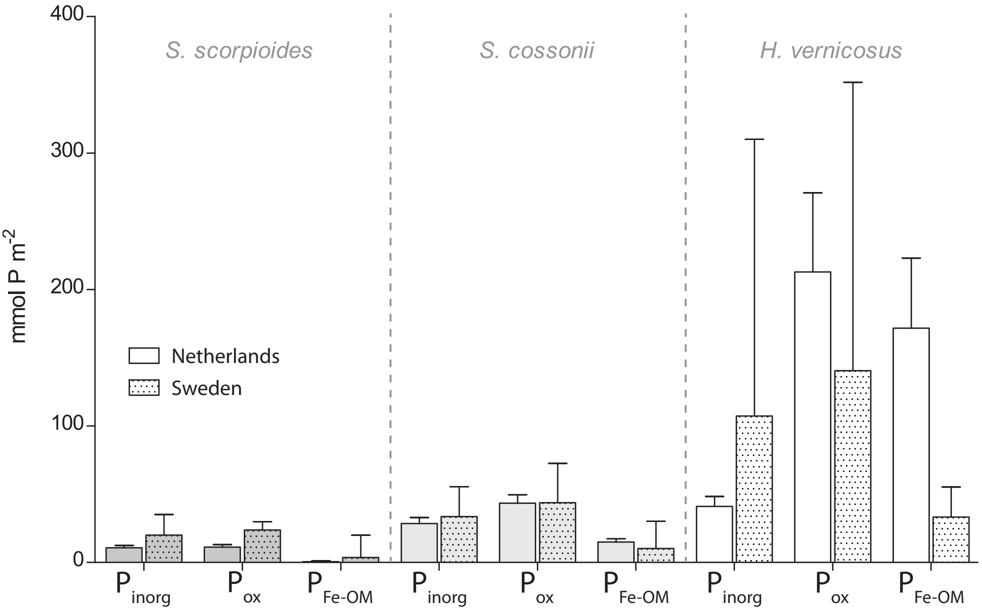

Figure 6.2 The inorganic P-content (P_{inorg}), concentrations of P bound to amorphous Fe and Al (P_{ox}), and the fraction of P potentially bound within Fe-OM complexes ($P_{Fe\text{-}OM}$). Sample means with standard deviations are indicated (*n* = 12).

Obviously, P-availability plays an important role in terms of the occurrence of the three brown mosses. Sites with *S. scorpioides* and *S. cossonii* were characterized by foliar N:P ratios above 16 g g^{-1}, while *H. vernicosus*-dominated sites showed foliar N:P ratios below 13.5 g g^{-1} (Table 6.5). In addition, foliar N:K ratios in the Dutch *H. vernicosus*-dominated site were around 2.3 g g^{-1}, while in all other sites the N:K ratio did not exceed 2.0 g g^{-1}.

Axis 2 of the CCA (explaining about 15.4% of the total variation in the dataset) most closely corresponds to porewater Ca-concentration and soil total Ca-content (Table 6.6; Figure 6.1), indicating that Ca-availability is the second important factor explaining the differences in occurrence of the three brown moss spp. Sites with *S. scorpioides* and *S. cossonii* were characterized by relatively high porewater Ca-concentrations and soil Ca-content compared to sites with *H. vernicosus* (Tables 6.3 and 6.4), although there was a large variation for each species. Porewater alkalinity showed similar, but less strong differences (Table 6.3). pH was not important for the differences in distribution of brown mosses (*t*-values<2.1) and was above 6.0 in all cases (Table 6.3).

Interestingly, the direction of the CCA arrow for Fe in porewater is nearly similar to that of total soil P and o-PO_4 in porewater, and opposite to porewater alkalinity (Figure 6.1). Rich fens with *H. vernicosus* were characterized by average porewater Fe-concentrations of 122 µmol L^{-1}, which were clearly higher than in rich fens dominated by *S. scorpioides* or *S. cossonii* (62 or 31 µmol L^{-1}; Table 6.3). Total soil Fe content showed even larger differences with 8.9 mol m^{-2} in rich fens with *H. vernicosus* versus 0.9 and 2.0 mol m^{-2} in rich fens with *S. scorpioides* and *S. cossonii*. These differences among brown mosses correspond to the P_{ox} measurements. Not only the crystalline and amorphous, inorganic Fe-fractions, but also the organic Fe-fractions were higher in the rich fens with *H. vernicosus* (Table 6.4).

In contrast to *S. scorpioides*- and *S. cossonii*-dominated soils, *H. vernicosus*-dominated soils were characterized by a relatively large fraction of P bound to amorphous Fe (P_{ox}; Figure 6.2). The considerable surplus of P_{ox} compared to P_{inorg} suggests a substantial share of $P_{Fe\text{-}OM}$, as described in paragraph 6.2. While in the *S. scorpioides*-dominated fens $P_{Fe\text{-}OM}$ accounted only for 1% of the total soil P-content and in *S. cossonii*-dominated fens only for 4%, for *H. vernicosus*-dominated fens it was 16% of the total soil P-content. On average, the $P_{Fe\text{-}OM}$ fraction even accounts for 50% of P_{ox} in *H. vernicosus*-dominated sites. The increase in Fe_{pyr} from *S. scorpioides* to *S. cossonii* and especially to *H. vernicosus* are in accordance with these estimates (Table 6.4).

In addition to nutrient-related differences, the level of the water table differed among the vegetation types. *S. scorpioides*-dominated fen sites were characterized by higher water tables of around +2.0 cm than sites with *S. cossonii* or *H. vernicosus*, where the average water table was around -1.2 cm (Table 6.4). The CCA diagram for all relevés confirms this, with S. scorpioides relevés clearly having the highest weighted averages for water table (Figure 6.1).

Ca and Fe, and nutrient availability

In Swedish rich fens, total soil Ca-content was negatively correlated with the P-content in plant tissue of vascular plants, and positively correlated with the foliar N:P ratio (Table 6.7). This negative correlation between soil Ca-content and P-availability was absent in the Netherlands (Table 6.8).

Fe seems to be more important than Ca in terms of P-availability, as total soil Fe-content showed positive correlations with the total soil P-content and with the P-concentrations in plant tissue for both the Netherlands and Sweden (Tables 6.7 and 6.8). In the Swedish fens, this increase in soil P-content with soil Fe-content was less strong than in the Dutch fens. In addition to tables 6.7 and 6.8, the estimated P-fraction in Fe-OM complexes ($P_{Fe\text{-}OM}$) showed a positive correlation with the soil Fe-content both in the Netherlands (r=0.90**) and in Sweden (r=0.39*). In the Netherlands, also porewater Fe-concentrations showed a positive correlation with P-contents in vegetation.

Table 6.7 Pearson correlation coefficients (*r*) between porewater concentrations (mol L^{-1}), soil concentrations (mol m^{-2}), biomass (g m^{-2}), concentrations in vegetation (g kg^{-1}) for all rich fen samples in Sweden ($n = 36$). Significant correlations are marked by dark grey table cells. Levels of significance are indicated as follows: *$P < 0.05$, **$P < 0.01$. p.w. = porewater.

Variable	Alkalinity p.w.	pH p.w.	Ca p.w.	Fe p.w.	$NH_4 + NO_3$ p.w.	o-PO_4 p.w.	Ca_{tot} soil	Fe_{tot} soil	P_{tot} soil	N_{tot} soil	Biomass	N veg	P veg	N:P veg
Alkalinity p.w.	1.00													
pH p.w.	0.73**	1.00												
Ca p.w.	0.99**	0.70**	1.00											
Fe p.w.	-0.26	-0.28	-0.44**	1.00										
$NH_4 + NO_3$ p.w.	0.04	-0.07	0.05	-0.15	1.00									
o-PO_4 p.w.	-0.31	-0.28	-0.30	0.18	-0.10	1.00								
Ca_{tot} soil	0.63**	0.65**	0.63**	-0.24	-0.08	-0.21	1.00							
Fe_{tot} soil	-0.23	0.07	-0.24	0.13	-0.10	0.31	-0.24	1.00						
P_{tot} soil	-0.11	-0.04	-0.07	0.01	-0.10	0.59**	-0.26	0.42*	1.00					
N_{tot} soil	-0.04	-0.40*	-0.01	-0.27	0.17	-0.24	-0.09	-0.39*	-0.07	1.00				
Biomass veg	-0.58**	-0.52**	-0.58**	0.46*	-0.14	0.50**	-0.40**	0.29*	0.06	-0.27	1.00			
N veg	0.17	0.36*	0.21	-0.02	-0.24	0.08	-0.09	0.36*	0.49**	-0.27	-0.40*	1.00		
P veg	-0.04	0.22	-0.01	0.06	-0.15	0.34*	-0.35*	0.38*	0.72**	-0.48**	0.13	0.82**	1.00	
N:P veg	0.17	-0.14	0.18	-0.19	0.23	-0.38*	0.36*	-0.39*	-0.53**	0.52**	-0.31	-0.71**	-0.86**	1.00

Table 6.8 Pearson correlation coefficients (r) between porewater concentrations (mol L^{-1}), soil concentrations (mol m^{-2}), biomass (g m^{-2}), concentrations in vegetation (g kg^{-1}) for all rich fen samples in the Netherlands (n = 36). Significant correlations are marked by dark grey table cells. Levels of significance are indicated as follows: *$P < 0.05$, **$P < 0.01$. p.w. = porewater.

Variable	Alkalinity p.w.	pH p.w.	Ca p.w.	Fe p.w.	$NH_4 + NO_3$ p.w.	o-PO_4 p.w.	Ca_{tot} soil	Fe_{tot} soil	P_{tot} soil	N_{tot} soil	Biomass	N veg	P veg	N:P veg
Alkalinity p.w.	1.00													
pH p.w.	0.74**	1.00												
Ca p.w.	0.74**	0.39*	1.00											
Fe p.w.	0.09	-0.02	-0.02	1.00										
$NH_4 + NO_3$ p.w.	-0.01	0.24	-0.13	-0.05	1.00									
o-PO_4 p.w.	-0.09	-0.15	-0.12	0.46**	-0.12	1.00								
Ca_{tot} soil	-0.20	-0.08	0.13	-0.25	-0.05	-0.23	1.00							
Fe_{tot} soil	-0.36*	-0.52**	0.04	0.21	-0.03	-0.02	0.21	1.00						
P_{tot} soil	-0.36*	-0.49**	-0.09	0.49**	0.00	0.18	0.17	0.90**	1.00					
N_{tot} soil	-0.52**	-0.50**	-0.13	0.37*	0.02	0.06	0.56**	0.72**	0.80**	1.00				
Biomass veg	0.16	-0.01	0.15	-0.22	-0.05	-0.26	-0.18	-0.08	-0.18	-0.27	1.00			
N veg	-0.22	-0.28	-0.03	0.74**	-0.07	0.41*	0.02	0.64**	0.79**	0.68**	-0.51**	1.00		
P veg	-0.17	-0.11	-0.06	0.76**	0.23	0.40*	-0.04	0.56**	0.76**	0.60**	-0.48**	0.90**	1.00	
N:P veg	0.22	-0.03	0.11	-0.32*	-0.26	-0.19	0.02	-0.40*	-0.48**	-0.27	0.24	-0.29	-0.71**	1.00

With regard to N-availability, correlations with Fe or Ca were less consistent (Tables 6.7 and 6.8). Although N-contents in plant tissue showed a positive correlation with soil Fe-contents for both countries, soil data showed clear differences. While in the Netherlands, soil N-contents strongly increased with the soil Fe-content, Sweden showed the opposite. Mineral N-concentrations in porewater (NH_4 + NO_3) showed no correlation with the Fe-content. Total soil Ca-content was positively correlated with the total soil N-content in the Netherlands, but not in Sweden. This discrepancy between soil and plant data suggests that plan N-uptake is less related to soil processes, and may rather be explained by physiological uptake processes.

Foliar N:P ratios showed a clear negative correlation with the soil Fe-content for both regions (Tables 6.7 and 6.8). While foliar N:P ratios were clearly above 20 g g^{-1} for most Fe-poor (and Ca-rich) fens, they decreased to values below 13.5 g g^{-1} for most Fe-rich fens. In addition to tables 6.7 and 6.8, foliar N:P ratios positively correlated with soil Ca_{tot}:P_{tot} ratios (r=0.20*), and negatively with soil Fe_{tot}:P_{tot} ratios (r=-0.21*).

In the Dutch rich fens, above-ground vegetation biomass neither correlated with soil Ca-content nor with soil Fe-content (Table 6.8). In the Swedish fens, however, aboveground biomass was affected by the soil Fe- and Ca-content in different ways. While the Ca-content showed a strong negative correlation, the soil Fe-content showed a positive correlation with vegetation biomass (Table 6.8). Also porewater Fe-concentrations showed a positive correlation with above-ground vegetation biomass in Sweden.

6.4. Discussion

The relative importance of Ca and Fe

P-availability

The relative abundances of Ca and Fe had differential effects on plant P-availability. In the Swedish rich fens, high soil Ca-contents obviously reduced P-availability. These findings correspond to the general idea of co-precipitation of PO_4 with calcite ($CaCO_3$) in calcareous rich fens (e.g. Boyer and Wheeler, 1989; Wassen et al., 1990). In the Netherlands, however, this correlation was absent, probably because soil Ca-contents were generally much lower than in Sweden. Moreover, soil Fe-content in general seems to be more important than soil Ca-content in terms of P-availability. Although P is bound to Fe compounds (e.g. Patrick and Khalid, 1974; Roden and Edmonds, 1997), soil Fe-content showed an obvious positive correlation with P-availability, giving rise to further discussion. We propose that the relatively weak binding of P within abundant Fe-OM complexes in rich fen soils together with high groundwater Fe discharge, inhibits the leaching of P from peat and leads to the accumulation of P that is still available to plants.

In rich fen peat soils, the high organic matter content can be expected to play an important role, as substantial amounts of Fe and P are present within Fe-OM complexes. Since the binding of P within soil Fe-OM complexes constitutes a reversible and relatively weak binding, part of this fraction is indirectly available through P-desorption by plant-mediated Fe complexation (release of chelating compounds) and/or rhizosphere acidification (Fixen and Grove, 1990; Marschner, 1995; Hinsinger, 2001). In Ca-rich and Fe-poor fens, the role of these Fe-OM complexes may very well be less important in terms of P-availability.

In addition, in Fe-rich soils, sulfate sorption onto Fe(oxy)hydroxides in the soil may pose a competitive reaction with binding of P. Under anaerobic field conditions, reduction of Fe and SO_4 may also lead to formation of FeS_x, strongly lowering the P-binding capacity (Smolders and Roelofs, 1993; Roden and Edmonds, 1997). The latter redox-related process may be less important in Ca-rich fens with a low soil Fe-content.

