



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.

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## 1. 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, as a result of 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 ammonium 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 towards a more acidic but less biodiverse, *Sphagnum*-dominated poor fen vegetation (Sjörs, 1950). Species-rich rich fens also require phosphorus (P) poor conditions (Kooijman and Paulissen, 2006) to prevent accelerated succession and outcompetition of rare species by graminoids (Kooijman, 1993; Cusell et al., 2013a).

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., accepted for publication). The subsequent mobilization of accumulated P may lead to additional eutrophication (Patrick and Khalid, 1974), especially under sulfate ( $\text{SO}_4$ ) rich conditions. Under anaerobic conditions, the reduction of iron (Fe) and  $\text{SO}_4$  leads to mobilization of Fe-bound orthophosphate (Caraco et al., 1989), and high  $\text{SO}_4$ -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 sub-aquatic sediments, with decreasing concentrations from water entry locations and main canals towards more isolated sites (van Wirdum, 1979; Lijklema, 1980; Craft and 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.

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.

## 2. Material and methods

### 2.1. Site description

National Park Weerribben-Wieden is a Ramsar site in the central part of the Netherlands (between 52°48' N–5°53' E and 52°38' N–6°08' E; Fig. 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 18 kgN ha<sup>-1</sup> year<sup>-1</sup> (1300 mol ha<sup>-1</sup> year<sup>-1</sup>; 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 Zuyderzee) in the west and moraine upland in the east (Haans and Hamming, 1962). This wetland changed dramatically between the 17th and 20th century due to 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 Zuyderzee were reclaimed and drained to develop agricultural polders (van Wirdum, 1990). 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, 1990). 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.

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 2000 and 2012, shows that the water input of the present wetland consists for 37% of rain water, for 15% of water from the adjacent upland and for 48% of water from lower lying agricultural polders (Cusell et al., 2013b). 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 and manure, 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, 1990). Previously, a 1D–2D model for surface water flows (SOBEK; Deltare systems, Delft, the Netherlands & ARCADIS, Apeldoorn, the Netherlands) was used to determine water flow patterns in National Park Weerribben-Wieden throughout the year (Cusell et al., 2013b). This model was based on

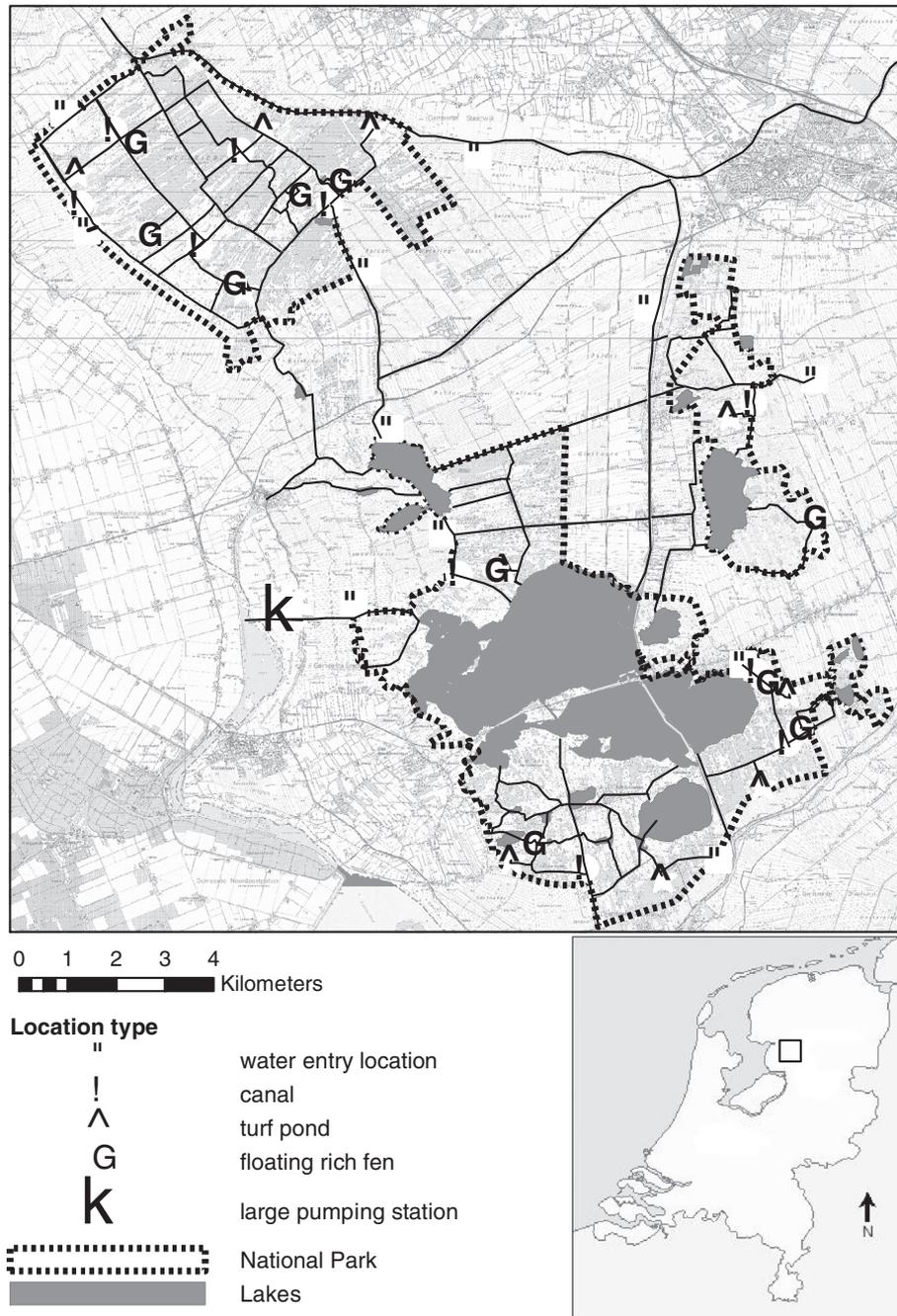


Fig. 1. Location and delimitation of the nature reserve Weerribben-Wieden. The position of the four different location types is indicated with different symbols.

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 (see Figs. S1 and S2 in Supplementary Information). 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, 1990) 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.

