The restoration of degraded iron-rich fens

Het herstel van gedegradeerde ijzerrijke laagvenen

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Working on a PhD dissertation may sometimes feel like an endless and lonely journey. At some point in time, we (i.e. PhD'ers) often come to the point where we ask ourselves why we are doing what we are doing, and whether our work is useful. Sometimes, so it seems, we need to reach the end to know the answer. And the answer is a cliché: the journey matters more than the destination. Along the way, I learned that one can only learn by doing, by trial and error, by blowing up samples, by learning from mistakes. I also learned that the work of a dissertation is not a lonely road, and it is certainly not the work of one single person. In contrast, the thesis that you now have in front of you is the final result of a combined effort of many people. Therefore, this section of "thank you" is probably the most important section of this thesis.

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Summary

Rich fens are mesotrophic moss- and helophyte-dominated wetlands that are influenced by year-round discharge of base-rich and often iron-rich water. Fens typically harbor many endangered plant species, and they can actively sequester carbon (as peat). Unfortunately they are also amongst the most threatened habitat types in the Northern hemisphere: most fens have either completely disappeared or have otherwise been severely degraded. Therefore, restoration of the remaining, often degraded, fens has become a priority. The major threats for the remaining fens are ongoing drainage and water table drawdown, nutrient enrichment (eutrophication), habitat loss and plant re-colonization constraints.

In this dissertation we investigated if (and how) we can restore (a) typical fen plant communities, (b) organic matter accumulation, and (c) nutrient limitation in degraded fens, and we identified various biogeochemical factors that may contribute to or hamper successful restoration. Since many of the fens in our primary study area, Western Europe, are characterized by high iron (Fe) contents we specifically focused on interactions between iron chemistry and the biogeochemical environment.

In Chapter 2 & 3 we investigated the effects of soil Fe content on the restoration potential of fens. We found that Fe plays an ambiguous role in fens: the capacity of Fe-compounds to bind phosphorus (P) results in an overall increase in P availability to plants in Fe-rich fens, as P is being "trapped" within the fen. Moreover, this Fe-bound P appears available for plant uptake. Next, we found that experimental rewetting of drained Fe-rich fens correlates with a high mobilization of organic carbon, inorganic carbon and ammonium in the pore water of peat, whereas such mass mobilization was not observed when drained Fe-poor fens were rewetted. Since these compounds are products of anaerobic decomposition of organic matter, this provides evidence for the "iron-redox-wheel", in which anaerobic decomposition of organic matter is coupled with iron(III)reduction. The overall result is that nutrient availability is often higher in Fe-rich fens than in Fe-poor fens, which stimulates productivity of the herb layer. A highly productive herb layer however is detrimental to typical endangered fen species, which are then outcompeted by fast-growing and often common species (Chapter 6). It remains uncertain how such Fe-related anaerobic decomposition compares to aerobic decomposition under drained conditions, as the latter may still be worse.

Shifts or variation in nutrient availability should impact the potential of fens to accumulate organic matter as well. In chapter 4 we provided evidence for interactive and contrasting effects of fen eutrophication on litter accumulation, litter quality and decomposition. Eutrophication altered fresh litter production ("productivity shift"), litter stoichiometry within the same species ("intraspecific shift"), overall litter stoichiometry of the vegetation under the prediction that low-competitive species are outcompeted by fast-growing competitors ("interspecific shift"), and litter decomposition rates due to an altered external environment (e.g., shifts in microbial activity; "exogenous shift"). The cumulative effect of these interactions will ultimately

co-determine whether peatlands remain sinks or become sources of carbon under eutrophic conditions.

Finally, since most degradation in fens has usually occurred in the top soil, removal of a degraded and eutrophied top peat layer was shown to improve conditions for rich fen restoration as it typically resulted in lower nutrient levels, higher groundwater levels, and an increased availability of base cations and light. Such conditions are generally beneficial for endangered rich fen communities (Chapter 5).

Samenvatting

Laagvenen en beekdalvenen zijn ondiepe moerassen die onder rechtstreekse invloed staan van uittredend mineraalrijk grondwater en een hoge bedekking hebben van helofyten en mossen. De nutriëntenbeschikbaarheid in ongestoorde laagvenen is laag tot intermediair, en de continue waterverzadiging leidt vaak tot accumulatie van koolstof opgeslagen in veen. Typisch voor ongestoord en mooi ontwikkeld laagveen is een dominantie van vaak erg zeldzame kleine zeggen (*Carex* spp.) en slaapmossen. Veel laagvenen hebben momenteel echter sterk te lijden onder menselijke invloeden zoals drainage, eutrofiëring en habitatfragmentatie. Dergelijke degradatie resulteert uiteindelijk in het verdwijnen van karakteristieke plantengemeenschappen en legt mogelijk ook een hypotheek op de capaciteit van deze venen om actief nieuw veen te accumuleren.

In dit proefschrift hebben we onderzocht of (en hoe) gedegradeerde beekdalvenen hersteld kunnen worden, en welke biogeochemische factoren herstel stimuleren danwel verhinderen. De nadruk lag hierbij zowel op abiotisch herstel (vb. nutriëntlimitatie, vernatting, accumulatie van organisch materiaal) alsook op biotisch herstel (het herstel van kleine zeggen- en slaapmosvegetatie). Omdat veel van de door ons onderzochte venen gevoed worden door ijzerrijk grondwater lag de nadruk in dit proefschrift eveneens op het doorgronden van interacties tussen ijzerrijkdom van het veen met de abiotische en biotische omgeving.

In hoofdstukken 2 & 3 hebben we de effecten onderzocht van een hoge ijzerrijkdom van veenbodems op de biogeo- en hydro-chemie van het veen. Een hoge ijzerrijkdom in bodems wordt vaak gekoppeld aan fosforbinding, wat tot de onvoorzichtige conclusie zou kunnen leiden dat een hoge ijzerrijkdom een lage fosforbeschikbaarheid voor planten garandeert. Uit ons onderzoek bleek echter het tegenovergestelde: net omdat fosfor gebonden wordt aan ijzermineralen, is de totale fosforvoorraad in ijzerrijke venen vaak veel hoger dan deze in ijzerarme venen. Met andere woorden lijkt het er op dat een hoge ijzerrijkdom van de bodem het uitspoelen van fosfor verhindert. Eveneens is gebleken dat deze grote fosforvoorraad beschikbaar is voor opname door planten. Vervolgens hebben we experimenteel aangetoond dat het opnieuw vernatten van verdroogd veen niet altijd leidt tot gewenste resultaten. Wanneer verdroogd ijzerrijk veen opnieuw werd vernat, correleerde dit met een zeer hoge mobilisatie van opgelost organisch koolstof, anorganisch koolstof en ammonium in het porievocht van het veen. Dergelijke mobilisatie vond in veel mindere mate plaats bij de vernatting van verdroogd ijzerarm veen. Deze resultaten wijzen op verdere afbraak van organisch materiaal in ijzerrijk veen na vernatting wat mogelijk deels te wijten is aan de werking van het “ijzer-redox-wiel”, waarbij anaerobe afbraak van organisch materiaal gekoppeld is aan ijzer(III)reductie. Het blijft echter onduidelijk of dergelijke anaerobe afbraak hoger is dan aerobe afbraak onder gedraineerde omstandigheden, en het is ook onduidelijk wat de vooruitzichten zijn op lange termijn.

Bovengenoemde ijzer-gestuurde processen resulteren in een relatief hogere nutriëntenbeschikbaarheid in ijzerrijke venen t.ov. ijzerarme venen, wat zich vertaalt in een productievere kruidlaag. Wanneer de biomassa van de kruidlaag in een veen echter te hoog en te dicht wordt dan verliezen vele zeldzame plantensoorten de concurrentiestrijd voor licht met snelgroeiende en vaak algemenere soorten, zoals aangetoond in hoofdstuk 6. Dit laatste proces ligt mogelijk aan de basis van het verdwijnen of afwezig zijn van vele bedreigde plantensoorten in, met name ijzerrijke, venen.

Verschuivingen in nutriëntenbeschikbaarheid in venen, of natuurlijke variatie daarin, beïnvloeden daarnaast het vermogen van venen om actief organisch materiaal te accumuleren. In hoofdstuk 4 is gebleken dat eutrofiëring interagerende en contrasterende effecten heeft op de vorming en afbraak van plantenstrooisel, wat aan de basis ligt van veenvorming. Enerzijds stimuleerde nutriëntenverrijking de biomassaproductie van planten en daarmee ook de input van vers strooisel, anderzijds nam de chemische kwaliteit van het strooisel en de afbreekbaarheid ook toe door deze verschuivingen. Tenslotte hadden de nutriëntengehaltes in de omgeving ook een rechtstreeks effect op afbraak, wellicht door terugkoppelingen met microbiële gemeenschappen, maar de richting van dit effect was dan weer afhankelijk van weefselkwaliteit. Uiteindelijk is het het cumulatieve netto effect van dergelijke interacties die bepalen of een laagveen nog actief koolstof accumuleert of eerder verliest, en dat kan beide richtingen op.

Tenslotte is het zo dat degradatie van veen doorgaans bovenaan het veenprofiel begint: de toplaag wordt namelijk het sterkst rechtstreeks beïnvloed door drainage en depositie van nutriënten. In hoofdstuk 5 hebben we aangetoond dat het afgraven van dergelijke gedegradeerde veenlaag tot enkele decimeters diepte vaak positieve effecten heeft op middellange termijn. Nutriëntengehaltes werden doorgaans verlaagd door de toplaag af te graven, terwijl grondwaterstanden, basenrijkdom van het porievocht, en lichtbeschikbaarheid hoger werden. Dergelijke omstandigheden dragen bij aan de ontwikkeling of het herstel van gemeenschappen van kleine zeggen en slaapmossen.

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General introduction

1.1 Rich fens

1.1.1 General characteristics

Rich fens are mesotrophic bryophyte- and helophyte-dominated wetlands that are influenced by year-round discharge of circumneutral base-rich water (Grootjans et al. 2006, Succow and Joosten 2001, Rydin and Jeglum 2013). Most fens are located in areas with high rates of groundwater discharge (seepage), but they also occur in areas that are predominantly affected by base-rich surface water. Since base-rich water has by definition relatively high concentrations of Ca^{2+}, Mg^{2+} and HCO_3^-, the pH of fens is generally well-buffered between 5.5 and 8. The soil of undrained fens is normally continuously waterlogged, and therefore predominantly anoxic. Such conditions hamper both presence and activity of mesofauna and microbiota, which correlates with low rates of organic matter decomposition. Therefore, many rich fens are peat- and carbon accumulating peatlands (= mires) with high soil organic matter contents (20 – 99 %).

This specific combination of abiotic conditions requires unique adaptations for plants in order to persist, and therefore rich fens harbor distinct plant communities. Undisturbed rich fens are typically covered by herbaceous communities of small and intermediate sedges (e.g. *Carex dioica* (L.), *C. diandra* (Schrank), *C. limosa* (L.), *C. lepidocarpa* (Tausch)), brown mosses (many species of the *Amblystegiaceae* family, *Scorpidium scorpioides* ((Hedw.) Limpr.), *Campylium stellatum* ((Hedw.) C.E.O. Jensen), but also *Tomentypnum nitens* (Hedw.) Loeske), and orchids (e.g. *Epipactis palustris ((*L.*) Crantz),* or *Dactylorhiza majalis* ((Rchb.) P.F.Hunt & Summerh.)).

1.1.2 Fen classification

"Fen" is a rather broad term that comprises a wide range of mesotrophic, typically moss- and helophyte-dominated freshwater wetlands. Several fen classification schemes have been proposed based on nutrient availability, vegetation structure and -composition, or pH and base saturation (Wheeler and Proctor 2000, Succow and Joosten 2001, Hajek et al. 2006). This large variation in classification schemes reflects the huge variation in and interactions between hydrological, hydro-chemical, geochemical and biological processes typically measured in fens (E.g. Fig. 1.1). For example, although most fens are peat-accumulating ecosystems, some are so calcareous that they accumulate tufa (= precipitation of Ca- and Mg-carbonates) rather than peat. The physiochemical characteristics of underlying mineral aquifers usually has a dominant impact on fen biogeochemistry as it co-determines the chemical composition of the water that feeds the fen. For example, some fens are fed by intermediately base-rich groundwater mixed with rainwater, whereas others are fed by strong upwelling of groundwater that is supersaturated with Ca^{2+}, Mg^{2+} and HCO_3^-. Such broad hydrogeochemical variation is captured in the variation in dominant plant communities: the former fens are often dominated by more "acidic" species and relatively few "true" rich fen species (e.g. moderately rich fens with the basophile peatmoss *Sphagnum teres* ((Schimp.) Ångström)) or *Warnstorfia exannulata* ((Schimp.) Loeske), whereas the latter fens are often dominated by, for example, communities of the *Caricion davallianae* alliance (e.g. extremely rich fens with *Carex davalliana* (Sm.), *Palustriella commutata* ((Hedw.) Ochyra)).

Figure 1.1: Some rich fen types. Left: Groundwater-fed percolation mire, dominated by *Carex lasiocarpa*, *Thelypteris palustris* and *Hamatocaulis vernicosus* (Rospuda, Poland), Surface water-fed terrestrialization mire, dominated by *Carex diandra*, *Scorpidium scorpioides* and *Campylium stellatum* (Weerribben, the Netherlands), Right: Groundwater-fed sloping mire, dominated by *Carex davalliana, Eriophorum latifolium* and *Palustriella commutata* (Alps, Switzerland).

1.2 Main threats and problems

Fens are amongst the most threatened habitat types in the Northern hemisphere: most fens have either completely disappeared or have otherwise been severely degraded over the past few centuries (Lamers et al. 2015). As a consequence, many of the typical plant species of small sedge and brown moss communities have also gone locally extinct, or they have become rare or endangered. Therefore, conservation and restoration of the remaining fens has become a top priority in nature conservation (van Diggelen et al. 2006). In the European Union, fens are now legally protected under the Habitats Directive (”Transition mires and quaking bogs” (H7140) but also ”Alkaline fens” (H7230)).

1.2.1 Drainage and water table drawdown

Drainage and water table drawdown are threatening many of the few remaining fens. Most drainage channels in Europe have been dug during the 19th-20th century, and the primary goal was to convert fens to economically productive environments. After drainage, fens were typically converted to agricultural land, or trees were planted for forestry. Water table drawdown can also be the result of groundwater abstraction which alters regional groundwater flows. Since fen soils usually contain large pools of reactive organic matter, the slightest alteration in hydrological conditions can have disproportional effects on fen chemistry and it can trigger a myriad of reactions. When fen soil is drained, intrusion of oxygen often increases the rate of organic matter decomposition and mineralization (Brouns et al. 2014), which eventually results in inorganic nutrient release (Grootjans et al. 1986). Moreover, peat oxidation triggers carbon loss (Laiho 2006), soil subsidence (Gambolati et al. 2006), regeneration of electron acceptors (Fenner et al. 2011), acidification (Beltman et al. 2001), leaching of base cations (Laiho et al. 1999), and eventually shifts in vegetation and loss of typical fen species (Malson et al. 2008). It remains questionable whether such biogeochemical shifts are reversible, at least in the short therm.

1.2.2 Eutrophication

Eutrophication, i.e. the increased availability of macronutrients (mainly N and P), is another significant threat to fens. Eutrophication can be a direct result of drainage coupled with peat mineralization, but it can also be due to external factors such as atmospheric nitrogen deposition, rising global temperatures, or the widespread use of artificial fertilizer and manure (van Diggelen et al. 2006, Lamers et al. 2015). Nowadays, one of the major challenges with respect to fens is understanding through which mechanisms nutrient enrichment affects vegetation structure and composition, organic matter accumulation and decomposition, and carbon fluxes. For example, eutrophication directly stimulates primary plant production (Kotowski et al. 2006, Hautier et al. 2009), which can lead to the competitive exclusion of small sedges and brown mosses by taller, faster-growing species due to light limitation (Kotowski et al. 2006). An increase in plant primary production may correlate with increased litter input rates, and it may directly and indirectly alter decomposition rates of plant litter and soil organic matter (Hessen et al. 2004). Eventually, although not very well known, eutrophication-induced biogeochemical shifts may fundamentally alter fen structure and functioning.

1.2.3 Biodiversity loss and dispersal limitation

The aforementioned trends of fen drainage, land use change and eutrophication have led to a widespread loss of typical fen species, overall loss of biodiversity, and local extinctions. The extinction trend is particularly clear for the smallest and most vulnerable rich fen species, e.g. *Carex dioica* or *Paludella squarrosa* ((Hedw.) Brid.) (Fig. 1.2). If species have gone extinct on a regional scale or can no longer disperse freely, then this has consequences for (1) long-term genetic viability of (relict) populations and (2) the ability of these species to (re-)colonize potentially suitable areas or restoration fens. In addition, fen degradation can lead to deterioration of the diaspore bank, and often only propagules of more common species persist (Klimkowska et al. 2010). Hence, many remaining fens are nowadays characterized by few typical fen species, or there is a gradual decline in numbers of vulnerable species due to ongoing fen degradation or genetic drift.

Figure 1.2: Some typical fen species that are severely threatened by habitat loss and fen degradation. Left: *Carex limosa*, Top-Right: *Carex dioica* (male plant), Bottom-right: *Paludella squarrosa*.

1.3 Fen restoration

An increasing number of restoration projects is initiated in Europe and North America in order to reverse the adverse effects of fen degradation. The first step in any restoration project is to restore hydrogeochemical processes and thereby to re-create favorable abiotic conditions (van Diggelen et al. 2006). In fens, restoration typically aims at re-installing the original water regime, lowering nutrient availability, re-initiating peat (and thus carbon) accumulation, and increasing the diversity of characteristic fen species. In this dissertation, we evaluate three techniques for fen restoration: rewetting, topsoil removal, and species (re-)introduction.

1.3.1 Rewetting

Fen rewetting, i.e. re-installing the original hydrological system, is usually the first step in the restoration of drained fens. Rewetting is usually accomplished by closing drainage ditches, by damming deep-draining rivers and canals, or by smart use of pumping stations. However, restoration of the initial hydrological regime is often notoriously difficult as fens rely on a constant discharge of nutrient-poor mineral-rich (ground-)water. Hence, rewetting by retention of rainwater or by flooding with river water is rarely adequate, as it tends to result in acidification or eutrophication respectively

(Wassen et al. 1990, Beltman et al. 1996). Moreover, rewetting of drained fens often leads to excessive mobilization of dissolved organic and inorganic carbon (DOC and DIC), ammonium (NH_4^+), and phosphate (PO_4^{3-}) into pore and surface water (Van Dijk et al. 2004, Zak and Gelbrecht 2007), at least in the short term. Nutrient levels and carbon effluxes therefore often remain high in rewetted fens.

1.3.2 Topsoil removal

Long-term fen drainage and degradation can alter biogeochemical conditions in the top soil to such an extent that successful fen restoration becomes notoriously difficult (see chapter 1.2.1), even after rewetting (Zak et al. 2010, Brouns et al. 2014). Eventually, degradation-induced biogeochemical alterations in the top soil may induce a shift towards different feedback mechanisms, forcing an ecosystem into an alternative degraded state (Suding et al. 2004). “Simple” restoration measures such as rewetting may then appear inadequate, and more rigorous measures for fen restoration may be required. In such cases, complete removal of the (degraded) top soil may be an option. Topsoil removal is a well-established measure in nature conservation and management on mineral soils (Allison and Ausden 2004, Olsson and Ödman 2014), but the effect of topsoil removal in fens is only documented fragmentarily (Patzelt et al. 2001, Klimkowska et al. 2007, Harpenslager et al. 2015), often with contrasting results.

1.3.3 Reintroduction

Many typical fen plant species (e.g. small sedges and brown mosses) often remain absent in restoration fens, even after successful abiotic restoration, whereas more common wetland species thrive (van Dijk et al. 2007, Malson et al. 2008, Aggenbach et al. 2013). This may be due to the “degradation legacy” of restoration fens in which long-term degradation has deteriorated diaspore banks and extirpated source populations of vulnerable species. Relict populations of vulnerable fen species, if still present, are often heavily fragmented, small or genetically impoverished (de Vere et al. 2009, Lamers et al. 2015). Hence, spontaneous recolonization of rewetted fens by target species often appears hampered by dispersal limitation or limited propagule viability (Cobbaert et al. 2004, Middleton et al. 2006). The success of fen restoration projects in terms of species diversity may be improved if abiotic restoration is combined with species (re-)introduction (Patzelt et al. 2001, Malson and Rydin 2007, Lamers et al. 2015).

1.4 Iron (Fe) - an underestimated element in fens?

Base-rich water that typically feeds fens is usually also rich in acidic cations such as iron (Fe^{3+}-Fe^{2+}). This is particularly so when groundwater has percolated through Fe-rich soil layers, e.g. sandy aquifers of maritime origin that contain large amounts of Fe-minerals such as glauconite (Vanranst and Deconinck 1983), before it enters the fen.

Discharge of Fe-enriched water should have a significant impact on fen functioning (Fig. 1.3). For example, retention of phosphorus (P) in wetland soils is often linked to iron chemistry as reactive surfaces of iron compounds provide sorption sites for dissolved inorganic P (Patrick and Khalid 1974, Zak et al. 2010), while formation of ferrous phosphate minerals serves as an additional P sink (Rothe et al. 2014). Such Fe-P binding hampers P mobilization into open water, and this may positively affect water quality of downstream catchments or co-determine in situ P availability to plants. Furthermore, evidence is emerging that the biogeochemical cycles of many elements (e.g. C, N, S,…) may be directly coupled to the “iron-redox wheel”, i.e. the alternate iron reduction-oxidation cycle (Fe^{3+}-Fe^{2+}) characteristic to fluctuating redox environments (Lovley 1987, Lindsay 1991, Davidson et al. 2003, Li et al. 2012). This is primarily because labile organic matter is an electron donor in the Fe-reduction reaction (Lovley and Phillips 1986, Lovley 1987), or in other words, Fe reduction upon flooding is coupled with anaerobic organic matter decomposition. In fens and wetlands, iron (Fe^{3+}-Fe^{2+}) is often the dominant reduction-oxidation couple, and concentrations of ferric iron may exceed concentrations of other potential electron acceptors such as sulfate SO_4^{2-} or nitrate NO_3^-. Moreover, ferrous iron may promote the activity of phenol oxidase (van Bodegom et al. 2005), which also results in higher rates of organic matter decomposition. Finally, high concentrations of ferrous iron are phytotoxic to plants (Snowden and Wheeler 1993), and many fens are characterized by pore water Fe concentrations that exceed such potentially phytotoxic (> 1000 µmolars) thresholds (Aggenbach et al. 2013). As such, iron may also have a potentially large effect on vegetation structure and composition in fens through direct toxicity effects.

Given that iron concentrations in fen soils and pore water can differ orders of magnitude among different fens (Aggenbach et al. 2013), it is somewhat surprising that a gradient in iron richness has, to our knowledge, never been incorporated in fen classification schemes.

Figure 1.3: Iron oxidation (red color) at the surface of a fen is indicative for discharge of Fe-rich groundwater (Zwarte Beek, Belgium).

1.5 Thesis aims and outline

The main research question of this dissertation is if and how we can restore (a) characteristic plant communities of small sedges and brown mosses and (b) organic matter accumulation and nutrient limitation in degraded rich fens, and which factors may contribute to or hamper restoration. Thereto, we aimed to investigate biogeochemical processes in fens with an emphasis on the effects of (gradients in) nutrient levels and iron richness on organic matter decomposition, nutrient availability and vegetation structure and composition. Furthermore, we investigated three different techniques of fen restoration: fen rewetting, topsoil removal and plant species (re-)introduction, which we relate to restoration-induced shifts in abiotic conditions and vegetation. In this dissertation we specifically focus on the restoration of iron-rich fens: recent observations have led to the hypothesis that past fen drainage has triggered excessive iron accumulation in the degraded top soil of fens, and these high iron concentrations may be a significant bottleneck for fen restoration (Aggenbach et al. 2013). Although it indeed appears that many Fe-rich fens are characterized by high nutrient levels and a low diversity of typical fen specialists (Aggenbach et al. 2013), the exact mechanisms behind the generally low restoration success of Fe-rich fens have remained unclear.

In **Chapter 2 & 3**, we investigate the effects of soil Fe content on the restoration potential of fens: in **Chapter 2** we elaborate on the ambiguous role of iron in P-binding and –limitation, and in **Chapter 3** we focus on the effects of rewetting on nutrient and carbon mobilization in Fe-poor and Fe-rich drained fens. In **Chapter 4** we investigate the interactive and contrasting effects of fen eutrophication on litter accumulation, litter quality and decomposition. Finally, we evaluate if topsoil removal (**Chapter 5**) and species (re-)introduction (**Chapter 6**) are effective measures for fen restoration, and we further identify abiotic bottlenecks constraints for restoration fens.

In **Chapter 7** we discuss and synthesize our findings and we evaluate the restoration potential of fens, with the emphasis on the biogeochemistry and restoration of Fe-rich fens. Finally, we provide management recommendations, and we identify priorities for future research.

1.6 References

Aggenbach, C. J. S., H. Backx, W. J. Emsens, A. P. Grootjans, L. P. M. Lamers, A. J. P. Smolders, P. J. Stuyfzand, L. Wolejko, and R. Van Diggelen. 2013. Do high iron concentrations in rewetted rich fens hamper restoration? Preslia 85:405-420.

Allison, M. and M. Ausden. 2004. Successful use of topsoil removal and soil amelioration to create heathland vegetation. Biological Conservation 120:221-228.

Beltman, B., T. Van den Broek, A. Barendregt, M. C. Bootsma, and A. P. Grootjans. 2001. Rehabilitation of acidified and eutrophied fens in The Netherlands: Effects of hydrologic manipulation and liming. Ecological Engineering 17:21-31.

Beltman, B., T. VanDenBroek, S. Bloemen, and C. Witsel. 1996. Effects of restoration measures on nutrient availability in a formerly nutrient-poor floating fen after acidification and eutrophication. Biological Conservation 78:271-277.

Brouns, K., J. T. A. Verhoeven, and M. M. Hefting. 2014. Short period of oxygenation releases latch on peat decomposition. Science of the Total Environment 481:61-68.

Cobbaert, D., L. Rochefort, and J. S. Price. 2004. Experimental restoration of a fen plant community after peat mining. Applied Vegetation Science 7:209-220.

Davidson, E. A., J. Chorover, & D. B. Dail. 2003. A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. Global Change Biology, 9(2), 228-236.

de Vere, N., E. Jongejans, A. Plowman, and E. Williams. 2009. Population size and habitat quality affect genetic diversity and fitness in the clonal herb Cirsium dissectum. Oecologia 159:59-68.

Fenner, N., R. Williams, H. Toberman, S. Hughes, B. Reynolds, and C. Freeman. 2011. Decomposition 'hotspots' in a rewetted peatland: implications for water quality and carbon cycling. Hydrobiologia 674:51-66.

Gambolati, G., M. Putti, P. Teatini, and G. G. Stori. 2006. Subsidence due to peat oxidation and impact on drainage infrastructures in a farmland catchment south of the Venice Lagoon. Environmental Geology 49:814-820.

Grootjans, A. P., Adema, E. B., Bleuten, W., Joosten, H., Madaras, M., and M. Janáková. 2006. Hydrological landscape settings of base-rich fen mires and fen meadows: an overview. Applied Vegetation Science, 9: 175-184.

Grootjans, A. P., P. C. Schipper, and H. J. Vanderwindt. 1986. Influence of Drainage on N-Mineralization and Vegetation Response in Wet Meadows .2. Cirsio-Molinietum Stands. Acta Oecologica-Oecologia Plantarum 7:3-14.

Hajek, M., M. Horsak, P. Hajkova, and D. Dite. 2006. Habitat diversity of central European fens in relation to environmental gradients and an effort to standardise fen terminology in ecological studies. Perspectives in Plant Ecology Evolution and Systematics 8:97-114.

Harpenslager, S. F., E. van den Elzen, M. A. R. Kox, A. J. P. Smolders, K. F. Ettwig, and L. P. M. Lamers. 2015. Rewetting former agricultural peatlands: Topsoil removal as a prerequisite to avoid strong nutrient and greenhouse gas emissions. Ecological Engineering 84:159-168.

Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for Light Causes Plant Biodiversity Loss After Eutrophication. Science 324:636-638.

Hessen, D. O., G. I. Agren, T. R. Anderson, J. J. Elser, and P. C. De Ruiter. 2004. Carbon, sequestration in ecosystems: The role of stoichiometry. Ecology 85:1179-1192.

Klimkowska, A., R. M. Bekker, R. Van Diggelen, and W. Kotowski. 2010. Species trait shifts in vegetation and soil seed bank during fen degradation. Plant Ecology 206:59-82.

Klimkowska, A., R. Van Diggelen, J. P. Bakker, and A. P. Grootjans. 2007. Wet meadow restoration in Western Europe: A quantitative assessment of the effectiveness of several techniques. Biological Conservation 140:318-328.

Kotowski, W., W. Thorig, R. van Diggelen, and M. J. Wassen. 2006. Competition as a factor structuring species zonation in riparian fens - a transplantation experiment. Applied Vegetation Science 9:231-240.

Laiho, R. 2006. Decomposition in peatlands: Reconciling seemingly contrasting results on the impacts of lowered water levels. Soil Biology & Biochemistry 38:2011-2024.

Laiho, R., T. Sallantaus, and J. Laine. 1999. The effect of forestry drainage on vertical distributions of major plant nutrients in peat soils. Plant and Soil 207:169-181.

Lamers, L. P. M., M. A. Vile, A. P. Grootjans, M. C. Acreman, R. van Diggelen, M. G. Evans, C. J. Richardson, L. Rochefort, A. M. Kooijman, J. G. M. Roelofs, and A. J. P. Smolders. 2015. Ecological restoration of rich fens in Europe and North America: from trial and error to an evidence-based approach. Biological Reviews 90:182-203.

Li, Y. C., S. Yu, J. Strong, and H. L. Wang. 2012. Are the biogeochemical cycles of carbon, nitrogen, sulfur, and phosphorus driven by the "Fe-III-Fe-II redox wheel" in dynamic redox environments? Journal of Soils and Sediments 12:683-693.

Lindsay, W. L. 1991. Iron oxide solubilization by organic matter and its effect on iron availability. Plant and soil, 130: 27-34.

Lovley, D. R., and E.J. Phillips. 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Applied and environmental microbiology 51: 683-689.

Lovley, D. R. 1987. Organic matter mineralization with the reduction of ferric iron: a review. Geomicrobiology Journal 5.3-4: 375-399.

Malson, K., I. Backeus, and H. Rydin. 2008. Long-term effects of drainage and initial effects of hydrological restoration on rich fen vegetation. Applied Vegetation Science 11:99-106.

Malson, K. and H. Rydin. 2007. The regeneration capabilities of bryophytes for rich fen restoration. Biological Conservation 135:435-442.

Middleton, B., R. van Diggelen, and K. Jensen. 2006. Seed dispersal in fens. Applied Vegetation Science 9:279-284.

Olsson, P. A. and A. M. Ödman. 2014. Natural Establishment of Specialist Plant Species after Topsoil Removal and Soil Perturbation in Degraded Calcareous Sandy Grassland. Restoration Ecology 22:49-56.

Patrick, W. H. and R. A. Khalid. 1974. Phosphate Release and Sorption by Soils and Sediments - Effect of Aerobic and Anaerobic Conditions. Science 186:53-55.

Patzelt, A., U. Wild, and J. Pfadenhauer. 2001. Restoration of wet fen meadows by topsoil removal: Vegetation development and germination biology of fen species. Restoration Ecology 9:127-136.

Rothe, M., T. Frederichs, M. Eder, A. Kleeberg, and M. Hupfer. 2014. Evidence for vivianite formation and its contribution to long-term phosphorus retention in a recent lake sediment: a novel analytical approach. Biogeosciences 11:5169-5180.

Rydin, H., Jeglum, J. K., and J.K. Jeglum. 2013. The biology of peatlands, 2e. Oxford university press.

Snowden, R. E. D. and B. D. Wheeler. 1993. Iron Toxicity to Fen Plant-Species. Journal of Ecology 81:35-46.

Succow, M. and H. Joosten. 2001. landschaftsökologische Moorkunde.

Suding, K. N., K. L. Gross, and G. R. Houseman. 2004. Alternative states and positive feedbacks in restoration ecology. Trends in Ecology & Evolution 19:46-53.

Van Bodegom, P. M., Broekman, R., Dijk, J., Bakker, C., & Aerts, R. 2005. Ferrous iron stimulates phenol oxidase activity and organic matter decomposition in waterlogged wetlands. Biogeochemistry, 76(1): 69-83.

van Diggelen, R., B. Middleton, J. Bakker, A. Grootjans, and M. Wassen. 2006. Fens and floodplains of the temperate zone: Present status, threats, conservation and restoration. Applied Vegetation Science 9:157-162.

Van Dijk, J., M. Stroetenga, L. Bos, P. M. Van Bodegom, H. A. Verhoef, and R. Aerts. 2004. Restoring natural seepage conditions on former agricultural grasslands does not lead to reduction of organic matter decomposition and soil nutrient dynamics. Biogeochemistry 71:317-337.

van Dijk, J., M. Stroetenga, P. M. van Bodegom, and R. Aerts. 2007. The contribution of rewetting to vegetation restoration of degraded peat meadows. Applied Vegetation Science 10:315-U314.

Vanranst, E. and F. Deconinck. 1983. Evolution of Glauconite in Imperfectly Drained Sandy Soils of the Belgian Campine. Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 146:415-426.

Wassen, M. J., A. Barendregt, A. Palczynski, J. T. Desmidt, and H. Demars. 1990. The Relationship between Fen Vegetation Gradients, Groundwater-Flow and Flooding in an Undrained Valley Mire at Biebrza, Poland. Journal of Ecology 78:1106-1122.

Wheeler, B. D. and M. C. F. Proctor. 2000. Ecological gradients, subdivisions and terminology of north-west European mires. Journal of Ecology 88:187-203.

Zak, D. and J. Gelbrecht. 2007. The mobilisation of phosphorus, organic carbon and ammonium in the initial stage of fen rewetting (a case study from NE Germany). Biogeochemistry 85:141-151.

Zak, D., C. Wagner, B. Payer, J. Augustin, and J. Gelbrecht. 2010. Phosphorus mobilization in rewetted fens: the effect of altered peat properties and implications for their restoration. Ecological Applications 20:1336-1349.

2

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

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Abstract

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. 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). 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. 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. 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 favorable 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 mmol L^{-1} and 15 mmol L^{-1} respectively.

2.1 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 & Vaithiyanathan 2009). In calcareous wetlands for example, P can be immobilized by precipitation with calcium (Ca) or adsorption to $CaCO_3$ (Boyer & Wheeler 1989; Richardson & Vaithiyanathan 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 favor 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.), *C. diandra* (Schrank), *C. lepidocarpa* (Tausch)) and brown mosses (mainly from the family *Amblystegiaceae*, e.g. *Hamatocaulis vernicosus* ((Mitt.) Hedenäs), *Scorpidium scorpioides* ((Hedw.) Limpr.), *Campylium stellatum* ((Hedw.) C.E.O. Jensen)), with high abundance in areas of low P availability (Boeye et al. 1997; Kooijman & Paulissen 2006).

We analyzed 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.

2.2 Materials and methods

2.2.1 Quantification of P limitation

Nutrient ratios of aboveground 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 (in 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).

2.2.2 Field survey

We collected soil, pore water and vegetation samples in 30 different fens (Table 2.1). 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 2.1) 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 μm, Rhizosphere Research Products, The Netherlands) attached to extension tubes (0-45 cm depth) or ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment, The Netherlands) (> 45 cm depth). In total, we made 49 vegetation relevées divided over 12 different fens in which we identified all vascular plant and bryophyte species (plot size > 2x2 m), and we collected aboveground herb biomass in a 0.4 x 0.4 m area (Table 2.1). Biomass was oven-dried at 70°C for 48 hours and weighed.

Table 2.1: Study sites with coordinates, country (BE = Belgium, NL = the Netherlands, PL = Poland), sampling depths, and the number of (1) soil samples with bulk chemistry measurements (= Bulk density, total Fe and P), (2) soil samples with oxalate-P measurements (Pox), and (3) vegetation (relevées and biomass) samples.

Site code	Longitude	Latitude	Country	Sampling depth (cm)	Soil samples bulk chemistry	Soil samples P_{ox}	Vegetation samples
BG	5.17667	51.21494	BE	0-10	2	0	0
LE	3.26744	51.15533	BE	0-10	2	0	0
MA	4.87344	50.84902	BE	0-10	7	7	7
TB	4.54467	50.90531	BE	0-10	1	0	0
ZB	5.28592	51.07978	BE	0-150	7	0	0
ZO	5.32522	51.09169	BE	0-300	11	0	0
BM	5.59675	52.00719	NL	0-50	5	0	0
DE	6.04575	50.87097	NL	0-25	2	0	0
ES	6.65922	52.87478	NL	0-50	5	0	0
EO	6.66669	52.88422	NL	0-10	2	0	0
GD	6.66781	53.02056	NL	0-200	15	4	0
HE	5.58133	52.00870	NL	0-10	4	4	4
HO	6.62956	52.90433	NL	0-10	4	3	4
HA	6.62750	52.91443	NL	0-10	4	4	4
KL	5.93439	50.78006	NL	0-25	2	0	0
LD	6.62803	53.01392	NL	0-50	19	11	10
LH	6.27711	52.64239	NL	0-50	6	4	4
LM	6.87536	52.34611	NL	0-10	2	0	0
LS	5.02189	51.68808	NL	0-10	2	0	0
MB	6.87007	52.44632	NL	0-10	3	3	3
PE	6.50346	53.17187	NL	0-10	8	6	8
RD	6.43581	52.62381	NL	0-10	2	0	0
BS	17.55217	53.89614	PL	0-100	12	4	0
BZ	22.60886	53.28650	PL	0-155	7	0	0
DZ	16.98933	53.52444	PL	0-200	15	5	0
GG	17.42639	54.35883	PL	0-10	2	0	0
LB	17.56011	54.02353	PL	0-100	14	4	1
LU	17.49678	54.02219	PL	0-50	11	3	1
RS	22.95367	53.90417	PL	0-200	9	0	1
SZ	23.35414	53.71533	PL	0-500	9	0	2

2.2.3 P uptake in a phytometer experiment

We conducted a phytometer experiment with *Carex rostrata* (Stokes) to additionally investigate (1) the interactive effects of soil P pools and –fractions and water regime (rewetted or drained) on plant P uptake and (2) rewetting prospects of drained fens in relation to Fe- and P chemistry. We chose *Carex rostrata* because (1) members of the Carex genus form a dominant plant group in fens, and (2) *C. rostrata* is a common species in the Northern hemisphere and grows in any fen type.

Soil core collection and mesocosm setup

We collected a total of 40 soil cores in drained parts of four degraded fens (LH, BM, ES, ZO). Sites covered a gradient of increasing Fe richness (means ± SD = 18 ± 5, 32 ± 6, 73 ± 15 and 525 ± 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 ten intact replicate soil cores of 45 cm x 12.5 cm (depth x diameter) at each location. We pushed hard-PVC tubes (diameter = 12.5 cm) of 50 cm length in the peat soil, after removing all aboveground 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 = ca. 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 N_2-deoxygenized water. The mesocosms were kept under these conditions for one 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 *Carex 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. 7 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 aboveground biomass. Biomass was oven-dried at 70°C for 48 hours and weighed.

2.2.4 Chemical analyses

Field survey

Pore water pH was measured directly in the field using portable equipment (WTW Multi 340i). Next, the samples were acidified with 0.7 mL 65% suprapure HNO_3 per 100 mL sample and analyzed on ICP (IRIS Intrepid II) for total dissolved P. Soil bulk density (in 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 (4h 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 HNO_3 (65%) and 1 mL H_2O_2 (30%) using a microwave labstation (Milestone srl). Additionally, we determined oxalate-P concentrations (Pox) for a subset of samples (Table 2.1), 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 hours, after which the extracts are filtered and analyzed with ICP. Total N, P and K concentrations (in mg g^{-1}) of biomass samples were determined by digesting 0.3 g oven-dried mill-ground plant material with H_2SO_4 (96%) , H_2O_2 (30%) and salicylic acid (Walinga et al. 1989). N and P were analyzed on a segmented flow analyzer (Skalar, Breda, The Netherlands) and K was analyzed 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 (NH_4Cl-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 (= NH_4Cl-P), reductant-soluble P (= NH_4Cl-P + BD-P), acid-soluble P (= NH_4Cl-P + BD-P + HCl-P). Foliar P and N concentrations (in mg g^{-1}) in *Carex rostrata* were determined as in the field survey.

2.2.5 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 (in 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: (1) vegetation N:P ratios (g g^{-1}), (2) foliar P concentrations (mg g^{-1}), and (3) 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 log 10-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 (1) vegetation N:P ratios, foliar P concentrations and vegetation P stocks and (2) 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 non-linear 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.

2.3 Results

2.3.1 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. 2.1a.) When using site averages, the trend remained similar (Linear regression: $F_{1,28} = 80.2$, $P < 0.001$, Fig. 2.1b). 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. 2.2a,b). Oxalate-P pools also correlated weakly with soil pools of aluminum (Al) (Fig. 2.2c). 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. 2.2d,e). Weight-based correlations are shown in Fig. S2.1 and Fig. S2.2 in Supporting Information.

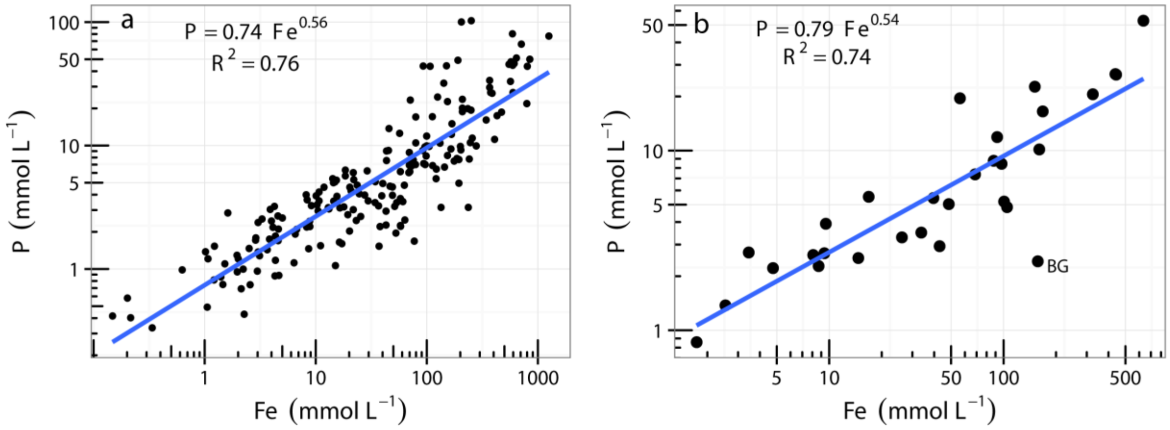

Figure 2.1: Log-log plots of P- versus Fe pools in fen soils (in mmol L^{-1}) for (a) all 194 soil samples collected at 30 study sites and at different depths (n = 194, ranging from 0-500 cm below the surface), and (b) independent averages of the 30 study sites. Regression lines are fitted with least-squares linear regression of the log-transformed variables.

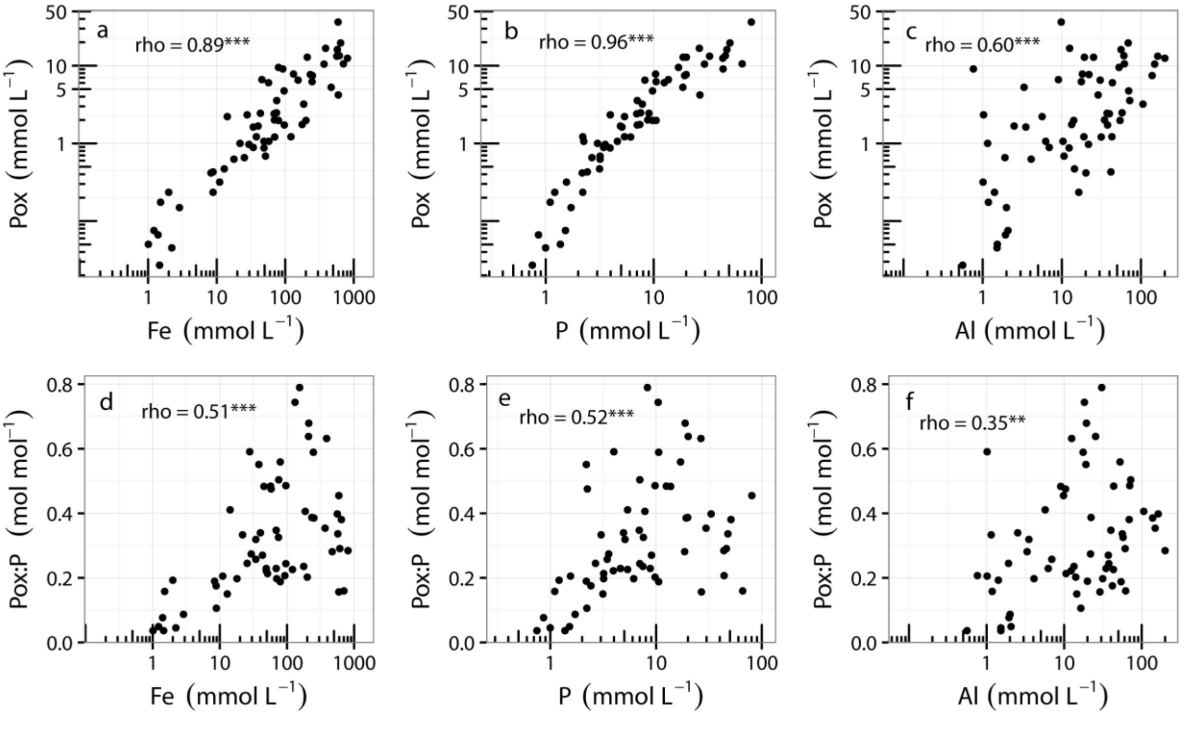

Figure 2.2: Correlations between soil pools of oxalate-P (Pox) and pools of (a) Fe, (b) P and (c) Al in fens (in mmol L^{-1}), and between the fraction oxalate-P P^{-1} (mol mol^{-1}) and soil pools of (d) Fe, (e) P and (f) Al in fens (n = 62 samples collected in 13 different fens between 0 – 200 cm depth). Spearman's rho values are listed together with their significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

2.3.2 Nutrient limitation

Soil P- and Fe pools and soil Fe:P ratios correlated negatively with vegetation N:P ratios (Fig. 2.3a,b,c). Whenever thresholds of 3.3 mmol P L^{-1} and 15 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. 2.3d,e,f) and in the total vegetation P stock (Fig. 2.3g,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, df = 47, P = 0.69) or vegetation N:P ratios (Spearman's rho = -0.10, df = 47, P = 0.49).

