

Floodplain rehabilitation and the importance of nitrogen dynamics for plant communities

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*Cover: 'Riviergezicht aan den Rijn bij Rhenen' (View on river Rhine near Rhenen) - B. Bueninck 1919 - Wolters, Groningen. Antique poster used in geography lessons in primary and secondary school, showing the river Rhine, already highly restrained at the start of the 20<sup>th</sup> century.*

# Floodplain rehabilitation and the importance of nitrogen dynamics for plant communities

Ecologisch herstel van uiterwaarden en de invloed van de stikstofhuishouding op plantengemeenschappen

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van rector magnificus, prof. dr. W.H. Gispen, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 24 januari 2007 des ochtends te 10.30 uur

door

Aarnout Martijn Antheunisse

geboren op 21 februari 1978 te 's-Heer Abtskerke

Promotoren: Prof. dr. J.T.A. Verhoeven  
Prof. dr. H.J. Laanbroek

De zon, het zand, de hitte en de rustige rivier  
De stilte en de droogte, en de leegte van dit hier  
De hemel en de aarde, de weidsheid van het land  
En de wijsheid van één man, dat is genoeg

Mooie dag - Bløf / Blauwe Ruis (Peter Slager)



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*Low discharge during August 2003 in the river Bug - Eastern Poland. Floodplains of this river have been sampled during the field survey, described in Chapter 2 (photo: Leon Lamers).*

# Chapter 1

## General introduction

### Introduction

This thesis comprises a set of studies on the effects of certain measures used in floodplain rehabilitation on herbaceous plant communities and their development, with special reference to soil nitrogen state and biogeochemical processes. The research focussed on restoration measures in non-tidal river floodplains, e.g. enhanced flooding by summer dike removal and also on measures in the tidal, estuarine reaches of temperate river systems, e.g. restoration of tidal range gradients. Non-tidal and tidal wetlands in river systems are known to be rather different in several controlling environmental factors; patterns in hydrology are opposite, with extremely regular and predictable flooding events in tidal floodplains (Verhoeven et al. 2001), but highly infrequent and irregular inundations in non-tidal river floodplains (Christensen & Christensen 2003). In addition, the incursion of seawater is, by definition, limited to the estuarine reaches, resulting in a gradient of brackish soil moisture conditions with effects on soil biogeochemistry (e.g. Rysgaard et al. 1999; Fear et al. 2005; Coci et al. 2005), soil physics (e.g. Bryan & Langston 1992; Paludan & Morris 1999) and biota (e.g. Hootsmans & Wiegman 1998; Lillebø et al. 2003; Deegan et al. 2005). On the other hand, the key biogeochemical processes, i.e. carbon and nitrogen conversion processes, are essentially the same in non-tidal and tidal floodplains, although other organisms might be responsible for the same process (Freitag et al. 2006). Furthermore, these two river compartments are strongly connected (one-way), with respect to nutrients, pollutants and biota (Vannote et al. 1980; Lorenz et al. 1997). In the synthesis, I discuss the findings of the separate studies and provide a broader framework with respect to these results, incorporating the consequences of my conclusions for successful ecological rehabilitation of non-tidal as well as tidal, estuarine floodplain systems.

## Temperate European floodplains in the last century

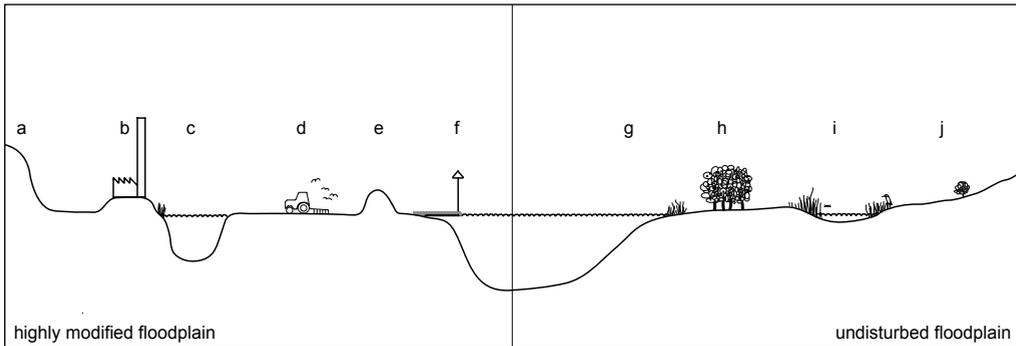
### *Characteristic values and functions of temperate river-floodplain systems*

Floodplains along the entire river continuum are characterised by high economical (Costanza et al. 1997) as well as ecological values (Nienhuis 2006; Smits et al. 2006). They provide various functions for society such as food production (agriculture), safeguarding the hinterland for flooding, water purification and nutrient retention (Tappin 2002; Neubauer et al. 2005). Furthermore, natural floodplains harbour highly diverse ecosystems with respect to plant communities and associated faunal assemblages. A large biodiversity can be maintained in these systems partly because of the presence of broad hydrologic gradients (Naiman et al. 1993; Moulton 1999; De Nooij et al. 2006). In addition, estuarine floodplain systems provide nursing grounds for numerous fish species (Zwolsman 1994) and foraging areas for large numbers of bird species (Ysebaert et al. 2000).

Natural floodplains include some of the most productive habitats in the world (Spink et al. 1998). Explanations for the high nutrient levels and corresponding high production have been discussed in the Flood Pulse concept (Junk et al. 1989). This widely accepted concept describes the effect of floods on river forelands in an unmodified river system (Tockner et al. 2000). In regulated rivers, however, the relation between river channel and floodplain has been strongly disturbed because of the fixation of the river bed and the creation of dikes and levees which reduce flooding frequency and duration (Lorenz et al. 1997). The economical importance of river channels and accompanying forelands for navigation, agricultural land-use, generation of hydro-energy and extraction of sand and gravel has resulted in heavily degraded river systems (Dynesius & Nilsson 1994; Lorenz et al. 1997; Nienhuis & Leuven 2001; Pedroli et al. 2002; Hohensinner et al. 2004). As a consequence, natural floodplain ecosystems are nowadays among the most threatened systems in the world (Tockner & Stanford 2002).

### *Degradation of ecological values of river floodplains*

River regulation and agricultural use of floodplains in Western-Europe have had a large impact on their biodiversity and other ecological values (Hohensinner et al. 2004). Hydrodynamic gradients have disappeared due to the construction of dikes and other flood defence measures (Figure 1). Rivers have been heavily loaded with nutrients and pollutants, such as heavy metals and organic compounds (Kooistra et al. 2004), and their adjacent floodplains covered by polluted, nutrient-rich sediments for decades. In addition, agricultural use of floodplain habitats has become very intensive locally with high fertiliser and manure applications (Lamers et al. 2006). The combination of these anthropogenic impacts has caused a decline in natural, species-rich floodplain plant communities (Pedroli et al. 2002). Threats and degradation of lowland river systems and their floodplains in the past centuries have not been restricted to the non-tidal stretches. Also the tidal, estuarine parts of river systems have been altered severely. In the Netherlands, parts of the Rhine-Meuse estuary were closed-off for enhanced flood defence after the major coastal flooding disaster of 1953 (Nienhuis et al. 1994; Smit et al. 1997; Tönis et al. 2002), with detrimental consequences for the ecological integrity of the originally tidal rivers: former shallow gradients with broad vegetation zones were reduced to steep slopes as erosion became dominant over sedimentation due to one-way water transport in the river systems (Smit et al. 1997). In addition to these physical alterations, eutrophication and pollution of tidal river systems was at least as intense in the tidal river systems (Struyf et al. 2004; Boesch 2004), which act as a sink for sediment and particulate pollutants transported by the rivers.



**Figure 1.** Cross-section of a highly modified (left) and an undisturbed floodplain-river system (right). Characteristics of a modified system: (a) winter dike protecting the fossil floodplain from flooding, (b) brickworks - high ground obstacle, (c) sand- or clay pit - deep water with sharp gradients, (d) intensive agricultural use, (e) summer dike or levee protecting the active floodplain from small flooding events and (f) groyne structures fixing the river channel for navigation. Characteristics of an undisturbed system: (g) active meandering summer bed with shallow gradients, (h) well-developed floodplain forest, (i) shallow side channel or oxbow lake with valuable riparian ecotones, (j) heterogeneous species-rich grasslands with only minor agricultural land use (grazing, hay-making).

#### *Land-use change and climatic developments affecting river discharge*

The number and size of peak discharges of the rivers Rhine and Meuse have increased in recent decades and is expected to rise even further (Middelkoop et al. 2001; Shabalova et al. 2003). The changing land-use in the river basins, related to urbanisation and impermeabilisation, drainage of agricultural lands, straightening of rivers and reducing the size of active floodplains by the construction of dams and levees is a major cause of increasing discharge quantities (Pfister et al. 2004). In addition to the effects of land-use change on hydrology and discharge quantities, changing climate and weather patterns are believed to enhance extreme flood events. Modelling studies forecast an effect of global climate change on the volume of maximum discharge and consequently on river flooding frequency. In Europe, it is expected that the frequency of summer inundations will increase in the coming century, through enhanced summer precipitation (Christensen & Christensen 2003; Kundzewicz et al. 2005). Furthermore, peak discharges during the main flooding period - late winter/early spring for these rivers - will be larger, resulting in a higher chance on catastrophic flood events. Recent extreme flood events along the Rhine and Meuse in 1993 and 1995, the Oder in 1997 and the Elbe in 2002 (Bischoff & Wolter 2001; Nienhuis & Leuven 2001; Oetken et al. 2005) might be an omen for these changing discharge patterns.

#### *Space for Rivers: enhanced flood protection and nature development*

Following the extreme discharge events in the winters of 1993 and 1995, the Dutch government launched the 'Space for Rivers' programme (*Ruimte voor de Rivier*), which aims at allowing the rivers to take more physical space to avoid emergencies during extremely high discharges (VROM & VWS 1996; Buijse et al. 2002). The strategy of only reinforcing and raising dikes was abandoned, as this was expected to be insufficient to protect the hinterland - the fossil floodplains - from flooding threats (Van Stokkom et al. 2005; Witter et al. 2006). Measures taken within the 'Space for Rivers' framework are aimed at a partial restoration of the connectivity between the main channel and its floodplains. Additional physical space is created by lowering the floodplains, digging artificial side channels and complete replacement of winter dikes (Buijse et al. 2002).

### *Floodplain rehabilitation efforts*

In recent decades rehabilitation efforts have been undertaken to re-instate at least part of the pre-degradation ecological values of lowland rivers and their floodplains. Since 1970, due to new legislation, the nutrient and pollution load of the river water and sediment of the largest western European rivers has decreased (Wolff 1978; Cals et al. 1998). Nevertheless, for the Dutch situation, concentrations are still much higher compared to background values, in particular nitrate and sulphate concentrations (Chapter 2; Lamers et al. 2006), notwithstanding the fact that EU and national legislation on fertiliser use have reduced the input of nutrients as diffuse pollution sources (Goodchild 1998).

Since 1995, many rehabilitation projects have been proposed or carried out aiming at the restoration of more natural hydrological conditions by increasing the connectivity between the river channel and the floodplains (Cals et al. 1998; Schropp & Bakker 1998; Simons et al. 2001; Baptist et al. 2004). In the Netherlands, the majority of these rehabilitation efforts is coupled to flood defence measures within the 'Space for Rivers' programme. In addition to a better flood protection, these measures create opportunities for ecological river restoration and nature development in floodplains, as areas within this programme are in general not suitable anymore for agricultural activities due to the enhanced connectivity with the river (Nienhuis & Leuven 2001; Hooijer et al. 2004; Pfister et al. 2004).

At present, many programmes are being initiated to recover the lost ecological values of estuaries and tidal river systems. These rehabilitation efforts concern the reintroduction of either tidal movement or seawater incursion or a combination of both (e.g. Chapter 5; Smit et al. 1997; Tanner et al. 2002). However, restoration in these formerly tidal rivers is less aiming at reducing levels of nutrients and pollutants as gradients of tidal range and salinity are thought control estuarine systems, irrespective of enhanced nutrient concentrations in water and sediment (Den Hartog 1974; Lamers et al. 2006).

## **Vegetation development in rehabilitated river floodplains**

### *Species-rich herbaceous plant communities as target vegetation*

Ecological rehabilitation of lowland floodplains mostly aims at restoring, at least partially, the natural geomorphological and hydrological processes in the river-floodplain system (De Nooij et al. 2006). Furthermore, establishment and development of diverse vegetation types are main goals of floodplain restoration projects (Clevering & Van Gulik 1997; Stromberg 2001; Bischoff 2002; Rohde et al. 2005). Although target vegetation types for floodplain restoration generally are not restricted to herbaceous species, I specifically focussed on grasslands and other herbaceous communities during my research.

This choice was based on the fact that species-rich floodplain grasslands have existed for centuries along the Western European rivers and are highly valuable in terms of biodiversity. Furthermore, the development of floodplain forests is not always desirable. Flood defence legislation does not allow the river floodplains to be covered in woods, as they obstruct water transport and may enhance rising water levels (Nienhuis & Leuven 2001). Herbaceous-vegetation targets for the lower, more frequently inundated parts of the floodplain are sedge marshes (*Caricetum gracilis*). In drier parts of the floodplain, flooded only 1 to 2 times a year, species-rich wet grasslands and hayfields are among the restoration goals (*Ranunculo-Alopecuretum geniculati*, *Fritillario-Alopecuretum pratensis*, *Sanguisorbo-Silactum* and *Lolio-Cynosuretum*) (Schaminée et al. 1996; Bal et al. 2001). Management, either by mowing or grazing by cattle, sheep or horses is often considered necessary for those vegetation systems, to prevent further succession towards softwood and hardwood floodplain forest (*Salicion*

albae and Fraxino-Ulmetum respectively). Under certain conditions, cyclic rejuvenation of the floodplain forests is an appropriate tool to keep the ecological values of the floodplain as high as possible within flood legislation restrictions (Baptist et al. 2004). In almost continuously inundated parts of the floodplain, valuable pioneer vegetation can develop during drawdown at low discharge, predominantly in the summer months (*Eleocharito acicularis*-*Limoselletum* and *Rumicetum maritimi*).

Typical plant communities characteristic for tidal floodplains comprise helophyte stands forming a zonation along a flooding gradient, with broad zones of *Phragmites australis* (Cav.) Steud. and *Bolboschoenus (Scirpus) maritimus* (L.) Palla (Coops et al. 1999). In addition, numerous species are adapted to a specific salt concentrations or large fluctuations in salinity and they occupy their own niche along the salinity gradient, with for instance communities dominated by *Spartina anglica* C.E. Hubb and *Puccinellia maritima* (Huds.) Parl. dominated at high salinities (Wetzel et al. 2004).

However, in many cases, rehabilitation measures have not led to the development of the targeted species-rich vegetation types, but instead plant communities with of a small number of common species tend to dominate rehabilitated floodplains (Chapter 2; Lamers et al. 2006).

#### *Possible constraints for vegetation development related to rehabilitation*

Several effects of floodplain rehabilitation have been reported which are potentially negative for performance and establishment of herbaceous plant species and hence for the overall community structure. In these cases, certain new physical, chemical or even biological conditions have been created through ecological restoration measures which were either not within tolerance of the desired group of plant species, or failed to provide conditions for dispersal and successful establishment of such species. Rehabilitation of floodplains by restoring the hydrological connectivity with the main river channel has large effects on flood regimes, i.e. the timing and duration of inundation. The locally occurring species, including the potential of the seed bank are likely not adapted to these altered flooding regimes, particularly when they include floods of long duration and floods in the summer period. Although the rehabilitation measures restore a more natural flood regime, it is possible that the historic situation of more natural flooding regimes had been altered too long ago, often more than a century, for characteristic species to survive (Van Eck et al. 2004; Leyer 2005). Hence, part of the target species typical for more natural floodplain conditions will have to re-establish themselves in order to make the ecological rehabilitation successful. The surface area of populations in which target species once occurred, has diminished drastically in the last century (Burkart 2001). This has strongly reduced the chance that seeds and other diaspores be transported to the newly rehabilitated floodplains (Bissels et al. 2004).

In addition to hydrological constraints, possible chemical bottlenecks can be identified as well, for instance the presence or accumulation of toxic substances in the soil. In most rehabilitation projects, recent sediments are not removed or covered by fresh topsoils. Decades of deposition of polluted sediments (Cals et al. 1998), for instance enriched with heavy metals through mining and other industrial activities, can have a serious impact on the performance of species (Oetken et al. 2005). Accumulation of other potentially toxic substances is also a realistic danger, as sulphate and ammonium concentrations were and are still high in western European rivers (Chapter 2). Sulphate will be reduced under anoxic conditions to sulphide, which is known to be a very severe plant toxin (Lamers et al. 1998; Lamers et al. 2001). Ammonium can also accumulate up to concentrations toxic for plant species under reduced soil conditions (Britto & Kronzucker 2002). In addition to toxic substances, sediments have been also highly enriched with nutrients, either by diffuse pollution or local agricultural practices (Admiraal et al. 1993; Van Oorschot 1994; Lamers et al. 2006).

## Nitrogen cycling in floodplain soils

### *Enhanced nutrient load as possible constraint for successful ecological development*

The long history of enrichment of sediments with nutrients is one of the major differences between more natural and impacted West European river systems (Lamers et al. 2006). Decades of accumulation caused by the deposition of enriched sediments are in most cases not tackled in floodplain rehabilitation projects. Therefore the focal point of this study was to determine whether plant growth in herbaceous floodplain communities was controlled by the availability of specific nutrients in the soil, and whether this was different for impacted and more natural river systems. Nitrogen, phosphorus and potassium are regarded as the most important nutrients controlling the growth of terrestrial plants (Güsewell 2004). Among these nutrients, nitrogen is most frequently limiting plant productivity in terrestrial ecosystems (Gorham 1991). It is susceptible to a series of microbe-mediated biogeochemical processes, which strongly affect the availability of nitrogen for plant growth, especially in floodplain soils subjected to alternating flooding and re-drying (Bowden 1986; Phillips 1999). Therefore, nitrogen received the most attention in the experiments and field studies presented in this thesis. Nevertheless, other potential constraints also receive attention as it is very unlikely that one specific constraint is always the true bottleneck.

### *Nitrogen biogeochemistry in floodplains: importance of hydrology*

Flooding, especially in periods with high temperatures, induces major changes in soil nitrogen biogeochemistry (McClain et al. 1998). In well-oxygenated soils, mineral nitrogen is mostly present in the form of nitrate, due to aerobic mineralisation and nitrification processes and the absence of denitrification (Bridgman et al. 1998; Olde Venterink et al. 2002). When soils become flooded, nitrate is rapidly denitrified or reduced to ammonium (Patrick Jr. & Reddy 1976; Baldwin & Mitchell 2000; Revsbech et al. 2005). Nitrification is inhibited by the lack of oxygen provoked by flooding, promoting ammonium accumulation (Haynes & Swift 1989; Phillips 1999). After the end of the inundation, when oxygen enters the soil, ammonium is nitrified and nitrate concentrations can reach high levels (Baldwin & Mitchell 2000). However, the largest fraction of soil nitrogen is present in organic form and has to be mineralised, either under oxic or anoxic conditions before it becomes available for plant uptake (Zak & Grigal 1991; Gale et al. 1992).

### *Effects of floodplain degradation and rehabilitation on nitrogen load and biogeochemistry*

I already mentioned that nitrogen loading is generally high in degraded floodplain systems, due to the history of many years of deposition of highly enriched sediments, diffuse pollution of river water or local agricultural practices (Lamers et al. 2006). On the other hand, degraded floodplains are often part of highly regulated river systems in which the hydrologic connection between the main river channel and the floodplain is restricted, including the lateral transport of sediment and nutrients (Chapter 2; Heiler et al. 1995; Tockner et al. 2000). In rehabilitated floodplains in which the connectivity with the main river is increased, flooding frequency and duration will be longer, resulting in higher deposition rates of nutrient-loaded sediment particles. However, I expect that the agricultural use and the history of enhanced nitrogen load of sediment and river is much more important in determining the total and available nitrogen concentration in the floodplain soil than the altered connectivity. Furthermore, frequent fluctuation of water levels in floodplains with a restored hydrological connection to the river channel will cause alternating oxic and anoxic conditions in the soil, promoting removal of nitrogen (Tanner et al. 1999).

*Importance of nitrogen for terrestrial floodplain vegetation*

In general, terrestrial floodplain vegetation is mostly thought to be limited in growth by the availability of nitrogen (Verhoeven et al. 1996; Van Oorschot et al. 1997; Ogden et al. 2002), although limitation by P, co-limitation or absence of limitation have been reported as well (Wassen et al. 1998). Therefore, changes in the availability of nitrogen for plant uptake could affect plant performance severely, thereby altering the community composition (Grime 1997). Considering that degradation and restoration of river floodplains is expected to have an effect on the availability of nitrogen, it can also be expected that plant growth and community structure will be heavily affected by these activities.

**Objectives of this study**

The main objective of this research is to find out in what way floodplain rehabilitation has affected vegetation development and establishment and test the existence of biogeochemical constraints for the development of species-rich herbaceous target vegetation types in rehabilitated floodplains. To reach the main objective, the following research questions were addressed:

1. Is there a difference in the limiting factor (i.e. nutrient availability) for plant growth in impacted, partially rehabilitated floodplains systems, compared to more natural floodplains in geographical reference river systems, in non-tidal as well as tidal reaches of the rivers?
2. Floodplain rehabilitation may incorporate enhancing the hydrologic connectivity between the river channel and its floodplains, resulting in a higher probability of summer inundation of the floodplains as a consequence. How is summer flooding affecting nutrient availability for plant growth and how does this relate to performance of vegetation in impacted, but restored floodplain systems, compared to vegetation in a more pristine floodplain with a continuous natural hydrology?
3. At present, rehabilitation of closed-off estuaries and former tidal rivers is aimed at restoring tidal movement and salinity gradients. In what way does rehabilitation affect biogeochemical processes in the floodplain soils and grassland vegetation performance in areas with a different history of estuarine influence and land use?
4. What is the importance of restoration of a tidal range versus restoration of salinity gradients for the development of target vegetation types in tidal, estuarine parts of river systems?
5. Knowing the constraints for successful ecological restoration of degraded floodplains, what additional measures are necessary to overcome these problems and reach the restoration targets with respect to vegetation development?

