

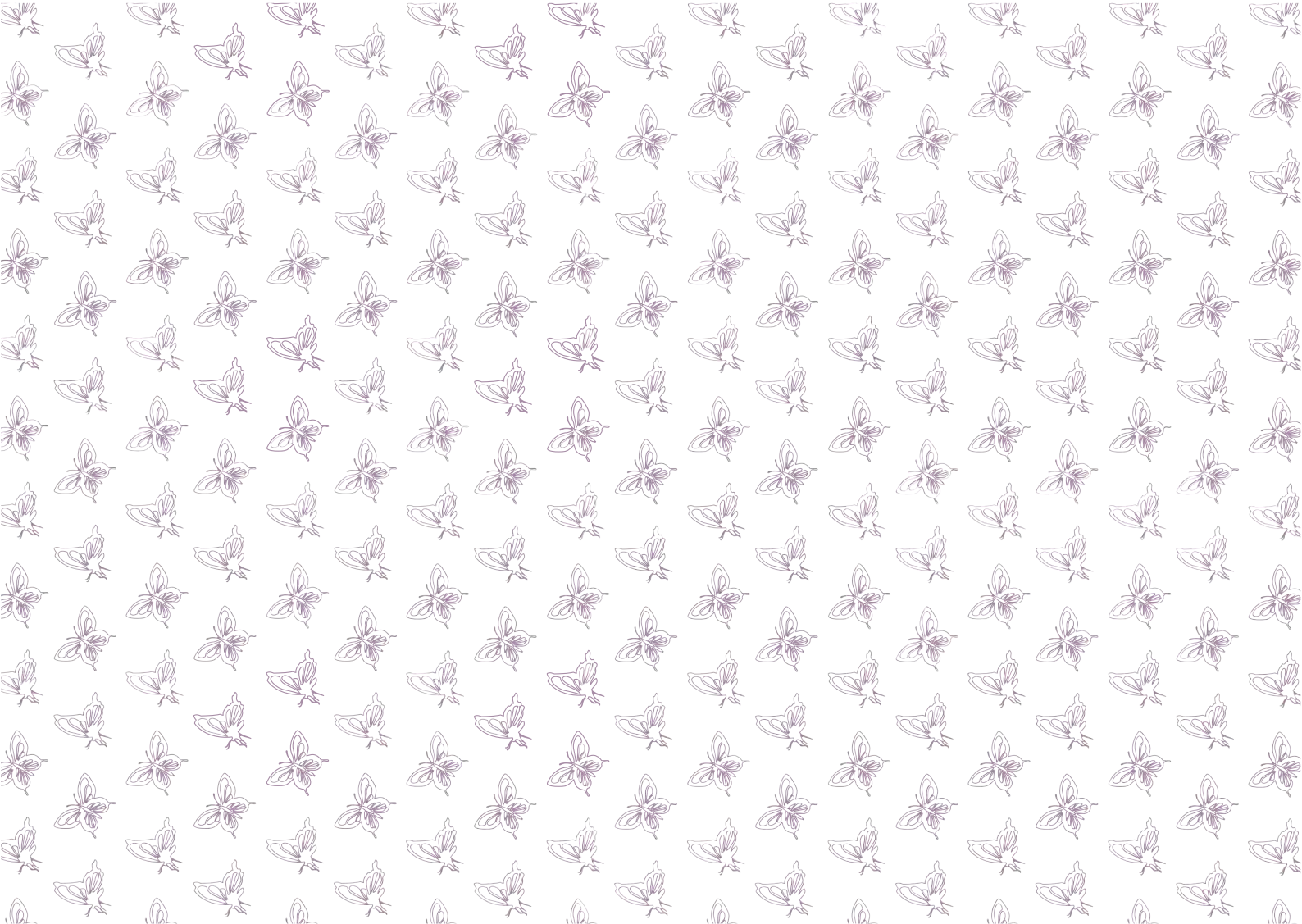
HUMAN IMPACT ON PEATLANDS: FROM BIOGEOCHEMICAL ISSUES TOWARDS SUSTAINABLE LAND USE OPTIONS

Author | J.M.H. van Diggelen



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The conservation and restoration of peatlands remain a challenge in an ever changing, turbulent and increasingly crowded world. I sometimes wonder why man always tries to interfere, instead of just let 'nature go its own way' and accept the (unfavourable) consequences. However, despite our relatively short existence, it was mostly mankind who induced this degradation of nature. Therefore, we should at least make an effort trying to change this. Hopefully this thesis will contribute to develop some good strategies to preserve peatlands in the long term.

J.M.H (José) van Diggelen



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1 | General introduction

Josepha M.H. van Diggelen

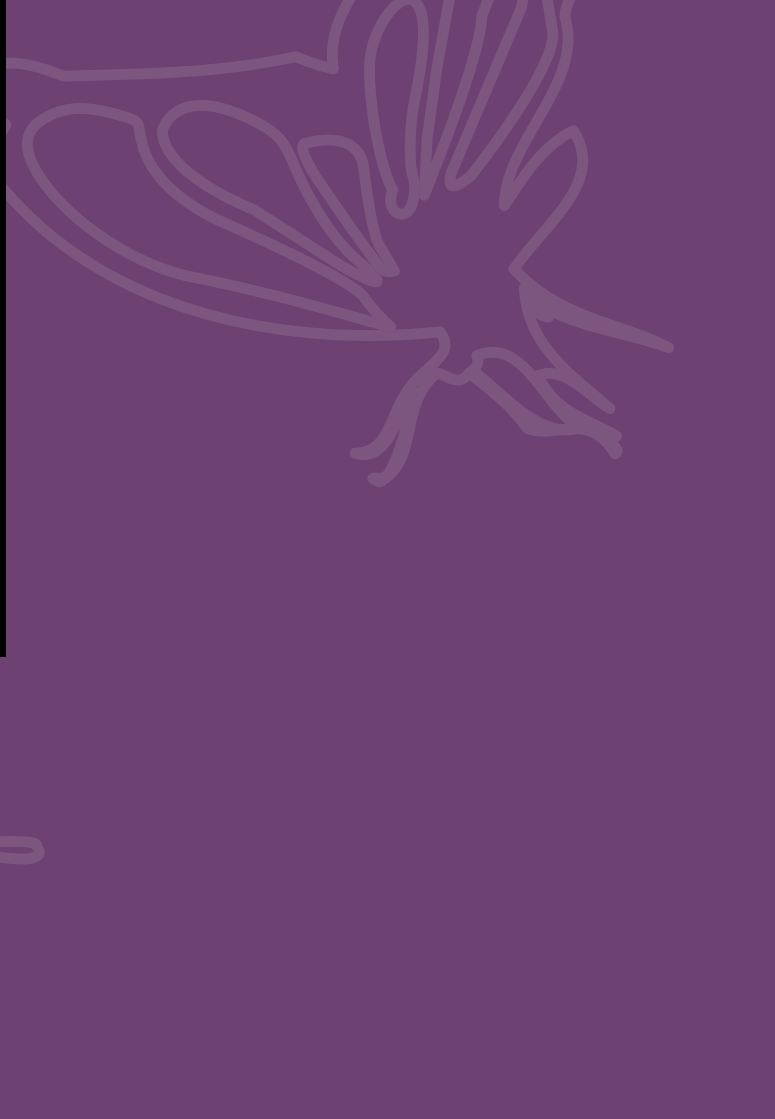
Peatlands

Wetlands, comprising saline, brackish and freshwater systems, can be found in all climatic zones of the world (Mitsch & Gosselink, 2000; Zedler & Kercher, 2005). They are characterised by permanent or temporary water-saturation due to stagnation of surface water, rainwater, or groundwater in poorly drained soils, leading to predominantly anaerobic soil conditions. Wetlands with highly organic soils (peatlands) are generally divided into ombrotrophic bogs and minerotrophic fens, but they also include tree- or shrub-dominated swamps and grass-dominated marshes (e.g. Mitsch & Gosselink, 2000; Wheeler & Proctor, 2000; Joosten & Clarke, 2002). The different types of peatlands are distinguished by plant composition and the extent of rainwater versus groundwater or surface water influence. Fens are typically dominated by graminoid species (including *Cyperaceae*), aquatic macrophytes and brownmosses, while bogs are characterised by *Sphagnum* species (Wheeler & Proctor, 2000; Lamers *et al.*, 2014). In peatlands with active peat formation (mires), anaerobic conditions decrease the activity of decomposing organisms by limited availability of oxygen (O_2), leading to a progressive accumulation of organic matter (peat) derived from decaying plant material. On average, vertical peat accumulation rates are 20-80 cm per 1000 years in bog mires (Walker, 1970; Moore & Bellamy, 1973), and 50-100 cm per 1000 years in fen mires (Malmer, 1975). However, much higher rates (2- or 3-fold higher) have also been found, largely depending on driving factors such as water regime, primary productivity and community composition, nutrient availability and temperature (Hemond, 1980; Nichols, 1983; Moore, 1989; Vitt *et al.*, 2009).

Next to vertical peat accumulation, terrestrialisation occurs from open water to floating peat mats, both in more acidic mires (bogs) and in mires fed by base-rich surface water and/or groundwater (rich fens). An ongoing succession of plant communities and concomitant peat accumulation leads to gradually increasing peat thickness, simultaneously decreasing the influence of base-rich water and increasing that of base-poor rainwater. This creates a biogeochemical buffering gradient including different successional vegetation stages from rich fen, via (*Sphagnum*-dominated) poor fen, towards eventually carr woodland (swamps) or bogs (Wheeler & Proctor, 2000; Grootjans *et al.*, 2006). As peatlands can provide a large range of habitat conditions, they may show a high diversity of plant and animal species, and are among the world's most productive ecosystems (Wheeler & Proctor, 2000). Worldwide, peatlands cover over 4 million km² in 180 countries (3% of the world's land area) and contain 30% of all global soil carbon (C) (Joosten & Clarke, 2002; Limpens *et al.*, 2008). Although they cover a relatively small land area at a global scale, they are very important for human welfare and provide a high variety of natural resources and services, such as C sequestration, but also nutrient cycling, filtering sediments of pollutants, regulating hydrology and controlling floods (Costanza *et al.*, 1997; Mitsch & Gosselink, 2000; Joosten & Clarke, 2002; Zedler & Kercher, 2005).

Human impact and the need for conservation

The world's human population is approximately 7 billion at present, and still increases with 200,000 people every month (Cohen, 2003). As the highest population densities can be found near wetlands, which is related to their multiple societal services, the anthropogenic impact and pressure on wetland ecosystems have strongly increased, and many natural peatlands have been converted into cultivated areas to improve agricultural resource management. This seriously threatens the current status of



Peatlands in the Netherlands

During the Holocene, the western part of The Netherlands became covered by marshland as a result of increased rainfall combined with poor drainage due to the marine clay subsoil. Several metres of peat (fen peat and eventually bog peat) accumulated on top of this clay soil as anaerobic soil conditions highly decreased decomposition rates (Pons, 1992). Since the early Middle Ages, humans managed to transform these marshlands into arable land by digging canals and ditches to enable drainage, and burning or cutting the original vegetation. From the 14th century onwards, peatland drainage was further enhanced by the construction of small dykes and the use of windmills. Severely altered hydrology as well as peat harvesting for turf caused a strong land subsidence of 2 metres or more since the early Middle Ages (Schothorst, 1977). Large coastal areas became increasingly influenced through inundations and seepage, by salt water from the Zuiderzee, an inland sea that was connected to the North Sea until the 1930s. This led to the accumulation of reduced sulphur (S) as iron sulphides (FeS₂) in the peat soils of Dutch coastal areas. The disconnection of the Zuiderzee and North Sea by the construction of a dam (Afsluitdijk) in 1932, and subsequently altered hydrological conditions led to a gradual desalinisation of coastal peatlands (Pons, 1992). As a result of their geohydrological setting and human interference, S-rich freshwater peatlands are located in coastal areas of the Netherlands, while freshwater peatlands less rich in S can be found more inland.

Currently, Dutch peatlands show a typical polder landscape, including a large network of waterways surrounding large drained peatland areas. These areas are predominantly used for agricultural purposes, but small areas are still managed as nature reserves. Drainage-based agricultural use of the peat soils led to the formation of peat meadows (Dutch: 'veenweides'), which are predominantly being managed as grasslands, inhabited by populations of meadow birds. These often migratory birds use the Dutch managed peat meadows as breeding areas on a large scale, and are nowadays protected as their populations are rapidly declining (Kleijn *et al.*, 2004). In areas with less anthropogenic influence where peat-forming and successional processes dominate, floating peat mats (quaking mires; in Dutch 'drijftillen' or 'kraggen') can still be found (Grootjans & van Diggelen, 1995; Lamers *et al.*, 2002b). These more or less natural, semi-terrestrial areas typically include a much higher botanical and zoological diversity than the agricultural areas. Nevertheless, both the semi-natural and agricultural landscapes nowadays have great European conservation value, and they also show the cultural heritage of a large part of the Netherlands. Intensive drainage has largely declined the relative peat cover in The Netherlands, and still leads to peat degradation and high land subsidence rates up to 1 cm per year in the remaining areas (Oude Essink *et al.*, 2010; Hoogland *et al.*, 2012). As a result, the soil surface of Dutch semi-natural peatland remnants is currently often situated higher than the intensively drained polders or urban areas surrounding, which has strongly altered their hydrology (figure 2). This has increased the risk of serious threats such as desiccation (water shortage), acidification, and eutrophication (Grootjans *et al.*, 1996; Koerselman & Verhoeven, 1992; Beltman *et al.*, 2001; Lamers *et al.*, 2002b; 2014).

Biogeochemical issues in peatlands Altered hydrology

As pointed out, hydrology has been severely altered in Dutch peatlands (figure 2), resulting in lowered water levels and water shortage (e.g. Middleton *et al.*, 2006a; Lamers *et al.*, 2002b; 2014; Cusell, 2014). Additional changes in hydrology are still to be expected, as it is predicted that global temperatures will increase with 1 to 5 °C in the 21st century, leading to changed precipitation regimes, sea level rise, and an increase of the number and severity of extreme weather events (Erwin, 2009; IPCC, 2014; Hay *et al.*, 2015). Peatlands are highly sensitive to lowered water levels, which

peatlands and their remaining resources and services such as hydrologic regulation, biodiversity and global C storage (e.g. Costanza *et al.*, 1997; Mitsch & Gosselink, 2000; Brinson & Malvárez, 2002; Zedler & Kercher, 2005; Bobbink *et al.*, 2006). Intensification of agriculture, drainage and peat excavation have led to a general reduction of organic C levels in many peat soils (Sleutel *et al.*, 2003; Bellamy *et al.*, 2005). Worldwide, more than 50%, and in more densely populated areas such as Europe, North America, and East Asia even more than 80%, of the wetlands have been lost or severely degraded (e.g. Joosten & Clarke, 2002; Laiho, 2006; Lamers *et al.*, 2014). During the 20th century, human activities such as cultivation and extraction of peat were carried out at a large scale in many fens and bogs in Europe. Nowadays, the highest relative cover of peat areas in Europe can be found in Finland, followed by Estonia, Ireland, Sweden and The Netherlands (figure 1; Montanarella *et al.*, 2006). The ongoing and increasing anthropogenic forcing, both in remnants of natural peatlands and in more cultivated peat areas, emphasises the importance of their conservation and restoration.

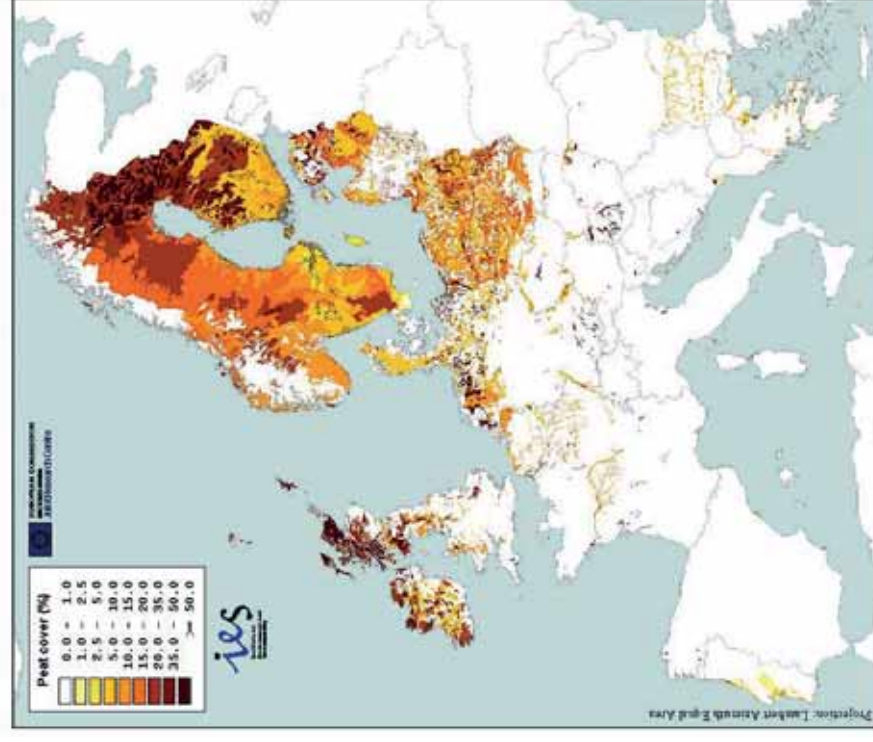


Figure 1. Relative cover (%) of peat and peat-topped soils in Europe (from: Montanarella *et al.*, 2006)

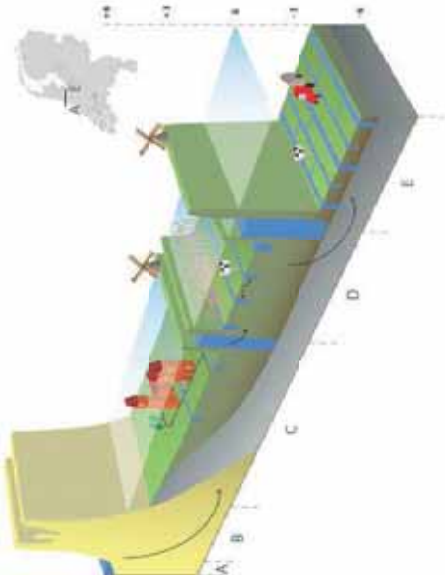


Figure 2. Schematic overview of a typical Dutch coastal peatland area, located several meters below sea water level (y-axis), and surrounded by urban and agricultural areas (made by G. van Dijk).

increase oxygenation of the peat soil and therefore stimulate the aerobic decomposition of organic matter. This leads to undesirable enhanced CO_2 emissions to the atmosphere and concomitant land subsidence (e.g. Kasimir-Klmedtsson *et al.*, 1997; Laiho, 2006; Lamers *et al.*, 2014; Mettrop *et al.*, 2014). Land subsidence of peat meadows leads to increasing risk of flooding, and additional costs for drainage (Verhoeven & Setter, 2009). The availability of O_2 in the peat is an important biogeochemical variable as it affects redox-sensitive microbially-mediated chemical processes in the soil. As such, water level changes directly interfere with carbon (C), sulphur (S), phosphorus (P) and nitrogen (N) cycling that highly determine water and soil quality and decomposition rates (Wheeler & Proctor, 2000; Lamers *et al.*, 2002b; 2014; Cusell, 2014).

Eutrophication

As nutrient availability regulates primary production and interspecific plant competition for light, an increasing availability is known to severely affect species composition and biodiversity in terrestrial or semi-terrestrial peatlands (Hogg *et al.*, 1995; Kooijman & Paulissen, 2006; Klimkowska *et al.*, 2007; Geurts *et al.*, 2009; Lamers *et al.*, 2002b; 2014), and also in aquatic systems (Smolders & Roelofs, 1995; Lamers *et al.*, 2002a,b). Primary production is generally N- and/or P-limited in (semi-) terrestrial and aquatic systems, freshwater and marine, and enrichment with either nutrient may induce a strong positive growth response (Elser *et al.*, 2007). Nutrient limitation usually prevents the dominance of fast-growing plant species, and increasing concentrations or changing N:P ratios resulting from eutrophication may alter plant community composition (Koerselman & Meuleman, 1996; Olde Venterink *et al.*, 2002). For instance, increased N availability is known to enhance the dominance of graminoids, which causes biodiversity loss in wetlands (Bobbink *et al.*, 1998).

There are different pathways of eutrophication in peatlands (figure 3). Fertilisation of peat meadows will directly increase nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations. During dry and often warm periods, additional water supplies coming from surrounding urban or agricultural areas are often used in an attempt to compensate for water shortage, which may directly lead to eutrophication as the external water usually contains increased concentrations of nutrients and other ions (Roelofs, 1991; Smolders & Roelofs, 1995). In contrast, during wet periods, dilution due to heavy rainfall and discharge of surplus water can also lead to a (temporary) decrease of nutrients in the surface water. Moreover, eutrophication is enhanced by increased atmospheric deposition of nitrogen

oxides (NO_x), ammonia (NH_3), ammonium (NH_4^+) and sulphur dioxide (SO_2), which also enhance soil and water acidification by forming nitric acid (HNO_3) and sulphuric acid (H_2SO_4) (Bobbink *et al.*, 1998). As a result, sulphate (SO_4^{2-}), NH_4^+ and NO_3^- will directly or indirectly lead to eutrophication. An indirect pathway is called 'internal eutrophication' as there is an increase of nutrients that are derived from the system itself (Smolders *et al.*, 2006a). Dissolved SO_4^{2-} concentrations may enhance P release both from anaerobic peat soils or underwater sediments, as microbial SO_4^{2-} reduction rates can be enhanced. As a result of SO_4^{2-} reduction, sulphide (S^{2-}) is formed that efficiently binds to Fe^{2+} and mobilises P (Lamers *et al.*, 2002a, Smolders *et al.*, 2006a). In addition to atmospheric deposition, SO_4^{2-} concentrations also become enhanced by the oxidation of reduced S deposits in the peat soil, or by the supply of SO_4^{2-} -rich water from surroundings. Finally, decomposition and concomitant nutrient mineralisation may also be enhanced by increased rates of microbially-mediated redox processes, using O_2 under aerobic conditions, or alternative electron acceptors such as NO_3^- or SO_4^{2-} under anaerobic conditions, which lead to the release of minerals such as N and P bound in the organic soil. In areas with seepage, SO_4^{2-} and NO_3^- levels may also increase as a result of the discharge of groundwater from surrounding forests or agricultural areas.

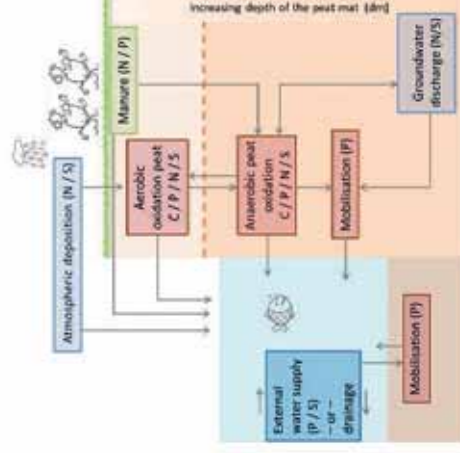


Figure 3. Schematic overview of pathways enhancing eutrophication in surface waters of peatlands (atmospheric deposition; aerobic and anaerobic peat oxidation; internal mobilisation in sediment; groundwater discharge; agricultural land use; external water).

Acidification

Soil desiccation due to water shortage is known to enhance acidification by the release of protons during aerobic oxidation processes in the soil (Lamers *et al.*, 1998b; Smolders *et al.*, 2006b), including the oxidation of reduced S deposits in formerly wet peat soils (Mylona, 1996; Lucassen *et al.*, 2002). The concentrations of base ions such as bicarbonate (HCO_3^-), calcium (Ca^{2+}) or magnesium (Mg^{2+}) determine the buffering capacity of the soil. When soil buffering is insufficient, soil pH decreases leading to a shift of plant and microbial composition. During terrestrialisation the influence of base-rich water in fens decreases as the thickness of the peat mat increases, which leads to natural succession of species-rich early-successional vegetation towards more acidic, species-poor late-successional vegetation (Verhoeven & Bobbink, 2001; Grootjans *et al.*, 2006). Although this gradual acidification is a natural process, atmospheric S and N deposition strongly accelerate succession towards late-successional stages that are often dominated by *Sphagnum*. This generates a loss of

typical fen species and a decrease of overall biodiversity in fens (Beltman *et al.*, 2001; Middleton *et al.*, 2006a; Lamers *et al.*, 2014).

Toxicity

Several compounds that are usually found in waterlogged, anaerobic peat soils such as NH_4^+ , S^{2-} or Fe^{2+} can accumulate to high concentrations and become toxic (Smolders *et al.*, 1995; Lucassen *et al.*, 2002; Lamers *et al.*, 2002a; 2013). In wet, anaerobic conditions NH_4^+ is the dominant N form and is easily taken up as a nutrient. At high levels, however, NH_4^+ may become toxic to several plant species (Britto & Kronzucker, 2002; Stevens *et al.*, 2011). Potentially toxic, reduced compounds may affect plant composition, although many wetland species have developed specialised adaptations to survive permanently or temporarily waterlogged soil conditions, and flooding. They are often able to oxidise their rhizosphere through O_2 release from the roots, thereby enhancing overall microbial activity and detoxifying reduced phytotoxins (Visser *et al.*, 2000b; Colmer, 2003).

Salinisation

As explained above, peatlands located in coastal areas of the Netherlands, which were historically influenced by the sea for longer time, have nowadays become freshwater wetlands. The ongoing strong land subsidence in these coastal areas may also enhance saline seepage water to intrude low-lying freshwater inland areas, leading to a shift towards more saline groundwater and surface water systems (Zedler & Kercher, 2005; Oude Essink *et al.*, 2010). Especially in relation to climate change and sea level rise, salinisation of freshwater systems is becoming a serious global problem (Nielsen *et al.*, 2003; Herbert *et al.*, in press). Increasing salinity severely affects biogeochemical cycling in freshwater systems, by increasing concentrations of ions such as chloride (Cl^-), sulphate (SO_4^{2-}), and calcium (Ca^{2+}) in surface water and soil pore water, which control P cycling (Carpenter & Smith, 1984; Gunnars & Blomqvist, 1997; Jordan *et al.*, 2008). This can have negative effects on water quality and biodiversity, and may lead to a further loss of freshwater peatlands (Nielsen *et al.*, 2003; Weston *et al.*, 2011). In agricultural areas an increasing salinity will also affect crop production or drink water quality for cattle (Bagley *et al.*, 1997; Williams, 2001). Therefore, salinisation is usually considered to be an undesirable development.

Dilemmas for peatland management

In more natural terrestrial and semi-terrestrial peatlands, most traditional management measures focus on conserving remnants of historic ecosystems or existing plant communities, usually by the restoration of the 'old' situation or by trying to increase the present surface area of rare vegetation. For example, mowing, grazing or burning have been used for decades as measures to promote slower-growing species by the reduction of light competition. Despite all efforts, however, these peatland areas are still degrading and biodiversity is strongly declining. As further degradation should be prevented, novel restoration measures are needed to conserve and restore peatlands. In areas where agricultural use is the main function, measures are needed to improve water quality and to decrease further land subsidence. As explained above, there are many threats to the functioning, biodiversity and water quality in peatlands, particularly in The Netherlands. Although peatlands may differ with respect to their main function (nature and/or agriculture), system type (aquatic, semi-terrestrial, or terrestrial), or status (good/poor quality), the biogeochemical processes explained apply to all these wetland types. The main issue, however, is the complexity of the interactions between atmosphere, soil, water and vegetation, and of soil and rhizosphere biogeochemistry including vegetation development and microbial-mediated processes. Moreover, with increasing population density and limited land area, nature managers and water managers also

have to comply with different stakeholder interests and ecosystem functions of peatlands (Lamers *et al.*, 2014). An important issue is the hydrological management of water levels as this leads to dilemmas. Water levels should be high to prevent land subsidence, while farmers prefer a lower water level to effectively use their land. As water management authorities (called water boards in the Netherlands) are usually responsible for water quality in aquatic systems, and landowners for terrestrial systems, it is a challenge to apply effective measures in the heterogeneous Dutch peatlands. Cooperation is achievable as long as the goals are matching. However, contrasting interests still lead to important management issues.

Objectives and outline of this thesis

Although there is ample knowledge about the main problems and threats in peatlands, the underlying controlling mechanisms and interactions between different processes are often still hypothetical or not yet well understood. In addition, a clear understanding of biogeochemical processes is essential to develop effective and sustainable management options, and to define realistic goals. This may help future prospects for the maintenance and restoration of peatlands and their high biodiversity.

The research included in this thesis was realised at B-WARE (Biogeochemical Water Management & Applied Research on Ecosystems) Research Centre, a spin-off company of the Aquatic Ecology and Environmental Biology Group and the Institute for Water and Wetland Research (IWWR) at the Radboud University Nijmegen. At B-WARE Research Centre, applied research is carried out to unravel key biogeochemical and ecological processes that control ecosystem functioning and are important for management (water management, nature management, ecological restoration). For this reason, the research presented in this thesis has an applied perspective in order to support and improve the management of both semi-natural and agricultural peatland systems, particularly for those in The Netherlands.

In this thesis, several issues including eutrophication (Chapter 2, 3, 5, 6), altered hydrology (Chapter 5 and 6), acidification (Chapter 3 and 5), and salinisation (Chapter 2), and their interactions with vegetation development (Chapter 3, 4, 5) or microbial transformations (Chapter 2 and 4) in peatlands will be discussed. The main objective is to provide more insight into controlling biogeochemical processes in P, N, S and Fe cycling and their effect on vegetation development and water quality, in order to identify key factors to enhance sustainable water management and land use options in (formerly) drained peatland systems. Chapter 2 describes how increased salinisation affects P cycling in S-rich, Dutch coastal peatlands. A controlled experimental set-up was used to study the driving mechanisms of P mobilisation at the sediment-water interface, and their interactions with salinity changes. A novel controlling mechanism was suggested, which may help to develop measures to improve water quality in peatlands. In chapter 3, the effects of increased NH_4^+ concentrations, and their interaction with pH, on the growth and development of two common wetland graminoids (*Juncus acutiflorus* and *Carex disticha*) in a hydroponic set-up are described. As biodiversity loss and degradation of wetland systems are often related to enhanced dominance of N-limited graminoids, results may contribute to improve future wetland management in order to conserve biodiversity. Chapter 4 provides an overview of interactions between belowground microbial activity and wetland vegetation composition, and how they affect N, S and Fe cycling in wetlands. Chapter 5 explores long-term effects of traditional management measures (summer mowing or burning after winter mowing) on vegetation development in an eutrophied and acidified fen. It also includes the effects of experimental liming in the field, and discusses its potential application as a measure for

the restoration of acidified rich fen vegetation. In chapter 6, the effects of hydrological changes (high or low surface water and groundwater levels) and their interactions with land subsidence, S, Fe and P biogeochemistry, and water quality in drained S-rich coastal peatlands are described. The resulting dilemmas for future water management and land use of drained peatlands will be discussed. Chapter 7 provides a synthesis which combines our results as described in this thesis with some additional unpublished results from my research obtained at B-WARE, in order to discuss some sustainable and effective management and land use options for conservation of both (semi-) natural and agricultural peatlands in the long term.



2 | New insights into phosphorus mobilisation from sulphur-rich sediments: Time-dependent effects of salinisation

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Maarten J. Schaafsma, Jan G.M. Roelofs, Alfons J.P. Smolders

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Abstract

Internal phosphorus (P) mobilisation from aquatic sediments is an important process adding to eutrophication problems in wetlands. Salinisation, a fast growing global problem, is thought to affect P behaviour. Although several studies have addressed the effects of salinisation, interactions between salinity changes and nutrient cycling in freshwater systems are not fully understood. To tackle eutrophication, a clear understanding of the interacting effects of sediment characteristics and surface water quality is vital. In the present study, P release from two eutrophic sediments, both characterized by high pore water P and very low pore water iron (Fe^{2+}) concentrations, was studied in a long-term aquarium experiment, using three salinity levels. Sediment P release was expected to be mainly driven by diffusion, due to the eutrophic conditions and low iron availability. Unexpectedly, this only seemed the driving mechanism in the short term (0-10 weeks). In the long term (>80 weeks), P mobilisation was absent in most treatments. This can most likely be explained by the oxidation of the sediment-water interface where Fe^{2+} immobilises P, even though it is commonly assumed that free Fe^{2+} concentrations need to be higher for this. Therefore, a controlling mechanism is suggested in which the partial oxidation of iron-sulphides in the sediment plays a key role, releasing extra Fe^{2+} at the sediment-water interface. Although salinisation was shown to lower short-term P mobilisation as a result of increased calcium concentration and oxygen availability, long-term P mobilisation by the interactions between sulphate reduction and oxygen availability. Our study showed time-dependent responses of sediment P mobilisation in relation to salinity, suggesting that sulphur plays an important role in the release of P from FeS_x -rich sediments, its biogeochemical effect depending on the availability of Fe^{2+} and O_2 .



Introduction

The eutrophication of surface waters is an urgent problem worldwide (Smith, 2003). Increased P concentrations have led to a strong decline of the biodiversity in freshwater wetlands, due to the resulting dominance of highly competitive macrophytes, and of algae and cyanobacteria, monopolising light (Smith, 2003; Lamers *et al.*, 2002a; Geurts *et al.*, 2009). Salinisation of freshwater systems has received increasing attention, especially in relation to climate change and sea level rise (Nielsen *et al.*, 2003). With increasing salinity, higher P concentrations are often found in surface waters (e.g. Carpenter and Smith, 1984; Gunnars and Blomqvist, 1997; Suzumura *et al.*, 2000; Jordan *et al.*, 2008), which may affect P cycling in freshwater systems. Therefore, salinisation is expected to enhance eutrophication in coastal, freshwater wetlands, leading to water quality deterioration and loss of biodiversity.