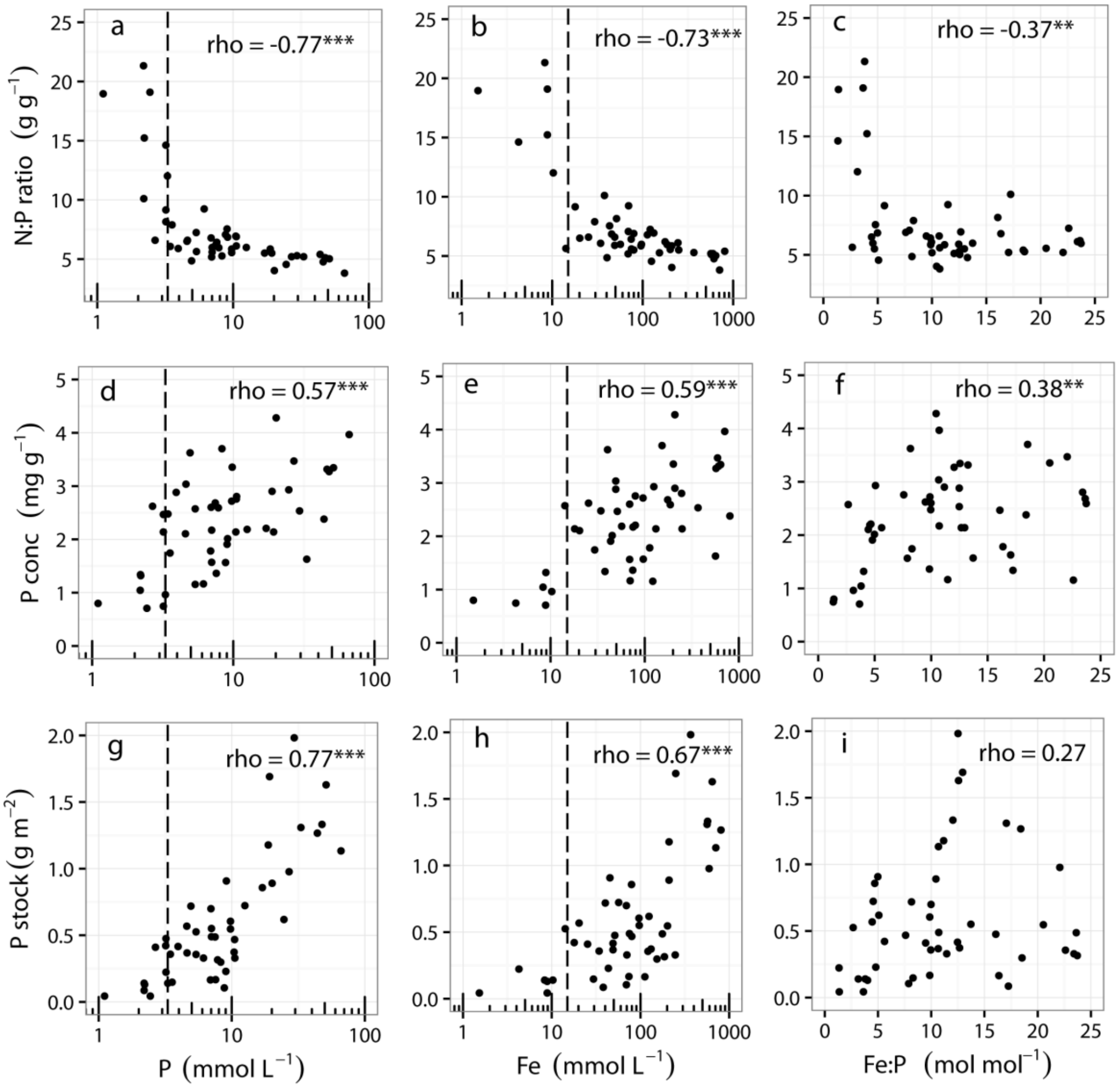

Figure 2.3: N:P ratios (g g^{-1}), P concentrations (mg g^{-1}), and P stocks (g m^{-2}) of fen vegetation in relation to top soil P pools (a, d and g), Fe pools (b, e and h), and Fe:P ratios (c, f and i) (n = 49 samples, collected in 12 different fens). P stocks were calculated as P concentrations * biomass. Dashed lines indicate thresholds of 3.3 mmol P L^{-1} and 15 mmol Fe L^{-1} above which P limitation is unlikely to occur. Spearman's rho values are listed together with their significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

2.3.3 Occurrence of endangered species

We recorded a total of 150 vascular plant species (including 40 endangered species, e.g. *Carex diandra*, *C. lepidocarpa* or *Pedicularis palustris* (L.)) and 33 bryophyte species (including 11 endangered species, e.g. *Campylium stellatum*, *Hamatocaulis 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 2.2). In addition, plots with large soil Fe- and P pools and high Fe:P ratios contained relatively fewer endangered species (Table 2.2).

Table 2.2: Spearman's correlation coefficients (rho) of the fraction of endangered fen species (herbs, bryophytes and total) versus 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. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

		Endangered species (%)		
		Vascular plants	Bryophytes	Total
Vegetation	N:P ratio (g g^{-1})	0.47***	0.44**	0.49***
	P conc (mg g^{-1})	-0.31*	-0.35*	-0.33*
	P stock (g m^{-2})	-0.37**	-0.36*	-0.40**
Soil	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*

2.3.4 P uptake in the phytometer experiment

Foliar P concentrations (in mg g^{-1}) in *Carex rostrata* were primarily determined by water levels (Fig. 2.4, 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 mmol L^{-1}) correlated with highest foliar P concentrations (Fig 2.4.: node 7 > node 6). In the drained mesocosms, large pools of acid-extractable P ("Acid P" > 1.6 mmol L^{-1}) correlated with higher foliar P concentrations (Fig. 2.4: 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 (Kruskal-Wallis chisquared = 31.5, 34.2 and 25.3 respectively, df = 3, $P < 0.001$). Foliar P concentrations and plant P stocks were lowest on the drained soils with small acid-extractable P pools (Acid $P < 1.6$ mmol L^{-1}; Fig. 2.4: Node 3), which corresponded with 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 mmol L^{-1}; Fig 2.4: Node 7), which corresponded with 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 g g^{-1}) only in some of the drained soils with small acid-extractable P pools (Adic P < 1.6 mmol L^{-1}; Fig. 2.4: Node 3).

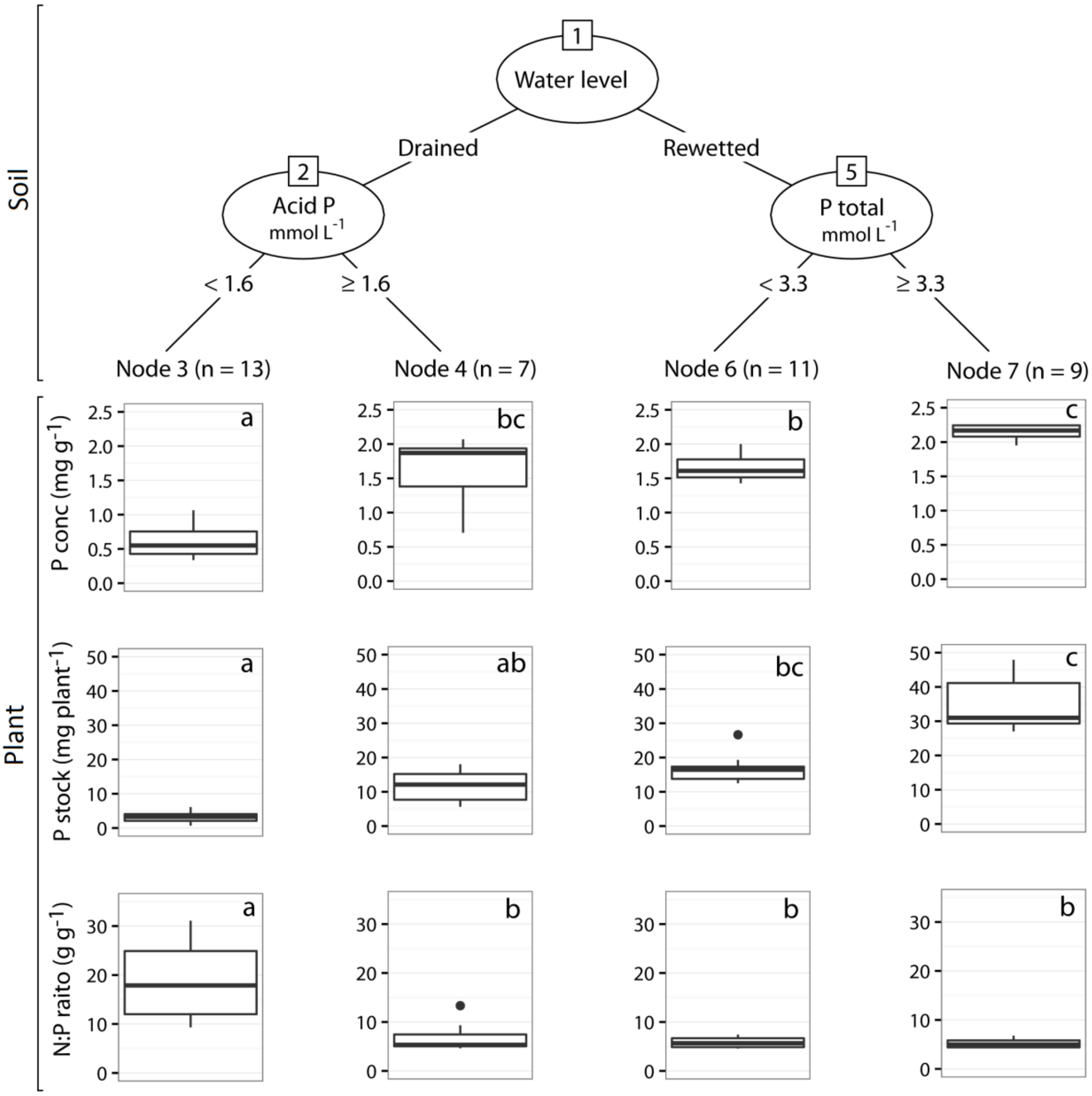

Figure 2.4: Regression tree of foliar P concentration (mg g^{-1}, top boxplots), plant P stock (mg plant^{-1}, middle boxplots) and N:P ratio (g g^{-1}, bottom boxplots) in aboveground biomass of *Carex rostrata* clones versus "water level" (drained or rewetted) and P variables of the soil (P variables 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 * plant biomass. The tree shows successive splitting and concomitant splitting criteria. The final nodes are visualized as boxplots of the variables, letters (abc) indicate significant differences between groups (P < 0.05).

2.4 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.

2.4.1 Iron as a "phosphorus trap"

The significant correlation between soil Fe- and P pools suggests that input, retention and accumulation of P in a 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 & Vaithiyanathan 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 2 sites only (TB and DZ), whereas the remaining 28 sites were non-calcareous (average pH between 5.5 and 7).

2.4.2 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 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 (1) the inverse relationship between soil pools of P and vegetation N:P ratios and (2) the positive correlation between soil P pools and P uptake by the vegetation. Whenever thresholds of 3.3 mmol P L^{-1} and 15 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 > 14-20 g g^{-1}) were counterintuitively only found in five Fe-poor fens with low soil Fe:P ratios (< 5 mol mol^{-1}) (Fig. 2.3c). 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 aboveground herb biomass at our sites (rho = -0.55, $P < 0.001$, Fig. S2.3 in Supporting information) suggests an overall increase in herb 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.

2.4.3 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 *Carex rostrata*. Individuals that were grown in the rewetted fen mesocosms had up to 4 x 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. 2.4). 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 may no longer occur 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 *Carex 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.

2.4.4 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. Although we acknowledge that Fe addition may have positive effects in turbid open water (Bakker, Van Donk & Immers 2015), 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. This mechanism may be particularly relevant in densely populated agricultural landscapes with high P loadings, where P easily ends up in fens through groundwater flow (Cusell et al. 2014). Furthermore, it likely that not all Fe-rich fens worldwide are by definition saturated with P, as some Fe-rich fens may still be dominated by small sedge and brown moss communities (pers. comm. H. Joosten). Although we did not encounter such Fe-rich and P-poor fens in Europe, it is possible that they may be more commonly found in in areas with very little or no agricultural activity in sparsely populated parts of the globe.

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 mmol L^{-1} 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, 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.

Acknowledgments

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2.5 References

Aggenbach, C.J.S., Backx, H., Emsens, W.J., Grootjans, A.P., Lamers, L.P.M., Smolders, A.J.P., Stuyfzand, P.J., Wolejko, L. & Van Diggelen, R. (2013) Do high iron concentrations in rewetted rich fens hamper restoration? Preslia, 85, 405-420.

Baken, S., Verbeeck, M., Verheyen, D., Diels, J. & Smolders, E. (2015) Phosphorus losses from agricultural land to natural waters are reduced by immobilization in iron-rich sediments of drainage ditches. Water Research, 71, 160-170.

Bakker, E.S., Van Donk, E. & Immers, A.K. (2015) Lake restoration by in-lake iron addition: a synopsis of iron impact on aquatic organisms and shallow lake ecosystems. Aquatic Ecology, 1-15.

Boeye, D., Verhagen, B., VanHaesebroeck, V. & Verheyen, R.F. (1997) Nutrient limitation in species-rich lowland fens. Journal of Vegetation Science, 8, 415-424.

Bolan, N.S. (1991) A Critical-Review on the Role of Mycorrhizal Fungi in the Uptake of Phosphorus by Plants. Plant and Soil, 134, 189-207.

Boyer, M.L.H. & Wheeler, B.D. (1989) Vegetation Patterns in Spring-Fed Calcareous Fens - Calcite Precipitation and Constraints on Fertility. Journal of Ecology, 77, 597-609.

Carlyle, G.C. & Hill, A.R. (2001) Groundwater phosphate dynamics in a river riparian zone: effects of hydrologic flowpaths, lithology and redox chemistry. Journal of Hydrology, 247, 151-168.

Corona, M.E.P., van der Klundert, I. & Verhoeven, J.T.A. (1996) Availability of organic and inorganic phosphorus compounds as phosphorus sources for Carex species. New Phytologist, 133, 225-231.

Crawley, M.J. (2005) Statistics: An introduction using R. Wiley.

Cusell, C., Kooijman, A., Fernandez, F., van Wirdum, G., Geurts, J.J.M., van Loon, E.E., Kalbitz, K. & Lamers, L.P.M. (2014) Filtering fens: Mechanisms explaining phosphorus-limited hotspots of biodiversity in wetlands adjacent to heavily fertilized areas. Science of the Total Environment, 481, 129-141.

Geurts, J.J.M., Smolders, A.J.P., Verhoeven, J.T.A., Roelofs, J.G.M. & Lamers, L.P.M. (2008) Sediment Fe : PO(4) ratio as a diagnostic and prognostic tool for the restoration of macrophyte biodiversity in fen waters. Freshwater Biology, 53, 2101-2116.

Gschwend, P.M. & Reynolds, M.D. (1987) Monodisperse ferrous phosphate colloids in an anoxic groundwater plume. Journal of Contaminant Hydrology, 1, 309-327.

Gusewell, S. (2004) N : P ratios in terrestrial plants: variation and functional significance. New Phytologist, 164, 243-266.

Hartland, A., Larsen, J.R., Andersen, M.S., Baalousha, M. & O'Carroll, D. (2015) Association of Arsenic and Phosphorus with Iron Nanoparticles between Streams and Aquifers: Implications for Arsenic Mobility. Environmental Science & Technology, 49, 14101-14109.

Jensen, H.S., Kristensen, P., Jeppesen, E. & Skytthe, A. (1992) Iron-Phosphorus Ratio in Surface Sediment as an Indicator of Phosphate Release from Aerobic Sediments in Shallow Lakes. Hydrobiologia, 235, 731-743.

Kaźmierczakowa, R., Bloch-Orłowska, J., Celka, Z., Cwener, A., Dajdok, Z., Michalska-Hejduk, D., Pawlikowski, P., Szczęśniak, E. & Ziarnek, K. (2016) Polska czerwona lista paprotników i roślin kwiatowych. Institute of Nature Conservation, PAS, Kraków.

Koerselman, W. & Meuleman, A.F.M. (1996) The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. Journal of Applied Ecology, 33, 1441-1450.

Kooijman, A.M. & Paulissen, M.P.C.P. (2006) Higher acidification rates in fens with phosphorus enrichment. Applied Vegetation Science, 9, 205-212.

Manning, P.G., Murphy, T.P. & Prepas, E.E. (1991) Intensive Formation of Vivianite in the Bottom Sediments of Mesotrophic Narrow Lake, Alberta. Canadian Mineralogist, 29, 77-85.

Ochyra, R. (1992) Czerwona Lista Mchów Zagrożonych Polsce (Red List of Threatened Mosses in Poland), 79-85. Polish Academy of Sciences.

Olde Venterink, H., Wassen, M.J., Verkroost, A.W.M. & de Ruiter, P.C. (2003) Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. Ecology, 84, 2191-2199.

Patrick, W.H. & Khalid, R.A. (1974) Phosphate Release and Sorption by Soils and Sediments - Effect of Aerobic and Anaerobic Conditions. Science, 186, 53-55.

Pawlikowski, P., Abramczyk, K., Szczepaniuk, A. & Kozub, L. (2013) Nitrogen:phosphorus ratio as the main ecological determinant of the differences in the species composition of brown-moss rich fens in north-eastern Poland. Preslia, 85, 349-367.

Reddy, K.R., Kadlec, R.H., Flaig, E. & Gale, P.M. (1999) Phosphorus retention in streams and wetlands: A review. Critical Reviews in Environmental Science and Technology, 29, 83-146.

Richardson, C.J. & Vaithiyanathan, P. (2009) 10 Biogeochemical Dynamics II: Cycling and Storage of Phosphorus in Wetlands. The Wetlands Handbook, 2 Volume Set, 228.

Rothe, M., Frederichs, T., Eder, M., Kleeberg, A. & Hupfer, M. (2014) Evidence for vivianite formation and its contribution to long-term phosphorus retention in a recent lake sediment: a novel analytical approach. Biogeosciences, 11, 5169-5180.

Schachtman, D.P., Reid, R.J. & Ayling, S.M. (1998) Phosphorus uptake by plants: From soil to cell. Plant Physiology, 116, 447-453.

Schwertmann, U. (1964) Differenzierung der Eisenoxide des Bodens durch Extraktion mit Ammoniumoxalat-Lösung. Zeitschrift für Pflanzenernährung, Düngung, Bodenkunde, 105, 194-202.

Siebel, H., Bijlsma, R.J. & Sparrius, L. (2012) Basisrapport voor de Rode Lijst Mossen 2012. BLWG Rapport 14. BLWG, Oude-Tonge.

Smolders, A.J.P., Lamers, L.P.M., Moonen, M., Zwaga, K. & Roelofs, J.G.M. (2001) Controlling phosphate release from phosphate-enriched sediments by adding various iron compounds. Biogeochemistry, 54, 219-228.

Sparrius, L., Odé, B. & Beringen, R. (2012) Basisrapport Rode Lijst Vaatplanten 2012 volgens Nederlandse en IUCN-criteria. FLORON Rapport 57. FLORON, Nijmegen.

Succow, M. & Joosten, H. (2001) landschaftsökologische Moorkunde.

Thormann, M.N., Currah, R.S. & Bayley, S.E. (1999) The mycorrhizal status of the dominant vegetation along a peatland gradient in southern boreal Alberta, Canada. Wetlands, 19, 438-450.

van Landuyt, W., Vanhecke, L. & Hoste, I. (2006) Rode lijst van de vaatplanten van vlaanderen en het Brussels Hoofdstedelijk Gewest. In: van Landuyt, W. et al. Atlas van de Flora van Vlaanderen en het Brussels Gewest. INBO en de nationale plantentuin van België, Brussel.

Walinga, I., van Vark, W., Houba, V.J.G. & van der Lee, J.J. (1989) Plant analysis procedures. Soil and Plant Analysis, Part 7. Agricultural University, Wageningen, 13-16.

Wassen, M.J., Venterink, H.O., Lapshina, E.D. & Tanneberger, F. (2005) Endangered plants persist under phosphorus limitation. Nature, 437, 547-550.

Welch, H.L., Kingsbury, J.A. & Coupe, R.H. (2010) Occurrence of phosphorus in groundwater and surface water of northwestern Mississippi. W: Proceedings of the 2010 Mississippi Water Resources Conference [online]. Raymond.[Dostęp 10.02. 2015]. Dostępny w Internecie: http://www.wrri. msstate. edu/pdf/welch10. pdf gy and Life Sciences in Falenty, pp. 80-86.

Zak, D., Gelbrecht, J., Wagner, C. & Steinberg, C.E.W. (2008) Evaluation of phosphorus mobilization potential in rewetted fens by an improved sequential chemical extraction procedure. European Journal of Soil Science, 59, 1191-1201.

Zak, D., Gelbrecht, J., Zerbe, S., Shatwell, T., Barth, M., Cabezas, A. & Steffenhagen, P. (2014) How helophytes influence the phosphorus cycle in degraded inundated peat soils - Implications for fen restoration. Ecological Engineering, 66, 82-90.

Zak, D., Wagner, C., Payer, B., Augustin, J. & Gelbrecht, J. (2010) Phosphorus mobilization in rewetted fens: the effect of altered peat properties and implications for their restoration. Ecological Applications, 20, 1336-1349.

2.6 Supporting information

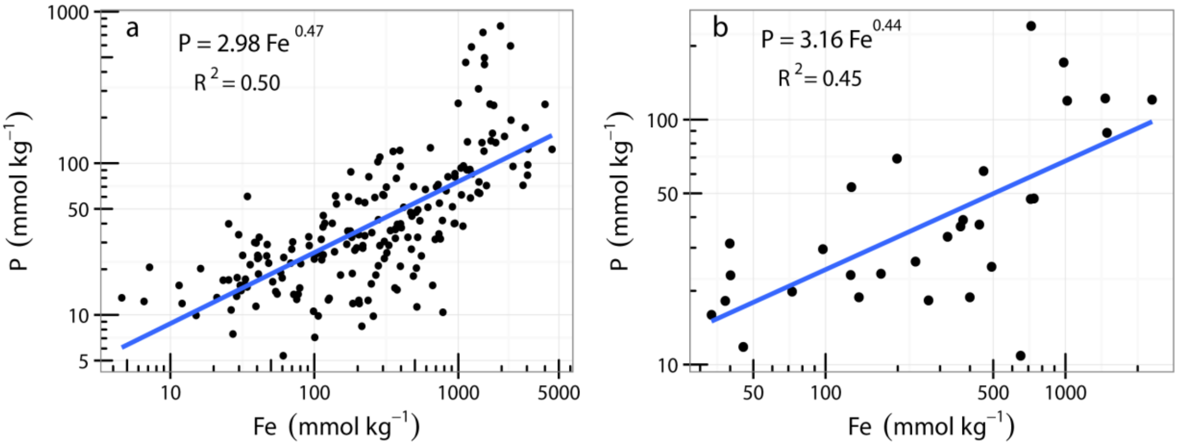

Figure S2.1: Log-log plots of P- versus Fe contents of fen soils (in mmol kg-1) for (a) all 194 soil samples collected at 30 study sites and at different depths (n = 194, ranging from 0-500 cm below the soil surface), and (b) independent averages of the 30 study sites. Regression lines are fitted with least-squares linear regression of the log-transformed variables.

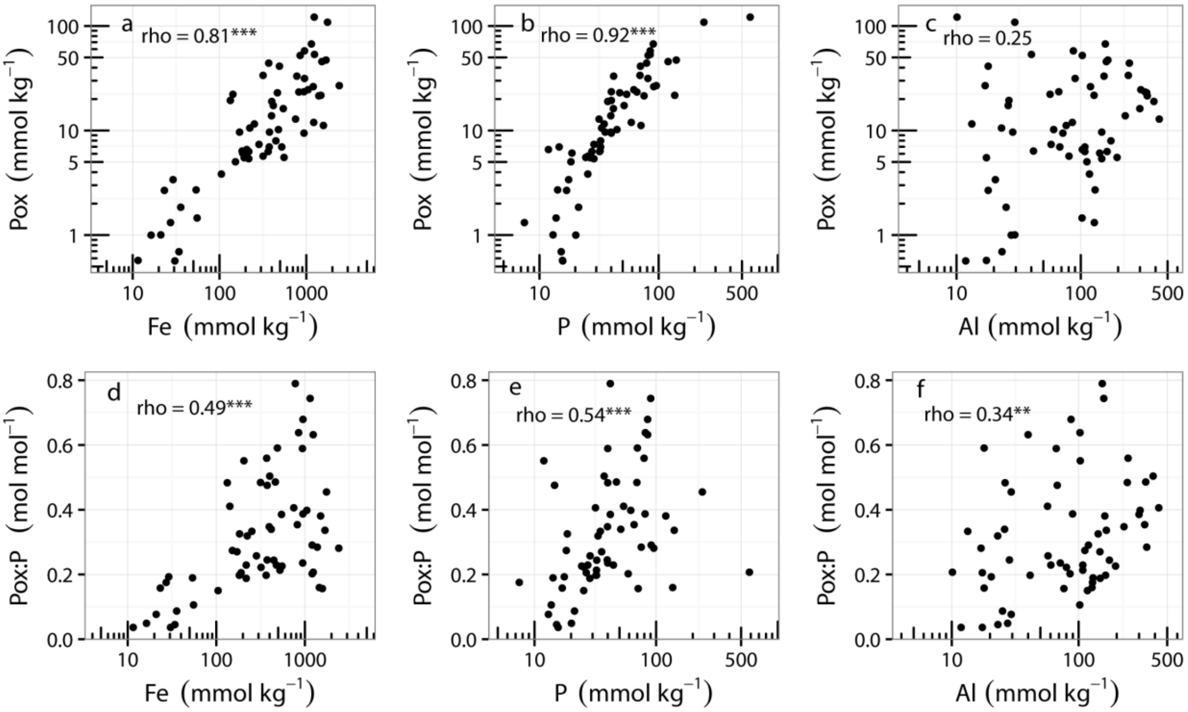

Figure S2.2: Correlations between soil contents of oxalate-P (Pox) and contents of (a) Fe, (b) P and (c) Al in fens (in mmol kg-1), and between the fraction oxalate-P P-1 (mol mol-1) and soil contents of (d) Fe, (e) P and (f) Al in fens (n = 62 samples collected in 13 different fens between 0 – 200 cm depth). Spearman's rho values are listed together with their significance (* P < 0.05, ** P < 0.01, *** P < 0.001).

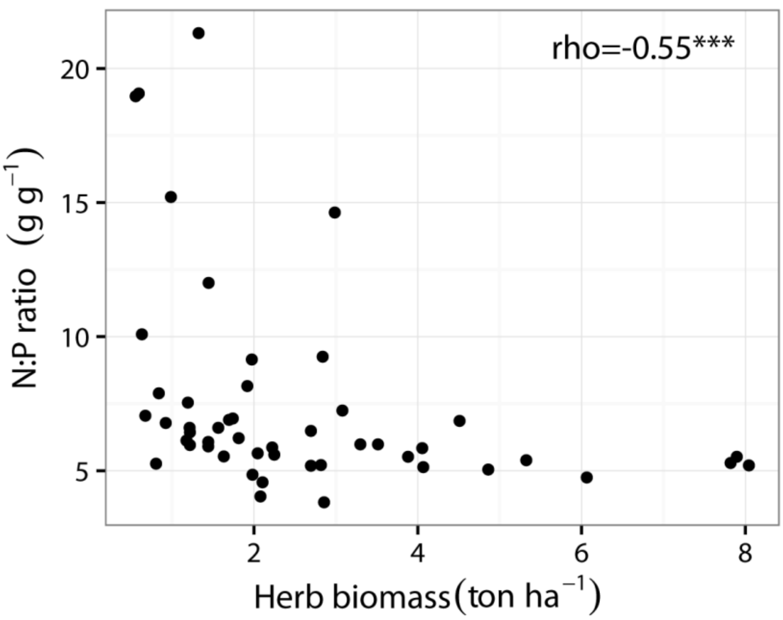

Figure S2.3: Correlation between N:P ratios (g g^{-1}) and aboveground biomass production (metric ton ha^{-1}) of the herb layer in fens (n = 49 samples collected in 12 different fens). Biomass is defined as the standing crop of the herb layer at time of sampling. *** indicates significance ($P < 0.001$).

3

Soil iron content as a predictor of carbon and nutrient mobilization in rewetted fens

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Abstract

Rewetted, previously drained fens often remain sources rather than sinks for carbon and nutrients. To date, it is poorly understood which soil characteristics stimulate carbon and nutrient mobilization upon rewetting. Here, we assess the hypothesis that a large pool of iron in the soil negatively affects fen restoration success, as flooding-induced iron reduction (Fe^{3+} to Fe^{2+}) causes a disproportionate breakdown of organic matter that is coupled with a release of inorganic compounds. We collected intact soil cores in two iron-poor and two iron-rich drained fens, half of which were subjected to a rewetting treatment while the other half was kept drained. Prolonged drainage led to the mobilization of nitrate (NO_3^-, > 1 mmol L^{-1}) in all cores, regardless of soil iron content. In the rewetted iron-rich cores, a sharp increase in pore water iron (Fe) concentrations correlated with concentrations of inorganic carbon (TIC, > 13 mmol L^{-1}) and dissolved organic carbon (DOC, > 16 mmol L^{-1}). Additionally, ammonium (NH_4^+) accumulated up to phytotoxic concentrations of 1 mmol L^{-1} in the pore water of the rewetted iron-rich cores. Disproportionate mobilization of Fe, TIC, DOC and NH_4^+ was absent in the rewetted iron-poor cores, indicating a strong interaction between waterlogging and iron-mediated breakdown of organic matter. Concentrations of dissolved phosphorus (P) rose slightly in all cores upon rewetting, but remained low throughout the experiment. Our results suggest that large pools of iron in the top soil of drained fens can hamper the restoration of the fen's sink-service for ammonium and carbon upon rewetting. We argue that negative effects of iron should be most apparent in fens with fluctuating water levels, as temporary oxygenation allows frequent regeneration of Fe^{3+}. We conclude that rewetting of iron-poor fens may be more feasible.

3.1 Introduction

Widespread drainage is compromising the ability of the world's groundwater-fed peatlands ("fens" hereinafter) to serve as sinks for nutrients and carbon (Kasimir-Klemedtsson et al. 1997, Lamers et al. 2015a). In response to drainage of organic soils, organic-bound nutrients are mineralized to inorganic mobile compounds (Olde Venterink et al. 2002, Zak and Gelbrecht 2007), thereby contributing to pore and surface water eutrophication and loss of typical biodiversity. At the same time, rates of carbon dioxide emission increase upon drainage, which significantly impacts the world's greenhouse gas budget (Kasimir-Klemedtsson et al. 1997). Finally, rising concentrations of dissolved organic carbon (DOC) in surface waters adjacent to degraded peatlands have been related to the destabilization of carbon pools within the peat (Freeman et al. 2004, Preston et al. 2011). These high DOC-loads have become subject to growing international concern as they cause ample environmental problems (Pastor et al. 2003, Holden 2005).

In response to the aforementioned trends, many countries are installing policies that aim to restore a significant portion of drained fens together with the vital ecosystem services that they provide. Fen restoration primarily focusses on the re-establishment of high groundwater levels, e.g. through ditch blockage. However, reports of excessive mobilization of dissolved organic and inorganic carbon, ammonium (NH_4^+), and phosphate (PO_4^{3-}) into pore and surface water upon rewetting indicate that successful fen restoration is not always guaranteed (Van Dijk et al. 2004, Zak and Gelbrecht 2007). The apparent difficulty in predicting restoration success by rewetting can be related to the complex and often diverse chemistry of fens: fens, much more than rainwater-fed bogs, vary greatly in cation, nutrient, electron acceptor and organic matter availability as well as pH and alkalinity. To date, the exact mechanisms and chemical characteristics that affect compound mobilization in drained peat soils are not well understood, which often results in an arbitrary or even random selection of fens that are listed as "suitable" for rewetting.

In this respect, past research has emphasized the importance of high concentrations of iron (Fe) in both soil and flooding water mainly because iron prevents mass mobilization of phosphate and reduced sulfur species into the pore water by providing sorption surfaces for both elements (Smolders et al. 1995, Geurts et al. 2008). Based on those insights, the rewetting of iron-rich fens could be considered a relatively safe choice. However, potential negative effects of large iron pools in organic soils are usually neglected. In unpolluted fens and wetlands, iron (Fe^{2+}-Fe^{3+}) forms a dominant reduction-oxidation couple (Lovley 1991, Clement et al. 2005), and ferric iron often exceeds concentrations of other potential electron acceptors such as SO_4^{2-} or NO_3^-. In fact, the "iron-redox wheel", in which iron alternately shifts between the ferric (Fe^{3+}) and ferrous (Fe^{2+}) state in fluctuating redox environments, is potentially of major importance for carbon and nutrient cycling as labile organic matter is an electron donor in the microbe-mediated Fe-reduction reaction (Lovley and Phillips 1986, Lovley 1991, Li et al. 2012). In a case study of a peatland that is influenced by Fe-inflow for example, an estimated 71.6% of anaerobic carbon mineralization was found to be directly coupled with Fe^{3+}-reduction, in contrast to only an estimated 6.7% in an upland bog that lacks Fe-inflow (Kusel et al. 2008). In order for such iron-redox wheel to persist, fluctuating water levels are a prerequisite. In this respect, it is key to realize that human-induced rewetting rarely results in stable water levels year round: wet winters often result in temporarily flooded conditions while occasional long dry summers cause the topsoil to dry out, even in rewetted fens (Richert et al. 2000, Grootjans et al. 2006). This is partly related to irreversible shifts in the local hydrological system (Grootjans et al. 2006), as well as to physical alterations in the peat soil due to past drainage (Evans et al. 1999, Schlotzhauer and Price 1999). These physio-chemical alterations imply that rewetted fens may be relatively vulnerable to iron-induced decomposition.

Besides the direct relationship between iron reduction and decomposition, iron can additionally stimulate organic matter mineralization through indirect pathways. For example, iron promotes the production and activity of the extracellulair enzyme phenol oxidase, which catalyzes the oxidation of decomposition-inhibiting phenolics (Van Bodegom et al. 2005, Fenner et al. 2011). Also, production of alkalinity coupled with Fe-reduction raises soil buffering (Lucassen et al. 2009, Aggenbach et al. 2013), thereby indirectly increasing rates of decomposition (Smolders et al. 2006).

As past fen drainage has been shown to concentrate iron in the degraded top soil (Aggenbach et al. 2013), the potential Fe-related soil reactivity in terms of carbon and nutrient mobilization in degraded fens could be substantial. The potential magnitude of such effect in peat soils has, however, never been documented. In this paper, we investigate if soil iron content is indeed a significant predictor of nutrient and carbon mobilization in drained fens upon rewetting. To test this hypothesis, we comparatively investigate the effects of experimental rewetting on four drained fens that vary in soil iron content.

3.2 Materials and methods

3.2.1 Study areas and soil core collection

We selected four fens in the Pleistocene part of the Netherlands and Belgium that strongly differ in soil iron content: two fens (BM and LH) had relatively low volumetric soil iron contents (31.5 and 18.3 mmol L^{-1} soil respectively), while two fens (ES and ZB) were iron-rich (72.6 and 525.1 mmol L^{-1} respectively, Table 3.1). All study sites were, historically, characterized by continuous upwelling of base-rich groundwater. Due to differences in geochemistry of the aquifers feeding the fens, the incoming groundwater of locations ES and ZB is relatively iron-enriched whereas the groundwater of BM and

LH is relatively iron-poor, which explains the observed differences in total soil iron pools. Over the past century, land use intensification coupled with drainage has led to lowered groundwater levels and thus degradation of the top peaty soil layers at all sites (Von Post Humification Index upper 50 cm > 8). Current vegetation is characterized by a high presence of fen meadow species such as *Caltha palustris*, *Carex panicea, Cirsium palustre*, *Galium palustre*, *Juncus spp.*, *Lotus uliginosus,* and *Holcus lanatus* in the herb layer and *Calliergon(ella) spp., Brachythecium spp.* and *Climacium dendroides* in the moss layer. To date, all sites are owned and protected by nature conservation agencies. We were granted permission to collect the peat cores from the following authorities: Staatsbosbeheer (sites BM and ES), Landschap Overijssel (site LH) and Natuurpunt (site ZB).

At the time of soil core collection (early February 2014), groundwater levels averaged 10-20 cm below the surface. Within each fen, we chose a central area with a peaty soil layer of > 50 cm thickness. We then collected ten intact replicate soil cores of 45 cm x 12.5 cm (depth x diameter) in a 2x4m grid at each location. To extract a core, we manually forced hard-PVC tubes (diameter = 12.5 cm) of 50 cm length in the peat soil after carefully removing aboveground vegetation. As the PVC tubes were sharpened at the bottom, it was fairly easy to cut through the peat without causing compaction. Next, we dug a narrow hole alongside the PVC tube which allowed us to close the tube’s bottom with a PVC-lid before actually removing the tube. This allowed us to collect a relatively undisturbed soil core without losing pore water, which was crucial to minimize peat oxidation during transport. Directly after removal, the top of the PVC tube was sealed with cling film.

Table 3.1: Soil characteristics. Soil bulk density (kg L^{-1}), organic matter content (%), Ammonium chloride-extractable P (NH_4Cl-P), bicarbonate-dithionite extractable Fe and P (BD-Fe and BD-P), hydrochloric acid-extractable Fe, Al, Ca and P (HCl-Fe, HCl-Al, HCl-Ca, and HCl-P), and total Fe and P contents (in mmol kg^{-1}) of the four study sites. Values (means ± SD) are based on samples (0-25 cm) collected in each of the 40 cores.

Parameter	Unit	Bennekomse Meent (BM)	Leijer Hooilanden (LH)	Elperstroom (ES)	Zwarte Beek (ZB)
Coordinates		52° 0'25.98"N; 5°35'48.80"E	52°38'32.71"N; 6°16'37.50"E	52°52'26.01"N; 6°39'32.96"E	51° 5'23.91"N; 5°19'10.69"E
Bulk density	kg L^{-1}	0.13 ± 0.02	0.07 ± 0.01	0.15 ± 0.05	0.27 ± 0.04
OM-content	%	41.7 ± 7.9	81.8 ± 1.4	44.6 ± 13.3	22.8 ± 3.5
NH_4Cl-P	mmol kg^{-1}	0.018 ± 0.003	0.054 ± 0.012	0.031 ± 0.021	0.019 ± 0.007
BD-P	mmol kg^{-1}	1.45 ± 0.55	2.72 ± 0.97	1.50 ± 0.43	1.55 ± 0.7
HCl-P	mmol kg^{-1}	2.81 ± 0.54	11.16 ± 3.05	9.23 ± 3.54	50.64 ± 17.85
Total P	mmol kg^{-1}	20.15 ± 3.6	44.85 ± 7.05	29.87 ± 12.81	144.8 ± 14.59
HCl-Al	mmol kg^{-1}	113.9 ± 23.7	128.7 ± 14.5	157.4 ± 37.0	108.3 ± 18.6
HCl-Ca	mmol kg^{-1}	126 ± 34.8	189.9 ± 37.3	99.1 ± 34.1	54.8 ± 11.6
BD-Fe	mmol kg^{-1}	31.5 ± 11.8	55.5 ± 18.1	185.6 ± 84.5	294.7 ± 42.2
HCl-Fe	mmol kg^{-1}	117.8 ± 29.1	174.4 ± 26.8	321.7 ± 112.7	778.1 ± 159.5
Total Fe	mmol kg^{-1}	258 ± 62	256 ± 36	537 ± 203	1960 ± 331

3.2.2 Experimental design and sampling

The 40 cores were placed in an unheated greenhouse where they were sheltered from direct sunlight. Average daily temperatures in the greenhouse ranged between 6.5 and 22.9°C (mean = 13.9 ± 3.8 °C) over the course of the experiment, which ran from February through June 2014. We horizontally inserted 10 cm permanent Rhizon pore water samplers (pore size 0.2 µm, Rhizosphere Research Products, the Netherlands) at three different depths in each core: 5, 15 and 25 cm below the peat surface. Each rhizon sampler was connected to a vacuum-syringe to allow the anaerobic extraction of pore water. Next, half of the soil cores received a rewetting treatment (water level at peat surface level), while the other half was drained (water level = 27 cm below the peat surface). As the water levels at the time of soil core collection averaged between 10-20 cm below the surface, the experimental water level manipulation not only triggered a rewetting effect but also a (more pronounced) drainage effect. Water level manipulation was accomplished by placing each PVC-tube (perforated at the bottom) in a separate larger tube that was prefilled with the required amount of stagnant N_2-deoxygenized artificial groundwater containing limited amounts of base minerals but no nutrients (i.e. N and P) or pollutants (i.e. S) (Fig. 3.1). This in order to simulate rewetting by unpolluted, minerotrophic groundwater. Characteristics of the artificial groundwater were (means of five samples ± SE, in µmol L^{-1}): pH = 7.0 ± 0.04, HCO_3^-= 988.3 ± 73.2, Ca^{2+} = 608.3 ± 57.6, K^+ = 18.1 ± 0.9, Na^+ = 198.6 ± 40.5, Mg^{2+} = 4.9 ± 0.7, Cl^- = 145.1 ± 25.6. In order to allow gas exchange between peat soil and the surrounding atmosphere while preventing plant growth, we made three 3 mm holes in each top lid. This is sufficient for gas exchange, but hampers plant growth due to light limitation. Water levels were manually kept stable throughout the duration of the experiment. The design was full-factorial so that each combination of location (n = 4) x water level treatment (n = 2) was replicated five times. After initiation of the experiment, we first allowed the cores to stabilize for four days. On day four, we collected the first set of 120 pore water samples which we considered the starting point reference (t = 0). Next, pore water samples were collected biweekly and analyzed for pH and EC using portable equipment (except t=8 weeks). Pore water samples collected at t = 0, t = 30 and t = 127 were stored air-tight at 4°C and transported to the lab for further chemical analysis.

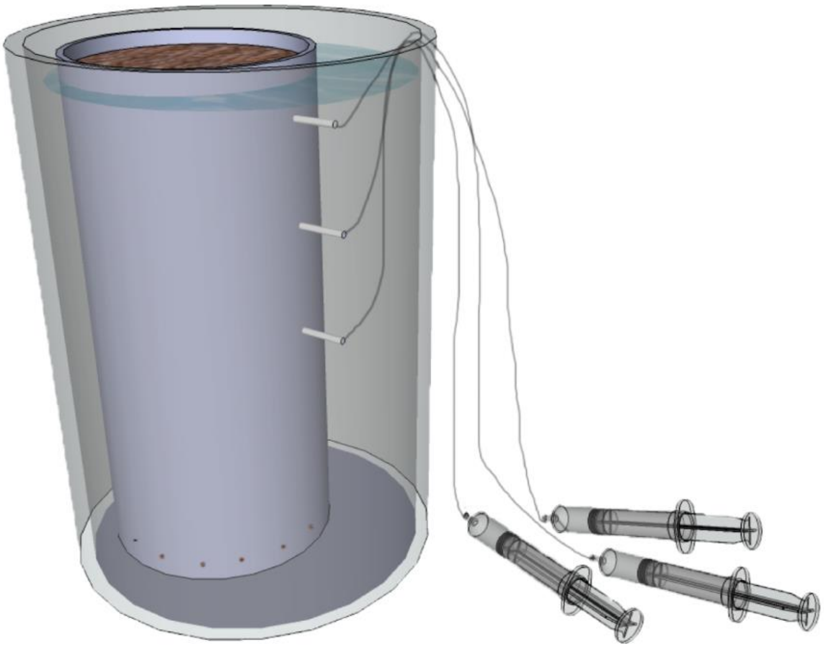

Figure 3.1: Experimental set-up. 40 intact vertical soil cores were collected in 4 drained fens using sharpened PVC tubes (45 x 12.5 cm), and were then placed in individual containers filled with stagnant de-oxygenized artificial groundwater. Tubes were perforated at the bottom to allow water inflow. Rhizons were placed at 5, 15 and 25 cm below the soil surface, and connected to vacuum-syringes. Half of the cores were rewetted to peat surface level, while the other half was kept moderately drained (water level 27 cm below peat surface level).

3.2.3 Chemical analyses

Total inorganic carbon (TIC) was analyzed in the lab on an Infrared Gas Analyzer (ABB Advance Optima). Concentrations of NH_4^+ and NO_3^- were determined colorimetrically on a continuous-flow Auto Analyzer system. Pore water subsamples were acidified by adding 0.7 ml 65 % suprapure HNO_3 per 100 ml sample and were analyzed with inductively coupled plasmaspectrometry (ICP, IRIS Intrepid II) for the following elements: Ca^{2+}, Mg^{2+}, K^+, Na^+, Fe_t, Mn_t, P_t, S_t, and Al_t. Dissolved Organic Carbon (DOC) was analyzed using a Shimadzu TOC-VCPH Total Organic Carbon Analyser (Shimadzu

Scientific Instruments, USA). Concentrations of CH_4 and H_2S were in the headspace of a 12 ml anaerobic glass vacuum tube that was prefilled with 0.5 ml 4% HCl, to which a subsample of 5 ml pore water was added. CH_4 concentrations were measured with a Hewlett-Packard 5890 gas chromatograph (Avondale, California) equipped with a flame-ionization detector and a Porapak Q column (80/100 mesh) operated at 120°C with N_2 as carrier gas. H_2S concentrations were determined using a Packard 438A gas chromatograph equipped with a Carbopack BHT100 column (40/60 mesh) and a flame photometric detector.
Mineral saturation indices were calculated based on pore water measurements of pH and the following dissolved species: Ca, Mg, Fe, Mn, Na, K, Cl, Al, S, P, C, and N using PhreeqC (Parkhurst and Appelo 1999).