In both the Dutch and the Swedish rich fens, foliar N:P ratios of vascular vegetation were clearly above 20 g g^{-1} in Fe-poor, Ca-rich fens, strongly indicating P-limitation (Koerselman and Meuleman, 1996; Güsewell and Koerselman, 2002; Olde Venterink et al., 2003). In most Fe-rich fens, foliar N:P ratios were much lower, showing values below 13.5 g g^{-1} in both countries. This indicates a relatively high P-availability and potential limitation of N, as was previously also demonstrated in a fertilization experiment in an Fe-rich fen (Cusell et al., 2014). These findings imply that particularly the abundance of Ca, rather than Fe, is important for P-limited vegetation types, while abundance of Fe, and therefore of P, may even result in potential limitation of N.

Species composition

While sites with *S. scorpioides* and *S. cossonii* were characterized by P-limitation, *H. vernicosus*-dominated rich fens were characterized by N-limitation due to the high availability of P, which has also been suggested in former research (Cusell et al. 2013; 2014; Pawlikowski et al., 2013). The overall bryophyte and vascular plant composition in the three different brown moss-dominated sites confirms the more eutrophic conditions at *H. vernicosus*-dominated sites (Kooijman, 1992).

Also the findings that sites with *S. cossonii*, and to a smaller extent sites with *S. scorpioides*, were characterized by relatively high Ca-richness compared to sites with *H. vernicosus*, are in accordance with previous findings (Hedenäs, 1989; Hedenäs and Kooijman, 1996; Štechová et al., 2008; Pawlikowski et al., 2013). In addition, Fe-rich conditions were previously related to vital and large populations of *H. vernicosus* (Štechová et al., 2012), and a rare occurrence or even absence of *S. scorpioides* and *S. cossonii* (Kooijman and Hedenäs, 1991). We here show for the first time that the relative abundances of Ca and Fe explain the plant-availability of P, and that Ca and Fe turn out to be very important drivers of species composition in rich fens through their differential effects on plant P-availability, rather than on N-availability.

Next to nutrient-related differences, potential toxic effects of NH_4 and/or Fe cannot be ruled out (e.g. Snowden and Wheeler, 1993; Lamers et al., 2015). Especially *S. scorpioides* has been reported to be extremely sensitive to NH_4 stress (Paulissen et al. (2004).

Also the finding that *S. scorpioides* relevés clearly had higher water tables compared to *H. vernicosus* is in accordance with previous findings by Štechová et al. (2012), who reported on the low tolerance of *H. vernicosus* to inundation.

Above-ground biomass production

In general, the above-ground vegetation biomass in the Dutch rich fens was 2.5 times higher than in the Swedish rich fens, which can primarily be attributed to the warmer climate and longer growing season in the Netherlands. In addition, species that were most common or even exclusively present in the Netherlands were mainly relatively eutrophic, fast-growing species. Especially the bryophyte layer, comprising *C. cordifolium* and *C. cuspidata* reflected more eutrophic conditions compared to Sweden (Hedenäs and Kooijman, 1996). The higher nutrient-uptake by the vegetation per m^2 could additionally support the idea that differences in production were due to differences in nutrient supply between the two countries. Also the finding that porewater DOC-concentrations in the Dutch sites were 6.5 times higher than in the Swedish sites strongly suggests higher decomposition rates, and hence higher turnover of nutrients. Differences in trophic conditions were, however, neither reflected in porewater nutrient concentrations, nor total nutrient concentrations in the soil, suggesting higher turnover rates and uptake rates rather than accumulation.

The relative influence of Ca and Fe on biomass production was different between the Netherlands and Sweden. In the Swedish fens, with weak anthropogenic forcing, the biomass was affected by the soil Fe- and Ca-content in different ways. Ca-content showed a strong negative correlation, as expected based on the principle of Ca-related precipitation resulting in reduced P-availability (Boyer and Wheeler, 1989), and hence reduced biomass (Wassen et al., 1990). However, soil Fe-content, and also porewater Fe-concentrations, showed a positive correlation with biomass in Sweden, presumably because of increased uptake of both P and N by the vegetation in Fe-rich fens.

In the Dutch rich fens, however, neither soil Ca-content nor soil Fe-content was correlated with above-ground vegetation biomass, which implies that there are other factors and/or processes important in the Netherlands. The longer growing season and the generally more eutrophic conditions in the Netherlands, and/or physiological constraints such as maximum growth rates could possibly play an important role. Additionally, co-limitation of K should be considered, since production rates were not enhanced in the Dutch Fe-rich fens, despite the relatively high P- and N-availability. A foliar N:K ratio of more than 2.1 g g^{-1} in the Dutch Fe-rich *H. vernicosus*-dominated sites may suggest co-limitation of K besides N (Olde Venterink et al., 2003), possibly limiting production in the Dutch Fe-rich fens. Finally, vascular plant species in the Netherlands comprised more graminoids, which may be mainly N-limited while other species are P-limited in the same vegetation. Graminoid encroachment may be induced by atmospheric NH_4 deposition (Verhoeven et al., 2011), which is much higher in the Netherlands than in Sweden (EMEP, 2014; Slootweg et al., 2014), possibly infirming correlations between Fe, Ca and nutrient limitation or above-ground biomass production in the Netherlands.

Implications for fen management

The differences between fens in areas with weak and with strong anthropogenic forcing turned out to be very important, also within the context of management. In the Netherlands a higher ANC is required than in central Sweden to maintain similar pH values above 6.0, which seems to be a critical value for rich fens (Kooijman, 2012). These findings are most probably related to the higher atmospheric N-deposition in the Netherlands compared to Sweden (EMEP, 2014; Slootweg et al., 2014). This emphasizes the problem of acidification of rich fens in countries with a high anthropogenic pressure, and hence the chance of a shift from minerotrophic brown mosses to *Sphagnum* spp. (Kooijman, 2012). Furthermore, the higher production rates in the Netherlands pose a threat to biodiversity because of the high competitive strength of fast growing species. Therefore, frequent mowing is essential to maintain a high biodiversity in Dutch rich fens. Finally, the relative roles of Ca and Fe strongly differ with respect to nutrient limitation and vegetation development in rich fens, and should therefore be included in studies relating vegetation development to geohydrological conditions.

6.5. Conclusions

Ca and Fe turned out to be important drivers of species composition in rich fens through their differential effects on plant P-availability, rather than on N-availability. Fens dominated by *S. scorpioides* or *S. cossonii* were characterized by high porewater Ca-concentrations and total soil Ca-contents, but low P-availability. In these fens, Ca-P precipitation explains P-limitation. In contrast, fens dominated by *H. vernicosus* were characterized by high Fe-, but also high P-availability in the soil, explaining N-limitation. The remarkable positive correlation between soil Fe-content and P-availability contrasts the idea that high Fe-contents automatically lead to low values of plant-available P. We instead propose that high groundwater Fe discharge leads to the accumulation of P that is still available to plants due to the relatively weak binding of P within abundant Fe-OM complexes. Whereas in the Swedish fens with weak anthropogenic forcing P-availability also regulates total biomass production, it only determines vegetation composition and type of nutrient limitation in the Netherlands, where above-ground biomass was 2.5 times higher. Furthermore, Dutch rich fens were more acidic than Swedish rich fens, which is probably related to the much higher atmospheric N-deposition.

We conclude that the relative roles of Ca and Fe strongly differ with respect to nutrient limitation and vegetation development in rich fens, and should therefore be included in studies relating vegetation development to geohydrological conditions.

Acknowledgements

The authors wish to thank Leen de Lange, Bert de Leeuw, and Leo Hoitinga for analytical assistance in the laboratory of the University of Amsterdam, and Geert Kooijman for assistance in selecting field sites and conducting the fieldwork. Per-Olof Nystrand at the County Administrative Board of Jämtland assisted with obtaining collecting permits (Dnr 522-2464-12) and indicating suitable sampling locations for *H. vernicosus*. This research was financially supported by Kennisnetwerk Ontwikkeling en Beheer Natuurkwaliteit (O+BN) of the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

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Chapter 7

Synthesis: an assessment of ecological benefits and drawbacks

7.1 Introduction

For the proper functioning of endangered biodiverse rich fens, protected as EU priority habitat *H7140A – Transition mires and quaking bogs (Quaking fens)*, base-rich and nutrient-poor (mesotrophic) conditions are required to prevent succession towards *Sphagnum*-dominated peatlands. Unfortunately, water- and soil quality of many European wetlands are negatively affected by changes in hydrology, eutrophication, acidification and toxicity, resulting in a decline in brown moss-dominated, biodiverse rich fens. During the past decades, water levels in European rich fen areas have often become constricted within narrow limits as a result of adjacent agricultural water management.

From a management perspective, the re-establishment of fluctuating water levels in non-pristine fens is considered, in order to optimize the generic ecological quality and to conserve and restore the vegetation in current brown moss-dominated rich fens. This chapter provides a synthesis, in which results and conclusions from the preceding chapters in combination with results from previous studies are discussed, summarized and integrated in an overview of potential ecological benefits and drawbacks from a management perspective (Figure 7.1).

The research was primarily focused on the biogeochemical effects of water table fluctuations in peat soils, as induced by changes in the surface water level, and their interaction with plant development. The effects of water table fluctuations were tested for different fen types, since biogeochemical soil characteristics may largely differ among different fen types, as influenced by surface water and/or current or former discharge of groundwater. Soil Ca- and Fe-contents turned out to be very important, and therefore a general distinction is made in this synthesis between Ca-rich fens with Fe-poor soils (soil Ca-content>240 mmol kg^{-1} d.w., soil Fe-content<35 mmol kg^{-1} d.w.; representative for rich fens in the Dutch peatland area Weerribben-Wieden) and rich fens with lower soil Ca content, but Fe-rich soils (soil Ca-content<190 mmol kg^{-1} d.w., soil Fe-content>180 mmol kg^{-1} d.w.; representative for rich fens in the Dutch peatland area Oostelijke Vechtplassen). Also the differences in responses among different vegetation types were tested, in particular between brown moss-dominated rich fen vegetation and *Sphagnum*-dominated vegetation. In addition, water quality appeared to be an important factor, especially when fens are inundated from time to time. Therefore, different water qualities

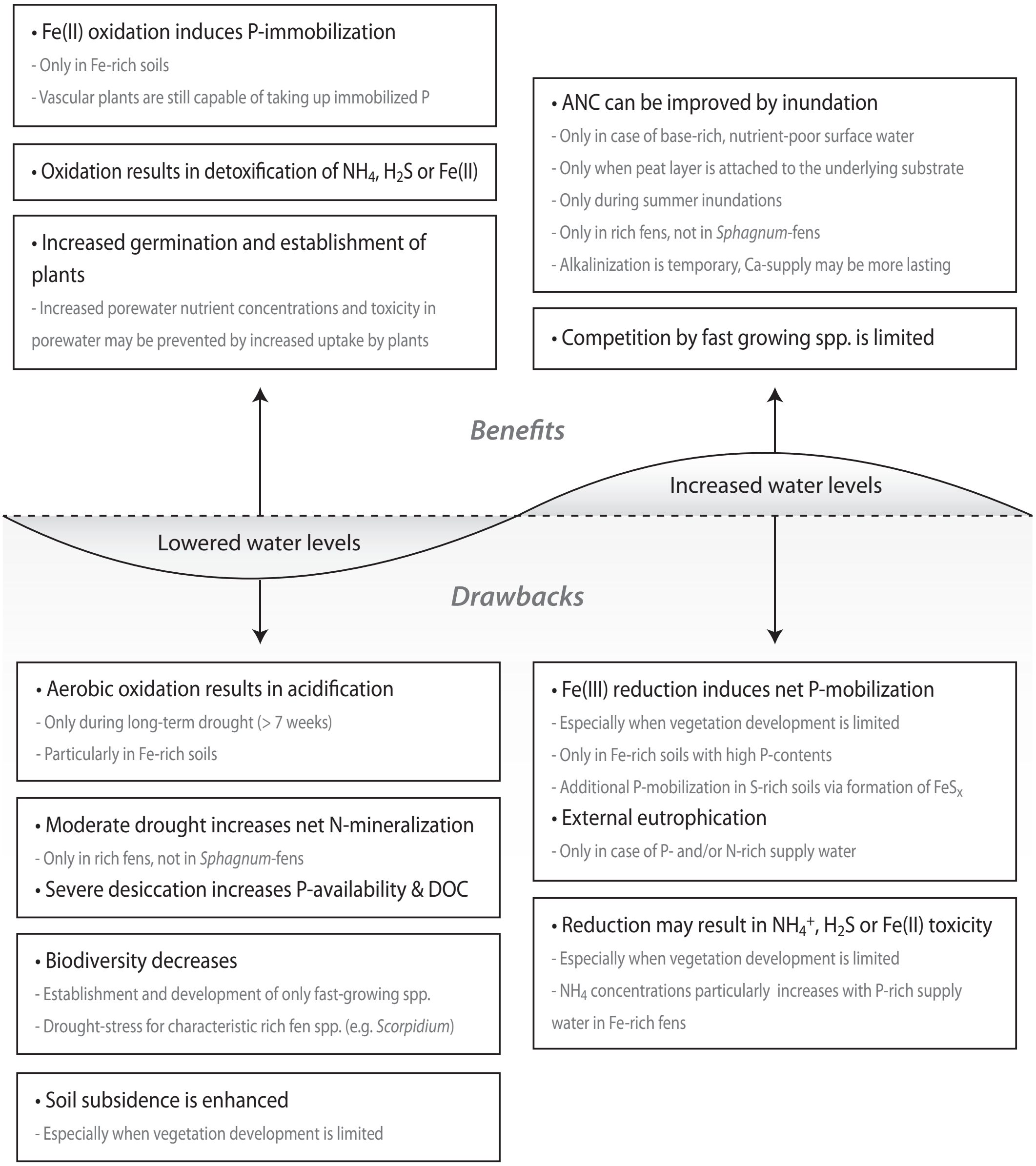

Figure 7.1 A summarizing overview of potential benefits and drawbacks of lowered and increased surface water levels, and hence changes in water tables in rich fen peat soils, on the level of site conditions (based on, and reformulated from Mettrop et al., 2012; Cusell et al., 2013; Cusell, 2014).

were included in the water level manipulation experiments. The potential benefits and drawbacks described in this synthesis are primarily based on the impact of fluctuating water levels on the environmental constraints as elucidated in Chapter 1. Following on from this, implications and recommendations are presented for different fen types to support water and nature management authorities in decision-making.

