


Restoration of endangered fen communities: the ambiguity of iron–phosphorus binding and phosphorus limitation

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Summary

1. Low phosphorus (P) availability limits plant biomass production in fens, which is a prerequisite for the persistence of many endangered plant species. We hypothesized that P limitation is linked to soil iron (Fe) content and soil Fe : P ratios as iron compounds provide binding sites for dissolved P, presumably reducing P availability to plants.

2. We sampled 30 fens in a trans-European field survey to determine how soil Fe pools relate to pools of P and Fe-bound P, and we measured vegetation P uptake and N : P ratio to assess where P limitation occurs. Next, we determined P uptake by *Carex rostrata* in experimental fen mesocosms to investigate interactive effects of soil Fe and P pools (and fractions) and water levels (drained or rewetted).

3. The field survey revealed that soil P pools correlate positively with soil Fe pools, regardless of fen degradation level, location or sampling depth. Moreover, soil Fe and P pools correlated positively with P uptake by the vegetation and negatively with vegetation N : P ratios. Generally, N : P ratios dropped below 10 g g⁻¹ whenever thresholds of 15 mmol Fe L⁻¹ soil and 3.3 mmol P L⁻¹ soil were exceeded. Endangered fen species mainly thrived in Fe- (and thus P-) poor fens.

4. The mesocosm experiment further showed that interactions between water levels and P pools determined plant P uptake: although fen rewetting led to an overall increase in P uptake, plants that had grown on drained Fe-rich soils with large acid-extractable P pools (> 1.6 mmol P_{acid} L⁻¹) could still sequester large quantities of P. Soil Fe : P ratio had no effect on P uptake.

5. *Synthesis and applications.* Our findings have important implications for the management and restoration of endangered fen communities. We demonstrated the existence of an iron–phosphorus (Fe–P) binding ambiguity in fens: large Fe pools ‘trap’ mobile P, thereby enhancing overall P availability to plants rather than diminishing it. For P limitation, we suggest an empirical threshold of < 3.3 mmol P L⁻¹ soil, which is mainly found in Fe-poor fens. Restoring fens by rewetting increases the relative availability of P and may not always result in favourable conditions for endangered fen communities. Rewetting of drained fens is most likely to be successful if soil P and Fe pools are well below 3.3 and 15 mmol L⁻¹ respectively.

Key-words: biodiversity, eutrophication, fen restoration, iron, iron chemistry, nutrient limitation, phosphorus, red list, water levels, wetlands

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Introduction

Phosphorus (P) limitation is an important prerequisite for the persistence of many low-productive wetland plant communities, and ongoing anthropogenic P enrichment triggers a drastic loss of endangered plant species (Olde Venterink *et al.* 2003; Wassen *et al.* 2005). The loss of vulnerable species can only be countered if we gain a better understanding of the complexity of factors influencing P limitation under natural conditions.

Retention and immobilization of P in wetlands is governed by complex interactions between biological and physio-chemical factors (Reddy *et al.* 1999; Richardson & Vaithyanathan 2009). In calcareous wetlands for example, P can be immobilized by precipitation with calcium (Ca) or adsorption to CaCO_3 (Boyer & Wheeler 1989; Richardson & Vaithyanathan 2009). In non-calcareous wetlands, which comprise the majority of freshwater wetlands, P retention is often linked to iron (Fe) chemistry. Reactive surfaces of soil Fe(oxy)-(hydr)-oxides provide an excess of sorption sites for dissolved inorganic P, with maximal sorption capacity at high redox potential (Patrick & Khalid 1974; Jensen *et al.* 1992; Smolders *et al.* 2001). Under anoxic conditions, formation of ferrous phosphate minerals serves as an additional P sink (Manning, Murphy & Prepas 1991; Rothe *et al.* 2014). Since Fe–P binding hampers P mobilization into pore and surface water, the soil Fe : P ratio is often used as a predictor of soil P retention capacity with high values indicating a low P mobilization risk (Jensen *et al.* 1992; Geurts *et al.* 2008; Zak *et al.* 2010). Moreover, high soil Fe : P ratios may correlate positively with the occurrence of endangered wetland plant species (Geurts *et al.* 2008), possibly because they benefit from a limited availability of easily available (dissolved) P.

The aforementioned observations on Fe–P binding led us to hypothesize that the discharge of Fe-rich groundwater as well as large Fe pools in wetland soils should favour conditions of P limitation through the formation of Fe–P complexes, or if we extrapolate, that P limitation is mainly a characteristic of Fe-rich rather than of Fe-poor wetlands. In this paper, we investigated how Fe chemistry and water levels relate to soil P pools and P limitation in groundwater-fed peatlands ('fens' hereinafter), which are protected by the European Habitats Directive (H7140). Undisturbed fens are typically covered by endangered plant communities dominated by small sedges (e.g. *Carex dioica* (L.), *Carex diandra* (Schrank) and *Carex lepidocarpa* (Tausch)) and brown mosses (mainly from the family *Amblystegiaceae*, e.g. *Hamatocaulis vernicosus* ((Mitt.) Hedenäs), *Scorpidium scorpioides* ((Hedw.) Limpr.) and *Campyllum stellatum* ((Hedw.) C.E.O. Jensen)), with high abundance in areas of low P availability (Boeye *et al.* 1997; Kooijman & Paulissen 2006).

We analysed the Fe–P vegetation relationship in fens by combining a trans-European field survey with a

phytometer experiment. We investigated how P pools in natural fen soils relate to soil Fe pools, and we hypothesized that P is mostly bound to amorphous Fe compounds. Second, we hypothesized that rooting plants can hardly utilize pools of Fe-bound P, and that endangered fen species are more abundant in Fe-rich fens.

Materials and methods

QUANTIFICATION OF P LIMITATION

Nutrient ratios of above-ground plant biomass are widely used as a diagnostic tool to evaluate which macronutrients (co-)limit vegetation growth (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003). Broadly, vegetation N : P ratios <10 (g g^{-1}) indicate nitrogen (N) limitation, whereas values >20 indicate P limitation (Gusewell 2004; Cusell *et al.* 2014). In other words, we assume no P limitation whenever the N : P ratio drops below 10 g g^{-1} , whereas values >10 indicate a gradual shift towards NP co-limitation (14–16) or P limitation (>20). For potassium (K), N : K values >2.1 and K : P values <3.4 indicate limitation (Olde Venterink *et al.* 2003).