## 2.2. 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* species 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. 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  $\text{SO}_4$ -concentrations on P-availability, samples were taken in the relative  $\text{SO}_4$ -rich western part of the nature reserve, where the former inland sea bordered the wetland, as well as in the relative  $\text{SO}_4$ -poor eastern part.

### 2.3. 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 sites were selected, with five sites 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).

#### 2.3.1. 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 0.01 M HCl down to pH 4.2. Turbidities of surface water samples were measured in NTU with a turbidimeter (Toho-Dentan, Tokyo, Japan), and samples were filtered through GF/C glass-fiber filters ( $\phi = 1.2 \mu\text{m}$ ; Whatmann, Brentford, UK). During the August sampling, concentrations of chlorophyll-a were determined spectrometrically after extracting the filter residues with ethanol (Nusch and Palme, 1975).

Subsequently, all water samples were divided into two subsamples, and 1% of concentrated  $\text{HNO}_3$  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  $\text{NH}_4$ ,  $\text{NO}_3$ ,  $\text{o-PO}_4$  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 ortho-phosphates ( $\text{o-PO}_4$ ). Ca and Cl-data were used to calculate the Ionic Ratio, which is equal to  $2 * [\text{Ca}] / (2 * [\text{Ca}] + [\text{Cl}])$  and can be regarded as an index for the relative importance of groundwater versus rainwater (van Wirdum, 1990).

#### 2.3.2. 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  $\text{NH}_4$ ,  $\text{NO}_3$  and  $\text{o-PO}_4$  were determined, as described in Section 2.3.1.

#### 2.3.3. 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.

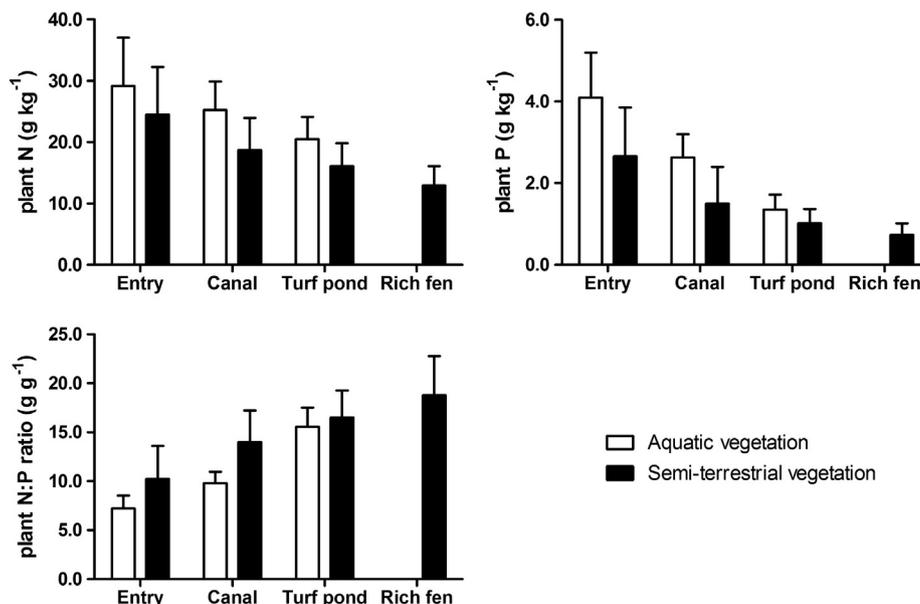


Fig. 2. N, P and N:P ratios in the tissue of aquatic vegetation (not present in rich fens) and aboveground semi-terrestrial vegetation. Sample means are given for western and eastern locations together, and error bars indicate standard deviations ( $n = 10$ ). Statistical information is given in Table 1.

**Table 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. Bold indicates  $p \leq 0.05$ . Different letters indicate significant differences between gradient positions for a particular variable, as determined by Tukey HSD post-hoc tests ( $n = 5$ ;  $p \leq 0.05$ ). See Fig. 2 for effect sizes of the plant N:P ratios.

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

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<sub>3</sub> (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.

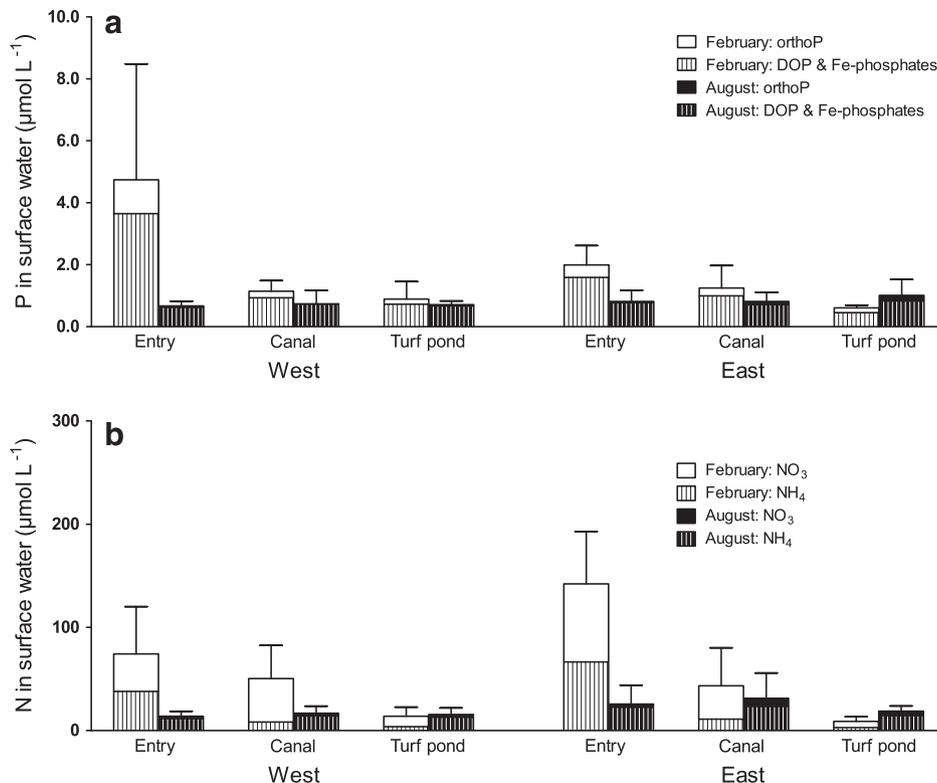
Concentrations of Fe- and Al-bound P ( $P_{FeAl}$ ) and Ca-bound P ( $P_{Ca}$ ) were determined according to the sequential extraction method of

Golterman (1996). After this sequential extraction, the remaining residue was digested with HNO<sub>3</sub> 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 ( $Fe_{ox}$  and  $Al_{ox}$ ) and  $Al_{ox-pyr}$  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).