Internal mobilisation of P from eutrophic aquatic sediments is an important process adding to eutrophication problems in wetlands (Bostrom *et al.*, 1988; Caraco *et al.*, 1989; Lamers *et al.*, 1998a; Smolders *et al.*, 2006a). The classic theoretical framework suggests that sufficiently high oxygen (O_2) concentrations in the surface water can prevent P release from the sediment (Einsele, 1936; Mortimer, 1941; 1942). According to this, the oxidation of dissolved iron (Fe^{2+}) in the sediment will result in the formation of iron oxides and hydroxides ($Fe(OH)_x$) at the sediment surface, effectively binding P and thereby preventing its release to the surface water. Under anaerobic conditions, these ferric compounds will be mobilised by Fe-reducing bacteria, and part of the P is released to the surface water.

Besides anaerobic conditions, increased sulphate (SO_4^{2-}) reduction rates are also known to be able to increase P mobilisation by decoupling Fe-P interactions at the sediment-water interface (Roelofs, 1991; Caraco *et al.*, 1993; Smolders and Roelofs, 1995; Smolders *et al.*, 2006a; Hupfer and Lewandowski, 2008; Geurts *et al.*, 2010). Sulphide (S^{2-}) binds efficiently to dissolved Fe^{2+} in sediment pore water, and most Fe^{2+} can become bound as iron sulphides (FeS_x) in the sediment, strongly decreasing Fe^{2+} sediment pore water concentrations (Hupfer and Lewandowski, 2008; Geurts *et al.*, 2010). Geurts *et al.* (2010) found that, in aerobic surface waters, P mobilisation from sediments with low pore water Fe:P ratios ($<1 \text{ mol mol}^{-1}$) was a linear function of sediment pore water P concentrations. As a result, one would expect a release of P irrespective of the O_2 concentration in the surface water of SO_4^{2-} enriched wetlands (Caraco *et al.*, 1989; Smolders *et al.*, 2001; Smolders *et al.*, 2006a; Hupfer and Lewandowski, 2008). In addition, dissolved P concentrations might further increase due to the enhanced anaerobic breakdown of organic matter linked to SO_4^{2-} reduction and concomitant mineralisation of P (Lamers *et al.*, 2002a; Smolders *et al.*, 2006a; Geurts *et al.*, 2010).

Salinisation of freshwater systems can enhance SO_4^{2-} reduction rates due to a higher SO_4^{2-} availability (Wetzel, 2001), which may strongly affect P mobilisation as described above. Moreover, increasing Cl^- and SO_4^{2-} concentrations might enhance P release from sediments by competition for anion binding sites (Caraco *et al.*, 1989; Beltman *et al.*, 2000). At the same time, an increase in salinity also leads to increased Ca^{2+} concentrations (Wetzel, 2001), which may result in the immobilisation of P by co-precipitation with Ca^{2+} and calcium carbonate ($CaCO_3$) (Bostrom *et al.*, 1988; House *et al.*, 1999; Nielsen *et al.*, 2003). Salinity changes affect a suite of biogeochemical processes in freshwater systems, where the net effect on P mobilisation is the combined result of these processes. Moreover, a time-dependent shift in dominance of each process on P release can be expected (Hupfer and Lewandowski, 2008). Most studies regarding P release focus on relative short-term effects ranging from one day to 90 days (Gunnars and Blomqvist, 1997; Smolders *et al.*, 2001; Geurts *et al.*, 2010;

Wu *et al.*, 2014), while long-term experiments are mostly lacking. In this paper we explore the time-dependent release of P from eutrophic sediments under different salinities, which is highly relevant regarding the worldwide interest in salinisation effects on freshwater wetland functioning.

To test time-dependent interactions between salinisation and P mobilisation, a controlled aquarium experiment was set up that lasted two years. Two FeS_x -rich sediments from a coastal freshwater wetland were subjected to three naturally occurring water types characterised by different salinities. Pore waters of the peat sediments were typically rich in P and S, and very poor in Fe, and the low total Fe:S ratios in the sediment suggested that most Fe was bound to reduced S (Lamers *et al.*, 2002a). In such sediments, a very high release of P from the sediment to the surface water can be expected, predominantly depending on pore water P concentrations (Caraco *et al.*, 1989; Smolders *et al.*, 2001; Smolders *et al.*, 2006a; Hupfer and Lewandowski, 2008; Geurts *et al.*, 2010). By monitoring biogeochemical changes in porewater and surface water under controlled conditions, we try to reveal how salinity affects short-term and long-term P release, in these type of sediments common for coastal wetlands.

Material and Methods

Sampling area

In this study, peat sediments were used from the coastal lowland fen area Wormer- and Jisperveld ($52^\circ 30' 42.7644''$; $4^\circ 52' 27.3756''$) in the Netherlands. Due to historic intrusion of brackish water, peat rich in minerals such as S, Ca and Fe has accumulated in this area. After more than 50 years of desalinisation resulting from altered hydrological conditions, it gradually became a freshwater system. The peatland comprises ca. 500 ha of open water and ca. 1660 ha of peat meadows, predominantly used for agricultural purposes and partly for nature conservation. Drainage is a standard procedure in this area, leading to peat decomposition and land subsidence. As a result, risks of flooding events and salinisation are increasing in this freshwater peatland.

Experimental design

On 18 March 2008, two types of submerged peat sediment were collected from a ditch at a depth of 0-20 cm (ca. 25 L in total), using a sediment multi sampler (Eijkelkamp Agrisearch Equipment). Although both sediments were relatively rich in organic S and P, they differed in P availability (sediment characteristics are given in Table 1).

Table 1. Characteristics of the two sediments used. Significant differences between treatments are indicated by different letters.

Sediment	Organic content %	Bulk Density $kg\ DW\ L^{-1}\ FW$	Total amounts bound to sediment									
			Total - P $mmol\ L^{-1}\ FW$	Org. - P $mmol\ L^{-1}\ FW$	Inorg. - P $mmol\ L^{-1}\ FW$	Total - Al $mmol\ L^{-1}\ FW$	Total - Ca $mmol\ L^{-1}\ FW$	Total - S $mmol\ L^{-1}\ FW$	Total - Fe $mmol\ L^{-1}\ FW$	Fe:S ratio $mol\ mol^{-1}$		
A	Mean	0.17	2.4 ^a	1.6	1.2 ^a	44.5	54.5	96.3	33.1	0.33 ^a		
	SEM	0.03	0.4	0.3	0.1	12.6	5.4	8.1	3.9	0.024		
B	Mean	0.13	4.3 ^b	2.6	2.3 ^b	43.4	44.0	83.6	38.3	0.46 ^b		
	SEM	0.02	0.3	0.1	0.1	4.6	0.8	4.7	2.3	0.001		

Intact peat cores from the same location as the main experiment were collected separately, to test the effects of aerobic versus anaerobic conditions of the surface water on P release. Water and sediment oxygen (O_2) profiles were measured, using a fixed fiber optical oxygen microsensor (optode) in combination with a Micro TX3 transmitter (PreSens Precision Sensing GmbH). The peat sediment cores were monitored during 18 weeks of either aerobic conditions similar to those of the main experiment, or anaerobic conditions by gently supplying N_2 to the surface water. During both aerobic and anaerobic conditions, P mobilisation rates were calculated from the linear increase in surface water P concentrations.

Chemical analyses

To monitor water quality, samples of surface water and pore water were collected every 2 months and analysed during the experiment. Pore water was collected anaerobically, using 30 mL vacuum bottles connected to Rhizon SMS-10 cm samplers that were fixed in the upper 10 cm of the sediment (Eijkelkamp Agrisearch Equipment). Disturbance of the sediment and water was minimised by the low frequency of sampling and small sample sizes (max. 25 mL). Sulphide concentrations were determined directly after the collection by fixing 10.5 mL pore water with 10.5 mL Sulphide Anti Oxidant Buffer (SAOB), and using an Orion sulphide-electrode and a Consort Ion meter (type C830) (Van Gemerden, 1984). The pH and alkalinity of all samples were measured within 24 hours after sampling, using a combined pH electrode (Radiometer) in combination with a TIM840 pH meter and a Titration Manager Titrablab Autoburette. Dissolved total inorganic carbon (TIC) was measured within 24 hours after sampling by injecting 0.2 mL pore water or surface water in a closed chamber containing 0.2 M H_3PO_4 solution, converting all dissolved TIC into CO_2 . A continuous gas flow (N_2) directly transports the CO_2 to an ABB Advance optima Infrared Gas Analyzer (IRGA) to measure total inorganic C concentrations. A calibration curve was made by injecting different volumes (0.1 – 1.0 mL) of 1.25 mM HCO_3^- solution. Prior to storage at 4°C until elemental analysis, 0.1 mL HNO_3 (65%) was added to 10 mL of each sample to prevent metal precipitation. Concentrations of dissolved Ca, Fe, P, S, and Al in these stored samples were measured using an Inductively Coupled Plasma Spectrophotometer (ICP IRIS Intrepid II XDL; Thermo Electron Corporation). Due to the anaerobic sampling of pore water, measured Fe predominantly consisted of dissolved Fe^{2+} rather than far less mobile Fe^{3+} . The remaining samples were stored at -20°C in order to determine the following ion concentrations colourimetrically on Auto Analyzer 3 systems (Bran and Luebbe): NO_3^- (Kamphake *et al.*, 1967), NH_4^+ (Grasshoff and Johansen, 1972), $ortho-PO_4^{3-}$ (Henriksen, 1965) and Cl^- (O'Brien, 1962). Na^+ and K^+ were determined with a Technicon Flame Photometer IV Control (Technicon Corporation). For both sediments gravimetric water contents were determined by drying for 48 h at 70 °C. Organic matter contents were estimated by loss on ignition for 4 h at 550 °C. A homogenized portion of 200 mg dry sediment was digested in 5 mL HNO_3 (65%) and 2 mL H_2O_2 (30%), using an Ethos 1 Advanced microwave digestion system (Milestone Inc.). Digestates were diluted and analysed by ICP as described above. In order to distinguish between the organic and inorganic P fraction, a P-fractionation procedure was carried out adapted after Golterman (1996).

Statistical analyses

For statistical analysis, SPSS Statistics for Windows (Version 21.0. IBM Corp. Armonk, NY; 2012) was used. To test for differences among treatments in sediment analyses (single measurements) or differences in calculated mobilisation rates, the General Linear Model (GLM) univariate procedure combined with Tukey's-b post-hoc test was used. To test for significant differences among treatments in repeated measurements, a GLM mixed model procedure was used. When significant differences between the two sediments were found, using a 2-way GLM mixed model with treatment as fixed

Table 2. Chemical composition of the surface water used for the different treatments (low, normal or high salinity).

Element	Low salinity Rain water $\mu\text{mol L}^{-1}$	Normal salinity Fresh water $\mu\text{mol L}^{-1}$	High salinity Brackish water $\mu\text{mol L}^{-1}$
Na^+	100	7000	85000
Cl^-	100	7000	85000
SO_4^{2-}	5	1500	5500
K^+	30	500	1000
Ca^{2+}	10	2000	2500
Mg^{2+}	10	1250	3750
HCO_3^-	0	4000	4000
NO_3^-	50	50	50
NH_4^+	50	50	50

To minimise O_2 intrusion, the sediments were stored anaerobically at 4°C in large, closed containers. The next day, 12 glass cylinders (diameter 15 cm, height 60 cm) were filled with 15 cm of sediment A and another 12 cylinders with 15 cm of sediment B. Next 40 cm of water was carefully poured on top of the sediments, avoiding re-suspension of sediment particles. Artificially composed surface water, based on site conditions (control treatment, Table 2), was used for all sediments during an acclimatisation period of 4 weeks. The experiment was carried out in the dark at a constant and environmentally relevant temperature of 15°C. To allow oxygen diffusion to the surface water, an open cylinder system was used.

After this acclimatisation period, three different surface water types were applied as salinity treatments: rainwater (low salinity; 100 $\mu\text{mol Cl L}^{-1}$), brackish water (high salinity; 85 mmol $Cl L^{-1}$) and freshwater (control; 7 mmol $Cl L^{-1}$). All treatments were artificially composed, based on field measurements (Table 2). Control water simulated water quality in the current conditions that exist in the wetland, brackish water composition was based on the historic conditions reported by Reijnders (1946). Rainwater quality equalled the chemical composition of atmospheric deposition as measured in the Netherlands (Boxman *et al.*, 2008). No P was added in order to be able to estimate the release of P from the sediment. For each treatment and sediment type, 4 replicates were used (24 cylinders in total).

Treatment solutions were stored in polyethylene containers (10 L), from which they were pumped into the cylinders using Masterflex L/S multichannel pumps (model 7535-08). The treatments were started by replacing the control water with the appropriate treatment water during 4 weeks, to ensure that all treatment solutions were added properly. Directly after treatment addition (week 10), stagnant conditions were created in order to measure short-term effects of P and S release from the sediment. Short-term mobilisation rates were calculated from the linear increase of the surface water P and S concentrations (0-10 weeks of the stagnant period). After a stagnant period of 26 weeks, pumps were running with a hydraulic retention time of 25 weeks for the treatment solutions during 48 weeks in order to maintain the appropriate treatment conditions. To measure long-term effects of salinity changes on the release of P and S from the sediment, pumps were stopped again (week 81) to create another stagnant period for 32 weeks. Long-term mobilisation rates were again calculated from the linear increase of the S and P concentrations in the surface water during this stagnant period.

factor, sediment as random factor and time as repeated measures, both sediments were analysed separately. In this separate model for sediments, time was used as repeated measures and treatment as fixed factor, with AR(1) heterogeneous as the covariance type. A Bonferroni post-hoc test was used to test for differences between treatments.

Ethics statement

This study was part of the National Research Programme 'Wormer- en IJperwater', funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV), within the framework of 'Nota Ruimte'. The water management authority 'Hoogheemraadschap Hollands Noorderkwartier' facilitated this programme and the nature management authority 'Natuurmonumenten' gave permission to take samples in their reserve.

Results

Pore water chemistry

As expected, pore water chemistry was strongly affected by changes in surface water salinity (Figure 1). Under brackish conditions, Na^+ showed a highly significant ($p < 0.005$) gradual increase in the pore water over time. For the low salinity and control treatment, no significant changes in pore water Na^+ concentrations occurred in sediment A, while Na^+ concentrations showed a significant decrease ($p < 0.05$) over time at a low salinity in sediment B. An interaction between treatment and sediment type was found for both Na^+ and S concentrations, which means that the treatments had a significant, but different, effect on the two sediments. Pore water S concentrations also showed a highly significant ($p < 0.005$) gradual increase at the high salinity treatment in both sediments (Figure 1). At a low salinity, S concentrations remained at a steady level while the control treatment showed a small, but not significant, increase. Moreover, no clear differences in sulphide concentrations were found between sediments or treatments (average values ranged between 0–50 $\mu\text{mol L}^{-1}$ for sediment A, and between 0–500 $\mu\text{mol L}^{-1}$ for sediment B; data not shown).

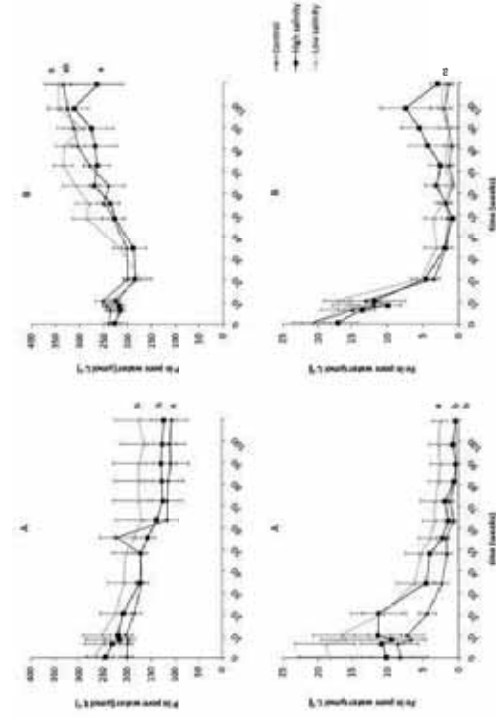


Figure 2. Phosphorus (P) and iron (Fe^{2+}) concentrations ($\mu\text{mol L}^{-1}$) in pore water of both sediments (A: left, B: right). Significant differences between treatments are indicated with different letters.

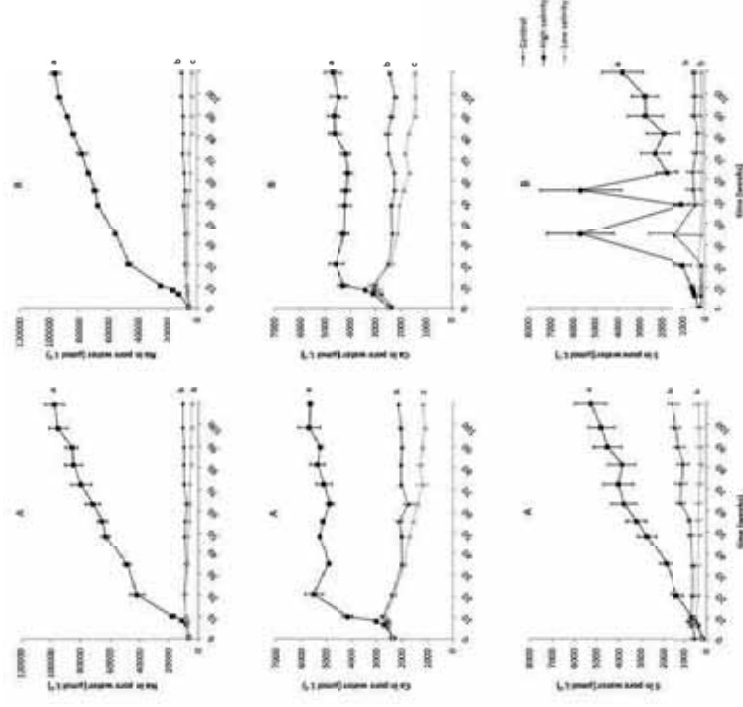


Figure 1. Sodium (Na^+), calcium (Ca^{2+}) and sulphur (S) pore water concentrations ($\mu\text{mol L}^{-1}$) in both sediments (A: left, B: right). Significant differences between treatments are indicated with different letters.

As a result of a higher salinity, Ca^{2+} was mobilised in the sediment, as shown by significantly ($p < 0.005$) increased pore water Ca^{2+} concentrations (Figure 1). This increase in the pore water, well above the added concentration of 2500 $\mu\text{mol L}^{-1}$ to the surface water, started directly after the onset of the high salinity treatment and Ca^{2+} concentrations remained at a steady high level during the course of the experiment. Dissolved Fe^{2+} concentrations were low and showed a gradual decrease for all treatments over time in both sediment types (Figure 2). A significantly higher ($p < 0.005$) Fe^{2+} concentration was found for the low salinity treatment at sediment A when compared to the control and higher salinity treatment. In contrast, no differences in pore water Fe^{2+} concentrations between treatments were found for sediment B. Pore water HCO_3^- concentrations were significantly higher ($p < 0.05$) under brackish conditions at sediment B, while no differences were found between treatments at sediment A (data not shown). Pore water P concentrations showed a gradual decrease at sediment A for all treatments. Moreover, a significantly ($p < 0.05$) stronger decrease of P in pore water was found for the control treatment, compared to the high and low salinity treatment at sediment A. In strong contrast, P concentrations showed a gradual increase in the pore water of sediment B for all salinity treatments (Figure 2), with the significantly ($p < 0.05$) lowest P concentrations in the high salinity treatment.

Surface water chemistry

A higher salinity led to gradually increased Na⁺ and S concentrations in the surface water, and showed significant (p<0.005) differences among all treatments, which eventually equalled the concentrations added (S: Figure 3; Na⁺: data not shown). For the low salinity treatment, however, S concentrations in the surface water reached much higher concentrations than the concentrations of the treatment water, which suggests S mobilisation from the sediment. These S mobilisation rates were calculated (Table 3) for both a short term, showing significantly (p<0.005) negative rates at a high salinity (high S consumption) for sediment A, and for a long term, still showing significantly (p<0.005) negative S mobilisation rates at a high salinity in both sediments. In the surface water, Ca²⁺ concentrations also increased and differed significantly (p<0.005) among all treatments for both sediments (data not shown). However, both the low and high salinity treatment led to much higher concentrations than the added concentrations.

In the low salinity and control treatment, P concentrations in the surface water increased directly after onset of the treatments (after 10 weeks; t=0). For the high salinity treatments, P concentrations of the surface water showed a strong and significant (P<0.05) decrease immediately after the onset of the treatments (after 10 weeks; t=0). After this temporary decrease, P concentrations started to increase gradually. As a result, significantly lower P concentrations (p<0.05) were found for the high salinity treatment compared to the low salinity treatment in both sediments at a short term (after 20 weeks; t=10), and a trend was found when compared to the control treatment (p<0.1) at sediment A. When P mobilisation rates were calculated for the short term, however, no differences among salinity treatments were found (Table 3).

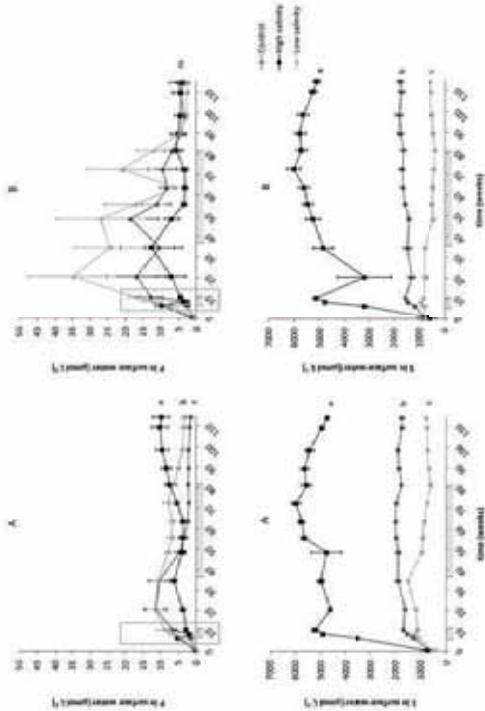


Figure 3. Phosphorus (P) and sulphur (S) concentrations (μmol L⁻¹) in the surface water above both sediments (A: left, B: right). Significant differences between treatments are indicated with different letters. Grey shadings under the x-axis indicate periods with through-flow (see Materials and Methods).

More than 80 weeks after the start of the experiment, P concentrations in the surface water above sediment A were significantly higher (p<0.01) at a high salinity (Figure 3), which was totally opposite to the short-term effect. Calculated P mobilisation rates were also significantly higher (p<0.05) with a high salinity compared to a low salinity at sediment A. For the control and low salinity treatment, P concentrations in surface water remained low, or even showed a decrease in the long term. At sediment B, however, no change of P in the surface water was found for any of the salinity treatments. The long-term P mobilisation rates with a high salinity were similar to the short-term rates at sediment A, while no long-term P mobilisation was observed for the low salinity and control treatments.

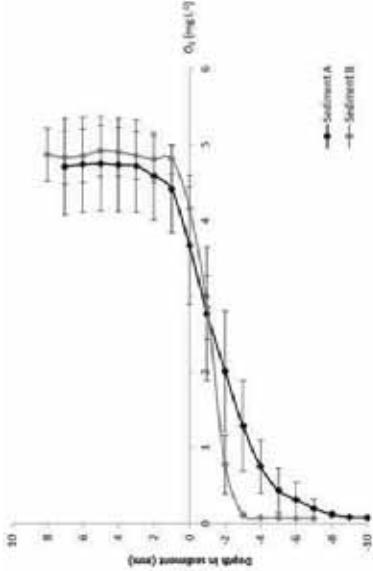


Figure 4. Oxygen (O₂) concentration (mg L⁻¹) profile per mm of both sediments (A and B), at the sediment-water interface during aerobic conditions.

Aerobic versus anaerobic surface water

The O₂ concentration profile (Figure 4) shows that under aerobic conditions, O₂ is still available in the sediment to an average depth of 7 mm (sediment A) and 3 mm (sediment B). The cores of both sediment A and B showed a significant (p<0.001) higher mobilisation rate of P during anaerobic conditions (Figure 5). At sediment A, P mobilisation was on average 3 times higher during anaerobic conditions compared to aerobic conditions, while this was almost 4 times higher at sediment B. These aerobic mobilisation rates were well within range of the short-term mobilisation rates found in the main experiment (control treatment; Table 3).

		P mobilisation (μmol m ⁻² day ⁻¹)		S mobilisation (μmol m ⁻² day ⁻¹)	
		short term	long term	short term	long term
High salinity	Mean	6.8	4.1 ^a	-1316.4 ^a	-1447.5 ^a
	SEM	1.6	2.4	86.7	243.0
	Mean	37.7	-1.1 ^{ab}	140.9 ^b	-87.0 ^b
Low salinity	Mean	14.3	0.6	216.0	86.4
	SEM	19.8	-4.0 ^b	-6.5	244.2 ^b
	SEM	12.9	2.3	425.0	69.2
High salinity	Mean	16.3	-2.9	-8536.5	-1121.7 ^a
	SEM	24.2	1.0	5474.0	141.3
	Mean	53.3	-2.2	-840.4	123.6 ^b
Control	Mean	36.6	2.5	517.8	91.1
	SEM	102.6	-13.1	-597.4	294.8 ^b
	SEM	74.3	6.5	236.3	83.3

Table 3. P and S mobilisation rates (μmol m⁻² day⁻¹) in the short term (10-30 weeks) and in the long term (80-110 weeks). Significant differences between treatments are indicated by different letters.

Discussion

Short-term effects (0 – 10 weeks)

P mobilisation

In the short term, no differences in net mobilisation rates of P were found among the different treatments. During this first stagnant period, moderate P mobilisation rates of 7–103 $\mu\text{mol m}^{-2} \text{d}^{-1}$ were found that fitted within the range of Geurts *et al.* (2010), who found mobilisation rates of 10–150 $\mu\text{mol m}^{-2} \text{d}^{-1}$ for sediments of which pore water Fe:P and total sediment Fe:S ratios were <1. Diffusion was most likely the main mechanism driving P release (Boström and Pettersson, 1982; Geurts *et al.*, 2010), since the sediments used in this experiment were not subjected to bioturbation or resuspension (Suzumura *et al.*, 2000), nor to a changed pH or temperature (Wu *et al.*, 2014). Moreover, both sediments were characterised by total Fe:S ratios below 0.5 (Table 1), which indicates that most Fe was bound to reduced S (Smolders *et al.*, 2001). Indeed, dissolved pore water Fe^{2+} concentrations were low in this study (and ranged between 0–20 $\mu\text{mol L}^{-1}$ for both sediments), and showed an even further decrease over time, resulting in very low pore water Fe:P ratios (<0.1) during the entire experimental period.

Salinity effects

Although increased salinity may lead to increased desorption of P from anion exchange sites (Caraco *et al.*, 1989), or by increased S^{2-} production and enhanced mineralisation rates (Lamers *et al.*, 2002a; Smolders *et al.*, 2006a), we did not find higher pore water P concentrations in the high salinity treatment. Instead, during the addition of the salinity treatments (between week 6 and 10), P concentrations in the surface water showed a short, strong drop for both sediments. This immediate drop of P observed upon a change of the surface water chemistry strongly points at a chemical, rather than a microbiological, explanation. It can most likely be explained by the co-precipitation of P with Ca^{2+} or CaCO_3 at the sediment-water interface (Boström *et al.*, 1988; Nielsen *et al.*, 2003), as Ca^{2+} concentrations directly and strongly increased in both surface and pore water upon the high salinity treatment (0–10 weeks). Accordingly, Suzumura *et al.* (2000) found a fast chemical P (im)mobilisation response within minutes, due to adsorption-desorption processes after a changed salinity. Van Dijk *et al.* (2013) found a similar immobilisation of P with increased salinity, explained by co-precipitation with Ca^{2+} in the sediment. Degassing of carbon dioxide (CO_2) and possibly also the presence of microbial mats (Dupraz *et al.*, 2009) may well have contributed to the precipitation of CaCO_3 at the sediment surface, as HCO_3^- concentrations were up to three times higher in pore water than in the surface water. After the initial drop of P, concentrations started to gradually increase, which shows that the short-term overall net P mobilisation to the surface water was higher than its immobilisation due to co-precipitation with Ca^{2+} .

Long-term effects (1.5 – 2 years)

P mobilisation

In contrast to the short-term results, and rather unexpectedly for eutrophic sediments, P mobilisation to the surface water was absent in 5 out of 6 treatments in the longer term (after 80 weeks). This is remarkable, as a strong net diffusive P release in both sediments was expected given the very low pore water Fe^{2+} concentrations and the still very high pore water P concentrations (Geurts *et al.*, 2010). Although a gradual decrease of P in the pore water of sediment A was observed, concentrations still remained sufficiently high for diffusive P release (>100 $\mu\text{mol L}^{-1}$) (Smolders *et al.*, 2001; Geurts *et al.*, 2010). Sediment Beven showed a gradual increase of pore water P concentrations during the experiment, without any increase of the P mobilisation to the surface water. Such results can only be explained by assuming that processes preventing net P release at the sediment-water

interface become active in the long term, at least under the conditions that were created during our experiment. Possible explanations for this phenomenon are: (1) precipitation of P with Fe^{3+} or $\text{Fe}(\text{OH})_x$ by the oxidation of the sediment surface (Einsele, 1936; Mortimer, 1941; 1942), (2) storage of P by the microbial community at the sediment surface during aerobic conditions (Deinema *et al.*, 1980; Hupfer and Uhlmann, 1991), (3) precipitation of P with calcium-minerals (Boström *et al.*, 1988; House *et al.*, 1999), although the latter would mainly be expected in the high salinity treatment.

An explanation for the lack of P release in the long term might be the uptake of P by microbial mats growing on top of the sediment (Deinema *et al.*, 1980; Hupfer and Uhlmann, 1991; Dupraz *et al.*, 2009). These mats can develop over time and might also benefit from stable sediment conditions that developed in the experimental set-up. However, our experiment was carried out in the dark, excluding photosynthetically active organisms, and no visible signs of such mats were observed. Nevertheless, the potential role of microbial sequestration of P on the long term cannot be ruled out. Most likely Fe redox cycling played a dominant role in the absence of P mobilisation, as was also indicated by the strongly increased P release under anaerobic conditions compared to aerobic conditions (Figure 5). It has been demonstrated that diffusive P release should be prevented under aerobic conditions if pore water Fe:P ratios are relatively high (at least >1) (Gunnars *et al.*, 2002; Blomqvist *et al.*, 2004; Geurts *et al.*, 2010). In our sediments, however, pore water Fe:P ratios were very unfavourable. Nevertheless, oxidation processes might be able to mobilise Fe^{2+} from FeS_x at a spatial micro-scale in the sediment surface at relatively low O_2 levels (Roden, 2012), catalysed by a oxidising microbes (Imhoff *et al.*, 1995). Our O_2 profiles showed that O_2 was available in the surface water and in the top millimetres of the sediment. The observed high S mobilisation rates in the low salinity treatment, where no S was added, indeed showed that SO_4^{2-} is being mobilised from the sediment by the oxidation of FeS_x . Simultaneously, Fe^{2+} thus becomes available to be oxidised (Roden, 2012), and is able to sequester dissolved P. So the intrusion of O_2 in reduced sediments may mobilise S bound Fe at a millimetre spatial scale, providing dissolved Fe^{2+} for the formation of ferric $\text{Fe}(\text{OH})_x$ at the sediment surface (Figure 6). This mechanism may very well explain the unexpected lack of P release from the sediments in the long term under aerobic conditions.

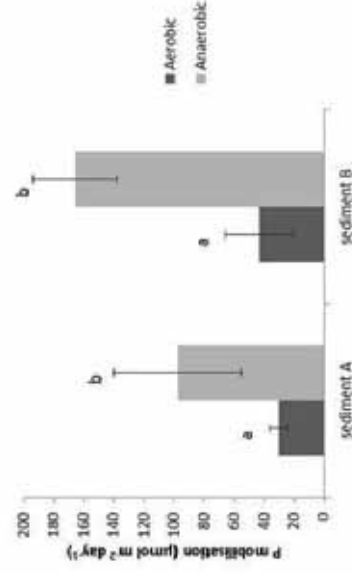


Figure 5. P mobilisation rates ($\mu\text{mol m}^{-2} \text{day}^{-1}$) during aerobic and anaerobic conditions for both sediment cores (A and B). Significant differences between treatments are indicated with different letters.

Our experimental set-up, without sediment disturbance and with relatively low biochemical O_2 demand (BOD) due to the absence of fresh organic matter input, will certainly have contributed to the long-term outcome of the experiment. Nevertheless, it seems plausible that it took a relatively long

time before the sediment surface became sufficiently oxidised, or before the microbial population was sufficiently developed, to completely prevent P mobilisation in the experiment. These results in the longer term may represent field situations with stable non-bioturbated FeS_x -rich sediments or sediments with stagnant, hypolimnetic water. During anaerobic conditions, P mobilisation was strongly enhanced (Figure 5), which clearly highlights the importance of O_2 availability to prevent P release. Field experiments are, therefore, necessary to validate our experimental results and suggested mechanism for the lack of P release from S-rich aquatic sediments.