**Thesis outline**

The research presented in this thesis was carried out within the TRIAS programme (TRIpartite Approach to Soil systems processes) funded by the Netherlands Organisation of Scientific Research (NWO). It is part of a larger project, completed in close cooperation with Roos Loeb (Radboud University Nijmegen) and Marzia Miletto (NIOO-KNAW) concerning biogeochemical constraints for development of species-rich vegetation in floodplain rehabilitation project (project nr. 835.80.010). The data in this thesis arose both from comparative field surveys (Chapter 2 and 4) and experimental ecological mesocosm

experiments (Chapter 3 and 5) (Odum 1984). Partly, the studies focussed on non-tidal, temperate river-floodplain systems and their specific issues concerning rehabilitation (Chapter 2 and 3), while other studies dealt with possible rehabilitation constraints for development of characteristic vegetation in tidal, estuarine reaches of Western European rivers (Chapter 4 and 5).

In Chapter 2, data are presented of a comparative field survey, carried out in non-tidal floodplains of the rivers Rhine, Meuse and Overijsselse Vecht (the Netherlands) and the rivers Odra, Bug and Narew (Poland). A correlation analysis of soil nutrient status, plant productivity and plant nutrient parameters was carried out using multivariate statistics to assess whether more natural and impacted floodplains show different relationships between plant growth and nutrient availability. (research question 1)

Chapter 3 describes an indoor mesocosm experiment in which soil monoliths with accompanying vegetation were subjected to a summer inundation. This type of inundation is supposed to become more frequent in the future, due to floodplain rehabilitation. It was tested whether nutrient availability in the soil was increased by a summer flooding. Furthermore, it was investigated if vegetation from a less disturbed floodplain, with a more natural hydrology (Dozdrowo, river Narew, Poland) was better adapted to summer inundation compared to vegetation from a recently rehabilitated floodplain (Steenwaard, river Rhine, the Netherlands). (research question 2)

A comparative field survey of estuarine tidal floodplains in Dutch and Irish rivers is presented in Chapter 4. Sample sites occupied by common helophytes were selected to assess which environmental parameters, related to salinity and tidal range, were correlated with presence and zonation of these species. Furthermore, the effects of human impact on the river system on sediment nutrient concentrations were assessed. (research questions 1 and 4)

In Chapter 5 a large-scale, outdoor mesocosm experiment is described. In this experiment soil monoliths with accompanying vegetation from a former estuarine floodplain (Haringvliet, the Netherlands) were subjected to an increase in tidal movement and salinity of flood water for more than one year. The main objective of this experiment was to assess what rehabilitation measure is most beneficial for vegetation performance. The effects on soil nitrogen processes and vegetation were investigated separately for semi-natural and agricultural grasslands to determine if higher nutrient concentrations in the agricultural soil would negatively effect estuarine restoration. (research questions 3 and 4)

Finally, Chapter 6 presents a synthesis and general discussion of the results presented in this thesis. With the most important biogeochemical constraints identified in the preceding chapters, additional restoration methods are presented and discussed, which could help to overcome the constraints for development of species-rich target vegetation types. (research question 5)





*The Emijksse Plaat, a nature restoration area along the river Waal, a distributary of the Rhine. Pioneer vegetation has established on the sandy shores (photo: Martijn Antheunisse)*

## Chapter 2

# Regional differences in nutrient limitation in floodplains of selected European rivers: implications for rehabilitation of characteristic floodplain vegetation

A. Martijn Antheunisse, Roos Loeb, Leon P.M. Lamers and Jos T.A. Verhoeven

*River Research and Applications*, 22: 1039-1055 (2006)

### Abstract

Extremely high river discharges in 1993 and 1995 along the Dutch rivers Rhine and Meuse have increased the public awareness of possible safety threats. As a result the 'Space for Rivers' programme was implemented, aiming at restoring physical space for the rivers in combination with ecological rehabilitation. However, the development of species-rich vegetation types in these floodplain areas is lagging behind restoration targets and biogeochemical constraints may play a vital role in this.

Biogeochemical, hydrological and vegetation data were collected in 111 plots in both rehabilitated and original floodplains in regulated and more pristine river systems in the Netherlands and Poland. Soil nutrient and soil pore water data were summarised by factor analysis and the subsequent principal components were compared to vegetation and hydrological data by correlation analysis. The correlation analyses between vegetation parameters including nutrient stoichiometry and the biogeochemical soil variables resulted in a remarkable difference between pristine and impacted river systems. The results suggest a clear N-limitation of plant growth in pristine floodplains, and apparent absence of limitation in regulated, impacted floodplains. In addition, results indicate that flooding events do not lead to one-way transport of sediment and nutrients from the river into the floodplains; rather they indicate that highly dynamic hydrologic conditions prevent soils from accumulating organic matter and nutrients.

This study shows that nutrient limitation in regulated floodplains shifted from distinctly N-limited plant growth to no nutrient limitation at all, probably due to decades of high fertiliser and manure application and nutrient input by the rivers during flooding. The consequence of our findings for rehabilitation activities is that it might be necessary to restore nitrogen limitation in floodplain systems in order to create opportunities for a species-rich floodplain vegetation, through nutrient removal by hay-making.

## Introduction

Natural floodplains include some of the most productive habitats in the world (Spink et al. 1998). Explanations for the high nutrient levels and corresponding high production in floodplains have been discussed by Junk, Bailey & Sparks (1989) in their Flood Pulse concept. This widely accepted concept describes the effect of floods on river forelands in an unmodified river system (Tockner et al. 2000). In regulated rivers, however, the relation between river channel and floodplain has been strongly disturbed because of the fixation of the river bed and the creation of dikes (also called levees) which reduce flooding frequency and duration (Lorenz et al. 1997). The economical importance of river channels and accompanying forelands for navigation, agricultural land-use, generation of hydro-energy and excavation of sand and gravel has resulted in heavily degraded river systems (Dynesius & Nilsson 1994; Lorenz et al. 1997; Nienhuis & Leuven 2001; Pedroli et al. 2002; Hohensinner et al. 2004). Nowadays, natural floodplain ecosystems are among the most threatened systems in the world (Tockner & Stanford 2002).

River regulation and intensive agricultural use of floodplains have severely altered both hydrodynamic and biogeochemical gradients (Hohensinner et al. 2004). Complex hydrodynamic gradients have disappeared due to the construction of dikes and other flood defence measures. Many regulated rivers have been heavily loaded with nutrients and pollutants and many floodplains have been covered by polluted, nutrient-rich sediments for decades. In addition, agricultural use of floodplain habitats, has become very intensive locally, with high fertiliser and manure applications (Lamers et al. 2006). The combination of these anthropogenic impacts caused a severe decline in the presence of natural, biodiverse floodplain vegetation types (Pedroli et al. 2002).

Human modification of river systems in the past centuries, in combination with predicted rise of sea levels and changing rain patterns caused by climate change, increases the possibility of high water levels (Hooijer et al. 2004; Pfister et al. 2004). Public and policy makers in western Europe have become increasingly aware of a continuous flooding threat, after extreme flood events along the Rhine and Meuse in 1993 and 1995, the Oder in 1997 and the Elbe in 2002 (Bischoff & Wolter 2001; Nienhuis & Leuven 2001; Oetken et al. 2005). The Dutch government launched the 'Space for Rivers' programme, which aims at allowing the rivers to take more physical space to avoid emergencies during extremely high discharges (VROM & V&W 1996; Buijse et al. 2002). Measures taken within the 'Space for Rivers' framework are aimed at partial restoration of the connectivity between the main channel and its floodplains. In addition to better flood protection, these measures create opportunities for ecological river restoration and nature development in floodplains (Nienhuis & Leuven 2001; Hooijer et al. 2004; Pfister et al. 2004). The development of species-rich characteristic vegetation types in rehabilitated areas in floodplains in the Netherlands has often, however, lagged behind restoration targets (Lamers et al. 2006).

The aim of this study was to evaluate the relation between floodplain biogeochemistry and floodplain vegetation in rehabilitated areas by assessing nutrient limitation and productivity in both natural and heavily impacted river systems. While other studies mostly took geomorphologic, hydrologic and dispersal issues into account (Schropp & Bakker 1998; Wolfert et al. 2001; Bischoff 2002; Pedroli et al. 2002), we also focussed on biogeochemical characteristics of the floodplain environment in our research.

We studied floodplains of six rivers in temperate Europe, both regulated and pristine rivers (Figure 1). The Rhine, Meuse, Vecht and Oder are characterised by a more confined flood pulse through diking and weiring, whereas the Narew and Bug have more natural flow characteristics (Dynesius & Nilsson 1994; Engelhardt et al. 1999; Bischoff & Wolter 2001;

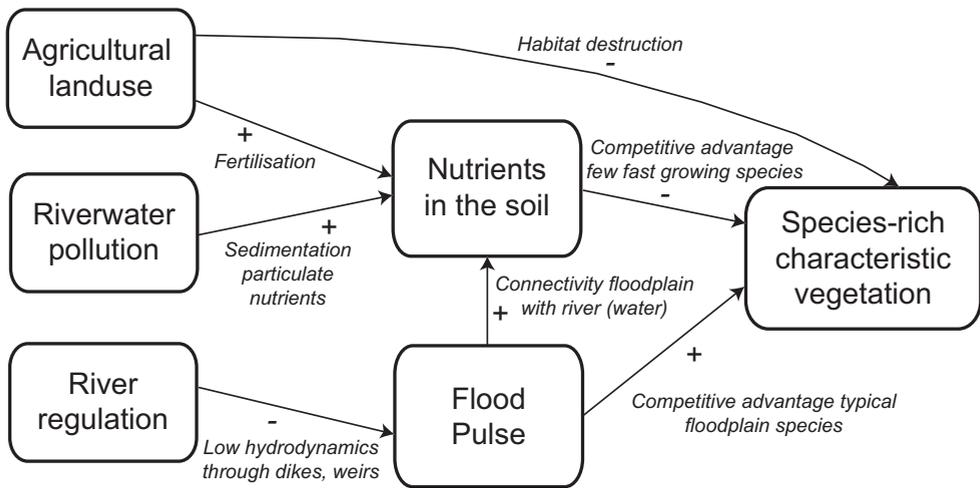


**Figure 1.** Map of West-Central Europe, showing the topographical location and main course of the six studied river systems (a) Vecht, (b) Rhine, (c) Meuse, (d) Oder, (e) Narew and (f) Bug. Additionally the Vistula (g), to which Narew and Bug are tributaries, is drawn; (●) indicate the studied floodplains.

Gielczewski 2003). In addition, the Bug and Narew have also not been affected by polluted sediments or intensive agricultural use. Species-rich characteristic floodplain vegetation types can still be found in floodplains of the Vecht, Narew and Bug (Dombrowski et al. 2002).

We hypothesised that Dutch floodplains are in general more nutrient-rich than Polish river forelands, because they have been more intensely used by farmers. Although the decrease in flood pulse by river regulation also decreased the connectivity between the floodplains and the river (Figure 2), thereby diminishing the deposition of nutrient-rich river sediments on the floodplain, the impact of intensive agricultural use of the floodplains is expected to be dominating and therefore to have created a much more nutrient-rich environment.

Additionally we hypothesised that plant growth is less limited by nutrients in more impacted floodplain systems and that the vegetation in those floodplains is much more productive. Although it is generally assumed that nitrogen is limiting plant growth in temperate floodplain systems, only few studies have been undertaken to test this (Van Oorschot 1994; Van Oorschot et al. 2000). Our expectation was that N limits plant growth in more pristine sites and that nutrients no longer limit plant growth in impacted floodplains.



**Figure 1.** Simplified conceptual model depicting the most important interactions between human impact and floodplain vegetation. Signs (+ / -) indicate increase or decrease in state (quantity) of environmental variables and increase or decrease in quantity and quality of species-rich target vegetation for management objectives

## Methods

### Study sites

The study was conducted in floodplains of six river systems in the Netherlands and Poland (Figure 1). The Rhine, the largest river in our study (Table 1), is constrained by dikes and groynes to a narrow channel and floodplain (Cals et al. 1998). Its two main distributaries, the Nederrijn and Waal, differ in that no weirs are present along the Waal, whereas the Nederrijn is regulated by three weirs, preventing very low water levels, but not affecting the height of levels at peak discharges (Van Geest et al. 2005). Over the period 1950-1970 the water became highly polluted by industrial and agricultural activities in the river basin (Wolff 1978). Although water quality has improved, pollution levels are still considerable in comparison to other rivers in this study (Table 2). The Meuse is the second largest river in the Netherlands. It is controlled by weirs and confined by dikes and groynes to guarantee year-round navigation (Admiraal et al. 1993; Schilderman et al. 1999; Pedrolí et al. 2002). The load of heavy metals

**Table 1.** Hydrological and geographical dimensions of the river systems in the present study. (Chbab 1996; Cals et al. 1998; Verhoeven et al. 1998; Bischoff & Wolter 2001; Dombrowski et al. 2002; Gielczewski 2003; Vervuren et al. 2003)

River	Type	Catchment area (km <sup>2</sup> )	Length (km)	Mean annual discharge (m <sup>3</sup> s <sup>-1</sup> )	Maximum discharge (m <sup>3</sup> s <sup>-1</sup> )
<i>The Netherlands</i>					
Rhine	glacier and rain-fed	185,000	1,320	2,200	12,600
Meuse	rain-fed	33,000	900	250	3,120
Vecht	rain-fed	3,780	176	9	86
<i>Poland</i>					
Oder	glacier and rain-fed	118,611	854	520	2,600
Bug	glacier and rain-fed	39,119	772	154	2,400
Narew	glacier and rain-fed	28,000	484	55	147

**Table 2.** Concentrations of most important chemical components used to characterise river water quality; N and P compounds indicate eutrophication and Na<sup>+</sup>, Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> indicate industrial pollution. Own data are based on low numbers of replicates, from n=1 for Narew up to n=8 for Rhine, replication is not known for the literature data.

River	N-NO <sub>3</sub> <sup>-</sup> (mg l <sup>-1</sup> )	N-NH <sub>4</sub> <sup>+</sup> (mg l <sup>-1</sup> )	P-PO <sub>4</sub> <sup>3-</sup> (mg l <sup>-1</sup> )	Cl <sup>-</sup> (mg l <sup>-1</sup> )	Na <sup>+</sup> (mg l <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> (mg l <sup>-1</sup> )
<i>The Netherlands</i>						
Rhine	2.14 <sup>b)</sup>	0.13 <sup>b)</sup>	0.11 <sup>b)</sup>	118.22 <sup>b)</sup>	72.82 <sup>b)</sup>	76.33 <sup>b)</sup>
Meuse	2.54 <sup>b)</sup>	0.17 <sup>b)</sup>	0.14 <sup>b)</sup>	50.47 <sup>b)</sup>	36.66 <sup>a)</sup>	65.30 <sup>b)</sup>
Vecht	0.56 <sup>b)</sup>	0.10 <sup>b)</sup>	0.05 <sup>b)</sup>	60.30 <sup>b)</sup>	41.76 <sup>a)</sup>	53.27 <sup>b)</sup>
<i>Poland</i>						
Oder	<0.07 <sup>a)</sup>	<0.10 <sup>a)</sup>	0.07 <sup>a)</sup>	161.18 <sup>a)</sup>	107.45 <sup>a)</sup>	99.33 <sup>a)</sup>
Bug	0.90 <sup>a)</sup>	0.40 <sup>a)</sup>	0.08 <sup>a)</sup>	22.96 <sup>a)</sup>	17.69 <sup>a)</sup>	40.52 <sup>a)</sup>
Narew	0.40 <sup>a)</sup>	0.05 <sup>a)</sup>	0.05 <sup>a)</sup>	18.74 <sup>a)</sup>	17.14 <sup>a)</sup>	23.10 <sup>a)</sup>

<sup>a)</sup> Own data, sampled in summer 2002 and 2003; <sup>b)</sup> Data from RIZA (2004), measurements in summer 2003; <sup>c)</sup> Data from IUCN office for Central Europe, measurements in summer 1999 (Dombrowski et al. 2002).

(pollution by mining) decreased since 1970, but inorganic nitrogen pollution increased over the last few decades (Vanderpoorten 1999). The Overijsselse Vecht (from this point called 'Vecht') is a relatively small river which drains part of Germany and the northern part of the Netherlands. The water level is significantly regulated by weirs in the Dutch part (Winter & Fredrich 2003). The water quality did not deteriorate to the degree reported for the Rhine and Meuse in 1970, but nutrient loads have been elevated (RIZA 2004).

The most polluted river in this study is the Oder, the boundary river between Germany and Poland. Part of the river basin is located in areas with highly polluting industries as coal mining, metal mining and metal smelting (Glasby et al. 2004). Again, this river has a history of intensive regulation; at the end of the 19<sup>th</sup> century, parts of the floodplain areas were transformed into a system of flood polders, which could be filled or emptied by pumping stations. After 1945 the system fell in disuse on the Polish shores and a natural flooding regime was established again (Engelhardt et al. 1999; Bischoff & Wolter 2001).

In this study the Narew and Bug are treated separately the Narew is actually a tributary of the Bug, before their confluence with the Vistula just north of Warsaw. The Bug is one of the few rivers in Europe retaining its original geo- and hydromorphologic character, both in the river bed and in the river forelands, although water quality did deteriorate over the last century (Dombrowski et al. 2002). The Narew is only partially regulated by a few navigational channels and a dam at the mouth creating the Zegrzynski Reservoir (Gielczewski 2003). Due to agricultural land use, nutrient concentrations in both the soil of the river forelands and the river itself are slightly elevated, but not as much as in the Dutch rivers or the Oder (Table 2). Within the floodplains of these rivers sampling sites were selected mainly based on their vegetation characteristics. We restricted ourselves to sites with herbaceous vegetation with a high chance of yearly inundation. Our dataset included sites ranging from helophyte marshlands to mown hay fields (Table 3) and showed quite some variation in species richness. Within a site, sampling points were laid out in different vegetation types along a height gradient representing a hydrodynamic gradient. Sample points were chosen in both rehabilitated floodplains, where parts of the embankments have been removed (Blauwe Kamer), the top soil layer has been excavated (Steenwaard) or a side channel has been dug (Ewijk) and sites where no restoration activities had been carried out. In total we sampled 38 points in the river forelands of the six rivers (Table 3). Per sampling point three replicate plots of 4 m<sup>2</sup> were laid out and sampled within a homogenous vegetation.

**Table 3.** Description of the sampling sites by diagnostic plant species, soil characteristics, natural history and hydrodynamics.