### 2.3.4. 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 bank, 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* species 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 soil concentrations by microwave digestion and CNS-analysis, as described in Section 2.3.3. Plant N:P ratios were calculated for the aquatic and



**Fig. 3.** Concentrations of o-PO<sub>4</sub> and soluble non-reactive P (NRP) (a), and NH<sub>4</sub> and NO<sub>3</sub> (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 given in Table 2.

semi-terrestrial vegetation on each site. Ratios in the semi-terrestrial vegetation were used as a bioassay indicator to determine which of both nutrients may limit plant growth (Koerselman and Meuleman, 1996; Güsewell, 2004). N:P ratios around  $15 \text{ g g}^{-1}$  ( $33 \text{ mol mol}^{-1}$ ) are indicative of balanced conditions, ratios of  $10 \text{ g g}^{-1}$  and lower suggest N-limitation, and ratios around  $20 \text{ g g}^{-1}$  or higher indicate P-limitation.

#### 2.4. 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

**Table 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. Bold indicates  $p \leq 0.05$ . Different letters indicate significant differences between gradient positions for a particular variable, as determined by Tukey HSD post-hoc tests ( $n = 5$ ;  $p \leq 0.05$ ). The Ionic Ratio is equal to  $2 * [\text{Ca}] / (2 * [\text{Ca}] + [\text{Cl}])$ . See Figs. 3, 4 and 5 for effect sizes.

	Gradient	East/west	Season	Gradient * Season	Entry	Canal	Turf pond
<i>Surface water</i>							
Turbidity	<b>0.000</b>	0.105	<b>0.000</b>	<b>0.023</b>	b	b	a
pH	0.587	0.868	<b>0.000</b>	0.978	a	a	a
Alkalinity	<b>0.000</b>	<b>0.011</b>	<b>0.020</b>	0.825	c	b	a
Ca	<b>0.002</b>	0.316	<b>0.005</b>	<b>0.015</b>	b	a	a
Fe	<b>0.000</b>	0.592	<b>0.000</b>	<b>0.000</b>	b	a	a
Al	<b>0.013</b>	0.683	<b>0.000</b>	<b>0.037</b>	b	ab	a
o-PO <sub>4</sub>	<b>0.018</b>	0.735	<b>0.000</b>	<b>0.001</b>	b	ab	a
NRP	<b>0.000</b>	0.250	<b>0.001</b>	<b>0.000</b>	b	a	a
total P	<b>0.000</b>	0.539	<b>0.000</b>	<b>0.000</b>	b	a	a
S	<b>0.005</b>	<b>0.003</b>	<b>0.012</b>	<b>0.030</b>	b	a	a
NO <sub>3</sub>	<b>0.004</b>	0.280	<b>0.000</b>	<b>0.001</b>	b	b	a
NH <sub>4</sub>	<b>0.000</b>	0.142	<b>0.016</b>	<b>0.000</b>	b	a	a
inorganic N	<b>0.000</b>	0.110	<b>0.002</b>	<b>0.000</b>	b	b	a
Ionic Ratio	<b>0.016</b>	0.201	<b>0.000</b>	<b>0.033</b>	b	ab	a
Chlorophyll-a	0.924	<b>0.004</b>	–	–	a	a	a
<i>Soil pore water</i>							
pH	<b>0.008</b>	0.261	<b>0.000</b>	0.742	b	b	a
Alkalinity	<b>0.006</b>	<b>0.001</b>	0.457	0.687	b	b	a
Ca	<b>0.000</b>	0.066	0.286	0.484	b	b	a
Fe	<b>0.001</b>	0.055	0.448	0.141	b	b	a
Al	0.310	0.068	0.052	0.921	a	a	a
o-PO <sub>4</sub>	<b>0.036</b>	<b>0.000</b>	0.482	0.405	ab	b	a
NRP	<b>0.002</b>	<b>0.001</b>	<b>0.036</b>	0.092	b	b	a
total P	<b>0.001</b>	<b>0.000</b>	<b>0.038</b>	0.671	b	b	a
S	0.433	<b>0.012</b>	0.168	0.822	a	a	a
Sulfide	0.068	0.904	<b>0.000</b>	0.268	a	a	a
NO <sub>3</sub>	0.794	0.594	0.102	0.452	a	a	a
NH <sub>4</sub>	<b>0.029</b>	<b>0.032</b>	0.950	0.957	b	b	a
inorganic N	0.191	<b>0.007</b>	0.829	0.792	a	a	a
Fe:o-PO <sub>4</sub>	<b>0.037</b>	<b>0.000</b>	0.147	0.246	b	ab	a

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.

### 3. Results

#### 3.1. N- and P-concentrations in plants

The 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. 2; Table 1). P-concentrations differed, however, more than N-concentrations. This led to different plant N:P ratios in the semi-terrestrial vegetation: from around  $10 \text{ g g}^{-1}$  near water entries to around  $15 \text{ g g}^{-1}$  in turf ponds and  $19 \text{ g g}^{-1}$  in rich fens. A similar gradient of increasing plant N:P ratios was found for the aquatic vegetation, although ratios of around  $8 \text{ g g}^{-1}$  near water entries and canals were lower than in the semi-terrestrial vegetation.

#### 3.2. P-fractions in floating rich fens

The high plant N:P ratios of about  $19 \text{ g g}^{-1}$  in rich fens were in accordance with soil proxies for P-availability in floating rich fens, such as low o-PO<sub>4</sub> concentrations of  $0.3\text{--}1.2 \mu\text{mol L}^{-1}$  in the soil pore water and low concentrations of plant-available P (P-Olsen) of  $3\text{--}8 \text{ mmol m}^{-2}$ . Total P-concentrations in floating fen soils were around  $100 \text{ mmol P per m}^2$ , of which 55% was non-labile organic P, 30% was Ca-bound P ( $P_{\text{Ca}}$ ) and only 15% was Fe- or Al-bound P ( $P_{\text{FeAl}}$ ). Hence, the  $P_{\text{Ca}}:P_{\text{FeAl}}$  ratio was rather high:  $2 \text{ mol mol}^{-1}$ . 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.