FIELD SURVEY

Study sites and sampling

We collected soil, pore water and vegetation samples in 30 different fens (Table 1). All fens are characterized by the discharge of base-rich groundwater (pH between 5.5 and 7), are predominantly non-calcareous, non-floating and some still have peat growth. Most fens are located in riverine valleys, but they are not flooded with river water. We sampled pristine (never drained) as well as degraded fens. Fens were scattered at low elevations (<160 m a.s.l.) across Belgium, the Netherlands and Poland. Soil samples ($n = 194$) were collected at various depths below the surface (0–500 cm, Table 1) using a knife (0–25 cm depth) or a Russian corer (>25 cm depth) and were collected pairwise: one set of samples was used for chemical analysis and the second for soil bulk density calculations. Pore water samples were collected at equal depths as the soil samples using Macrorhizon filters (pore size = $0.15 \mu\text{m}$, Rhizosphere Research Products, The Netherlands) attached to extension tubes (0–45 cm depth) or ceramic soil moisture samplers (Eijkkelkamp Agrisearch Equipment, The Netherlands) (>45 cm depth). In total, we made 49 vegetation relevés divided over 12 different fens in which we identified all vascular plant and bryophyte species (plot size $>2 \times 2$ m), and we collected above-ground herb biomass in a 0.4×0.4 m area (Table 1). Biomass was oven-dried at 70°C for 48 h and weighed.

P UPTAKE IN A PHYTOMETER EXPERIMENT

We conducted a phytometer experiment with *Carex rostrata* (Stokes) to additionally investigate (i) the interactive effects of soil P pools (and fractions) and water regime (rewetted or drained) on plant P uptake and (ii) rewetting prospects of drained fens in relation to Fe and P chemistry. We chose *C. rostrata* because (i) members of the *Carex* genus form a dominant plant

Table 1. Study sites with coordinates, country (BE = Belgium, NL = the Netherlands, PL = Poland), sampling depths and the number of (i) soil samples with bulk chemistry measurements (=Bulk density, total Fe and P), (ii) soil samples with oxalate-P measurements (P_{ox}) and (iii) vegetation (relevées and biomass) samples

Site code	Site name	Longitude	Latitude	Country	Sampling depth (cm)	Soil samples bulk chemistry	Soil samples P_{ox}	Vegetation samples
BG	Buitengoor	5.17667	51.21494	BE	0–10	2	0	0
LE	Leiemeersen	3.26744	51.15533	BE	0–10	2	0	0
MA	Malendriesbeekvallei	4.87344	50.84902	BE	0–10	7	7	7
TB	Torfbroek	4.54467	50.90531	BE	0–10	1	0	0
ZB	Zwarte Beek – West	5.28592	51.7978	BE	0–150	7	0	0
ZO	Zwarte Beek – East	5.32522	51.09169	BE	0–300	11	0	0
BM	Bennekomse Meent	5.59675	52.00719	NL	0–50	5	0	0
DE	Dentchenbag	6.04575	50.87097	NL	0–25	2	0	0
ES	Elperstroom – Reitma	6.65922	52.87478	NL	0–50	5	0	0
EO	Elperstroom – Oosterma	6.66669	52.88422	NL	0–10	2	0	0
GD	Gasterense Diep	6.66781	53.02056	NL	0–200	15	4	0
HE	Hellen	5.58133	52.00870	NL	0–10	4	4	4
HO	Holmers	6.62956	52.90433	NL	0–10	4	3	4
HA	Halkenbroek	6.62750	52.91443	NL	0–10	4	4	4
KL	Klitserbeek	5.93439	50.78006	NL	0–25	2	0	0
LD	Looner Diep	6.62803	53.01392	NL	0–50	19	11	10
LH	Leijer Hooilanden	6.27711	52.64239	NL	0–50	6	4	4
LM	Lemselermaten	6.87536	52.34611	NL	0–10	2	0	0
LS	Langstraat	5.02189	51.68808	NL	0–10	2	0	0
MB	Mosbeek	6.87007	52.44632	NL	0–10	3	3	3
PE	Peizermade	6.50346	53.17187	NL	0–10	8	6	8
RD	Reestdal	6.43581	52.62381	NL	0–10	2	0	0
BS	Bagno Stawek	17.55217	53.89614	PL	0–100	12	4	0
BZ	South Biebrza	22.60886	53.28650	PL	0–155	7	0	0
DZ	Dobrzynka	16.98933	53.52444	PL	0–200	15	5	0
GG	Gogolewko	17.42639	54.35883	PL	0–10	2	0	0
LB	Lubon West	17.56011	54.02353	PL	0–100	14	4	1
LU	Lubon East	17.49678	54.02219	PL	0–50	11	3	1
RS	Rospuda	22.95367	53.90417	PL	0–200	9	0	1
SZ	Biebrza – Szusalewo	23.35414	53.71533	PL	0–500	9	0	2

group in fens and (ii) *C. rostrata* is a common species in the Northern hemisphere and grows in any fen type.

Soil core collection and mesocosm set-up

We collected a total of 40 soil cores in drained parts of four degraded fens (LH, BM, ES and ZO). Sites covered a gradient of increasing Fe richness (means \pm SD = 18 \pm 5, 32 \pm 6, 73 \pm 15 and 525 \pm 93 mmol Fe L⁻¹ soil respectively). Within each fen, we chose a central peaty area dominated by fen (meadow) species such as *Carex lasiocarpa* (Ehrh.), *Cirsium palustre* ((L.) Scop.), *Caltha palustris* (L.), *Juncus acutiflorus* (Ehrh. ex Hoffm.), *Climacium dendroides* ((Hedw.) F. Weber & D. Mohr.) and *Calliergonella cuspidata* ((Hedw.) Loeske). In February 2014, we collected 10 intact replicate soil cores of 45 cm \times 12.5 cm (depth \times diameter) at each location. We pushed hard-PVC tubes (diameter = 12.5 cm) of 50 cm length in the peat soil, after removing all above-ground vegetation, to extract a core. The cores were transported to an unheated greenhouse where half of the cores received a rewetting treatment (water level = surface level), whereas the other half was kept drained (water level = c. 30 cm below surface). Water level manipulation was accomplished by placing each PVC tube (perforated at the bottom) in a separate container that was prefilled with the required amount of stagnant N₂-deoxygenized water. The mesocosms were kept under these

conditions for 1 year before initiation of the actual experiment. Early March 2015, we collected two composite soil samples per mesocosm from the following three depths: 5, 15 and 25 cm, whereby one sample was used for bulk density calculation and the other was used for chemical analysis.