7.2 Higher incidence of lowered water levels

Potential benefits

Temporary drought may have a beneficial limiting effect on phosphorus (P)-availability, but this is highly dependent on the chemical composition of the peat soil. In rich fens with Fe-rich soils, temporary drought may be beneficial for P-limited vegetation types to some extent, since high rates of Fe-oxidation and subsequent Fe-P precipitation in the soil (Patrick and Khalid, 1974; Richardson, 1985) can temporarily reduce P-availability in porewater. Fe-related precipitation of P from the surface water can be important for development of aquatic vegetation in open water by the reduction of algal development, and hence for the rejuvenation of early stages of terrestrialization. However, in the context of preserving current rich fens, Fe-related P-immobilization in the soil may have totally different effects. As elucidated in Chapter 6, total soil Fe-content in rich fens is positively correlated with total soil P-content and P-concentration in plant tissue, and negatively correlated with foliar N:P ratios in vascular plants. In the most Fe-rich fens, N:P ratios were even below 13.5 g g^{-1}, indicating potential nitrogen (N)-limitation and excess of P. This remarkable positive correlation between soil Fe-content and P-availability contrasts the idea that high Fe-contents automatically lead to low values of plant-available P. Being true for the surface water in aquatic systems, this does not hold for peat soils in rich fens. Instead, high groundwater Fe discharge may lead to the accumulation of P that is still available to plants due to the relatively weak binding of P within abundant Fe-OM (Organic Matter) complexes. Enhanced P-immobilization during temporary droughts in Fe-rich fens is therefore not necessarily beneficial, which is not only an important finding from a management perspective, but moreover of great interest in terms of the general understanding of biogeochemical processes in peatlands. In addition, Ca-rich fens with low Fe-contents do not show a response in porewater P-availability to increased oxygen availability, because immobilization of P by co-precipitation with calcite is not induced or affected by aerobic conditions.

Aerobic oxidation during aeration can result in detoxification of NH_4^+, sulfide and Fe(II). Especially after a long period with anaerobic conditions, NH_4^+ concentrations can decrease during episodes with lowered water tables. For rich fen bryophytes, known to be sensitive to NH_4^+ toxicity (Paulissen et al., 2004; Verhoeven et al., 2011), occasional oxidation-induced detoxification may therefore be important.

In addition, a period of drought early in the growing season can considerably stimulate germination and establishment of vascular plants, leading to reduced porewater nutrient concentrations. Increased plant development, and hence increased plant activity, probably leads to increased radial oxygen loss (ROL) from roots (Lamers et al., 2012), stimulating nitrification in the rhizosphere, and increased uptake of N. Also P-consumption by plants can be strongly enhanced by increased growth rates in early spring. This can tone down the stimulating effect of P-availability on microbial activity (Amador and Jones, 1995; White and Reddy 2000), and hence mitigate toxicity of NH_4^+, Fe(II), sulfide and/or organic acids.

In addition to the effects on the level of site conditions as shown in Figure 7.1, surface water level fluctuations can have a major impact on a landscape scale. Allowing lowered surface water levels can result in a reduced need for external input of nutrient-rich water in the entire wetland (Coops and Hosper, 2002; Jaarsma et al., 2008; Schep et al., 2012). This is actually not a direct drought-induced effect, but primarily an effect of hydrological isolation. Moreover, this effect is strongly dependent on area-specific hydrological conditions and water quality. In De Weerribben, for instance, a short period in summer with water level drawdown of about 5 cm, did not seem to affect flow rates, flow directions and the chemical composition of surface waters (Cusell, 2014).

Potential drawbacks

One of the main risks with regard to drought is the stimulation of aerobic oxidation processes, which may lead to decreased soil acid neutralizing capacity (ANC) (Stumm and Morgan, 1996), and subsequently to vegetation changes. Also this effect strongly differs among fens with different biogeochemical characteristics. In Ca-rich fens with low soil Fe-contents, the effects of drought-induced oxidation and acidification are small, because Ca is not redox sensitive and changes in pH can be buffered (Stumm and Morgan, 1996). In rich fens with Fe-rich soils, however, acidification can be enhanced by Fe(II) oxidation, and hence pH values can drop to values below 6.0 after a drought period of 10 weeks, which is considered a critical pH value for rich fens dominated by brown mosses (Kooijman, 2012). During short-term drought (7 weeks), however, ANC remained sufficiently high in both rich fen types to prevent a severe drop in pH.

Lowering of pH as a result of a lowered water table is assumed to be temporary. When the water table is increased again, most of the protons produced are consumed due to the anaerobic reduction of alternative electron acceptors. However, a temporary decrease in pH may lead to suitable conditions for dominance by *Sphag-*

num-species that further acidify the habitat, which in turn can lead to severe decline of the rich fen bryophyte vegetation.

As a result of moderate drought of about 10 weeks, in which only the oxygen availability increases but the peat soil remains moist, carbon respiration rates can increase, but only in rich fens and not in *Sphagnum*-dominated fens. In the latter fen type, decomposition is probably not only limited by oxygen deficiency, but also by the high concentrations of phenolic compounds in *Sphagnum*-litter (Van Breemen, 1995; Aerts et al., 2001). Rich fens presumably contain lower concentrations of phenolic compounds. Also, the low pH in *Sphagnum*-fens may have been relatively unfavorable for the degradation of phenolic material (Pind et al., 1994). In rich fens, stimulation of phenol oxidase activity by aeration may therefore lead to phenol-concentrations that are low enough for other degradative enzymes in rich fens to be active, such as glucosidase and phosphatase (Freeman et al., 2004), resulting in increased respiration upon aeration in rich fens.

An important question with respect to vegetation is: does this increase in decomposition rates upon 10 weeks of aeration in rich fens also result in increased nutrient availability? Aeration only resulted in increased net N-mineralization in rich fens. Net N-mineralization rates in *Sphagnum*-fens were, however, not affected by increased availability of oxygen, which is attributed to differences in concentrations of phenolic compounds and degradative enzymes, like for respiration. No significant differences in net P-release were detected during aeration, even though respiration rates increased. This may be caused by the fact that released P can be bound immediately after mineralization, for example as Fe-phosphates or Ca-phosphates. Especially the formation of Fe(III) oxides in rich fens with Fe-rich soils may reduce net P-mobilization during aeration, as explained in the previous section.

Upon severe drought of about 10 weeks, i.e. total desiccation, DOC production increased considerably, which may be related to die-off of microbes as a result of water shortage, by which cellular constituents are released. This idea was indeed supported by a decreasing microbial C mass upon total desiccation. In contrast to aeration, full desiccation led to an enormous increase of net P-release. High microbial mortality resulting from drought, as supported by the reduced microbial biomass C upon desiccation, may have resulted in a net increase of extractable o-PO_4 concentrations.

A high drought incidence can also have direct effects via drought stress in vascular plants and bryophytes. As a result, typical wetland plant communities may be replaced by vegetation favored by drier conditions (Lamers et al., 2015). Since rates of N-mineralization and P-immobilization by Fe(III) compounds both increase, the encroachment of dryland graminoid species may be stimulated at the expense of characteristic brown moss and slow-growing vascular species (Verhoeven et al., 2011; Cusell et al., 2014). Subsequently, increased biomass production can lead to a less diverse species composition and may offer less room and light for rich fen mosses. In addition, drought may lead to favorable conditions for *Sphagnum* spp. at the expense of brown mosses. While *Sphagnum* spp. are well able to tolerate both water shortages and acid conditions (Rochefort et al. 1990), vitality of brown mosses such as *Scorpidium* spp. strongly decreases during 7 weeks of drought.

Drought can also lead to subsidence of the peat soil surface, particularly when preceded by inundation. This suggests that subsidence is not solely due to reduced buoyancy by release of entrapped gas bubbles (Strack et al., 2006), or increased decomposition rates as a result of aeration. Presumably, subsidence is further induced by the reduced vegetation development during prior inundation, which led to inhibited root growth and lower stability of the peat soil.

Lowered water levels: conclusions and implications for management

All in all, the potential drawbacks seem to be more important than the potential benefits of temporary lowered surface water levels, and related lowered water tables in the peat soil. The combined effects of enhanced acidification (particularly in Fe-rich soils), increased nutrient mineralization, direct drought-stress for brown mosses, improved conditions for *Sphagnum* spp., and increased biomass production by fast-growing species will strongly hamper the development of protected brown moss vegetation in rich fens. A drought-induced vegetation shift from rich fen vegetation (H7140A; Figure 1.1) to *Sphagnum*-dominated fens (H7140B), as described in Chapter 1, poses a major threat for the conservation of rich fen biodiversity. Long-term (>7 weeks) aeration and especially desiccation of the top 10 cm of the soil in rich fens should therefore be avoided.

7.3. Higher incidence of increased water levels

Potential benefits

In terms of counteracting acidification of rich fens, shallow short-term summer inundation with base-rich surface water can be an efficient measure. In contrast to winter inundation, raising surface water levels in summer, when evapotranspiration rates are high, results in infiltration, and hence an increase of ANC. Secondly, internal alkalinity generation, as a result of anaerobic microbial redox processes (Stumm and Morgan, 1996), is enhanced by higher temperatures in summer. The latter effect will be temporary, since aerobic oxidation during subsequent droughts can lead to re-acidification. The first process of infiltration of Ca-rich water, however, may contribute to a lasting increase in the peat soil ANC, as the ANC is not only determined by the amount of bicarbonate in porewater in the circum-neutral pH range, but also by the saturation of Ca and Mg at the adsorption complex in the pH range below (Stumm and Morgan, 1996). The ability of rich fen soils to exchange H^+ for Ca^{2+} from the adsorption complex, and thereby buffer porewater pH, will be highly beneficial to counteract acidification during subsequent periods of drought in particular, when bicarbonate has been largely consumed and base cation exchange against H^+ initiates.

In *Sphagnum*-dominated fens, however, ANC remains relatively low during summer inundation with base-rich surface water, presumably due to exchange of Ca^{2+} from inundation water for H^+. In addition, *S. palustre* is well able to endure periods of inundation. Even with base-rich inundation water, which was assumed to cause problems since *Sphagnum* spp. are generally associated with and adapted to acidic conditions, *S. palustre* thrived remarkably well. Short-term summer inundation with base-rich water as a measure seems therefore only efficient in places where base-rich conditions still prevail. At the point when *Sphagnum* spp., which are able to acidify its environment, have already made their entry over a large surface area, short-term inundation is less effective. Therefore, short-term summer inundation is primarily considered a preventive measure, in order to maintain current brown moss-dominated rich fens.

Short-term summer inundation as a measure is, however, only considered beneficial under specific conditions. First, raising the surface water level has the strongest effect when the peat layer is attached to the underlying substrate by roots, because otherwise inundation is difficult due to peat buoyancy. Raising water levels in ditches and canals without actual inundation will only gradually affect the fens, and often only increases ANC in the first couple of meters as lateral water movement through the peat soil is very slow due to its low hydrological conductivity (Lamers et al., 2015). In floating *Sphagnum*-dominated fens, raising surface water levels does not result in inundation, because the buoyant peat follows changes in surface water levels and the water table in the peat soil remains unaltered. This, however, may be different in floating rich fens. As shown by aerial footage of the National Park Weerribben-Wieden (Cusell, 2014), inundation of floating rich fens with *Scorpidium* spp. is not uncommon. In the case of floating rich fens, the origin of inundation water and the mechanism by which inundation takes place is still subject to debate. In young fens, seepage from beneath the floating peat mat may be involved, but in later stages, flooding of surface water from adjacent ditches over the peat mat is more likely. Since these questions are relevant for management, it is important that further research is conducted on flooding in floating rich fens.

Inundation may have additional effects on vegetation development. A wet period in early spring, resulting in reduced vegetation development, is not necessarily detrimental for mesotrophic peatlands. Competition by fast growing species may be limited this way, which may eventually result in increased biodiversity. In addition, inundation, or at least waterlogging, turned out to be vital for rich fen bryophytes.

Potential drawbacks

During inundation, P-availability may increase as a result of net P-mobilization (internal eutrophication) due to Fe reduction (Patrick and Khalid, 1974). Especially in Fe-rich soils with high P-contents, this rapid anaerobic P-mobilization can be severe. Moreover, high sulfate reduction rates and formation of iron sulfides (FeS_x) may lead to decreased P-binding capacity of the peat sediment, and hence additional P-mobilization in S-rich soils (Caraco et al., 1989; Smolders and Roelofs, 1993; Lamers et al., 1998). This net internal P-mobilization may be lower upon inundation after a period of several weeks of drought in spring, as a result of increased growth rates and P-consumption by plants. In rich fens with high Ca-contents, however, net P-mobilization during inundation is relatively low. This can be explained by the fact that most P is bound to Ca, which is not sensitive to oxidation-reduction processes (Stumm and Morgan, 1996).

Increased surface water influence, as a result of inundation, can also lead to higher nutrient inputs (external eutrophication) (e.g. Koerselman and Verhoeven, 1992; Wassen et al., 1996). In relatively nutrient-poor (mesotrophic) fens adjacent to agricultural areas, external P-input can be highly detrimental (Lamers et al., 2015). This effect may also strongly depend on biogeochemical characteristics of the peat soil, in which particularly a high soil Ca-content, and to a lesser extent a high Fe-content, can be beneficial because of P-immobilization.

Anaerobic conditions can also lead to the formation of potential phytotoxins such as NH_4^+, sulfide, Fe(II) and/or organic acids to plants, depending on soil chemistry (Lamers et al., 2015). Plant growth can strongly be hampered during inundation in early spring shortly after winter, when the vegetation has had little chance to develop and ROL is still low, due to accumulation of these toxins. In Fe-rich fens, inundation with P-rich water can lead to porewater Fe-concentrations over 1000 µmol L^{-1}, and NH_4^+ concentrations well over 100 µmol L^{-1}, a level above which toxic effects can seriously damage bryophyte vegetation under summer conditions (Paulissen et al., 2004; Verhoeven et al., 2011). In Ca-rich fens, the relatively high pH presumably stimulates nitrification in the topsoil (Wild et al., 1971), and NH_4^+ concentrations generally do not exceed 100 µmol L^{-1} (e.g. Rochefort and Vitt, 1988; Kooijman and Westhoff, 1995).

