Preventing acidification and eutrophication in rich fens: Water level management as a solution?

Casper Cusell

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1

General introduction

1. General introduction

This study was initiated in order to investigate the biogeochemical and ecological effects of allowing more variation in surface water levels in freshwater wetlands that currently show more or less constant water levels. According to the Ramsar (1971) definition, f*reshwater wetlands are "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing*". They often consist of a mosaic of aquatic, semi-aquatic and terrestrial vegetation types, among which biodiverse rich fens (*Scorpidio-Caricetum diandrae*) with many highly threatened species including vascular plants, such as *Liparis loeselii* (L.) Rich. and *Parnassia palustris* L., and bryophytes such as *Scorpidium scorpioides* (Hedw.) Limpr., *Scorpidium cossonii* (Schimp.) Hedenäs and *Hamatocaulis vernicosus* (Mitt.) Hedenäs. These brownmoss-dominated rich fens are protected under the European Habitat Directive (transition mires and quaking bogs, H7140).

Rationale for this thesis

A water management decision to allow a more fluctuation of surface water levels in National Park Weerribben-Wieden, which is a large and protected freshwater wetland in the Netherlands, was dismissed by the Council of State (Raad van State 2007). The Council held that the potential negative effects on endangered species and protected habitat types, such as rich fens, had not been sufficiently investigated. Following this judgment, it was decided to study the biogeochemical and ecological effects of lowered and raised surface water levels on the ecological functioning of this nature reserve, with emphasis on the preservation and restoration of brownmoss-dominated rich fens. This ultimately resulted in the present thesis, which especially focusses on the processes of eutrophication, alkalinity generation and acidification in relation to the introduction of more fluctuating water tables in rich fens.

Rich fens as biodiversity hotspots in freshwater wetlands

Brownmoss-dominated rich fens are characterized by base-rich and nutrient-poor conditions (Sjörs 1950; van Wirdum 1991; Kooijman 1993a; Wheeler & Proctor 2000). They can be situated directly on base-rich substrates, such as marl soils, but most Dutch rich fens depend on groundwater and/or surface water flows from surrounding base-rich areas for their base supply (van Wirdum 1993). Such base-rich water receiving fens are often part of much larger minerotrophic freshwater wetlands, that consist of a mosaic of open water, aquatic vegetation, semi-aquatic vegetation, rich fen, poor fen, carr woodland and/or bog vegetation. Although the terrestrialization of minerotrophic surface waters may follow different successional pathways, the following types are commonly found in successive phases of terrestrializing Dutch

Fig. 1.1. Visualization of the different successional stages during the terrestrialization from open waters to carr woodland: (a) open water, (b) aquatic vegetation of *Characeae*, (c) emerged aquatic vegetation of *Stratiotes aloides*, (d) helophyte vegetation, (e) floating rich fen, (f) transition from a rich fen with *Scorpidium scorpioides* (right) to a poor fen with *Sphagnum palustre* (left), (g) bog vegetation and (h) carr. (pictures: R. van Leeuwen & C. Cusell)

turbaries (peatlands used for peat extraction) (e.g. Segal 1966; van Wirdum 1995; Fig. 1.1): (a) open water, (b) submerged aquatic plants such as *Chara hispida* L., *Chara virgata* Kützing and *Nitella flexilis* L., (c) emerged aquatic plants such as *Stratiotes aloides* L., (d) helophyte vegetation with root-mat forming stands of *Typha angustifolia* L., *Phragmites australis* (Cav.) Steud. and *Thelypteris palustris* Schott, (e) rich fen with a moss-layer dominated by brownmosses, (f) *Sphagnum*-dominated poor fen, (g) bog vegetation and (h) carr woodland. These final, terrestrial vegetation types (e – g) can be present on floating and non-floating peat soils, depending on the depth of the sandy or clayey soil below the peat.

During the first stage of terrestrialization, in which helophyte vegetation forms a root mat, vegetation is in direct contact with minerotrophic surface water. Contact to minerotrophic water is also decisive for the fen type that develops on the root mat. The input of base-rich water may occur through the discharge of groundwater (Koerselman *et al*. 1990), by the seepage of surface water from beneath the floating root mat (van Wirdum 1991) and/or through flooding from adjacent surface waters (Barendregt *et al*. 2004; Cusell *et al*. 2013; Fig. 1.2). The contact with base-rich water will, however, gradually decrease during succession, due to peat formation. This leads to a transition from rich fens toward more acid but less biodiverse, *Sphagnum*-dominated poor fens and bogs with moss species such as *Sphagnum fallax* (H.) Klinggr., *Sphagnum palustre* L. and eventually hummock-forming species such as *Sphagnum magellanicum* Brid. (e.g. Sjörs 1950; Du Rietz 1954).

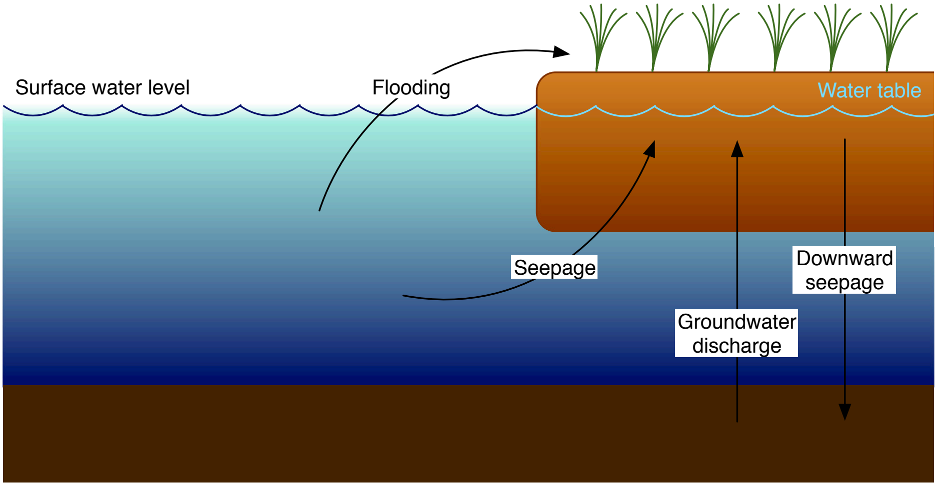


Fig. 1.2. The main flow patterns of water in a floating fen.

Rich fens in National Park Weerribben-Wieden

In this thesis, the main focus is on the management of brownmoss-dominated rich fens in the Ramsar area "National Park Weerribben-Wieden" (between 52°48' N – 5°53' E and 52°38' N – 6°08' E; Fig. 1.3), with "De Weerribben" in the north and "De Wieden" in the south. This is a large wetland area that still contains a fair number of well-developed brownmoss-dominated rich fens. It is a European hotspot for endangered mosses, vascular plants, dragonflies and butterflies.

The present nature reserve of about 95 km^2 was once part of a very large wetland system of about 15000 km^2 in the west of the Netherlands, bordered by dunes in the west and moraine upland in the east (Berendsen 2011). In this broad zone of 50 – 90 km, active peat formation started at the offset of the Subboreal (around 5000 years BP), when the rise in sea-level leveled off, because of cooler and drier conditions than in the previous Atlantic period (Zagwijn 1986; Pons 1992). At the location of the present nature reserve, peat was formed on top of some meters of eolian cover sands (Formation of Boxtel), which lay on top of a more than 100 m thick package of other sandy layers that also included discontinuous, less permeable layers (DINOloket 2014). It appears from soil surveys (Veenenbos 1950; Haans & Hamming 1962) that two dome-shaped bogs of ombrotrophic *Sphagnum*-peat developed away from the main rivers in this area. Finally, these bogs covered most of the area, while minerotrophic fens with *Carex*-, *Phragmites*- and woody (carr) peats continued to grow in and along river beds.

A period of sea transgression started around 2600 years BP, at the start of the Subatlantic (van Geel *et al.* 1998). This transgression resulted in the cessation of active peat growth and the development of the inland sea Zuiderzee (Berendsen 2011). Between 1000 and 1300 AD, dikes were built to stop the expansion of the Zuiderzee near the western border of the present nature reserve (van Wirdum 1991). In the Late Middle Ages, people started to superficially extract peat. The wetland changed dramatically between the 17th and 20th century as a consequence of extensive peat extraction, during which peat was also collected from below the groundwater level. This started around 1600 in the south (De Wieden) and ended around 1920 in the north (De Weerribben). This land use resulted in a large number of abandoned turbaries, in which open waters developed into new floating fens, especially after a large pumping station (Stroink) was put into operation in 1920 to effectively decrease surface water level variations to ca. 10 cm around a predetermined level (van Wirdum 1991). In areas with limited peat thickness, e.g. close to the moraine upland in the east part, non-floating fens developed.

During the 20th century, most of the original wetland area and parts of the Zuiderzee were reclaimed and drained to become polders for agricultural use (van Wirdum 1991). Nowadays, the remaining wetland of about 9500 ha has an average surface level of 0.3 – 0.6 m below mean sea level (BMSL). Surface water levels are maintained at 0.73 – 0.83 m BMSL throughout the year. The surrounding polders have lower surface

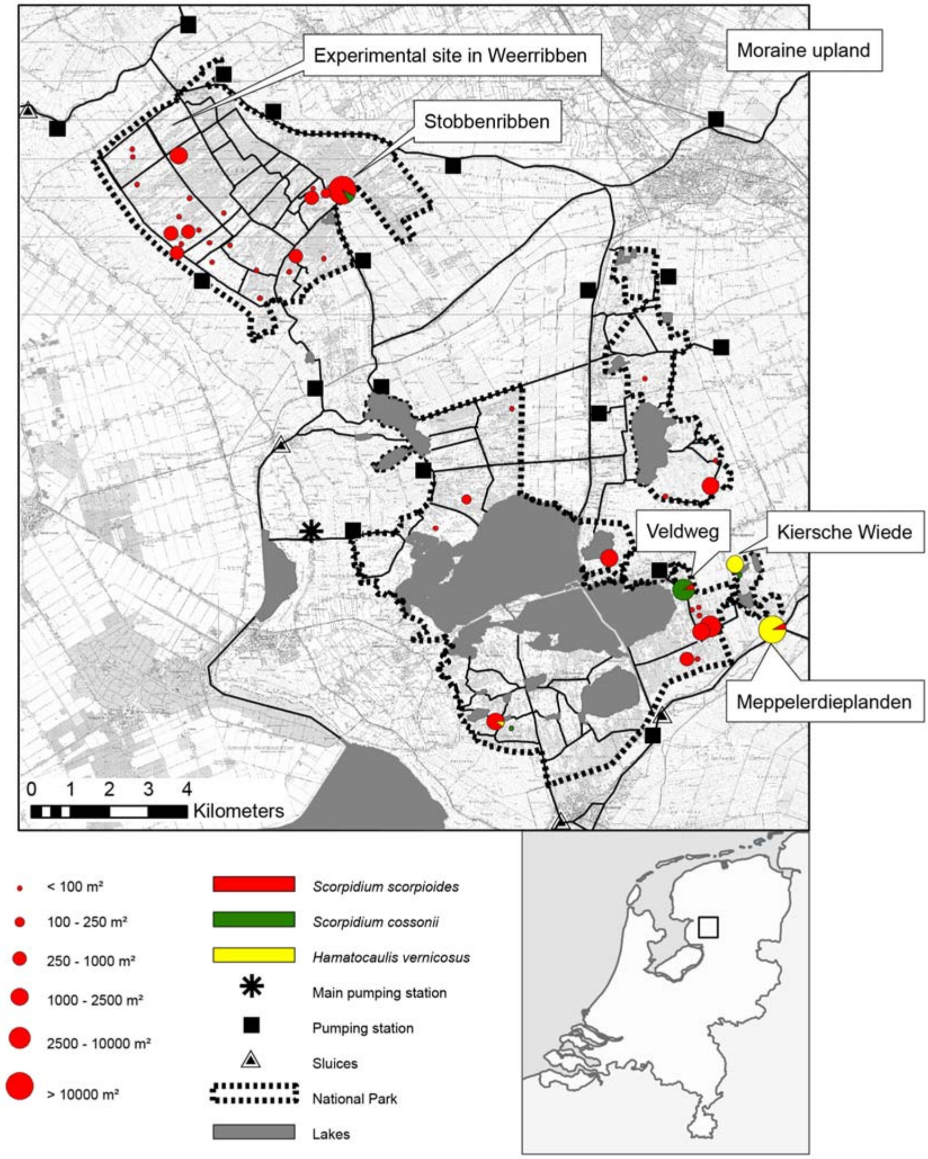


Fig. 1.3. The occurrence and abundance of *Scorpidium scorpioides*, *Scorpidium cossonii* and *Hamatocaulis vernicosus* in National Park Weerribben-Wieden, and the location of the National Park in the Netherlands (between 52°48' N – 5°53' E and 52°38' N – 6°08' E).

levels of 1.0 – 2.5 m BMSL. These polders, which are drained by about 30 pumping stations (Fig. 1.3) to various lower water levels of 1.5 – 3.0 m BMSL, discharge excess water into the higher lying wetland (van Wirdum 1979). As a consequence of these reclamations, there is almost no direct discharge of groundwater anymore in National Park Weerribben-Wieden. The remaining wetland has become a groundwater recharge area (van Wirdum 1991). Presently, surface water levels in the wetland reserve itself are regulated by one main pumping station at the western border of the nature reserve (Fig. 1.3), which removes water during wet periods, and sporadically pumps water in during pronounced dry periods. In the past, other locations have been used to pump water in, and this occurred more often (Groeneweg & van Wirdum 2004).

Water balance studies for National Park Weerribben-Wieden show that the water input of the present wetland consists of rainwater (about 35%), drainage water from the adjacent upland, including the Steenwijker Aa (about 20%), and water pumped in from lower lying agricultural polders (about 45%) (Jol & Laseur 1982; Balirwa 1993; Cusell *et al.* 2013). Mean annual precipitation is about 800 mm (KNMI 2014). The discharge of polder water is about 50% smaller in summer than in winter, due to the precipitation surplus in winter and the evapotranspiration surplus during large parts of the summer. Hence, the water composition in the present wetland is largely determined by the land use of the surrounding polders and by the season. During the second half of the 20th century, when new polders were created and manure was being used excessively, the inputs of polder water led to severely increased N- and P-inputs into the National Park (Jol & Laseur 1982; Cusell *et al.* 2013). For N, atmospheric deposition was an important additional input. Although the present estimated deposition of about 19 kg N ha^{-1} $year^{-1}$ (1350 mol ha^{-1} $year^{-1}$; RIVM 2012) is lower than in many other Dutch areas, it is still above published values for the critical N-deposition of about 10 and 17 kg N ha^{-1} $year^{-1}$ for *Sphagnum*-dominated poor fens (H7140B) and *Scorpidium*-dominated rich fens (H7140A), respectively (van Dobben & van Hinsberg 2008; Bobbink & Hettelingh 2011).

Risks for rich fens: acidification, eutrophication and toxicity

The extent of biodiverse, brownmoss-dominated rich fens remained about equal in National Park Weerribben-Wieden during the past 10 – 15 years (Pommer 2011). The total cover of this fen type, however, only comprises less than 0.5% of the National Park area. *Scorpidium scorpioides* is still present in 41 fens, but only eight of these sites have a cover exceeding 0.1 ha (Fig. 1.3). *Scorpidium cossonii* and *H. vernicosus* are very rare, and only occur in four fens. The main site with *H. vernicosus* is, in fact, a clay-rich floodplain fen in a hydrological different area. Many other wetlands in Western Europe even showed a strong decline in the occurrence of brownmoss species and the cover of brownmoss-dominated rich fens in recent decades (Berg & Wiehle 1992; Kooijman 1992; JNCC 2007; Paulissen *et al.* 2013).

As mentioned above, the cover of rich fens may decline due to natural succession of former rich fens toward *Sphagnum*-dominated fens (Clapham 1940; Mörnsjö 1969; Kuhry *et al.* 1993). Hydrological isolation from base-rich water and/or increasing peat thickness due to peat accumulation (van Wirdum 1991; van Diggelen *et al.* 1996) will eventually lead to enlarged relative influence of hardly buffered rainwater, leading to low acid neutralizing capacity (ANC) and stimulating the succession toward *Sphagnum*-dominated fens (van Wirdum *et al.* 1992). Human-induced acidification (through airborne deposition of N and S) and eutrophication have, however, stimulated this succession in many cases.

Absence of rejuvenation of floating fens

Despite the excavation of many new turf ponds in recent decades, new formation of rich fens through terrestrialization of open waters has almost not occurred in the Netherlands during the past 50 years (e.g. Lamers *et al.* 2002). Although the causes are not yet entirely clear and may differ between different areas, the absence of newly formed rich fens may be attributed to toxicity of sulfide and/or NH_4 in soil pore waters of abandoned turf ponds (Roelofs 1991; Smolders & Roelofs 1993; Lamers *et al.* 2013). In addition, P-eutrophication of the surface water and banks may have played a role (e.g. Schindler 1977; Koerselman & Verhoeven 1995; Søndergaard *et al.* 2001; Lamers *et al.* 2002). If P-loading is excessive in surface waters, the growth of highly productive phytoplankton will be stimulated, which will eventually lead to turbid surface waters and the disappearance of submerged aquatic macrophytes (e.g. Scheffer *et al.* 1993; Janse 2005). In addition, highly productive, species-poor vegetation may develop on the banks of turf ponds (Lamers *et al.* 2002). Such eutrophication can be a consequence of excessive external P-inputs (e.g. Bootsma *et al.* 1999; Jeppesen *et al.* 2005), but can also be caused by accelerated internal P-mobilization through increased SO_4-inputs (Boström *et al.* 1982; Caraco *et al.* 1989; Roelofs 1991). Finally, Cuppen *et al.* (1997) and Fairchild *et al.* (1998) have also shown that several macrophytes are sensitive to high herbicide concentrations in surface waters. It is, however, unclear whether this has been important for the succession of Dutch wetlands.

Fortunately, the situation in Dutch wetlands seems to have slightly improved during the past 15 – 25 years. Although there has not been any new development yet of rich fens with *S. scorpioides* in National Park Weerribben-Wieden, the area of well-developed aquatic vegetation with *Stratiotes aloides* and initial stages of terrestrializing vegetation has increased (Pommer 2011). The cover of *Stratiotes aloides* probably already started to increase around 1985 (van Wirdum 1991).

Acidification

As long as there is no certainty that terrestrialization will lead to the formation of new rich fens, it is very important to conserve and restore the existing ones. As mentioned, rich fens depend on the input of base-rich water to prevent or slow down the succession to *Sphagnum*-dominated fens. If there is sufficient supply of base-rich water to buffer the soil pH between 5.5 and 8.0 (e.g. Sjörs 1950), rich fens can persist for decades, sometimes even for centuries (e.g. O'Connell 1981; van Wirdum 1991). In order to maintain a pH of above 5.5, the input of acid buffering substances, such as HCO_3, should be approximately equal to or larger than the input and production of acidifying substances. In National Park Weerribben-Wieden, where almost no groundwater discharge occurs anymore, the input of sufficient base-rich water through seepage of surface water from beneath the floating root mat and/or through flooding, is therefore of uppermost importance for the persistence of rich fens in this area (van Wirdum 1991; Barendregt *et al.* 2004; Cusell *et al.* 2013).

An additional input of acidifying compounds via atmospheric deposition (NH_x, NO_y and SO_x) can have a profound effect on the stability of rich fens (Gorham *et al.* 1987; Sjörs & Gunnarsson 2002). High total acid deposition presumably led to the local extinction of *S. scorpioides* in Dutch weakly-buffered fens during the 1960s (Kooijman & Westhoff 1995). According to Kooijman (2012), high atmospheric deposition rates have also affected the pH of well-buffered fens in the Netherlands. At similar alkalinities and Ca-concentrations in soil pore waters, Dutch fens with *S. scorpioides* showed lower pH-values then reference fens in Sweden, Poland and Ireland.

Eutrophication

In addition to sufficient input of base-rich water, site conditions should be relatively nutrient-poor to conserve the existing brownmoss-dominated rich fens (Kooijman 1993a; Hájek *et al.* 2006; Kooijman & Paulissen 2006). Field studies, based on plant N:P-, N:K- and K:P-ratios in the aboveground vegetation (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003), and fertilization experiments show that brownmoss-dominated rich fens can be limited by N (e.g. Boeye *et al.* 1997; Štechová *et al.* 2008; Pawlikowski *et al.* 2013), P (e.g. Verhoeven & Schmitz 1991; Boeye *et al.* 1997; Kooijman 2012; Pawlikowski *et al.* 2013), or a combination of both. So, depending on the environmental conditions, additional inputs of N and P may have profound effects on brownmoss-dominated rich fens. They can lead to increased aboveground biomass production of highly competitive vascular plants, which often results in reduced light availability for slow-growing vascular plants and mosses (Kotowski & van Diggelen 2004) and a decrease of species richness (Grime 1979; Wheeler & Shaw 1991). Furthermore, *S. scorpioides* may be replaced by *Calliergonella cuspidata* (Hedw.) Loeske under P-rich conditions (Meijer & de Wit 1955; van Wirdum 1991; Kooijman 1993b). This is problematic, since even under wet conditions, in which their apices were just above the water table, *C. cuspidata* plants were more readily overgrown by large acidifying *Sphagnum* spp. such as *S. squarrosum* Crome and *S. palustre*, than *S. scorpioides* (Kooijman & Bakker 1995).

Toxicity

Rich fens with *S. scorpioides* may also be sensitive to sulfide- and NH_4-toxicity. Paulissen *et al.* (2004) found that NH_4-concentrations of 100 µmol L^{-1} are potentially toxic to *S. scorpioides*. In addition, Verhoeven *et al.* (2011) showed in a field experiment that four years of high NH_4-deposition (35 kg N ha^{-1} $year^{-1}$) seriously damaged rich fens with *S. scorpioides*, while similar fluxes of NO_3-deposition had no effect. Under base-rich conditions, part of the NH_4 will be nitrified, but nitrification rates will drop with decreasing pH (Painter 1970; Wild *et al.* 1971). If NH_4-toxicity is important, this may therefore primarily be expected in acidified fens that show low nitrification rates (Kooijman & Paulissen 2006; Kooijman 2012). Potential effects of enhanced sulfide concentrations on rich fens have not yet been examined, but experiments have shown that the aboveground biomass production of some characteristic species, such as *Equisetum fluviatile* L., *Menyanthes trifoliata* L. and *Thelypteris palustris*, decreases at sulfide concentrations above 50 – 150 µmol L^{-1} (Geurts *et al.* 2009).

Development of carr woodland

In Western Europe, the cessation of mowing in fens often leads to the development of carr woodland dominated by alder or willow (e.g. Godwin 1934; Wiegers 1992). Several studies in the Netherlands (Bakker *et al.* 1994; van Diggelen *et al.* 1996; van Belle *et al.* 2006) suggest that the decline in mowing activity between the 1950s and 1970s has resulted in large-scale development of carr woodland within 10 – 20 years. Regular mowing therefore appears to be a crucial management measure for the preservation of rich fens by resetting succession, particularly since new development of rich fens is hardly taking place anymore.

Allowing more fluctuating surface water levels

In wet regions dominated by agriculture, such as the Netherlands, surface water levels are strictly controlled in order to support a variety of services such as drainage for agriculture, freshwater supply and flood protection. This generally implies more constant water levels, suppressing or even inverting seasonal and incidental meteorological variation. Under these conditions, the re-introduction of more fluctuating surface water levels has been proposed as one of the management tools to improve the water quality in wetlands (e.g. Mitsch & Gosselink 2007) and to counteract acidification and eutrophication of fens (Grootjans *et al.* 2001; Loeb *et al.* 2008a). There are, however, also examples where the implementation of lowered and raised water tables led to a stimulation of acidification, eutrophication and/or toxicity in fens (e.g. Lamers *et al.* 2002).

Although the impacts of a more fluctuating surface water level regime on brownmoss-dominated rich fens have not yet been examined in a systematic and comprehensive study, there is a number of studies that examined the influence of lowered and raised water tables on specific processes and/or on other fen types. The literature results are described in the following sections and have been combined into one scheme (Fig. 1.4), showing the possible impacts.

Effects on surface water quality

Lowered surface water levels during periods with an evapotranspiration surplus will lead to reduced input of water into wetlands. Such a reduction of water inputs may lead to more ombrotrophic (less Ca-rich) conditions in surface waters, as has been described by Groeneweg & van Wirdum (2004) for surface waters in National Park Weerribben-Wieden. Since surface waters in Europe often contain high nutrient concentrations as a result of intensive agricultural land use around wetlands, lowered

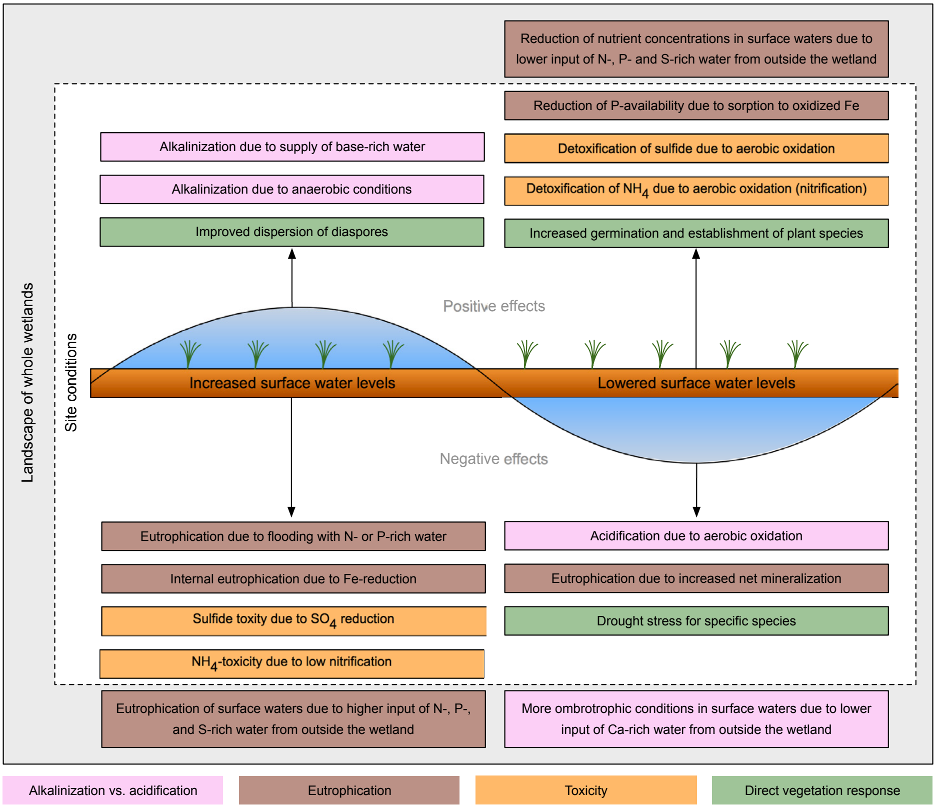


Fig. 1.4. The potential effects of lowered and raised surface water levels on rich fens without groundwater discharge. Effects on the level of the entire wetland and on the level of site conditions are depicted.

water tables will presumably also result in a beneficial reduction of N-, P- and S-inputs (Coops & Hosper 2002; Jaarsma *et al.* 2008; Schep *et al.* 2012). In contrast, raised surface water levels may lead to eutrophication of surface waters, when the water level rise is accompanied by an increased input of N- and P-rich water into a wetland (e.g. Bollens 2000).

The effect of lowered water levels on site conditions

Episodes of water level drawdown will not only influence the ecohydrochemical conditions in the surface waters of the wetland, but are also likely to have an impact on site conditions in fens, including rich fens. Temporary desiccation of the peat surface may result in reduced P-availability in relatively Fe-rich fens, due to sorption of P to oxidized Fe(III) and Fe(III) oxides and hydroxides, as has been described by Lamers *et al.* (1998a) for a mesotrophic wetland meadow with *Caricion nigrae*. Furthermore, oxidation during episodes with lowered water tables may also detoxify previously produced sulfide (Connell & Patrick 1969) and NH_4 (e.g. Williams 1974) in fens.

However, the lowering of surface water levels can also have adverse effects for fens. It may induce eutrophication by the stimulation of mineralization rates as a result of higher oxygen intrusion into soils, as has been recorded in several different fen types (Grootjans *et al.* 1986; Updegraff *et al.* 1995; Bridgham *et al.* 1998; Olde Venterink *et al.* 2002a). Depending on the site conditions, lowered surface water levels may thus result in either decreased or increased nutrient availability. If the ANC is insufficient to compensate for the acid produced, water level drawdown may also lead to undesired lowering of the pH in topsoils as a result of aerobic oxidation processes (microbial redox processes using oxygen as an electron acceptor). This has for instance been observed in a mesotrophic wetland meadow with *Caricion nigrae* (Lamers *et al.* 1998a) and in several carr woodlands (Lucassen *et al.* 2002). Finally, prolonged periods with lowered surface water levels may also lead to drought stress in vascular plants and mosses, as has been described for *S. scorpioides* (Boryslawski 1978) and other rich fen species (Mälson *et al.* 2008).

The effect of raised water levels on site conditions

In wetlands without groundwater discharge, such as National Park Weerribben-Wieden, a temporary rise of the surface water level may be the only way to enhance the ANC in the upper part of fen soils. The ANC of topsoils can be increased by a higher influx of Ca and HCO_3. It seems reasonable to assume that raised water levels may lead to flooding with base-rich surface water and/or to enhanced seepage of base-rich water from beneath the floating root mat, due to an increased upward pressure (van Wirdum 1991; Barendregt *et al.* 2004; Cusell *et al.* 2013). On the other hand, raised surface water levels may also lead to internal microbial alkalinity generation as a result of NO_3-, Fe(III)- and/or SO_4-reduction under anaerobic conditions (e.g. Gambrell & Patrick

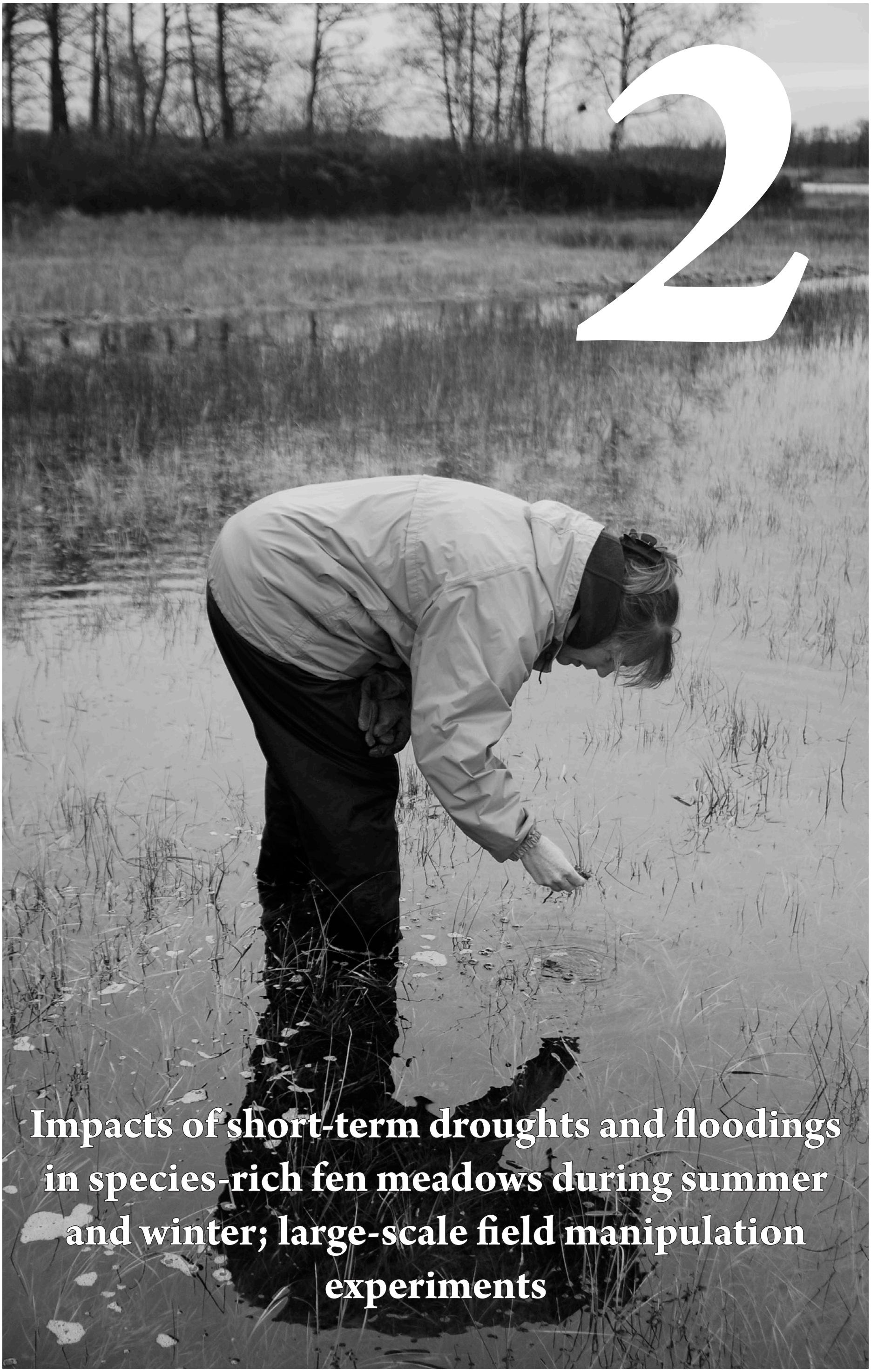
1978; Baker *et al.* 1986) in the soil. However, this increase in ANC will be temporary, since aerobic oxidation during subsequent episodes with lower water tables will lead to re-acidification, as shown in mesotrophic wetland meadows and riverine hay meadows (Lamers *et al.* 1998a; Loeb *et al.* 2008a)

A rise in surface water levels may, however, also result in undesired eutrophication in fens due to higher P- and N-inputs during flooding (Wassen & Joosten 1996; Mitsch & Gosselink 2007). In addition, flooding may lead to internal P-mobilization in fens as a result of Fe(III)-reduction (e.g. Patrick & Khalid 1974). This may especially occur when flooding water contain high levels of SO_4, as Lamers *et al.* (1998b) showed in a mesocosm experiments with sods from a mesotrophic wetland meadow with *Caricion nigrae*. Furthermore, anaerobic conditions during inundation of fens may stimulate other undesired processes such as NH_4-accumulation (e.g. Reddy & Patrick 1984) and sulfide production (e.g. Lamers *et al.* 1998b, 2013).

Aims and outline of this thesis

In conclusion, previous research suggests that lowered as well as raised surface water levels may potentially have both positive and negative impacts on rich fens. For the restoration and preservation of these biodiverse and endangered fens, it is of great importance to get a better understanding of all these interacting processes. Therefore, the main objective of this thesis is to determine the potential effects of both lowered and raised surface water levels on the biogeochemical and ecological functioning of fens, with the emphasis on preservation and restoration of brownmoss-dominated rich fens. The focus is on the processes of eutrophication, alkalinity generation and acidification in relation to fluctuating water levels in the Dutch National Park Weerribben-Wieden. These processes are studied at several spatial scales, from site conditions to the entire wetland area. Furthermore, special attention is paid to the type of nutrient limitation in brownmoss-dominated rich fens in relation to their edaphic characteristics, since the effect of specific nutrient inputs may well be determined by these characteristics.

Chapter 2 describes and explains the biogeochemical responses found during short-term (2 weeks) episodes of surface water level rise (during winter and summer) and drawdown (during summer) in large-scale field experiments, including floating and non-floating fen sites with several vegetation types. Since longer periods of inundation and water level drawdown could not be tested in the field, mesocosm experiments have been performed in the laboratory. Chapter 3 discusses the impacts of long-term (31 weeks) lowered and raised water tables on rich fen mesocosms, as tested under winter and summer conditions. Apart from the biogeochemical responses, vegetation responses have also been examined in this study. Chapter 4 reports about a long-term laboratory peat incubation experiment to study the effects of aeration (oxygen intrusion) and desiccation (oxygen intrusion plus water shortage) on mineralization and acidification rates in peat from poor fens and rich fens. To gain more insight into the effects of more fluctuating surface water levels at the entire wetland scale, Chapter 5 focusses on mechanisms that explain changes in nutrient availability along a gradient from water entry locations to more isolated rich fens. Next, Chapter 6 evaluates the consequences of N- and P-addition on different rich fen types in a fertilization experiment in the field to determine potential effects of flooding with nutrient-rich water and to unravel the potential causes for N- and/or P-limitation in rich fens. In Chapter 7, results of all studies are integrated and discussed. Implications for nature management and water management, and suggestions for future research, are included.



2

Impacts of short-term droughts and floodings in species-rich fen meadows during summer and winter; large-scale field manipulation experiments

2. Impacts of short-term droughts and floodings in species-rich fen meadows during summer and winter; large-scale field manipulation experiments

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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, short-term water level fluctuations have been postulated to restore the acid neutralizing capacity (ANC) by inundation and to reduce P-eutrophication during episodes with lower water levels.

This is the first study which tests this hypothesis in large-scale field manipulation experiments in protected base-rich fens, *Calliergonella*-dominated fens and *Sphagnum*-dominated fens. Five different experiments were performed: two weeks of raised levels (+10 cm) in a floating and a non-floating fen during winter, two weeks of high levels during summer in a non-floating fen, and two 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 in fens or on soil biogeochemistry. For non-floating fens, raised surface water levels led to flooding in all vegetation types, without affecting the nutrient concentrations. Although redox potentials decreased immediately in the upper part of soils, ANC was generally not enhanced in winter, due to the lack of infiltration into the waterlogged soils. In summer, in contrast, ANC increased as a result of higher temperatures and evapotranspiration, which led to enhanced infiltration of inundation water and to microbial alkalinity generation. Short-term lowering of surface water levels in summer led to lower water tables in non-floating fens, but only if precipitation rates were low. ANC and nutrient concentrations were, however, not affected at all.

Synthesis and applications: Our results show that the biogeochemical effects of short-term surface water level fluctuations strongly depend on peat buoyancy, water saturation of soils, season and weather. This explains why short-term floodings in winter are often not a suitable measure to restore the ANC of fens, while short-term floodings in summer do lead to an increase of the ANC. Short-term droughts do not affect the ANC or nutrient availability. These results are not only important for the hydrological management of fens, but also have future implications since short-term extreme weather events will occur more frequent, due to climate change.

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). These species-rich fens are protected under the European Habitats Directive (transition mires, type H7140) and often comprise many threatened vascular plants and bryophytes such as *Liparis loeselii* (L.) Rich. and *Scorpidium scorpioides* (Hedw.) Limpr. In recent decades many rich fens have been lost in Europe, due to land use change (Kooijman 1992; JNCC 2007; Paulissen *et al.* 2013). Part of this decline is caused by natural succession to *Sphagnum*-dominated fens (e.g. Clapham 1940; Gorham *et al.* 1987; Kuhry *et al.* 1993), but anthropogenic forces, including high N-deposition rates, have presumably accelerated this succession in many cases. Although no experimental studies have been carried out yet, field studies indicate that atmospheric deposition may lead to accelerated acidification of rich fens (Gorham *et al.* 1987; Gunnarsson *et al.* 2000; Kooijman 2012). In addition, P-eutrophication can accelerate succession of P-limited rich fens to *Sphagnum*-dominated fens (Kooijman 1993a; Kooijman & Paulissen 2006).

In wetlands with strongly regulated surface water levels as a result of agriculture in the surroundings, one of the proposed management tools to counteract acidification and P-eutrophication is the re-introduction of more fluctuating surface water levels. Raised surface water levels may result in an increase of the alkalinity, pH, and/or Ca-concentrations in soil pore waters (Loeb *et al.* 2008a). The acid neutralizing capacity (ANC) can be increased by microbial-induced anaerobic reduction of Fe(III), SO_4 and/or NO_3 in wet soils (Gambrell & Patrick 1978; Baker *et al.* 1986), which will be temporary since aerobic oxidation during subsequent episodes with lower water tables in fens can lead to the opposite process of acidification (Lamers *et al.* 1998a; Loeb *et al.* 2008a). In addition, ANC can also increase more permanently by infiltration of base-rich surface water during inundation. A rise in surface water levels may, however, also result in eutrophication due to higher P- and N-inputs during flooding (Wassen *et al.* 1996) and/or increased internal P-mobilization by Fe(III)- and/or SO_4-reduction (Patrick & Khalid 1974; Lamers *et al.* 1998b). The latter process depends on the P-concentrations in the soil and its type of binding (Loeb *et al.* 2008b).

Episodes with lowered surface water levels will lead to reduced input of water into wetlands, because less surface water is needed to compensate for water losses. Since surface waters in Europe often contain high nutrient concentrations, due to intensive agricultural land use around wetlands, lowered water tables in fens will presumably result in a reduction of N- and P-inputs (Coops & Hosper 2002). At the same time, however, lowered water tables can also stimulate net mineralization rates, due to higher oxygen availability in fen soils (Grootjans *et al.* 1986; Updegraff *et al.* 1995; Bridgham *et al.* 1998; Olde Venterink *et al.* 2002a). Furthermore, water level drawdown may result in acidification as a consequence of aerobic oxidation processes (Lamers *et al.* 1998a; Lucassen *et al.* 2002), which are microbial-induced redox processes that consume oxygen.

All mechanisms mentioned above have been studied intensively in mesocosm and incubation experiments, but none of these studies examined the net effect of all water-level related processes in a field experiment. This is the first study in which the physical and biogeochemical responses of short-term (two weeks) surface water level rises (during winter and summer) and drawdowns (during summer) have been tested for several years in large-scale field experiments in protected base-rich fens, *Calliergonella*-dominated fens and *Sphagnum*-dominated poor fens. The questions addressed in this study were: (a) what are the changes in water table and biogeochemical responses as a result of short-term (two weeks) changes in surface water level, (b) do these responses differ between floating and non-floating fens, and (c) do the responses to raised surface water levels in non-floating fens differ between winter and summer conditions? The answers to these questions will not only be important for the hydrological management of fens, but are also likely to have future implications since short-term periods with intense precipitation or drought will occur more frequent in many parts of the world, due to climate change (e.g. Bronstert 2003; Kundzewicz *et al.* 2006). Our expectation for (a) was that raised surface water levels lead to an increased ANC, but also to P-eutrophication. In contrast, lowered surface water levels are expected to lead to acidification and eutrophication, due to increased mineralization rates. For (b), we expect that the effects on biogeochemistry are largest in non-floating fens, since water tables will presumably fluctuate more in these fens than in floating fens. For (c), we expect that the increase in ANC will be larger in summer than in winter floodings, because of higher infiltration rates and/or higher microbial alkalinity generation.

Materials and methods

Experimental design

Three summer-mown experimental fen sites in the Dutch Ramsar area "National Park Weerribben-Wieden" were used to determine the biogeochemical effects of short-term rises and decreases of the surface water level: a floating fen site in "De Weerribben" (WEE; 52°47' N, 5°55' E) and two non-floating fen sites in "Kiersche Wiede" (KW; 52°42' N, 6°08' E) and "Veldweg" (VW; 52°42' N, 6°07' E). The floating WEE-fen had a buoyant 70 – 90 cm thick peat layer, floating above a sandy substrate at 250 cm below soil surface. It comprised three vegetation types: (a) fen type where *Calliergonella cuspidata* (Hedw.) Loeske dominated the moss layer (**Call**; *Caricion nigrae – Carex nigra-Agrostis canina* type), (b) poor fen type where *Sphagnum palustre* L. and *Sphagnum fallax* (H.) Klinggr. dominated the moss layer (**Sph**; *Caricion nigrae – Pallavicinio-Sphagnetum typicum* type), and (c) moor type with *Erica tetralix* L. and *S. palustre* (**Moor**; *Oxycocco-Ericion – Sphagno palustris-Ericetum* type). In contrast, the non-floating KW- and VW-fens were firmly connected to the lower sandy substrate, which was found at a depth of 60 – 90 cm. In addition to the three mentioned vegetation types in the WEE-area, the KW- and VW-areas also contained some rich fens (**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.

The present surface water level of the National Park, situated below sea level, 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. In this study, five experiments were conducted to evaluate the biogeochemical effects of lowered and raised surface water levels on the different fen types mentioned (Table 2.1). In the WEE- and KW-areas, experimental floodings and water level drawdowns were enabled 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 two 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 in the WEE-fen and between 2008 and 2011 in the KW-fen. Technical constraints made it impossible to raise surface water levels in the WEE-area in 2008 and 2011. 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 two weeks (3.5 – 4 mm/day) resulted in surface water levels of 0.73 m BMSL. In this case, surface water levels were thus not manipulated by pumps and just equaled the levels in the entire National Park. Finally, surface water levels were lowered by 15 cm for two 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**).

Table 2.1. Schematic overview of the five field manipulation experiments.

	Area	Fen type	Month	Treatment of two 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

Sampling

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

Before and after the treatment, soil pore water samples of the upper 10 cm of the soil were collected anaerobically by connecting vacuumed plastic syringes (50 mL) to soil moisture samplers (Rhizons SMS 10 cm, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). The first 10 mL of each sample was discarded to exclude stagnant sampler water. Similar samples were also collected in July 2008 to determine the initial biogeochemical conditions for all fen sites, before any treatment had started.

Since raised surface water levels led to flooding of the KW-area (experiment 4), we also collected the flooding water above the vegetation for this experiment. After one week of flooding, iodated polyethylene bottles of 100 mL were used to collect the flooding water in 2009, 2010 and 2011. At the same moment, surface water samples were taken in five adjacent ditches that supply the flooding water.

Plant species composition was also recorded in the subplot of 2 x 2 m in July 2008 (before the start of any treatment) and July 2011 (after the treatments). All vascular plant and bryophyte species were recorded, and cover values were estimated as percentages. These results will not be discussed further, since no significant developments were found during the short period of three years, except for a trend to higher abundance of *Sphagnum* spp., i.e. *S. palustre* and *S. fallax* (Cusell *et al.* 2013).

Chemical analyses

The pH-values of all water samples were measured, and alkalinities were determined by titration to pH 4.2, using 0.01M HCl. Next, surface water and flooding water samples were filtered through GF/C glass-fiber filters (Ø = 1.2 μm; Whatmann, Brentford, UK). Subsequently, all samples were divided into two subsamples, and 1% of concentrated HNO_3 (P.A. quality) was added to one of these subsamples to avoid metal precipitation. Both subsamples were stored in the dark at -24 °C until further analysis. 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 the KW-fen 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 minutes. The E_m was measured as the potential between a sensor tip and a reference electrode, 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_m being the measured potential and E_{ref} being the potential of the reference probe to the standard hydrogen probe of 228 mV.

Statistical analyses

Statistical analyses were performed in SPSS for Windows (SPSS 20.0.0, IBM, Armonk, USA). A two-way ANOVA with 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, before any treatment had started. Since fen sites differed in terms of biogeochemistry and the ability to float, subsequent analyses were performed separately for the five different experiments. Because the 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, two or three consecutive measurements were used to determine contrasts between the conditions right before, during and right after the change in surface water levels. The differences between measurements directly before and after the treatment were used as response variables in the linear mixed models. Differences between vegetation types and years, whenever significant in the mixed model, were further examined by comparing their estimated marginal means in a LSD post-hoc test.

In experiment 2, where increases of the surface water level led to flooding in the KW-area, 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 flooding water had a homogenous composition or differed between the vegetation types in the KW-area. The second model used a categorised 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 pore waters during floodings.

Results

Initial conditions

At the fen sites studied, water tables were significantly higher in base-rich Scor- and Call-vegetation than in more acidic Sph- and Moor-vegetation, with mean depths of 5 – 10 cm and 15 – 23 cm below the surface in July (Fig. 2.1, Table 2.2). As expected, initial pH-values of 5.6 – 6.3 in soil pore waters for Scor- and Call-vegetation were

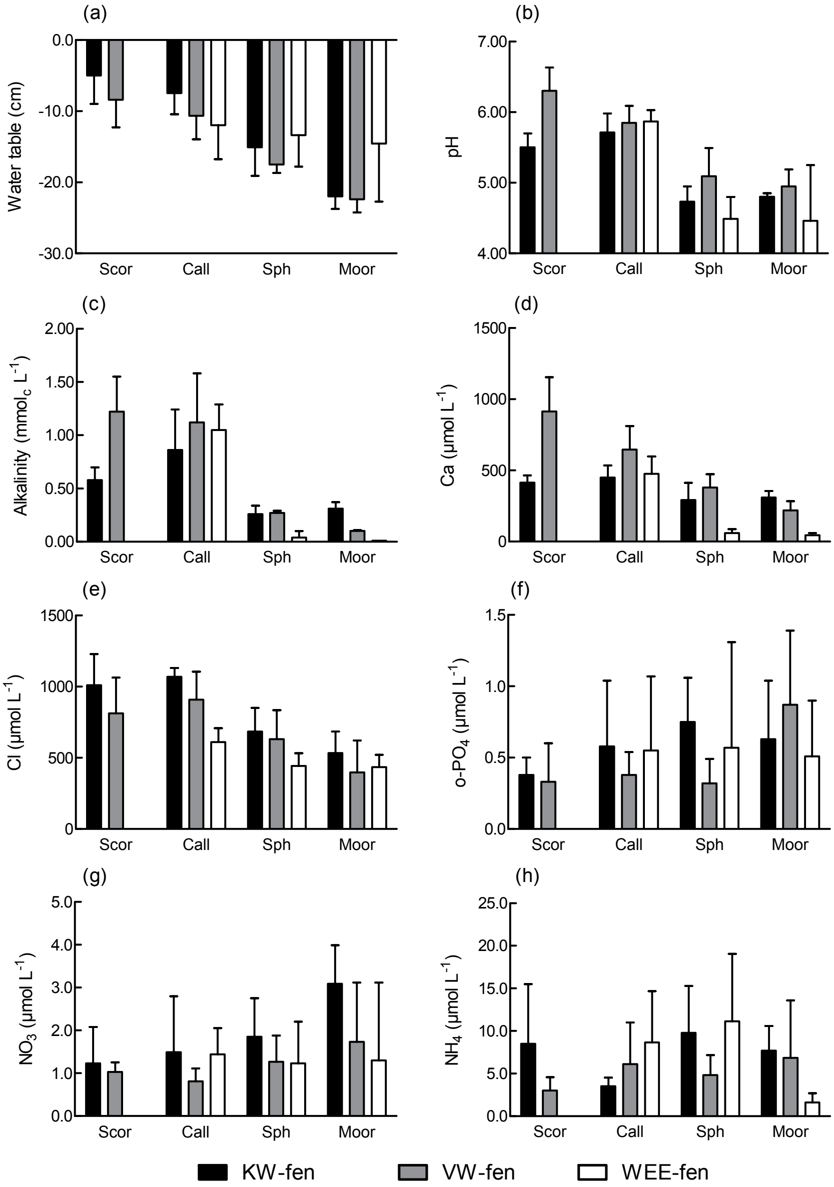

Fig. 2.1. Water table (a), pH (b), alkalinity (c) and concentrations of Ca (d), Cl (e), o-PO$_4$ (f), NO$_3$ (g) and NH$_4$ (h) in the soil pore waters of four vegetation types (Scor = fen with a moss-layer dominated by *Scorpidium cossonii* or *Hamatocaulis vernicosus*, Call = fen with a moss-layer dominated by *Calliergonella cuspidata*, Sph = fen with a moss-layer dominated by *Sphagnum palustre*, moor with *Erica tetralix* and *Sphagnum palustre*) in three fens. Sample means are given 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 Table 2.2.

also significantly higher than in Sph- and Moor-vegetation, where mean pH-values of about 4.7 were measured. 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 $\mu mol_c\ L^{-1}$, Ca-concentrations of around 500 and 200 $\mu mol\ L^{-1}$ and Cl-concentrations of around 900 and 500 $\mu mol\ L^{-1}$. It was, however, remarkable that the VW-fen showed higher pH-values, alkalinities and Ca-concentrations than the other two fen sites, which was especially the case in base-rich vegetation types, as indicated by significant interaction effects of area and vegetation type. In contrast, concentrations of o-PO_4, NO_3 and NH_4 did not differ between vegetation types or fen sites. These concentrations were low in the soil pore waters of all vegetation types, with concentrations below 1, 3 and 10 $\mu mol\ L^{-1}$, respectively.

Table 2.2. Effects of fen site, vegetation type and their interaction on chemical variables in the pore water at the start of the experiment in July 2008. KW = non-floating fen in Kiersche Wiede, VW = non-floating fen in Veldweg, WEE = floating fen in Weerribben, Scor = fen with a moss-layer dominated by *Scorpidium cossonii* or *Hamatocaulis vernicosus*, Call = fen with a moss-layer dominated by *Calliergonella cuspidata*, Sph = fen with a moss-layer dominated by *Sphagnum palustre*, moor with *Erica tetralix* and *Sphagnum palustre*.

	Fen site	Veg	Fen site * Veg	KW	VW	WEE	Scor	Call	Sph	Moor
water table	1.83	13.46**	1.57	a	a	a	b	b	a	a
pH	6.32**	29.46**	2.58*	a	b	a	b	b	a	a
alkalinity	2.46	21.50**	2.61*	a	a	a	b	b	a	a
Ca	10.44**	17.50**	4.32**	b	c	a	c	b	a	a
Fe	2.63	0.83	2.19	a	a	a	a	a	a	a
S	1.64	1.10	2.32	a	a	a	a	a	a	a
Cl	6.72**	10.50**	1.02	b	b	a	b	b	a	a
o-PO4	0.32	0.95	0.62	a	a	a	a	a	a	a
NH4	0.83	0.94	1.95	a	a	a	a	a	a	a
NO3	2.20	1.42	0.62	a	a	a	a	a	a	a

F-ratios with their level of significance: * $P \leq 0.05$, ** $P \leq 0.01$. Different letters indicate significant differences ($P \leq 0.05$) between treatments, n.s. = not significant.

Experiment 1: Raised surface water levels in a floating fen during winter

As expected, a rise of surface water levels by 10 cm had almost no effect on the water tables in the floating fen soils (Fig. 2.2, see Table S2 in Supporting Information of online article). Along with this limited change in water tables, none of the measured biogeochemical variables was significantly changed for any of the vegetation types.