Plant manipulation, experimental design and harvest

In summer 2014, we collected one small individual plant of *C. rostrata* from a field site (GD). The individual was transplanted into a 1-L pot (on universal potting soil) in a greenhouse nursery. During the following months, we started a procedure of vegetative cloning in which we kept splitting the individual into two separate viable plants whenever the plant had reached a sufficiently large size, and we did the same for all of the newly formed individuals. Each newly formed individual was transplanted into a separate 1-L pot. In autumn 2014, the procedure had resulted in a total of 40 genetically identical individuals. Early March 2015, we clipped all plants at 0.5 cm above the surface. Seven days later, after all plants had resprouted, plants were removed from their nursery pots and roots were rinsed. Next, the 40 plants were randomly allocated over the 40 mesocosms. Plants were grown for one growing season (until end of August 2015, $t = 152$ days), after which we collected all above-ground biomass. Biomass was oven-dried at 70 °C for 48 h and weighed.

CHEMICAL ANALYSIS

Field survey

Pore water pH was measured directly in the field using portable equipment (WTW Multi 340i; WTW, Weilheim, Germany). Next, the samples were acidified with 0.7 mL 65% suprapure HNO₃ per 100 mL sample and analysed on ICP (IRIS Intrepid II) for total dissolved P. Soil bulk density (kg L⁻¹) was determined by oven-drying (105 °C) soil samples with known volumes to constant mass. Organic matter content (%) was determined by loss-on-ignition (4 h 550 °C). Fe, P, Ca and Al concentrations (in mmol kg⁻¹ dry mass) of soil samples were determined on ICP-OES after digesting 0.2 g of oven-dried and mill-ground soil with 4-mL HNO₃ (65%) and 1-mL H₂O₂ (30%) using a microwave labstation (Milestone srl). Additionally, we determined oxalate-P concentrations (P_{ox}) for a subset of samples (Table 1), which is a measure for P bound to amorphous or less crystalline Fe and Al compounds (Schwertmann 1964). In short, fresh soil samples are shaken in an ammonium-oxalate (16.1 g L⁻¹)/oxalic acid (10.9 g L⁻¹) solution (pH = 3) in full darkness for 2 h, after which the extracts are filtered and analysed with ICP. Total N, P and K concentrations (mg g⁻¹) of biomass samples were determined by digesting 0.3 g oven-dried mill-ground plant material with H₂SO₄ (96%), H₂O₂ (30%) and salicylic acid (Walinga *et al.* 1989). N and P were analysed on a segmented flow analyser (Skalar, Breda, The Netherlands) and K was analysed on ICP (Thermo Fisher, Franklin, MA, USA).

Phytometer experiment: sequential P extractions

We conducted sequential extractions on fresh soil samples from the experimental mesocosms to determine concentrations of ammonium-chloride extractable P (NH₄Cl-P), bicarbonate-dithionite extractable P (BD-P) and hydrochloric acid-extractable P (HCl-P) (Zak *et al.* 2008). P concentrations of the chemical extracts were determined using the molybdenum blue method after acid digestion (Zak *et al.* 2008). We defined the different P pools in the soil as follows: desorbable P (=NH₄Cl-P), reductant-soluble P (=NH₄Cl-P + BD-P) and acid-soluble P (=NH₄Cl-P + BD-P + HCl-P). Foliar P and N concentrations (mg g⁻¹) in *C. rostrata* were determined as in the field survey.

DATA ANALYSIS

All analyses were done in R 3.2.3 (R Development Core Team 2016), and significance was accepted at $P < 0.05$ for all tests. To calculate element pool sizes (mmol L⁻¹) in the soil, we multiplied element concentrations (mmol kg⁻¹) with bulk densities (kg L⁻¹) of the corresponding samples. We investigated P uptake by natural fen vegetation and *Carex* clones using three proxies: (i) vegetation N : P ratios (g g⁻¹), (ii) foliar P concentrations (mg g⁻¹) and (iii) plant P stocks [i.e. corrected for biomass production (expressed in mg P m⁻² in natural fen vegetation and mg P plant⁻¹ in the phytometer experiment)].

Field survey

We tested if soil P pools in fens can be predicted from soil Fe pools using least-squares linear regression. We first used all soil data (i.e. from all sites and depths, $n = 194$ samples) and then re-ran the test using only site averages (correcting for pseudo-replicates, $n = 30$).

Prior to the analyses, we log₁₀-transformed Fe and P values to attain a normal distribution and homoscedasticity of the errors. We used Spearman's rank correlation to test if soil pools of oxalate-P and the fractions oxalate-P P⁻¹ correlate with soil pools of Fe, P and Al, and to test if vegetation N : P ratios, foliar P concentrations and vegetation P stocks correlate with total dissolved P, soil Fe pools, P pools and Fe : P ratios of the top soil (0–10 cm). Finally, we calculated the fraction of endangered vascular plant and bryophyte species (number of endangered species divided by total species count) in each of the vegetation relevées, which we correlated with (i) vegetation N : P ratios, foliar P concentrations and vegetation P stocks and (ii) soil Fe and P pools and Fe : P ratios. A species was considered endangered if it was listed on the red list (status 'vulnerable', 'declining' or worse) of either the Netherlands (Siebel, Bijlsma & Sparrius 2012; Sparrius, Odé & Beringen 2012), Flanders (van Landuyt, Vanhecke & Hoste 2006) or Poland (Ochyra 1992; Kaźmierczakowa *et al.* 2016).