Chemical characterization of the soils (Table 3.1) was done by sequential extractions on fresh soil to determine ammonium-chloride extractable P (NH_4Cl-P) (=desorbable P), bicarbonate-dithionite extractable Fe and P (BD-Fe and BD-P (=reductant-soluble Fe and P)) and hydrochloric acid extractable Fe, Al, Ca, and P (HCl-Fe, HCl-Al, HCl-Ca, and HCl-P (=acid-soluble Fe, Al, Ca and P)) (Zak et al. 2008). Fe, Al, and Ca concentrations of the chemical extracts (BD and/or HCl) were determined using inductively coupled plasma optical emission spectrometry (ICP-OES)I (Cap 6000 series, Thermo Fischer), and P concentrations of the chemical extracts were determined using the molybdenum blue method after acid digestion (Zak et al. 2008). Precision and accuracy were better than 5% for Fe, Al, and Ca analysis and the detection limit was 2, 3 or 4 µmol respectively. Total soil Fe and P was determined on ICP-OES after digesting 200 mg of oven-dried and ground soil with 4 mL HNO_3 (65%) and 1 mL H_2O_2 (30%) using a microwave labstation (Milestone srl). Organic matter content (% dry weight) was determined by loss on ignition (4h at 550°C).

3.2.4 Data analyses

We used Linear Mixed-Effect Modeling with REML estimation in SPSS (IBM SPSS Statistics 20) to assess the effects of soil iron content and water level on pore water concentrations of dissolved Fe, carbon (TIC, DOC and CH_4) and macronutrients (NH_4^+, NO_3^-, total dissolved P). To this purpose, we made two groups based on soil iron content (treatment = “Iron content”): the two locations with mean soil iron content > 50 mmol L^{-1} were classified as iron-rich sites (sites ES and ZB), and the remaining two locations with soil iron content < 50 mmol L^{-1} as iron-poor sites (sites BM and LH). For each of these two groups, one half of the replicate cores was fully rewetted while the other half was drained (treatment = “Water level”). This setup resulted in a total of four different groups for data analysis. To correct for random between-site variation as well as for differences in means between sites, we added “Site ID (ES, ZB, BM or LH)” as a random factor nested within the treatment “iron content”. Although we collected data at different points in time, we did not add “Time” as an additional factor in the model for two reasons. First, visual data exploration reveals that the effect of "Time" is merely strengthening (i.e. differences between treatments become more pronounced as time progresses). Second, adding "Time" as a factor to the model would make the model unnecessarily complicated, as this would imply 3-way interactions between "Time", "Iron content" and "Water level". Hence, we simplify the Mixed Model by comparing treatment differences both at the start as well as at the end of the experiment (n = 0 and n = 127 days respectively) with tests for 2-way interactions between the fixed factors "Iron content" and "Water level". We expect that the magnitude of the effect of rewetting ("Water level") on carbon and nutrient mobilization depends on soil iron content, with higher carbon and nutrient mobilization in the rewetted iron-rich soil cores. For statistical analyses, values of the three pore water subsamples per soil core were averaged to attain column averages as well as to avoid pseudo-replication.

To further verify whether iron reduction is directly coupled with the mobilization of TIC, DOC and NH_4^+ after rewetting, we used Spearman's rank correlation to compare the change in concentrations of total dissolved iron (ΔFe) from t=0 days to t=127 days with ΔTIC, ΔDOC and ΔNH_4^+. Correlations were conducted for the 20 rewetted cores and the 20 partly drained cores separately. Significance was accepted at the $p < 0.05$ level.

3.3 Results

3.3.1 pH and conductivity

Pore water pH in the peat columns remained relatively stable between 6.0 and 6.7 for all groups (Fig. 3.2a). Over time, the rewetted soil cores gradually attained higher pH values than the drained soil cores. Highest final pH values were reached in the rewetted iron-rich cores, despite the fact that initial pH values at the start of the experiment were lowest in this group. Rewetting had a significant positive effect on pH values, but no interaction between water level and soil iron content was found (Table 3.2). Electrical conductivity (EC) in the partly drained soil cores as well as in the rewetted iron-poor soil cores remained between 200 and 600 $\mu S\ cm^{-1}$ (Fig. 3.2b). EC in the rewetted iron-rich soil cores however more than doubled over time, with the strongest increase during the first 70 days after rewetting. After 120 days, EC levelled around values of 1000 $\mu S\ cm^{-1}$. We found a significant interaction effect between water level and iron content with significantly higher EC-values in rewetted, iron-rich soil cores (Table 3.2).

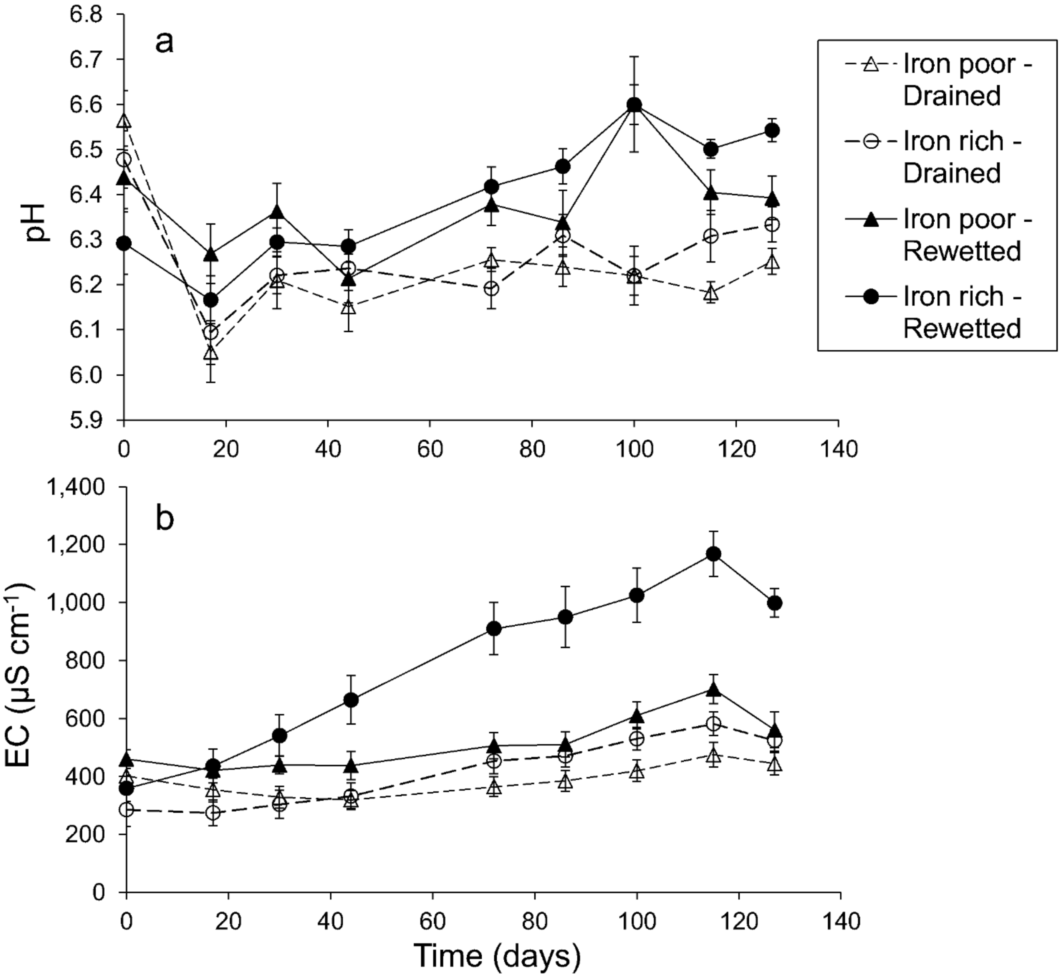

Figure 3.2: Changes in pore water pH and electrical conductivity (EC) in 40 soil cores that differ in experimental water level treatment (rewetted or drained) and initial soil iron content (high or low). Soil cores were classified into 4 groups: rewetted iron-poor fens (n=10 cores from 2 sites), drained iron-poor fens (n=10 cores from 2 sites), rewetted iron-rich fens (n=10 cores from 2 sites), and drained iron-rich fens (n=10 cores from 2 sites). Dots represent group means ± SE.

Table 3.2: Output of the linear mixed-effect models. The models included two fixed factors "Water level (rewetted or drained)" and "Iron content (low or high)" and were corrected for the random factor "Site ID" (ZB, ES, BM or LH), with tests for interactions between soil iron content and water level. Dependent variables are mean pore water pH, EC, and concentrations of total dissolved iron (Fe), total inorganic carbon (TIC), dissolved organic carbon (DOC), methane gas (CH_4), ammonium (NH_4^+), nitrate (NO_3^-) and total dissolved phosphorus (P) measured at the start (t = 0 days) and at the end of the experiment (t = 127 days).

Dependent variable	**Fixed factor**	**0 days**			**127 days**		
		df	**F-value**	**P-value**	**df**	**F-value**	**P-value**
pH (μmol L^{-1})	Water level	**1,34**	**6.65**	**0.014**	**1,34**	**28.92**	**<0.001**
	Iron content	1,2	0.71	0.488	1,2	2.21	0.276
	Water level * Iron content	1,34	0.23	0.633	1,34	1.10	0.301
EC (μS cm^{-1})	Water level	**1,34**	**8.00**	**0.008**	1,34	49.94	<0.001
	Iron content	1,2	0.46	0.569	1,2	6.12	0.132
	Water level * Iron content	1,34	0.10	0.754	**1,34**	**18.42**	**<0.001**
Fe (μmol L^{-1})	Water level	1,34	0.94	0.339	1,34	58.80	<0.001
	Iron content	1,2	0.13	0.752	1,2	20.68	0.045
	Water level * Iron content	1,34	0.00	0.979	**1,34**	**35.71**	**<0.001**
TIC (μmol L^{-1})	Water level	**1,34**	**6.84**	**0.013**	1,34	374.36	<0.001
	Iron content	1,2	0.75	0.479	1,2	6.33	0.128
	Water level * Iron content	1,34	1.56	0.220	**1,34**	**34.53**	**<0.001**
DOC (μmol L^{-1})	Water level	1,34	0.31	0.580	1,34	96.52	<0.001
	Iron content	1,2	0.01	0.943	1,2	1.48	0.347
	Water level * Iron content	1,34	0.26	0.612	**1,34**	**25.37**	**<0.001**
CH_4 (μmol L^{-1})	Water level	1,34	0.48	0.494	**1,34**	**6.61**	**0.014**
	Iron content	**1,2**	**127.78**	**<0.001**	1,2	0.03	0.872
	Water level * Iron content	1,34	1.22	0.276	1,34	0.02	0.889
NH_4^+ (μmol L^{-1})	Water level	1,34	1.45	0.237	1,34	69.69	<0.001
	Iron content	1,2	3.26	0.213	1,2	5.68	0.14
	Water level * Iron content	1,34	1.00	0.324	**1,34**	**29.33**	**<0.001**
NO_3^- (μmol L^{-1})	Water level	1,34	7.47	0.010	**1,34**	**314.32**	**<0.001**
	Iron content	1,2	8.00	0.106	1,2	0.11	0.772
	Water level * Iron content	**1,34**	**9.04**	**0.005**	1,34	0.29	0.591
P (μmol L^{-1})	Water level	1,34	0.30	0.587	**1,34**	**40.98**	**<0.001**
	Iron content	1,2	1.40	0.359	1,2	0.96	0.43
	Water level * Iron content	1,34	0.23	0.632	1,34	0.98	0.329

3.3.2 Carbon and nutrient mobilization

At the start of the experiment (t = 0 days) the factors “water level” and “iron content” had no significant effect on pore water concentrations of dissolved Fe, DOC, NH_4^+ and P (Table 3.2, Fig. 3.3), indicating that hydrochemical conditions in all four groups were relatively similar. In the iron-poor soil cores, initial concentrations of CH_4 were higher, while there was an interaction effect of water level with iron content for concentrations of NO_3^- (Table 3.2, Fig. 3.4), with slightly higher NO_3^- concentrations in the drained Fe-rich cores (in the order of a few µmolars). Initial concentrations of TIC were slightly higher in all rewetted cores.

At the end of the experiment (t = 127 days), the drained soil cores had responded to further desiccation in a similar manner regardless of soil iron content (Fig. 3.3). Prolonged drainage (> 30 days) led to mass mobilization of NO_3^- (> 1 mmol L^{-1}) at all locations (Fig. 3.4), and this effect was independent of soil iron content (Table 3.2).

Experimental rewetting led to a significant increase in pore water concentrations of Fe, TIC, DOC, NH_4^+, P and CH_4 (Table 3.2, Fig. 3.3). However, hydrochemical conditions in the rewetted iron-rich soil cores differed markedly from the conditions in the rewetted iron-poor soil cores (Table 3.2, Fig. 3.3). For the variables Fe, TIC, DOC, and NH_4^+, we found strong positive interactions between water regime and soil iron content: concentrations of Fe, TIC, DOC and NH_4^+ increased in response to rewetting, but the magnitude of this effect was much stronger in the iron-rich soil cores. Concentrations of both Fe and NH_4^+ reached more than 1 mmol L^{-1} in the rewetted iron-rich soil cores, while the accumulation of TIC and DOC had almost doubled compared to the rewetted iron-poor soil cores, reaching final mean concentrations of >13 mmol L^{-1} and >15 mmol L^{-1} respectively. Final CH_4 concentrations did not differ between iron-rich and iron-poor soil cores and had increased after rewetting, but we observed a delay in CH_4 accumulation in the rewetted iron-rich soil cores (Fig. 3.3e). Pore water concentrations of total dissolved P increased slightly after rewetting, but in the iron-rich soil cores an initial increase to concentrations of 4.0 µmol L^{-1} was followed by a gradual decrease to concentrations of 2 µmol L^{-1} (Fig. 3.3f). Initial mean (t = 0 days) concentrations of total dissolved sulfur (S_t) were low in all treatments (< 100 µmol L^{-1} , results not shown), while concentrations of sulfide remained below detection limit (< 0.1 µmol L^{-1}) throughout the course of the experiment (results not shown).

In addition, the change in concentrations of dissolved Fe throughout the experiment in the rewetted cores (ΔFe) correlated positively with ΔTIC (rho = 0.86, df = 18, $p < 0.001$, Fig. 3.5a), ΔDOC (rho = 0.85, df = 18, $p < 0.001$, Fig. 3.5b), and ΔNH_4^+ (rho = 0.82, df = 18, $p < 0.001$, Fig. 3.5c). In the drained soil cores ΔFe also correlated with ΔTIC (rho = 0.69, df = 18, $p < 0.001$, Fig. 3.5a) and ΔDOC (rho = 0.78, df = 18, $p<0.001$, Fig. 3.5b), but not with ΔNH_4^+ (rho = 0.08, df = 18, p = 0.7, Fig. 3.5c).

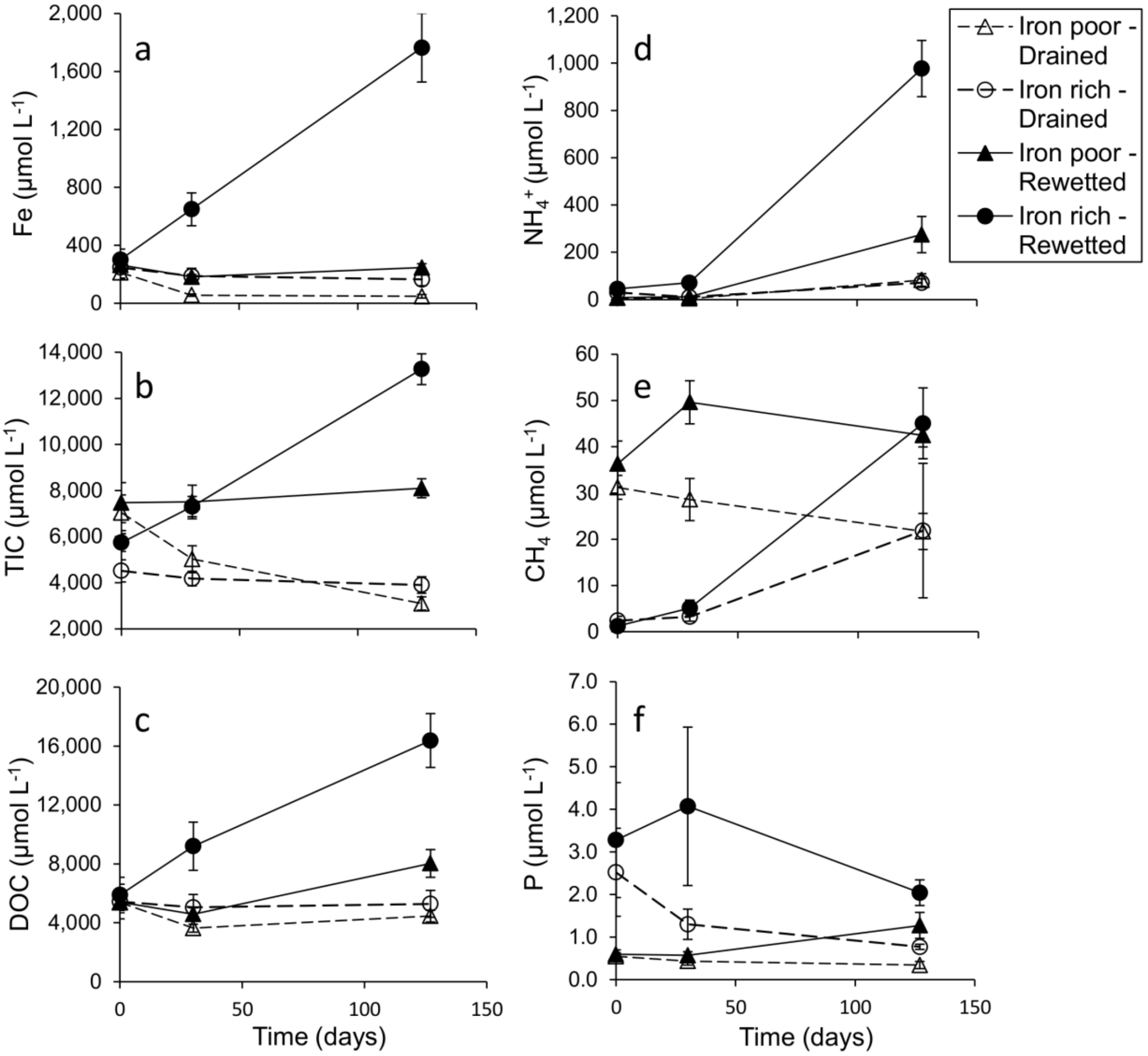

Figure 3.3: Mobilization of (a) dissolved iron, (b) total inorganic carbon, (c) dissolved organic carbon, (d) ammonium, (e) methane gas and (f) total dissolved phosphorus over time (t = 0, 30 and 127 days) in the pore water of 40 soil cores that differ in experimental water level treatment (rewetted or drained) and initial soil iron content (high or low). Soil cores were classified into 4 groups: rewetted iron-poor fens (n=10 cores from 2 sites), drained iron-poor fens (n=10 cores from 2 sites), rewetted iron-rich fens (n=10 cores from 2 sites), and drained iron-rich fens (n=10 cores from 2 sites). Dots represent group means ± SE.

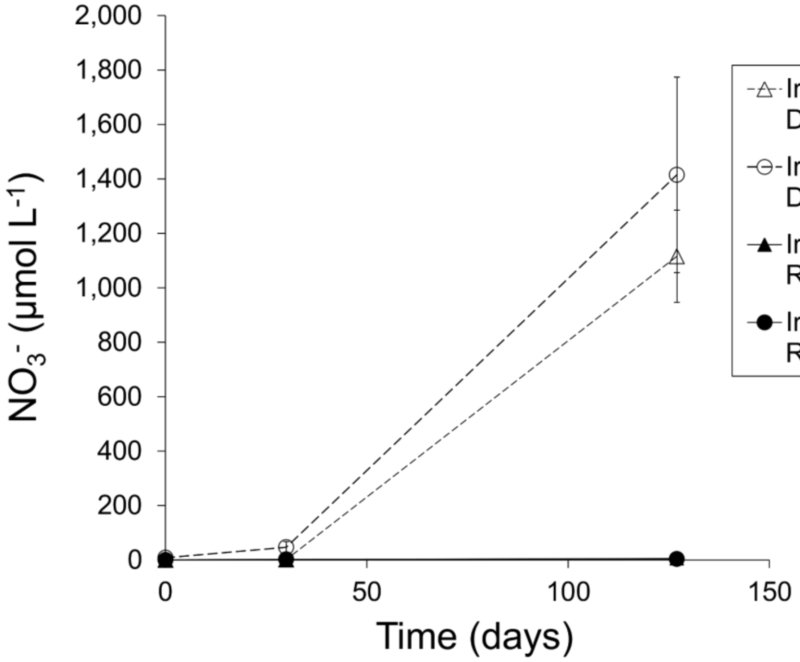

Figure 3.4: Mobilization of nitrate (NO_3^-) over time (t = 0, 30 and 127 days) in the pore water of 40 soil cores that differ in experimental water level treatment (rewetted or drained) and initial soil iron content (high or low). Soil cores were classified into 4 groups: rewetted iron-poor fens (n=10 cores from 2 sites), drained iron-poor fens (n=10 cores from 2 sites), rewetted iron-rich fens (n=10 cores from 2 sites), and drained iron-rich fens (n=10 cores from 2 sites). Dots represent group means ± SE.

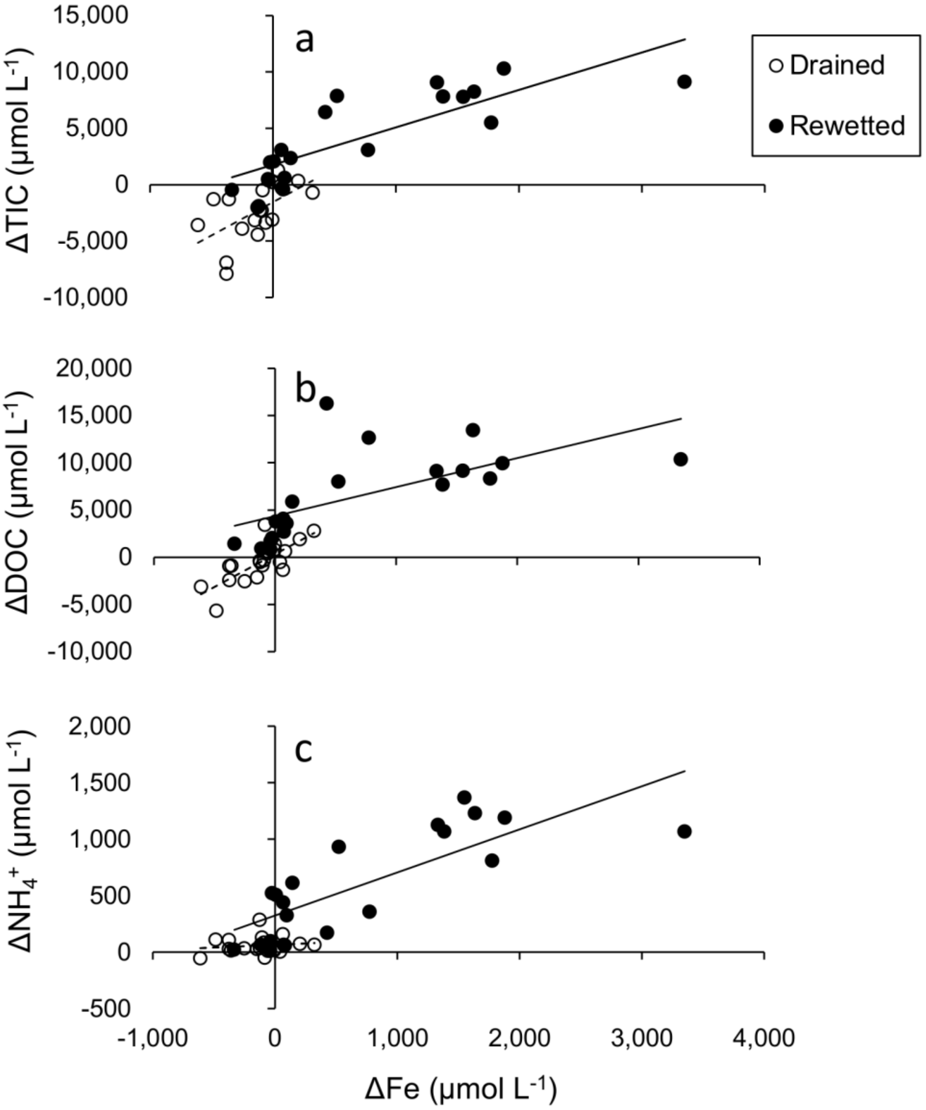

Figure 3.5: Relationship between the change in pore water Fe concentrations (ΔFe) and the change in concentrations of (a) total inorganic carbon (ΔTIC), (b) dissolved organic carbon (ΔDOC) and (c) ammonium (ΔNH_4^+) (in µmol L^{-1}) in 20 rewetted and 20 drained soil cores over 127 days (n = 4 sites).

3.3.3 Mineral saturation indices

After 127 days, saturation for siderite ($FeCO_3$) was found in all of the rewetted soil cores, with supersaturation in the iron-rich cores. Supersaturation for rhodochrosite ($MnCO_3$) was found only in a subset of rewetted iron-rich cores, but not in the iron-poor cores. Vivianite ($Fe(II)_3(PO_4)_2.8H_2O$) precipitation was predicted in some (but not all) of the rewetted iron-rich soil cores. Slight supersaturation for calcite ($CaCO_3$) was sporadically found in the cores with highest pH values (> 6.4), which only occurred in some alkaline layers of the rewetted iron-rich cores of site ES. Ca-P precipitation as hydroxyapatite ($Ca_5(PO_4)_3(OH)$) was unlikely due to strong undersaturation.

3.4 Discussion

Rewetting of drained organic soils unambiguously triggered the mobilization and accumulation of NH_4^+, TIC and DOC into the pore water, but this rewetting effect was disproportionately stronger in fens with large iron pools, suggesting a strong iron-mediated breakdown of organic matter (Lovley and Phillips 1986, Li et al. 2012). Concentrations of dissolved P however only rose slightly, and, in the iron-rich cores, had again dropped to low levels at the end of the experiment, indicating a P sink.

3.4.1 Nutrient (P, NO_3^- and NH_4^+) mobilization

Peatland drainage increases mineralization rates of organic matter. In response, organic-bound nutrients are converted into inorganic mobile ions, which is why drainage of wet soils is coupled with eutrophication (Olde Venterink et al. 2002). In our dataset, this eutrophication effect corresponds with the observed increase in concentrations of NO_3^- up to > 1 mmol L^{-1}. Conversely, accumulation of NO_3^- did not occur in the rewetted soil cores, as no nitrification of ammonium can take place under anaerobic conditions. However, although rewetting can be an effective mechanism to prevent aerobic decomposition and accumulation of nitrate in organic soils, rewetting does not necessarily lower nutrient availability in general. Compared to the drained soil cores, we measured slightly higher concentrations of dissolved P and much higher concentrations of ammonium in the rewetted cores. For P, this apparent discrepancy is directly related to the differences in redox-state between both water level treatments. Higher concentrations of dissolved P in the rewetted cores can be linked to anaerobic reduction processes in which organic matter and organic-P are mineralized, as well as to the well-known redox-sensitive dissolution of P from amorphous Fe-(hydr)oxides under anoxic conditions (Patrick and Khalid 1974, Lucassen et al. 2005, Zak et al. 2010). The latter mechanism is particularly relevant in drained and degraded groundwater-fed fens, in which a large part of the inorganic P pool is already iron-bound (Zak and Gelbrecht 2007, Aggenbach et al. 2013). Although we had expected a mass release of P in the rewetted iron-poor soils in particular, pore water P concentrations only rose to low concentrations of 1.3 μmol L^{-1}. In the rewetted iron-rich cores, an initial increase in total dissolved P during the first (t = 30 d) phase of rewetting was soon followed by a decrease in concentrations of dissolved P to 2 μmol L^{-1}. Computed oversaturation for vivianite in some of the rewetted iron-rich cores at the end of the experiment suggests vivianite precipitation under anoxic iron-rich conditions, which can be an effective sink for soluble P but is generally slow to obtain equilibrium (Murphy et al. 2001, Heiberg et al. 2010, Heiberg et al. 2012). Additionally, other phases than iron compounds may have controlled the solubility of P in the pore water after rewetting, as P can be resorbed to redox-insensitive compounds such as aluminium oxides (Sakadevan and Bavor 1998, Walpersdorf et al. 2013). It remains unclear to what extent this occurred in our study. Finally, it should be noted that we measured total P in the pore water and not orthophosphate-P. Therefore, we cannot exclude that part of the P is actually within DOM. If so, then this means that our results of P-release could be an overestimation of actual inorganic P release. Concentrations of inorganic P may thus be even lower than the results reported here. These results suggest that pore water P mobilization upon fen rewetting can remain well below eutrophic thresholds, which is in contrast with most studies on fen rewetting (Zak et al. 2010, van de Riet et al. 2013). We relate this primarily to the low S loads in our study sites, as sulfates and sulfides enhance internal eutrophication (Smolders et al. 2006).

In contrast to P, NH_4^+ concentrations rose to potentially phytotoxic levels (1 mmol L^{-1}) in the iron-rich rewetted cores, while remaining considerably lower in the rewetted iron-poor cores (< 0.3 mmol L^{-1}). As inflow and outflow of NH_4^+ as well as plant uptake was prevented in our closed experiment, only in situ NH_4^+ production/mobilization and transformation/adsorption plays a role. At least two nonexclusive processes must be taken into account in order to explain the observed interaction effect between rewetting and soil iron content on NH_4^+ accumulation. First, a disproportionately high accumulation of NH_4^+ in the iron-rich soil cores can be related to the iron-mediated anaerobic breakdown of organic matter, which triggers the conversion of organic-N to inorganic-N and releases $NH4^+$ into the pore water. Here, reduced nitrification rates prevent NH_4^+ transformation (Russow et al. 2013). The positive correlations between pore water accumulation of Fe and NH_4^+ as well as Fe and TIC in our dataset indeed suggest such iron-mediated breakdown of organic matter coupled with NH_4^+ release, as all compounds are products of anaerobic decomposition processes. Second, we explored the possibility that a larger quantity of mineral NH_4^+ was, by chance, already adsorped to the adsorption complex of the two iron-rich fens. Rewetting with artificial groundwater increases competition for soil exchange sites and may stimulate NH_4^+ release from the soil (Lucassen et al. 2006). However, previously collected field data on KCl-extractable NH_4^+ at all four study sites indicates that the amount of NH_4^+ that can be released from the adsorption complex of one liter of soil equals an estimated 0.98 and 0.97 mmol in the iron-poor sites (site BM and LH respectively) and 1.07 and 0.54 mmol in the iron-rich sites (site ES and ZB respectively). Therefore, the differential response of iron-rich and iron-poor fens is unlikely to be explained by desorption. Finally, nitrate reduction is another well-known mechanism for NH_4^+ production (Burgin and Hamilton 2007), and this reaction can be mediated by iron compounds (Hansen et al. 1996). However, this pathway is unlikely in our experiment as this would require a large pool of nitrate. In our study, initial pore water NO_3^- concentrations in the peat cores were only in the order of a few µmolars, while field data on KCl-extractable NO_3^- suggests values close to or even below detection limit at all study sites.

3.4.2 Carbon (DOC, TIC and CH_4) production

Throughout the experiment, mean pore water DOC concentrations in the drained and rewetted iron-poor soil cores remained within the range of 3.5-9.1 mmol L^{-1}, with slightly elevated concentrations in the latter. In the rewetted iron-rich soil cores however, DOC accumulation reached high mean levels of 16 mmol L^{-1} (> 192 mg L^{-1}).

Such link between iron concentrations and disproportionate DOC mobilization has also been observed in the field (Hughes et al. 1998, Fenner et al. 2011). We see two nonexclusive reasons for the positive correlation between DOC mobilization and soil iron content in the rewetted soil cores. First, the same mechanism that explains NH_4^+-mobilization affects DOC mobilization as well, i.e. the iron-mediated decomposition of organic matter. As DOC is a product of decomposition (Kalbitz et al. 2000), increased rates of litter decomposition (either directly or indirectly) coupled with a large pool of soil iron should stimulate DOC release into the pore water. Second, large quantities of DOM can be adsorbed to Fe(III)(hydro)xides under oxic conditions (Lal et al. 1997, Riedel et al. 2013), so that Fe(III)-reduction triggers the dissolution of Fe-DOC coagulates. As pointed out in recent research (Riedel et al. 2013), such Fe-DOC coagulation in oxic soil layers serves as a barrier to DOC efflux from semi-terrestrial environments, but such barrier disappears upon rewetting. Although the quantitative contribution of each process to DOC-accumulation in rewetted Fe-rich soils cannot be disentangled in our experiment, it is nonetheless clear that large-scale rewetting of drained iron-rich fens (in contrast to iron-poor fens) triggers a strong mobilization of DOC, thereby increasing DOC fluxes towards adjacent water catchments.

In non-calcareous fens (including our study sites), high concentrations of TIC are mainly related to in-situ anaerobic reduction processes coupled with organic matter decomposition (Smolders et al. 2006). Upon rewetting, hydrogen ions are consumed during anaerobic decomposition while HCO_3^- and CO_2 (TIC) are produced, and waterlogged conditions prevent rapid CO_2-degassing towards the atmosphere. In our experiment, excessive accumulation of TIC and a sharp rise in pH in the rewetted iron-rich cores, but to a lesser extent in the rewetted iron-poor cores, again points towards iron-induced anaerobic decomposition of organic matter in rewetted soils. For degraded iron-rich fens in particular, a considerable part of TIC production is indeed directly coupled with iron reduction (Aggenbach et al. 2013). Likewise, the reduction of nitrates or sulphates also produces TIC and alkalinity (Smolders et al. 2006, Geurts et al. 2009), but given the low mean pore water concentrations of these variables at the start of the experiment (< 9 µmol L^{-1} and < 100 µmol L^{-1} respectively), this is unlikely to explain excessive TIC production in our soil cores. It should be noted however that we only measured carbon accumulation in the pore water, but not total carbon fluxes towards the atmosphere. As oxygen is the most favorable electron acceptor, total carbon emissions (in the form of CO_2) are most likely highest in all of the drained cores. Here, the generally rapid degassing of CO_2 in aerated soils prevents CO_2-accumulation in the pore water, so that flux measurements would be needed to estimate actual C-loss to the atmosphere.

Organic soils that have been subject to prolonged drainage generally require a longer time lag before significant CH_4-accumulation, as electron acceptors have had sufficient time to regenerate (Estop-Aragones and Blodau 2012). Although methanogenesis can occur on a micro-scale despite the presence of energetically favorable electron acceptors, significant CH_4 production is expected to only take place after sequential consumption of these electron acceptors by micro-organisms (Achtnich et al. 1995, Fenner et al. 2011). In our dataset, we observe a clear (an estimated ± 50-100 days) delay in methane accumulation in the rewetted iron-rich cores but not in the iron-poor cores, which, again, suggests that ferric iron is being reduced as an energetically favorable electron acceptor.

3.4.3 Management implications

In the past, it has been emphasized that high concentrations of iron in soil and flooding water are beneficial for the restoration of wet ecosystems as iron prevents mobilization of phosphates and sulfides into pore and surface water (Smolders et al. 1995, Geurts et al. 2008, Zak et al. 2010). This is particularly relevant in heavily degraded P-eutrophied or S-polluted wetlands that are characterized by very low soil Fe:S and Fe:P ratios (Smolders et al. 2006, Zak and Gelbrecht 2007). In unpolluted mesotrophic fens however, the P- and S-binding service of iron may be of much less importance for the vitality of the fen system. In our experiment for example, concentrations of sulfide remained below detection limit both in iron-rich and iron-poor fens, while concentrations of dissolved P never reached eutrophic thresholds. Under such conditions, positive effects of iron may be overshadowed by negative effects. In this respect, many restored fens are, despite rewetting, characterized by seasonal fluctuations in water levels (and thus redox conditions, (Richert et al. 2000, Grootjans et al. 2006)) and large pools of accumulated iron in the top soil (Aggenbach et al. 2013). Under such conditions, the iron redox cycle in which iron alternately shifts between the ferric (Fe^{3+}) and ferrous (Fe^{2+}) state can cause a continuous positive feedback on organic matter mineralization coupled with nutrient and carbon mobilization (Li et al. 2012). Here, repeated mass mobilization of TIC, DOC and NH_4^+ in each rewetting cycle can be expected to impact the functioning of the fen ecosystem and downstream systems. Although our data do not allow for an accurate prediction of how much carbon is released or mineralized with each oxidation/rewetting cycle, we can make a rough estimate based on several assumptions. We assume that a major part of DOC and TIC in soil water is the product of organic matter decomposition and associated processes like iron reduction. According to a simplified calculation (e.g. disregarding CO_2 emissions towards the atmosphere) about 0.8% of total carbon in iron-rich cores and 0.17% of total carbon in iron-poor cores must be dissolved to reach the final mean sum of DOC and TIC concentrations of 18 mM or 3.5 mM (ΔTIC + ΔDOC), respectively. This estimate is based on i) different organic matter content and dry bulk density for iron-rich soils (means = 0.326 kg:kg, 0.21 kg L^{-1}) vs. iron-poor soils (means = 0.617 kg:kg, 0.1 kg L^{-1}) (Table 3.1), ii) the assumption that one litre of water corresponds roughly with one litre of soil, and iii) that organic matter has an average C fraction of 0.4 (kg:kg). Accordingly, one litre of soil of the iron-rich fens holds on average 27 g C and of the iron-poor fens holds on average 25 g C compared to ΔDOC+ΔTIC concentrations of 216 mg C L^{-1} (18 mM) and 42 mg C L^{-1} (3.5 mM) in pore water of iron-rich cores vs. in iron-poor cores which equates to percentage details given above. Therefore, if the goal of a fen rewetting project is to restore a low-productive fen that provides vital ecosystem services such as long-term carbon sequestration and nutrient retention, we suggest that the rewetting of unpolluted drained iron-poor fens should deserve priority over the rewetting of drained iron-rich fens.

3.4.4 Conclusions

The biogeochemical effects of rewetting in drained fens are often unpredictable and site-dependent, and many rewetted fens may remain sources rather than sinks for carbon and nutrients, at least in the short term. In our comparative study, soil cores collected in drained iron-rich fens were characterized by a high mobilization of Fe, TIC, DOC and NH_4^+ in the pore water after rewetting, while mobilization was lower in soil cores collected in drained iron-poor fens. Concentrations of dissolved P remained well below eutrophic thresholds, indicating a P sink even in rewetted fens. Our results suggest that high iron pools in organic soils interact with water regime, and rewetting stimulates strong iron-mediated organic matter mineralization coupled with carbon and inorganic nutrient (NH_4^+) mobilization. However, we do not claim that drained Fe-rich fens should therefore not be rewetted: if the goal is to restore a potentially peat-accumulating fen rather than a fen meadow, then complete rewetting is without exception a first and crucial step as a drained fen can never accumulate peat. In the short term, rewetting may however be more effective in (unpolluted) iron-poor fens.

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3.5 References

Achtnich, C., F. Bak, and R. Conrad. 1995. Competition for Electron-Donors among Nitrate Reducers, Ferric Iron Reducers, Sulfate Reducers, and Methanogens in Anoxic Paddy Soil. Biology and Fertility of Soils 19:65-72.

Aggenbach, C.J.S., Backx, H., Emsens, W.J., Grootjans, A.P., Lamers, L.P.M., Smolders, A.J.P., Stuyfzand, P.J., Wolejko, L. & Van Diggelen, R. (2013) Do high iron concentrations in rewetted rich fens hamper restoration? Preslia, 85, 405-420.

Burgin, A. J. and S. K. Hamilton. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Frontiers in Ecology and the Environment 5:89-96.

Clement, J. C., J. Shrestha, J. G. Ehrenfeld, and P. R. Jaffe. 2005. Ammonium oxidation coupled to dissimilatory reduction of iron under anaerobic conditions in wetland soils. Soil Biology & Biochemistry 37:2323-2328.

Estop-Aragones, C. and C. Blodau. 2012. Effects of experimental drying intensity and duration on respiration and methane production recovery in fen peat incubations. Soil Biology & Biochemistry 47:1-9.

Evans, M. G., T. P. Burt, J. Holden, and J. K. Adamson. 1999. Runoff generation and water table fluctuations in blanket peat: evidence from UK data spanning the dry summer of 1995. Journal of Hydrology 221:141-160.

Fenner, N., R. Williams, H. Toberman, S. Hughes, B. Reynolds, and C. Freeman. 2011. Decomposition 'hotspots' in a rewetted peatland: implications for water quality and carbon cycling. Hydrobiologia 674:51-66.

Freeman, C., N. Fenner, N. J. Ostle, H. Kang, D. J. Dowrick, B. Reynolds, M. A. Lock, D. Sleep, S. Hughes, and J. Hudson. 2004. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. Nature 430:195-198.

Geurts, J. J. M., J. M. Sarneel, B. J. C. Willers, J. G. M. Roelofs, J. T. A. Verhoeven, and L. P. M. Lamers. 2009. Interacting effects of sulphate pollution, sulphide toxicity and eutrophication on vegetation development in fens: A mesocosm experiment. Environmental Pollution 157:2072-2081.

Geurts, J. J. M., A. J. P. Smolders, J. T. A. Verhoeven, J. G. M. Roelofs, and L. P. M. Lamers. 2008. Sediment Fe : PO(4) ratio as a diagnostic and prognostic tool for the restoration of macrophyte biodiversity in fen waters. Freshwater Biology 53:2101-2116.

Grootjans, A. P., E. B. Adema, W. Bleuten, H. Joostn, M. Madaras, and M. Janakova. 2006. Hydrological landscape settings of base-rich fen mires and fen meadows: an overview. Applied Vegetation Science 9:175-184.

Hansen, H. C. B., C. B. Koch, H. NanckeKrogh, O. K. Borggaard, and J. Sorensen. 1996. Abiotic nitrate reduction to ammonium: Key role of green rust. Environmental Science & Technology 30:2053-2056.

Heiberg, L., C. B. Koch, C. Kjaergaard, H. S. Jensen, and H. C. B. Hansen. 2012. Vivianite Precipitation and Phosphate Sorption following Iron Reduction in Anoxic Soils. Journal of Environmental Quality 41:938-949.

Heiberg, L., T. V. Pedersen, H. S. Jensen, C. Kjaergaard, and H. C. B. Hansen. 2010. A Comparative Study of Phosphate Sorption in Lowland Soils under Oxic and Anoxic Conditions. Journal of Environmental Quality 39:734-743.

Holden, J. 2005. Peatland hydrology and carbon release: why small-scale process matters. Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences 363:2891-2913.

Hughes, S., B. Reynolds, S. A. Brittain, J. A. Hudson, and C. Freeman. 1998. Temporal trends in bromide release following rewetting of a naturally drained gully mire. Soil Use and Management 14:248-250.

Kalbitz, K., S. Solinger, J. H. Park, B. Michalzik, and E. Matzner. 2000. Controls on the dynamics of dissolved organic matter in soils: A review. Soil Science 165:277-304.

Kasimir-Klemedtsson, A., L. Klemedtsson, K. Berglund, P. Martikainen, J. Silvola, and O. Oenema. 1997. Greenhouse gas emissions from farmed organic soils: a review. Soil Use and Management 13:245-250.

Kusel, K., M. Blothe, D. Schulz, M. Reiche, and H. L. Drake. 2008. Microbial reduction of iron and porewater biogeochemistry in acidic peatlands. Biogeosciences 5:1537-1549.

Lal, R., J. M. Kimble, R. F. Follett, and B. A. Stewart. 1997. Soil processes and the carbon cycle. CRC Press.

Lamers, L. P. M., M. A. Vile, A. P. Grootjans, M. C. Acreman, R. van Diggelen, M. G. Evans, C. J. Richardson, L. Rochefort, A. M. Kooijman, J. G. M. Roelofs, and A. J. P. Smolders. 2015. Ecological restoration of rich fens in Europe and North America: from trial and error to an evidence-based approach. Biological Reviews 90:182-203.

Li, Y. C., S. Yu, J. Strong, and H. L. Wang. 2012. Are the biogeochemical cycles of carbon, nitrogen, sulfur, and phosphorus driven by the "Fe-III-Fe-II redox wheel" in dynamic redox environments? Journal of Soils and Sediments 12:683-693.

Lovley, D. R. 1991. Dissimilatory Fe(Iii) and Mn(Iv) Reduction. Microbiological Reviews 55:259-287.

Lovley, D. R. and E. J. P. Phillips. 1986. Organic-Matter Mineralization with Reduction of Ferric Iron in Anaerobic Sediments. Applied and Environmental Microbiology 51:683-689.

Lucassen, E. C. H. E. T., A. J. P. Smolders, G. Boedeltje, P. J. J. van den Munckhof, and J. G. M. Roelofs. 2006. Groundwater input affecting plant distribution by controlling ammonium and iron availability. Journal of Vegetation Science 17:425-434.

Lucassen, E. C. H. E. T., A. J. P. Smolders, L. P. M. Lamers, and J. G. M. Roelofs. 2005. Water table fluctuations and groundwater supply are important in preventing phosphate-eutrophication in sulphate-rich fens: Consequences for wetland restoration. Plant and Soil 269:109-115.

Lucassen, E. C. H. E. T., P. Spierenburg, R. G. A. Fraaije, A. J. P. Smolders, and J. G. M. Roelofs. 2009. Alkalinity generation and sediment CO2 uptake influence establishment of Sparganium angustifolium in softwater lakes. Freshwater Biology 54:2300-2314.

Murphy, T., A. Lawson, M. Kumagai, and C. Nalewajko. 2001. Release of phosphorus from sediments in Lake Biwa. Limnology 2:119-128.

Olde Venterink, H., T. E. Davidsson, K. Kiehl, and L. Leonardson. 2002. Impact of drying and re-wetting on N, P and K dynamics in a wetland soil. Plant and Soil 243:119-130.

Parkhurst, D. L. and C. A. J. Appelo. 1999. User's guide to PHREEQC (version 2) - A computer program for speciation, batch-reaction, one-dimenstional transport, and inverse geochemical calculations: US Geological Survey Water-Resources Investigations Report 99 - 4259. 312 p.

Pastor, J., J. Solin, S. D. Bridgham, K. Updegraff, C. Harth, P. Weishampel, and B. Dewey. 2003. Global warming and the export of dissolved organic carbon from boreal peatlands. Oikos 100:380-386.

Patrick, W. H. and R. A. Khalid. 1974. Phosphate Release and Sorption by Soils and Sediments - Effect of Aerobic and Anaerobic Conditions. Science 186:53-55.

Preston, M. D., M. C. Eimers, and S. A. Watmough. 2011. Effect of moisture and temperature variation on DOC release from a peatland: Conflicting results from laboratory, field and historical data analysis. Science of the Total Environment 409:1235-1242.

Richert, M., O. Dietrich, D. Koppisch, and S. Roth. 2000. The influence of rewetting on vegetation development and decomposition in a degraded fen. Restoration Ecology 8:186-195.

Riedel, T., D. Zak, H. Biester, and T. Dittmar. 2013. Iron traps terrestrially derived dissolved organic matter at redox interfaces. Proceedings of the National Academy of Sciences of the United States of America 110:10101-10105.