Site <sup>1)</sup>	Location	Diagnostic species <sup>2)</sup>	Floodplain type
<i>The Netherlands</i>			
Meuse			
NMK1	51°9' N, 5°50' E	<i>Leersia oryzoides</i>	bank connected deep floodplain water
NMM1	51°49' N, 5°35' E	<i>Carex elata</i>	bank isolated floodplain water
NMM2		<i>Glyceria maxima</i>	
Rhine			
NRB1	51°57' N, 5°36' E	<i>Carex acutiformis</i>	bank connected side channel
NRB2		<i>Mentha aquatica</i>	bank isolated side channel
NRE1	51°53' N, 5°44' E	<i>Cirsium arvense</i>	sandy levee
NRE2		<i>Persicaria hydropiper</i>	bank connected side channel
NRO1	51°51' N, 5°53' E	<i>Glyceria maxima</i>	bank isolated floodplain water
NRO2		<i>Iris pseudacorus</i>	marshy floodplain
NRO3		<i>Mentha aquatica</i>	
NRS1	51°58' N, 5°12' E	<i>Limosella aquatica</i>	bank connected side channel
NRS2		<i>Plantago major</i>	
NRS3		<i>Bidens tripartita</i>	
Overijsselse Vecht			
NVH1	52°33' N, 6°7' E	<i>Fritillaria meleagris</i>	unprotected floodplain grassland
NVH2		<i>Caltha palustris</i>	
NVH3		<i>Carex acuta</i>	marshy floodplain
NVH4		<i>Glyceria maxima</i>	
NVS1	52°35' N, 6°6' E	<i>Carex acuta</i>	terrestrialised side channel
NVS2		<i>Phragmites australis</i>	
<i>Poland</i>			
Bug			
PB1	52°37' N, 21°36' E	<i>Atriplex patula</i>	sandy levee
PB2		<i>Sparganium erectum</i> subsp. <i>neglectum</i>	
PB3		<i>Glyceria maxima</i>	
PB4	52°38' N, 21°42' E	<i>Sparganium emersum</i>	(connected) side channel
PB5		<i>Schoenoplectus lacustris</i>	
PB6		<i>Carex acuta</i>	
Narew			
PN1	53°8' N, 22°9' E	<i>Achillea ptarmica</i>	unprotected floodplain grassland
PN2		<i>Phalaris arundinacea</i>	
PN3		<i>Glyceria maxima</i>	marshy floodplain
PN4	53°8' N, 22°9' E	<i>Carex acuta</i>	bank isolated side channel
PN5		<i>Glyceria maxima</i>	
PN6		<i>Agrostis stolonifera</i>	isolated side channel
Oder			
PO1	53°20' N, 14°32' E	<i>Glyceria maxima</i>	bank connected side channel
PO2		<i>Carex species</i>	
PO3		<i>Carex acuta</i>	marshy floodplain
PO4		<i>Phragmites australis</i>	floodplain reedswamp
PO5	53°16' N, 14°29' E	<i>Geum rivale</i>	high protected grounds
PO6		<i>Cirsium oleraceum</i>	

<sup>1)</sup> The first letter of the site code depicts the land (N = Netherlands, P = Poland), the second letter the river basin (M=Meuse, R=Rhine, V=Vecht, B=Bug, N=Narew, O=Odra), the third letter in the Dutch dataset indicates the specific location (K=Koningsteen, M=Megen, B=Blauwe Kamer, E=Ewijk, O=Oude Waal, (R)S=Steenwaard, H=Huis den Doorn, (V)S=Streukel), the number indicates the series of permanent quadrats

<sup>2)</sup> Species nomenclature follows that of Van der Meijden (1996)

Table 3. Continued

Site <sup>1)</sup>	Nature development	Management & landuse	Hydrodynamics		
			FD <sup>2)</sup>	FF <sup>3)</sup>	FT <sup>4)</sup>
<i>The Netherlands</i>					
Meuse					
NMK1	protected area, no measures taken	no frequent management	5	4	4
NMM1	-	no frequent management	2	2	2
NMM1			2	2	2
Rhine					
NRB1	protected area, no measures taken	extensive grazing	4	3	4
NRB2	summerdike removed (1992)		4	3	3
NRE1	lowered (1989)	extensive grazing	3	3	5
NRE2			4	4	4
NRO1	protected area, no measures taken	no frequent management	4	2	2
NRO2		frequent mowing and grazing	4	3	3
NRO3			4	3	3
NRS1	lowered (1997)	grazing (cattle and geese)	5	5	4
NRS2			4	4	4
NRS3			5	4	4
Overijsselse Vecht					
NVH1	protected area, no measures taken	two-yearly mown	2	2	2
NVH2			3	2	2
NVH3			3	3	3
NVH4			4	3	3
NVS1	protected area, no measures taken	no frequent management	5	4	4
NVS2		reed culture	5	4	4
<i>Poland</i>					
Bug					
PB1	'pristine'	no frequent management <sup>3)</sup>	4	4	4
PB2			5	5	5
PB3			5	5	5
PB4	'pristine'	no frequent management <sup>3)</sup>	5	4	4
PB5			5	4	4
PB6			4	3	4
Narew					
PN1	'pristine'	extensive grazing <sup>3)</sup>	2	1	2
PN2			2	2	2
PN3			3	2	3
PN4	'pristine'	no frequent management <sup>3)</sup>	3	3	3
PN5			4	3	4
PN6			5	3	4
Oder					
PO1	no measures taken	no frequent management <sup>3)</sup>	3	3	4
PO2			2	3	4
PO3			2	2	3
PO4			2	2	2
PO5	no measures taken	grazing and burning <sup>3)</sup>	1	1	1
PO6			1	1	1

<sup>3)</sup> Based on single field observations (August 2003), actual management scheme might differ marginally

<sup>4)</sup> Flood duration in classes of average flood duration (days per year) based on quadrat height and water level data (RIZA 2004) from the period 1990-1999 for the Dutch sites and estimations based on floodplain characteristics and within site differences for the Polish sites, classes are: 1: 0-5 d y<sup>-1</sup>, 2: 6-20 d y<sup>-1</sup>, 3: 21-40 d y<sup>-1</sup>, 4: 41-100 d y<sup>-1</sup>, 5: 100+ d y<sup>-1</sup> inundation

<sup>5)</sup> Flood frequency in classes of average number of flooding events per year (f y<sup>-1</sup>) based on quadrat height and water level data (RIZA 2004) from the period 1990-1999 for the Dutch sites and estimations based on floodplain characteristics and within site differences for the Polish sites, classes are: 1: 0-1 f y<sup>-1</sup>, 2: 2-3 f y<sup>-1</sup>, 3: 4-6 f y<sup>-1</sup>, 4: 7-15 f y<sup>-1</sup>, 5: 16+ f y<sup>-1</sup>

<sup>6)</sup> Floodplain type in classes indicate increasing hydrodynamic connectivity of the specific floodplain type with the main river channel, classes signify 1: high protected ground, 2: bank isolated floodplain water, 3: winter bed, 4: bank connected side channel, 5: 1 bank summer bed

*Field methods*

We used a modified Braun-Blanquet abundance / coverage estimation scale to describe the vegetation (Barkman et al. 1964). The species with the highest coverage or most differentiating character was used to initially characterise the vegetation (Table 3 - *diagnostic species*).

The elevation of the Dutch permanent quadrats was obtained by levelling the heights to reference points with a known absolute height, by using a digital leveller (TOPCON DL-103 AF, Tokyo, Japan). We used NAP-points (Dutch Ordnance Level) or local water gauges with a NAP indication as reference points. The nearest river water gauge was identified for every study site and hourly water levels in the period 1990-1999 (RIZA 2004) were used to calculate average inundation duration and flooding frequency per year. These values were converted to hydrodynamic classes in which the sites were distributed evenly (Table 3). In study sites without direct connection between the river and the floodplain, levels were corrected for height of summer dikes and sluices. Extensive water gauge readings were not available for the Polish sampling points, instead relative heights of the plots to present river water levels (August 2003) were measured and mainly within-site (river) differences were used to estimate in which hydrodynamic class the sampling points would fit best.

Dutch sites were sampled once in the period from June to September 2002, Polish sites were sampled in August 2003. Soil samples were collected using PVC tubes (diameter 3 cm, length 12 cm). The tubes were hammered into the soil and carefully removed, stored at 4°C and processed as quickly as possible, but not later than 2 weeks after collection. Soil pore water samples were collected using hollow PVC tubes with ceramic, porous cups (Eijkkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) by creating a vacuum through a syringe. It was not possible to sample pore water in soils low in moisture content. River water was collected in 200 ml PVC flasks. In every quadrat the aboveground biomass of 0,04 m<sup>2</sup> was cut about 1 cm above soil surface and stored at 4°C till further analysis for maximally three weeks.

*Laboratory methods*

Extractable concentrations of ammonium and nitrate were determined with a 0.2 M KCl-extraction (Houba et al., 1989). 10 g of fresh material was weighed, 100 ml of extraction solution was added and the sample was shaken for one hour at 100 rpm. After centrifuging (4 minutes 4000 rpm) the supernatant was filtered and stored at -20°C until concentrations N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup> were determined colourimetrically on a continuous flow analyser (Skalar SA-40).

An estimate of plant available orthophosphate was determined using the ammonium lactate-acetic acid extraction (Houba et al. 1989). To extract orthophosphate, 10 g of fresh material was weighed, 100 ml (0.1 M) ammonium lactate-acetic acid was added and the suspension was shaken for four hours at 100 rpm. After centrifuging (4 minutes 4000 rpm) the supernatant was filtered and stored at -20°C until the concentration P-PO<sub>4</sub><sup>3-</sup> was determined colourimetrically on a continuous flow analyser (Skalar SA-40). Soil moisture content was determined gravimetrically after drying 20 g of fresh soil during 24 hours at 105°C. Organic matter content was determined by loss on ignition of dry ground soil at 550°C for 2.5 hours.

Total soil nitrogen, phosphorus and potassium concentrations were determined with a salicylic acid thiosulphate modification of the Kjeldahl digestion (Bremner & Mulvaney 1982). N and P concentrations were determined colourimetrically on a continuous flow analyser (Skalar SA-40) and K concentrations by flame emission spectroscopy.

The pH in soil pore water and surface water samples was determined with a standard KCl pH-electrode and alkalinity was estimated by titrating part of the sample down to pH 4.2 using 0.01 M HCl. The concentration of free sulphide was determined in a 10 ml subsample fixed

immediately after collection with sulphide antioxidant buffer containing sodium hydroxide, sodium EDTA and ascorbic acid. A sulphide ion-specific Ag electrode and a double junction calomel reference electrode were used. The concentrations of orthophosphate, nitrate (plus nitrite), ammonium and chloride in the water samples were measured colourimetrically with Technicon AA II systems, using ammonium-molybdate, hydrazine sulphate, salicylate and ferriammonium sulphate, respectively. Sodium and potassium were determined by flame photometry (Technicon Flame Photometer IV). Total concentrations of calcium, magnesium, iron, aluminium, manganese, zinc, sulphur and silicon in the samples were determined by inductively coupled plasma emission spectrometry (Jarrell Ash IL Plasma-200) (Lamers et al. 1998).

The aboveground biomass was sorted in living and senescent fractions, dried at 70°C for 48 hours and then weighed. The living fraction was ground and stored for further analysis. Per sample 150 mg was used to determine nitrogen, phosphorus and potassium concentrations of the biomass with a salicylic acid thiosulphate modification of the Kjeldahl digestion (Bremner & Mulvaney 1982). N and P concentrations were determined colourimetrically on a continuous flow analyser (Skalar SA-40) and K concentrations by flame emission spectroscopy.

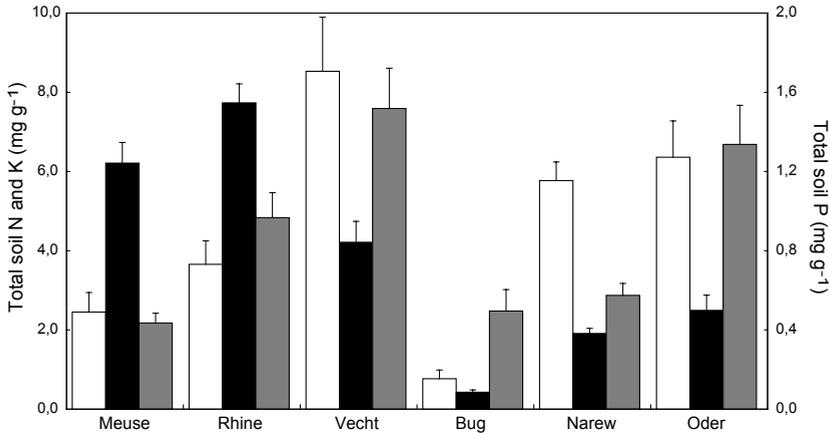
#### *Statistical analysis*

Four outlying sites (plots), identified by a hierarchical cluster analysis, were removed from further analysis. Principal components were determined using the Factor Analysis procedure in SPSS 10.0.05, the initial solution was varimax-rotated and the most suitable, in terms of explanatory power and interpretation, number of factors was extracted and scores were saved per sampling plot. Missing values, occurring in soil moisture data were replaced by the mean. Original variables were correlated to the extracted factors by calculating Pearson's correlation coefficients. The majority of the original and derived variables was not normally distributed and variance between groups was not homogenous. Transformation of the variables did not improve this, therefore non-parametric tests (Kruskal-Wallis) were performed to test for differences between river types. Differences in mean values of plant variables for regulated and pristine rivers were tested using a T-test.

## **Results**

The soils of the floodplains studied differed significantly in total nutrient pools between rivers (Kruskal-Wallis tests for soil N:  $\chi^2=66.1$ ,  $p<0.001$ , for soil P:  $\chi^2=42.3$ ,  $p<0.001$  and for soil K:  $\chi^2=86.6$ ,  $p<0.001$ ) (Figure 3). Total soil nitrogen was highest for the Vecht and lowest for the Bug. The pattern for total soil phosphorus was often the same as for soil nitrogen. Soil potassium was clearly higher for Dutch rivers and especially low for the Bug.

A factor analysis with all soil nutrient and soil pore water data reduced the original 27 variables to four principal components (Table 4). The strong reduction resulted in a relatively low explanatory value of the components (51 percent combined) and low communalities of several variables ( $\text{NO}_3^-$  and  $\text{S}^{2-}$ , both in soil pore water). The first principal component (or factor) was characterised by the positive loading of several soil pore water anions and cations associated with more reduced soil conditions ( $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ) and explained 16 percent of the variance. The second factor was associated with nitrogen richness of the soil (extractable N, total soil N and  $\text{NH}_4^+$  in soil moisture, soil organic matter and soil water content), and explained 15 percent of the variance. The third factor was positively loaded by  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{Zn}^{2+}$  in soil pore water and soil total P, indicators of anthropogenic surface water



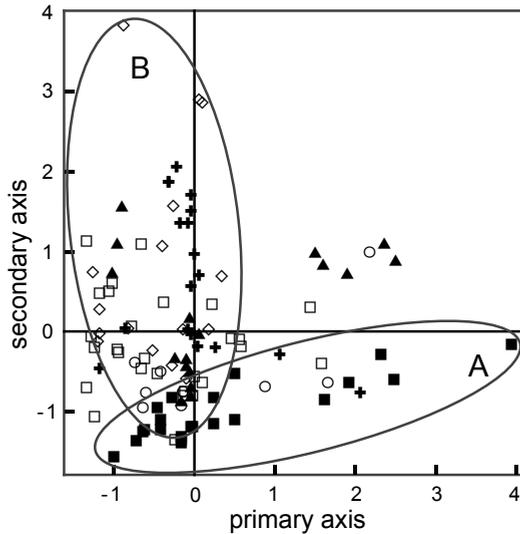
**Figure 3.** Total nutrient concentration of the topsoil in  $\text{mg g}^{-1}$  dry weight for N (white), K (black) and P (grey) per studied river. Error bars indicate + 1.0 s.e.

**Table 4.** Factor loading of the principal components with soil and soil pore water variables and the communalities; proportion of the total variance of the original variable accounted for by the four factors. Factor one is interpreted by soil redox state, factor two by nitrogen availability, factor three by indicators of industrial pollution and factor four by buffer capacity.

Original variables	Loading of principal components with original variables				Communality
	Factor 1	Factor 2	Factor 3	Factor 4	
pore water $\text{Fe}^{2+}/3+$	0.885	0.121			0.806
pore water $\text{Mn}^{2+}$	0.821				0.689
pore water Si	0.791		-0.255		0.709
pore water alkalinity	0.672			0.393	0.612
pore water $\text{PO}_4^{3-}$	0.637	0.171	-0.173		0.472
extractable $\text{PO}_4^{3-}$	0.527		0.299	-0.193	0.407
pore water $\text{NH}_4^+$	0.419			0.115	0.194
soil organic matter		0.900			0.822
extractable N		0.869			0.760
soil water content	0.202	0.868			0.803
extractable $\text{NO}_3^-$	-0.222	0.754			0.621
total soil N		0.655	0.198	-0.239	0.525
extractable $\text{NH}_4^+$	0.264	0.367	0.160		0.230
pore water $\text{NO}_3^-$		-0.165	-0.150	0.127	0.073
pore water $\text{Na}^+$			0.840	0.271	0.783
pore water $\text{Cl}^-$			0.800	0.248	0.705
v water $\text{Zn}^{2+}$	0.123		0.777	-0.146	0.644
total soil P	0.120	0.304	0.581	-0.403	0.607
total soil K	0.268	0.129	0.333		0.200
pore water free $\text{S}^{2-}$		-0.148	-0.186		0.066
pore water $\text{Ca}^{2+}$	0.384			0.793	0.777
pore water $\text{Mg}^{2+}$	0.328		0.424	0.774	0.887
pore water $\text{SO}_4^{2-}$	-0.248			0.587	0.416
pore water $\text{K}^+$	0.111		0.322	0.536	0.408
pore water pH	-0.100	-0.170	0.145	0.491	0.301
pore water $\text{Al}^{3+}$	-0.118			-0.313	0.120
pore water P			0.248	-0.299	0.169
% variance explained	16	15	12	8	

pollution and explained 12 percent of the variance. The fourth factor was characterised by several indicators of soil buffer capacity ( $\text{Ca}^{2+}$  in soil pore water and pH of soil pore water), but explained only 8 percent of the variance.

The positioning of the sample sites on the first two principal components revealed two groups of sample points per river (Figure 4). Almost all sample points in floodplains of the Vecht scored negative on factor 1 and all sample points of the Bug scored low on factor 2.



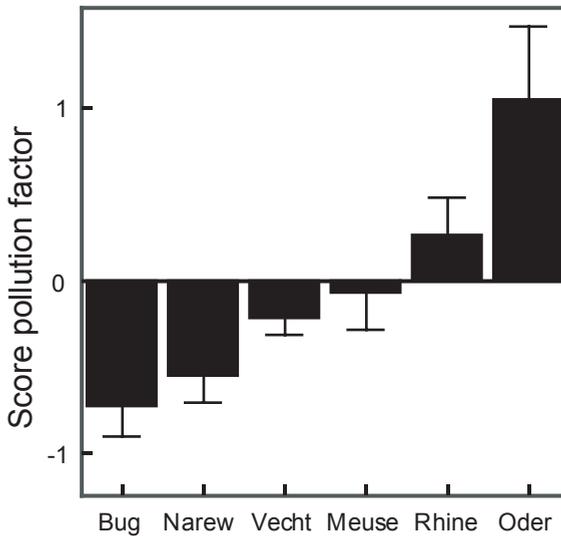
**Figure 4.** Position of sample points on factor 1, reflecting a gradient of more reduced conditions (horizontal axis) and factor 2, interpreted as a gradient of nitrogen availability (vertical axis) of the factor analysis on the combined soil and soil pore water data. Open circles (○) represent sample points in Meuse floodplains, open squares (□) Rhine, open diamonds (◇) Vecht, filled squares (■) Bug, filled triangles (▲) Narew and bold cross (✦) Oder. The horizontal oval (A) encompasses all the Bug sites, which are generally low in nitrogen availability, and the vertical oval (B) all the Vecht sites, therefore generally with less reduced soils. Other groups have broader position ranges on these two principal axes.

Sample sites of the other rivers were not grouped by these factors. A comparison of the mean position per river on factor 3 yielded significant differences (Kruskal-Wallis test,  $\chi^2=46.6$ ,  $p<0.001$ ) (Figure 5), with high values for Rhine and Oder and low values for Narew and Bug.

Variables describing the hydrodynamics (flooding characteristics) of the sample sites (Table 3, last 3 columns) correlated significantly with factor 1 and 2, whereas there were no significant correlations with factor 3 and 4 (Table 5). Correlations of flooding frequency and duration with factor 1 were all positive, suggesting that scores for this component indicates reduced sediment conditions. In contrast, the correlations of flooding frequency and floodplain type with factor 2 were significantly negative, indicating that with less yearly flooding events, soils show higher organic matter content and nitrogen availability.

**Table 5.** Pearsons bivariate correlation coefficients of hydrodynamic variables and principal components of the factor analysis on the combined soil and soil pore water data: \*  $p<0.05$ ; \*\*\*  $p<0.001$ . See Table 4 for the interpretation of the extracted factors.

Hydrodynamic variable	Factor 1	Factor 2	Factor 3	Factor 4
Flood duration	0.334***	-0.144	-0.062	0.075
Flood frequency	0.228*	-0.231*	-0.020	0.171
Floodplain type	0.237*	-0.379***	0.083	0.159



**Figure 5.** Mean score per river on component 3 (pollution) of the factor analysis on the combined soil and soil pore water data. Error bars indicate + or - 1.0 s.e.

In addition to a factor analysis on the combined soil and soil pore water data, a separate analysis on the 9 soil variables was performed (Table 6). The three resulting principal components explained 72 percent of the variance. The first principal component had high factor loadings of both available and total nitrogen and soil organic matter, and explained 38 percent of the variance. The second factor comprised the two phosphorus variables and explained 19 percent of the variance. The third factor had high loadings for extractable ammonium and total potassium and explained 15 percent of the variance. This factor analysis separated almost completely the most important nutrients for plant growth (N, P and K). We correlated quantitative and qualitative plant (nutrient) variables with these three factors.

**Table 6.** Factor loading of the principal components with soil variables and the communalities; proportion of the total variance of the original variable accounted for by the three factors. Factor one signifies nitrogen content of the soil, factor two phosphorus content and in factor three ammonium and potassium are grouped.

Original variables	Loading of principal components with original variables			Communality
	Factor 1	Factor 2	Factor 3	
soil organic matter	0.914			0.844
extractable NO <sub>3</sub> <sup>-</sup>	0.851	-0.241		0.785
extractable N	0.808		0.477	0.881
soil water content	0.752	0.378	0.242	0.768
total soil N	0.684	0.406	0.197	0.671
extractable PO <sub>4</sub> <sup>3-</sup>	-0.136	0.788	0.188	0.675
total soil P	0.316	0.721	-0.160	0.646
extractable NH <sub>4</sub> <sup>+</sup>		0.396	0.784	0.782
total soil K		-0.145	0.612	0.400
% variance explained	38	19	15	

**Table 7.** Pearsons bivariate correlation coefficients of quantitative and qualitative plant variables and principal components of the factor analysis soil data only, separately for pristine rivers (max n=38) and regulated rivers (max n=72); \* p<0.05; \*\* p<0.01; \*\*\* p<0.001. See Table 6 for the interpretation of the extracted factors.