Increased water levels: conclusions and implications for management

In contrast to drought, periods of inundation with base-rich water in summer can be favorable in order to structurally improve the porewater ANC. Both supply of base-rich water and internal soil alkalinization in the topsoil of Ca-rich fens that lack sufficient HCO_3- and Ca-buffering can be important to prevent acidification. In rich fens with Ca-rich soils and low Fe-contents, the potential benefits of temporary inundation outweigh potential drawbacks, since short-term inundation is not harmful in terms of P-mobilization. In rich fens with Fe-rich soils, however, inundation should be prevented shortly after winter, when vegetation development, hence P-consumption by plants, is still limited. Especially inundation with P-rich water seems to stimulate microbial activity, despite Fe-related precipitation of P, resulting in NH_4 and/or Fe(II) toxicity. For Fe-rich fens in agricultural areas, this may well generate a friction between preventing acidification and N-eutrophication during drought on the one hand, and preventing external eutrophication and accumulation of toxins during inundation on the other hand. Therefore, improved water quality is a primary requirement in these fens.

7.4 Concluding remarks

This synthesis identifies the potential benefits and drawbacks of re-establishment of fluctuating water levels in non-pristine fens in order to conserve and restore the vegetation in current brown moss-dominated, biodiverse rich fens. Area-specific chemical properties of peat soils and surface water, as determined by the geohydrological setting in the landscape, turned out to strongly determine the responses to surface water level-induced water table fluctuations in the peat soil. Especially the Ca- and Fe-contents of peat soils proved to be very important factors. In addition, the timing of temporary drought or inundation in the growing season turned out to be important, because of the strong interaction between biogeochemical processes and vegetation development. These findings not only contribute to the general biogeochemical and ecological understanding of water level-induced processes in rich fens, but are also valuable to support water and nature management authorities in decision-making.

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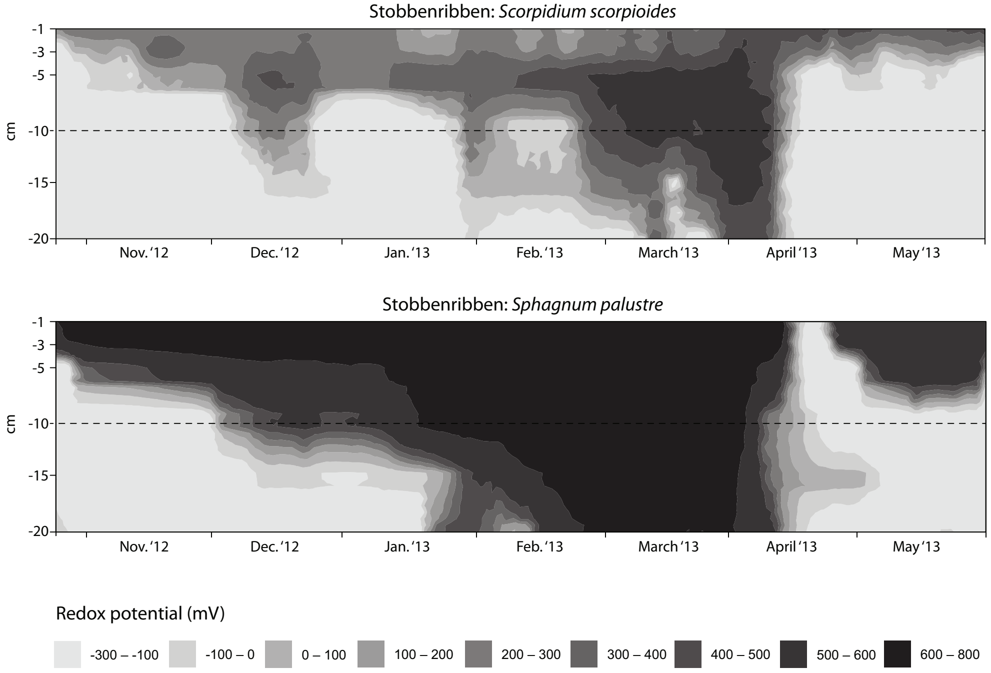
Supplementary data

Appendix A



Appendix A In both a rich fen and a *Sphagnum*-dominated fen in the area Stobbenribben, the redox potential (E_h) in the upper 20 cm was measured over a period of seven months in order to assess the extent to which oxygen availability increases as a result of drought. Permanently installed redox electrodes, connected to a Hypnos data logger (MVH Consult, Leiden, the Netherlands) were used to record E_h at -1 cm, -3 cm, -5 cm, -10 cm, -15 cm, and -20 cm, every 15 minutes (Vorenhout et al., 2011). The redox profiles indicate that periods of more than two months of drought can have major impacts on the redox potential in the upper 10 cm of the soil in both rich fens and *Sphagnum*-fens. The relatively dry spring of 2013 led to a significant increase of E_h, up to the time that long-term precipitation led to a significant decrease of E_h in April 2013. Especially in *Sphagnum*-fens the redox potential was affected by drought, as E_h values of +600 mV were measured up to a depth of -20 cm. These data indicate that long periods of drought do occur in intact peatlands, and therefore long-term laboratory incubation results, as reported in chapter 2, are representative and useful for assessing the effects of drought in the field.

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Appendix B

Appendix B.1 Effects of fen site, vegetation type and their interaction on chemical variables in porewater at the start of the experiment in July 2008. *F*-ratios (ANOVA) are shown with their level of significance and the results of Tukey HSD post-hoc tests.

Start of the experiment in July 2008	Veg*Area				Area						Veg						
	df denom.	df num.	*F*	*P*	df num.	*F*	*P*	KW	VW	WEE	df num.	*F*	*P*	Scor	Call	Sph	Moor
Water table	43	5	1.57	0.188	2	1.83	0.172	a	a	a	3	13.46	0.000	b	b	a	a
pH	42	5	2.58	0.040	2	6.32	0.004	a	b	a	3	29.46	0.000	b	b	a	a
Alkalinity	40	5	2.61	0.039	2	2.46	0.098	a	a	a	3	21.50	0.000	b	b	a	a
Ca	41	5	4.32	0.003	2	10.44	0.000	b	c	a	3	17.50	0.000	c	b	a	a
Fe	43	5	2.19	0.073	2	2.63	0.063	a	a	a	3	0.83	0.486	a	a	a	a
S	40	5	2.32	0.061	2	1.64	0.207	a	a	a	3	1.10	0.359	a	a	a	a
Cl	41	5	1.02	0.419	2	6.72	0.003	b	b	a	3	10.50	0.000	b	b	a	a
o-PO_4	42	5	0.62	0.689	2	0.32	0.727	a	a	a	3	0.95	0.427	a	a	a	a
NH_4	42	5	1.95	0.106	2	0.83	0.445	a	a	a	3	0.94	0.428	a	a	a	a
NO_3	42	5	0.62	0.689	2	2.20	0.124	a	a	a	3	1.42	0.253	a	a	a	a

Appendix B.2 Effects of water level treatment on the water tables and chemical variables in porewater of different vegetation types during successive years for the five different field experiments. As response variables, the differences between the measurements right after and right before water level treatment were used. For the water levels, the differences between the measurements during and right before the increase in surface water level were also tested. *F*-ratios (linear mixed models) are shown with their level of significance. Differences between vegetation types were further examined by comparing their estimated marginal means in a LSD post-hoc tests. Experiment 1 = surface water level increase in a floating fen (WEE-area); experiment 2 = surface water level increase in a non-floating fen (KW-area); experiment 3 = wet periods in July, with about 50 mm of rain in two weeks (3.5–4 mm/day) in a non-floating fen (VW-area); experiment 4 = surface water level decrease in a floating fen (WEE-area); experiment 5 = surface water level decrease in a non-floating fen (KW-area). For NH_4 and NO_3 in experiment 3, a one-way ANOVA was used to determine significant differences between vegetation types, since NH_4 and NO_3 were not measured well in 2009.

Experiment 1		Veg*Year				Year						Veg						
	Period	df num.	df denom.	*F*	*P*	df num.	df denom.	*F*	*P*	2009	2010	df num.	df denom.	*F*	*P*	Call	Sph	Moor
Water level	start	2	10.1	1.36	0.301	1	10.1	1.06	0.328	a	a	2	10.9	2.58	0.121	a	a	a
	during-start	2	10.6	0.06	0.947	1	10.9	0.03	0.859	a	a	2	9.4	10.95	0.004	a	a	b
	end-start	2	10.2	2.68	0.116	1	10.3	1.58	0.236	a	a	2	10.7	0.88	0.445	a	a	a
pH	end-start	2	9.2	1.09	0.375	1	9.2	0.80	0.393	a	a	2	10.2	0.80	0.474	a	a	a
Alkalinity	end-start	2	7.6	3.72	0.075	1	7.7	4.89	0.060	a	a	2	8.9	0.46	0.674	a	a	a
Ca	end-start	2	11.0	0.57	0.584	1	11.0	0.14	0.712	a	a	2	11.1	0.10	0.904	a	a	a
Fe	end-start	2	10.0	2.70	0.115	1	10.1	0.22	0.652	a	a	2	10.4	0.22	0.810	a	a	a

S	end-start	2	8.9	0.01	0.989	1	8.9	4.97	0.053	a	a	2	8.9	1.09	0.379	a	a	a
Cl	end-start	2	7.6	0.01	0.987	1	7.9	4.50	0.065	a	b	2	6.9	0.40	0.683	a	a	a
o-PO$_4$	end-start	2	9.5	1.70	0.223	1	9.5	1.70	0.223	a	a	2	10.4	2.07	0.176	a	a	a
NH$_4$	end-start	2	8.0	1.72	0.239	1	8.0	2.40	0.160	a	a	2	8.1	1.74	0.235	a	a	a
NO$_3$	end-start	2	8.6	0.45	0.654	1	8.6	0.44	0.523	a	a	2	8.5	3.27	0.088	a	a	a

Experiment 2		Veg*Year				Year								Veg							
	Period	df num.	df denom.	*F*	*P*	df num.	df denom.	*F*	*P*	2008	2009	2010	2011	df num.	df denom.	*F*	*P*	Scor	Call	Sph	Moor
Water level	start	6	15.5	2.12	0.109	2	15.5	157.44	0.000		c	b	a	3	16.2	10.22	0.001	b	b	a	a
	during-start	6	14.6	3.25	0.031	2	14.6	115.99	0.000		a	b	c	3	15.1	8.29	0.002	c	bc	a	ab
	end-start	6	15.7	0.88	0.529	2	15.8	136.72	0.000		a	b	c	3	16.2	1.25	0.324	a	a	a	a
pH	end-start	9	13.7	4.11	0.010	3	13.8	12.46	0.000	b	b	b	a	3	14.7	13.99	0.000	c	c	b	a
Alkalinity	end-start	9	8.4	7.80	0.003	3	8.5	28.35	0.000	a	ab	a	b	3	14.5	3.76	0.035	b	ab	a	a
Ca	end-start	9	15.0	3.54	0.015	3	15.1	13.82	0.000	b	b	a	c	3	16.0	0.35	0.789	a	a	a	a
Fe	end-start	9	11.4	1.93	0.148	3	11.5	8.22	0.003	b	b	a	b	3	8.6	1.38	0.313	a	a	a	a
S	end-start	9	11.9	2.72	0.055	3	11.4	1.27	0.332	a	a	a	a	3	14.5	1.42	0.276	a	a	a	a
Cl	end-start	9	15.7	7.18	0.000	3	15.5	70.64	0.000	ab	a	b	c	3	16.7	1.27	0.318	a	a	a	a
o-PO$_4$	end-start	9	13.5	1.30	0.321	3	13.1	12.79	0.000	ab	a	b	a	3	14.1	0.88	0.474	a	a	a	a
NH$_4$	end-start	9	7.0	2.40	0.131	3	6.9	2.39	0.156	a	a	a	a	3	10.6	1.05	0.409	a	a	a	a
NO$_3$	end-start	9	15.9	1.70	0.171	3	15.9	2.70	0.080	a	a	a	a	3	16.1	1.72	0.203	a	a	a	a

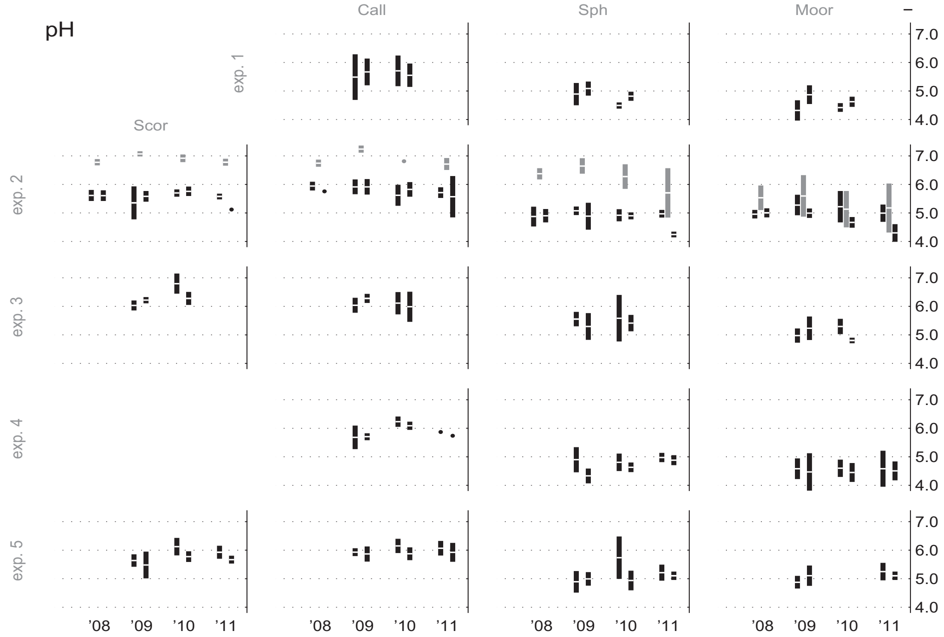
Experiment 3		Veg*Year				Year						Veg							
	Period	df num.	df denom.	*F*	*P*	df num.	df denom.	*F*	*P*	2008	2009	df num.	df denom.	*F*	*P*	Scor	Call	Sph	Moor
Water level	start	3	16.0	2.21	0.126	1	16.0	289.96	0.000	b	a	3	16.0	15.26	0.000	c	b	a	a
	during-start	3	16.0	3.13	0.055	1	16.0	1.61	0.222	a	a	3	16.0	1.15	0.361	a	a	a	a
	end-start	3	16.0	2.70	0.080	1	16.0	0.35	0.565	a	a	3	16.0	0.99	0.424	a	a	a	a
pH	end-start	3	15.4	3.01	0.062	1	15.9	1.03	0.325	a	a	3	16.8	2.24	0.122	a	a	a	a
Alkalinity	end-start	3	10.9	4.04	0.037	1	10.9	16.87	0.002	b	a	3	10.9	11.94	0.001	b	b	a	a
Ca	end-start	3	12.8	3.88	0.049	1	12.8	8.48	0.012	b	a	3	11.0	11.17	0.001	b	bc	ab	a
Fe	end-start	3	13.7	3.86	0.034	1	13.7	25.55	0.000	b	a	3	13.3	6.10	0.008	ab	bc	c	a
S	end-start	3	13.5	0.36	0.784	1	13.5	0.35	0.561	a	a	3	14.6	0.67	0.584	a	a	a	a
Cl	end-start	3	10.8	1.13	0.380	1	10.9	21.71	0.001	a	b	3	10.3	2.43	0.124	a	a	a	a
o-PO$_4$	end-start	3	15.7	1.45	0.266	1	15.8	1.40	0.255	a	a	3	13.2	1.63	0.231	a	a	a	a
NH$_4$	end-start											3	11.0	0.43	0.739	a	a	a	a
NO$_3$	end-start											3	11.0	1.36	0.306	a	a	a	a