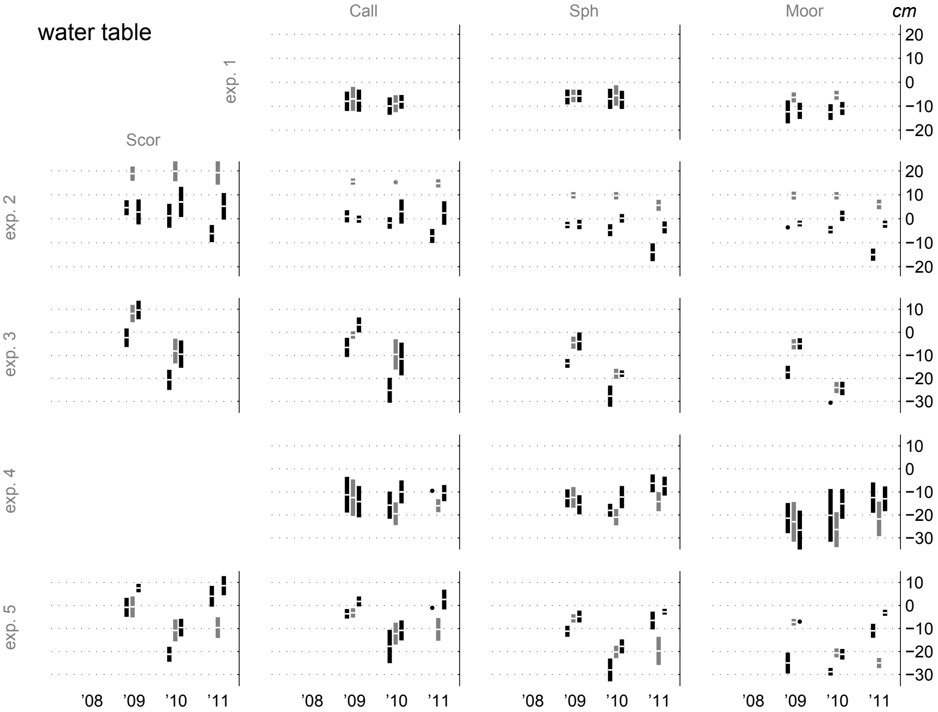

Fig. 2.2. Effect of five surface water level treatments on the water table (given in cm above/below the fen surface) in four vegetation types during the monitored years. Water tables were measured two days before (black lines at the left of each triplet), during (grey lines) and two days after the treatments (black lines at the right of each triplet). Sample means (white centers of a line) are given with their standard deviations (n = 5). Scor = fen with a moss-layer dominated by *Scorpidium cossonii* or *Hamatocaulis vernicosus*, Call = fen with a moss-layer dominated by *Calliergonella cuspidata*, Sph = fen with a moss-layer dominated by *Sphagnum palustre*, moor with *Erica tetralix* and *Sphagnum palustre*, exp. 1 = floating WEE-fen during raised surface water levels in winter, exp. 2 = non-floating KW-fen during raised surface water levels in winter, exp. 3 = non-floating VW-fen during raised surface water levels in summer, exp. 4 = floating WEE-fen during lowered surface water levels in summer, exp. 5 = non-floating KW-fen during lowered surface water levels in summer. Statistical information is provided in Table S2 (Supporting Information of online article).

Experiment 2: Raised surface water levels in a non-floating fen during winter

The rise in surface water levels by 10 cm during the treatment periods in November led to flooding in all vegetation types during all four years (Fig. 2.2, see Table S2). Water table rises were largest in 2011, when initial water tables were lowest with 5 – 15 cm below the surface, and smallest in 2009, when initial water tables were highest with levels around the fen surface. In 2009, most Scor- and Call-vegetation was even inundated at the start of the treatment. Furthermore, water tables raised significantly more in Scor- and Call-vegetation than in Sph- and Moor-vegetation.

In samples of flooding water, concentrations of the inert Cl-anion did not differ between vegetation types during any of the monitored years, and were equal to the concentrations in the adjacent ditches that supply the flooding water (Fig. 2.3). Concentrations of o-PO_4, NH_4 and NO_3 did also not differ between vegetation types and were low with values of 0.05, 3 and 2 μmol L^{-1}, respectively. In contrast, alkalinities and Ca-concentrations did significantly differ in flooding water samples, with alkalinities of around 900 and 500 μmol_c L^{-1} and Ca-concentrations of about 500 and 200 μmol L^{-1} above Scor- and Call-vegetation versus Sph- and Moor-vegetation. Also, the pH decreased significantly from about 7.0 in ditches to 6.4 in standing flooding water above Scor- and Call-vegetation to about 5.4 in flooding water above Moor-vegetation.

Before the floodings, Cl-concentrations in soil pore waters were lower than in the flooding water above the vegetation in all four monitored years (Fig. 2.4). However, the higher Cl-concentrations in flooding waters only led to increased pore water concentrations of Cl during the floodings of 2010 and 2011, and these effects differed significantly between vegetation types, as indicated by the interaction effect of area and vegetation type (see Table S2). In Scor- and Call-vegetation, pore water concentrations of Cl only increased significantly during the flooding of 2011, while Sph- and Moor-vegetation showed significantly increased Cl-concentrations in 2010 and 2011. This clearly shows that the flooding water did not always infiltrate. An additional analysis showed that Cl-concentrations only increased in soil pore water when the start water tables were lower than 5 cm below the soil surface (Table 2.3).

Table 2.3. Effect of water table on the Cl-infiltration into soil pore waters of the KW-fen during floodings in 2009, 2010 and 2011 (experiment 4).

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

Mean values and standard deviations for the differences between the Cl-concentrations just after and before the floodings. Different letters indicate significant differences between water level categories ($P \leq 0.05$).

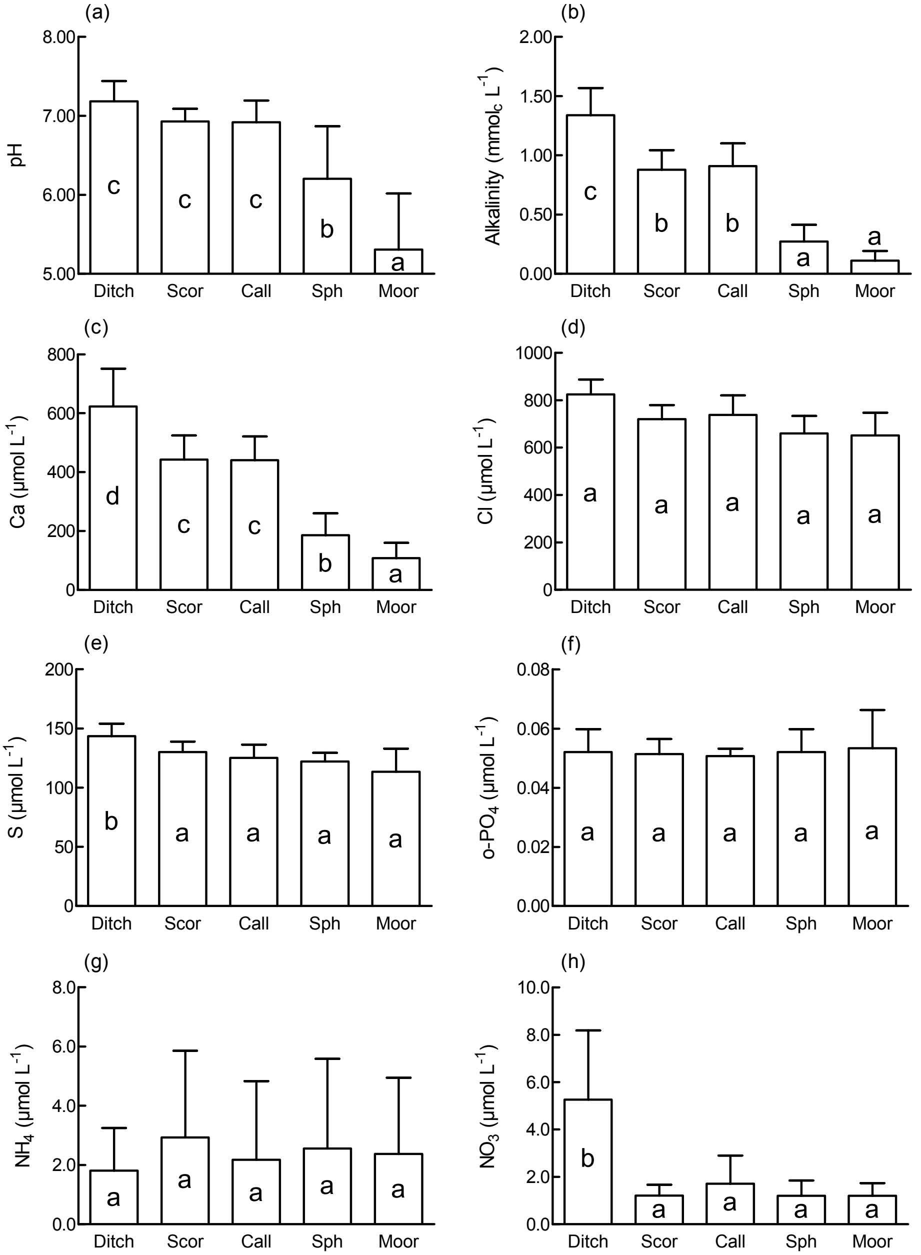

Fig. 2.3. pH (a), alkalinity (b) and concentrations of Ca (c), Cl (d), S (e), o-PO$_4$ (f), NO$_3$ (g) and NH$_4$ (h) in the surface water of adjacent ditches, which supply the flooding water, and the flooding water above four vegetation types in the KW-fen (experiment 2). See the caption of Fig. 2.1 for a description of the abbreviations. Sample means for 2009, 2010 and 2011 are given with their standard deviations (n = 15). Different letters indicate significant differences between vegetation types ($P \leq 0.05$).

In line with the absence of infiltration in 2008 and 2009, flooding had almost no biogeochemical effect in 2008 and 2009. In contrast, biogeochemical effects were observed during the flooding of 2011, when infiltration occurred in all vegetation types. The response to the flooding in 2011 did, however, differ between vegetation types. Redox potentials (E_h) decreased almost immediately in the Sph- and Moor-vegetation, where E_h decreased from about +600 to -100 mV in the upper 12 to 18 cm of the soils, respectively (Fig. 2.5). On the other hand, E_h was only slightly affected in Scor-soils, because nearly the entire profile already showed anaerobic conditions, with E_h-values of around -200 mV, before the flooding. In these soils, E_h only changed slowly from around 300 to -200 mV in the upper 2 cm of the soil. In contrast, alkalinities and Ca-concentrations only increased significantly in soil pore waters of Scor- and Call-vegetation, with 350 $\mu mol_c L^{-1}$ and 150 $\mu mol L^{-1}$, and remained equal in soil pore waters of Sph- and Moor-vegetation, as indicated by the interaction effect of area and

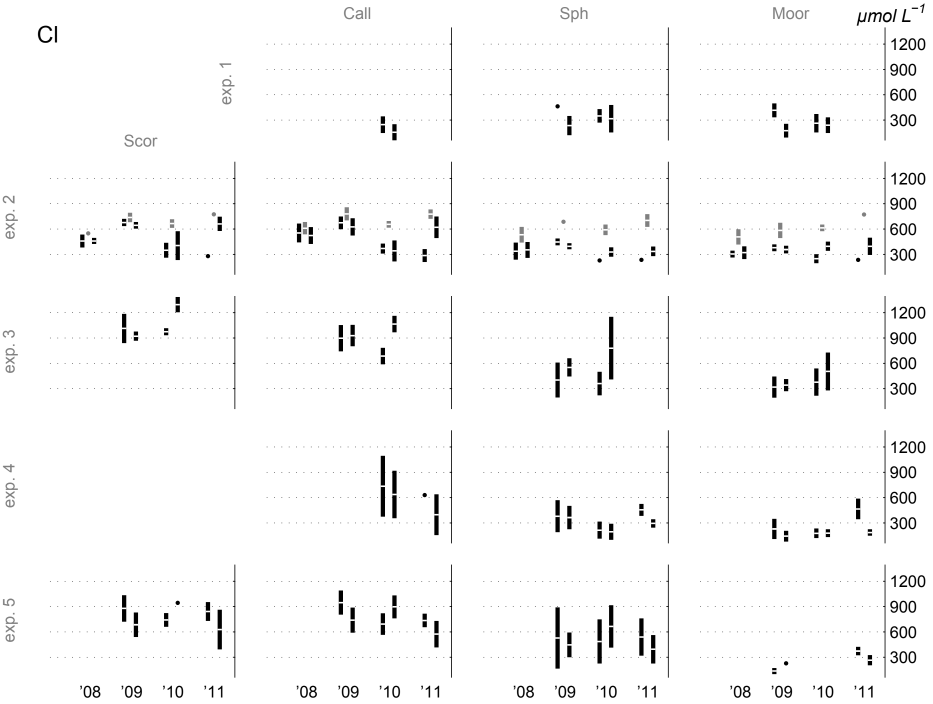

Fig. 2.4. Effect of five surface water level treatments on the Cl-concentrations ($\mu mol L^{-1}$) in four vegetation types during the monitored years. Concentrations were measured two days before (black lines at the left of each triplet), during (grey lines; only in experiment 2) and two days after the treatments (black lines at the left of each triplet). See the caption of Fig. 2.2 for a description of the abbreviations. Sample means (white centers of a line) are given with their standard deviations (n = 5). Statistical information is provided in Table S2 (Supporting Information of online article).

vegetation type (Figs. 2.6 & 2.7, see Table S2). Finally, the flooding of 2011 had no effect on Fe-, S-, o-PO_4, NH_4- and NO_3-concentrations in soil pore waters of any vegetation type (Appendix A, see Table S2).

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 (Fig. 2.2, see Table S2), 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 all soils. In 2009, this rise resulted in flooding with surface water in Scor- and Call-vegetation, while lower (surface) water levels at the start of the 2010-treatment prevented inundations.

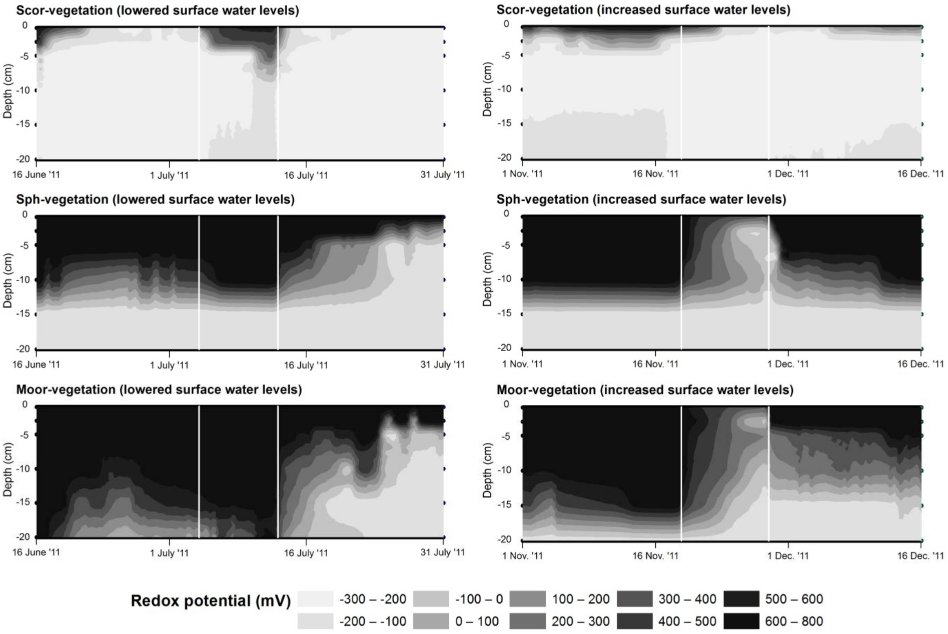

Fig. 2.5. Redox potentials (E_h) in the upper 20 cm of the soil (vertical scale) in three vegetation types of the KW-fen (Scor = fen with a moss-layer dominated by *Hamatocaulis vernicosus*, Sph = fen with a moss-layer dominated by *Sphagnum palustre*, moor with *Erica tetralix* and *Sphagnum palustre*) 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 with lowered (-15 cm; left) and raised (+10 cm; right) surface water levels. For interpolation, ordinary kriging was applied in ArcGIS (ArcMap 10.0, ESRI, Redlands, USA).

The raised surface water levels in 2009 and 2010 had no effect on pH or o-PO_4, NH_4- and NO_3-concentrations in soil pore waters (Appendix A, see Table S2). In contrast, alkalinities and Ca-concentrations in soil pore waters increased during the wet periods in both years. These increases were, however, higher in 2009 than 2010 and effects differed between vegetation types, as indicated by the interaction effect of area and vegetation type (Figs. 2.6 & 2.7). In 2009, alkalinities and Ca-concentrations increased stronger in the flooded Scor- and Call-vegetation than in the non-flooded Sph- and Moor-vegetation, with increases in alkalinities of around 1900 $\mu mol_c L^{-1}$ and 300 $\mu mol L^{-1}$ and increases in Ca-concentrations of around 450 $\mu mol_c L^{-1}$ and 80 $\mu mol L^{-1}$, respectively. Non-flooded Scor- and Call-vegetation in 2010 showed significantly smaller increases in alkalinities (about 600 $\mu mol_c L^{-1}$) and Ca-concentrations (about 150 $\mu mol L^{-1}$), while Sph- and Moor-vegetation showed similar increases in alkalinities (about 250 $\mu mol_c L^{-1}$) and Ca-concentrations (about 80 $\mu mol L^{-1}$) in 2010 as in 2009. Furthermore, Cl-concentrations increased by about 300 $\mu mol L^{-1}$ in soil pore waters of all vegetation types during the non-flooded situation in 2010, while Cl-concentrations remained similar in 2009.

In 2009 and 2010, raised surface water levels led to decreased S-concentrations and increased Fe-concentrations in soil pore waters of all vegetation types (Appendix A, see Table S2). In all vegetation types, S-concentrations decreased with 50 – 150 $\mu mol L^{-1}$ in both years, while Fe-concentrations increased significantly more in 2009 than 2010, with 50 – 115 $\mu mol L^{-1}$ and 15 – 25 $\mu mol L^{-1}$, 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 (Fig. 2.2, see Table S2). Water tables were hardly affected by the treatments in July 2010 and July 2011, which were characterised by a precipitation surplus of 1.0 – 1.5 mm day^{-1} at the end of the treatment period (weather station Marknesse: KNMI 2014). In contrast, water tables were significantly lowered in all soils after the treatment in July 2009, when there was an evaporation surplus of about 2.5 mm day^{-1} during the treatment period. It should, however, be noted that it was a small decrease of only 4 cm. In addition to the limited change in water tables, none of the measured biogeochemical conditions changed in the soil pore water of any of the vegetation types (see Table S2), except that Cl- and S-concentrations decreased significantly in all vegetation types in 2011.

Experiment 5: Lowered surface water levels in a non-floating fen during summer

Before the start of the treatments, water tables significantly differed among the three monitored years (Fig. 2.2, see Table S2), 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 – -25 cm) and 2011 (+5 – -10 cm). In 2011, most Scor-vegetation was even inundated at the start of the treatment.

Lowering of surface water levels by about 15 cm only led to significantly lower water tables in July 2011 (Fig. 2.2, see Table S2). Despite the decrease of surface water levels in ditches, water tables raised in July 2009 and 2010, due to rather heavy rainfall. These raised surface water levels in 2009 and 2010 had no effect on pH and Fe-, o-PO_4, NH_4- and NO_3-concentrations in soil pore waters (Appendix A, see Table S2). The inundated locations with Scor- and Call-vegetation did, however, show significantly increased alkalinities (about 500 μmol_c L^{-1}) in their soil pore waters in 2009, as indicated by the interaction effect of area and vegetation type (Fig. 2.6), while Ca-concentrations did not change (Fig. 2.7) and Cl-concentrations even decreased during this treatment (Fig. 2.4).

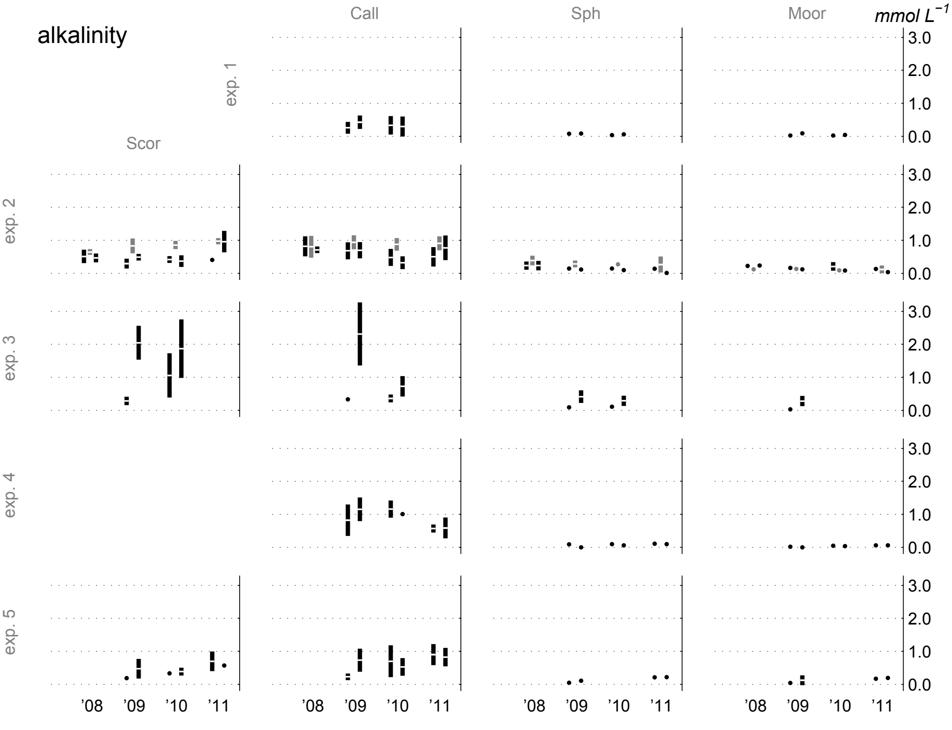

Fig. 2.6. Effect of five surface water level treatments on the alkalinities ($mmol_c$ L^{-1}) in four vegetation types during the monitored years. Values were measured two days before (black lines at the left of each triplet), during (grey lines; only in experiment 2) and two days after the treatments (black lines at the left of each triplet). See the caption of Fig. 2.2 for a description of the abbreviations. Sample means (white centers of a line) are given with their standard deviations (n = 5). Statistical information is provided in Table S2 (Supporting Information of online article).

Although surface water levels were also raised by 4 – 6 cm after the treatment in July 2011, due to two days of rainfall (about 25 mm day^{-1}) after the end of the treatment, the lowered surface water levels did lead to lower water tables during the treatment (Fig. 2.2, see Table S2). During this treatment, water tables were lowered by 10 – 15 cm in all vegetation types. These lowered water tables led to an increase of the redox potential (E_h) from around -200 to +500 mV in the upper 5 cm of Scor-soils (Fig. 2.5). In contrast, E_h did not change in the upper 10 cm of Sph- and Moor-soils, since E_h was already above +600 mV in these topsoils before the start of the treatment. In all vegetation types, E_h decreased immediately during the two days of rainfall after the end of the treatment. However, during the episode of lowered E_h, no changes in pH, alkalinity, Ca-concentrations or nutrient concentrations were observed in the soil pore waters of any of the vegetation types (see Table S2).

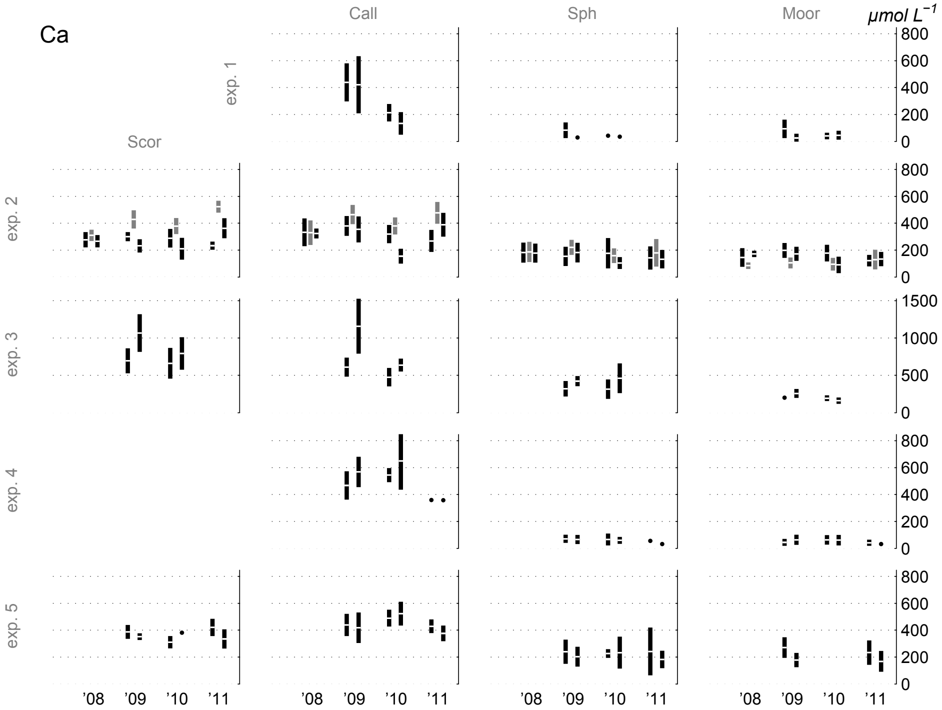

Fig. 2.7. Effect of five surface water level treatments on the Ca-concentrations (μmol L^{-1}) in four vegetation types during the monitored years. Concentrations were measured two days before (black lines at the left of each triplet), during (grey lines; only in experiment 2) and two days after the treatments (black lines at the left of each triplet). Sample means (white centers of a line) are given with their standard deviations (n = 5). See the caption of Fig. 2.2 for a description of the abbreviations. Statistical information is provided in Table S2 (Supporting Information of online article).

Discussion

Water tables in floating fens hardly depend on surface water levels

In floating fens, water tables changed only a few centimeters during raised (+10) and lowered (-15) surface water levels, but these changes were caused by weather conditions (precipitation and evapotranspiration) rather than by treatments. As hypothesized, fluctuations in surface water levels had almost no effect on water tables in floating fens with *Calliergonella*- and *Sphagnum*-dominated vegetation, since the buoyant peat followed the surface water levels. This was not only the case during short-term experiments of two weeks, but also occurred during a similar surface water level rise of three months (field observation of C. Cusell). As a result of the limited change in water tables, ANC and nutrient concentrations in soil pore waters did not change during the field experiments, not even after three months of lowered or raised surface water levels (Cusell *et al.* 2013).

It has, however, been reported that lowered surface water levels may 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* spp. (Cusell *et al.* 2013). Such rich fens are usually located at or below the water table, instead of clearly above, like *Sphagnum*-dominated fens. Although there is still debate about the origin of this inundation water, which may be seepage of surface water from beneath the floating root mat (van Wirdum 1991) or flooding of surface water (Cusell *et al.* 2013), it is clear that floating rich fens may get inundated when surface water levels get sufficiently high. The absence of inundation in our floating fens may thus solely be caused by the limited surface water level rise of only 10 cm and the high buoyancy of the floating fens studied, but may also reflect the absence of rich fens with *Scorpidium* spp. in these floating fens.

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 drawdowns in summer (-15 cm), since weather conditions also affected the water tables in these fens. Water tables only dropped once during the three monitored treatments, which was the only year in which the treatment period coincided with 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. During this water table drop of about 12 cm in non-floating fens, redox potentials (E_h) increased from around -200 to +500 mV in the upper 5 cm of Scor-soils, which indicates that the lowered water tables led to the entry of oxygen into these soils (e.g. Gambrell & Patrick 1978; Rowell 1981). In Sph- and Moor-vegetation, oxygen availability in topsoils was already high at the start of the treatment, as shown by the initial E_h-levels of above +600 mV. The intrusion of oxygen in the upper part of soils during two weeks of lowered surface water levels did, however, not lead to acidification or eutrophication. It is, however, well known that longer episodes with lowered water tables can stimulate net mineralization rates (Grootjans *et al.* 1986; Bridgham *et al.* 1998; Olde Venterink *et al.* 2002a) and acidification by aerobic oxidation processes (Lamers *et al.* 1998a; Lucassen *et al.* 2002).

Flooding of non-floating fens

In non-floating fens, which did not follow the surface water level, high water tables clearly led to inundation, due to fixation of these fens to the sandy substrate. This is supported by diver data (Cusell *et al.* 2013). The high Cl-concentrations in the inundation water compared to the soil pore waters showed that this inundation water originated from the adjacent ditches. Plots with highest water tables before the treatment, which were often dominated by *Scorpidium* spp. or *H. vernicosus*, showed the largest rise in water tables during these floodings. This is presumably because rich fens are usually situated in depressions which are 5 – 10 cm lower than the surface of *Sphagnum*-dominated vegetation, where water tables often bulge somewhat.

Effect of winter flooding on the ANC depends on infiltration rates

The absence of change in Cl-concentrations in soil pore waters during most winters, despite higher Cl-concentrations in the flooding water, shows that there was no or hardly any infiltration into the waterlogged soils. Infiltration only occurred when water tables were lower than 5 cm below the surface before the start of the flooding. This is in accordance with Hooijer (1996) and Banach *et al.* (2009), who also found absence of or limited infiltration of flooding water in waterlogged riverine floodplain fens.

Under non-infiltrating conditions in winter, two weeks of flooding had no effect on the alkalinity and Ca-concentration of soil pore waters. Neither infiltration of HCO_3, Ca and Mg (ANC input) nor increased anaerobic reduction rates in the peat (internal ANC generation) occurred. However, longer-term inundations during winter can lead to both forms of ANC-increase in waterlogged (rich) fen soils, as was demonstrated in a mesocosm experiment (Chapter 3).

Under infiltrating conditions during the flooding in the winter of 2011, alkalinity and Ca-concentration in soil pore water did increase by 50 – 100% during two weeks of flooding, but only in Scor- and Call-soils. Since alkalinity and Ca-concentration increased at a ratio of 2:1 in these plots, the increase in alkalinity is probably mainly caused by the infiltration of Ca- and HCO_3-rich flooding water, and not by microbial-induced anaerobic reduction processes. Despite redox potentials (E_h) of below -200 mV throughout the soil profile of Scor-vegetation, at which Fe(III)- and SO_4-reduction will lead to internal alkalinity generation (e.g. Ponnamperuma 1984; Mitsch & Gosselink 2007), unchanged Fe- and SO_4-concentrations in soil pore waters support the idea of limited occurrence of microbial alkalinity generation. This is most probably caused by the low temperatures in winter and the subsequent low microbial activity (Loeb *et al.* 2008a).

For Sph- and Moor-vegetation, flooding led to an immediate decrease of E_h from values around +600 mV, indicative of the presence of oxygen (e.g. Gambrell & Patrick 1978; Rowell 1981), to around -100 mV. Similar to Scor-soils, these anaerobic conditions did not result in internal alkalinity generation. However, unlike Scor-vegetation, infiltration of the flooding water did also not lead to an increase of alkalinities and Ca-concentrations in soil pore waters of Sph- and Moor-vegetation. This was related to lower alkalinities and Ca-concentrations in the water layer above Sph- and Moor-vegetation compared to Scor- and Call-vegetation. The difference in flooding water composition at a relatively short distance (10 – 20 meters) can only be explained by the exchange of Ca^{2+} for H^+ between flooding water and the adsorption complex of living mosses and their peat. This process has already been described regularly for non-flooded conditions in *Sphagnum*-dominated fens (Clymo 1963; Kooijman & Bakker 1994; Soudzilovskaia *et al.* 2010). Acidification (alkalinity consumption) of flooding water will mainly occur at *Sphagnum*-dominated sites, because adsorption complexes of *Scorpidium* spp. and *C. cuspidata* are probably already saturated with Ca before the flooding, while adsorption complexes of *Sphagna* often contain a fair amount of H^+.

Effect of flooding on the ANC depends on season

The increase of alkalinities and Ca-concentrations in soil pore waters after two weeks of flooding of non-floating fens with Scor- and Call-vegetation was much larger in summer than in winter, even when infiltration occurred in winter. Under summer conditions, alkalinity and Ca-concentration in Scor- and Call-soils increased by about 1900 $\mu mol_c\ L^{-1}$ and 450 $\mu mol\ L^{-1}$ instead of 350 $\mu mol_c\ L^{-1}$ and 150 $\mu mol\ L^{-1}$ in winter. This clear seasonal difference can likely be explained by higher evapotranspiration in summer, which facilitates the infiltration of base-rich flooding water.

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 flooding 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. In these cases, increases in Ca- and Cl-concentrations cannot be caused by infiltration and are probably due to evaporative concentration. In addition, alkalinity production is higher during floodings in summer than in winter as a result of increased microbial alkalinity generation in the warmer peat soil, due to the absence of oxygen and use of alternative electron acceptors such as SO_4 and Fe(III). The simultaneous decrease in SO_4-concentrations (SO_4-reduction) and increase in Fe-concentrations (mobilization of Fe(II)) indeed support this theory (Stumm & Morgan 1996). It must, however, be noted that increase in alkalinity by concentration and microbial-induced anaerobic reduction processes may well be temporary since aerobic oxidation during subsequent episodes with lower water tables in fens can lead to the opposite process of acidification (Lamers *et al.* 1998a; Loeb *et al.* 2008a).

Short floodings 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 flooding water contained very low o-PO_4 concentrations of 0.05 µmol L^{-1}, excluding the occurrence of P-eutrophication by high P-inputs (external eutrophication). Other studies show that flooding 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, the field experiments also showed no evidence of increased internal P-mobilization in waterlogged soils during two weeks of flooding, Several other experiments have, however, shown that prolonged inundation may well lead to internal P-mobilization in waterlogged soils (Patrick & Khalid 1974; Loeb *et al.* 2008a), especially in P-rich fens (Chapter 3) and with SO_4-rich flooding water (Lamers *et al.* 1998b). In the present study, the duration of two weeks seems to be sufficiently short and P-concentrations in flooding seem to be sufficiently low to induce P-eutrophication. However, P-eutrophication can certainly occur during floodings when soil quality and/or surface water quality are insufficient.

Implications for the management of biodiverse fens

Rich fens, which contain many threatened vascular plants and bryophytes, can only persist under well-buffered and nutrient-poor conditions (e.g. Sjörs 1950; Wheeler & Proctor 2000; Kooijman & Paulissen 2006). In wetlands with more or less stable 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.

Our large-scale field experiments suggest that two 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 inundation. Although some studies report inundations of floating fens with *Scorpidium* spp. (O'Connell 1981; van Wirdum 1991; Cusell *et al.* 2013), this study showed that a limited rise in surface water levels did not lead to inundation of the floating *Sphagnum*-dominated fens. As a consequence, ANC and nutrient concentrations did not change in these floating *Sphagnum*-dominated fens. In contrast, in non-floating fens, two weeks of raised surface water levels did lead to flooding. The ANC, however, only increased when base-rich flooding water was able to infiltrate into the soil. In winter, this only occurred when the water table was lower than 5 cm below the surface before the start of the treatment. In summer, infiltration is facilitated by higher evapotranspiration, which is supported by the outcome of long-term mesocosm experiments (Chapter 3). 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 will be temporary since aerobic oxidation during subsequent episodes with lower water tables can lead to acidification (Lamers *et al.* 1998a; Loeb *et al.* 2008a).

Two weeks of moderate summer drought as a result of lowered surface water levels (-10 cm) did not have severe effect on biogeochemical conditions, and the hypothesized acidification or eutrophication did not occur. Longer periods with lowered surface water levels can, however, stimulate net mineralization and acidification. It is therefore advised to prevent longer periods of low water tables as much as possible.

Overall, it can be concluded that short periods of raised surface water level and inundation can be profitable for rich fens as long as surface waters are nutrient-poor and infiltration occurs. This management tool is most suitable for non-floating fens and may best be used in summer when temperatures and evapotranspiration are higher than in winter. Short-term periods with intense precipitation, which are very likely to occur more frequent in future, due to climate change (e.g. Bronstert 2003; Kundzewicz *et al.* 2006), can thus have a positive effect on rich fens, especially if it occurs in summer. On the other hand, periods with low water tables should be prevented as much as possible.

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3

Impacts of water level fluctuation on mesotrophic rich fens: acidification versus eutrophication

3. Impacts of water level fluctuation on mesotrophic rich fens: acidification versus eutrophication

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Abstract

Surface water levels in areas with intensive agriculture have often been strictly controlled for decades. Recently, more natural fluctuating water levels have been propagated to improve the ecological quality of wetlands in these areas. This study investigated the effects of surface water level fluctuations on protected base-rich mesotrophic fens during winter and summer.

We used a mesocosm approach to simulate water level fluctuations under winter and summer conditions (light and temperature) to test the effects of water table on the biogeochemistry and vegetation of two highly endangered fen types with the brownmosses *Scorpidium scorpioides* and *Hamatocaulis vernicosus* as dominant mosses.

Both species showed decreased photosynthetic capacity at lower water tables. These water tables also resulted in decreased alkalinity, due to aerobic oxidation processes in the moss layer. High water tables led to increased pH and alkalinity, due to anaerobic reduction processes and infiltration of base-rich water.

For the P-limited *S. scorpioides* mesocosms, high water tables did not lead to P-mobilization or to significant changes in biomass production, P-uptake and N:P-ratios. However, for the mesocosms with *H. vernicosus*, where P-limitation was not obvious, high water tables did result in P-mobilization and increased P-availability, due to Fe(III)-reduction. The lower P-mobilization for *S. scorpioides* appeared to be related to lower total soil P-content and higher Fe:P- and Ca:P-ratios. Although high water tables resulted in NH_4-accumulation under winter conditions, this did not happen under summer conditions, making NH_4-toxicity unlikely.

Synthesis and applications. Our results suggest that low water tables could hamper protected brownmosses in rich fens, especially during the summer. In contrast, inundations may have a positive effect on rich fens by increasing the alkalinity. However, inundations may lead to eutrophication, due to internal P-mobilization in soils with a high total P-content and low Fe:P-ratio. Therefore, we recommend that soils with high total P-content and low Fe:P-ratios should not be flooded.

Introduction

Surface water levels in pristine wetlands vary with meteoric and groundwater balances in and around these wetlands (Baker *et al.* 2009). However, water levels in most non-pristine land systems are rather strictly controlled in view of a variety of human interests, including agriculture and flood protection. The surface waters in most Northwest European wetlands are managed for this control, which usually implies more constant water levels. This is accomplished by a water supply from rivers and storage basins during dry periods, which frequently has a questionable water quality. In addition, large-scale drainage and groundwater abstractions have resulted in lowered potential groundwater levels and reduced exfiltration of groundwater into the remaining wetlands (Grootjans & ten Klooster 1980; van Wirdum 1991; van Diggelen *et al.* 2006; van Loon 2010).

In the situation described, artificial supply of water in dry seasons reduces the risks of desiccation and acidification by aerobic oxidation processes (e.g. Roelofs 1991), and decreases the influence of base-poor rainwater (van Wirdum 1991). At the same time, however, the surface water inflow may lead to eutrophication as a result of high P- and N-inputs (Koerselman *et al.* 1990; Wassen *et al.* 1996; Bollens 2000; Olde Venterink *et al.* 2002b) or nutrient mobilization through high SO_4-concentrations (Patrick & Khalid 1974; Caraco *et al.* 1989; Roelofs 1991; Lamers *et al.* 1998b). Solving problems regarding acidification and desiccation may thus lead to unwanted eutrophication. Hydrological isolation of fens (e.g. by building dams) can provide a solution to exclude eutrophic water. However, this often accelerates acidification because of the enlarged relative influence of rainwater, which does not have buffering capacity (e.g. van Wirdum, 1991).

This friction between preventing acidification and eutrophication in wetlands under severe human pressure is especially problematic for mesotrophic base-rich fens, which are protected under the European Habitats Directive (transition mires and quaking bogs, H7140). Both acidification and eutrophication can result in fast transformation of these species-rich communities, with many endangered brownmosses such as *Scorpidium scorpioides* (Hedw.) Limpr. and *Hamatocaulis vernicosus* (Mitt.) Hedenäs, being replaced by more acid and species-poor *Sphagnum*-dominated vegetation (Sjörs 1950; van Wirdum 1991, 1993; Kooijman & Bakker 1995; Wheeler & Proctor 2000; Kooijman & Paulissen 2006).

As neither the isolation of specific conservation areas nor the input of polluted surface water seems satisfying, alternative management options are needed. Allowing a more varying surface water level regime has recently been advocated to improve the ecological water quality of wetlands and to reduce management costs (Loeb *et al.* 2008a). This may cause water tables to decrease in fens during dry periods, while inundation of fens with base-rich water may occur during wetter periods. However, these more natural surface water levels may also have unwanted side-effects such as increased net mineralization and acidification by aerobic oxidation processes during periods of decreased water levels (Oomes *et al.* 1997; Lamers *et al.* 1998a; Lucassen *et al.* 2002; Olde Venterink *et al.* 2002a; Geurts *et al.* 2010). In periods with high surface water levels and inundations, the increased nutrient load of supplied surface water may lead to eutrophication and P-mobilization, as well as to sulfide and NH_4-toxicity as a result of anaerobic reduction processes (Smolders & Roelofs 1996; Lamers *et al.* 1998b). The re-introduction of more varying surface water level regime in wetland systems must therefore be considered in view of the potentially most sensitive, but also most valuable, rich fens.

We used a mesocosm approach to test the impact of low and high water tables in rich fens, and unravel different processes like acidification and eutrophication. The aim was to analyze the effects of water table on biogeochemistry and vegetation development under summer and winter conditions in two characteristic types of rich fen with moss layers dominated by either *H. vernicosus* or *S. scorpioides*. The experiment was set up using intact soil cores, including the bryophyte and vascular vegetation. The questions addressed, were: (a) what are the responses of pore water chemistry and vegetation to high and low water tables, (b) what are the responses to increased NH_4- and SO_4-concentrations in the supply water, and (c) how are these responses influenced by season. Our hypothesis for (a) was that low water tables would reduce the growth of *H. vernicosus* and *S. scorpioides,* and increase the acidity and nutrient concentrations in the pore water. In contrast, periods of inundation with base-rich water would increase the concentration of base cations and alkalinity in the pore water, and may lead to eutrophication by internal P-mobilization. For (b), we hypothesized that high NH_4- or SO_4-concentrations in the inundation water lead to NH_4 or sulfide toxicity. Our investigation did not allow for separate analysis of the partly antagonistic, temperature and light controlled processes for hypothesis (c).

Materials and methods

Experimental design

Intact soil cores (upper 30 cm) including living mosses and higher plants were collected in 64 PVC columns (radius * length = 9.5 cm * 50 cm) in April 2009. They were collected from two summer-mown rich fens in National Park Weerribben-Wieden (the Netherlands), which is a Ramsar area that receives special protection in view of the European Habitats Directive. We collected 24 cores in "Kiersche Wiede" (52°42' N, 6°8' E) where *H. vernicosus* dominated the moss layer (H-cores; *Caricion nigrae – Carex nigra-Agrostis canina* type), and 40 cores in "Stobbenribben" (52°47' N, 5°59' E) where *S. scorpioides* dominated the moss layer (S-cores; *Caricion davallianae – Scorpidium-Carex diandra* type). Fewer cores with *H. vernicosus* were collected, because it concerns one of the few locations in the Netherlands with this EU-habitat directive species.

At the day of collection, all cores were placed for two weeks in a climate room at 4 °C with a relative air humidity of 50–60% and a light level of 150 μmol m^{-2} s^{-1} (Philips MGR 102, 400 W; 12 hours day^{-1}). After this, a second acclimatization period of three weeks started, in which half of the cores (12 H-cores and 20 S-cores) were randomly selected and transported to a climate room at 18 °C and 16 hours of light per day (summer conditions). The other half stayed at 4 °C and 12 hours of light per day (winter conditions). Water tables were maintained at surface level during both acclimatization periods, corresponding to the field situation.

The experiment started after the second acclimatization period (week 0). In both climate rooms, 12 H- and S-cores randomly received three different treatments: a control treatment with water table at the surface (0), a treatment with a 15 cm lower water table (-15) and a treatment with a 15 cm higher water table (+15) (n = 4). Table 3.1 shows the chemical composition of the supplied inundation water. The remaining 16 cores with *S. scorpioides* were used to test for possible NH_4 and sulfide toxicity during high water tables under winter and summer conditions, by raising the water tables with NH_4-enriched or SO_4-enriched water (+15N and +15S) (n = 4). NH_4- and SO_4-inputs represented the quality of surface water in agricultural areas adjacent to National Park Weerribben-Wieden. Every three weeks, standing water was refreshed entirely to compensate for changing concentrations in the surface water caused by infiltration, plant uptake and/or exchange between water and soil.

During the experiment, water tables were checked twice weekly and replenished with demineralized water when necessary to compensate for evapotranspiration. Unfortunately, due to leakages we had to exclude one control core (0) under summer conditions and one core with low water table (-15) under winter conditions, both collected from the H-fen, from analyses (n = 3 for both).

Table 3.1. Chemical composition of the inundation water.

	Added to	**Concentration (μmol L^{-1})**
$CaCl_2.2H_2O$	All inundation water	1500
$MgCl_2.6H_2O$	All inundation water	300
KCl	All inundation water	100
$NaHCO_3$	All inundation water	2000
NH_4Cl	Inundation with NH_4-addition	100
Na_2SO_4	Inundation with SO_4-addition	1000

Sampling

Permanent soil moisture samplers (Rhizons SMS 10 cm, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) were inserted to collect pore water at two depths: one in the moss layer (-1 cm) and the other in the soil (-10 cm). Samples were taken anaerobically by connecting vacuumed plastic syringes of 50 mL. The first 10 mL of each sample was discarded to exclude stagnant sampler water. Moss pore water samples were taken in weeks 0, 5, 12, 21 and 31. Soil pore water samples were taken more often in weeks 0, 3, 5, 8, 12, 15, 21, 27 and 31, because most changes were expected in this layer. Samples from week 0 were taken after the acclimatization periods, just before starting the treatments.

At the end of the experiment, the photosynthetic yield at the apex of three randomly selected individuals of *H. vernicosus* or *S. scorpioides* were measured for each core (Junior-PAM fluorometer; Heinz Walz GmbH, Effeltrich, Germany) to get an indication of their vitality. Fluorescence yields were determined after 30 minutes of dark adaptation as $(F_m – F_0)/F_m$, where F_0 is the minimum fluorescence of chlorophyll at a low intensity of light needed to keep the reaction centers of photosynthetic system II open, while F_m is the maximum fluorescence at an intense light pulse. Subsequently, all aboveground plant biomass was harvested and separated into four groups: *Carex* spp. (predominantly *C. elata* All., *C. lasiocarpa* Ehrh., *C. rostrata* Stokes and *C. oederi oedocarpa* (Andersson) Lange), *Juncus* spp. (in particular *Juncus subnodulosus* Schrank), grass spp. (principally *Phragmites australis* (Cav.) Steud., *Agrostis canina* L. and *Calamagrostis stricta* Koeler) and a rest group (e.g. *Equisetum fluviatile* L., *Galium palustre* L., *Menyanthes trifoliata* L. and *Utricularia minor* L.). Dry weights were determined after drying at 70 °C until constant weight. Finally, soil samples of the uppermost 10 cm were taken.

Chemical analyses

Immediately after sampling, sulfide concentrations in the pore water were measured with a sulfide ion-specific Ag-electrode (Orion Research, Beverly, USA) and a double junction calomel reference electrode in 10.5 mL pore water with 10.5 mL sulfide antioxidant buffer (van Gemerden 1984). The pH-values were measured, and alkalinities were determined by titration to pH 4.2 using 0.01M HCl. After dividing all samples into two subsamples, 1% of concentrated HNO_3 was added to one of them to prevent metal precipitation. Both subsamples were stored in iodated polyethylene bottles at -24 °C until further analysis. Total concentrations of Ca, Fe, Mn and S were measured in the acidified subsamples by ICP-OES (Optima 3000 XL, PerkinElmer, Waltham, USA). NH_4, NO_3, o-PO_4, Cl and dissolved organic matter (DOC) concentrations were analyzed colorimetrically with continuous flow auto-analyzers (Skalar Analytical BV, Breda, the Netherlands).

Dry weight and gravimetric moisture content of the soil samples, collected from the uppermost 10 cm at the end of the experiment, were determined by drying at 70 °C until constant weight. To determine total element concentrations in soils and plants (all plant groups together), dried and ground samples were digested for 17 min with 4 mL HNO_3 (65%) and 1 mL H_2O_2 (30%) (Milestone srl, Sorisole, Italy). Digestates were analyzed by ICP-OES, as described above. N-contents in plant material were determined with a Carlo Erba NA1500 elemental analyzer (Thermo Fisher Scientific, Waltham, USA). Vascular plant N:P-ratios were calculated on dry weight basis.

Statistical analyses

Statistical analyses were performed using SPSS for Windows (version 20.0, SPSS, Chicago, USA). QQ-plots were used to assess the normality of the distribution of the residuals of all measured chemical variables. All variables except pH and alkalinity, were log(x+1)-transformed to compensate for skewness and kurtosis. A three-way ANOVA with Tukey HSD post-hoc test has been performed to determine significant differences due to season, water table or source area at the start of the experiment. Some variables differed between both source areas, but there were no significant differences for season and water table at the start (Table 3.2). In the subsequent analyses, the results of H- and S-cores were tested as two separate data sets, to determine the effect of season and water table for both types of fen. As sub-replicates were taken several times from the same cores, a linear mixed model with time as random-effect was used to determine the response to fixed factors (season and water table) for both fen types (West *et al.* 2007). This model was used instead of GLM repeated measures, because the required assumption of independent and identically distributed residual errors was violated. Differences between water tables, whenever significant in the linear mixed model, were further examined by comparing their estimated marginal means in a Bonferroni post-hoc test (correction for multiple comparisons). To further examine interaction effects of season and water table, pair-wise comparisons, with a 95% confidence interval, of the final measurements were used.

A three-way ANOVA with Tukey HSD post-hoc test was performed to determine the effect of treatments on variables that were only measured at the end of the experiment: total soil concentrations, nutrient contents of plant tissue, biomass of plant groups and fluorescence yield of mosses. These variables were not log-transformed, because QQ-plots indicated normality of distribution for the residuals of all these variables.

Table 3.2. Effects of source area, season, water table and their interactions on chemical variables in the pore water of the moss layer (-1 cm) and soil (-10 cm) at the start of the experiment.

	Area	**Season**	**Table**	**Area * Season**	**Area * Table**	**Season * Table**	**Area * Season * Table**
Moss pore water							
pH	58.06*	0.38	0.03	0.03	0.14	0.53	2.21
Alkalinity	57.61*	0.35	0.31	0.01	0.75	0.07	2.18
Ca	94.89*	0.40	0.58	0.03	1.62	0.47	1.20
Cl	15.87*	1.54	1.27	2.66	1.77	1.04	2.10
Fe	30.67*	0.08	4.88	0.17	6.58*	0.21	0.03
S	0.12	3.32	0.24	0.21	1.05	1.08	1.19
Sulfide	6.87	4.94	0.82	4.43	0.04	0.11	0.12
o-PO_4	7.63*	0.13	0.55	0.01	0.48	1.26	1.36
NO_3	2.04	0.17	4.99	0.04	1.37	0.46	0.32
NH_4	0.59	1.10	1.49	1.33	0.91	0.40	0.07
DOC	4.35	6.51	4.23	0.97	2.16	1.92	0.01
Soil pore water							
pH	60.80*	1.97	0.01	1.41	0.28	0.39	1.87
Alkalinity	34.73*	3.58	0.98	0.30	0.53	0.24	3.56
Ca	51.36*	1.61	1.15	0.03	0.70	0.63	1.56
Cl	14.49*	1.33	0.33	1.95	1.28	0.39	1.44
Fe	1.21	0.01	1.95	0.09	0.10	1.20	0.02
S	1.55	2.15	3.54	0.31	2.29	0.36	1.63
Sulfide	5.66	7.11	0.39	7.13	0.01	0.08	0.12
o-PO_4	11.07*	1.78	1.32	2.45	1.37	1.31	1.31
NO_3	0.00	0.11	0.07	0.11	0.08	0.03	0.03
NH_4	5.34	0.92	0.80	1.70	0.48	0.49	0.94
DOC	2.98	2.43	0.38	0.51	0.23	0.02	1.77

F-ratios with their level of significance: * $P \leq 0.01$.

Results

pH and acid buffering

Initial values

At the start of the experiment, the pH differed significantly between cores from fens with *H. vernicosus* (H-cores) and *S. scorpioides* (S-cores), having mean values of 5.8 and 6.3 respectively (Fig. 3.1, Table 3.2). Alkalinity and Ca-concentrations also differed between both core types, with initial alkalinities of about 0.4 and 0.9 $\text{mmol}_c\ L^{-1}$ and Ca-concentrations of around 400 and 1000 $\mu\text{mol}\ L^{-1}$, for H- and S-cores respectively.