Plant variables	Narew, Bug (pristine)			Oder, Rhine, Meuse, Vecht (regulated)		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
peak standing crop (g m <sup>-2</sup> )	0.552***	-0.016	0.009	0.203	0.257*	-0.253*
N concentration biomass (mg g <sup>-1</sup> )	-0.037	-0.094	0.265	0.245	-0.140	0.085
P concentration biomass (mg g <sup>-1</sup> )	-0.321	0.253	0.205	-0.291*	-0.140	0.230
K concentration biomass (mg g <sup>-1</sup> )	-0.165	0.000	0.204	-0.226	-0.101	0.293*
N:P ratio biomass	0.275	-0.348*	0.005	0.639***	-0.066	-0.206
N:K ratio biomass	0.048	-0.201	-0.161	0.443***	-0.118	-0.192
P:K ratio biomass	-0.124	0.204	-0.062	-0.048	-0.119	-0.033
N in standing crop (mg m <sup>-2</sup> )	0.545**	-0.130	0.052	0.286*	0.184	-0.179
P in standing crop (mg m <sup>-2</sup> )	0.385*	-0.011	0.011	-0.010	0.274*	-0.137
K in standing crop (mg m <sup>-2</sup> )	0.396*	-0.032	0.041	0.066	0.272*	-0.116

Correlations between the plant variables and the three factors of the soil data set were calculated separately for the more pristine rivers (Bug and Narew) and the regulated, impacted rivers (Oder, Rhine, Meuse and Vecht, Table 7). For the pristine rivers, peak standing crop was strongly positively correlated with factor 1. No significant correlations were found for factors 2 and 3. With respect to nutrient stoichiometry in plant tissue, only one significant correlation was present: N:P ratio correlated negatively to factor 2. N, P and K in standing crop correlated positively to factor 1. For the regulated rivers a different picture emerged. Peak standing crop was positively correlated with factor 2 and negatively with factor 3. P concentration in plant tissue correlated negatively to factor 1 and K concentration positively to factor 3. Strong, positive correlations were present for N:P and N:K ratios in plant tissue with factor 1. N in standing crop correlated positively to factor 1 and P and K in standing crop were positively correlated with factor 2.

Mean values of N, P and K concentrations and ratios between these values in living plant material were compared between regulated and pristine floodplain sites. Pristine sites were significantly lower in K tissue concentration and significantly higher in P:K ratio than regulated sites. Overall peak standing biomass was also significantly lower in pristine sites (Table 8).

**Table 8.** Plant productivity and nutrient concentrations; mean values for regulated (max n = 72) and pristine river floodplains (max n = 38) separately and results T-test for equality of mean (2-tailed), \* p<0.05; \*\*p<0.01.

Plant variables	Values ± s.e.		Statistics	
	Regulated	Pristine	T-value	P-value
peak standing crop (g m <sup>-2</sup> )	862 ± 86	526 ± 62	2.723	0.008**
N concentration biomass (mg g <sup>-1</sup> )	16.45 ± 0.68	16.31 ± 1.04	0.102	0.919
P concentration biomass (mg g <sup>-1</sup> )	2.72 ± 0.15	2.73 ± 0.18	0.050	0.960
K concentration biomass (mg g <sup>-1</sup> )	19.11 ± 0.96	15.63 ± 1.18	2.215	0.029*
N:P ratio biomass	6.69 ± 0.34	6.38 ± 0.33	0.586	0.559
N:K ratio biomass	1.03 ± 0.08	1.16 ± 0.08	1.333	0.186
P:K ratio in biomass	0.15 ± 0.00	0.18 ± 0.01	2.996	0.004**

## Discussion

The most important finding in this study is the relation of standing crop and stoichiometric vegetation parameters (nutrient ratios) to the biogeochemical soil variables in the floodplains studied. We found strongly positive correlations of peak standing crop to the soil nitrogen component for the pristine river floodplains, indicating that plant is limited by nitrogen. This was confirmed by our analysis of tissue nutrient ratios in aboveground living biomass. These positive correlations of peak plant biomass to soil nitrogen were absent for regulated, impacted river systems, suggesting that primary productivity is no longer controlled by nitrogen in these impacted floodplains.

### *Plant tissue signals*

Nitrogen and phosphorus are generally considered as the most important limiting factors for plant growth in terrestrial ecosystems (Güsewell 2004). Concentrations of these nutrients in living biomass and N:P ratios in plant tissue have been used frequently in recent studies to assess limitation or co-limitation by these nutrients (Koerselman & Meuleman 1996; Verhoeven et al. 1996; Boeye et al. 1997; Willby et al. 2001; Güsewell et al. 2003). We found values of tissue N comparable to those found in an extensive survey of European wetlands (Willby et al. 2001), but P values were somewhat higher, both for regulated and pristine floodplain systems. Therefore the mean N:P ratio in plant tissue was well below the critical N:P value of 15 for all sites and not a single value exceeded 15. These results suggest that if there is limitation in growth by one of these nutrients, nitrogen is the most likely limiting factor for plant growth in all sites, more pristine as well as more impacted floodplain systems (Verhoeven et al. 1996).

### *Relation between floodplain vegetation and biogeochemical variables*

The factor analysis with 9 soil variables strongly separated N from P variables, both in the total nutrient pools and in the extractable fractions, in two principal factors with high explanatory power (38 and 19 percent). In the analysis for pristine rivers, peak standing crop and N standing crop correlated highly significantly to factor 1, suggesting an overall N-limitation for plant growth, which is supported by the N:P ratios we found in living tissue. A similar relationship was not found in a correlation analysis for regulated, impacted floodplain sites, indicating an absence of nitrogen limitation in these systems. This conclusion is confirmed by the positive correlations of both N:P and N:K ratios to the soil nitrogen factor, as this shows that nitrogen becomes less limiting (increasing ratios) as soil nitrogen increases. In addition to this evidence for absence of N-limitation, there is a rather low, but positive correlation of peak standing crop to the soil factor indicating soil P richness. Although this could indicate limitation by P, we assume this is not the case as the floodplain environments we studied are all highly enriched with phosphorus (Figure 3) and tissue N:P ratios are well below the critical ratio of 15.

The control of plant growth by N-limitation was also recognised in several other studies in less impacted floodplains, e.g. semi-arid Australian river floodplains (Ogden et al. 2002), and English riverine ecosystems (Van Oorschot et al. 1997), although some floodplains of the pristine Polish Bierbza were found not to be limited by nutrients (Wassen et al. 1998).

### *Total nitrogen, phosphorus and potassium pools*

Mean values found for total soil N and P concentrations ( $4.53 \pm 0.41 \text{ mg g}^{-1}$  and  $0.92 \pm 0.07 \text{ mg g}^{-1}$  respectively) were comparable with other studies concerning river forelands of both regulated and pristine river systems (Spink et al. 1998; Van Oorschot et al. 1998; Verhoeven et

al. 1998). In contrast to our expectations, the floodplain soils of regulated rivers did not differ in a consistent way in total soil N and P from those along more pristine rivers. This suggests that if there had been a decrease in nutrient retention by confinement of the flood pulse, this is either obscured by an increased input by agricultural land use, or not large enough compared to the total nutrient pool in the floodplain soils to yield significant differences. Van Oorschot (1994) also found no consistent difference in total soil N and P in impacted and more pristine floodplains of the river Torridge in Devon, England. In our dataset, mean values for total N and P were lower for river forelands in the Meuse and Bug, probably as a result of the sediment characteristics of these rivers. The sediment carried by these rivers is more sandy than that of the other rivers in this study, which is reflected in the lower organic matter content and associated N and P.

The only total soil nutrient pool showing higher values in regulated than in pristine rivers was the potassium pool. However, the low total soil K concentration of the regulated Oder indicates that regulation may not be the key factor explaining differences in floodplain K richness. The flood pulse may have counteracting effects on the K concentration of floodplain soils. Particle-bound K will be deposited during a flooding event leading to an increase in soil K (Malisauskas & Sileika 2001). On the other hand, as K adsorption to clay particles increases with drainage of the soil (Olde Venterink et al. 2002), conditions during flooding might increase the exchangeable K fraction and propagate leaching of K, resulting in a decrease of total K in the soil. The presence or absence of a history of intensive agricultural land use is probably more important than the degree of regulation in explaining differences in K concentration. Fertilisation intensity has been much higher for Dutch floodplain systems than any of the other floodplains studied (Jongman 1992; Van Dijk et al. 1995).

#### *Environmental variables in relation to water quality and hydrodynamics*

The sample points in the various river systems studied were well mixed in a biplot with the first two factors of the principal component analysis with both soil and soil pore water (Figure 4). This implies that the floodplains in the river systems were not different from each other with respect to the two principle components with the highest explanatory value. Some patterns did emerge, with the Vecht scoring negatively on factor 1, indicating sample sites in Vecht river forelands had less reduced soils, and Bug scoring negatively on factor 2 indicating relatively low soil nutrient status for its sandy floodplains. Factor 3, associated with industrial and non-point pollution of the river (Admiraal et al. 1993), did separate the sample points of the river systems (Figure 5). The Oder and Rhine had the highest scores and the Narew and Bug the lowest scores, which confirms the classification in pristine and regulated, impacted rivers, based on measurements of ion concentrations in surface water (Table 2).

The hydrodynamic variables correlated significantly with the first two principal components. Hydromorphology and more specifically the hydrological connectivity between river and floodplain is commonly recognised as a very important factor in river ecology (Junk et al. 1989; Lorenz et al. 1997; Buijse et al. 2002). We found positive correlations of hydrodynamic variables, especially flooding duration, to factor 1, indicating degree of anoxia. Frequency of flooding and floodplain type, a measure for general connectivity, correlated negatively to factor 2, characterised by soil organic matter and soil nitrogen. This contradicts the general opinion that flooding leads to an input of organic material and nutrients (e.g. Junk et al. 1989; Heiler et al. 1995; Malisauskas & Sileika 2001). However, it has also been found that flooding can lead to net export of nutrients, dissolved organic matter and plant material back to the main river channel (Baldwin & Mitchell 2000), especially when human interference is present (Thoms 2003). In addition, under dynamic conditions only coarser, mineral sediments are more likely to be deposited. The absence of a relation between hydrodynamic

variables and industrial pollution (factor 3) suggests that an increase of hydrological connectivity of a floodplain through rehabilitation measures in polluted river systems is not a priori a constraint for soil quality and subsequent development of target vegetation (Buijse et al. 2002).

*Consequences for ecological rehabilitation and bottlenecks*

Our results suggest that due to regulation and human impact, nutrient limitation in the river forelands we studied shifted from a distinct limitation by nitrogen to a situation with absence of N-limited plant growth. Excess input of nutrients into the soil is obviously the most important cause. We can put this conclusion forward with reasonable confidence, although we have compared river reaches or distributaries, rather than whole river basins. Our study focussed specifically on river floodplain habitats and the way these are affected by measures related to flooding risk minimisation ('Space for Rivers'). Therefore, we have selected our sites in the lower reaches of river basins, where floodplains are a major feature of the stream valley. By selecting river floodplains with different degrees of regulation and intensity of agricultural use, we have been able to demonstrate that these human impacts have led to changes in the biogeochemical controls of floodplain productivity. In the Water Framework Directive of the EU, ecological standards (e.g. the Good Ecological Potential or GEP) will be the basis for new water quality policies (European Union 2000). The differences found in this study may be useful for developing standards for the GEP in the lower reaches of river basins, as far as floodplain habitats are concerned. As the approach has never intended to generalise data for whole river basins, scaling up to that level will not be possible with the current data set.

The hypothesis that a decrease in nutrient input by a diminishing flood pulse effect would be insignificant compared to input of nutrients by human activities, i.e. farming and fertilising (Figure 2), is supported by our findings. Furthermore, we were not able to find but a few target species as stated by the Dutch government (Bal et al. 1995) in restored floodplain sites. We did find target vegetation in river floodplains of the pristine Bug and Narew and in regularly mown floodplains of the Vecht in a nature reserve without a history of intensive agriculture (data not shown). This is an indication that biogeochemical alterations of the floodplain habitat form a constraint in the rehabilitation of species-rich floodplain vegetation.

The consequence of our findings for rehabilitation activities is that it might be necessary to restore nitrogen limitation in floodplain systems in order to create opportunities for a species-rich floodplain vegetation. Haymaking is a way to remove nutrients from the system (Prach 1996).

Already, there is a long list of investigated possible constraints for development of vegetation types consisting of target species in floodplains. Amongst others dispersal constraints received a lot of attention, as the area of target species diminished drastically in the last century (Bischoff 2002), preventing seeds and other diaspores to be transported to the newly rehabilitated river forelands (Bissels et al. 2004). Also possible hydrodynamic constraints have been studied thoroughly in different studies (Casanova & Brock 2000; Van Eck 2004). From this study, we can add increased nutrient availability and resulting shift in nutrient limitation, as a possible constraint for development of vegetation types consisting of target species in floodplains. Therefore, it is advisable and necessary to include nutrient availability in pre- and post-rehabilitation monitoring studies to optimise the success of ecological restoration in floodplains.

## Acknowledgements

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*Soil surface of a soil monolith from the experiment described in Chapter 3, directly after clipping. On the right hand side of the photograph the end of a rhizon soil moisture sampler is visible, which were used to obtain soil pore water (photo: Martijn Antheunisse)*

## Chapter 3

# Short-term response of soil nutrient dynamics and herbaceous riverine plant communities to summer inundation

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*Wetlands, accepted (2006)*

### Abstract

Rare, infrequent inundations of river floodplains during the growing season are known to have a stronger impact on plant distribution than yearly floods in winter and early spring. This may be explained by (1) a strong, direct effect of flooding on the more fully developed plants in summer, and/or (2) stronger effects mediated by soil nutrient availability, because of a much more active microbial community which will react on the soil saturation as a result of the flood. In a mesocosm experiment we tested whether herbaceous vegetation performance and soil nutrient dynamics from a strongly modified Dutch floodplain along the Rhine and a more pristine Polish floodplain along the Narew responded differently to a 14-day summer inundation.

Flooding had a negative impact on plant biomass on the Dutch floodplain soils, while biomass remained unchanged on the Polish soils. This is probably related to the absence of summer flooding in the strongly regulated Rhine floodplains, while summer flooding is still common along the Narew. One month after the inundation a decrease in species diversity (Shannon's H) in both systems was observed. This decrease was caused by better performance of a few graminoid species, leading to a higher degree of dominance in the vegetation. Soil pore water phosphate concentrations were higher in Dutch soils and reached the highest levels during the inundation. Nitrate concentrations were also generally higher in the Dutch soils, only during inundation concentrations in both soil types were very low. Differences in nutrient availability between soils were reflected in aboveground biomass tissue concentrations, but biomass response was apparently not related to nutrient availability.

We conclude that ecological rehabilitation measures in strongly modified river systems should take into account that summer inundation might lead to the disappearance of species which are not adapted to this water regime.

## Introduction

In temperate river floodplain systems, floods occur predominantly in winter, due to seasonal rain and melting of snow and glaciers (Nilsson et al. 1991). In addition to these frequent winter inundations, flooding also occurs occasionally in summer (Sykora et al. 1988). Regular winter floods are less important in determining species distributions in floodplains than the occasional summer floods (Klimesova 1994; Siebel & Blom 1998). There are indications that flooding events during the growing season are a major controlling factor in vegetation zonation in temperate floodplain systems (e.g. Van de Steeg & Blom 1998; Vervuren et al. 2003; Van Eck et al. 2004).

Recent developments in the management of river systems as well as global climate change are expected to affect the frequency of extreme summer floods. Flow patterns of nearly all large European rivers and accompanying floodplains have been altered dramatically in the 20<sup>th</sup> century by the construction of summer dikes, that prevent inundation of the floodplain in summer (Dynesius & Nilsson 1994; Tockner & Stanford 2002; Leyer 2005). Although flooding during periods of high discharge is not always prevented by these measures, the floodplain area subject to both regular winter and occasional summer inundations has been strongly reduced. In the past decade, water resource managers have become aware of the negative effects of very constrained river systems on flood risk at very high discharge (Nienhuis & Leuven 2001). Plans have been developed to allow the rivers more physical space, amongst others by removing summer levees and displacing dikes (VROM & V&W 1996; Buijse et al. 2002). This will expose larger parts of the floodplains to flooding events during the growing season (Blom et al. 1990). In addition, several modelling studies forecast an effect of global climate change on river flooding frequency. In Europe, it is expected that the frequency of summer inundations will increase in the coming century, through enhanced summer precipitation (Christensen & Christensen 2003; Kundzewicz et al. 2005).

Studies in which the importance of summer flooding for species distribution was addressed, demonstrated direct relations between the occurrence of flooding and the presence of plants with adaptations promoting survival in flooded conditions (Klimesova 1994; Vervuren et al. 2003; Van Eck et al. 2005). Oxygen deficiency is probably most important during summer flooding (Crawford & Braendle 1996), but the decrease of underwater light availability may also be a constraint, especially in turbid rivers (Blom et al. 1994; Vervuren et al. 2003). In this chapter we elaborate on the effect of summer inundation on the vegetation, both through direct effects on plant performance and through effects mediated by soil processes, and evaluated their implications for ecological rehabilitation practices in floodplains. Flooding, especially in periods with high temperatures, induces major changes in soil biogeochemistry (McClain et al. 1998), modifying the availability of the two most important nutrients for plant growth, phosphorus and nitrogen (Güsewell 2004). In well-oxygenated soils, mineral nitrogen is mostly present in the form of nitrate, due to mineralisation and nitrification processes and the absence of denitrification (Bridgham et al. 1998; Olde Venterink et al. 2002). When soils become flooded, nitrate is rapidly denitrified or reduced to ammonium (Patrick Jr. & Reddy 1976; Baldwin & Mitchell 2000; Revsbech et al. 2005). Nitrification is inhibited by the lack of oxygen provoked by flooding, promoting ammonium accumulation (Haynes & Swift 1989; Phillips 1999), even up to concentrations toxic for plants (Britto & Kronzucker 2002). After inundation subsides, when oxygen enters the soil, ammonium is nitrified and nitrate concentrations can reach high levels (Baldwin & Mitchell 2000).

Phosphorus dynamics in the soil are also dependent on redox state and associated

bacterial transformations, although more indirectly, through iron and sulphur cycling (Baldwin & Mitchell 2000). Mineral phosphorus is predominantly present in floodplain soils in adsorptive forms of phosphate with amorphous iron or aluminium oxides and clay minerals, as co-precipitants with  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  and  $\text{Ca}^{2+}$ , depending on specific sediment characteristics (McClain et al. 1998; Lamers et al. 2001). Sediment and flood-water characteristics will determine whether phosphate is released during inundation. Iron adsorptive phosphate will become available through sulphide accumulation in the soil as a result of sulphate reduction, and subsequent pyrite formation (Koerselman et al. 1993; Lamers et al. 1998; Lamers et al. 2006). Aluminium-associated and calcium-bound phosphate is less likely to become available, as these complexes are not influenced by changing redox conditions (Olde Venterink et al. 2002; Shenker et al. 2005). During the re-drying phase, the redox processes are simply reversed and available phosphate will decrease again (Lamers et al. 2006).

The scope of this study was to elucidate the effects of floods during the growing season on two different soil-vegetation floodplain systems. We selected one floodplain system along a highly modified river, where summer flooding is very unlikely to occur, due to the presence of weirs and summer levees. The other river foreland was situated in a river basin with a more natural topography and subsequent hydrological regime with a higher probability of summer inundation. A mesocosm experiment was carried out in which soil monoliths and vegetation were subjected to experimental summer inundation. We used intact monoliths with soil and accompanying vegetation, in contrast to other studies, where plants were studied in pots without inter- and/or intra specific interactions and possibly important biogeochemical actors (e.g. Blom et al. 1994; Klimesova 1994).

We expected that summer flooding would lead to higher biomass production in monoliths from the impacted site by increased nutrient availability in the soil compared to the more pristine system. This higher productivity, in addition to lower performance of the occurring plant species known to be less-adapted to summer inundation, would lead to a decrease in species diversity. This decrease was expected to be absent in the monoliths from the natural site. Additionally, we hypothesised that flooding with Rhine water, with higher nitrate and sulphate concentrations, would lead to higher biomass production in monoliths of both origins and subsequent lower diversity compared to flooding with Narew water.

## Methods

### *Experimental design*

To test the hypotheses a mesocosm experiment was designed in the greenhouse in which soil monoliths (dimensions: 35x25x12 cm) with accompanying vegetation were subjected to a 14-day inundation period at summer (water) temperature after a preceding three-month growing period.

The experiment was started in November 2003. It was situated in the greenhouse facilities of Utrecht University, the Netherlands. Average temperature did not drop below approximately 18°C. An artificial daily photoperiod of at least 16 hours was maintained throughout the experiment, drying out of soils was prevented by regular watering with osmotic water. Soil was collected in two river floodplain areas. Twelve monoliths were taken from the grazed 'Steenwaard' floodplain (51°58' N, 5°12' E) along the river Lek, a distributary of the Rhine, the Netherlands, which is a partly rehabilitated floodplain area along a highly regulated river. Soil nutrient characteristics are summarised in Table 1. Eight monoliths were collected in a grazed and frequently mown floodplain of the more pristine river Narew (Poland) near the village of Dozdrowo in a protected landscape park (53°8' N, 22°9' E), where

**Table 1.** Soil nutrient characteristics of the sites where the monoliths have been collected (n=6,  $\pm$  s.e.). Total nutrient pools have been determined using a modified Kjeldahl-digestion and extractable nutrients with a 0.2 M KCl extraction (N) or a 0.1 M ammonium lactate-acetic acid extraction (P). (Chapter 2). P-values result from an independent samples T-test, \*:  $p < 0.05$ , \*\*:  $p < 0.01$  and \*\*\*:  $p < 0.001$  between Steenwaard and Dozdrowo samples.