Russow, R., N. Tauchnitz, O. Spott, S. Mothes, S. Bernsdorf, and R. Meissner. 2013. Nitrate turnover in a peat soil under drained and rewetted conditions: results from a [N-15]nitrate-bromide double-tracer study. Isotopes in Environmental and Health Studies 49:438-453.

Sakadevan, K. and H. J. Bavor. 1998. Phosphate adsorption characteristics of soils, slags and zeolite to be used as substrates in constructed wetland systems. Water Research 32:393-399.

Schlotzhauer, S. M. and J. S. Price. 1999. Soil water flow dynamics in a managed cutover peat field, Quebec: Field and laboratory investigations. Water Resources Research 35:3675-3683.

Smolders, A. J. P., L. P. M. Lamers, E. C. H. E. T. Lucassen, G. Van der Velde, and J. G. M. Roelofs. 2006. Internal eutrophication: How it works and what to do about it - a review. Chemistry and Ecology 22:93-111.

Smolders, A. J. P., R. C. Nijboer, and J. G. M. Roelofs. 1995. Prevention of sulphide accumulation and phosphate mobilization by the addition of iron(II) chloride to a reduced sediment: An enclosure experiment. Freshwater Biology 34:559-567.

Van Bodegom, P. M., R. Broekman, J. Van Dijk, C. Bakker, and R. Aerts. 2005. Ferrous iron stimulates phenol oxidase activity and organic matter decomposition in waterlogged wetlands. Biogeochemistry 76:69-83.

van de Riet, B. P., M. M. Hefting, and J. T. A. Verhoeven. 2013. Rewetting Drained Peat Meadows: Risks and Benefits in Terms of Nutrient Release and Greenhouse Gas Exchange. Water Air and Soil Pollution 224.

Van Dijk, J., M. Stroetenga, L. Bos, P. M. Van Bodegom, H. A. Verhoef, and R. Aerts. 2004. Restoring natural seepage conditions on former agricultural grasslands does not lead to reduction of organic matter decomposition and soil nutrient dynamics. Biogeochemistry 71:317-337.

Walpersdorf, E., C. B. Koch, L. Heiberg, D. W. O'Connell, C. Kjaergaard, and H. C. B. Hansen. 2013. Does vivianite control phosphate solubility in anoxic meadow soils? Geoderma 193:189-199.

Zak, D. and J. Gelbrecht. 2007. The mobilisation of phosphorus, organic carbon and ammonium in the initial stage of fen rewetting (a case study from NE Germany). Biogeochemistry 85:141-151.

Zak, D., C. Wagner, B. Payer, J. Augustin, and J. Gelbrecht. 2010. Phosphorus mobilization in rewetted fens: the effect of altered peat properties and implications for their restoration. Ecological Applications 20:1336-1349.

4

Eutrophication triggers contrasting multilevel feedbacks on litter accumulation and decomposition in fens

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Abstract

Eutrophication is a major threat for the persistence of nutrient-poor fens, as multilevel feedbacks on decomposition rates could trigger carbon loss and increase nutrient cycling. Here, we experimentally investigate the effects of macronutrient (NPK) enrichment on litter quality of six species of sedge (*Carex* sp.), which we relate to litter decomposition rates in a nutrient-poor and nutrient-rich environment. Our research focused on four levels: we examined how eutrophication alters (1) fresh litter production (“productivity shift”), (2) litter stoichiometry within the same species (“intraspecific shift”), (3) overall litter stoichiometry of the vegetation under the prediction that low-competitive species are outcompeted by fast-growing competitors (“interspecific shift”), and (4) litter decomposition rates due to an altered external environment (e.g., shifts in microbial activity; “exogenous shift”). Eutrophication triggered a strong increase in fresh litter production. Moreover, individuals of the same species produced litter with lower C:N and C:P ratios, higher K contents, and lower lignin, Ca and Mg contents (intraspecific shift), which increased litter decomposability. In addition, species typical for eutrophic conditions produced more easily degradable litter than did species typical for nutrient-poor conditions (interspecific shift). However, the effects of nutrient loading of the external environment (exogenous shift) were contradictory. Here, interactions between litter type and ambient nutrient level indicate that the (exogenous) effects of eutrophication on litter decomposition rates are strongly dependent of litter quality. Moreover, indicators of litter quality only correlated with decomposition rates for litter incubated in nutrient-poor environments, but not in eutrophic environments. This suggests that rates of litter decomposition can be uncoupled from litter stoichiometry under eutrophic conditions. In conclusion, our results show that eutrophication affects litter accumulation and –decomposition at multiple levels, in which stimulatory and inhibitory effects interact. The cumulative effect of these interactions ultimately determine whether peatlands remain sinks or become sources of carbon under eutrophic conditions.

4.1 Introduction

More than an estimated one-quarter of the world's soil carbon is stored in peatlands (Joosten and Clarke 2002, Moore 2002). The prerequisite for net peat accumulation is a continuous input of fresh plant litter followed by partial decomposition (and long-term storage) of that litter (i.e., peat). As macronutrients are fixed in recalcitrant structures of the growing peat matrix, (inorganic) nutrient availability is generally low in peatlands (Joosten and Clarke 2002), which facilitates the presence of many rare, slow-growing plant species. However, widespread drainage coupled with peat mineralization, atmospheric nitrogen deposition, rising global temperatures, and the use of artificial fertilizer and manure is causing severe eutrophication of peatlands worldwide (van Diggelen et al. 2006, Lamers et al. 2015). To date, one of the major challenges is understanding how nutrient enrichment affects organic matter accumulation, decomposition, and net carbon fluxes in peatlands (Bragazza et al. 2006), as eutrophication-induced shifts in the carbon balance are likely.

Eutrophication directly stimulates primary (and thus fresh litter) production (Kotowski et al. 2006, Hautier et al. 2009), and it may directly and indirectly alter decomposition rates of fresh litter and soil organic matter (Hessen et al. 2004). However, many studies of peatland eutrophication are biased by a (too) narrow scope, e.g. only focusing on exogenous effects of nutrient enrichment on decomposition (e.g. Bragazza et al. 2006), which has often resulted in oversimplified conclusions with respect to potential peat accumulation. In our paper, we argue that eutrophication-induced shifts occur at least at four different levels in the process of peat formation. First, an increase in nutrient availability stimulates plant primary production (Hautier et al. 2009), and the concomitant increase in fresh litter production should directly increase rates of peat accumulation. In this paper, we refer to this as a "productivity shift." Second, nutrient enrichment may affect litter stoichiometry (e.g., litter quality indicators such as C:N ratios, C:P ratios, and lignin or cellulose contents) within individual plants of the same species (Aerts and de Caluwe 1997, Gusewell 2004). Such altered within-species stoichiometry can affect decomposition rates of freshly produced litter: a low litter C:N ratio or low lignin concentration often correlates with fast decay (Taylor et al. 1989, Gessner and Chauvet 1994). We refer to this eutrophication-induced within-species stoichiometric shift as an "intraspecific shift." Third, eutrophication may not only affect an individual plant's stoichiometry, it also affects the structure and composition of complete plant communities. If macronutrients are in ample supply, low-competitive, stress-tolerant plant species are generally outcompeted by taller, more competitive species (Kotowski et al. 2006, Hautier et al. 2009). Such changes in the composition of the vegetation can directly influence decomposition rates, as the quality of the litter (of the plant community as a whole) often changes as well (Hobbie 1992, Ward et al. 2015). This community level response to eutrophication is referred to as an "interspecific shift." Fourth, eutrophication affects the composition, biomass, and behavior of microbial communities in the soil (Wright et al. 2009, Kaštovská et al. 2012, Chen et al. 2014), which should again be mirrored in litter decomposition rates. To date, the exogenous effect of eutrophication on litter decomposition has remained unclear (Knorr et al. 2005). While some studies report high litter decomposition rates in eutrophic environments (Qualls and Richardson 2000, Bragazza et al. 2006, Kominoski et al. 2015), others report neutral or even opposite patterns (Craine et al. 2007, Hobbie 2008). The contrasting results led to the emergence of two seemingly competing theories with respect to effects of external nutrient supply on litter decomposition (Craine et al. 2007, Chen et al. 2014). The first theory of "stoichiometric decomposition" generally predicts higher decomposition rates with increasing exogenous nutrient input (the latter leading to, for example, lower soil and substrate C:N ratios; Hessen et al. 2004), whereas the second theory of "microbial nutrient mining" emphasizes the existence of a guild of micro-organisms that uses labile C as an energy source to decompose recalcitrant organic matter in order to gain access to organically bound nutrients (Moorhead and Sinsabaugh 2006). The latter theory predicts lower decomposition rates when the easily available exogenous nutrient pool increases, i.e. in response to eutrophication (Craine et al. 2007, Chen et al. 2014). We refer to this fourth eutrophication-induced shift as an "exogenous shift."

In the present study, we investigated how macronutrient (NPK) enrichment affects litter production, litter quality, and litter decomposition rates in groundwater-fed peatlands ("rich fens" hereafter). Undisturbed rich fens are oligo-to mesotrophic, waterlogged wetlands in which the peat-forming communities mainly consist of bryophytes and low-productive, stress-tolerant, small sedges of the *Carex* genus (Wheeler and Proctor 2000). In Europe and North America, eutrophication has affected a large proportion of the remaining fens (Lamers et al. 2015), leading to a loss of biodiversity and the replacement of small sedges by taller, more competitive sedges (Wheeler and Proctor 2000, van Diggelen et al. 2006). The majority of fen and peatland studies that addressed this eutrophication effect on ecosystem functioning have solely focused on effects of N enrichment (Aerts and de Caluwe 1997, Bragazza et al. 2012). Only relatively few studies experimented with P enrichment (Qualls and Richardson 2000, Sarneel et al. 2010) or the combined effects of NPK enrichment (Carfrae et al. 2007, Xing et al. 2011, Kaštovská et al. 2012). This has large implications for a good understanding of a potential shift in ecosystem functioning, since eutrophication of fens (in contrast to rainwater-fed bogs that are mainly affected by atmospheric N deposition) usually corresponds with a combined increase in pools of both inorganic nitrogen and phosphorus (Emsens et al. 2015, Lamers et al. 2015), as well as potassium if fertilizers are at the origin of the eutrophication (Wheeler 1983). Here, we aimed to disentangle the direction and magnitude of the different NPK eutrophication-induced shifts on litter decomposition, and related these results to actual peat accumulation.

We selected six species of *Carex* that are naturally found along a fertility gradient in fens, and we varied NPK availability to quantify changes in litter production and litter quality. Litter quality was subsequently related to actual rates of litter decomposition in standardized nutrient-poor and nutrient-enriched decomposition beds. We hypothesized that (1) eutrophication increases plant biomass and litter production (productivity shift), (2) eutrophication increases litter quality and decomposability within the same species (intraspecific shift), (3) species typical for nutrient-enriched habitats produce litter that is more easily decomposable (interspecific shift), (4) eutrophic environments stimulate litter decomposition rates as predicted by stoichiometric theory, regardless of litter quality (exogenous shift). We thus expected that eutrophication causes a multilevel shift in a fen's carbon balance by installing positive feedbacks on rates of litter production, litter quality, litter decomposition, and nutrient cycling. Theoretically, such feedbacks could hamper peat accumulation and further contribute to eutrophication, eventually turning a peatland from sink to source of carbon.

4.2 Materials and methods

4.2.1 Species selection and classification

We selected six species of *Carex* that are typically found in fens and wet organic soils of the Northern hemisphere: *Carex paniculata*, *C. appropinquata*, *C. lepidocarpa, C. nigra*, *C. diandra*, and C. echinata. The selected species occupy different parts of a fen along a fertility gradient. Therefore, we ranked the species according to their natural occurrence from eutrophic to oligotrophic conditions. We based this ranking on the species' revised Ellenberg values for nitrogen following Hill et al. (1999), in which *Carex paniculata* (6) > *C. appropinquata* (4) > *C. diandra* (3) > *C. lepidocarpa* (2) = *C. nigra* (2) = *C. echinata* (2). Note that Ellenberg-N values represent the general productivity of the system in which the species optimally occurs rather than solely N loading of the system (Klaus et al. 2012). Generally, low-competitive (low Ellenberg-N) species have their natural optimum in nutrient-depleted environments (Kotowski et al. 2006), which can be linked to their high nutrient use efficiency (Aerts 1999). In contrast, competitive (high Ellenberg-N) species only thrive in more eutrophic environments as they trade off nutrient use efficiency with rapid growth and foliar expansion (Aerts 1999). Although the latter strategy allows competitors to maximize light interception under eutrophic conditions (thereby outcompeting low-competitive species), it simultaneously hampers long-term survival in nutrient-depleted environments (Aerts 1999, Kotowski et al. 2006).

4.2.2 Germination and transplantation

In spring 2013, cold-moist stratified *Carex* seeds were germinated on moist filter paper in an incubator under a fluctuating day : night regime (24–15°C, 12:12 h photoperiod). After germination, seedlings were transported to a greenhouse nursery. Here, seedlings were temporarily grown on a moist mixture of standard potting soil and white sand to allow an optimal initial growth and establishment to an approximate height of 10 cm. After seven weeks, 180 healthy individuals (30 seedlings of each species) were carefully removed from their nursery pots and roots were rinsed to remove adherent soil particles. Seedlings were then transplanted into the experimental mesocosms.

4.2.3 Experimental design

Our study had a full-factorial design in which 30 seedlings of each of the six species were divided (in monocultures) over a total of 60 experimental mesocosms (volume 5 L), resulting in three (sub-replicate) seedlings of one species per replicate mesocosm. Next, one-half of the mesocosms received a nutrient-rich treatment while the other half was kept nutrient poor. In total, this setup resulted in five replicate mesocosms per species per nutrient treatment (2 nutrient levels × 6 species [3 individuals per mesocosm] × 5 replicate mesocosms). Mesocosms were placed in an unheated greenhouse (to prevent N deposition) in full daylight, and had a 2-cm layer of river gravel at the bottom. The remaining volume of the mesocosms was filled with clean white sand. Next, five multichannel peristaltic pumps (Masterflex 7521-57, Cole-Parmer, Chicago, Illinois, USA, each with 12 separate connections connected to light-sealed water reservoirs containing tap water) were connected to the 60 mesocosms. In each mesocosm, water from the reservoirs was pumped in at a constant rate of 0.18 L d^{-1} to compensate for evapotranspirational loss and to guarantee a constant basic supply of essential base cations, trace elements, and minimal amounts of nutrients (tap water characteristics in Table S4.1 in Supporting information). The inlets of the pumps were placed at the bottom of the mesocosms, in the river gravel, to ensure an equal distribution of water and nutrients as well as to simulate groundwater upwelling typical for fens. A water outlet was placed at the top of the mesocosms to allow runoff of excess water. When evapotranspiration exceeded water input (e.g., on warm and sunny days), demineralized water was added to keep the mesocosms continuously waterlogged as in natural fens. Extra macronutrients (NPK) were injected biweekly directly into the mesocosm inlets, using a 2mL syringe, as dissolved KNO_3 (N and K) and KH_2PO_4 (P and K) from stock solutions so that total amounts of N, P, and K allocated to each mesocosm at the end of the growing season (n = 119 d) equaled 363.1, 21.1, and 959.8 mg, respectively, for the nutrient-rich treatment and 58.8, 3.8, and 88.6 mg, respectively, for the nutrient-poor treatment. These nutrient quantities were based on the range of values reported by Gusewell and Gessner (2009) who performed comparable mesocosm experiments with different species of *Carex* under different nutrient supplies. In our study, “nutrient-poor” implies conditions of severe macronutrient limitation whereas “nutrient-rich” implies a non-limiting macronutrient availability (for the selected species). In other words, the nutrient-poor treatment more closely resembles the natural range of the low Ellenberg-N species (e.g., *C. echinata* or *C. lepidocarpa*), whereas the nutrient-rich treatment more closely resembles the natural range of the higher Ellenberg-N species (e.g., *C. paniculata* or *C. appropinquata*). Every two weeks, the mesocosms were spatially randomized.

4.2.4 Morphological measurements and plant harvest

During the growing season, we determined the specific leaf area (SLA) of all plants. Next to a species’ Ellenberg-N value, SLA indices are also used as a proxy for plant-growth strategy: fast-growing, competitive species (typical for eutrophic systems) tend to have higher SLA indices than slow-growing, stress-tolerant species (typical for nutrient-limited systems; Westoby 1998). For SLA determination, we randomly collected one fresh, fully expanded, and illuminated leaf per individual plant in the middle of the day at the end of August 2013. Next, we cut out a 5–6 cm fragment from the middle of the leaf, and immediately calculated the surface area of one side of the fragment by combining digital photography with image-processing software ImageJ (Schneider et al. 2012). Next, all fragments were oven dried (70°C) for 24 h and weighed. SLA was calculated as leaf area divided by dry mass (cm^2 g^{-1}). We used mesocosm averages of SLA (three sub-replicates) for further data analysis. From October onward, senesced leaves (litter) were collected every week, rinsed, air dried, and stored in dry paper bags. At the end of the growing season (November 2013, n = 119 d), we counted the total number of living leaves per individual plant (leaf count), and we averaged the lengths of the two longest leaves per plant (leaf length). Next, the mesocosms were placed in a dark cooling room to simulate winter conditions and to initiate rapid leaf senescing. Mid-January 2014, all sedges were removed from the cooling room and aboveground (senesced) biomass was harvested and carefully rinsed with deionized water to remove any adherent soil particles. Per mesocosm, litter from the final harvest was mixed with the senesced leaves that had been collected in the weeks before. Next, all biomass was dried at 45°C and then weighed (g). We used this 45°C-dried rather than 70°C-dried material in subsequent decomposition experiments as this more closely resembles natural material for decomposition. To estimate total (70°C-dried) biomass per mesocosm, subsamples (1.4–1.9 g) of each 45°C-dried homogenized sample were oven-dried at 70°C to calculate a conversion factor per sample. Total biomass was then calculated by multiplying this conversion factor with the mass of the total collected 45°C-dried material. We only report the latter biomasses per plant (total biomass per mesocosm/3 plants) in this paper. The 70°C-dried litter was then ground using a rotary mill (Retsch zm 200, Retsch, Haan, Germany) and used for chemical analysis.

4.2.5 Chemical analyses

We made a selection of chemical (plant litter) variables that are most commonly used as indicators of litter quality: N, P, K, C, lignin, and cellulose content (and mass ratios between these variables). Additionally, we determined litter Ca and Mg contents as these variables may also correlate with decomposition rates. Total N, P, K, Ca, and Mg contents were determined following Walinga et al. (1989), in which ground samples were digested with H_2SO_4, salicylic acid and H_2O_2. N and P were analyzed on a segmented flow analyzer (Skalar, Breda, the Netherlands); K, Ca, and Mg were analyzed on ICP (Thermo Fisher, Franklin, Massachusetts, USA). Total C contents were analyzed though combustion of oven-dried and mill-ground plant material, using a CN-analyzer (Flash 2000, Thermo Fisher Scientific, Waltham, Massachusetts, USA). We used the van Soest method to analyze plant material for alpha-cellulose ("cellulose" hereinafter) and ADF-lignin content ("lignin" hereinafter; Van Soest 1963). In this method, cetyltrimethylammonium-bromide (CTAB) is added to 0.5–1 g of ground plant material and heated, which dissolves proteins. Samples were then rinsed, dried at 105°C, and weighed. Next, 72% sulfuric acid (H_2SO_4) was added to dissolve cellulose, after which samples were again rinsed, dried, and weighed. Mass loss, corrected for initial mass of the sample, was used to calculate cellulose content. Finally, lignin was removed from the samples by ashing the remaining material at 550°C for 4 h. Again, mass loss was used to calculate lignin content. Due to unforeseen procedural errors during the heating phase and sulfuric-acid phase, respectively, we lost two cellulose and three lignin samples (out of the 60 samples). Contents are reported in mg g^{-1} dry mass.

4.2.6 Decomposition experiments

Decomposition experiments were run in 10 closed artificial decomposition beds (57 cm long × 39 cm wide × 28 cm high). Each bed was filled with 25 kg of limed (25 g $CaCO_3$) clean white sand, and inoculated with 1 L fresh peat soil from a *Scorpidium scorpioides*-dominated rich fen (collected in the Weerribben, the Netherlands; 52°47′02.4″ N 5°58′58.8″ E) to attain a decomposer community typical for rich fens. Beds were placed in a basement with relatively stable temperatures (minimum–maximum range between 15.2°C and 19.9°C) in full darkness. To allow establishment of the decomposer community, mesocosms had been established six months prior to initiation of the decomposition experiment. Water levels were manually kept at surface level with a mixture of 90% demineralized water and 10% tap water, the latter to provide a minimal supply of essential trace and base elements (Table S4.1 in Supporting information) to mimic rich fen conditions. Soil pH in the decomposition beds equaled 7.2 ± 0.04 (measured with a HI 99121 portable pH meter [Hanna Instruments, Woonsocket, Rhode Island, USA]). After five months (one month prior to actual litter incubation), one-half of the beds (five) were heavily eutrofied by mixing 200 g slow-release NPK fertilizer (17-9-11 Substral Osmocote Scotts Benelux, Sint-Niklaas, Belgium) with the soil. The other one-half of the decomposition beds was kept nutrient poor; soil was also “mixed” to guarantee an equal treatment but no fertilizer was added. Dried plant litter (0.82 ± 0.08 g [mean ± SD]) was manually cut into 5-cm fragments and then put in litter bags. As plants from the nutrient-poor treatment had not produced sufficient biomass for incubation, we were restricted to incubating litter from plants that had grown under the nutrient-rich treatment. Ten replicate litterbags of each of the six species (two litter samples were collected from each of the 30 nutrient-rich mesocosms) were divided pairwise over the nutrient-poor and nutrient-rich decomposition beds. Additionally, we incubated one piece of standard (alpha-)cellulose filter paper (0.78 ± 0.006 g) in each mesocosm. The litter bags (8 × 5.5 cm) were made from polyester netting with mesh size 325 µm (TopZeven, Haarlem, the Netherlands). We chose this fine mesh size, as the use of a too large mesh size in narrow-leaved grasses causes unacceptable litter loss through the maze (Aerts et al. 2003). Litter bags were placed horizontally on the soil surface and were then pushed 2–3 mm into the top soil to guarantee contact of the plant litter with surrounding soil and pore water; this is also the zone where leaf litter would be deposited under natural conditions. All litterbags were retrieved after 116 d (0.32 yr). Litter samples were carefully rinsed with demineralized water, dried (45°C), and remaining mass (g) was determined. To correlate litter decomposition rates with measures of litter quality, we calculated decomposition constants k for each litter replicate from a single-exponential decay model (Wider and Lang 1982), which has been proven to be a solid indicator for decomposition rates (Hobbie 2008):

$$\ln(M_t/M_0) = -k*t$$

where Mt is the mass at time t, M0 is the initial mass, k is the decay constant, and t is time (yr).

4.2.7 Data analysis

We analyzed our data using linear mixed effect (LME) modelling with REML in SPSS 22 (IBM Corp. 2013), in which we treated Species (1–6) as well as Nutrient level (rich or poor) as fixed-effect predictors. Data were log-transformed wherever this resulted in a better approximation of the normal distribution of the model residuals. Next, we ran separate models to test for the effects of species and nutrient level on each of the following response variables: total (litter) biomass, leaf count, leaf length, SLA, litter C:N and C:P ratios, litter contents of cellulose, lignin, Ca, Mg, and K, and litter decomposition constants (k). In each model, we always tested for possible interactions between the two fixed factors. If interactions were nonsignificant, we additionally ran a model that only included the main effects of the two predictors. In this paper, we only report the outcome of these main-effect models if the significance of any of the parameters had changed. Finally, we ran Spearman correlation tests to correlate (untransformed) widely used litter quality indicators (N, P, C:N, C:P, lignin, cellulose, lignin : N, lignin : P, K, Ca, Mg), as well as Ellenberg-N values and SLA indices, with actual litter decomposition constants (k). Correlation tests were run for the nutrient-poor and nutrient-enriched decomposition beds separately. For all tests, significance was accepted at $P < 0.05$.

4.3 Results

4.3.1 Plant morphology

Plants grown in the nutrient-rich treatment produced more (factor 1.7–2.5×) and longer (factor 1.1–2.5×) leaves compared to the nutrient-poor treatment. Total aboveground litter production per plant (biomass) more than doubled (by a factor of 2.2–5.2×; Table 4.1) in the nutrient-rich treatment. The fixed-effect model indicated significant interaction effects between species and nutrient treatment for biomass production, leaf count, and leaf length (Table S4.2 in Supporting information), indicating that the magnitude of the positive effect of nutrient enrichment on litter production was species dependent. Specific leaf area (SLA), however, was not affected by nutrient treatment, but differed significantly between species (Table S4.2 in Supporting information).

Table 4.1: Plant leaf length, leaf count, total aboveground biomass, and specific leaf area (SLA) of the study species (averages ± SD) under low and high nutrient treatments at the end of the experiment (n = 5).

Species and nutrient treatment	Leaf length (cm)		Leaf count (n)		Total aboveground biomass (g)		Specific Leaf Area (SLA, cm^2 g^{-1})	
	Average	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
C. paniculata								
Low	25.6	3.4	30.1	8.2	0.65	0.14	297	32
High	36	4.3	51.7	9.5	1.73	0.38	315	34
C. appropinquata								
Low	16.1	1.5	24.9	5.6	0.48	0.13	298	27
High	39.5	5.9	60.8	11.3	2.45	0.74	292	24
C. diandra								
Low	23.2	1.3	35.7	10.6	0.57	0.08	201	31
High	31.6	4.5	64.1	18.3	1.43	0.53	206	18
C. lepidocarpa								
Low	14.1	0.9	36.1	7	0.92	0.14	300	27
High	17.9	1.3	78.3	11.2	2.03	0.14	289	25
C. nigra								
Low	16.4	1.7	22.9	3.5	0.45	0.04	252	51
High	21.4	2.4	50.1	20.4	1.07	0.11	260	34
C. echinata								
Low	15.5	1.9	60.8	4.3	0.8	0.18	215	21
High	17.9	2.5	120.8	5.8	2.16	0.5	226	20

4.3.2 Litter chemistry

Under the nutrient-rich treatment, *Carex* species produced litter with lower C:N ratios, C:P ratios, and lignin contents, while cellulose contents remained unaltered and only differed between species (Figure 4.1; Table S4.3 in Supporting information). Additionally, litter Ca and Mg contents were significantly lower in the high-nutrient treatment, while K contents increased (Figure 4.2; Table S4.3 in Supporting information). Significant interaction effects between "species" and "nutrient level" were present for litter C:N ratios, cellulose contents, and K and Mg contents (Table S4.3 in Supporting information). When we re-ran all models without interaction terms to test for the main effects of species and nutrient levels, all main effects retained their significance.

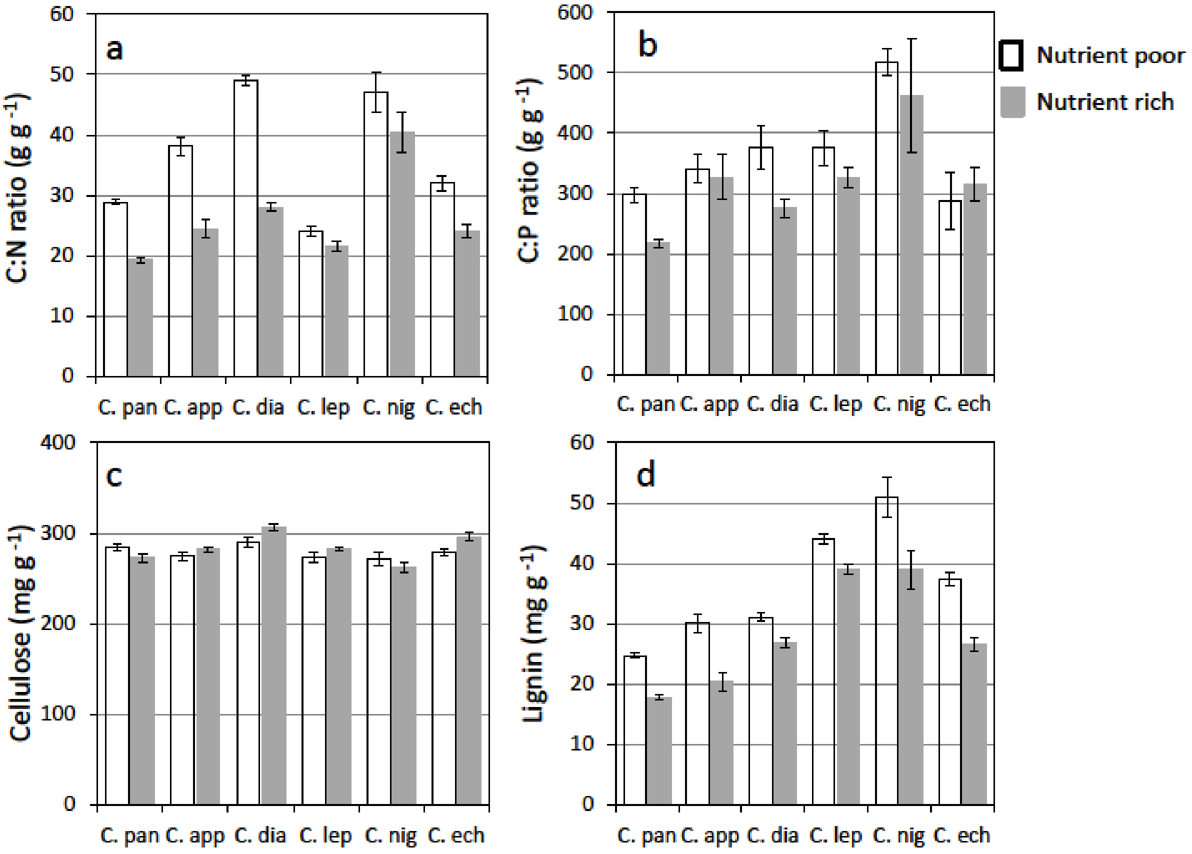

Figure 4.1: Effects of nutrient availability on leaf litter (a) C:N ratios, (b) C:P ratios, (c) cellulose, and (d) lignin content of six species of *Carex*: *C. paniculata, C. appropinquata, C. lepidocarpa, C. nigra, C. diandra,* and *C. echinata*. Bars represent mean ± SE (n = 5).

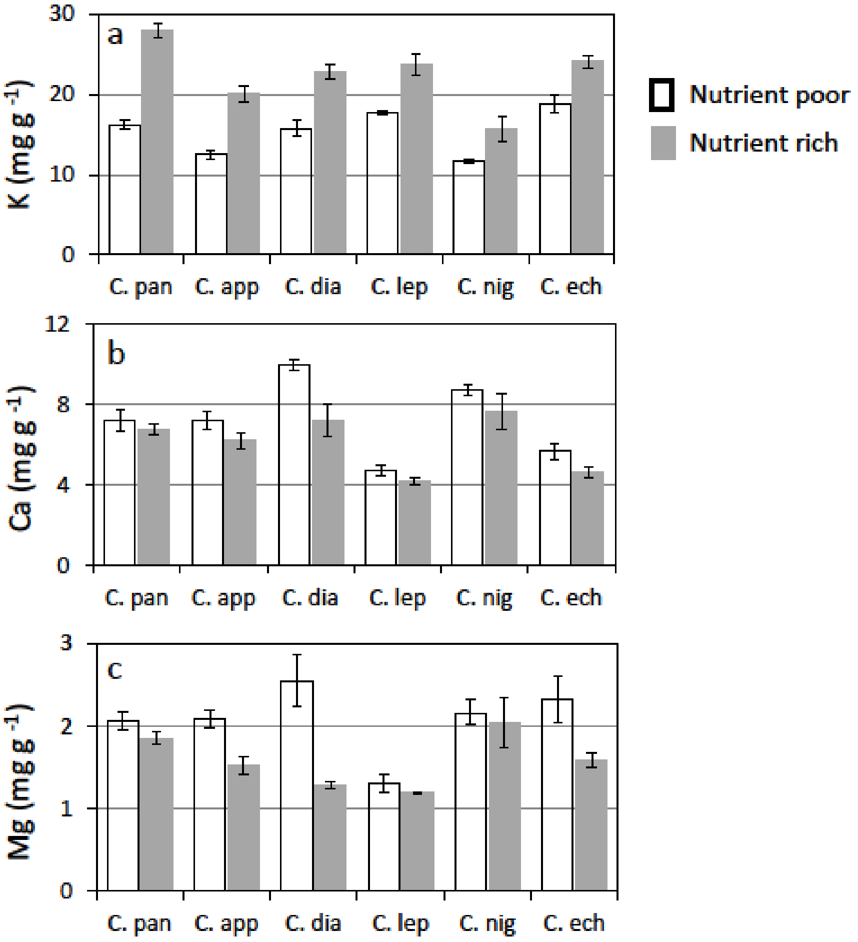

Figure 4.2: Effects of nutrient availability on leaf litter (a) K, (b) Ca, and (c) Mg content of six species of *Carex*. Bars represent mean ± SE (n = 5).

4.3.3 Decomposition rates

At the end of the decomposition experiment (n = 116 d), soil pH in the nutrient-enriched decomposition beds had dropped slightly (6.3 ± 0.1), while pH in decomposition beds without nutrient addition had risen (8.0 ± 0.1). Nutrients that had been released from the slow-release fertilizer into the pore water of the nutrient-rich decomposition beds had accumulated to final hypertrophic concentrations of (in mg L^{-1}): PO_4^{3}-P: 348 ± 80; NH_4-N: 2,430 ± 135; NO_3-N, 2,243 ± 48. *Carex* litter in the nutrient-enriched decomposition beds had decomposed approximately twice as slow as litter in the nutrient-poor beds (Figure 4.3; Table S4.4 in Supporting information). In contrast, the cellulose paper had a seemingly higher decomposition constant in the nutrient-enriched beds, but this difference was nonsignificant (Mann-Whitney U test, P = 0.15). The fixed-effect model indicated a significant interaction effect between species (including the cellulose paper) and nutrient level on the decomposition constant k (Table S4.4 in Supporting information). The main effects remained significant when omitting the interaction term, indicating that both species and nutrient treatment determine the rate of litter decomposition.

Finally, we correlated litter decomposition constants k with indicators of litter quality in the nutrient-poor and the nutrient-rich decomposition beds separately. For the nutrient-poor decomposition beds, correlations were significantly negative for litter C:N, C:P, lignin:N, lignin:P ratios as well as lignin contents, while litter K, N, and P content correlated positively with the decomposition constants (Table 4.2). Moreover, both the species' Ellenberg-N values and SLA indices correlated positively with the decomposition constants in the nutrient-poor decomposition beds, indicating a higher decomposability of litter from competitive (high Ellenberg-N, high SLA) species. Correspondingly, the species' Ellenberg-N values correlated with SLA indices (Spearman's rho = 0.304, n = 60, P = 0.018). However, in the nutrient-enriched decomposition beds, none of the litter quality indicators correlated with the decomposition constants, and neither did Ellenberg-N values or SLA indices (Table 4.2). Finally, litter cellulose, Ca, and Mg content did not correlate with the decomposition constants, and this pattern was consistent in both nutrient treatments (Table 4.2).

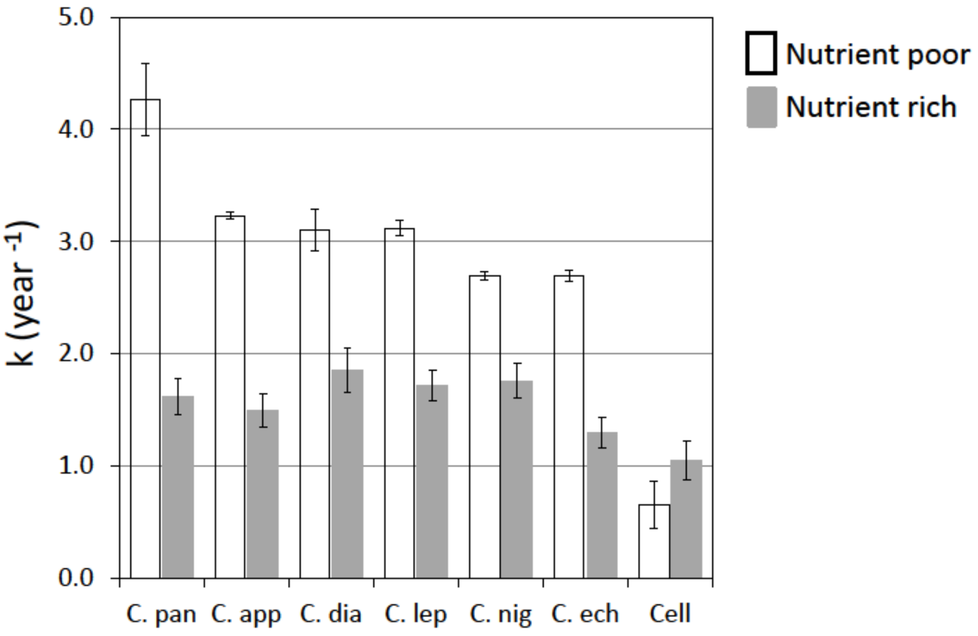

Figure 4.3: Decomposition constants k (yr^{-1}) of litter of six *Carex* species grown under nutrient-rich conditions and cellulose paper ("Cell"). Litter was incubated pairwise in nutrient-poor (white bars) and nutrient-rich (gray bars) decomposition beds. Bars represent mean ± SE (n = 5).

Table 4.2: Spearman's correlations between initial litter quality parameters and decomposition constants k of *Carex* leaf litter incubated in nutrient-poor (left) and nutrient-rich (right) decomposition beds.

	Nutrient-poor beds k (n = 30)		**Nutrient-rich beds k** (n = 30)	
Parameter	**Spearman's rho**	***P* value**	**Spearman's rho**	***P* value**
N (mg g^{-1})	**0.57**	**0.001**	-0.16	0.412
P (mg g^{-1})	**0.5**	**0.005**	-0.05	0.787
C:N ratio (g g^{-1})	**-0.55**	**0.002**	0.17	0.376
C:P ratio (g g^{-1})	**-0.49**	**0.006**	0.07	0.718
Lignin (mg g^{-1})	**-0.41**	**0.034**	0.27	0.171
Lignin:N ratio (g g^{-1})	**-0.53**	**0.005**	0.33	0.094
Lignin:P ratio (g g^{-1})	**-0.43**	**0.027**	0.18	0.38
K (mg g^{-1})	**0.47**	**0.009**	-0.23	0.215
Ca (mg g^{-1})	0.07	0.709	0.18	0.338
Mg (mg g^{-1})	0.03	0.88	0.12	0.516
Cellulose (mg g^{-1})	-0.08	0.678	-0.08	0.688
Ellenberg-N ranking	**0.74**	**< 0.001**	0.03	0.865
Specific Leaf Area (SLA, cm^2 g^{-1})	**0.43**	**0.018**	0.11	0.58

4.4 Discussion

We investigated how eutrophication of fens by primary nutrients (NPK) affects litter production, litter stoichiometry, and litter decomposition rates of six species of *Carex*. The effects of eutrophication were contrasting: although *Carex* plants grown on eutrophic soils produced more than double the amount of fresh litter (productivity shift), a concomitant increase in litter quality both within (intraspecific shift) and between (interspecific shift) species clearly stimulated litter decomposition rates. However, the stimulant effect of such eutrophication-induced higher litter quality on decomposition rates only held if litter was deposited in nutrient-poor (exogenous) environments. In eutrophic environments, decomposition rates were uncoupled from litter stoichiometry, and *Carex* decomposition rates were twice as low (exogenous shift). This suggests a dominant role of the external environment on litter decomposition rates.

4.4.1 Productivity shifts

It is well known that eutrophication causes an increase in primary biomass production and concomitant competition for light (Kotowski et al. 2006, Hautier et al. 2009). In response, rates of fresh litter input toward the peat soil increase correspondingly. In our data set, plants grown in the nutrient-rich treatments had produced 2.2–5.2 times more litter (in g dry mass) by the end of the experiment than plants grown in the nutrient-poor treatment. Such significant nutrient-induced increase in primary production may favor peat accumulation and thus carbon sequestration (Oechel et al. 2000).

4.4.2 Plant litter quality shifts and litter decomposition rates.

Apart from the direct positive effects of eutrophication on fresh litter production, indirect effects of eutrophication on rates of litter decomposition co-determine whether fens will be long-term net sinks or sources of carbon. Nutrient additions can affect litter stoichiometry within plants of the same species (Aerts and de Caluwe 1997, Gusewell 2004). Our study showed that indicators of high litter quality (i.e., low C:N and C:P ratios, high K contents, and low lignin contents) were indeed significantly altered in response to nutrient enrichment. Individuals of the same species that had grown in the nutrient-rich mesocosms consistently produced higher-quality litter than individuals grown in the nutrient-poor mesocosms. Stoichiometric intraspecific shifts occurred in all of the six *Carex* species, and appeared to be independent of the species' natural (optimal) occurrence along a fertility gradient (Ellenberg-N values). Moreover, species with the highest Ellenberg-N values (e.g., *C. paniculata*, *C. appropinquata*) generally produced more easily degradable litter than did species with lower Ellenberg-N values (e.g., *C. echinata*, *C. nigra*). According to plant-strategical theory (Westoby 1998), fast-growing, competitive species should primarily invest in rapid expansion of (photosynthetic) leaf area rather than investing in structural strength and defensive degradation-inhibiting compounds. Indeed, we found that the species' Ellenberg-N values correlated positively with SLA indices, which indicates rapid expansion of assimilation-related tissue in competitive plants. Accordingly, specific leaf area (SLA) of the plants correlated positively with litter decomposition constants, in line with other research (Cornelissen and Thompson 1997, Gusewell and Verhoeven 2006). Since fen eutrophication triggers a plant-strategical shift from slow-growing, small-sedge communities toward communities dominated by tall, competitive sedges and helophytes (Wheeler and Proctor 2000), the concomitant interspecific shift in vegetation composition coupled with shifts in dominant plant strategies and overall tissue quality will expectedly accelerate rates of litter decomposition and nutrient cycling (Cornwell et al. 2008).

4.4.3 Exogenous environment shifts and litter decomposition

Decomposition rates in the nutrient-enriched decomposition beds were, for the litter of the *Carex* species, approximately twice as low as in the nutrient-poor decomposition beds. Since litter was of equal quality in both treatments (pairwise incubations), such pronounced difference can only be due to differences in the external environment. This implies that the effect of the external environment on decomposition processes can easily outweigh the previously described intra-and interspecific litter quality shifts. However, we found a strong interaction effect between litter type (*Carex* species and cellulose filter paper) and nutrient treatment on decomposition rate, indicating that the effect of external eutrophication on litter decomposition is highly dependent of litter type and quality. In contrast to the *Carex* litter, the cellulose filter paper decomposed slightly faster (although nonsignificant, $P = 0.15$) in the nutrient-enriched mesocosms. Such litter-dependent response to fertilization has been suggested by other researchers as well (Sinsabaugh et al. 2002, Moorhead and Sinsabaugh 2006).

Although we did not quantify microbial activity nor qualify the composition of microbial communities, we can discuss our results in the light of existing knowledge. Primarily, our results are in accordance with the concept of “microbial nutrient mining” (Moorhead and Sinsabaugh 2006, Craine et al. 2007). This theory predicts that, under nutrient-limited conditions, slow-growing microbial “miner” guilds will thrive, as they specialize in retrieving nutrients that are stored in recalcitrant organic matter (in our case in the *Carex* litter; Moorhead and Sinsabaugh 2006). Microorganisms that do relatively well under nutrient-poor conditions are presumably slow-growing K strategists, including many fungi (Fontaine et al. 2003, Chen et al. 2014). Consequently, the rate of decomposition under such conditions should primarily depend on how easily the organic matter can be decomposed (i.e., the relative amount of defensive and recalcitrant structures in the material), which is in line with our observed correlations between litter quality indicators (e.g., C:N and C:P ratios, lignin contents, K contents) and litter decay constants in the nutrient-poor decomposition beds. Interestingly, we found that litter decomposition rates were uncoupled from litter stoichiometry in the nutrient-enriched decomposition beds. Here, none of the well-known litter quality indicators correlated with the decomposition constants. If overall nutrient availability is high, difficult-to-retrieve organically bound nutrients no longer need to be mined from recalcitrant organic matter. Here, we expect a microbial shift toward dominance of guilds of fast-growing opportunistic competitors (mainly bacteria) that thrive on the readily available inorganic nutrients and on soluble polymers from fresh litter (Fontaine et al. 2003, Moorhead and Sinsabaugh 2006). Presumably, such “eutrophic” communities consist of rapidly reproducing r strategists (Chen et al. 2014), which could (under these conditions) outcompete K strategists (Fontaine et al. 2003). Indeed, changes in microbial community due to nutrient (mainly nitrogen) amendment have been linked to a suppressed activity of lignin-degrading enzymes and a stimulated activity of cellulose-degrading enzymes (Fog 1988). An increase in external nutrient availability should thus lead to a decreased breakdown of lignified organic matter and an increased breakdown of cellulosic organic matter (Sinsabaugh et al. 2002), which is in line with our observation of relatively faster decomposition of the cellulose filter paper but slower decomposition of lignified *Carex* litter in the nutrient-rich decomposition beds.

Finally, we emphasize that we investigated the effects of ambient nutrient levels on litter decomposition based on two extremities (no nutrient addition and hypertrophic nutrient levels), and we did not investigate the effects of all possible nutrient levels between these two extremities. The final nutrient concentrations released by the Osmocote fertilizer can be considered hypertrophic and resemble conditions of heavy anthropogenic NPK fertilization without subsequent runoff, uptake, or dilution of nutrients (as is the case in a closed mesocosm). It is thus possible that different or less pronounced patterns emerge under field conditions, or if a different range of ambient nutrient levels is applied. The latter supposition requires future investigation. Taken together, our results suggest that, in oligotrophic ecosystems, microorganism vigor can be nutrient-limited (or, at least, nutrient–carbon co-limited). Despite widespread belief that soil microorganisms are usually C limited, the concept of microbial macronutrient limitation is not new (Egli 1991, Schimel and Weintraub 2003). Under eutrophic conditions, potential nutrient limitation is evidently lifted (Fig. 4.4), so that microbial vigor is determined mainly by the availability of high-quality labile carbon (e.g., sugars and cellulose).