High water tables

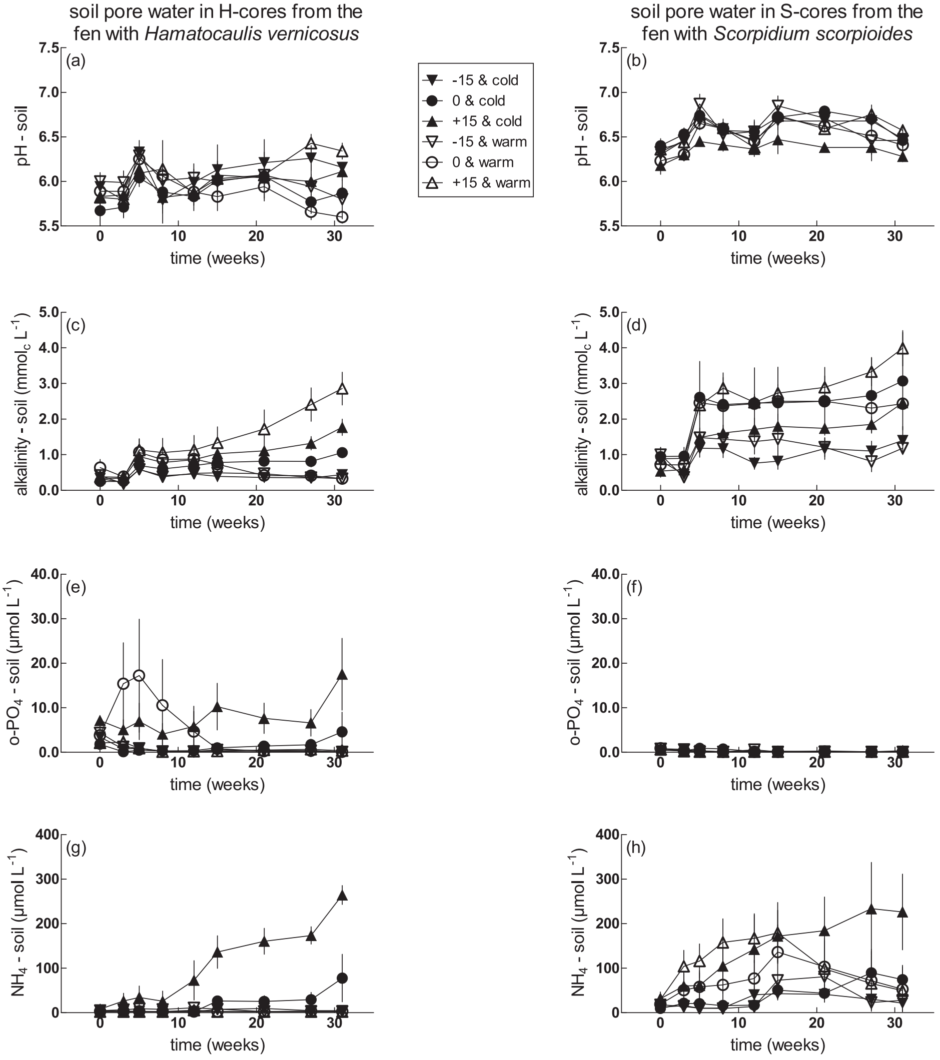
High water tables led to a continuous increase of Cl-concentrations in both moss and soil layers of H- and S-cores (Figs. 3.1 & 3.2, Tables 3.3 & 3.4). A similar pattern was found for Ca-concentrations in H- and S-cores. In inundated H-cores, Cl- and Ca-concentrations in the soil pore water increased especially under summer conditions, as indicated by interaction effects of season and water table (see Table S2 in Supporting Information of online article).

Alkalinity increased in most H- and S-cores with high water tables compared to control cores (Figs. 3.1 & 3.2, Tables 3.3 & 3.4), although alkalinity also increased in many control cores. Unlike Cl- and Ca-concentrations, which increased immediately in cores with high water tables, alkalinity stayed constant during the first three weeks of inundation. After three weeks, however, alkalinity increased sharply in the subsequent two weeks from 0.4 to 1.1 $\text{mmol}_c\ L^{-1}$ in H-cores and from 0.9 to 2.5 $\text{mmol}_c\ L^{-1}$ in S-cores. The pH also increased between weeks three and five from 5.8 to 6.2 in H-cores and from 6.3 to 6.7 in S-cores. After five weeks, alkalinity kept raising in all inundated cores, though less fast, while pH did not change anymore in H- and S-cores.

High water tables also resulted in significantly lower S-concentrations in the moss layer, especially after three to five weeks (Figs. 3.1 & 3.2, Tables 3.3 & 3.4). In contrast, Fe-concentrations in the moss layer increased, but only under winter conditions (see Tables S2 & S3 in Supporting Information of online article).

Low water tables

In the moss layer, alkalinity showed a significant decrease from 0.5 to 0.2 $\text{mmol}_c\ L^{-1}$ in H-cores, and from 1.0 to 0.5 $\text{mmol}_c\ L^{-1}$ in S-cores (Figs. 3.1 & 3.2, Tables 3.3 & 3.4). This was accompanied by significantly decreased Fe-concentrations in S-cores and significantly increased S-concentrations in both H- and S-cores. In the moss layer, S-concentrations particularly increased under summer conditions, as shown by interaction effects of season and water table for both H- and S-cores (see Tables S2 & S3). It was only under these summer conditions in H-cores that low water tables led to decreased pH-values in the moss layer. For S-cores, low water tables did not affect the pH in the moss layer. In the soil pore water, pH was not affected for either H- or S-cores.


soil pore water in H-cores from the fen with *Hamatocaulis vernicosus*
soil pore water in S-cores from the fen with *Scorpidium scorpioides*
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
-15 & cold
0 & cold
+15 & cold
-15 & warm
0 & warm
+15 & warm
pH - soil
alkalinity - soil (mmol$_c$ L^{-1})
o-PO$_4$ - soil (µmol L^{-1})
NH$_4$ - soil (µmol L^{-1})
time (weeks)
0
10
20
30
5.5
6.0
6.5
7.0
7.5
0.0
1.0
2.0
3.0
4.0
5.0
0.0
10.0
20.0
30.0
40.0
0
100
200
300
400

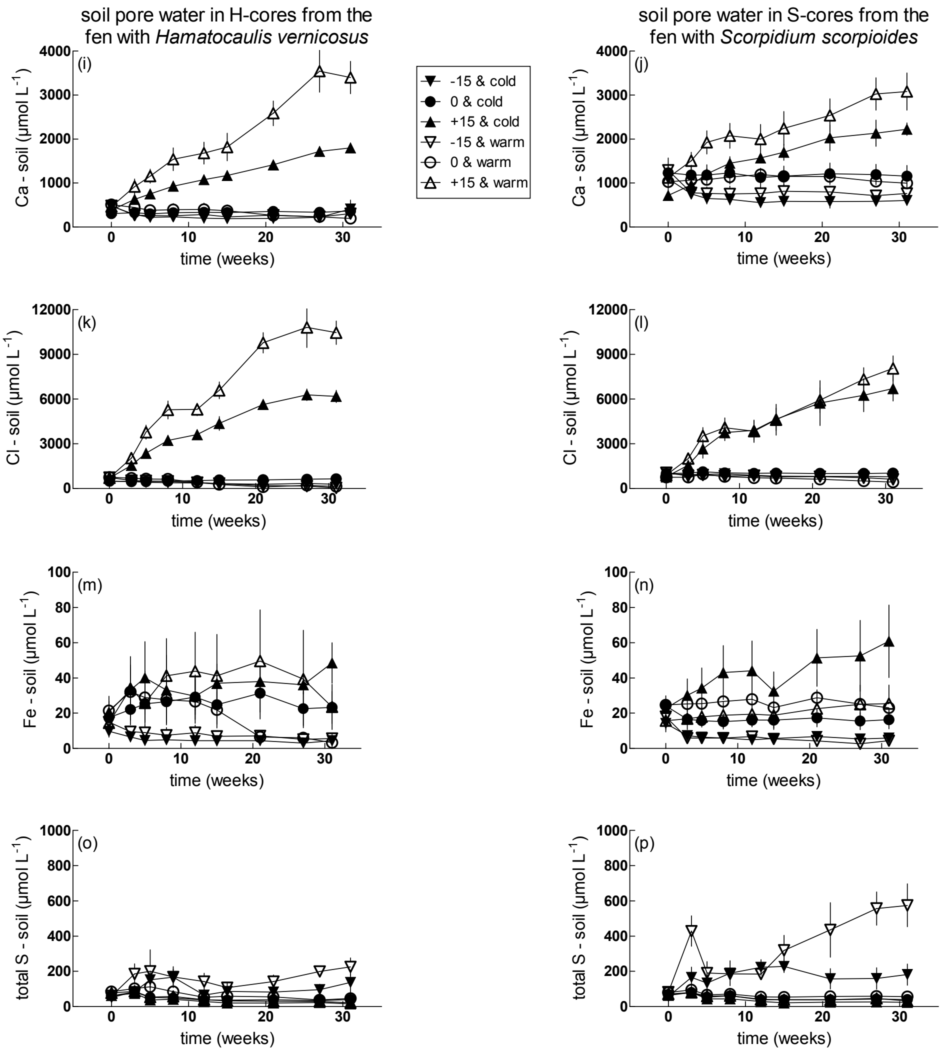
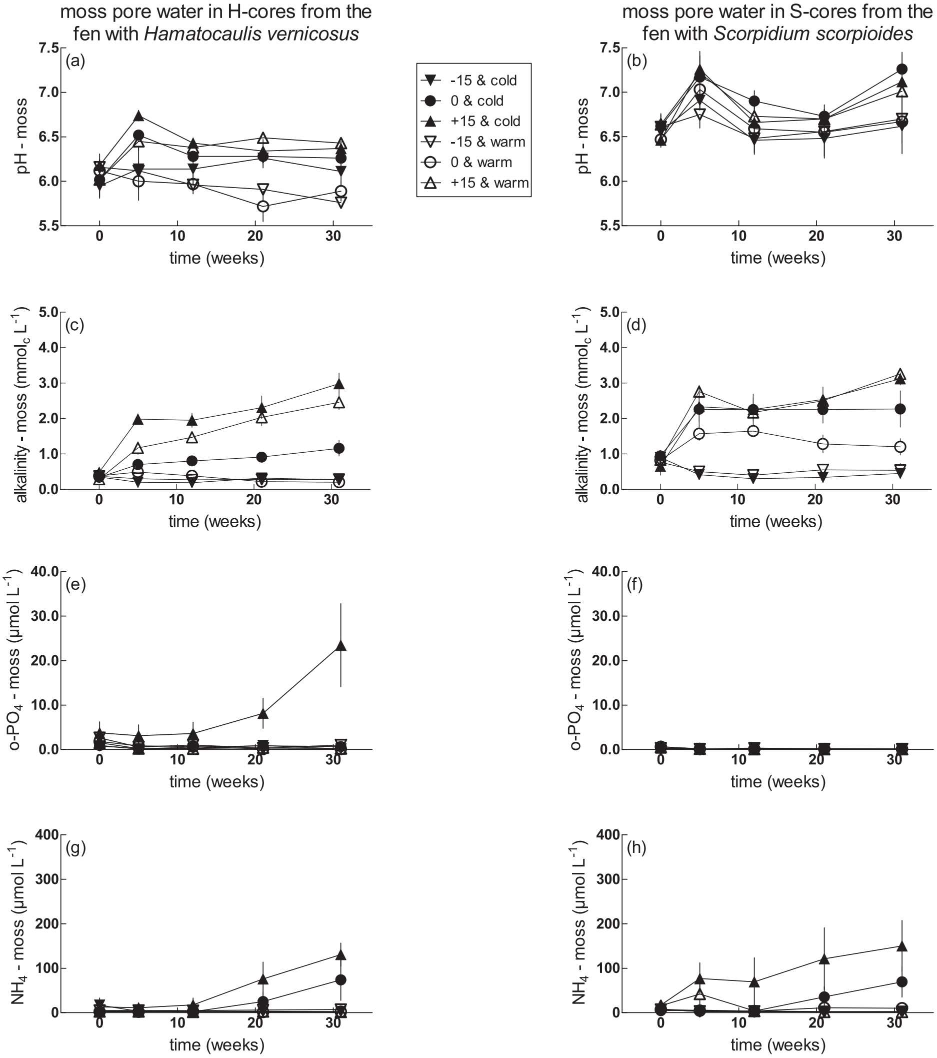


Fig. 3.1. pH, alkalinity, o-PO$_4$, NH$_4$-, Ca-, Cl-, Fe- and S-concentrations in the soil pore water during 31 weeks of raised (+15), control (0) and lowered (-15) water tables in H- and S-cores with *H. vernicosus* and *S. scorpioides*. Sample means are given and error bars indicate standard errors (n = 3-4).


moss pore water in H-cores from the fen with *Hamatocaulis vernicosus*
moss pore water in S-cores from the fen with *Scorpidium scorpioides*
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
▼ -15 & cold
● 0 & cold
▲ +15 & cold
▽ -15 & warm
○ 0 & warm
△ +15 & warm
pH - moss
alkalinity - moss (mmol$_c$ L^{-1})
o-PO$_4$ - moss (µmol L^{-1})
NH$_4$ - moss (µmol L^{-1})
time (weeks)
5.5
6.0
6.5
7.0
7.5
0.0
1.0
2.0
3.0
4.0
5.0
0.0
10.0
20.0
30.0
40.0
0
100
200
300
400
0
10
20
30

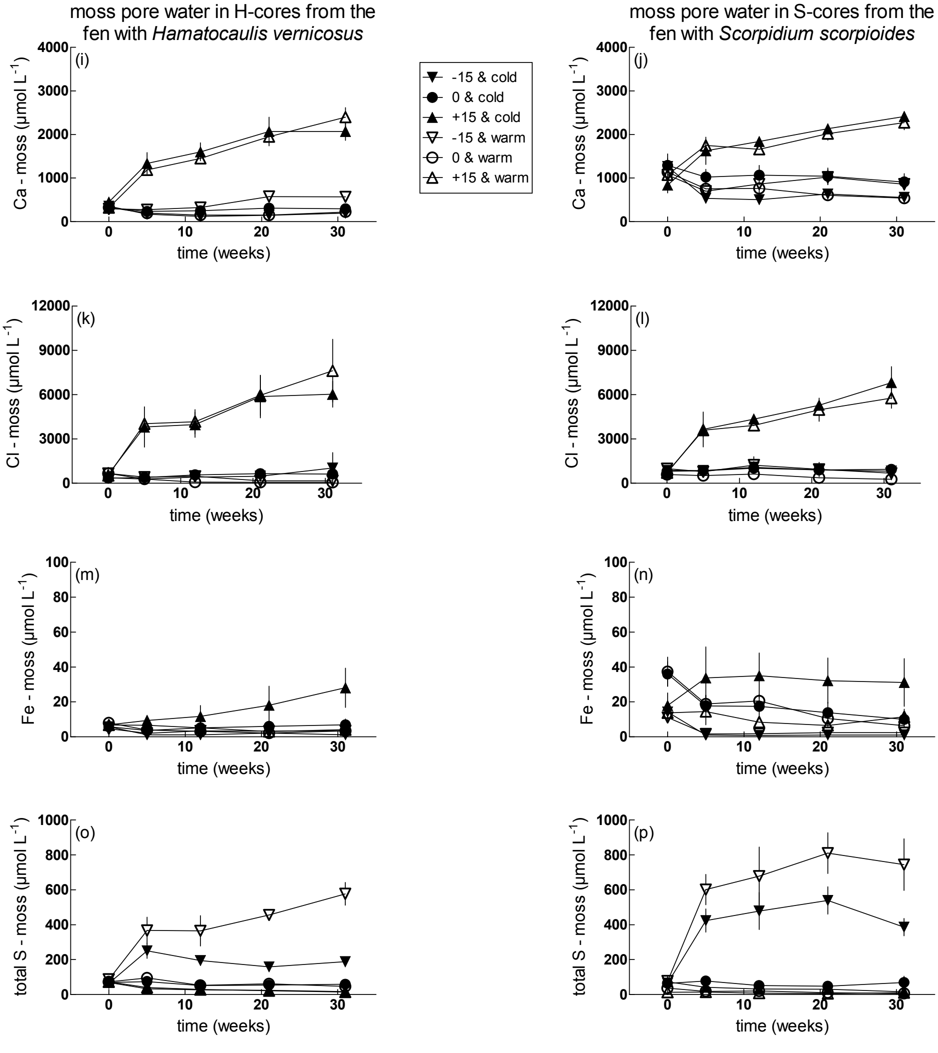


Fig. 3.2. pH, alkalinity, o-PO_4, NH_4-, Ca-, Cl-, Fe- and S-concentrations in the moss pore water during 31 weeks of raised (+15), control (0) and lowered (-15) water tables in H- and S-cores with *H. vernicosus* and *S. scorpioides*. Sample means are given and error bars indicate standard errors (n = 3-4).

Table 3.3. Effects of season, water table and interaction effects on chemical variables in the pore water of the moss layer (-1 cm) and soil (-10 cm) of H-cores with *Hamatocaulis vernicosus*.

	Season	**Table**	**Season * Table**	**-15**	**0**	**15**
Moss pore water						
pH	13.11**	24.28**	4.02*	a	a	b
Alkalinity	9.10**	81.65**	4.59*	a	b	c
Ca	0.04	130.21**	1.50	a	a	b
Cl	0.01	169.60**	0.97	a	a	b
Fe	2.03	11.05**	4.25*	a	a	b
S	55.28**	217.39**	61.37**	c	b	a
Sulfide	0.11	2.34	2.58	a	a	a
o-PO_4	1.76	3.95*	5.21*	a	a	b
NO_3	0.20	0.22	4.24*	a	a	a
NH_4	5.61*	3.54*	7.36**	a	ab	b
DOC	7.22**	7.44**	5.01*	b	ab	a
Soil pore water						
pH	0.69	1.29	0.15	a	a	a
Alkalinity	4.14	3.45*	1.26	a	a	b
Ca	9.08**	71.40**	3.89*	a	a	b
Cl	1.90	100.03**	3.70*	a	a	b
Fe	0.60	0.83	3.24	a	a	a
S	16.77**	13.42**	3.19	b	a	a
Sulfide	2.38	2.11	1.76	a	a	a
o-PO_4	9.80**	6.22**	7.13**	a	a	b
NO_3	1.85	1.81	2.33	a	a	a
NH_4	0.48	4.49*	4.61*	a	ab	b
DOC	0.68	0.15	5.96**	a	a	a

Significant differences between water tables are indicated by different letters. See Table S2 (Supporting Information of online article) for further information about interaction effects. F-ratios with their level of significance: * $P \leq 0.05$ and ** $P \leq 0.01$.

Table 3.4. Effects of season, water table and interaction effects on chemical variables in the pore water of the moss layer (-1 cm) and soil (-10 cm) of S-cores with *Scorpidium scorpioides*.

	Season	**Table**	**Season * Table**	**-15**	**0**	**15**
Moss pore water						
pH	1.02	1.61	2.82	a	a	a
Alkalinity	0.72	100.09**	6.97**	a	b	c
Ca	0.28	126.92**	3.07	a	a	b
Cl	1.59	159.01**	0.79	a	a	b
Fe	4.69*	7.30**	3.88*	a	ab	b
S	6.23*	112.76**	6.04**	c	b	a
Sulfide	1.63	0.59	2.53	a	a	a
o-PO_4	0.79	2.24	2.73	a	a	a
NO_3	0.07	67.32**	0.14	b	a	a
NH_4	21.31**	11.20**	9.41**	a	ab	b
DOC	10.90**	12.78**	7.84**	b	a	a
Soil pore water						
pH	0.21	0.36	1.54	a	a	a
Alkalinity	3.80	19.63**	2.44	a	b	b
Ca	3.88	32.74**	0.71	a	b	c
Cl	0.37	75.98**	1.42	a	a	b
Fe	0.91	4.55*	0.84	a	b	b
S	0.52	14.15**	1.80	b	a	a
Sulfide	0.51	2.17	3.16	a	a	a
o-PO_4	0.89	1.01	0.50	a	a	a
NO_3	0.73	6.86**	0.38	b	a	a
NH_4	0.15	6.78**	5.55*	a	ab	b
DOC	19.14**	3.03	2.72	a	a	a

Significant differences between water tables are indicated by different letters. See Table S2 (Supporting Information of online article) for further information about interaction effects. F-ratios with their level of significance: * $P \leq 0.05$ and ** $P \leq 0.01$.

Table 3.5. Chemical soil composition at the end of different treatments in H- and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides*.

	Fe_t * (mmol kg^{-1} dw)	**Ca_t *** (mmol kg^{-1} dw)	**P_t *** (mmol kg^{-1} dw)	**Fe_t:P_t *** (mol mol^{-1})	**Ca_t:P_t *‡** (mol mol^{-1})
H. vernicosus					
-15 & cold	21.2 (4.4)	273 (37)	35.0 (1.1)	0.61 (0.12)	7.8 (1.0)
0 & cold	28.4 (10.7)	275 (54)	34.0 (3.3)	0.84 (0.23)	8.2 (2.0)
+15 & cold	27.0 (5.1)	340 (63)	34.5 (5.2)	0.79 (0.06)	9.9 (1.6)
-15 & warm	24.2 (5.9)	301 (24)	36.2 (4.4)	0.66 (0.09)	8.4 (0.6)
0 & warm	21.5 (4.0)	275 (77)	36.2 (4.3)	0.69 (0.11)	6.8 (2.2)
+15 & warm	20.2 (5.5)	372 (70)	28.1 (6.7)	0.72 (0.09)	12.0 (3.4)
S. scorpioides					
-15 & cold	48.1 (21.4)	466 (60)	21.9 (3.7)	2.19 (1.00)	21.5 (3.1)
0 & cold	59.3 (15.8)	443 (15)	20.9 (1.7)	2.88 (0.92)	21.3 (1.9)
+15 & cold	47.0 (16.9)	422 (22)	19.3 (2.6)	2.41 (0.72)	22.1 (2.0)
-15 & warm	55.0 (23.7)	461 (39)	22.5 (3.7)	2.36 (0.71)	20.7 (2.0)
0 & warm	72.3 (7.0)	445 (56)	21.1 (2.2)	3.44 (0.35)	21.1 (1.9)
+15 & warm	34.6 (21.9)	435 (36)	18.2 (2.0)	1.84 (1.00)	22.2 (1.6)

Mean values and standard deviations (n = 3-4). Significant differences ($P \leq 0.01$) between source areas, seasons and water tables are indicated by *, † and ‡ respectively.

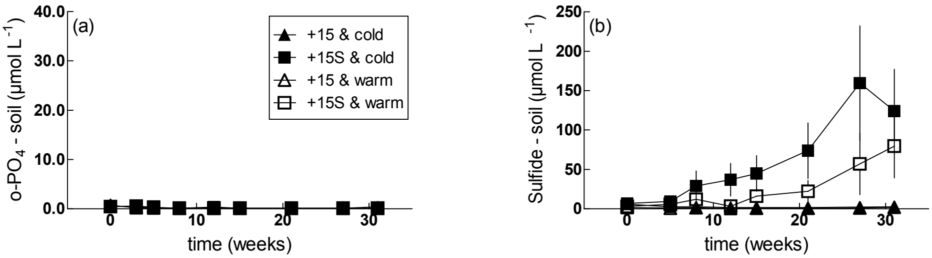


Fig. 3.3. Sulfide and o-PO_4 concentrations in the soil pore water during 31 weeks of raised water tables with either 0 or 1000 µmol L^{-1} SO_4 in S-cores with *Scorpidium scorpioides*. Sample means are given and error bars indicate standard errors (n = 4).

Nutrients

Initial concentrations

At the start of the experiment, o-PO_4 concentrations significantly differed between H- and S-cores in the moss layer and soil (Figs. 3.1 & 3.2, Table 3.2). H-cores contained about 1.0 µmol L^{-1} in the moss layer and about 6.0 µmol L^{-1} in the soil pore water, while S-cores showed much lower o-PO_4 concentrations of about 0.2 and 0.6 µmol L^{-1} in the moss layer and soil pore water, respectively. Also, the total P-content of soils was significant higher in H-cores, leading to significantly lower total Fe:P- and Ca:P-ratios (Table 3.5).

High water tables

High water tables led to a significantly increase of o-PO_4 concentrations in the pore water (moss and soil) of H-cores, but only under winter conditions as shown by season * water table interactions (Figs. 3.1 & 3.2, see Table S2). In contrast, o-PO_4 concentrations in S-cores were not affected by high water tables, not even in cores with extra SO_4-input (Fig. 3.3).

Low water tables

In neither H- nor S-cores did low water tables influence the o-PO_4 concentrations in the pore water (Figs. 3.1 & 3.2, Tables 3.3 & 3.4). Low water tables did, however, lead to significantly increased DOC-concentrations in the moss layer of both core types, but only under summer conditions (see Table S2 & S3). Low water tables also led to significantly increased NO_3-concentrations in S-cores, but not in H-cores.

Potential toxic effects of NH_4 and sulfide

NH_4-accumulation only occurred in cores with high water tables and only under winter conditions, as shown by the significant interaction effects of season and water table, for both H- and S-cores (Figs. 3.1 & 3,2, see Tables S2 & S3). For both core types, concentrations of 100 – 150 µmol L^{-1} in the moss pore water and 200 – 300 µmol L^{-1} in the soil pore water were reached under winter conditions. Extra NH_4-input into S-cores with high water levels did, however, not lead to a further increase. Here, NH_4-concentrations in the pore water were already higher than in the supplied water.

Sulfide concentrations in both cores were, in contrast, not affected by water table. They stayed low in almost all cores, with values below 2 µmol L^{-1}. Strong sulfide accumulation, to levels of 200 µmol L^{-1}, only occurred in SO_4-enriched S-cores, and especially under winter conditions (Fig. 3.3).

Table 3.6. N- and P-contents and N:P-ratios of the aboveground vascular plant tissue at the end of the experiment for H- and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides.*

	Plant N (g kg^{-1} dp)	**Plant P *† (g kg^{-1} dp)**	**Plant N:P *† (g g^{-1})**
H. vernicosus			
-15 & cold	6.4 (1.3)	2.35 (0.08)	2.7 (0.5)
0 & cold	10.8 (2.3)	2.89 (0.29)	3.7 (0.5)
+15 & cold	13.7 (9.4)	3.57 (1.12)	3.6 (2.0)
-15 & warm	8.3 (0.5)	0.69 (0.10)	12.2 (1.9)
0 & warm	10.4 (3.4)	0.72 (0.11)	15.2 (7.0)
+15 & warm	24.4 (3.6)	0.70 (0.24)	38.2 (15.6)
S. scorpioides			
-15 & cold	12.1 (5.3)	0.83 (0.19)	14.4 (4.6)
0 & cold	11.7 (3.1)	0.84 (0.29)	14.6 (4.3)
+15 & cold	10.4 (3.5)	1.11 (0.32)	9.5 (2.8)
-15 & warm	16.8 (5.0)	0.55 (0.26)	34.0 (14.8)
0 & warm	13.7 (7.6)	0.42 (0.08)	36.6 (29.5)
+15 & warm	19.2 (1.3)	0.46 (0.18)	46.1 (14.5)

Mean values and standard deviations (n = 3-4). Significant differences between source areas and seasons are indicated by * and †, respectively ($P \leq 0.01$).

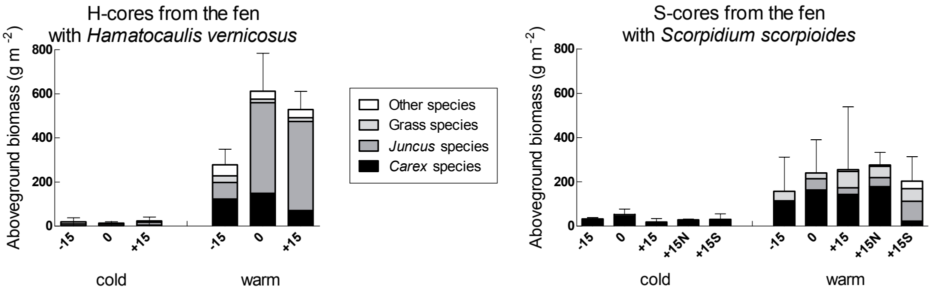


Fig. 3.4. Aboveground biomass of different vegetation groups after 31 weeks of high (+15), control (0) and low (-15) water tables in H- and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides,* including inundated S-cores to which NH_4 (100 µmol L^{-1}; +15N) or SO_4 (1000 µmol L^{-1}; +15S) was added. Sample means are given and error bars indicate standard deviations (n = 3-4).

Vegetation response

As expected, the aboveground biomass at the end of the experiment was significantly higher under summer than winter conditions (Fig. 3.4). Furthermore, the more eutrophic H-cores had a significantly higher total biomass than S-cores. Water table treatments did not influence the total biomass, except for H-cores with low water tables under summer conditions, which showed a decrease. This was mainly caused by lower biomass of *Juncus* spp. Addition of NH_4 at high water tables, which only occurred in S-cores, did also not affect aboveground biomass, but SO_4-addition led to a strong decline of *Carex* spp. and an increase of *Juncus* and grass spp.

Different water tables did not lead to differences in plant N- and P-contents, nor in plant N:P-ratios (Table 3.6). However, the source area of the core and season seemed to be important. In accord with the higher nutrient status of H-cores, the vegetation contained significantly higher P-contents in control H-cores compared to S-cores, leading to N:P-ratios of about 15 and 37, respectively. The higher P-contents and aboveground biomass of H-cores resulted in a much higher P-consumption compared to S-cores, especially under summer conditions (Table 3.7). Plant N:P-ratios were also significantly affected by season. Values were significantly higher under summer than winter conditions for both core types, due to significantly lower P-contents, but equal N-contents. The ratios increased from 4 to 15 in control H-cores, and from 15 to 37 in control S-cores.

Water table treatments clearly influenced the vitality of mosses. Low water tables led to a significantly lower fluorescence yield of between 0.25 and 0.35 for both mosses, compared to a yield of about 0.50 in control cores (Table 3.7). These values indicate that *H. vernicosus* and *S. scorpioides* were vital in control cores, but significantly less vital at low water tables. Inundation, in contrast, did not lead to vitality changes. Both species showed a fluorescence yield of between 0.50 and 0.65 under both seasonal conditions. Under summer conditions, growth rates were higher than in control cores, with many individuals growing more than 15 cm in 31 weeks to position their apices above the water table (results not shown). The addition of NH_4 in S-cores with high water tables did not influence the fluorescence yield of *S. scorpioides*, but SO_4-enrichment led to a significant decrease.

Discussion

Differences between both fen types

Cores with *H. vernicosus* (H-cores) not only showed lower pH, alkalinity and Ca-concentrations than cores with *S. scorpioides* (S-cores), which is consistent with previous studies (e.g. Hedenäs 2003; Štechová *et al.* 2008), but also higher P-availability. Low P-availability in S-cores corresponded with strong P-limitation, as indicated by high plant N:P-ratios of around 35 in control S-cores under summer conditions (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003; Güsewell 2004). This is supported by significantly lower plant N:P-ratios under winter than summer conditions, suggesting that P became less readily available than N during the growing season.

Table 3.7. Vitality of two moss species, as indicated by their fluorescence yield $(F_m\text{-}F_o)/F_m$, and P-amounts in aboveground vascular plant tissue (P-concentration * biomass ha^{-1}) at the end of the experiment for H- and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides*.

	$(F_m\text{-}F_0)/F_m$ of mosses †‡	P in aboveground biomass *† ($mol\ ha^{-1}$)
H. vernicosus		
-15 & cold	0.42 (0.11)	22 (12)
0 & cold	0.54 (0.15)	16 (6)
+15 & cold	0.58 (0.08)	39 (23)
-15 & warm	0.26 (0.11)	62 (18)
0 & warm	0.21 (0.03)	143 (45)
+15 & warm	0.48 (0.05)	104 (41)
S. scorpioides		
-15 & cold	0.29 (0.06)	9 (2)
0 & cold	0.62 (0.12)	10 (7)
+15 & cold	0.55 (0.07)	7 (6)
+15N	0.66 (0.06)	
+15S	0.40 (0.03)	
-15 & warm	0.27 (0.11)	26 (26)
0 & warm	0.43 (0.12)	31 (16)
+15 & warm	0.60 (0.05)	32 (29)
+15N	0.61 (0.04)	
+15S	0.36 (0.18)	

Mean values and standard deviations (n = 3-4). Significant differences between source areas, seasons and water tables are indicated by *, † and ‡ respectively ($P \leq 0.01$).

Risks of low surface water levels

At low water tables, aerobic oxidation of Fe(II) and sulfide led to decreased alkalinity in the moss layer of both fens. This process was also described by Loeb *et al.* (2008a) for soils of riverine meadows. Under summer conditions, this decrease in buffer capacity led to a significant lowering of the pH in the moss layer of H-cores from 6.0 to 5.5, while S-cores still showed sufficient buffer capacity to prevent such a decrease of pH. Furthermore, increased DOC-concentrations under summer conditions suggested an increase in decomposition rates in both core types with low water table (Oomes *et al.* 1997; Olde Venterink *et al.* 2002a). Concentrations of o-PO_4 remained, however, low. In H-cores, this was probably due to increased P-uptake by plants. In the strongly P-limited S-cores, this is presumably caused by P-sorption to oxidized Fe or Ca (Patrick & Khalid 1974; Koerselman *et al.* 1993).

A low fluorescence yield for *H. vernicosus* and *S. scorpioides* in treatments with low water tables suggest that both mosses do not like long periods of drought, which corresponds to other studies (Boryslawski 1978; Mälson *et al.* 2008). Although temporary desiccation and acidification seem to be a rather common phenomenon in rich fens (e.g. Kooijman & Whilde 1993; Štechová & Kučera 2007), long periods of drought may lead to the disappearance of brownmosses (den Held *et al.* 1992; van Diggelen *et al.* 2006).

Benefits and risks of high surface water levels

High vitality of both mosses during inundation suggest that long periods of high water tables can be beneficial to *H. vernicosus* and *S. scorpioides*. Inundation with base-rich water led to an increase of soil buffer capacity. The sharp increase in alkalinity and pH after three weeks of inundation was at least partly caused by anaerobic reduction processes, as S-concentrations decreased simultaneously (Smolders *et al.* 2006; Loeb *et al.* 2008a). Apart from internal alkalinity generation, increasing Cl- and Ca-concentrations also suggested continuous infiltration of HCO_3-rich water, which seemed to be highest under summer conditions, when infiltration was easier as a result of higher evapotranspiration. The lack of increase in alkalinity and pH during the first three weeks of inundation, despite the immediate infiltration of base-rich inundation water at the start of the experiment, was probably caused by the consumption of HCO_3 through aerobic oxidation processes (Loeb *et al.* 2008a).

Reducing conditions may not only lead to desired alkalinization, but also to undesired P-mobilization (e.g. Patrick & Khalid 1974). The effect of high water tables on net P-availability differed between both core types. P-availability remained low in strongly P-limited cores with *S. scorpioides*, as can be concluded from low o-PO_4 concentrations and high plant N:P-ratios, while o-PO_4 concentrations increased in inundated cores with *H. vernicosus*. Relatively low total soil Fe:P- and Ca:P-ratios in H-cores compared to S-cores made them more sensitive to P-mobilization (Smolders

& Roelofs 1993; Zak *et al.* 2004; Geurts *et al.* 2008). Concentrations of o-PO_4, however, only increased in H-cores under winter conditions, because mobilized o-PO_4 was rapidly taken up by plants under summer conditions, as can be concluded from the increased P-consumption of plants.

In addition to internal alkalinity generation and P-mobilization, reduced conditions may also lead to high sulfide and NH_4-concentrations (e.g. Lamers *et al.* 1998b). Despite SO_4-reduction in all inundated cores, Fe-concentrations seemed to be high enough to bind most produced sulfide and prevent toxic effects (Smolders & Roelofs 1993, 1996; van der Welle *et al.* 2006). This was, however, not the case in SO_4-enriched inundated S-cores. Sulfide concentrations up to levels of 200 µmol L^{-1} were not only toxic to *S. scorpioides*, but also resulted in a vegetation change from dominance of *Carex* spp. to *Juncus* and grass spp. This is in accordance with results found by Lamers *et al.* (1998b), who found a similar change already at sulfide levels of about 20 µmol L^{-1}.

NH_4-concentrations, in contrast, did increase in all inundated cores as a result of anaerobic reduction processes, but only under winter conditions. Despite high NH_4-concentrations of well above 100 µmol L^{-1}, a level above which toxic effects can be expected for brownmosses under summer conditions (Paulissen *et al.* 2004), none of the mosses showed a decreased fluorescence yield, probably due to lack of growth. Although anaerobic microbial activity must have been higher under summer conditions (e.g. Dunfield *et al.* 1993), increased plant activity of graminoid species, such as *Carex elata* and *Carex rostrata* (Visser *et al.* 2000), *Phragmites australis* (e.g. Gries *et al.* 1990) and *Juncus* spp. (Visser *et al.* 2000) probably led to increased radial oxygen loss (ROL) from roots, which stimulates nitrification and prevents NH_4-accumulation. Increased influence of ROL under summer conditions is supported by relatively low concentrations of CH_4 and Mn (results not shown), Fe and sulfide.

Implications for fen conservation

Rich fens can only persist under mineral-rich and nutrient-poor conditions (Sjörs 1950; van Wirdum 1991; Wheeler & Proctor 2000; Kooijman & Paulissen 2006). Managers of rich fens should therefore focus on the preservation and restoration of these conditions (e.g. Grootjans *et al.* 2006). More varying surface water levels have recently been advocated as a successful management tool (Loeb *et al.* 2008a). However, our mesocosm experiment showed that more varying water levels may be risky for certain sensitive and endangered types of rich fen.

Periods with low surface water levels should be prevented as much as possible to prevent desiccation, acidification and eutrophication of brownmoss-dominated rich fens (e.g. Mälson *et al.* 2008). This study shows that such periods may indeed lead to increased net mineralization and acidification by aerobic oxidation processes, especially during the summer.

By contrast, periods of inundation with base-rich water led to mineral supply and soil alkalinization in rich fens. This study shows that infiltration of base-rich water will especially occur during the summer, when infiltration is easier as a result of high evapotranspiration. However, P-concentrations in the inundation water should be low to prevent eutrophication and concomitant species loss, especially in fen types in which P strongly limits biomass production. This may require additional measures such as P-stripping of the surface water. In addition, inundation water should also contain low SO_4-concentrations to prevent sulfide toxicity to characteristic moss and *Carex* spp. Finally, soils with high total P-content and low Fe:P-ratios should not be flooded, because this will increase the risk of internal P- mobilization and increased P-availability, even without external loading.

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4

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

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

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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 (a) anaerobic, waterlogged conditions, (b) aerobic, moist conditions, characteristic for moderate drought in which oxygen intrusion takes place, and (c) 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 C-loss 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 in both fen types. This may be due to microbial mortality 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*-dominated 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.

Introduction

Acidification and eutrophication are considered a threat to N- and P-limited, minerotrophic base-rich fens, which are generally called rich fens (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 & Patrick 1974; Loeb *et al.* 2008a) 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 micro-organisms that require electron acceptors other than oxygen (McLatchey & Reddy 1998). This leads to the sequential reduction of NO_3, Fe(III) and SO_4, and finally methanogenese (Stumm & Morgan 1996; Mitsch & Gosselink 2007), 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 B), and aerobic oxidation processes may prevail. This may lead to acidification as a result of the use of oxygen (Stumm & 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 & Wheatley 1988; Bridgham *et al.* 1998; Updegraff *et al.* 1995; Olde Venterink *et al.* 2002a; 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.* 2002a).

Decomposition and mineralization may also be affected by the acid neutralizing capacity (ANC) of a peatland (Verhoeven *et al.* 1988, 1990; Kooijman & Hedenäs 2009). It has been generally assumed that the conditions for litter decay and nutrient turnover are more favorable in mineral-rich 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*-dominated 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 & Hedenäs 2009). In addition, net N- and P-mineralization do not necessarily increase at higher pH-values, and often increase from rich fens to poor fens (Verhoeven *et al.* 1988, 1990; Bridgham *et al.* 1998; Scheffer *et al.* 2001; Kooijman & Hedenäs 2009). Additional experimental

Fig. 4.1. The three different research sites in the Netherlands: Stobbenribben (52°47’ N, 6°0’ E), Kiersche Wiede (52°42’ N, 6°8’ E) and Binnenpolder Tienhoven (52°10’ N, 5°6’ E).

research is therefore needed to assess whether the ANC in fens also affects the changes induced by aeration and desiccation. Although O_2-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 (a) anaerobic, moist conditions, (b) aerobic, moist conditions, which are characteristic for moderate drought in which oxygen intrusion takes place, and (c) 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*-dominated fens, due to differences in microbial immobilization characteristics. The following responses are discussed in this paper: (a) acidification as a result of oxygen intrusion, (b) changes in C-mineralization, (c) changes in net N-mineralization, and (d) changes in net P-release. In addition, implications for the hydrological management of both rich fens and *Sphagnum*-dominated fens are discussed.

Materials and methods

Sampling

Peat soil samples were collected from three sites in the Netherlands (Fig. 4.1): Stobbenribben (ST), Kiersche Wiede (KW) and 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 area "National Park Weerribben-Wieden", in which rich fens are fed by a supply of base-rich, 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 a dominance of *Sphagnum* spp.

Peat samples were collected in November 2011 and kept at field moisture content. From each of the three sites, five samples were collected from a mineral-rich, brownmoss-dominated plot, and five from an ombrotrophic, *Sphagnum*-dominated plot (n = 30). Rich fen plots 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., which is unable to survive in calcareous water (Clymo & Hayward 1982), indicates relatively ombrotrophic conditions.

In Stobbenribben and Binnenpolder Tienhoven, rich-fen samples were collected from plots dominated by *S. scorpioides*, and in Kiersche Wiede from *H. vernicosus* dominated plots. All samples in *Sphagnum*-dominated fens were collected from plots dominated by *S. palustre*, which were situated within 25 meter from the rich fen plots. 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 design and chemical analyses

Three different conditions were simulated during incubation: (a) anaerobic (moist) incubation for 69 days, (b) aerobic (moist) incubation for 62 days and (c) 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. Samples were incubated under field-moist conditions with 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 (855 Series, Plas-Labs Inc., Lansing, USA), filled with inert Ar-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 (Vario EL Cube, Elementar, Hanau, Germany). Furthermore, portions of 250 mg dry peat soil were digested for 50 minutes in a microwave (Multiwave, Perkin Elmer, Waltham, USA) 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-OES (Optima 3000 XL, PerkinElmer, Waltham, USA) (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, using a solid(g):liquid(g)-ratio of 1:10. After two hours of shaking, pH was measured. 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), o-PO_4 and 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*-dominated fens, because the *Sphagnum*-peat absorbs much solution. After one hour of shaking in 100 mL bottles, extraction solutions were collected by using Rhizon SMS soil moisture samplers (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), which were connected to vacuum serum bottles. Concentrations were measured by using continuous flow auto-analyzers (Skalar Analytical BV, Breda, the Netherlands). 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 o-PO_4 concentrations between initial samples and incubated samples.

Microbial C and N were determined by chloroform fumigation extraction (Jenkinson & 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, 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 & 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 4.1). 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 & 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.

Table 4.1. 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} day^{-1}
Q	Respiration (CO_2-emission)	μmol C kg^{-1} day^{-1}
NC_m	N:C-ratio in microbial biomass	mol N mol C^{-1}
NC_s	N:C-ratio in substrate	mol N mol C^{-1}
D	Denitrification	μmol N kg^{-1} day^{-1}
Calculated variables		
eC	Microbial growth efficiency	mol C mol C^{-1}
GN	Gross N-release	μmol N kg^{-1} day^{-1}
I	N-immobilization	μmol N kg^{-1} day^{-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 $eN = eC * (NC_m/NC_s)$

Statistical analyses

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

Results

Initial soil and microbial characteristics

The initial soil characteristics before incubation clearly differed between both fen types for many variables (Table 4.2). As expected, pH-values were significantly higher in rich fens than in *Sphagnum*-dominated fens. The effect of location on pH was the strongest for rich fens, considering a significant interaction effect of location * fen type. 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*-dominated fens, resulting in significantly lower C:N- and C:P-ratios in rich fen peat. Total P-concentrations were the lowest in the ST, resulting in significantly higher C:P- and N:P-ratios. The rich fen soils were also characterized by significantly higher total concentrations of Ca, 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 effect of location * fen type. Fe-concentrations were also significantly higher in rich fen peat, 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 effect of location * fen type. The effect of location on concentrations of extractable NH_4 was significant, and this effect was the strongest for rich fens, considering a significant interaction effect of location * fen type. Also extractable NO_3-concentrations differed between locations, and the effect of location was the strongest for rich fens, considering a significant interaction effect of location * fen type. 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. In addition, bulk density was 2-3 times higher in rich fens, while gravimetric soil moisture content was twice as high in *Sphagnum*-dominated fens.

Anaerobic CO_2-production per kg dry peat soil at t = 0 did not differ significantly between both fen types or between locations (Table 4.2). However, if expressed

Table 4.2. Initial characteristics of the peat soil and microbial biomass at t = 0 at the different research sites. ST = Stobbenribben, KW = Kiersche Wiede, BPT = Binnenpolder Tienhoven. Positive fluxes indicate release.

	Rich fen			*Sphagnum*-dominated fen		
	ST	**KW**	**BPT**	**ST**	**KW**	**BPT**
	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_t (g kg^{-1} dw) †	464 (2)	482 (1)	331 (6)	470 (4)	454 (2)	471 (2)
N_t (g kg^{-1} dw) *†	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_t (g kg^{-1} dw) *†	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_t (g kg^{-1} dw) *†	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_t (g kg^{-1} dw) *†	1.3 (0.0)	2.0 (0.0)	17.0 (0.7)	1.1 (0.0)	6.0 (0.2)	1.9 (0.2)
Ca_t:Fe_t (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 (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 (g g^{-1}) *†	823 (22)	480 (14)	295 (21)	1451 (43)	652 (15)	902 (7)
Substrate N:P (g g^{-1}) *†	31.1 (0.8)	22.4 (0.7)	14.6 (1.0)	35.1 (1.1)	15.3 (0.5)	21.0 (2.2)
ext. NH_4 (mg kg^{-1} dw) *†	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} dw) *†	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} dw)	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)
Gravimetric moisture content (%) *	982 (58)	1421 (75)	889 (156)	2404 (263)	1747 (438)	1910 (63)
Microbial C (mg g^{-1} dw) *†	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 at t = 0 (mgC kg^{-1} day^{-1})	316 (77)	344 (97)	359 (35)	424 (60)	370 (31)	350 (75)
Anaerobic CO_2-flux at t = 0 (gC dm^{-3} day^{-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 at t = 0 (mgC gC_m^{-1} day^{-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 at t = 0 (mgC kg^{-1} day^{-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)

Mean values and standard deviations (n = 5). Significant differences between rich fen peat and *Sphagnum*-peat and research sites are indicated by * and † respectively ($P \leq 0.05$).

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, due to the lower bulk density of *Sphagnum*-peat. In contrast, when expressed per mass of microbial C, respiration was significantly higher (factor 1.7 on average) in the *Sphagnum*-dominated fens, as the total concentration of microbial C was significantly higher in rich fen peat. 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*-dominated fens than in rich fens. 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 4.3. Treatments had a significant effect on H^+-concentrations. Both aeration and desiccation led to a net increase of H^+-concentrations, hence to significant lowering of the pH (Fig. 4.2). Overall, the net increase of H^+-concentrations during incubation was greater in *Sphagnum*-dominated fens than in rich fens. However, lowering of the pH was higher in rich fens. As indicated by a significant interaction effect of fen type * treatment, the effect of aeration and desiccation on pH was higher in rich fens. In the ST-location the effect of aeration and desiccation on pH was less strong than in the other locations.

C-cycling

During incubation, the overall effect of fen type and treatment on the CO_2-emission per kg dry peat soil were significant and considering a significant interaction effect of fen type * treatment, the effect of treatment was stronger for rich fens (Table 4.3). 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*-dominated fens (Fig. 4.3). As the overall concentration of microbial C in mass per kg dry peat was on average two times lower in *Sphagnum*-dominated fens, overall CO_2-emission per mass unit microbial C was on average 1.5 times higher in *Sphagnum*-dominated 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*-dominated 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*-dominated 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.

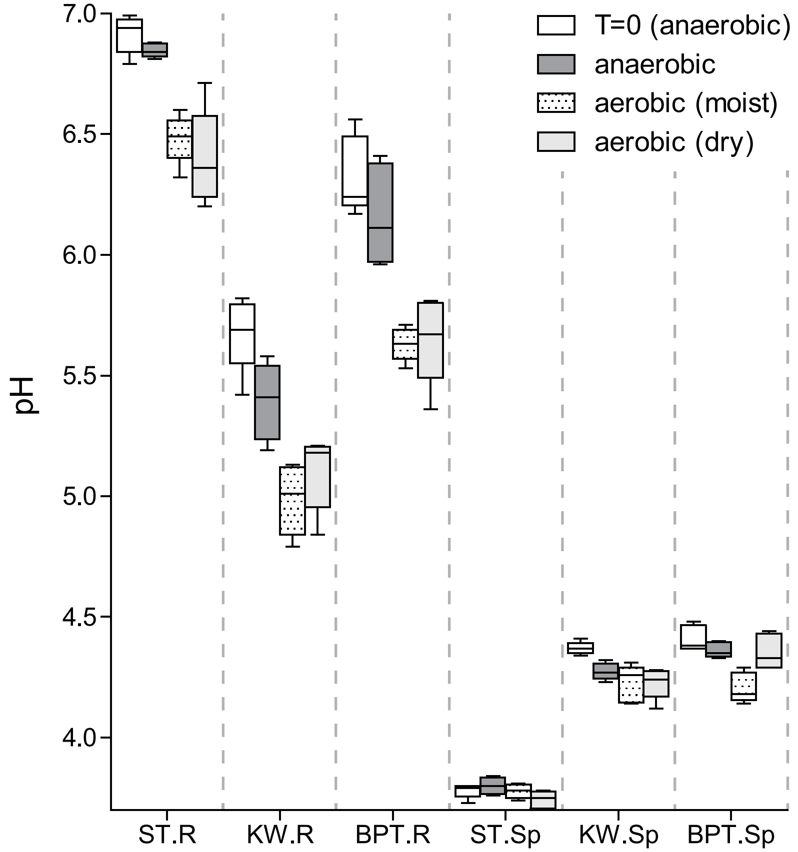

Fig. 4.2. Boxplots showing values of soil pH-H_2O from the different research sites for the different treatments (n = 5). ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-dominated fen, KW.Sp = Kiersche Wiede *Sphagnum*-dominated fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-dominated 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 4.3.

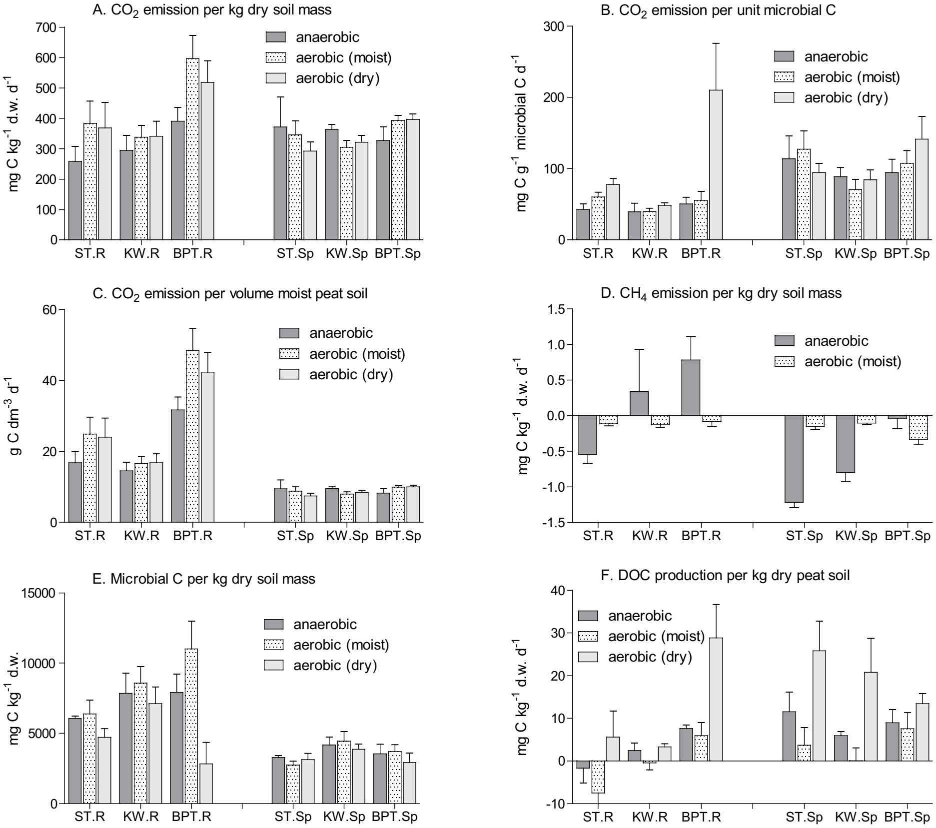

Fig. 4.3. Average fluxes of CO_2 (a, b, c), fluxes of CH_4 (d), microbial C (e) and DOC-production (f) under anaerobic, moist aerobic and dry aerobic conditions for samples from the different research sites (n = 5). Positive fluxes indicate release. ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-dominated fen, KW.Sp = Kiersche Wiede *Sphagnum*-dominated fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-dominated fen. Sample means are given and error bars indicate standard deviations. Significant effects are indicated in Table 4.3.

Table 4.3. 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.

	Fen type	**Treatment**	**Location**	**Fen type * Treatment**	**Fen type * Location**	**Treatment * Location**	**Anaerobe (moist)**	**Aerobe (moist)**	**Aerobe (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 dw)	13.7**	9.7**	39.1**	13.2**	15.5**	5.1*	a	b	b
CO_2-flux (per C_{micro})	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 dw)	282.9**	35.6**	21.4**	24.3**	3.4*	12.5**	b	c	a
CH_4-flux (per kg dw)	79.8**	3.1	40.0**	53.1**	1.4	50.1**	n.s.	n.s.	-
DOC-production (per kg dw)	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-min. (per kg dw)	149.1**	33.4**	173.5**	62.9**	14.3**	21.4**	a	b	a
net N-min. (per C_{micro})	267.7**	7.5**	126.6**	23.0**	42.4**	18.7**	a	b	a
net N-min. (per dm^2)	13.2	45.1**	119.2**	65.2**	6.9**	20.8**	a	c	b
N_2-flux (per kg dw)	65.1**	-	105.8**	-	27.8**	-	-	-	-
gross N-min. (per kg dw)	546.5**	13.7**	102.7**	13.2**	86.9**	6.4**	a	c	b
gross N-min. (per C_{micro})	58.6**	33.5**	51.7**	26.4**	36.9**	23.4**	a	a	b
N-immobilization (per kg dw)	633.1**	8.6**	164.2**	3.1	70.3**	10.9**	a	b	b
N-immobilization (per C_{micro})	167.1**	44.5**	92.6**	16.9**	30.1**	19.9**	a	a	b
net P-release (per kg dw)	0.7	351.4**	8.7**	0.8	14.3**	13.6**	b	a	c
net P-release (per C_{micro})	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

F-ratios with their level of significance: * $P \leq 0.05$, ** $P \leq 0.01$. Different letters indicate significant differences ($P \leq 0.05$) between treatments, n.s. = not significant.