#### 3.3. Spatial nutrient distribution from water entry locations to turf ponds

##### 3.3.1. Surface water gradients: February vs. August

Measurements in February showed significantly higher o-PO<sub>4</sub> and total soluble P-concentrations in the surface waters of entry locations than in turf ponds (Fig. 3; Table 2). Values for o-PO<sub>4</sub> were rather low at all sites, with concentrations below  $1 \mu\text{mol L}^{-1}$ . Total soluble P-concentrations were higher, with levels up to  $5 \mu\text{mol L}^{-1}$  near water entry locations. Concentrations of soluble non-reactive P (NRP), including dissolved organic phosphorus (DOP) and small ( $<1.2 \mu\text{m}$ ) colloidal Fe-phosphates, were therefore about 5 times larger than concentrations of o-PO<sub>4</sub>. In August, concentrations of all P-fractions in the surface water

**Table 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  $2 * [\text{Ca}] / (2 * [\text{Ca}] + [\text{Cl}])$ . 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 <sub>4</sub>	−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 <sub>3</sub>	0.121	0.562	0.138	0.285
NH <sub>4</sub>	−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

\*  $p \leq 0.1$ .

\*\*  $p \leq 0.05$ .

did, however, not differ between water entries, canals and turf ponds. Concentrations of total P and o-PO<sub>4</sub> were low throughout the nature reserve, and chlorophyll-a concentrations were almost everywhere below 20 µg L<sup>-1</sup>.

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 concentrations of o-PO<sub>4</sub>, soluble non-reactive P (NRP) and total soluble P in the surface water on the other hand (Table 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<sup>-1</sup> and 15–25 NTU, compared to 20–30 µmol L<sup>-1</sup> and 3–5 NTU in turf ponds (Figs. 3 & 4; Table 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. 4; Table 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<sup>-1</sup> 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<sup>-1</sup>, 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<sup>-1</sup> respectively, while significantly higher concentrations of above 30 and 500 µmol L<sup>-1</sup> were measured near water entry locations in February. High S-concentrations were only found at the western side of the nature reserve.

3.3.2. Soil pore water gradients

Total soluble P and o-PO<sub>4</sub> concentrations in the soil pore water decreased significantly from water entries to turf ponds (Fig. 5; Table 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<sub>4</sub> concentrations, resulting in significantly lower Fe:o-PO<sub>4</sub> ratios in turf ponds than near water entries.

Soil pore water concentrations of o-PO<sub>4</sub> were below 3 µmol L<sup>-1</sup> in the east, while much higher concentrations of 5–8 µmol L<sup>-1</sup> were found in the west (Fig. 5; Table 2). Since soluble Fe-concentrations did not differ between the west and east, significantly lower Fe:o-PO<sub>4</sub> ratios of 1–40 mol mol<sup>-1</sup> were found in the west than in the east, where ratios of 40–300 mol mol<sup>-1</sup> were found. Low Fe-concentrations in the soil pore water of turf ponds resulted in the lowest Fe:o-PO<sub>4</sub> ratios of