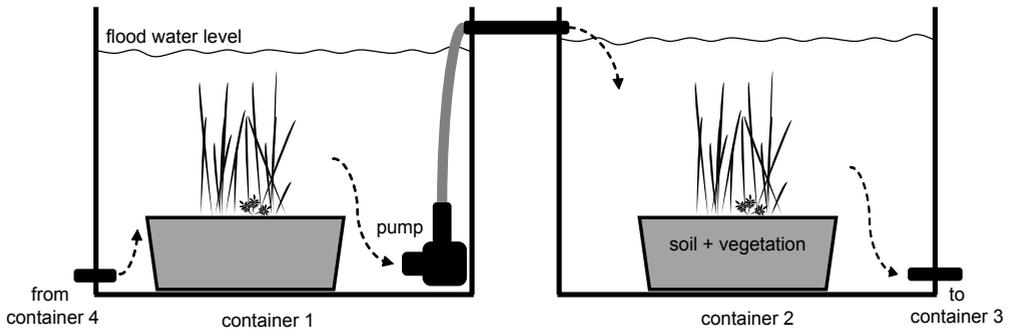
Location	Total nutrients (mg g <sup>-1</sup> )			Extractable nutrients (mg kg <sup>-1</sup> )		
	N	P	K	N-NO <sub>3</sub> <sup>-</sup>	N-NH <sub>4</sub> <sup>+</sup>	P-PO <sub>4</sub> <sup>3-</sup>
Steenwaard (NL):	3.41 $\pm$ 0.79	0.97 $\pm$ 0.14	7.92 $\pm$ 0.65	10.20 $\pm$ 2.36	0.01 $\pm$ 0.01	105.62 $\pm$ 31.30
Dozdrowo (PL):	4.47 $\pm$ 0.41	0.45 $\pm$ 0.02	1.82 $\pm$ 0.16	2.63 $\pm$ 0.81	1.16 $\pm$ 0.15	16.02 $\pm$ 4.42
p-value	0.262	0.014*	0.000***	0.013*	0.001**	0.035*

aboveground biomass was sampled during monolith-collection for comparison. Both sites held a herbaceous vegetation, with fewer species per monolith for the impacted site than for the more pristine site. Monoliths from the Steenwaard were dominated by *Alopecurus geniculatus* L. and *Ranunculus repens* L., whereas the vegetation of the monoliths from Dozdrowo was richer in species, with dominance of *Achillea ptarmica* L., *Carex acuta* L. and *R. repens* (Table 2). Weighted Ellenberg N scores (Ellenberg et al. 1992) for these two vegetation types were 4.9 for the Dozdrowo and 6.8 for Steenwaard vegetation. Soils differed predominantly in extractable nutrients, whereas total nitrogen and phosphorus showed a higher similarity. The river water showed lower concentrations of soluble nitrogen and indicators for industrial pollution in the Narew than in the Rhine river (Chapter 2).

Monoliths were collected using a tailor-made stainless steel box with sharpened edges and subsequently transferred to openwork HDPE containers with dimensions 365x265x129 mm. Nylon cloth was placed in the containers to prevent washing out of soil particles. Before

**Table 2.** Plants species in the two soil-vegetation systems and their average coverage before the summer inundation treatment, species with an average cover of 0.1 percent or lower have been omitted (Dozdrowo n=8, Steenwaard n=12).

Species	average coverage	
	Dozdrowo vegetation	Steenwaard vegetation
Dicotyls	36.3	13.9
<i>Achillea ptarmica</i>	6.1	
<i>Caltha palustris</i>	1.0	
<i>Cardamine pratensis</i>	1.8	
<i>Galium palustre</i>	6.6	
<i>Myosotis scorpioides</i>	1.5	
<i>Pimpinella saxifraga</i>	3.3	
<i>Polygonum persicaria</i>		3.6
<i>Ranunculus repens</i>	6.6	6.7
<i>Rorripa amphibia</i>	3.1	
<i>Rumex acetosa</i>		2.5
<i>Sonchus arvensis</i>		0.7
<i>Stellaria graminea</i>	5.6	
<i>Trifolium repens</i>		0.3
<i>Veronica serpyllifolia</i>	0.7	
Monocotyls	11.4	17.5
<i>Agrostis stolonifera</i>	3.4	
<i>Alopecurus geniculatus</i>		14.5
<i>Carex acuta</i>	4.8	
<i>Eleocharis palustris</i>	0.9	0.9
<i>Poa pratensis</i>	2.4	2.1



**Figure 1.** Schematic lateral view depicting part of the experimental inundation setup in which soil monoliths and vegetation were submerged. Dotted arrows indicate the water current, maintained by a pump in one container and gravity in the other three per water system, as four containers were connected with PVC tubing.

collecting, the aboveground biomass was clipped. Dozdrowo monoliths were collected in August 2003, and transported to the Netherlands in 4 days and afterwards stored at 4°C under low light conditions until the start of the experiment. Steenwaard monoliths were collected in September 2003 and immediately stored at similar conditions. Four soil moisture samplers (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) were placed in each monolith in order to collect a representative sample of soil pore water. The inundation started after a growth period of almost three months (87 days) and lasted for a period of 14 days. Four monoliths of each type were subjected to an inundation with Rhine or Narew-type water. Additionally, four monoliths from the Dutch site were not inundated and were monitored as a control. During the inundation, soil and vegetation were submerged almost completely, unless stem elongation or growth led to plant parts emerging. During flooding, monoliths were placed in watertight open top HDPE containers with dimensions 60x40x40 cm. A volume of approximately 75 litres of flood water per container was used. Containers were connected per treatment (four replicates) to prevent changes in water chemistry within a treatment and to facilitate maintenance of a water current by pumping with a commercially available pond-pump (LIBEL 600) from one container to the other (Figure 1). The water displacement was approximately 150 litres per hour, to prevent anoxia in the water column. Available information on river water quality was used to prepare Rhine and Narew water types (Chapter 2). Several salts were added to osmotic water to reach comparable concentrations in the flood water (Table 3). The pH was brought to near neutral values by adding a concentrated citric acid solution.

**Table 3.** Concentrations of added an- and cat-ions in Narew and Rhine type flood water used in the experiment.

	Concentrations of added ions (mg l <sup>-1</sup> )							
	Ca <sup>2+</sup>	Cl <sup>-</sup>	K <sup>+</sup>	Na <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>	Zn <sup>2+</sup>
<i>Water type:</i>								
Narew type	64.99	15.01	3.50	15.02	2.00	0.15	20.01	0.02
Rhine type	75.03	85.00	4.49	50.04	9.98	0.15	55.02	0.05

### *Sampling*

We used a modified Braun-Blanquet abundance / coverage estimation scale to describe the vegetation (Barkman et al. 1964) using the nomenclature of Heukels' Flora (Van der Meijden 1996). Vegetation diversity was determined by calculating Shannon's Index (H), with coverage data for the pre-inundation measurements, and with aboveground biomass data per species for the post-inundation measurement (Shannon & Weaver 1949).

Total aboveground biomass was sampled 28 days after the end of the inundation. The harvested material was sorted in living fractions per species and a senescent fraction, dried at 70°C for 48 hours, weighed and stored until further analysis. It turned out to be necessary to group the samples per monolith as the size of samples of individual species was too small to analyse. The living fraction was ground and stored for further analysis. Per sample 150 mg was used to determine nitrogen, phosphorus and potassium concentrations of the biomass with a salicylic acid thiosulphate modification of the Kjeldahl digestion (Bremner & Mulvaney 1982). N and P concentrations were determined colourimetrically on a continuous flow analyser (SKALAR SA-40, Breda, the Netherlands) and K concentrations by flame emission spectroscopy.

Soil pore water samples were collected by connecting 60 ml vacuum infusion flasks to the soil moisture samplers. The four samples per monolith were combined within one hour, two by two and transferred to 25 ml plastic scintillation flasks. One combination sample was frozen immediately (-20°C). The other combination sample for iron (II) analysis was acidified with 0.25 ml 10 M HCl in 25 ml to prevent precipitation of iron and also frozen until further analysis. Surface water during the inundation was sampled with 100 ml PVC flasks, transported to the lab and treated similarly as the soil pore water samples. All stored water samples were thawed thoroughly before analysis. Non-acidified samples were analysed for multiple cat- and anions, including nitrate, phosphate, sulphate and potassium, using a continuous flow analyser or flame emission spectroscopy (SKALAR SA-40, Breda, the Netherlands). Acidified samples were analysed for Fe<sup>2+/3+</sup> only on the continuous flow analyser.

### *Statistical analysis*

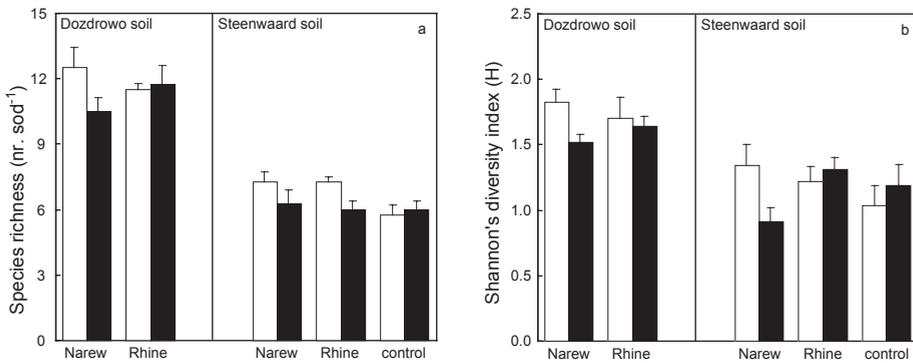
Statistical analysis was performed with the statistical package SPSS 12.0.1 (Chicago Ill, USA). Initial differences between soil and vegetation were tested with independent samples T-Tests (two-tailed). Within soil type, differences between control and inundation treatments were tested with a one-way ANOVA followed by Tukey post-hoc test to determine homogenous subsets. Treatment effects on soil pore water composition and plant nutrient parameters were tested with a two-way multivariate ANOVA.

## **Results**

### *Vegetation composition, productivity and tissue nutrients*

Directly preceding the inundation treatment, species richness and species diversity (Shannon's Index) was significantly higher in the vegetation from the Polish floodplain (T-Test, T=9.53 p=0.000 and T=4.51 p=0.000 respectively, Figure 2a-b). One month after inundation ended, this initial difference was still present (Table 4). In both soil types and especially when flooded with Narew type water, diversity decreased significantly (ANOVA, F=5.341 p=0.030) species richness was not significantly lower (ANOVA, F=1.615 p=0.217).

Total aboveground biomass one month after inundation was significantly higher in the Dozdrowo vegetation (Figure 3a, Table 4), and reached values similar to the field control. Steenwaard monoliths one month after inundation showed lower biomass than the non-



**Figure 2.** Species richness (a) and Shannon's diversity index (H) (b) for herbaceous vegetation per monolith for the different inundation treatment. White bars indicate values just before the inundation treatment and black bars depict numbers one month post-inundation. 'Narew' and 'Rhine' indicate flooding with either Narew or Rhine type water, a non-flooding control is present for Steenwaard soil. Error-bars indicate + 1 s.e., n=4, refer to Table 4 and text for the presence of significant treatment effects.

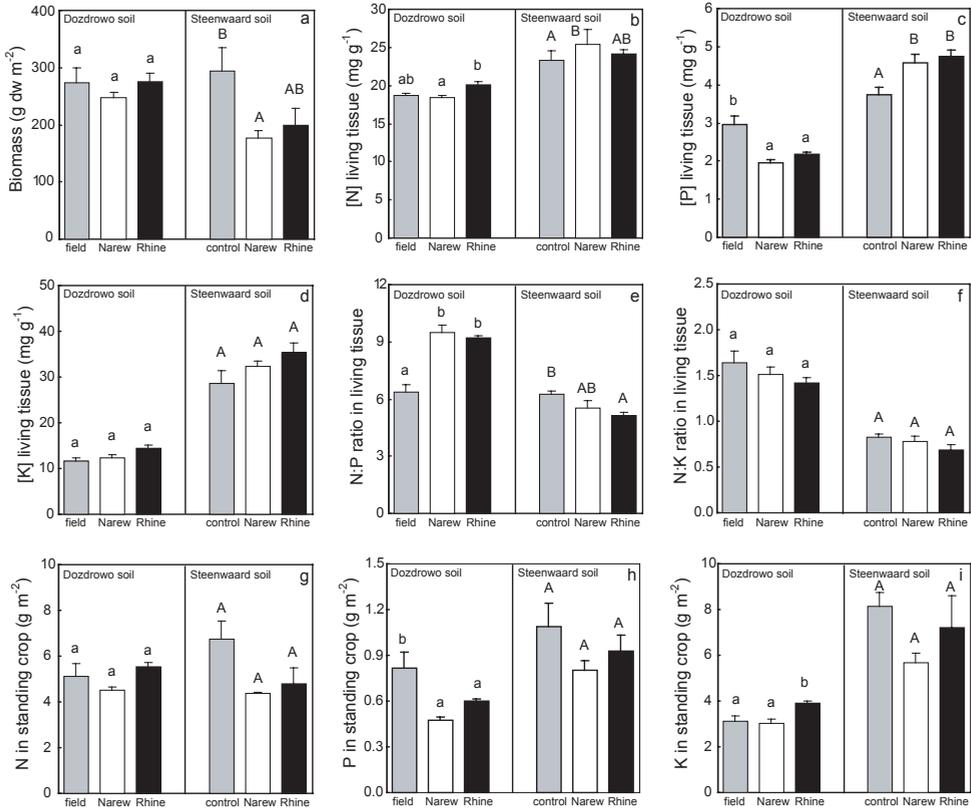
flooded control; the difference was significant for flooding with Narew water.

Concentrations of nitrogen, phosphorus, and potassium were all significantly higher in living aboveground Steenwaard vegetation, compared to vegetation on Dozdrowo soils (T-Test,  $T=3.25$   $p=0.023$ , T-Test,  $T=2.72$   $p=0.042$  and  $T=5.48$   $p=0.003$  respectively). One month after the inundation ended these initial differences were still present (Figure 3b-d, Table 4). Phosphorus concentrations were lower in Dozdrowo vegetation compared to the field control and higher in the Steenwaard vegetation compared to the non-flooded control. Concentrations N, P and K did not differ significantly between inundation with Narew or Rhine water, within Dozdrowo or Steenwaard soils (Table 4).

Living aboveground tissue N:P ratio was not significantly different between Dozdrowo field data (control) and Steenwaard control (T-Test,  $T=0.31$   $p=0.771$ , Figure 3e). One month after the inundation treatment ended the N:P ratio had increased in vegetation from the

**Table 4.** Outcome of multiple two-way ANOVAs concerning vegetation and plant parameters one month after the inundation event. Significant differences between experimental treatments (monolith origin, flood water type or interaction effects) are indicated by: \*  $p<0.05$ , \*\*  $p<0.01$  and \*\*\*  $p<0.001$ .

	Monolith origin	Flood water type	Interaction (M x F)
<i>Vegetation composition</i>			
Species density	0.000***	0.459	0.237
Shannon's H	0.000***	0.012*	0.137
<i>Production</i>			
Biomass (g dw m <sup>-2</sup> )	0.002**	0.172	0.902
<i>Nutrient concentration in aboveground living biomass</i>			
Nitrogen (mg g <sup>-1</sup> )	0.000***	0.836	0.192
Phosphorus (mg g <sup>-1</sup> )	0.000***	0.225	0.758
Potassium (mg g <sup>-1</sup> )	0.000***	0.083	0.736
N:P	0.000***	0.229	0.809
N:K	0.000***	0.161	0.968
<i>Nutrients in standing crop</i>			
Nitrogen (g m <sup>-2</sup> )	0.239	0.070	0.442
Phosphorus (g m <sup>-2</sup> )	0.000***	0.066	0.958
Potassium (g m <sup>-2</sup> )	0.002**	0.135	0.684



**Figure 3.** Aboveground biomass (a), tissue N, P, K concentrations (b, c, and e), N:P and N:K ratios in living biomass (f and g), and N, P and K standing crop (h, i, and j) for all treatments one month after the inundation. White bars indicate inundation with Narew-type water and black bars flooding with Rhine type water. Grey bars, control values, for Narew monoliths are derived from field data. For the Steenwaard, a genuine control is present. Resulting homogenous groups of one-way ANOVA, and subsequently Tukey post-hoc testing on Dozdrowo and Steenwaard soils separately, are indicated in roman (a-ab-b) for Dozdrowo soils and italic capitals (A-AB-B) for Steenwaard soils. Error-bars indicate + 1 s.e., n=4 (3 for Narew field control), refer to Table 4 for the presence of significant treatment effects.

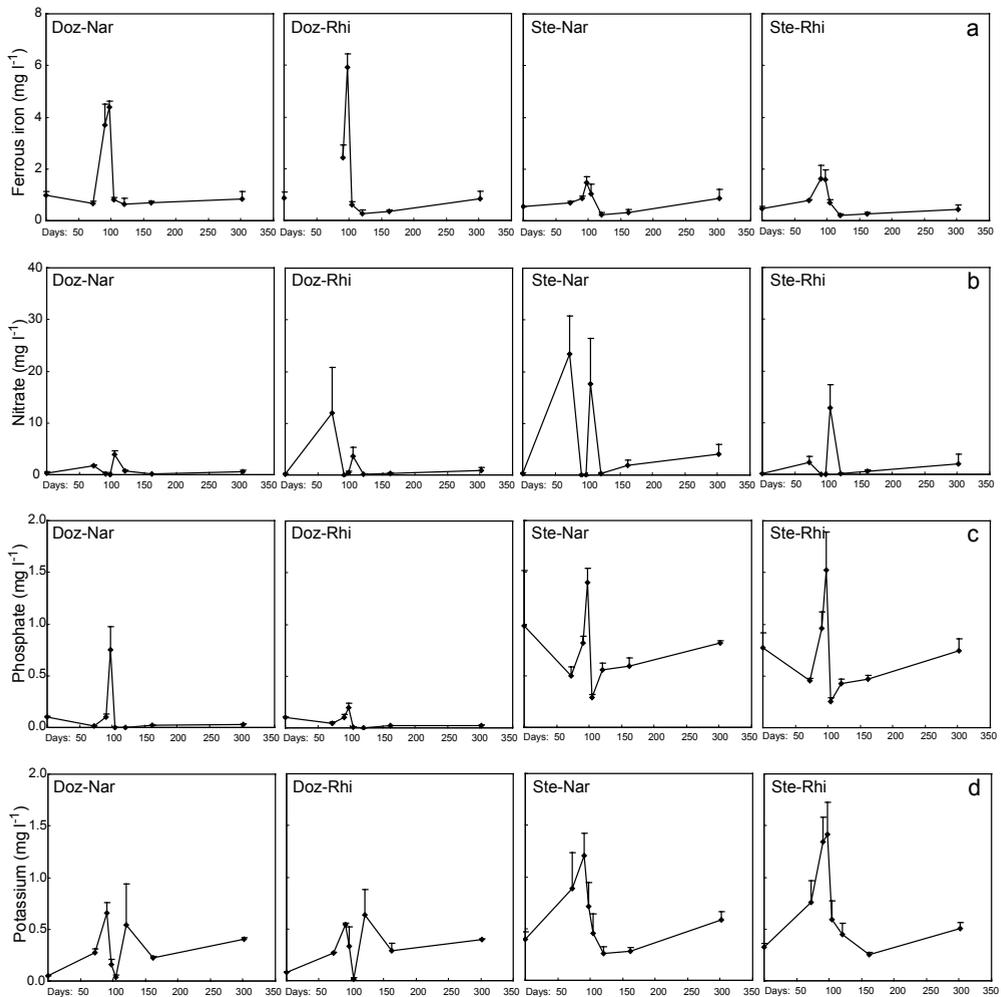
Dozdrowo monoliths. The N:P ratio in Steenwaard vegetation decreased significantly in the Rhine-water inundation treatment. Initially, N:K ratio was almost twice as high in Dozdrowo vegetation (T-Test,  $T=7.00$   $p=0.001$ , Figure 3f) and the difference in ratio had not changed one month after inundation ended (Table 4). No significant difference between inundation with Narew or Rhine water type was found for nutrient ratios.

Before inundation, potassium standing stock was significantly higher for the Steenwaard vegetation compared to Dozdrowo vegetation (T-Test,  $T=6.96$   $p=0.001$ , Figure 3i), while nitrogen and phosphorus standing stock did not differ (T-Test,  $T=1.57$   $p=0.176$ , and  $T=1.38$   $p=0.224$  respectively, Figure 3g-h). One month after the inundation ended, phosphorus as well as potassium standing stocks were significantly higher in the Steenwaard vegetation (Table 4). Nutrients in standing stock showed a non-significant trend to be higher when inundated with Rhine-type water compared to flooding with Narew water. ANOVAs followed by Tukey post-hoc tests within soil-type only showed a significant difference for potassium standing stock in the Dozdrowo vegetation.

*Dynamics of important ions in soil pore water*

In Figure 4 soil pore water concentrations of iron, nitrate, phosphate and potassium during the experiment are presented. Differences between soil types and inundation water type were tested using a two-way ANOVA at four different sample moments in time (Table 5).

Iron concentrations reached the highest levels during inundation in all treatments. Before and especially during inundation, concentrations were significantly higher in Dozdrowo soils. Flooding with Rhine water led to higher iron levels in the soil pore water during inundation compared to flooding with Narew water. Nitrate concentrations in the soil pore water showed a high temporal as well as spatial variation. Nitrate disappeared in all treatments during inundation and increased again rapidly directly afterwards with significantly higher concentrations in Steenwaard monoliths. Phosphate concentrations in soil pore water were



**Figure 4.** Composition of soil pore water per treatment, with respect to concentrations of ferrous iron (a), nitrate, (b) phosphate (c) and potassium (d) during the experiment. ‘Doz’ are values for soil with Dozdrowo origin and ‘Ste’ for Steenwaard soils. ‘Nar’ indicates inundation treatment with Narew water and ‘Rhi’ with Rhine water. Inundation commenced at day 87 and was prolonged for 14 days. Error-bars indicate + 1 s.e., n=4 (or less in case of missing values).