4.4.4 Critical remarks on extrapolation: below-vs. aboveground decomposition

It should be noted that we only investigated eutrophication-induced shifts in aboveground herb litter production and chemistry, whereas bryophytes and belowground roots significantly contribute to peat formation in fens (Saarinen 1996). The litter bags in our decomposition experiment were buried in the top layer of the waterlogged soil at the oxic/anoxic interface, where aboveground litter would be deposited under natural conditions. In this zone however, oxygen depletion is less severe than in deeper fully anoxic peat layers, which implies that in our experiment fungal growth may not have been hampered by oxygen depletion. Indeed, typical fungal degraders of recalcitrant polymers (“nutrient miners”) are rarely extracted from deep anoxic peat layers as they generally cannot survive anoxia (Thormann 2006). This has important implications with respect to the generalization of our results: although it is likely that (fungal) recalcitrant polymer degraders could have thrived on the litter in our experiment, they would probably have been excluded if litter had been buried deeper within the anoxic soil. Therefore, we suggest that future research on peat accumulation should also focus on the quantification and qualification of root production, root decay, and decomposer communities in deeper, fully anoxic peat layers. Finally, although our research focused on groundwater-fed peatlands in the Northern hemisphere, environmentally induced shifts in the quantity and quality of plant litter should also play a key role in peat formation in a broader range of peatland types, e.g., tropical peatlands (Sjögersten et al. 2014).

4.4.5 Cumulative effects of eutrophication on peat accumulation and carbon sequestration

We have shown that fen eutrophication triggers contrasting multilevel effects on litter accumulation and decomposition. When nutrient-poor fens are enriched with nutrients, e.g., in response to anthropogenic disturbances, a strong increase in rates of fresh litter production combined with generally lower rates of litter decomposition (the latter due to likely shifts in microbial activity) should favor peat accumulation and thereby carbon sequestration. This implies that if both conditions of high rates of fresh litter input and high exogenous nutrient availability are satisfied, decomposer communities do not decompose “older” more recalcitrant litter to gain nutrients, resulting in its net accumulation. However, we also found that eutrophication increases plant litter quality and thereby the overall degradability of litter. Indeed, individuals of the same species produced higher-quality tissue, and a shift from small, stress-tolerant, plant species toward fast-growing competitors increases overall tissue quality of the vegetation. The latter (intra-and interspecific) litter-quality shifts should, on the other hand, lead to higher decomposition rates in eutrophic systems, contrary to the aforementioned findings. In the end, these contrasting results suggest that oligotrophic, as well as eutrophic, fens can accumulate peat and sequester carbon, but we hypothesize that the underlying mechanisms are different: peat accumulation in nutrient-poor fens is primarily due to a high recalcitrance (e.g., higher lignin content) of fresh litter and organic matter, whereas peat accumulation in nutrient-rich fens is due to high rates of fresh litter input combined with decreased nutrient-mining by microbial communities (Figure 4.4). These predictions are supported by field observations that peat layers of groundwater-fed fens can either consist of small sedge and bryophyte fragments (indicating peat accumulation under nutrient limitation), or, on the other extreme, of tall sedge and reed fragments (indicating peat accumulation under higher nutrient availability; Succow and Joosten 2001).

Finally, we should note that our results only apply to non-drained fens, as we did not manipulate water levels. Drainage triggers rapid peat and litter mineralization and concomitant eutrophication, and permanent aerobic conditions will hamper peat formation regardless of nutrient levels (Lamers et al. 2015). Conclusively, it is clear that eutrophication can affect litter (and eventually peat) accumulation and decomposition at multiple levels, in which stimulatory and inhibitory effects may simultaneously interact. We urge future studies to take these multiple levels into account.

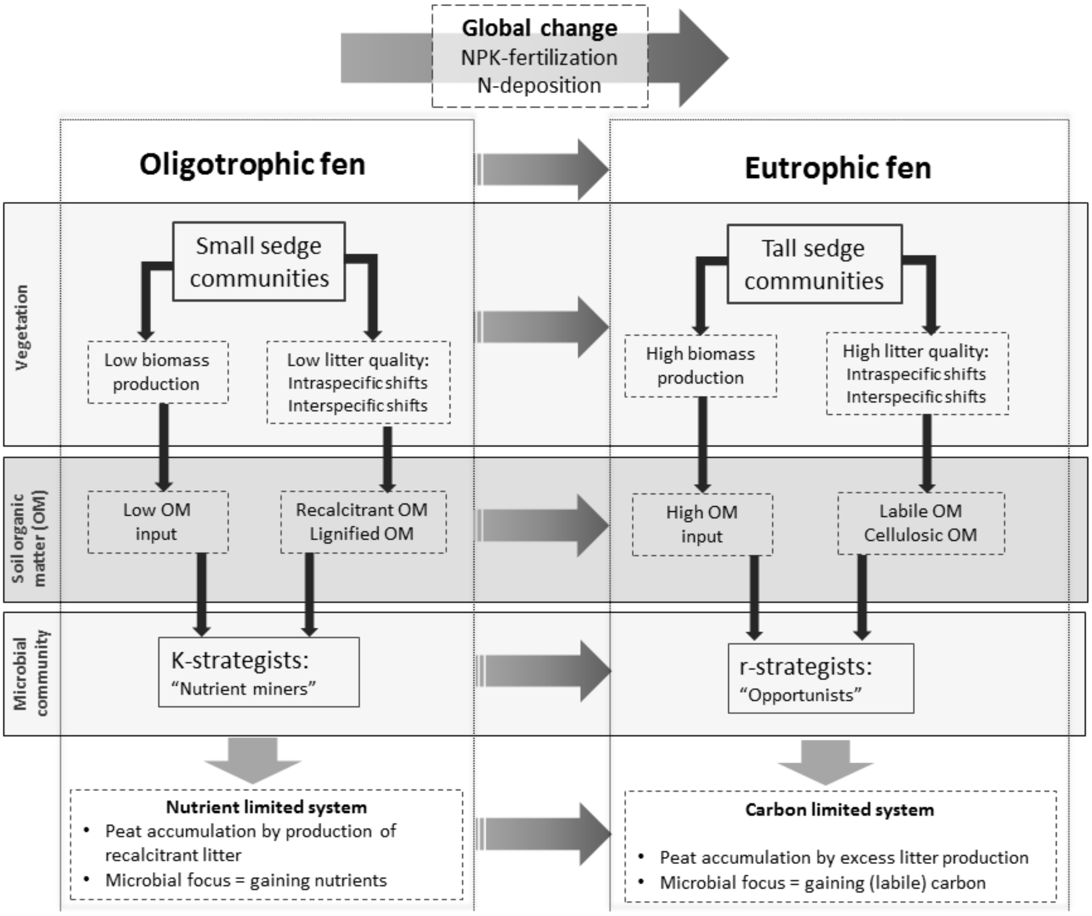

Figure 4.4: Simplified hypothetical flowchart showing contrasting pathways for peat accumulation in oligotrophic (left) and eutrophic (right) fens. Eutrophication causes a decline in the area covered by oligo- and mesotrophic small-sedge fens in favor of eutrophic tall-sedge fens.

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4.5 References

Aerts, R. 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. Journal of Experimental Botany 50:29–37.

Aerts, R., and H. de Caluwe. 1997. Nutritional and plant-mediated controls on leaf litter decomposition of Carex species. Ecology 78:244–260.

Aerts, R., H. de Caluwe, and B. Beltman. 2003. Plant community mediated vs. nutritional controls on litter decomposition rates in grasslands. Ecology 84:3198–3208.

Bragazza, L., C. Freeman, T. Jones, H. Rydin, J. Limpens, N. Fenner, N, T. Ellis, R. Gerdol, M. Hájek, T.Hájek, P. Iacumin, L. Kutnar, T. Tahvanainen, and H. Toberman. 2006. Atmospheric nitrogen deposition promotes carbon loss from peat bogs. Proceedings of the National Academy of Sciences USA 103:19386–19389.

Bragazza, L., A. Buttler, J. Habermacher, L. Brancaleoni, R. Gerdol, H. Fritze, P. Hanajik, R. Laiho, and D. Johnson. 2012. High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. Global Change Biology 18:1163–1172.

Carfrae, J. A., L. J. Sheppard, J. A. Raven, I. D. Leith, and A. Crossley. 2007. Potassium and phosphorus additions modify the response of Sphagnum capillifolium growing on a Scottish ombrotrophic bog to enhanced nitrogen deposition. Applied Geochemistry 22:1111–1121.

Chen, R. R., M. Senbayram, S. Blagodatsky, O. Myachina, K. Dittert, X. G. Lin, E. Blagodatskaya, and Y. Kuzyakov. 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Global Change Biology 20:2356–2367.

Cornelissen, J. H. C., and K. Thompson. 1997. Functional leaf attributes predict litter decomposition rate in herbaceous plants. New Phytologist 135:109–114.

Cornwell, W. K., et al. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11:1065–1071.

Craine, J. M., C. Morrow, and N. Fierer. 2007. Microbial nitrogen limitation increases decomposition. Ecology 88:2105–2113.

Egli, T. 1991. On multiple-nutrient-limited growth of microorganisms, with special reference to dual limitation by carbon and nitrogen substrates. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 60:225–234.

Emsens, W. J., C. J. S. Aggenbach, A. J. P. Smolders, and R. van Diggelen. 2015. Topsoil removal in degraded rich fens: Can we force an ecosystem reset? Ecological Engineering 77:225–232.

Fog, K. 1988. The effect of added nitrogen on the rate of Decomposition of organic-matter. Biological Reviews of the Cambridge Philosophical Society 63:433–462.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: A question of microbial competition? Soil Biology & Biochemistry 35:837–843.

Gessner, M. O., and E. Chauvet. 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75:1807–1817.

Gusewell, S. 2004. N: P ratios in terrestrial plants: variation and functional significance. New Phytologist 164:243–266.

Gusewell, S., and M. O. Gessner. 2009. N: P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Functional Ecology 23:211–219.

Gusewell, S., and J. T. A. Verhoeven. 2006. Litter N: P ratios indicate whether N or P limits the decomposability of graminoid leaf litter. Plant and Soil 287:131–143.

Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324:636–638.

Hessen, D. O., G. I. Agren, T. R. Anderson, J. J. Elser, and P. C. De Ruiter. 2004. Carbon, sequestration in ecosystems: the role of stoichiometry. Ecology 85:1179–1192.

Hill, M. O., J. Mountford, D. Roy, and R. G. H. Bunce. 1999. Ellenberg's indicator values for British plants. ECOFACT Volume 2 Technical Annex. Institute of Terrestrial Ecology, Huntingdon, UK.

Hobbie, S. E. 1992. Effects of plant-species on nutrient cycling. Trends in Ecology & Evolution 7:336–339.

Hobbie, S. E. 2008. Nitrogen effects on decomposition: a five-year experiment in eight temperate sites. Ecology 89:2633–2644.

Joosten, H., and D. Clarke. 2002. Wise use of mires and peatlands. International Mire Conservation Group, 304 pp, Jyväskylä, Finland.

Kaštovská, E., T. Picek, J. Bárta, J. Mach, T. Cajthaml, and K. Edwards. 2012. Nutrient addition retards decomposition and C immobilization in two wet grasslands. Hydrobiologia 692:67–81.

Klaus, V. H., T. Kleinebecker, S. Boch, J. Muller, S. A. Socher, D. Prati, M. Fischer, and N. Holzel. 2012. NIRS meets Ellenberg's indicator values: prediction of moisture and nitrogen values of agricultural grassland vegetation by means of near-infrared spectral characteristics. Ecological Indicators 14:82–86.

Knorr, M., S. D. Frey, and P. S. Curtis. 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86:3252–3257.

Kominoski, J. S., A. D. Rosemond, J. P. Benstead, V. Gulis, J. C. Maerz, and D. W. Manning. 2015. Low-to-moderate Nitrogen and phosphorus concentrations accelerate microbially driven litter breakdown rates. Ecological Applications 25:856–865.

Kotowski, W., W. Thorig, R. van Diggelen, and M. J. Wassen. 2006. Competition as a factor structuring species zonation in riparian fens—a transplantation experiment. Applied Vegetation Science 9:231–240.

Lamers, L. P. M., M. Vile, A. Grootjans, M. Acreman, R. van Diggelen, M. Evans, C. Richardson, L. Rochefort, A. Kooijman, J. Roelofs, A. Smolders. 2015. Ecological restoration of rich fens in Europe and North America: from trial and error to an evidence-based approach. Biological Reviews 90:182–203.

Moore, P. D. 2002. The future of cool temperate bogs. Environmental Conservation 29:3–20.

Moorhead, D. L., and R. L. Sinsabaugh. 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs 76:151–174.

Oechel, W. C., G. L. Vourlitis, S. J. Hastings, R. C. Zulueta, L. Hinzman, and D. Kane. 2000. Acclimation of ecosystem CO_2 exchange in the Alaskan Arctic in response to decadal climate warming. Nature 406:978–981.

Qualls, R. G., and C. J. Richardson. 2000. Phosphorus enrichment affects litter decomposition, immobilization, and soil microbial phosphorus in wetland mesocosms. Soil Science Society of America Journal 64:799–808.

Saarinen, T. 1996. Biomass and production of two vascular plants in a boreal mesotrophic fen. Canadian Journal of Botany 74:934–938.

Sarneel, J. M., J. J. M. Geurts, B. Beltman, L. P. M. Lamers, M. M. Nijzink, M. B. Soons, and J. T. A. Verhoeven. 2010. The effect of nutrient enrichment of either the bank or the surface water on shoreline vegetation and decomposition. Ecosystems 13:1275–1286.

Schimel, J. P., and M. N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry 35:549–563.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Sinsabaugh, R. L., M. M. Carreiro, and D. A. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60:1–24.

Sjögersten, S., C. R. Black, S. Evers, J. Hoyos-Santillan, E. L. Wright, and B. L. Turner. 2014. Tropical wetlands: A missing link in the global carbon cycle? Global Biogeochemical Cycles 28:1371–1386.

Succow, M., and H. Joosten. 2001. Landschaftsökologische Moorkunde. E. Schweizerbart´sche Verlagsbuchhandlung, Stuttgart, Germany.

Taylor, B. R., D. Parkinson, and W. F. J. Parsons. 1989. Nitrogen and lignin content as predictors of litter decay-rates— a microcosm test. Ecology 70:97–104.

Thormann, M. N. 2006. Diversity and function of fungi in peatlands: a carbon cycling perspective. Canadian Journal of Soil Science 86:281–293.

van Diggelen, R., B. Middleton, J. Bakker, A. Grootjans, and M. Wassen. 2006. Fens and floodplains of the temperate zone: present status, threats, conservation and restoration. Applied Vegetation Science 9:157–162.

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. Journal of the Association of Official Agricultural Chemists 46:829–835.

Walinga, I., W. van Vark V. J. G. Houba, J. J. and van der Lee 1989. Plant analysis procedures. Pages 13–16 *in* Soil and plant analysis, Part 7, Department of Soil Science and Plant Nutrition Agricultural University, Wageningen, the Netherlands.

Ward, S. E., K. H. Orwin, N. J. Ostle, M. J. I. Briones, B. C. Thomson, R. I. Griffiths, S. Oakley, H. Quirk, and R. D. Bardgett. 2015. Vegetation exerts a greater control on litter decomposition than climate warming in peatlands. Ecology 96:113–123.

Westoby, M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. Plant and Soil 199:213–227.

Wheeler, B. D. 1983. Vegetation, nutrients and agricultural land-use in a North Buckinghamshire Valley fen. Journal of Ecology 71:529–544.

Wheeler, B. D., and M. C. F. Proctor. 2000. Ecological gradients, subdivisions and terminology of north-west European mires. Journal of Ecology 88:187–203.

Wider, R. K., and G. E. Lang. 1982. A critique of the analytical methods used in examining decomposition data obtained from litter bags. Ecology 63:1636–1642.

Wright, A. L., K. R. Reddy, and R. Corstanje. 2009. Patterns of heterotrophic microbial activity in eutrophic and oligotrophic peatlands. European Journal of Soil Biology 45:131–137.

Xing, Y., J. Bubier, T. Moore, M. Murphy, N. Basiliko, S. Wendel, and C. Blodau. 2011. The fate of 15N-nitrate in a northern peatland impacted by long term experimental nitrogen, phosphorus and potassium fertilization. Biogeochemistry 103:281–296.

4.6 Supporting information

Table S4.1: Chemical characteristics of the tap water used in the mesocosm experiment to grow Carex shoots.

Element	Value	Unit
pH	7.2	
P	0.03	mg L^{-1}
NO_3^-	11.7	mg L^{-1}
NH_4^+	0.02	mg L^{-1}
K	3.7	mg L^{-1}
Na	33.0	mg L^{-1}
Ca	63.0	mg L^{-1}
Mg	7.1	mg L^{-1}
S	17.4	mg L^{-1}
Cl	46	mg L^{-1}
Fe	0.02	mg L^{-1}
Mn	0.001	mg L^{-1}
Al	0.033	mg L^{-1}
Cu	0.006	mg L^{-1}
B	0.045	mg L^{-1}
Zn	0.009	mg L^{-1}
Ni	0.002	mg L^{-1}
Se	0.002	mg L^{-1}

Table S4.2: Output of the fixed-effect model on the interactive effects of "species" and "nutrient level" on litter biomass production (g), leaf count (n), leaf length (cm), and specific leaf area (SLA, $cm^2\ g^{-1}$).

Effect	Dependent variable	d.f.	F-value	p-value
Species		5,48	12.0	**<0.001**
Nutrient level	Biomass [log10(x+1)]	1,48	300.5	**<0.001**
Species * Nutrient level		5,48	5.1	**<0.001**
Species		5,48	33.6	**<0.001**
Nutrient level	Leaf count	1,48	163.7	**<0.001**
Species * Nutrient level		5,48	4.1	**0.004**
Species		5,48	45.0	**<0.001**
Nutrient level	Leaf length	1,48	128.8	**<0.001**
Species * Nutrient level		5,48	16.0	**<0.001**
Species		5,48	20.7	**<0.001**
Nutrient level	SLA	1,48	0.28	0.602
Species * Nutrient level		5,48	0.34	0.888

Table S4.3: Output of the fixed-effect model on the interactive effects of “species” and “nutrient level” on litter C:N and C:P ratios, as well as contents of lignin, cellulose, K, Ca and Mg.

Effect	Dependent variable	d.f.	F value	P value
Species	C:N	5,48	37.4	**<0.001**
Nutrient level		1,48	85.3	**<0.001**
Species * Nutrient level		5,48	5.6	**<0.001**
Species	C:P [log10(x)]	5,48	6.2	**<0.001**
Nutrient level		1,48	5.2	**0.027**
Species * Nutrient level		5,48	1.1	0.375
Species	Lignin	5,45	23.6	**<0.001**
Nutrient level		1,45	27.0	**<0.001**
Species * Nutrient level		5,45	0.7	0.595
Species	Cellulose	5,46	9.6	**<0.001**
Nutrient level		1,46	2.8	0.099
Species * Nutrient level		5,46	3.1	**0.018**
Species	K	5,48	25.4	**<0.001**
Nutrient level		1,48	168.7	**<0.001**
Species * Nutrient level		5,48	4.3	**0.003**
Species	Ca	5,48	24.6	**<0.001**
Nutrient level		1,48	18.3	**<0.001**
Species * Nutrient level		5,48	1.7	0.154
Species	Mg [log10(x)]	5,48	9.6	**<0.001**
Nutrient level		1,48	32.8	**<0.001**
Species * Nutrient level		5,48	3.9	**0.005**

Table S4.4: Output of the fixed-effect model on the interactive effects of “species” (including cellulose paper) and “nutrient level” on decomposition constant “k”.

Effect	Dependent variable	d.f.	F-value	p-value
Species	Decomposition constant "k"	6,56	32.2	**<0.001**
Nutrient level		1,56	216.3	**<0.001**
Species * Nutrient level		6,56	15.8	**<0.001**

5

Topsoil removal in degraded rich fens: can we force an ecosystem reset?

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Abstract

Global land-use intensification and drainage has altered the biogeochemical properties of many peatlands, and concomitant eutrophication has led to a loss of low-competitive fen species. We investigated the hypothesis that removal of a degraded and eutrophied top peat layer, thereby exposing an underlying peat layer, can improve conditions for rich fen communities. We studied the long-term (3-18 years) effects of past topsoil removal in six rich fens in Western Europe by comparing topsoil removal plots with (untouched) control plots. Overall, topsoil removal plots were characterized by lower bulk densities and soil nutrient pools of P and KCl-extractable NH_4^+, while organic matter contents and soil C:N ratios were higher. Pore water concentrations of NO_3^- and NH_4^+ were lower in the topsoil removal plots, while concentrations of base cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and HCO_3^- were higher. Furthermore, lower nutrient levels appeared to restrict herb biomass production in the topsoil removal plots, so that optimized light conditions led to the establishment of light-demanding target species and a significant increase in bryophyte cover. Multivariate analysis revealed that most variation in vegetation assembly was due to higher groundwater levels in the topsoil removal plots, closely followed by a higher relative light intensity (RLI) at surface level, lower pore water nutrient (NH_4^+) concentrations, and higher concentrations of base cations. We conclude that topsoil removal can be an effective mechanism to “reset” a degraded peatland to its initial state of nutrient limitation, base saturation and high availability of light, thereby improving the conservation prospects of endangered rich fen communities.

5.1 Introduction

The global intensification of land use, a sharp increase in the use of artificial fertilizer, and an increase in anthropogenic nitrogen deposition is compromising the functioning of many nutrient-limited ecosystems, both in the aquatic and (semi-)terrestrial environment (Matson et al. 1997, Phoenix et al. 2006). As many endangered species are adapted to nutrient-poor habitats, eutrophication is considered a major threat to global species diversity (Wassen et al. 2005, Hautier et al. 2009).

Groundwater-fed peatlands (henceforth "rich fens") are particularly vulnerable to land-use intensification and eutrophication. Typically, pristine rich fens are characterized by continuously wet, base-rich and mesotrophic conditions and are dominated by low-competitive small sedges and brown mosses (Grootjans et al. 2006, Malson and Rydin 2007). In Europe, most of the pristine rich fens have disappeared due to land use change, while degradation of the remaining fens leads to the gradual replacement of typical fen species with general wetland species (Kooijman 1992, Lamers et al. 2015). Therefore, both the conservation and restoration of the many degraded fens is a top priority for the long-term protection of this habitat type together with its typical species (van Diggelen et al. 2006). This is now legally acknowledged through the EU's Habitats Directive (Romão 1996).

In comparison with mineral soils, peatland eutrophication is a more complex process as nutrient enrichment is not necessarily related to an increased input from external sources alone (Bragazza et al. 2009). As peat soil mainly consists of reactive organic matter, the slightest alterations in hydrological conditions can have disproportional effects on fen chemistry. In this respect, water table drawdown is considered one of the major threats to rich fens (van Diggelen et al. 2006, Lamers et al. 2015). Drawdown can be triggered through construction of local drainage ditches, but it can also be the result of alterations in regional hydrology (e.g. increased rates of groundwater abstraction and consequent reduction of regional seepage fluxes (van Diggelen et al. 2006)). When peat soil is drained, intrusion of oxygen becomes a driving force for increased rates of organic matter decomposition and mineralization (Brouns et al. 2014), which eventually results in nutrient release and eutrophication (Grootjans et al. 1986). Moreover, peat oxidation triggers carbon loss (Laiho 2006, Brouns et al. 2014), soil subsidence (Gambolati et al. 2006), regeneration of electron acceptors (Fenner et al. 2011), acidification (Beltman et al. 2001, Cusell et al. 2013), leaching of base cations (Laiho et al. 1999), shifts in vegetation assembly and a loss of typical biodiversity (Malson and Rydin 2007, Malson et al. 2008). Long-term peat degradation can alter biogeochemical conditions to such an extent that successful fen restoration becomes notoriously difficult, even after rewetting (Zak et al. 2010, Brouns et al. 2014, Zak et al. 2014). Eventually, it is likely that the sum of these biogeochemical alterations in the top soil can trigger a shift towards a system that is governed by a different set of feedbacks, possibly forcing the peatland towards an alternative degraded state (Suding et al. 2004).

In this study, we test the hypothesis that removal of the top layer of (disturbed) peat to restore rich fen ecosystems can trigger a system “reset” (i.e. after topsoil removal, biogeochemical conditions will become similar to initial pristine conditions), and that it improves conditions for the establishment of target plant communities. Although topsoil removal is a well-established measure in nature conservation on mineral soils (Allison and Ausden 2004, Olsson and Ödman 2014), the effect of topsoil removal in rich fens (in which an underlying peat layer is exposed) is only documented fragmentarily (Patzelt et al. 2001, Klimkowska et al. 2007, Klimkowska et al. 2015), often with contrasting results. We believe this is due to the difficulty of predicting the effects of exposing an underlying high-quality peat layer after topsoil removal, as rapid peat mineralization and concomitant re-eutrophication is not unlikely (Brouns et al. 2014). To empirically test the effects of topsoil removal on abiotic conditions and rich fen development, we conducted a comparative field study in which we analyzed the mid-term (3-18 years) effects of past topsoil removal in six rich fens.

5.2 Materials and methods

5.2.1 Study sites

Topsoil removal is an uncommon restoration measure for peatlands. Therefore, study site selection was determined mainly by the availability of suitable locations. Sites were included for sampling only if they met the following criteria: (1) peat-formation had started directly on mineral soils, thus excluding floating mires; (2) the peatland was fed by base-rich groundwater; (3) all peatlands were drained in the past, having led to topsoil degradation (Von Post Humification topsoil > 8); (4) topsoil removal has taken place > 3 years ago and the area has re-vegetated; (5) after topsoil removal, a new underlying peat layer was exposed (excluding locations where all peat was removed down to the mineral subsoil); and (6) control plots (plots in which topsoil removal has not taken place) were available in the vicinity of the topsoil removal plots, to allow a meaningful comparison. In total, we located six fens in the Netherlands and Belgium that met all criteria (Table 5.1, Figure 5.1). All sites have a history of agriculture that included haymaking and (limited) fertilization, while two of the sites (DA and PE) had also been rewetted by canal and ditch blocking.

Table 5.1: Study site location with coordinates, along with time (yr) since topsoil removal, and average depth (cm) of topsoil removal.

Study site	Coordinates	Time since topsoil removal (yr)	Average depth of topsoil removal (cm)
Leijer Hooilanden (LH)	52°38'33.73"N ; 6°16'43.23"E	10	15
Hellen (HE)	52° 0'33.94"N ; 5°34'48.93"E	10	30
Drentsche Aa (DA)	53° 0'49.65"N ; 6°37'45.88"E	18	20
Holmers (HO)	52°54'12.41"N ; 6°37'45.83"E	11	30
Peizermade (PE)	53°10'19.33"N ; 6°30'12.19"E	3	30
Malendriesbeekvallei (MA)	50°50'56.48"N ; 4°52'24.39"E	5	25

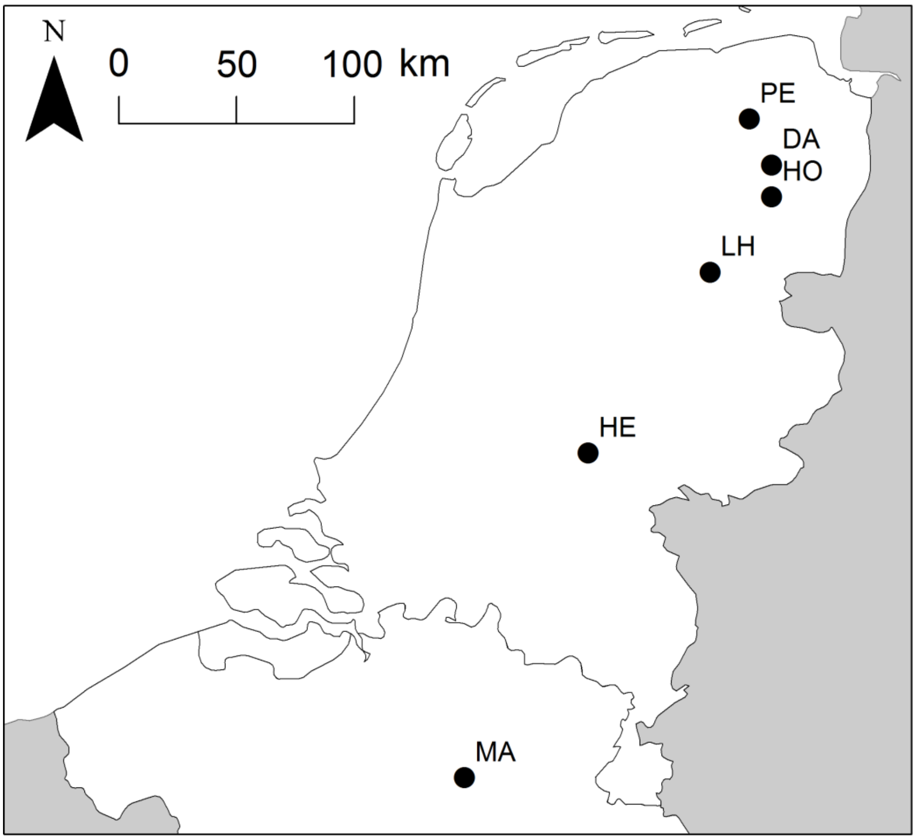

Figure 5.1: Geographical spread of the study sites in Belgium and the Netherlands.

5.2.2 Sampling design

In June 2013, within each study site we selected eight plots of 2 m x 2 m, resulting in a total of 48 plots. Four replicate plots per site were selected randomly in the zone where topsoil removal had taken place, and four replicate control plots were selected on nearby spots where the degraded topsoil had been left untouched. In each plot we recorded the cover of all species using the Londo scale (Londo 1976). An Accupar LP-80 ceptometer (Decagon Devices Inc., Pullman, WA, USA) was used to measure relative light intensity (RLI) below the vegetation at surface level (= percentage of incoming photosynthetically active radiation as measured with a reference sensor above the canopy (Kotowski and van Diggelen 2004)), averaged over 4 measurements per plot. This is an important measure as a sufficiently high availability of light (indicating limited herb productivity and thus less competition) is crucial for rich fen communities (Kotowski and van Diggelen 2004, Kotowski et al. 2006). To ascertain that a high relative light intensity correlates with a decreased productivity at our study sites, we harvested the above-ground herb biomass in a randomly-placed sub-plot of 0.4 m x 0.4 m within each of the larger plots. Next, four soil sub-samples per plot were taken from the upper 10 cm of the peat soil, and mixed into one homogeneous sample. Separate soil samples were taken for bulk density calculations. Element pool sizes in the soil are an indication of total long-term availability, but soil-bound elements are only partially plant-available. We therefore additionally collected pore water samples from the upper 10 cm of the soil in each plot using macro-rhizon samplers with a pore size of 0.15µm (Rhizosphere Research Products, the Netherlands). Elements dissolved in the pore water are directly plant-available, but concentrations are much more subject to temporal variations. Pore water samples were stored at 4° until further treatment.

5.2.3 Chemical analyses

We measured pH and EC of all pore water samples directly in the field using portable field equipment (WTW multi 340i). Total inorganic carbon (TIC) was analyzed on an Infrared Gas Analyzer (ABB Advance Optima): the resulting values were used to calculate HCO_3^- concentrations. Concentrations of NH_4^+ and NO_3^- were determined on an Auto Analyzer 3 system (Bran+Luebbe) using ammonium molybdate, hydrazine sulphate and salicylate. Pore water sub-samples were acidified by adding 0.7 ml 65 % suprapure HNO_3 per 100 ml sample and were analyzed on ICP (IRIS Intrepid II) for the following elements: Ca, Mg, K, Na, Fe, Mn, P, S, and Al.

Soil samples for bulk density (10x10x10 cm) were dried (72 h at 105°C) and weighed; results were expressed in kg * L^{-1}. Soil organic matter content (%) was determined by loss-on-ignition for 4 h at 550 °C. KCl-extractions and ammonium-oxalate extractions (in darkness) on moist soil allowed the determination of pH_{KCl}, NO_3-N, NH_4-N and P-oxalate (P_{ox}) respectively. The latter is a measure for reactive soil-P bound to amorphous components including Fe and Al. Oven-dry soil sub-samples (48 h at 70 °C) were homogenized and ground in liquid N. C and N contents (%) were determined with a

Carlo Erba NA1500 elemental analyser (Thermo Fisher Scientific). 200 mg of soil was digested with 4 mL HNO_3 (65%) and 1 mL H_2O_2 (30%) using a microwave labstation (Milestone srl) to measure total Ca, Mg, K, P, S, Fe, Al, and Mn with ICP. Values were calculated for dry weight soil. Above-ground herb biomass was oven-dried (48h at 70°C) and weighed; results were converted to tons per hectare.

5.2.4 Data analyses

As soil bulk density differs strongly between different fens, content of soil chemical variables was expressed per volume unit (mmol * L^{-1}). Values of KCl-extractable NO_3-N were largely below the detection limit and were, therefore, excluded from further analyses.

Before statistical analysis, environmental data were checked for normality based on visual inspection of histograms and normal Q-Q plots. If needed, data were transformed using either Logarithmic, Square root, or Inverse transformations to attain approximate normal distributions. Species cover values from vegetation relevées were converted into percentages.

To test for the main effects of topsoil removal on biogeochemical parameters, we ran a mixed-effect model using Restricted Maximum Likelihood (REML) estimation in which we treated the factor "topsoil removal" (no = 0, yes = 1) as a fixed effect and "study site" (LH, HE, DA, HO, PE or MA) as a random effect. The latter was a deliberate choice as our study sites can be considered a collection of random samples drawn from a (theoretically) large pool of rich fens to which we would like to extrapolate (Bennington and Thayne 1994). This model, therefore, allowed to test for the main effects of the treatment "topsoil removal" while correcting for inter-site variation, in which we are not interested.

Vegetation data were stored in Turboveg 2.75 (Hennekens and Schaminee 2001). Next, data were exported to the JUICE software (Tichy 2002) in order to link Ellenberg light indicator values to each of the species in the dataset. For each plot, we then calculated an average "species' light requirement index (henceforth "SLRI")". These indices were obtained by averaging the mean Ellenberg light indicator values for all individual species that were found within the same plot. To have a measure for restoration success after topsoil removal, we counted the number of target species per plot. A target species met at least one of the following two criteria: (1) the species is listed on the "red list" of either the Netherlands (van der Meijden et al. 2000) or Flanders (van Landuyt et al. 2006), or (2) the species can be considered typical for small-sedge and brown-moss rich fen vegetation in Western Europe. A list with typical species had been constructed in advance. This list is based on a broad assessment of rich fen relevées in which we included all species with a frequency of > 20% in the relevées, combined with rare low-frequency species that are considered highly characteristic for rich fens (Schaminee et al. 1995).

The relationship between plant communities and abiotic characteristics in plots with and without topsoil removal was analyzed through multivariate analysis. First, species cover values were Log10(x+1) transformed. We then ran a Detrended Correspondence Analysis to determine the total length of the gradient (length > 5). Next, abiotic variables were selected by forward stepwise selection in a Canonical Correspondence Analysis (CCA) and a significance test based on permutations ($p < 0.05$ at 499 permutations). To account for site effects, permutations were restricted by adding the blocking factor “site” as a covariable. We used pore water variables rather than soil variables (pH and concentrations of dissolved HCO_3^-, Al, Ca, Fe, K, Mg, Na, Mn, P, S, NH_4^+ and NO_3^-) in the ordination because these are most relevant to rooting plants. Additionally, we included groundwater levels (“water level”) and relative light intensity at surface level (“RLI”). Extra variables were only added to the model if they showed no strong correlation with any of the preceding variables (Inflation factor < 20).

Univariate statistical analyses were performed in SPSS 20 (SPSS Inc.), for multivariate analyses we used CANOCO for Windows 4.5 (ter Braak and Šmilauer 2002).

5.3 Results

5.3.1 Peat and pore water chemistry

Large differences in soil properties were found between plots with and without topsoil removal. Overall, the topsoil removal plots are characterized by a higher soil organic matter content ($F_{1,41} = 36.94$, $p < 0.001$) and lower bulk density ($F_{1,41} = 30.26$, $p < 0.001$) (Table 5.2), both of which are strongly correlated (Spearman’s rho = -0.865, df = 10, $p < 0.001$). This observed decrease in bulk density correlates with a decrease in total pool size of all minerals, with the exception of stocks of Ca, K and S which remained unaltered. Interestingly, the most notable effects were found for total nutrient pool sizes: pools of total-P and oxalate extractable-P were drastically lower in topsoil removal plots at all sites (total pool size up to > 6 times lower (P-total: $F_{1,41} = 141.9$, $p < 0.001$, P_{ox}: $F_{1,41} = 118.4$, $p < 0.001$)), while soil C:N-ratios increased ($F_{1,41} = 71.52$, $p < 0.001$) and KCl-extractable NH_4^+-stocks decreased ($F_{1,41} = 13.82$, $p < 0.001$).

Table 5.2: Results of the mixed-effect model for the effects of topsoil removal on topsoil chemistry (mmol*L^{-1}), corrected for study site. NS = not significant, * P <0.05, ** P<0.01, *** P<0.001, and, -- = decrease at all sites, - = decrease at most sites, 0 = no change , + increase at most sites, ++ = increase at all sites.

Effect	Dependent variable	d.f.	F-value	P-value	Direction
Topsoil removal	pH $_{KCl}$	1, 41	18.55	***	+
	NH_4-N	1,41	13.82	***	-
	P-tot	1,41	141.9	***	--
	P_{ox}	1,41	118.4	***	--
	C:N	1,41	71.52	***	++
	S	1,41	0.10	NS	0
	K	1,41	2.16	NS	0
	Ca	1,41	0.46	NS	0
	Mg	1,41	8.15	**	-
	Fe	1,41	29.66	***	-
	Al	1,41	14.92	***	-
	Mn	1,41	47.18	***	--
	Bulk density	1,41	30.26	***	-
	OM-content	1,41	36.94	***	++

While mineral stocks in the soil were equal or lower in the topsoil removal plots than in the control plots, mineral concentrations in the pore water followed an opposite trend (Table 5.3). Concentrations of base cations Ca ($F_{1,41} = 20.77$, $p < 0.001$), Mg ($F_{1,41} = 15.85$, $p < 0.001$), Na ($F_{1,41} = 58.47$, $p < 0.001$) and K ($F_{1,41} = 10.25$, $p < 0.01$) as well as concentrations of HCO_3^- ($F_{1,41} = 34.58$, $p < 0.001$) and dissolved Fe ($F_{1,41} = 4.98$, $p < 0.05$) were generally higher in the topsoil removal plots, correlating with increased groundwater levels ($F_{1,41} = 116.85$, $p < 0.001$) and a higher pH ($F_{1,41} = 15.79$, $p < 0.001$). Furthermore, inorganic nitrogen concentrations (NO_3^- and NH_4^+) decreased ($F_{1,41} = 27.71$, $p < 0.001$ and $F_{1,41} = 16.75$, $p < 0.001$ respectively), but concentrations of dissolved phosphorus remained unaltered ($F_{1,41} = 2.52$, $p > 0.05$).

Table 5.3: Results of the mixed-effect model for the effects of topsoil removal on pore water chemistry ($\mu mol * L^{-1}$), corrected for study site. NS = not significant, * P <0.05, ** P<0.01, *** P<0.001, and, -- = decrease at all sites, - = decrease at most sites, 0 = no change , + increase at most sites, ++ = increase at all sites.

Effect	**Dependent variable**	**d.f.**	**F-value**	**P-value**	**Direction**
Topsoil removal	pH	1, 41	15.79	***	+
	HCO_3^-	1,41	34.58	***	+
	NH_4^+	1,41	16.75	***	-
	NO_3^-	1,41	27.71	***	-
	P	1,41	2.52	NS	0/-
	S	1,41	29.54	***	--
	Na	1,41	58.47	***	+
	K	1,41	10.25	**	+
	Ca	1,41	20.77	***	++
	Mg	1,41	15.85	***	++
	Fe	1,41	4.98	*	+
	Al	1,41	5.52	*	-
	Mn	1,41	0.59	NS	0
	groundwater level	1,41	116.85	***	++

5.3.2 Floristic response to topsoil removal

In total, we registered 116 species of vascular plants (5 were identified to genus level) and 16 species of bryophytes (2 to genus level). 37 of these species (28 %) were classified as typical of rich fens and 15 (11 %) were red list species. As most of the red list species were also classified as typical fen species (e.g. *Carex diandra*, *Menyanthes trifoliata,…)*, there were 38 "target species" in our dataset (29 % of total species count). The remaining 71 % were considered general wetland or meadow species with a much broader amplitude (e.g., *Juncus effusus*, *Mentha aquatica*,…).

Compared with the control plots, topsoil removal plots have a lower herb biomass at all study sites ($F_{1,41}$ = 72.54, p < 0.001, Table 5.4), which correlates with increased light intensity (RLI) at the surface level (Pearson's r: -0.842, df = 10, p < 0.001, Figure 5.2). Consequently, we found an increase in bryophyte cover ($F_{1,41}$ = 19.58, p < 0.001) and SLRI ($F_{1,41}$ = 28.43, p < 0.001) in the topsoil removal plots. Both biodiversity (as defined by the total number of species) as well as the fraction of target species was higher in all of the topsoil removal plots ($F_{1,41}$ = 45.59, p < 0.001 and $F_{1,41}$ = 37.63, p < 0.001 respectively).

Table 5.4: Results of the mixed-effect model for the effects of topsoil removal on total herb biomass, total number of species, moss cover, fraction of target species, and the species' light requirement index (SLRI) per plot. NS = not significant, * P <0.05, ** P<0.01, *** P<0.001, and, -- = decrease at all sites, - = decrease at most sites, 0 = no change, + increase at most sites, ++ = increase at all sites.

Effect	Dependent variable	d.f.	F-value	P-value	Direction
Topsoil removal	Herb biomass (t ha^{-1})	1,41	72.54	***	--
	N° of species	1,41	45.59	***	++
	SLRI	1,41	28.43	***	++
	Bryophyte cover (%)	1,41	19.58	***	+
	Target species (%)	1,41	37.63	***	++

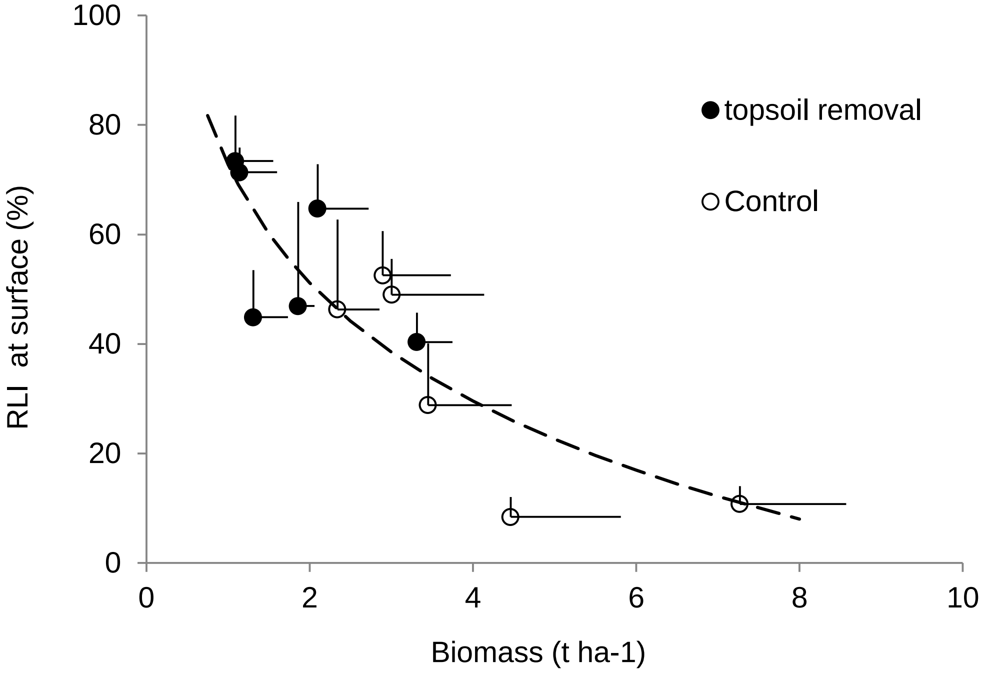

Figure 5.2: Relationship between average herb biomass (t ha^{-1}) and relative light intensity at the soil surface (%), grouped for plots with and without topsoil removal ($y = -31.1 * \ln(x) + 72.8$; $R^2 = 0.72$). Dots represent averages per site and treatment, bars represent standard deviations.

Canonical Correspondence Analysis resulted in a total of eight significant variables that partly explain variation in vegetation assemblages between the plots (Figure 5.3): groundwater levels, RLI, and pore water concentrations of NH_4^+, S, Ca, K, Al and Mn . Axis 1 and 2 combined explain 42.1% of the total species-environment relation. Species-environment correlations with axis 1 (eigenvalue = 0.49) and axis 2 (eigenvalue = 0.35) equaled 0.95 and 0.93 respectively (total inertia = 6.09). The plots with and without topsoil removal were largely separated along the horizontal axis (axis 1), which correlated best with groundwater levels (r = - 0.88), RLI (r = - 0.50) and pore water concentrations of NH_4^+ (r = 0.60), S (r = 0.54), Ca (r = - 0.43), Al (r = 0.35) and Mn (r = - 0.26). Some separation can also be observed along the vertical axis (axis 2), which correlates with pore water concentrations of K (r = -0.74; generally higher in the topsoil removal plots).