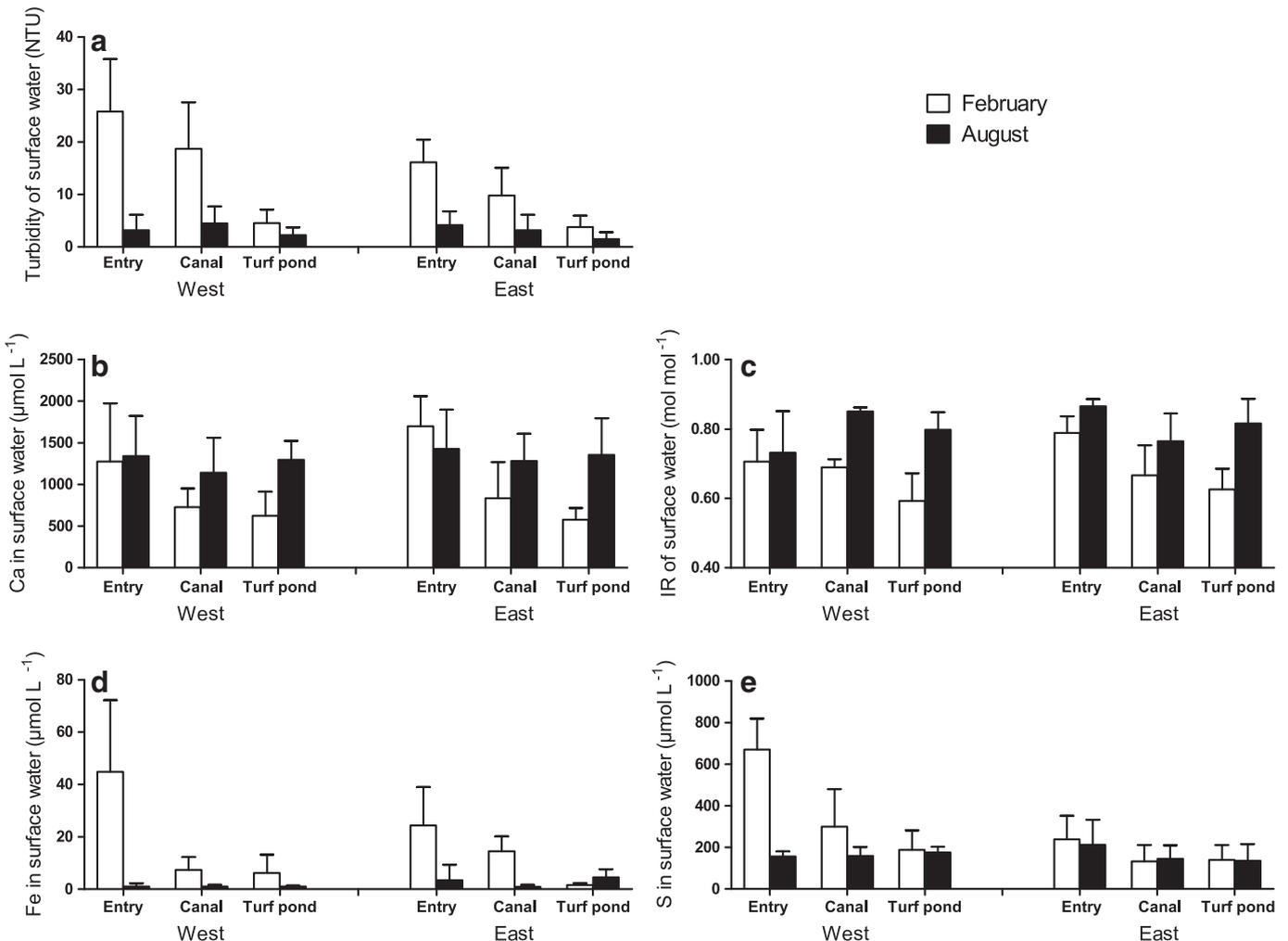
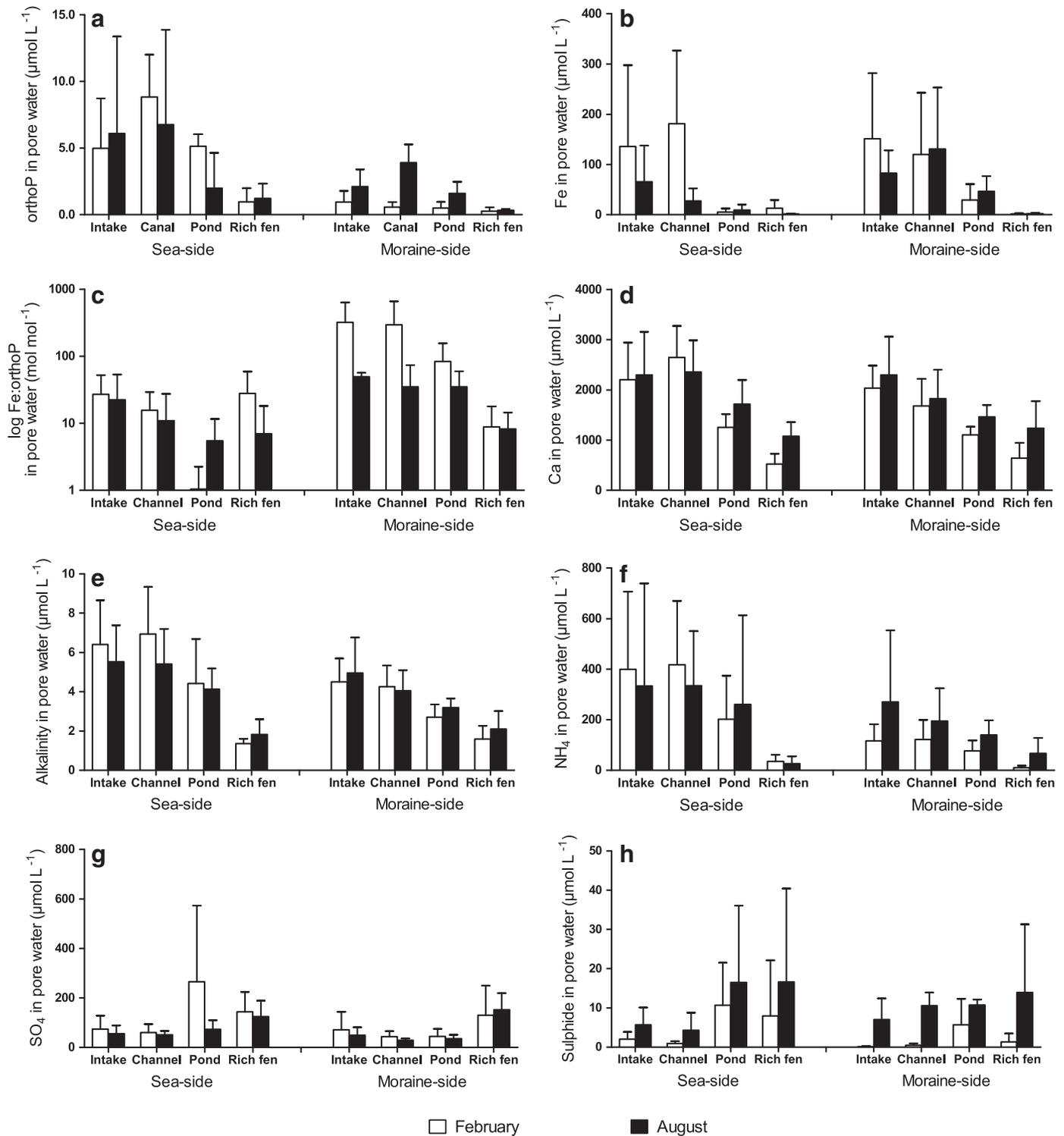


Fig. 4. Turbidity (a), Ca-concentration (b), Ionic Ratio (c), and concentrations of Fe (d) and S (e) in the surface water of entry locations, canals and turf ponds, in February and August. The Ionic Ratio is equal to 2 \* [Ca] / (2 \* [Ca] + [Cl]). Sample means are given and error bars indicate standard deviations (n = 5). Statistical information is given in Table 2.

1–8 mol mol<sup>-1</sup> in western turf ponds. Also, alkalinity of soil pore water differed significantly between the west and east, with values of 4–7 mmol<sub>c</sub> L<sup>-1</sup> in the west and 3–5 mmol<sub>c</sub> L<sup>-1</sup> in the east. High NH<sub>4</sub><sup>+</sup> concentrations, up to levels of 400 μmol L<sup>-1</sup>, 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<sup>-1</sup> throughout the nature reserve.

### 3.3.3. Soil quality gradients

Total P-concentrations in sub-aquatic sediments were significantly higher near eastern water entry locations than in turf ponds (Fig. 6; Table 4) with 200–700 versus 100–200 mmol m<sup>-2</sup>, 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<sup>-1</sup> throughout the area.



**Fig. 5.** Concentrations of o-PO<sub>4</sub> (a) and Fe (b), Fe:o-PO<sub>4</sub> ratios (c), Ca (d), alkalinity (e), and concentrations of NH<sub>4</sub> (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<sub>4</sub> ratios are given on a log-transformed scale. Sample means are given and error bars indicate standard deviations (n = 5). Statistical information is given in Table 2.