Phytometer experiment

We constructed a regression tree with foliar P concentration as the dependent variable to investigate how total plant P uptake relates to various soil P fractions and water levels. Regression trees allow the capture of potential nonlinear effects while simultaneously providing an easy display of potential high-order interactions between the main explanatory variables (Crawley 2005). The complexity parameter was set to a value of 0.05 to maximize the discriminative power of the model. We included the following explanatory variables: water level (Categorical: 'Drained' or 'Rewetted'), soil pools of desorbable P, reductant-soluble P, acid-soluble P, total P (in mmol P L⁻¹ soil) and soil Fe : P ratio (mol mol⁻¹). Finally, we performed a Kruskal–Wallis (KW) test followed by a Dunn's *post hoc* test with Bonferroni correction to flag significant differences between the groups formed at the final nodes of the regression tree. Additionally, we used KW to test for differences between plant P stocks and N : P ratios between the final node groups.

Results

SOIL FE AND P POOLS

Soil P pools correlated positively with soil Fe pools in fens, irrespective of study site or sampled soil depth (linear regression: $F_{1,192} = 616.5$, $P < 0.001$, Fig. 1a). When using site averages, the trend remained similar (linear regression: $F_{1,28} = 80.2$, $P < 0.001$, Fig. 1b). Omitting the outlier site 'BG' increased the predictive power of the model (linear regression: $F_{1,27} = 131.4$, $R^2 = 0.83$, $P < 0.001$). In addition, soil pools of oxalate-P correlated positively with pools of Fe and P (Fig. 2a,b). Oxalate-P pools also correlated weakly with soil pools of aluminium (Al) (Fig. 2c). The fraction oxalate-P P⁻¹ increased with increasing P and Fe pool size: low oxalate-P fractions (<0.15 mol mol⁻¹) were only found in Fe- and P-poor fens (Fig. 2d,e). Weight-based correlations are shown in Fig. S1 and Fig. S2, Supporting Information.

NUTRIENT LIMITATION

Soil P and Fe pools and soil Fe : P ratios correlated negatively with vegetation N : P ratios (Fig. 3a–c). Whenever

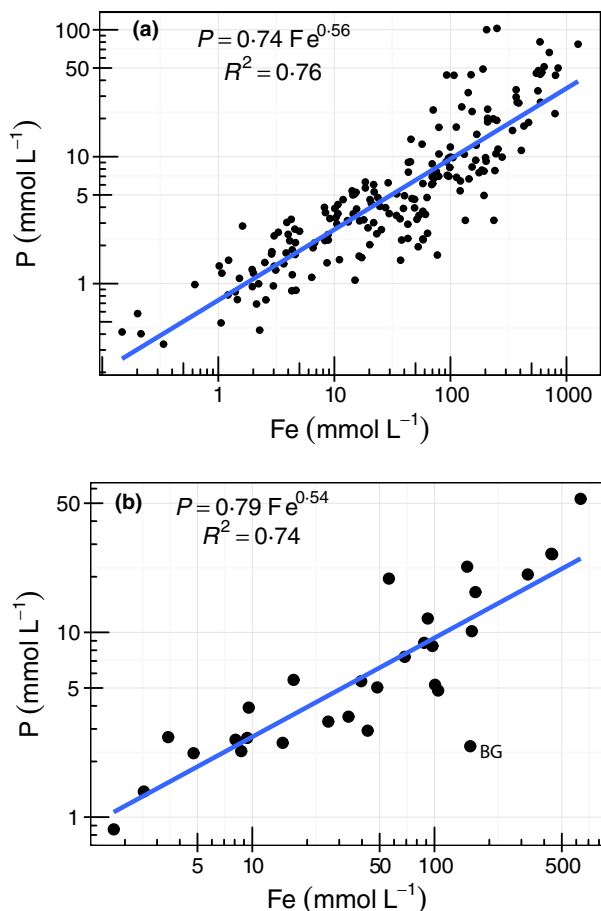


Fig. 1. Log–log plots of P vs. Fe pools in fen soils (in mmol L^{-1}) for (a) all 194 soil samples collected at 30 study sites and at different depths ($n = 194$, ranging from 0 to 500 cm below the surface) and (b) independent averages of the 30 study sites. Regression lines are fitted with least-squares linear regression of the log-transformed variables.

thresholds of $3.3 \text{ mmol P L}^{-1}$ and $15 \text{ mmol Fe L}^{-1}$ were exceeded, vegetation N : P ratios dropped below 10. Overall, N : P ratios indicated P limitation in three plots, NP co-limitation in two plots and N limitation in the remaining plots. P concentrations in the vegetation (Fig. 3d,e,f) and in the total vegetation P stock (Fig. 3g,h, i) correlated positively with soil P and Fe pools and soil Fe : P ratios. Evidence for K limitation was only found in one plot at site DA (N : K and K : P ratios = 2.3 and 2.5 respectively).

Concentrations of dissolved P in pore water did not correlate with foliar P concentrations (Spearman's $\rho = 0.06$, d.f. = 47, $P = 0.69$) or vegetation N : P ratios (Spearman's $\rho = -0.10$, d.f. = 47, $P = 0.49$).

OCCURRENCE OF ENDANGERED SPECIES

We recorded a total of 150 vascular plant species (including 40 endangered species, e.g. *C. diandra*, *C. lepidocarpa* or *Pedicularis palustris* (L.)) and 33 bryophyte species (including 11 endangered species, e.g. *C. stellatum*, *H. vernicosus*,

Fissidens adianthoides (Hedw.)). The proportion of endangered vascular plant and bryophyte species per plot varied between 0% and 58% and correlated positively with vegetation N : P ratios and negatively with vegetation P concentrations and stocks (Table 2). In addition, plots with large soil Fe and P pools and high Fe : P ratios contained relatively fewer endangered species (Table 2).

P UPTAKE IN THE PHYTOMETER EXPERIMENT

Foliar P concentrations (mg g^{-1}) in *C. rostrata* were primarily determined by water levels (Fig. 4, first split): rewetting generally led to an increase in foliar P concentrations. In addition, the size of the soil P pool co-determined foliar P concentrations. In the rewetted mesocosms, large P pools ('P Total' $>3.3 \text{ mmol L}^{-1}$) correlated with highest foliar P concentrations (Fig. 4: node 7 > node 6). In the drained mesocosms, large pools of acid-extractable P ('Acid P' $>1.6 \text{ mmol L}^{-1}$) correlated with higher foliar P concentrations (Fig. 4: node 4 > node 3). The final model explained 76% of the variation in P concentrations. Desorbable P, reductant-soluble P and soil Fe : P ratio had no additional explanatory effect on foliar P concentrations.