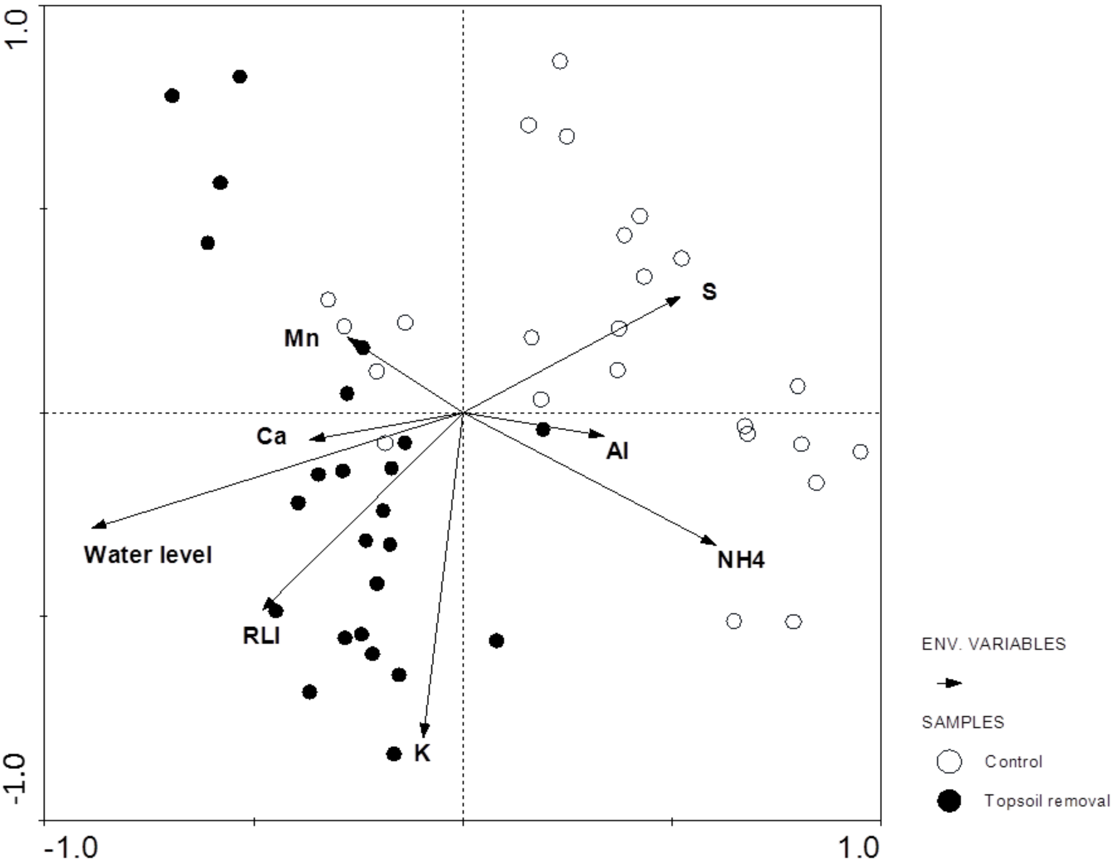

Figure 5.3: CCA-biplot showing significant ($p < 0.05$) explanatory environmental variables (vectors) in relation to species composition in the 48 study plots (dots). Chemical variables were measured in the pore water, "Water level" = groundwater level and "RLI" = relative light intensity at surface level. Plots are grouped into topsoil removal or no topsoil removal (= "Control"). Study site was included as a blocking factor in the analysis (site blocks not shown in figure).

5.4 Discussion

Our results show that removal of a degraded and eutrophied top peat layer can considerably improve geochemical conditions for rich fen development. Nutrient availability (either N, P or both) was much lower in the topsoil removal plots than in control plots, whereas light availability (RLI) and base cation (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) concentrations in the pore water were higher, correlating with higher groundwater levels. In accordance, bryophyte cover and the number of rich fen target species was higher.

5.4.1 Peat and pore water chemistry

In our study, hydro-geochemical conditions in the top soil differed markedly between plots with and without topsoil removal. One of the inherent effects of topsoil removal is the concomitant lowering of the soil surface level and rise in relative groundwater levels, which we observed in all sites. The resulting increased influence of base-rich groundwater leads to a higher base availability and alkalinity (Boeye et al. 1995, Lamers et al. 2015), which corresponds with our observation of higher pore water concentrations of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and HCO_3^{-} in the topsoil removal plots. Correspondingly, we observed significantly higher pH_{KCl}-values in the topsoil removal plots (ranging from 5.2 to 6.3).

Topsoil removal led to a drastic decline in nutrient concentrations: concentrations of nitrogen were lower both in the peat soil (lower NH_4^{+}-N, higher C:N ratios) and in the pore water (NH_4^{+} and NO_3^{-}) of the topsoil removal plots, and a similar distinct pattern was found for soil pools of total-P and oxalate-extractable P. Lower concentrations of nitrate after topsoil removal can be due to complete removal of the eutrophied top layer, but it can also be a consequence of wetter (more anoxic) conditions, which enhances denitrification by micro-organisms (Whitmire and Hamilton 2005). Under such permanently wet conditions however, reduced nitrification rates can lead to ammonium accumulation (Paulissen et al. 2005). One would therefore expect that the observed higher groundwater levels in the topsoil removal plots would correlate with higher concentrations of NH_4^{+}, but we see the opposite. We assume that the higher NH_4^{+}-concentrations in the control plots can be explained by (past) peat mineralization followed by the release and adsorption of ammonium to the cation exchange complex of the soil. In the topsoil removal plots, such accumulated soil-bound NH_4^{+} is removed together with the mineralized topsoil. Also, the increased input of base cations by groundwater after topsoil removal enhances the desorption of NH_4^{+} from the soil adsorption complex, allowing NH_4^{+} to be washed out (Lucassen et al. 2006).

Concentrations of total phosphorus in the pore water were not lower in the topsoil removal plots, but we find up to six times lower soil pools of total-P and oxalate-P. Phosphorus is relatively immobile and tends to accumulate in an inorganic form in the top soil of degraded or fertilized fens (Graham et al. 2005, Zak et al. 2010), particularly when iron is abundant (Aggenbach et al. 2013). Such P-enriched layer is generally easily removed with topsoil removal, as is well known from restoration projects on mineral soils (Allison and Ausden 2004). The discrepancy between lower P-concentrations in the top soil but unaltered P-concentrations in the pore water of the topsoil removal plots is possibly the result of lowered redox potentials that facilitate P-release in the form of PO_4^{3}-P to the pore water (Zak et al. 2010, van de Riet et al. 2013).

5.4.2 Floristic response to topsoil removal

We observed an increase in biodiversity as well as in the fraction of target species in all topsoil removal plots. Moreover, target species that were already present in the control plots are nearly always found in the topsoil removal plots as well, indicating activation of the seed bank after topsoil removal or rapid recolonization from nearby areas.

The multivariate analysis indicated that vegetation composition in the topsoil removal plots is strongly related to high groundwater levels, low pore water concentrations of NH_4^+, a high availability of light (RLI), and high concentrations of base cations Ca^{2+} and K^+. Higher groundwater levels correlated with higher concentrations of base cations and HCO_3^-. A continuous supply of both HCO_3^- (ensuring a high alkalinity and a buffered pH) and base cations is essential for rich fen species, which are vulnerable to acidification and base leaching (van Diggelen et al. 1996, Grootjans et al. 2006, Cusell et al. 2013). In this respect, a slight increase in concentrations of potassium may be of particular importance as K leaches relatively easily from degraded peat soils, thereby hampering fen restoration (van Duren et al. 1997). As many of the control plots were still suffering from (slight) drainage, drainage-related processes such as low base availability or occasional drought-stress may co-explain the limited occurrence of target species in the control plots. Furthermore, the low nitrogen levels (especially NH_4^+) in the topsoil removal plots are equally important for the establishment of rich fen communities: many rich fen species are easily outcompeted by competitive helophytes in N-enriched systems (Verhoeven et al. 2011), while ammonium accumulation can be phytotoxic to target species (Paulissen et al. 2005). Finally, the CCA unravels the significant effect of relative light intensity (RLI) on target community establishment, which is inversely related to productivity and nutrient availability. Site averages for the topsoil removal plots always exceeded 40% of RLI at surface level, whereas RLI in some of the control plots approached the 5% threshold of light compensation where respiration exceeds photosynthesis in herbs (Larcher 2003). Under such conditions, light stress becomes a strong environmental filter for fen vegetation (Kotowski and van Diggelen 2004). For typical rich fen communities of small sedges and brown mosses, thresholds lie generally around 40-60 % of RLI (Kotowski and van Diggelen 2004, Kotowski et al. 2006). Consequently, we observed a higher fraction of light-demanding target species and an increase in bryophyte cover in the topsoil removal plots.

5.4.3 Site effects

We did not analyze different sites separately, but it appears that the strength of the effect of topsoil removal is somewhat site dependent. Moreover, depth of topsoil removal as well as time since topsoil removal varied between sites, and this may affect the outcome as well (Klimkowska et al. 2007). We have too few study sites to statistically disentangle all the different site effects that affect the magnitude of restoration success, but visual inspection of our data combined with the CCA-analysis suggests that higher groundwater levels and lower nutrient stocks play a major role, with most successful results on (previously) drained locations with a heavily-mineralized top soil (location LH, HE, HO and MA). The two locations that had been rewetted before topsoil removal (DA, PE) showed a less distinct, albeit positive, response to topsoil removal.

5.4.4 Topsoil removal as a restoration strategy for organic soils

Topsoil removal on peat soils (thereby exposing an underlying peat layer) is an uncommon practice in nature restoration (but see Patzelt et al. 2001, Klimkowska et al. 2009, Klimkowska et al. 2015), but our results show that it can significantly improve conditions for rich fen development. It should be noted that we cannot ascertain that topsoil removal triggers a complete "ecosystem reset" to pristine conditions, as this requires complete knowledge of the conditions prior to degradation. However, it is clear that hydro-geochemical conditions in the degraded fens shift towards conditions that are, at least, more typical for pristine rich fens (see Aggenbach et al. 2013), and that target species respond positively. At the same time, a complete removal of a degraded peat layer is irreversible and not without risk. Moreover, the removed peat may be a considerable source of CO_2 when it oxidizes. We provide a simple decision flow-chart with criteria that should be met before topsoil removal is implemented (Figure 5.4). First, topsoil removal is evidently not required if the target rich fen vegetation is already present. Second, if other restoration measures deserve priority (for rich fens this includes rewetting with minerotrophic water (van Diggelen et al. 2006)), then topsoil removal should be considered only if (1) rewetting is not possible or (2) nutrient (N or P) pools are so high that typical rich fen species are outcompeted by non-target species. Finally, if topsoil removal is expected to negatively affect any neighboring area of high ecological value (e.g. through drainage effects), then potential gains in the restoration area must be balanced with potential losses in the neighboring area.

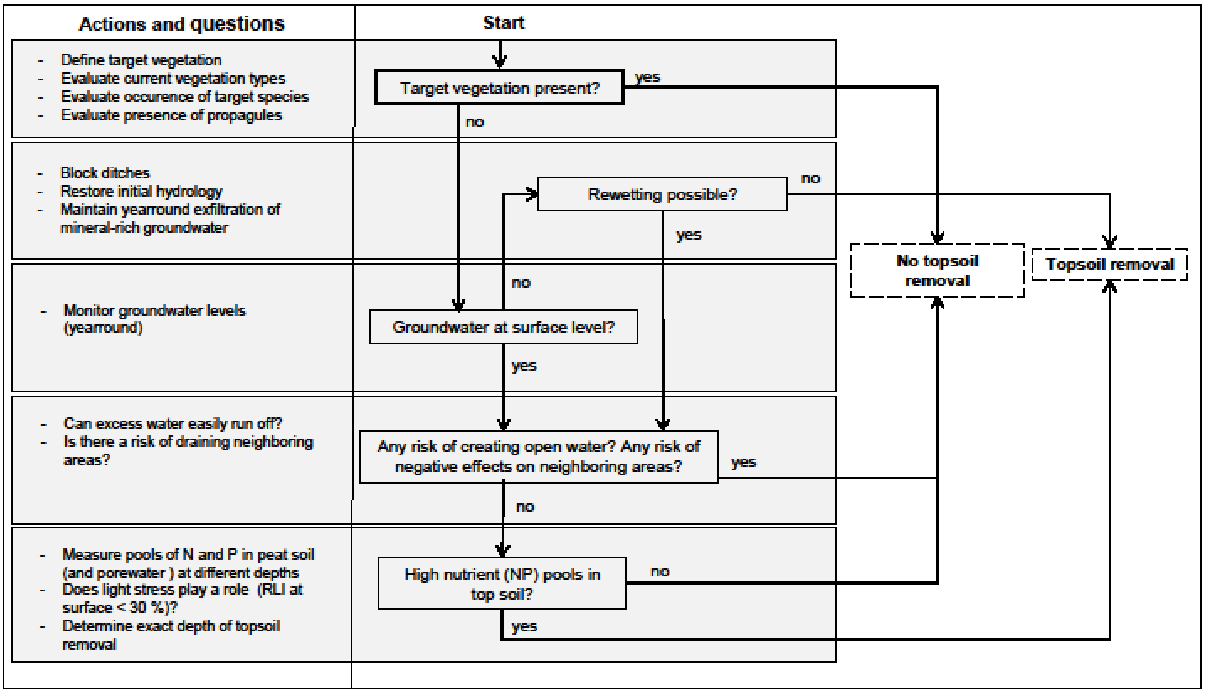

Figure 5.4: Decision flow-chart with criteria on when to consider topsoil removal in degraded rich fens.

5.4.5 Conclusions

Our study has shown that topsoil removal on degraded peat soils can significantly improve conditions for rich fen development. We suggest that the best results are to be expected in areas where raising groundwater levels to the surface level is not possible, and where topsoil removal leads to immediate rewetting. Removal of the degraded top layer exposes an underlying peat layer of better physio-chemical quality with lower bulk density and less nutrients. Generally, target species respond relatively fast. We propose that topsoil removal should be more frequently considered in degraded groundwater-fed peatlands. As most peatlands in Europe are already in a stage of severe degradation, such drastic measures may be crucial to improve the conservation prospects of these endangered habitats.

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5.5 References

Aggenbach, C. J. S., H. Backx, W. J. Emsens, A. P. Grootjans, L. P. M. Lamers, A. J. P. Smolders, P. J. Stuyfzand, L. Wolejko, and R. Van Diggelen. 2013. Do high iron concentrations in rewetted rich fens hamper restoration? Preslia 85:405-420.

Allison, M. and M. Ausden. 2004. Successful use of topsoil removal and soil amelioration to create heathland vegetation. Biological Conservation 120:221-228.

Beltman, B., T. Van den Broek, A. Barendregt, M. C. Bootsma, and A. P. Grootjans. 2001. Rehabilitation of acidified and eutrophied fens in The Netherlands: Effects of hydrologic manipulation and liming. Ecological Engineering 17:21-31.

Bennington, C. C. and W. V. Thayne. 1994. Use and Misuse of Mixed-Model Analysis of Variance in Ecological-Studies. Ecology 75:717-722.

Boeye, D., D. Vanstraaten, and R. F. Verheyen. 1995. A Recent Transformation from Poor to Rich Fen Caused by Artificial Groundwater Recharge. Journal of Hydrology 169:111-129.

Bragazza, L., Buttler, A., Siegenthaler, A., Mitchell, E.A., 2009. Plant litter decomposition and nutrient release in peatlands. In: Baird, A.J., Belyea, L.R., Comas, X., Reeve, A.S., Slater, L.D. (Eds.), Carbon cycling in Northern peatlands. Geophysical Monograph Series, 184: 99–110

Brouns, K., J. T. A. Verhoeven, and M. M. Hefting. 2014. Short period of oxygenation releases latch on peat decomposition. Science of the Total Environment 481:61-68.

Cusell, C., L. P. M. Lamers, G. van Wirdum, and A. Kooijman. 2013. Impacts of water level fluctuation on mesotrophic rich fens: acidification vs. eutrophication. Journal of Applied Ecology 50:998-1009.

Fenner, N., R. Williams, H. Toberman, S. Hughes, B. Reynolds, and C. Freeman. 2011. Decomposition 'hotspots' in a rewetted peatland: implications for water quality and carbon cycling. Hydrobiologia 674:51-66.

Gambolati, G., M. Putti, P. Teatini, and G. G. Stori. 2006. Subsidence due to peat oxidation and impact on drainage infrastructures in a farmland catchment south of the Venice Lagoon. Environmental Geology 49:814-820.

Graham, S. A., C. B. Craft, P. V. McCormick, and A. Aldous. 2005. Forms and accumulation of soil P in natural and recently restored peatlands - Upper Klamath Lake, Oregon, USA. Wetlands 25:594-606.

Grootjans, A. P., E. B. Adema, W. Bleuten, H. Joosten, M. Madaras, and M. Janakova. 2006. Hydrological landscape settings of base-rich fen mires and fen meadows: an overview. Applied Vegetation Science 9:175-184.

Grootjans, A. P., P. C. Schipper, and H. J. Vanderwindt. 1986. Influence of Drainage on N-Mineralization and Vegetation Response in Wet Meadows .2. Cirsio-Molinietum Stands. Acta Oecologica-Oecologia Plantarum 7:3-14.

Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for Light Causes Plant Biodiversity Loss After Eutrophication. Science 324:636-638.

Hennekens, S. M. and J. H. J. Schaminee. 2001. TURBOVEG, a comprehensive data base management system for vegetation data. Journal of Vegetation Science 12:589-591.

Klimkowska, A., D. J. Elst, and A. P. Grootjans. 2015. Understanding long-term effects of topsoil removal in peatlands: overcoming thresholds for fen meadows restoration. Applied Vegetation Science 18: 110-120.

Klimkowska, A., W. Kotowski, R. van Diggelen, A.P. Grootjans, P. Dzierza, and K. Brzezinska. 2009. Vegetation re-develoment after fen meadow restoration by topsoil removal and hay transfer. Restoration Ecology 18: 924-933.

Klimkowska, A., R. Van Diggelen, J. P. Bakker, and A. P. Grootjans. 2007. Wet meadow restoration in Western Europe: A quantitative assessment of the effectiveness of several techniques. Biological Conservation 140:318-328.

Kooijman, A. M. 1992. The Decrease of Rich Fen Bryophytes in the Netherlands. Biological Conservation 59:139-143.

Kotowski, W., W. Thorig, R. van Diggelen, and M. J. Wassen. 2006. Competition as a factor structuring species zonation in riparian fens - a transplantation experiment. Applied Vegetation Science 9:231-240.

Kotowski, W. and R. van Diggelen. 2004. Light as an environmental filter in fen vegetation. Journal of Vegetation Science 15:583-594.

Laiho, R. 2006. Decomposition in peatlands: Reconciling seemingly contrasting results on the impacts of lowered water levels. Soil Biology & Biochemistry 38:2011-2024.

Laiho, R., T. Sallantaus, and J. Laine. 1999. The effect of forestry drainage on vertical distributions of major plant nutrients in peat soils. Plant and Soil 207:169-181.

Lamers, L. P. M., M. A. Vile, A. P. Grootjans, M. C. Acreman, R. van Diggelen, M. G. Evans, C. J. Richardson, L. Rochefort, A. M. Kooijman, J. G. M. Roelofs, and A. J. P. Smolders. 2015. Ecological restoration of rich fens in Europe and North America: from trial and error to an evidence-based approach. Biological Reviews 90:182-203.

Larcher, W. 2003. Physiological plant ecology: ecophysiology and stress physiology of functional groups. Springer.

Londo, G. 1976. Decimal Scale for Releves of Permanent Quadrats. Vegetatio 33:61-64.

Lucassen, E. C. H. E. T., A. J. P. Smolders, G. Boedeltje, P. J. J. van den Munckhof, and J. G. M. Roelofs. 2006. Groundwater input affecting plant distribution by controlling ammonium and iron availability. Journal of Vegetation Science 17:425-434.

Malson, K., I. Backeus, and H. Rydin. 2008. Long-term effects of drainage and initial effects of hydrological restoration on rich fen vegetation. Applied Vegetation Science 11:99-106.

Malson, K. and H. Rydin. 2007. The regeneration capabilities of bryophytes for rich fen restoration. Biological Conservation 135:435-442.

Matson, P. A., W. J. Parton, A. G. Power, and M. J. Swift. 1997. Agricultural intensification and ecosystem properties. Science 277:504-509.

Olsson, P. A. and A. M. Ödman. 2014. Natural Establishment of Specialist Plant Species after Topsoil Removal and Soil Perturbation in Degraded Calcareous Sandy Grassland. Restoration Ecology 22:49-56.

Patzelt, A., U. Wild, and J. Pfadenhauer. 2001. Restoration of wet fen meadows by topsoil removal: Vegetation development and germination biology of fen species. Restoration Ecology 9:127-136.

Paulissen, M. P. C. P., L. E. Besalu, H. De Bruijn, P. J. M. Van der Ven, and R. Bobbink. 2005. Contrasting effects of ammonium enrichment on fen bryophytes. Journal of Bryology 27:109-117.

Phoenix, G. K., W. K. Hicks, S. Cinderby, J. C. I. Kuylenstierna, W. D. Stock, F. J. Dentener, K. E. Giller, A. T. Austin, R. D. B. Lefroy, B. S. Gimeno, M. R. Ashmore, and P. Ineson. 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. Global Change Biology 12:470-476.

Romão, C. 1996. Interpretation manual of European Union habitats. European Commission.

Schaminee, J. H. J., E. J. Weeda, and V. Westhoff. 1995. De vegetatie van Nederland 2: Plantengemeenschappen van wateren, moerassen en natte heiden, Opulus Press, Uppsala/Leiden. 358 pp.

Suding, K. N., K. L. Gross, and G. R. Houseman. 2004. Alternative states and positive feedbacks in restoration ecology. Trends in Ecology & Evolution 19:46-53.

ter Braak, C. and P. Šmilauer. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Section on Permutation Methods. Microcomputer Power, Ithaca, New York.

Tichy, L. 2002. JUICE, software for vegetation classification. Journal of Vegetation Science 13:451-453.

van de Riet, B.P., Hefting, M.M., Verhoeven, J.T.A., 2013. Rewetting drained peat meadows: risks and benefits in terms of nutrient release and greenhouse gas exchange. Water Air and Soil Pollution 224: 1440-1452.

van der Meijden, R., B. Odé, C. L. G. Groen, F. J. Witte, and D. Bal. 2000. Bedreigde en kwetsbare vaatplanten in Nederland. Basisrapport met voorstel voor de Rode Lijst. Gorteria 26:85-208.

van Diggelen, R., B. Middleton, J. Bakker, A. Grootjans, and M. Wassen. 2006. Fens and floodplains of the temperate zone: Present status, threats, conservation and restoration. Applied Vegetation Science 9:157-162.

van Diggelen, R., W. J. Molenaar, and A. M. Kooijman. 1996. Vegetation succession in a floating mire in relation to management and hydrology. Journal of Vegetation Science 7:809-820.

van Duren, I. C., D. Boeye, and A. P. Grootjans. 1997. Nutrient limitations in an extant and drained poor fen: implications for restoration. Plant Ecology 133:91-100.

van Landuyt, W., L. Vanhecke, and I. Hoste. 2006. Rode lijst van de vaatplanten van vlaanderen en het Brussels Hoofdstedelijk Gewest. In: van Landuyt, W. et al. Atlas van de Flora van Vlaanderen en het Brussels Gewest. INBO en de nationale plantentuin van België, Brussel.

Verhoeven, J. T. A., B. Beltman, E. Dorland, S. A. Robat, and R. Bobbink. 2011. Differential effects of ammonium and nitrate deposition on fen phanerogams and bryophytes. Applied Vegetation Science 14:149-157.

Wassen, M. J., H. O. Venterink, E. D. Lapshina, and F. Tanneberger. 2005. Endangered plants persist under phosphorus limitation. Nature 437:547-550.

Whitmire, S. L. and S. K. Hamilton. 2005. Rapid removal of nitrate and sulfate in freshwater wetland sediments. Journal of Environmental Quality 34:2062-2071.

Zak, D., J. Gelbrecht, S. Zerbe, T. Shatwell, M. Barth, A. Cabezas, and P. Steffenhagen. 2014. How helophytes influence the phosphorus cycle in degraded inundated peat soils - Implications for fen restoration. Ecological Engineering 66:82-90.

Zak, D., C. Wagner, B. Payer, J. Augustin, and J. Gelbrecht. 2010. Phosphorus mobilization in rewetted fens: the effect of altered peat properties and implications for their restoration. Ecological Applications 20:1336-1349.

6

Competition for light drives endangered species loss in fens: an introduction experiment

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Abstract

Many endangered plant species remain absent in rewetted, previously drained fens. We performed a 3-year introduction experiment with endangered fen species (10 *Carex*- and 6 bryophyte species) in 4 hydrologically restored fens to investigate which factors hamper establishment and survival. *Carices* were introduced both as adults and seedlings, mosses as gametophyte mats. Introductions were done on (initially) bare soil to exclude excessive competition for light during the first year: this allowed us to discriminate between the species' fundamental and realized niches. Overall first year survival of the introduced plants was high in all fens (mean survival = 96%), indicating that abiotic conditions were within the species' fundamental niches. However, survival analysis revealed that a decrease in relative light intensity (RLI) at the soil surface during consecutive years (indicating an increase in biotic competition for light) explained high mortality rates in most species. As a result, overall final survival was lowest in the two most productive (low light) fens (mean survival = 38%), while most transplants had persisted in the two less productive (high light) fens (mean survival = 79%). In accordance, taller and faster-growing species had a higher overall survivability as they were able to outgrow light limitation, suggesting asymmetric competition, and they thus occupied a broader realized niche than smaller and slower-growing species. Light limitation also drove the loss of 5 out of 6 bryophyte species. Overall, adult *Carex* plants performed better than seedlings and bryophytes. We conclude that both dispersal limitation and asymmetric competition for light may explain the loss of endangered species in rewetted fens. Although species reintroduction can be feasible in hydrologically restored fens, we propose a minimum empirical threshold of 30 % relative light intensity at the soil surface.

6.1 Introduction

Rich fens are groundwater-fed wetlands with high pH that are typically covered by plant communities of small sedges (*Carex* spp.) and brown mosses (mainly of the *Amblystegiaceae* family) (Grootjans *et al.* 2006; Rydin & Jeglum 2013). In the Northern hemisphere, many fens have suffered from severe drainage, land use change and degradation (Lamers et al. 2015). Therefore, conservation and restoration of the remaining fens has become a top priority in nature conservation (van Diggelen et al. 2006).

Fen restoration is usually focused on hydrological restoration (i.e. the restoration of high water levels and a continuous groundwater discharge with appropriate chemistry) (Mälson, Backeus & Rydin 2008; Zak *et al.* 2010). However, many typical fen species often remain absent at restoration sites, despite successful rewetting, and are replaced by more common wetland species (van Dijk *et al.* 2007; Mälson, Backeus & Rydin 2008; Aggenbach *et al.* 2013). This trend is particularly clear for the smallest and most vulnerable rich fen species, e.g. *Carex dioica* (L.), *C. limosa* (L.), *C. lepidocarpa* (Tausch)), *Tomentypnum nitens* (Hedw.) Loeske, *Scorpidium scorpioides* ((Hedw.) Limpr.), or *Campylium stellatum* ((Hedw.) C.E.O. Jensen)). The marked absence of typical fen species in rewetted fens may have at least three nonexclusive reasons.

First, rewetted fens have a "degradation legacy" in which long-term drainage has deteriorated diaspore banks and extirpated source populations of vulnerable species. Relict populations, if still present, are often heavily fragmented, small or genetically impoverished (de Vere *et al.* 2009; Lamers *et al.* 2015). Hence, spontaneous recolonization of rewetted fens by target species often appears to be hampered by dispersal limitation or limited propagule viability (Cobbaert, Rochefort & Price 2004; Middleton, van Diggelen & Jensen 2006).

Second, abiotic conditions in rewetted fens have usually been altered in comparison to pristine fens (Zak *et al.* 2010; Aggenbach *et al.* 2013), and conditions may have become hostile to some fen species. If so, then the abiotic environment no longer overlaps with the species' fundamental niche. Fen species may for example be vulnerable to prolonged soil drainage and concomitant base leaching and acidification (Cusell *et al.* 2013; van Diggelen *et al.* 2015), or they can be negatively affected by high concentrations of potential phytotoxins (e.g. iron (Fe^{2+}), hydrogen sulfide (H_2S), or ammonium (NH_4^+)) that often accumulate upon fen rewetting (Paulissen *et al.* 2004; Geurts *et al.* 2009; Aggenbach *et al.* 2013).

Finally, many rewetted fens are characterized by high nutrient mobilization rates and nutrient levels (van Dijk *et al.* 2007; Zak *et al.* 2010; van de Riet, Hefting & Verhoeven 2013). Although high nutrient availability is usually not directly harmful to plants, the concomitant increase in plant primary production sets off strong biotic competition for light and growing space and eventually determines the realized niche of a species. Under eutrophic conditions, small and low-competitive species are easily outcompeted by taller and fast-growing competitors (Tilman 1988; Kotowski *et al.* 2006; DeMalach, Zaady & Kadmon 2017).

In this study, we performed a species introduction experiment in four rewetted fens to investigate mechanisms behind the loss of endangered fen species. Our setup allowed us to discriminate between direct abiotic constraints on species establishment and vigor (i.e. fundamental niche), constraints imposed by nutrient-driven competition for light and growing space (i.e. realized niche), and constraints on dispersal and (re-)colonization potential. We argue that dispersal limitation and competition for light may explain the widespread loss of endangered species in rewetted fens.

6.2 Materials and methods

6.2.1 Study sites

We selected four rich fens in the Netherlands: Langstraat (LS: 51°41'1.06"N; 4°58'27.53"E), Holmers (HO: 52°54'9.85"N; 6°37'47.32"E), Drentsche Aa (DA: 53°1'13.88"N; 6°40'10.28"E), and Peizermade (PE: 53°10'5.26"N; 6°30'18.43"E). All fens have been drained in the past, but high groundwater levels were restored > 10 years ago by closing drainage ditches (sites DA, HO and PE) and/or by removing several decimeters of degraded top soil (sites LS and HO). The soils of sites DA and PE were predominantly peat soils (> 40 cm peat), site HO had a shallow (±10 cm) peat layer on top of a silty mineral soil, whereas site LS had peaty sand. All sites are now owned by nature conservation agencies: permits for field experiments were granted by Staatsbosbeheer (LS, HO, DA) and Natuurmonumenten (PE).

6.2.2 Study species and propagule collection

In 2012 and 2013 we collected ripe seeds of 10 species of sedge (*Carex* spp.) and gametophyte mats of 6 species of bryophyte (Table 6.1). The sedges were typical small- to medium-sized fen species, while most of the bryophytes can be broadly classified as “brown mosses” (Mainly the *Amblystegiaceae* family, but also *Meesiaceae* and *Calliergonaceae*). All species are typical for well-developed fens in large parts of the Northern hemisphere, and are endangered (red list “vulnerable” or worse) or have disappeared in the Netherlands.

Due to past drainage and deterioration of most fens in the Netherlands (Lamers et al. 2015), many fen species have either gone regionally extinct, or, are only left in small relict populations with limited viability. Therefore, we also collected propagules in other European countries with larger populations. We aimed to minimize the impact of propagule collection: for sedges we collected small amounts of ripe seeds, and for mosses we collected one or two living gametophyte mats (15 x 15 cm) per species. The use of gametophyte fragments is a well-established method for bryophyte reintroduction (Mälson & Rydin 2007; Graf & Rochefort 2010).

Table 6.1: *Carex* and bryophyte species in the experiment with country of propagule collection (Sw = Sweden, Sl = Slowakia, Pl = Poland, Nl = The Netherlands, Sp = Spain). Numbers represent the total number of introduced individuals (all sites combined).

***Carex* species**	**N adult plants**	**N seedlings**	**Donor country**
Carex pulicaris (L.)	32	32	Sw
Carex davalliana (Sm.)	32	32	Sl, Pl
Carex dioica (L.)	31	32	Sw
Carex limosa (L.)	32	32	Sw
Carex appropinquata (Schumach.)	32	32	Nl, Sw
Carex diandra (Schrank)	32	32	Sp
Carex lepidocarpa (Tausch)	32	32	Pl, Sp
Carex flava (L.)	0	32	Sl, Pl
Carex chordorrhiza (Ehrh. ex L.f.)	12	12	Sw
Carex lasiocarpa (Ehrh.)	0	4	Sw
Total	235	272	
Bryophyte species	**N bryophyte mats**		
Scorpidium scorpioides ((Hedw.) Limpr.)	32		Nl
Calliergon giganteum ((Schimp.) Kindb.)	32		Sl, Sw
Scorpidium revolvens ((Sw.) Rubers)	32		Sw
Campylium stellatum ((Hedw.) C.E.O. Jensen)	32		Nl
Tomentypnum nitens ((Hedw.) Loeske)	32		Sl, Sw
Paludella squarrosa ((Hedw.) Brid.)	12		Sw
Total	172		

6.2.3 Preparation of plant material

Carex seeds received a cold-moist stratification treatment in full darkness (4°C) for a minimum of 3 months to break seed dormancy. Next, seeds were germinated on moist filter paper in an incubator under a fluctuating day-night regime (24/15°C, 12/12h photoperiod). Half of the plants were germinated in spring 2013 and transported to a greenhouse nursery: these plants were considered “adult” at the time of introduction into the field in spring 2014 (height 18 ± 7 cm; leaf count 56 ± 30 (mean ± SD)). The other half of the plants were germinated in spring 2014 and transplanted as "seedlings" in the experiment two weeks later (height 10 ± 4 cm, leaf count 11 ± 6 (mean ± SD)). In total, we aimed to introduce a total of 32 seedlings and 32 adult plants per *Carex* species, but low germination rates in some species resulted in a smaller sample size for some species (and zero adults in *C. flava* and *C. lasiocarpa*; Table 6.1).

The bryophytes were cultivated in plastic containers (39 x 28 x 14 cm) prior to introduction. The containers were filled with a layer of limed clean white sand, and demineralized water was regularly added to keep the sand waterlogged. All species gradually expanded within the containers, with the exception of *Paludella squarrosa* which survived but did not expand. Before introduction into the field, the bryophyte mats were separated into 32 smaller mats per species (20.3 ± 9.3 cm^2; mean ± SD). The limited growth of *Paludella squarrosa* resulted in only 12 mats for this species.

6.2.4 Field experiment

Mid-April 2014, all individual sedges (seedlings and adults) and bryophyte mats were equally divided over and introduced at the four study sites. At each site we selected an area of 4.5 x 9.5 m in which 8 experimental plots of 1 x 1 m were established (Fig. S6.1 in Supporting information). The experimental area was unmown and not grazed by livestock for the duration of the experiment. Preparation of the experimental plots was as follows: first, we cut the existing vegetation. Next, we manually removed the top 10 cm of the soil (in sods), tilted the sods upside down, and then pressed the sods back into the hole. The outer 25 cm edge of each plot was covered with root canvas. By doing so, we created a 0.5 x 0.5 m patch of bare light-saturated soil without leaving a depression. The reasoning for starting from a patch of bare soil rather than introducing plants into existing (closed) vegetation was because we wanted to separate potential direct negative effects of the abiotic environment (fundamental niche) from indirect negative effects of competition for light and growing space with an (existing) herb layer (realized niche). By the initial removal of biotic competition, we were thus able to (temporarily) delay competition-induced mortality.

Each experimental plot was further subdivided in 25 (10 x 10 cm) sub-plots (Fig. S6.1 in Supporting information). Individual plants were assigned to each sub-plot so that each plot always contained 2 individuals (1 seedling and 1 adult) of each sedge species and 1 gametophyte-mat of each bryophyte species. Sedges were planted directly into the soil, and bryophyte mats were simply pressed on top of the soil. A small wooden label was pushed into the soil at 1 cm from each individual plant to facilitate future recovery.

Plant performance and species richness

The field experiment was monitored six times in total divided over three growing seasons: April 2014 (week 0), May 2014 (week 3), August 2014 (week 16), May 2015 (week 54), August 2015 (week 68), and July 2016 (week 111). We checked survival of each individual sedge or bryophyte mat: the criterion for plant survival was the presence of photosynthetically active (green) tissue. For surviving sedges, we additionally measured the height (cm) of each individual plant (from the base of the stem to the highest living leaf, excluding inflorescences). At each visit we also recorded total species richness per plot, including both introduced target species as well as naturally (re-)appearing non-target species.

Abiotic conditions

We collected pore water samples in all experimental plots once per growing season (2014, 2015 and 2016) using MacroRhizon samplers (filter size = 0.15 µm, Rhizosphere Research Products, The Netherlands). pH of the pore water was measured directly in the field using portable equipment (WTW Multi 340i, WTW, Weilheim, Germany). Total inorganic carbon (TIC) was determined on an infrared gas analyzer (ABB Advance Optima): HCO_3^- concentrations were calculated based on pH. Concentrations of NH_4^+ and NO_3^- were determined on an Auto Analyzer 3 System (Bran + Luebbe). Pore water subsamples were acidified with 0.7 ml 65% suprapure HNO_3 per 100 ml sample and analyzed on ICP (IRIS Intrepid II) for total dissolved concentrations of Ca, Mg, K, Na, Fe, Mn, P, and S, and Al.

In spring 2014, we installed one perforated PVC filter tube at each site which we provided with a water level logger (CTD-diver; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) to automatically log groundwater levels bi-daily during the experiment.

We determined relative light intensity (RLI) near the soil surface of each plot at each visit using an Accupar LP-80 ceptometer (Decagon Devices Inc., Pullman, WA, USA), which measures photosynthetically active radiation (PAR, in $\mu mol\ m^{-2}\ s^{-1}$). In short, a horizontal bar fitted with multiple PAR sensors was horizontally inserted just above the soil or moss surface below the herb layer, while a reference light sensor was simultaneously logging the amount of incoming PAR at approximately 1 m above the vegetation. Relative light intensities (in % PAR) at soil surface level can then be calculated. This we repeated in three positions per plot, and we averaged the values to attain one representative RLI value per plot. RLI below the herb layer is a good proxy for the amount of biotic competition for light: low RLIs indicate that most incoming PAR is captured and utilized by the vegetation, and are typical below a dense and highly productive herb layer (Kotowski & van Diggelen 2004).

6.2.5 Data analyses

All analyses were performed in R 3.2.3 (R Development Core Team 2016). Significance was accepted at $p < 0.05$.

Pore water chemistry values of the three sampling seasons were averaged: statistical analyses were based on these mean values (one value per plot). To visualize abiotic (dis-)similarity between different sites and plots, we performed a principal components analysis based on the correlation matrix (PCA, centered and scaled) including the following variables: concentrations of dissolved Na, K, Mg, Ca, N-inorg (NO_3^--N + NH_4^+-N), S, Al, Fe, P, Mn, and HCO_3^- (all log10-transformed), pH and RLI. For RLI, we used the “final” values per plot obtained at the end of the experiment (July 2016). Groundwater dynamics were quantified with cumulative relative frequency curves.

We analyzed plant survival in two ways using the R package *survival* (Therneau & Lumley 2016). First, we investigated differences in plant survival between the three plant functional groups (adults and seedlings of *Carex*, bryophytes) among the four study sites (LS, HO, DA and PE), regardless of species. Second, we analyzed survival of the plants grouped per species versus RLI, regardless of plant functional group or study site. We chose this approach as one survival model that includes all factors (Survival versus site, functional group, species and RLI) was statistically not possible. Differences in survival distributions of the three plant groups among study sites were tested with log-rank tests: results were visualized with Kaplan-Meier curves. Cox Proportional Hazard models (CoxPH) were used to test for the effect of (final) RLI on survival probabilities of the 16 different species. We report the hazard ratio as a parameter that quantifies survival: hazard ratios < 1 indicate decreasing death risk with increasing RLI whereas hazard ratios > 1 indicate increasing death risk with increasing RLI. In addition to CoxPH, we used logistic regression (logit-link) to estimate the survival probability of each species at the end of the experiment versus RLI, in which survival (value = 1) or death (value = 0) of an individual plant was plotted against the corresponding RLI-value of the experimental plot. We did this to extract inflection points for all species, i.e. the RLI below which survival probability of a species was < 50 %.

Finally, we calculated two proxies that define *Carex*-species morphology and growth strategy, which we compared with survival fractions of the species at the end of the experiment: (1) maximum adult height (cm) and (2) seedling relative height increment rate (cm week^{-1}). Maximum height was defined as the mean height of the 25% tallest (living) individuals of the species at the end of the experiment, height increment rate is the growth of (living) sedges during week 3-16, excluding the first three weeks (week 0-3) to overcome potential negative effects of transplantation. As survival fractions are bounded between 0 and 1, we used Beta-regression (with logit-link) (Cribari-Neto & Zeileis 2010).

6.3 Results

6.3.1 Abiotic conditions

Principal components analysis revealed the clustering of the plots based on pore water chemistry and final RLI (Fig. 6.1). Combined, PC1 and PC2 accounted for 70.9% of the total variance (41.4 and 29.5 % respectively). Variables with high loadings on PC1 were Fe, HCO_3^-, Na, Mn, P and RLI (loadings -0.39, -0.38, 0.38, -0.37, -0.36, and 0.29 respectively); variables with high loadings on PC2 were Al, inorganic N, S and pH (loadings = 0.46, 0.45, 0.42, and -0.38 respectively).

On the site-level, sites PE and DA were characterized by relatively high pore water concentrations of dissolved P, Fe, HCO_3^- and Mn, whereas RLI was low (Fig. 6.1, Table S6.1 in Supporting information). Conversely, sites LS and HO generally had lower pore water concentrations of dissolved P, Fe, HCO_3^- and Mn, but RLI was much higher. Furthermore, site HO was somewhat distinct by having low concentrations of inorganic N and Al, and highest pH. Pore water pH, however, was above 6 at all sites (Table S6.1 in Supporting information).

Groundwater levels in the fens fluctuated between 29.8 cm below and 20.3 cm above soil surface. The sites differed in duration of inundations (water level was at or above surface 8% of the time at LS, 34 % at PE, 35 % at DA, and 60 % at HO; Fig. S6.2 in Supporting information). Deep inundation (> 15 cm) as well as deep water level drawdown (< -20 cm) was uncommon and mainly restricted to winter (max = 3 % of time at HO) and summer (max = 6% of time at HO) respectively.

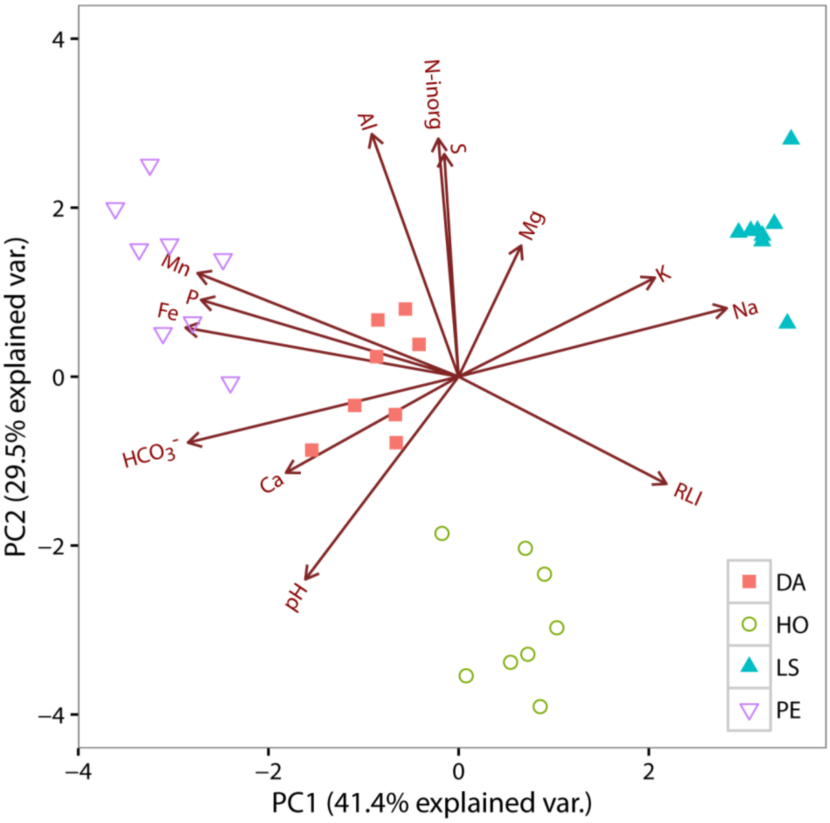

Figure 6.1: Principal components analysis (PCA) of the experimental plots based on pore water chemistry and relative light intensity (RLI, values from last sampling round). Symbols represent the plots, arrows represent the abiotic variables. "N-inorg" equals NH_4^+-N + NO_3^--N. Plots are grouped per study site (HO, LS, PE and DA).

6.3.2 RLI, species richness and survival probabilities

RLI averaged about 87 % at the start of the experiment and gradually decreased over time (Fig. 6.2a). At the end of the experiment, RLI was still relatively high for sites LS and HO (> 30 %) and much lower for sites DA and PE (< 15 %). The gradual decrease in RLI corresponded with an initial increase in total species richness (including the introduced species, Fig. 6.2b). The initial increase in species richness was followed by a strong decrease at sites DA and PE (the sites with the lowest final RLI), so that final species richness was highest at sites LS and HO (the sites with the highest final RLI).

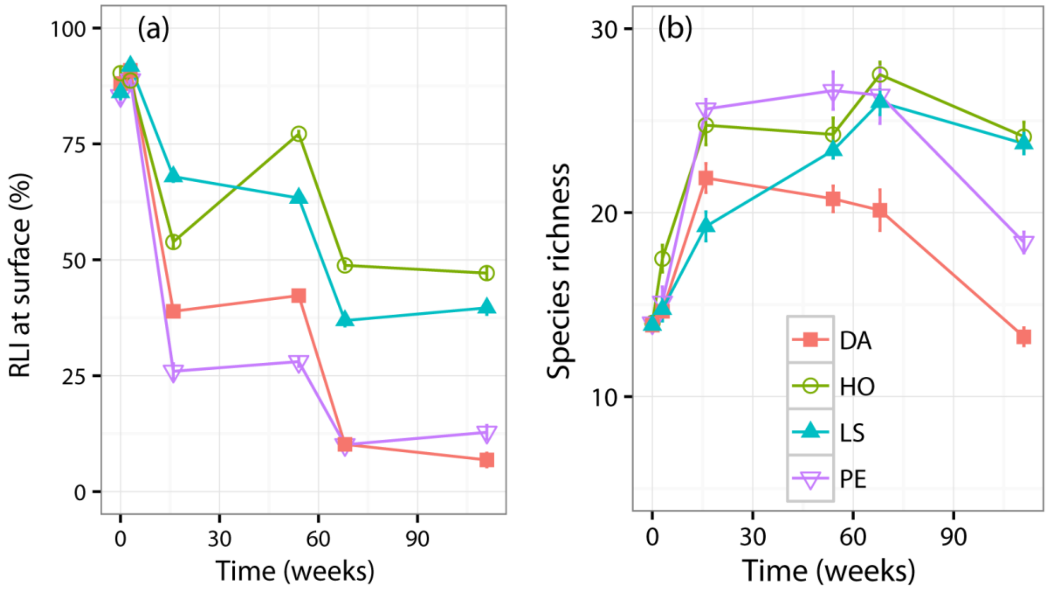

Figure 6.2: Evolution of (a) relative light intensity (RLI in %) and (b) total species richness in the experimental plots. Data are grouped per study site (HO, LS, PE and DA), vertical bars indicate standard errors.

Survival distributions of the introduced plants across sites were similar for all three functional groups: overall survival of adult plants, seedlings and bryophytes was highest in sites LS and HO and lowest in sites PE and DA (Fig. 6.3abc, Table S6.2 in Supporting information).

Survival distributions also differed among the three functional groups: overall, adult plants had a higher survival than seedlings and bryophytes (log-rank χ^2 = 16.8, df = 1, p < 0.001 and χ^2 = 20.8, df = 1, p < 0.001 respectively), but seedlings and bryophytes had equal survival probabilities (log-rank χ^2 = 0.1, df = 1, p = 0.72).