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 ( $\text{Al}_{\text{ox-pyr}}$  and  $\text{Fe}_{\text{ox-pyr}}$ ), while turf ponds were dominated by organically bound Al and Fe ( $\text{Al}_{\text{pyr}}$  and  $\text{Fe}_{\text{pyr}}$ ) (Fig. 7; Table 4). The selective extractions of P showed a similar pattern (Fig. 6). 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 ( $P_{\text{Ca}}$ ) and especially Fe- and Al-bound P ( $P_{\text{FeAl}}$  and  $P_{\text{ox}}$ ) than the turf pond soils. The  $P_{\text{Ca}}:P_{\text{FeAl}}$  ratios were rather low in all sub-aquatic sediments with values between 0.1 and 0.3 mol mol<sup>-1</sup>.

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. 8; Table 4). Values decreased from 15–80 mmol m<sup>-2</sup> (with a median of 35) near water entries to 5–8 mmol m<sup>-2</sup> 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 ( $P_{\text{FeAl}}$  and  $P_{\text{ox}}$ ). P-Olsen and plant N:P ratios are less correlated with Ca-bound P, while organic P ( $P_{\text{org}}$ ) did not correlate at all (Table 5).

## 4. Discussion

### 4.1. 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<sup>-1</sup>, to turf ponds with

dense stands of *S. aloides*, where ratios of 15 g g<sup>-1</sup> were found. Clear P-limited conditions, however, only occurred in rich fens with *Scorpidium*-species, as indicated by semi-terrestrial plant N:P ratios of around 19 g g<sup>-1</sup> (Koerselman and Meuleman, 1996; Güsewell, 2004). These P-limited conditions correspond with conditions found for other rich fens (Bedford et al., 1999; Kooijman and Hedenäs, 2009).

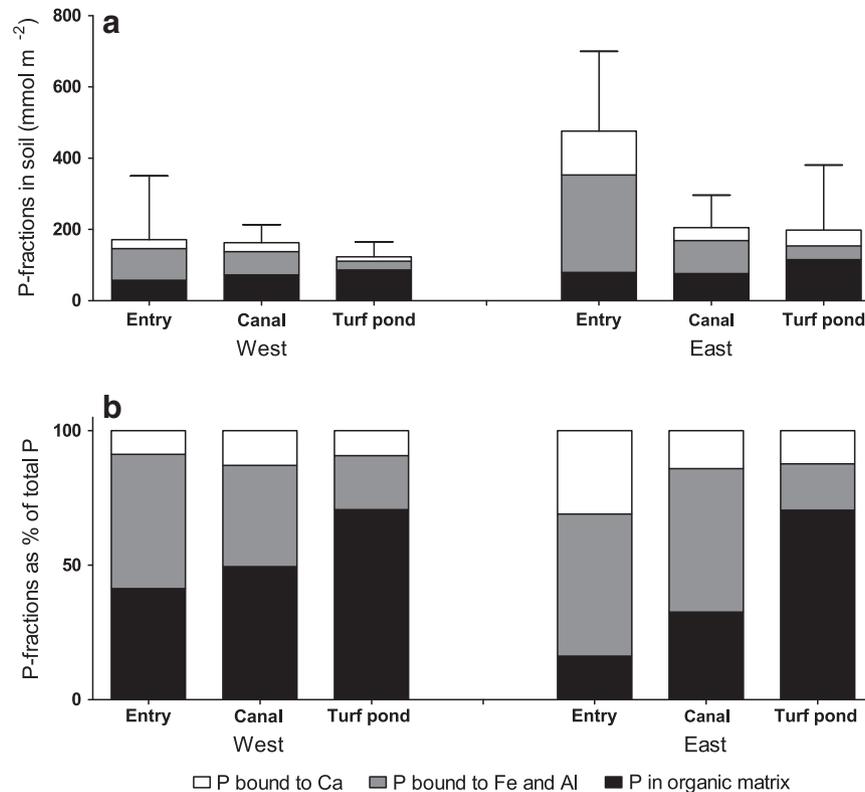
P-Olsen, a soil proxy for plant P-availability, also decreased along this gradient. Concentrations of plant-available P were very low in turf ponds and floating rich fens, with values of 3–8 mmol m<sup>-2</sup>, given the fact that Lamers et al. (accepted for publication) defined a critical concentration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

### 4.2. 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. 9). The combined examinations of surface waters, soil pore waters and sub-aquatic sediments enabled a much better understanding of the filtering mechanisms involved.

#### 4.2.1. Variations in surface water quality

N and P-concentrations in the surface water were higher near water entry locations than in turf ponds, as a result of high nutrient concentrations in the agricultural polder water. Such nutrient gradients have also been found in other studies (van Wirdum, 1979; Craft and 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



**Fig. 6.** 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 m<sup>2</sup> in the uppermost 10 cm of the soil, while relative values to total P are given in graph (b) (n = 5). Statistical information is given in Table 4.

**Table 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. Bold indicates  $p \leq 0.05$ . Different letters indicate significant differences between gradient positions for a particular variable, as determined by Tukey HSD post-hoc tests ( $n = 5$ ;  $p \leq 0.05$ ). See Figs. 6, 7 and 8 for effect sizes of the Fe- and P-fractions.

	Gradient	East/west	Gradient * East/west	Entry	Canal	Turf pond
<i>Totals</i>						
Ca <sub>t</sub>	0.173	0.181	0.791	a	a	a
Fe <sub>t</sub>	<b>0.047</b>	0.115	0.169	b	ab	a
Al <sub>t</sub>	<b>0.001</b>	0.060	<b>0.002</b>	b	a	a
P <sub>t</sub>	<b>0.010</b>	<b>0.040</b>	<b>0.041</b>	b	ab	a
N <sub>t</sub>	0.076	0.126	0.055	a	a	a
S <sub>t</sub>	0.057	0.121	0.979	a	a	a
Ca <sub>t</sub> :Fe <sub>t</sub>	0.553	0.478	0.057	a	a	a
Ca <sub>t</sub> :P <sub>t</sub>	0.183	0.759	0.301	a	a	a
Fe <sub>t</sub> :P <sub>t</sub>	0.315	0.647	0.100	a	a	a
<i>P-fractions</i>						
P <sub>org</sub>	0.482	0.546	0.818	a	a	a
P <sub>Ca</sub>	<b>0.003</b>	<b>0.015</b>	<b>0.011</b>	b	a	a
P <sub>FeAl</sub>	<b>0.025</b>	0.904	0.961	b	ab	a
P <sub>ox</sub>	<b>0.006</b>	<b>0.048</b>	0.064	b	a	a
P <sub>org</sub> :P <sub>t</sub>	<b>0.000</b>	0.123	0.550	a	a	b
P <sub>Ca</sub> :P <sub>t</sub>	0.105	0.186	0.107	a	a	a
P <sub>FeAl</sub> :P <sub>t</sub>	<b>0.000</b>	0.987	0.862	b	b	a
P <sub>Ca</sub> :P <sub>FeAl</sub>	0.171	0.518	0.758	a	a	a
P-Olsen	<b>0.033</b>	0.108	0.298	b	ab	a
<i>Fe-fractions</i>						
Fe <sub>ox</sub>	0.356	0.562	0.264	a	a	a
Al <sub>ox</sub>	0.089	0.083	0.206	a	a	a
Fe <sub>ox</sub> -Fe <sub>pyr</sub>	0.306	0.720	<b>0.024</b>	a	a	a
Al <sub>ox</sub> -Al <sub>pyr</sub>	0.131	0.510	0.997	a	a	a
Fe <sub>pyr</sub>	0.344	0.609	0.439	a	a	a
Al <sub>pyr</sub>	0.181	0.086	0.126	a	a	a
Fe <sub>pyr</sub> :Fe <sub>ox</sub>	<b>0.003</b>	0.412	0.212	a	ab	b
Al <sub>pyr</sub> :Al <sub>ox</sub>	<b>0.013</b>	0.396	0.425	a	ab	b