**Table 5.** Differences in concentrations of ferrous iron, nitrate, phosphate and potassium in soil pore water at four sample moments during the experiment; at start of the experiment (day 1), during the inundation (day 97), seven days and almost 200 days after inundation (day 104 and 302 respectively). A multivariate two-way ANOVA was used to test for significant differences between soil origin (Doz - Dozdrowo and Ste - Steenwaard) and inundation water type (Nar - Narew and Rhi - Rhine) simultaneously. Data were log-transformed if necessary, \*:  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ . No significant interaction effects were found.

Soil pore water component:	ferrous iron	nitrate	phosphate	potassium
<i>Soil origin effect</i>				
pre-inundation	Doz > Ste *	Doz = Ste	Doz < Ste *	Doz < Ste ***
during inundation	Doz > Ste ***	Doz = Ste	Doz < Ste ***	Doz < Ste **
short term post-inundation	Doz = Ste	Doz < Ste *	Doz < Ste ***	Doz < Ste **
long term post-inundation	Doz = Ste	Doz = Ste	Doz < Ste ***	Doz < Ste*
<i>Inundation water type effect</i>				
pre-inundation	Nar = Rhi	Nar = Rhi	Nar = Rhi	Nar = Rhi
during inundation	Nar < Rhi *	Nar = Rhi	Nar = Rhi	Nar = Rhi
short term post-inundation	Nar = Rhi	Nar = Rhi	Nar = Rhi	Nar = Rhi
long term post-inundation	Nar = Rhi	Nar = Rhi	Nar = Rhi	Nar = Rhi

higher in Steenwaard soils during the entire experiment. Especially after the inundation little phosphate was present in the soil pore water of Dozdrowo monoliths. In all treatments, levels increased during inundation and decreased afterwards. Soil pore water potassium concentrations were also higher in Steenwaard soils at all four sample points. Potassium almost disappeared in Dozdrowo soils during inundation and increased again afterwards. The decrease in Steenwaard monoliths was less dramatic.

## Discussion

Our results show large differences in response to summer flooding between the two soil-vegetation types and almost no effect of differences in the quality of the floodwater. The different responses of the floodplain types investigated are directly relevant to discussions on rehabilitation strategies for modified river floodplains systems (Palmer et al. 2005; Lamers et al. 2006).

### *Methodical issues and solutions*

A mesocosm experiment at this scale can never address all geomorphologic and biological interactions which take place in the field situation (Carpenter 1996; Huston 1999). We have neglected possible effects of sedimentation, which often occurs during inundation and may provide extra nutrients and organic matter (Steiger et al. 2003). Furthermore, the experimental units were placed in conditions quite different from the field environment, with a possible effect on existing competition balance. To prevent accumulative non-treatment effects, the experiment was set-up to investigate soil and vegetation response on the short term only and we particularly addressed differences between the strongly modified and more pristine floodplains systems. Therefore, despite the shortcomings, we consider the results of this experiment useful to gain a better understanding of effects of summer inundation on floodplain soils and associated herbaceous vegetation types.

### *Plant community performance and vegetation composition*

One month after the inundation, aboveground biomass was significantly reduced on Steenwaard soil, while biomass of Dozdrowo vegetation did not show this reduction and was

comparable to the field situation. Aboveground biomass was not different between the two studied vegetation types without summer flooding (Steenwaard control compared with Dozdrowo field data). These results confirm our hypothesis that plant communities from pristine floodplains are better adapted to summer inundation, compared to the plant communities from modified river system (Nilsson et al. 1997).

However, both types showed a lower diversity index ( $H$ ) after the inundation ended. This was caused by an increased dominance of both *Carex acuta* and *Agrostis stolonifera* in the Dozdrowo soils and *Alopecurus geniculatus* in the Steenwards soils. These plant species are known to exhibit typical physiological adaptations to inundation (Gilbert et al. 2003), for instance the ability to bring oxygen in the soil by aerenchymatous tissue (Visser et al. 2000). The species-richness on the monoliths remained unchanged after the summer inundation treatment. It is possible that either the duration of inundation or the regeneration time after the flood was not sufficiently long to completely remove species from the system through changes in competitive balance. Other authors (Van Eck et al. 2004) also found that a two-week summer inundation only affected biomass in most studied species and did not result in direct mortality.

Although there was no effect of the water type on biomass production of the two vegetation-soil systems, there was a stronger effect of flooding with Narew water on Shannon's  $H$ . These results contrast our hypothesis on effect of flood water quality. We expected diversity to decline in vegetation flooded with Rhine water by enhanced biomass production. However, an effect of water chemistry on productivity was absent and the monoliths flooded with Narew water had lower diversity compared to those flooded with Rhine water. This is probably due to the interference of algal growth with higher plant performance in the more eutrophic Rhine water. During the inundation an algal bloom occurred in containers with Rhine water, reducing the amount of light available for photosynthesis in the submerged vegetation (Blom et al. 1994). The species increasing their dominance after inundation with Narew water probably have benefited from the clarity of this water, while plant growth was slightly hampered by algae in the treatment with Rhine water.

#### *Availability of nutrients*

The nutrient-rich nature of the Steenwaard floodplain, which is the result of a long history of agricultural land-use with extensive manure and fertiliser applications (Lamers et al. 2006) is clearly visible in the higher soil pore water phosphate and potassium concentrations. Nitrate concentrations in soil pore water were highly variable, both temporally and spatially, which is rather common in wetland soils (Boerner & Koslowsky 1989; Dent & Grimm 1999; Villar et al. 1999; Baldwin et al. 2005). Despite the high variability, a general pattern of decrease during inundation and increase after drainage occurred in all treatments, probably as a result of alternating activity of denitrifying and nitrifying bacteria (Tanner et al. 1999; Baldwin & Mitchell 2000).

The phosphate mobilisation during inundation and the decreasing solubility after re-drying in all treatments confirms our expectations (e.g. Lamers et al. 1998; Baldwin & Mitchell 2000; Van Dijk et al. 2004). The lower iron concentrations in Steenwaard soil during inundation compared to Dozdrowo soil indicate interaction of iron and sulphate with phosphate availability, in addition to a straightforward difference in (adsorbed) mineral phosphate (McClain et al. 1998). Pre-inundation soil pore water sulphate concentrations were higher in Steenwaard soils compared to Dozdrowo soils (appr. 500 vs. 100 mg l<sup>-1</sup>, data not shown). Under anoxic conditions, sulphate is reduced to sulphide (Laanbroek 1990), which will bind to iron that was previously complexed with phosphate, resulting in non-soluble FeS or FeS<sub>2</sub> (Smolders & Roelofs 1993; Lamers et al. 1998). This process explains the lower soluble

iron and higher dissolved phosphate concentrations in Steenwaard soil pore water during inundation.

Potassium concentrations decreased during submergence, although this nutrient is known to be released during inundation by reduced physical adsorption to clay particles (Mengel 1982; Zeng & Brown 2000). We have to consider another process responsible for the net decrease. Dilution of soil pore water by the flood water can be ruled out, as potassium concentrations in floodwater in our experiment were always at least twice as high compared to soil pore water (Table 3). Potassium ions might have been exchanged for other cations adsorbed onto the clay particles immediately after flooding, thereby decreasing the potassium concentration in soil pore water. The increase after the end of the inundation may be explained by an elevated mineralisation rate as more organic matter is available (Grootjans et al. 1985; Olde Venterink et al. 2002) possibly in combination with a reversal of the process responsible for the decrease during inundation.

#### *Tissue nutrient concentrations, productivity and nutrient limitation*

The higher overall availability of phosphorus, potassium and nitrogen in Steenwaard soils was very well reflected in nutrient concentrations in aboveground living biomass; higher concentrations of all nutrients were found in Steenwaard vegetation compared to Dozdrowo plants, before as well as after the inundation treatment. Nutrient uptake is often determined directly by availability in the soil (Pegtel et al. 1996). As the decreasing availability of phosphate after inundation was reflected in lower tissue P concentrations in Dozdrowo soils, and the enhanced mobilisation during inundation was mirrored in higher P concentrations in Steenwaard soils, our data showed a direct relation between phosphorus availability and uptake by plants.

Ratios of nutrients in aboveground plant tissue have been widely used to assess nutrient limitation in terrestrial vegetation systems (e.g. Verhoeven et al. 1996; Bedford et al. 1999; Willby et al. 2001; Wassen et al. 2005). The N:P ratios found in this research are all clearly below the critical level of 15, and indicating that nitrogen rather than phosphorus availability has been limiting plant growth in both floodplain systems (Koerselman & Meuleman 1996). N:K ratios and P:K ratios (not shown) indicate that limitation by potassium is very unlikely (Olde Venterink et al. 2003c). In the field study described in Chapter 2, it was found that herbaceous vegetation of natural Polish floodplains was also growth-limited by nitrogen whereas plants in impacted Dutch sites were not clearly limited in their growth by nutrient availability in the soil.

Although all N:P ratios in our dataset indicate that nitrogen has limited plant growth, this does not mean that the hampered growth after flooding is due to stronger N limitation. In Steenwaard soils, nitrogen concentrations in vegetation of flooded treatments were higher compared to the control, whereas biomass was lower. Stronger nitrogen limitation would have led to lower nitrogen concentrations. The lower biomass production on monoliths from the modified Steenwaard floodplain is probably caused by the absence of plant species optimally adapted to inundation (Blom et al. 1990; Ernst 1990; Blom et al. 1994; Crawford & Braendle 1996). An effect of nutrient availability cannot be ruled out that easily for the Polish vegetation. One month after the inundation ended there was no effect on production, but the N:P ratio had increased significantly and on a longer term phosphorus might become limiting plant growth instead of nitrogen as phosphorus availability remained very low after inundation.

*Consequences for floodplain rehabilitation management*

The objective of this experiment was to obtain a better understanding of the effects of summer inundation on floodplains along a highly modified and a more pristine river. We clearly demonstrated that inundation during the growing season had a higher impact on vegetation from the highly modified floodplain, because it is normally protected from summer flooding, and plants species in the vegetation lacked specific physiological adaptations to flooding (Van Eck et al. 2004). Therefore, rehabilitation measures in those systems should take into account that valuable, less-well adapted plant communities might lose species as a result of the rehabilitated water regime. Due to a history of absence of inundation in the growing season, community composition shifted from flood-tolerant to flood-sensitive. Particularly in floodplains with high nutrient availability, these communities can be easily out-competed by fast-growing, well-adapted graminoid species and result in a loss of biodiversity over time.

**Acknowledgements**

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*The tidal-river Fergus, part of the Shannon estuary in western Ireland. In this area, near the town of Clarecastle, soil, water and vegetation was sampled along a height transect in the summer of 2004 as part of the field campaign described in Chapter 4. (photo: Rutger Zeijpveld)*

## Chapter 4

# Restoration of natural hydrological and salinity gradients in tidal rivers; consequences for helophyte vegetation

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*Estuarine, Coastal and Shelf Science, accepted (2006)*

### Abstract

In Europe and the US many estuaries and tidal river systems have been degraded by human exploitation during the last century. Due to (partial) damming of estuaries, the MTR (Mean Tidal Range) decreased and related hydrological gradients disappeared, together with salinity gradients. At present, restoration schemes aim at rehabilitating natural hydrology and gradients in salinity to restore the characteristic flora and fauna communities of these habitats. In Western Europe, extensive helophyte vegetation stands are among the targets of these projects. The success of these initiatives relies on a sound understanding of the relation between helophyte zonation and gradients in tidal amplitude and salinity. This study compared such relations in tidal river stretches in the Netherlands and Ireland.

Soil characteristics, hydrological conditions and vegetation parameters were determined in 87 plots within the MTR of six tidal river in Ireland and the Netherlands, assumed to be different in historical nutrient loading. Part of the sampled plots were vegetated by plant communities dominated by *Phragmites australis* (Cav.) Steud., and *Bolboschoenus* (*Scirpus*) *maritimus* (L.) Palla, highly characteristic for tidal river systems. These plots were given specific attention in the analyses as the aforementioned helophytes are characteristic for European tidal river systems and important for nutrient cycling in the ecosystems.

In contrast to expectations, Irish floodplain sediments were more nutrient-rich than those in Dutch tidal rivers. This was most likely caused by soil and sediment differences, leading to more organic and associated organic nutrients in sediments of the Irish locations. However, stoichiometric analysis showed that Dutch sediments were relatively enriched in phosphorus. No effect of nutrient availability was found on plant productivity. The zonation and productivity of the selected helophyte plant species was only related to the tidal amplitude, not to salinity. In addition, salinity was positively correlated with tissue nutrient concentration of *P. australis*.

Our results stress the importance of the rehabilitation of a natural hydrology gradient when a tidal river system is to be restored with extensive stands of characteristic helophyte species as restoration target. A distinct salinity gradient is of lesser importance, although the interaction between a marine water influence and phosphate availability - lower at higher salinities - should therefore receive special attention during planning of rehabilitation measures.

## Introduction

Tidal rivers and associated floodplains are amongst the most valuable ecosystems of the world. They supply numerous goods and services (Costanza et al. 1997). Estuarine systems play a major role in water purification and nutrient retention (Tappin 2002; Neubauer et al. 2005), provide nursing grounds for numerous fish species (Zwolsman 1994) and foraging areas for large numbers of bird species (Ysebaert et al. 2000). Furthermore, tidal river systems with their characteristic salinity gradient are a habitat for many characteristic, specifically adapted plant species. Large stands of helophytic species are often found (e.g. *Phragmites australis* (Cav.) Steud. and *Bolboschoenus (Scirpus) maritimus* (L.) Palla) (Coops et al. 1996; Clevering & Van Gulik 1997; Chambers et al. 2003). These are known to play an important role in nutrient retention and therefore capable to enhance the water quality (Krolikowska 1997; Coveney et al. 2002).

In the last century, the quantity and ecological quality of tidal river systems has decreased rapidly, due to regulation of rivers, pollution and economic use of the wetland systems (De Jonge & De Jong 2002). At the same time, non-point pollution increased rapidly, thereby increasing nutrient loading of the river water (Struyf et al. 2004; Boesch 2004). In the Netherlands, parts of the Rhine-Meuse estuary were closed-off for enhanced sea defence (Nienhuis et al. 1994; Smit et al. 1997), with detrimental consequences for the tidal rivers. The area of tidal flats diminished and shallow gradients with broad vegetation zones were reduced to steep slopes as erosion became dominant over sedimentation due to one-way water transport in the river systems (Smit et al. 1997). Due to all these changes in tidal rivers, large stands of helophytes have disappeared, leading to a local loss of biodiversity and reduced functioning of the ecosystem (Tonis et al. 2002; Nienhuis et al. 2002).

At present, many programmes are initiated to recover some of the lost ecological values of estuaries and tidal river systems, both in Europe and America. These rehabilitation efforts concern the reintroduction of either tidal movement or seawater incursion or a combination of both (e.g. Smit et al. 1997; Perez et al. 2000; Tanner et al. 2002; Hassett et al. 2005). In this framework, thorough knowledge on relations between environmental factors and performance of specific helophytes is indispensable when these characteristic vegetation types are amongst the targets of restoration, which is often the case in Western Europe (Coops et al. 1999).

The Rhine-Meuse estuary in the Netherlands has been severely altered through the construction of flood control structures (Rigley & Rijsberman 1994; Storm et al. 2005). The brackish tidal reaches have been replaced by freshwater habitats with a much smaller MTR than in the original situation. There are plans to restore in part the brackish conditions and the tidal action (Driesprong et al. 1998; Kerkhofs et al. 2005). We have compared some remaining tidal parts, mainly freshwater dominated, but also some mesohaline sites in the Rhine-Meuse estuary with brackish tidal habitats in several Irish river systems, including the Shannon-Fergus estuary.

The objective of our study was to unravel the effects of the environmental variables on performance and zonation of two selected helophyte plant species, *P. australis* and *B. maritimus*. These plant species are well adapted to alternating wet and dry periods (Coops et al. 1999). Both are clonal, perennial species with extensive rhizomes, which makes them resilient to environmental changes and strong competitors. Furthermore, these helophytes thrive in anoxic sediments as aerenchymatous tissue in stems can provide the roots and surrounding soil with oxygen (Ernst 1990). *P. australis* is often mentioned as a plant species characteristic for freshwater conditions, but extensive stands are also described for polyhaline tidal estuarine systems (Lissner & Schierup 1997; Chambers et al. 2003). The species is notorious for its

**Table 1.** Relationships between important parameters of the physical environment in the intertidal zone of tidal river systems and human impact with soil nutrient characteristics.

soil characteristics	physical environment		human impact <i>diffuse eutrophication</i> <sup>f</sup>
	<i>tide</i> <sup>d</sup>	<i>salinity</i> <sup>e</sup>	
SOM	more SOM high in tidal zone due to lower stream velocity and flood marks <sup>d</sup>	more SOM in sites with higher salinity due to reduced decomposition rate <sup>e</sup>	-
Total soil N	associated with SOM <sup>f</sup>	associated with SOM <sup>f</sup>	increase <sup>g</sup>
Total soil P	more P in sediments higher in tidal zone due to higher sedimentation rates	-	very large increase <sup>g</sup>
P availability	P more available lower in tidal zone due to on average lower redox conditions	phosphate more bound to Ca at higher salinities due to pH and Ca <sup>2+</sup> availability <sup>h</sup>	more available <sup>g</sup>
N availability	NH <sub>4</sub> <sup>+</sup> more available lower in tidal zone than NO <sub>3</sub> <sup>-</sup> due to longer periods of anoxia	-	more available <sup>g</sup>

<sup>d</sup> Daily influence of tide, depending on MTR and vertical distance to MHW

<sup>e</sup> Salinity of the soil moisture as integrative value year-round river water salinity

<sup>f</sup> Water and sediment nutrient load by long term and surplus fertilisation in the river basins

<sup>g</sup> Lillebø et al. (2006)

<sup>h</sup> Mendelsohn et al. (1999)

<sup>i</sup> Morse et al. (2004)

<sup>j</sup> Lamers et al. (2006)

<sup>k</sup> Padulan & Morris (1999)

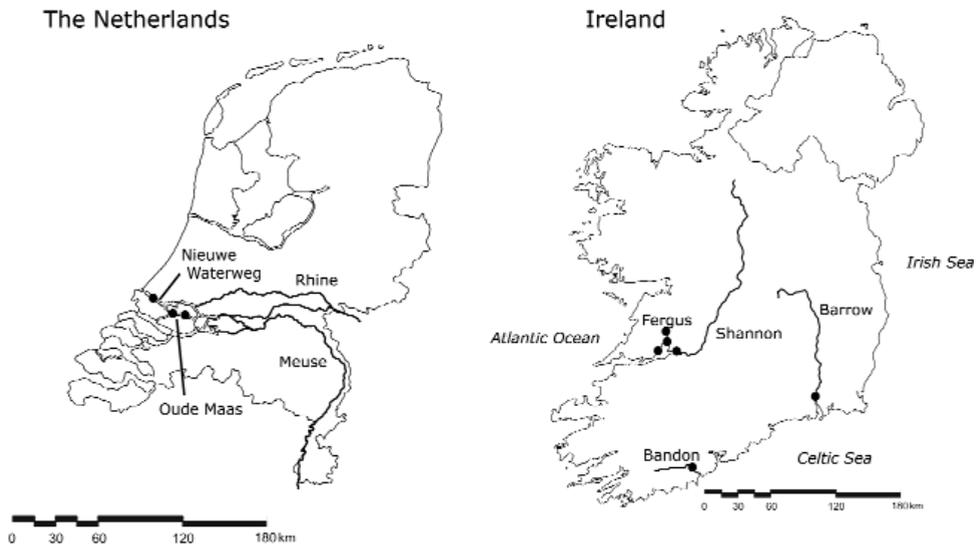
invasive behaviour in (tidal) river systems and estuaries on the east coast of the US (Chambers et al. 1999). In Europe, it occupies the upper part of the intertidal zone compared to other helophyte species (Coops et al. 1999). *B. maritimus* is a species with a broader ecological amplitude for salinity conditions, it is known to persist long after the influence of sea-water has been reduced (Weeda et al. 1994). It was our specific goal to investigate how the limits of occurrence of these two helophyte species in terms of salinity, MTR and soil nutrient richness would compare with current and future conditions in the Rhine-Meuse estuary.

In numerous studies, the relations between the most important physical environmental variables in tidal river systems (tide and salinity), human impacts by extensive non-point pollution on one hand and nutrient status of the sediments on the other hand have been investigated (Table 1). Soil organic matter content and total nitrogen and phosphorus concentrations in the floodplain soils were found to be higher under less dynamic conditions close to MHW (Morse et al. 2004; Lillebø et al. 2006) and at higher salinities (Mendelsohn et al. 1999). Total as well as available nutrient concentrations are higher in floodplains of (tidal) river systems with a history of diffuse pollution (e.g. Flindt et al. 1999; Coelho et al. 2004; Lamers et al. 2006). However, the relative importance of the main environmental variables in tidal river systems along combined tidal and salinity gradients is less well understood. Apart from the water-sediment interactions, there are also direct effects of both salinity and tide on plant growth and zonation of characteristic helophyte plant communities (Coops et al. 1996). We wanted to test whether the presence and abundance of the two helophyte species is related to tidal and salinity gradients and soil nutrient richness. We expected SOM, total N and total P to be higher in the tidal zone, SOM and total N to be higher at higher salinities and total and available nutrients to be higher in the Dutch river floodplains that have been more

severely impacted by nutrient-rich sediments than the Irish floodplains. We expected that the higher levels of both total and available nutrients in the Dutch sediments have resulted in higher plant biomass production. In addition we hypothesised that the quantity and forms of available nitrogen and phosphorus species would be related to variables operating on a small scale, such as daily flood duration and average salinity of soil moisture. We hypothesised that daily flood duration, determined by location in the intertidal zone, is the most important parameter in determining zonation and occurrence of the two helophyte plant species. Finally, we expected an effect of salinity on distribution and productivity on the two major helophyte species *P. australis* and *B. maritimus* (Hootsmans & Wiegman 1998).