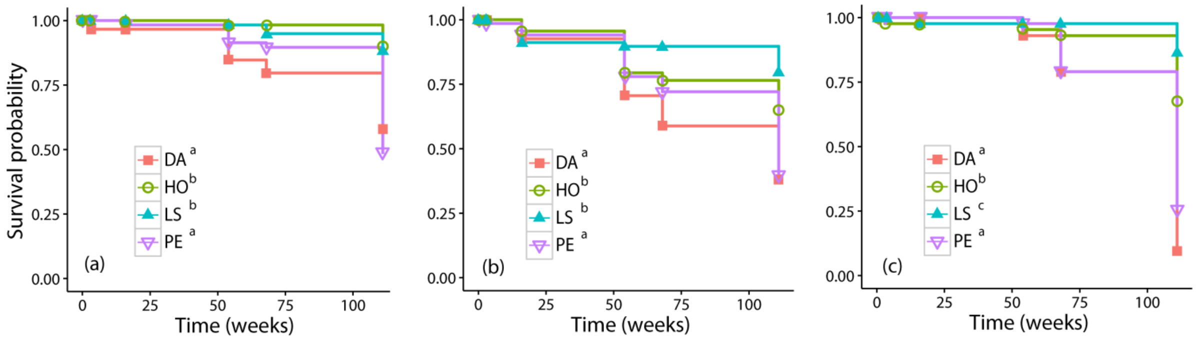

Figure 6.3: Kaplan-Meier survival curves of individual (a) adult *Carex* plants (9 species), (b) *Carex* seedlings (9 species), and (c) bryophytes (6 species) through time. Data are grouped per study site (HO, LS, PE and DA), superscripts (abc) indicate significant differences between groups ($P < 0.05$).

6.3.3 Species survival versus RLI and species morphology

CoxPH revealed that 11 out of 16 species (6 *Carex* and 5 bryophyte species) had a higher survival probability with increasing RLI (Hazard ratios < 1, Table 6.2). Of the remaining four *Carex* species, two species (*C. appropinquata* and *C. diandra*) had a lower (albeit non-significant) survival probability with increasing RLI (Hazard ratio > 1, Table 6.2), one species (*C. lasiocarpa*) was unaffected by RLI (no deaths), whereas one species (*C. flava*) had a higher but non-significant survival probability with increasing RLI. For the bryophytes, we found no significant effect of RLI on *Paludella squarrosa*: 10 out of 12 individuals had eventually died regardless of RLI.

For the species that were significantly affected by RLI, logistic regression models further revealed the RLI-inflection points per species (i.e. RLI at which the species' survival probability equals 50 %), which varied between 0 and 50.5 % (Table 6.2, Fig. S6.3).

Table 6.2: Results of Cox Proportional-Hazard models (hazard ratios) and Logistic Regression models (odds ratios) of survival probability of the introduced plants (grouped per species) versus relative light intensity (RLI, in %). For CoxPH: Hazard ratios <1 indicate decreased hazard (longer survival) with increasing RLI, hazard ratios > 1 indicate increased hazard (shorter survival) with increasing RLI. Inflection points (50%-survival thresholds) are also indicated (in % RLI). Asterisks indicate significance with *p < 0.05, **p < 0.01, *** p < 0.001.

	CoxPH Survival	**Logistic regression**	
	Hazard ratio	**Odds ratio**	**Inflection point (RLI (%))**
Carex pulicaris	0.98**	1.08**	50.5
Carex davalliana	0.97**	1.05**	19.0
Carex dioica	0.94***	1.12***	30.7
Carex limosa	0.94***	1.11***	33.2
Carex appropinquata	1.06	0.94	NA
Carex diandra	1.00	1.00	NA
Carex lepidocarpa	0.93*	1.09*	0.0
Carex flava	0.85	1.24	NA
Carex chordorrhiza	0.96**	1.12*	44.5
Carex lasiocarpa	No deaths	No deaths	
Scorpidium scorpioides	0.94***	1.10***	37.8
Scorpidium revolvens	0.91***	1.14***	23.7
Campylium stellatum	0.95***	1.08**	40.9
Tomentypnum nitens	0.93**	1.11**	19.4
Calliergon giganteum	0.95*	1.07*	10.8
Paludella squarrosa	0.98	NA	NA

Overall final survival of the *Carex* species (cumulative of the four study sites) was higher for tall (Fig. 6.4a, Table S6.3) and fast-growing species (Fig. 6.4b, Table S6.3) than for small, slow-growing species. In accordance, the three *Carex* species that were not affected by low RLI (*C. appropinquata*, *C. diandra* and *C. lasiocarpa*, Table 6.2) were also the tallest species with highest relative height increment rates (Fig. 6.4). Maximum heights of the *Carex* species correlated positively with their relative height increment rates (rho = 0.70, df = 8, p = 0.03).

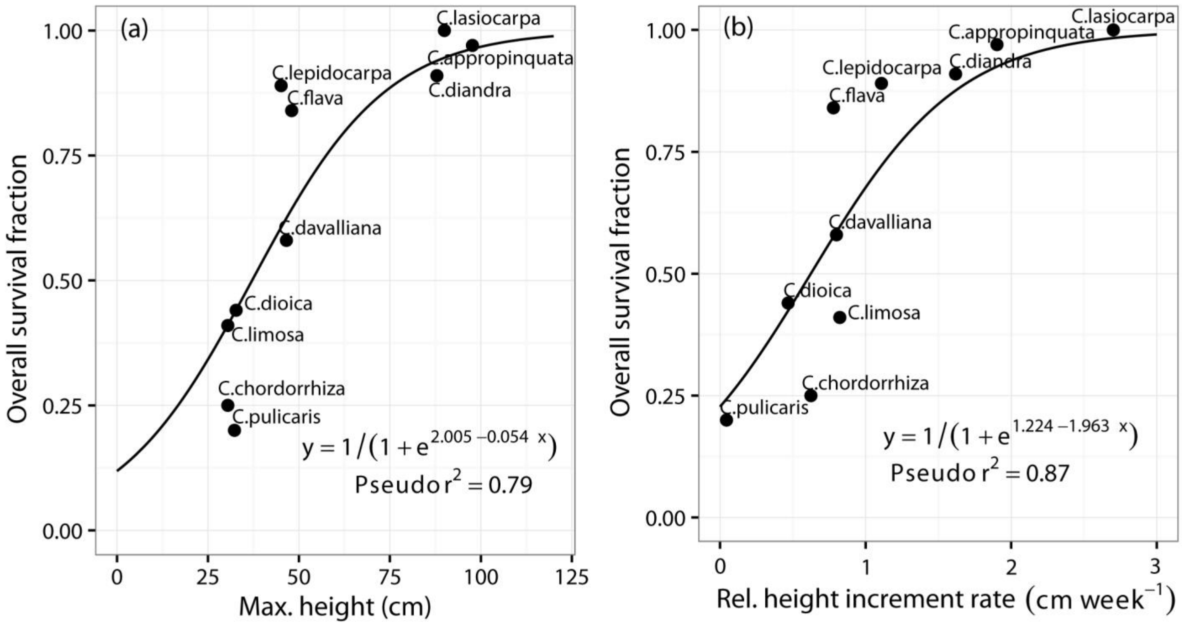

Figure 6.4: Overall survival fraction per *Carex* species at the end of the experiment (four study sites combined) in relation to (a) maximum height (cm) and (b) relative height increment rate (cm week^{-1}) of the corresponding species. Maximum height is the mean height of the 25% tallest (living) individuals per species at the end of the experiment, relative height increment is the net height increment of seedlings during the first growing season after introduction (13 weeks). Models were fitted using Beta-regression (logit-link).

6.4 Discussion

We delayed (excessive) biotic competition by initiating species introduction on patches of bare light-saturated fen soil. Hence, we were able to evaluate target species survival while discriminating between direct effects of the abiotic environment and indirect biotic effects: the underlying assumption is that rapid (first-year) mortality would be related to an unfavorable or hostile abiotic environment (the fundamental niche), whereas delayed (second- and third-year) mortality would be related to increasing biotic competition for light and growing space (the realized niche). The high first-year survival in the absence of heavy biotic competition suggests that abiotic conditions were within the fundamental niches of most introduced fen species. Final survival was highest in the low-productive fens, and twice as low in the more productive fens. Hence, excessive competition for light in rewetted fens appears to restrict target species survival. In low-productive fens, dispersal limitation may be a second important bottleneck.

6.4.1 Degradation legacy

Widespread drainage, land use change and habitat fragmentation cannot be compensated for simply by restoring water regime if target species have gone extinct or can no longer disperse freely. Hence, typical fen species can be absent simply because they are no longer able to naturally (re-)colonize a fen after hydrological restoration (Kooijman, Beltman & Westhoff 1994; Cobbaert, Rochefort & Price 2004; Middleton, van Diggelen & Jensen 2006). In addition, relying on vegetation recovery starting from a viable diaspore bank can be troublesome as well since drainage or degradation will deteriorate such banks, and often only seeds of common species persist (Klimkowska *et al.* 2010). The role of dispersal limitation in an area can easily be verified: if manual species introduction is successful in the long term (i.e. species can establish, propagate and eventually form a healthy population), then this is a solid indicator for dispersal limitation for those particular species in that area. The relatively high survival rates that we observed at some sites confirms the hypothesis of dispersal limitation as a bottleneck in fen restoration. It is becoming increasingly clear that the success of fen or wetland restoration projects in terms of species diversity can only be maximized if abiotic restoration is combined with species (re-)introduction (Patzelt, Wild & Pfadenhauer 2001; Mälson & Rydin 2007; Lamers *et al.* 2015), especially in densely populated and fragmented regions.

6.4.2 Abiotic constraints and fundamental niches

At least two direct abiotic constraints may fundamentally hamper the development of small sedge and brown moss communities. First, high and stable water levels are essential, and prolonged deep drainage may directly cause death or limited performance of vulnerable species (Manukjanova, Stechova & Kucera 2014). Also, prolonged drainage can indirectly limit survival of fen species: concomitant oxidation reactions lead to H^+ production, acidification and base cation leaching (Van Haesebroeck *et al.* 1997; Lamers, Van Roozendaal & Roelofs 1998), and this often induces a shift from rich fen species towards *Sphagnum* domination (Soudzilovskaia *et al.* 2010; Kooijman *et al.* 2016). However, since our study sites had been successfully rewetted in the past, groundwater levels were within the range for small sedge and brown moss communities (Goebel 1996), and pore water pH (> 6), HCO_3^- (> 2 mmol L^{-1}) and Ca (> 1 mmol L^{-1}) concentrations were sufficiently high (Table S6.1 in Supporting information). Water levels, pH and base availability are therefore unlikely to have had a negative impact on survival in our experiment.

Second, rewetted fens can be rich in potential phytotoxins that can hamper survival. For instance, high concentrations of H_2S and NH_4^+ as well as reduced forms of metals such as Fe^{2+} and Mn^{2+} can cause necrosis and death within weeks (Snowden & Wheeler 1993; Paulissen *et al.* 2004; Geurts *et al.* 2009). Two study sites (DA and PE) were characterized by pore water concentrations of dissolved iron that were far above potentially phytotoxic thresholds (> 2 mmol Fe L^{-1}) (Snowden & Wheeler 1993; Aggenbach *et al.* 2013). However, a very high first year survival of the introduced *Carices* and bryophytes (mean survival = 96 %) rules out phytotoxicity as a relevant factor in our experiment. Overall, these results suggest that abiotic conditions were, at least in the short term, largely within the target species' fundamental niches at all sites.

6.4.3 Biotic competition and realized niches

As time progressed, the introduced plants grew taller while the pre-existing natural vegetation gradually recolonized the (initially) bare soil experimental plots. As a result, biotic competition for light and growing space increasingly affected performance of the introduced plants, eventually constraining their survival (realized niche). Survival analysis showed that 11 out of the 16 species were negatively affected by low relative light intensities at soil surface level (RLI). For the *Carices*, the only four species that were not affected by RLI were also the tallest species with more rapid growth and high overall survival (*Carex flava, C. diandra, C. appropinquata* and *C. lasiocarpa*). It is likely that taller and faster-growing species were simply able to outgrow light limitation that is typically found near the soil surface of productive sites, thereby producing even more shade for the smaller species which seemed to lack the competitive ability to cope with light stress. This pattern confirms the theory that interspecific competition is dependent on species-specific traits and is therefore highly asymmetrical (Gaudet & Keddy 1988; DeMalach, Zaady & Kadmon 2017). The RLI-inflection points of the *Carex* species that were negatively affected by light limitation, i.e. the RLI value at which the species' survival probability drops below 50 %, varied between 0 % (*C. lepidocarpa*) and 50.5 % (*C. pulicaris*) with an average of 29.7 %.

For the bryophytes, 5 out of 6 species were negatively affected by low RLI values. Bryophytes generally cannot compete for light with a herb layer due to obvious morphological constraints, and therefore a too dense herb layer is assumed to be a major bottleneck for many bryophyte species (van der Wal, Pearce & Brooker 2005). The RLI-inflection points of the bryophyte species varied between 10.8 % (*Calliergon giganteum*) and 40.9 % (*Campylium stellatum*), with an average of 26.5 %. The loss of one species (*Paludella squarrosa*) could not be explained by light limitation, as it also largely disappeared in the low-productive fens. Although the lack of statistical significance in this species may be related to a limited sample size (n = 12) or poor vitality at the moment of introduction, the loss of this species may also be linked to other, currently unidentified, factors.

We did not investigate which exact plant-functional mechanisms explain the negative impact of low RLI on plants' performance. However, average RLI close to the surface of the two productive fens was less than 15%, which is generally insufficient for shade-avoiding plants to reach their light saturation point (Larcher 2003), i.e. the light intensity at which photosynthetic rates are maximal. Hence, direct photosynthetic constraints imposed by the limited availability of photosynthetically active radiation in the lower zones of the more productive fens are likely to explain the limited vigor and survival of the small-stature plant species.

6.4.4 Productivity shifts in rewetted fens?

We have empirically shown that most typical fen species on average disappear below values of 25-30% RLI at soil surface level, which corresponds with an estimated herb standing crop > 4 metric ton ha^{-1} (Emsens *et al.* 2015). This value however is based on an overall average of all species included in this study, and some species may require higher RLI values to persist. Kotowski et al. (2006) for example showed that natural small sedge and brown moss vegetation often has a RLI at surface level closer to 60%, which corresponds with an estimated herb standing crop < 1.5-2 metric ton ha^{-1} (Emsens *et al.* 2015). This indicates that our empirical estimate may be too optimistic and lie at the boundary of the tolerance range.

Unfortunately, herb productivity in many remaining fens in the Northern hemisphere is often well above such low values (Emsens *et al.* 2015; Emsens *et al.* 2017). In degraded and rewetted fens for example, nutrient mobilization rates and nutrient pools can be orders of magnitude higher than in pristine fens (Zak *et al.* 2010; Aggenbach *et al.* 2013). At the same time, high levels of anthropogenic N deposition in combination with NP-inflow from agricultural fertilizers is threatening the few remaining low-productive fens (Koerselman, Bakker & Blom 1990; Cusell *et al.* 2014). Such nutrient-driven shifts in herb productivity and thus light limitation act as an environmental filter to the benefit of fast-growing and often more common species (Kotowski & van Diggelen 2004). In our study, RLI correlated negatively with pore water concentrations of P and Fe (Spearman's rho = -0.43 ($P = 0.02$) and -0.69 ($P < 0.001$) respectively, results not shown), thereby supporting recent observations that productivity in fens is often highest in P- and Fe-rich fens (Emsens *et al.* 2017).

6.4.5 Study limitations

Some limitations of our experiment need to be addressed. First, we did not study germination and establishment success from seeds or sporophytes. Moreover, plants were deliberately introduced on (initially) bare soil but not within dense existing vegetation, meaning that we created priority effects that may have favored early survival. Hence, we can draw no conclusions with respect to germination, nor to establishment success within existing vegetation. Second, we monitored survival over a relatively short time span, whereas long-term survival can be affected by other factors as well. For example, irregular climatic extremes such as extreme drought can also cause local extinctions of vulnerable species. Such extremes have for example been shown to impact species distributions in other ecosystem types (Vervuren, Blom & de Kroon 2003; Stampfli & Zeiter 2004). Third, we did not study genotypic variation among donor populations, although genetic within-species differences could affect reintroduction outcomes (Zedler 2000). This may have blurred outcomes of this experiment as we used donor material from a variety of climatologically distinct regions. Finally, we did not investigate the potential role of belowground competition, nor did we investigate the potential effect of differences in light availability on (shifts in) biomass allocation to roots and shoots. Aboveground competition is often assumed to be more important for steering community structure and composition than belowground competition (Twolan-Strutt and Kenny 1996, Hautier et al. 2009), especially in more productive environments. However, belowground competition may play an increasingly important role under low nutrient levels (Kotowski et al. 2006), and could therefore be an important regulating factor in the low-productive fens.

6.4.6 Management recommandations for fen restoration

We identified dispersal limitation and excessive competition for light as two important driving mechanisms behind the ongoing loss of endangered fen species. Although dispersal limitation can be lifted by targeted (re-)introduction, light limitation due to a too productive herb layer is more difficult to tackle. A solution to increase light availability in degraded fens may be to completely remove the eutrophied top soil prior to introduction (Emsens et al. 2015), or to suppress the productivity of the herb layer and gradually deplete nutrient pools by regular mowing (Middleton, Holsten & van Diggelen 2006; Hajkova, Hajek & Kintrova 2009).

Overall, we emphasize that reintroduction of low-competitive fen species are only promising if certain conditions are met. First, abiotic conditions should be within the fundamental niche of the target species, i.e. relatively stable groundwater levels near the soil surface and a sufficiently high base cation availability and pH ($pH_{\text{pore water}} > 6$). Second, nutrient availability and concomitant competition for light should be sufficiently low so that growth conditions lie within the realized niche of vulnerable fen species. As a rule of thumb for small sedge and brown moss communities we propose a minimum empirical threshold of 30 % RLI at soil surface level, which corresponds with an estimated herb standing crop < 4 metric ton dry weight ha^{-1}.

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6.5 References

Aggenbach, C.J.S., Backx, H., Emsens, W.J., Grootjans, A.P., Lamers, L.P.M., Smolders, A.J.P., Stuyfzand, P.J., Wolejko, L. & Van Diggelen, R. (2013) Do high iron concentrations in rewetted rich fens hamper restoration? Preslia, 85, 405-420.

Cobbaert, D., Rochefort, L. & Price, J.S. (2004) Experimental restoration of a fen plant community after peat mining. Applied Vegetation Science, 7, 209-220.

Cribari-Neto, F. & Zeileis, A. (2010) Beta Regression in R. Journal of Statistical Software, 34, 1-24.

Cusell, C., Kooijman, A., Fernandez, F., van Wirdum, G., Geurts, J.J.M., van Loon, E.E., Kalbitz, K. & Lamers, L.P.M. (2014) Filtering fens: Mechanisms explaining phosphorus-limited hotspots of biodiversity in wetlands adjacent to heavily fertilized areas. Science of the Total Environment, 481, 129-141.

Cusell, C., Lamers, L.P.M., van Wirdum, G. & Kooijman, A. (2013) Impacts of water level fluctuation on mesotrophic rich fens: acidification vs. eutrophication. Journal of Applied Ecology, 50, 998-1009.

de Vere, N., Jongejans, E., Plowman, A. & Williams, E. (2009) Population size and habitat quality affect genetic diversity and fitness in the clonal herb Cirsium dissectum. Oecologia, 159, 59-68.

DeMalach, N., Zaady, E. & Kadmon, R. (2017) Light asymmetry explains the effect of nutrient enrichment on grassland diversity. Ecology Letters, 20, 60-69.

Emsens, W.J., Aggenbach, C.J.S., Smolders, A.J.P & van Diggelen, R. (2015) Topsoil removal in degraded rich fens: Can we force an ecosystem reset? Ecological Engineering, 77, 225-232.

Emsens, W.J., Aggenbach, C.J.S., Smolders, A.J.P., Zak, D. & van Diggelen, R. (2017) Restoration of endangered fen communities: the ambiguity of iron-phosphorus binding and phosphorus limitation. Journal of Applied Ecology - in press.

Gaudet, C.L. & Keddy, P.A. (1988) A Comparative Approach to Predicting Competitive Ability from Plant Traits. Nature, 334, 242-243.

Geurts, J.J.M., Sarneel, J.M., Willers, B.J.C., Roelofs, J.G.M., Verhoeven, J.T.A. & Lamers, L.P.M. (2009) Interacting effects of sulphate pollution, sulphide toxicity and eutrophication on vegetation development in fens: A mesocosm experiment. Environmental Pollution, 157, 2072-2081.

Goebel, W. (1996) Klassifikation überwiegend grundwasserbeeinflusster Vegetationstypen. Wirtschafts-und Verlagsgesellschaft Gas und Wasser.

Graf, M.D. & Rochefort, L. (2010) Moss Regeneration for Fen Restoration: Field and Greenhouse Experiments. Restoration Ecology, 18, 121-130.

Grootjans, A.P., Adema, E.B., Bleuten, W., Joostn, H., Madaras, M. & Janakova, M. (2006) Hydrological landscape settings of base-rich fen mires and fen meadows: an overview. Applied Vegetation Science, 9, 175-184.

Hajkova, P., Hajek, M. & Kintrova, K. (2009) How can we effectively restore species richness and natural composition of a Molinia-invaded fen? Journal of Applied Ecology, 46, 417-425.

Hautier, Y., P.A. Niklaus, & A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324: 636-638.

Klimkowska, A., Bekker, R.M., Van Diggelen, R. & Kotowski, W. (2010) Species trait shifts in vegetation and soil seed bank during fen degradation. Plant Ecology, 206, 59-82.

Koerselman, W., Bakker, S.A. & Blom, M. (1990) Nitrogen, Phosphorus and Potassium Budgets for 2 Small Fens Surrounded by Heavily Fertilized Pastures. Journal of Ecology, 78, 428-442.

Kooijman, A.M., Beltman, B. & Westhoff, V. (1994) Extinction and Reintroduction of the Bryophyte Scorpidium-Scorpioides in a Rich-Fen Spring Site in the-Netherlands. Biological Conservation, 69, 87-96.

Kooijman, A.M., Cusell, C., Mettrop, I.S. & Lamers, L.P.M. (2016) Recovery of target bryophytes in floating rich fens after 25 yr of inundation by base-rich surface water with lower nutrient contents. Applied Vegetation Science, 19, 53-65.

Kotowski, W., Thorig, W., van Diggelen, R. & Wassen, M.J. (2006) Competition as a factor structuring species zonation in riparian fens - a transplantation experiment. Applied Vegetation Science, 9, 231-240.

Kotowski, W. & van Diggelen, R. (2004) Light as an environmental filter in fen vegetation. Journal of vegetation science, 15, 583-594.

Lamers, L.P.M., Van Roozendaal, S.M.E. & Roelofs, J.G.M. (1998) Acidification of freshwater wetlands: Combined effects of non-airborne sulfur pollution and desiccation. Water Air and Soil Pollution, 105, 95-106.

Lamers, L.P.M., Vile, M.A., Grootjans, A.P., Acreman, M.C., van Diggelen, R., Evans, M.G., Richardson, C.J., Rochefort, L., Kooijman, A.M., Roelofs, J.G.M. & Smolders, A.J.P. (2015) Ecological restoration of rich fens in Europe and North America: from trial and error to an evidence-based approach. Biological Reviews, 90, 182-203.

Larcher, W. 2003. Physiological plant ecology: ecophysiology and stress physiology of functional groups. Springer.

Mälson, K., Backeus, I. & Rydin, H. (2008) Long-term effects of drainage and initial effects of hydrological restoration on rich fen vegetation. Applied Vegetation Science, 11, 99-106.

Mälson, K. & Rydin, H. (2007) The regeneration capabilities of bryophytes for rich fen restoration. Biological Conservation, 135, 435-442.

Manukjanova, A., Stechova, T. & Kucera, J. (2014) Drought survival test of eight fen moss species. Cryptogamie Bryologie, 35, 397-403.

Middleton, B., van Diggelen, R. & Jensen, K. (2006) Seed dispersal in fens. Applied Vegetation Science, 9, 279-284.

Middleton, B.A., Holsten, B. & van Diggelen, R. (2006) Biodiversity management of fens and fen meadows by grazing, cutting and burning. Applied Vegetation Science, 9, 307-316.

Patzelt, A., Wild, U. & Pfadenhauer, J. (2001) Restoration of wet fen meadows by topsoil removal: Vegetation development and germination biology of fen species. Restoration Ecology, 9, 127-136.

Paulissen, M.P.C.P., van der Ven, P.J.M., Dees, A.J. & Bobbink, R. (2004) Differential effects of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen input. New Phytologist, 164, 451-458.

R Development Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rydin, H. & Jeglum, J.K. (2013) The biology of peatlands, 2e. Oxford University Press.

Snowden, R.E.D. & Wheeler, B.D. (1993) Iron Toxicity to Fen Plant-Species. Journal of Ecology, 81, 35-46.

Soudzilovskaia, N.A., Cornelissen, J.H.C., During, H.J., van Logtestijn, R.S.P., Lang, S.I. & Aerts, R. (2010) Similar cation exchange capacities among bryophyte species refute a presumed mechanism of peatland acidification. Ecology, 91, 2716-2726.

Stampfli, A. & Zeiter, M. (2004) Plant regeneration directs changes in grassland composition after extreme drought: a 13-year study in southern Switzerland. Journal of Ecology, 92, 568-576.

Therneau, T.M. & Lumley, T. (2016) Package ‘survival’. Verze.

Tilman, D. (1988) Plant strategies and the dynamics and structure of plant communities. Princeton University Press.

Twolan-Strutt, L., & P.A. Keddy. 1996. Above-and Belowground Competition Intensity in Two Contrasting Wetland Plant Communities. Ecology, 77(1): 259-270.

van de Riet, B.P., Hefting, M.M. & Verhoeven, J.T.A. (2013) Rewetting Drained Peat Meadows: Risks and Benefits in Terms of Nutrient Release and Greenhouse Gas Exchange. Water Air and Soil Pollution, 224.

van der Wal, R., Pearce, I.S.K. & Brooker, R.W. (2005) Mosses and the struggle for light in a nitrogen-polluted world. Oecologia, 142, 159-168.

van Diggelen, J.M.H., Bense, I.H.M., Brouwer, E., Limpens, J., van Schie, J.M.M., Smolders, A.J.P. & Lamers, L.P.M. (2015) Restoration of acidified and eutrophied rich fens: Long-term effects of traditional management and experimental liming. Ecological Engineering, 75, 208-216.

van Diggelen, R., Middleton, B., Bakker, J., Grootjans, A. & Wassen, M. (2006) Fens and floodplains of the temperate zone: Present status, threats, conservation and restoration. Applied Vegetation Science, 9, 157-162.

van Dijk, J., Stroetenga, M., van Bodegom, P.M. & Aerts, R. (2007) The contribution of rewetting to vegetation restoration of degraded peat meadows. Applied Vegetation Science, 10, 315-U314.

Van Haesebroeck, V., Boeye, D., Verhagen, B. & Verheyen, R.F. (1997) Experimental investigation of drought induced acidification in a rich fen soil. Biogeochemistry, 37, 15-32.

Vervuren, P.J.A., Blom, C.W.P.M. & de Kroon, H. (2003) Extreme flooding events on the Rhine and the survival and distribution of riparian plant species. Journal of Ecology, 91, 135-146.

Zak, D., Wagner, C., Payer, B., Augustin, J. & Gelbrecht, J. (2010) Phosphorus mobilization in rewetted fens: the effect of altered peat properties and implications for their restoration. Ecological Applications, 20, 1336-1349.

Zedler, J.B. (2000) Progress in wetland restoration ecology. Trends in Ecology & Evolution, 15, 402-407.

6.6 Supporting information

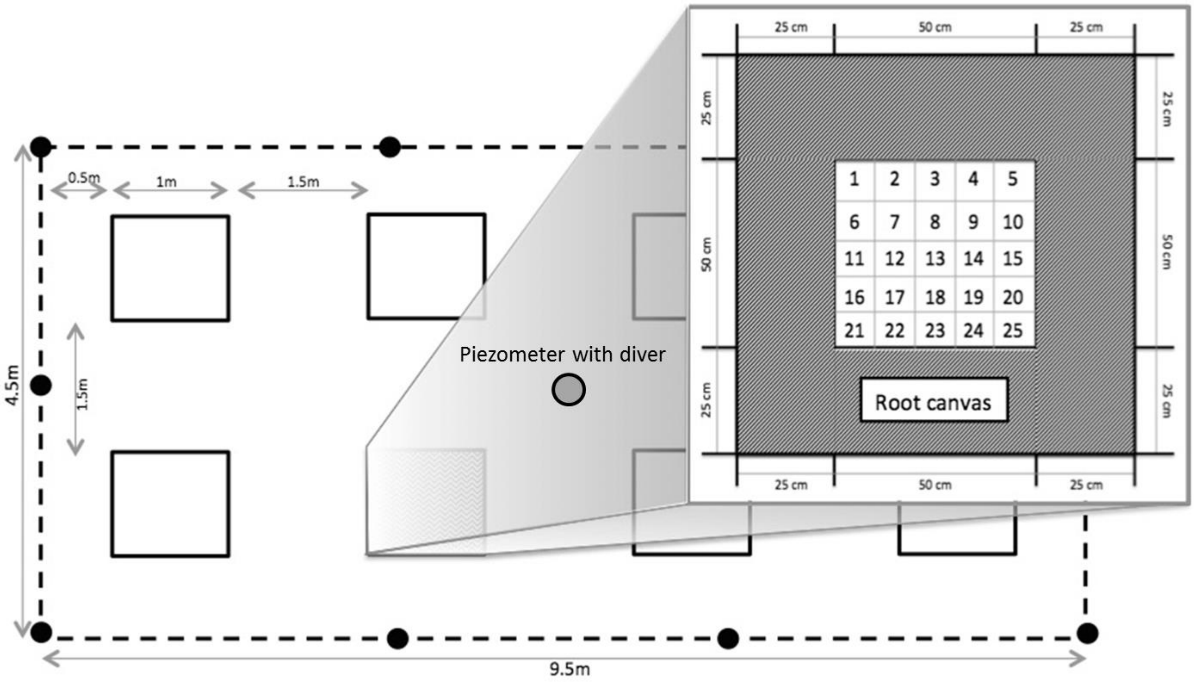

Figure S6.1: Experimental design of the field experiment. A total of eight (1 x 1 m) experimental plots was established at each of the four study sites. Each experimental plot was further subdivided in 25 (10 x 10 cm) blocks in which one individual plant (sedge or moss mat) was introduced.

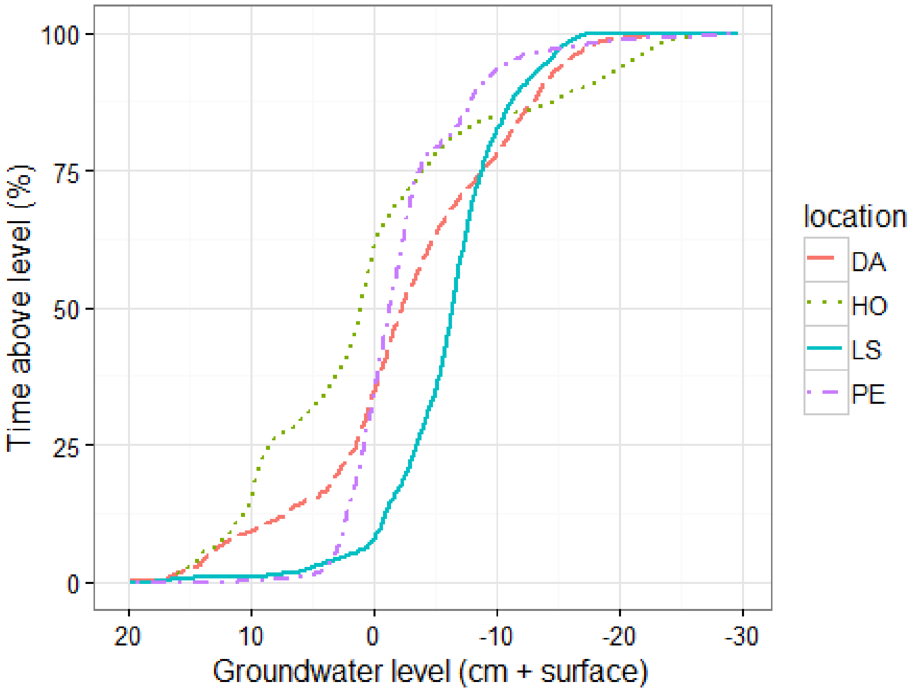

Figure S6.2: Cumulative relative frequency curves of groundwater levels at the four study sites (DA, HO, LS and PE). Data were collected over three consecutive years.

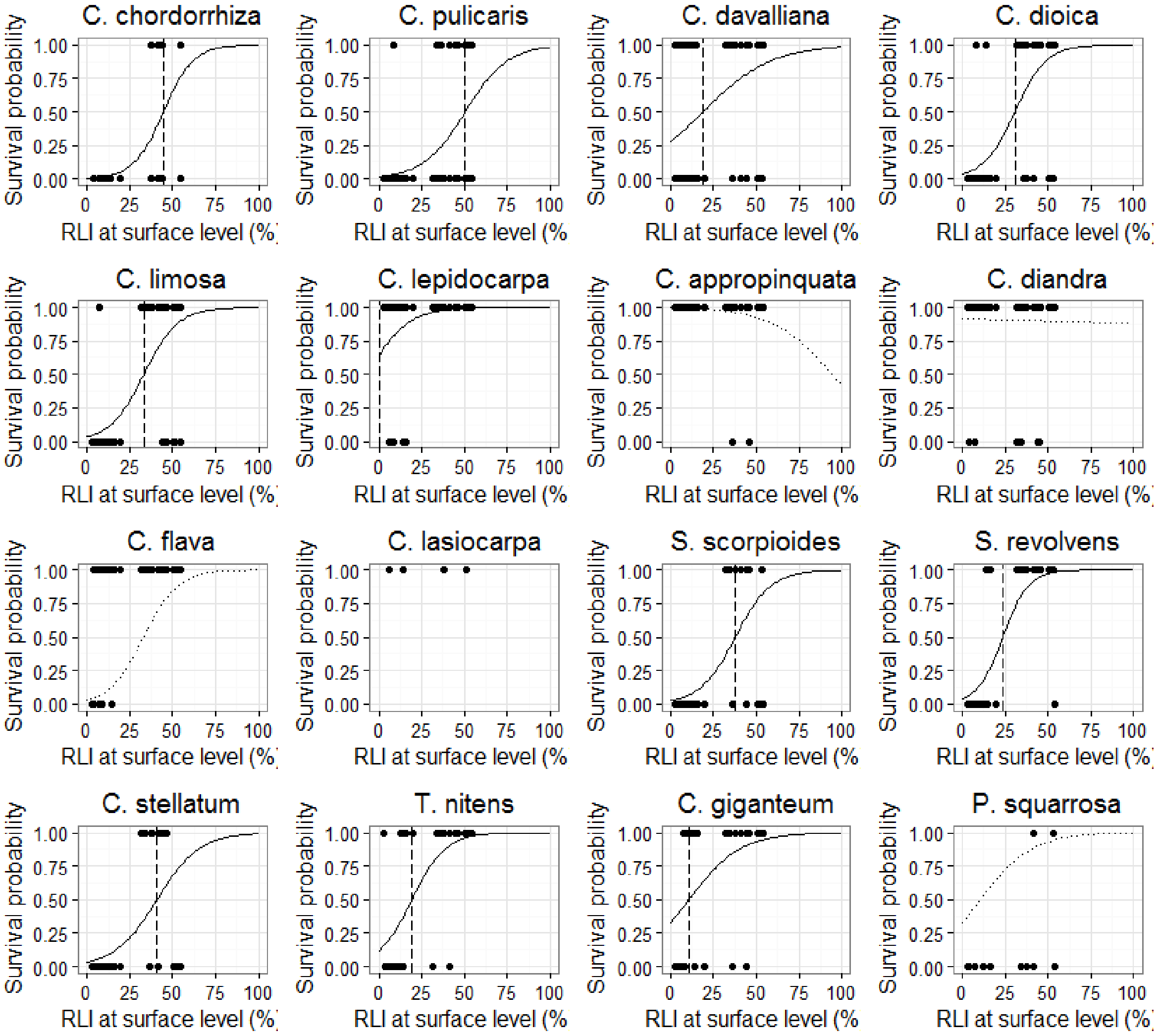

Figure S6.3: Logistic regression curves of survival probabilities of introduced fen species versus relative light intensity (RLI in %) at the soil surface level. Solid curves indicate significant regression models, dotted curves indicate non-significant regression models. Vertical dashed lines indicate the inflection points (i.e. RLI at which survival probability equals 50 %).

Table S6.1: Pore water chemistry and relative light intensities (at soil surface level, RLI) in the experimental plots at the four study sites. Chemical values are based on three sampling rounds (growing seasons of 2014, 2015 and 2016). RLI's are based on three measurements per plot in the final experimental year (2016).

		pH		NO_3^-		NH_4^+		Na		Al		Ca		Fe		K		Mg		Mn		P		S		HCO_3^-		RLI	
				(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(µmol L^{-1})		(%)	
Site	**Plot**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
PE	1	6.6	0.2	3.5	1.7	33.1	47.8	376	84	6.7	2.9	2358	594	2497	1044	93.8	138.2	291	64	43.1	26.0	28.1	9.0	59.8	16.8	4503	461	19.7	14.8
PE	2	6.5	0.1	10.6	16.1	29.4	21.5	361	70	8.7	5.0	2151	271	2694	1209	39.8	12.1	240	4	36.9	12.6	23.1	13.0	95.9	60.6	5209	1463	7.2	0.7
PE	3	6.6	0.2	3.9	2.8	51.0	35.0	333	129	12.7	9.0	2422	423	2965	354	47.5	45.2	282	66	42.1	7.8	48.2	18.6	95.7	7.5	5956	1255	12.5	7.3
PE	4	6.4	0.1	2.3	3.0	50.9	71.0	327	119	7.7	3.5	2620	1007	2084	1522	20.3	20.3	275	125	24.7	14.3	62.6	46.5	83.9	10.7	5579	1770	14.5	6.4
PE	5	6.4	0.3	1.9	0.6	8.7	10.3	311	41	6.7	3.0	1823	1033	1094	1163	14.0	6.1	187	97	30.7	23.1	9.9	7.8	53.7	10.1	3857	2276	8.9	4.1
PE	6	6.4	0.1	2.7	1.5	26.6	26.6	327	47	8.0	2.0	2094	663	2293	930	7.8	9.7	226	52	34.0	13.7	13.6	10.0	55.5	11.2	4650	795	16.0	11.3
PE	7	6.5	0.2	2.6	2.7	16.8	16.2	348	40	5.9	1.7	2534	216	1790	435	34.2	26.4	285	35	31.1	8.1	12.7	7.5	64.7	14.1	5967	2203	11.0	2.1
PE	8	6.6	0.1	20.6	26.7	78.2	100.9	341	90	8.7	3.0	3213	832	2330	1511	144.3	115.6	371	77	59.2	41.9	16.6	8.4	100.6	36.8	6597	1329	12.5	7.9
DA	1	6.6	0.1	1.0	0.7	46.7	64.4	530	79	4.4	2.8	1498	360	2318	359	74.0	111.1	283	85	28.9	24.4	1.6	0.5	20.1	5.3	4331	2087	4.2	2.0
DA	2	6.6	0.1	1.5	0.7	43.7	51.0	541	54	3.8	1.0	1513	431	2326	459	69.5	94.0	306	121	26.5	17.6	2.3	2.8	19.0	6.7	3794	1128	8.1	6.6
DA	3	6.6	0.1	1.0	1.0	8.1	3.4	547	32	4.5	2.3	1672	240	2978	330	26.3	36.9	286	48	20.8	11.0	3.5	2.0	18.0	3.6	5859	1821	13.9	2.7
DA	4	6.6	0.1	1.5	1.1	13.6	9.8	579	44	3.0	1.4	1361	265	2017	314	32.2	32.5	226	50	17.0	3.3	2.3	1.4	15.7	4.4	4432	1306	8.4	3.9
DA	5	6.5	0.1	0.8	0.3	11.1	8.4	568	31	3.1	2.0	1395	383	1859	555	30.4	36.4	236	56	17.2	4.4	3.6	1.4	24.9	11.5	3969	1313	7.4	3.7
DA	6	6.6	0.1	1.0	0.9	11.2	9.7	593	79	3.9	2.4	1572	481	2523	814	41.3	55.2	268	94	20.3	11.3	4.3	0.5	16.9	4.6	4727	1521	3.6	1.1
DA	7	6.5	0.1	1.0	0.5	27.2	33.1	597	83	3.3	2.4	1423	388	1986	1274	47.5	62.6	263	68	18.0	5.9	3.8	1.5	22.1	9.9	3658	590	6.1	3.7
DA	8	6.5	0.1	1.1	0.7	26.2	36.8	609	46	3.3	1.7	1420	455	2048	261	45.4	64.2	235	65	18.0	5.2	5.5	4.4	21.7	11.9	4467	1742	2.8	
HO	1	6.6	0.0	0.5	0.3	13.3	20.6	477	128	0.6	0.5	2683	2422	141	127	93.7	122.4	296	290	11.3	12.5	3.4	4.5	19.0	9.5	4150	2002	44.1	10.2
HO	2	6.6	0.1	0.7	0.7	6.8	5.6	470	88	1.0	0.5	2064	787	184	47	21.4	10.8	215	91	6.8	0.8	1.6	0.8	12.9	5.4	4483	765	46.1	18.0
HO	3	6.6	0.2	0.4	0.5	13.2	20.0	501	138	0.9	0.4	2601	2255	152	213	78.4	104.6	262	242	8.7	10.2	1.4	0.7	15.7	9.4	4360	2309	41.3	5.8
HO	4	6.7	0.2	0.7	1.1	3.8	3.3	470	81	0.4	0.5	2127	1471	147	221	38.1	41.7	223	174	6.1	8.5	1.5	1.0	13.1	6.6	4022	2067	36.2	10.2
HO	5	6.7	0.2	0.4	0.4	6.1	8.4	454	124	0.5	0.5	2549	2319	164	217	46.2	54.9	274	244	13.0	16.4	1.1	0.8	16.4	7.1	4136	2184	53.1	12.0
HO	6	6.8	0.3	0.4	0.4	5.0	4.8	495	94	0.5	0.5	2577	1748	223	199	52.3	73.3	262	191	15.0	16.4	3.0	4.3	13.9	6.2	4391	1583	51.4	24.9
HO	7	6.7	0.0	0.8	1.1	27.3	43.0	485	133	0.6	0.4	3073	1661	387	246	79.5	116.2	318	221	15.7	12.1	3.7	4.4	19.5	7.9	5343	830	54.3	22.1
HO	8	6.7	0.2	0.5	0.6	12.4	19.5	435	81	0.4	0.2	1810	1260	93	125	55.5	76.9	200	156	9.0	12.7	5.0	6.9	17.1	9.5	3218	1264	50.5	16.5
LS	1	6.4	0.3	0.5	0.4	26.0	42.3	727	193	4.4	1.3	1172	630	72	59	88.0	119.8	249	157	4.9	4.6	1.1	0.5	57.2	25.2	2197	268	41.4	4.5
LS	2	6.2	0.2	0.5	0.4	41.9	69.9	878	310	3.7	1.7	1398	1017	85	128	154.4	212.7	317	257	10.0	14.0	1.5	0.4	233.7	200.7	2130	838	37.8	4.2
LS	3	6.3	0.2	0.6	0.4	26.0	42.1	953	186	4.7	1.6	1521	763	109	84	181.4	206.9	353	203	8.2	5.4	1.4	0.4	58.8	33.7	2999	724	37.6	9.6
LS	4	6.2	0.1	0.5	0.4	47.6	80.1	791	233	4.6	1.9	1260	726	78	64	111.3	142.5	279	189	9.7	8.6	1.5	0.5	51.8	22.3	2488	685	45.3	5.5
LS	5	6.2	0.1	0.6	0.6	35.0	56.8	750	184	6.1	1.4	1151	587	53	22	100.1	125.4	245	147	8.6	9.7	2.3	1.3	88.1	31.7	2120	119	44.1	9.2
LS	6	6.3	0.2	0.6	0.5	33.1	53.1	984	272	5.8	1.1	1365	947	108	116	129.8	184.3	297	237	10.5	9.9	1.3	0.4	47.9	5.5	2378	497	31.7	2.4
LS	7	6.3	0.1	0.6	0.6	34.8	54.7	1016	278	4.5	0.8	1514	848	101	90	183.6	222.5	356	235	12.7	7.9	1.1	0.2	36.7	9.8	2990	207	34.2	1.6
LS	8	6.3	0.2	0.6	0.2	42.4	70.5	890	291	5.0	3.4	1489	1062	100	98	138.7	180.7	331	269	12.9	13.3	1.7	0.4	38.3	6.4	2700	467	45.1	7.7

Table S6.2: Comparison of survival distributions between different study sites (DA, HO, LS and PE) within functional groups (adult plants, seedlings, bryophytes). Values indicate χ2 values from a long-rank test (df = 1). Asterisks indicate statistical significance with *p < 0.05, **p < 0.01, *** p < 0.001.

	Adult (*Carex*)			**Seedlings (*Carex*)**			**Bryophytes**		
	Survival			**Survival**			**Survival**		
Study site	HO	LS	PE	HO	LS	PE	HO	LS	PE
DA	16.5***	13.9***	0.20	8.8**	22.9***	0.50	27***	47.5***	2.0
HO		0.10	23.4***		3.50	6.3*		4.2*	14.4***
LS			19.1***			20.0***			31.0***

Table S6.3: Coefficients (± SE) of the two beta-regression models (logit-link) for maximum height of the species (model 1) and relative height increment rate of the seedlings (model 2). Asterisks indicate statistical significance with *p < 0.05, **p < 0.01, *** p < 0.001.