1.5  $\mu\text{mol L}^{-1}$ , 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 Text S1 and Table S1 in Supplementary Information). During this period, concentrations of o-PO<sub>4</sub> 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  $\mu\text{mol L}^{-1}$  around 1980, to much lower concentrations of 0.5–1.5  $\mu\text{mol L}^{-1}$  around 2000. This decrease in o-PO<sub>4</sub> 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  $\mu\text{g L}^{-1}$  in the summer of 1980 to 20–40  $\mu\text{g L}^{-1}$  in the summer of 2002 (see Table S1). For this period, similar patterns have been found in a nearby lake district (Maasdam and 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; Cusell et al., 2013b). 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, 1990). 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., 2013b). 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., 2013b). 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.

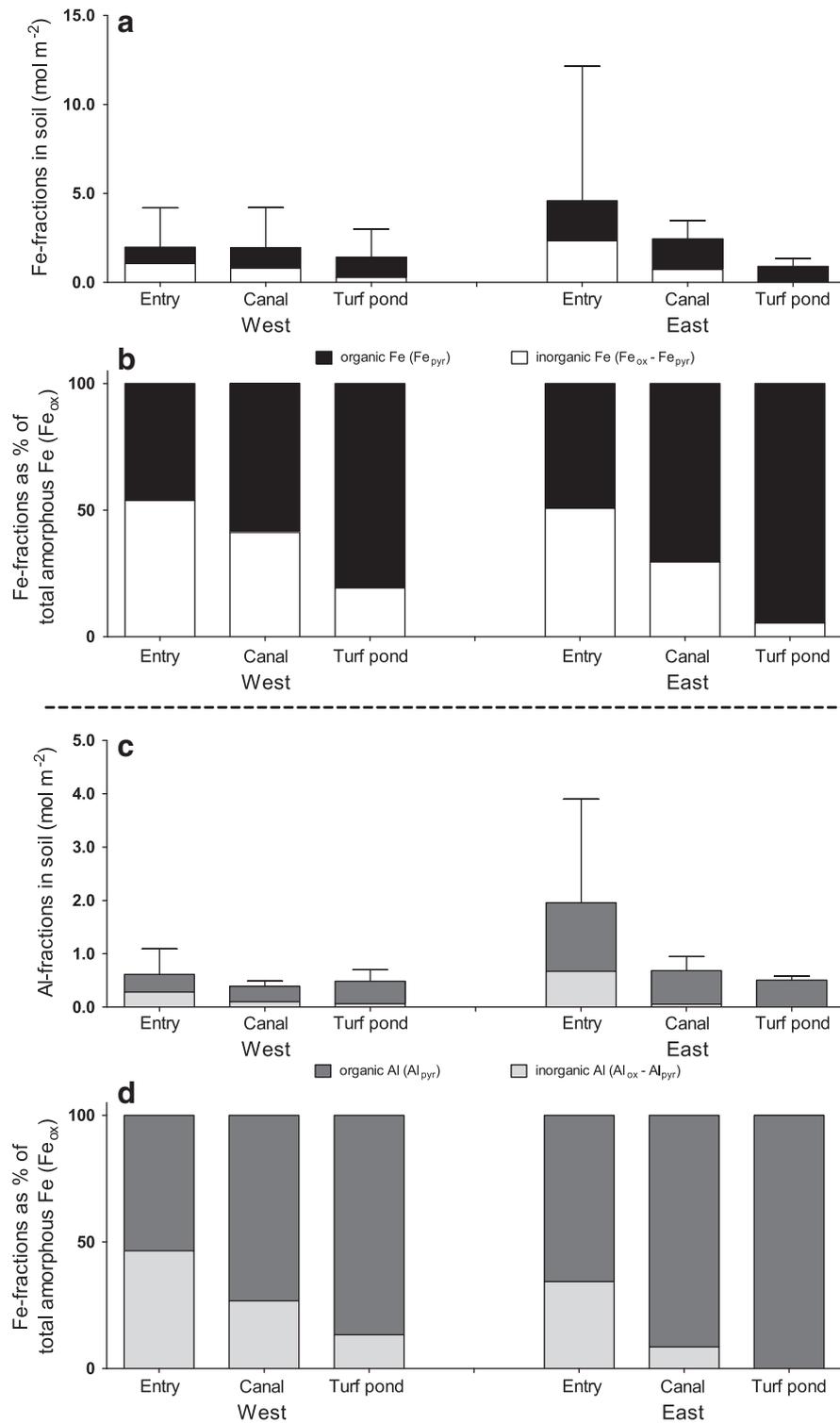
#### 4.2.2. Filtering mechanisms and spatial differences in soil quality

One important P-filtering mechanism in wetlands is the assimilation and accumulation of organic material (Howard-Williams, 1985; Richardson and 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 underwater peat soils due to 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 the sorption of phosphates 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 seepage in the deeper lying polders around the nature reserve (van Wirdum, 1990), 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 seepage (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 and 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 the relatively high Fe:o-PO<sub>4</sub> ratios of above 10 mol mol<sup>-1</sup> 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<sub>4</sub>-rich surface water may lead to P-mobilization as a result of Fe-reduction and/or increased mineralization rates (Caraco et al., 1989; Lamers et al., 1998; Smolders et al., 2006). This is indeed supported by the measurement of higher concentrations of o-PO<sub>4</sub> in the soil pore waters of these locations, which were above the critical value of 5  $\mu\text{mol L}^{-1}$  (Geurts et al., 2008). Some