Salinity effects

For the high salinity treatment, one of the sediments showed an increase of the surface water P concentration also in the long term (80 weeks). In saline or estuarine systems, P is often found to be easily released from soil particles (Carpenter and Smith, 1984; Suzumura *et al.*, 2000), and dissolved P concentrations are usually higher with increasing salinity (Gunnars and Blomqvist, 1997; Blomqvist *et al.*, 2004; Jordan *et al.*, 2008). At a high salinity, SO_4^{2-} concentrations increased in both surface water and pore water and a considerable part may be reduced deeper in the sediment, since it was not released to the surface water (Figure 6). Produced S^{2-} will react with O_2 and interfere with the oxidation of FeS_x or again immobilise Fe^{2+} . As expected, the net mobilisation of Fe^{2+} will be less, leading to insufficient formation of ferric $\text{Fe}(\text{OH})_3$ to prevent the release of P to the surface water (Hupfer and Lewandowski, 2008; Geurts *et al.*, 2010). This decoupling of the Fe and P cycle (Caraco *et al.*, 1989) at a micro-scale diminishes the P-binding capacity at the water-sediment interface. In sediment A, O_2 penetrated deeper into the sediment, suggesting that less O_2 was consumed, less FeS_x was oxidised, and less Fe^{2+} was mobilised. This may partly explain the long-term release of P from sediment A in the high salinity treatment. Desorption of P from ferric $\text{Fe}(\text{OH})_x$ due to the high Cl⁻ concentrations (Caraco *et al.*, 1989; Beltman *et al.*, 2000) might have increased this effect.

Implications for water management

Although the mobilisation of P from the S-rich and relatively Fe-poor sediments (typical for coastal wetlands) was mainly driven by diffusion, the build-up of a stable oxidised sediment surface may have prevented the release of P under the experimental conditions. We hypothesise that the oxidation of FeS_x in the sediment surface delivers the Fe^{2+} necessary for the precipitation of P at the sediment-water interface (Figure 6). Disturbance of the sediment-water interface due to wind, ebullition of gases from the sediment, and bioturbation can, however, prevent this build-up of a protective Fe-rich sediment surface and potentially increase the release of P (Boström and Pettersson, 1982; Boström *et al.*, 1988). Although such processes might also mix the sediment surface with O_2 and have an opposite effect. Moreover, our results indicate that an increased salinity may lead to a long-term P release, probably by interfering with the Fe^{2+} mobilisation due to increased SO_4^{2-} reduction rates in the anaerobic sediment. They also point out that sediments may react differently upon increased salinity. Therefore, O_2 and BOD, but also the actual concentration of SO_4^{2-} play a key role in the mobilisation of P from FeS_x -rich sediments. This might have important implications for water management and nature management of eutrophic peatlands in relation to salinisation.

More research, especially field measurements, is necessary to further confirm the experimental results we found for these FeS_x -rich sediments. Our experiment was carried out at 15 °C and without the continuous input of reactive organic material. Warmer conditions, e.g. during warm episodes in summer will lead to increased mineralisation rates, and also to higher O_2 consumption rates and lower solubility of O_2 . Especially when there is a high input of reactive organic matter, this will lead to strongly decreased O_2 concentrations in the surface water, which may prevent adequate oxidation of the sediment surface. Under such conditions this biogeochemical mechanism is expected to fail, leading to strong P mobilisation from the sediment as was shown in this study and also found by Smolders *et al.* (2006a). As a result, floating-leaved species, or floating beds of algae or cyanobacteria may develop, which will further decrease the O_2 concentrations in the surface water and enhance sediment P mobilisation. This explains why FeS_x -rich sediments that show very high dissolved P concentrations and low dissolved Fe^{2+} concentrations tend to show a high P release mainly in summer, which has important implications for water management.

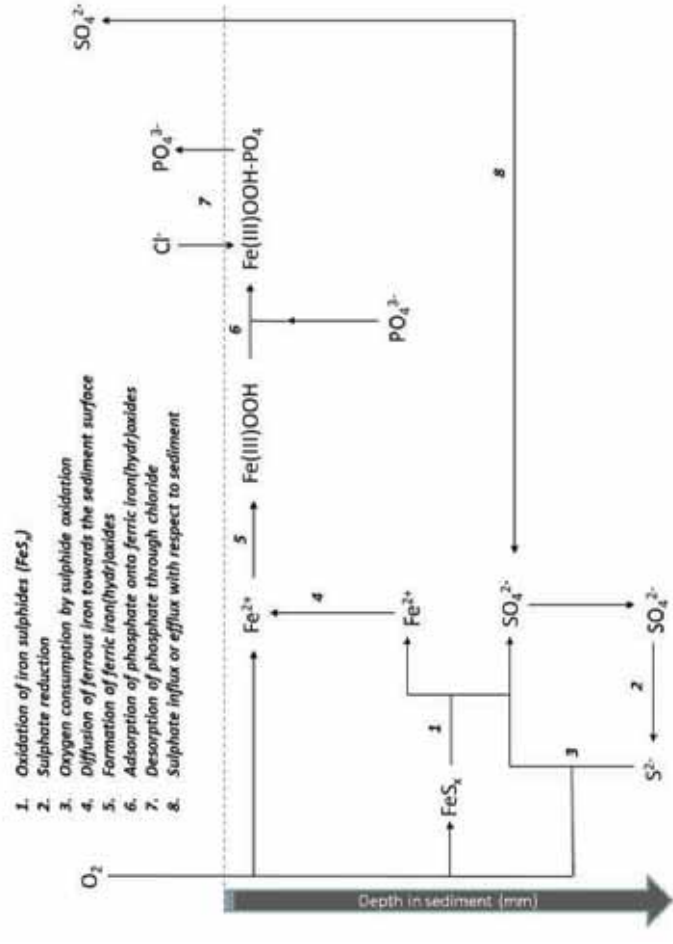


Figure 6. Schematic overview of the proposed mechanism, showing key processes in the upper millimetres of the S-rich, peat sediments involved in P mobilisation. Salinisation leads to an increased SO_4^{2-} influx, affecting Fe diffusion to the sediment surface, enabling increased P mobilisation in the longer term.

Conclusions

Low pore water $\text{Fe}:\text{P}$ ratios indicated a decoupling of the Fe and P cycle. Although these FeS_x -rich sediments were expected to release significant amounts of P by diffusion, this only seemed to be the case in the short term under aerobic conditions.

Increased salinity led to co-precipitation of P with Ca^{2+} in the short term, lowering actual P concentrations. However, short-term P mobilisation rates were found to be similar for all treatments, regardless of salinity.

Our experimental results suggest that the classic theoretical framework of oxidative conditions in the surface water that prevent P release from the sediment, may also hold in sediments showing unfavourable total Fe:S ratios but high FeS_x concentrations. In our FeS_x -rich, eutrophic sediments, typical for coastal wetlands, O_2 availability still seemed to be the most important determinant of sediment P release, at least under stable sediment conditions.

We suggest a controlling mechanism in which the partial oxidation of FeS_x mobilises sufficient Fe^{2+} at micro-scale for the precipitation of P at the sediment-water interface.

Next to O_2 , SO_4^{2-} plays a key role in P mobilisation, as high concentrations may counteract the oxidising effect by immobilising Fe^{2+} . In the longer term, an increased salinity may, as a result, lead to P mobilisation despite oxidation of the sediment surface.



3 | Differential responses of two wetland graminoids to high ammonium at different pH values

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Abstract

Enhanced soil ammonium (NH_4^+) concentrations in wetlands often leads to graminoid dominance, but species composition largely varies. Although NH_4^+ is readily taken up as a nutrient, several wetland species are known to be sensitive to high NH_4^+ concentrations or even suffer from toxicity, particularly at low soil pH. More knowledge about differential graminoid responses to high NH_4^+ availability in relation to soil pH can help to understand vegetation changes and to maintain wetland biodiversity. The responses of two wetland graminoids, *Juncus acutiflorus* and *Carex disticha*, to high (2 mmol L^{-1}) versus control ($20 \mu\text{mol L}^{-1}$) NH_4^+ concentrations were therefore tested in a controlled hydroponic set up, at two pH values (4 and 6). A high NH_4^+ concentration did not change total biomass for both species at either pH, but increased carbon allocation to the shoots, increased P uptake, and led to K and Ca limitation, depending on pH treatment. More than 50% of N taken up by *C. disticha* was invested in N-rich amino acids with a decreasing C:N ratio, while this was only 10% for *J. acutiflorus*. Although both species appeared to be well adapted to high NH_4^+ loadings in the short term, *C. disticha* showed a higher extent of classic detoxifying responses that are early warning indicators for decreased tolerance in the long term. In general, the efficient above-ground biomass allocation, P-uptake, and N detoxification explain the competitive strength of wetland graminoids at the expense of overall biodiversity at high NH_4^+ loadings.



Introduction

Increased nitrogen (N) availability is known lead to biodiversity loss and degradation of ecosystem functions, often related to enhanced dominance of N-limited graminoids (Bobbink *et al.*, 1998). For several decades, N availability in wetlands has been greatly enhanced by the increased input of the mobile N-form nitrate (NO_3^-) leaching from surrounding agricultural land (Britto & Kronzucker, 2002; Miller & Cramer, 2004; Sutton *et al.*, 2011) and forests (Dise *et al.*, 1995), by enhanced atmospheric N deposition (Bobbink *et al.*, 1998), and by increased decomposition of organic soil with concomitant N mineralisation (Lamers *et al.* 2001; Geurts *et al.*, 2010). In wetland soils, NO_3^- is generally only present near the soil surface during relatively wet or waterlogged conditions. Instead, the more immobile NH_4^+ is the dominant N form under anaerobic conditions due to limited oxygen availability, leading to low NH_4^+ oxidation rates, increased NO_3^- losses by denitrification, and dissimilatory NO_3^- reduction to NH_4^+ (DNRA; Burgin & Hamilton, 2007). A highly increased N input may therefore lead to significantly increased NH_4^+ concentrations in such wetland systems (Britto & Kronzucker, 2002; Miller & Cramer, 2004), affecting the competition among species and leading to vegetation changes.

Although NH_4^+ is readily taken up as a nutrient, various species from dry, moist and wetland ecosystems appear to be sensitive to high NH_4^+ concentrations, and this N form can even become toxic (Van Katwijk *et al.*, 1997; Britto & Kronzucker, 2002; Stevens *et al.*, 2011). Several typical sensitivity symptoms have regularly been found when plants were exposed to high NH_4^+ concentrations, including suppressed growth (or even mortality), lower photosynthesis, a decrease in root-shoot ratio, decreased uptake of cations and increased uptake of anions, and increased metabolic costs due to assimilation of free amino acids (Britto & Kronzucker, 2002; Tylová *et al.*, 2008; Van der Heide *et al.*, 2008; Christensen *et al.*, 2011; Stevens *et al.*, 2011; Fritz *et al.*, 2014). However, tolerance to NH_4^+ appears to differ widely among species and even among plants belonging to the same species (Cruz *et al.*, 2011).

It is often assumed that plants naturally growing in NH_4^+ rich, acidic systems are more tolerant to high NH_4^+ concentrations, whereas acid-sensitive species from more buffered systems are more sensitive to NH_4^+ (De Graaf *et al.*, 1998; Britto *et al.*, 2001; Britto & Kronzucker, 2002; Van den Berg *et al.*, 2005). Additionally, NH_4^+ toxicity is often found to be related to a low pH of the rhizosphere, while in more buffered conditions in the rhizosphere toxicity symptoms are often minimized or even absent (Findenegg, 1987; Lucassen *et al.*, 2002; Van den Berg *et al.*, 2005). Therefore, wetland species adapted to waterlogged, buffered conditions, may be expected to cope well with strongly increased NH_4^+ levels in soil pore water (concentrations in the millimolar range) as long as the pH is in the neutral range. However, due to differences in their growth responses and tolerances, species (including graminoids) are expected to show differential responses.

In the present study we therefore tested the responses and tolerances to greatly enhanced NH_4^+ availability for two graminoid species that often dominate vegetation in wet grasslands and marshes, Sharp flowered rush (*Juncus acutiflorus*) and Brown sedge (*Carex disticha*). Firstly, we tested whether these wetland species indeed prefer the uptake of NH_4^+ rather than NO_3^- . Secondly, their responses and tolerances to strongly increased NH_4^+ concentrations were tested under neutral and acidic conditions, by quantifying several factors that control NH_4^+ tolerance of wetland graminoids. As both species naturally occur on buffered soils, we expected high NH_4^+ loadings to become toxic only at a low pH, while at neutral pH a fertilizing effect was expected. *J. acutiflorus* is often found under more nutrient-rich conditions than *C. disticha*, and increased dominance of *J. acutiflorus* at the expense of smaller sedges such as *C. disticha* has been regularly observed in wet

meadows affected by increased N input (personal observations). As dominance of graminoids often affects biodiversity in wetlands, more knowledge of differential responses by graminoid species to highly increased NH_4^+ availability from agricultural inputs may help to understand vegetation changes and to maintain wetland biodiversity.

Material and Methods

Experimental design

Test species

In this research, two very common graminoid spp. for Europe, Sharp flowered rush - *Juncus acutiflorus* and Brown sedge - *Carex disticha*, were selected. These species usually occur on moist or wet soils in grasslands, sedge fens, and dune valleys. The soils in these habitats are generally buffered at a pH of 5 - 6, and can be characterized as mesotrophic to moderately eutrophic. In the Netherlands, the soils of well-developed vegetation, in which both species are present but not dominant, contain NH_4^+ concentrations ranging from 10 to 20 $\mu\text{mol L}^{-1}$ (unpublished field data). Both species are known to have special adaptations such as root aerenchyma and radial oxygen loss (ROL) from the roots to function well in waterlogged conditions, although the spatial ROL pattern differs between the species (Lamers *et al.*, 2013).

Plant collection

Before the start of the experiments, sods with *C. disticha* and *J. acutiflorus* were collected from a wet meadow in a nature reserve near Nijmegen, the Netherlands (De Bruuk, 51°46' N; 5°53' E) in mid June 2009 (N sensitivity exp.) and 2011 (N uptake exp.). Ramets of both species were carefully taken from the sods. The roots of equally sized individuals were cut off and the remaining shoots were put into an aerated $\frac{1}{8}$ Hoagland nutrient solution (Hoagland & Arnon, 1950) in order to develop new roots to a length of approximately 2 cm.

N uptake experiment

The preferential N form (NO_3^- or NH_4^+) for both plant species was explored in an N uptake experiment. For this experiment, a hydroponic system was used with 1.5 L opaque plastic containers. After adjustment of 7 days in aerated $\frac{1}{8}$ Hoagland nutrient solution at either pH 4 or pH 6 (containing both NO_3^- and NH_4^+), plants were provided with the following nutrient solution: 100 $\mu\text{mol L}^{-1}$ Ca^{2+} , 100 $\mu\text{mol L}^{-1}$ Mg^{2+} , 200 $\mu\text{mol L}^{-1}$ K^+ , 100 $\mu\text{mol L}^{-1}$ SO_4^{2-} , 200 $\mu\text{mol L}^{-1}$ PO_4^{3-} , 0.8 $\mu\text{mol L}^{-1}$ Mn^{2+} , 0.8 $\mu\text{mol L}^{-1}$ H_3BO_3 , 0.7 $\mu\text{mol L}^{-1}$ Zn^{2+} , 0.27 $\mu\text{mol L}^{-1}$ Fe (applied as Fe-EDTA), 0.2 $\mu\text{mol L}^{-1}$ Cu^{2+} , 0.008 $\mu\text{mol L}^{-1}$ Mo (De Graaf *et al.*, 1998), with both NO_3^- (20 $\mu\text{mol L}^{-1}$) and NH_4^+ (20 $\mu\text{mol L}^{-1}$), at either pH 4 or pH 6 ($n=5$). A permanent air flow through the nutrient solution prevented anaerobic conditions, and 1-cyanguanidine (1% molar concentration of the N concentration) was added to the nutrient solution to inhibit nitrification (Van den Berg *et al.*, 2005). The experiment was performed in a climate controlled room with a 16:8 hours day:night regime, at an average temperature of 20°C during the day and 17°C during night, a relative humidity of 50-70%, and irradiance of 300-400 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Three individuals of the same species were placed in each plant container by using polystyrene trays for flotation. After 21 days, either $\text{Na}^{15}\text{NO}_3$ or $^{15}\text{NH}_4\text{Cl}$ (1% molar concentration of total N) were added to determine uptake rates of both N forms by the different species. After 2 hours of labeling, plants were harvested and uptake rates were calculated from the accumulation of enriched N in this period.

N sensitivity experiment

After an acclimatisation period of 3 weeks in aerated $1/8$ Hoagland nutrient solution, plants of both species were exposed to four different treatments in a full factorial designed hydroponic system, differing in N concentration and pH (n=4). Two individuals of the same species were placed in each plant container using polystyrene trays for flotation and to prevent algal growth. A permanent air flow through the nutrient solution prevented anaerobic conditions. In total, 32 plant containers were used. The experiment was performed in a climate controlled room with similar conditions as described above, and lasted for eight weeks. Two different nutrient solutions were used as treatments: $20 \mu\text{mol L}^{-1} \text{NH}_4^+$ and $2 \text{mmol L}^{-1} \text{NH}_4^+$ at either pH 4, representing acidified conditions, or pH 6, representing buffered conditions. Concentrations of micro-nutrients were similar to those in the former experiment (see above) for all four solutions. The pH of the nutrient solution was set using $1.2 \text{mol L}^{-1} \text{HCl}$ and $1 \text{mol L}^{-1} \text{NaOH}$ and pH was checked daily and adjusted when required. The nutrient solution in each container was continuously refreshed by pumping at a rate of 1.5L day^{-1} from a separate 25 L opaque storage tank, filled with weekly refreshed nutrient solution. In order to prevent nitrification, 1-cyanguanidine (1% molar concentration of NH_4^+) was added to all storage tanks (Van den Berg *et al.*, 2005). During the experiment, growth rates of roots and shoots were monitored by measuring their maximum lengths. After eight weeks, all plants were harvested.

Chemical analysis

For both experiments, fresh weight of all root and shoot material was determined directly after harvesting and for the N sensitivity experiment roots were scanned to determine total root length using WinRHIZO (Regent Instruments Inc., Canada). In addition, the number of leaves was counted for each individual plant. In the N sensitivity experiment, a fresh subsample (1 g) of all shoots was directly frozen at -80°C for amino acid analysis. Free amino acids were extracted using ethanol according to Van Dijk & Roelofs (1988). Twenty amino acids were quantified by measuring fluorescence after precolumn derivation with 9-Fluorenylmethyl-Chloroformate (FMOC-Cl) and measured with Norvaline as the internal standard using HPLC (Model 920-LC; Varian Liquid Chromatography, USA). Another fresh subsample of the shoot (2cm^2) was used to determine chlorophyll concentrations directly after harvest, using ethanol extraction (Wintermans & De Mots, 1965) and a spectrophotometer (Model UV-1205; Shimadzu, Japan).

Root and shoot material was dried for 24 hours at 70°C to determine dry weight (biomass). Total nutrient concentrations in roots and shoots were measured by digesting 200 mg of dried, homogenised material in sealed Teflon vessels in an Ethos D microwave lab station Terminal 20 (Milestone Inc., USA) with 4 mL nitric acid (65% v/v) and 1 mL hydrogen peroxide (30% v/v). This material was then analysed for total concentrations of Ca, K, Mg, P and S using Inductively Coupled Plasma emission spectrophotometry (ICP, Iris Intrepid II; Thermo Fisher Scientific, USA). In order to measure C and N concentrations, 4 mg of dried, homogenised root and shoot material was transferred to tin cups and combusted in a CNS elemental analyser (Model EA NA1500; Carlo Erba Instruments, Italy). For the N uptake experiment, the isotopic N composition in the dry plant tissue was determined using an elemental analyser (Model EA 1110; Thermo Fisher Scientific, USA) coupled to an Isotopic Ratio Mass Spectrometer (IRMS, Model Finnigan Delta Plus; Thermo Fisher Scientific, USA). Total uptake rates were calculated from enrichment of ^{15}N in plant tissue, after correction of the ^{15}N background due to natural abundance and the dilution factor of the added amount of ^{15}N .

Statistical analysis

Data of sub-replicates from the 2 or 3 plants growing in one container were pooled, leaving 4 or 5 replicates per treatment (sensitivity experiment and uptake experiment, respectively). Data are expressed as mean $\pm$ S.E.M. If necessary, data were transformed logarithmically to meet the criteria of ANOVA testing and all data were analysed using the SPSS 16.0 package (SPSS Inc., USA). A one way ANOVA was carried out to test for possible differences in chemical composition, root:shoot ratio and biomass, using treatment type as class variables. A two-way ANOVA was used to test for interactions between pH and N treatments.

Results

Uptake NH_4^+ versus NO_3^-

Both *C. disticha* and *J. acutiflorus* were able to take up NH_4^+ as well as NO_3^- at both pH 4 and pH 6 when both N forms were available (figure 1), but both species showed higher uptake rates for NH_4^+ than for NO_3^- ($p < 0.05$). The rates for NH_4^+ uptake were 2-3 times those for NO_3^- in *C. disticha* and only 1.5 times in *J. acutiflorus*. In this experiment, pH neither affected N uptake nor N allocation (results not shown) in both species.

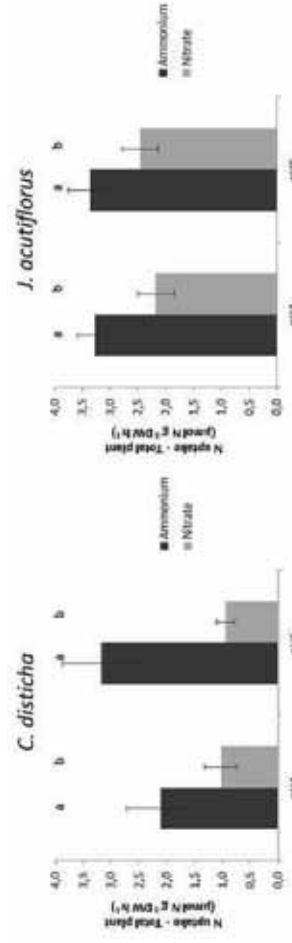


Figure 1. Uptake rates for either NO_3^- or NH_4^+ ($\mu\text{mol N g}^{-1} \text{total DW h}^{-1} \pm \text{SEM}$) at two pH values for *C. disticha* and *J. acutiflorus*, when both N forms were available at $20 \mu\text{mol L}^{-1}$. Significant differences between the treatments are indicated by different letters.

Effects low versus high NH_4^+

Biomass production

None of the treatments in the N sensitivity experiment led to mortality of individual plants. For both species, increased NH_4^+ concentrations did not change total plant biomass (figure 2). For *C. disticha*, shoot biomass significantly ($p < 0.001$) increased at $2 \text{mmol L}^{-1} \text{NH}_4^+$, while root biomass significantly ($p < 0.001$) decreased. Shoot biomass showed a higher increase at pH 6 than at pH 4 ($p < 0.03$). As a result, root:shoot ratios of *C. disticha* were found to be significantly ($p < 0.001$) lower at $2 \text{mmol L}^{-1} \text{NH}_4^+$ and significantly ($p < 0.01$) lower at pH 6. For *J. acutiflorus*, a significant interaction ($p < 0.03$) between pH and high NH_4^+ was found in shoot biomass. This led to an increase of shoot biomass in the high NH_4^+ treatment compared to the low NH_4^+ treatment at pH 4, while at pH 6 no significant differences were found. Root biomass was significantly ($p < 0.001$) lower at high NH_4^+ concentrations, both at pH 4 and pH 6. As a result, a significant ($p = 0.05$) interaction between high NH_4^+ and pH was found for the root:shoot ratio, which was mostly caused by a decrease of root biomass (pH 4 and pH

Figure 2. Total biomass (g DW; $\pm$ SEM) of *C. disticha* and *J. acutiflorus* after growing on 20 μ M L^{-1} NH_4^+ or 2 mmol L^{-1} NH_4^+ at either pH 4 or pH 6, divided into shoot biomass, rhizome biomass and root biomass. Significant differences are indicated by different letters, * indicates a significant interaction between pH and N effects.



Table 1. Concentrations of total C, N, P, K, Fe, Ca, Mg, N:P ratio, and N:K ratio ($\pm$ SEM) in shoots and roots of *C. disticha* and *J. acutiflorus* grown on 20 $\mu\text{mol L}^{-1}$ NH_4^+ , or 2 mmol L^{-1} NH_4^+ , at either pH 4 or pH 6.



an interaction effect ($p < 0.05$) between NH_4^+ and pH, and an increase in shoots only with high NH_4^+ ($p < 0.001$). For *J. acutiflorus*, pH and NH_4^+ showed an interaction ($p < 0.0015$) in shoot tissue, with the lowest K concentrations at high NH_4^+ and low pH. In root tissue of *J. acutiflorus*, K concentrations were also significantly lower ($p < 0.001$) at high NH_4^+ , regardless of pH. This led to increased N:K ratios ($p < 0.001$) in roots of *J. acutiflorus*, with an interaction effect ($p = 0.05$) between increased NH_4^+ and low pH. Iron (Fe) concentrations in shoot and root tissue of *C. disticha* showed no differences between treatments (table 1). Also *J. acutiflorus* showed no differences in Fe concentrations in roots. Although an interaction ($p < 0.05$) between N and pH treatments was found for shoots of this species, no clear pattern could be observed. In shoots of both species, significantly lower concentrations of calcium (Ca) were found at high NH_4^+ ($p < 0.001$) and at low pH ($p < 0.01$). In root tissue, lower ($p < 0.001$) Ca concentrations were found only for *C. disticha* at low pH, regardless of the N treatment. Magnesium (Mg) concentrations in root tissue of both species were also reduced ($p < 0.001$) at high NH_4^+ and at a low pH ($p < 0.001$). In shoot tissue, significantly lower ($p < 0.05$) Mg concentrations were only found at a low pH, but no differences between NH_4^+ treatments were found. When the dilution effect due to an increase of shoot biomass at high NH_4^+ was considered, calculations may show whether certain cations became limited for this treatment during the experiment (Timmer and Stone, 1978; De Graaf et al., 1998). According to these calculations, Ca became limited at pH 4 for both species, and K became limited only for *J. acutiflorus* at both pH values.

Table 2. Free amino acid concentrations ($\mu\text{mol N g}^{-1}$ DW) in shoots of *C. disticha* and *J. acutiflorus*, grown on $20 \mu\text{mol L}^{-1} \text{NH}_4^+$ or $2 \text{ mmol L}^{-1} \text{NH}_4^+$ at either pH 4 or pH 6.

<i>Carex disticha</i>									
		$\text{NH}_4^+ 20 \mu\text{M}$		$\text{NH}_4^+ 2 \text{ mM}$		p value			
		pH 4	pH 6	pH 4	pH 6	N	pH	N	pH
Total AA's	$\mu\text{mol N g}^{-1}$ DW	51 ± 8	53 ± 7	680 ± 81	497 ± 70	0.00	ns	0.00	ns
N-rich AA's	$\mu\text{mol N g}^{-1}$ DW	2.9 ± 1.0	7.1 ± 3.1	497 ± 74	371 ± 66	0.00	ns	0.00	ns
Asparagine	$\mu\text{mol N g}^{-1}$ DW	0.8 ± 0.4	4.0 ± 3.1	487 ± 73	365 ± 65	0.00	ns	0.00	ns
Aspartic acid	$\mu\text{mol N g}^{-1}$ DW	3.3 ± 0.3	3.3 ± 0.5	0.5 ± 0.5	1.2 ± 0.7	0.00	ns	0.00	ns
Glutamine	$\mu\text{mol N g}^{-1}$ DW	0.9 ± 0.3	1.2 ± 0.3	0 ± 0	0 ± 0	0.00	ns	0.00	ns
Glutamic acid	$\mu\text{mol N g}^{-1}$ DW	12.6 ± 0.8	11.8 ± 1.8	11.1 ± 1.5	9.4 ± 1.2	ns	ns	ns	ns
Arginine	$\mu\text{mol N g}^{-1}$ DW	0.2 ± 0.1	0.3 ± 0.1	6.2 ± 1.0	3.2 ± 1.3	0.00	ns	0.05	ns
Alanine	$\mu\text{mol N g}^{-1}$ DW	4.5 ± 0.5	4.1 ± 0.6	18.4 ± 2.9	14.4 ± 1.8	ns	ns	ns	ns
Serine	$\mu\text{mol N g}^{-1}$ DW	6.0 ± 1.3	5.8 ± 1.3	83.3 ± 7.5	46.4 ± 8.9	0.00	ns	0.00	ns

<i>Juncus acutiflorus</i>									
		$\text{NH}_4^+ 20 \mu\text{M}$		$\text{NH}_4^+ 2 \text{ mM}$		p value			
		pH 4	pH 6	pH 4	pH 6	N	pH	N	pH
Total AA's	$\mu\text{mol N g}^{-1}$ DW	84 ± 9	64 ± 5	164 ± 19	217 ± 21	0.00	ns	0.03	0.03
N-rich AA's	$\mu\text{mol N g}^{-1}$ DW	8.2 ± 1.1	6.5 ± 1.2	40 ± 9	91 ± 11	0.00	ns	0.01	0.01
Asparagine	$\mu\text{mol N g}^{-1}$ DW	1.0 ± 0.3	0.8 ± 0.4	23 ± 5	61 ± 5	0.00	0.03	0.01	0.01
Aspartic acid	$\mu\text{mol N g}^{-1}$ DW	5.1 ± 0.5	3.9 ± 0.2	9.3 ± 1.2	8.3 ± 1.5	0.00	ns	0.00	ns
Glutamine	$\mu\text{mol N g}^{-1}$ DW	2.5 ± 0.8	1.7 ± 0.4	21 ± 5	24 ± 12	0.00	ns	0.00	ns
Glutamic acid	$\mu\text{mol N g}^{-1}$ DW	12.9 ± 1.3	12.1 ± 0.7	20 ± 4	23 ± 2	0.00	ns	0.00	ns
Arginine	$\mu\text{mol N g}^{-1}$ DW	0.6 ± 0.2	0.3 ± 0.0	1.0 ± 0.4	1.7 ± 1.1	ns	ns	ns	ns
Alanine	$\mu\text{mol N g}^{-1}$ DW	4.8 ± 0.6	2.9 ± 0.1	10.0 ± 0.8	12.2 ± 3.2	0.01	ns	0.01	ns
Serine	$\mu\text{mol N g}^{-1}$ DW	5.2 ± 0.6	4.0 ± 0.6	11.9 ± 2.4	14.1 ± 1.8	0.00	ns	0.00	ns

N allocation to free amino acids

Both species responded to enhanced NH_4^+ concentrations by significantly ($p < 0.001$) increasing their total free amino acid concentrations in shoot tissue (table 2). The strong correlation ($R^2 = 0.87$) between the total free amino acids concentration and the total N concentration in the shoot tissue (figure 3) highlights the investment of both species into N-rich amino acids to store available N. The strongest response was shown by *C. disticha*, with 73–75% of the total amino acid concentration that consisted of N-rich amino acids at the high NH_4^+ treatment. The dominant N-rich free amino acid was asparagine, which accounted already for 71–73% of the total free amino acid concentrations. At the same time, other free amino acids such as glutamine and aspartic acid showed a significant ($p < 0.001$) decrease in the shoot tissue of *C. disticha*. Although *J. acutiflorus* showed a similar response to enhanced NH_4^+ , this was much less extreme than in *C. disticha*. *J. acutiflorus* also showed a significantly increase ($p < 0.001$) in total free amino acid concentrations (table 2). However, only 29–41% of the total free amino acid concentration was invested in N-rich amino acids ($p < 0.01$), which consisted mostly of asparagine (14–28%) and glutamine (11–12%). The total-N concentration and the free amino acid concentration in *J. acutiflorus* increased less than in *C. disticha* (figure 3). Most of the total N is located in the shoot tissue of both species (figure 4), showing strong significant interactions ($p < 0.01$) between high NH_4^+ and pH. However, *C. disticha* distributed significantly more N ($p < 0.001$) towards the roots in response to high NH_4^+ , while this response was absent for *J. acutiflorus*.



Figure 3. Correlation between total-N concentrations ($\mu\text{mol N g}^{-1}$ DW) in shoots of *C. disticha* and *J. acutiflorus*, and their N concentrations as free amino acids ($\mu\text{mol N g}^{-1}$ DW).

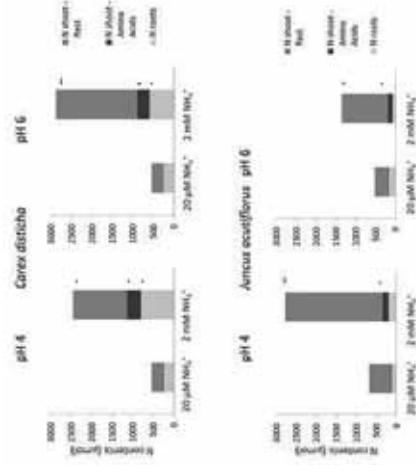


Figure 4. Total-N contents (μmol) in plants of *C. disticha* or *J. acutiflorus*, divided in roots, in free amino acids in the shoot, and in the rest of the shoot, after growing on low ($20 \mu\text{mol L}^{-1}$) or high (2 mmol L^{-1}) NH_4^+ concentrations at either pH 4 or pH 6. * indicates a significant increase due to the N treatment, ** indicates a significant increase due to an interacting effect of the N and pH treatment.

Discussion

Nutrient and C allocation

The uptake of NO_3^- is an energy demanding process for plants, because it has to be reduced to NH_4^+ before assimilation (Marschner, 1995; Miller & Cramer, 2004). Because NH_4^+ uptake is energetically more favourable, and both species often occur in wet, relatively NH_4^+ rich environments, a preference of NH_4^+ was to be expected. We indeed found that particularly *C. disticha*, and to a lesser extent also *J. acutiflorus*, preferentially take up NH_4^+ . Although NH_4^+ uptake was higher, NO_3^- was also taken up readily, especially by *J. acutiflorus*. In anoxic wetland soils, NH_4^+ is the most common N species present, but radial oxygen loss enhances oxidation of the rhizosphere (Visser *et al.*, 2000b; Colmer, 2003) leading to enhanced nitrification around the root surfaces (Engelaar *et al.*, 1995; Armstrong & Armstrong, 2001). With this rhizosphere modifying strategy, wetland plants might facilitate the uptake of either NO_3^- or NH_4^+ (Chang *et al.*, 2010; Konnerup & Brix, 2010). Interestingly, *J. acutiflorus*, which showed the highest affinity for NO_3^- , also has the highest radial oxygen loss (Lamers *et al.*, 2013). In addition, high affinities for NO_3^- in addition to NH_4^+ are also beneficial for both species when occurring in riparian wetlands.