Experiment 4		Veg*Year				Year							Veg						
	Period	df num.	df denom.	*F*	*P*	df num.	df denom.	*F*	*P*	2009	2010	2011	df num.	df denom.	*F*	*P*	Call	Sph	Moor
Water level	start	4	9.7	1.31	0.331	2	9.5	25.03	0.000	a	a	b	2	10.9	1.91	0.194	a	a	a
	during-start	4	10.3	0.40	0.807	2	10.3	15.01	0.001	b	a	a	2	9.4	19.20	0.000	b	b	a
	end-start	4	10.0	0.32	0.857	2	9.9	15.89	0.001	a	c	b	2	9.7	0.18	0.842	a	a	a
pH	end-start	4	8.9	0.54	0.712	2	8.8	0.24	0.789	a	a	a	2	11.6	0.73	0.504	a	a	a
Alkalinity	end-start	4	7.7	9.10	0.005	2	7.9	10.18	0.006	b	a	b	2	10.2	1.96	0.190	a	a	a
Ca	end-start	4	9.7	1.20	0.371	2	9.5	3.88	0.058	a	a	a	2	11.1	3.04	0.088	a	a	a

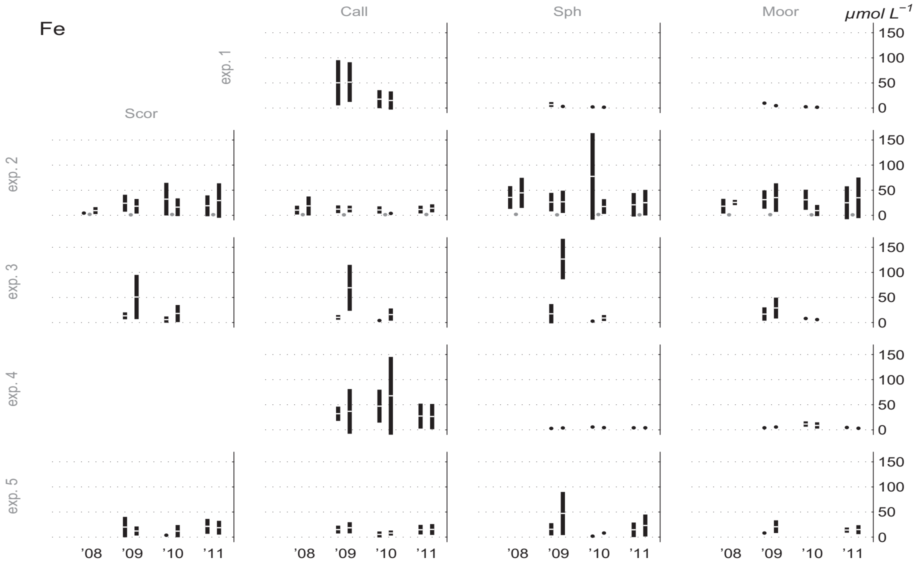
Fe	end-start	4	4.6	0.81	0.574	2	4.6	0.26	0.781	a	a	a	2	10.0	0.40	0.680	a	a	a
S	end-start	4	7.1	3.08	0.091	2	7.5	5.15	0.039	ab	b	a	2	7.2	0.56	0.597	a	a	a
Cl	end-start	4	8.5	22.79	0.000	2	8.5	22.81	0.000	c	b	a	2	4.9	14.84	0.009	b	a	a
o-PO_4	end-start	4	9.9	6.40	0.008	2	9.9	4.60	0.039	b	a	ab	2	10.6	2.85	0.102	a	a	a
NH_4	end-start	2	8.7	2.07	0.184	1	10.7	0.11	0.742		a	a	2	8.9	3.38	0.081	a	a	a
NO_3	end-start	2	10.2	0.26	0.777	1	10.5	2.73	0.128		a	a	2	10.4	0.04	0.961	a	a	a

Experiment 5		Veg*Year				Year							Veg							
	Period	df num.	df denom.	*F*	*P*	df num.	df denom.	*F*	*P*	2009	2010	2011	df num.	df denom.	*F*	*P*	Scor	Call	Sph	Moor
Water level	start	6	15.7	9.11	0.000	2	15.8	209.47	0.000	b	a	c	3	16.0	25.74	0.000	c	c	b	a
	during-start	6	16.0	31.83	0.000	2	16.0	167.02	0.000	b	c	a	3	16.4	6.97	0.003	a	a	a	b
	end-start	6	13.7	8.20	0.001	2	13.7	26.90	0.000	b	b	a	3	16.1	5.53	0.008	ab	a	a	b
pH	end-start	5	10.5	1.09	0.419	2	11.1	2.87	0.099	a	a	a	3	12.2	0.60	0.624	a	a	a	a
Alkalinity	end-start	5	14.4	3.10	0.042	2	17.4	8.59	0.003	b	a	a	3	7.8	0.63	0.614	a	a	a	a
Ca	end-start	5	13.4	0.64	0.672	2	13.4	4.19	0.039	a	b	a	3	10.5	0.30	0.823	a	a	a	a
Fe	end-start	5	9.1	1.48	0.287	2	11.1	1.61	0.244	a	a	a	3	10.0	1.39	0.302	a	a	a	a
S	end-start	5	6.0	1.50	0.317	2	8.1	2.68	0.128	a	a	a	3	3.3	11.62	0.029	b	b	b	a
Cl	end-start	5	15.0	1.73	0.189	2	18.8	63.01	0.000	a	b	a	3	16.2	2.31	0.114	a	a	a	a
o-PO_4	end-start	5	10.4	1.99	0.162	2	10.6	1.61	0.244	a	a	a	3	9.0	1.84	0.210	a	a	a	a
NH_4	end-start	2	13.4	0.14	0.669	1	14.0	0.35	0.564		a	a	3	12.4	1.29	0.320	a	a	a	a
NO_3	end-start	2	8.2	1.06	0.390	1	8.6	3.86	0.083		a	a	3	14.6	2.76	0.080	a	a	a	a

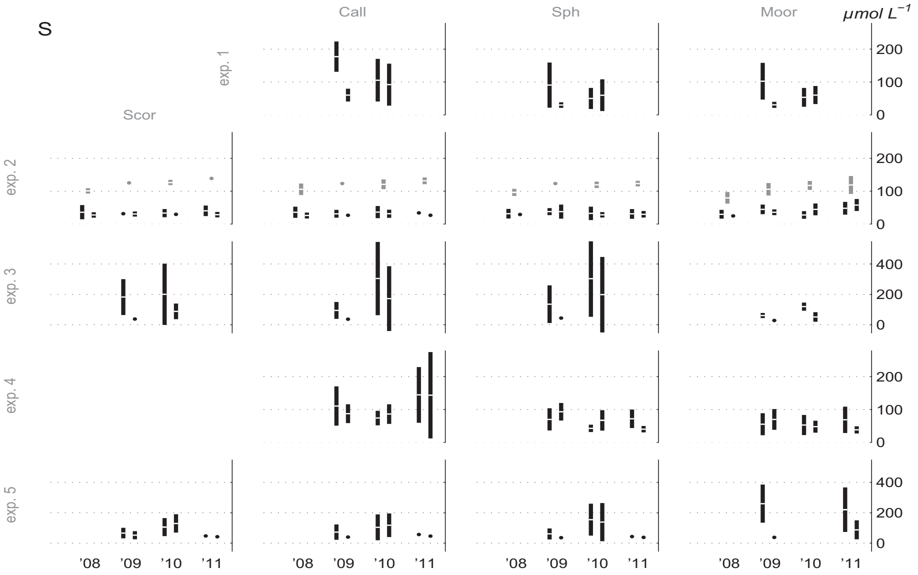
Appendix C



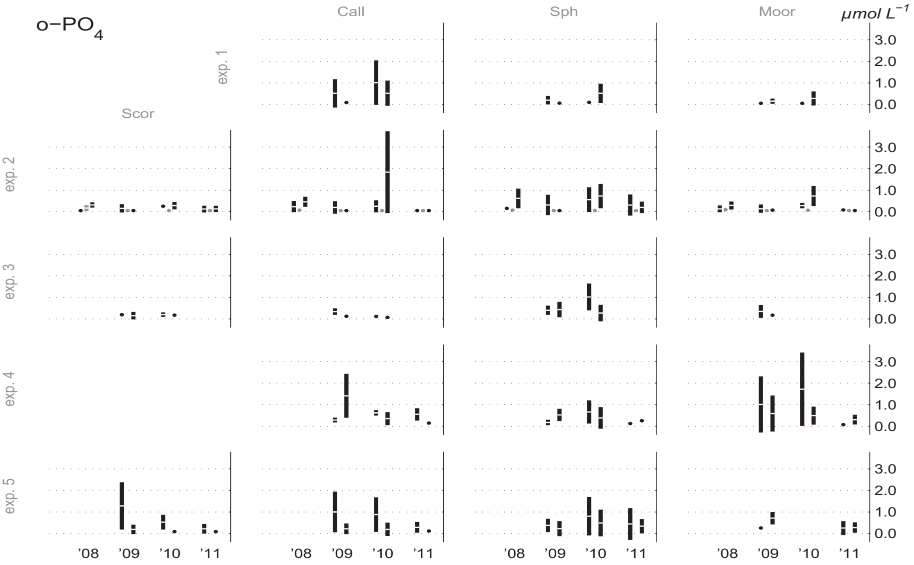
Appendix C.1 Effect of five surface water level treatments on the pH in four vegetation types, as measured 2 days before (black lines at the left of each triplet), during (grey lines) and 2 days after the treatments (black lines at the right of each triplet). Sample means (white centres of a line) are shown with their standard deviations ($n = 5$). Scor = fen dominated by *Scorpidium cossonii* or *Hamatocaulis vernicosus*, Call = fen dominated by *Calliergonella cuspidata*, Sph = fen dominated by *Sphagnum palustre*, Moor with *Erica tetralix* and *Sphagnum palustre*. Experiment 1 = floating WEE-fen during raised surface water levels in winter, experiment 2 = non-floating KW-fen during raised surface water levels in winter, experiment 3 = non-floating VW-fen during raised surface water levels in summer, experiment 4 = floating WEE-fen during lowered surface water levels in summer, experiment 5 = non-floating KW-fen during lowered surface water levels in summer. Statistical information is provided in Table S2.



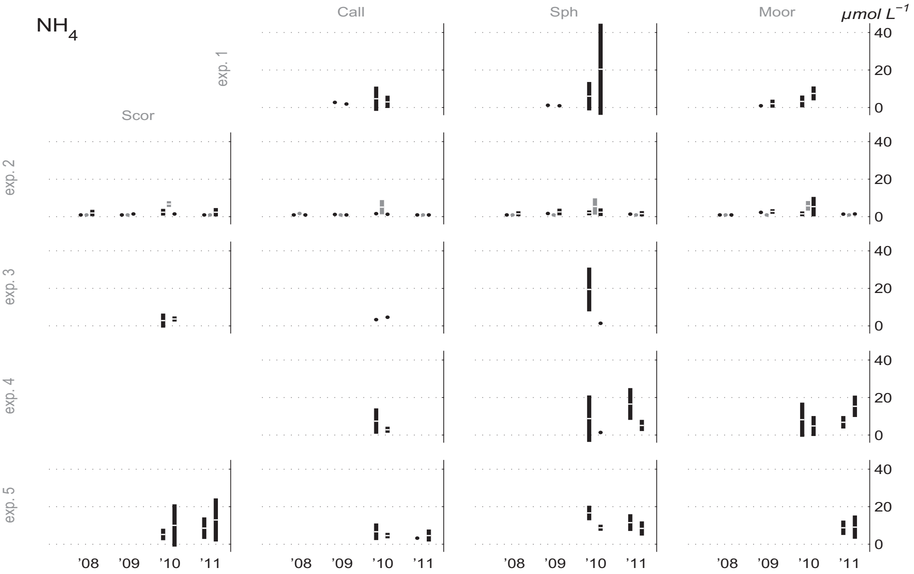
Appendix C.2 Effect of five surface water level treatments on the Fe-concentrations (µmol L-1) in four vegetation types. See the caption of Appendix C.1 for abbreviations and further information. Statistical information is provided in Table S2.



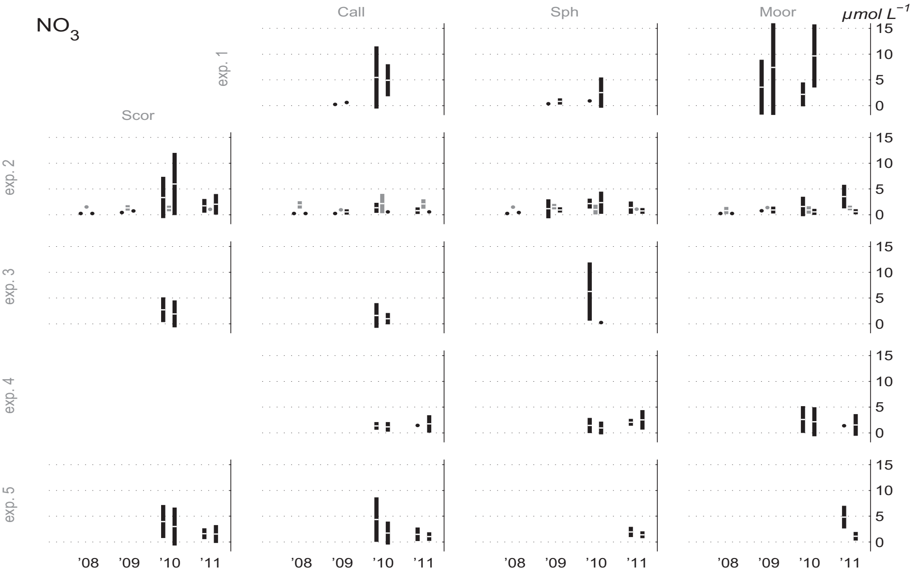
Appendix C.3 Effect of five surface water level treatments on the total S-concentrations (µmol L-1) in four vegetation types. See the caption of Appendix C.1 for abbreviations and further information. Statistical information is provided in Table S2.