## Methods

In August and September 2005, vegetation types in the intertidal zone of six tidal river stretches in the Netherlands and Ireland (Figure 1) were sampled to assess soil, hydrodynamic and vegetation characteristics over a salinity gradient. Oligohaline sites were mostly present in locations in the Netherlands where river basins are large, resulting in year-round relative high discharges, whereas mesohaline sites were restricted to tidal rivers in Ireland, where discharges are lower.



**Figure 1.** Geographical distribution of the studied tidal river stretches and sample sites (●) in the Netherlands and Ireland.

### Study sites

Overall, 87 plots were sampled in the intertidal zone (Table 2). The vegetation present in these plots ranged from distinct terrestrial vegetation types dominated by *Epilobium hirsutum* L. close to the high water line, helophyte monocultures, i.e. *Schoenoplectus lacustris* (L.) Palla, lower in the tidal zone and unvegetated sediments close to the low water line. Plots were selected mainly according to the presence of homogeneous vegetation patches, but in addition we aimed at sampling vegetation types in transects along height gradients, reflecting ranges in

**Table 2.** Number of sampled plots (replicates) per vegetation types within the studied river stretches. Vegetation types are named after the dominant plant species; Eh=*Epilobium birsutum*, Fr=*Festuca rubra*, Pa=*Phragmites australis*, Pm=*Puccinellia maritima*, Bm=*Bolboschoenus maritimus*, Sl=*Schoenoplectus lacustris/tabernaemontani*, Sa=*Spartina anglica* and Un=unvegetated

River	Study site	Coordinates	Dominant plant species							
			Eh	Fr	Pa	Pm	Bm	Sl	Sa	Un
Oude Maas (NL) id.	Beerenplaat	51°50' N, 4°22' E	3	-	3	-	3	3	-	3
	Heinenoord	51°49' N, 4°30' E	-	-	3	-	-	3	-	3
Nieuwe Waterweg (NL)	Rozenburg	51°54' N, 4°14' E	-	-	3	-	-	3	-	3
Barrow (IRL)	Ballinlaw	52°18' N, 7°01' W	-	-	3	-	3	-	3	-
Bandon (IRL)	Skangore	51°44' N, 8°37' W	-	3	3	-	3	-	-	3
Fergus (IRL) id.	Knockanima	52°48' N, 8°57' W	-	-	3	-	3	3	-	3
id.	Roscliff	52°43' N, 9°03' W	-	-	-	-	-	-	3	3
id.	Ing West	52°46' N, 8°57' W	-	-	-	-	-	-	3	-
Shannon (IRL)	Inishcullin	52°42' N, 8°51' W	-	-	3	3	3	-	3	-

hydrodynamic conditions within one study site (Figure 2). Within the group of 87 plots, 39 were sampled in vegetation types strongly dominated by one of the target helophytes; *P. australis* and *B. maritimus*.



**Figure 2.** Typical helophyte vegetation zonation in tidal river stretch of the Barrow (Ireland), with *B. maritimus* growing in the foreground, with laid-out sample grid, *P. australis* at the back higher up the tidal gradient and *S. anglica* on the right part of the photograph occurring in the lowest vegetated part of the tidal zone (photo: R. Zeijpveld).

*Sampling and analysis*

Plant communities were sampled in triplicate plots of 4 m<sup>2</sup> for multiple soil and vegetation parameters. Replicates within one vegetation type were laid out perpendicular to the hydrological gradient. We used a modified Braun-Blanquet abundance / coverage estimation scale to describe the vegetation (Barkman et al. 1964). In every quadrat the aboveground biomass of 0.16 m<sup>2</sup> was cut about 1 cm above soil surface and stored at 4°C till further analysis for maximally three weeks. The relative elevation of the quadrats was obtained by levelling the heights to estimated high water level at the site, by using a digital leveller (TOPCON DL-103 AF, Tokyo, Japan). Mean tidal range (MTR: MHW-MHL) was determined visually by discolouration on helophyte stems and later cross-checked with online databases (RIZA 2004). The estimated MTR was used in combination with the relative height of the plots to the high-water mark (RHP) to calculate a hydrodynamic score (1-RHP/MTR). Values close to 0 indicate high tidal influence and more dynamic conditions as plots were near MLW, whereas values close to 1 indicate the opposite with plots located near MHW.

Per plot, three soil samples (ø 3.5 cm, length 10 cm) were collected with a foil sampler (Eijkelpkamp Agrisearch Equipment, Giesbeek, the Netherlands) and stored at 4°C. Further processing was done on a mixture of three samples per plot, not later than two weeks after collection.

The clipped biomass was sorted in living and senescent fractions, dried at 70°C for 48 hours and then weighed. The living fraction was ground and stored for further analysis. Per sample 150 mg was used to determine nitrogen and phosphorus concentrations of the biomass with a salicylic acid thiosulphate modification of the Kjeldahl digestion (Bremner & Mulvaney 1982).

Extractable concentrations of ammonium and nitrate were determined with a 0.4 M KCl-extraction (Houba et al. 1989). 10 g of fresh material was weighed, 100 ml of extraction solution was added and the sample was shaken for one hour at 100 rpm, and pH was measured. After centrifuging (4 minutes 4000 rpm) the supernatant was filtered and stored at -20°C until concentrations N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup> were determined. An estimate of plant-available phosphate was determined using both the ammonium lactate-acetic acid extraction (Houba et al. 1989) and the Olsen extraction with sodium bicarbonate (Bray & Kurtz 1945). For the ammonium lactate-acetic acid extraction 5 g of fresh material was weighed, 100 ml (0.1 M) of the extraction solution was added and the suspension was shaken for four hours at 100 rpm. After centrifuging (4 minutes 4000 rpm) the supernatant was filtered and stored at -20°C until the concentration P-PO<sub>4</sub> was determined. For the Olsen extraction, 3 g of fresh material was weighed, 50 ml 0.5 M NaHCO<sub>3</sub> solution was added and the suspension was shaken for 30 minutes at 100 rpm. After centrifuging (4 minutes 4000 rpm) the supernatant was filtered. P-PO<sub>4</sub><sup>3-</sup> concentrations were determined colourimetrically (spectrophotometer, Shimadzu UV-120-01, Kyoto, Japan) after formation of phosphomolybdenum-blue complex by adding ammonium molybdate and potassium antimonyltartrate (Houba et al. 1989). In addition, a soil extraction with demineralised water was performed to measure concentration of chloride in the soil. 10 g of fresh material was weighed, 100 ml of demineralised water was added and the sample was shaken for one hour at 100 rpm. After centrifuging (4 minutes 4000 rpm) the supernatant was filtered and stored at -20°C until the concentration of Cl<sup>-</sup> was determined. Total soil nitrogen and phosphorus concentrations were determined with a salicylic acid thiosulphate modification of the Kjeldahl digestion (Bremner & Mulvaney 1982). Concentrations of N (nitrate, ammonium) and P (phosphate) and other ions in all digests and extractions (except phosphate in the Olsen extractions) were determined colourimetrically on a continuous flow analyzer (SA-40, Skalar Analytical, Breda, the Netherlands). Soil moisture content was determined gravimetrically after drying 20 g of fresh soil during 24

hours at 105°C. Organic matter content was determined by loss on ignition of dry ground soil at 550°C for 2.5 hours.

#### Data analysis and statistics

Relations between environmental variables and plant variables were tested with regression and correlation analysis. We categorised the soil salinity parameter to facilitate statistic analysis of variance of plant productivity and nutrient variables. Extractable chloride was used to classify the sample locations in terms of salinity. Concentrations were calculated for the soil moisture and converted to salinity values. Class boundaries were deviating from standard fresh, oligohaline, mesohaline and polyhaline categories, to prevent uneven distribution of cases. A distinct division in two groups was detected. Therefore a simple two-group classification was used: 'oligohaline' for sites with a salinity lower than 5, and 'mesohaline' if soil moisture salinity was higher than 5.

The statistical software package SPSS 12.0.1 (SPSS Inc., Chicago Ill., USA) was used for statistical analysis. In linear regression analysis all independent variables were entered and missing values, although rarely occurring, were replaced by the mean value of the total variable. In most analyses of variance, group sizes were very unequal often leading to heterogeneity in the data. In those cases, where the general assumption for performing an ANOVA was lacking and transformations did not improve this, the non-parametrical Kruskal-Wallis was used.

**Table 3.** River stretches studied in the field survey and their average yearly discharges as reported in literature. Mean tidal range (MTR) is based on field data and literature. Mixed samples per plot were used to assess average soil moisture salinity, soil organic matter content (SOM), total (Kjeldahl) soil nitrogen (N) and phosphorus (P) and their mass ratios (SOM:N, SOM:P and N:P) per river stretch. Homogenous groups are indicated (per variable) with uppercase letters (A-B-C), resulting from non-parametric post-hoc testing following a Kruskal-Wallis test, with highly significant differences ( $p < 0.001$  level) for all tested parameters.

Tidal river stretch	Discharge $m^3 s^{-1}$	MTR $m$	Salinity ‰	SOM %	
Oude Maas (NL)	650 <sup>a)</sup>	1.2 <sup>a)</sup>	0.16±0.02 A	3.73±0.66 A	
Nieuwe Waterweg (NL)	1400 <sup>b)</sup>	1.6 <sup>b)</sup>	8.43±0.72 B	1.27±0.07 A	
Barrow (IRL)	40 <sup>b)</sup>	1.0	14.21±1.22 B	12.56±1.01 B	
Bandon (IRL)	15 <sup>b)</sup>	1.3	7.74±0.31 AB	22.10±2.87 BC	
Fergus (IRL)	10 <sup>b)</sup>	1.8	13.16±1.18 B	10.32±0.47 B	
Shannon (IRL)	175 <sup>a)</sup>	1.3	11.95±0.64 B	15.13±1.02 B	
	N $mg g^{-1}$	P $mg g^{-1}$	SOM:N <i>ratio</i>	SOM:P <i>ratio</i>	N:P <i>ratio</i>
Oude Maas (NL)	1.23±0.27 A	0.60±0.09 A	38.6±2.8 A	60±7 A	1.86±0.30 A
Nieuwe Waterweg (NL)	0.30±0.06 A	0.42±0.06 A	50.2±5.4 AB	33±30 A	0.74±0.13 A
Barrow (IRL)	2.69±0.25 AB	0.63±0.07 AB	47.5±2.5 AB	205±13 B	4.31±0.14 B
Bandon (IRL)	5.36±0.54 B	0.83±0.05 B	40.3±1.8 AB	278±43 B	6.62±0.19 B
Fergus (IRL)	2.17±0.15 AB	0.59±0.03 AB	49.7±2.4 B	179±7 B	3.69±0.19 AB
Shannon (IRL)	3.34±0.38 B	0.73±0.04 B	47.8±2.2 AB	210±15 B	4.56±0.45 B

<sup>a)</sup> Rijkswaterstaat 2004 <http://www.waterstat.nl>

<sup>b)</sup> Office of Public Works 2006 <http://www.opw.ie/hydro>

<sup>c)</sup> SAGE 2006 <http://www.sage.wisc.edu/riverdata>

## Results

### *Site characteristics and soil nutrients*

The river systems studied differed strongly in average discharge between the two countries (Table 3), with values on average one magnitude higher for Dutch rivers. The Oude Maas (NL) was the only genuine freshwater tidal river in the dataset with an average salinity of 0.16. Almost all the other rivers were significantly higher in salt concentrations. Soil organic matter (SOM) was higher in Irish river systems, as well as the (organic) nitrogen concentration in the soil. The difference in total phosphorus pool was less pronounced, but again higher values were found in Irish river systems. SOM to soil N ratios were rather equal across river stretches, only in sediments of the Oude Maas the ratio was significantly lower compared to those of the Fergus river. Two clear country-specific groups were found for SOM to soil P ratios, with significantly higher values for the Irish tidal river sediments. In addition, the ratio between total nitrogen and phosphorus was significantly higher in Irish sediments, except for the Fergus river. In the Nieuwe Waterweg this ratio was lower than 1.

Two independent parameters of the physical environment were used to explain variation in soil nutrient parameters, i.e. tidal height (location in the intertidal range) and soil moisture salinity (Table 4). Highly significant regression models were found for soil organic matter and available P (lactate). Significant models were also found for total soil N and nitrate availability. The location of sites in the intertidal zone was explanatory for soil organic matter content, total soil N and the type of nitrogen availability, with a positive correlation for nitrate and a negative one (although not significant) for ammonium. Soil moisture salinity correlated positively to soil organic matter and less strongly to total soil N. A highly negative relation was present between soil moisture salinity and available P (lactate).

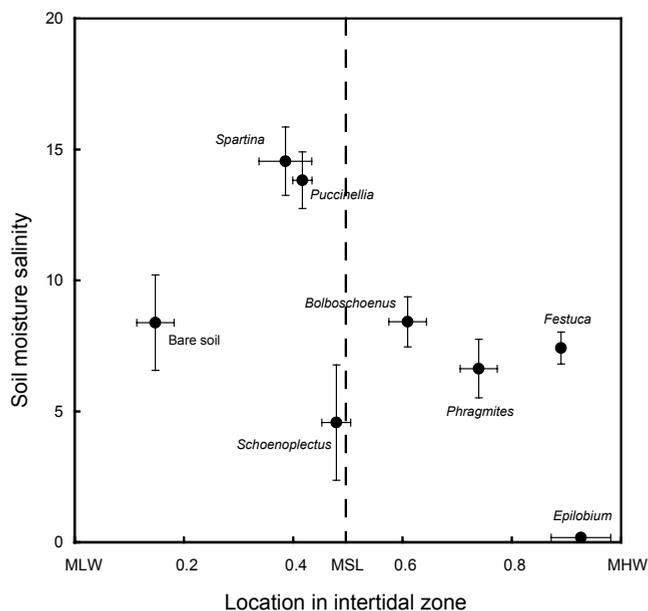
**Table 4.** Model R-squared values and beta standardised coefficients of linear regressions of selected soil (nutrient) parameters with hydrological gradient score (location in MTR) and salinity in soil moisture entered as explanatory, independent variables. Missing values were replaced by mean, no outliers were removed (n=87), n.s. non significant, \* p<0.05, \*\* p<0.01 and \*\*\* p<0.001 indicates level of significance of the regression model and the coefficients.

Dependent variable	Model R <sup>2</sup>	Beta standardised coefficients	
		<i>tide</i>	<i>salinity</i>
SOM	0.174***	0.304**	0.368**
Total soil N	0.104*	0.287**	0.234*
Total soil P	0.006 <sup>ns</sup>	0.078 <sup>ns</sup>	-0.002 <sup>ns</sup>
P-Olsen	0.010 <sup>ns</sup>	-0.004 <sup>ns</sup>	0.101 <sup>ns</sup>
P-Lactate	0.182***	-0.117 <sup>ns</sup>	-0.440***
N-NO <sub>3</sub> <sup>-</sup> (KCl)	0.118**	0.354**	0.106 <sup>ns</sup>
N-NH <sub>4</sub> <sup>+</sup> (KCl)	0.032 <sup>ns</sup>	-0.170 <sup>ns</sup>	-0.027 <sup>ns</sup>

### *Vegetation and productivity*

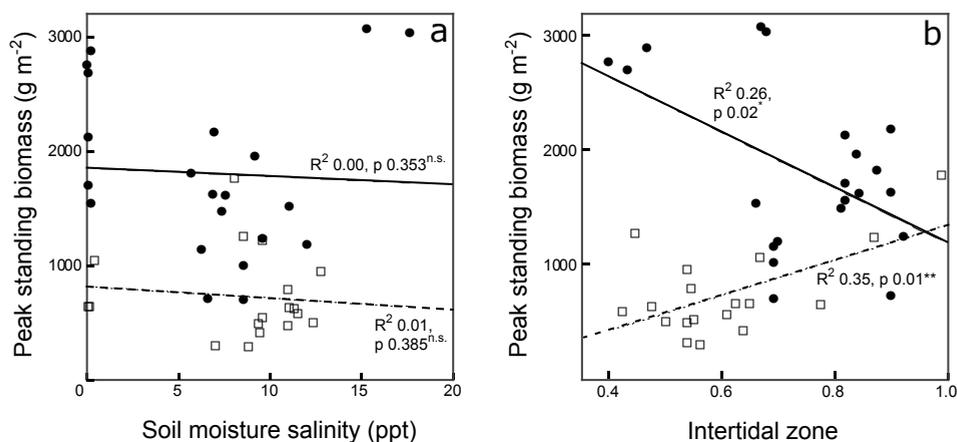
The distribution of the plant communities sampled along the salinity gradient and the occupied zone in the tidal gradient is summarised in Figure 3. In general, there was higher variance within vegetation types for soil moisture salinity, compared to location in the intertidal zone (average standard error was 18 percent for salinity and 8 for location in tidal zone). Vegetated sediments were restricted to approximately the upper 70 percent of the MTR, in which helophytes show a clear zonation from *Schoenoplectus spp.* on the lower part, through *B. maritimus* to *P. australis* higher up in the tidal zone. *Spartina anglica* C.E. Hubb and *Puccinellia maritima* (Huds.) Parl. occupy more or less the same point in the tidal zone - salinity continuum. *Festuca rubra* L. and *E. hirsutum* were both found close to MHW, but *E. hirsutum* occurred at low soil pore water (0.2) salinity and *F. rubra* at mesohaline conditions (7.4).

The relation of vegetation with soil nutrients was investigated by a linear regression



**Figure 3.** Distribution of the sampled vegetation types in the intertidal-salinity continuum, ranging from Mean Low Water level (MLW) to Mean High Water level (MHW). Similar sample points were labelled by genus of dominant species in the vegetation (Table 2). Error bars indicate  $\pm$  s.e.m, N varies from 3 to 21.

analysis of soil organic matter, total soil N and P and available N and P as explanatory variables and peak standing biomass as dependent. This regression did not yield a significant model ( $R^2 = 0.12$ ) and also no significant correlations of individual soil nutrient parameters and peak standing biomass were found.



**Figure 4.** Peak standing biomass of *P. australis* (●) and *B. maritimus* (□) stands with respect to measured salinity in soil moisture (a) and location of the helophyte stands in the intertidal zone (b), from MLW (0) to MHW (1). Linear regression lines are fitted for *P. australis* (straight) and *B. maritimus* (dotted).  $R^2$  and p-values for significance of correlations (Pearson's Coefficient) are given for each helophyte species separately.

**Table 5.** Results of multiple analysis of variance tests for quantitative vegetation parameters between the selected helophyte vegetation types and within these types with respect to the two salinity classes (O=oligohaline, M=mesohaline). Data were transformed if assumptions for ANOVA were not met.

Vegetation parameter	Between	Within		<i>B. maritimus</i>	
	<i>p</i> -value	<i>P. australis</i>	<i>salinity groups</i>	<i>p</i> -value	<i>salinity groups</i>
Biomass (mg dw m <sup>-2</sup> )	0.000*** <sup>a)</sup>	0.051 <sup>n.s.</sup> <sup>a)</sup>	O=M	0.815 <sup>n.s.</sup> <sup>c)</sup>	O=M
N concentration (mg g <sup>-1</sup> )	0.017*	0.016*	O<M	0.859 <sup>n.s.</sup> <sup>c)</sup>	O=M
N:P ratio	0.012* <sup>b)</sup>	0.064 <sup>n.s.</sup>	O=M	0.483 <sup>n.s.</sup>	O=M
N standing stock (mg m <sup>-2</sup> )	0.000*** <sup>a)</sup>	0.725 <sup>n.s.</sup>	O=M	0.529 <sup>n.s.</sup>	O=M

<sup>a)</sup> Original data ln-transformed

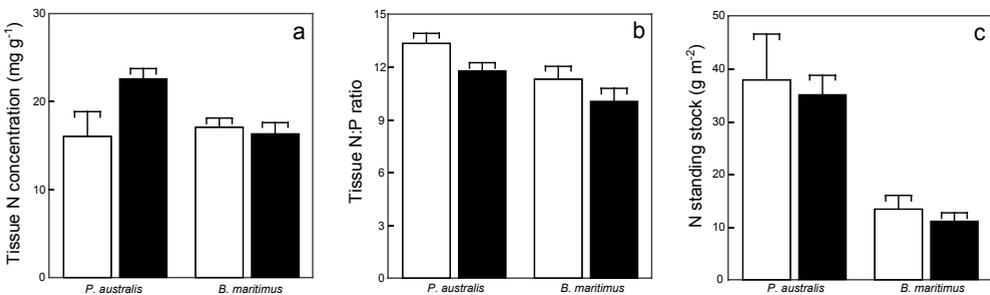
<sup>b)</sup> Original data square transformed

<sup>c)</sup> Non-transformable data: non-parametric Kruskal-Wallis test used instead

### *P. australis* and *B. maritimus*

Plant communities dominated by either *P. australis* or *B. maritimus* were not distributed differently along the salinity gradient (Figure 3, T-test:  $t=1.191$ ,  $p=0.241$ ). Peak standing biomass was higher for *P. australis* vegetation compared to *B. maritimus* stands (1809 vs. 381 g m<sup>-2</sup>) (T-test:  $t=5.855$ ,  $p<0.001$ ), but peak standing biomass of neither of both helophytes was affected by salinity (Figure 4a, Table 5). On average, plant communities dominated by *P. australis* were located higher in the intertidal zone ( $0.71\pm 0.03$ ) compared to vegetation types consisting of *B. maritimus* ( $0.61\pm 0.03$ ) (T-test:  $t=2.716$ ,  $p<0.01$ , Figure 2). In Figure 4b it is clear that there is overlap in distribution of both species. Furthermore peak standing biomass is dependent on the location in the intertidal zone. *P. australis* biomass is lowest at MHW, whereas *B. maritimus* communities are least productive at MLW.