Surprisingly, total biomass of both species was not affected at the high NH_4^+ treatment (2 mmol L^{-1}), while total biomass of other wetland species such as *Acorus calamus* (Vojtkova *et al.*, 2004) and *Glyceria maxima* (Tylova *et al.*, 2008) decreased as a result of sensitivity to high NH_4^+ loadings (resp. 14.8 & 3.7 mmol L^{-1}). In our experiments, there was neither a negative (toxic), nor a positive (nutrient) effect. Instead, *J. acutiflorus* and *C. disticha* only showed modified biomass allocation, in the form of a decreased root:shoot ratio as result of high NH_4^+ . This ability to change only biomass allocation, without decreasing total biomass, suggests high NH_4^+ tolerance. Such a tolerance to high NH_4^+ loadings in wetland graminoids was so far only found for *Phragmites australis* (Tylová *et al.*, 2008; Engloner, 2009). Given the low N:P ratios in the above-ground biomass (<14 g g^{-1}) that we found for both species, it seems likely that growth was N-limited in the low NH_4^+ treatments (Wassen *et al.*, 1995; Koerselman & Meuleman, 1996; Olde Venterink *et al.*, 2002). However, at the high NH_4^+ treatments, this seems very unlikely. Additionally, as higher NH_4^+ availability increased P uptake, and N:P ratios remained below this critical value, P limitation was even more unlikely. However, high NH_4^+ levels decreased K concentrations in the shoot, leading to increased N:K ratios. The fact that these ratios were >2.1 indicate that growth may have become (co-)limited by K at high NH_4^+ (Olde Venterink *et al.*, 2002), as was confirmed for *J. acutiflorus* using the method of Timmer & Stone (1978) and De Graaf *et al.* (1998). These results are in agreement with Tylová *et al.* (2008), who also found induced K shortage in the wetland species *Glyceria maxima* as a result of high NH_4^+ levels. Although total biomass was not affected, *J. acutiflorus* yielded a lower shoot biomass as might be expected with high NH_4^+ at pH 6. Although it is difficult to explain this response from our results, previous research suggested that cation deficiency might indeed be responsible for reduced growth of *J. acutiflorus* (Smolders *et al.*, 1997).

pH effects

A low pH was expected to have a negative effect on cation uptake at increasing NH_4^+ levels (Findenegg, 1987; Lucassen *et al.*, 2002; Van den Berg *et al.*, 2005). A higher proton concentration impairs the uptake of NH_4^+ , because it is accompanied by the release of protons to the rhizosphere (Miller & Cramer, 2004). Lucassen *et al.* (2002) found that the wetland species *Cirsium dissectum* suffered from severe growth reduction and even mortality at high NH_4^+ at pH 4, but not at pH 6, due to internal acidification of the roots. In contrast, growth nor NH_4^+ uptake rates of both tested graminoids were significantly affected by low pH in our study. It has been suggested that the uptake of NH_4^+ is most

favourable when co-transported with anions, since the assimilation of these anions could enhance rhizosphere alkalisation (Britto & Kronzucker, 2002). Although increasing S concentrations with increasing NH_4^+ concentration were found earlier for *Gentiana pneumonanthe*, *Calluna vulgaris*, *Deschampsia flexuosa* (Van den Berg *et al.*, 2005) and in *Ricinus communis* (Van Beusichem *et al.*, 1988), we did not find such effect here (data not shown). Uptake of other anions such as bicarbonate (HCO_3^-) could have caused a similar effect. Since we used a nitrification inhibitor, NO_3^- concentrations are unlikely to have induced this response.

Nutrient or toxin?

Biomass and resource allocation reflects the plasticity of plants to adapt to new situations, and to be able to compete with other fast growing species under more nutrient-rich conditions. Both species were able to increase their competitive strength with respect to light acquisition, at a lower root biomass, showing high C efficiency. Investing less C, while additional N is demanded for chlorophyll synthesis during biomass allocation to the shoot (Van Dijk & Roelofs, 1988), seems adaptively beneficial with highly increased NH_4^+ levels. This result shows that N eutrophication does not have to lead to higher total biomass production of plants *per se* in order to increase their competitive strengths. It also suggests that the positive aboveground response that is generally found as a result of eutrophication, without considering belowground responses, may reflect changed biomass allocation rather than growth stimulation. Especially if other nutrients, such as K, become (co-)limiting as a result of enhanced uptake of NH_4^+ . On the other hand, such changes in plant morphology and physiology as a result of increased NH_4^+ might still negatively affect plant fitness in the long term. The observed low root:shoot ratio in combination with impaired uptake per unit root biomass may reduce the uptake rates of other essential nutrients, and make plants more vulnerable to desiccation (Marschner, 1995; De Graaf *et al.*, 1998). Although a reduced root:shoot ratio is recognised as a symptom of NH_4^+ toxicity (Britto & Kronzucker, 2002; Stevens *et al.*, 2011), its occurrence alone may not certify actual toxicity. However, deficient plant Ca and K concentrations found for both graminoids in response to enhanced NH_4^+ uptake (Van Beusichem *et al.*, 1988; Britto & Kronzucker, 2002; Tylova *et al.*, 2008; Stevens *et al.*, 2011), and strongly increased concentrations of free (N-rich) amino acids in their shoot tissue (Marschner, 1995; Smolders *et al.*, 2000; Britto & Kronzucker, 2002; Miller & Cramer, 2004; Stevens *et al.*, 2011), do indicate that both species suffer from N overload. NH_4^+ is taken up by passive diffusion, but it also has to be assimilated immediately because of its toxicity when accumulating in plant tissue. Consequently, uptake of NH_4^+ directly results in a high demand of C skeletons during assimilation (Miller & Cramer, 2004).

Differential responses

High accumulation of free N-rich amino acids can be a good indicator for N saturation in plants (Van Dijk & Roelofs, 1988; Rabe, 1990; Näsholm *et al.*, 1994; Smolders *et al.*, 2000; Tomassen *et al.*, 2003). In our study, *C. disticha* stored much more total-N (up to 50%) in the form of free amino acids in above-ground biomass than *J. acutiflorus* (10%). Smolders *et al.* (1996) found extremely high accumulation of total-N (82–97%) as free amino acids, mostly as asparagine, in phosphorus-deficient *Stratiotes aloides* subjected to high NH_4^+ levels. In our study, *C. disticha* also predominantly invested in the amino acid asparagine, which is known to be a storage compound and the major transport compound of N from the root to the leaves (Mifflin & Lea, 1977; Lam *et al.*, 1996; Lea *et al.*, 2007). However, its metabolic role is minor, as only the amide group is used in the assimilation of proteins (Mifflin & Lea, 1977). Asparagine can readily accumulate at high rates when plants are growing in a N-rich medium, especially when an other mineral ion, probably K or Ca in our study, becomes limited for growth (Rabe, 1990; Lea *et al.*, 2007). In contrast, *J. acutiflorus* did accumulate

a greater variety of free N-rich amino acids in addition to asparagine, including glutamine, glutamic acid, alanine and serine. Glutamine synthesis is the initial step from which other amino acids that are used in the metabolism of developing plant parts are synthesized (Mifflin & Lea, 1977). This may indicate that although free amino acid concentrations are increasing, *J. acutiflorus* still seems to be coping relatively well with increased NH_4^+ uptake, while *C. disticha* appears to be more sensitive. As growth was not enhanced, however, *J. acutiflorus* might additionally have accumulated non amino acid N compounds that were not measured in our study. Cruz *et al.* (2011) already suggested that plant tolerance to NH_4^+ may differ among species. Our results indeed showed differential responses between specific wetland adapted graminoids. Tylová *et al.* (2008) suggested that different responses between wetland species upon high NH_4^+ could be related to their rooting strategy in hypoxic soils. The deeper rooting strategy and a higher ROL (Lamers *et al.*, 2013) might enhance NH_4^+ tolerance of *J. acutiflorus*, although this was not studied in this research. Our results imply that in addition to increased growth rates, differential tolerances to high NH_4^+ concentrations among graminoids may lead to changes in composition, in our case a higher competitive strength of *J. acutiflorus* and a lower competitive strength of *C. disticha* in the long term.

Conclusions

The wetland graminoids *J. acutiflorus* and *C. disticha* appeared to be well adapted to a highly increased NH_4^+ concentration and showed high tolerance in the short term. They both showed high C efficiency, allocating their biomass towards aboveground plant tissue without any change in total biomass. This efficient C allocation response enhances their competitive strength upon N enrichment, which will lead to changes in plant composition at the expense of wetland biodiversity. In addition, both species showed several well-known response mechanisms in order to detoxify high NH_4^+ loadings, including decreased root:shoot ratios, increased allocation of N into free amino acids, and indications for cation (K, Ca) deficiency. However, differential responses were found, as *C. disticha* showed a higher extent of classic detoxifying responses than *J. acutiflorus*. These are early warning indicators for a decreased tolerance to high NH_4^+ loadings in the long term. Even though a high tolerance was observed in the short term, it can be expected that the plasticity and adaptive ability with respect to high NH_4^+ loadings may decrease the competitive strength of *C. disticha*, and presumably also of *J. acutiflorus*, in the long term.



4 | Microbial transformations of nitrogen, sulfur and iron dictate vegetation composition in wetlands: a review

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Abstract

The majority of studies on rhizospheric interactions between microbial communities and vegetation focus on pathogens, mycorrhizal symbiosis, and/or carbon transformations. Although the biogeochemical transformations of nitrogen (N), sulfur (S) and iron (Fe) have profound effects on plants, these effects have received far less attention. This review is meant for microbiologists, biogeochemists and plant scientists interested in the imminent rhizospheric interactions between microbial communities and wetland vegetation. It includes a call for transdisciplinary research by providing a number of challenging topics for future research. Firstly, all three elements are plant nutrients, and microbial activity significantly changes their mobility and availability. Secondly, microbial oxidation with oxygen supplied by radial oxygen loss (ROL) from roots in wetlands causes acidification, while reduction using alternative electron acceptors leads to generation of alkalinity, affecting pH in the rhizosphere and hence plant composition. Thirdly, reduced species of all three elements may become phytotoxic. In addition, Fe cycling is tightly linked to that of S and phosphorus (P). As water level fluctuations are very common in wetlands, rapid changes in the availability of oxygen and alternative terminal electron acceptors will result in strong changes in the prevalent microbial redox reactions, with significant effects on plant growth. Depending on geological and hydrological settings, these interacting microbial transformations change the conditions and resource availability for plants, which are both strong drivers of vegetation development and composition by changing relative competitive strengths. Conversely, microbial composition is strongly driven by vegetation composition. Therefore, the combination of microbiological and plant ecological knowledge is essential to understand the biogeochemical and biological key factors driving heterogeneity and total (i.e., microorganisms and vegetation) community composition at different spatial and temporal scales. As N and S inputs have drastically increased due to anthropogenic forcing and Fe inputs have decreased at a global scale, this combined approach has become even more urgent.



Introduction

Plant ecologists have long been focusing mainly on above-ground processes, while a significant part of the driving mechanisms of ecosystem functioning is taking place below-ground (Waisel *et al.*, 1996). This strongly pleads for studies including biogeochemical transformations in soils and the important interactions between their key players: microorganisms and plants. This paper will focus on the roles of microbial conversions of the elements nitrogen (N), sulfur (S) and iron (Fe), with respect to their major role in the functioning and succession of plant communities in wetlands. The rationale lies in the fact that they can all be converted to chemical species that can serve as nutrients, as terminal electron acceptors and electron donors, and can also be toxic. We hope in this review to show the fascinating biogeochemical interactions between plant communities and microbial communities, and encourage microbiologist and plant ecologists to further integrate their disciplines in the hidden half of the ecosystem (Figure 1). Wetland overall communities (i.e., the integration of micro- and macrocommunities) provide excellent examples for this complex interaction playground, the rhizosphere. We define the rhizosphere as that part of the soil where plant roots significantly interact with soil biological and chemical processes, at the scale of 10^{-4} m or less to 10^{-1} m. One should realize that because the root systems of plants can be very large, up to 112 km^2 of soil (Robinson *et al.*, 2003) and reaching a depth between 10^{-3} m and 10^1 m (or even deeper for trees; Canadell *et al.*, 1996). Therefore, a major part of the upper soil of all vegetated wetlands can be defined more or less as a potential rhizosphere. This urges the use of an integrated approach of microbiologists and plant ecologists, in order to understand wetland biogeochemistry, biodiversity and resilience with respect to ecosystem changes. The purpose of this paper is to show how microbial activity related to the biogeochemical transformation of N, S and Fe also has profound effects on the activity and competitive strength of plants in wetlands. In this way, they shape the structure, succession and biodiversity of vegetation.

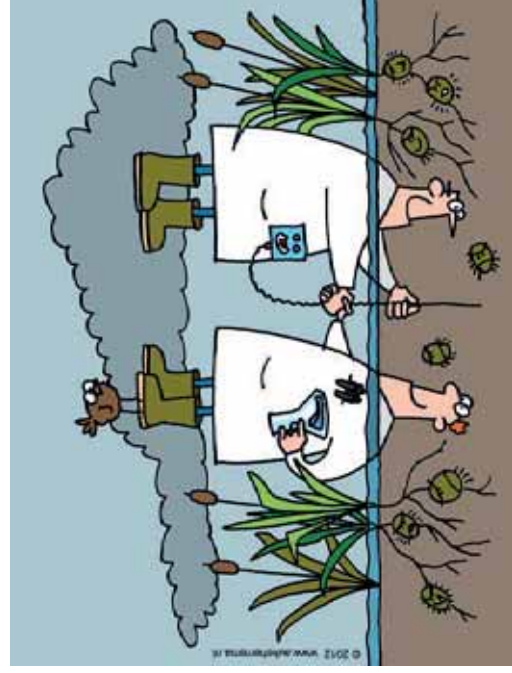


Figure 1. Belowground scientific collaboration in wetlands: microbiology meets plant ecology. Cartoon by Auke Herrema.

Heterogeneity in soils drives biodiversity and functioning

Unvegetated wetland soils, such as deeper parts of lake sediments or sediments of turbid shallow lakes (with insufficient light for underwater plant photosynthesis) are relatively homogeneous, except for the sediment-water boundary layer that generally shows a steep redox profile related to the successive depletion of terminal electron acceptors (Wetzel, 2001). One of the interesting characteristics of vegetated soils, and particularly of wetland soils, is their heterogeneity in space and time (Paul & Clark, 1989; Conrad & Frenzel, 2003; Bodelier, 2011). Microbiological batch experiments, even those containing multiple organisms, hardly resemble these soils. One of the challenges in microbial ecology is therefore to include this heterogeneity in resources (nutrients, substrates, electron acceptors) and conditions (e.g., oxygenation level, pH) under experimentally controlled conditions. Even under apparently stable conditions, the outcome of competition for one or several nutrients may be chaotic (Huisman & Weissing, 1999), but heterogeneity is even more relevant, ecologically speaking, because it facilitates the formation of multiple niches and generates larger fluctuations in conditions over time. As such, heterogeneity strongly affects competition between species, and diversity will differ from that under homogeneous conditions. Competitive exclusion, occurring under homogeneous, stable conditions, can be prevented by spatial and temporal heterogeneity, both for microorganisms and for macroorganisms.

In soils with plants (almost all soils in the world), heterogeneity increases as a result of rhizospheric effects (Marschner, 1995) and three-dimensional gradients (changes in concentrations with distance) at the micron-millimeter scale can be very steep. Different consortia of microbial species are present inside roots, on the root surface, and occur at different distances from roots, all of which are related to root exudate concentrations, oxygen availability, nutrient availability and pH (Brune *et al.*, 2000; Bodelier, 2003; Bodelier *et al.*, 2006). Plant roots even influence soil temperature, with significant effects on microbial activity (Boone *et al.*, 1998). Rhizospheric interactions between microbes and plants have been studied to a large extent with respect to pathogens, mycorrhizal symbiosis, and carbon transformations.

As a result of heterogeneity, the availability of electron acceptors (e.g., O_2 , NO_3^- , Fe^{3+} , SO_4^{2-}), nutrients and toxins can differ greatly at even the micro-scale (Bodelier *et al.*, 2006). These small-scale differences in electron acceptor availability explain the co-existence of different microbial groups, without outcompeting one another due to thermodynamic competitive advantage. In addition, the rapid transformation of reduced products by microbial re-oxidation, loss to the atmosphere (e.g., N_2) or chemical sequestration (e.g., Fe_x) may lead to favorable thermodynamic conditions that are incomparable to those in closed batch systems.

Changes in plant species composition will strongly affect microbial communities in the rhizosphere. Especially in wetlands, where soils are periodically or constantly flooded or waterlogged, radial oxygen loss (ROL) from plant roots has an extreme impact on rhizospheric microbial communities, locally converting conditions in the wetland soil to those normally found in oxygenated upland soil. Using microelectrodes, Frenzel *et al.* (1992) showed that rice plants were well able to oxygenate the soil. Oxygen is transported through aerenchyma in the stolons and roots by diffusion or mass flow, and the extent and type of aerenchyma formation differs to a large extent among species (Jackson & Armstrong, 1999; Visser *et al.*, 2000a,b; Voosenek *et al.*, 2006). As a consequence, the spatial pattern of ROL, also strongly varies among wetland species, due to differences in lignin and/or suberin deposits in the exodermis of their roots that act as a barrier prevent loss of O_2 in the upper soil layer to ensure O_2 supply to the active root tips (Armstrong, 1978; Laan *et al.*, 1989;

Colmer, 2003), and thereby prevent root anaerobiosis (Lambers, 1976; Vartapetian & Jackson, 1997; Jackson & Armstrong, 1999). This means that steep changes in oxygen availability, and hence in microbial communities, will prevail in the rhizosphere, in contrast to anaerobic bulk soil, depending on plant species composition. For this reason, microbial communities in wetland soils may be much more diverse than in upland soils. The physiological ability to oxidize their rhizospheres differs among plant species, which may not only facilitate conspecifics but also heterospecifics. Next to this spatial heterogeneity, there will also be a strong temporal heterogeneity in the rhizosphere due to changes in temperature, plant presence, plant activity, and water table. Particularly in riparian wetlands, where water tables fluctuate frequently, both resource availability and conditions can vary widely, providing challenges and opportunities for microbes.

Nutrient cycling and uptake in the rhizosphere

For both heterotrophic and autotrophic organisms including plants, sufficient nutrient acquisition is essential to transform the carbon acquired into growth and reproduction and safeguard the fitness of their populations. The rates of transformation of dead organic material into simple organic compounds and minerals, carried out by fauna and microorganisms, strongly determine the growth rates of plants. At high nutrient availability, fast-growing and highly competitive species will generally dominate at the expense of slow-growing species and monopolize light and space. As the quality of the dead organic matter strongly differs between plant species, decomposition (carbon mineralization) and the mineralization of the macronutrients N, P, potassium (K), and S, and of micronutrients including Fe and manganese (Mn), will depend on the species composition. Higher nutrient concentrations in dead material may stimulate mineralization rates, in this way providing a positive feedback (Hobbie, 1992; Aerts & Chapin, 2000). This will, however, depend on the nature of nutrient limitation of the decomposition rate, which can be limited either by N, P, or both. Although the C:N ratio of organic matter has traditionally been used as a quantitative indication of (potential) decomposition rates, the C:P ratio may be a better indicator for locations that show a high availability of N, e.g., as a result of high atmospheric N input. However, studies on the effect of organic matter quality on decomposition and nutrient mineralization rates still show much inconsistency (Kalbitz *et al.*, 2000). Note that the rates of vegetation growth and microbial decomposition do not necessarily have to be limited by the same element. Plants are able to actively forage for nutrients, leading to selective root placement at spots enriched with nutrients (Hutchings & De Kroon, 1994; Hodge 2004). In this way, not only nutrient availability and its heterogeneity, but also traits and plasticity of plants with respect to root foraging strategies will drive belowground competition, vegetation diversity and biomass production (De Kroon *et al.*, 2012). This means that there is a complex interaction between plant species composition and microbial communities including fungi, bacteria and archaea. For technical reasons related to root sampling, many interesting characteristics of this hidden, but very important aspect of plant communities (Waisel *et al.*, 1996) are yet to be discovered. New techniques including soil scanning in large-scale rhizotrons (Figure 2) enable an underground view on plant communities, which can be coupled to e.g. fluorescence in situ hybridization (FISH) techniques visualizing the locations of different microbial groups or species, microarrays to rapidly check for known sequences, polymerase chain reaction (PCR) based community analyses and metagenomics (full community genomics), to gain insight into rhizospheric microbe-plant communities and interactions. Next generation DNA sequencing technologies can be expected to make rhizosphere community analyses much faster, cheaper and easier, enabling for instance multiple comparisons between different plant species in one vegetation type, and between conspecifics under different environmental conditions.



Figure 2. The Nijmegen Phytotron facility, and a scan image of the root scanner in transparent tubes in the soil (inlay).

The three elements that we focus on here, N, S and Fe, all are essential nutrients for the metabolism and growth of both plants and microorganisms. These elements are predominantly taken up by plants from the soil via the roots, as only few plant species may take up high amounts of nutrients via the leaves. An exception are aquatic macrophytes that take up nutrients from the surface water, especially when soil concentrations are low or when the plants lack roots. On a quantitative base, N is the most important macronutrient (Marschner, 1995; Larcher, 2003), essential for the synthesis of amino acids, amines and peptides (including proteins). As the availability and uptake of this element is a major factor limiting biomass production in many ecosystems (Vitousek & Howarth, 1991; Larcher *et al.*, 2003), its availability and mobility are important drivers of species composition and biodiversity. Enhanced N availability changes biodiversity and heterogeneity, both above- and below-ground. Due to selective root placement, spots enriched in N contain more roots. For plants including Fabaceae (formerly called Leguminosae), the symbiotic relation with rhizobial strains of a variety of genera of Proteobacteria including *Rhizobium*, attracted by the exudation of phenolics and aldonic acids from roots, is of vital importance for atmospheric dinitrogen (N_2) fixation in soils that are low in N (Dakora & Phillips, 2002). For non-Fabaceae plants including the tree and shrub genera *Alnus*, *Casuarina*, *Ceanothus*, and *Myrica*, atmospheric N_2 is supplied by *Frankia* Actinobacteria. The cyanobacterial symbiont *Anabaena* provides atmospheric N to water ferns (*Azolla* spp.). The yearly amounts of nitrogen provided by these symbionts can be as high as 100 kg N ha^{-1} or more, which is similar to the highest anthropogenic airborne N fluxes reported. As a result, these plant species have a strong competitive advantage over other species under N limitation, especially under riparian conditions with coupled nitrification and denitrification. Note

that N limitation will also occur when P levels are high and P is no longer limiting biomass production, which explains the dominance of the *Azolla-Anabaena* complex and free living N-fixing aquatic cyanobacteria under high P loads (Lamers *et al.*, 2002b). In addition to the above-mentioned 'usual suspects', a large phylogenetic variety of free-living and symbiotic microorganisms including other Proteobacteria, Archaea, and Verrucomicrobia is now known to be able to fix atmospheric dinitrogen gas (Kneip *et al.*, 2007; Khadem *et al.*, 2010; Reed *et al.*, 2011).

Although less frequently, low availability of S may also hamper plant growth (Dijkshoorn & Van Wijk, 1967; Marschner, 1995). Like N, S is a vital constituent for protein structuring. While N and S are macronutrients, Fe is considered a micronutrient (average N:S:Fe in plant shoots is 500:15:1 on a molar basis; Marschner, 1995). The availability of Fe is essential for photosynthesis, as it is a cofactor for chlorophyll synthesis. To acquire Fe, plants have developed different mechanisms through evolution that facilitate uptake (Jones, 1998; Dakora & Phillips, 2002; Hell & Stephan, 2003). Gramineaceous plants exude metal chelating amino acids called phyto-siderophores, which are also important for the uptake of other micronutrient metals such as Mn, zinc (Zn) and copper (Cu). Non-gramineaceous monocots, dicots and bacteria release protons in the rhizosphere, or exude reducing and chelating compounds such as phenolics (Marschner, 1995; Schmidt, 1999). Soil Fe is, however, also strongly linked to phosphorus (P) availability, as both elements form a variety of chemical complexes, making P less available. Therefore, mechanisms to increase Fe availability may also be regarded as P mobilizing mechanisms (Bolan, 1991), next to the infection with mycorrhizal fungi.

Plants and microbes may compete for N, S, and P, depending on their relative availabilities (Hobbie, 1992), and competition models should therefore include this competition, yet few studies have. Especially at low nutrient levels, microbial uptake may significantly hamper nutrient uptake by plants, and hence reduce growth rates. As a result, they may even be able to increase the competitive advantage of slow-growing plant species and influence biodiversity. Not only competition at short-term, but also release rates from the microbial pool should be accounted for. As an example, selective root placement may be much slower than microbial colonization of a nutrient-rich patch, but subsequent release of N and P from the microbial pool can be effectively intercepted by these roots (Hodge *et al.*, 2000).

Belowground carbon turnover

The die-off of plant rhizomes, roots and above-ground litter, and their shredding by macro-, meio- and microfauna strongly determines the availability of organic substrate for fermentation and the availability of organic electron donors (e.g., lactate, acetate, succinate, propionate) for a variety of microbial anaerobic and aerobic redox processes. The decomposability of dead plant material greatly varies between species, not only due to varying N and P concentrations, but also as a result of varying concentrations of recalcitrant compounds such as lignin, and of potentially toxic secondary metabolites including other phenolic compounds and alkaloids. Consequently, plant species composition of a vegetation type has a great impact on decomposition rates and nutrient cycling (Hobbie, 1992). Decomposition rates of graminoid species such as grasses (Gramineae) and sedges (Cyperaceae) are, for instance, much faster than those of peat mosses (*Sphagnum*), which contain high concentrations of polyphenolics and pectin-like polysaccharides (Verhoeven & Toth, 1995; Hajek *et al.*, 2011). Even though net primary production of *Sphagnum* can, for instance, not compete with the highest production rates of vascular plants, low decomposition rates may still lead to fast peat accumulation. Next to species effects on dead organic matter (OM), microbial breakdown can also vary within one particular species. High atmospheric deposition rates of N

may, for instance, decrease the concentration of polyphenols in *Sphagnum* peat and concomitantly increase decomposition rates, with consequences for net C sequestration (Bragazza *et al.*, 2007). The actual decomposition rates are regulated by the interaction between the nutrient (N, P) concentrations in the OM, recalcitrant or toxic compounds, and soil conditions. Peat accumulation (i.e., sequestration of atmospheric C) depends on the precarious balance between production and decomposition, and high production rates are generally coupled to higher decomposition rates as a result of organic matter quality.

Phenolic compounds have been shown to block the decomposition in peatlands to a large extent by inhibiting the activity of various crucial microbial enzymes in carbon transformation (Freeman *et al.*, 2001). Oxygen intrusion during low water tables activates phenol oxidase activity, lowering phenol concentrations and thereby stimulating overall decomposition. In addition, oxygen acts as the most favorable electron acceptor, stimulating decomposition rates by a factor of two or more. ROL from roots can be expected to have the same impact. A spectacular example is the large-scale rhizosphere oxidation by the vascular peatland plant species *Astelia pumila* and *Donatia fascicularis*, that may look tiny above-ground but possess extensive root systems that strongly stimulate decomposition rates to a depth of more than one meter (Fritz *et al.*, 2011). As a result of low nutrient levels in the pristine Patagonian peatlands where these species occur, carbon is preferentially allocated to root production. The strong soil oxygenation also nullifies methane emission by the inhibition of methanogenic communities and/or the stimulation of methanotrophic communities. In oligotrophic lakes, small-leaved (so-called isoetid) species with relatively large root systems have a similar effect on decomposition rates (Smolders *et al.*, 2002b). The growth rates of facultatively aerobic, heterotrophic bacteria including *Pseudomonas chlororaphis* are significantly enhanced under flooded conditions by ROL of emergent macrophytes such as *Glyceria maxima* and *Oryza sativa* (Bodelier *et al.*, 1997; 1998; Gilbert and Frenzel, 1998), demonstrating the strong effect root systems may have on microbial composition and processes.

Microbial soil acidification affects plants

The cycling of N, Fe and S in the soil is not only determined to a large extent by microbial activity, but also by uptake, release and transformations of species of these elements due to root activity. Plants can change the pH of the rhizosphere by the exudation of protons, organic acids, HCO_3^- and OH^- (Dakora & Phillips, 2002). By the excretion of root exudates, acids and oxygen, plants control microbial resource availability, concentrations of oxygen as a potential toxin and electron acceptor, and pH in the rhizosphere. As a result of ROL or temporary water table draw-down (e.g., in riparian wetlands), pH can be strongly lowered due to microbial and chemical oxidation of N, S, Fe, generating acid (oxygen literally means acid generator). This may even lower the availability and uptake of redox-sensitive metals (e.g., Fe, Mn) or increase the availability and uptake of pH-sensitive metals that are not redox-sensitive (e.g., aluminum, Al). The pH change by the oxidation of ammonium and nitrite by nitrifying proteobacteria (e.g., the genera *Nitrosomonas*, *Nitrosospira*, *Nitrobacter*, *Nitrospira*) or crenarchaea, reduced S including sulfides to S and SO_4^{2-} by S oxidizing proteobacteria (e.g., *Beggiatoa*, *Thiobacillus*), and ferrous iron to ferric iron (e.g., *Acidithiobacillus ferrooxidans*, *Gallionella ferruginea*) not only influences microbial communities, but also plant communities. Most plant species are sensitive to low soil pH values (pH<5), not only by direct effects on membrane physiology, but also indirectly, by Al toxicity and by the impairment of the uptake of potassium (K), calcium (Ca) and magnesium (Mg) (Marschner, 1995). Although Al is one of the most common metals in soils, its toxicity is completely dependent on its mobility, which is strongly regulated by pH. The pH in the rhizosphere may be much lower than that of the bulk

soil. The regulation of pH is a combined effect of ROL activity, rhizospheric oxygen consumption, proton loss by roots (e.g., for NH_4^+ uptake), availability of electron donors like N, Fe and S, and the acid neutralizing capacity (ANC) of the soil. Depending on the pH range (from 8 down to 3), ANC is provided by bicarbonate and carbonate, exchange of base cations such as Ca and Mg at soil cation exchange sites, and the dissolution of Fe- and Al-oxides and hydroxides and silicates. Where bulk soil will be buffered under anaerobic conditions by the microbial reduction of N, manganese (Mn), Fe, and S (all generating alkalinity, i.e., consuming H^+), local oxidation by ROL will reverse this process. As such, high ROL activity can mimic the lowering of the water table in wetlands at a very local, but crucial scale. Under flooded or waterlogged conditions, most bulk soils are buffered around pH 6-6.5 by the $\text{CO}_2\text{-HCO}_3^-$ buffering mechanism. As microbial decomposition (generating inorganic carbon) is generally coupled to microbial mineralization of P and N, microbially-derived HCO_3^- concentrations can even be used as a proxy for nutrient availability in freshwater and marine soils (Van der Heide *et al.*, 2010).

Nitrogen: a mobile nutrient that may become toxic

Either the availability of electron donors or the availability of electron acceptors determines the nature and rates of microbial redox reactions in wetland soils. In the rhizosphere of plants showing high rates of ROL, oxidation of N, S, and Fe takes place (Bodelier, 2003). This implies that there will be a strong gradient in microbial communities at the microscale, from close to the roots towards the bulk soil. Ammonium (NH_4^+) produced in anaerobic spots is oxidized to nitrate/nitrite ($\text{NO}_3^-/\text{NO}_2^-$) by N oxidizing communities, presumably mainly Crenarchaeota (Leininger *et al.*, 2006; Jetten, 2008). Such local redox processes create a variety of N species, which have effect on plant performance. Although plants can use oxidized inorganic N (NO_3^-), reduced inorganic N (NH_4^+), and organic N (amino acids) as N source, plants adapted to soils with low pH and low redox potential prefer NH_4^+ (Marschner, 1995). For plants adapted to soils with higher pH, it has been shown that NH_4^+ can be toxic to a variety of plant species, mainly by leading to nutrient imbalances (K, Mg deficiency) or internal acidification in roots. Aquatic macrophytes prefer either NH_4^+ or NO_3^- ; depending on the pH of the surface water where the species mainly occurs (Roelofs *et al.*, 1984). Most rooting wetland plants are evolutionary adapted to NH_4^+ uptake, although NH_4^+ may also be taken up after rhizospheric oxidation to NO_3^- . Plants of riparian wetlands, however, are known to face problems at a high $\text{NH}_4^+ : \text{NO}_3^-$ ratio in acidified soil during lower water tables in the growing season (Lucassen *et al.*, 2003; Van den Berg *et al.*, 2005). Increased anthropogenic NH_4^+ inputs, as a result of ammonia (NH_3) emissions in regions with intensive cattle breeding, have for this reason led to the decrease of sensitive species and loss of biodiversity (Stevens *et al.*, 2011). In riparian wetlands with a low soil ANC, acidification due to high N and S inputs increases the $\text{NH}_4^+ : \text{NO}_3^-$ ratio due to a decrease of the activity of N oxidizers. In soils that have been acidic for longer time, nitrification rates may be higher due to the development of acid-resistant N reducers (De Boer & Kowalchuck, 2001). In soils with a high pH (>8.5), a significant proportion of NH_4^+ (ammonium) is present as ammonia (NH_3 ; $\text{pK} = 9.25$), which is highly toxic to plant roots (Schenk & Wehrmann, 1979). Similarly, high pH values of surface water, e.g. as a result of aquatic photosynthesis ($\text{pH} > 10$), can lead to NH_3 toxicity to submerged vegetation and fauna in N loaded aquatic systems (Thurston *et al.*, 1981). The actual toxicity of N depends on its accumulation in plant tissues and becomes higher under P-limited conditions due to extra accumulation. By the production of N-rich free amino acids, plants are able to detoxify excess internal N, but only to a certain level (Tomassen *et al.*, 2003).