Appendix C.4 Effect of five surface water level treatments on the 0-PO$_4$ concentrations (µmol L^{-1}) in four vegetation types. See the caption of Appendix C.1 for abbreviations and further information. Statistical information is provided in Table S2.

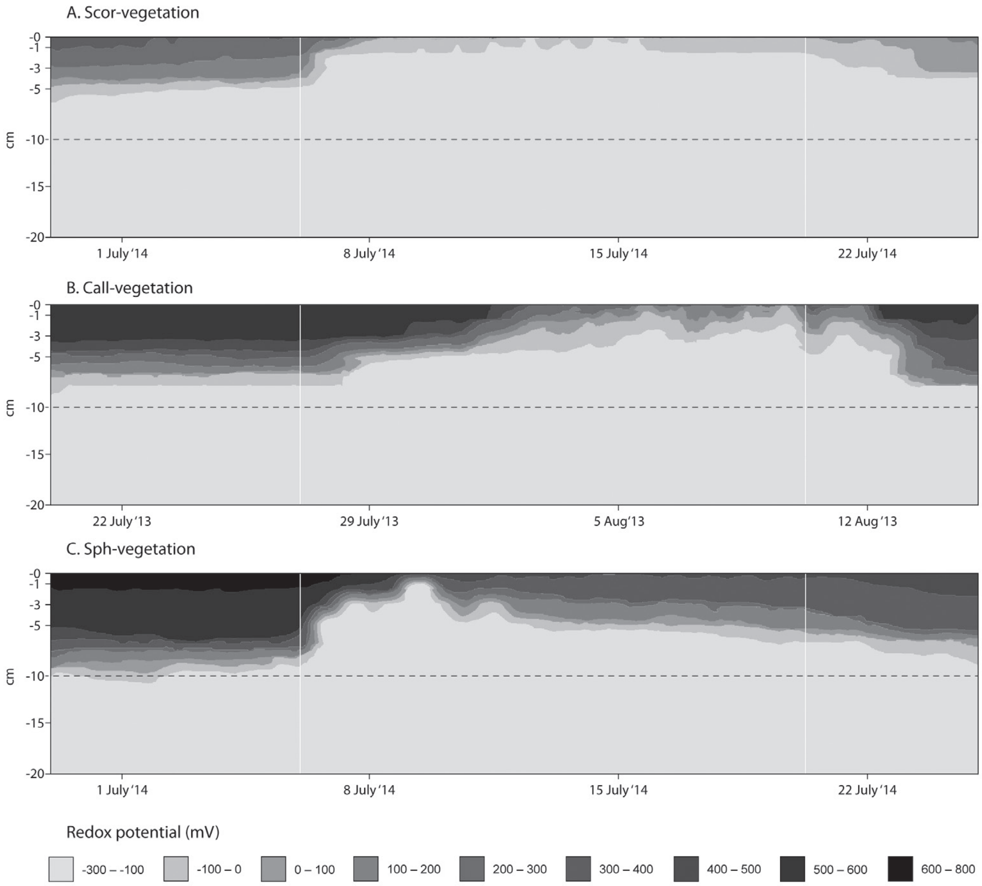


Appendix C.5 Effect of five surface water level treatments on the NH$_4$ concentrations (µmol L^{-1}) in four vegetation types. See the caption of Appendix C.1 for abbreviations and further information. Statistical information is provided in Table S2.



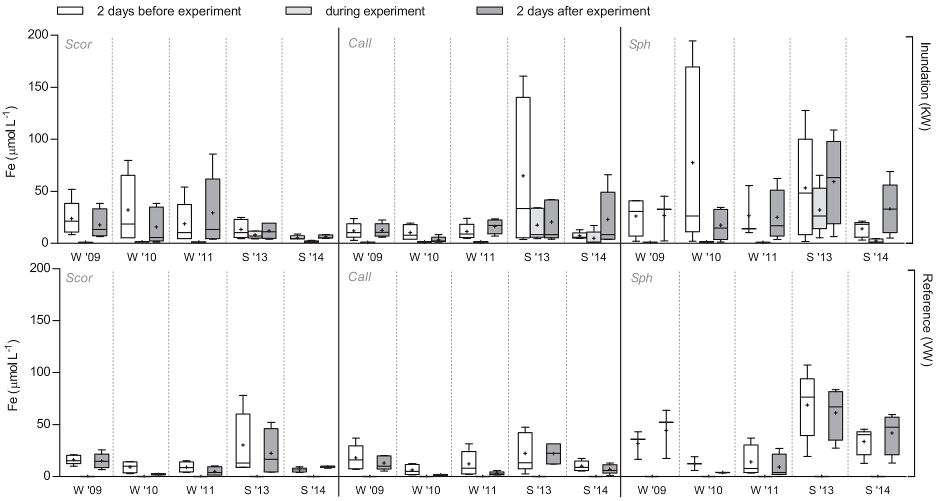
Appendix C.6 Effect of five surface water level treatments on the NO_3 concentrations (μmol L^{-1}) in four vegetation types. See the caption of Appendix C.1 for abbreviations and further information. Statistical information is provided in Table S2.

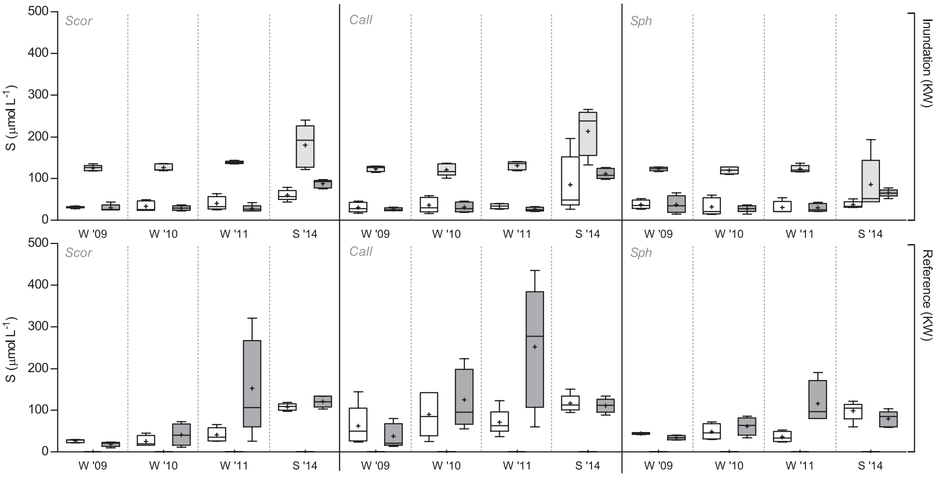
Appendix D

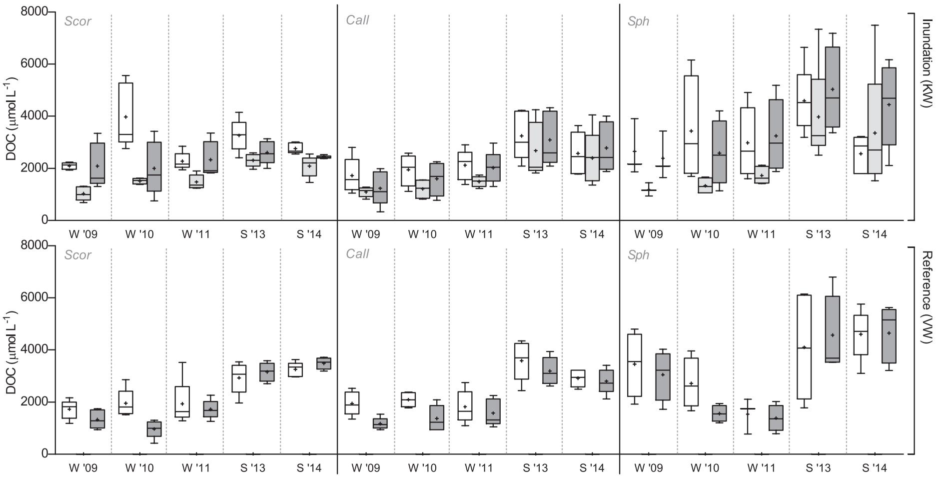


Appendix D Redox potentials (E_h) in the upper 20 cm of the soil in the three vegetation types during summer inundation in the KW-fen in 2013 and 2014. Scor (A) = fen dominated by *Hamatocaulis vernicosus*, Call (B) = fen dominated by *Calliergonella cuspidata*, and Sph (C) = fen dominated by *Sphagnum palustre*. The vertical white lines indicate the initiation and end of the treatment period. For interpolation, ordinary kriging was applied in ArcGIS (ArcMap 10.0, ESRI, Redlands, USA).

Appendix E


2 days before experiment
during experiment
2 days after experiment
Fe (µmol L^{-1})
0
50
100
150
200
Scor
Call
Sph
W '09
W '10
W '11
S '13
S '14
Inundation (KW)
Reference (VW)


S (µmol L^{-1})
0
100
200
300
400
500
Scor
Call
Sph
W '09
W '10
W '11
S '14
Inundation (KW)
Reference (KW)



Appendix E Fe-, S- and DOC-concentrations per vegetation type in pore water 2 days before the experiment, in inundation water during the experiment, and in pore water 2 days after the experiment. Sample means with standard deviations are indicated (*n* = 5). Statistical information is provided in Table 5.3. Scor = fen dominated by *Scorpidium cossonii* or *Hamatocaulis vernicosus*, Call = fen dominated by *Calliergonella cuspidata*, Sph = fen dominated by *Sphagnum palustre*. KW = Kiersche Wiede (experimental fen site), VW = Veldweg (reference fen site).

SUMMARY

Chapter 1

For the proper functioning of endangered biodiverse rich fens, base-rich and nutrient-poor (mesotrophic) conditions are required to prevent succession towards *Sphagnum*-dominated peatlands. Unfortunately, water- and soil quality of many European wetlands are negatively affected by changes in hydrology, eutrophication, acidification and toxicity, resulting in a decline in brown moss-dominated rich fens that show high vascular plant diversity. During the past decades, water levels in European rich fen areas have often become constricted within narrow limits as a result of adjacent agricultural water management. Currently, the re-establishment of fluctuating water levels in non-pristine fens is being considered from a management perspective, in order to conserve and restore the required conditions and target vegetation in current brown moss-dominated rich fens. The main objective of this research was to assess the potential ecological benefits and drawbacks of both lowered and increased surface water levels, as a management tool in different rich fen types, in order to support water and nature management authorities in decision-making.

Chapter 2

The lowering of surface water levels can result in lowered water tables in peat soils. To get a better understanding of the separate effects of aeration (oxygen intrusion) during moderate drought and desiccation (oxygen intrusion plus water deficiency) during severe drought, a long-term incubation experiment was conducted. Both rich fen peat samples and *Sphagnum*-peat samples were included to quantify the rates of decomposition, net N-mineralization, net P-release, denitrification, and the partitioning of C, N and P in soils and microbial biomass. The soil samples were incubated under (1) anaerobic, waterlogged conditions, (2) aerobic, moist conditions, characteristic for moderate drought in which oxygen intrusion takes place, and (3) aerobic, desiccated conditions to simulate severe drought.

Under anaerobic, waterlogged conditions, net N-mineralization rates per mass dry peat soil and per microbial C mass were much higher (on average 10 times) in *Sphagnum*-peat than in peat from rich fens, probably caused by higher microbial N-demand and N-immobilization in rich fens. The response upon aeration differed greatly between rich fen peat and *Sphagnum*-peat. Aeration resulted in a considerable decrease of the pH in rich fen peat. Furthermore, aeration led to increased respiration and net N-mineralization rates in the rich fen peat, while these rates did not change for *Sphagnum*-peat. The absence of aeration effects in *Sphagnum*-dominated fens suggests that decomposition rates are more strongly determined by litter quality than by oxygen intrusion. Upon further desiccation, both net P-release and DOC production, which remained unchanged upon aeration, increased significantly for both fen types. This may be due to microbial die-off and/or a change in microbial composition.

The strong decrease in pH and the strong response to aeration in rich fens compared to *Sphagnum*-fens, as well as the strong increase in P-availability upon further desiccation in both fen types, have important implications for peatland management as they indicate that particularly rich fens are highly sensitive to drought.

Chapter 3

To gain more detailed insight into the influence of vegetation development during water level manipulations with different water qualities, and the importance of chemical soil characteristics, mesocosm experiments were conducted. The interactive effects of water level fluctuation and P-enrichment under controlled summer conditions were examined, using peat cores including vegetation from three fens differing in biogeochemical characteristics. Peat cores, including vegetation, were collected from (1) a rich fen with a low Fe-content, (2) a rich fen with a high soil Fe-content, and (3) a mineral-poor fen with a high soil S-content.

The effects of fluctuating water levels on biogeochemistry and vegetation appeared to be highly dependent on peat chemistry, and more important than the effects of P-enrichment. Only when plant growth was stimulated by a favorable water level regime, P-enrichment led to increased P-consumption by plants. In rich fens with a high soil Ca-content (1), 7 weeks of a lowered water table (-15 cm) did not lead to a drop in pH. However, soil subsidence, increased N-availability and decline of the rich fen bryophyte *Scorpidium scorpioides* give cause to concern. 7 weeks of subsequent inundation (+15 cm) offered possibilities for restoration in these fens, since alkalinity and Ca-concentrations increased, while soil P-mobilization did not occur. Even P-enrichment did not result in increased P-availability, presumably due to Ca-related precipitation of P. In rich fens with a high soil Fe-content (2), soil subsidence, increased N-availability, decline of the rich fen bryophyte *Calliergon giganteum*, plus acidification due to Fe-oxidation, pose serious threats during drought. Shallow inundation, however, is also harmful in these Fe-rich fens, especially directly after mowing and with P-rich water, because plant growth was hampered, presumably by toxicity of NH_4^+ and/or Fe(II). In mineral-poor fens with a high soil S-content (3), shallow inundation should be avoided, because of tremendous internal P-mobilization. Vitality of the dominant bryophyte *Sphagnum palustre*, however, was not affected. Low water tables affected neither vegetation, nor biogeochemistry, showing resistance to short-term drought in these fens.