N-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*-dominated fens than in the rich fens (Fig. 4.4, Table 4.3). 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 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*-dominated 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*-dominated fens, both expressed per kg dry peat soil mass, and per microbial C-mass (Fig. 4.5, Table 4.3). Estimated microbial N-immobilization was considerably higher in rich fens than in *Sphagnum*-dominated 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 (Fig. 4.4, Table 4.3). According to a significant interaction effect 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 volume peat soil. In *Sphagnum*-dominated 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 effect 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 effect of fen type * treatment on microbial N-immobilization per kg dry peat soil was detected, 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 (Fig. 4.4). 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 efficiency 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 C-mass increased considerably especially at the BPT rich 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.

P-cycling

The overall effect of treatment on net P-release was significant (Fig. 4.6, Table 4.3). 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 entirely, 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*-dominated fens, as indicated by a non-significant interaction effect 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 as a consequence of 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.

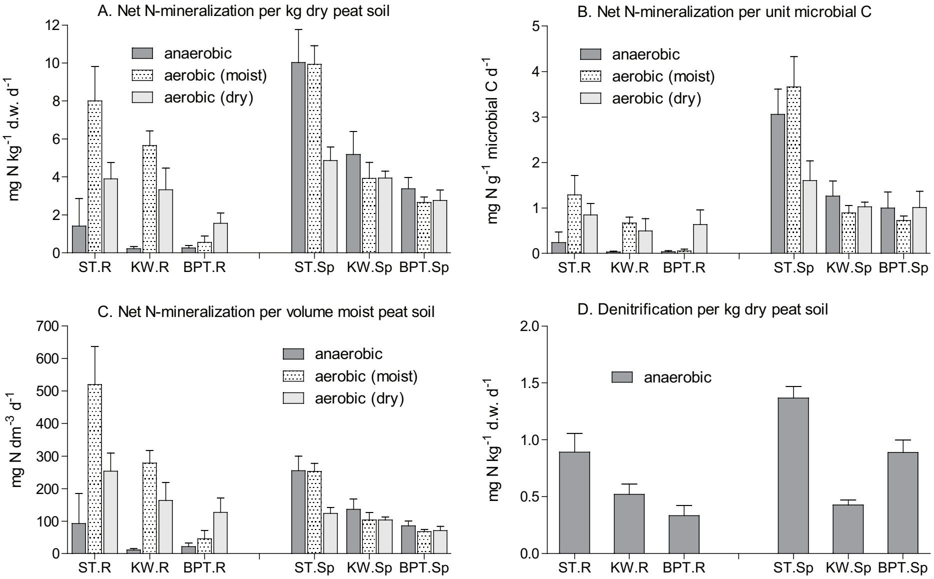

Fig. 4.4. Rates of net N-mineralization (a, b, c) under anaerobic, moist aerobic and dry aerobic conditions, and rates of anaerobic denitrification (d) for samples from the different research sites (n = 5). ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-dominated fen, KW.Sp = Kiersche Wiede *Sphagnum*-dominated fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-dominated fen. Sample means are given and error bars indicate standard deviations. Significant effects of fen type and treatment are indicated in Table 4.3.

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 (Stumm & Morgan 1996), 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 raised again, most of the protons produced will most likely be consumed, due to the anaerobic reduction of alternative electron acceptors (Loeb *et al.* 2008a). 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.

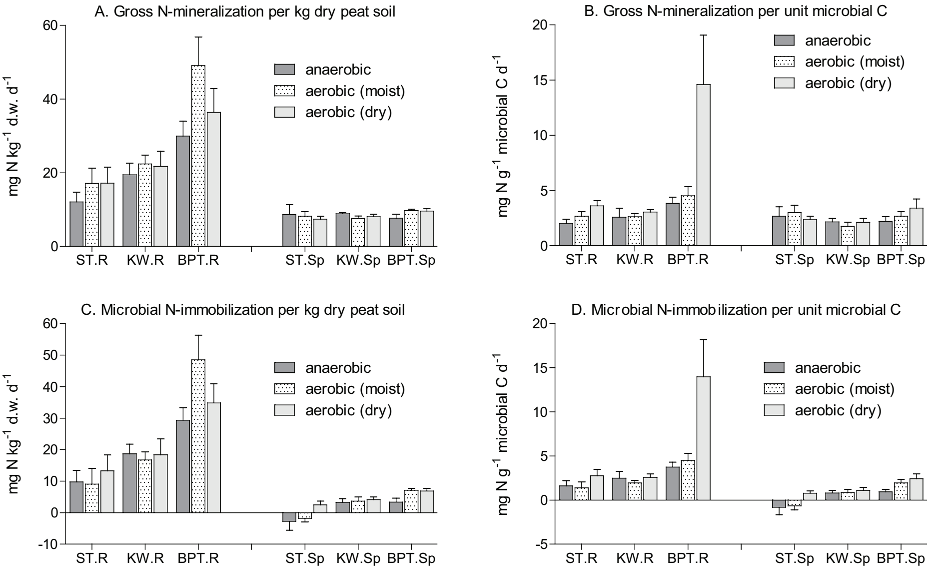

Fig. 4.5. Rates of gross N-mineralization (a, b) and N-immobilization (c, d) for samples from the different research sites under different incubation conditions (n = 5). ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-dominated fen, KW.Sp = Kiersche Wiede *Sphagnum*-dominated fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-dominated fen. Sample means are given and error bars indicate standard deviations. Significant effects of fen type and treatment are indicated in Table 4.3.

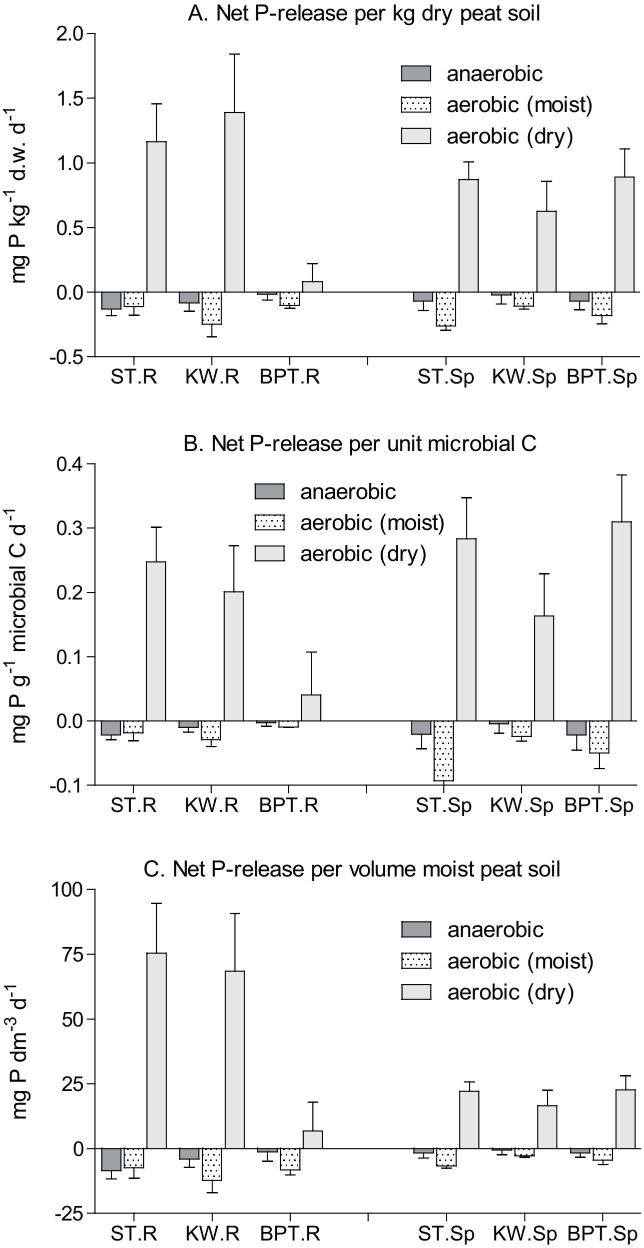

Fig. 4.6. Rates of net P-release for samples from the different research sites under different incubation conditions (n = 5). ST.R = Stobbenribben rich fen, KW.R = Kiersche Wiede rich fen, BPT.R = Binnenpolder Tienhoven rich fen, ST.Sp = Stobbenribben *Sphagnum*-dominated fen, KW.Sp = Kiersche Wiede *Sphagnum*-dominated fen, BPT.Sp = Binnenpolder Tienhoven *Sphagnum*-dominated fen. Sample means are given and error bars indicate standard deviations. Significant effects of fen type and treatment are indicated in Table 4.3.

Anaerobic respiration in rich fens versus *Sphagnum*-dominated fens

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 & 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 micro-organisms 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 sulfatases (Freeman *et al.* 2001), leading to reduced breakdown of organic matter (Fenner & Freeman 2011). However, in view of the fact that anaerobic respiration rates per kg did not differ between fen types 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*-dominated fens.

The respiration rate per unit microbial C was lower in rich fens than in *Sphagnum*-dominated fens, which may be pH-related. Enwall *et al.* (2007) found a negative correlation between soil pH and the microbial metabolic quotient (*q*CO2; respiration-to-biomass ratio), indicating a decreased efficiency of heterotrophic micro-organisms to convert organic C into microbial biomass in rather acidic soils. This would explain the high respiration rate per unit microbial C in *Sphagnum*-dominated fens. Moreover, this lower respiration rate per unit microbial C in rich fens seems to be compensated for by a larger number of micro-organisms 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; Danevčič *et al.* 2010). Low or even negative emission rates may reflect CH_4-oxidation by microbial communities associated with living and dead mosses (Raghoebarsing *et al.* 2005; Liebner *et al.* 2011), and may also reflect suppression of methanogenese by other electron acceptors, which yield higher amounts of Gibbs free energy (Stumm & Morgan 1996). High soil N-concentrations have frequently been linked to decreases in methanogenese (Bridgham & Richardson 1992; Bender & Conrad 1994). This may to some extent explain the persistent negative CH_4-emission rates for both rich fen and *Sphagnum*-dominated 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 (Raghoebarsing *et al.* 2005) is reflected in this study.

Respiration response of aeration and desiccation differs between fens

C-respiration generally increases upon aeration of peat soils (e.g. Moore & Knowles 1989; Freeman *et al.* 1993; Oechel *et al.* 1998; Blodau & Moore 2003; Danevčič *et al.* 2010; Fenner & 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 & Ghani 1995). Upon desiccation, the microbial soil respiration per unit microbial C increased in the rich fens, indicating reduced microbial efficiency. In the *Sphagnum*-dominated 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*-dominated 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 & Freeman 2011), aeration did not lead to increased respiration rates in *Sphagnum*-dominated fens, probably because *Sphagnum*-litter contained too many phenolic compounds. Rich fens presumably contain lower concentrations of phenolic compounds. Also, the acidic pH of *Sphagnum*-dominated 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 C 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 & McDonald 1992; Olde Venterink *et al.* 2002a), but in our study respiration did not increase. The increased DOC-production may instead be related to mortality of microbes, due to 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*-dominated fens

To connect plant production 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*-dominated 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 & 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 more favorable in mineral-rich than in mineral-poor wetlands (Bayley *et al*. 2005). Also, net N-mineralization rates per microbial C-mass were higher in *Sphagnum*-dominated fens than in rich fens, which correspond well with previous experiments carried out by Kooijman & 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 dissimilatory NO_3-reduction to NH_4 (DNRA) or anaerobic NH_4-oxidation (anammox) (Burgin & 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 & Reddy 2009).

The higher anaerobic net N-mineralization per kg dry peat, per microbial C-mass and per volume peat soil in *Sphagnum*-dominated fens cannot be explained by differences in gross N-mineralization either, as estimated gross N-mineralization was higher in rich fens than in *Sphagnum*-dominated 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 & Groffman 2007). In this study, estimated microbial N-immobilization rates were significantly higher in rich fens than in *Sphagnum*-dominated fens, which probably explains the differences in net N-mineralization rates between fen types. Microbial decomposition of organic matter is regulated by a variety of heterotrophic bacteria and fungi (Coulson & 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 & 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 (Iverson, 1977), and fungal activity becomes more dominant (Winsborough & Basiliko 2010). Bacteria generally have a lower biomass C:N-ratio and a higher N-demand than fungi (Hassink *et al.* 1993; Robertson & 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 of wetlands is generally assumed to result in an increase of net N-mineralization rates per kg peat (Grootjans *et al.* 1985, 1986; Williams & Wheatley 1988; Bridgham *et al.* 1998; Updegraff *et al.* 1995; Olde Venterink *et al.* 2002a; 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. This not only occurred due to increased microbial biomass, but also because of increased net N-mineralization per unit microbial C. Interestingly, net N-mineralization rates in *Sphagnum*-dominated 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. During aeration and desiccation, concentrations of phenolic compounds may only get low enough in rich fens, allowing other enzymes to be active and resulting in increased net N-mineralization. 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, P-release is also influenced by redox-sensitive processes of chemical P-binding. For P it has been shown by Olde Venterink *et al.* (2002a) 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 & Khalid 1974; Richardson 1985) or Ca-phosphates (Boyer & Wheeler 1989; Reddy *et al.* 1993). Since the mobilization and immobilization of Fe-phosphates is redox-sensitive (Patrick & 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. Microbial mortality resulting from drought, as supported by the reduced microbial C-biomass 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*. 2008a), a temporary decrease in pH may lead to suitable conditions for dominance by *Sphagnum* spp. 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 P- and N-availability, and leads to lower C-sequestration rates (Turetsky 2003). 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 entirely 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 & 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*-dominated fen in Stobbenribben, indicate that such periods of drought are common in the upper 10 cm of the soil (see Appendix B).

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.

Conclusions

Our results show that under anaerobic conditions, net N-mineralization rates are lower in rich fens than in *Sphagnum*-dominated 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*-dominated 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*-dominated 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*-dominated fens, not only because of direct adverse effects on wetland vegetation, but also because of severe increase of P-availability, leading to rapid succession.

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5

Filtering fens: Mechanisms explaining phosphorus-limited hotspots of biodiversity in wetlands adjacent to heavily fertilized areas

5. Filtering fens: Mechanisms explaining phosphorus-limited hotspots of biodiversity in wetlands adjacent to heavily fertilized areas

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Abstract

The conservation of biodiverse wetland vegetation, including that of rich fens, has a high priority at a global scale. Although P-eutrophication may strongly decrease biodiversity in rich fens, some well-developed habitats do still survive in highly fertilized regions, due to nutrient filtering services of large wetlands. The occurrence of such nutrient gradients is well-known, but the biogeochemical mechanisms that determine these patterns are often unclear. We therefore analyzed chemical speciation and binding of relevant nutrients and minerals in surface waters, soils and plants along such gradients in the large Ramsar nature reserve Weerribben-Wieden in the Netherlands.

P-availability was lowest in relatively isolated floating rich fens, where plant N:P-ratios indicated P-limitation. P-limitation can persist here despite high P-concentrations in surface waters near the peripheral entry locations, because only a small part of the P-input reaches the more isolated waters and fens. This pattern in P-availability appears to be primarily due to precipitation of Fe-phosphates, which mainly occurs close to entry locations as indicated by decreasing concentrations of Fe- and Al-bound P in the sub-aquatic sediments along this gradient. A further decrease of P-availability is caused by biological sequestration, which occurs throughout the wetland as indicated by equal concentrations of organic P in all sub-aquatic sediments.

Our results clearly show that the periphery of large wetlands does indeed act as an efficient P-filter, sustaining the necessary P-limitation in more isolated parts. However, this filtering function does harm the ecological quality of the peripheral parts of the reserve. The filtering mechanisms, such as precipitation of Fe-phosphates and biological uptake of P, are crucial for the conservation and restoration of biodiverse rich fens in wetlands that receive eutrophic water from their surroundings. This seems to implicate that biodiverse wetland vegetation requires larger areas, as long as eutrophication has not been seriously tackled.

Introduction

Many minerotrophic freshwater wetlands show large heterogeneity at the landscape scale, as they consist of a mosaic of open water, aquatic vegetation, semi-aquatic vegetation, rich fen, poor fen and swamp forest, due to the ongoing process of succession and terrestrialization. In the first half of the 20th century, biodiverse floating rich fens with *Scorpidium scorpioides* (Hedw.) Limpr. developed in mesotrophic waters on root mats of helophytes such as *Typha angustifolia* L. and *Phragmites australis* (Cav.) Steud (e.g. van Wirdum 1995). However, due to eutrophication, sulfide toxicity and/or NH_4-toxicity in these waters, new formation of rich fens has hardly occurred during the past decades in countries such as the Netherlands (van Wirdum 1979; Roelofs 1991; Smolders *et al.* 2003). The sustainability of the present rich fen remnants strongly depends on sufficient input of base-rich water to avoid fast succession toward a more acidic but less biodiverse, *Sphagnum*-dominated poor fen vegetation (Sjörs 1950). Species-rich rich fens also require P-poor conditions (Kooijman & Paulissen 2006) to prevent accelerated succession and outcompetition of rare species by graminoids (Kooijman 1993b; Chapter 3).

Most European wetlands, however, receive water from areas with intensive agricultural land use, leading to a high supply of N and P by surface water and/or groundwater (e.g. Koerselman *et al.* 1990). This may have resulted in the accumulation of P in sub-aquatic sediments during the past decades (Lijklema 1980; Lamers *et al.* 2014). The subsequent mobilization of accumulated P may lead to additional eutrophication (Patrick & Khalid 1974), especially under SO_4-rich conditions. Under anaerobic conditions, the reduction of Fe(III) and SO_4 leads to mobilization of Fe-bound P (Caraco *et al.* 1989), and high SO_4-concentrations may stimulate organic matter decomposition and P-mineralization (Drever 1997). Despite this large-scale eutrophication, remnants of sensitive and biodiverse habitat types, such as rich fens, still exist in some of the wetlands involved. This is probably caused by the spatial differentiation of N- and P-concentrations in the surface water and sediments, with decreasing concentrations from water entry locations and main canals towards more isolated sites (van Wirdum 1979; Lijklema 1980; Craft & Richardson 1993).

So far, little attention has been paid to the mechanisms that determine this gradient in larger wetlands. In this study, it is hypothesized that not all incoming P will be transported by surface water flows to the most isolated parts of wetlands, because part of the P-input will precipitate rather close to the entry locations. Furthermore, P-uptake by vascular plants, algae and micro-organisms, which occurs throughout wetlands, will presumably lead to a further decrease of P-concentrations in the surface water, especially during the growing season. These P-fractions accumulate in sub-aquatic sediments, where they may be stored permanently or become available again by desorption, dissolution and mineralization, posing a secondary risk of P-eutrophication.

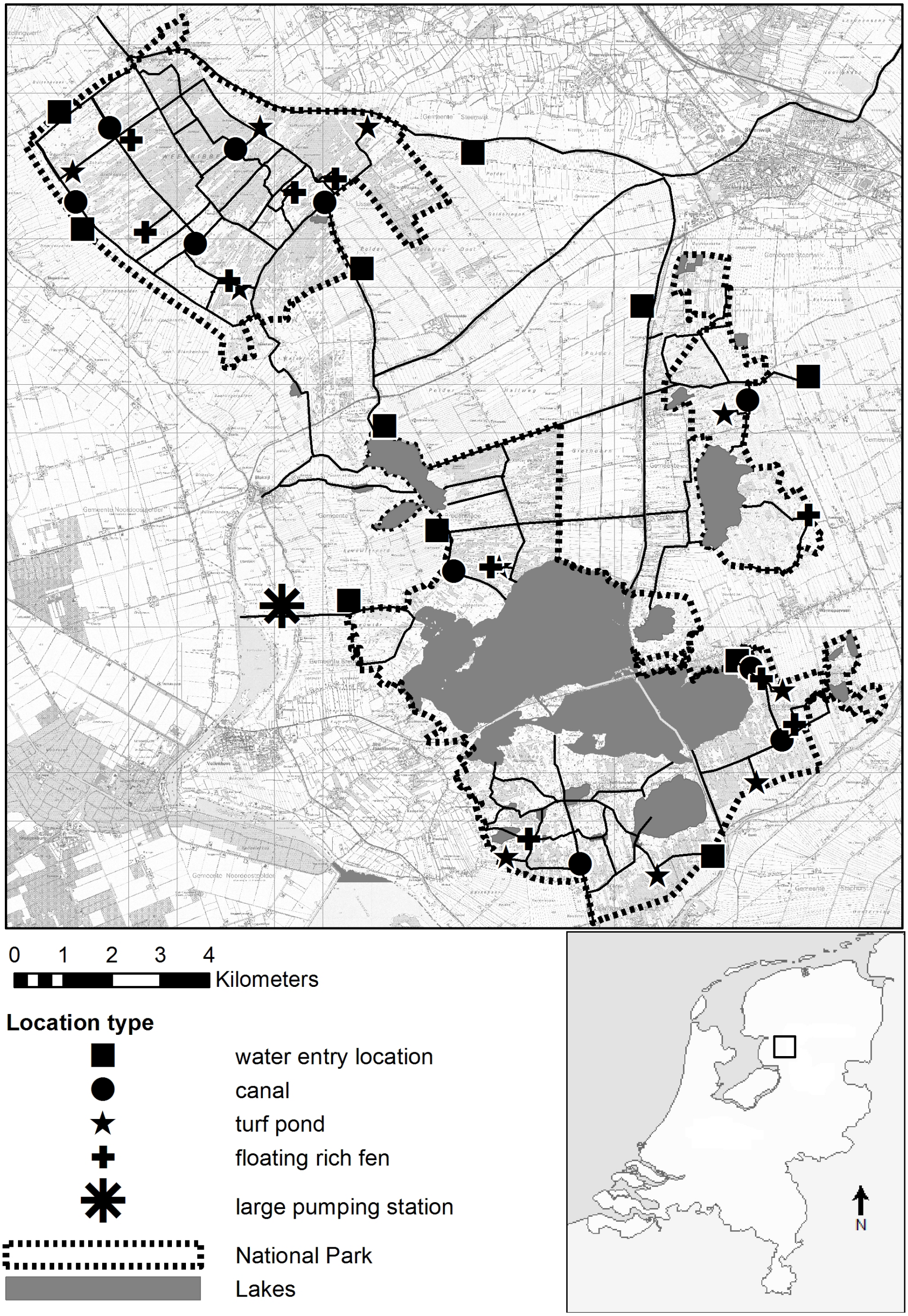

Fig. 5.1. Location and delimitation of the nature reserve Weerribben-Wieden (between 52°48' N – 5°53' E and 52°38' N – 6°08' E). The position of the four different location types is indicated with different symbols.

In order to identify the role of these biogeochemical mechanisms, we conducted a landscape-scale study in the Dutch National Park Weerribben-Wieden, a large wetland surrounded by heavily fertilized agricultural lands, which still comprises biodiverse rich fen vegetation with *S. scorpioides*. Our main aim was to determine which mechanisms explain the change in nutrient availability, especially for P, in waters and soils along a gradient from water entry points in the periphery to more isolated vegetation sensitive to eutrophication. These insights are not only important to understand the functioning of wetlands that receive high anthropogenic nutrient inputs, which is the case for many European and American wetlands that lie in the vicinity of agricultural areas, but also to understand nutrient biogeochemistry in more pristine wetlands.

Materials and methods

Site description

National Park Weerribben-Wieden is a Ramsar area in the central part of the Netherlands (between 52°48' N – 5°53' E and 52°38' N – 6°08' E; Fig. 5.1), characterized by a maritime temperate climate with a mean annual precipitation of about 800 mm (KNMI 2014). There is a precipitation surplus in winter, while large parts of the summer (April – August) are generally characterized by an evapotranspiration surplus. The atmospheric N-deposition is about 19 kg N ha^{-1} $year^{-1}$ (1350 mol ha^{-1} $year^{-1}$; RIVM 2012), which is lower than the national average.

Until the 17th century, this area was part of a large wetland that was bordered by an inland sea (the Zuiderzee) in the west and moraine upland in the east (Haans & Hamming 1962). This wetland changed dramatically between the 17th and 20th century as a consequence of peat excavation, which was carried out below the groundwater level, and the subsequent terrestrialization of abandoned turf ponds (van Wirdum 1995). During the 20th century, most of the original wetland area and parts of the Zuiderzee were reclaimed and drained to develop agricultural polders (van Wirdum 1991). The remaining wetland of about 9500 ha was conserved to serve as a regional water storage basin. In this basin, substantial terrestrialization occurred during the first half of the 20th century, and a mosaic of open canals, lakes and turf ponds with aquatic and semi-aquatic vegetation developed, including poor fen, rich fen and swamp forest.

The remaining wetland has an average surface level of 0.3 – 0.6 m below mean sea level (BMSL), and surface water levels are maintained at 0.73 – 0.83 m BMSL throughout the year. The surrounding polders are lower with surface levels of 1.0 – 2.5 m BMSL (van Wirdum 1991). These polders, which are drained by about 30 pumping stations to maintain various lower water levels of 1.5 – 3.0 m BMSL, discharge excessive water volumes into the higher lying wetland. Water levels in the wetland reserve itself are regulated by one main pumping station, which removes water during wet periods and sporadically pumps water in during pronounced dry periods.

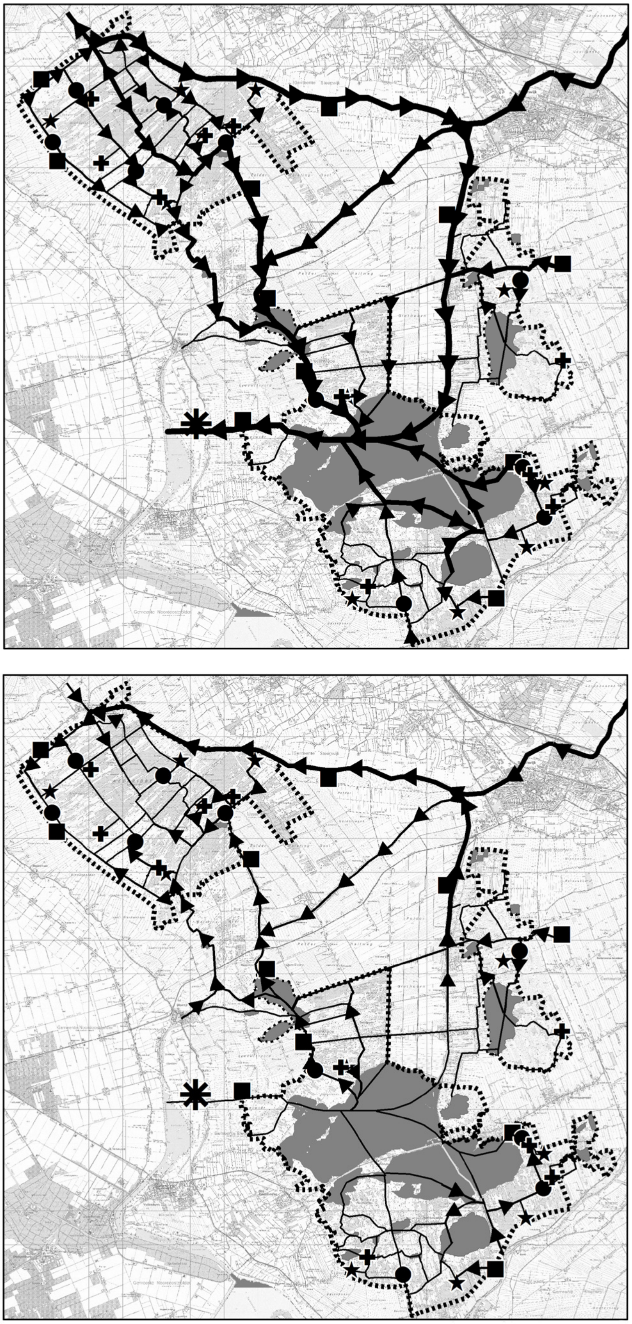

Fig. 5.2. Patterns of surface water flow in National Park Weerribben-Wieden during a period with a precipitation surplus (top) and an evapotranspiration surplus (bottom). The magnitude and direction of each flow is based on a 1D-2D model for surface water flows (SOBEK; de Lange 2011; Cusell *et al.* 2013).

The annual water balance for National Park Weerribben-Wieden, which is based on the activities of pumping stations and data about precipitation and evapotranspiration between 2001 and 2012, shows that the water input of the present wetland consists for 37% of rainwater, for 15% of water from the adjacent upland and for 48% of water from lower lying agricultural polders (Cusell *et al*. 2013). The discharge of polder water is about 50% smaller in summer than in winter. Hence, the composition of water in the remaining wetland is largely determined by the land use of the surrounding polders and the season. During the second half of the 20th century, when arable lands and meadows received excessive amounts of fertilizer, these inputs of polder water led to severely increased N- and P-inputs into the National Park (van Wirdum 1979).

The specific amounts and distribution of these inputs differ, however, substantially throughout the year (van Wirdum 1991). Previously, a 1D-2D model for surface water flows (SOBEK; Deltares systems, Delft, the Netherlands & ARCADIS, Apeldoorn, the Netherlands) was used to determine water flow patterns in National Park Weerribben-Wieden throughout the year (see Appendix C for a description of this model). This model was based on the activities of all pumping stations, precipitation and evapotranspiration rates, infiltration and seepage rates, and data of surface water levels in the National Park and surrounding polders. The model shows a complex flow pattern that may change direction several times during the year (Fig. 5.2). In periods with a precipitation surplus, mostly during winter, the surplus flows from relatively isolated locations in the Nature Park to the periphery, where it is discharged into the large Lake IJsselmeer to the west of the reserve. In contrast, an evapotranspiration surplus, mostly occurring in summer, leads to an opposite flow pattern, with a surface water flow from the periphery to the more isolated areas. Van Wirdum (1979, 1991) also reported this seasonal effect. Nearly constant surface water levels in the National Park and adjacent polders indicate that this reverse in flow direction is caused by local weather conditions, rather than by changing water levels in polders.

Investigated spatial gradient

To gain a better understanding of the biogeochemical mechanisms that act in rich fens, site conditions of floating rich fens were studied in this protected habitat. These floating fens consist of a buoyant peat layer of 0.5 – 0.7 m with a soil pore water pH of 6.2 – 7.5. They can be classified as *Caricion davallianae* (*Scorpidium* - *Carex diandra* type), and were dominated by several *Carex* spp. and brown mosses such as *S. scorpioides*.

The biogeochemical functioning of rich fens is determined not only by local site conditions, but also by the nutrient concentrations in the supplied base-rich surface water. Since the nutrient quality of this surface water is probably influenced by all kinds of filtering mechanisms between locations where nutrient-rich water enters the nature reserve and the rich fens, we examined this connective surface water system. A large array of water and soil variables were examined along a spatial gradient for (a) water entry locations at the periphery of the nature reserve, (b) intermediary canals and (c) more isolated turf ponds close to floating rich fens (Fig. 5.1). Water entries and canals are surface waters with vegetation that can generally be classified as a species-poor *Myriophyllo-Nupharetum*, while turf ponds are characterized by a *Stratiotetum* with dense stands of *Stratiotes aloides* L. The pH of the surface water in these aquatic systems was 7.0 – 7.7. To examine the potential effects of different SO_4-concentrations on P-availability, samples were taken in the relative SO_4-rich western part of the nature reserve, where the former inland sea bordered the wetland, as well as in the relative SO_4-poor eastern part.

Sampling and chemical analyses

Water, soil and vegetation samples were collected at all four types of locations. For each location type, ten representative and homogeneous plots were selected, with five plots in the west and five in the east of the wetland. Distances to the nearest water entry location were determined for each location using ArcGIS (ArcGIS 10.0, ESRI, Redlands, CA).

Surface water samples

Surface water samples were collected from water entry locations, canals and turf ponds in February and August 2009, on two consecutive days. All large pumping stations were active on a daily base in February 2009, while they were largely inactive in August 2009. Iodated polyethylene bottles of 500 mL were used to collect surface water at a depth of 10 cm below the surface.

The pH-values were measured immediately after sampling, and alkalinities were determined by titration with 0.01M HCl down to pH 4.2. Turbidities of surface water samples were measured in NTU with a turbiditymeter (Toho-Dentan, Tokyo, Japan), and samples were filtered through GF/C glass-fiber filters (ø = 1.2 μm; Whatmann, Brentford, UK). During the August sampling, concentrations of chlorophyll-a were determined spectrometrically after extracting the filter residues with ethanol (Nusch & Palme 1975).

Subsequently, all water samples were divided into two subsamples, and 1% of concentrated HNO_3 was added to one to avoid metal precipitation. Both subsamples were stored in the dark in iodated polyethylene bottles until further analysis. Non-acidified subsamples were stored at 4 °C, and acidified subsamples were stored at -24 °C. In the non-acidified subsamples, concentrations of NH_4, NO_3, o-PO_4 and Cl were analyzed colorimetrically by continuous flow auto-analyzers (System 3, Bran+Luebbe, Norderstedt, Germany). Total soluble Ca-, Mg-, K-, Fe-, P- and S-concentrations were measured in the acidified subsamples by ICP-OES (IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA). Total soluble P consisted of soluble non-reactive P (NRP) and o-PO_4. Ca- and Cl-data were used to calculate the Ionic Ratio, which is equal to $2^*[Ca]/(2^*[Ca] + [Cl])$ and can be regarded as an index for the relative importance of groundwater versus rainwater (van Wirdum 1991).

Soil pore water samples

Soil pore water samples were collected in February and August 2009. For water entry locations, canals and turf ponds, soil pore waters were anaerobically collected by connecting vacuumed plastic syringes (50 mL) to ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). The samplers were installed in the upper 10 cm of the soil, which mainly consisted of highly decomposed organic sludge. In the floating rich fens, pore water samples were collected from the upper 10 cm of the floating soil, which mainly contained peat of *S. scorpioides*. These samples were taken similarly, but with permanent soil moisture samplers with a hydrophilic porous polymer filter (Rhizons SMS 10 cm, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). The first 10 mL of all soil pore waters were discarded to exclude stagnant sampler water.

Immediately after sampling, 10.5 mL soil pore water was fixed with 10.5 mL sulfide antioxidant buffer (van Gemerden 1984), and sulfide concentrations were measured on the same day with a sulfide ion-specific Ag-electrode and a double junction calomel reference electrode (Orion Research, Beverly, USA). In addition, pH-values, alkalinities and concentrations of total soluble Ca, Mg, K, Fe, P, S, and of NH_4, NO_3 and o-PO_4 were determined, as described above.

Soil samples

Soil samples were taken in February 2009. For aquatic locations, samples of the upper 10 cm were taken with a metal sediment corer. For floating fens, samples of the uppermost 10 cm were collected with a saw. The living moss-layer was excluded. All soil samples were transported in airtight bags, and stored at 4 °C in the dark until further analysis.

Dry weights and gravimetric moisture contents of soil samples were determined by drying at 70 °C until constant weight. Bulk densities were calculated to be able to express all soil variables on an area basis. The remaining soil was quickly frozen in liquid nitrogen, freeze-dried and ground. These samples were digested by microwave (Anton Paar, Graz, Austria) destruction with HNO_3 (65%) and HCl (37%) (Bettinelli *et al.* 1989). Digestates were diluted and total Ca-, Fe-, Al-, S- and P-concentrations were measured by ICP-OES. A CNS analyzer (Vario EL, Elementar, Hanau, Germany) was used to determine total C- and N-concentrations.

Concentrations of Fe- and Al-bound P (P_{FeAl}) and Ca-bound P (P_{Ca}) were determined according to the sequential extraction method of Golterman (1996). After this sequential extraction, the remaining residue was digested with HNO_3 and HCl (as described above) to determine the concentration of organic P (P_{org}). This organic P-pool mainly consists of refractory non-labile organic P, since most labile organic P will be extracted from the soil samples during the preceding sequential extraction. Furthermore, concentrations of P bound to amorphous Fe and Al (P_{ox}) were determined by oxalate extraction in the dark (Schwertmann 1964). Amorphous Fe- and Al-concentrations were also measured in these extracts. Amorphous Fe and

Al (Fe_{ox} and Al_{ox}) consist of non-crystalline inorganic Fe- and Al(hydr)oxides ($Fe_{ox\text{-}pyr}$ and $Al_{ox\text{-}pyr}$) and organic Fe- and Al-complexes (Fe_{pyr} and Al_{pyr}). To distinguish between these fractions, organic Fe- and Al-complexes were determined by pyrophosphate extraction (Wada 1989). Finally, the plant-available P-fractions in soils were measured by Olsen extraction (1954).

Vegetation samples

Vegetation samples for chemical analysis were collected in August 2009. For aquatic locations, separate samples were taken for the aquatic vegetation and the aboveground semi-terrestrial helophyte vegetation on the shore, while aboveground standing biomass was sampled in the floating rich fens. Water entry locations and canals mainly contained *Nuphar lutea* L., *Ceratophyllum demersum* L. and *Elodea nuttallii* Planch., and turf ponds were dominated by *Stratiotes aloides*, while the terrestrial vegetation at these sites was dominated by *Typha angustifolia* and *Phragmites australis.* The floating rich fens only contained semi-terrestrial species, and were dominated by small *Carex* spp. such as *C. lasiocarpa* Ehrh., *C. diandra* Schrank, *C. oederi oedocarpa* (Andersson) Lange and *C. rostrata* Stokes.

Vegetation samples were rinsed with demineralized water to exclude soil and algal particles, and dried at 70 °C until constant weight. Ground, dry samples were used to determine total concentrations by microwave digestion and CNS-analysis, as described above. Plant N:P-ratios were calculated for the aquatic and semi-terrestrial vegetation on each plot. Ratios in the semi-terrestrial vegetation were used as a bioassay indicator to determine which of both nutrients may limit plant growth (Koerselman & Meuleman 1996; Güsewell 2004). N:P-ratios around 15 $g\ g^{-1}$ (33 $mol\ mol^{-1}$) are indicative of balanced conditions, ratios of 10 $g\ g^{-1}$ and lower suggest N-limitation, and ratios around 20 $g\ g^{-1}$ or higher indicate P-limitation.

Statistical analyses

Statistical analyses were performed using SPSS for Windows (SPSS 20.0.0, IBM, Armonk, NY). Normality of residuals was tested with QQ-plots and a Kolmogorov-Smirnov test. All data that were not normally distributed were log(x+1)-transformed to improve their fit to a normal distribution. Rich fens were explicitly not included in this gradient, because they are characterized by floating soils instead of sub-aquatic sediments, which make them subject to different redox and mineralization conditions. Differences between rich fens in the western and eastern part of the reserve were analyzed with a one-way ANOVA ($P \leq 0.05$). For the gradient from water entry locations to turf ponds, differences between gradient position, west and east, and season in chemical properties of the surface water and soil pore water were examined by a three-way ANOVA ($P \leq 0.05$). Differences between east and west, and gradient position in chemical properties of soil and vegetation were determined with a two-way ANOVA ($P \leq 0.05$). Tukey HSD post-hoc tests ($P \leq 0.05$) were used to determine significant differences between the individual means of the gradient positions.

To gain more insight into the effect of distance from water entries on N- and P-concentrations in the surface water, Pearson correlation coefficients were calculated between concentrations in the surface water and the distance to the nearest entry locations of nutrient-rich water. This was carried out separately for canals and turf ponds.

Results

N- and P-concentrations in plants

N- and P-concentrations in the vegetation tissues, both in aquatic and aboveground semi-terrestrial vegetation, were highest near water entry locations and lowest in turf ponds and floating rich fens (Fig. 5.3, Table 5.1). P-concentrations differed, however, more than N-concentrations. This led to different plant N:P-ratios in the semi-terrestrial vegetation: from around 10 g g^{-1} near water entries to around 15 g g^{-1} in turf ponds and 19 g g^{-1} in rich fens. A similar gradient of increasing plant N:P-ratios was found for the aquatic vegetation, although ratios of around 8 g g^{-1} near water entries and canals were lower than in the semi-terrestrial vegetation.

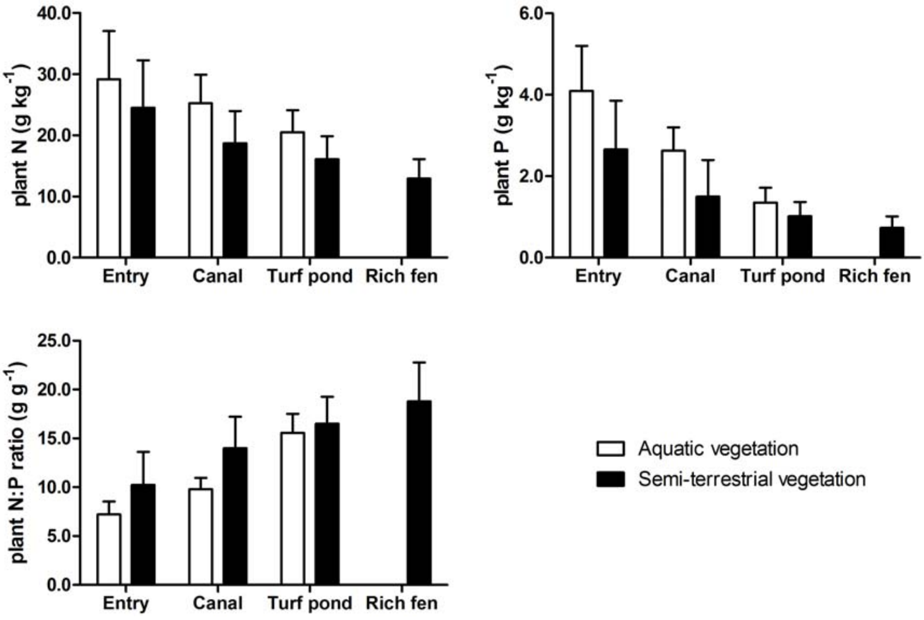

Fig. 5.3. N-content, P-content and N:P-ratios in the tissue of aquatic vegetation (not present in rich fens) and aboveground semi-terrestrial vegetation. Sample means are given for western and eastern locations together, and error bars indicate standard deviations (n = 10). Statistical information is provided in Table 5.1.

P-fractions in floating rich fens

The high plant N:P-ratios of about 19 g g^{-1} in rich fens were in accordance with soil proxies for P-availability in floating rich fens such as low o-PO_4 concentrations of 0.3 – 1.2 µmol L^{-1} in the soil pore water and low concentrations of plant-available P (P-Olsen) of 3 – 8 mmol m^{-2}. Total P-concentrations in floating fen soils were around 100 mmol P per m^2, of which 55% was non-labile organic P, 30% was Ca-bound P (P_{Ca}) and only 15% was Fe-or Al-bound P (P_{FeAl}). Hence, the P_{Ca}:P_{FeAl}-ratio was rather high: 2 mol mol^{-1}. This corresponds to much higher total Ca-concentrations than Fe- and Al-concentrations in floating fen soils, leading to total Ca:Fe-ratios of about 14 mol mol^{-1}.

Table 5.1. *P*-values for the effects of gradient position (water entry, canal, turf pond and floating rich fen), east/west position and the gradient * west/east position interaction on plant nutrient concentrations in aquatic vegetation and semi-terrestrial vegetation, as tested by two-way ANOVA's.

	Gradient	East/West	Gradient * East/West	Entry	Canal	Turf pond	Rich fen
Aquatic vegetation							
C plant	**0.018**	0.822	0.275	a	b	ab	-
N plant	**0.003**	0.143	0.057	b	ab	a	-
P plant	**0.000**	0.068	0.058	c	b	a	-
C:N plant	**0.000**	0.157	0.408	a	b	c	-
C:P plant	**0.000**	0.931	0.087	a	b	c	-
N:P plant	**0.000**	0.771	0.189	a	b	c	-
Semi-terrestrial vegetation							
C plant	**0.000**	0.781	0.449	a	b	b	b
N plant	**0.000**	0.860	0.069	b	ab	a	a
P plant	**0.000**	0.863	0.183	b	a	a	a
C:N plant	**0.000**	0.605	0.170	a	ab	bc	c
C:P plant	**0.000**	0.986	0.723	a	ab	bc	c
N:P plant	**0.000**	0.761	0.761	a	ab	bc	c

Bold values indicate $P \leq 0.05$. Different letters indicate significant differences between gradient positions for a particular variable, as determined by Tukey HSD post-hoc tests ($n = 5$; $P \leq 0.05$).

Spatial nutrient distribution from water entry locations to turf ponds

Surface water gradients: February versus August

Measurements in February showed significantly higher o-PO_4 and total soluble P-concentrations in the surface waters of entry locations than in turf ponds (Fig. 5.4, Table 5.2). Values for o-PO_4 were rather low at all plots, with concentrations below 1 µmol L^{-1}. Total soluble P-concentrations were higher, with levels up to 5 µmol L^{-1} near water entry locations. Concentrations of soluble non-reactive P (NRP), including DOP and small (< 1.2 µm) colloidal Fe-phosphates, were therefore about 5 times larger than concentrations of o-PO_4. In August, concentrations of all P-fractions in the surface water did, however, not differ between water entries, canals and turf ponds. Concentrations of total P and o-PO_4 were low throughout the nature reserve, and chlorophyll-a concentrations were almost everywhere below 20 µg L^{-1}.

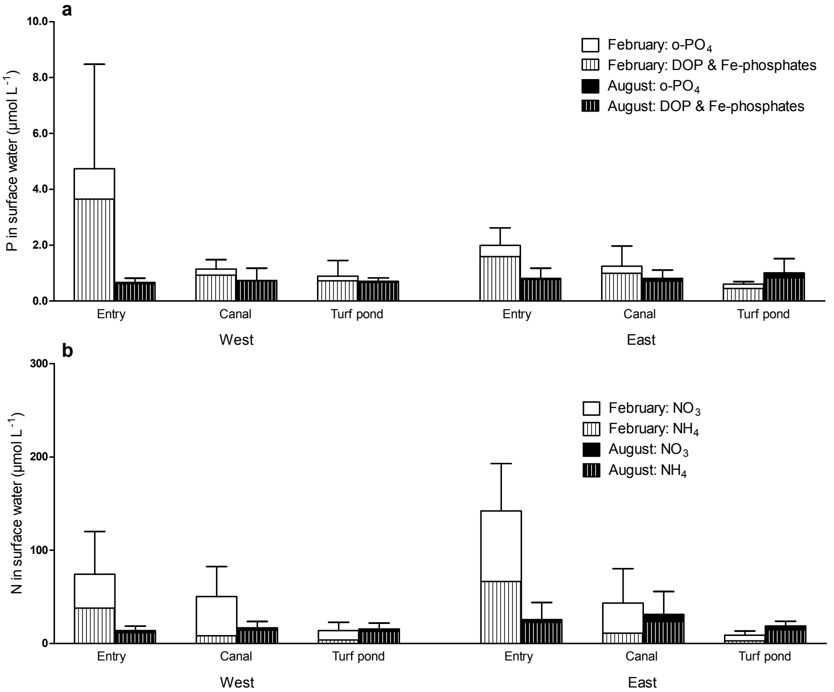

Fig. 5.4. Concentrations of o-PO_4 and soluble non-reactive P (NRP) (a), and NH_4 and NO_3 (b) in the surface water of entry locations, canals and turf ponds, in February and August. Sample means are given and error bars indicate standard deviations (n = 5). Statistical information is provided in Table 5.2.

Table 5.2. *P*-values for the effects of gradient position (water entry, canal and turf pond), east/west position, season (February and August) and the gradient * season interaction on some chemical variables in the surface water and soil pore water, as tested by three-way ANOVA's. Other potential interactions were also tested, but did not occur. The Ionic Ratio is equal to 2*[Ca]/(2*[Ca] + [Cl]).

	Gradient	East/West	Season	Gradient * Season	Entry	Canal	Turf pond
Surface water							
Turbidity	**0.000**	0.105	**0.000**	**0.023**	b	b	a
pH	0.587	0.868	**0.000**	0.978	a	a	a
Alkalinity	**0.000**	**0.011**	**0.020**	0.825	c	b	a
Ca	**0.002**	0.316	**0.005**	**0.015**	b	a	a
Fe	**0.000**	0.592	**0.000**	**0.000**	b	a	a
Al	**0.013**	0.683	**0.000**	**0.037**	b	ab	a
o-PO_4	**0.018**	0.735	**0.000**	**0.001**	b	ab	a
NRP	**0.000**	0.250	**0.001**	**0.000**	b	a	a
total P	**0.000**	0.539	**0.000**	**0.000**	b	a	a
S	**0.005**	**0.003**	**0.012**	**0.030**	b	a	a
NO_3	**0.004**	0.280	**0.000**	**0.001**	b	b	a
NH_4	**0.000**	0.142	**0.016**	**0.000**	b	a	a
inorganic N	**0.000**	0.110	**0.002**	**0.000**	b	b	a
Ionic Ratio	**0.016**	0.201	**0.000**	**0.033**	b	ab	a
Chlorophyll-a	0.924	**0.004**	-	-	a	a	a
Soil pore water							
pH	**0.008**	0.261	**0.000**	0.742	b	b	a
Alkalinity	**0.006**	**0.001**	0.457	0.687	b	b	a
Ca	**0.000**	0.066	0.286	0.484	b	b	a
Fe	**0.001**	0.055	0.448	0.141	b	b	a
Al	0.310	0.068	0.052	0.921	a	a	a
o-PO_4	**0.036**	**0.000**	0.482	0.405	ab	b	a
NRP	**0.002**	**0.001**	**0.036**	0.092	b	b	a
total P	**0.001**	**0.000**	**0.038**	0.671	b	b	a
S	0.433	**0.012**	0.168	0.822	a	a	a
Sulfide	0.068	0.904	**0.000**	0.268	a	a	a
NO_3	0.794	0.594	0.102	0.452	a	a	a
NH_4	**0.029**	**0.032**	0.950	0.957	b	b	a
inorganic N	0.191	**0.007**	0.829	0.792	a	a	a
Fe:o-PO_4	**0.037**	**0.000**	0.147	0.246	b	ab	a

Bold indicates $P \leq 0.05$. Different letters indicate significant differences between gradient position, as determined by Tukey HSD post-hoc tests (n = 5; $P \leq 0.05$).

The spatial decrease found for P-concentrations in February was supported by a significant negative correlation between the distance of a specific canal or turf pond from its nearest water entry location on the one hand, and the concentrations of o-PO_4, soluble non-reactive P (NRP) and total soluble P in the surface water in February on the other hand (Table 5.3). Such correlations were, however, not found in August, which also indicated that there was no P-gradient present in the surface water during the growing season.

The soluble inorganic N-concentrations and turbidity of the surface water were also relatively high near water entries and canals in February, with values of about 50 – 150 µmol L^{-1} and 15 – 25 NTU, compared to 20 – 30 µmol L^{-1} and 3 – 5 NTU in turf ponds (Figs. 5.4 & 5.5, Table 5.2). However in August, similar to P-concentrations, N-concentrations and turbidity were low throughout the nature reserve.

These clear differences in spatial patterns between February and August measurements were also found for the concentrations of soluble Ca, Fe, Al and S in the surface water, and for the Ionic Ratios (Fig. 5.5, Table 5.2). Just like the soluble N- and P-concentrations, these concentrations did not differ significantly within the nature reserve in August, while clear gradients were measured from water entries to turf ponds in February. In August, Ca-concentrations and Ionic Ratios were relatively high throughout the area, with values of about 1400 µmol L^{-1} and 0.8, respectively. In February, however, Ca-concentrations were significantly lower in canals and turf ponds in February, with concentrations of 500 – 800 µmol L^{-1}, which also led to significantly lower Ionic Ratios of about 0.6. Soluble Fe- and S-concentrations in the surface water showed reverse patterns. Fe- and S-concentrations were low throughout the reserve in August, with concentrations of about 5 and 150 µmol L^{-1} respectively, while significantly higher concentrations of above 30 and 500 µmol L^{-1} were measured near water entry locations in February. High S-concentrations were only found at the western side of the nature reserve.

Soil pore water gradients

Total soluble P and o-PO_4 concentrations in the soil pore water decreased significantly from water entries to turf ponds (Fig. 5.6, Table 5.2). A similar decrease was found for the soluble Fe-, Al- and Ca-concentrations. The Fe-concentrations in the soil pore water decreased even faster than the o-PO_4 concentrations, resulting in significantly lower Fe:o-PO_4 ratios in turf ponds than near water entries.

Soil pore water concentrations of o-PO_4 were below 3 µmol L^{-1} in the east, while much higher concentrations of 5 – 8 µmol L^{-1} were found in the west (Fig. 5.6, Table 5.2). Since soluble Fe-concentrations did not differ between the west and east, significantly lower Fe:o-PO_4 ratios of 1 – 40 mol mol^{-1} were found in the west than in the east, where ratios of 40 – 300 mol mol^{-1} were found. Low Fe-concentrations in the soil pore water of turf ponds resulted in the lowest Fe:o-PO_4 ratios of 1 – 8 mol mol^{-1} in western turf ponds. Also, alkalinity of soil pore water differed significantly between the west and east, with values of 4 – 7 $mmol_c$ L^{-1} in the west and 3 – 5 $mmol_c$ L^{-1} in the east. High NH_4-concentrations, up to levels of 400 µmol L^{-1}, were also only found in the west of the nature reserve. Soluble sulfide concentrations in the soil pore water did, however, not differ between both sides, and were below 15 µmol L^{-1} throughout the nature reserve.

Soil quality gradients

Total P-concentrations in sub-aquatic sediments were significantly higher near eastern water entry locations than in turf ponds (Fig. 5.7, Table 5.4) with 200 – 700 versus 100 – 200 mmol m^{-2}, respectively. A similar decrease was found for total Al- and Fe-concentrations. Total Fe:P-, Ca:P- and Ca:Fe-ratios did not differ among sub-aquatic sediments, and were around 20, 25 and 1.4 mol mol^{-1} throughout the area.