Finally, foliar P concentrations, plant P stocks and N : P ratios differed between the final node groups (KW chi-squared = 31.5, 34.2 and 25.3 respectively, d.f. = 3, $P < 0.001$). Foliar P concentrations and plant P stocks were lowest on the drained soils with small acid-extractable P pools (acid P $<1.6 \text{ mmol L}^{-1}$; Fig. 4: Node 3), which corresponded to the Fe-poor soils. In contrast, foliar P concentrations and plant P stocks were highest on the rewetted soils with large soil P pools (P total $>3.3 \text{ mmol L}^{-1}$; Fig. 4: Node 7), which corresponded to the Fe-rich soils. Foliar P concentrations and plant P stocks were equal on drained P-rich soils (Node 4) and rewetted P-poor soils (Node 6). Vegetation N : P ratios indicated P limitation (N : P $>20 \text{ g g}^{-1}$) only in some of the drained soils with small acid-extractable P pools (acid P $<1.6 \text{ mmol L}^{-1}$; Fig. 4: Node 3).

Discussion

We investigated whether large soil pools of Fe and high soil Fe : P ratios limit P availability to plants due to strong Fe–P binding. The field survey revealed that Fe-rich fens are P-rich fens, both in terms of P pool size as well as in the magnitude of P uptake by plants. These results were supported by the phytometer experiment, in which we additionally demonstrated the effect of water levels. Overall, endangered fen species were most abundant in Fe-poor fens.

IRON AS A 'PHOSPHORUS TRAP'

The significant correlation between soil Fe and P pools suggests that input, retention and accumulation of P in a

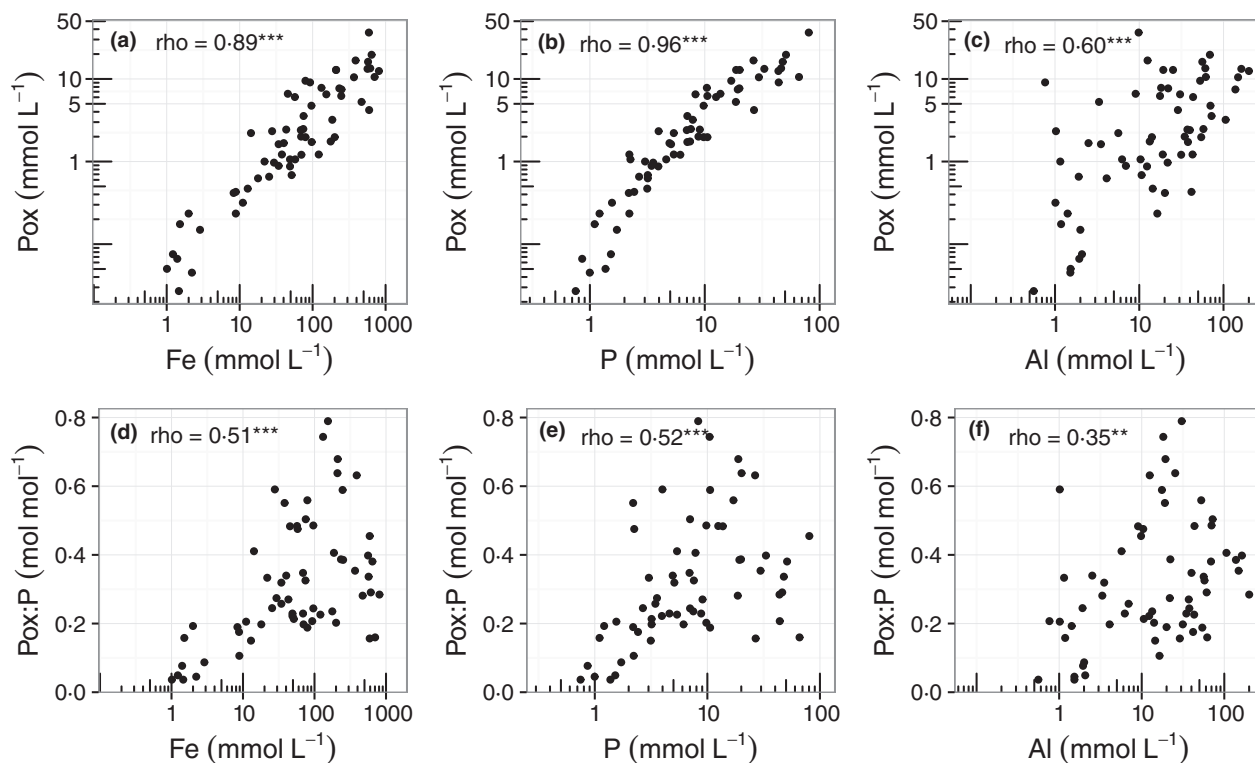


Fig. 2. Correlations between soil pools of oxalate-P (P_{ox}) and pools of (a) Fe, (b) P and (c) Al in fens (mmol L^{-1}), and between the fraction oxalate-P P^{-1} (mol mol^{-1}) and soil pools of (d) Fe, (e) P and (f) Al in fens ($n = 62$ samples collected in 13 different fens between 0 and 200 cm depth). Spearman's rho values are listed together with their significance ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

fen system are tightly connected to input, retention and accumulation of Fe. We see two main nonexclusive mechanisms for such strong Fe–P relationship.

First, it is likely that Fe-enriched anaerobic groundwater that feeds Fe-rich fens is, naturally, also P-enriched. P can be transported with water flow in the form of dissolved (inorganic) phosphates that can originate from the reduction/dissolution of Fe–P minerals elsewhere (Patrick & Khalid 1974; Carlyle & Hill 2001; Welch, Kingsbury & Coupe 2010), or the movement of colloidal Fe–P (=suspended mobile Fe–P solids of size >1 nm and <1 μm), even in aquifers (Gschwend & Reynolds 1987; Hartland *et al.* 2015).