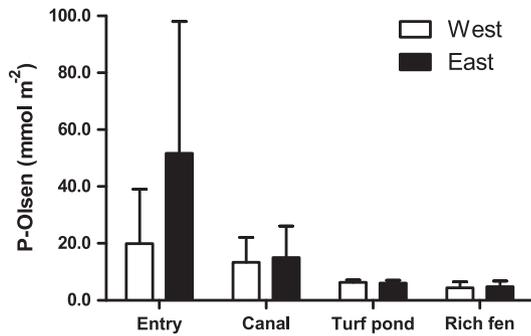


**Fig. 7.** Concentrations of inorganic and organic bound Fe and Al in the sub-aquatic sediments of water entry locations, canals and turf ponds. Graphs a and c show means, with their standard deviations as error bars, of the Fe- and Al-concentrations per  $\text{m}^2$  in the uppermost 10 cm of the soil, while relative values to total amorphous Fe and Al are given in graphs b and d ( $n = 5$ ). Statistical information is given in Table 4.

P-mobilization may also occur in western turf ponds. Here, Fe:o- $\text{PO}_4$  ratios were below  $10 \text{ mol mol}^{-1}$ , and Fe was primarily present as organically bound Fe, which is less capable of adsorbing P than inorganic solid Fe compounds (Koenings and 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 and Khalid, 1974), especially during warm episodes and with

high sulfate loads, when Fe is reduced and no longer able to prevent P mobilization to the water layer.

The occurrence of these 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 alkalization (Schindler et al., 1986; Roelofs, 1991). In addition, hampered nitrification, increased dissimilatory nitrate reduction



**Fig. 8.** Concentrations of plant-available P (P-Olsen) in the (underwater) 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 given in Table 4.

to ammonium (DNRA) and/or increased mineralization probably led to higher  $\text{NH}_4$ -concentrations in the soil pore waters of these locations (Lamers et al., 1998). Sulfide did, however, hardly accumulate to levels that are known to be toxic to a number of freshwater macrophytes (above  $10 \mu\text{mol L}^{-1}$ ; Lamers et al., 2013), most likely due to efficient binding to Fe (Smolders and Roelofs, 1996; van der Welle et al., 2006).

**Table 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***	-
$P_{\text{org}}$	-0.075	0.072
$P_{\text{Ca}}$	-0.180*	0.394***
$P_{\text{FeAl}}$	-0.244***	0.749***
$P_{\text{ox}}$	-0.318***	0.904***
$P_{\text{t}}$	-0.275***	0.598***

\*  $p \leq 0.1$ .

\*\*  $p \leq 0.05$ .

\*\*\*  $p \leq 0.01$ .

#### 4.3. Low P-availability in floating rich fens

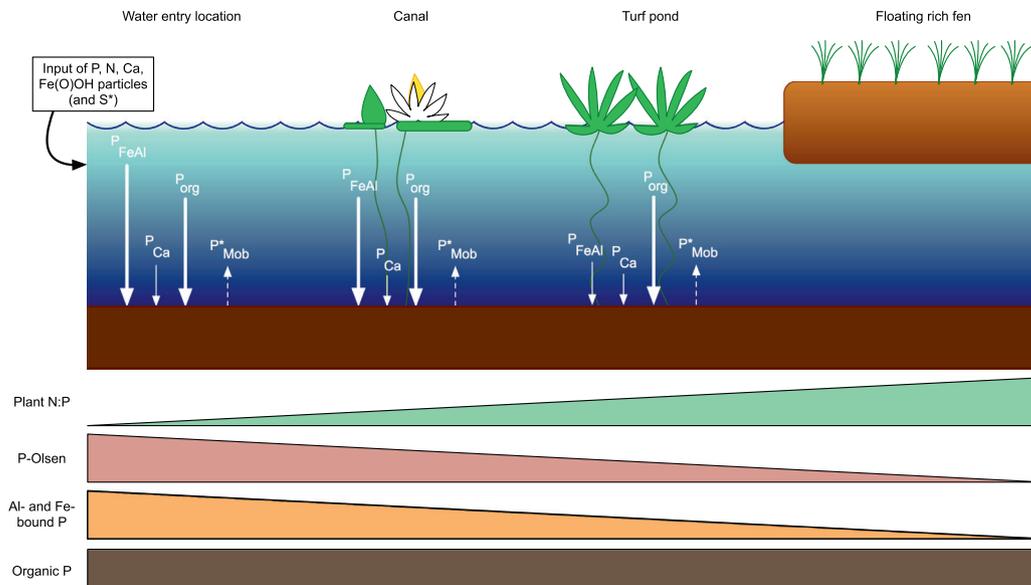
The soil characteristics of floating rich fens seem to lead to limited P-availability, as indicated by low concentrations of P-Olsen per unit volume. In these soils relatively little P was bound to Fe and Al, and relatively much to Ca and organic material compared to the sub-aquatic sediments. This was 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 phosphorus to calcium 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 proceeds towards more isolated parts, as it certainly did in the past in the studied nature reserve (van Wirdum, 1990).

#### 5. 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. 9). 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.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2014.02.032>.



**Fig. 9.** 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  $\text{SO}_4$ -rich western part of the studied nature reserve than at the east side.

## Conflict of interest

None of the authors has a conflict of interest.

## Acknowledgment

We wish to thank Leo Hoitinga, Ton van Wijk, Piet Wartenbergh and Roy Peters for their assistance with chemical analyses, Ties Luijendijk for his illustration (Fig. 9), and Koos Verstraten for critically reviewing the manuscript. We also want to thank State Forestry (SBB) and Natuurmonumenten for giving their kind permission to take samples in their nature reserves, and Water Board 'Reest & Wieden' for providing their datasets. This study was funded by the Province of Overijssel, Water Board 'Reest & Wieden' and the Dutch Ministry of Economic Affairs, Agriculture and Innovation as a part of the National Research Program 'Ontwikkeling + Beheer Natuurkwaliteit' (OBN).

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