Along with the decrease in total Al-, Fe- and P-concentrations, selective extraction spectra of Al, Fe and P also changed in these sub-aquatic sediments. Near water entries, most extractable Al and Fe in the sub-aquatic sediments was present as inorganic Al and Fe (Al_{ox-pyr} and Fe_{ox-pyr}), while turf ponds were dominated by organically bound Al and Fe (Al_{pyr} and Fe_{pyr}) (Fig. 5.8, Table 5.4). The selective extractions of P showed a similar

Table 5.3. Pearson correlations between the distance of canals and turf ponds from entry locations of nutrient-rich water and some chemical variables in the surface water of these canals and turf ponds. The Ionic Ratio is equal to 2*[Ca]/(2*[Ca] + [Cl]). The correlations were separately calculated for the measurements in February and August (n = 10).

	February		**August**	
	Canal	**Turf pond**	**Canal**	**Turf pond**
Turbidity	0.058	0.073	0.008	0.063
pH	-0.080	-0.434	0.569*	0.096
Alkalinity	-0.076	-0.627*	0.027	0.162
Ca	0.085	-0.416	0.266	0.070
Fe	-0.591*	-0.782**	0.058	-0.308
o-PO_4	-0.573*	-0.704**	-0.092	-0.216
NRP	-0.570*	-0.694**	0.035	-0.259
total P	-0.569*	-0.640**	0.031	-0.249
S	-0.234	-0.257	-0.388	0.153
NO_3	0.121	0.562	0.138	0.285
NH_4	-0.649**	-0.073	0.143	-0.046
inorganic N	-0.119	-0.209	0.167	-0.255
Ionic Ratio	0.173	-0.599*	-0.079	0.115
Chlorophyll-a	-	-	-0.054	0.287

* $P \leq 0.1$ and ** $P \leq 0.05$

pattern (Fig. 5.7). Although absolute amounts of non-labile organic P did not differ significantly between the different locations along the gradient, most extractable P in the sub-aquatic sediments of turf ponds was present as organic P (about 70%), while water entry locations and canals only contained 15 – 50% organic P. The sub-aquatic sediments near water entries and canals contained significantly more Ca-bound P (P_{Ca}) and especially Fe- and Al-bound P (P_{FeAl} and P_{ox}) than the turf pond soils. The P_{Ca}:P_{FeAl}-ratios were rather low in all sub-aquatic sediments with values between 0.1 and 0.3 mol mol^{-1}.

These changes in extractable P-fractions indicate higher P-availability in the sub-aquatic sediments near water entries than in turf ponds, which is supported by significantly higher concentrations of plant-available P-Olsen (Fig. 5.9, Table 5.4). Values decreased from 15 – 80 mmol m^{-2} (with a median of 35) near water entries to 5 – 8 mmol m^{-2} in turf ponds. This decrease is in accordance with higher plant N:P-ratios, and is highly correlated with decreasing concentrations of Fe-bound P (P_{FeAl} and P_{ox}). P-Olsen and plant N:P-ratios are less correlated with Ca-bound P, while organic P (P_{org}) did not correlate at all (Table 5.5).

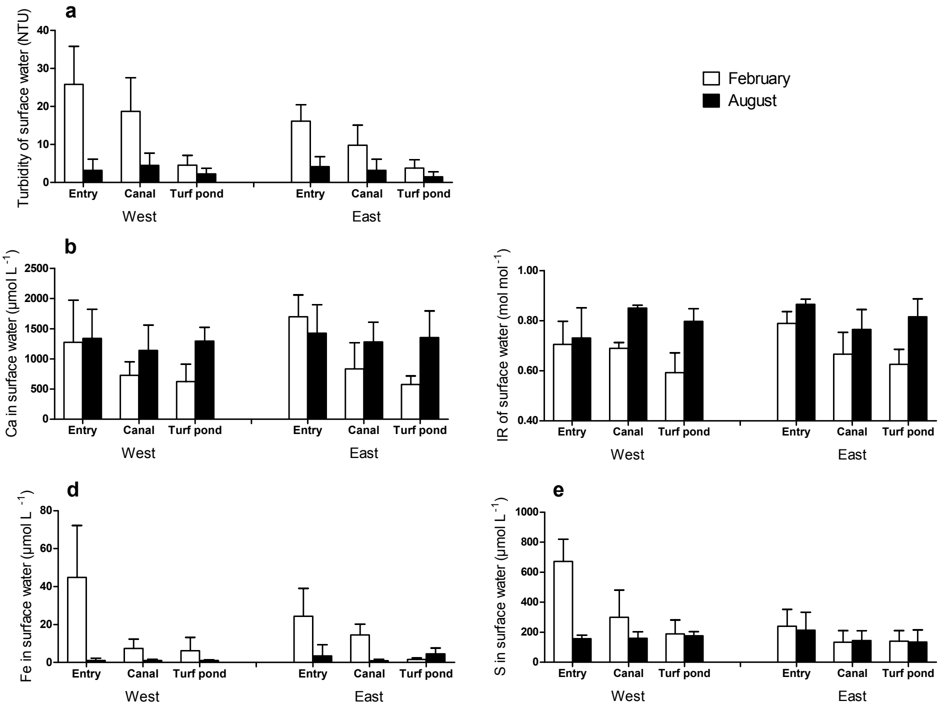

Fig. 5.5. Turbidity (a), Ca-concentration (b), Ionic Ratio (c), and concentrations of Fe (d) and S (e) in the surface water of entry locations, canals and turf ponds, in February and August. The Ionic Ratio is equal to 2*[Ca]/(2*[Ca] + [Cl]). Sample means are given and error bars indicate standard deviations (n = 5). Statistical information is provided in Table 5.2.

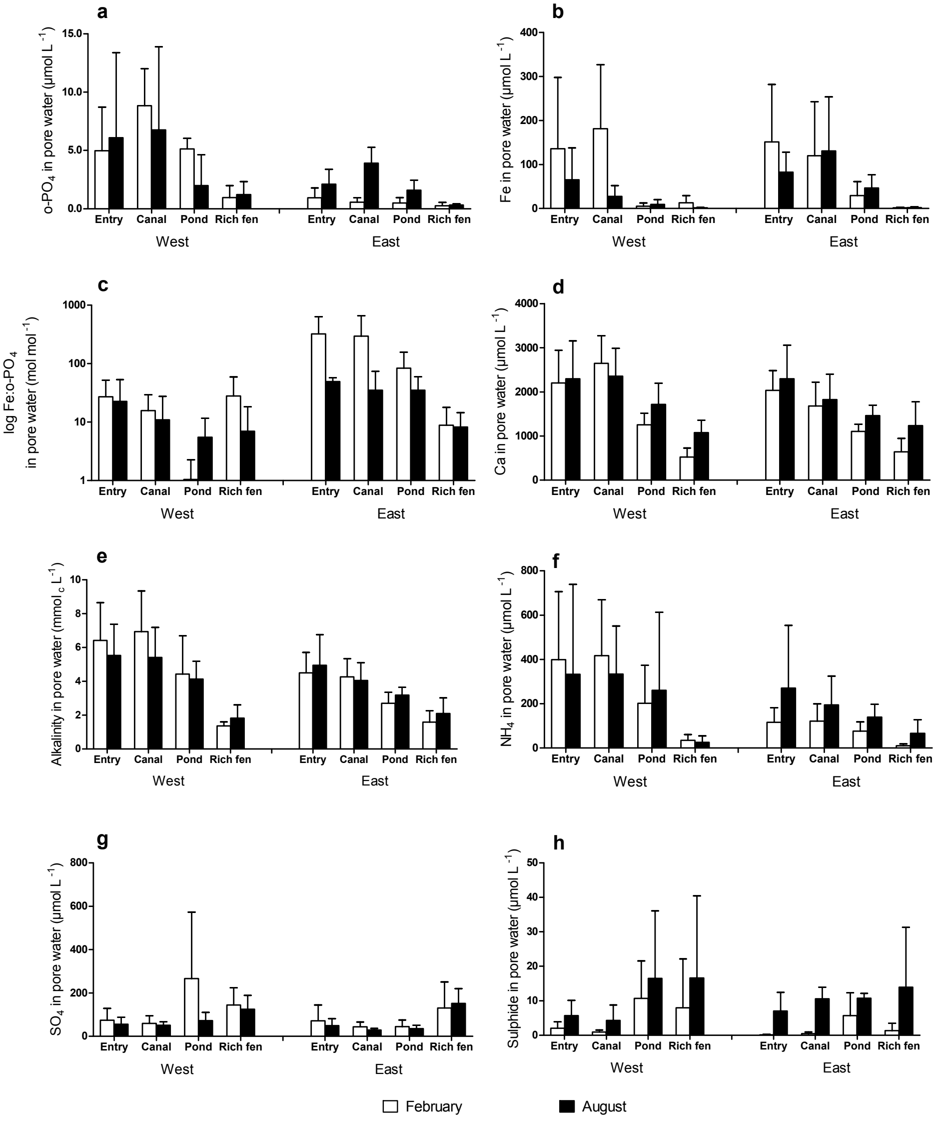

Fig. 5.6. Concentrations of o-PO_4 (a) and Fe (b), Fe:o-PO_4 ratios (c), Ca (d), alkalinity (e), and concentrations of NH_4 (f), S (g) and sulfide (h) in the soil pore water of entry locations, canals and turf ponds, in February and August. Note that Fe:o-PO_4 ratios are given on a log-transformed scale. Sample means are given and error bars indicate standard deviations (n = 5). Statistical information is provided in Table 5.2.

Table 5.4. *P*-values for the effects of gradient position (water entry, canal and turf pond), east/west position and the gradient * east/west position interaction on some soil chemical variables, as tested by two-way ANOVA's.

	Gradient	East/West	Gradient * East/West	Entry	Canal	Turf pond
Totals						
Ca_t	0.173	0.181	0.791	a	a	a
Fe_t	**0.047**	0.115	0.169	b	ab	a
Al_t	**0.001**	0.060	**0.002**	b	a	a
P_t	**0.010**	**0.040**	**0.041**	b	ab	a
N_t	0.076	0.126	0.055	a	a	a
S_t	0.057	0.121	0.979	a	a	a
$Ca_t:Fe_t$	0.553	0.478	0.057	a	a	a
$Ca_t:P_t$	0.183	0.759	0.301	a	a	a
$Fe_t:P_t$	0.315	0.647	0.100	a	a	a
P-fractions						
P_{org}	0.482	0.546	0.818	a	a	a
P_{Ca}	**0.003**	**0.015**	**0.011**	b	a	a
P_{FeAl}	**0.025**	0.904	0.961	b	ab	a
P_{ox}	**0.006**	**0.048**	0.064	b	a	a
$P_{org}:P_t$	**0.000**	0.123	0.550	a	a	b
$P_{Ca}:P_t$	0.105	0.186	0.107	a	a	a
$P_{FeAl}:P_t$	**0.000**	0.987	0.862	b	b	a
$P_{Ca}:P_{FeAl}$	0.171	0.518	0.758	a	a	a
P-Olsen	**0.033**	0,108	0.298	b	ab	a
Fe-fractions						
Fe_{ox}	0.356	0.562	0.264	a	a	a
Al_{ox}	0.089	0.083	0.206	a	a	a
Fe_{ox}-Fe_{pyr}	0.306	0.720	**0.024**	a	a	a
Al_{ox}-Al_{pyr}	0.131	0.510	0.997	a	a	a
Fe_{pyr}	0.344	0.609	0.439	a	a	a
Al_{pyr}	0.181	0.086	0.126	a	a	a
$Fe_{pyr}:Fe_{ox}$	**0.003**	0.412	0.212	a	ab	b
$Al_{pyr}:Al_{ox}$	**0.013**	0.396	0.425	a	ab	b

Bold indicates $P \leq 0.05$. Different letters indicate significant differences between gradient positions, as determined by Tukey HSD post-hoc tests ($n = 5$; $P \leq 0.05$).

Table 5.5. Pearson correlations between variables that are indicative for the nutrient availability of vascular plants (plant N:P-ratio and P-Olsen) and some P-fractions in the soil. Results from all studied locations were combined in this analysis (n = 40).

	Plant N:P ratio	**P-Olsen**
P-Olsen	-0.317***	-
P_{org}	-0.075	0.072
P_{Ca}	-0.180*	0.394***
P_{FeAl}	-0.244***	0.749***
P_{ox}	-0.318***	0.904***
P_t	-0.275***	0.598***

* $P \leq 0.1$, ** $P \leq 0.05$ and *** $P \leq 0.01$

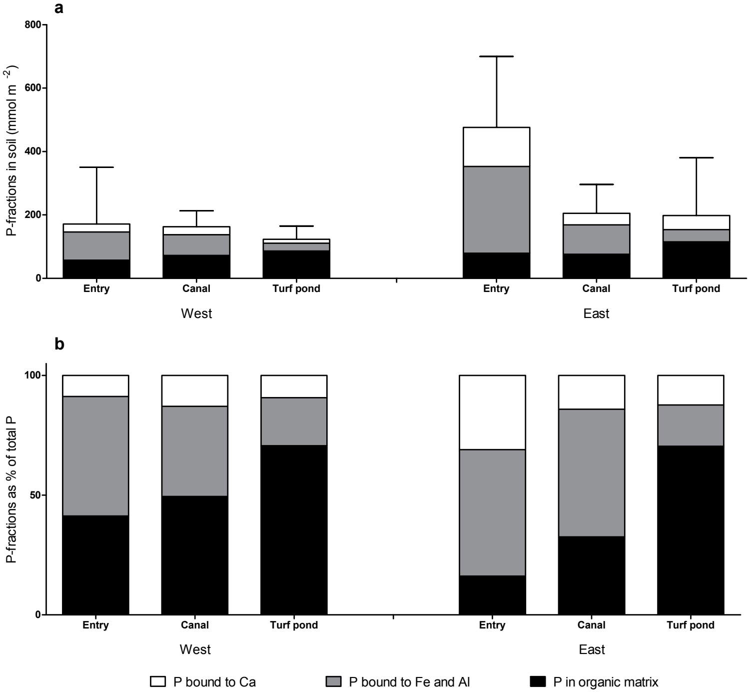

Fig. 5.7. Concentrations of P bound to Fe and Al, Ca and organic matter in the sub-aquatic sediments of water entry locations, canals and turf ponds. Graph (a) shows the means, with their standard deviations as error bars, of the P-concentrations per m² in the uppermost 10 cm of the soil, while relative values to total P are given in graph (b) (n = 5). Statistical information is provided in Table 5.4.

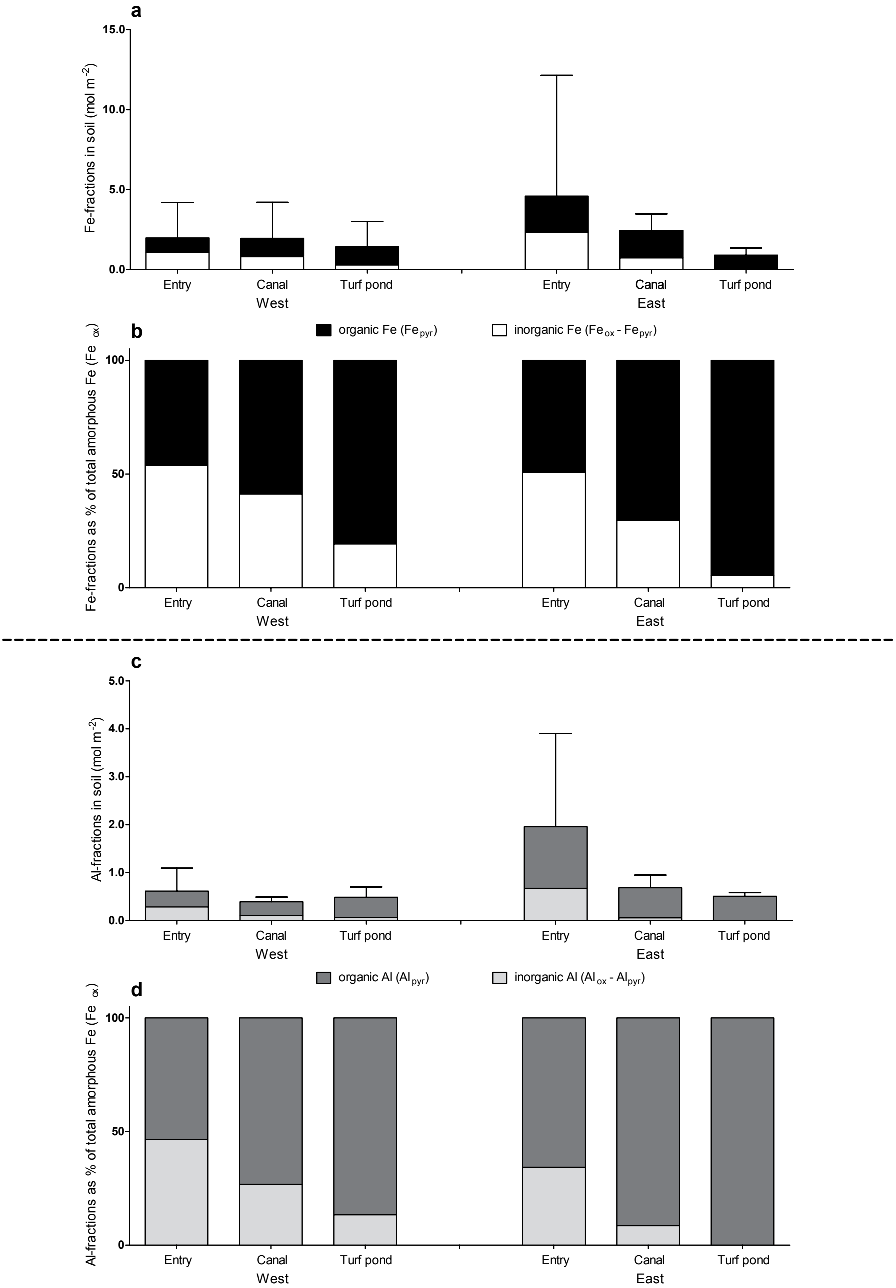

Fig. 5.8. Concentrations of inorganic and organic bound Fe and Al in the sub-aquatic sediments of water entry locations, canals and turf ponds. Graphs a and c show means, with their standard deviations as error bars, of the Fe- and Al-concentrations per m^2 in the uppermost 10 cm of the soil, while relative values to total amorphous Fe and Al are given in graphs b and d (n = 5). Statistical information is provided in Table 5.4.

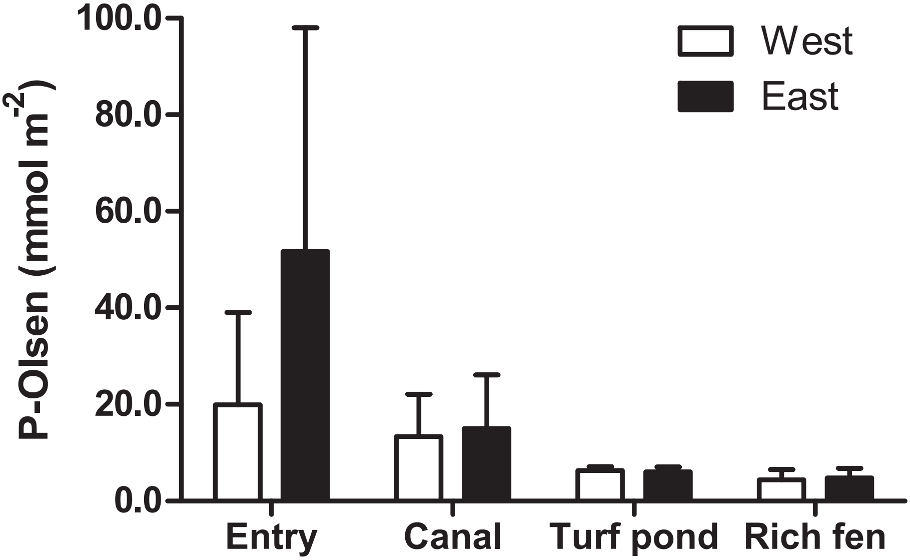

Fig. 5.9. Concentrations of plant-available P (P-Olsen) in the (sub-aquatic) soils of water entry locations, canals, turf ponds and rich fens. Sample means are given and error bars indicate standard deviations (n = 5). Statistical information is provided in Table 5.4.

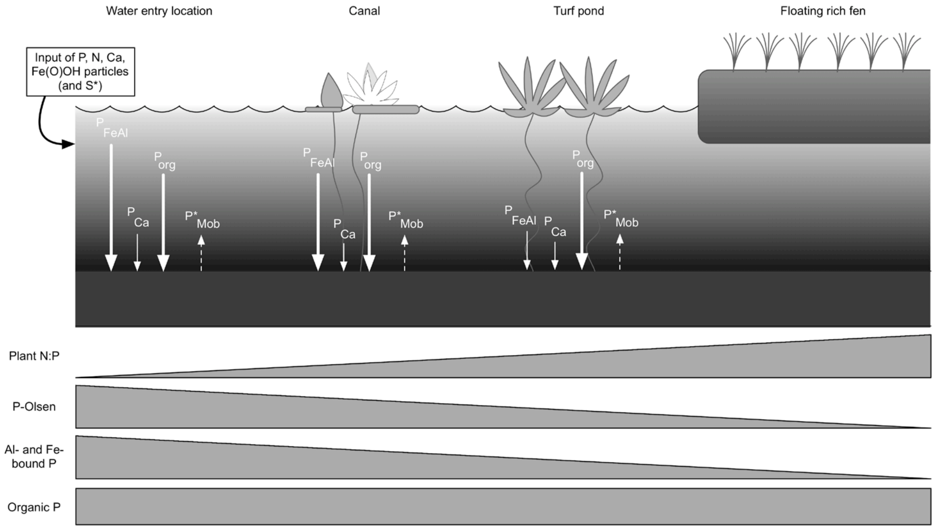

Fig. 5.10. A conceptual model for large wetlands with high P-inputs. It shows the importance of relevant P-mechanisms at different locations. Fe-inputs (as suspended Fe-precipitates) were high in the wetland studied. * P-mobilization was higher at the SO_4-rich western part of the studied nature reserve than at the east side.

Discussion

Spatial gradients in nutrient availability for plant

N:P-ratios in aquatic and semi-terrestrial vascular plants clearly indicated a decrease in P-availability from water entries with *Nuphar lutea* and *Elodea nuttallii*, where ratios were 8 – 10 g g^{-1}, to turf ponds with dense stands of *Stratiotes aloides*, where ratios of 15 g g^{-1} were found. Clear P-limited conditions, however, only occurred in rich fens with *Scorpidium* spp., as indicated by semi-terrestrial plant N:P-ratios of around 19 g g^{-1} (Koerselman & Meuleman 1996; Güsewell 2004). These P-limited conditions correspond with conditions found for other rich fens (Bedford *et al.* 1999; Kooijman & Hedenäs 2009).

P-Olsen, a soil proxy for plant P-availability, also decreased along this gradient. Concentrations of plant-available P were very low in turf ponds and floating rich fens, with values of 3 – 8 mmol m^{-2}, given the fact that Lamers *et al.* (2014) defined a critical concentration for P-Olsen of 250 – 500 µmol L^{-1} (25 – 50 mmol m^{-2}) for high biodiversity in unfertilized terrestrial fens.

Potential mechanisms that cause spatial gradients in P-availability

It was hypothesized that the distinctive patterns in P-availability were mainly related to filtering mechanisms within the nature reserve. Not all the incoming P will be transported to the most isolated parts of wetlands, because these mechanisms will lead to storage of P in sub-aquatic sediments (Fig. 5.10). The combined examinations of surface waters, soil pore waters and sub-aquatic sediments enabled a much better understanding of the filtering mechanisms involved.

Variations in surface water quality

N- and P-concentrations in the surface water were higher near water entry locations than in turf ponds, due to high nutrient concentrations in the agricultural polder water. Such nutrient gradients have also been found in other studies (van Wirdum 1979; Craft & Richardson 1993; Reddy *et al.* 1999). Strikingly, this study shows that these distinct nutrient gradients were only present in February 2009, when all large pumping stations were active, and not in August 2009, when most of these pumping stations were inactive. Total soluble P-concentrations in the surface water were only above the critical concentration of 1.5 µmol L^{-1}, below which the cover of red list macrophytes increases sharply (Geurts *et al.* 2008), during the winter measurement, and only near entry locations and canals.

The same pattern of high P-concentrations in winter and low P-concentrations in summer was also observed in a historical dataset (from 1980 to 2002) of six canals within the nature reserve (see Appendix D & Table S1 in Supporting Information of online article). During this period, concentrations of o-PO_4 also showed a significant long-term decrease from potentially problematic (with respect to the enhanced risk of algal blooms) levels of 1.0 – 2.5 μmol L^{-1} around 1980, to much lower concentrations of 0.5 – 1.5 μmol L^{-1} around 2000. This decrease in o-PO_4 concentrations was found for all seasons, with lowest concentrations during summers. This decrease coincided with a significant decrease in chlorophyll-a concentrations from high levels of 50 – 120 μg L^{-1} in the summer of 1980 to 20 – 40 μg L^{-1} in the summer of 2002. For this period, similar patterns have been found in a nearby lake district (Maasdam & Claassen 1998).

The differences between winter and summer conditions are probably caused by lower nutrient uptake by plants and algae and larger inputs of nutrient-rich water from agricultural polders during the winter (Toor *et al.* 2004; Rip 2007; Schoumans & Groenendijk 2000). The observed differences between winter and summer conditions are most probably enlarged by the flow pattern of surface water in National Park Weerribben-Wieden (van Wirdum 1979, 1991). In periods with a precipitation surplus, mostly during winter, a surplus of rainwater flows from relatively isolated locations to the periphery of the National Park (Cusell *et al.* 2013). This effectively prevents the transport of nutrient-rich input water to more isolated locations within the reserve. In contrast, a surplus of evapotranspiration, which mostly occurs during summer, leads to an opposite flow pattern, with supply of surface water from the periphery to the more isolated areas (Cusell *et al.* 2013). This stimulates large-scale mixing throughout the area, but does not lead to increased N- and P-concentrations inside the nature reserve, because relatively little nutrient-rich water is discharged from polders into the wetland during dry periods.

Filtering mechanisms and spatial differences in soil quality

One important P-filtering mechanism in wetlands is the assimilation and accumulation of organic material (Howard-Williams 1985; Richardson & Marshall 1986; Reddy *et al.* 1999). This P-accumulation consists of easily decomposable P and refractory, non-labile organic P (Reddy *et al.* 1999; Søndergaard *et al.* 2001). This study suggests that the accumulation of refractory organic P in the upper layer of the sub-aquatic sediment is approximately the same throughout the nature reserve, since concentrations of refractory organic P did not differ throughout the nature reserve. This P-pool, which contributes to 20 – 40% of the total P in sub-aquatic sediments of water entry locations and about 80% in turf pond soils, can only become available through mineralization (Paul 2007). Since decomposition and net mineralization rates are usually rather small in sub-aquatic peat soils as a result of anaerobic conditions (e.g. Geurts *et al.* 2010), this refractory organic P-pool will not have a large influence on the P-availability. This is supported by the absence of correlation between P-Olsen and refractory organic P in the sub-aquatic sediments.

A second important filtering mechanism is P-sorption to Al- and Fe-compounds, and the precipitation of colloidal Al- and Fe-phosphates. These mechanisms have already been described for some time (e.g. Mortimer 1941; Golterman 1995). Generally, however, the spatial context at a landscape scale was not considered.

Water entry locations were not only the main input of N and P, but also contributed significantly to the input of Al, Fe and Ca. The high inputs of Al, Fe and Ca at the entry locations are due to groundwater discharge in the deeper lying polders around the nature reserve (van Wirdum 1991), which are the source areas of this entry water. Fe-inputs probably mainly occur as suspended FeO and Fe(OOH) precipitates, as these are easily formed in oxic surface waters that are fed by groundwater discharge (Tuhela *et al.* 1997). These inputs have led not only to higher Al- and Fe-concentrations in the sub-aquatic sediments near water entries compared to turf ponds, but also to a similar pattern for Fe- and Al-bound P. This indicates that Al- and Fe-induced accumulation of P mainly occurs near water entry locations and canals. Lijklema (1980) suggested similar accumulation patterns in National Park Weerribben-Wieden during the 1970s. This means that wetlands that show high P-inputs without concomitant high inputs of Fe and Al are probably more sensitive to eutrophication.

Although the binding of P to Fe prevents P-mobilization to the water layer (e.g. Patrick & Khalid 1974), many plants are still capable of taking up P from accumulated Fe-phosphates in soils (Marschner 1995). The relatively high concentrations of plant-available P-Olsen and low plant N:P-ratios near water entry locations and canals indeed indicate that the P-availability in National Park Weerribben-Wieden is at least partly determined by the concentration of Al- and Fe-bound P in the soil. Hence, the precipitation of Al- and Fe-phosphates near entry locations of nutrient-rich water seems to locally increase the P-availability in soils, while it simultaneously reduces the transfer of P towards turf ponds.

According to relatively high Fe:o-PO_4 ratios of above 10 mol mol^{-1} in the soil pore waters of most locations, P-mobilization rates to the surface water should be low throughout the nature reserve (Zak *et al.* 2004; Geurts *et al.* 2008). Some P-mobilization to the surface water may, however, occur near water entry locations and canals at the west side of the nature reserve, where inputs of SO_4-rich surface water may lead to P-mobilization as a result of Fe(III)-reduction and/or increased mineralization rates (Caraco *et al.* 1989; Lamers *et al.* 1998b; Smolders *et al.* 2006). This is indeed supported by the measurement of higher concentrations of o-PO_4 in the soil pore waters of these locations, which were above the critical value of 5 μmol L^{-1} (Geurts *et al.* 2008). Some P-mobilization may also occur in western turf ponds. Here, Fe:o-PO_4 ratios were below 10 mol mol^{-1}, and Fe was primarily present as organically bound Fe, which is less capable of adsorbing P than inorganic solid Fe-compounds (Koenings & Hooper 1976; Borggaard *et al.* 2005). Therefore, it should be kept in mind that previously accumulated Fe-phosphates may still be remobilized under anaerobic conditions (Patrick & Khalid 1974), especially during warm episodes and with high SO_4-loads, when Fe is reduced and no longer able to prevent P-mobilization to the water layer.

The occurrence of these anaerobic reduction processes near water entry locations and canals at the west side of the nature reserve was also shown by higher alkalinity in the soil pore waters, as a consequence of internal alkalinity generation (Schindler *et* *al.* 1986; Roelofs 1991). In addition, hampered nitrification, increased dissimilatory NO_3-reduction to NH_4 (DNRA) and/or increased mineralization probably led to higher NH_4-concentrations in the soil pore waters of these locations (Lamers *et al.* 1998b). Sulfide did, however, hardly accumulate to levels that are known to be toxic to some freshwater macrophytes (above 10 μmol L^{-1}; Lamers *et al.* 2013), most likely due to efficient binding to Fe (Smolders & Roelofs 1996; van der Welle *et al.* 2006).

Low P-availability in floating rich fens

The soil characteristics of floating rich fens seem to lead to limited P-availability, as indicated by low concentrations of P-Olsen per unit volume. In these soils relatively little P was bound to Fe and Al, and relatively much to Ca and organic material compared to the sub-aquatic sediments. This was probably caused by the relatively low concentrations of Fe and Al in the floating rich fens, since Ca-concentrations were similar to those of the sub-aquatic sediments. Although the exact binding capacity of P to Ca is difficult to estimate for peat soils, this redox insensitive P-fraction will probably only become available after acidification (Golterman 1998). Furthermore, the organic P-fraction will only become available through the relatively slow processes of mineralization (Bridgham *et al.* 1998). Since this study showed that P-availability is mainly correlated with Fe- and Al-bound P, the small amount of Fe- and Al-bound P in rich fens presumably explains the low P-availability.

In addition, efficient P-filtering in the periphery of the nature reserve seems to be crucial for maintaining low P-availability in more isolated parts and thus for the existence and persistence of rich fens within wetlands that are influenced by P-inputs. To have this effect, wetland reserves should be large enough. There may, however, still be a risk that the border of the more eutrophic and less biodiverse periphery of wetlands slowly precedes towards more isolated parts, as it certainly did in the past in the studied nature reserve (van Wirdum 1991).

Conclusions

Our study showed that the periphery of large wetlands acts as an efficient P-filter through the precipitation of Fe-phosphates, biological uptake of P and dilution of surface water by rainwater (Fig. 5.10). These filtering mechanisms are presumably crucial for the existence and persistence of biodiverse rich fens with *S. scorpioides* in wetlands that receive eutrophic water from their surroundings, which is the case in many European and American wetlands that lie in the vicinity of agricultural areas. This filtering comes, however, at the expense of the ecological quality of the periphery and a reduction of nutrient inputs is therefore strongly recommended.

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6

Nitrogen or phosphorus limitation in rich fens? - Edaphic differences explain contrasting results in vegetation development after fertilization

6. Nitrogen or phosphorus limitation in rich fens? - Edaphic differences explain contrasting results in vegetation development after fertilization

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Abstract

Many rich fens are threatened by high nutrient inputs, but the literature is inconsistent with respect to the type of nutrient limitation and the influence of edaphic characteristics. We performed experiments with N- and P-fertilization in three endangered rich fen types: floating fen with *Scorpidium scorpioides*, non-floating fen with *Scorpidium cossonii*, floodplain fen with *Hamatocaulis vernicosus*. In addition, K-fertilization was performed in the floodplain fen.

The floodplain fen showed no response to P-addition, but N- and K-addition led to grass encroachment and decline of moss cover and species richness. In contrast, in the P-limited floating fen with *S. scorpioides,* P-addition led to increased vascular plant production at the expense of moss cover. *Scorpidium scorpioides*, however, also declined after N-addition, presumably due to NH_4-toxicity. The fen with *S. cossonii* took an intermediate position, with NP co-limitation.

These striking contrasts corresponded with edaphic differences. The N-limited fen showed low Ca:Fe-ratios and labile N-concentrations, and high concentrations of plant-available P and Fe-bound P. The P-limited fen showed an opposite pattern with high Ca:Fe-ratios and labile N-concentrations, and low P-concentrations. This implies that edaphic characteristics dictate the nature of nutrient limitation, and explain contrasting effects of N- and P-eutrophication in different fens.

Introduction

Rich fens are well-buffered and nutrient-poor peatland habitats that occur at a soil pH of 5.0 – 8.0 (e.g. Sjörs 1950). These types of peatlands are generally species-rich and are protected under the European Habitats Directive (transition mires and quaking bogs, type H7140). They generally comprise a large number of rare vascular plants and bryophytes. In Europe, most rich fens are dominated by endangered brownmosses such as *Scorpidium scorpioides* (Hedw.) Limpr. and *Scorpidium cossonii* (Schimp.) Hedenäs, *Hamatocaulis vernicosus* (Mitt.) Hedenäs.

In the past decades, many rich fens have been lost in NW-Europe (Kooijman 1992; JNCC 2007). Although part of this decline can be explained by natural succession towards less biodiverse, *Sphagnum*-dominated fens (Clapham 1940; Sjörs 1950), anthropogenic acidification and eutrophication have accelerated this succession (e.g. Gorham *et al.* 1987; Kooijman & Paulissen 2006). Inputs of nutrients may well increase aboveground biomass production of highly competitive vascular plants, which often results in reduced light availability for slow-growing plants (Kotowski & van Diggelen 2004) and a decrease of species richness (Grime 1979; Wheeler & Shaw 1991; Janssens *et al.* 1998). In yearly-mown rich fens, P and/or N are limiting biomass production (e.g. Boeye *et al.* 1997). The occurrence of P (co-)limitation is often caused by (1) low inputs of P-rich water, (2) chemical P-adsorption by Ca (Boyer and Wheeler 1989) or Fe (Zak et al. 2010) and/or (3) an annual mowing regime (Koerselman et al. 1990).

Water pollution (N and P) and increased atmospheric deposition of N may thus have profound effects on the vegetation development of rich fens (e.g. Kooijman 1993a; Verhoeven *et al.* 2011). Likewise, inundation with surface water may be problematic for rich fens when the water contains too high levels of nutrients. The response of rich fen types to different nutrient inputs is, however, not straightforward, and may depend on the type of nutrient limitation. Field studies, based on plant N:P-, N:K- and P:K-ratios in the aboveground vegetation, and fertilization experiments in rich fens showed that the growth of vascular plants may be limited by N (Vermeer 1986; Boeye *et al.* 1997; Olde Venterink & Vittoz 2008; Pawlikowski *et al.* 2013), P (Boyer & Wheeler 1989; Verhoeven & Schmitz 1991; Kooijman 1993b; Wassen *et al.* 1995; Beltman *et al.* 1996; Boeye *et al.* 1997; Rozbrojová *et al.* 2008; Pawlikowski *et al.* 2013), or a combination of both. The type of limitation does not seem to be caused by difference in management strategies, since all fens were yearly mown and unfertilized. Although most of these studies did not focus on the biogeochemical conditions in the soil, it seems reasonable to assume that soil conditions have a strong influence on the type of nutrient limitation (Olde Venterink et al. 2002b).

The aim of this study was therefore to explicitly link the effects of fertilization to the biogeochemical soil conditions in several rich fens, distinguishing between a floating fen soil, a non-floating fen soil and a clay-rich riverine soil. The main question addressed in this study was: Do effects of increased nutrient inputs in rich fens, especially of N and P, depend on edaphic conditions? We hypothesized that rich fen types with low P-availability in the soil and soil pore water encounter P-limitation. In this case, P-inputs are expected to lead to increased aboveground biomass, decreased light availability and concomitant changes in the composition of vascular plants and bryophytes. Rich fens with relatively low N-concentrations in the soil will, on the other hand, be N-limited, making them vulnerable for increased N-inputs.

Materials and methods

Experimental design

The fertilization experiment was performed in three summer-mown rich fens in the Dutch Ramsar area “National Park Weerribben-Wieden”: Stobbenribben (ST; 52°47’ N, 5°59’ E), Veldweg (VW; 52°42’ N, 6°07’ E) and Meppelerdieplanden (MDL; 52°41’ N, 6°09’ E). Plots in the ST-fen, where *S. scorpioides* dominated the moss layer, were classified as *Caricion davallianae* (*Scorpidium - Carex diandra* type). This area is a complex of quaking fens (van Wirdum 1991), containing about 0.8 m thick floating peat soils. Plots in the VW-fen were also classified as *Caricion davallianae* (*Scorpidium - Carex diandra* type), but the moss layer was dominated by *S. cossonii*. This fen consists of a fixed, non-floating peat soil of 0.5 – 1.0 m thick. The MDL-area, where *H. vernicosus* dominated the moss layer, was classified as *Caricion nigrae* (*Carex nigra - Agrostis canina* type). This area is a riverine fen meadow with a thin organic layer of 5 – 10 cm on top of fluvial clay. So, these fens contained more or less similar vegetation types despite clearly differing soils.

Five blocks of 4 x 4 m were selected in each fen, which were divided into four subplots of 2 x 2 m: a control, and three subplots with N-, P- and NP-fertilization. For N-fertilization, slow release granules of NH_4NO_3 (Osmocote 38+0+0, Scotts International B.V., Geldermalsen, the Netherlands) were applied at a dose of 600 kg N ha^{-1} $year^{-1}$. For P-fertilization, a dose of 70 kg P ha^{-1} jr^{-1} was added as slowly-soluble $CaHPO_4$. These doses equal the present fertilization intensity in Dutch agricultural grasslands (Schils and Snijders 2004; Lamers et al. 2006), and should thus be regarded as the maximal potential eutrophication which could occur in these fens. Similar loads have been recorded in riparian zones (Hefting et al. 2006) and in wetlands that are located within agricultural catchments (Verhoeven et al. 2006). These very high additions were used to be sure that nutrient limitations could be detected clearly within the relatively short fertilization period of three years. The addition of fertilizers started in April 2010, and was repeated in the early springs of 2011 and 2012. All subplots were mown yearly by the end of August.

After the first fertilization treatment, preliminary results of nutrient concentrations in the aboveground vegetation (results not shown) indicated that there may be K-limiting conditions in the MDL-area with *H. vernicosus*. To test this, two additional subplots (2 x 2 m) with K- and NPK-fertilization were added to all five blocks in the MDL-area. These subplots were only fertilized in the early springs of 2011 and

2012. Slow release granules of K_2SO_4 (Osmocote 0+0+42, Scotts International B.V., Geldermalsen, the Netherlands) were applied at a dose of 250 kg K ha^{-1} year^{-1}.

Sampling

Just before the first fertilization treatment in April 2010, a saw was used to collect soil samples of the uppermost 10 cm of each subplot. Apart from this, a small steel core of exactly 100 mL was used to sample the uppermost 10 cm of each soil for bulk density analysis. In both cases, the living moss-layer was excluded. All soil samples were transported in air-tight bags, and stored in the dark at 4 °C until further analysis. Furthermore, soil pore water samples were anaerobically collected at a depth of 10 cm by connecting vacuumed plastic syringes (50 mL) to permanent soil moisture samplers (Rhizons SMS 10 cm, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). Soil pore waters were also collected in May 2012, which was one month after the third fertilization treatment. The first 10 mL of soil pore water was discarded to exclude stagnant sampler water.

For each subplot, peak standing aboveground biomass was collected in August 2012, which was the third season after the start of the experiment. This was done in quadrats of 25 x 25 cm in the middle of each subplot. In addition, light availability (photosynthetically active radiation, PAR; 400 – 700 nm) at the soil surface was determined in August 2012, before the vegetation was cropped. PAR-measurements were performed in triplicate using a small line-sensor at the soil surface and above the vegetation, and mean values were calculated for each subplot (Veer & Kooijman 1997). Measurements were carried out on cloudless days around noon. Light availability at the soil surface was calculated as a fraction of the light intensity above the vegetation.

Next, plant species composition was recorded for each subplot of 2 x 2 m in August 2012 (before mowing). All vascular plant and bryophyte species were listed, and cover values were estimated as percentages. To examine the effect of light availability on the cover of bryophytes, bryophytes were again recorded in December 2012 (after mowing). Nomenclature of vascular plants was according to van der Meijden (2005), while Siebel & During (2006) was used for the nomenclature of bryophytes. The covers of vascular plants and mosses in August were used to calculate Shannon diversity indices, in which both the number and the evenness of species are included (Shannon 1948).

Chemical analyses

The pH-values of soil pore water samples were measured, and alkalinities were determined by titration to pH 4.2, using 0.01M HCl. Subsequently, samples were divided into two subsamples. To avoid metal precipitation, 1% of concentrated HNO_3 was added to one of these subsamples, and both were stored in the dark at -24 °C until further analysis. Total concentrations of soluble Ca, Fe, Al and P were measured in acidified subsamples by ICP-OES (IRIS Intrepid II, Thermo Electron Corporation, Franklin, USA). Concentrations of NH_4, NO_3, o-PO_4 and K were analyzed colorimetrically in non-acidified subsamples with continuous flow auto-analyzer systems (Bran + Luebbe, Norderstedt, Germany).

To determine dry weights and gravimetric moisture contents, soil samples were dried at 70 °C until constant weight was reached, and bulk densities were calculated. Since bulk densities differed significantly between the clay-rich MDL-soils and the peaty ST- and VW-soils, soil results are reported per L wet soil instead of per unit mass, because plant production and nutrient availability for roots are mainly based on the amount of nutrients in a specific root-volume. Organic matter content was determined by loss-on-ignition after heating the samples for four hours at 550 °C. The remaining soil was quickly frozen in liquid nitrogen and freeze-dried. After grinding, soil samples were digested by microwave (Anton Paar, Graz, Austria) destruction using HNO_3 (65%) and HCl (37%) (Bettinelli *et al.* 1989). Digestates were analyzed by ICP-OES (Optima 3000 XL, PerkinElmer, Waltham, USA) to determine Ca-, K-, Fe-, Al- and P-concentrations. N-concentrations were determined with a CNS analyzer (Vario EL, Elementar, Hanau, Germany). Sequential extractions of Golterman (1996) were used to determine concentrations of Fe- and Al-bound P (P_{Fe}), Ca-bound P (P_{Ca}) and organic P (P_{org}). All P-fractions were measured by ICP-OES. Furthermore, plant available P-fractions were determined by Olsen extraction (Olsen *et al.* 1954) and concentrations of labile NH_4, NO_3 and K were analyzed colorimetrically with continuous flow auto-analyzer in NaCl extracts (35 g of fresh soil was shaken for one hour in 100 mL of 0.2 M NaCl).

Aboveground vegetation samples were dried for 72 hours at 70 °C to determine dry weights. Samples from 2012 were ground to determine total P- and K-concentrations by microwave digestion, and total N-concentrations by CNS-analysis, as described above. N:P-, N:K- and K:P-ratios in the shoots of vascular plants were calculated to determine which of the three nutrients may limit plant growth, 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

Statistical analyses were performed with SPSS for Windows (SPSS 20.0.0, IBM, Armonk, NY). Covers of vascular plants were combined into plant functional groups, i.e. grasses, *Phragmites australis* (Cav.) Steud., *Carex* spp., *Juncus* spp. and other species. For all measured variables, normality of distributions was tested with QQ-plots and a Kolmogorov-Smirnov test. All variables that were not normally distributed were log(x+1)-transformed to improve their fit to a normal distribution. Since a split-plot design has been used, linear mixed models ($P \leq 0.05$) with blocks as random factor (Heck *et al.* 2010) were performed to determine the response of measured variables to the fixed factors location (floating ST-fen with *S. scorpioides*, non-floating VW-fen with *S. cossonii* and riverine MDL-fen with *H. vernicosus*) and treatment (control, N-, P- and NP-fertilization). Differences between locations and fertilization treatments, whenever significant in the linear mixed model, were further examined by comparing their estimated marginal means in a LSD post-hoc test ($P \leq 0.05$). Initial differences in soil characteristics between the three locations were analyzed in a similar way, but with only location as fixed factor. Since each specific moss species was only present at one of the locations, results should be interpreted as differences between these locations, and not as differences between the different moss species or habitats.

The effects of two years of K-fertilization in the MDL-fen with *H. vernicosus* were tested separately. Linear mixed models, with blocks as random factor, and LSD post-hoc tests ($P \leq 0.05$) were used to determine the response to K- and NPK-fertilization compared to controls in the MDL-fen.

Results

Initial soil conditions in the fen types

The initial pH of soil pore water was higher for the floating ST-fen with *S. scorpioides* than for the other two fen types, 6.8 versus 6.1 (Table 6.1). Alkalinity, Ca- and K-concentrations were also higher in the ST-fen, with initial alkalinities around 3.4 versus 1.8 $\text{mmol}_c\ L^{-1}$, Ca-concentrations of about 1750 versus 850 $\mu\text{mol}\ L^{-1}$, and K-concentrations around 70 versus 7 $\mu\text{mol}\ L^{-1}$. In contrast, initial Al- and Fe-concentrations were highest in the soil pore waters of the MDL-fen with *H. vernicosus*, while lowest Al- and Fe-concentrations were found in the ST-fen with *S. scorpioides*. Although initial o-PO_4 concentrations were equally low in all fens, around 0.1 $\mu\text{mol}\ L^{-1}$, total P-concentrations in the soil pore water were also significantly higher in the MDL-fen with *H. vernicosus* than in both other fens. NO_3- and NH_4-concentrations were low in all fens, showing mean concentrations of about 8 and 4 $\mu\text{mol}\ L^{-1}$, respectively.

In addition to differences in soil pore water quality, other soil properties also differed between the three fens studied. The riverine MDL-fen with *H. vernicosus*, which was characterized by a clay-dominated soil below a thin organic layer of around 10 cm, had a significantly higher dry and wet bulk density (Table 6.1). Only 50% of the total dry weight consisted of organic material in the topsoil of this MDL-fen, while the soil of the VW- and ST-fens consisted for about 90% of organic material. The clay-rich topsoils of the MDL-fen contained significantly more total Ca, and especially more Al and Fe. Lowest total Al- and Fe-concentrations were found in the floating ST-fen. This led to total Ca:Fe-ratios of 0.5, 10 and 30 for the riverine MDL-fen with *H. vernicosus*, the non-floating fen with *S. cossonii* and the floating fen with *S. scorpioides*.

Also, total P-concentrations were 10 times higher in the soil of the MDL-fen than in the other soils, with lowest concentrations occurring in the floating ST-fen with *S. scorpioides* (Table 6.1). Concentrations of Fe- and Al-bound P (P_{Fe}), Ca-bound P (P_{Ca})

Table 6.1. Initial soil pore water and soil characteristics in the three rich fens. ST = floating fen in Stobbenribben with *Scorpidium scorpioides*, VW = non-floating fen in Veldweg with *Scorpidium cossonii*, MDL = riverine fen in Meppelerdieplanden with *Hamatocaulis vernicosus*, ws = field-wet soil.

	ST *S. scorpioides*	**VW** *S. cossonii*	**MDL** *H. vernicosus*
Soil pore water			
pH	6.81 (0.30)^{B}	6.15 (0.21)^{A}	6.04 (0.21)^{A}
alkalinity (mmol$_c$ L^{-1})	3.40 (0.72)^{B}	1.81 (0.50)^{A}	1.76 (0.60)^{A}
Ca (μmol L^{-1})	1660 (383)^{B}	943 (334)^{A}	770 (184)^{A}
K (μmol L^{-1})	71.5 (26.6)^{B}	9.9 (13.2)^{A}	3.1 (3.4)^{A}
Al (μmol L^{-1})	0.3 (0.3)^{A}	0.8 (0.6)^{B}	25.3 (5.1)^{C}
Fe (μmol L^{-1})	4.4 (2.9)^{A}	22.0 (17.6)^{B}	543.9 (218.8)^{C}
o-PO$_4$ (μmol L^{-1})	0.10 (0.06)^{A}	0.10 (0.11)^{A}	0.11 (0.06)^{A}
total P (μmol L^{-1})	0.84 (0.41)^{A}	0.65 (0.38)^{A}	3.35 (1.47)^{B}
NO$_3$ (μmol L^{-1})	8.2 (1.4)^{A}	8.5 (2.5)^{A}	7.4 (3.9)^{A}
NH$_4$ (μmol L^{-1})	3.6 (0.7)^{A}	4.4 (2.3)^{A}	6.8 (3.7)^{A}
inorganic N (μmol L^{-1})	11.8 (2.1)^{A}	12.8 (3.8)^{A}	13.3 (4.9)^{A}
General soil characteristics			
dry bulk density (g L^{-1})	55 (7)^{A}	60 (10)^{A}	189 (22)^{B}
wet bulk density (g L^{-1})	646 (485)^{A}	669 (60)^{A}	761 (62)^{B}
organic material (%)	91 (1)^{B}	89 (3)^{B}	47 (6)^{A}
Total soil concentrations			
Ca$_t$ (mmol L^{-1} ws)	43.8 (3.7)^{A}	43.4 (6.9)^{A}	63.1 (8.2)^{B}
K$_t$ (mmol L^{-1} ws)	1.5 (0.3)^{A}	1.7 (0.5)^{A}	11.6 (2.4)^{B}
Al$_t$ (mmol L^{-1} ws)	1.6 (0.3)^{A}	9.0 (7.4)^{B}	242.6 (55.1)^{C}
Fe$_t$ (mmol L^{-1} ws)	1.7 (0.6)^{A}	6.3 (4.4)^{B}	116.9 (36.6)^{C}
N$_t$ (mmol L^{-1} ws)	71 (17)^{A}	86 (23)^{A}	236 (25)^{B}
P$_t$ (mmol L^{-1} ws)	1.3 (0.1)^{A}	2.6 (0.8)^{B}	17.0 (2.4)^{C}
N$_t$:P$_t$ (mol mol^{-1})	48.7 (10.4) ^{B}	37.4 (14.0) ^{B}	13.7 (1.8)^{A}
Fe$_t$:P$_t$ (mol mol^{-1})	1.3 (0.5)^{A}	2.2 (1.1)^{A}	6.8 (1.5)^{B}
Ca$_t$:P$_t$ (mol mol^{-1})	32.5 (5.1)^{C}	17.2 (3.6)^{B}	3.7 (0.1)^{A}
Ca$_t$:Fe$_t$ (mol mol^{-1})	30.0 (14.6)^{C}	9.7 (5.3)^{B}	0.6 (0.1)^{A}
P-fractions in the soil			
P$_{org}$ (mmol L^{-1} ws)	1.07 (0.47)^{A}	1.90 (1.56)^{A}	8.78 (1.94)^{B}
P$_{Ca}$ (mmol L^{-1} ws)	0.12 (0.05)^{A}	0.44 (0.30)^{A}	4.88 (2.87)^{B}
P$_{Fe}$ (mmol L^{-1} ws)	0.12 (0.05)^{A}	0.24 (0.14)^{A}	3.29 (0.34)^{B}
P-Olsen (mmol L^{-1} ws)	0.11 (0.07)^{A}	0.15 (0.10)^{A}	0.42 (0.06)^{B}
P$_{org}$:P$_t$ (mol mol^{-1})	80 (1)^{B}	72 (7)^{B}	53 (5)^{A}
P$_{Ca}$:P$_t$ (mol mol^{-1})	10 (1)^{A}	17 (7)^{A}	28 (8)^{B}
P$_{Fe}$:P$_t$ (mol mol^{-1})	10 (2)^{A}	11 (3)^{A}	19 (3)^{B}
K and N-concentrations in NaCl-extracts			
K (mmol L^{-1} ws)	0.313 (0.085)^{B}	0.060 (0.095)^{A}	0.013 (0.021)^{A}
NO$_3$ (mmol L^{-1} ws)	0.031 (0.028)^{B}	0.008 (0.004)^{A}	0.011 (0.004)^{A}
NH$_4$ (mmol L^{-1} ws)	0.123 (0.031)^{C}	0.075 (0.023)^{B}	0.031 (0.017)^{A}
inorganic N (mmol L^{-1} ws)	0.154 (0.047)^{C}	0.083 (0.026)^{B}	0.042 (0.019)^{A}

Mean values and standard deviations (n = 20). Different letters indicate significant differences between fens ($P \leq 0.05$).

and organic P (P_{org}) were also significantly higher in the MDL-fen with *H. vernicosus,* as well as the concentrations of plant available P. Concentrations of P-Olsen were around 400 μmol L^{-1} in the MDL-fen, while concentrations of only 100 – 150 μmol L^{-1} were found in the VW- and ST-fen. In the MDL-fen a relatively large part of total P consisted of inorganic P_{Fe} and P_{Ca} (about 19 and 28%), while only 53% of the total P was present as organic P. Organic P did, in contrast, contribute to 70-80% of the total P in the ST- and VW-fens, where both P_{Fe} and P_{Ca} only contributed to only10 – 15% of total P.