	Model coefficient (logit link)	**SE**
Model 1		
Intercept	-2.005***	0.573
Maximum height (cm)	0.054***	0.011
Model 2		
Intercept	-1.224***	0.398
Relative height increment rate (cm week^{-1})	1.963***	0.400

7

Synthesis

7.1 Iron-rich fens: a distinct fen type?

Research on fen biogeochemistry and -classification has primarily focused on gradients and variation in nutrient availability, water type, vegetation, base saturation and pH. Results from this dissertation suggest that high concentrations of iron (Fe^{3+}-Fe^{2+}) in fen soils and pore water should not be ignored in terms of its effect on fen functioning and vegetation structure and composition. Despite frequent use of the terms “Fe-rich” and “Fe-poor” throughout this dissertation, we never specified when exactly a fen should be regarded as Fe-rich rather than Fe-poor. This is because it would be arbitrary to set threshold values that distinguish between both fen types: we are looking at broad gradients in Fe richness rather than at distinct classes. Overall, total soil Fe pools and pore water concentrations of dissolved Fe can differ several orders of magnitude between and within fens: Fe-poor fens can contain soil Fe pools as low as 1 mmol L^{-1} and pore water Fe concentrations < 5 µmol L^{-1}, whereas Fe-rich fens can contain soil Fe pools as much as 1000 mmol L^{-1} and pore water Fe concentrations > 4000 µmol L^{-1}. In the field, a visually distinct red coloration of the top soil due to iron oxidation is often a useful indication that one is no longer in the very Fe-poor spectrum, but we note that such red coloration can already be visible in the moderately Fe-rich range. As a rule of thumb, based on our research, we believe that both top soil Fe pools and pore water Fe concentrations should minimally exceed 50 mmol L^{-1} and and 500 µmol L^{-1} respectively for a fen to be classified as “Fe-rich”. Although the latter are arbitrary values, mobilisation of DOC, TIC and NH_4^+ was much less pronounced in rewetted peat soils with Fe pools < 50 mmol L^{-1} (**Chapter 3**). Moreover, fens with soil Fe pools > 50 mmol L^{-1} were mostly also P-rich and fairly productive (> 3.3 mmol P L^{-1}, **Chapter 2:** Fig. 2.1b). These values imply that many fens that have been defined as “Fe-rich” in other studies would actually still be in the relatively Fe-poor spectrum (e.g. Stechova et al. 2012).

7.1.1 Nutrient chemistry

In **Chapter 2**, we have shown that Fe contents of natural fen soils correlate positively with soil phosphorus (P) contents. This pattern of Fe-P co-accumulation was so universal that it occurred independent of fen degradation level (pristine or rewetted), location (Benelux or Poland), or soil sampling depth (from 0 to 500 cm below the surface). These results suggest that input, retention and accumulation of P in a fen are tightly connected to input, retention and accumulation of Fe. We relate this to a “Fe-P trapping” mechanism in Fe-rich fens: any P that enters the fen is retained in the soil due to sorption to amorphous Fe compounds or through the formation of ferrous phosphates such as vivianite (Patrick and Khalid 1974, Rothe et al. 2014). Another non-exclusive explanation for this pattern may be that there is a coupled inflow of Fe and P in fens, e.g. if Fe-rich groundwater that discharges in a fen is also P-rich. Although we did not investigate the exact mechanisms behind such Fe-P co-accumulation in detail our study supports the hypothesis that Fe-rich fens are often P-rich fens, at least in continental Europe.

In **Chapter 3**, we conducted a mesocosm experiment to investigate the effects of fen rewetting on nutrient and carbon mobilization in pore water of drained Fe-rich and –poor soils. We observed a disproportionate mobilization of dissolved Fe, total inorganic carbon (TIC), dissolved organic carbon (DOC) and ammonium (NH_4^+) in the pore water of the rewetted Fe-rich fens, whereas mobilization of these compounds was significantly lower in the rewetted Fe-poor fens. Overall, there was a strong correlation between pore water concentrations of dissolved Fe and concentrations of DOC, TIC and NH_4^+. Concentrations of NH_4^+ in the Fe-rich fen soils even reached eutrophic and potentially phytotoxic thresholds (> 1 mmol L^{-1}). Although we need to be cautious when interpreting these correlative patterns, such results suggest an interaction between fen rewetting and Fe-mediated breakdown of organic matter. This may also imply that fen rewetting does not necessarily lead to restoration of the sink service of fens for carbon and nutrients, at least not in the short term.

In **Chapter 4**, we experimentally investigated how fen eutrophication with macronutrients (N, P and K) affects primary plant and litter production, litter quality and decomposition of six wetland *Carex* species. Fen eutrophication may be the result of internal processes (e.g. fen drainage and degradation) or nutrient input from external sources (e.g. inflow of NPK-polluted water or N deposition), but high nutrient levels can also be related to in situ fen characteristics (e.g. high Fe contents correlate with a higher availability of P and NH_4^+, **Chapters 2 & 3**). We found that eutrophication affects litter accumulation and –decomposition at multiple levels, in which stimulatory and inhibitory effects interact. Although eutrophication strongly increased primary (and thus fresh litter) production as expected, a concomitant increase in tissue quality of the vegetation correlated with a higher litter decomposability. Such contrasting effects of eutrophication on litter input and decomposition can, in theory, outweigh each other. Direct effects of altered nutrient levels in the external environment on litter decomposition rates were unpredictable and seemed dependent of litter chemistry. Overall, these observations led to the novel hypothesis that both mesotrophic and eutrophic fens can accumulate peat, but that the underlying biogeochemical mechanisms may be different.

7.1.2 Vegetation structure and composition

The dominant effect of iron on fen (nutrient) chemistry and functioning (**Chapters 2 & 3**) is reflected in the structure and composition of the vegetation of Fe-rich fens (Figure 7.1). Our field survey for example revealed that numbers of endangered (red list) species are often high and low in Fe-poor and Fe-rich fens respectively (**Chapter 2**). We found that this may primarily be due to the generally large P pools and high P availability in Fe-rich fens: P limitation limits herb biomass production, which is crucial for the persistence of many endangered species (Boeye et al. 1997, Wassen et al. 2005). This is because a highly productive herb layer in fens typically results in the competitive exclusion of small-stature and vulnerable fen species, usually as a direct consequence of asymmetric light limitation (Kotowski et al. 2006, DeMalach et al. 2017). The negative correlation between vegetation N:P ratios and standing herb biomass in our study indeed suggested an overall increase in herb productivity from P- towards N limitation, which correlated with an increase in fen Fe richness. In other words, the vegetation of Fe-rich fens was more often high-productive and N-limited rather than low-productive and P-limited. Although N limitation can also limit primary production (Venterink et al. 2003, Pawlikowski et al. 2013), rates of N deposition in the modern landscape often exceed critical loads (Morris 1991, Kooijman 2012), and this may have increased the relative importance of P limitation for the persistence of low-productive communities. We note that the observed high herb productivity in Fe-rich fens may not solely be a result of the larger soil P pools, but it may also be co-explained by the stimulant effect of iron reduction or iron-induced phenol oxidase activity on organic matter decomposition and concomitant NH_4^+ release (**Chapter 3**).

In **Chapter 6** we provided experimental evidence for these correlative observations by monitoring survival of 679 transplants (of 16 endangered species) of small sedges and brown mosses in four restoration fens. As expected, 3-year survival was highest (mean = 79 %) in the low-productive fens. Here, we were able to directly relate the high mortality of target species in the Fe-rich fens to a highly productive herb layer and concomitant asymmetric competition for light. We found that on average at least 30 % of incoming photosynthetically active radiation (PAR) needs to penetrate through the vegetation canopy and reach the soil surface to ascertain long-term survival of most small and vulnerable fen species. This value corresponds with an estimated (herb) standing crop < 4 metric tons ha^{-1} (**Chapter 5**). Unfortunately, herb productivity in many remaining fens in the Northern hemisphere is often well above such low values (**Chapters 2 & 5**).

Vegetation structure and composition of Fe-rich fens is possibly also governed by direct toxicity effects of high concentrations of ferrous iron in pore water (> 1000 μmolars L^{-1}) (Wheeler et al. 1985, Snowden and Wheeler 1993). Wetland plants generally have an array of functional mechanisms and morphological adaptations that allow them to cope with metal toxicity (e.g. rhizosphere oxidation through radial oxygen loss (ROL) or internal coping mechanisms (Laan et al. 1989, Deng et al. 2009)). Interspecific differences in Fe tolerance levels will therefore directly affect the occurrence and abundance of different plant species. In theory, the more Fe-tolerant species will be most likely to thrive in Fe-rich fens due to an enhanced competitive ability, whereas Fe-intolerant species should perish. In **Chapter 6** however, the very high first-year survival of the transplants in the Fe-rich fens suggests that direct Fe toxicity does not limit growth nor survival of many of the characteristic species of small sedges and brown mosses. This implies that if Fe-tolerance plays a role, it is more likely to facilitate than to hamper these characteristic species.

Finally, it is plausible that the abovementioned species-specific adaptations to cope with high concentrations of ferrous iron have a regulating effect on the biogeochemistry of Fe-rich fens. For example, high rates of radial oxygen loss from plant roots may affect biogeochemical conditions in the rhizosphere and top soil (Flessa 1994, van Bodegom et al. 2005a). We hypothesize that the presence of such an “oxygen pump” in an otherwise completely anoxic fen soil should increase rates of organic matter mineralization and nutrient mobilization on a micro-scale. This may be due to the direct effect of oxygen release through plant roots, but it can also be the result of anaerobic decomposition when Fe^{2+} in the rhizosphere is oxidized to Fe^{3+} (e.g. as root iron plaques) and thereafter (again) reduced to Fe^{2+} (Weiss et al. 2005). These plant-functional mechanisms are likely to further increase rates of organic matter mineralization and nutrient cycling in Fe-rich fens.

Figure 7.1: Typical view of a Fe-rich fen (top soil Fe pool > 50 mmol L^{-1}). Characteristic is the (often) relatively high productivity of the herb layer, which is usually dominated by intermediate and tall sedges (e.g. *Carex rostrata*, *C. aquatilis*), *Equisetum spp*., *Menyanthes trifoliata*, *Comarum palustre* and *Ranunculus lingua*. The moss layer is typically a mixture of *Calliergonella cuspidata* and *Calliergon* spp., with patches of *Climacium dendroides* or *Plagiomnium* spp. Iron oxidation is usually clearly visible at the soil surface (red color), and the peat is often highly decomposed (even in deep layers).

7.1.3 Hypothetical summary: the biogeochemical functioning of Fe-rich fens

In this section we combine existing knowledge with results from this thesis, and we briefly summarize the biogeochemical functioning of Fe-rich fens. In summary, we postulate that the following biogeochemical mechanisms dominate in Fe-rich fens:

1) The iron-redox wheel (Fig. 7.2), which is highly active in fluctuating redox environments in organic wetland soils (Lovley and Phillips 1986, Lindsay 1991, Davidson et al. 2003, Li et al. 2012). The iron-redox wheel is coupled to the biogeochemical cycles of carbon, nitrogen and phosphorus through direct effects on anaerobic decomposition but also through reductive dissolution and desorption of Fe-bound P upon each reduction cycle (**Chapter 2 & 3**). The redox wheel in Fe-rich fens can be triggered both on a macro-scale (e.g. fluctuating water levels) and on a micro-scale (e.g. radial oxygen loss in the rhizosphere).

2) Fe-P co-accumulation. Fe-rich fens are often P-rich fens, which we attribute to “P trapping” and P retention by Fe compounds. Much of this Fe-bound P appears plant-available (**Chapter 2**), especially under anoxic conditions.

3) Fe toxicity. Although we found no evidence of Fe toxicity for species of small sedges and brown mosses (**Chapter 6**), Fe toxicity is a well-known mechanism in wetlands that causes necrosis and death in many (mainly Fe-intolerant) plant species (Snowden and Wheeler 1993). Fe toxicity is therefore very likely to affect vegetation structure and composition in fens.

4) High phenol oxidase activity. Studies suggest that high concentrations of ferrous iron may promote activity of the enzyme phenol oxidase (van Bodegom et al. 2005b, Wang et al. 2017). Phenol oxidase catalyzes the oxidation and removal of phenolics, meaning that a high activity may stimulate rates of organic matter decomposition. Phenol oxidase may therefore be an additional explanation for the high mobilization of dissolved carbon and ammonium that we observed upon rewetting drained Fe-rich fens (**Chapter 3**).

We hypothesize that these biogeochemical mechanisms result in the following characteristics of wet Fe-rich fens:

1) Relatively high rates of organic matter decomposition and nutrient cycling, directly related to:

   - The iron-redox wheel (anaerobic decomposition coupled with Fe reduction) (**Chapter 3**, Li et al. 2012)
   - The generally more eutrophic environment of Fe-rich fens, which increases plant litter quality and thus litter decomposability (**Chapter 4**)
   - High phenol oxidase activity

2) Productive herb layer and strong competition for light. This can be related too:

- High rates of organic matter decomposition and concomitant (inorganic) nutrient mobilization (**Chapters 2 & 3**)
- Fe-P co-accumulation and a high P availability (**Chapter 2**)

3) High potential for alternate acidification and alkalinity generation under fluctuating water levels:

   - Rewetting of drained Fe-rich fens generates large amounts of HCO_3^- (Aggenbach et al. 2013), thereby raising pH (**Chapter 2**)
   - Drainage or oxidation of wet Fe-rich fens generates acidity through ferrous iron oxidation

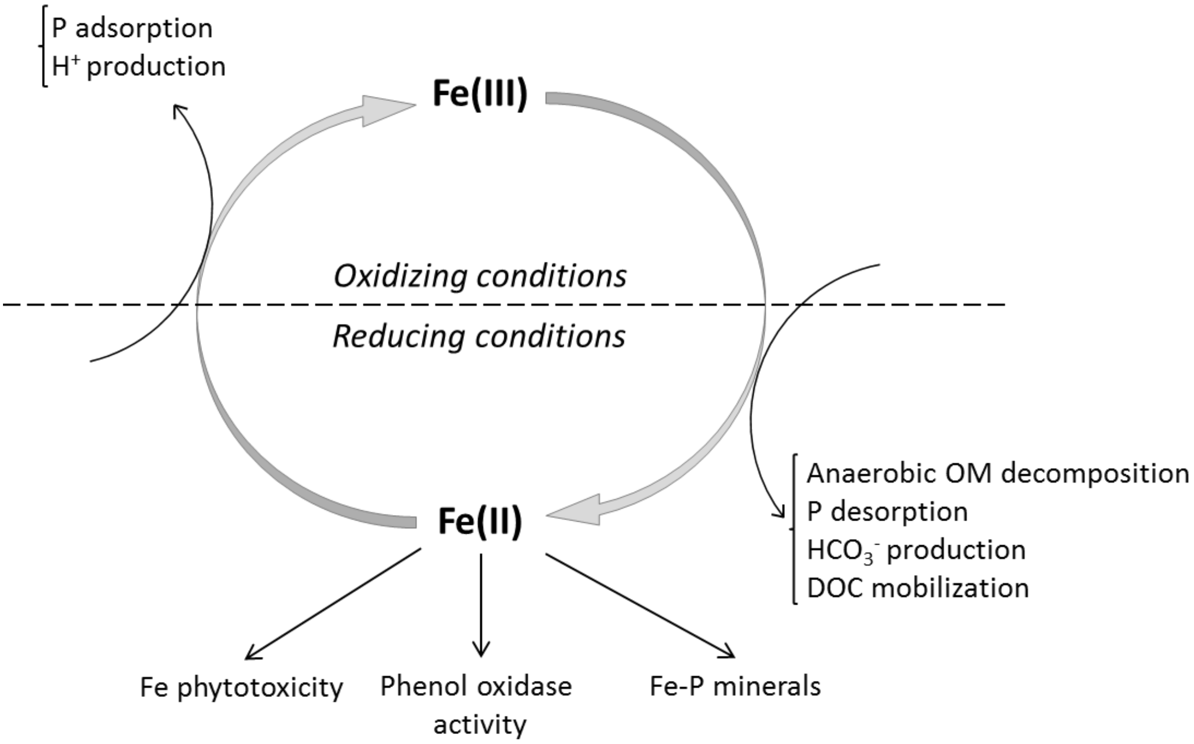

Fig. 7.2: Simplified representation of the main biogeochemical processes in fens that are directly connected to the iron-redox wheel. Note that the redox wheel also affects biogeochemical cycling of other elements (e.g. S and N), which has not been incorporated in this scheme.

7.2 Fen restoration

7.2.1 Rewetting

Fen rewetting, i.e. re-installing the original hydrological regime, is typically the first step in the restoration of drained fens. However, rewetting of drained fens often leads to high mobilization of dissolved organic and inorganic carbon (DOC and DIC), ammonium (NH_4^+), and phosphate (PO_4^{3-}) into pore water of the top soil layer (Van Dijk et al. 2004, Zak and Gelbrecht 2007), at least in the early stages of rewetting.

In **Chapter 3** we have shown that such excessive release of compounds upon rewetting correlates with soil Fe content: rewetting of drained Fe-rich fen soils led to a much higher mobilization of NH_4^+, DOC and TIC in pore water in comparison to rewetting of drained Fe-poor soils. Possibly, this is related to the anaerobic Fe-mediated decomposition of organic matter (i.e. the “iron redox wheel”) (Lovley and Phillips 1986, Li et al. 2012, Knorr 2013) or the increased activity of phenol oxidase (van Bodegom et al. 2005b), although the release of DOC upon rewetting may also be due to other biogeochemical factors such as the dissolution of Fe-DOC coagulates (Riedel et al. 2013).

In **Chapter 2**, we additionally tested for the interactive effects of fen water levels (drained or rewetted) and soil Fe- and P contents on P uptake by individual clones of *Carex rostrata*. As expected, individuals of *Carex rostrata* had a much higher P uptake when grown on rewetted soils, which we relate to the reductive dissolution and desorption of Fe-bound P under low redox potential (Patrick and Khalid 1974, Zak et al. 2010), in which P is either mobilized or less firmly sorbed to Fe compounds and therefore more easily accessible for plant uptake. Overall, P uptake by *Carex rostrata* was largest in the rewetted Fe-rich soils, as the Fe-rich soils also contained the largest P pools.

Based on the results from **Chapter 2 & 3** we postulate that rewetting of drained Fe-poor fens may have a higher success rate if the goal is to restore peat-accumulating, mesotrophic fens that are dominated by small sedge and brown moss communities. Rewetting of drained Fe-rich fens is more likely to lead to development of eutrophic (P- and NH_4^+-rich) fens with a more productive herb layer.

However, we do not claim that drained Fe-rich fens should therefore not be rewetted: if the goal is to restore a potentially peat-accumulating fen rather than a fen meadow, then complete rewetting is without exception a first and crucial step. Peat can only accumulate under waterlogged conditions. Moreover, most research (including our own) on fen rewetting is based on short-term measurements. It is likely that long-term (decades to centuries) and stable rewetting may gradually result in a more feasible environment for small sedge and brown moss communities. At this point nobody knows what centuries of rewetting will do to the biogeochemistry of degraded and previously drained fens, but it is plausible that dissolved compounds such as DOC, ammonium, ferrous iron and phosphates will be gradually washed out with water flow, and that constant waterlogging will reinstate peat accumulation which may gradually result in a more feasible top soil environment.

7.2.2 Topsoil removal

Sometimes “simple” restoration measures such as rewetting seem insufficient for the restoration of species-rich and peat-accumulating mesotrophic fens, especially in the short term. This may be due to the fact that long-term drainage and degradation can severely alter biogeochemical conditions in the top soil. Moreover, many remaining fens have been impacted by global land-use intensification and have a history of fertilization (Lamers et al. 2015). In **Chapter 5** we investigated the hypothesis that complete removal of a degraded and eutrophied top peat layer, thereby exposing an underlying peat layer, can improve conditions for rich fen development. By comparing topsoil removal plots with control plots in six degraded fens, we showed that topsoil removal generally led to smaller soil pools of Fe, P and KCl-extractable NH_4^+, while organic matter contents and soil C:N ratios were higher. Moreover, topsoil removal evidently led to higher groundwater levels, and this correlated with higher concentrations of base cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and HCO_3^- in pore water. In response we observed lower herb biomass in the topsoil removal plots, which correlated with a higher bryophyte cover and the establishment of light-demanding fen species. This led to the conclusion that topsoil removal in drained and degraded fens can improve the prospects of fen restoration, which is supported by an increasing amount of evidence (Harpenslager et al. 2015, Zak et al. 2015, Zak et al. 2017).

7.2.3 Species (re-)introduction

Many endangered plant species (e.g. small sedges and brown mosses) remain absent in restoration fens, even after topsoil removal or fen rewetting, whereas taller and more common wetland species often thrive (van Dijk et al. 2007, Malson et al. 2008, Aggenbach et al. 2013). Results from our transplantation experiment (**Chapter 6**) suggest that (re-)colonization of restoration fens by typical fen species may be hampered by dispersal limitation: 3-year survival of the transplants was very high in two low-productive fens. This indicates that despite apparently suitable abiotic conditions in some fens, many target species may simply be absent because they are not able to (re-)colonize the fen. In the remaining two Fe-rich fens most transplants gradually disappeared due to a too high herb productivity, which led to asymmetric light limitation. Here, the generally small and slow-growing fen species were simply not able to outgrow light limitation. We conclude that manual species (re-)introduction can bypass the bottleneck of dispersal limitation in restoration fens, but for small sedge and brown moss communities this is only feasible if overall herb productivity is sufficiently low. We propose that at least 30 % of total incoming photosynthetically active radiation (PAR) needs to reach soil surface level.

7.2.4 Mowing and grazing

We did not investigate the effects of mowing or grazing, yet we want to briefly discuss the potential of both measures for fen restoration. Both measures are typically applied to open up the vegetation, to prevent tree encroachment, or to gradually deplete nutrient pools (mowing followed by hay removal). What we must realize however is that most ecosystems in lowland Europe in which these measures are commonly applied are part of a semi-natural and anthropogenic landscape. The natural climax vegetation would otherwise simply be forest, albeit possibly with some grazer- or river-induced patchiness. Many (though not all) groundwater-fed fens on the contrary are natural systems that have historically been able to persist for thousands of years, often largely independent of anthropogenic practices, and therefore we should be skeptical with respect to introducing mowing or grazing as a "restoration" strategy in fens. With respect to grazing or browsing, we believe that drawbacks outweigh the potential benefits. First, grazing and browsing typically results in redistribution (or increased recycling rates) of nutrients rather than in a nutrient efflux. Second, cattle (especially large ungulates) can severely damage a waterlogged peat soil by trampling and this often results in a loss of characteristic fen species (pers. obs. WJ Emsens, Stammel et al. 2003, Bergamini et al. 2008). With respect to mowing, things are a bit more complicated. First, hay removal can be a significant pathway for nutrient removal, and this may for example partly compensate for the modern-day high N deposition rates. However, P pools in (mostly) Fe-rich fens are often so high (10-100 mmol P L^{-1} soil, **Chapter 2**) that centuries of consistent haymaking would be required to reach the target of 3.3 mmol P L^{-1} for the upper 30 cm of the peat soil. Therefore, although regular opening up of the vegetation can somewhat reduce the performance of strong competitor species and increase overall light availability (Opdekamp et al. 2012), mowing will not always lead to the restoration of a nutrient-poor environment within a reasonable time span. Second, mowing can be used as an effective strategy to combat encroachment of trees and reed. Drained and rewetted fens, much more so than pristine fens, are often prone to rapid encroachment of alder, birch and willow as topsoil structure and hydrological regime have usually been altered. However, we need to be aware that the use of heavy machinery on waterlogged organic soils also causes soil compaction and destroys microtopography (Kotowski et al. 2013). The former may eventually even further facilitate tree encroachment, whereas the latter will result in the loss of valuable small-scale variation in habitat. Finally, it is worth mentioning that pristine fens are not necessarily devoid of trees: sparsely scattered trees can be a common sight.

7.3 Uncertainties and recommendations

Results from this dissertation have given some more insight in fen biogeochemistry as well as in the restoration potential of degraded fens, but our results also generated new research questions and introduced new uncertainties.

7.3.1 Uncertainties and recommendations for science

The first uncertainty relates to the correlative character of most research presented in this dissertation, as correlation does not equal causation. Although we disentangled many different processes that may govern hydrogeochemical processes and vegetation composition in Fe-rich and -poor fens, we cannot ascertain that our observations are always the direct result of (natural?) gradients in Fe richness. For example, it may be that large soil pools of Fe are an artifact of long-term fen drainage and degradation, as fen drainage triggers carbon loss and an increase in soil bulk density but at circumneutral pH it also triggers concomitant immobilization and accumulation of Fe in the aerated top soil (Fig. 7.3, Aggenbach et al. 2013). If so, then gradients in overall Fe richness of fens may not only reflect natural gradients in Fe concentrations of groundwater that feeds the fens, but it may also be due to gradients in fen "degradation level" and altered peat properties. The latter factor alone may greatly affect biogeochemical processes in fens (Zak et al. 2010), regardless of soil iron content. Nonetheless, it is highly plausible that both factors (fen degradation level and "natural" iron richness) interact and possibly even strengthen each other's adverse effects. Related to this uncertainty, another question that has remained partially unanswered is whether Fe-rich fens can be considered a distinct fen type that is "naturally" more eutrophic, decomposed and relatively species-poor (in terms of endangered fen species), or if these fens have simply been more heavily affected by fen drainage and degradation: high iron contents may then be a symptom rather than a cause. Regardless of the exact mechanism, it is clear that extremely Fe-rich fens (soil Fe pool > 50-100 mmol L^{-1}) are not often covered by low-productive communities of small sedges and brown mosses.

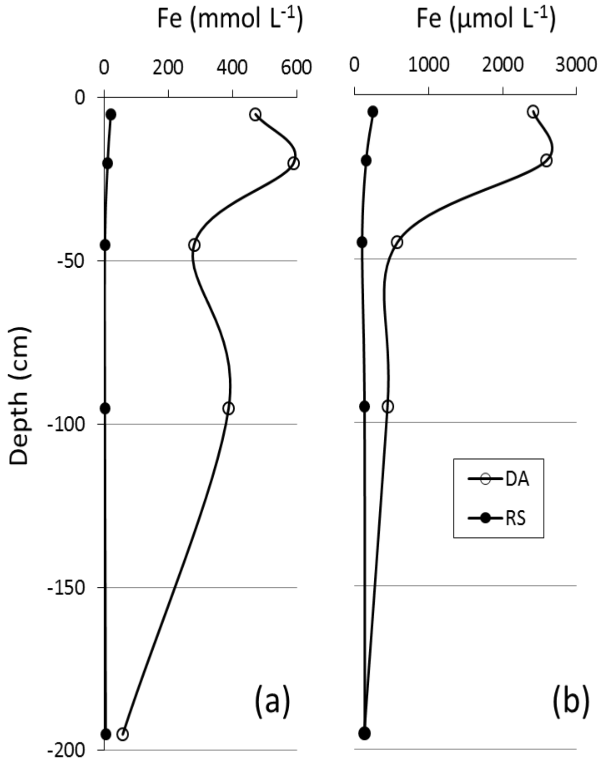

Figure 7.3: Depth profiles of total Fe (a) soil pools (mmol L^{-1}) and (b) pore water concentrations (µmol L^{-1}) in the peat layer of two fens with contrasting histories. Site DA is a rewetted fen with a long history of deep drainage and degradation (Drentsche Aa, Gasterense diep, the Netherlands), whereas site RS is a relatively undisturbed fen (Rospuda valley, Poland) (Emsens et al. *unpublished data*).

Second, many of the processes that we studied (e.g. Fe reduction, organic matter decomposition, P limitation) are mediated or influenced by microbial activity. Although we made an effort to also explain observations from a microbial approach (e.g. **Chapters 3 & 4**), we did not quantify nor qualify microbial (functional) diversity (e.g. using Phospholipid-derived fatty acids (PLFA) or Next-Gen DNA Sequencing). Therefore, all microbiology-related insights presented in this thesis are hypothetical rather than empirical. We believe that microbiology is the next frontier in (future) fen and peatland research, and therefore we urge microbial wetland researchers to upscale micro-scale results to the ecosystem level. Extremely relevant and interesting would be to quantify and qualify differences or shifts in microbial community and -activity between Fe-rich and Fe-poor fens, between pristine and degraded fens, between P-limited or N-limited fens, or before and after implementation of different fen restoration techniques.

Third, we did not investigate belowground [plant] biotics. This is relevant, as peat accumulation in fens typically follows a different pathway than peat accumulation in, for example, ombrotrophic bogs. In bogs, peat accumulation occurs through horizontal layering due to upward growth and partial decay of (mainly) *Sphagnum* in the top soil, whereas peat accumulation in fens is much more determined by partial decay of roots and rhizomes that grow downward into the old peat matrix. The latter also implies that aboveground herb litter is probably only of minor importance for peat accumulation in fens. In **chapter 4** we unfortunately only investigated aboveground litter production and litter quality, and therefore we suggest that future research should primarily focus on the quantification and qualification of root production, root decay, but also on decomposer communities in deeper, fully anoxic peat layers. Related to this, we investigated how aboveground competition for light affects survival of characteristic fen species (**chapter 6**) and plant community structure and composition (**chapter 5**), but we did not investigate belowground competition (e.g. rhizosphere competition for nutrients). Little is known about belowground competition in fens, and we do not know if (and how) it plays a significant role. In general, the effect of belowground competition on plant community composition and plant vigor is considered of limited importance compared to the effect of aboveground competition (Twolan-Strutt and Kenny 1996, Hautier et al. 2009), especially in more productive environments. However, it is not unlikely that belowground competition starts to play an increasingly important role with decreasing nutrient supplies (Kotowski et al. 2006), and therefore its relevance for rich fens may be underestimated or overlooked. Quantification and qualification of these belowground processes will be, together with microbial research, the next big step forward in fen research.

7.3.2 Uncertainties and recommendations for fen management and restoration

The three restoration techniques that we investigated in this dissertation (i.e. rewetting (**Chapters 2 & 3**), topsoil removal (**Chapter 5**), and species (re-)introduction (**Chapter 6**) had variable outcomes: restoration success in terms of species diversity, nutrient availability and –mobilization, and carbon release seemed highly dependent on overall fen biogeochemistry. We can however draw some general conclusions with respect to fen restoration techniques, and we highlight some uncertainties.

(1) The effect of topsoil removal in rich fens (in which an underlying peat layer is exposed) is only documented fragmentarily (Patzelt et al. 2001, Klimkowska et al. 2010, Klimkowska et al. 2015, Zak et al. 2015), and different studies typically report contrasting results. In our study however, removal of a heavily degraded top peat layer always had overall neutral to positive effects on fen chemistry and vegetation structure and –composition, and we never registered clearly adverse effects (**Chapter 5**). This thus suggests that sudden exposition of a "fresh" peat layer after topsoil removal does not necessarily have a long-lasting negative impact on fens.

Nonetheless, we must emphasize that we only investigated a select set of (mainly biochemical) variables on a local (plot) scale, while there are other factors that should be taken into account when considering topsoil removal as a restoration measure. Fen restoration projects are therefore normally embedded in a larger landscape context, thereby also including the topography and hydrology of the system as a whole (Zak et al. 2017). For example, topsoil removal in one area can negatively affect other connected areas if it results in drainage of the latter. Vice versa, topsoil removal can fail if it leads to accumulation and stagnation of open water in the topsoil removal area, thereby "restoring" a pond rather than a fen. Therefore, we strongly urge nature managers to be very cautious when considering topsoil removal in fens: it cannot be undone. Finally, removal of large volumes of peat may also result in a large CO_2 efflux to the atmosphere when the removed peat layer is left to oxidize.

(2) Rewetting, e.g. by closing drainage ditches or also by topsoil removal, is without exception always a crucial first step in the restoration of drained fens: pristine fens are characterized by (ground-)water levels close to soil surface level for most of the year, and such waterlogging is a prerequisite for peat accumulation. However, if fens have been drained for a long time or if they have accumulated large pools of Fe, then fen rewetting may initially lead to high mobilization rates of carbon (DOC and TIC) and NH_4^+ in pore water (**Chapter 3**), and P will become less limiting for plant growth due to reductive dissolution and desorption of the redox-sensitive P fraction (**Chapter 2**). As a result, the herb layer in rewetted fens often remains fairly productive. Although we did not investigate long-term (= several decades to centuries) effects of rewetting, we are convinced that the combination of continuously stable water levels at soil surface level and patience may eventually lead to more suitable abiotic conditions for peat accumulation and the development of typical fen communities. At some point after rewetting the bulk of electron acceptors (including Fe^{3+}) should have been consumed which may result in decreased rates of organic matter decomposition, whereas dissolved nutrients and DOC may be gradually "flushed" out. The latter supposition, however, is merely hypothetical as we will need long-term studies to confirm that such shifts are taking place in long-term rewetted fens.

(3) Plant species (re-)introduction is usually not considered to be a crucial step in fen restoration, and nature managers are often reluctant to do so. Although we agree that endangered species should ideally be able to re-colonize a fen by natural dispersal mechanisms after abiotic fen restoration, this is in practice not always the case. Many wetland species indeed recolonize rewetted fens, but these are typically more common species (e.g. the sedges *Carex rostrata*, *C. lasiocarpa* or *C. demissa* or the bryophytes *Calliergonella cuspidata* or *Climacium dendroides*). Endangered fen species (e.g. *Carex dioica*, *C. pulicaris*, *C. lepidocarpa* or the bryophytes *Hamatacoulis vernicosus, Scorpidium scorpioides* or *Tomentypnum nitens*), in contrast, rarely seem able to naturally (re-)colonize restoration fens. One of the possible explanations is obvious: most of these species have become very rare due to (past) widespread fen degradation, or in worst cases they have gone completely extinct on a regional scale. Add to that problems of genetic impoverishment and -drift of relict populations, as well as ongoing habitat fragmentation and -loss, and it may become clear why these species are often absent in rewetted fens. We believe that targeted (re-)introduction of these species may then be a solution, but only if abiotic conditions are presumably suitable (so preferably in combination with other restoration techniques such as rewetting and/or topsoil removal). We should however specifically mention that we did not study genotypic variation within the donor material used in **Chapter 6**, although genetic within-species differences could affect reintroduction outcomes (Zedler 2000). We therefore advise conservationists and scientists to take genetics into account before conducting actual large-scale re-introduction.

7.4 References

Aggenbach, C. J. S., H. Backx, W. J. Emsens, A. P. Grootjans, L. P. M. Lamers, A. J. P. Smolders, P. J. Stuyfzand, L. Wolejko, and R. Van Diggelen. 2013. Do high iron concentrations in rewetted rich fens hamper restoration? Preslia 85:405-420.

Bergamini, A., M. Peintinger, S. Fakheran, H. Moradi, B. Schmid, & J. Joshi. 2009. Loss of habitat specialists despite conservation management in fen remnants 1995–2006. Perspectives in Plant Ecology, Evolution and Systematics, 11(1): 65-79.

Boeye, D., B. Verhagen, V. VanHaesebroeck, and R. F. Verheyen. 1997. Nutrient limitation in species-rich lowland fens. Journal of Vegetation Science 8:415-424.

Davidson, E. A., J. Chorover, & D. B. Dail. 2003. A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. Global Change Biology, 9(2): 228-236.

DeMalach, N., E. Zaady, and R. Kadmon. 2017. Light asymmetry explains the effect of nutrient enrichment on grassland diversity. Ecology Letters 20:60-69.

Deng, H., Z. H. Ye, and M. H. Wong. 2009. Lead, zinc and iron (Fe2+) tolerances in wetland plants and relation to root anatomy and spatial pattern of ROL. Environmental and Experimental Botany 65:353-362.

Flessa, H. 1994. Plant-Induced Changes in the Redox Potential of the Rhizospheres of the Submerged Vascular Macrophytes Myriophyllum-Verticillatum L and Ranunculus-Circinatus L. Aquatic Botany 47:119-129.

Harpenslager, S. F., E. van den Elzen, M. A. R. Kox, A. J. P. Smolders, K. F. Ettwig, and L. P. M. Lamers. 2015. Rewetting former agricultural peatlands: Topsoil removal as a prerequisite to avoid strong nutrient and greenhouse gas emissions. Ecological Engineering 84:159-168.

Hautier, Y., P.A. Niklaus, & A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324: 636-638.

Klimkowska, A., W. Kotowski, R. van Diggelen, A. P. Grootjans, P. Dzierza, and K. Brzezinska. 2010. Vegetation Re-development After Fen Meadow Restoration by Topsoil Removal and Hay Transfer. Restoration Ecology 18:924-933.

Klimkowska, A., D. J. D. van der Elst, and A. P. Grootjans. 2015. Understanding long-term effects of topsoil removal in peatlands: overcoming thresholds for fen meadows restoration. Applied Vegetation Science 18:110-120.

Knorr, K. H. 2013. DOC-dynamics in a small headwater catchment as driven by redox fluctuations and hydrological flow paths - are DOC exports mediated by iron reduction/oxidation cycles? Biogeosciences 10:891-904.

Kooijman, A. M. 2012. Poor rich fen mosses': atmospheric N-deposition and P-eutrophication in base-rich fens. Lindbergia 35:42-52.

Kotowski, W., W. Thorig, R. van Diggelen, and M. J. Wassen. 2006. Competition as a factor structuring species zonation in riparian fens - a transplantation experiment. Applied Vegetation Science 9:231-240.

Kotowski, W., Jabłońska, E., & Bartoszuk, H. 2013. Conservation management in fens: Do large tracked mowers impact functional plant diversity?. Biological conservation, 167: 292-297.

Laan, P., A. Smolders, C. W. P. M. Blom, and W. Armstrong. 1989. The Relative Roles of Internal Aeration, Radial Oxygen Losses, Iron Exclusion and Nutrient Balances in Flood Tolerance of Rumex Species. Acta Botanica Neerlandica 38:131-145.

Lamers, L. P. M., M. A. Vile, A. P. Grootjans, M. C. Acreman, R. van Diggelen, M. G. Evans, C. J. Richardson, L. Rochefort, A. M. Kooijman, J. G. M. Roelofs, and A. J. P. Smolders. 2015. Ecological restoration of rich fens in Europe and North America: from trial and error to an evidence-based approach. Biological Reviews 90:182-203.

Li, Y. C., S. Yu, J. Strong, and H. L. Wang. 2012. Are the biogeochemical cycles of carbon, nitrogen, sulfur, and phosphorus driven by the "Fe-III-Fe-II redox wheel" in dynamic redox environments? Journal of Soils and Sediments 12:683-693.

Lindsay, W. L. 1991. Iron oxide solubilization by organic matter and its effect on iron availability. Plant and soil, 130: 27-34.

Lovley, D. R. and E. J. P. Phillips. 1986. Organic-Matter Mineralization with Reduction of Ferric Iron in Anaerobic Sediments. Applied and Environmental Microbiology 51:683-689.

Malson, K., I. Backeus, and H. Rydin. 2008. Long-term effects of drainage and initial effects of hydrological restoration on rich fen vegetation. Applied Vegetation Science 11:99-106.

Morris, J. T. 1991. Effects of Nitrogen Loading on Wetland Ecosystems with Particular Reference to Atmospheric Deposition. Annual Review of Ecology and Systematics 22:257-279.

Opdekamp, W., O. Beauchard, H. Backx, F. Franken, T.J. Cox, R. Van Diggelen, & P. Meire .2012. Effects of mowing cessation and hydrology on plant trait distribution in natural fen meadows. Acta oecologica, 39, 117-127.

Patrick, W. H. and R. A. Khalid. 1974. Phosphate Release and Sorption by Soils and Sediments - Effect of Aerobic and Anaerobic Conditions. Science 186:53-55.

Patzelt, A., U. Wild, and J. Pfadenhauer. 2001. Restoration of wet fen meadows by topsoil removal: Vegetation development and germination biology of fen species. Restoration Ecology 9:127-136.

Pawlikowski, P., K. Abramczyk, A. Szczepaniuk, and L. Kozub. 2013. Nitrogen:phosphorus ratio as the main ecological determinant of the differences in the species composition of brown-moss rich fens in north-eastern Poland. Preslia 85:349-367.

Riedel, T., D. Zak, H. Biester, and T. Dittmar. 2013. Iron traps terrestrially derived dissolved organic matter at redox interfaces. Proceedings of the National Academy of Sciences of the United States of America 110:10101-10105.

Rothe, M., T. Frederichs, M. Eder, A. Kleeberg, and M. Hupfer. 2014. Evidence for vivianite formation and its contribution to long-term phosphorus retention in a recent lake sediment: a novel analytical approach. Biogeosciences 11:5169-5180.

Snowden, R. E. D. and B. D. Wheeler. 1993. Iron Toxicity to Fen Plant-Species. Journal of Ecology 81:35-46.

Stammel, B., K. Kiehl, & J. Pfadenhauer, J. 2003. Alternative management on fens: Response of vegetation to grazing and mowing. Applied Vegetation Science, 6(2): 245-254.

Stechova, T., J. Kucera, and P. Smilauer. 2012. Factors affecting population size and vitality of Hamatocaulis vernicosus (Mitt.) Hedenas (Calliergonaceae, Musci). Wetlands Ecology and Management 20:329-339.

Twolan-Strutt, L., & P.A. Keddy. 1996. Above-and Belowground Competition Intensity in Two Contrasting Wetland Plant Communities. Ecology, 77(1): 259-270.

van Bodegom, P. M., M. de Kanter, C. Bakker, and R. Aerts. 2005a. Radial oxygen loss, a plastic property of dune slack plant species. Plant and Soil 271:351-364.

van Bodegom, P. M., Broekman, R., Dijk, J., Bakker, C., & Aerts, R.. 2005b. Ferrous iron stimulates phenol oxidase activity and organic matter decomposition in waterlogged wetlands. Biogeochemistry 76.1: 69-83.

Van Dijk, J., M. Stroetenga, L. Bos, P. M. Van Bodegom, H. A. Verhoef, and R. Aerts. 2004. Restoring natural seepage conditions on former agricultural grasslands does not lead to reduction of organic matter decomposition and soil nutrient dynamics. Biogeochemistry 71:317-337.

van Dijk, J., M. Stroetenga, P. M. van Bodegom, and R. Aerts. 2007. The contribution of rewetting to vegetation restoration of degraded peat meadows. Applied Vegetation Science 10:315-U314.

Venterink, H. O., M. J. Wassen, A. W. M. Verkroost, and P. C. de Ruiter. 2003. Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. Ecology 84:2191-2199.

Wang, Y., Wang, H., He, J. S., & Feng, X. 2017. Iron-mediated soil carbon response to water-table decline in an alpine wetland. Nature communications, 8, 15972.

Wassen, M. J., H. O. Venterink, E. D. Lapshina, and F. Tanneberger. 2005. Endangered plants persist under phosphorus limitation. Nature 437:547-550.

Weiss, J. V., D. Emerson, and J. P. Megonigal. 2005. Rhizosphere iron(III) deposition and reduction in a Juncus effusus L.-dominated wetland. Soil Science Society of America Journal 69:1861-1870.

Wheeler, B. D., M. M. Alfarraj, and R. E. D. Cook. 1985. Iron Toxicity to Plants in Base-Rich Wetlands - Comparative Effects on the Distribution and Growth of Epilobium-Hirsutum L and Juncus-Subnodulosus Schrank. New Phytologist 100:653-669.

Zak, D. and J. Gelbrecht. 2007. The mobilisation of phosphorus, organic carbon and ammonium in the initial stage of fen rewetting (a case study from NE Germany). Biogeochemistry 85:141-151.

Zak, D., T. Goldhammer, A. Cabezas, J. Gelbrecht, R. Gurke, C. Wagner, H. Reuter, J. Augustin, A. Klimkowska, and R. McInnes. 2017. Top soil removal reduces water pollution from phosphorus and dissolved organic matter and lowers methane emissions from rewetted peatlands. Journal of Applied Ecology In press.

Zak, D., N. Meyer, A. Cabezas, J. Gelbrecht, R. Mauersberger, B. Tiemeyer, C. Wagner, and R. McInnes. 2015. Topsoil removal to minimize internal eutrophication in rewetted peatlands and to protect downstream systems against phosphorus pollution: a case study from NE Germany. Ecological Engineering.

Zak, D., C. Wagner, B. Payer, J. Augustin, and J. Gelbrecht. 2010. Phosphorus mobilization in rewetted fens: the effect of altered peat properties and implications for their restoration. Ecological Applications 20:1336-1349.

Zedler, J. B. 2000. Progress in wetland restoration ecology. Trends in Ecology & Evolution 15:402-407.

List of publications (A1)

Emsens, W.J., Aggenbach, C.J.S., Rydin, H., Smolders, A.J.P., & van Diggelen, R. Competition for light drives endangered species loss in fens: an introduction experiment. *Submitted.*

Emsens, W.J., Aggenbach, C.J.S., Smolders, A.J.P., Zak, D., & van Diggelen, R. 2017. Restoration of endangered fen communities: the ambiguity of iron-phosphorus binding and phosphorus limitation. *Journal of Applied Ecology – in press.*

Emsens, W.J., Schoelynck, J., Grootjans, A. P., Struyf, E., & van Diggelen, R. 2016. Eutrophication alters Si cycling and litter decomposition in wetlands. *Biogeochemistry* 130: 289–299.

Emsens, W.J., Aggenbach, C.J.S., Grootjans, A.P., Nfor, E.E., Schoelynck, J., Struyf, E., & van Diggelen, R. 2016. Eutrophication triggers contrasting multilevel feedbacks on litter accumulation and decomposition in fens. *Ecology* 97: 2680-2690.

Emsens, W.J., Aggenbach, C. J.S., Schoutens, K., Smolders, A.J.P., Zak, D., & van Diggelen, R. 2016. Soil iron content as a predictor of carbon and nutrient mobilization in rewetted fens. *PloS One* 11(4) : e0153166.

Emsens, W.J., Aggenbach, C.J.S., Smolders, A.J.P., & van Diggelen, R. 2015. Topsoil removal in degraded rich fens: Can we force an ecosystem reset? *Ecological Engineering 77* : 225-232.

Aggenbach, C.J.S., Backx, H., **Emsens, W.J.**, Grootjans, A.P., Lamers, L. P., Smolders, A. J.P., ... & van Diggelen, R. 2013. Do high iron concentrations in rewetted rich fens hamper restoration? *Preslia* 85 : 405-420.

Conferences

August 24-28, 2015: SER world conference on ecological restoration (Manchester, UK). Oral presentation.

April 13-16, 2015: HydroEco world conference (Vienna, Austria). Oral presentation.

Curriculum Vitae

Willem-Jan Emsens was born in Turnhout, Belgium, 1987. He obtained his BSc (2009) and MSc (2011) in Forest and Nature Conservation at Wageningen University, the Netherlands, where he specialized in ecology. He did his MSc thesis on seed dispersal by mammals in the Panamanian rainforest under supervision of Dr. Patrick Jansen and Dr. Roland Kays, in cooperation with the Smithsonian Tropical Research Institute. After that he re-specialized in vegetation science, eco-hydrology and wetland biogeochemistry, and in 2012 he started working on his PhD thesis “The restoration of degraded iron-rich fens” at the Ecosystem Management Research group of Antwerp University, Belgium, under supervision of prof. Rudy van Diggelen. In 2017, he was stationed at the Plant Ecology and Evolution group of Uppsala University (under supervision of prof. Håkan Rydin), Sweden, to study biogeochemical processes in pristine raised bogs.