It has been shown that increased availability of $\text{NO}_3^-/\text{NO}_2^-$ by increased nitrification rates in the rhizosphere may speed up denitrification in the anaerobic shell around the root zone with sufficient

oxygen supply (Reddy *et al.*, 1989; Arth *et al.*, 1998). In addition, higher rates of anaerobic ammonium oxidation (anammox) or dissimilatory nitrate reduction to ammonium (DNRA) can be expected to increase at the oxic/anoxic interface (Jetten *et al.*, 1998; Burgin and Hamilton, 2007; Hu *et al.*, 2011). The uptake of NH_4^+ or NO_3^- by plant roots may, however, lower denitrification rates in freshwater and marine wetlands by reducing the N supply (Bodelier *et al.*, 1996; Weish *et al.*, 2000). For vegetation, the rates of these N conversions are important, as most of the gaseous reaction products N_2 and N_2O leaves the soil compartment and is no longer available (except through microbial N_2 refixation). N_2 production by N reduction is strongly reduced by lowering of pH from 7 to 4, but the (net) $\text{N}_2\text{O}:\text{N}_2$ production ratio shows an exponential increase in this transect as a result of the inhibition of N_2O reduction at low pH (Van den Heuvel *et al.*, 2011). The heterogeneity with respect to N_2O emission rates can rather be explained by differences in soil structure affecting gas transport than by microbial composition (Van den Heuvel *et al.*, 2009), and probably also by vegetation composition. As such, N biogeochemistry is far more complex and variable than that of P, which is only released to the atmosphere in very small quantities as phosphine (Wetzel, 2001). In riparian wetlands, the water table strongly dominates N cycling and availability. N dynamics are influenced by microbial activity at all spatial scales, from the rhizosphere (plant N availability) through the community level (competition between species under N limitation) to the ecosystem level (N exchange with the surroundings, including the atmosphere). In addition, there is high variability at the temporal scale, mostly coupled to seasonal change and water table fluctuations (Neubauer *et al.*, 2005).

High inputs of NO_3^- in wetland soils are known to inhibit microbial Fe reduction for thermodynamical reasons (hence N reducers outcompete Fe reducers). This may not only lead to Fe deficiency in vegetation (Smolders *et al.*, 1993), but also to reduced mobilization and availability of P in the rhizosphere (Lucassen *et al.*, 2004). A significant part of soil P is sequestered by Fe, and ferrous (Fe^{2+}) iron phosphate complexes are more soluble than ferric (Fe^{3+}) iron phosphate compounds (Richardson, 1985; Scheffer & Schachtschabel, 2002). For the vegetation involved, increased N inputs may have contrasting effects, depending on the actual nutrient limiting the rate of biomass production. For N-limited systems, biomass production will increase and plant biodiversity will generally decrease by out-competition of slow-growing species. For P-limited systems, productivity may become lower and biodiversity become increased as a result of lower Fe reduction rates, leading to lower P availability. The latter is the reason why P-loaded lakes have even been treated with NO_3^- in order to change the eutrophic state, dominated by algae, to a less eutrophic state in which underwater vegetation receives more light (Rippl & Eiseltoová, 2010).

Toxicity of S and Fe, and their interactions with P availability

Fe reduction by bacteria, including sulfate reducing bacteria (e.g., *Geobacter sulfurreducens*), and archaea converts ferric compounds into ferrous iron (Lovley & Phillips, 1988; Coleman *et al.*, 1993; Lovley *et al.*, 2004; Weiss *et al.*, 2004), which is much more mobile and is preferentially taken up by plants. The reduction of ferric iron, produced as a result of ROL, may suppress other anaerobic processes including methanogenesis (Frenzel *et al.*, 1999). Although Fe is an essential micronutrient, high levels of Fe may become phytotoxic. This toxicity may even become visible above-ground as necrotic spots on the leaves of wetland plants (Lucassen *et al.*, 2000). The oxidation of Fe by ROL may detoxify Fe due to the microbial or chemical precipitation of Fe hydroxides in the soil (Armstrong, 1978; Bientfait, 1989). However, excess Fe in combination with relatively low ROL can generate problems for plants due to Fe plaque formation on the root surface or by Fe deposition in the root apoplast (Taylor *et al.*, 1984; Smolders & Roelofs, 1996; Emerson *et al.*, 1999). These incrustations include Fe oxides (e.g. ferrihydrite), Fe oxyhydroxides (e.g. goethite), Fe carbonates (e.g. siderite), and Fe phosphates

(e.g. vivianite), microbially-derived organic-metal complexes, bacterial biofilms, and co-deposition of other metals such as manganese, copper, zinc and lead (Batty et al., 2000; Hansel et al., 2001). Root physiology can be negatively affected by this plaque that acts as a physical barrier and interferes with P metabolism (Snowden & Wheeler, 1995). An interesting aspect of Fe hydroxide precipitation on microbes is the fact that they act as nanowires shuttling electrons in microbial communities (Lovely, 2006; Rabaey et al., 2007), and also electrically connect them to plant roots.

The activity of sulfate reducing prokaryotes (SRP) is also of high relevance to vegetation development. Dissimilatory sulfate reducers include both Bacteria (e.g., *Desulfovibrio*, *Desulfobacter*) and Archaea (e.g., *Archaeoglobus*) producing sulfide (H_2S). Whereas oxidized states of S (SO_4^{2-} , S) are hardly toxic to plants, H_2S is highly toxic by inhibiting the activity of cytochrome c oxidase in mitochondria, leading to a subsequent blocking of energy production in roots, and by inhibiting other metal-containing enzymes (Koch et al., 1990; Raven & Scrimgeour, 1997). The rhizospheric oxidation of S (Figure 3) by microbial activity of S oxidizers including *Beggiatoa* detoxifies H_2S , and ROL activity of plants will therefore moderate the sensitivity of plants to sulfide. All three pH-dependent species of sulfide (H_2S , HS⁻ and S²⁻, $pK_1=7.2$; $pK_2=13.7$) seem to be equally toxic to plants, but for most anaerobic soils the gaseous H_2S will be prevailing due to $HCO_3^-CO_2$ buffering around pH 6-6.5. For marine wetlands receiving sufficient organic matter to keep electron donor concentrations high, H_2S production affects the growth of marine and brackish water macrophytes in the millimolar range. The phytotoxin is known to hamper the establishment and growth of the two main mangrove genera *Avicennia* and *Rhizophora* (McKee, 1993) and of seagrasses including *Thalassia testudinum* and *Zostera marina* (Koch et al., 2007; Frederiksen et al., 2008; Holmer et al., 2009). Fluctuations in ROL of seagrass plants lead to low H_2S concentrations during daytime and increased concentrations during nighttime. At the end of the growing season decreasing ROL, higher microbial O_2 consumption and H_2S toxicity in concert induce the die-off of vegetation in brackish wetlands (Azzoni et al., 2001).

Although S concentrations are generally lower in freshwater wetlands, high input rates due to volcanic activity or other geological conditions, and as a result of S pollution of the atmosphere, groundwater and surface water have raised SO_4^{2-} concentrations at a global scale. In anaerobic freshwater wetland soils, SO_4^{2-} reduction may therefore become a dominant process next to Fe reduction (Lamers et al., 1998a), while its rate can be determined by the availability of either electron donors or SO_4^{2-} (Lamers et al., 2002a). Sulfide concentrations in the range of 10-100 $\mu mol L^{-1}$ are known to be toxic to the roots of freshwater plants (Tanaka et al., 1968; Armstrong et al., 1996; Smolders and Roelofs, 1996; Lamers et al., 1998a; Geurts et al., 2009). Like for marine plants, H_2S toxicity to roots can



Figure 3. Radial oxygen loss (ROL) of *Juncus acutiflorus* is shown by the oxidation of added H_2S in an anaerobic rhizotron with living plants and natural organic wetland soil. This was confirmed by the use of oxygen microelectrodes. Yellowish-white haloes of S^{2-} and S precipitates have been formed by microbial S oxidation. Lamers & Janssen, unpublished results.

be prevented by the activity of sulfur bacteria using O_2 from the roots (Joshi & Hollis, 1977). In addition, volatile S compounds including H_2S and, after its methylation, dimethylsulfide and methanethiol are released to the atmosphere (Lomans et al., 2002). As the thermodynamical characteristics of Fe reduction and S reduction do not differ very much, these conversions take place in the same soils, and FeS_x precipitation occurs (Lamers et al., 2002a). By this sequestration the concentration of both potential phytotoxins is reduced (Van der Welle et al., 2006), and plants may even become Fe deficient (Smolders & Roelofs, 1993).

Already in the 50s of the former century, the prominent role of S in aquatic nutrient biogeochemistry was described (Ohle, 1954). As SO_4^{2-} will serve as a terminal electron acceptor, it will speed up overall anaerobic decomposition. Increased inputs of SO_4^{2-} may additionally mobilize Fe bound P in the soil, due to its reduction to H_2S competing for Fe binding sites (Sperber, 1958; Caraco et al., 1989; Roden & Edmonds, 1997; Lamers et al., 1998a; Smolders et al., 2006a) or mobilization of P from polyphosphates by sulfur bacteria (Golterman, 2001). Whether or not sulfate-induced eutrophication occurs, depends on the levels of P in the sediment and its type of binding (Lamers et al., 2002a; Loeb et al., 2008; Geurts et al., 2008). The large spatial and temporal heterogeneity in O_2 supply (as determined by the balance between local ROL and local oxygen consumption) enables the coupling of oxidation and reduction of the same element (S, Fe, N) at a small-scale in the rhizosphere. In this way, consortia of microorganisms using the same element can coexist in wetland soils, but the directions of the transformations are governed by plant physiology and soil characteristics.

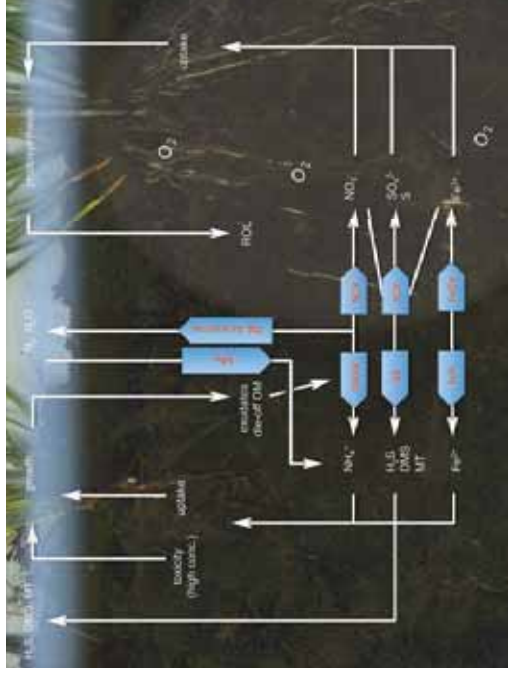


Figure 4. Microbe-plant interactions in the biogeochemistry of N, S and Fe in wetland soils. Losses of volatile N and S compounds to the atmosphere also occur through plant tissues, which has not been depicted here. See text for further explanation. DNRA: dissimilatory nitrate reduction to ammonium; SR: sulfate reduction; FeR: iron reduction; NOX: N oxidation to NO_3^- ; SOX: S oxidation; FeOX: Fe oxidation; DN: denitrification; Nfix: N_2 fixation; Anammox: anaerobic ammonium oxidation; ROL: radial oxygen loss (by roots).

In addition to the use of organic electron donors, the reduction of one of the three elements can be coupled to the oxidation of the other (chemolithotrophy). Sulfide, both dissolved and as FeS_2 deposits in the soil can be used as an electron donor by proteobacteria such as *Thiobacillus denitrificans* coupled to the use of NO_3^- (Haaijer *et al.*, 2006; 2007; Burgin & Hamilton, 2008). Even in peat soils rich in organic compounds, NO_3^- pulses are still able to stimulate to chemolithotrophic denitrification while organotrophic denitrification rates were very high (Lamers, unpublished results). For plants, this means that the detoxification of sulfide extends beyond the direct influence of ROL, and that S-rich situations may show lower N-availability. At the landscape scale, N pollution of the catchment will mobilize S from subsoil FeS_2 deposits, and the resulting S pollution of groundwater will lead to P pollution in wetlands where this polluted water is discharged. In this way, there is a microbial 'S bridge' between N and P at this hydrological scale (Smolders *et al.*, 2010). In wetland soils, partial H_2S oxidation to S^0 and pyrite (FeS_2) can also be coupled to Fe reduction (Thamdrup *et al.*, 1994), with consequences for the availability and toxicity of both elements to plants, and for the availability of P.

Global changes of N, S and Fe biogeochemistry

As a result of anthropogenic forcing, the input of all three elements, N, S and P, into wetlands has strongly changed at a global scale (Vitousek *et al.*, 1997; Schlesinger, 1997). As a result of urbanization, industrialization and intensive farming, surface waters of many wetlands have become polluted by N and P (Carpenter *et al.*, 1998). In contrast to P, increased N inputs (NO_x and NH_4) are also the result of atmospheric deposition, which has strongly increased in industrialized regions and in regions with intensive farming (Vitousek *et al.*, 1997; Bobbink *et al.*, 1998). Whereas S deposition rates in Europe have been reduced by 80-90% as a result of legislation, they are strongly increasing in other parts of the world. In fast-developing regions such as Asia (particularly China), SO_x emissions may soon equal the combined emissions of North America and Europe as a result of the large-scale use of coal as a fuel (Shah *et al.*, 2000). In addition to N and S pollution, many wetlands have been drained for land use change (Lamers *et al.*, in press), which has not only resulted in the loss of wetlands, but also in significant changes in the hydrochemistry of wetlands that have not suffered from complete dehydration. Lowering of groundwater tables, also due to the extraction of drinking water and industrial process water, has decreased the influence of groundwater in wetlands to a large extent. As a result, the discharge of anaerobic, Fe-rich groundwater has decreased. This means that the role of Fe in regulating vegetation composition, P availability and sulfide detoxification in wetlands has been changed (Lamers *et al.*, in press). In combination with increased inputs of N, S and P, large-scale changes of wetland communities (microbial communities and their vegetation) have resulted, and ecosystem services such as C sequestration, flood protection, water storage and biodiversity have been altered. The resilience of ecosystems to global change depends to a large extent on the responses microbial communities and their positive and negative feedbacks with respect to these changes. For this reason, changes in microbial composition and activity should be incorporated in ecosystem models (Treseder *et al.*, 2012).

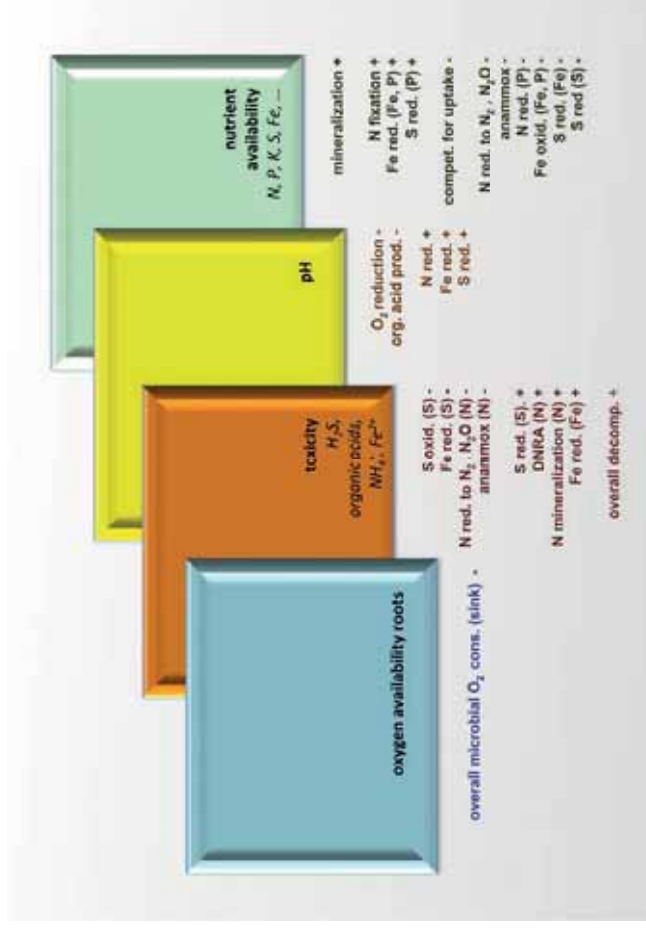


Figure 5. Four filters created by microbial activities related to N, S and Fe biogeochemistry influence the occurrence and functioning of wetland plant species, and their interspecific competition.

A positive influence on the parameter mentioned on the filter frame is denoted by +, a negative by -.

Table 1. Examples of challenging fundamental and applied ecological questions regarding plant-microbe communities in wetlands (and other ecosystem types). In addition to correlative research, experimental research in the field and lab is essential to determine causal relationships. The fast development of powerful and cheaper molecular techniques will facilitate comparative and experimental community studies.

Landscape/ecosystem • Geochemistry, microbial communities and vegetation development • Biogeochemical controls of carbon dynamics (primary production-decomposition feed-backs) • Impacts of anthropogenic hydrological changes (e.g. change from discharge of Fe-rich groundwater to recharge) on the interactions between microbial communities and vegetation • Pollution effects (e.g. N pollution groundwater, P pollution surface water, increased airborne N and S) on plant-microbe interactions • Climate change effects on landscape biogeochemistry • Microbial ecology of conservation biology • Microbial ecology of restoration ecology	Ecosystem • Role of microorganisms in vegetation succession and development • Role of vegetation in the succession and dynamics of microbial communities • Role of soil heterogeneity (e.g. nutrients, organic substrates, toxins, temperature, electron acceptors) in the functioning of plant-microbe communities, including the competition between microorganisms • Biodiversity in plant-microbe communities: niche differentiation, disturbance, fluctuation, neutral theory • Biodiversity of plant-microbe communities and ecosystem functioning and resilience • Microbial consortia in three-dimensional rhizosphere gradients • Rhizosphere community dynamics (e.g. diurnal, seasonal) • Host specificity (e.g. comparison of species, same species under different environmental conditions) • Competition between plants based on their microbial communities (e.g. rhizosphere community as a plant trait) • Competition between microbes as a result of plant community changes • Dispersal of microbes and plants • Climate change effects (temperature, rainfall, CO₂) and plant-microbe interactions • Optimization of water purification in constructed wetlands based on the manipulation of soil-plant-microbe interactions	Ecophysiology • Microbial controls on nutrient and water uptake by plants • Gas (e.g. CO₂, CH₄, N₂, N₂O) exchange in the plant-microbe continuum • Selective root placement and microbial communities • Permanent or periodical anaerobiosis and microbe-plant interactions • Microbial manipulation of phytotoxic compounds (including agricultural crops) • Generation of electricity in planted wetland soils
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General conclusion

The biogeochemical cycling of N, S and Fe shows strong interactions in microbe-plant communities at various levels, from rhizosphere to landscape (Figure 4). The composition and activity of microbial communities dictate vegetation composition to a large extent by their strong effects on four important drivers (belowground environmental filters) of the growth, composition and biodiversity of wetland vegetation, namely root oxygen availability, phytotoxicity, pH, and nutrient availability (Figure 5). Microbe-plant interactions provide challenging and complex new topics for highly interesting ecological research, in which not only correlative, but especially also mesocosm experiments under controlled conditions are carried out by a consortium of specialists. The fast development of new and much cheaper molecular techniques opens up a whole new world of rhizosphere research, in which basic and applied ecological issues on plant-microbe communities (Table 1) can be addressed that were impossible or much more difficult in the recent past. In addition, new students should be challenged and trained to address ecological topics in this multi-disciplinary way. Especially on a rapidly changing globe, it is vital that both microbiologists and plant ecologists meet in the hidden half of plant communities to better understand ecosystem changes at multiple levels.

5 | Restoration of acidified and eutrophied rich fens: long-term effects of traditional management and experimental liming

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Abstract

Rich fens are known for their high botanical diversity encompassing many endangered species. For decades, several management measures, including mowing and burning, have been applied to maintain a high biodiversity by means of slowing down the natural succession from calcareous rich fens to acidic poor fens or woodland. In this study, we assessed the long-term effects of these traditional management measures, and explored the effectiveness of liming as a measure to restore rich fen vegetation. Effects of summer mowing, and of burning after winter mowing, were assessed by comparing current (2013) and historical (1967) vegetation data. Effects of experimental liming, using different levels of lime addition (0, 1000, 2000, and 4000 kg Dolokal/ha), were monitored in the field during 7.5 years. Summer mowing led to more acidic and nutrient-poor conditions as indicated by a shift from rich to poor fen vegetation, including a well-developed bryophyte cover dominated by *Sphagnum* with some threatened species. Burning (after winter mowing) counteracted acidification but increased nutrient availability, as indicated by dominance of vascular species characteristic of productive tall-herb grasslands and a sparse bryophyte cover with common species. We conclude that the traditional measures were unable to maintain rich fen composition in the long term. Given the fact that the restoration of hydrological conditions, favouring rich fens, is not always feasible, liming could be an alternative to counteract acidification and improve rich fen conditions in the short term. This measure, however, appeared to be unsustainable as the re-establishment and dominance of *Sphagnum* spp. seriously complicated the development of rich fen vegetation in the longer term.



Introduction

Fens show a high habitat and species diversity, and are home to many rare and endangered plant species (Bootsma *et al.*, 2002; Bedford & Godwin, 2003). As they show a natural terrestrialisation from open water into different successional stages of vegetation composition, fens often comprise a myriad of vegetation types. The formation of floating mats and the subsequent increase in peat thickness leads to increased influence of base-poor rainwater, and reduced influence of base-rich surface water and/or groundwater, which still remains dominant in the margins and deeper in the peat mat. This process creates a biogeochemical gradient that enhances the development of different successional stages from rich fen (dominated by Cyperaceae and brown mosses), via poor fen (with or without hummock forming *Sphagnum* species), towards eventually carr woodland (Verhoeven & Bobbink, 2001; Grootjans *et al.*, 2006). Rich fens are generally more species-rich than poor fens or woodlands. These earlier successional stages are, however, seriously threatened and biodiversity has strongly declined in many rich fens as a result of fast succession to either poor fens or woodlands (Beltman *et al.*, 2001; Middleton *et al.*, 2006a,b; Lamers *et al.*, 2002b, 2014).

Due to anthropogenic influences including major changes in hydrology, agricultural pollution of groundwater and surface water, and increased atmospheric deposition of sulphur and nitrogen, many fens have degraded as a result of concomitant desiccation, acidification and eutrophication (Hogg *et al.*, 1995; Kooijman & Paulissen, 2006; Klimkowska *et al.*, 2007; Lamers *et al.*, 2002b, 2014). This has significantly changed the rate, course and outcome of succession, resulting in accelerated transition of brownmoss dominated rich fens to *Sphagnum* dominated poor fens (van Diggelen *et al.*, 1996; Kooijman & Paulissen, 2006; Bobbink *et al.*, 2010; Lamers *et al.*, 2014). If *Sphagnum* spp. become dominant, acidification is accelerated by their high rain water retention, cation exchange and active excretion of uronic and phenolic acids, enhancing peat accumulation (Hemond, 1980; Verhoeven & Liefveld, 1997; Bootsma *et al.*, 2002). In addition, eutrophied fens often show a low species diversity as slow-growing vascular plant- and bryophyte species, adapted to low nutrient concentrations, are outcompeted by tall-growing, highly productive species (Hogg *et al.*, 1995; Bobbink *et al.*, 1998; Bobbink *et al.*, 2010; Cusell *et al.*, 2014).

To counteract these anthropogenic effects, and in particular to maintain the original lifespan of early successional vegetation, such fens depend on active management including mowing, grazing or burning (Hogg *et al.*, 1995; Middleton *et al.*, 2006a). For decades, mowing has been a traditional management tool in European fens. The removal of biomass and resulting increase of light availability is thought to increase biodiversity, but only if species are still present or able to disperse or germinate (Middleton *et al.*, 2006a). However, few studies have tried to explore long-term effects of mowing management on vegetation development in fens (van Diggelen *et al.*, 1996; van Belle *et al.*, 2006). Burning after winter mowing can be useful to remove litter, and fire is also thought to slightly raise the soil pH and base-cation concentrations (Raison, 1979). However, not much is known about the potential role of burning in maintaining rich fen biodiversity (Middleton *et al.*, 2006a).

Acidification is recognised an important issue in fens nowadays. More intrusive measures, such as top-soil removal have enhanced acidification due to stagnation of rainwater, re-establishment of *Sphagnum* (Beltman *et al.*, 1996b; 2001), and the exposure of the formerly reduced peat soil to oxygen that may oxidise reduced sulphur (Mylona, 1996). A more recent measure to counteract acidification is the application of lime, which may improve base-rich conditions by increasing acid buffering, and preventing *Sphagnum* spp. from becoming dominant (Beltman *et al.*, 2001; Dorland

et al., 2004). In this way, base-rich conditions may be restored, enabling minerotrophic rich fen species to re-establish (Beltman *et al.*, 1996a; 2001; Patzelt *et al.*, 2001). However, an increase in soil pH may at the same time stimulate peat decomposition and increase mineralisation (Ono, 1991; Smolders *et al.*, 2002a). As only few studies have explored the effectiveness of liming on vegetation and biogeochemistry, in our study we experimentally tested this measure to restore rich fen vegetation from *Sphagnum*-dominated poor fen vegetation, by applying different levels of lime and monitoring the development over a period of 7.5 years. In addition, we assessed the long-term effects of traditional management measures aimed at maintaining species richness in fens. Effects of summer mowing, and of burning after winter mowing, were assessed by comparing current (2013) with historical (1967) vegetation data. Our results will be discussed with respect to their implications for future management to restore rich fen vegetation in fens.

Material and Methods

Research area

The research was carried out in the wetland reserve and Natura 2000 area the 'Nieuwkoopse Plassen' in The Netherlands (52°9' N, 4°49' E). This area is characterised by alternating ridges of peat and peat extraction ponds, with an extensive network of canals and ditches throughout the reserve, as well as several large shallow fen lakes (den Held *et al.*, 1992). The current vegetation types, representative of different successional stages found on peat mats, can only be maintained by human interference, as areas without management have all developed into carr woodland (Wiegers, 1992). The surface level is ± 1.5 metres below sea level, which is 0.4 to 4 metres higher than surrounding agricultural areas that have subsided due to drainage. As a result, the Nieuwkoopse Plassen reserve has become an infiltration area dependent on precipitation and relatively nutrient-rich, buffered surface water from the river the *Oude Rijn* to maintain sufficiently high surface water levels.

Comparing current with historical vegetation data

Sampling strategy

The vegetation of the Nieuwkoopse Plassen was investigated by den Held in 1967 (den Held, 1970), by recording the cover and composition of bryophytes and vascular plant species in randomly selected plots throughout the reserve. In 2013, 49 of these historic fen sites were reassessed. The selection was based on their current management type (23 summer-mown, and 26 winter-mown and burned), the vegetation composition in 1967 (equal distribution of the different successional stages) and the accuracy by which the sites could be found again. Selected sites ranged in size from 2 to 30 m² (average size of 10 m²). It was not known exactly how long summer mowing and burning after winter mowing had been carried out since 1967. We do know, however, that the measures had been carried out annually for at least 10 years before the start of this part of the research.

Vegetation assessment

Composition and cover of bryophytes (April-May, 2013) and vascular plants (June-July, 2013) were assessed using the ordinal scale used by den Held (1970): codes 1-4 represent <5% cover with 1: 1 individual, 2: 2-5 individuals; 3: 6-50 individuals, 4: >50 individuals; 5: 5-12% cover; 6: 13-25% cover; 7: 26-50% cover; 8: 51-75% cover; 9: 76-100% cover. The numbers of individuals for categories 1-4 were adjusted to account for the differences in size between the relevés by multiplying them with a factor calculated as the log of the area of the relevé, divided by the log of the standard relevé area (4 m²). The nomenclature follows van der Meijden (2005) for vascular plants and Siebel & During (2006)

for bryophytes, but *Sphagnum capillifolium* and *Sphagnum rubellum* were considered one species. Additional information about red-list status and common habitats was gained from Synbiosys (version 2.5.8. Alterra, Wageningen).

Liming experiment

Experimental set up

In October, 2006, 12 plots of 3x3 meters were created on a thick, (formerly) floating mat, from which the top soil (10–30 cm) had been removed in the winter of 2000–2001. At start of the experiment, the mat had been (re-)acidified and become dominated by *Sphagnum* species. Vegetation was mown during summer every year, which continued during the experimental period. Liming treatments were randomly distributed over these plots (3 replicates for each treatment): control, 1000 kg/ha lime (1k), 2000 kg/ha lime (2k), and 4000 kg/ha lime (4k). The amount of lime for each treatment was equally spread over the plots as a powder (Dolokali; CaCO₃ 84%, MgCO₃ 10%, MgO 5%, <0,16 mm).

To assess treatment effects on soil chemistry, a pooled soil sample (2–3 subsamples) of the upper 10 cm was collected from each plot just before the treatment (t=0), and at t= 0.5, 1, 2, 2.5, 4.5, and 7.5 years. Additionally, at t= 4.5 and t=7.5 years, soil pore water was collected anaerobically, using vacuum syringes (60 ml) connected to ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment) that were placed in-situ. Soil samples and pore water samples were stored overnight at 4°C until further analysis. The composition of the bryophytes in the plots was assessed at t=0.5, 1.5, 2.5, 4.5, and 7.5 years, the vascular plant composition at t=0.5, 1.5, 2.5, and 4.5 years, using the Londo method (Londo, 1975). Afterwards, this scale was converted to the scale used by den Held (1970).

Chemical analysis

The bio-available amount of phosphorus (P) in the soil was measured by water extraction using 17.5 g of fresh soil shaken (2 hours) with 50 mL MilliQ. To determine the bio-available amount of nitrate (NO₃⁻), ammonium (NH₄⁺) and potassium (K⁺), a soil extraction was performed using 50 mL of 0.2 M NaCl shaken (2 hours) with 17.5 g fresh soil. The soil pH was determined in the latter extract, using a combined pH electrode (Radiometer) and a TIM840 pH meter. The amounts of calcium (Ca²⁺) and magnesium (Mg²⁺) bound to the soil adsorption complex were determined by extracting fresh soil (amount equal to 2.5 g dry weight) with 200 mL of SrCl₂ solution (0.2 M, 1 hour). Digestates and soil extracts were diluted where necessary and analysed for cation- and nutrient concentrations by ICP and AA (see below). The pH values of pore water samples were measured within 24 hours after sampling as explained above. Dissolved total inorganic carbon (TIC) was measured within 24 hours after sampling by injecting 0.2 mL pore water or surface water in a closed chamber containing 0.2 M H₃PO₄ solution, converting all dissolved TIC into CO₂. A continuous gas flow (N₂) directly transports the CO₂ to an ABB Advance optima Infrared Gas Analyzer (IRGA) to measure TIC. The pore water pH was used to calculate the partition of dissolved CO₂ and HCO₃⁻ concentrations in the pore water. Prior to storage at 4°C until further elemental analysis by ICP, 0.1 mL 65% HNO₃ was added to 10 mL of each pore water sample to prevent metal precipitation. The remaining pore water samples were stored at -20°C until measurement of nutrient concentrations by AA (see below). To determine concentrations of Ca²⁺, Mg²⁺, P and other cations an Inductively Coupled Plasma Spectrophotometer (ICP; Thermo Electron corporation IRIS Intrepid II XDL) was used. The following ion concentrations were determined colourimetrically on Auto Analyzer 3 systems (Bran and Luebbe): NO₃⁻ (Kamphake et al., 1967), NH₄⁺ (Grasshoff & Johanssen, 1972), and phosphate (PO₄³⁻; Henriksen, 1965). K⁺ concentrations were determined with a Technicon Flame Photometer IV Control (Technicon Corporation).

Statistical analyses

Vegetation survey

Changes in vegetation in relation to the traditional management types were investigated using the indirect gradient analysis Detrended Correspondence Analysis (DCA). This analysis was performed for bryophytes and vascular plants separately, using CANOCO (Version 4.55; ter Braak & Šmilauer, 1998). The relevés were classified based on their species composition using WinTwins (Version 2.3; Hill, M.O.). Default settings were used, except that cut levels were set to 1, 2, 3, 4, 5, 6, 7, 8 and 9, according to the ordinal cover and abundance scale used for the vegetation assessments. Clusters up to third division were taken into account. A similar analysis was performed for investigating the changes in vegetation caused by the different liming treatments. However, instead of a DCA, a Principal Components Analysis (PCA) was performed as the maximum length of gradient was close to 3.0 (Table 1; ter Braak & Šmilauer, 1998).

Table 1. Statistical analyses of the effects of traditional management measures on vegetation development over 46 years (DCA; Figure 1 and suppl. data) and the effects of experimental liming on vegetation development (PCA; Figure 5 and suppl. data).

	Summer mowing & Burning		Liming	
	Bryophytes	Vascular plants	Bryophytes	Vascular plants
Length of gradient (DCA)	5.428	4.322	2.977	1.336
Total variation (SD)	5.345	6.127	1.031	0.735
Axis 1 variation	12.9 %	10.0 %	42.3 %	34.1 %
Axis 2 variation	6.3 %	5.1 %	15.3 %	22.2 %

Biogeochemical analyses

For statistical analysis of the chemical results from the liming experiment, SPSS Statistics for Windows (Version 21.0; IBM Corp.) was used. Differences among treatments were tested using a GLM mixed model. Time was used as repeated measure and treatment as fixed factor, with AR(1) heterogeneous as the covariance type. A Bonferroni post-hoc test was used to test for differences between treatments. Differences between treatments at a fixed moment were analysed with ANOVA, using Tukey's-b posthoc test. Data was transformed with logarithm or square root when residuals were not normally distributed.