Second, fens with large pools of Fe and high soil Fe : P ratios may function as 'P traps', meaning that any inorganic P that enters the fen system (e.g. through water inflow or anthropogenic P fertilization) or becomes available due to mineralization is retained due to P sorption to amorphous Fe compounds or the formation of ferrous phosphates such as vivianite (Patrick & Khalid 1974; Rothe *et al.* 2014). The presence of such Fe–P trapping mechanism in natural Fe-rich wetland soils has been suggested in previous research, and also depends on physio-chemical soil characteristics like pH and redox potential (Aggenbach *et al.* 2013; Cusell *et al.* 2014; Baken *et al.* 2015). In our dataset, a strong positive correlation between Fe pools and pools of oxalate-P indeed indicates that P is effectively retained by amorphous Fe compounds. This is also supported by the positive correlation between soil Fe pools and the proportion of oxalate-P on total P. Low proportions ($<15\%$) are only found in Fe-poor

fens, in which P is presumably largely fixed in refractory organic matter (Aggenbach *et al.* 2013). Although oxalate-P also includes P bound to amorphous redox-insensitive Al compounds, weak correlations between soil pools of oxalate-P and Al indicated the lesser importance of Al in the fens under investigation.

Finally, we have to consider that P can also be immobilized by adsorption to CaCO_3 or precipitation with Ca, but this process mainly dominates in calcareous wetlands (pH $>7-8$) (Boyer & Wheeler 1989; Richardson & Vaithianathan 2009). Here, P may remain largely unavailable for plant uptake regardless of soil Fe content. However, calcareous fens are relatively rare in lowland Europe (Succow & Joosten 2001). We for instance registered small patches of CaCO_3 precipitation at two sites only (TB and DZ), whereas the remaining 28 sites were non-calcareous (average pH between 5.5 and 7).

P LIMITATION AS A CHARACTERISTIC OF FE-POOR FENS

Although P is most easily taken up by plants as dissolved inorganic phosphate, concentrations of dissolved P did not correlate with foliar P concentrations or vegetation N : P ratios. This suggests that, for rooting plants, pools of (or fractions of) soil-bound inorganic P are a more important determinant of P availability, probably because plants have an array of functional mechanisms to mobilize P from the soil matrix (Bolan 1991; Schachtman, Reid & Ayling 1998). For instance, the Fe-bound P pool, which

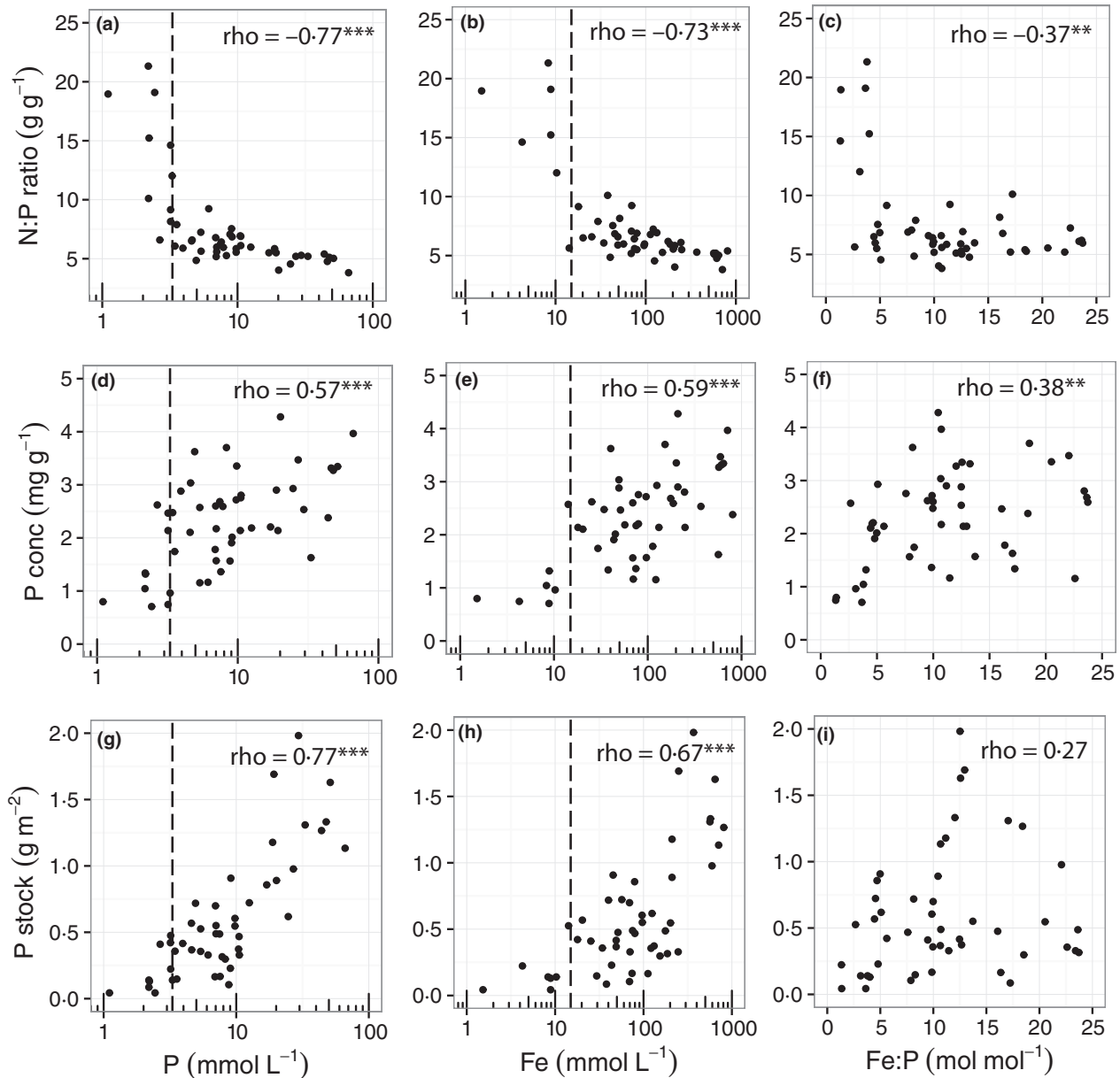


Fig. 3. N : P ratios (g g^{-1}), P concentrations (mg g^{-1}) and P stocks (g m^{-2}) of fen vegetation in relation to top soil P pools (a, d and g), Fe pools (b, e and h) and Fe : P ratios (c, f and i) ($n = 49$ samples, collected in 12 different fens). P stocks were calculated as P concentrations \times biomass. Dashed lines indicate thresholds of $3.3 \text{ mmol P L}^{-1}$ and $15 \text{ mmol Fe L}^{-1}$ above which P limitation is unlikely to occur. Spearman's rho values are listed together with their significance ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