Total N- and K-concentrations were also significantly higher in the soil of the MDL-fen than in the other soils, with concentrations that were about 3 and 7 times higher, respectively (Table 6.1). Despite these higher total N- and K-concentrations in the MDL-fen, concentrations of labile NO_3, NH_4 and K were lowest in this MDL-fen with *H. vernicosus* and highest in the floating ST-fen with *S. scorpioides*.

Effects of N- and P-fertilization

Soil pore water

In all fens, N-fertilization led to increased concentrations of soluble inorganic N (NO_3 + NH_4) in the soil pore water compared to the controls (Table 6.2). There were, however, differences in NO_3- and NH_4-responses of the three fens, as indicated by the interaction effects of location and fertilization treatment for NO_3 and NH_4 (Fig. 6.1). In the riverine MDL-fen with *H. vernicosus,* NO_3-concentrations increased sharply after N-fertilization to levels of about 180 μmol L^{-1}, while NH_4-concentrations remained low (2 – 10 μmol L^{-1}). In contrast, N-fertilization led to a sharp increase of NH_4-concentrations to levels of around 500 μmol L^{-1} in the ST- and VW-fens with *S. scorpioides* and *S. cossonii,* while NO_3-concentrations remained low in these fens with concentrations of 0.5 – 5 μmol L^{-1}. It was also on these two locations that N-fertilization resulted in significantly higher alkalinities compared to the controls. Combined fertilization of N and P led to similar results for alkalinity, and for NO_3- and NH_4-concentrations in the soil pore water of all fens.

Surprisingly, three years of P-fertilization did not lead to increased o-PO_4 and total soluble P-concentrations in the soil pore water of the MDL-fen with *H. vernicosus* (Fig. 6.1, Table 6.2). Here, concentrations of o-PO_4 remained as low as in control subplots, at 0.1 – 0.6 μmol L^{-1}, while much higher concentrations of 3 – 5 μmol L^{-1} were found in the P-fertilized subplots of the ST- and VW-fens with *S. scorpioides* and *S. cossonii*. Combined fertilization of N and P had, however, no effect on the concentrations of o-PO_4 and total soluble P, not even in the soil pore water of the ST- and VW-fens.

Table 6.2. Effect of location (Loc) and fertilization treatment (Treat) on various chemical variables in the soil pore water and aboveground vegetation in 2012, after three years of N-, P- or NP-fertilization. ST = floating fen in Stobbenribben with *Scorpidium scorpioides*, VW = non-floating fen in Veldweg with *Scorpidium cossonii*, MDL = riverine fen in Meppelerdieplanden with *Hamatocaulis vernicosus*.

	Loc	Treat	Loc x Treat	ST	VW	MDL	con-trol	N	P	NP
Soil pore water										
pH	38.23**	0.27	1.22	b	a	a	a	a	a	a
Alkalinity	49.65**	3.73*	1.54	c	b	a	a	b	a	b
Ca	83.76**	0.69	1.54	c	b	a	a	a	a	a
K	15.62**	4.02*	5.12**	b	a	a	b	b	a	b
Al	78.18**	0.07	1.21	a	b	c	a	a	a	a
Fe	17.94**	0.42	1.50	a	b	c	a	a	a	a
o-PO_4	2.74	15.43**	6.93**	a	a	a	a	a	b	a
total P	2.10	22.54**	5.77**	a	a	a	a	a	b	a
NO_3	3.92*	4.52**	3.23*	a	a	b	ab	c	a	bc
NH_4	40.60**	59.38**	11.95**	b	b	a	a	b	a	b
inorganic N	10.68**	53.23**	3.22*	b	b	a	a	b	a	b
NO_3:NH_4	3.94*	3.29*	3.21*	a	a	b	ab	c	a	bc
Chemical composition aboveground vegetation										
K	40.43**	1.57	1.88	b	a	a	a	a	a	a
N	16.72**	100.38**	0.99	a	a	b	a	b	a	b
P	25.51**	148.03**	2.31	a	a	b	a	a	b	b
N:P	19.93**	270.62**	4.89**	c	b	a	c	d	a	b
N:K	95.06**	66.58**	6.55**	a	b	c	a	b	a	b
K:P	91.78**	150.71**	7.05**	c	b	a	b	b	a	a
Vegetation characteristics										
Aboveground biomass	4.32*	47.89**	3.64**	a	ab	b	a	b	b	c
Light intensity at soil surface	13.74**	119.31**	19.08**	b	a	a	d	b	c	a
Species richness	26.25**	60.37**	1.19	a	b	c	b	a	b	a
Shannon-index	20.93**	42.08**	1.23	a	b	b	b	a	b	a
Cover vascular plants	3.75	27.40**	5.98**	a	a	a	a	b	b	c
Cover grasses	16.90**	3.89*	1.41	a	b	c	a	ab	b	b
Cover *Phragmites*	23.30**	1.32	6.07**	b	a	a	a	a	a	a
Cover *Carex* spp.	2.50	6.36**	3.10*	a	a	a	a	b	a	b
Cover *Juncus* spp.	1.52	13.56**	2.19	a	a	a	a	b	a	b
Cover other plants	37.26**	16.97**	0.26	a	b	c	bc	a	c	b
Cover mosses in summer	2.74	116.36**	3.38*	a	a	a	d	b	c	a
Cover *Scor./Ham.* in summer	12.59**	161.20**	23.89**	a	a	b	d	b	c	a
Cover *Call.* in summer	3.95*	29.06**	2.47	a	ab	b	b	b	c	a
Cover *Scor./Ham.* in winter	1.06	81.10**	14.19**	a	a	a	c	b	b	a
Cover *Call.* in winter	19.20**	27.25**	8.95**	a	b	b	a	b	c	b

F-ratios with their level of significance are given (n = 5; * $P \leq 0.05$ and ** $P \leq 0.01$). Different letters indicate significant differences between locations and fertilization treatment ($P \leq 0.05$).

Nutrient concentrations in aboveground vascular vegetation

In the control subplots, the aboveground vascular vegetation in ST- and VW-fens contained less N and P than in the MDL-fen (Table 6.2). Plant N:P-ratios of 14 – 17 g g^{-1} seem to indicate co-limiting conditions of N and P for biomass production in the non-floating VW-fen with *S. cossonii,* while plant N:P-ratios of about 22 g g^{-1} were indicative for P-limiting conditions in the floating ST-fen with *S. scorpioides* (Fig. 6.2). In the riverine MDL-fen with *H. vernicosus,* where the aboveground vascular vegetation contained significantly more N and especially P, plant N:P-ratios of about 9 g g^{-1} indicated N-limiting conditions. Not only N, but also K seemed to be co-limiting in this fen, as indicated by plant N:K-ratios of 2.8 g g^{-1} and K:P-ratios of 3.2 g g^{-1}.

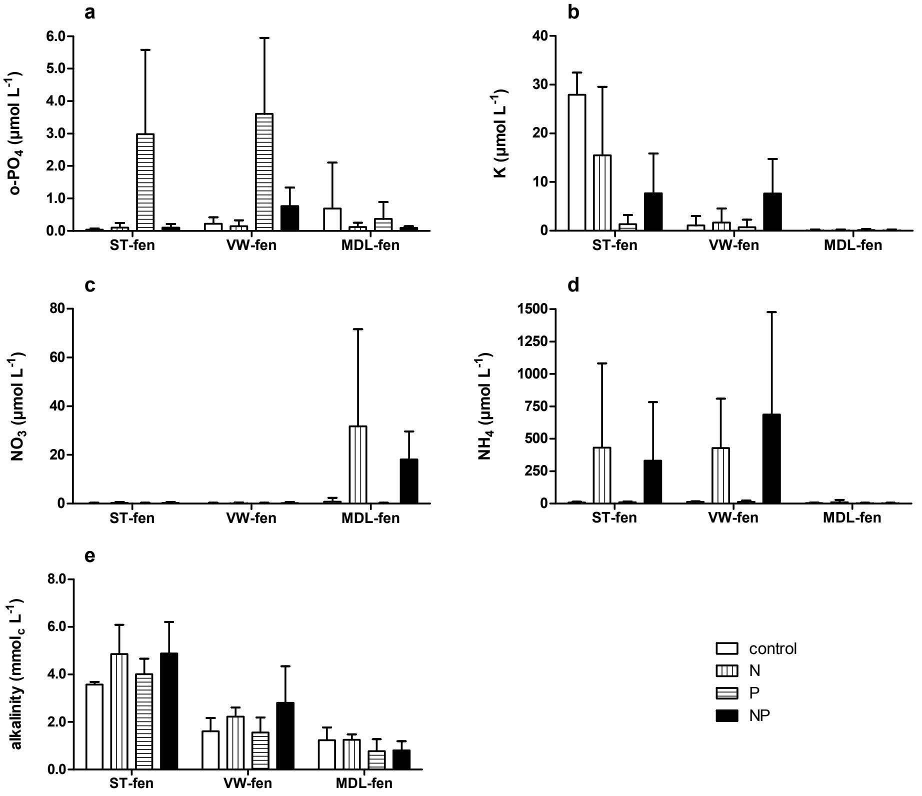

Fig. 6.1. Concentrations of o-PO_4 (a), K (b), NO_3 (c) and NH_4 (d), and alkalinity in the soil pore water of the three fens after three years of N-, P- and NP-fertilization. Sample means are given and error bars indicate standard deviations (n = 5). ST = floating fen in Stobbenribben with *Scorpidium scorpioides,* VW = non-floating fen in Veldweg with *Scorpidium cossonii,* MDL = riverine fen in Meppelerdieplanden with *Hamatocaulis. vernicosus.* Statistical information is provided in Table 6.2.

Three years of N- and NP-fertilization led to significantly increased N-concentrations and N:K-ratios in the aboveground vegetation of all fens studied (Fig. 6.2, Table 6.2). Similarly, P- and NP-fertilization led to significantly increased P-concentrations and decreased K:P-ratios in the aboveground vegetation of the three fens. Furthermore, N-fertilization resulted in a significant increase of plant N:P-ratios in all studied fens, while NP- and especially P-fertilization resulted in significantly lower plant N:P-ratios.

Biomass production and light availability

In control subplots without fertilization, aboveground biomass was lowest in the floating ST-fen with *S. scorpioides* (150 – 250 g m^{-2}) and highest in the riverine MDL-fen with *H. vernicosus* (350 – 450 g m^{-2}). Fertilization clearly led to an increase of the aboveground biomass and decreased light availability at the soil surface, but the three fens responded differently upon N and P-addition (Fig. 6.3, Table 6.2). Three years of N-fertilization led to increased biomass and decreased light availability in the MDL-fen with *H. vernicosus* and the VW-fen with *S. cossonii*, while no significant effects of N-fertilization were found in the ST-fen with *S. scorpioides*. In contrast, P-fertilization led to increased biomass and reduced light availability in the VW- and ST-fens, while no effects on biomass productivity and light availability were found in P-fertilized subplots of the MDL-fen. In all fens, three years of combined NP-fertilization resulted in the largest biomass production and the lowest light availability at the soil surface.

Vegetation composition

Fertilization also affected species composition of different fen types (Fig. 6.4, Table 6.2). In the riverine MDL-fen, the cover of grasses, such as *Calamagrostis canescens* (Weber) Roth, *Phalaris arundinacea* L. and *Glyceria fluitans* (L.) R.Br., increased significantly in N- and NP-fertilized subplots, while other characteristic fen species, such as *Myosotis laxa* Lehm., *Pedicularis palustris* L. and *Juncus filiformis* L., disappeared entirely. Also the bryophyte composition changed. After three years of fertilization, *H. vernicosus* and *C. cuspidata* had almost disappeared in N- and NP-fertilized subplots in August, and in December (after mowing) only *C. cuspidata* was found. The biodiversity, determined as the species richness and the Shannon diversity index, decreased in these subplots (Fig. 6.5). In contrast, the vascular plant and bryophyte composition did not change significantly in MDL-subplots that only received P.

In the non-floating VW-fen, both N- and P-fertilization resulted in an increase of the total cover of vascular plants from about 70% in control plots to 95 – 100% in fertilized subplots (Fig. 6.4, Table 6.2). Three years of N- or NP-fertilization led to encroachment of grasses and *Carex* spp., such as *Agrostis canina* L., *Calamagrostis canescens* and *Carex elata* All., while other characteristic fen species, such as *Myosotis laxa*, *Pedicularis palustris* and *Utricularia intermedia* Hayne, disappeared. In addition, the cover of *S. cossonii* declined, while the cover of *C. cuspidata* increased significantly in both N- and NP-fertilized subplots. The increase of *C. cuspidata* was only found in December (after mowing), and not in August. As a result of these changes in species composition, the biodiversity decreased significantly in the N- and NP-fertilized subplots of the VW-fen (Fig. 6.5, Table 6.2). In contrast, species richness and Shannon diversity did not change in VW-subplots that were only fertilized with P. Although the cover of the grass *Agrostis canina* increased significantly after three years of P-fertilization, this had no effect yet on the overall species composition of vascular plants (Fig. 6.4, Table 6.2). The bryophyte composition was, however, significantly changed; almost all *S. cossonii* had disappeared and the cover of *C. cuspidata* had sharply increased, especially in December (after mowing).

In the floating ST-fen with *S. scorpioides*, three years of fertilization led to a significantly increased cover of vascular plants from about 70% in control plots to 100% in P- and NP-fertilized subplots, which was mainly caused by the increased abundance of *Carex elata* (Fig. 6.4, Table 6.2). In contrast, the cover of *S. scorpioides* sharply decreased after three years of P- or NP-fertilization. They had partly been replaced by *C. cuspidata*, which showed high cover especially in P-fertilized subplots during the winter (after mowing). Despite the increased cover of *Carex elata* and the decline of *S. scorpioides* in P-fertilized subplots, species richness and Shannon's diversity index had not changed significantly (Fig. 6.5, Table 6.2). Species richness and the diversity index had, however, decreased in the NP-fertilized subplots, due to the disappearance of characteristic fen species such as *Carex diandra* Schrank, *Liparis loeselii* (L.) Rich. and *Pedicularis palustris*. A similar decrease in species diversity and in the cover of endangered vascular plants and mosses was found in subplots that had only been fertilized with N (Figs. 6.4 & 6.5, Table 6.2).

The effects of K-fertilization in the riverine MDL-fen with *H. vernicosus*

K-fertilization in the riverine MDL-fen with *H. vernicosus* had no significant effect on soil pore water chemistry, not even on K-concentrations (Table 6.3). Also, soil pore water characteristics did not differ between NPK- and NP-fertilized subplots.

The aboveground biomass was, however, significantly larger in K-fertilized than in the control subplots of the MDL-fen, with values of about 500 versus 300 g m^{-2} (Table 6.3). This led to a significantly lower light availability in the K-fertilized subplots. In addition, K-fertilization resulted in an increase of the total vascular plants cover, especially of *Carex disticha* Huds., and a decrease of the total moss cover, especially of *H. vernicosus*. Overall species richness decreased significantly in these subplots, since some characteristic species, such as *Pedicularis palustris* and *Juncus filiformis*, had disappeared after two years of K-fertilization.

NPK-fertilization resulted in even more aboveground biomass than the K-treatment. Levels of about 1000 g m^{-2} were comparable to NP-fertilized subplots in this fen. The effect of NPK-fertilization on the species composition of vascular plants and mosses equaled the effect of NP-fertilization: less species, almost no mosses and a higher cover of grasses, especially of *Calamagrostis canescens* and *Phalaris arundinacea*.

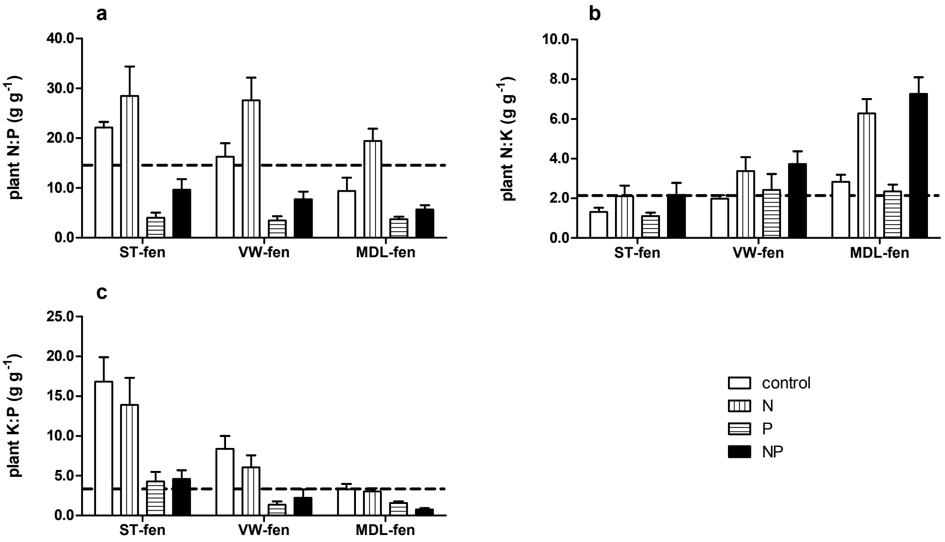

Fig. 6.2. N:P (a), N:K (b) and K:P (c) ratios in the aboveground tissue of the vascular vegetation after three years of N-, P- and NP-fertilization. Sample means are given and error bars indicate standard deviations (n = 5). The dotted lines indicate thresholds for nutrient limitation (Olde Venterink *et al.* 2003), 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, K-limitation or co-limitation of NK if K:P < 3.4 and N:K > 2.1. ST = floating fen in Stobbenribben with *Scorpidium scorpioides,* VW = non-floating fen in Veldweg with *Scorpidium cossonii,* MDL = riverine fen in Meppelerdieplanden with *Hamatocaulis vernicosus*. Statistical information is provided in Table 6.2.

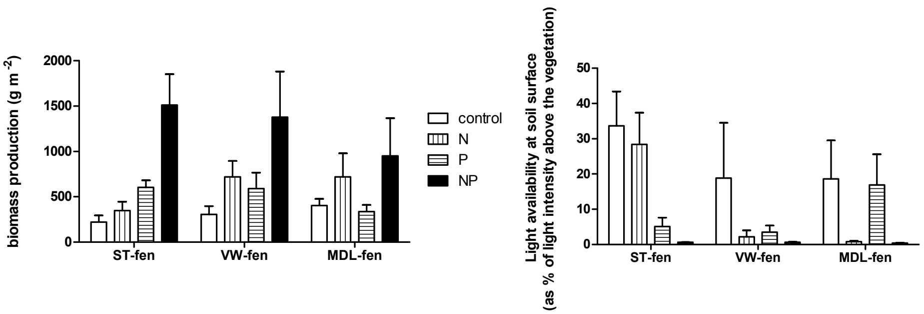

Fig. 6.3. Aboveground biomass production (left) and relative light (PAR) intensity at soil surface (right) after three years of N-, P- or NP-fertilization. Sample means are given and error bars indicate standard deviations (n = 5). ST = floating fen in Stobbenribben with *Scorpidium scorpioides,* VW = non-floating fen in Veldweg with *Scorpidium cossonii,* MDL = riverine fen in Meppelerdieplanden with *Hamatocaulis vernicosus.* Statistical information can be found in Table 6.2.

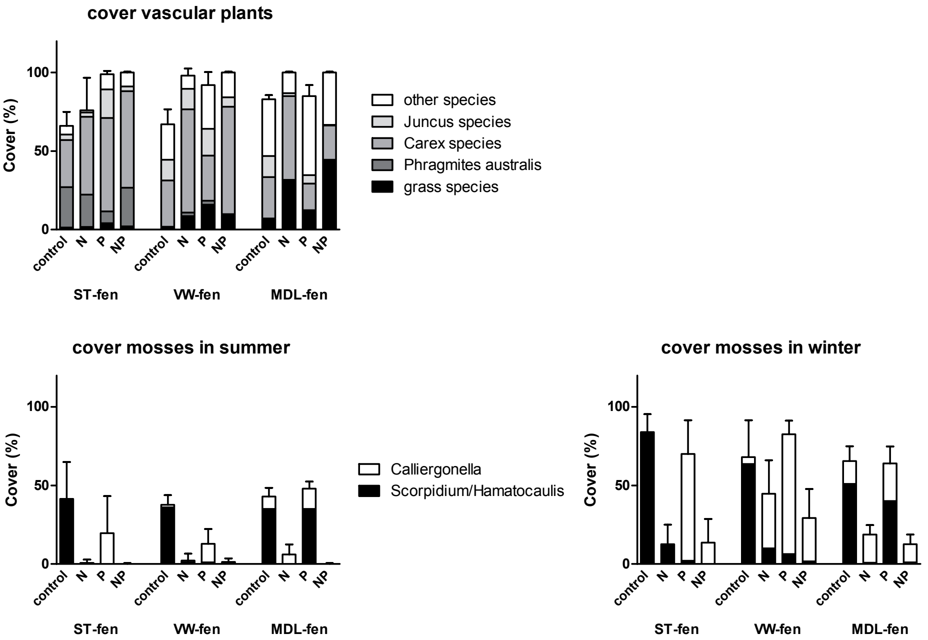

Fig. 6.4. Cover of vascular plants in August (above) and mosses in August (below, left) and December (below, right) after three years of N-, P- or NP-fertilization. The vascular plants are combined into plant functional groups. Sample means are given and error bars indicate standard deviations (n = 5). ST = floating fen in Stobbenribben with *Scorpidium scorpioides*, VW = non-floating fen in Veldweg with *Scorpidium cossonii*, MDL = riverine fen in Meppelerdieplanden with *Hamatocaulis vernicosus*. Statistical information is provided in Table 6.2.

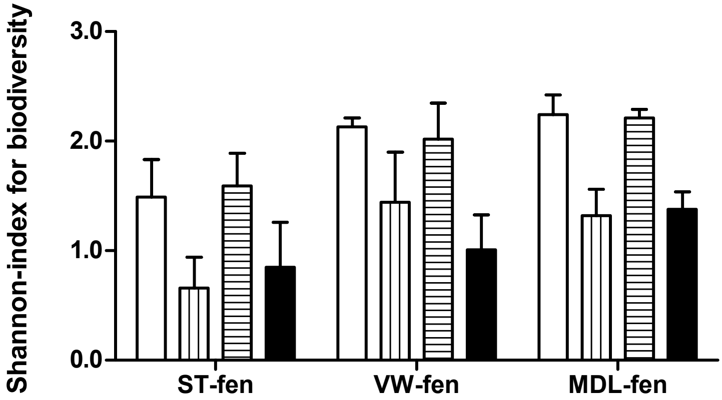

Fig. 6.5. Shannon diversity index after three years of N-, P- or NP-fertilization. Sample means are given and error bars indicate standard deviations (n = 5). ST = floating fen in Stobbenribben with *Scorpidium scorpioides*, VW = non-floating fen in Veldweg with *Scorpidium cossonii*, MDL = riverine fen in Meppelerdieplanden with *Hamatocaulis vernicosus*. Statistical information is provided in Table 6.2.

Table 6.3 Effect of fertilization treatment in the MDL-fen with *Hamatocaulis vernicosus* on chemical variables in the soil pore water and aboveground vegetation in 2012, after two years of K-, NP- or NPK-fertilization.

	control	**K-fertilization**	**NP-fertilization**	**NPK-fertilization**
Soil pore water				
K (µmol L^{-1})	0.1 (0.1)^{A}	4.8 (10.0)^{A}	0.1 (0.1)^{A}	3.9 (5.3)^{A}
Vegetation characteristics				
Aboveground biomass (g m^{-2})	316 (72)^{A}	503 (63)^{B}	990 (391)^{C}	1341 (494)^{C}
Light intensity at soil surface (%)	18.6 (10.9)^{C}	8.6 (3.0)^{B}	0.4 (0.1)^{A}	0.4 (0.1)^{A}
Species richness (per 4 m^{2})	17.2 (2.4)^{C}	13.0 (2.4)^{B}	10.6 (1.1)^{AB}	8.4 (1.1)^{A}
Shannon-index	2.24 (0.18)^{B}	2.03 (0.23)^{B}	1.38 (0.16)^{A}	1.25 (0.36)^{A}
Cover vascular plants (%)	82 (3)^{A}	92 (3)^{B}	100 (0)^{C}	100 (0)^{C}
Cover grasses (%)	7 (5)^{A}	13 (12)^{A}	45 (24)^{B}	55 (29)^{B}
Cover *Carex* spp. (%)	26 (12)^{A}	45 (19)^{A}	22 (24)^{A}	23 (28)^{A}
Cover *Juncus* spp. (%)	13 (9)^{B}	8 (7)^{B}	0 (0)^{A}	0 (0)^{A}
Cover other plants (%)	36 (11)^{A}	26 (11)^{A}	33 (18)^{A}	22 (20)^{A}
Cover mosses in summer (%)	44 (6)^{C}	30 (7)^{B}	0 (0)^{A}	0 (0)^{A}
Cover *H. vernicosus* in summer (%)	35 (4)^{C}	18 (10)^{B}	0 (0)^{A}	0 (0)^{A}
Cover *C. cuspidata* in summer (%)	9 (7)^{B}	12 (5)^{B}	0 (0)^{A}	0 (0)^{A}
Cover *H. vernicosus* in winter (%)	51 (4)^{C}	24 (7)^{B}	1 (1)^{A}	1 (1)^{A}
Cover *C. cuspidata* in winter (%)	15 (7)^{A}	15 (4)^{A}	12 (4)^{A}	11 (3)^{A}

Mean values and standard deviations (n = 5). Different letters indicate significant differences between fertilization treatments ($P \leq 0.05$).

Discussion

Different types of nutrient limitation

Our study showed profound differences in nutrient limitation among the three fens studied. The floating ST-fen with *S. scorpioides* was clearly P-limited, since aboveground biomass production only increased after P- and NP-addition, and not after N-addition. P-limited conditions are confirmed by high plant N:P-ratios of about 22 g g^{-1} in the control subplots of the ST-fen (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003; Güsewell 2004). These results are consistent with previous fertilization experiments (Verhoeven & Schmitz 1991; Kooijman 1993b; Boeye *et al.* 1997) and plant nutritional studies (Kooijman & Hedenäs 2009; Kooijman 2012) in rich fens with *S. scorpioides*.

In contrast to the floating ST-fen, the aboveground biomass production in the non-floating VW-fen with *S. cossonii* not only increased after P-addition, but also after N-addition. This co-limitation of N and P was supported by plant N:P-ratios of 14 – 17 g g^{-1} in the control subplots (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003; Güsewell 2004).

Finally, the riverine MDL-fen with *H. vernicosus* was co-limited by N and K, as indicated by increased biomass production after N- or K-addition, and the lack of response to P-addition. This was supported by relatively low N:P-, high N:K- and low K:P-ratios in the aboveground vegetation of the control MDL-subplots (Olde Venterink *et al.* 2003). The occurrence of N-limitation in the MDL-fen was consistent with results of Olde Venterink & Vittoz (2008), who found N-limiting conditions in the last remnant of *H. vernicosus* and *Saxifraga hirculus* L. in Switzerland. Co-limitation of K at locations with *H. vernicosus* has not been described previously, but has been found in meadows with *Cirsio–Molinietum* (de Mars *et al.* 1996) and *Calthion palustris* vegetation (van Duren *et al.* 1997; van Duren & Pegtel 2000), especially when these meadows had been drained or hay cropped for a long time. In addition, Loeb *et al.* (2009) found co-limitation of N and K in mown river floodplains with *Arrhenatherion* grasslands containing *Fritillaria meleagris* L.

The nature of nutrient limitation is determined by edaphic differences between fen types

The striking differences in response to fertilization among the three fen types can be explained by clear differences in the initial biogeochemical characteristics of their soils. The availability of labile N and K was lowest in the NK-limited MDL-fen, intermediate in the NP-limited VW-fen and highest in the P-limited ST-fen. In contrast, concentrations of plant-available P (P-Olsen) and total P showed a reverse pattern with relatively high concentrations in the NK-limited MDL-fen and low concentrations in the NP- and P-limited fens. The relatively high P-concentrations measured in the NK-limited MDL-fen with *H. vernicosus* are consistent with other studies (Štechová *et al.* 2008; Pawlikowski *et al.* 2013). So, N-availability in the soil was relatively low in fens with (co-)limitation of N, while P-availability was relatively low in fens with (co-)limitation of P.

Higher P-availability in the riverine NK-limited MDL-fen with *H. vernicosus* is presumably caused by high concentrations of Fe-bound P. Although P can easily be bound to Fe (e.g. Patrick & Khalid 1974), many vascular plants are still able to take up this Fe-bound P (Marschner 1995). These high concentrations of Fe-bound P coincided with rather high Al- and Fe-concentrations in the soil and soil pore water of the NK-limited MDL-fen. This is in accord with previous studies in vital populations of *H. vernicosus* that also found high Fe-concentrations in soil pore waters of these fens (Hedenäs & Kooijman 1996; Štechová *et al.* 2012; Pawlikowski *et al.* 2013). In contrast, the NP- and P-limited fens predominantly contained organic P, which is part of the organic matrix and can only become available through mineralization (Paul 2007). Since net P-mineralization will be rather low under the anaerobic and calcareous conditions in rich fens with *S. cossonii* and *S. scorpioides* (Kooijman & Hedenäs 2009; Chapter 4), P-availability will be limited in these fens.

Changes in interspecific competition and vegetation development due to fertilization

Responses to P-fertilization

Three years of P-fertilization had no effect yet on the species richness and Shannon's diversity indexes of any of the fens studied. This was expected for the NK-limited MDL-fen, but biomass production should increase and species diversity should decline after long-term P-addition in the NP- and P-limited fens (e.g. Wassen *et al.* 2005). It is, however, very likely that species diversity eventually decreases in the P-fertilized subplots of the NP- and P-limited fens, since the cover of some fast-growing species, such as *Agrostis canina* and *Carex elata*, increased after three years of fertilization. Furthermore, the increase in *Calliergonella cuspidata* and the simultaneous sharp decrease in *S. scorpioides* and *S. cossonii* show that bryophyte composition had already changed in these two fens. This change in bryophyte cover, which was especially observed in December when aboveground vegetation was mown and light availability was not restricted, is typical of P-eutrophication in P-limited rich fens (Kooijman 1993b). The replacement of *Scorpidium* spp. by *C. cuspidata* may eventually result in a succession to *Sphagnum*-dominated fens, since *C. cuspidata* is more rapidly overgrown by large acidifying *Sphagnum* spp., such as *S. squarrosum* Crome and *S. palustre* L., than *S. scorpioides* (Kooijman & Bakker 1995).

Responses to N-fertilization

In contrast to P-fertilization, species richness declined in all fens after three years of N-fertilization. This decrease in species diversity may be explained by two mechanisms (Verhoeven *et al.* 2011): (a) an increase of the aboveground biomass production as a result of N-addition, which leads to a decline of the light availability at the soil surface, and (b) toxic effects as a consequence of NH_4-addition. This study shows that different mechanisms may act in different rich fen types.

As expected, aboveground biomass production and light availability did not change in N-fertilized subplots of the P-limited ST-fen with *S. scorpioides*. In this case, the decrease in species diversity was presumably due to high NH_4-concentrations in the soil pore water of about 350 µmol L^{-1}. These high NH_4-concentrations were probably partly caused by the dissolution of NH_4NO_3-granules, but possibly also by dissimilatory NO_3-reduction to NH_4 (DNRA) under anaerobic conditions (Rütting *et al.* 2011). The latter may also explain the increased alkalinity in these N-fertilized subplots (Stumm & Morgan 1996). Under these NH_4-rich conditions, the cover of *S. scorpioides* also decreased sharply, which is in accord with experimental studies on NH_4-toxicity (Paulissen *et al.* 2004).

In the NP-limited VW-fen with *S. cossonii*, N-fertilization resulted both in an increased aboveground biomass production and in high NH_4-concentrations. In this case, it was impossible to separate the effects of both mechanisms and to point out which one was responsible for the disappearance of endangered species and the decline of *S. cossonii*. An additional study, in which not only the fertilization treatments but also the addition of light to the fen understory is taken into account, as carried out by Hautier *et al.* (2009) in grassland communities, may elucidate the contributions of both mechanisms.

N-fertilization did, however, not lead to high NH_4-concentrations in the soil pore waters of the NK-limited MDL-fen with *H. vernicosus*. Instead, NO_3-concentrations were raised in this fen. Presumably due to aerobic NH_4-oxidation (nitrification) during pronounced periods of relatively low water tables, as described in other studies (Williams & Wheatley 1974; Grootjans *et al.* 1985; Neill 1995). The disappearance of endangered indicator species and the declined cover of *H. vernicosus* in N- and K-fertilized subplots of the NK-limited MDL-fen should, therefore, be attributed to the increased biomass production and resulting decrease in light availability.

Implications for fen management

This study clearly shows that the biogeochemical conditions of fens have a major impact on the nature of nutrient limitation. Fens with low concentrations of available N in the soil will often be (co)limited by N, while fens with low plant-available P will often be (co)limited by P. Since the conservation and restoration of biodiverse rich fens strongly depends on the nature of nutrient limitation (Verhoeven *et al.* 1996; Lamers *et al.* 2002; Lamers *et al.* 2014), management approaches (including source reduction) should be based on the biogeochemical conditions in a fen system. Otherwise, a succession to more acid *Sphagnum*-dominated fens may occur (e.g. Kooijman & Paulissen 2006).

Since the aboveground biomass production in N-limited fens will depend on N-inputs, the composition of vascular plants and bryophytes will not be very sensitive to P-addition. In contrast, these systems are presumably sensitive to atmospheric N-deposition (Koerselman *et al.* 1990; Verhoeven & Schmitz 1991; Bobbink *et al.* 1998; Wassen & Olde Venterink 2009), enhanced net N-mineralization during pronounced periods with low water tables (Grootjans *et al.* 1985; Williams & Wheatley 1988; Chapter 4) and inundations with N-rich surface water.

This study, however, appears to indicate that critical N-loads (Bobbink *et al.* 1998) differ between different fen types. N-inputs will presumably have less impact on the biomass production of P-limited rich fens (e.g. Verhoeven & Schmitz 1991), unless there is concomitant P-eutrophication. Despite less effect on biomass production, it should be born in mind that long-term atmospheric deposition may results in high NH_4-concentrations that can be toxic to some of the endangered vascular plants and brownmosses in these P-limited fens (Verhoeven *et al.* 2011).

An increase in P-input is, however, the main risk for most P-limited rich fens. This will not only lead to enlarged aboveground biomass production, but may also change the species-rich bryophyte cover by replacing calcicolous mosses, such as *S. cossonii*, *S. scorpioides* and *C. stellatum*, by more eutrophic or acid mosses, such as *C. cuspidata*, *S. squarrosum* and *S. palustre* (Kooijman 1993b; Kooijman & Bakker 1995). So, these P-limited fen types will presumably be affected by enhanced net P-mineralization during long periods of severe drought (Chapter 4) and regular inputs of P-rich inundation water during short-term flooding events (Olde Venterink *et al.* 2003). Also, internal P-mobilization during long-term inundations may be problematic for relative Fe-rich P-limited fens (Lamers *et al.* 2002; Zak *et al.* 2010; Chapter 3), especially when the supplied water is SO_4-rich (Lamers *et al.* 1998b; Lamers *et al.* 2002).

Acknowledgements

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7

General discussion

7. General discussion

The main focus of my thesis was to determine the influence of fluctuating surface water levels on the biogeochemistry and ecology of biodiverse rich fens. The research was carried out in the Dutch Ramsar area "National Park Weerribben-Wieden" that still includes a substantial area of biodiverse, brownmoss-dominated rich fens. These rich fens, characterized by well-buffered (with respect to acidification) and relatively nutrient-poor (mesotrophic) conditions, are hotspots for the diversity of highly threatened vascular plants and bryophytes (Sjörs 1950; van Wirdum 1991; Kooijman 1993a). The area of brownmoss-dominated rich fens is, however, declining in many West European wetlands (e.g. Kooijman 1992; JNCC 2007; Paulissen *et al.* 2013) as a consequence of the natural succession toward *Sphagnum*-dominated fens (e.g. Clapham 1940; van Wirdum 1991; van Diggelen *et al.* 1996), and human-induced acidification and eutrophication (e.g. Gorham *et al.* 1987; Kooijman & Paulissen 2006). In addition, rejuvenation of new rich fens is hardly taking place. Terrestrialization of open waters has not yet resulted in the formation of new rich fens in the Netherlands during the past decades (e.g. Lamers *et al.* 2002), even in areas where habitat quality seems to have improved. Transplantation experiments showed that bryophyte key species, such as *Scorpidium scorpioides* (Hedw.) Limpr., *Scorpidium cossonii* (Schimp.) Hedenäs and *Hamatocaulis vernicosus* (Mitt.) Hedenäs, remained vital after more than 10 months in initial terrestrializing vegetation (Cusell *et al.* 2013; Fig. 7.1), indicating that dispersal may be an additional problem. As long as the exact constraints for the terrestrialization and development of biodiverse rich fens are still unclear, it is even more important to conserve and restore the vegetation in existing brownmoss-dominated rich fens.

In densely populated lowland regions dominated by agriculture, such as the Netherlands, surface water levels are strictly controlled. This generally implies more or less constant surface water levels, suppressing or even inverting meteorological variation. Under these conditions, the re-introduction of more fluctuating surface water levels has been proposed as one of the management tools to improve the water quality in wetlands (e.g. Mitsch & Gosselink 2007) and to counteract potential effects of acidification and eutrophication of fens (Grootjans *et al.* 2001; Loeb *et al.* 2008a). Lowered as well as raised surface water levels may, however, also have negative impacts on fens (e.g. Lamers *et al.* 2002). In this thesis, the main objective was therefore to determine the potential effects of both lowered and raised surface water levels on the ecohydrological and biogeochemical functioning of fens, with a strong emphasis on the preservation and restoration of endangered, brownmoss-dominated rich fens. This study focused on the processes of eutrophication, alkalinity generation and acidification at several spatial scales, from site conditions to the entire wetland. The key issues discussed are (a) landscape ecology in relation to regional hydrochemistry,

(b) water table dynamics in fens, (c) alkalinization versus acidification, (d) nutrient dynamics and (e) the accumulation of potentially toxic compounds (Fig. 7.2). Next to the outcomes of the studies presented in the previous chapters, additional information, especially about landscape-scale processes, has been integrated in this chapter.

Regional ecohydrology and biogeochemistry of National Park Weerribben-Wieden

To thoroughly understand the functioning of rich fens and the potential impact of fluctuating surface water levels, it is not sufficient to only examine biogeochemical habitat conditions inside fens. It is also vital to understand the geohydrological and ecological functioning of the entire wetland complex and its surroundings, since local conditions are often strongly determined by regional processes. To gain a better understanding of the present exchange of nutrients and bases between the area and its surroundings, water balances and mass balances for P, N and Ca were estimated, based on the activities of pumping stations and data about precipitation and evapotranspiration between 2001 and 2012 (Cusell *et al.* 2013). In addition, an existing 1D-2D SOBEK-model (ARCADIS 2004) for surface water flows in National Park Weerribben-Wieden was updated to simulate water flows and P-, N- and Ca-fluxes in the north of the nature reserve (De Weerribben). A more detailed description of the SOBEK-model can be found in Appendix C.

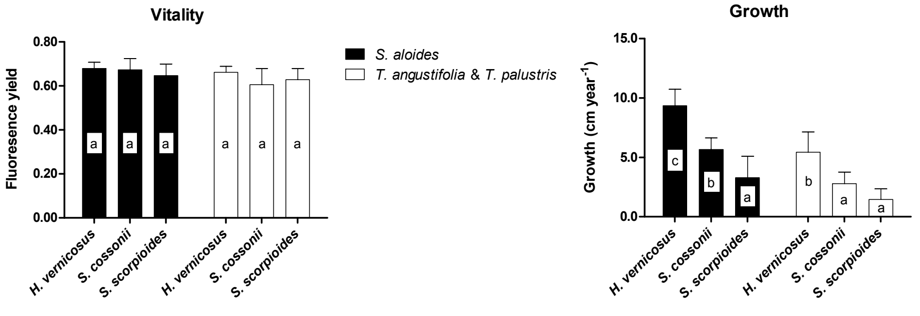
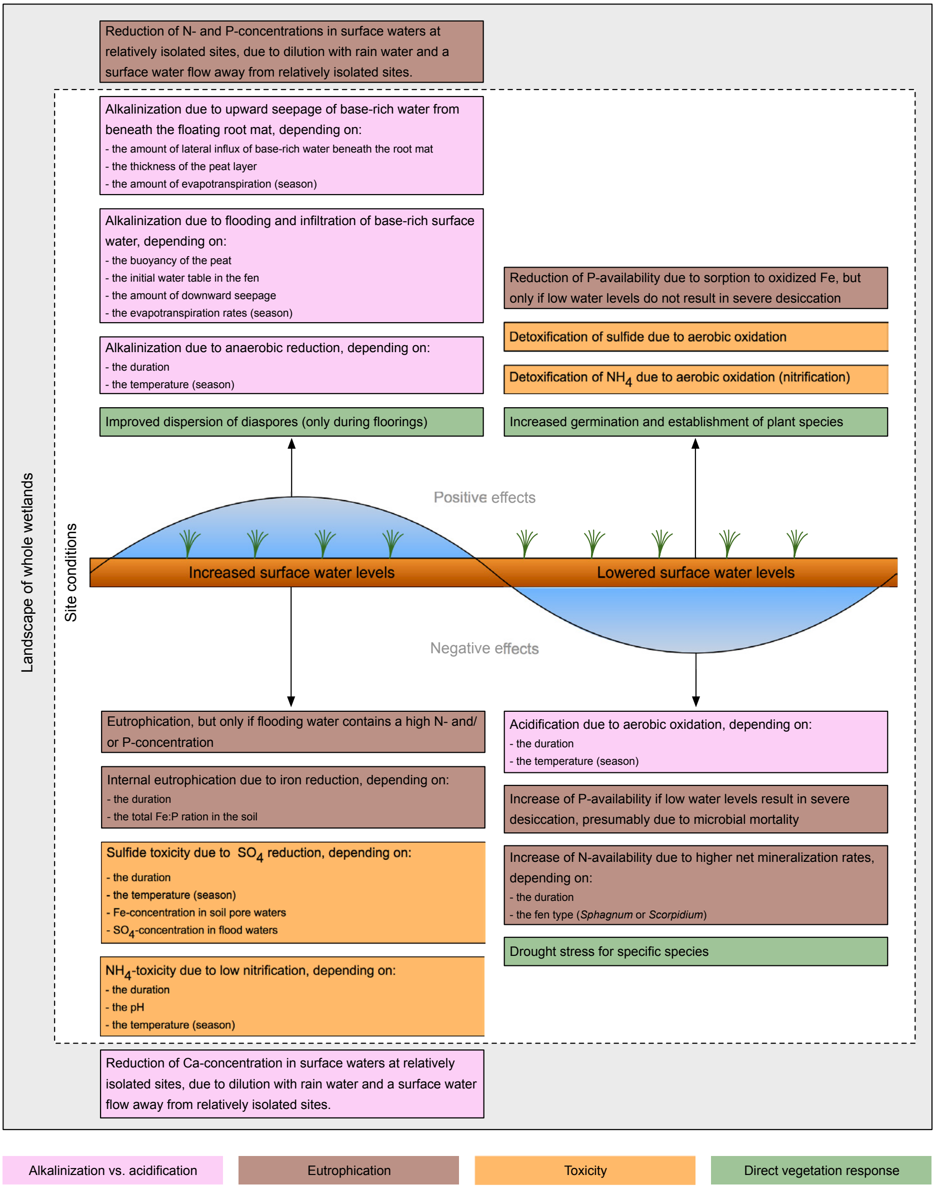


Fig. 7.1. Vitality and growth of *Scorpidium scorpioides, Scorpidium cossonii* and *Hamatocaulis vernicosus* after more than10 months of transplantation into floating mats of *Stratiotes aloides* plants (black) and terrestrializing helophyte vegetation with *Typha angustifolia* and *Thelypteris palustris* (white). Transplantation was carried out according to Kooijman (1993c) and Kooijman & Whilde (1993). Growth rates were determined relative to a velcro strip, which marked the start length at 3 cm below the shoot apex, according to Rochefort & Vitt (1988). Vitality was measured as the fluorescence yield $(F_m\text{-}F_0)/F_m$ (see Chapter 3 for the applied method), which indicates vital conditions above values of about 0.4 (Maxwell & Johnson 2000). Sample means are given and error bars indicate standard deviations (n = 15). Significant differences (one-way ANOVA and LSD post-hoc test) are indicated by different letters.



Reduction of N- and P-concentrations in surface waters at relatively isolated sites, due to dilution with rain water and a surface water flow away from relatively isolated sites.

Alkalinization due to upward seepage of base-rich water from beneath the floating root mat, depending on:
- the amount of lateral influx of base-rich water beneath the root mat
- the thickness of the peat layer
- the amount of evapotranspiration (season)

Alkalinization due to flooding and infiltration of base-rich surface water, depending on:
- the buoyancy of the peat
- the initial water table in the fen
- the amount of downward seepage
- the evapotranspiration rates (season)

Alkalinization due to anaerobic reduction, depending on:
- the duration
- the temperature (season)

Improved dispersion of diaspores (only during floorings)

Reduction of P-availability due to sorption to oxidized Fe, but only if low water levels do not result in severe desiccation

Detoxification of sulfide due to aerobic oxidation

Detoxification of NH_4 due to aerobic oxidation (nitrification)

Increased germination and establishment of plant species

Eutrophication, but only if flooding water contains a high N- and/ or P-concentration

Internal eutrophication due to iron reduction, depending on:
- the duration
- the total Fe:P ration in the soil

Sulfide toxicity due to SO_4 reduction, depending on:
- the duration
- the temperature (season)
- Fe-concentration in soil pore waters
- SO_4-concentration in flood waters

NH_4-toxicity due to low nitrification, depending on:
- the duration
- the pH
- the temperature (season)

Acidification due to aerobic oxidation, depending on:
- the duration
- the temperature (season)

Increase of P-availability if low water levels result in severe desiccation, presumably due to microbial mortality

Increase of N-availability due to higher net mineralization rates, depending on:
- the duration
- the fen type (*Sphagnum* or *Scorpidium*)

Drought stress for specific species

Reduction of Ca-concentration in surface waters at relatively isolated sites, due to dilution with rain water and a surface water flow away from relatively isolated sites.

Fig. 7.2. Schematic representation of potential effects of lowered and raised surface water levels on rich fens without groundwater discharge. Effects on the level of the entire wetland and on the level of site conditions are depicted.

Since mass balances and the SOBEK-model could not be validated sufficiently (Cusell *et al.* 2013), results should be interpreted with some caution. For this reason, I focused on the general processes instead of on the exact concentrations and fluxes. Although the information from the balances and SOBEK-models has not been included in the previous chapters, it is important to summarize some of the results, because they give a better understanding of the ecohydrological functioning of the nature reserve. In addition, these model results can be coupled to the results of Chapter 5 about nutrient behavior at a landscape-scale.

Sources and distribution of nutrients

Mass balances show that about 70% of the total P-input of the nature reserve originates from adjacent polder areas (Fig. 7.3), lower-lying agricultural lands that need active water management. This outcome corresponds with those of earlier studies by Jol & Laseur (1982) and Balirwa (1993). For N, almost half of the input originates from the polders and the other half from the atmospheric N-deposition of about 19 kg N ha^{-1} year^{-1}. The P- and N-inputs from polders are 2 – 3 times higher in winter than in summer, due to higher discharges of polder water into the reserve during winter and lower nutrient concentrations in polder water during summer (Cusell *et al.* 2013).

Despite these high fluxes, nutrient concentrations in the pore water of rich fens are low throughout the year with o-PO_4, NH_4- and NO_3-concentrations of about 0.3, 8 and 5 µmol L^{-1} (Chapters 2, 5 & 6). Higher nutrient inputs in the periphery of the nature reserve in winter do not automatically lead to higher nutrient concentrations in turf ponds and ditches near rich fens, which are located in relatively isolated parts of the nature reserve (Table 7.1). This is partly caused by changes of water flow patterns throughout the year, as could be inferred from measurements in the field and the SOBEK-run for the "actual" surface water level regime (Cusell *et al.* 2013; Fig. 7.4; Chapter 5). Although P- and N-inputs from the polders are high in winter, the precipitation surplus results in outward directed surface water flows, from the relatively isolated parts of the nature reserve to large canals and the periphery of the nature reserve. This appears to effectively prevent the transport of nutrient-rich input water to more isolated locations inside the reserve. In contrast, a surplus of evapotranspiration, which mostly occurs during summer, leads to an opposite flow pattern from the periphery to the more isolated areas. This appears to stimulate large-scale water mixing throughout the area, but does not lead to increased N- and P-concentrations in the vicinity of rich fens, because relatively small amounts of nutrient-rich water are discharged from polders into the wetland during these dry periods.

Filtering processes in surface waters lead to a further reduction of nutrient loads towards relatively isolated locations (Chapter 5). One of these important mechanisms is the assimilation and accumulation of nutrients by plants and algae during the growing season and the storage of these nutrients in dead organic matter (Howard-Williams 1985; Richardson & Marshall 1986; Reddy *et al.* 1999). For P, additional important

Table 7.1. Total inputs of Ca, P and inorganic N through pumping stations at water entry locations in February and August (based on mass balances from 2001 till 2012), and concentrations of Ca, total P and inorganic N in the surface water of water entry locations, canals and turf ponds in February and August (based on Chapter 5).

	February			August		
	Entry	*Canal*	*Turf pond*	*Entry*	*Canal*	*Turf pond*
Distance to nearest entry location (m)	0 (0)	2027 (1374)	3447 (1355)	0 (0)	2027 (1374)	3447 (1355)
Ca						
Input (10^3 kmol month^{-1})	21.8 (8.3)			19.4 (13.9)		
Surface water (µmol L^{-1}) *†	1486 (569)	774 (297)	547 (272)	1367 (426)	1249 (565)	1436 (453)
total P						
Input (10^3 kmol month^{-1}) *	0.082 (0.035)			0.044 (0.032)		
Surface water (µmol L^{-1}) *†	3.4 (2.9)	1.9 (1.9)	0.7 (0.4)	0.7 (0.3)	0.9 (0.5)	0.9 (0.4)
inorganic N						
Input (10^3 kmol month^{-1}) *	1.43 (0.60)			0.47 (0.32)		
Surface water (µmol L^{-1}) *†	108 (58)	47 (32)	16 (16)	20 (14)	24 (18)	25 (21)

Mean values and standard deviations (n = 12 for inputs; n = 10 for surface waters). Significant differences between season and gradient locations are indicated by * and †, as determined by Tukey HSD post-hoc tests ($P \leq 0.05$).

filtering mechanisms are the sorption of P to Al/Fe-compounds and the precipitation of colloidal Al- and Fe-phosphates (Chapter 5), as was also found by Lijklema (1980). This mainly happens in the vicinity of the pumping stations, because these stations not only represent the main input of N and P, but also of Fe, due to groundwater discharge in the deeper lying polders around the nature reserve and the subsequent pumping of Fe-rich water into the reserve.

The flow patterns and filtering mechanisms described strongly influence the spatial distribution of nutrient availability in the National Park (Chapter 5). Increasing N:P-ratios in aquatic and semi-terrestrial vascular plants clearly indicate a decrease in P-availability (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003) from the pumping stations at the periphery of the nature reserve, where ratios were about 10 g g^{-1}, to more isolated turbaries and rich fens, where ratios of 15 and 19 g g^{-1} were

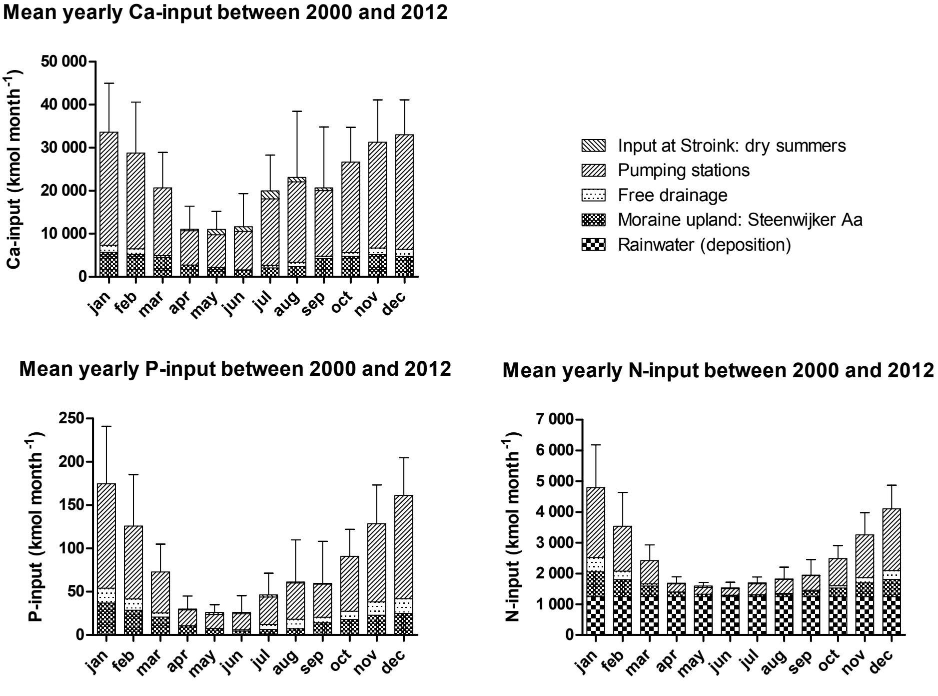


Fig. 7.3. Mass balances for Ca, P and N, indicating the contribution of various sources for National Park Weerribben-Wieden during different seasons. Balances were calculated on the basis of the activities of pumping stations, concentrations in the polder water, precipitation and evapotranspiration rates, and rates of groundwater discharge or downward seepage in the National Park and polders. The N-deposition rate was set on 19 kg N ha^{-1} $year^{-1}$ (RIVM 2012), which remained unchanged throughout the year. Rates of P- and Ca-deposition were calculated by multiplying the amount of precipitation in a month with a mean measured P-concentration of 0.02 $\mu mol\ L^{-1}$ and Ca-concentration of 7.63 $\mu mol\ L^{-1}$ (RIVM 2012).

found in semi-terrestrial plants. So, along this gradient, the availability of P appears to decrease more than that of N, which is presumably caused by a combination of sorption and precipitation of Al/Fe-phosphates near the periphery of the nature reserve and a constant input of N by atmospheric deposition throughout the wetland.