Results

Historical references

In 1967, different successional fen stages were present, ranging from young successional stages with species characteristic of rich fens, to older stages with a dominance of *Sphagnum* characteristic of poor fens. In 46 of the historic relevés *Sphagnum palustre* or *Sphagnum fallax* was the dominant bryophyte species (avg. code 6; supplementary data), but additional species indicated differences in abiotic conditions. The first group of historic relevés (n=6) contained bryophytes characteristic for more buffered conditions such as *Pellia* species (avg. code 5) or *Brachythecium rutabulum* (code 2 or 3). Vascular plants of rich fens such as *Hammarbya paludosa* and *Liparis loeselii* were present

Except for the 1k treatment, all lime treatments still showed significantly higher ($p=0.005$) pH values over time than the control (figure 2; upper panel). The exchangeable Ca^{2+} and Mg^{2+} concentrations of the soil matrix increased strongly ($p<0.005$) for all lime treatments with, as expected, the highest concentrations for the highest lime treatment (4k) (figure 2; lower panel). The 1k treatment showed a strong drop after 4.5 years, while in all other lime treatments exchangeable Ca^{2+} and Mg^{2+} concentrations showed a gradual decrease over time but were still significantly higher ($p<0.001$). After 7.5 years, concentrations were still significantly higher ($p<0.01$) in the 4k treatment. Accordingly, the buffer capacity of the pore water, as reflected in dissolved HCO_3^- concentrations, were shown to be significantly ($p<0.01$) increased for the 4k treatment after 4.5 years, while after 7.5 years no differences between treatments were found anymore. CO_2 concentrations in the pore water showed no clear differences between lime treatments after both 4.5 and 7.5 years (data not shown).

Effects of liming on nutrient availability

The amount of bio-available P became slightly, but not significantly, higher for the lime treatments compared to the control over time (figure 3; upper panel). Plant available nitrogen (N) became significantly ($p<0.05$) higher in the 4k treatment over time, when compared to the control (figure 3; lower panel), with NH_4^+ accounting for more than 81% of the available N. The much lower plant available NO_3^- concentrations in the soil showed no differences between treatments. Moreover, plant available K^+ concentrations showed no significant differences between lime treatments over time (data not shown). Similarly, PO_4^{3-} , NO_3^- , NH_4^+ and K^+ concentrations in pore water did not differ among treatments after 4.5 and 7.5 years either, and all remained relatively low (data not shown).

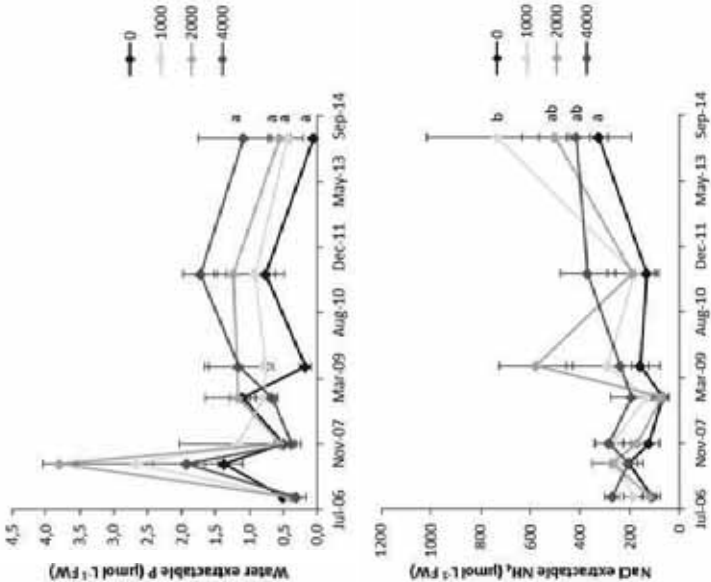


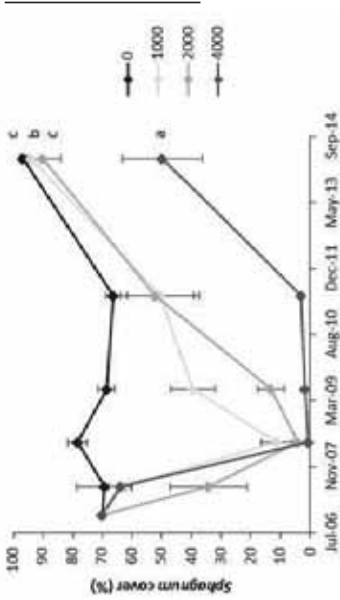
Figure 3. Long-term effects of lime addition (0, 1000, 2000, 4000 kg/ha) on available nutrient concentrations in the soil of the experimental plots (P and N). Error bars indicate SEM values, different letters indicate significant differences between treatments.

Effects of liming on vegetation development

In all lime treatments, *Sphagnum* spp. died and reached the lowest cover 1.5 years after liming (<10% cover; figure 4). From this moment on, re-growth of *Sphagnum* started in accordance with the lime treatment, resulting in the lowest cover in the 4k treatment and the highest cover in the 1k treatment. Liming did, however, not have a clear effect on vascular plant composition (suppl. data). The bryophyte composition in the limed plots over 7.5 years could be divided into 4 main groups (figure 5). The first group ($n=13$) mainly consisted of 4k lime plots, characterised by a low cover of *S. palustre* (11 relevés; code 2-4) and *Polytrichum commune* (10 relevés; code 1-2), and the presence of rich fen species like *Aneura pinguis* (10 relevés; code 2), *Bryum pseudotriquetrum* (9 relevés; code 2) and *Riccardia chamaedryfolia* (4 relevés; code 2) (rich fen; figure 5). Moreover, *Liparis loeselii*, a characteristic rich fen orchid, was found after 4k lime addition in 3 relevés (code 1-2). The second group (poor fen with *P. commune*; figure 5) consisted of 9 relevés that were all characterised by *S. palustre* (avg. code 5) and *P. commune* (avg. code 3), and the presence of *S. fallax* in 6 relevés (code 2). In 3 of these relevés, only these three bryophyte species were present. The 26 relevés of the third group (poor fen with liverworts; figure 5) were all dominated by *S. palustre* (avg. code 7) with a co-dominance of *S. fallax* in 24 relevés (avg. code 4), *S. rubellum* (18 relevés; avg. code 4) and the liverworts *C. connivens* (15 relevés; avg. code 3) and *K. pauciflora* (14 relevés; avg. code 2). The last group (bog; figure 5) was again dominated by *S. palustre* (11 relevés; avg. code 8), *S. fallax* (9 relevés; avg. code 3), and *S. rubellum* (10 relevés; avg. code 3), and a relatively high abundance of *C. macrostachya* (7 relevés; avg. code 3), *K. pauciflora* (4 relevés; avg. code 3), and the ombrotrophic hummock species *S. papillosum* (6 relevés; avg. code 4) and *S. magellanicum* (8 relevés; avg. code 3).

The vegetation composition in the plots changed during the time-span of the experiment. All plots, including the control, were characterised by poor fen vegetation just after the start of the experiment (0.5 years). The control plots, two 1k and one 2k limed plots maintained a poor fen vegetation throughout 4.5 years. However, one 1k plot, and two 2k plots were characterised by a rich fen composition after 1.5 years, and subsequently returned into a poor fen composition. The 4k plots had developed into a rich fen vegetation after 1.5 years, which remained throughout 4.5 years of monitoring. After 7.5 years, however, all control and limed plots were characterised by a *Sphagnum* bog composition.

Figure 4. Cover of *Sphagnum* species (%) over time in the experimental plots with different amounts of lime addition (0, 1000, 2000, 4000 kg/ha). Error bars indicate SEM values, different letters indicate significant differences between treatments.



extra N input by mineralisation enhances its accumulation. The observed increase of P availability was not significant, however, this might be due to the small number of replicates in our experiment as a trend was visible. Increased nutrient availability might be unfavourable for plant composition and biodiversity of rich fens in the long term, since it may enhance dominance of highly competitive species (Boeye *et al.*, 1997; Bobbink *et al.*, 1998; Olde Venterink *et al.*, 2002; Cusell *et al.*, in press), and accelerate succession towards *Sphagnum*-dominated poor fens (Kooijman & Paulissen, 2006). Even though the soil still showed buffered conditions after 7.5 years, *Sphagnum* became dominant again in the 4k lime treatment, which provided a strong positive feedback due to its active peat formation and acidification, raising the soil surface and decreasing the buffering of the top soil (Malmer *et al.*, 1994; Lamers *et al.*, 2000; Fritz *et al.*, 2014). Increasing dominance of *Sphagnum* species may therefore have interfered with the germination and establishment of rich fen species.

Implications for future management of rich fens

Even without management, vegetation changes will occur due to natural succession, but these will be strongly influenced by atmospheric N deposition, eutrophication and/or climatic change (Hogg *et al.*, 1995; Malmer *et al.*, 2005; Kooijman & Paulissen, 2006; Bobbink *et al.*, 2010; Lamers *et al.*, 2014). Changes in succession rate have been found to be related to management (Bakker *et al.*, 1994), with an accelerated development of old successional stages at the expense of early successional rich fen vegetation (van Diggelen *et al.*, 1996; Kooijman & Paulissen, 2006; Bobbink *et al.*, 2010; Lamers *et al.*, 2014). Although terrestrialisation can take decades, the transformation of one successional fen stage into the next can take only 11–12 years (Bakker *et al.*, 1994; Verhoeven & Bobbink, 2001), depending on the level of eutrophication. Moreover, the development of late successional stages is often indirectly correlated with increasing thickness of the peat mat, due to a changing hydrology. This raises the question whether it is feasible to restore early successional rich fen stages on thick, old peat mats.

In our study, none of the traditional management measures were able to maintain or re-establish the historic (1967) successional vegetation gradient, nor similar species richness. We indeed found that changes in vegetation over the last 46 years were strongly driven by eutrophication and acidification, but also that they could be clearly linked to the effects of different management options. Although liming, as an alternative, was found to enhance rich fen development in the very short term, *Sphagnum* showed a rapid expansion accelerating succession towards poor fen and bog vegetation. In our experiment, re-establishment of *Sphagnum* may have been favoured by the use of relatively small plots and the fact that *Sphagnum* had not been removed before lime application. When liming is used at a larger scale and directly after top-soil removal, dominance of *Sphagnum* might be postponed. Repeated addition of lime might be another option, although the effectiveness and possible negative effects are yet to be tested. Newly formed floating mats might be favourable to promote development of rich fen vegetation. However, given the fact that terrestrialisation is a significant problem faced in the conservation of fens (Geurts *et al.*, 2009; Lamers *et al.*, 2014), management will still call for management and restoration measures to restore rich fen vegetation on older peat mats. Alternatively, management goals could be adapted to the condition, targeting for the development of a bryophyte-rich poor fen vegetation, as developed in our summer mowing regime.

Slightly acidic conditions and the presence of *Sphagnum* species not necessarily inhibit the occurrence of rich fen vegetation per se, as we already found a vertical soil gradient with slightly acidic conditions in the top soil and buffered conditions deeper in the soil in 1967. However, *Sphagnum* dominance and (re-) acidification, and thereby accelerated succession from rich fen to poor fen, has to be slowed down. The only sustainable option to achieve this seems to lie in the restoration of original hydrological conditions in the top soil, for example by allowing buffered groundwater or surface water to infiltrate the top soil (van Diggelen *et al.*, 1996; Beltman *et al.*, 2001; Lamers *et al.*, 2014), or by regular inundation with base-rich surface water, although possible eutrophication issues should be taken into account (Cusell *et al.*, 2013). In conclusion, we found that the management measures summer mowing, burning after winter mowing, and liming were all unable to restore rich fens in the long term.



6 | How to choose between evils:

The biogeochemistry of drained coastal peatlands causes a management dilemma

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Submitted

Abstract

For centuries, drainage-based water management has been applied in peatlands worldwide to facilitate their agricultural use, leading to large-scale deterioration. Strongly enhanced aerobic peat decomposition in these drained peatlands causes high CO_2 emission and severe land subsidence. However, this hydrological management also severely affects other biogeochemical processes controlling soil and water quality. In coastal areas, pore water SO_4^{2-} concentrations are generally high in the unsaturated zone of the peat soil, due to oxidation of the large inorganic S pool. As a result, drained coastal peatlands form a highly underestimated source of SO_4^{2-} to adjacent surface waters, which may indirectly enhance eutrophication processes. In order to halt aerobic peat decomposition, the rewetting of drained agricultural peat soils by establishing permanently high groundwater levels has been advocated. However, this often leads to enhanced CH_4 fluxes, which may rival the greenhouse effects of previous CO_2 emissions during aerobic conditions. Moreover, the rewetting of former agricultural soils leads to enhanced mobilisation of P to the groundwater in the short term, directly enhancing surface water eutrophication. We propose, however, that reduced Fe co-discharged into surface waters during rewetting may alleviate the extent of eutrophication. The fact that both options (drainage vs. rewetting) have their own problems causes a serious dilemma for the water management in drainage-based coastal peatlands. At the same time, there is a growing call for sustainable use of subsiding coastal peatland regions and for the re-evaluation of future policy. As water management is directly related to land use, the trade-off analysis should not only be based on the geohydrological setting of coastal peatlands and former land use, but also on potential ecosystem services linked to different water management models. Although the continuation of drainage-based water management enables traditional land use, it also enhances land subsidence and severe SO_4^{2-} mobilisation. Meanwhile, there are several promising land use options for rewetted peatland areas, and a permanent high groundwater management is expected to both enhance net C sequestration and limit eutrophication in the long term. Therefore, we suggest that permanent rewetting to either restore natural wetlands or enable paludiculture (wet crop production) is the most sustainable water management strategy in drained coastal peatlands.



Figure 1. Peatlands drained for the purpose of palm oil production in Indonesia (photo left; <http://www.wetlands.org/News/Pressreleases/tabid/60/ID/2253/Billion-euros-for-Indonesian-nature-carbon-loss-great-effort-big-risk.aspx>), sugar production in Florida (photo middle; <http://www.circuloofblue.org/waternews/2008/world/north-america/sugarcane-plant-production-to-remain-as-bid-to-save-everglades-is-scaled-back>), and agricultural grassland in The Netherlands (photo right; J.M.H. van Diggelen).

Introduction

Peatlands are extensively being used for anthropogenic purposes such as peat extraction and crop production, in both coastal and inland regions worldwide (e.g. Joosten & Clarke, 2002; Zedler & Kercher, 2005; Verhoeven & Setter, 2009). Large parts have gradually been converted into managed grasslands on peat soil used for cattle grazing and hay-making (peat meadows; Bakker, 1989), or into plantations for the production of crops such as potato, oil palm or sugarcane (Fargione *et al.*, 2008; Couwenberg *et al.*, 2010; Koh *et al.*, 2011). To enable this agricultural use of peatlands, continuous large-scale drainage is necessary, which generally requires an extended network of waterways and canals (Figure 1). The drainage-based, intensive land use has a huge impact on its local and regional environment, as it strongly affects hydrology and related biogeochemical processes resulting in rapid peatland deterioration (e.g. Joosten & Clarke, 2002; Laiho, 2006; Lamers *et al.*, 2014). Drainage greatly increases the oxygenation and subsequent aerobic decomposition of peat soils. Once important carbon (C) sinks, the nowadays cultivated and drained peatlands have been converted into strong C sources with net emission rates ranging between 80 and 880 g C m⁻² y⁻¹ (Kasimir-Klmedtsson *et al.*, 1997; Lamers *et al.*, 2014). As a result of drainage and enhanced decomposition, severe land subsidence rates of 8–10 mm y⁻¹ in Western Europe (Oude Essink *et al.*, 2010; Hoogland *et al.*, 2012), 25–75 mm y⁻¹ in the United States (Galloway *et al.*, 1999) or up to 50–150 mm y⁻¹ in Asia have been observed (Syvitski *et al.*, 2009), requiring a progressively lower drainage base to enable current land use.

Meanwhile, the global mean sea level is rising at an increasing rate (IPCC, 2014; Hay *et al.*, 2015). Due to ongoing subsidence, flooding risks have severely increased in drained peatlands, especially in coastal areas, compromising their flood protection service (Zedler & Kercher, 2005; Verhoeven & Setter, 2009; Herbert *et al.*, in press). Moreover, aerobic peat decomposition enhances mineralisation, leading to high nutrient fluxes to groundwater and adjacent surface waters (van Beek *et al.*, 2004; Zak *et al.*, 2008; Geurts *et al.*, 2010). Drained, agriculturally used peatlands highly contribute to nutrient loading of adjacent surface waters via superficial run-off, shallow or lateral flow of drained groundwater from the topsoil, and leaching of groundwater from the reduced subsoil (van Beek *et al.*, 2007). In coastal areas, peatlands are generally rich in reduced sulphur compounds (FeS_x) and alterations in hydrology will strongly affect sulphur (S) cycling. The oxygenation of terrestrial S-rich peat soils will lead to highly

increased sulphate (SO₄²⁻) concentrations in pore water, groundwater and surface water. Enhanced SO₄²⁻ concentrations may well induce P mobilisation from underwater organic sediments, a process also known as internal eutrophication, which is problematic given the stimulation of algal growth at the expense of submerged biodiversity (Corelli, 1998; Lamers *et al.*, 1998a; 2002a; Smolders *et al.*, 2006a). As a result, drainage-based agriculture not only enhances land subsidence and greenhouse gas (GHG) emission, but also eutrophication-related problems in coastal peatlands.

In an attempt to reduce the severe issues involved with drainage-based peatland use, the re-establishment of higher groundwater levels has been suggested as an alternative management option for drained peatlands (Joosten *et al.*, 2012; Grootjans *et al.*, 2012). High groundwater levels will strongly decrease peat decomposition and carbon dioxide (CO₂) emission rates by limiting the availability of oxygen (O₂) as the thermodynamically most favourable terminal electron acceptor in the breakdown of organic matter (Laiho, 2006). In addition, phenol oxidase activities, regulating decomposition rates, are lower during anaerobic conditions (Freeman *et al.*, 2004). However, rewetting of drained and fertilised peatlands may also lead to high methane (CH₄) emission rates, compromising the advantage with respect to total GHG emission (e.g. Moore & Knowles, 1989; Hendriks *et al.*, 2007). In addition, it may lead to strongly enhanced phosphorus (P) mobilisation by the stimulation of microbial iron (Fe) reduction and concomitant release of Fe bound P, significantly increasing P concentrations in groundwater and surface water (Aldous *et al.*, 2007; Zak *et al.*, 2010; Cabezas *et al.*, 2013; Van de Riet *et al.*, 2013; Kinsman-Costello *et al.*, 2014). As both types of groundwater level management (low versus high water levels) have their own issues concerning GHG emission and eutrophication, this generates a serious dilemma for water management, nature management and policy with respect to large coastal peatland areas. In this paper we will therefore discuss the complex biogeochemical interactions among S, Fe, and P of (formerly) drained coastal peatlands as governed by either groundwater level management model, and discuss the emerging dilemmas in terms of preferred water management and future policy for a more sustainable use of subsiding coastal peatland regions.

Biogeochemistry of drainage-based peatlands; the business as usual model Effects on soil quality

In the terrestrial parts of peatlands, a saturated zone (phreatic zone) and an unsaturated zone (vadose zone) can be distinguished. The saturated zone permanently remains below groundwater level, while the unsaturated zone is exposed to O₂ when the groundwater level drops. Even without drainage, groundwater level fluctuations at the interface of the saturated and unsaturated peat zone are common due to seasonal variations in temperature and precipitation. Drainage significantly lowers average groundwater levels, subsequently increasing the depth of the unsaturated zone (frequently more than 50 cm below soil surface), while seasonal groundwater level fluctuations still occur at the interface of the saturated and unsaturated peat zone. This drainage-based water management highly increases oxygenation of the peat soil, which leads to enhanced aerobic peat decomposition, higher GHG emission and land subsidence (e.g. Moore & Knowles, 1989; Joosten & Clarke, 2002; Joosten *et al.*, 2012). As a result of O₂ penetrating into the peat matrix, aerobic microbial communities that use organic C compounds as an electron donor are stimulated, leading to a net release of CO₂ and N₂O (Kasimir-Klmedtsson *et al.*, 1997; Berglund & Berglund, 2011). In addition, decomposition rates are depending on environmental conditions such as temperature and pH, the composition of the microbial community and other decomposers, peat soil composition, and nutrient availability (Laiho, 2006).

Long-term exposure to aerobic conditions significantly enhance CO₂ emission rates, however, aerobic rates show a 2-fold increase in the unsaturated peat zone when compared to the saturated peat zone (figure 2). The long-term aerobic decomposition has led to irreversible changes in peat characteristics of the unsaturated zone, including not only a decrease in cellulose, lignin (Laiho, 2006) and phenolic compounds (Freeman *et al.*, 2004), but also highly increased P availability (figure 3), decreased lignin : P ratios (Tomassen *et al.*, 2004), and higher dissolved organic C concentrations (DOC; Zak *et al.*, 2010). These changes may all contribute to the acceleration of decomposition rates.

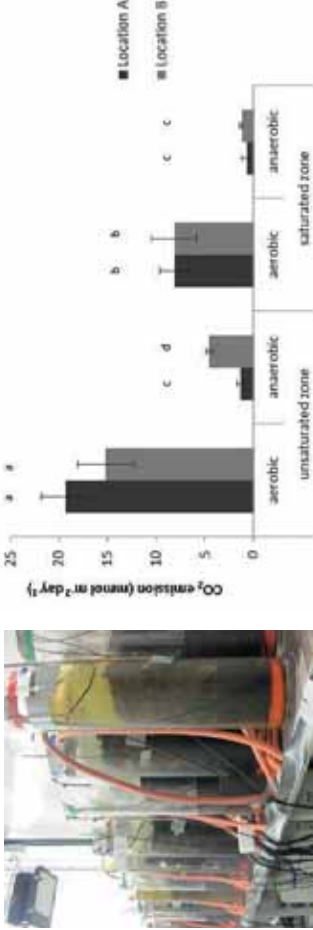


Figure 2. CO₂ emission rates (mmol m⁻² day⁻¹) for peat cores collected in the saturated and unsaturated peat zone from two drained coastal peatland areas in The Netherlands (Wormer-IJsserveld; 52° 30' 37"; 4° 51' 46"), incubated under aerobic and anaerobic conditions. Significant differences are indicated by different characters. CH₄ emission rates were negligible in this experiment (van Diggelen, unpublished results).

Ongoing land subsidence and adjusted water level management will lead to the gradual exposure of deeper, formerly saturated peat zones to oxygenation. Although nutrient contents are generally much lower in the saturated peat zone (figure 3), nutrient availability will be increased through mineralisation. This creates a positive feedback, and aerobic decomposition will become a self-stimulating process over time in the unsaturated zone as long as acidification due to aerobic oxidation processes and the production of organic acids is prevented by sufficient buffering. This is likely, as alternating aerobic and anaerobic episodes generate buffering capacity through increasing HCO₃⁻ concentrations (Smolders *et al.*, 2006b). In addition, lime is occasionally added to drained peatlands in agricultural use to prevent acidification (Bertrand *et al.*, 2007).

Drainage-based water management not only changes the characteristics of the unsaturated peat soil, but it also leads to condensation of mineral components (Zak *et al.*, 2010; Aggenbach *et al.*, 2013). Due to peat oxygenation, high amounts of immobile, reduced FeS_x and organic S present in the unsaturated zone of coastal areas will be prone to aerobic oxidation. This primarily results in the formation of relatively mobile Fe²⁺ and SO₄²⁻ (Smolders *et al.*, 2006b; Haaijer *et al.*, 2007). SO₄²⁻ easily becomes discharged, resulting in a net loss of S from the unsaturated zone. Consequently, S does not accumulate in this zone and the total S pool is generally only slightly higher in the unsaturated peat zone compared to the saturated zone (figure 3). Mobilised Fe²⁺, however, becomes oxidised in the lower parts of the unsaturated soil (20-40 cm below soil surface) where it precipitates as amorphous Fe oxides and hydroxides (FeOOH). This process leads to a strong accumulation of Fe

in this zone (figure 3). As oxidised Fe compounds have a high P sorption capacity, the mobility of P is limited in the unsaturated zone as long as aerobic conditions prevail (Lucassen *et al.*, 2005; Griffioen, 2006; Aggenbach *et al.*, 2013). Consequently, strong accumulation of P is also found in the unsaturated zone of the peat soil (figure 3), which may for an important part originate from P released during historic peat mineralisation, and for another part from the excessive use of manure and fertiliser for decades, if not centuries (Sharpley *et al.*, 1994; van Beek *et al.*, 2004; 2007).

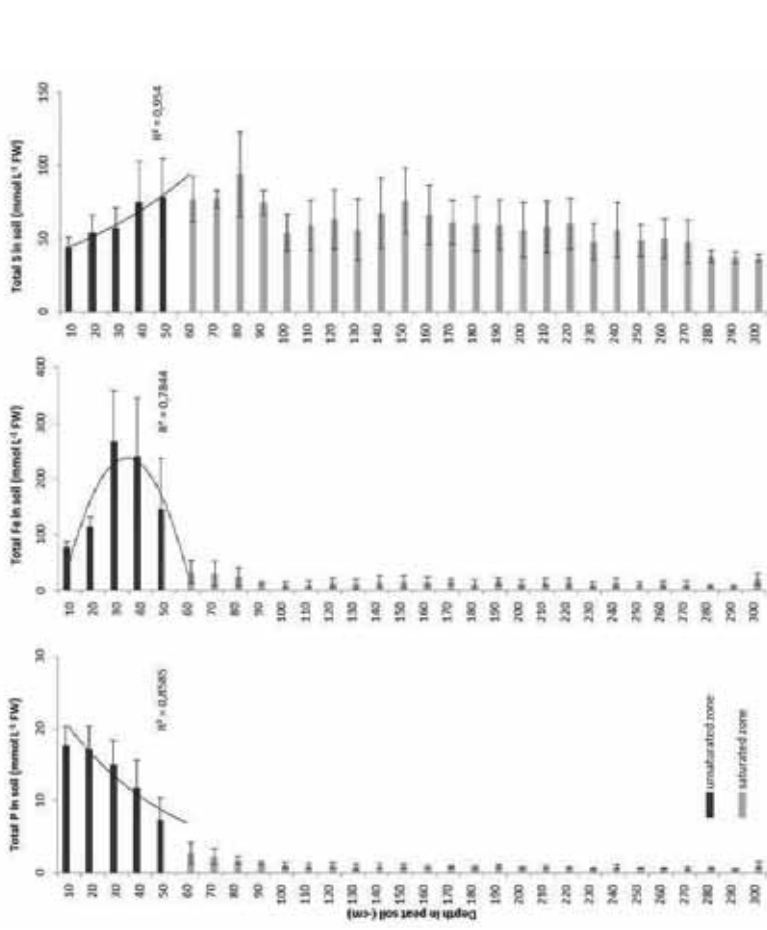


Figure 3. Depth profiles showing average total concentrations of phosphorus (P), iron (Fe) and sulphur (S) (mmol L⁻¹ FW; ± SEM), measured in the unsaturated (0 - 50 cm; black bars) and saturated zone (>60 cm; grey bars) of drained, agricultural areas (n=4) in a coastal peatland in the Netherlands (Wormer-IJsserveld; 52° 30' 37"; 4° 51' 46").

Effects on the quality of adjacent surface waters

Weather conditions and hydrological water flows in the peat highly determine nutrient discharge towards surrounding surface water. During dry periods there is hardly any water flowing towards surrounding canals. Surface water may even infiltrate into the peat soil when groundwater levels have dropped in summer and water levels in the canals are maintained high by external supply of surface water to diminish further water shortage. Evaporation in dry and warmer periods enhances

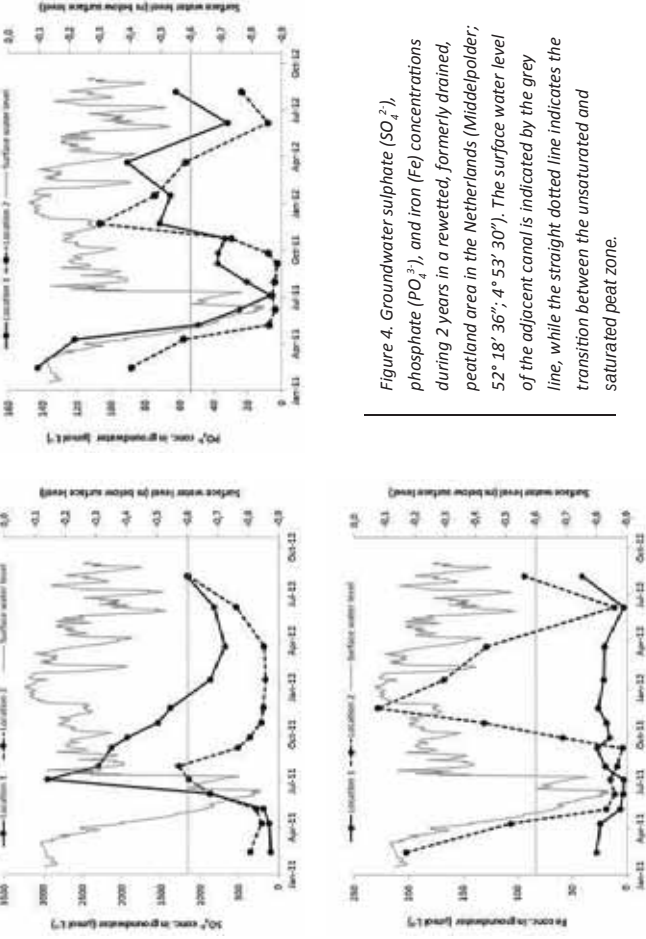


Figure 4. Groundwater sulphate (SO_4^{2-}), phosphate (PO_4^{3-}), and iron (Fe) concentrations during 2 years in a rewetted, formerly drained, peatland area in the Netherlands (Middelpolder; $52^\circ 18' 36''$, $4^\circ 53' 30''$). The surface water level of the adjacent canal is indicated by the grey line, while the straight dotted line indicates the transition between the unsaturated and saturated peat zone.

desiccation of the topsoil during low groundwater levels, also enhancing aerobic oxidation of Fe^{2+} and thus the formation of SO_4^{2-} and poorly soluble $\text{Fe}(\text{OH})_3$. Consequently, P concentrations in the groundwater remain low due to P adsorption to $\text{Fe}(\text{OH})_3$ (Griffioen, 2006; Smolders *et al.*, 2006b), while SO_4^{2-} concentrations strongly increase (figure 4). In the occasion of heavy rainfall after a dry period, about 90% of the surplus precipitation becomes discharged towards adjacent surface waters through the unsaturated peat zone (van Beek *et al.*, 2004). Consequently, leaching of SO_4^{2-} will temporarily lead to a strong increase of its concentrations in adjacent canals or lakes, where it may indirectly lead to eutrophication through the mobilisation of P from the sediment (figure 6). In this process, SO_4^{2-} is reduced in the sediment as an alternative electron acceptor during microbial decomposition of organic matter. Sulphide (H_2S) is formed, which efficiently decouples Fe - P interactions at the sediment-water boundary, internally mobilising phosphate (PO_4^{3-}) and increasing pore water H_2S concentrations (Caraco *et al.*, 1989; Smolders & Roelofs, 1995; Smolders *et al.*, 2006a; Lamers *et al.*, 2013). In aquatic systems P is often limiting for production, and its increased availability will strongly enhance eutrophication related dominance of fast growing primary producers such as duckweed or algae (Roelofs, 1991; Corell, 1998; Smolders & Roelofs, 1995). In conclusion, drained peat soils in coastal areas appear to be a very significant source of SO_4^{2-} , enhancing internal eutrophication of surface waters.

Raised groundwater levels as an alternative management model Effects on soil quality

As an alternative management option, high groundwater levels are advocated in order to prevent further peat degradation, and have been shown to significantly decrease CO_2 emission (figure 2; Couwenberg *et al.*, 2010; Joosten *et al.*, 2012; Grootjans *et al.*, 2012). On the other hand, CH_4 emissions may increase following anaerobic conditions, which can partly counteract the beneficial effect of higher groundwater levels with respect to global warming potential (Moore & Knowles, 1989; Hendriks *et al.*, 2007; Harpenslager *et al.*, *subm.*). Nevertheless, various studies have shown that net GHG emissions are still reduced after rewetting (van de Riet *et al.*, 2013; Hendriks *et al.*, 2007; Hendriks *et al.*, 2008), which is also expected in S-rich coastal peatlands as high SO_4^{2-} reduction rates will inhibit CH_4 production (Gauci *et al.*, 2004). Anaerobic conditions will strongly enhance SO_4^{2-} and $\text{Fe}(\text{OH})_3$ reduction in permanently wet peatlands. As a result, groundwater SO_4^{2-} concentrations will remain relatively low and dissolved Fe^{2+} concentrations will increase (figure 4).