is much larger in Fe-rich fens, may actually be accessible to some fen species (Corona, van der Klundert & Verhoeven 1996; Zak *et al.* 2014). This supposition is supported by (i) the inverse relationship between soil pools of P and vegetation N : P ratios and (ii) the positive correlation between soil P pools and P uptake by the vegetation. Whenever thresholds of $3.3 \text{ mmol P L}^{-1}$ and $15 \text{ mmol Fe L}^{-1}$ soil were exceeded, N : P ratios sharply dropped below 10 g g^{-1} , indicating N limitation. In our study, N was the primary limiting nutrient at the majority of sites. Indications for P limitation (N : P $> 14\text{--}20 \text{ g g}^{-1}$) were counterintuitively only found in five Fe-poor plots with low soil Fe : P ratios ($< 5 \text{ mol mol}^{-1}$) (Fig. 3c). P

availability in fens seems thus primarily determined by the size of the (Fe-bound) P pool in the soil, and not by soil Fe : P ratios. The nature of nutrient limitation was also reflected in the overall scarcity of endangered (red list) plant species at the Fe-rich/N-limited study sites, which supports the observation that endangered species are often scarce in P-rich environments (Boeye *et al.* 1997; Olde Venterink *et al.* 2003; Wassen *et al.* 2005). We did not further investigate the mechanisms that explain the low abundance of endangered species in P-rich fens, but a negative correlation between vegetation N : P ratios and above-ground herb biomass at our sites ($\rho = -0.55$, $P < 0.001$, Fig. S3) suggests an overall increase in herb

Table 2. Spearman's correlation coefficients (ρ) of the fraction of endangered fen species (herbs, bryophytes and total) vs. vegetation N : P ratios (g g^{-1}), P concentrations (mg g^{-1}) and P stocks (g m^{-2}) and soil Fe pools, P pools and Fe : P ratios ($n = 49$ plots, sampled in 12 different fens)

	Endangered species (%)		
	Vascular plants	Bryophytes	Total
<i>Vegetation</i>			
N : P ratio (g g^{-1})	0.47***	0.44**	0.49***
P concentration (mg g^{-1})	-0.31*	-0.35*	-0.33*
P stock (g m^{-2})	-0.37**	-0.36*	-0.40**
<i>Soil</i>			
Fe (mmol L^{-1})	-0.53***	-0.53***	-0.55***
P (mmol L^{-1})	-0.65***	-0.47***	-0.67***
Fe : P (mol mol^{-1})	-0.27	-0.51***	-0.29*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

productivity from P towards N limitation. Although N-limited systems can also be low productive, they tend to occupy a broader productivity gradient than P-limited sites (Olde Venterink *et al.* 2003; Pawlikowski *et al.* 2013). Hence, the relative scarcity of endangered species in many P-rich/N-limited fens could be a direct consequence of excessive biotic competition. Evidence of K limitation was only found in one plot of the field survey, suggesting that it plays a less important role in fens.

THE IMPORTANCE OF WATER LEVELS

Results from the phytometer experiment supported the outcome of the field survey, and additionally revealed another important factor which we could not disentangle in the field: the effect of water levels. In the regression tree, the factor 'water level' (rewetted or drained) was the main explanatory variable for differences in foliar P concentrations in *C. rostrata*. Individuals that were grown in the rewetted fen mesocosms had up to 4× higher foliar P concentrations than individuals from the drained mesocosms. This pattern can be related to the well-known reductive dissolution and desorption of Fe-bound P under anoxic conditions and low redox potential (Patrick & Khalid 1974; Zak *et al.* 2010), in which P is either mobilized or, at least, less firmly sorbed to Fe compounds. Such conditions evidently increase the relative availability of P to plants. In the rewetted mesocosms, the soil P pool was the best predictor of foliar P concentration with a cut-off value set at 3.3 mmol P L^{-1} for distinction between the two final node groups (Fig. 4). However, although sedges from the Fe- and P-poor rewetted soils had a significantly lower foliar P concentration and P stock than sedges from the Fe- and P-rich rewetted soils, their growth was still limited by N rather than P (N : P ratios <10). This suggests that the maximum threshold of 3.3 mmol P L^{-1} obtained from the field survey may still be an overestimation: in rewetted degraded fens, P limitation possibly occurs at even lower soil P thresholds.

Finally, we measured relatively high foliar P concentrations and plant P stocks in plants that were grown on the drained Fe- and P-rich soil mesocosms. This indicates that the oxidative environment with concomitant strong Fe-P binding was insufficient to trigger P limitation, as also shown by the low N : P ratios (<10). In other words, plants were still able to utilize the large P pool despite soil aeration. Here, a plant functional apparatus for the uptake of Fe- and soil-bound P may be in place, possibly e.g. a mutualistic relationship with mycorrhizal fungi that excrete phosphatase enzymes (Bolan 1991; Schachtman, Reid & Ayling 1998). Although small quantities of fungal hyphae have been observed on *C. rostrata* rootlets (Thormann, Currah & Bayley 1999), the ecological relevance for this species is unclear. In the drained mesocosms, the acid-extractable P pool rather than the total P pool was the best predictor of P uptake. Again, soil Fe : P ratio had no effect on P uptake, which supports conclusions from the field survey.

MANAGEMENT RECOMMENDATIONS FOR FEN RESTORATION AND CONSERVATION

Our findings have important implications for the management and restoration of endangered fen communities of small sedges and brown mosses. Primarily, we have shown that large soil pools of Fe correspond with large pools of plant-available P, indicating that P is generally not a limiting nutrient in Fe-rich fens.

Based on our observations, we discourage the practice of anthropogenic Fe addition to decrease the P mobilization potential of fen soils for restoration. Although we acknowledge that Fe addition may have positive effects in turbid open water (Bakker, van Donk & Immers 2016), we urge managers to remain cautious: adverse effects of Fe addition may only become visible after decades. High soil Fe : P ratios and large Fe pools function as 'traps' for mobilized P, and this Fe-bound P pool appears largely available to rooting wetland plants.