Sources and distribution of bases

Mass balances show that about 85% of Ca originates from polder areas under the current conditions (Fig. 7.3). Especially deep polders, which show strong discharge of base-rich groundwater (van Wirdum 1991), are important and represent 50 – 60% of the total Ca-input. Probably, the deep polders have already influenced the Ca-

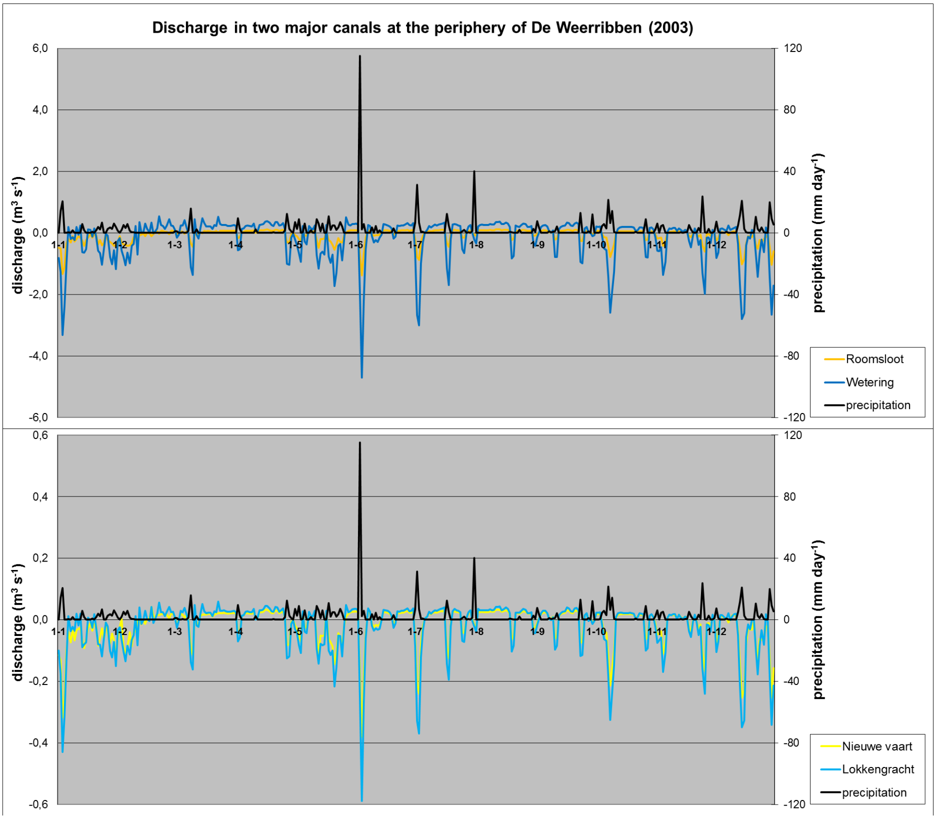


Fig. 7.4. The relationship between precipitation and discharge in two major canals at the periphery of De Weerribben (above) and in two minor canals in the center of De Weerribben (below), as calculated by SOBEK for the "actual" surface water level regime in 2003. Positive discharges correspond to a water flow from the periphery to the more isolated areas, while negative discharges correspond to an opposite water flow from relatively isolated parts of the nature reserve to large canals and the periphery of the nature reserve.

balance since the reclamations in the 1920 – 1950s. The rivers and canals that drain the relatively base-rich moraine upland at the eastern side of the nature reserve are only responsible for about 15% of the total Ca-input. Under the current conditions, it is therefore not desirable to completely isolate the reserve from the polders, even though they are also responsible for a large part of the N- and P-inputs.

As for P and N, Ca-inputs are highest in winter (Fig. 7.3), but most of this Ca will not reach the more isolated sites with rich fens. In winter, the precipitation surplus and the surface water flow away from the relatively isolated parts of the nature reserve (Cusell *et al.* 2013; Fig. 7.4; Chapter 5) prevents mass transport of nutrient-rich and base-rich input water to more isolated locations. In summer, when nutrient inputs from polders are lower than in winter, Ca-inputs from polders are still significant as a

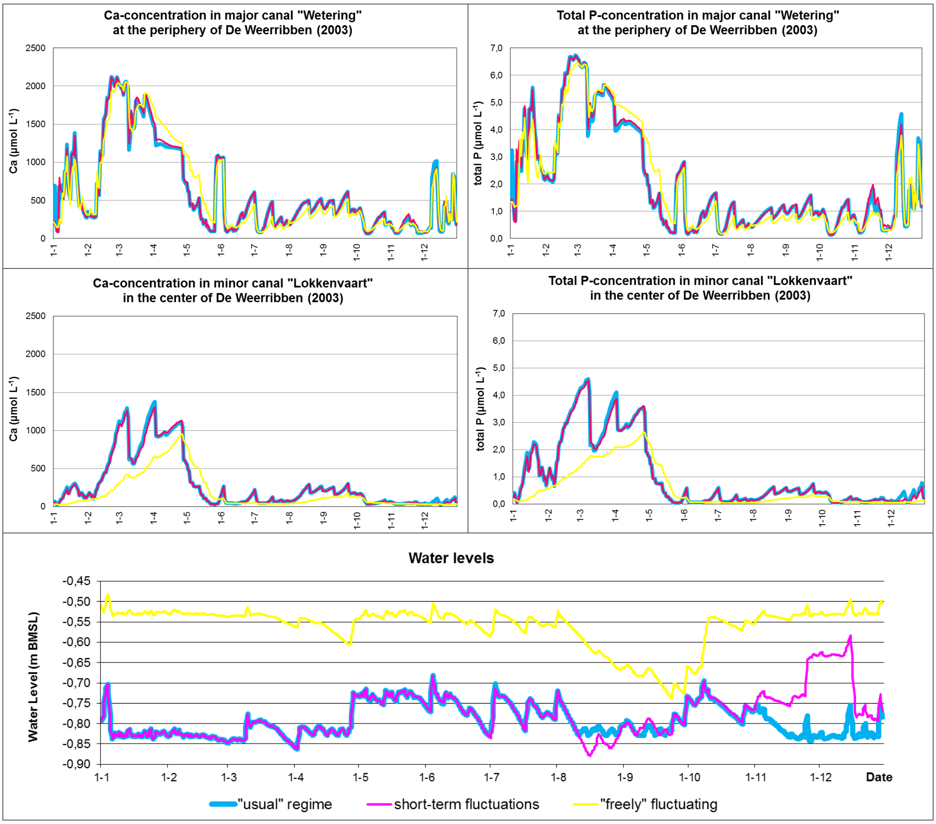


Fig. 7.5. Effect of two fluctuating surface water level regimes (short-term decrease in summer & short-term rise in November – pink; freely fluctuating between 0.53 and 0.93 m BMSL – yellow) on the water level (below), the Ca- and total P-concentration in a major canal at the periphery of De Weerribben (above) and a minor canal in the center of De Weerribben (middle), as calculated by SOBEK. The runs were based on the weather conditions in 2003, a year which was characterized by a very dry summer (KNMI 2014). Additional examples, which support the mentioned patterns, can be found in de Lange (2011) and Cusell *et al.* (2013).

result of the ongoing discharge of groundwater in these polders. These Ca-inputs are, however, only high during relatively wet summers, when polders still have to discharge quite a lot of water into the wetland (Cusell *et al.* 2013). Subsequently, the overall evapotranspiration surplus in summer leads to a flow pattern towards isolated sites, and thus to large-scale mixing of relatively nutrient-poor and Ca-rich water throughout the entire nature reserve (Cusell *et al.* 2013; Fig. 7.4; Chapter 5).

Raised surface water levels

The main objective of my thesis was to determine the influence of fluctuating surface water levels on rich fens. In order to predict the ecohydrological consequences of more fluctuating water levels on a landscape-scale, three surface water level regimes have been tested in SOBEK: (a) the "actual" regime with water levels between 0.73 and 0.83 m below mean sea level (BMSL), (b) a regime with short-term periods of raised water levels in summer (0.63 m BMSL) and lowered water levels in winter (0.88 m BMSL), and (c) a regime with freely fluctuating water levels throughout the year which may vary between 0.53 and 0.93 m BMSL. A more detailed description of these runs can be found in Appendix C.

According to SOBEK-simulation (b), short-term (one week) surface water level rises of about 10 cm during heavy rainfall in winter had almost no effect on flow rates and flow directions of the surface water in De Weerribben (de Lange 2011; Cusell *et al.* 2013). High precipitation may lead to higher surface water levels, but just as for the present "actual" water level regime, the water flowed away from the relatively isolated parts of De Weerribben to large canals and the periphery of the reserve. Nutrient and Ca-concentrations in surface water slightly increased in the canals at the periphery, due to higher discharges of polder water, but slightly decreased at more isolated locations near rich fens, due to dilution with rainwater (Fig. 7.5).

SOBEK-run (c), with larger and longer surface water level fluctuations during natural droughts and wet periods, led to raised water levels of 0.53 m BMSL during most of the year (Fig. 7.5). Only in summer, when there was no precipitation surplus, surface water levels lowered to 0.73 m BMSL, but they were still higher than in the present "actual" water level regime. According to the model, raised surface water levels throughout most of the year would not influence nutrient and Ca-concentrations in surface waters at the periphery of De Weerribben (Fig. 7.5). However, in the more isolated sites, nutrient concentrations decreased with 50 – 80% in the model, due to dilution with rainwater. Conditions at the more isolated sites not only became less eutrophic, which generally is an ecological benefit, but also more ombrotrophic (Fig. 7.5). Ca-concentrations may decrease 20 – 70%, which will be harmful to rich fens. Model studies of Borren *et al.* (2012) to other Dutch fen areas also show that the introduction of fluctuating surface water levels may lead to increased influence of rainwater, and thus to a decrease in both nutrient and base concentrations.

Lowered surface water levels

Lower surface water levels during dry periods with an evapotranspiration surplus will generally lead to a reduction of the input of nutrient-rich water into wetlands (Coops & Hosper 2002; Jaarsma *et al*. 2008; Schep *et al*. 2012). However, SOBEK-simulation (b) indicates that a short-term (one week) surface water level drawdown of about 5 cm in summer, as the Water Management Authority Reest & Wieden wanted to introduce in the nature reserve, has almost no effect in De Weerribben (Fig. 7.5). Flow rates, flow directions and the chemical composition of surface waters did not change significantly by this short change. In addition, mass balances show that under the present conditions less than 1% of the total N- and P-inputs are derived from the main inlet station at Stroink during dry episodes (Fig. 7.3). This corresponds to low N- and P-concentrations in the supplying lake (Vollenhovermeer) during such dry episodes (de Vries 2011). So, a short period with short-term, slightly lowered surface water levels does not seem to affect the ecohydrological functioning of the nature reserve.

Water table dynamics in fens

Raised surface water levels

During short-term (two weeks) surface water level rises of about 10 cm, water tables responded differently in floating and non-floating fens (Chapter 2). Inundation only occurred in non-floating fens. These inundations were due to flooding from nearby ditches, as shown by homogenously distributed Cl-concentrations in inundation water that were much higher than in soil pore water. In contrast, similar experiments had almost no effect on the water tables in floating fens with *Sphagnum*-dominated vegetation, since these floating soils followed surface water levels. This lack of response was not only found after two weeks of high surface water levels, but also during a similar field treatment of three months (data not shown). Such stable water tables have also been reported for floating subarctic and temperate fens (Roulet 1991; van Wirdum 1991).

In order to estimate the potential effect of raised surface water levels on water tables in the entire National Park Weerribben-Wieden, aerial footage of the entire nature reserve were made (Cusell *et al.* 2013). The films, which showed the visible part of the light spectrum, were shot from a small airplane on September 1, 2010, when surface water levels were 2 cm higher than the presently used maximum of -0.73 m BMSL (Waterschap Reest & Wieden 2014). Although it was difficult to observe whether inundation took place in winter-mown fens as a result of limited visibility of the soil surface, it was clear that inundation of fens occurred throughout the nature reserve but not in all fens (Fig. 7.6).

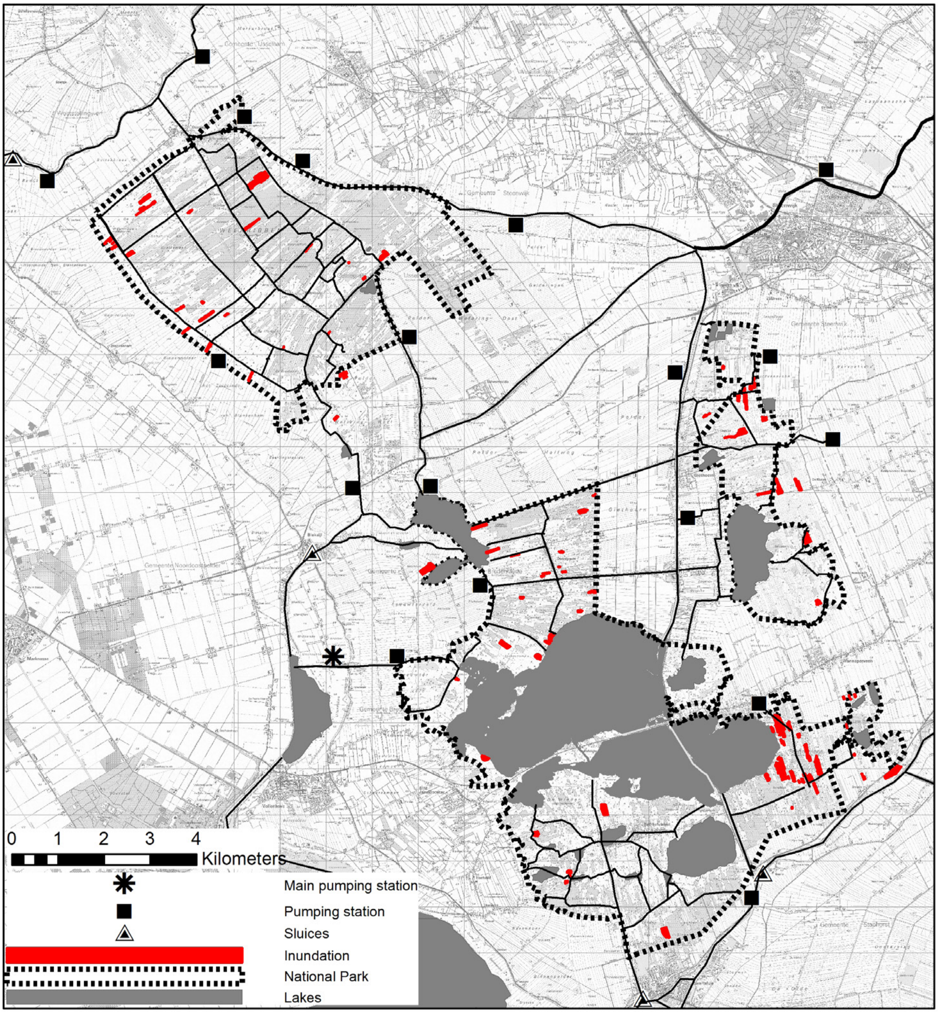


Fig. 7.6. Sites in National Park Weerribben-Wieden (between 52°48' N – 5°53' E and 52°38' N – 6°08' E) for which inundation was certain during the high surface water levels on September 1, 2010, based on an aerial survey. Surface water levels were about 2 cm higher than the presently used maximum of -0.73 m BMSL (Waterschap Reest & Wieden 2014).

In the southeast of the reserve, where peat ponds are shallow and fens are generally firmly attached to the sandy subsoil, many fens were inundated. Although it appeared that most floating fens, covering 80 – 90% of the terrestrialized part of the nature reserve, were not inundated, a small part of these floating fens was still inundated, including all major floating rich fens with *Scorpidium* spp. Inundation of floating rich fens is certainly not uncommon, and has also been reported by others (O'Connell 1981; van Wirdum 1991). However, most *Sphagnum*-dominated fens, which cover most of the nature reserve, did not seem to be inundated in the aerial survey, although the observations may have been affected by higher vegetation in these winter-mown fens. The observed absence of inundation of *Sphagnum*-dominated, poor fen vegetation near rich fens with *Scorpidium* spp. (Fig. 7.7) supports this idea of limited occurrence of inundation of other *Sphagnum*-dominated fens in the area.

While it is clear that even floating rich fens with *Scorpidium* spp. are inundated during high surface water levels, there is still debate about the origin of this inundation water. In areas without groundwater discharge, such as National Park Weerribben-Wieden, inundation may be caused by seepage of water from beneath the floating root mat (van Wirdum 1991), accumulation of rainwater and/or flooding by surface water (Barendregt *et al.* 2004; Cusell *et al.* 2013). For rich fen vegetation, it is presumably irrelevant how the minerotrophic water is supplied to the top layer, as long as it is base-rich and mineral-poor. The mechanism is, however, important for nature managers, since it will presumably affect their management strategies for the conservation and restoration of floating rich fens, e.g. using active "irrigation" by discharging surface water on top of the fens, lowering of fen shores, applying small ditches within fen systems and allowing raised surface water levels. Therefore, future research should focus on the hydrological mechanisms involved in different types of floating fens. Continuous tracer analyses, e.g. using temperature or Cl-concentration as a tracer, detailed analysis of continuous diver data of water levels, and/or the use of water flow sensors should therefore be considered.

Fig. 7.7. The occurrence of inundation in two rich fens (left: Kikkerlanden; right: Reeënweg) during high surface water levels on September 1, 2010, as shown by these stills of aerial footage. In both areas, inundation almost exclusively occurred in the *Scorpidium*-dominated vegetation (encircles zones). Almost all *Sphagnum*-dominated vegetation was not inundated.

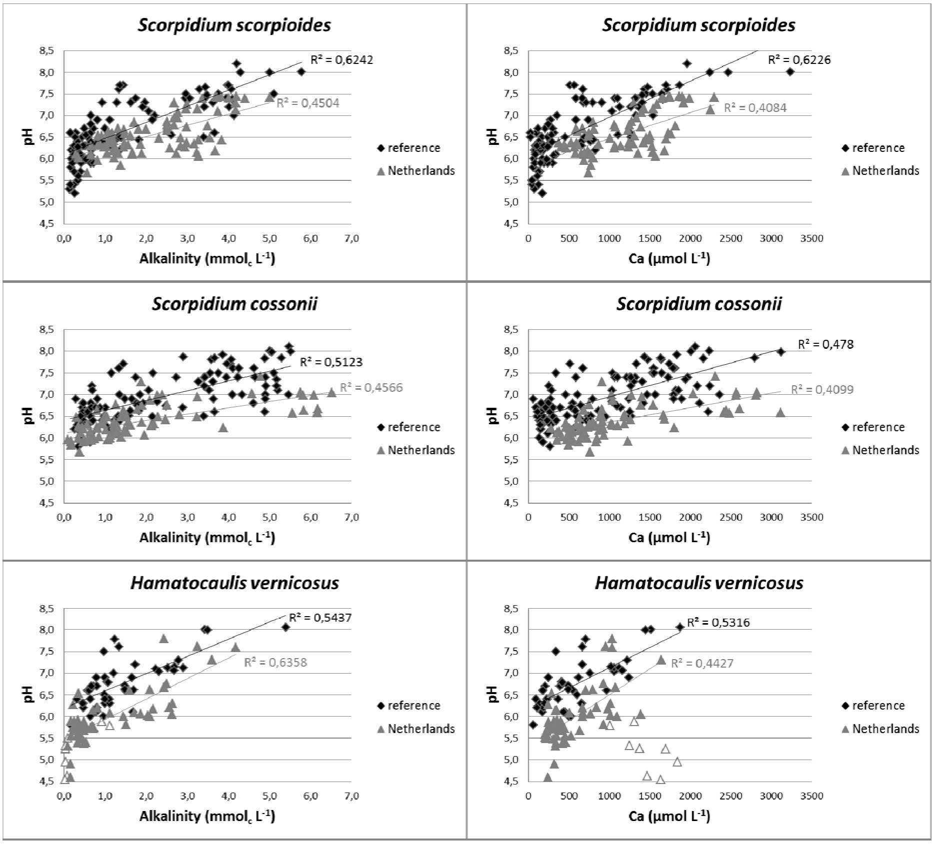


Fig. 7.8. Relation between Ca-concentrations, alkalinities and pH-values in soil pore waters of habitats with *Scorpidium scorpioides*, *Scorpidium cossonii* and *Hamatocaulis vernicosus* in the Netherlands and in European reference sites with lower atmospheric deposition. Reference sites were sampled between 1991 and 1997 throughout Sweden (n = 84, 89 and 29; unpublished data of Kooijman & Hedenäs), in 2010 throughout Poland and Slovakia (n = 3, 7 and 4; unpublished data of Kooijman & Pawlikowski), in 2011 in Sèche de Gimel, Switzerland (n = 3 for *H. vernicosus*; unpublished data Cusell) and in 2012 in Jämtland, Sweden (n = 12, 12 and 12; Neijmeijer 2013). In the Netherlands, samples were collected between 2008 and 2011 (n = 80, 82 and 68). Eight samples with *H. vernicosus* (open triangles) were excluded for the trend-line in the Netherlands. These samples differed, because water tables were low during sampling, which presumably not only resulted in acidification but also in desorption of Ca from the adsorption complex.

Lowered surface water levels

In the field experiments, water tables did not respond uniformly to two weeks of manipulated surface water level drawdown (-15 cm) in summer (Chapter 2). In both non-floating and floating fens, water tables only dropped when there was an evapotranspiration surplus during the treatment period. Under such conditions, water tables dropped 10 – 15 cm in non-floating fens, while levels only dropped 4 – 6 cm in floating fens, due to soils that partly moved down with the surface water drawdown (unpublished diver data). However, in wet summer periods, water tables did not decrease during manipulated surface water level drawdowns. In non-floating fens, water tables were even raised a few centimeters under these wet conditions. The response of water tables in fens therefore seems to be more affected by weather conditions, especially by the balance between precipitation and evapotranspiration, than by the surface water levels in adjacent ditches. Similar results were found in other Dutch fens with low rates of groundwater discharge and downward seepage (Schep *et al.* 2012). However, under natural conditions, lowered surface water levels will often be the result of dry episodes, and will thus often coincide with lower water tables in fens, especially in non-floating fens.

Alkalinization versus acidification

It is generally accepted that acidification will eventually lead to succession of biodiverse rich fens toward species-poorer *Sphagnum*-dominated fens. This may be caused by natural peat accumulation and concomitantly reduced influence of minerotrophic water. However, anthropogenically increased atmospheric deposition (NH_x, NO_y and SO_x) may well accelerate the acidification of rich fens (Gorham *et al.* 1987; Kooijman 2012). Dutch fens show significantly lower pH-values than reference fens in Sweden, Poland and Switzerland, where present potential acid deposition equals 800 – 1500 mol_c ha^{-1} $year^{-1}$ (Schöpp *et al.* 2003) instead of the average potential acid deposition of about 3000 mol_c ha^{-1} $year^{-1}$ in the Netherlands (Kros *et al.* 2008). For example, it seems that alkalinities and Ca-concentrations should be about 4000 and 2000 µmol L^{-1} (showing a stoichiometrical balance of HCO_3^- and Ca^{2+}) to reach a pH of 7.0 in Dutch fens with *S. scorpioides*, while reference fens only needed concentrations of about 2600 and 1300 µmol L^{-1} (ditto) to reach this pH (Fig. 7.8). Similar relations were found for fens with *S. cossonii* and *H. vernicosus*.

It is, furthermore, noteworthy to mention that none of the Dutch fens with *Scorpidium* spp. were characterised by weakly-buffered conditions (Fig. 7.8). This is in accordance with a study of Kooijman & Westhoff (1995), who showed that *S. scorpioides* became locally extinct in Dutch weakly-buffered fens during the 1960s. This is not surprising, since Vitt & Kuhry (1992) and Gunnarsson *et al.* (2000) already showed that rich fens in the pH range of 5.0 – 5.8 are the ones most sensitive to acidification. In this pH-range, the exchange of Ca and Mg from adsorption complexes becomes an important acid buffering mechanism, which implies that the base saturation of the CEC becomes very important, especially when HCO_3 becomes depleted in the soil pore water (Lamers *et al.* 2014).

Raised surface water levels

One way to enhance the input of base-rich water into the top layer of fen soils is a temporary rise of surface water levels. The effect of raised surface water levels, however, clearly depends on the buoyancy of a fen, the season, the vegetation type, the infiltration rates and the duration of flooding (Chapters 2 & 3, Cusell *et al.* 2013).

In the field manipulation experiments (Chapter 2), alkalinities and Ca-concentrations did not change in soil pore waters of floating fens with *Sphagnum*-dominated vegetation, due to the limited change in water tables in the fens, not even after three months (data not shown). There are, however, quite a few floating rich fens in National Park Weerribben-Wieden that do get inundated. As mentioned above, these inundations may be caused by flooding with base-rich surface water (Barendregt *et al.* 2004; Cusell *et al.* 2013), accumulation of rainwater and/or by seepage of base-rich water, which is supplied from the surface water system through preferential flow channels beneath the floating root mat (van Wirdum 1991). For the latter, it seems obvious that the thicker the floating peat layer is, the more difficult it will be for base-rich surface water to reach the peat surface (van Wirdum 1991, 1993). Furthermore, seepage of base-rich water presumably depends to a large extent on evapotranspiration rates, because this is the most obvious driver for upward flows. Since evapotranspiration surpluses in the Netherlands only occur in summer, upward seepage will probably not be of much importance in winter. The occurrence of flooding with base-rich surface water will, on the other hand, largely depend on the buoyancy of the peat and the potential lag between the surface water level rise and the upward seepage in fens. EC-profiles in the soil of several important rich fen sites in National Park Weerribben-Wieden indeed seem to indicate that flooding occurs in many of the floating rich fens with *S. scorpioides*, since EC-values are often higher in the surface layers of the peat than below (Bresjer 2013; Cusell *et al.* 2013; Fig. 7.9). Moreover, many of the floating fens studied showed local depressions along their borders, where ditch water and inundation water were directly connected during periods with high surface water levels. Although there is still discussion about the exact interpretation of the data (Cusell *et al.* 2013), it is obvious that flooding with surface water can indeed contribute to the supply of base-rich water in floating rich fens. However, as mentioned above, the exact contribution of the different potential hydrological mechanisms in floating fens deserves more attention in future research.

In non-floating fens, raised surface water levels will lead to flooding as soon as the surface water level becomes higher than the more or less fixed peat layer. In summer, two weeks of flooding resulted in a clear increase of the ANC in non-floating rich fens (Chapter 2). Alkalinity and Ca-concentration in soil pore waters increased by 1900 and

450 µmol L^{-1}, respectively. A large part of this increase was caused by the infiltration of base-rich flooding water. The ratio of 4:1 between the increase in alkalinity and Ca, the simultaneous decrease in S-concentrations and the increase in Fe-concentrations, however, showed that the increase in alkalinity was not merely caused by infiltration of base-rich surface water, but also by internal generation of HCO_3 as a result of the use of other electron acceptors (including Fe(III) and SO_4) than oxygen in microbial redox reactions (Stumm & Morgan 1996). It must, however, be noted that increases in alkalinity by internal alkalinization are often temporary, since aerobic oxidation during subsequent periods with lower water tables will result in acidification, consuming alkalinity (Lamers *et al.* 1998a; Mettrop *et al.* unpublished data).

In contrast, under winter conditions, short-term flooding of non-floating fens often did not lead to an increase of the ANC, due to lower temperatures and evapotranspiration rates (Chapter 2). Redox potentials were sufficiently low, with values below -100 mV in the entire soil profile of all fen types studied, at which Fe(III)- and SO_4-reduction may lead to internal alkalinity generation (e.g. Gambrell & Patrick 1978; Ponnamperuma 1984; Mitsch & Gosselink 2007). Nevertheless, significant production of HCO_3 has not been recorded, most probably due to low anaerobic microbial activity in winter and the short period of flooding. In addition to this absence of internal alkalinity production in winter, the short-term field experiments showed that substantial infiltration or diffusion of base-rich flooding water was impossible in winter, because the fen soils were already entirely waterlogged before the flooding

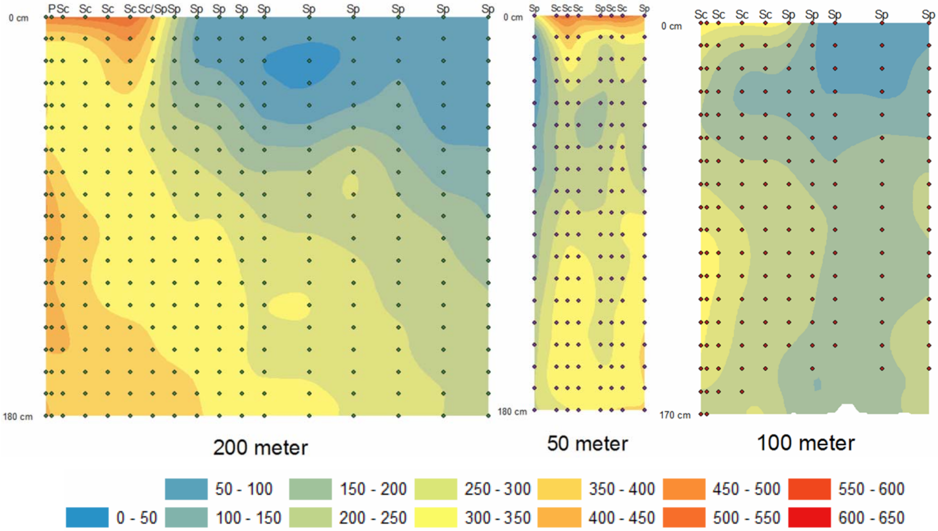


Fig. 7.9. Examples of EC-profiles in depth (0 – 180 cm) in three floating rich fens after a period of heavy rainfall in April/May 2013 (Bresjer 2013): Stobbenribben – fen A (left), Wiedegat (middle) and Kikkerlanden (right). Sc = site dominated by *Scorpidium scorpioides*, P = site dominated by *Calliergonella cuspidata* and Sp = site dominated by *Sphagnum* spp.

(Chapter 2). This is in accordance with studies of Hooijer (1996) and Banach *et al.* (2009) in riverine floodplain fens, who also found absence or limited occurrence of infiltration under waterlogged conditions.

Infiltration of base-rich flooding water is, however, possible under winter conditions, but only if the soil is not entirely waterlogged before flooding. During the field manipulation experiments in winter, performed on a site with little recharge (downward seepage), infiltration only occurred when the initial water table was lower than 5 cm below the soil surface (Chapter 2). Under such conditions, alkalinity and Ca-concentration in soil pore water increased by about 350 and 150 µmol L^{-1} (ratio 2.3:1) in rich fens. This is however a much smaller increase than in summer, when infiltration is enhanced by high evapotranspiration, and alkalinity and Ca-concentration increased by 1900 and 450 µmol L^{-1}. The relatively low increase in ANC during flooding in winter is probably caused by lower temperatures, and subsequently lower evapotranspiration and microbial activity. In *Sphagnum*-dominated fens, alkalinity and Ca-concentration were not increased at all after flooding, even when infiltration occurred, due to low alkalinity and Ca-concentration in the flooding water above *Sphagnum*-dominated peat. These lower concentrations above *Sphagnum*-dominated fens, compared to rich fens, are presumably caused by the longer distance from the ditch and the exchange of Ca^{2+} for H^+ between flooding water and the adsorption complexes of living *Sphagnum*-mosses in this transect.

In contrast to the absence of alkalinization in waterlogged soils during short-term winter flooding of non-floating fens in the field, waterlogged rich fen soils may show an increase in alkalinity, Cl- and Ca-concentrations on the longer term, as shown by the mesocosm experiment (Chapter 3). Although the increase in concentrations was significantly lower under winter than under summer conditions, this indicates that long-term inundation in winter will eventually lead to infiltration of base-rich flooding water into fen soils, even when soils are waterlogged.

Lowered surface water levels

Episodes with lowered water tables will stimulate acidification (the production of H^+ and the decrease of ANC; van Breemen *et al.* 1983) in the upper layer of fen soils as a consequence of aerobic oxidation processes, which are microbial redox processes that use oxygen as a terminal electron acceptor. Whether or not this leads to a change in pH depends on the buffer capacity of the soil. Two weeks of experimental surface water level drawdown in the field (-15 cm) did, however, not affect the pH or alkalinity of soil pore waters (Chapter 2). In floating fens, the alkalinity and pH did not change due to the limited change in water tables, not even after three months of lowered surface water levels (data not shown). In non-floating fens, experimental water level drawdown did lead to an increase of redox potentials (E_h) from around -200 to +500 mV in the upper 5 cm of the soils, which indicates that the lowered water levels led to the intrusion of oxygen into these soils (e.g. Gambrell & Patrick 1978). In *Sphagnum*-dominated fens, oxygen availability in topsoils was already high at the start of the treatment, as shown by the initial E_h-levels of above +600 mV. The presence of oxygen in the upper part of soils of both vegetation types did, however, not lead to decreasing pH-values or alkalinities within two weeks.

However, in the longer-term mesocosm (Chapter 3) and incubation experiments (Chapter 4), aeration (oxygen intrusion) did lead to aerobic acidification. In the mesocosms with rich fen vegetation, low water tables (-15 cm) led to a significant decrease in alkalinity and pH after 5 – 10 weeks, albeit only in the dry moss layer at 1 cm depth and not in the wetter soil layer at 10 cm depth. In the incubation experiment, about 10 weeks of aeration and further desiccation also led to decreased pH-values in rich fens and *Sphagnum*-dominated fens. It must, however, be noted that the pH will most probably increase again during subsequent rise of the water table (Loeb *et al.* 2008a; Mettrop *et al.* unpublished data), but it may affect the vegetation during water table drawdown.

Nutrient dynamics

In addition to sufficient input of base-rich water, concentrations of plant-available nutrients should not be too high in rich fen soils (Kooijman 1993a; Hájek *et al.* 2006; Kooijman & Paulissen 2006). Since a decrease or rise in water level may well influence nutrient availability in peat soils, nutrient dynamics were also examined in this thesis. Fens may vary in their response to different nutrient inputs, and results indicate that edaphic characteristics dictate the nature of nutrient limitation (Chapter 6).

In the fertilization experiment, the floodplain fen with *H. vernicosus* was limited by N and K (Chapter 6). The soil of this fen showed low concentrations of labile N and relatively high concentrations of plant-available P and Fe-bound P, as compared to fens with *S. scorpioides* or *S. cossonii*. These results correspond well with those for fens with *H. vernicosus* in NW-Poland and Czech Republic that also show N-limitation under relatively P- and Fe-rich conditions (Stechová *et al.* 2008, 2012; Pawlikowski *et al.* 2013). Although the precipitation of Fe-phosphates has been reported to diminish P-mobilization in fens (e.g. Zak *et al.* 2004), recent studies show a positive correlation between P-availability and Fe-concentrations in rich fens, indicating that higher Fe-concentrations do not necessarily result in low P-availability (Neijmeijer 2013; Pawlikowski *et al.* 2013; Chapter 6). Apparently, many plants are able to take up this Fe-bound P, as has also been reported by Marschner (1995).

In the floating fen with *S. scorpioides*, in contrast, vascular plant production was clearly limited by P (Chapter 6), which is consistent with the outcomes of other studies in rich fens with *S. scorpioides* (Verhoeven & Schmitz 1991; Kooijman 1993b, 2012; Beltman *et al.* 1996; Boeye *et al.* 1997; Kooijman & Hedenäs 2009; Pawlikowski *et al.* 2013). These P-limited conditions in rich fens with *S. scorpioides* can most likely be explained by significantly lower total P-concentrations and higher total Ca:Fe-ratios in the soil compared to the floodplain fen with *H. vernicosus*, which resulted in low concentrations of plant-available P and Fe-bound P in the soil. The effect of Fe-rich versus Ca-rich soil conditions on the P-availability of different rich fen types should be further studied, as this may imply an important role for sufficient Ca-inputs next to potential buffering effects. In addition to increased vascular plant production, P-fertilization also led to the replacement of *S. scorpioides* by *Calliergonella cuspidata* (Hedw.) Loeske. This effect, which has been described in detail by Kooijman (1993b), can be problematic since even under wet conditions, in which the apices were just above the water table, *C. cuspidata* is more readily overgrown by large acidifying *Sphagnum* spp. than *S. scorpioides* (Kooijman & Bakker 1995).

Apparently, contrasting effects of N-, K- and P-fertilization in different fens are at least partly determined by edaphic differences. The effect of high N- and P-inputs on rich fen vegetation, for instance due to altered surface water level regimes, will thus not only depend on the extent of the input, but also on local soil conditions.

Raised surface water levels

In the field manipulation experiments (Chapter 2), nutrient availability was not affected by two weeks of experimentally raised surface water levels (+10 cm). In both floating and non-floating fens, concentrations of NO_3, NH_4, o-PO_4 and total soluble P were not changed in the soil pore water, not even under flooded conditions in winter or summer. The timespan of two weeks was probably too short to induce P-mobilization. However, during longer-lasting mesocosm experiments with rich fen soils, P-mobilization did occur, but the effects depended on edaphic characteristics (Chapter 3). In strongly P-limited mesocosms with *S. scorpioides*, eight months of inundation had no effect on the P-availability and P-concentrations, possibly due to low total P and high total Fe:P- and Ca:P-ratios in the soil. In the slightly more eutrophic mesocosms with *H. vernicosus*, P-availability and P-concentrations increased significantly after 2 – 3 months of inundation. The latter fen type, in which aboveground biomass production was not strongly limited by P, had relatively low total Fe:P- and Ca:P-ratios in the soil, indicative of higher P-availability.

An additional factor preventing eutrophication in the field experiments were the low concentrations of N and P in the surface water of the adjacent ditches, both in winter and summer (Chapter 2). Studies in riverine fens show, however, that flooding may well result in eutrophication when P- and N-inputs are high (Wassen & Joosten 1996; Mitsch & Gosselink 2007). Several short-term fertilization experiments also show severe impacts on *Scorpidium*-dominated rich fens (Vermeer 1986; Verhoeven & Schmitz 1991; Kooijman 1993b; Boeye *et al.* 1997; Chapter 6). Although future research should focus more on the longer-lasting effects of less extreme nutrient inputs during flooding of both N- and P-limited rich fens, several field observations already indicate that such less extreme, but longer-lasting inputs, may still have severe effects on brownmoss-dominated rich fens. For example, van Wirdum (1991) indicated that local flooding with relatively nutrient-rich water in Stobbenribben (one of the largest and most biodiverse rich fens in National Park Weerribben-Wieden) led to a eutrophic zone with domination of *Calliergonella cuspidata* near the supplying ditches of this rich fen. This *Calliergonella*-zone was larger in the 1970s than in the 1990s (Schouwenberg 2000; Barendregt *et al.* 2004), when part of this eutrophic *Calliergonella*-zone along the ditches was replaced by *S. scorpioides*. These authors attributed this replacement to an improvement of the surface water quality in the supplying ditch. A further reduction of the eutrophic *Calliergonella*-zone, in favor of *Scorpidium*-dominated rich fen vegetation, has occurred in the last decade (Cusell *et al.* 2013).

Lowered surface water levels

Along with the limited change in water table, nutrient concentrations in soil pore water did not change during the field manipulation experiments with lowered surface water levels (-15 cm) in floating fens (Chapter 2), not even after three months of lowered surface water levels (data not shown). There was also no evidence of eutrophication or oligotrophication after two weeks of lowered water levels in non-floating fens.

However, on the longer term, it is likely that lowered water levels will stimulate mineralization rates, as has been recorded in different fen types (e.g. Grootjans *et al.* 1986; Chapter 4). The incubation experiments showed that several weeks of oxygen intrusion (aeration) can indeed lead to increased soil respiration and net N-mineralization in rich fens (Chapter 4). In contrast to rich fen peat, decomposition and net N-mineralization rates were not influenced by oxygen intrusion in *Sphagnum*-peat. This is in accordance to studies of Basiliko *et al.* (2008) and Brouns *et al.* (2014), who also found no change in decomposition rates after aeration of oligotrophic *Sphagnum*-peat. Decomposition of such oligotrophic *Sphagnum*-peat is probably not only limited by oxygen deficiency, but also by litter quality and by the higher concentrations of phenolic compounds than in rich fen peat (van Breemen 1995; Freeman *et al.* 2004). In all fens, aeration is likely to stimulate the activity of phenol oxidase, which is the enzyme involved in the breakdown of phenolic compounds (Freeman *et al.* 2001; Fenner & Freeman 2011). The differences found may be caused by much higher concentrations of phenolic compounds in oligotrophic *Sphagnum*-litter than litter from rich fens, although concentrations in eutrophic *Sphagnum*-peat may also be lower (Brouns et al. 2014).

Without severe desiccation, oxygen intrusion resulted in net P-immobilization, both in *Sphagnum*-dominated fens and rich fens (Chapter 4). The absence of net P-release upon aeration is presumably due to the direct binding of released P after mineralization, for example to oxidized Fe(III) and to Fe(III) oxides and hydroxides (e.g. Patrick & Khalid 1974; Lucassen *et al.* 2005). Severe desiccation (oxygen intrusion plus water shortage), however, resulted in an enormous increase of the net P-release, in both *Sphagnum*-peat and rich fen peat. This was probably caused by microbial mortality. Such an increase in P-availability can have a large impact on the development of fens, especially in P-limited rich fens.

Toxicity

Raised surface water levels

One of the potential negative effects of inundation is sulfide toxicity due to increased SO_4-reduction rates, especially under SO_4-rich conditions (e.g. Lamers *et al.* 1998b). Mesocosm experiments showed that eight months of inundation with SO_4-enriched water (1000 μmol L^{-1}) led to sulfide concentrations of about 100 – 200 μmol L^{-1} in soil pore water of rich fens (Chapter 3), which are toxic levels to several plant species (Lamers *et al.* 2013). These concentrations were not only toxic for *S. scorpioides*, but possibly also contributed to the observed vegetation change from dominance of *Carex* spp. to dominance of *Juncus* and grass spp. This corresponds to findings of Lamers *et al.* (1998b) and Geurts *et al.* (2009), who showed similar changes at sulfide concentrations of respectively 20 and 50 – 150 μmol L^{-1}. When inundation water was SO_4-poor, however, sulfide concentrations remained below 3 μmol L^{-1} (Chapter 3). Similarly, in the field, almost none of the sampled fens showed concentrations above 5 μmol L^{-1} (Chapters 2 and 5), not even during periods with flooding. So, as long as surface waters do not contain high SO_4-concentrations, which is the case for most of National Park Weerribben-Wieden (Chapter 5), sulfide toxicity does not seem to be a major risk during inundation.

In addition to sulfide, NH_4 can also be toxic to bryophytes and vascular plants in rich fens (Verhoeven *et al.* 2011; Chapter 6). Several field studies show, however, that NH_4-concentrations are generally low in soil pore water of rich fens with *Scorpidium* spp., and almost never exceed 100 μmol L^{-1} (e.g. Rochefort & Vitt 1988; Kooijman & Westhoff 1995; Neijmeijer 2013), a concentration above which Paulissen *et al.* (2004) found toxic effects for *S. scorpioides*. This is even the case in Dutch rich fens, exposed to high N-deposition rates of above 20 kg N ha^{-1} $year^{-1}$ (e.g. Paulissen *et al.* 2004, Chapter 5). These low NH_4-concentrations in brownmoss-dominated rich fens are presumably caused by the high pH, which stimulates nitrification (Wild *et al.* 1971).

Flooding with NH_4-rich surface water and/or NH_4-accumulation due to limited nitrification under anaerobic conditions (Williams 1974; Reddy & Patrick 1984) may, however, lead to high NH_4-concentrations in soil pore water of flooded fens. NH_4-concentrations were not increased after two weeks of flooding in the field experiments, both in winter and summer (Chapter 2). In contrast, eight months of inundation did lead to higher NH_4-concentrations of 200 – 300 μmol L^{-1} in mesocosms with rich fen vegetation, but only under winter conditions (Chapter 3). However, brownmosses and vascular vegetation seemed to be unaffected by these increased NH_4-concentrations under winter conditions, probably due to their low metabolism under these conditions. Although anaerobic microbial activity must have been higher under summer conditions (e.g. Dunfield *et al.* 1993), NH_4-accumulation was presumably prevented by increased photosynthesis of *Phragmites australis* (Cav.) Steud., *Carex* and *Juncus* spp. This probably led to increased radial oxygen loss (ROL) from their roots (Gries *et al.* 1990; Visser *et al.* 2000), which stimulated nitrification and prevented NH_4-accumulation in summer. So, even under inundated conditions, NH_4-toxicity appears to be no problem in brownmoss-dominated rich fens.

Lowered surface water levels

NH_4 and sulfide may be detoxified as a result of aerobic oxidation when an episode with low water tables succeeds a period with anaerobic conditions. Although this mechanism has not been studied in this thesis, studies in riverine hay meadows (Loeb *et al.* 2008a), grasslands (van de Riet *et al.* 2013) and rich fens (Mettrop *et al.* unpublished data) show that NH_4-concentrations can indeed decrease during episodes with lowered water tables.

Implications for fen management

Brownmoss-dominated rich fens only persist under well-buffered and rather nutrient-poor conditions. Apart from an adequate mowing regime, managers of rich fens should therefore also focus on the preservation and restoration of these conditions. It is advised to avoid long periods of low surface water levels as much as possible to prevent direct drought effects on plants, soil acidification and eutrophication as a result of higher mineralization. In contrast, the introduction of temporarily raised surface water levels may be beneficial to rich fen areas without groundwater discharge, but nature and water management should pay attention to the following issues:

- The rise in surface water levels must lead to inundation with base-rich water. For this reason, it is important to determine which surface water levels are needed to get inundation of the non-floating and/or floating fens involved.
- The inundation water should not be eutrophic. If nutrient concentrations are too high, several management options can be used to reduce the P-input at the source, such as the disconnection of polders and P-stripping at the periphery of the nature reserve, e.g. at the pumping stations. However, before implementation of such measures, it is of utmost importance to also evaluate the impact on the Ca-input and the base status of the wetland.
- In summer, inundation and flooding seem to be more beneficial than in winter for rich fens, due to (a) higher Ca-concentrations in surface waters near the fens, (b) slightly lower o-PO_4 concentrations in surface waters near the fens and (c) higher evapotranspiration rates, which increase infiltration. Potential effects of drowning should, however, also be taken into account, especially in summer when organisms are active (e.g. Runhaar *et al.* 2004).

General conclusions

My thesis focusses on the potential beneficial and harmful effects of lowered and raised surface water levels on the ANC and nutrient status of brownmoss-dominated rich fens (Fig. 7.2). Temporarily raised surface water levels may have a positive effect on rich fens, but only under specific conditions. First of all, a rise in surface water levels should lead to inundation. Most non-floating fens in National Park Weerribben-Wieden get flooded during surface water level rises of about 15 cm. Field observations suggest that floating *Scorpidium*-dominated fens are also inundated at such surface water levels. Water level rises of about 15 cm do, however, not lead to inundation of most *Sphagnum*-dominated floating fens, since they move up with the surface water level. As a consequence, the ANC and nutrient concentrations will not change in these *Sphagnum*-dominated floating fens.

Secondly, the inundation water must be base-rich. In areas without groundwater discharge, the base-richness of the surface water is therefore an important factor. Under the present conditions in National Park Weerribben-Wieden, Ca-concentrations in the surface water near rich fens are significantly higher in summer than in winter. Model calculations suggest that the evapotranspiration surplus in summer leads to a surface water flow from Ca-sources at the periphery of the nature reserve towards more isolated sites, and thus to large-scale mixing of relatively base-rich water throughout the nature reserve. In contrast, higher surface water levels in winter will very likely lead to a greater rainwater influence at relatively isolated sites, and thus to less influence of base-rich surface water near rich fens.

Thirdly, flooding will only lead to an increase of the ANC when the base-rich inundation water is able to infiltrate into the soil. In winter, when evapotranspiration is low and soils are often waterlogged, infiltration or diffusion of base-rich water is often impossible during short-term inundations of one or two weeks. Under these conditions, infiltration and improvement of buffer capacity will presumably only occur if there is a fair amount of downward seepage. In summer, in contrast, infiltration occurs much easier due to higher evapotranspiration. Higher temperatures in summer will also stimulate the internal alkalinity generation, although such increases in alkalinity will often be temporary, due to acidification during subsequent periods with lower water tables.

Fourthly, inundation water should not contain high nutrient concentrations. Special attention has been paid to processes that determine P-availability in rich fens, since most rich fens in the studied nature reserve are P-limited and should therefore not be inundated with P-rich water. Despite rather high P-inputs by pumping stations at the periphery of the nature reserve, mainly in winter, the current concentrations of o-PO_4 are low throughout the year in the surface water near rich fens. This can be explained by several filtering processes in the nature reserve, including the assimilation and accumulation of organic matter, the sorption of P to Al/Fe-compounds and the precipitation of colloidal Al- and Fe-phosphates. Moreover, model calculations suggest that raised surface water levels will even lead to slightly lower concentrations of o-PO_4 in the surface water near relatively isolated rich fens due to dilution with rainwater. However, inundation may lead to internal P-mobilization in fens with high total P-content and low Fe:P-ratios in the soil, especially during longer periods of inundation.

Although no negative effects were found during two weeks of lowered surface water levels in the field manipulation experiments, longer-term surface water level drawdown in summer may have severe effects in rich fens. In addition to direct drought effects for mosses, soil pH may decrease due to aerobic oxidation. In addition, several weeks of complete desiccation presumably leads to a large net P-release. Moreover, model calculations show that the hypothetical advantages of reduced nutrient inputs during one week of lowered surface water levels in summer are not important in De Weerribben under the present conditions, as a result of low N- and P-concentrations in Vollenhovermeer, which supplies water during such dry episodes. This may, however, be different in other wetlands when the supplied water from outside the reserve contains high nutrient concentrations.

In conclusion, I have been able to unravel the differential effects of water table fluctuation in rich fens in relation to surface water quality, peat quality and season. This thesis not only contributes to more fundamental biogeochemical and ecological issues related to surface water level fluctuations, but is also useful for decision support in the hydrological management of rich fens.

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Appendices

Appendix A – Site conditions during lowered and raised surface water levels in the field manipulation experiments

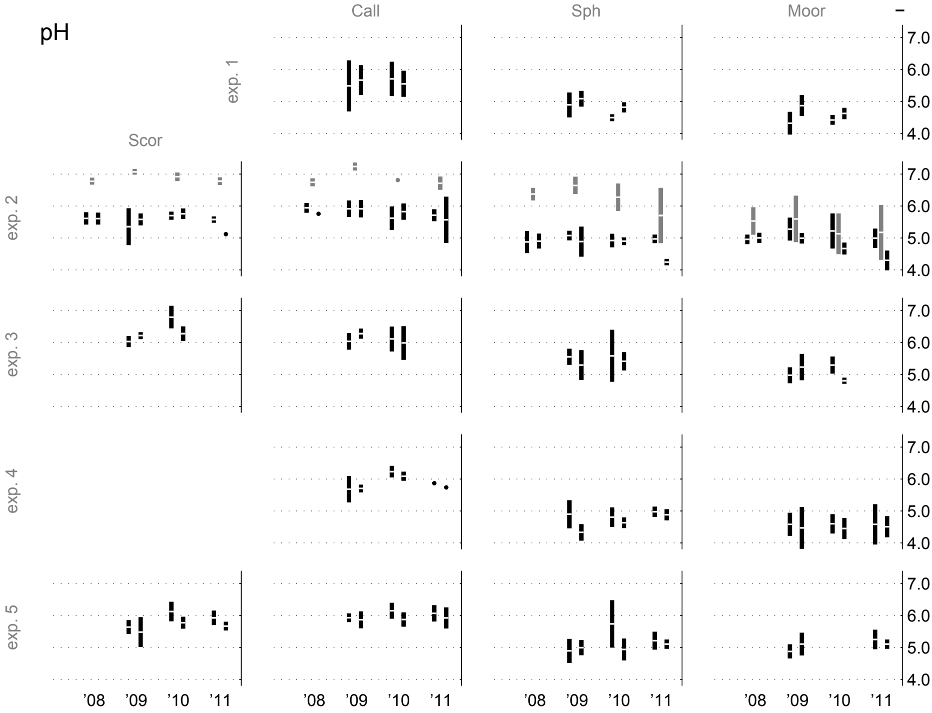


Fig. A1. Effect of five surface water level treatments on the pH in four vegetation types during the monitored years. Water tables were measured two days before (black lines at the left of each triplet), during (grey lines) and two days after the treatments (black lines at the right of each triplet). Sample means (white centers of a line) are given with their standard deviations (n = 5). Scor = fen with a moss-layer dominated by *Scorpidium cossonii* or *Hamatocaulis vernicosus*, Call = fen with a moss-layer dominated by *Calliergonella cuspidata*, Sph = fen with a moss-layer dominated by *Sphagnum palustre*, moor with *Erica tetralix* and *Sphagnum palustre*, exp. 1 = floating WEE-fen during raised surface water levels in winter, exp. 2 = non-floating KW-fen during raised surface water levels in winter, exp. 3 = non-floating VW-fen during raised surface water levels in summer, exp. 4 = floating WEE-fen during lowered surface water levels in summer, exp. 5 = non-floating KW-fen during lowered surface water levels in summer.