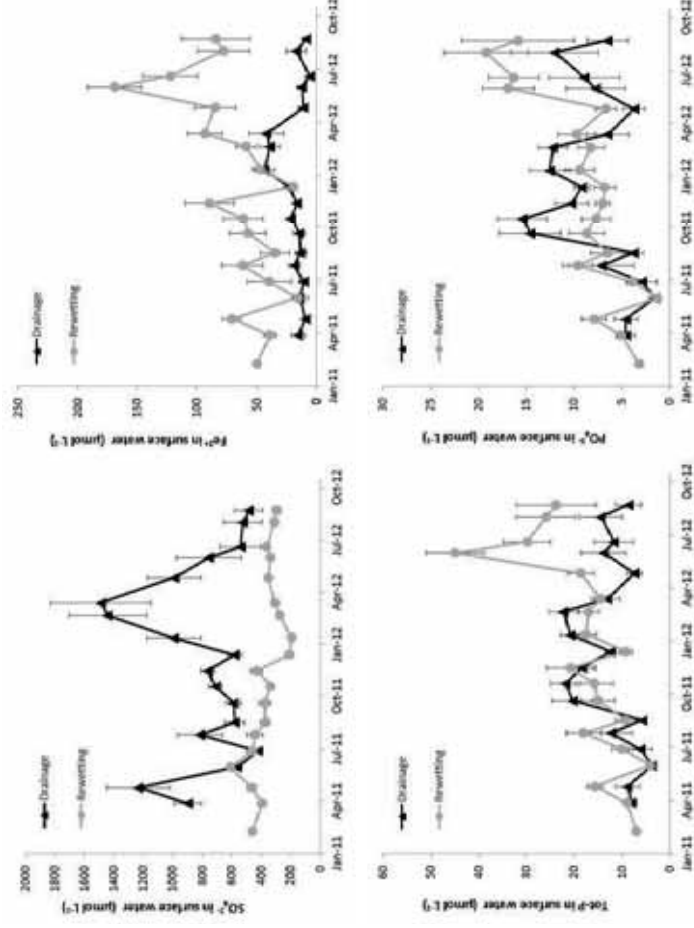


Figure 5. Average surface water sulphate (SO_4^{2-}), iron (Fe^{2+}), phosphate (PO_4^{3-}) and phosphorus (Tot-P) concentrations during 2 years in canals surrounding drained (black line) versus rewetted (grey line) coastal peatland areas ($n=4$) in the Netherlands (Ronde Hoep; $52^\circ 16' 04''$, $4^\circ 54' 00''$). The central area of this peatland was rewetted in 2011, while a drainage-based management was maintained in the rest of the area.

Another important issue after rewetting of the unsaturated peat zone is the fact that P becomes strongly mobilised to the pore water and groundwater (Aldous *et al.*, 2007; Zak *et al.*, 2008; 2010; Cabezas *et al.*, 2013; van de Riet *et al.*, 2013). The release of inorganic P, predominantly in the form of PO_4^{3-} , is found to be a rapid process (within days) in which P is mobilised by anaerobic reduction of Fe(III)OOH to which PO_4^{3-} was bound under aerobic conditions (Lamers *et al.*, 1998a; Cabezas *et al.*, 2013; Kinsman-Costello *et al.*, 2014). Microbial cell lysis because of an osmotic shock from the transition of aerobic to anaerobic conditions may add to P release (Turner & Haygarth, 2001; van Dijk *et al.*, 2004). During these processes following rewetting, both P and Fe will dissolve (figure 4; Lucassen *et al.*, 2005; van de Riet *et al.*, 2013; Kinsman-Costello *et al.*, 2014), although part of the Fe will also become re-immobilised by precipitation as FeS_2 (figure 6). The rates of Fe and P mobilisation are expected to depend on the concentrations of Fe and P accumulated in the unsaturated zone before the groundwater level increases (figure 3).

Effects on the quality of adjacent surface waters

The discharge of dissolved P towards adjacent surface waters becomes strongly enhanced after rewetting of drained peatlands, and is an important source of eutrophication (e.g. van Dijk *et al.*, 2004; Zak *et al.*, 2010; Kinsman-Costello *et al.*, 2014). Co-discharge of dissolved Fe with P, however, might be an important and relatively underestimated process in the actual contribution to nutrient loading after rewetting (figure 5). Permanently high groundwater levels will increase drainage through shallow pathways, enhancing nutrient transport from the former unsaturated peat zone, and decreasing the potential high contribution from the saturated peat soil (van Beek *et al.*, 2007). Additionally, as inundation of the peat soil is usually allowed with a high groundwater management, this increases storage capacity leading to enhanced rainwater retention and dilution of nutrients. In general, groundwater movement will be limited compared to drainage-based systems, and transport of shallow P (and Fe)-rich groundwater towards adjacent surface waters will occur predominantly during heavy rainfall.

We suggest that the concomitant discharge of dissolved Fe^{2+} upon high water levels induces co-precipitation of P in surface waters, following Fe oxidation in the surface water, alleviating the effects of P discharge. So, once in the surface water, P may get (temporarily) immobilised in the sediment by co-precipitation with Fe^{3+} (Smolders *et al.*, 2006a; Geurts *et al.*, 2008; Lamers *et al.*, 2013). As a result, surface water P concentrations are not as high as might be expected based on often extremely high groundwater P concentrations, although eutrophic conditions usually still prevail (figure 5). The extent of this effect depends on the concentration of dissolved Fe in phreatic groundwater, which may vary due to local soil conditions (figure 4). In general, we expect relatively high dissolved Fe:P ratios in the unsaturated peat zone to partly prevent the accumulation of P in the surface water. Moreover, supply of relatively Fe-rich peat particles from the unsaturated peat zone, directly entering adjacent waterways, may also enhance immobilisation of P in the surface water. Co-transport of Fe from the peat soil is therefore proposed to act as a P filter in surface water.

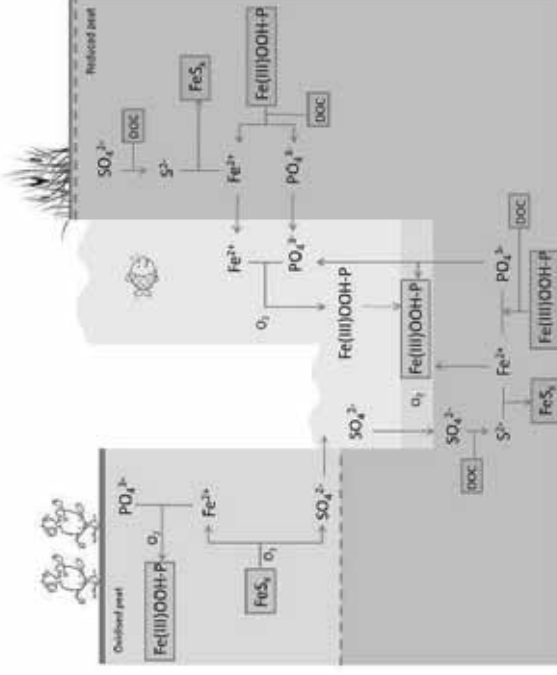


Figure 6. Schematic overview of interactions between P, Fe and S cycling for drained (left; low groundwater levels) and rewetted (right; high groundwater levels) coastal peatlands, and the resulting effects for adjacent surface water. See text for further explanation.

Tackling the water management dilemma in coastal peatlands

The choice between evils: raising the groundwater level

In this paper we focused on the shifts of dominant biogeochemical interactions among Fe, S and P in the unsaturated zone of coastal peatlands that, unlike changes in GHG emissions (Moore & Knowles, 1989; Kasimir-Klemmedtsson *et al.*, 1997; Hendriks *et al.*, 2007), received much less attention in literature. As both drainage-based groundwater management and the restoration of a permanently high groundwater level directly or indirectly leads to eutrophication issues in (formerly) agricultural coastal peatlands, this generates a large problem for water management authorities that have to comply with strict water quality criteria. The regular drainage-based water management model with seasonally fluctuating low groundwater levels strongly stimulate Fe and S oxidation–reduction cycles, which leads to the enhancement of both aerobic and anaerobic decomposition. As a result, alternating episodes of high SO_4^{2-} and P release will continue, which may additionally lead to constant or periodical accumulation of highly toxic H_2S (Lamers *et al.*, 2013). In hydrological nutrient flow models, the calculations of P loading from drained peat soils are usually based on a general P flux (van Beek *et al.*, 2004; 2007; Hendriks *et al.*, 2008). However, concentrations of S and Fe during reduced and oxidised conditions, their enhancing or inhibiting effect on P eutrophication, and groundwater movement in the saturated and unsaturated zone should also be taken into account to determine the actual contribution of nutrient loading to the aquatic system. Especially considering climate change and sea level rise, current drainage-based land use and water management is considered unsustainable, not only with respect to GHG emissions and soil subsidence, but also with respect to the resulting poor water quality in the long term.

Although the re-establishment of permanently high surface water and phreatic groundwater levels in formerly drained, agricultural peatlands leads to severe P mobilisation, in coastal areas it may be less harmful to water quality than previously thought, due to the proposed alleviating co-mobilisation of dissolved Fe^{2+} with P, but also because of more limited water movement towards the surface water

(Aldous *et al.*, 2007; Kinsman-Costello *et al.*, 2014). In addition, when high groundwater levels remain stable, mobilised P will be partially re-immobilised by the formation of reduced amorphous Fe(II) OOH in the longer term (Vepraskas & Faulkner, 2001). Maintenance of high groundwater levels after rewetting is, however, difficult in formerly drained peatlands, which are nowadays often infiltrating systems. This can be achieved by retaining rainwater, which additionally leads to nutrient dilution in surface waters. During long dry periods, however, the input of external water is often still needed, which may form an additional source of eutrophication (Roelofs, 1991; Smolders & Roelofs, 1995; Burgin & Hamilton, 2008). Nevertheless, permanent high water levels will limit GHG emission in S-rich coastal areas and enhance net C sequestration, reduce land subsidence, improve flooding protection, and will probably also improve water quality prospects in the longer term compared to the current low water levels. Therefore, despite eutrophication risks as a result of rewetting in the short term, we still consider restoration of permanently high surface water and groundwater levels the most sustainable management option for coastal peatlands.

Land use options for rewetted peatlands

Raising groundwater levels in formerly drained, agricultural peatlands, will induce significant problems regarding land use options. Traditional agricultural land use will be strongly affected when drained peatlands get permanently rewetted. High soil P levels, however, are of environmental rather than agronomic concern, particularly in areas of intensive agricultural use (Sharpley *et al.*, 1994). A more sustainable option for coastal peatlands would be to also find alternative agricultural land use options in combination with raised groundwater levels and limited eutrophication. Paludiculture, which involves the cultivation of wetland plants for commercial use, has been shown to be a successful alternative in rewetted peatlands (Joosten & Clarke, 2002; Joosten *et al.*, 2012). This agricultural use of rewetted peatlands will also enhance P uptake by the cultivated wet crops, limiting its discharge to adjacent surface waters. Of course the suggested changes need support of land owners and other stakeholders involved, which may be a delicate issue. A trade off analysis for decision-making should be based on the geohydrological setting of coastal peatlands, land use options and profits, but also potential ecosystem services resulting from different water management models.

To limit the availability and mobilisation of P in the case of wetland restoration, removal of the nutrient-rich topsoil before rewetting may be a good option (Smolders *et al.*, 2007; Emsens *et al.*, 2015). Topsoil removal will directly enhance the restoration of permanent high groundwater levels, and anaerobic conditions in combination with low P availability will strongly decrease decomposition and nutrient mineralisation rates, creating mesotrophic conditions. However, these conditions will also have important consequences for land use. The lowered areas with restored high groundwater levels and decreased nutrient content, will be most suitable for development of (semi-)natural peatlands. Resulting undesired elimination of the seed bank can effectively be counteracted by hay transfer after topsoil removal (Patzelt *et al.*, 2001; Hölzel & Otte, 2003; Klimkowska *et al.*, 2010). This will enhance the establishment of biodiverse peatland vegetation, promoting re-growth of peat in the longer term. As topsoil removal directly lowers the soil surface, this measure is often considered undesirable in subsiding peatlands. However, the removed P-rich soil can still be used in near areas to raise drained and subsided peat soils, facilitating traditional dry agricultural use.

Conclusions

We highlighted the key roles of S and Fe cycling in P mobilisation and eutrophication of (formerly) drained coastal peatlands, and discussed the emerging management dilemmas in terms of preferred water management and future policy for a more sustainable use of subsiding coastal regions. Although the re-establishment of permanently high groundwater levels directly leads to P eutrophication, at least in the short term, these effects on surface water quality might be temporary and partly alleviated by co-transport of Fe. Moreover, it is expected to decrease GHG emission and enhance net C sequestration in the long term, and there are several promising land use alternatives for rewetted areas. Continuation of drainage-based water management, on the other hand, enables traditional land use, but also enhances severe SO₄²⁻ mobilisation, CO₂ emission and land subsidence. Therefore, we suggest that permanent rewetting of (formerly) drained coastal peatlands seems to be the most sustainable water management strategy in the long term.



7 | General discussion and synthesis

Josepha M.H. van Diggelen

Peatlands and their land use

The main objective of my thesis was to provide more insight into a number of important biogeochemical processes controlling the functioning of anthropogenically modified peatlands, in order to contribute to their sustainable future management. Worldwide, the anthropogenic forcing on wetland ecosystems is strong and still increasing (e.g. Mitsch & Gosselink, 2000; Brinson & Malvárez, 2002; Zedler & Kercher, 2005). Especially peatlands are seriously under pressure, due to issues such as altered hydrology (water shortage), eutrophication, and acidification (e.g. Grootjans *et al.*, 1996; Koerseman & Verhoeven, 1992; Joosten & Clarke, 2002; Lamers *et al.*, 2014). At a landscape level, peatlands comprise a suite of different ecosystem types, such as semi-terrestrial quaking mires, aquatic turf ponds, and terrestrial peat meadows, resulting in high habitat diversity which makes them potentially biodiverse systems (Wheeler & Proctor, 2000). In the Netherlands and many other countries, peatlands were historically used for peat excavation on a relatively large scale, while non-excavated areas were converted to agricultural lands. As a result, drainage-based water management has become common practice in these peatlands and their surroundings. Nowadays a large part of the terrestrial peat areas in the Netherlands are predominantly used as grasslands for agricultural purposes, while smaller (semi-)terrestrial areas are managed as nature reserves. In the natural reserves, vegetation succession including peat formation still occurs, hosting endangered species that also present important European conservation values (Grootjans & van Diggelen, 1995; Lamers *et al.*, 2002b). This type of land use created a typical landscape that shows the cultural heritage of a large part of the Netherlands (figure 1).

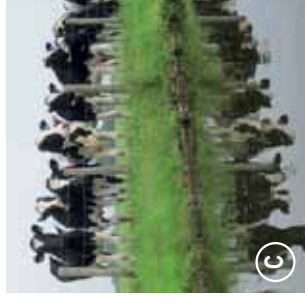
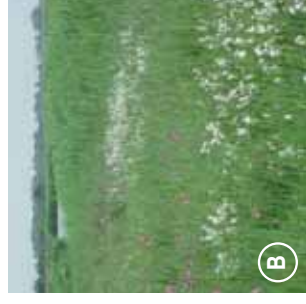


Figure 1. Typical Dutch fen landscape (Nieuwkoopse plassen; Chapter 5), comprising terrestrial and semi-terrestrial areas, surrounded by a network of aquatic areas incl. ditches, canals and turf ponds (A). In a Dutch coastal peatland (Wormer- and Jisperveld), terrestrial and semi-terrestrial areas are either managed to conserve natural, biodiverse habitats (B) or to support agricultural use (C).
Photo credits: J. Arkesteijn (A); J.M.H. van Diggelen (B-C).

Drainage-based peatland management

As discussed in this thesis, drainage-based land use affects several redox-sensitive processes that induce undesired alterations such as accelerated peat decomposition, eutrophication and acidification, which have a large impact on both (semi-)natural and agricultural peatlands (Chapter 5 and 6). Oxygen (O_2) availability is one of the main drivers that controls a number of biogeochemical processes involved in carbon (C), sulphur (S), iron (Fe), phosphorus (P) and nitrogen (N) cycling, affecting biotic and abiotic processes, ecosystem functioning and biodiversity (Chapter 4). Hydrological changes including fluctuating water levels are therefore expected to initiate a whole chain of these redox-related processes (Chapter 6). As the ecosystem will be affected at different levels, not only key biogeochemical mechanisms will be discussed in this synthesis, but also their interactions with vegetation development and ecosystem functioning. In the last section of this synthesis, these findings will be discussed in terms of potential, sustainable management options for peatlands.

Drainage-based land use severely changes local and regional hydrology and limits the supply of base-rich groundwater, which often also leads to water shortage and acidification in adjacent (semi-) natural systems (Bootsma *et al.*, 2002; Cusell *et al.*, 2013). In these more natural systems, the ongoing vegetation succession from rich fen to poor fen, and eventually bogs or carr woodland, is largely depending on the increasing vertical peat accumulation in the root mat and subsequent changing input of base-rich water. This eventually creates a vertical soil gradient leading to the accumulation of rainwater at the peat surface, facilitating the transition from fen towards bog vegetation (Koerselman *et al.*, 1990; van Diggelen *et al.*, 1996). As a result of decreased groundwater influence and greatly enhanced *Sphagnum* growth under non-buffered, acidifying conditions, natural succession is strongly accelerated towards late-successional vegetation types, which leads to a loss of the early-stage, biodiverse rich-fen vegetation types (Chapter 5).

Another important issue resulting from ongoing drainage-based land use is that the C-sink function of peatlands is seriously threatened, as enhanced oxygenation of the topsoil (unsaturated peat zone) significantly increases aerobic decomposition rates and leads to severe land subsidence (e.g. Joosten & Clarke, 2002; Sleutel *et al.*, 2003; Bellamy *et al.*, 2005; Laiho, 2006; Lamers *et al.*, 2014). As a result, not only the global warming potential of peatlands, but also flooding risks and additional drainage costs have dramatically increased. A high water level management is often advocated as an alternative to limit CO_2 emission and peat degradation (Chapter 6; Joosten *et al.*, 2012; Grootjans *et al.*, 2012). However, as long-term drainage has irreversibly changed peat characteristics such as topsoil P accumulation especially in agricultural but also in (semi-)natural peatlands, rewetting may lead to several other problems such as enhanced eutrophication and CH_4 emission (Chapter 6; Zak *et al.*, 2010; Harpenslager *et al.*, *subm.*).

Eutrophication: P and N dynamics

Biomass production in peatlands may in general be limited by either P or N, or by both. The mobility and plant availability of P is largely controlled by S and Fe cycling, in which O_2 availability plays a very important role (Chapter 2, 4 and 6; Smolders *et al.*, 2001; Lamers *et al.*, 2002a; Hupfer & Lewandowski, 2008; Geurts *et al.*, 2009). Different P mobilisation rates can be expected for peatlands that are either S-rich (coastal areas) or S-poor (inland areas), mainly depending on the presence and availability of Fe and P. Einsele (1936) and Mortimer (1941; 1942) already described the effectiveness of sufficiently high O_2 concentrations in the surface water to prevent P release from underwater sediments. However, for coastal peatlands we found that despite relatively low dissolved Fe and high SO_4^{2-} concentrations in underwater sediments, the oxidation of FeS_x compounds at a millimetre

spatial scale may still provide sufficient dissolved Fe for the formation of ferric $Fe(O)OH$ at the sediment surface (Chapter 2). As a result, there can still be efficient immobilisation of P in the long term (figure 2). In absence of O_2 , high NO_3^- concentrations may also increase dissolved SO_4^{2-} and Fe^{2+} concentrations by the anaerobic oxidation of FeS_x compounds in the peat soil (Haaijer *et al.*, 2007; Burgin & Hamilton, 2008; Smolders *et al.*, 2010). This highlights the role of FeS_x -rich sediments typical for coastal systems, which may act as an additional Fe source for potential P immobilisation. Inland systems are generally poor in FeS_x compounds, and P exchange is therefore predominantly controlled by the actual Fe:P ratio of the sediment porewater (Smolders *et al.*, 2006a; Geurts *et al.*, 2010). For both inland and coastal systems, however, P mobilisation is expected to be enhanced with increasing surface water SO_4^{2-} concentrations that may result from increased salinisation (Chapter 2; Herbert *et al.*, *in press*), drainage of SO_4^{2-} from oxidising S-rich peat meadows (Chapter 5) or the use of SO_4^{2-} rich surface water to compensate for water shortage (Roelofs, 1991).

In (semi-)terrestrial systems, plant-available N is derived from mineralisation, surface water and groundwater input, atmospheric deposition and N_2 -fixing micro-organisms (Britto & Kronzucker, 2002; Miller & Cramer, 2004). However, N availability can be greatly enhanced by increased atmospheric N deposition (Bobbink *et al.*, 1998), high nitrate (NO_3^-) discharge via polluted groundwater (Dise *et al.*, 1995; Sutton *et al.*, 2011), increased ammonium (NH_4^+) mineralisation as a result of increased decomposition of organic soils, and directly from the fertilisation of peat meadows (van Beek *et al.*, 2007). In the absence of O_2 in anaerobic wetland soils, NO_3^- is the most favourable electron acceptor for the oxidation of organic matter. Increased N levels stimulate anaerobic decomposition and nutrient mineralisation at the interface of the saturated and unsaturated peat zone (figure 2), where the coupling between nitrification and denitrification leads to a net loss of gaseous N_2 .

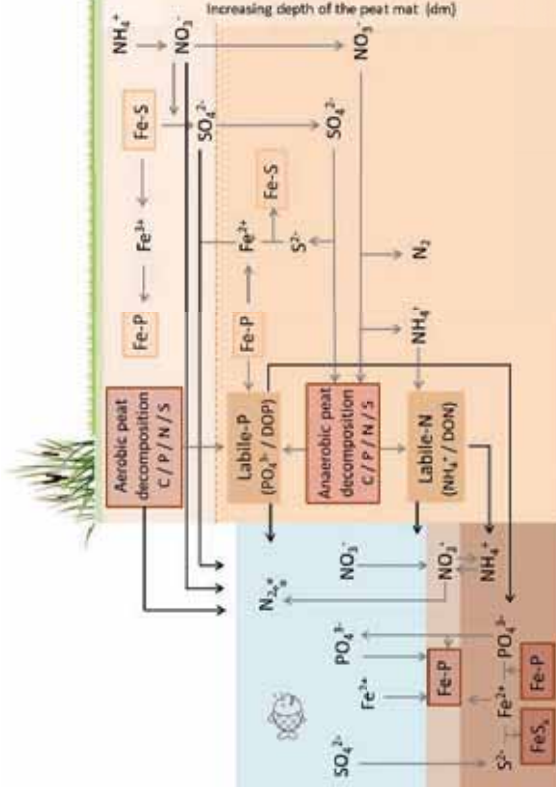


Figure 2. Schematic overview of dominant processes in S, Fe, P and N cycling (grey arrows) and nutrient fluxes (black arrows) in drained peatland soils and adjacent surface waters.

Dissolved nutrients in surface waters of drained Dutch peatland areas show distinct annual fluctuations (figure 3), where the net concentrations are a result of several alternating processes. During the growing season, rates of microbially-mediated NO_3^- reduction increase due to higher temperatures. This leads to higher N losses to the atmosphere, and as a result surface water N concentrations are generally low during this period, while P concentrations are still high due to internal mobilisation and enhanced SO_4^{2-} reduction (Chapter 2; Smolders *et al.*, 2006a). The highest P concentrations resulting from internal eutrophication are usually found when temperatures are also highest (July and August; figure 3). From September until February, microbially-mediated reduction processes will be limited as a result of decreasing temperatures, enhancing accumulation of SO_4^{2-} and NO_3^- concentrations in the surface water. In addition, generally high rainfall during this period will lead to the discharge of SO_4^{2-} and NO_3^- from adjacent desiccated peat meadows shortly after the dry season (figure 3), however, rainfall leads to a net dilution of P in this wet period (Chapter 6). To prevent high groundwater levels or even flooding, surplus precipitation and dissolved nutrients are effectively pumped out of the lowland peat meadow areas during heavy rainfall. By the resulting lack of rainwater retention during this wet period, supply of external water from surrounding surface water systems becomes necessary to prevent desiccation when evapotranspiration exceeds precipitation. This, however, may lead to an additional input of P and SO_4^{2-} , increasing the risk of eutrophication (Roelofs, 1991; Lamers *et al.*, 2002a).

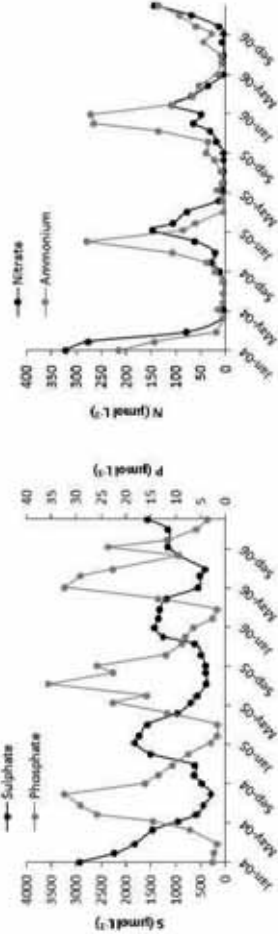


Figure 3. Seasonally fluctuating surface water nutrient levels (PO_4^{3-} , SO_4^{2-} , NO_3^- , NH_4^+) in a peat meadow area near Oudewater, The Netherlands (Data from water management authority 'De Stichtse Rijnlanden'). Similar results have been found for other peatland areas throughout the Netherlands (data not shown).

Vegetation development and biodiversity

In agricultural peatlands, relatively high surface water P concentrations are often found (figure 3), while the threshold for freshwater systems is only $<2 \mu\text{mol L}^{-1}$ to obtain well-developed submerged vegetation with clear water (Geurts *et al.*, 2008). At high nutrient levels, a clear water state is often only temporarily stable or even absent (Scheffer, 1989). It is, however, questionable whether it is feasible to aim for P-limitation in peatlands that have been drained and in agricultural use for centuries (Chapter 6). In aquatic systems, wind enhances water turbulence, which may have a positive effect by directly increasing O_2 availability at the sediment surface, enhancing P immobilisation (Chapter 2). However, at the same time re-suspension of peat particles can occur which (temporarily) increases surface water turbidity. As a result, light penetration decreases and establishment and growth of macrophytes is inhibited (e.g. Barko *et al.*, 1982; Scheffer, 1989). The re-suspension of peat particles is a large problem in shallow aquatic systems found in many Dutch peatlands, as the top of sediments

is often covered with highly degraded, easily re-suspended, organic matter (sludge). The effect of artificial lowering water turbulence on vegetation development was experimentally monitored in normally very turbid surface waters with relatively high P concentrations ($>3 \mu\text{mol L}^{-1}$) in a Dutch coastal peatland (van Diggelen *et al.*, 2013). Decreased turbidity directly led to the establishment of either favourable *Chara* spp, despite relatively high P concentrations in the surface water, or to the development of undesired floating algae (figure 4). Rooting macrophytes can limit water turbulence and prevent re-suspension of particles by sealing the sediment surface, and also take up nutrients preventing the accumulation of P and N in the surface water. In addition, the established *Chara* spp. are known to excrete allelochemicals, preventing algal dominance (Berger & Schagerl, 2004), which might promote a new stable state with clear water even at higher nutrient levels. In relation to climate change and sea level rise, increasing risks of salinisation in freshwater systems will affect P concentrations in surface waters (Chapter 2). In addition, a higher surface water salinity might help to inhibit algal development by osmotic changes, and promote the development of a stable clear water state at higher P concentrations with typical submerged, brackish vegetation (Smolders *et al.*, 2003).

Increased nutrient availability is a very important driver of biodiversity loss in aquatic and (semi-) terrestrial systems, as it will enhance primary production and induce a change in plant community composition due to dominance of highly competitive species (Koerselman & Meuleman, 1996; Olde Venterink *et al.*, 2002; Elser *et al.*, 2007). The presence of endangered species and species richness in fens is often found to be negatively related to increased P availability (Olde Venterink *et al.*, 2003; Koijman & Paulissen, 2006). Increased N availability may enhance graminoid dominance in (semi-) terrestrial systems, which generally causes a loss of biodiversity due to light limitation (Bobbink *et al.*, 1998; Olde Venterink, 2002; Cusell *et al.*, 2014a,b). High N availability was shown to significantly enhance aboveground biomass, at the expense of belowground biomass, for the graminoid species *Carex disticha* and *Juncus acutiflorus* (Chapter 3).



Figure 4. Inhibition of organic matter resuspension in a large coastal peatland in the Netherlands (Warmer-IJsperveld) leads to clear water and development of either *Chara* spp. (B) or floating algae (C). Photo credits: J. Graafland (A); J.M.H. van Diggelen (B-C)

Rhizosphere effects on C and N cycling

The net C exchange of peatlands with the atmosphere depends on the balance between peat accumulation and decomposition rates, and both may be affected by eutrophication. A large suite of microbially-mediated processes determine aerobic and anaerobic decomposition rates in relation to litter quality and nutrient availability (Chapter 4). When anaerobic conditions prevail, nitrification is blocked and denitrification will be decoupled, which may lead to high accumulation of NH_4^+ in the groundwater. In addition, P concentrations are generally also enhanced due to anaerobic reduction of Fe(III)OOH to which PO_4^{3-} is bound under aerobic conditions (Chapter 2; Cabezas *et al.*, 2013; Kinsman-Costello *et al.*, 2014). As the quality of graminoid litter will be affected due to enhanced NH_4^+ levels (Chapter 3), in combination with the generally high nutrient availability, this may promote anaerobic decomposition rates. A large rhizotron experiment with *Carex disticha* and *Juncus acutiflorus* indeed showed net CO_2 sequestration at normal nutrient levels (control), while bare peat soils showed CO_2 loss (figure 5). Experimentally enhanced NH_4^+ influx to the soil pore water, however, caused a shift from a net C-sink to a net C-source for the vegetated soils as well, due to higher decomposition rates (van Diggelen, unpublished results).

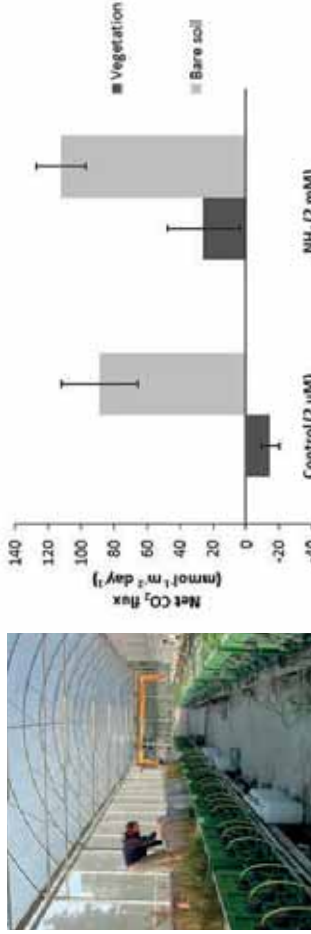


Figure 5. Three-year rhizotron experiment to test effects of enhanced NH_4^+ (2 mmol L^{-1}) and control concentrations (20 μmol L^{-1}) on net CO_2 fluxes from anaerobic peat soils covered with vegetation (*Carex disticha* and *Juncus acutiflorus*), as compared to bare soil (J.M.H. van Diggelen, unpublished results). Photo credit: L.P.M. Lamers.

Vascular plant species may stimulate aerobic decomposition in anaerobic peat soils by oxidising the rhizosphere through radial oxygen loss (ROL), as was found in oligotrophic peatlands (Fritz *et al.*, 2011). The oxidation of NH_4^+ in the rhizosphere can, however, not only stimulate denitrification in adjacent anaerobic parts of the soil, but may theoretically also enable anaerobic NH_4^+ oxidation (anammox), both leading to higher N losses to the atmosphere (Kartal *et al.*, 2007; Zhu *et al.*, 2010). In the rhizosphere of *Carex* vegetation of the above-mentioned experiment, anammox bacteria were indeed observed (figure 6). In this way, wetland-adapted plant species are able to create local niches for specific rhizosphere microbial populations, affecting N cycling (Chapter 4). In addition, both N enrichment and rhizosphere oxidation by vascular plants are able to enhance respectively anaerobic and aerobic CH_4 oxidation (Bodelier & Laanbroek, 2004; Strous & Jetten, 2004; Raghoebarsing *et al.*, 2006). On the other hand, rhizosphere oxidation by ROL may also enhance CO_2 emission from root

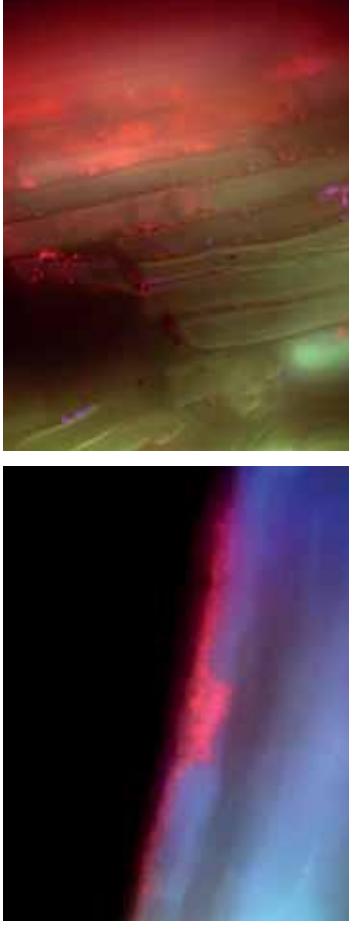


Figure 6. Fluorescence in situ hybridization (FISH) micrographs of a root sample from *Carex disticha* (collected from the rhizotron experiment, grown on anaerobic peat soil with $2 \text{ mmol L}^{-1} \text{NH}_4^+$), using probes Cren 537 & Cren 599 (Crenarchaea), AMX 820, BS 820, ScaMa 820 & DH1 820 (anammox) and Cy5: Eub mix (Dains *et al.*, 1999). Images show the abundance of microbial communities at the root surface (left; red colour) and the presence of anammox within these communities (right; purple colour) (J.M.H. van Diggelen & M.C. Schmid, unpublished results).

respiration, aerobic decomposition and mineralisation of root exudates (Crow & Wieder, 2005). These two examples show that belowground plant-soil interactions play an important role with respect to C and N cycling, and call for further research to explain the variation in C fluxes in different peatland types in relation to eutrophication.