Given the strong mediating effect of soil chemistry, risks and benefits of re-establishment of fluctuating water levels with clean or P-rich water need to be considered for different fen types separately, as based on their peat chemistry, in water and nature management.

Chapter 4

Large-scale field manipulation experiments were conducted in rich fens with characteristic rich fen mosses and poor fens with *Sphagnum*. Five different experiments were conducted: 2 weeks of raised levels (+10 cm) in a floating and a non-floating fen during winter, 2 weeks of high levels in a non-floating fen during summer, and 2 weeks of lowered levels (-15 cm) in a floating and a non-floating fen during summer.

For floating *Sphagnum*-fens, both lowered and raised surface water levels in adjacent ditches did not show any effect on water tables in the peat soil, soil acid neutralizing capacity (ANC) or nutrient levels in fens. For non-floating fens, short-term droughts did not affect ANC or nutrient availability. Furthermore, raised surface water levels led to inundation in all vegetation types, without affecting nutrient concentrations or vegetation composition. Although redox potentials decreased immediately in upper soils, ANC was generally not enhanced in winter due to limited infiltration into the waterlogged soils. During a wet period in summer, in contrast, ANC increased due to enhanced infiltration as a result of higher evapotranspiration.

In conclusion, temporary raised surface water levels in winter are not very effective to enhance ANC in non-floating fens with base-rich surface water. In summer, however, the effect may be more effective.

Chapter 5

The main objective of the study in this chapter was to test whether short-term (2 weeks) inundation is more effective in summer than in winter to restore the ANC in the upper 10 cm of non-floating peat soils, as hypothesized in Chapter 4. Large-scale field experiments were conducted for two years in a fen with rich fen vegetation and *Sphagnum*-vegetation in summer, and the results were compared to the results of previous three years of winter inundation in the same area (Chapter 4).

Winter inundation did not result in an increase of porewater ANC, because infiltration was inhibited in the waterlogged peat and evapotranspiration rates were relatively low. Also, low temperatures limited microbial alkalinity generation. In summer, however, when temperature and evapotranspiration rates were higher, inundation did result in increased porewater Ca- and HCO_3-concentrations, but only in areas with characteristic rich fen bryophytes. This increase was not only due to stronger infiltration into the soil, but also to higher microbial alkalinity generation under anaerobic conditions. In contrast, porewater ANC did not increase in *Sphagnum*-plots as a result of the ability of *Sphagnum* spp. to actively acidify their environment. In both rich and poor fens, flooding-induced P-mobilization remained sufficiently low to safeguard P-limited vegetation. NO_3^- and NH_4^+ dynamics showed no considerable changes either.

In conclusion, short-term summer inundation with base-rich and nutrient-poor surface water is considered beneficial in the management of rich fens, and much more effective than winter inundation.

Chapter 6

In rich fens, Ca- and Fe- concentrations show large variations. The relative importance of Ca and Fe, particularly in relation to the availability of P for rich fen vegetation, is however largely unknown. To elucidate this, the relation between vegetation characteristics and peat chemistry was examined in 24 stands of rich fen vegetation: 12 in the Netherlands (strong anthropogenic forcing) and 12 in central Sweden (weak anthropogenic forcing). In addition, specific habitat preferences of three typical brown moss spp. were assessed.

Ca and Fe turned out to be important drivers of species composition in rich fens through their differential effects on plant P-availability. Fens dominated by *Scorpidium scorpioides* or *S. cossonii* were characterized by high porewater Ca-concentrations and total soil Ca-contents, but low P-availability. In these Ca-rich, but Fe-poor fens, foliar N:P ratios of vascular vegetation were above 20 g g^{-1}, indicating P-limitation due to Ca-P precipitation. In contrast, fens dominated by *Hamatocaulis vernicosus* were characterized by high porewater Fe-concentrations and total soil Fe-contents, but also relatively high P-availability. Total soil Fe-content showed a positive correlation with total soil P-content and P-concentration in plant tissue, and a negative correlation with foliar N:P ratios. N:P ratios in these fens were even below 13.5 g g^{-1}, indicating potential N-limitation and excess of P. The remarkable positive correlation between soil Fe-content and P-availability contrasts the general idea that high Fe-contents automatically lead to low values of plant-available P by sequestration. Instead, it is proposed that high groundwater Fe discharge leads to the accumulation of P that is still available to plants due to the relatively weak binding of P within abundant Fe-OM (Organic Matter) complexes. Furthermore, total biomass production was regulated by plant P-availability in Sweden. In the Netherlands, however, where above-ground biomass was 2.5 times higher, only the vegetation composition was regulated by plant P-availability. Finally, Dutch rich fens were more acidic than Swedish, which is probably related to the much higher atmospheric deposition.

In conclusion, the relative roles of Ca and Fe strongly differ with respect to nutrient limitation and vegetation development in rich fens, and should therefore be included in studies relating vegetation development to geohydrological conditions.

Chapter 7

This chapter provides a synthesis, in which results and conclusions from the preceding chapters in combination with results from previous studies are discussed, summarized and integrated in an overview of potential ecological benefits and drawbacks of water level fluctuations from a management perspective.

The potential drawbacks of temporary lowered surface water levels, and hence lowered water tables in the peat soil, seem to be more important than the potential benefits. The combined effects of enhanced acidification (particularly in Fe-rich soils), increased nutrient mineralization, direct drought-stress for brown mosses, improved conditions for *Sphagnum* spp., and increased biomass production by fast-growing species will strongly hamper the development of protected brown moss vegetation in rich fens. Long-term (>7 weeks) aeration and especially desiccation of the top 10 cm of the soil in rich fens should therefore be avoided.

In contrast to drought, periods of inundation with base-rich water in summer can be favorable in order to structurally improve the porewater ANC. Both infiltration of base-rich water and internal soil alkalinization in the topsoil of Ca-rich fens, that lack sufficient HCO_3- and Ca-buffering, can be important to prevent acidification. In rich fens with Ca-rich soils and low Fe-contents, the potential benefits of temporary inundation outweigh potential drawbacks. In rich fens with Fe-rich soils, however, inundation should be prevented shortly after winter, when vegetation development, hence P-consumption by plants, is still limited. Especially inundation with P-rich water seems to stimulate microbial activity, despite Fe-related precipitation of P, resulting in NH_4^+ and/or Fe(II) toxicity. For Fe-rich fens in agricultural areas, this may well generate a friction between preventing acidification and N-eutrophication during drought on the one hand, and preventing external eutrophication and accumulation of toxins during inundation on the other hand. Therefore, improved water quality is a primary requirement in these Fe-rich fens in agricultural areas.

In conclusion, area-specific chemical properties of peat soils and surface water, as determined by the geohydrological setting in the landscape, turned out to strongly determine the responses to surface water level-induced water table fluctuations in the peat soil. Especially the Ca- and Fe-contents of peat soils are important factors. In addition, the timing of temporary drought or inundation in the growing season turned out to be important, because of the strong interaction between biogeochemical processes and plant development. These findings not only contribute to the general biogeochemical and ecological understanding of water level-induced processes in rich fens, but are also valuable to support water and nature management authorities in decision-making.

SAMENVATTING

Hoofdstuk 1

Basenrijke trilveen, een beschermd habitattype binnen het Natura 2000-netwerk onder de noemer 'Overgangs- en trilvenen; H7140A', wordt gekenmerkt door grote soortenrijkdom en de aanwezigheid van rode lijst soorten zoals Rood, Geel en Groen schorpioenmos. Nutriënt-arme omstandigheden en een hoge basenrijkdom in de bovenste laag van de veenbodem, als gevolg van contact met basenrijk grond- en/of oppervlaktewater, zijn zeer belangrijk om betreffende soorten te behouden. Niet alleen in Nederland, maar in Europa is helaas sprake van een sterke achteruitgang van het aandeel en de gesteldheid van basenrijke trilvenen. Als gevolg van hydrologische ingrepen, verzuring, eutrofiëring en toxiciteit kunnen gunstige omstandigheden ontstaan voor veenmossen (*Sphagnum*-soorten) en dreigt snelle successie richting veenmosrietland. Er zal dus iets moeten gebeuren om basenrijke trilvenen op een duurzame manier in stand te houden.

Als gevolg van intensieve regulering van de waterstand in regio's met zowel veengebieden als landbouw is er niet of nauwelijks meer sprake van fluctuaties in het waterpeil van het oppervlaktewater. Zodoende is ook de fluctuatie van de waterstanden in de veenbodem van trilveen-habitats zeer beperkt, terwijl peilfluctuaties hier mogelijk juist van waarde zijn om de negatieve effecten van verzuring, eutrofiëring en toxiciteit te reduceren. Het hoofddoel van dit onderzoek was daarom het inzichtelijk maken van de mogelijke voor- en nadelen van herinvoering van een fluctuerend waterpeil als maatregel om doelvegetaties te behouden in verschillende typen basenrijke trilvenen.

Hoofdstuk 2

Verlaagde waterstanden in het oppervlaktewater kunnen leiden tot verlaagde waterstanden in de veenbodem. Om meer inzicht te krijgen in de afzonderlijke effecten van zuurstoftoetreding en uitdroging in zowel basenrijke veenbodems met schorpioenmossen als in veenbodems gedomineerd door *Sphagnum*, is een incubatie-experiment uitgevoerd in het laboratorium. Gedurende ca. 9 weken zijn bodemmonsters geïncubeerd onder (1) anaerobe, waterverzadigde omstandigheden, (2) aerobe, veldvochtige omstandigheden, waarbij alleen sprake was van zuurstoftoetreding, en (3) aerobe, uitgedroogde omstandigheden, waarbij naast zuurstoftoetreding ook uitdroging een rol speelde.

Onder anaerobe, waterverzadigde omstandigheden bleek de netto N-mineralisatie vele malen hoger in veenbodems met *Sphagnum*-mossen dan in basenrijkere schorpioenmos-venen. Oorzaak hiervan is de hogere microbiële N-immobilisatie in basenrijke trilveenbodems onder anaerobe omstandigheden. Ook de respons op zuurstoftoetreding bleek noemenswaardig te verschillen tussen beide veentypen. Een toename in de zuurstofbeschikbaarheid resulteerde in een daling in pH en een flinke toename van de netto N-mineralisatie in de basenrijke schorpioenmosvenen. In de *Sphagnum*-venen was dit echter niet het geval, waarschijnlijk omdat de mineralisatiesnelheid in dit veentype sterker wordt bepaald door de strooiselkwaliteit dan door de zuurstofbeschikbaarheid. Voor wat betreft de P-beschikbaarheid traden geen veranderingen op onder zuurstofrijke omstandigheden. Uitdroging daarentegen bleek te leiden tot een sterke toename in beschikbaar P in beide veentypen, met als mogelijke verklaring dat watertekorten hebben geleid tot microbiële sterfte en/of een verschuiving in de samenstelling van de microbiële populatie.

Zowel de sterke toename in N-beschikbaarheid en daling in pH bij verhoogde zuurstofbeschikbaarheid in basenrijke trilvenen als de sterke toename in P-beschikbaarheid bij uitdroging in beide veentypen, impliceren dat langdurige perioden van droogte dienen te worden voorkomen. Met name voor basenrijke trilvenen is dit van essentieel belang.

Hoofdstuk 3

Om meer inzicht te krijgen in de invloed van vegetatie-ontwikkelingen tijdens veranderingen in de waterstand met verschillende waterkwaliteiten, en bovenal de invloed van verschillende chemische bodemeigenschappen nader te bestuderen, zijn mesocosmos-experimenten uitgevoerd. Hierbij zijn zomercondities gesimuleerd in een laboratorium kweekcel en zijn veenkolommen bemonsterd uit (1) een basenrijke veenbodem met een laag Fe-gehalte, (2) een basenrijke veenbodem met hoog Fe-gehalte, en (3) een basenarme veenbodem met een hoog S-gehalte.

De effecten van waterstandsfluctuaties op biogeochemische processen en vegetatie-ontwikkeling bleken in hoge mate afhankelijk van de bodemchemische samenstelling. Ook bleek de uitwerking van verschillende waterstanden van grotere invloed dan de P-beschikbaarheid in het toegediende water. Enkel in het geval dat plantengroei werd gestimuleerd door een gunstig verloop van de waterstand leidde extra toediening van P tot een verhoogde P-consumptie door planten. In basenrijke veenbodems met een hoog Ca-gehalte en een lage Fe-concentratie (1) resulteerde een 7 weken durende peilverlaging van 15 cm niet tot een daling in de pH. Er was echter wel sprake van noemenswaardige bodemdaling, een toename in de N-beschikbaarheid en een aanzienlijke afname in vitaliteit van Rood schorpioenmos. Een hieropvolgende periode van 7 weken met verhoogde waterstanden (+15 cm), waarbij inundatie optrad, bood mogelijkheden om de negatieve effecten van droogte te herstellen, aangezien de alkaliniteit in het bodemvocht toenam en van P-mobilisatie uit de bodem geen sprake was. Zelfs additie van P via het toegediende water leidde niet tot verhoogde o-PO_4 concentraties in het bodemvocht vanwege de binding van P in deze Ca-rijke veenbodems. In basenrijke veenbodems met een hoog Fe-gehalte (2) leidde een periode met lage waterstanden eveneens tot bodemdaling, verhoogde N-beschikbaarheid, een afname van de vitaliteit van in dit geval Reuzenpuntmos, en bovenal verzuring als gevolg van Fe-oxidatie. Hieropvolgende verhoogde waterstanden boden voor deze bodems echter weinig herstellende mogelijkheden. Vooral direct nadat de vegetatie gemaaid was, en vooral bij P-rijk inundatiewater, werd de plantengroei aanzienlijk geremd, vermoedelijk als gevolg van toxische concentraties aan NH_4^+ en/of Fe(II) in het bodemvocht. In basenarme bodems met een hoog S-gehalte bleek inundatie eveneens een grote negatieve uitwerking te hebben vanwege enorme P-mobilisatie uit de bodem. Vitaliteit van de op deze bodems voorkomende *Sphagnum*-soorten werd echter niet beïnvloed, ongeacht het waterstandsregiem of de waterkwaliteit.

Concluderend kan worden gesteld dat, gezien het zeer bepalende effect van de bodemchemie, de mogelijke risico's en voordelen van waterstandsfluctuaties vanuit water- en natuurbeheer afzonderlijk dienen te worden overwogen voor veentypen met verschillende bodemchemische samenstelling.