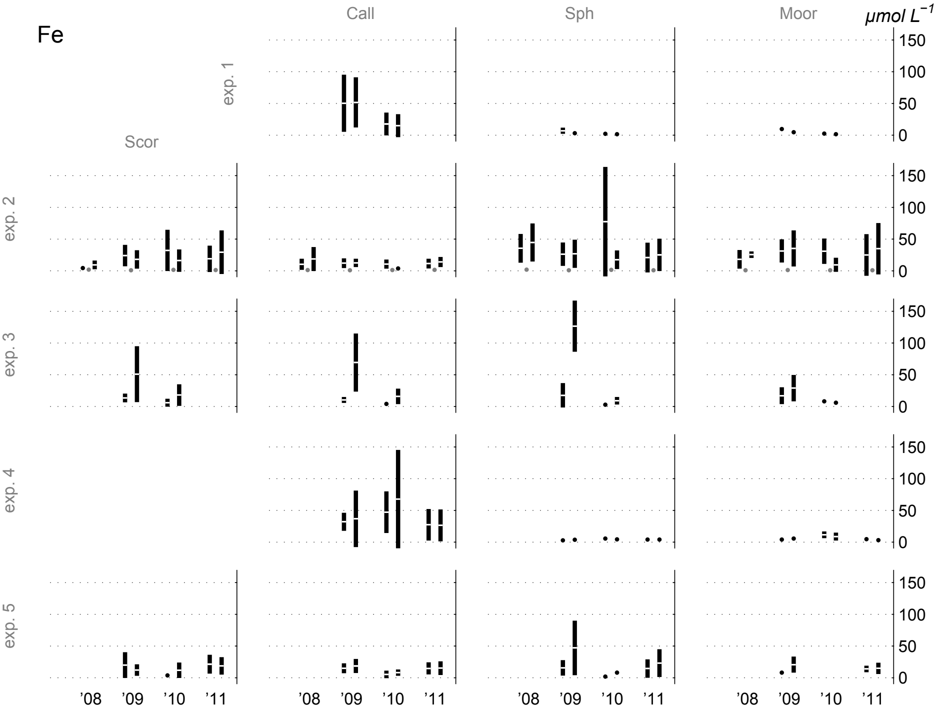


Fig. A2. Effect of five surface water level treatments on the concentration of Fe (μmol L^{-1}) in four vegetation types during the monitored years. Concentrations were measured two days before (black lines at the left of each triplet), during (grey lines) and two days after the treatments (black lines at the right of each triplet). See the caption of Fig. A1 for a description of the abbreviations. Sample means (white centers of a line) are given with their standard deviations (n = 5).

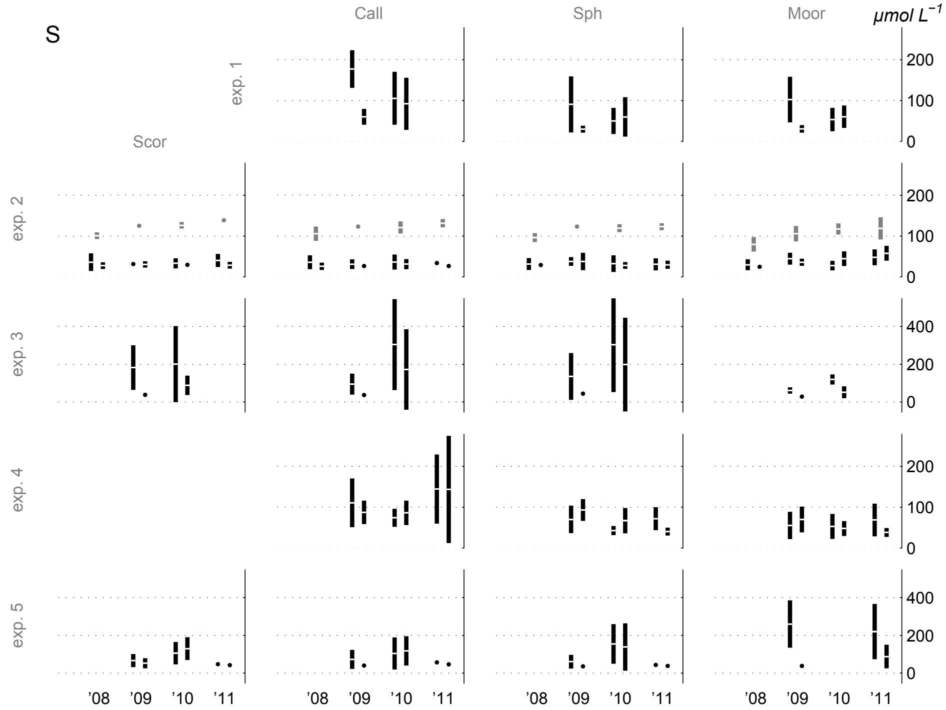


Fig. A3. Effect of five surface water level treatments on the total S-concentration (μmol L^{-1}) in four vegetation types during the monitored years. Concentrations were measured two days before (black lines at the left of each triplet), during (grey lines) and two days after the treatments (black lines at the right of each triplet). See the caption of Fig. A1 for a description of the abbreviations. Sample means (white centers of a line) are given with their standard deviations (n = 5).

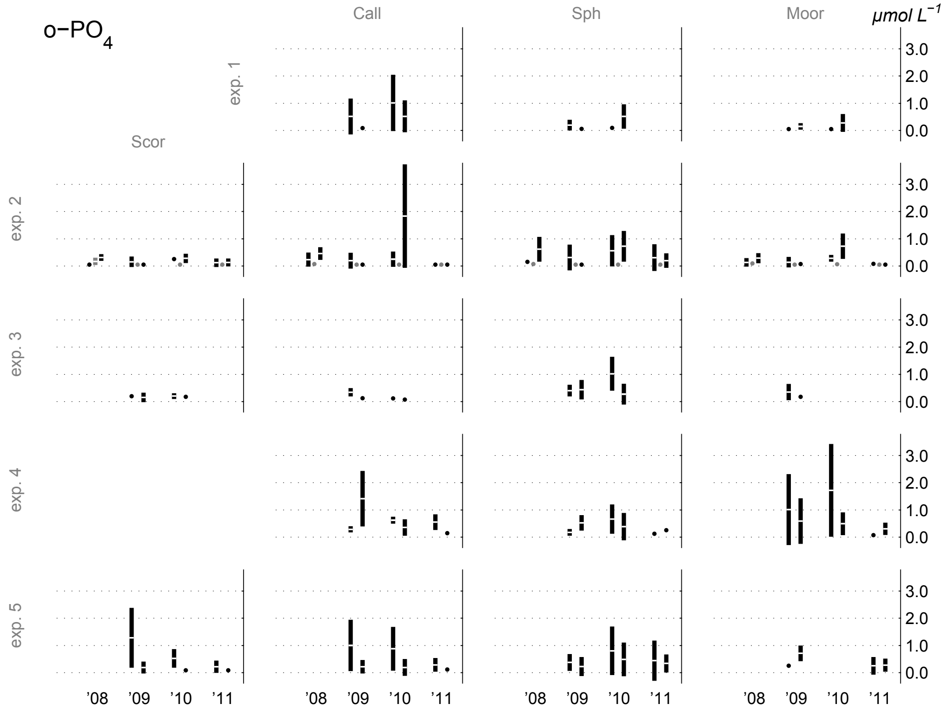


Fig. A4. Effect of five surface water level treatments on the concentration of o-PO_4 (µmol L^{-1}) in four vegetation types during the monitored years. Concentrations were measured two days before (black lines at the left of each triplet), during (grey lines) and two days after the treatments (black lines at the right of each triplet). See the caption of Fig. A1 for a description of the abbreviations. Sample means (white centers of a line) are given with their standard deviations (n = 5).

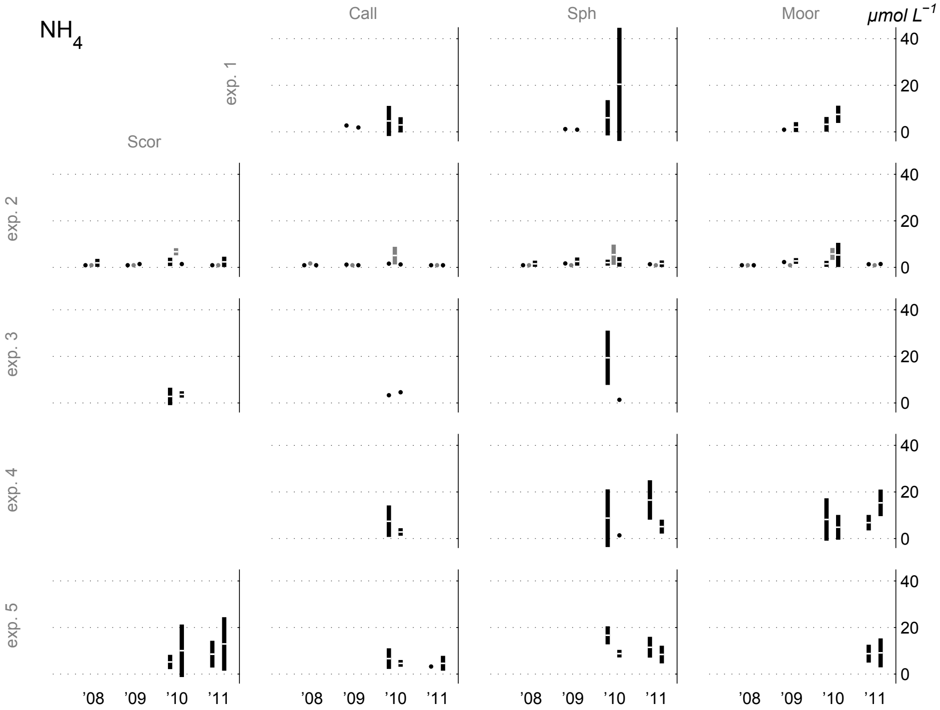


Fig. A5. Effect of five surface water level treatments on the NH_4-concentration (μmol L^{-1}) in four vegetation types during the monitored years. Concentrations were measured two days before (black lines at the left of each triplet), during (grey lines) and two days after the treatments (black lines at the right of each triplet). See the caption of Fig. A1 for a description of the abbreviations. Sample means (white centers of a line) are given with their standard deviations (n = 5).

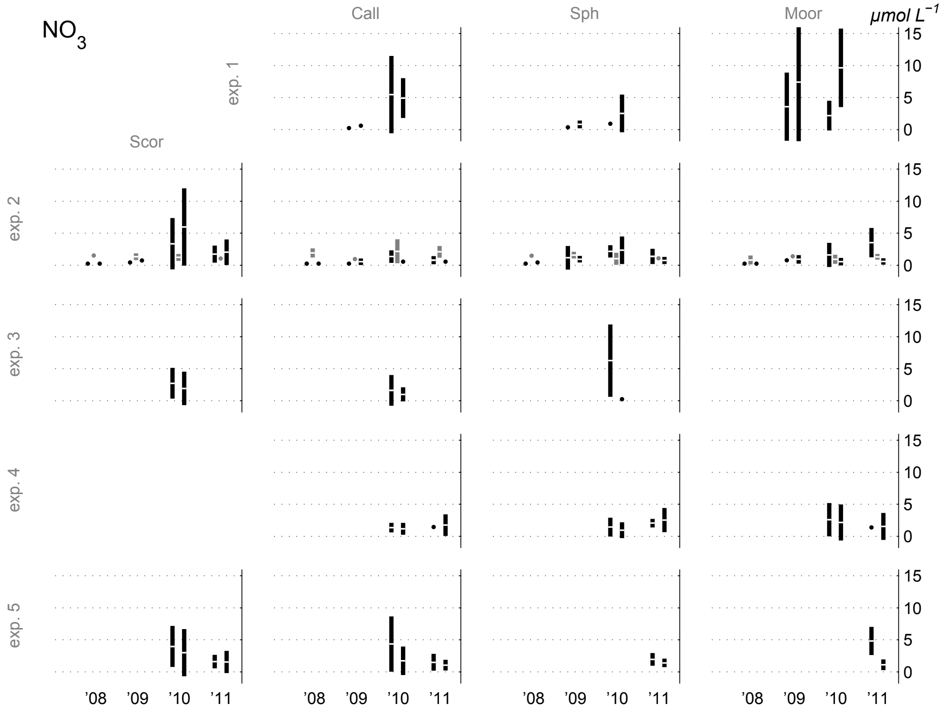
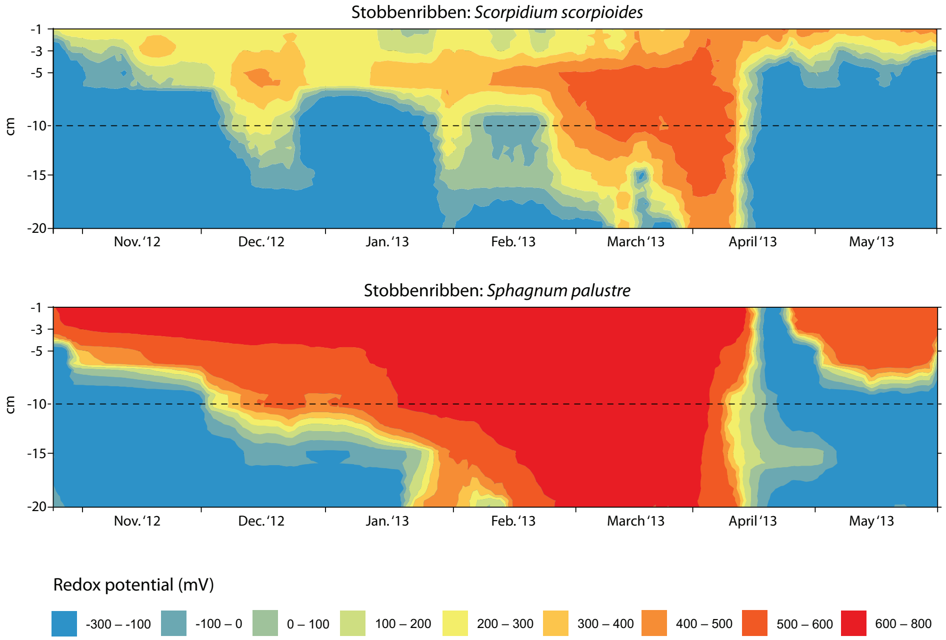


Fig. A6. Effect of five surface water level treatments on the NO_3-concentration (µmol L^{-1}) in four vegetation types during the monitored years. Concentrations were measured two days before (black lines at the left of each triplet), during (grey lines) and two days after the treatments (black lines at the right of each triplet). See the caption of Fig. A1 for a description of the abbreviations. Sample means (white centers of a line) are given with their standard deviations (n = 5).

Appendix B – Redox measurements in Stobbenribben

In both a rich fen and a *Sphagnum*-dominated fen in the area Stobbenribben (52°47’ N, 5°59’ E), 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 the E_h at -1, -3, -5, -10, -15 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 E_h in the upper 10 cm of the soil in both rich fens and *Sphagnum*-dominated 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*-dominated fens the E_h 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 conducted in Chapter 4, are representative and useful for assessing the effects of drought in the field.



Appendix C – Methods and materials SOBEK-model

General description SOBEK-model

SOBEK is a 1D-2D flow-model that suites for the integral simulation of hydrological processes in surface water systems (Deltares 2014). The surface water system is divided in a large number of connected segments, which all have their own length, width and depth. At each time step, all water flows into and out the segment are calculated by solving the full Saint-Venant equations, i.e. in- and outputs from adjacent surface water segments, groundwater discharge or downward seepage, precipitation and evapotranspiration (ARCADIS 2004). At locations where the surface is in contact with surface water from outside the modeled area, boundary flows are also calculated. These boundary flows can be freely flowing, such as in- and outputs of rivers, but can also be regulated, such as pumping stations and sluices. By solving the water flows into and out each segment, the model can calculate water flow directions, water flow speeds and residence times. On top of that, the model labels all incoming boundary flows and precipitation, enabling the calculation of influence-fractions of boundaries at each segment. By multiplying these water fractions with nutrient concentrations at the boundary, nutrient concentrations can be calculated for each segment.

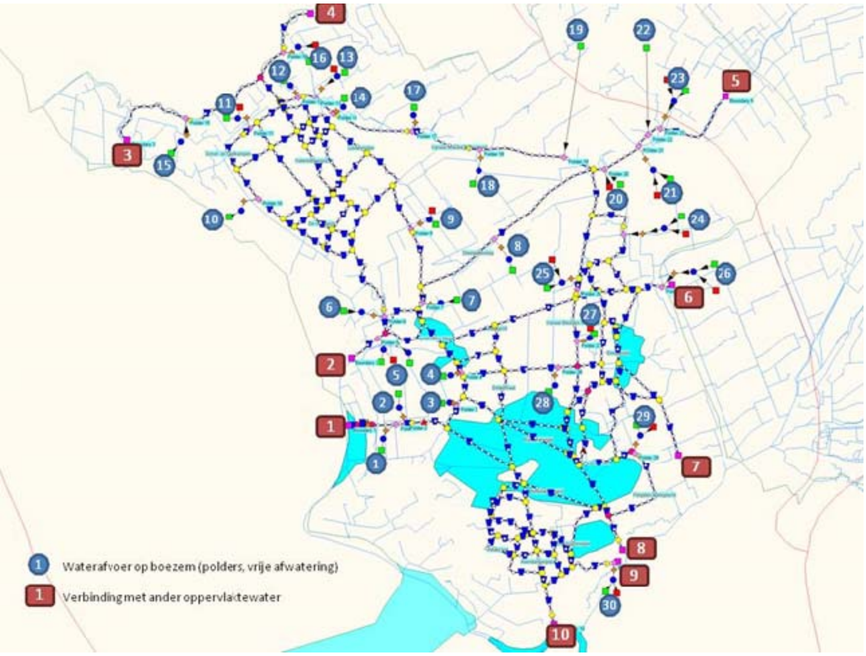


Fig. C1. A scheme of the SOBEK-model of National Park Weerribben-Wieden (between 52°48' N – 5°53' E and 52°38' N – 6°08' E). Small white circles are individual segments, red blocks and blue circles are boundary sites

SOBEK-model for National Park Weerribben-Wieden

An existing SOBEK-model for National Park Weerribben-Wieden (ARCADIS 2004; Fig. C1) was updated to simulate water and nutrient flows in the north of the nature reserve (De Weerribben). The updated model contained 40 boundaries. Most of them were pumping stations from polders (most of the blue circles), whose inputs into the National Park were regulated by the pump capacity and the target water levels. Other boundaries were free drainage from polders (blue circles 19 till 23), sluices (most of the red squares), main pumping station Stroink that regulates the surface water levels in the National Park (red square 1) and the supplying river Steenwijker Aa which forms the main input of water from the adjacent upland (red square 5). The water input through sluices was estimated from their activity, the in- and outputs of pumping station Stroink were regulated by the surface water levels in the National Park, and the input through the Steenwijker Aa was based on overflow measurements.

The model determined the volume of water and the chemical composition of each segment on a daily basis. Therefore, the following input data had to be supplied on a daily basis for each segment of the model (de Lange 2011):

- Precipitation and evapotranspiration in 2003: All model runs were carried out for the hydrological situation of 2003, since all basis data in the existing model originated from this year. 2003 was characterized by a very dry summer and a usual, rather wet winter (KNMI 2014). Evapotranspiration rates were adjusted to specific evaporation factors depending on vegetation, urban circumstances and the presence of open water.
- Rates of local groundwater discharge or downward seepage: Based on Snepvangers *et al.* (2007) rates were given to the segments in the National Park and to all adjacent polders. In contrast to the former model of ARCADIS (2004), in which these rates were constant throughout the nature reserve, rates differed between locations in the new model.
- The sizes of all segments
- The water level regime for the National Park and the adjacent polders: The regimes differed between polders.
- The pump capacity of all pumping stations: The capacities differed between pumping stations. In contrast to the former model of ARCADIS (2004), water inputs from pumping station Stroink during dry episodes were introduced into the model.
- The concentrations of P, N and Ca in precipitation water: Data of Geert Kooijman (2001) from three locations in De Weerribben have been used.
- P, N and Ca in surface waters of all boundaries: To calculate the P-, N- and Ca-concentrations for each segment, concentrations in the surface water of boundaries should be entered into the model. Data of the Water Management Authority Reest & Wieden was used, and supplemented with some personal data. To get representative concentrations per day, data of different years (2003-2010) have been compressed

into one year. Subsequently, equations of polynomial trend lines have been used to calculate the concentrations on a day basis (see an example in Fig. C2). Although more detailed data was used than in the former ARCADIS-model (2004), there were still polders with a lack of data. For these polders, concentrations of P, N and Ca were equal to Wetering (deep polders) or Blankenham (shallow polders).

At the start of each run, all water in each segment was entirely derived from the segment itself. In order to get reasonable influence-fractions for the water in each segment on January 1, it was decided to use the input data of 2003 for two consecutive model years. The results reported are always from the second model year.

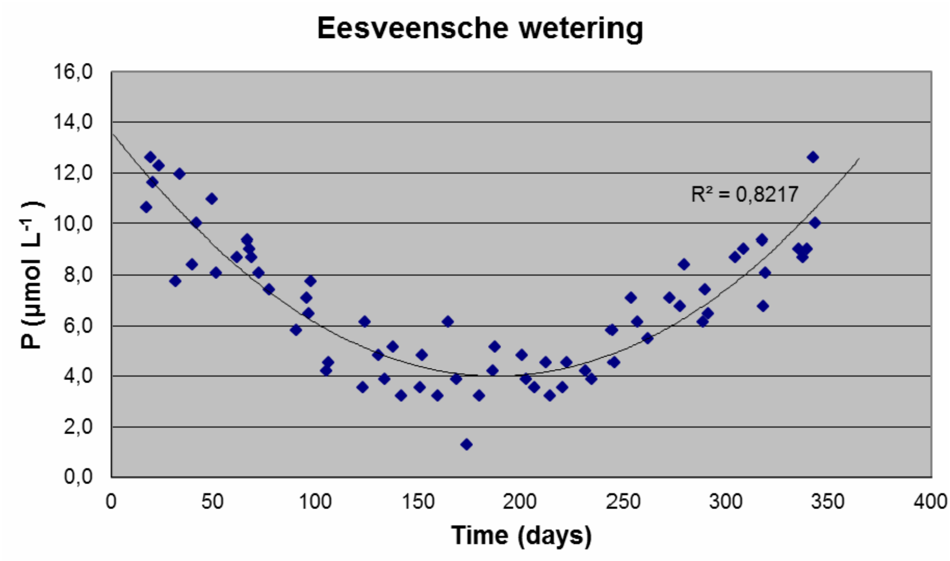


Fig. C2. Total P-concentrations in surface waters of Eesveensche Wetering. Polynomial trend line is plotted on the basis of data from the Water Management Authority Reest & Wieden (2003-2010).

Modeled scenarios

Three scenarios have been run in SOBEK to predict the consequences of a few possible water level regimes, namely:

- Actual surface water level regime: Fluctuation between 0.73 and 0.83 BMSL was allowed, but target levels differed
  - January 1 – March 1: target water level was 0.83 m BMSL.
  - March 1 – April 1: water level rises to 0.73 m BMSL.
  - April 1 – October 25: target water level was 0.73 m BMSL.
  - October 25 – November 15: water level falls to 0.83 m BMSL.
  - November 15 – December 31: target water level was 0.83 m BMSL.

- A regime with short-term periods of lowered and raised surface water levels. The Water Management Authority Reest & Wieden wanted to introduce this regime in 2007. This decision was, however, reversed in court (Raad van State 2007), because potential negative effect were not sufficiently investigated. This SOBEK-run was carried out to determine potential negative and positive effects of such a more fluctuating water regime on the ecohydrological functioning of the entire wetland.
  - January 1 – March 1: target water level is -0.80 m BMSL.
  - March 1 – April 1: water level rises to -0.73 m BMSL.
  - April 1 – May 1: target water level is -0.73 m BMSL.
  - May 1 – September 15: target water level is -0.73 m BMSL, but the water level may decrease twice during this period to -0.88 m BMSL. Each time a maximum of seven days under the water level of -0.83 m BMSL is allowed. Pumping station Stroink gets active after five days under or at the water level of -0.88 m BMSL. It stays active till the target water level of -0.83 m BMSL is reached.
  - September 15 – November 1: target water level is -0.73 m BMSL.
  - November 1 – December 1: target water level is -0.73 m BMSL, but the water level may rise up to -0.63 m BMSL once a year, for a maximum of seven days. Afterwards the target level of -0.73 m BMSL is reached within 14 days.
  - December 1 – January 1: water level falls to -0.80 m BMSL.
- A regime with larger and longer surface water level fluctuations, in order to evaluate long-term effects:
- January 1 – January 1: water level can fluctuate between 0.53 m and 0.93 m BMSL. If these levels are exceeded water inlet or outlet occurred at pumping station Stroink.

Appendix D – Nutrient concentrations in canals between 1981 and 2000

Materials and methods

Monthly surface water data, obtained between 1981 and 2000 (except during ice cover) in six canals, were used to provide insight into the long-term development of the surface water quality. These canals were equally distributed over the nature reserve. Local Water Management Authorities collected the surface water samples in iodated polyethylene bottles of 500 mL at a depth of 10 cm below the water surface. Bottles were transported in dark boxes.

Surface water samples were filtered through a GF/C glass-fiber filter (1.2 μm; Whatmann, Brentford, UK) at the day of sampling, and stored in the dark in iodated polyethylene bottles at 4 °C until further analysis. Filters were stored under similar conditions. Filtered subsamples were used to measure concentrations of o-PO_4 colorimetrically by continuous flow auto-analyzers. Chlorophyll-a concentrations were determined for three canals, as described in section 2.3.1 of the article.

Statistical analyses

Multiple linear regressions in combination with ANOVA were used to determine the statistical significance of trends ($P \leq 0.05$) during the last decades. Seasonal fluctuations were taken into account by using a hierarchical model. The first hierarchical level consisted of the predictor year, the second level consisted of dummy predictors for seasons and the third level consisted of dummy predictors for different canals. Starting from the model with only year, the other levels (respectively seasons and canals) were added and model improvement was evaluated through ANOVA (Davis 2002). A model including all terms appeared to be the most appropriate. The independent predictors were examined for multicollinearity, and collinearity tolerances were acceptable.

Results

Results of the multiple linear regressions are shown in Table S1, which can be found in the Supporting Information of the online article. Differences between winter (blue) and summer (red) measurements are visualized in the figures below.

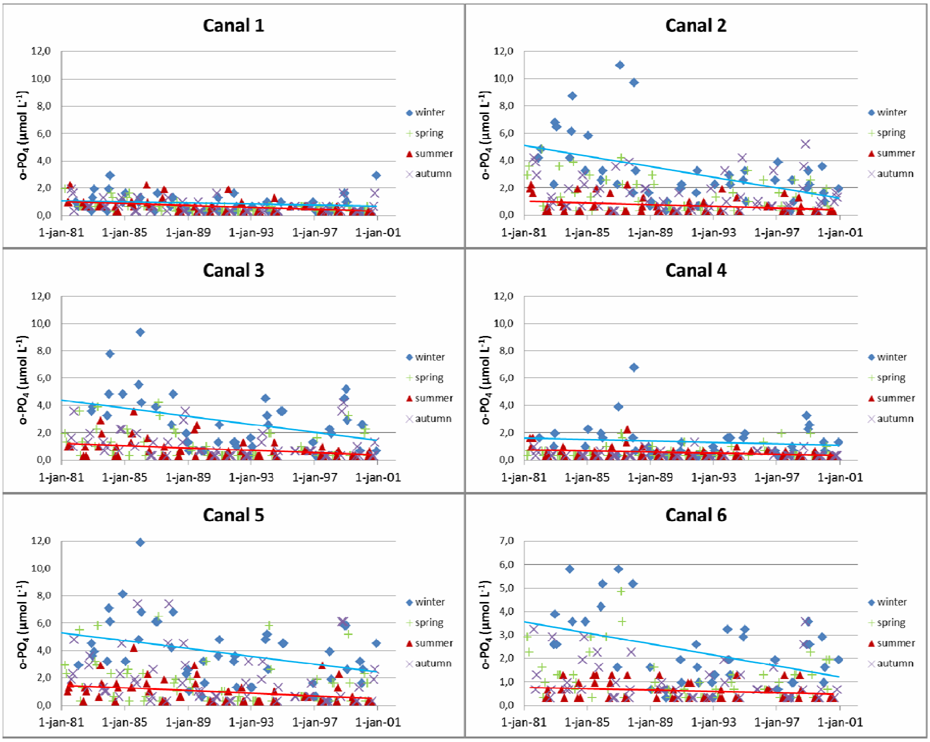


Fig. D1. Concentration of o-PO$_4$ (visualized per season) in the surface waters of six canals between 1981 and 2000. Linear regression lines were calculated for winter (blue) and summer (red) conditions.

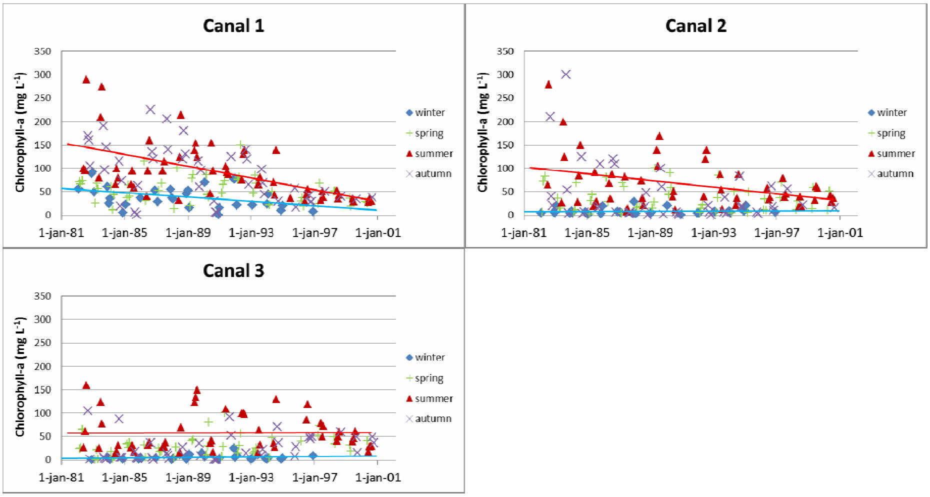


Fig. D2. Concentration of chlorophyll-a (visualized per season) in the surface waters of three canals between 1981 and 2000. Linear regression lines were calculated for winter (blue) and summer (red) conditions.

Summary

Summary

For my thesis, I examined the effects of the re-introduction of fluctuating surface water levels in freshwater wetlands. These systems, and especially the rich fen wetlands that I focused on, often consist of a mosaic of aquatic, semi-aquatic and terrestrial vegetation types, including many biodiverse communities with high numbers of endangered species. Rich fens are protected under the European Habitat Directive (transition mires and quaking bogs, type H7140), and are characteristic for well-buffered (with respect to acidity) and nutrient-poor conditions. Next to overall eutrophication, high NH_4-concentrations may specifically be toxic to bryophytes and vascular plants in rich fens. For the preservation and restoration of rich fens, it is therefore important to prevent acidification, eutrophication and toxicity.

In densely populated, lowland regions that are dominated by agriculture, such as the Netherlands, surface water levels are kept at an almost constant level throughout the year. Under such conditions, the re-introduction of more fluctuating surface water levels has been propagated to improve water quality in wetlands and to counteract potential effects of acidification, eutrophication and toxicity in fens. Lowered as well as raised surface water levels may, however, also stimulate these adverse processes. Therefore, the main objective of this thesis has been to determine the potential beneficial and harmful effects of lowered and raised surface water levels on the biogeochemical and ecological functioning of fens, with a strong emphasis on the preservation and restoration of endangered brownmoss-dominated rich fens. The studies have been performed in the Dutch National Park Weerribben-Wieden, which is a large and protected Ramsar freshwater wetland in the Netherlands, in which groundwater discharge hardly occurs. Studies have been carried out at two spatial scales: at the regional level of the entire wetland as well as at the level of site conditions of plants.

Chapter 2 The biogeochemical effects of short-term (two weeks) surface water level rises (+ 10 cm; during winter and summer) and drawdowns (-15 cm; during summer) were tested in large-scale field manipulation experiments in floating and non-floating fen sites. The results suggest that two weeks of summer drought do not have a severe effect on biogeochemical conditions, both in floating and non-floating fens.

Two weeks of raised surface water levels may, on the other hand, counteract acidification in base-rich fens, but only if this leads to flooding and infiltration into the peat soil. Flooding only occurred in non-floating fens, and was absent in floating fens. However, flooding in non-floating fens did not automatically result in an increase of the acid neutralization capacity (ANC) of the soil. In most winters, infiltration of base-rich flooding water did not occur, because soils were already waterlogged

before the rise of the surface water level. The ANC increased much easier during summer, because higher temperatures and subsequent higher evapotranspiration led to enhanced infiltration of base-rich flooding water. In addition, internal alkalinity generation was higher due to higher microbial activity in the soil. Furthermore, low N- and P-concentrations in the flooding water at the field locations prevented any eutrophication during the floodings, both during winter and summer. In conclusion, the field experiments suggest that temporary increases of surface water levels may be a suitable management tool to enhance the ANC in non-floating fens, especially in summer, as long as nutrient concentrations are not too high in the flooding water and infiltration is possible.

Chapter 3 Since longer periods of inundation and water level drawdown could not be tested in the field, mesocosm experiments have been performed in the laboratory. The impact of long-term (31 weeks) lowered and raised water levels on the biogeochemistry and vegetation of rich fen mesocosms were tested under winter and summer conditions. The results show that long episodes with lowered water levels will lead to decreased alkalinity due to aerobic oxidation processes in the moss layer. In addition, lowered water levels hampered the vitality of brownmosses in rich fens, especially under summer conditions. Long lasting periods with low water levels therefore seem to be harmful to rich fen vegetation.

In contrast, long-term inundation had a positive effect on rich fens by increasing the ANC due to microbial reduction processes and infiltration of base-rich water. The effect was significantly larger under summer conditions, which is presumably caused by the larger evapotranspiration and higher microbial activity. P-rich fens should, however, be handled with caution, since internal P-mobilization may occur during long-term inundations in soils that contain high total P-content and low Fe:P-ratios. Although inundation also resulted in the accumulation of NH_4, the occurrence of NH_4-toxicity was unlikely because concentrations only increased under winter conditions, when plants are less sensitive. Under summer conditions, radial oxygen loss (ROL) from active plant roots presumably stimulated nitrification and prevented NH_4-accumulation.

Chapter 4 A long-term laboratory incubation experiment was performed, to test the effects of (a) anaerobic, waterlogged conditions, (b) aerobic, moist conditions which are characteristic for aeration (oxygen intrusion) and (c) aerobic, desiccated conditions (oxygen intrusion plus water shortage). Mineralization and acidification rates were studied in peat from *Sphagnum*-dominated poor fens and rich fens. Under anaerobic conditions, net N-mineralization rates were much higher in the acid *Sphagnum*-peat than in the base-rich peat from rich fens, while gross N-mineralization rates were significantly higher in the rich fen peat. This is caused by higher microbial N-demand and N-immobilization in rich fen peat. The response upon aeration differed greatly between rich fen peat and *Sphagnum*-peat. In rich fen peat, aeration resulted in a significant decrease of the pH. In addition, aeration led to increased net N-mineralization rates in the rich fen peat, but not in *Sphagnum*-peat. The absence of aeration effects in *Sphagnum*-peat indicates that mineralization rates are more strongly determined by litter quality than by oxygen intrusion. Upon further desiccation, net P-release, which remained unchanged upon aeration, increased significantly in peat from both fen types, probably due to microbial mortality and/or a change in microbial composition. 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, suggest that prolonged periods with drought have severe effects, especially on rich fens.

Chapter 5 To gain more insight into the effect of more fluctuating water levels and related nutrient characteristics at a wetland landscape scale, chemical speciation and binding of relevant nutrients have been analyzed in surface waters, soils and plants along a gradient from water entry locations to more isolated rich fens. Based on plant N:P-ratios, P-availability was lowest in relatively isolated rich fens, which were characterized by P-limited conditions. P-limitation can persist here despite relatively high P-inputs at the peripheral entry locations, because only a small part of these inputs reaches the more isolated waters and fens. This pattern in P-availability is mainly caused by the precipitation of Fe-phosphates, which primarily occurs close to entry locations as indicated by high concentrations of Fe- and Al-bound P in the sub-aquatic sediments near pumping stations, and by biological sequestration, which occurs throughout the wetland as indicated by equal concentrations of organic P in all sub-aquatic sediments. Therefore, these results clearly show that the periphery of large wetlands act as an efficient P-filter, sustaining the necessary P-limitation in more isolated parts.

Chapter 6 To test the nature of nutrient limitation in relation to soil characteristics, fertilization experiments with N, P and K were carried out in different rich fen types. In this way potential eutrophicating effects of flooding with nutrient-rich water can be coupled to specific nutrients involved. There were strong contrasts among the responses of the three rich fens. A floodplain fen with *Hamatocaulis vernicosus* showed no response to P-addition, but N- and K-addition led to grass encroachment and decline of moss cover and species richness. In contrast, a floating fen with *Scorpidium scorpioides* was clearly limited by P, while vascular plant production in a non-floating fen with *Scorpidium cossonii* was co-limited by N and P. In the latter two fens, P-fertilization also resulted in the replacement of *Scorpidium* spp. by *Calliergonella cuspidata*, which may be problematic since *C. cuspidata* is easily overgrown by large acidifying *Sphagnum* spp. The cover of *Scorpidium* spp., however, also declined after extreme N-addition, which was presumably caused by NH_4-toxicity.

These striking contrasts among fens corresponded with clear edaphic differences. The N-limited *Hamatocaulis*-fen showed low total Ca:Fe-ratios and labile

N-concentrations in the soil, and high concentrations of plant-available P and Fe-bound P. The P-limited conditions in rich fens with *Scorpidium* spp. is presumably caused by the significantly higher total Ca:Fe-ratios in the soil compared to the N-limited floodplain fen with *Hamatocaulis vernicosus*, which resulted in low concentrations of plant-available P and Fe-bound P in the soil. Although precipitation of Fe-phosphates is reported to diminish P-availability in fens, this study shows a positive correlation between P-availability and Fe-concentrations in rich fen soils, indicating that higher Fe-concentrations do not necessarily result in lower P-availability, as plants are able to mobilize this Fe-bound P. In conclusion, the experiment clearly shows that edaphic characteristics dictate the nature of nutrient limitation in rich fens.

Chapter 7 This synthesis integrates and discusses all results and management implications of the previous chapters, with the emphasis on the potential beneficial and harmful effects of lowered and raised surface water levels on the ANC and nutrient status of brownmoss-dominated rich fens. Since brownmoss-dominated rich fens only persist under well-buffered and rather nutrient-poor conditions, managers of rich fens should focus on the preservation and restoration of these conditions. Although no negative effects were found during two weeks of lowered water levels in field experiments, it is nevertheless recommended to prevent prolonged periods with low water levels as much as possible. If soils become too dry, net N-mineralization will increase and pH will decrease due to aerobic oxidation processes. In addition, several weeks of full desiccation will presumably lead to drought stress in *Scorpidium*-mosses and to a large net P-release.

Raised surface water levels, on the other hand, may be beneficial to rich fens, but only under specific conditions. Firstly, a rise in surface water levels should lead to actual inundation with base-rich water. In National Park Weerribben-Wieden, most non-floating fens and *Scorpidium*-dominated floating fens get flooded during surface water level rises of about 15 cm. Similar rises do, however, not lead to inundation of most *Sphagnum*-dominated floating fens, since they migrate with the surface water levels. Secondly, inundation water should not contain too much nutrients. If surface water is eutrophic, the type of nutrient limitation will determine the effect of specific nutrient inputs on rich fens. Thirdly, the base-rich and nutrient-poor inundation water must be able to infiltrate into the soil. In winter, when evapotranspiration is low and soils are generally waterlogged, infiltration is often impossible. In summer, infiltration of Ca-rich water and internal alkalinity generation are much more likely due to higher temperatures and evapotranspiration.

As a general conclusion, I have been able to unravel the differential effects of water table fluctuation in rich fens in relation to surface water quality, peat quality and season, by the combination of correlative field research, large-scale and smaller scale field manipulation experiments, climate room and peat incubation experiments. In this way, my thesis not only contributes to more fundamental biogeochemical and ecological issues related to surface water level fluctuations, but can also be used for decision support in the hydrological management of rich fens.

Nederlandstalige samenvatting

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In deze thesis staat het effect van de invoering van flexibelere waterstanden op Nederlandse laagvenen centraal, waarbij vooral is gekeken naar de effecten op soorten- en basenrijke trilvenen. Basenrijk trilveen is een beschermd habitattype (overgangs- en trilvenen; H7140) dat veel rode lijst soorten bevat, waaronder het karakteristieke mos Rood schorpioenmos. Deze trilvenen komen onder goed gebufferde (wat betreft pH) en nutriëntarme condities voor. Verder kunnen veel mossen en vaatplanten in trilvenen slecht tegen hoge NH_4-concentraties. Voor het behoud en herstel van beschermde trilvenen is het dus van belang om verzuring, eutrofiering en NH_4-toxiciteit te voorkomen.

In dichtbevolkte en laaggelegen gebieden met veel landbouw, zoals Nederland, zijn de waterstanden in het oppervlaktewater vaak streng gereguleerd, waardoor deze vrijwel niet fluctueren. Onder deze omstandigheden wordt het invoeren van een meer flexibel peilregime, met lagere waterstanden in de zomer en hogere waterstanden in de winter, vaak als oplossing gezien voor de ecologische ontwikkeling van veengebieden. De waterkwaliteit zou verbeteren en de verzuring, eutrofiëring en toxiciteit van veenkragges zou worden tegengegaan. In theorie kunnen verlaagde en verhoogde waterstanden echter ook nadelig effecten hebben, en juist tot verzuring en eutrofiëring leiden. Het bepalen van de potentiële voor- en nadelen van verlaagde en verhoogde waterpeilen voor het biogeochemisch en ecologisch functioneren van venen is dan ook het belangrijkste doel van dit onderzoek, waarbij vooral is gekeken naar de effecten op beschermde basenrijke trilvenen.

De verschillende studies zijn in het Nederlandse Natura2000-gebied “Nationaal Park Weerribben-Wieden” uitgevoerd. Dit is een groot laagveengebied waar nauwelijks kwel van grondwater optreedt. Tijdens de onderzoeken is zowel gekeken naar invloeden op standplaatscondities als op de regionale schaal van het gehele laagveengebied.

Hoofdstuk 2 De biogeochemische effecten van kortdurende (twee weken) peilverhogingen (+ 10 cm; in de winter en zomer) en -verlagingen (-15 cm; in de zomer) zijn in grootschalige veldexperimenten getest. Dit is gedaan op locaties met drijvende en niet-drijvende venen.

Zowel in drijvende als niet-drijvende venen leiden twee weken durende zomerdroogtes waarschijnlijk niet tot significante biogeochemische veranderingen. Daarentegen kunnen twee weken met verhoogde waterstanden wel degelijk een effect hebben. Door de lage N- en P-concentraties in het oppervlaktewater van de onderzoeksgebieden is er tijdens de peilverhogingen nergens eutrofiëring opgetreden, maar er is in sommige gevallen wel een aanrijking van basen waargenomen, waardoor de zuur neutraliserende capaciteit (ZNC) van de veenbodem toenam. Dit gebeurt echter alleen als de peilverhoging leidt tot overstroming met basenrijk oppervlaktewater, en dit overstromingswater ook daadwerkelijk kan infiltreren.

Overstroming trad echter alleen op in de niet-drijvende venen, en dus niet in de drijvende onderzoeksgebieden (die grotendeels gedomineerd werden door veenmossen). Toch nam ook in niet-drijvende venen de ZNC tijdens de meeste peilverhogingen in de winter niet toe, doordat de kragges al waterverzadigd waren voor de peilverhoging en het basenrijke overstromingswater dus niet kon infiltreren. In de zomer kon het overstromingswater wel infiltreren door de hogere evapotranspiratiesnelheden, waardoor de ZNC makkelijker toenam. Bovendien was de interne alkaliniteitsproductie tijdens overstromingen in de zomer hoger door de hogere microbiële activiteit in de kragges.

Hoofdstuk 3 Aangezien er in het veld geen langere peilwijzigingen uitgevoerd konden worden, zijn er mesocosmos-experimenten in het laboratorium uitgevoerd. Het effect van langdurige (31 weken) peilverlagingen en -verhogingen op de biogeochemie en vegetatie van trilvenen is zowel onder winter- als zomercondities onderzocht.

Uit de mesocosmos-experimenten bleek dat langdurige peilverlagingen leiden tot een afname van de buffercapaciteit (alkaliniteit) in de moslaag door aerobe oxidatie processen. Deze peilverlagingen leidden ook tot een vitaliteitafname van trilveenmossen, zoals Rood en Geel schorpioenmos, vooral bij zomercondities. Het lijkt dan ook verstandig om langdurige peilverlagingen nabij trilvenen zo veel mogelijk te voorkomen.

Daarentegen hadden langdurige inundaties een positief effect op trilvenen, doordat de ZNC toenam als gevolg van microbiële reductieprocessen en infiltratie van basenrijk inundatiewater. Het effect was significant groter onder zomerse dan winterse condities, wat waarschijnlijk veroorzaakt werd door de hogere evapotranspiratiesnelheden en microbiële activiteit. Bij P-rijke venen moet echter wel worden opgepast met inundaties, omdat langdurige inundatie van kragges met hoge totaal P-concentraties en lage Fe:P-ratios kan leiden tot P-mobilisatie, en dus tot eutrofiëring. Tenslotte leidden de langdurige peilverhogingen niet tot NH_4-toxiciteit.

Hoofdstuk 4 Om de effecten van verschillende vocht- en aeratietoestanden op de mineralisatie- en verzuringsnelheden in veenmos- en schorpioenmos-gedomineerde veen te bepalen, is een langdurig (ca. 9 weken) incubatie-experiment uitgevoerd in het laboratorium. In het experiment is gekeken naar de gevolgen van (a) anaerobe waterverzadigde condities, (b) aerobe veldvochtige condities (alleen zuurstof toetreding) en (c) aerobe uitgedroogde condities (zuurstof toetreding en watertekort).

Onder anaerobe condities was de netto N-mineralisatie veel hoger in het zure veenmosveen dan in het basenrijke schorpioenmosveen, terwijl de bruto N-mineralisatie juist significant hoger was in het basenrijke veen. Dit werd veroorzaakt door de hogere microbiële N-immobilisatie in het schorpioenmosveen. Ook de reactie op aeratie en verdere uitdroging verschilde sterk tussen beide veentypen. In schorpioenmosveen leidden beide behandelingen tot een daling van de pH en een toename van de netto N-mineralisatie, terwijl dit niet het geval was in het veenmosveen. Het uitblijven van effecten in het veenmosveen komt waarschijnlijk doordat de mineralisatie van dit veen sterker wordt bepaald door de strooiselkwaliteit dan door de beschikbaarheid van zuurstof.

Aeratie onder veldvochtige omstandigheden had geen effect op de o-PO_4 concentraties in het veen, maar verdere verdroging leidde wel tot een sterke toename van de P-beschikbaarheid in zowel veenmosveen als schorpioenmosveen. Watertekort leidde vermoedelijk tot sterfte van micro-organismen en/of een verschuiving in de microbiële populatie. Zowel deze toename in P-beschikbaarheid bij vergaande verdroging, als de verzuring en de toename van de netto N-mineralisatie bij aeratie van schorpioenmosveen, doen sterk vermoeden dat langdurige droogtes ernstige effecten kunnen hebben op veensystemen, en vooral op trilvenen.

Hoofdstuk 5 Om het effect van flexibelere waterstanden niet alleen op het standplaatsniveau maar ook op landschappelijke schaal te kunnen begrijpen, is er onderzoek gedaan naar de chemische speciatie en binding van alle relevante nutriënten die de N- en P-beschikbaarheid in het Nationaal Park Weerribben-Wieden zouden kunnen beïnvloeden. Over een gradiënt van poldergemalen tot meer geïsoleerde locaties, waar ecologisch goed ontwikkelde petgaten en trilvenen liggen, is gekeken naar de verschijningsvormen van deze nutriënten. Dit is zowel in het oppervlaktewater, het bodemvocht, de bodem als de vegetatie gedaan.

De N:P-ratio's van de vaatplanten lieten zien dat de P-beschikbaarheid het hoogst was nabij poldergemalen en in de grote kanalen van het Nationaal Park en het laagst in de relatief geïsoleerde trilvenen. Hier was sprake van P-gelimiteerde condities. Deze afname van de P-beschikbaarheid is mogelijk door neerslag van Fe-fosfaten, wat vooral nabij poldergemalen optreedt, zoals blijkt uit de hoge concentraties aan Fe- en Al-gebonden P in deze onderwaterbodems. Ook treedt overal in het Nationaal Park biologische vastlegging van P op. De resultaten uit dit onderzoek tonen dus aan dat de buitenrand van grote veengebieden kan functioneren als een efficiënte nutriëntenfilter in gebieden die te maken hebben met (behoorlijk) hoge P-inputs, waardoor er wel degelijk P-gelimiteerde trilvenen kunnen voorkomen op relatief geïsoleerde locaties.

Hoofdstuk 6 Om de potentiële effecten van nutriëntrijk overstromingswater te bepalen, zijn bemestingsexperimenten uitgevoerd in drie basenrijke venen met verschillende bodems: een drijvend trilveen met Rood schorpioenmos, een niet-drijvend veen met Groen schorpioenmos en een kleirijk veen met Geel schorpioenmos op een overstromingsvlakte. In dit onderzoek is gekeken naar het verband tussen de effecten van nutriëntentoevoeging (N, P, K en combinaties) en de bodem.

P-toevoeging had geen effect op het kleirijke veen met Geel schorpioenmos, maar N- en K-toevoeging leidden tot vergrassing en een afname van de mosbedekking en de soortenrijkdom. Het drijvende trilveen met Rood schorpioenmos bleek daarentegen duidelijk P-gelimiteerd te zijn, en reageerde dan ook juist sterk op P-toevoeging, terwijl de biomassaproductie in het niet-drijvende veen met Groen schorpioenmos gestuurd werd door co-limitatie van N en P. In deze laatste twee venen leidde P-toevoeging niet alleen tot een verhoogde biomassaproductie, maar werden de schorpioenmossen ook vervangen door Gewoon puntmos. In deze venen nam de bedekking van schorpioenmossen overigens ook af in de subplots met N-toevoeging. In dit geval kwam dit echter niet door een verhoogde biomassaproductie, maar waarschijnlijk door NH_4-toxiciteit.

De verschillende reacties op toevoeging van nutriënten tussen de drie venen kwamen overeen met duidelijke verschillen in de bodemsamenstelling. De bodem van het NK-gelimiteerde veen met Geel schorpioenmos werd gekenmerkt door lage totaal Ca:Fe-ratios en concentraties aan labiel N, terwijl de concentraties Fe-gebonden P en plantbeschikbaar P hoog waren. De P-limitatie in het trilveen met Rood schorpioenmos is waarschijnlijk juist veroorzaakt door relatief hoge totaal Ca:Fe-ratios in de bodem, waardoor de concentraties aan Fe-gebonden P en beschikbaar P laag waren. Hoewel binding van P aan Fe vaak als positief wordt beschouwd, omdat de P-beschikbaarheid zou afnemen, liet deze studie echter een significant positief verband zien tussen de P-beschikbaarheid en de Fe-concentraties in bodems. Dit komt zeer waarschijnlijk doordat veel planten in staat zijn om Fe-gebonden fosfaten te mobiliseren, en op te nemen.

Hoofdstuk 7 In de synthese zijn alle resultaten van het onderzoek samengevoegd en bediscussieerd, waarbij vooral is gekeken naar de potentiële effecten van verlaagde en verhoogde oppervlaktewaterstanden op de ZNC en nutriëntenstatus van schorpioenmos-gedomineerde basenrijke venen. Verder wordt ingegaan op de gevolgen van het voorliggende onderzoek voor het natuurbeheer. Aangezien schorpioenmosvenen alleen voorkomen onder voldoende gebufferde (wat pH betreft) en tamelijk nutriëntarme condities, is vooral gekeken naar het effect van verschillende waterstanden op deze condities.

Hoewel er tijdens de twee weken durende peilverlagingen in het veldexperiment geen negatieve effecten zijn opgetreden, worden langdurige periodes met verlaagde waterstanden toch afgeraden. Bij langdurigere aeratie van kragges zal de netto N-mineralisatie waarschijnlijk toenemen, en kan er verzuring optreden. Daarnaast kan verdergaande verdroging leiden tot droogtestress bij schorpioenmossen, en tot een sterke toename van de hoeveelheid beschikbaar P in de kragges.

Verhoogde waterstanden kunnen daarentegen een positief effect hebben op basenrijke venen, maar alleen onder specifieke condities. Ten eerste zou een verhoging van de waterstand moeten leiden tot inundatie met basenrijk water. In Nationaal Park Weerribben-Wieden raken de meeste niet-drijvende venen, maar ook de drijvende schorpioenmosvenen overstroomd als de oppervlaktewaterstand ca. 15 cm stijgt. Soortgelijke stijgingen leiden echter niet tot inundaties bij de drijvende veenmosgedomineerde venen (die het meest voorkomen in het natuurgebied), doordat deze kragges met het waterpeil meebewegen. Ten tweede mag het inundatiewater niet te veel nutriënten bevatten. Als het oppervlaktewater eutroof is, zal het effect afhangen van het type nutriëntlimitatie in het basenrijke veen en de samenstelling van het eutrofe water. Ten derde moet er wel infiltratie van het basenrijke en nutriëntarme inundatiewater optreden. In de winter, wanneer er weinig verdamping optreedt en de meeste kraggebodems al waterverzadigd zijn, is infiltratie vaak onmogelijk. In de zomer is de kans op infiltratie van basenrijk water en interne microbiële alkaliniteitsproductie echter een stuk groter door de hogere temperaturen en evapotranspiratiesnelheden.

Uiteindelijk is het dus gelukt om de verschillende biogeochemische effecten van flexibelere waterstanden op basenrijke venen te bepalen d.m.v. een combinatie van correlatief veldonderzoek, grote en kleine veldexperimenten, en incubatie- en kolomexperimenten in het laboratorium. De resultaten van deze onderzoeken hebben niet alleen bijgedragen aan de kennisontwikkeling over fundamentele biogeochemische en ecologische vragen omtrent het laten fluctueren van oppervlaktewaterstanden nabij basenrijke venen, maar vormen ook een leidraad voor het beheer hiervan.

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Dankwoord

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