Challenges for conservation and restoration management

As described in this thesis, gradients and fluctuations in water level, buffering capacity, nutrient availability and salinity in peatlands affected by anthropogenic forcing all have large effects on ecosystem functioning and biodiversity. The complexity of their interacting effects make it difficult to distinguish one main driver which can be manipulated as a management tool. For the restoration of peatlands, however, the key measure will be the restoration of wet conditions, which basically means to abandon traditional drainage-based water management. Unfortunately this is not easy, since there is a heritage of peatland systems that have extensively been drained for centuries. The severely altered conditions, including unfavourable hydrological setting, high nutrient availability and irreversibly changed soil characteristics, negatively affect biogeochemical processes that enhance eutrophication, even after rewetting (Chapter 6). Although high groundwater management may be considered unfavourable by some stakeholders, as current drainage-based water management is generally the fundament of customary agricultural land use, the socio-economic benefits in the longer term are high (Costanza *et al.*, 1997; Zedler & Kercher, 2005). Wetland ecosystem services such as flood protection, water purification and storage, especially in relation to higher extreme weather incidence and sea level rise, are all negatively affected by drainage-based water management, and urge the consideration of alternative management options (Joosten & Clarke, 2002).

Although there are opportunities to incorporate peatland restoration and management into sustainable environmental policy, defining realistic goals is often difficult. For example, should the main focus be on improving water quality, or on limiting land subsidence, or maybe on enhancing biodiversity? And in the case of restoration, is it wise to hold on to historic references, or is it better to explore current possibilities and chances (Chapter 5)? It seems impossible to meet all goals simultaneously, and conflicting interests of different stakeholder groups can lead to management dilemmas (Chapter 6). Therefore, it is recommended to assign different specific goals to different peatland areas, after which the management type can be applied which best suits the chosen goals. A good management strategy in (formerly) drained peatlands seems to be the separation of agricultural and (semi-)natural areas, as eutrophication-related issues often arise when combined. When both land use types are preferred within the same area, the construction of hydrological buffer zones between both areas should be considered. In the next section, different management scenarios will be discussed.

Management scenarios (Semi-)natural areas

Current nature management in (semi-)natural areas is often aiming at the fixation of particular successional stages, in order to maintain high biodiversity in the short term. The traditional mowing of floating peat mats (quaking mires) that formed after terrestrialisation of turf ponds led to the development of several very biodiverse vegetation types (Grootjans & van Diggelen, 1995; van Diggelen *et al.*, 1996; Lamers *et al.*, 2002b). Nowadays these different vegetation types are rapidly declining due to accelerated succession as a result of anthropogenic eutrophication and acidification, despite the management measures applied (Kooijman & Paulissen, 2006). Summer mowing was shown to enhance the development of late-successional stages (poor fens), which is considered less favourable as early successional stages (rich fens) have become rare and encompass a much higher biodiversity (Chapter 5). Burning after winter mowing was also found to be inadequate as it enhances eutrophication. The low effectiveness of current management highlights the need for novel, alternative measures.

The most important issue is the strongly altered hydrology of (semi-)natural fen areas, which led to a decline of intruding groundwater and surface water, and an increase of rainwater. In addition, accelerated succession strongly decreased peat inundation by surface water (Cusell *et al.*, 2013). Rewetting of (semi-)natural peatland areas may enhance buffering in the peat mat due to internal alkalinity generation under anaerobic conditions and increased intrusion of buffered water, strongly depending on weather conditions (Chapter 6; Lucassen *et al.*, 2005; Cusell *et al.*, 2013; Mettrop *et al.*, 2014). However, due to the often irreversibly altered hydrological setting, some peatlands may become infiltrating systems after rewetting, enhancing rain water dominance and therefore late-successional vegetation types in the long term. Therefore, those areas where intrusion of groundwater will still be present after rewetting are the most promising ones for restoration of peatland biodiversity. That is, as long as nutrient concentrations also remain sufficiently low after rewetting (Kooijman & Bakker, 1995). Regular flooding of fen areas by buffered surface water might be an alternative to prevent acidification and help to fixate different successional vegetation stages, at least in the short term (Bootsma *et al.*, 2002; Cusell *et al.*, 2013), but in many areas surface water quality is compromised and resulting nutrient loads are likely to lead to strong eutrophication and concomitant biodiversity loss of the vegetation.

Agricultural areas

For agricultural areas, one option is to maintain business as usual and deal with its consequences such as land subsidence and a high nutrient input. In S-rich coastal peatlands, alternating episodes of high SO_4^{2-} and P release will continue with drainage-based water management, leading to severe eutrophication in adjacent surface waters (Chapter 6). In S-poor systems, however, SO_4^{2-} -induced eutrophication is expected to be limited with a drainage-based water management, leaving sufficient Fe available for P-binding. Therefore, drainage can be expected to have a less negative effect on surface water quality in S-poor systems. In S-rich systems, the unfavourable Fe:P ratio (Chapter 2) may be balanced by the artificial addition of Fe into ditches or lakes, optimising water quality (Geurts *et al.*, 2011). The relative contribution of agricultural peat meadows to total nutrient loading of surface waters should be taken into account when evaluating the effects of drainage-based water management in these systems. If the reduction of this nutrient source is expected to only result in a minor improvement of the water quality, drainage-based water management could be maintained. However, this will still lead to undesired ongoing greenhouse gas emission and land subsidence, which might not only be undesired from an environmental point of view, but also become financially unbeneficial due to increasing drainage costs in the long term.

An alternative and much more sustainable agricultural use of peatlands is paludiculture, which involves the rewetting of drained peat soils and cultivation of wetland plants for commercial use (Joosten *et al.*, 2012). This will strongly inhibit aerobic peat decomposition, and the cultivated wet crops will take up a large part of the mobilised P after rewetting of formerly drained, agricultural peat meadows. This may even limit P discharge to adjacent surface waters (Chapter 6). However, whichever alternative for current practice is chosen, agricultural use will always be strongly affected after permanent rewetting. Therefore, this change in land use needs the support of landowners, which may be a delicate issue. To enhance rewetting of peatlands, compensating landowners when converting to paludiculture might have to be incorporated in policy if wet crop production is not economically feasible yet.

Many agriculturally used peatland areas are currently situated at lower levels in the landscape than (semi-)natural areas as a result of land subsidence, which may even promote the intrusion of groundwater in these areas unlike in the higher situated nature areas. As drainage costs are increasing, and agricultural use may become unprofitable, this will favour the transition of these lands towards (semi-)natural areas. However, the removal of the P-rich topsoil in formerly agricultural or degraded areas is essential in order to enhance biodiversity and lower decomposition rates in this process (Chapter 6; Ensens *et al.*, 2015; van de Riet *et al.*, 2013; Harpenslager *et al.*, subm.). Topsoil removal may enhance groundwater influence, soil buffering and development of early-successional minerotrophic vegetation. Although soil removal from an already subsiding system may appear undesirable, the remaining oligotrophic subsoil would provide more suitable conditions for peat regrowth than the actual nutrient-rich topsoil. The removed topsoil should subsequently be used in drained, subsiding peatlands that are still in agricultural use, promoting maintenance of traditional agriculture on these fields by increasing the soil surface. Moreover, it will allow the establishment of higher groundwater levels in restored peatlands without increasing flooding risks of the surrounding agricultural area.

General conclusion

The deterioration of peatlands, as a result of human impact such as altered hydrology (water shortage), eutrophication and acidification, is controlled by underlying biogeochemical processes in relation to the locally prevailing conditions. In my thesis, I pointed out that knowledge of the driving biogeochemical factors and processes is not only essential to understand ecosystem changes, but also to define effective management strategies for peatlands in the long term.





8 | References

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9 | Nederlandse samenvatting

Laagveen

Over de hele wereld komen 'wetlands' voor in zowel zout-, brak- en zoetwatergebieden. Ze worden gekenmerkt door permanent of tijdelijk natte omstandigheden als gevolg van stagnant oppervlaktewater, regenwater en/of grondwater. Door de natte, zuurstofloze (anaerobe) omstandigheden wordt de afbraak van plantenmateriaal geremd, waardoor er in de bodem ophoping van dit (gedeelte)lijk afgebroken organisch materiaal plaatsvindt en veenvorming optreedt. Wetlands met een organische bodem worden daarom veen- of moerasgebieden genoemd, die doorgaans onderverdeeld worden in ombrotroof hoogveen en minerotroof laagveen. Het verschil hier tussen wordt vooral bepaald door de mate van grondwater- versus regenwaterinvoer en de verschillen in vegetatie hierdoor. Laagvenen hebben zich gevormd in relatief gebufferd water dat is ontstaan door vermenging van regenwater met grondwater en/of oppervlaktewater. Er is sprake van relatief hoge concentraties aan opgeloste zouten en een relatief hoge pH waarde (neutrale zuurgraad). Hoogveen kent een veel zuurder milieu, omdat er vooral invloed van regenwater is en weinig zuurbuffering. In laagveensystemen zijn grasachtige planten, waterplanten en bruinmossen karakteristiek, terwijl hoogveen vooral uit veenmossoorten bestaat.

In veengebieden waar natuur nog de vrije hand heeft, zijn vaak overgangen van verschillende vegetatietypen te zien die worden beïnvloed door de dikte van het veenpakket en de samenstelling van het water. Een toename in de dikte van het veen gaat namelijk gepaard met een afname van grondwater-, en een toename van regenwaterinvoer. Hierdoor ontstaat er een biogeochemische gradiënt in de buffering van de bodem, die vervolgens beïnvloedt welke vegetatietypen hierop kunnen voorkomen. Onderbasenrijke (gebufferde) omstandigheden wordt de vorming van laagveenvegetaties gestimuleerd, en hoe groter de invloed van ongebufferd regenwater, hoe verder de ontwikkeling naar hoogveenvegetaties wordt gestimuleerd. Deze successie in vegetatie als gevolg van de toename van de dikte van het veenpakket is een natuurlijk proces, die zorgt voor een grote variatie aan habitats (leefgebieden) in veengebieden. Hierdoor is er vaak een grote diversiteit aan planten- en diersoorten te vinden in veengebieden, en behoren ze tot de meest productieve ecosystemen ter wereld. Wereldwijd bedekken veengebieden een relatief klein landoppervlak (4 miljoen km² verdeeld over 180 landen), maar hierin is wel 30% van de totale bodemkoolstof ter wereld vastgelegd. Bovendien bieden ze diverse natuurlijke functies en voordelen in o.a. nutriëntenkringloop, de regulatie van waterstroming, en als buffers bij overstromingen.

Menselijke invloed

Door de grote voordelen die wetlands te bieden hebben, zijn er ook relatief hoge dichtheden van mensen te vinden om en nabij veengebieden overal ter wereld. Omdat de menselijke bevolking nog steeds groeiende is, neemt de druk op veengebieden ook nog steeds toe. Grote delen van de veengebieden zijn wereldwijd in gebruik genomen ten behoeve van menselijke voorzieningen zoals brandstof (turfwinning) en voedsel (agrarisch gebruik). Wereldwijd is er al meer dan 50%, tot zelfs 80% in dichtbevolkte delen zoals Europa, Noord-Amerika en Oost-Azië, aan veengebied verloren gegaan of sterk achteruit gegaan. De historisch veenontginning, drainage en intensivering van agrarisch gebruik vormen een serieuze bedreiging voor de natuurlijke functies van veengebieden. Nederland heeft nog een van de (relatief) grootste bedekkingen laagveen in Europa, wat het belang van behoud en/of herstel van de overgebleven laagveengebieden benadrukt.



In Nederland is het laagveenlandschap het laagst gelegen, en komt vooral voor in het westelijke en noordelijke deel van Nederland. Door historische vervening, oftewel turfwinning, is er tegenwoordig in de polders van het laagveengebied nog steeds vaak een typisch patroon te zien van langgerekte rechthoekige petgaten (dit zijn relatief ondiepe, uitgegraven stukken veen) met hiertussen de legakkers waar het uitgegraven veen (turf) op te drogen werd gelegd. Om vroeger de vervening, en later het agrarisch gebruik van veenweiden mogelijk te maken was steeds verdere ontwatering (drainage) van het laagveengebied noodzakelijk, waardoor er uiteindelijk een heel netwerk van sloten, kanalen, boezemwateren en gemalen in en rondom de aangrenzende polders is ontstaan. Door windwerking en golfslag zijn er uiteindelijk smalle legakkers weggeslagen, waardoor ook vaak ondiepe grote meren zijn ontstaan. Binnen dit aquatische netwerk liggen tegenwoordig de terrestrische delen van het landschap, met hierop de veenweides die veelal in agrarisch gebruik zijn, en oude legakkers of veenweides met bloemrijke hooilanden, schraallanden en blauwgraslanden. Er zijn ook nog (semi-)terrestrische delen in de huidige laagveengebieden die een meer natuurlijke functie hebben. Voorbeelden hiervan zijn petgaten of luwe sloten waar nieuwe veenvorming plaatsvindt of heeft plaatsgevonden (verlanding), met een hierop verschillende stadia aan verlandingsvegetaties zoals trilveen, hoogveen, rietlanden en broekbossen.

De voortdurende drainage van de laagveengebieden, en ook van omringende polders, hebben echter geleid tot diverse problemen zoals veranderingen in de hydrologie (watertekort in de warme perioden en een sterke afname van grondwaterinvoer), eutrofiëring (een toename aan nutriënten door o.a. uitspoeling vanuit agrarisch land, inlaatwater en atmosferische depositie), en verzuring (als gevolg van watertekort of door een toename van regenwaterinvoer). Het eigenlijke doel van drainage is het verlagen van het waterpeil voor agrarisch gebruik van de veenweiden, maar dit heeft ook waterpeilfluctuaties en zuurstofinbrenging in de veenbodem tot gevolg. Zuurstof is de belangrijkste sleutel voor de aansturing van redox-gevoelige processen die betrokken zijn in de kringloop van o.a. koolstof (C), zwavel (S), ijzer (Fe), fosfor (P) en stikstof (N). Deze biogeochemische processen hebben een grote invloed op zowel de biodiversiteit als het functioneren van zowel de (semi-)natuurlijke als agrarische delen van het veengebied. Dit komt doordat ze leiden tot diverse problemen zoals eutrofiëring of verzuring, maar ook veenafraak en bodemdaling. Een aantal van deze door mensen geïnitieerde problemen en onderliggende biogeochemische processen worden in meer detail besproken in de verschillende hoofdstukken die in dit proefschrift beschreven staan. Bovendien wordt er aan de hand van deze kennis een doorkijk gemaakt naar mogelijkheden voor duurzaam beheer, om de overgebleven laagveengebieden te behouden of te herstellen in de toekomst.

H2: Eutrofiëring van oppervlaktewater door fosformobilisatie

Het is bekend dat eutrofiëring van oppervlaktewater flink kan worden gestimuleerd door de bijdrage van fosfor (P) mobilisatie uit de onderwaterbodem in veengebieden (nalevering van P; interne eutrofiëring). Vooral gebieden die rijk zijn aan zwavel (S) in de bodem, bijvoorbeeld waar invloed van zout of brak water is (of is geweest), speelt dit een grote rol omdat S nauw verbonden is met de kringloop van P en ijzer (Fe). De invloed van zout of brak water neemt overal ter wereld steeds verder toe door onttrekking van zoet grondwater, droogte en stijging van de zeespiegel. De interactie tussen veranderingen in saliniteit (zoutgehalte) van het oppervlaktewater en eutrofiëring zijn echter nog niet goed bekend. Daarom is er een aquariumexperiment uitgevoerd met eutrofe onderwaterbodems, die hoge P- en zeer lage Fe-concentraties bevatten, en erboven oppervlaktewater met 3 verschillende saliniteitniveaus. Er werd verwacht dat de hoeveelheid P die vrijkomt uit de onderwaterbodem zou worden gestuurd door diffusie, omdat de Fe-beschikbaarheid zo laag is. Dit bleek echter alleen op de korte termijn (0-10 weken) het geval, terwijl er verrassend

genoeg op de lange termijn (>80 weeks) geen P nalevering uit de onderwaterbodem meer bleek te zijn bij een normale of lage saliniteit. Dit komt zeer waarschijnlijk doordat de toplaag van de onderwaterbodem oxideert bij de overgang van het water naar het sediment op de lange termijn, waarbij Fe^{2+} wordt geoxideerd en hierbij efficiënt aan P bindt. Theoretisch zou de concentratie opgelost Fe^{2+} hoger moeten zijn om dit effect te bereiken. Daarom stellen we een hypothetisch mechanisme voor waarbij extra Fe^{2+} wordt vrijgemaakt uit de oxidatie van ijzersulfiden die in de zwavelrijke onderwaterbodems aanwezig zijn. Onder stabiele condities kan er op de lange termijn in de toplaag van de bodem voldoende zuurstof indringen die deze oxidatie mogelijk maakt.

Een toename van de saliniteit zorgde op de korte termijn voor een afname van de P-concentratie in het oppervlaktewater, wat zeer waarschijnlijk te wijten is aan de plotseling hogere calciumconcentraties in brak water. Op de lange termijn nam de P-nalevering echter toe onder brakke condities, ondanks de aanwezige zuurstofrijke toplaag van de bodem. Wanneer de saliniteit in het oppervlaktewater toeneemt, neemt ook sulfaat toe wat in de anaerobe onderwaterbodem wordt gereduceerd tot sulfide. Dit sulfide bindt vervolgens aan het vrijgekomen Fe^{2+} in de toplaag, waardoor er minder P wordt gebonden en dit alsnog naar het oppervlaktewater verdwijnt. Een hoge saliniteit doet hiermee het positieve effect van het zuurstof in de toplaag teniet. Dit experiment laat zien dat S een belangrijke rol speelt in het vrijkomen van P uit zwavelrijke onderwaterbodems, waarbij de mate van P mobilisatie afhankelijk is van de interactie tussen sulfaatreductie en de ijzer- en zuurstofbeschikbaarheid.

H3: Verschillende effecten van N-eutrofiëring op grasachtige soorten

Eutrofiëring van wetlands in de vorm van een toename van ammonium (NH_4^+) concentraties in de bodem leidt vaak tot een flinke toename van grasachtige soorten, maar de soortensamenstelling vertoont doorgaans grote verschillen. Ondanks het feit dat planten NH_4^+ makkelijk kunnen opnemen als een nutriënt, zijn diverse wetlandsoorten gevoelig voor hoge concentraties, of wordt NH_4^+ zelfs giftig bij een lage pH in de bodem. Door meer inzicht te krijgen in de verschillen tussen grasachtige soorten in hun respons op een toename in NH_4^+ beschikbaarheid, in relatie tot pH, kunnen veranderingen in vegetatiesamenstelling beter worden begrepen. Daarom hebben we twee wetlandsoorten, Veldrus (*Juncus acutiflorus*) en Tweerijige zegge (*Carex disticha*), vergeleken in een experiment waarbij ze konden groeien bij hoge (2 mmol L^{-1}) versus normale (20 $\mu\text{mol L}^{-1}$) NH_4^+ concentraties, bij pH 4 of pH 6. Het bleek dat de totale biomassa van beide grassoorten niet toenam bij hoge concentraties, maar ze vertoonden wel een duidelijke toename van verdeling van biomassa richting de spruit. Deze efficiënte C allocatie geeft een voordeel in de competitie om licht met andere soorten wanneer de N beschikbaarheid toeneemt in wetlands, wat kan leiden tot veranderingen in plantensamenstelling ten koste van de biodiversiteit.

Beide soorten lijken op de korte termijn goed bestand tegen hoge NH_4^+ concentraties, maar desondanks vertonen ze wel algemeen bekende ontgiftingsmechanismen zoals een afgenomen wortelspruit verhouding, indicaties voor kalium- en calciumlimitatie, en een toegenomen aanmaak van vrije, stikstofrijke aminozuren. Dit zijn vroeger aanwijzingen dat ze een minder hoge tolerantie hebben voor hoge NH_4^+ concentraties op de lange termijn, met name voor de zegge die duidelijk meer van deze ontgiftingsreacties vertoonde. Er kan op de lange termijn worden verwacht dat het aanpassend vermogen van deze soort afneemt bij aanhoudend hoge NH_4^+ concentraties. Meer dan 50% van het opgenomen N werd geïnvesteerd in de aanmaak van N-rijke aminozuren, terwijl dit slechts 10% was bij Veldrus. Deze verschillen in gevoeligheid kunnen mogelijk de veranderingen in dominantie tussen verschillende grasachtigen (grassen, zeggen, russen) verklaren wanneer de NH_4^+ beschikbaarheid toeneemt. In het algemeen wordt de sterke competitiekracht van grasachtigen

benadrukt door de efficiënte allocatie van biomassa, de goede P-opname en efficiënte N-ontgifting, in ieder geval op de korte termijn, die vaak leidt tot een afname van de wetlandbiodiversiteit bij een toename van NH_4^+ .

H4: Interacties tussen planten en micro-organismen in de kringloop van S, Fe en N

Verschillende biogeochemische processen die plaatsvinden in de bodem worden gereguleerd door microbiële processen, waarbij zuurstof een grote rol speelt. In anaerobe (zuurstofarme) bodems is de diffusie van zuurstof erg laag, maar dit kan worden gefaciliteerd door de aanwezige vegetatie die speciale aanpassingen hebben om in de natte, anaerobe bodems te overleven. De interacties in de rhizosfeer (de directe wortelomgeving) tussen microbiële gemeenschappen en wetlandvegetatie zijn zeer divers, maar hier focussen we op de biogeochemische omzettingen van stikstof (N), zwavel (S) en ijzer (Fe). Omdat deze processen op het grensgebied liggen van verschillende onderzoeksdisciplines, biedt het leuke en uitdagende mogelijkheden voor toekomstig onderzoek en samenwerking. Bovendien heeft de menselijke invloed wereldwijd de aanvoer van N en S drastisch verhoogd, terwijl die van Fe is verlaagd, waardoor deze gecombineerde aanpak van onderzoek extra belangrijk is geworden.

Alle drie de elementen zijn nutriënten voor planten, en hun mobiliteit en beschikbaarheid kan duidelijk worden beïnvloed door microbiële activiteit. Bovendien is, zoals in H2 al beschreven, de Fe-kringloop nauw verbonden aan die van S en P. Door veelvoorkomende waterstandsfluctuaties in wetlands zijn er snelle overgangen tussen het gebruik van zuurstof en alternatieve elektronenacceptoren (oxidatoren), wat ook schommelingen veroorzaakt in de dominante microbiële redoxreacties. Ook stimuleert de vegetatie zelf de beschikbaarheid van zuurstof door transport van zuurstof via de wortels naar de wetlandbodem, wat leidt tot microbiële oxidatie en vervolgens tot verzuring, terwijl microbiële redoxprocessen met alternatieve elektronenacceptoren juist tot buffering leiden. Hierdoor wordt de pH sterk bepaald door de dominante microbiële processen, wat de vegetatie samenstelling behoorlijk kan beïnvloeden. De drie elementen hebben ook met elkaar gemeen dat ze na reductie allemaal toxisch kunnen worden voor planten. Afhankelijk van de geologische en hydrologische omstandigheden veranderen deze microbiële omzettingen van de abiotische omstandigheden en voedselbeschikbaarheid voor planten. Dit bepaald in sterke mate de ontwikkeling en samenstelling van de vegetatie, doordat de competitie tussen soorten veranderd wordt. Aan de andere kant wordt ook de microbiële samenstelling sterk beïnvloed door de vegetatiesamenstelling, waardoor de interactie nog eens wordt benadrukt. Daarom is de gecombineerde kennis van microbiologie en plantenecologie essentieel om biogeochemische en biologische sleutelfactoren te zoeken die de heterogeniteit en totale samenstelling van gemeenschappen (micro-organismen en vegetatie) op zowel ruimtelijke als tijdsafhankelijke schaal bepaalt.

H5: Beheeropties voor het behoud van verzuurde trilveenvegetaties

In meer natuurlijke delen van het laagveengebied zijn het vooral trilveenvegetaties die bekend staan om hun hoge botanische diversiteit, inclusief veel bedreigde soorten. Deze trilveenvegetaties gaan echter hard achteruit in Nederland door de sterke afname aan grondwaterinvoer en het gebrek aan nieuwe verlanding. Diverse beheersmaatregelen, inclusief maaien en branden, worden al tientallen jaren toegepast met als doel het trilveenstadium langer te behouden en de natuurlijke successie richting veenmosrietland of broekbos te vertragen. De lange-termijneffecten van deze traditionele maatregelen zijn beoordeeld in dit onderzoek, door vegetatiegegevens uit 2013 te vergelijken met gegevens uit 1967. Bovendien is de effectiviteit van de relatief nieuwe maatregel bealking experimenteel getest om trilveenvegetaties te herstellen in verzuurde systemen. Dit

veldexperiment is gedurende 7,5 jaar gevolgd, waarbij verschillende hoeveelheden kalk (0, 1000, 2000, and 4000 kg Dolokal/ha) werden uitgestrooid op stukken bodem van 3x3m.

Zomermaaien bleek te leiden tot meer zure en nutriënt-arme omstandigheden, wat werd aangetoond door een verschuiving van trilveen- naar veenmosrietlandvegetaties, inclusief een goed ontwikkelde mosbedekking gedomineerd door veenmossoorten met een aantal rode-lijstsoorten. Branden (na wintermaaien) leek verzuring goed tegen te gaan, maar bleek wel tot een ongewenste toegenomen nutriëntenbeschikbaarheid te leiden. Dit werd geïndiceerd door dominantie van hogere planten die karakteristiek zijn voor productieve graslanden, en een schaarse mosbedekking met algemene soorten. Hieruit kan worden geconcludeerd dat deze traditionele maatregelen op de lange termijn niet voldoende in staat zijn om de trilveenvegetaties in stand te houden. Omdat de hydrologische omstandigheden vaak onomkeerbaar veranderd zijn en hierdoor de grondwaterinvoer niet te herstellen was, zou bekalking op korte termijn een alternatief kunnen zijn om verzuring tegen te gaan en trilveenvegetaties te bevorderen. Deze maatregel bleek echter niet duurzaam op de lange termijn, omdat de hervestiging van veenmossoorten de ontwikkeling van trilveenvegetaties erg bemoeilijkt.

H6: Beheeropties voor het duurzaam gebruik van gedraineerde veenweiden

Wereldwijd worden veengebieden al zeer lang gedraineerd om agrarisch gebruik te bevorderen, wat op grote schaal heeft geleid tot een drastische achteruitgang van de natuur- en waterkwaliteit in deze gebieden. Aerobe (zuurstofrijke) veenafbraak wordt hierdoor sterk gestimuleerd, wat hoge kooldioxide (CO_2) emissie en flinke bodemdaling tot gevolg heeft. Dit op drainage gebaseerde waterbeheer heeft bovendien invloed op andere biogeochemische processen die de bodem- en waterkwaliteit beïnvloeden. In kustgebieden zijn sulfaatconcentraties in het grondwater over het algemeen hoog door de oxidatie van de grote hoeveelheid aanwezig anorganisch S in de onverzadigde (niet constant natte) bovenlaag van de veenbodem. Hierdoor vormen gedraineerde veengebieden aan de kust vaak een ongekend grote bron van sulfaat voor aangrenzende oppervlaktewateren. Dit sulfaat kan in het oppervlaktewater vervolgens weer indirect leiden tot eutrofiëring, zoals in H2 uitgelegd. Om de nadelige effecten van veenoxidatie tegen te gaan, worden voornamelijk gedraineerde agrarische veenbodems weer vernet door permanent hoge grondwaterpeilen in te voeren. Dit leidt echter vaak tot een toename van methaan (CH_4) fluxen, wat mogelijk zelfs de broeikasgasuitstoot van het voormalige CO_2 onder gedraineerde omstandigheden evenaart. Bovendien leidt vernatting van voormalige agrarische bodems tot een (tijdelijke) toename van P-mobilisatie in het grondwater van de veenweide, wat vervolgens kan uitspoelen naar omliggende sloten en daar tot directe eutrofiëring van het oppervlaktewater leidt. Tijdens het proces van P-mobilisatie na vernatting komt er echter ook gereduceerd ijzer (Fe) vrij, dat vervolgens mee uitspoelt naar het oppervlaktewater. We verwachten dat dit co-transport van Fe en P in het oppervlaktewater de mate van eutrofiëring beperkt, afhankelijk van de hoeveelheid Fe die mee uitspoelt naar de sloot.

Het feit dat zowel drainage als vernatting leiden tot diverse problemen met betrekking tot eutrofiëring en uitstoot van broeikasgas, zorgt voor een serieus dilemma bij waterbeheerders die te maken hebben met (voornamelijk) gedraineerde, zwavelrijke veengebieden. Tegelijkertijd is er een steeds verder toenemende behoefte aan duurzaam gebruik en een herziening van het beleid van dalende veengebieden in kustregio's. Omdat waterbeheer direct is gerelateerd aan landgebruik, zou de afweging voor een nieuw beleid niet alleen gebaseerd moeten worden op de huidige geohydrologische ligging van de gebieden of het voormalig landgebruik, maar ook op de potentiële diensten die het ecosysteem te bieden heeft als gevolg van verschillende beheeropties. Voortzetting van drainage-gebaseerd waterbeheer heeft als voordeel dat het traditionele landgebruik mogelijk blijft, maar het leidt ook tot

verdere bodemdaling en flinke sulfaatmobilisatie. Daartegenover staan veelbelovende mogelijkheden voor het gebruik van vernatte veengebieden, zoals natte landbouw (paludicultuur) of het herstellen van natuurlijke wetlands, en verwachten we dat een permanent hoge grondwaterstand op de lange termijn zal leiden tot een netto vastlegging van koolstof en minder uitspoeling van P. Daarom denken we dat permanente vernatting de meest duurzame waterbeheerstrategie is in (voormalig) gedraineerde zwavelrijke veengebieden.

Tot slot

Zoals beschreven in dit proefschrift hebben veranderingen in de hydrologie, nutriëntenbeschikbaarheid, buffering en saliniteit, als gevolg van menselijke invloed, grote effecten op het functioneren en de biodiversiteit van veengebieden. Al deze factoren worden gestuurd door onderliggende biogeochemische processen die samenhangen met de lokale omstandigheden. De complexiteit van de onderliggende interacties maakt het moeilijk om slechts een draaiknop aan te wijzen om veengebieden te herstellen. Er is echter een essentiële actie om mee te starten, en dat is het herstel van natte condities. Ik heb in mijn proefschrift niet alleen laten zien dat kennis over sturende biogeochemische factoren en processen inzicht geeft om veranderingen in het ecosysteem beter te begrijpen, maar ook dat deze kennis nodig is om goede en effectieve beheerstrategieën te bepalen om veengebieden te herstellen en behouden op de lange termijn.



10 | Curriculum vitae



My name is Josepha Maria Hendrika (José) van Diggelen, and I was born on May 15th, 1982 in Reek, The Netherlands. I lived and grew up in Reek, where I also went to primary school.

After I had completed my secondary education (Merlet College, Grave) in 2000, I finished my propaedeutics-exam for Higher Laboratory Education (HLO) in 2001, Nijmegen. Hereafter, I started the first 2 years of my Biology studies at Utrecht University, and completed my doctoral exam at the Radboud University Nijmegen in 2007. During my first internship I experimentally studied the effects of salinisation in peatland area Iiperveld, The Netherlands, at the department of Aquatic Ecology and Environmental Biology (Radboud University Nijmegen). My second internship was completed overseas, at the School of Plant Biology (University of Western Australia), where I studied specialised adaptations of endemic plant species that grow on extremely dry granite rocks. After my examination in 2007 I directly started working at B-WARE Research Centre in Nijmegen. I am working as a consultant, and mainly focus on applied ecological and biogeochemical research in peatland areas throughout the Netherlands. Several projects and research programmes that I was involved in during the last 8 years have contributed to the different papers and reports listed below.

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11 | Dankwoord

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het motiverend als er soms wordt geïnformeerd naar je status of dat er even een hart onder de riem wordt gestoken, en hiervoor wil ik iedereen van mijn collega's bij B-WARE en van de Ecologie-groep voor zover ik die nog niet bij naam had genoemd allemaal bijzonder voor bedanken! Dit geldt ook buiten de werkmuren, want alle lieve vrienden, familie en kennissen binnen en buiten de Reek zijn mij ontzettend waardevol en tot steun geweest in deze periode. De gezellige (al dan niet 'biologen') klets, lol, drankjes en muziek op z'n tijd, maar ook de serieuzere gesprekken hoop ik met jullie allemaal nog lang voort te kunnen zetten! In het bijzonder wil ik hier 'ons' pap en mam toch even noemen, en ook mijn dames Geertje en Jodi. Fijn dat jullie onvoorwaardelijk in mijn leven zijn.

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Onderzoekcentrum B-WARE

Onderzoekcentrum B-WARE B.V. is een spin-off bedrijf van de afdeling Aquatische Ecologie & Milieubiologie, welke deel uitmaakt van het onderzoeksinstituut IWWR (Institute for Water and Wetland Research) van de Radboud Universiteit Nijmegen. B-WARE werd in november 2002 opgericht en combineert de expertise van zuiver wetenschappelijk en toegepast universitair onderzoek. Kennisontwikkeling en -uitwisseling tussen de Universiteit en B-WARE is het uitgangspunt en staat borg voor hoogwaardig toegepast onderzoek, noodzakelijk voor het oplossen van praktijkvragen van opdrachtgevers.

Specialisatie

De werkwijze bestaat uit de combinatie van analyses in de veldsituatie en het opzetten en uitvoeren van veldexperimenten en experimenten onder gecontroleerde laboratoriumcondities, naast deskresearch. De verkregen resultaten dragen direct bij aan het ontwikkelen van nieuwe inzichten binnen het natuur- en waterbeheer en worden bijvoorbeeld toegepast bij natuurherstel en –ontwikkeling, en waterberging. Deze werkwijze maakt het mogelijk dat de adviezen bij uitvoering in de praktijk een grote kans van slagen hebben. Er wordt onderzoek verricht aan sturende processen in onder meer hoogvenen, laagvenen, meren, plassen, vennen, beekdalen, heiden, soortenrijke graslanden, polders en broekbossen.

Voor wie?

Onderzoekcentrum B-WARE verricht onderzoek voor ministeries, provincies, provinciale landschappen, gemeenten, particuliere terreinbeheerders en natuur- beschermingsorganisaties, waterschappen en adviesbureaus.