Hoofdstuk 4

Om een betere vertaalslag te kunnen maken van laboratorium-resultaten naar de veldsituatie zijn grootschalige veldexperimenten uitgevoerd. De uitwerking van peilverlaging (-15 cm) gedurende twee weken in de zomer en peilverhoging (+10 cm) gedurende twee weken in zowel zomer als winter is nader onderzocht op lokaties met zowel drijvende als niet-drijvende trilveenkraggen, en in verschillende vegetatietypen.

In drijvende kraggen met *Sphagnum* had zowel peilverlaging als peilverhoging in het oppervlaktewater geen effect op de waterstand in de veenbodem. Evenmin werd de zuur neutraliserende capaciteit (ZNC) of de nutriëntbeschikbaarheid beïnvloed. Ook in niet-drijvende veenkraggen bleek een tijdelijke peilverlaging in geen van de vegetatietypen van belang. Een verhoogde waterstand in het oppervlaktewater leidde in alle vegetatietypen tot inundatie, waarbij de nutriëntbeschikbaarheid en de vegetatie-compositie niet werd beïnvloed. In het geval van winterinundatie bleef ook in de niet-drijvende kraggen de ZNC onveranderd vanwege de geringe infiltratie van het basenrijke inundatiewater. Tijdens een natte periode in de zomer daarentegen nam de ZNC wel toe, vermoedelijk vanwege hogere infiltratie van het inundatiewater bij hogere evapotranspiratiesnelheden.

De uitkomsten laten zien dat tijdelijk verhoogde waterstanden in de winter niet erg effectief blijken als het gaat om verhoging van de ZNC via oppervlaktewater in niet-drijvende venen. In de zomer lijkt deze maatregel echter een stuk lucratiever te zijn.

Hoofdstuk 5

In dit hoofdstuk staat de vraag centraal of zomerinundaties daadwerkelijk uitkomst bieden als het gaat om het tegengaan van verzuring in niet-drijvende venen. Voortbordurend op de uitkomsten van Hoofdstuk 4 zijn in twee opeenvolgende jaren grootschalige veldexperimenten uitgevoerd, waarbij waterstanden in oppervlaktewater gedurende twee weken zijn verhoogd (+15 cm) in de zomer. De resultaten voor zowel basenrijke vegetatietypen als voor *Sphagnum*-vegetatie zijn vergeleken met de resultaten van voorgaande drie jaren van winterinundatie in hetzelfde gebied (Hoofdstuk 4).

Waar winterinundatie geen uitwerking had, bleek tijdelijke inundatie in de zomer inderdaad te leiden tot een hogere ZNC op plekken met karakteristieke schorpioenmos-vegetatie. Niet alleen infiltratie van basenrijk oppervlaktewater was hoger vanwege hogere evapotranspiratie in de veenkragge. Ook was er sprake van een hogere interne alkaliniteitsproductie onder anaerobe omstandigheden bij een hogere temperatuur in de zomer. Op plekken met *Sphagnum* was er geen sprake van een toename in ZNC, vermoedelijk vanwege het vermogen van *Sphagnum*-mossen om hun directe omgeving te verzuren.

Concluderend kan worden gesteld dat tijdelijke inundatie met basenrijk en nutriëntarm water in de zomer, in tegenstelling tot in de winter, perspectieven biedt voor beheer als het gaat om het tegengaan van verzuring in basenrijke trilvenen.

Hoofdstuk 6

Tussen verschillende typen basenrijke venen kunnen Ca- en Fe-gehalten in de veenbodem sterk variëren. Zoals al bleek uit het onderzoek van Hoofdstuk 3, kunnen deze verschillen in bodemchemie van groot belang zijn, zeker als het gaat om de P-beschikbaarheid voor vegetatie. Om hier een beter inzicht in te krijgen is de relatie tussen bodemeigenschappen en vegetatie-eigenschappen nader onderzocht in 24 basenrijke veenlokaties, waarvan 12 in Nederland (gekenmerkt door een hoge anthropogene invloed) en 12 in centraal Zweden (gekenmerkt door een lage anthropogene invloed). Daarnaast zijn de specifieke verschillen in habitat-vereisten tussen Rood, Groen en Geel schorpioenmos nader in beeld gebracht.

De gehalten aan Ca en Fe bleken zeer belangrijke factoren als het gaat om P-beschikbaarheid op verschillende manieren. Trilvenen met Rood en Groen schorpioenmos werden gekenmerkt door hoge Ca-concentraties in zowel bodemvocht als in de veenbodem, maar een lage P-beschikbaarheid. Getuige N:P ratio's in bovengrondse biomassa van de vaatplanten in deze Ca-rijke, maar Fe-arme venen was hier sprake van P-limitatie als gevolg van Ca-P precipitatie. In venen met Geel schorpioenmos daarentegen was sprake van relatief hoge Fe-concentraties in zowel bodemvocht als in de veenbodem, maar ook een relatief hoge P-beschikbaarheid. Het Fe-gehalte vertoonde een positieve correlatie met het P-gehalte in de veenbodem en in het plantenmateriaal, en een negatieve correlatie met de N:P ratio in de bovengrondse biomassa van de vaatplanten. N:P ratio's in deze Fe-rijke venen getuigden zelfs van N-limitatie en een overmaat aan P. Deze opmerkelijke positieve correlatie tussen het Fe-gehalte en de P-beschikbaarheid is in tegenstelling met het algemeen gangbare idee dat hoge Fe-gehalten per definitie leiden tot een lage P-beschikbaarheid voor planten. Sterker nog: een hoge aanvoer van Fe-rijk grondwater kan leiden tot accumulatie van P wat beschikbaar is voor planten vanwege de relatief zwakke binding van P binnen veelvuldig aanwezige complexen van Fe met organische stof. Verder bleek dat de totale productie van biomassa in de Zweedse venen werd gereguleerd door de P-beschikbaarheid, terwijl in de Nederlandse venen, waar sprake was van veel hogere bovengrondse biomassa, alleen de soortencompositie werd gereguleerd door de P-beschikbaarheid. Tenslotte bleken de Nederlandse venen ook in hogere mate te zijn verzuurd dan de Zweedse venen, waarschijnlijk vanwege de hogere atmosferische depositie in Nederland.

De rol van Ca en Fe blijkt dus zeer verschillend te zijn als het gaat om P-limitatie en de ontwikkeling van vegetatie in basenrijke venen. Dit onderscheid is belangrijk om te maken als het gaat om de relatie tussen vegetatie-ontwikkeling en geohydrologische condities.

Hoofdstuk 7

Het laatste hoofdstuk voorziet in een synthese, waarin de resultaten en conclusies van de voorgaande hoofdstukken, in combinatie met resultaten van andere onderzoeken, inzichtelijk worden gemaakt en de mogelijke voor- en nadelen van waterstandsfluctuaties vanuit beheersperspectief tegen elkaar worden afgewogen.

De mogelijke nadelen van tijdelijke peilverlaging in het oppervlaktewater, en dus verlaging van de waterstand in de veenbodem, bleken van veel groter belang dan de mogelijke voordelen. De combinatie van verzuring (vooral in Fe-rijke veenbodems), verhoogde mineralisatie, directe droogte-stress voor beschermde mossoorten, gunstige omstandigheden voor *Sphagnum*-mossen, en verhoogde biomassa productie door relatief snel groeiende soorten zal de ontwikkeling van basenrijke trilveen-vegetaties beslist niet ten goede komen. Langdurige zuurstoftoetreding (>7 weken) en bovenal uitdroging van de bovenste 10 cm van de veenbodem dient dan ook te worden voorkomen.

Verhoogde waterstanden kunnen echter wel gunstig uitpakken onder bepaalde condities. Tijdelijke inundatie met basenrijk oppervlaktewater in de zomer biedt kansen voor het verhogen van de ZNC in de bovenste laag van de veenkragge. Zowel door infiltratie van dit oppervlaktewater als door interne alkaliniteitsproductie onder anaerobe omstandigheden kan op deze manier een belangrijke bijdrage worden geleverd aan het tegengaan van verzuring. In trilvenen met Ca-rijke en Fe-arme veenbodems wegen deze mogelijke voordelen zwaarder dan de mogelijke nadelen. In trilvenen met Fe-rijke bodems daarentegen kan inundatie voor noemenswaardige belemmeringen zorgen, vooral vroeg in het groeiseizoen als de vegetatie-ontwikkeling, en dus de P-consumptie door planten, nog niet goed op gang is gekomen. Vooral inundatie met P-rijk water kan, ondanks precipitatie van Fe met P, resulteren in NH_4^+ en/of Fe(II) toxiciteit door het sterke stimulerende effect op de microbiële activiteit. Voor Fe-rijke trilvenen in gebieden met veel landbouw doet zich om deze redenen een lastige overweging voor. Aan de ene kant dient verdroging, verzuring en mineralisatie als gevolg van lage waterstanden te worden voorkomen, en aan de andere kant vormen externe eutrofiëring en toxiciteit als gevolg van hoge waterstanden een belemmering. Een verbeterde waterkwaliteit is daarom in Fe-rijke veengebieden met landbouw een eerste vereiste voordat door middel van inundatie verdere progressie kan worden geboekt.

De resultaten van dit onderzoek benadrukken het belang van gebiedsspecifieke chemische bodemeigenschappen en de waterkwaliteit, zoals bepaald door de geohydrologische positie in het landschap, als het gaat om de uitwerking van peilfluctuaties in basenrijke trilvenen. Met name de Ca- en Fe-gehalten in de veenbodem zijn hierbij van belang. Daarbij komt dat, in verband met de sterke interactie tussen biogeochemie en vegetatie-ontwikkeling, de timing in het groeiseizoen van tijdelijke peilverlaging of peilverhoging van grote invloed is. Deze bevindingen zijn niet alleen belangrijk binnen de fundamentele ontwikkeling van biogeochemische en ecologische kennis over de uitwerking van peilfluctuaties in basenrijke trilvenen, maar zijn ook van grote waarde vanuit beheersperspectief.

DANKWOORD

De voltooiing van dit promotieonderzoek bleek geen sinecure. Zonder de hulp en bijdragen van de hieronder genoemde personen zou dit proefschrift dan ook niet tot stand zijn gekomen. Een dankwoord is daarom op zijn plaats.

Als eerste wil ik dit dankwoord richten aan Annemieke Kooijman en Leon Lamers. Annemieke, jij hebt als dagelijks begeleider en copromotor vanuit de Universiteit van Amsterdam een grote rol gespeeld bij zowel de opzet, uitvoering als rapportage van alle onderzoeksonderdelen. Ik heb op verschillende gebieden veel van je kunnen leren. Onze tussentijdse besprekingen over de onderzoeksresultaten hebben de verschillende hoofdstukken in dit proefschrift sterk verbeterd. Daarnaast zijn jouw adviezen aangaande het schrijven van gecompliceerde materie in een gestructureerde tekst met een duidelijke boodschap voor mij van grote waarde geweest. Ook wil ik niet onvermeld laten dat ik mijn aanstelling binnen IBED aan de Universiteit van Amsterdam hoofdzakelijk aan jouw inzet te danken heb. Leon, ook jij bent van grote waarde geweest bij de totstandkoming van dit proefschrift, als dagelijks begeleider en inmiddels ook als promotor vanuit de Radboud Universiteit Nijmegen. Niet alleen heb je mij van een grote hoeveelheid nuttige en opbouwende kritiek voorzien, maar ook heeft het aanstekelijke enthousiasme, waarmee jij een onderzoeksopzet vormgeeft en waarmee je resultaten duidt, ertoe bijgedragen dat ik steevast met plezier en inspiratie aan het werk ben geweest. En onze gezamenlijke passie voor de dakhaas vergrootte het werkplezier.

Henry Hooghiemstra, mijn promotor, en Frank Hammecher, bedrijfsvoerder van IBED, wil ik bedanken voor ondersteuning op de momenten waarop dat nodig was. Verder wil ik de volgende commissieleden bedanken voor het beoordelen van het manuscript: Ab Grootjans, Karsten Kalbitz, Koos Verstraten, Lars Hedenäs, Pim de Voogt, Wim Admiraal en Winnie Rip.

Graag wil ik Casper Cusell uitvoerig bedanken voor praktische en inhoudelijke hulp. Het was erg zinvol en prettig om met jou op ieder moment te kunnen overleggen. Met name de autoritten heen en weer naar Noordwest-Overijssel waren hiervoor een uitstekende gelegenheid. Jouw inspirerende adviezen hebben ervoor gezorgd dat ik gemotiveerd bleef als het even tegen zat.

Voorts dank ik de volgende laboratorium medewerkers voor alle hulp die zij mij hebben geboden in de vorm van labwerkzaamheden en analyses: Leo Hoitinga, Leen de Lange, Peter Serné, Bert de Leeuw (allen UvA), en Jelle Eygensteyn (RUN).

Ook het werk dat studenten hebben verzet is van groot belang geweest bij de afronding van de verschillende onderzoeksonderdelen. Met name wil ik de volgende personen bedanken voor hun toegewijde inzet: Melchior Rutte, Tessa Neijmeijer, Marta Picella en Silvia di Liddo.

Mijn dank gaat ook uit naar Natuurmonumenten, Staatsbosbeheer en Landschap Noord-Holland voor het herhaaldelijk beschikbaar stellen van motorboten en het verlenen van toestemming voor monstername en plaatsing van meetinstallaties op hun terrein. In het bijzonder wil ik Rosalie Martens en Arco Lassche van Natuurmonumenten bedanken, zonder wier medewerking de grootschalige peilopzet in het Kiersche Wiede niet had kunnen plaatsvinden.

Dit onderzoeksproject maakt deel uit van het nationale onderzoeksprogramma OBN (Ontwikkeling en Beheer Natuurkwaliteit), financieel ondersteund door het Ministerie van Economische Zaken. Graag wil ik alle betrokkenen in het onderzoeksconsortium en alle leden van het deskundigenteam Laagveen- en zeekleilandschap bedanken voor hun bijdragen binnen het project.

Mijn dank gaat verder uit naar Geert Kooijman, Michel Vorenhout en alle collega's binnen IBED. Met name (oud-)kamergenoten Erik, Bodo en Leena wil ik bedanken voor een gezellige werksfeer.

Mijn ouders Janneke en Rémy, mijn broer Lennart, en overige familie, vrienden en vriendinnen wil ik graag bedanken voor steun, belangstelling, en aangename afleiding. Willemijn en Niels, ik vind het erg leuk dat jullie willen optreden als paranimf. Gezien de enorm lange tijd dat wij drieën elkaar kennen heeft dat voor mij een bijzondere betekenis.

Tot slot wil ik Nikki bedanken voor steun, vrolijkheid en veldassistentie (zelfs bij slecht weer). Ik ben blij met jou en ik wil je nooit meer kwijt.