Finally, we have elucidated the importance of water levels. Many fens in the Northern hemisphere have a drainage history, and fen rewetting projects are being installed to reverse the loss of endangered species. However, rewetting increases the relative availability of P to plants due to the redox-sensitive dissolution and desorption of Fe-bound P. Therefore, rewetting may push a fen even further away from P limitation, and many endangered species may not be able to establish. We argue that rewetting of drained fens is most likely to be successful if soil P and Fe pools are well below 3.3 and 15 mmol L^{-1} respectively. Conversely, rewetting of Fe-rich and P-rich fens may not always result in the restoration of P-limited systems. In such cases, topsoil removal prior to rewetting can be an effective restoration measure (Emsens *et al.* 2015), but the prerequisite is that the bulk of Fe and P has accumulated in the top soil. Alternatively, rewetting of Fe- and P-rich fens could result in the development of valuable nutrient-rich habitats such as peat-accumulating tall sedge communities.

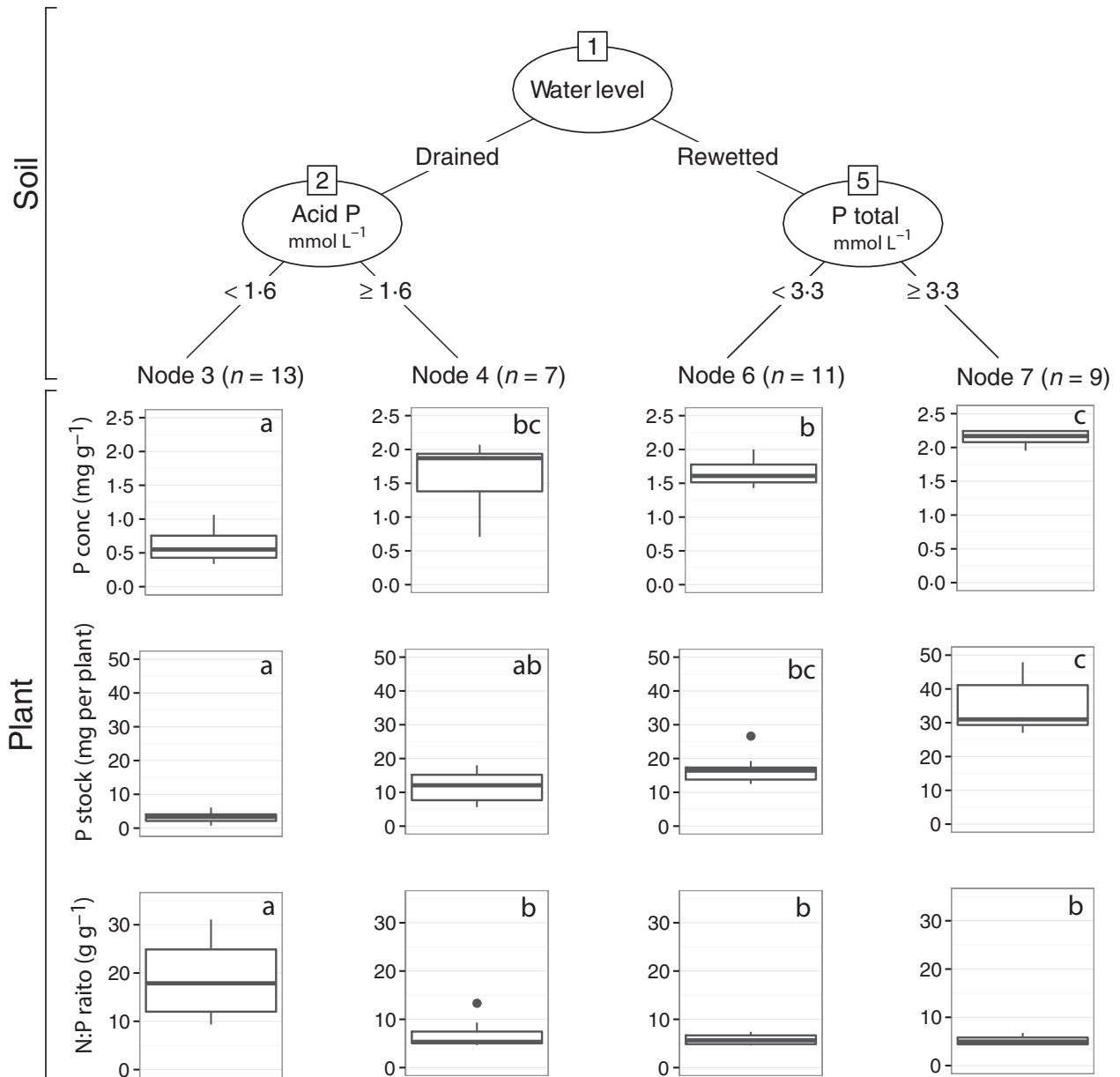


Fig. 4. Regression tree of foliar P concentration (mg g^{-1} , top box plots), plant P stock (mg per plant , middle box plots) and N : P ratio (g g^{-1} , bottom box plots) in above-ground biomass of *Carex rostrata* clones vs. 'water level' (drained or rewetted) and P variables of the soil (P variables in the model = pools of desorbable P, reductant-soluble P, acid-soluble P (= 'Acid P'), total P and soil Fe : P ratios). Plant P stocks were calculated as P concentrations \times plant biomass. The tree shows successive splitting and concomitant splitting criteria. The final nodes are visualized as box plots of the variables, and letters (abc) indicate significant differences between groups ($P < 0.05$).

Authors' contributions

W.J.E., C.J.S.A., A.J.P.S. and R.vD. designed methodology; W.J.E., C.J.S.A., D.Z., A.J.P.S. and R.vD. collected the data; W.J.E. analysed the data; and W.J.E. led the writing of the manuscript. All authors contributed to the drafts and gave approval for publication.

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Data accessibility

Data have been archived in the Dryad Digital Repository <https://doi.org/10.5061/dryad.37k44> (Emsens *et al.* 2017).

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Supporting Information

Details of electronic Supporting Information are provided below.

Fig. S1. Soil P vs. Fe contents.

Fig. S2. Soil oxalate-P vs. Fe, P and Al contents.

Fig. S3. Vegetation N : P ratios vs. biomass.