Nitrogen concentration was generally higher in *P. australis* living aboveground tissue compared to *B. maritimus*. Especially in a more saline environment the highest concentrations were found (Figure 5a, Table 5). N:P ratio in the biomass was lower in *B. maritimus* stands, but for both species no significant differences were found between stands from the two salinity classes (Figure 5b, Table 5). Nitrogen standing stock was significantly higher for *P. australis* communities compared to *B. maritimus* stands. No differences between salinity groups were found for both species (Figure 5c, Table 5).



**Figure 4.** Tissue N (a), N:P ratio in living biomass (b) and N standing stock (c) for vegetation stands of *P. australis* and *B. maritimus*. White bars represents samples from oligohaline sites, black from mesohaline sites. See text for salinity calculations and class boundaries and Table 5 for statistical testing of differences between and within helophyte plant communities. N = variable ranging from 3 to 11.

## Discussion

Two main results can be derived from this field study: (1) The expected differences in anthropogenic impact by eutrophication between the floodplains in Dutch and Irish tidal rivers were not detected directly by our data. We found higher concentrations of total nitrogen and phosphorus in the floodplain sediments of Irish tidal rivers, which had been expected to be less impacted. A further analysis of relative abundance of N and P compared to soil organic matter content revealed that Dutch sediments were relatively enriched in phosphorus and (for the Oude Maas plots) also nitrogen. (2) The distribution and productivity of the dominant helophyte plant species in the brackish zone of these estuaries was much more influenced by the hydrodynamic gradient than by salinity of the root zone.

### *Between river sediment differences*

The hypotheses resulting from the interaction scheme of soil characteristics and the physical environment (Table 1) are partly confirmed by our results. Soil organic matter and total nitrogen concentrations were, indeed, higher closer to MHW, under less dynamic conditions. Higher up in the tidal zone more sedimentation occurs due to reduced flow velocity and general more dense vegetation (Lillebø et al. 2006). Furthermore, soil organic matter and nitrogen concentrations were positively related to higher salinity levels in soil moisture. This might be attributed to slower decomposition as salinity could have inhibited the growth of decomposers (Reice & Herbst 1982; Mendelsohn et al. 1999). The hypothesis regarding the negative relationship between salinity and P availability is confirmed by our data. The explanation is that phosphate is more bound to  $\text{Ca}^{2+}$  ions at more neutral soil moisture pH levels, characteristic for brackish and saline conditions (Zwolsman 1994; Paludan & Morris 1999).

Differences in sediment composition, with respect to organic matter and total nutrient concentrations between floodplains in Irish and Dutch rivers, were opposite to our expectations. We expected a higher nutrient load and nutrient concentration in Dutch sediments, as the river basins of the rivers Rhine (Nieuwe Waterweg) and Meuse (Oude Maas) have a long history of intensive land use (Lamers et al. 2006), whereas the basins of the Irish rivers are much less impacted (Foy et al. 2002). However, we found low concentrations for the Dutch situation and higher values in Irish sediments.

Differences in climate, physical river dimensions or sediment quality of the studied river basins, either determined by natural or by anthropogenic actors, might be responsible for these unexpected results. Although the sites studied are more or less on the same longitude (Table 2) an effect of climatic difference cannot be ruled out directly. Temperature fluctuations throughout the year are somewhat smaller in Ireland due to higher Atlantic influence. It is however, not likely that this would have such a profound impact on sediment composition.

The river systems of the two countries differ clearly in physical dimensions, with mean yearly discharge on average more than one order of magnitude higher in the Dutch tidal river stretches (Table 3). This is a direct consequence of the fact that Ireland is an island: river basins are always rather limited in size and discharge. Although discharge is lower, this does not necessarily mean that stream velocity, negatively related to deposition rates of sediment and organic matter (Olde Venterink et al. 2003b), is also proportionally lower. River bed profiles are generally shallower in Ireland and the slope of the river is higher, promoting relatively high stream velocity. Therefore this difference is also not likely to be causing our unexpected result. Higher soil organic matter and associated total (organic) nitrogen and phosphorus concentrations in the Irish river systems may be related to a country-specific difference in soil type. The soil in Irish basins is generally more organic due to the extensive bog systems, which

still can be found in large areas of the country (Huang 2002), but are absent in river basins of Rhine and Meuse (Pfister et al. 2004). This will result in higher POM and DOM in water of the Irish rivers and consequently also higher concentrations of organic matter in floodplain sediments and associated nitrogen and phosphorus concentrations. Although total N and P concentrations are lower in the Dutch sediments, evidence for human induced eutrophication is present. The (mass) ratios of SOM total soil N and P clearly show that the Dutch sediments are relatively enriched in phosphorus, and to a lesser extent, nitrogen (Table 3), with on average ratios more than four times lower in the Dutch sediments for phosphorus. Especially, this macro-nutrient is a persistent indicator of diffuse pollution in sediments (Lamers et al. 2006).

#### *Zonation of plant communities*

Intertidal elevation was the most important factor in determining the distribution of the plant communities studied, while salinity of the root zone was less important within the range investigated (Figure 3). There are also no indications that any of the nutrient-related variables control the distribution of the vegetation types in the tidal gradient. It is remarkable that *P. maritima* and *S. anglica* occupy more or less the same ecological niche with respect to tide and salinity, while these species were never found co-existing or even bordering each other in this field study. From observations in the field we conclude that grazing by cattle is very important for the presence or absence of these species in our study areas. *P. maritima* is very well adapted to this type of disturbance (Tessier et al. 2003). The zonation of the three helophyte species along the tidal gradient is in agreement with distribution patterns reported in the literature, with *P. australis* high up in the tidal zone, *B. maritimus* at intermediate heights, and *Schoenoplectus spp.* closest to the low water mark (e.g. Coops et al. 1996; Clevering et al. 1996; Coops et al. 1999). Our results, however, show their distribution in relation to height in the tidal gradient and root zone salinity in greater detail for a number of contrasting river systems.

#### *Common helophyte species*

The helophyte plant species *P. australis* and *B. maritimus* not only differ in the way they are distributed along the tidal gradient, there is also a differential effect of the height in the tidal gradient on their productivity. *P. australis* stands are highest in biomass at locations closest to MLW, whereas *B. maritimus* dominated communities have optimum productivity near MHW. This productivity response contrasts with their relative distribution along the tidal gradient. Possibly, *P. australis* is only able to survive in the highly dynamic zone by investing in biomass to outgrow prolonged inundation, occurring during neap tides (Coops et al. 1996). *B. maritimus* was found to be hampered slightly in productivity at more dynamic conditions lower in the tidal zone. Although peak biomass is higher under less dynamic conditions, the species will be out-competed higher up in the tidal zone by *P. australis* (if present), as the latter species can reach much higher stands and therefore monopolise PAR (Lenssen et al. 1999). Although we expected an additional effect of a salinity gradient on the distribution and performance of *P. australis* and *B. maritimus*, this was not shown by the results (Figure 3a). Vegetation types consisting of either species were found along the entire salinity gradient. Moreover, overall productivity across the gradient was not significantly different for both species. We found an effect of root zone salinity on nitrogen concentration in aboveground biomass of *P. australis*. Elevated nitrogen concentrations have been reported for this species when growing under salt stress (Hartzendorf & Rolletschek 2001). Plants, adapted to grow under saline conditions are capable to counteract negative osmosis effects by accumulating amino acids, especially proline, in their tissue (Parida & Das 2005). This results in overall higher N concentrations.

The fact that no significant differences were found in N:P ratio in *P. australis* biomass and

nutrient standing stock between the salinity classes is an indication that nitrogen is most likely to be limiting *P. australis* productivity. The extra demand for nitrogen to deal with salinity effects can not be counteracted by enhanced uptake of nitrogen. The type of limitation is supported by the range of the N:P ratios in this study, which are all indicative for nitrogen-controlled plant growth (Koerselman & Meuleman 1996). Not a single effect of salinity on either productivity or tissue nitrogen concentration of *B. maritimus* was found. This suggests that this helophyte is a more salt-tolerant helophyte, compared to *P. australis*. Still, it is able to thrive also under freshwater conditions. This is caused by the relative species poverty and associated low competition pressure in the intertidal area. *P. australis* is less adapted to the longer daily flood duration and therefore in most cases confined to higher parts of the intertidal zone (Coops et al. 1999; Chambers et al. 2003).

#### *Consequences for tidal river rehabilitation*

From this study we can conclude that tidal dynamics are of overwhelming importance compared to other environmental parameters in the distribution and zonation of plant species, especially for the helophyte species *P. australis* and *B. maritimus*. When extensive stands of these species, which perform valuable ecosystem services such as water quality enhancement and habitat for fish and waterfowl (Gopal 1999; Boedeltje et al. 2001), are amongst the restoration targets of a tidal river rehabilitation project, it is of vital importance to aim at recreating a natural hydrodynamic gradient. The introduction of a limited oscillatory water movement may not be sufficient to restore broad zones of different characteristic plant species. The rehabilitation of a sufficiently wide tidal amplitude is just as important, first of all because a larger amplitude will affect a more extensive part of the shore, but also larger differences in water depth fluctuations will have characteristic effects on species interactions, as some species are better adapted to prolonged inundation than others (Crawford & Braendle 1996; Coops et al. 1996; Coops et al. 1999; Lenssen et al. 1999). Our study further suggests that restoration of the salinity gradient is less important for creating this wide helophyte zone, at least for salinities not surpassing 15. Furthermore, differential concentrations of total and available nutrients in the sediment, a result of either specific basin characteristics or anthropogenic sources were not found to be affecting the presence and zonation of helophyte species. Although sediments in Dutch tidal river stretches are relatively enriched in phosphorus, this is not expected to hinder vegetation development as productivity was not found to be controlled by nutrient availability in the studied river systems. These results lead to the conclusion that tidal dynamics and associated hydrological gradients are solely controlling vegetation composition and zonation in floodplains of tidal river systems and should be given priority in estuarine rehabilitation projects.

#### **Acknowledgements**

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*Collecting soil monoliths in the Beninger Slikken nature reserve on the shores of Lake Haringvliet for the mesocosm experiment, described in Chapter 5. (Photo: Susan Sollie)*

## Chapter 5

# Response of nitrogen dynamics in semi-natural and agricultural grassland soils to experimental variation in tide and salinity

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### Abstract

In the framework of rehabilitation efforts to enhance the ecological value of closed-off estuaries, we studied the effects of restoring a tidal movement and seawater incursion on soil nitrogen conversion rates and vegetation response of semi-natural and agricultural grasslands in an outdoor mesocosm experiment. Intact soil monoliths including vegetation were collected in June 2004 on two locations on the shores of the Haringvliet lagoon in the south-western part of the Netherlands, which used to be a well-developed estuary before closure in 1970. For more than one year, soil monoliths were continuously subjected to a full-factorial combination of tidal treatment [stagnant, tidal (0.2 m amplitude)] and water type [freshwater, oligohaline (salinity = 3)]. Soil, soil moisture and water nitrogen concentrations were monitored for a year, as well as vegetation response and nitrogen conversion rates in the soil.

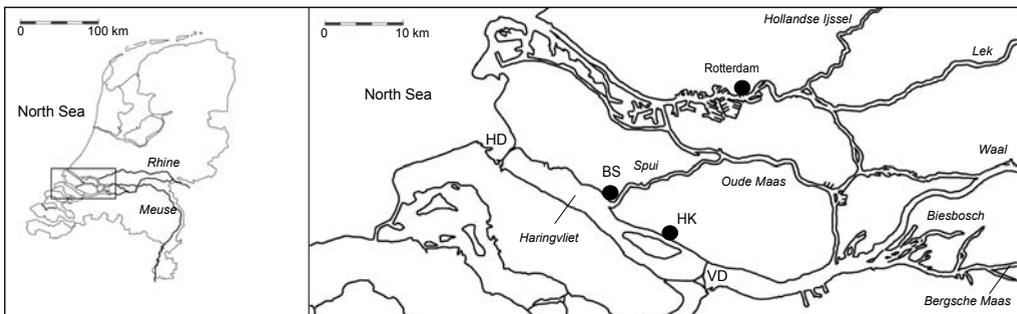
As expected, nitrogen mineralisation rates were enhanced by the tidal treatment in comparison with the stagnant treatment. Denitrification rates however, were much less affected by tide and were even lower in the tidal treatments after three months in the agricultural grassland soils, implying that in general, soils were more oxic in the tidal treatments. Oligohaline treatments had virtually no effect on soil nitrogen conversion rates compared to freshwater treatments. Vegetation performance, however, was lower under saline conditions, especially in the semi-natural grassland. No further significant differences in response to the tidal and oligohaline treatments were found between the two soils although they differed strongly in soil characteristics.

We conclude that if the rehabilitation measures in the former Haringvliet estuary are carried out as planned, drastic changes in soil nitrogen processes and vegetation composition will not occur.

## Introduction

Estuarine systems are amongst the most valuable ecosystems in the world (Costanza et al. 1997). They provide a range of goods and services; for example, they play a major role in water purification and nutrient retention of large river systems (Tappin 2002; Neubauer et al. 2005; Gribsholt et al. 2005). In the last century, these systems have become globally threatened by river regulation (Ibanez et al. 1996; Coops et al. 1999), pollution (Bryan & Langston 1992; Hawkins et al. 2002), eutrophication (Flindt et al. 1999; Conley 1999; Kemp et al. 2005) and even complete closure of estuaries by dams for safety reasons or freshwater supply (Tenbrinke et al. 1994; Smit et al. 1997).

In the Netherlands the rivers Rhine and Meuse used to enter the North Sea in a combined estuary with a freshwater tidal marshland area in the upper reach (Biesbosch), unique in size for Europe (Zonneveld 1960), and a oligohaline part close to the sea (Haringvliet: Tönis et al. 2002) (Figure 1). In 1970, the estuary was closed-off as part of the 'Deltaplan' invoked after the catastrophic 1953 flood (Nienhuis 1993). As a consequence, the Haringvliet became a large freshwater lagoon with a river discharge function for Rhine and Meuse. The original salinity gradient disappeared as the sluices in the Haringvliet Dam (North Sea side) are only opened during low tide to discharge river water (Withagen 2000). The tidal amplitude decreased from 2 meters before closure to only 0.2 meter maximum, as there is still a hydraulic connection with the North Sea through the Spui (Figure 1). These changes in the physical and chemical environment had a very large effect; the surface of tidal flats decreased dramatically and sedimentation-erosion equilibriums were disturbed, resulting in very steep banks with narrow gradients (Tönis et al. 2002). Plant species specifically adapted to oligohaline water, salinity fluctuations and frequent submergence (i.e. *Cochlearia officinalis* L., *Althaea officinalis* L.), were replaced by plants, characteristic for hypertrophic conditions, i.e.



**Figure 1.** Location of the Haringvliet estuary within the Netherlands and local tributaries and distributaries of the rivers Rhine and Meuse. A limited hydraulic connection between the Haringvliet and the North Sea was maintained after closure through the Spui. BS - Beninger Slikken is the semi-natural grassland sampling site and the agricultural grassland was sampled at HK - Hitsertse Kade. The two dams closing off the Haringvliet estuary are indicated with HD - Haringvliet Dam, 1970 and VD - Volkerak Dam, 1969.

*Urtica dioica* L. and *Epilobium hirsutum* L. (Withagen 2000).

At present, there is a wide recognition with the general public, policymakers and ecologists, that the former Haringvliet-Biesbosch estuary had very high ecological values compared with the current situation (Rigley & Rijsberman 1994). A management plan to regain some of the original values by restoring part of the sea-influence, has been developed and thoroughly scrutinised for negative side effects on safeguarding the hinterland and agricultural practices in the area (Driesprong et al. 1998). This plan includes measures to open up the sluices for two-way tidal currents; initially the sluices in the Haringvliet will be set only 'ajar' by 2008 (Kalsbeek 2004). Elaborate Environmental Impact Assessment Reports were

compiled in which effects of restoration on sedimentation-erosion equilibriums, water quality, fauna and vegetation were investigated by literature studies (e.g. Driesprong et al. 1998). The effects of reintroducing tide and a salinity gradient on nutrient cycling in the (new) intertidal zones were given less attention, and to our knowledge, this has not been studied thoroughly before in estuarine systems in a restoration context.

In this study, we investigated the effects of tide and salinity on nitrogen dynamics in grasslands in the former estuary in order to be able to predict the future effects of restoration efforts. As many of the original marshlands have been cultivated and are currently in agricultural use, we compared semi-natural grasslands (which have changed from a oligohaline estuarine to a freshwater riverine environment) with agricultural grasslands (which have been fertilised and drained for at least 100 years). Both grassland types are envisaged to be rehabilitated by allowing more tidal influence and increased salinity. We studied the response of these two systems to renewed tidal and oligohaline conditions in an experimental approach with partly controlled environmental conditions. Soil-vegetation units (monoliths) from two locations in the Haringvliet area (Figure 1) were transferred to outdoor mesocosm with controlled conditions with respect to tidal inundation and water salinity.

We expected biogeochemical nitrogen conversion rates to be higher in soils subjected to a tidal water regime due to a continuous fluctuation in oxygen supply, promoting both anaerobic (denitrification and dissimilatory nitrate reduction to ammonium) and aerobic processes (aerobic mineralisation and nitrification) (e.g. Verhoeven et al. 1998; Flindt et al. 1999; Eriksson et al. 2003). In addition, we hypothesised that at least at the start of the experiment conversion rates would be lower in soils treated with oligohaline water compared to freshwater, as the microbial communities present are assumed to be specifically adapted to freshwater conditions and therefore would perform less under oligohaline conditions (Coci et al. 2005). This effect was expected to be less strong in the soil from semi-natural grassland, in which the legacy of sea-influence might still be present in the composition of microbial communities after 35 years compared to the agricultural grasslands, where rain and drainage will have flushed out the soils for a period of at least 100 years. For similar reasons, we expected the semi-natural grassland vegetation to adapt better to oligohaline conditions relative to vegetation from the agricultural grassland.

## Methods

An outdoor mesocosm experiment in which soil monoliths from the Haringvliet area were subjected to salinity and tidal treatments for over one year was designed to test the hypotheses. The experiment was set up at the experimental facilities of Radboud University Nijmegen, the Netherlands.

### *Study sites: soil monolith origin*

Soil monoliths were taken from two areas in the former Haringvliet estuary (Figure 1). The Beninger Slikken area (51°47' N, 4°13' E) used to consist of tidal flats with oligohaline marshland and helophyte vegetation before 1970. Nowadays, it is a protected freshwater marshland and the original vegetation is partly replaced by a semi-natural grassland characteristic for more freshwater conditions. The Hitsertse Kade area (51°44' N, 4°21' E) is an embanked narrow polder, fringing the Eendragstpolder, which was diked already in 1653 (Allewijn 1989). It has been in agricultural use (as a pasture) for at least the last century. Nonetheless, this small polder might be flooded at present at high water levels in the Haringvliet during winter.

The soils from both locations were rather different, with significantly higher nitrogen availability in the agricultural grassland. The soil from the semi-natural grassland was higher in organic matter content and in soil moisture. Initial bulk density values are not available, but measurements three weeks after the start of the experiment indicated no difference. Furthermore, the more recent history of marine influence on the semi-natural grassland compared to the agricultural grassland was clearly visible in soil moisture sulphate and sodium concentrations (Table 1). As the Hitsertse Kade is in use as a pasture, vegetation comprised primarily forage grasses; *Agrostis stolonifera* L. and *Poa trivialis* L. were found in all monoliths whereas *Lolium perenne* L. was co-dominant in some of the vegetation units: mean species number was only  $3 \pm 1$  per soil monolith. Vegetation from the semi-natural grassland was more diverse (mean species number was  $15 \pm 2$ ), with many dicotyledonous species. *A. stolonifera*, *Mentha aquatica* L., *Myosotis scorpioides* L., *P. trivialis*, and *Potentilla anserina* L. were species with high abundance. *Pulicaria dystenterica* (L.) Bernh., *Glaux maritima* L. and *Althaea officinalis* L., diagnostic species for vegetation types of (former) oligohaline conditions (Weeda et al. 1994), were still present in several soil monoliths.

**Table 1.** Initial extractable nitrogen concentrations and soil physical characteristics in semi-natural grassland (SemN) and agricultural (Agr) soil, collected in May and June 2004 respectively. In addition extractable  $SO_4^{2-}$  and  $Na^+$  concentrations as indicators of a history of sea-influence are given. P-values result from an independent samples T-test, \*:  $p < 0.05$  and \*\*\*  $p < 0.001$  between grassland types. Mean values  $\pm$  s.e.m (standard error),  $n=16$ .

Soil	extractable nitrogen (mg N kg <sup>-1</sup> )			physical soil conditions				marine indicators (mg kg <sup>-1</sup> )	
	N-NO <sub>3</sub> <sup>-</sup>	N-NH <sub>4</sub> <sup>+</sup>	combined	SOM(%) <sup>1)</sup>	<i>n</i> <sup>2)</sup>	bulk <sup>3)</sup>	pH	SO <sub>4</sub> <sup>2-</sup>	Na <sup>+</sup>
SemN	2.4±0.5	3.8±0.6	6.1±0.6	14.7±0.6	0.70±0.03	1.25±0.04	7.65±0.03	1574±611	101±10
Agr	13.1±1.5	0.3±0.3	13.4±1.5	10.4±0.3	0.26±0.01	1.27±0.04	7.63±0.01	28±2	8±2
p-values	0.000***	0.000***	0.000***	0.000***	0.000***	0.597 <sup>n.s.</sup>	0.560 <sup>n.s.</sup>	0.023*	0.000***

<sup>1)</sup> Soil organic matter content (%)

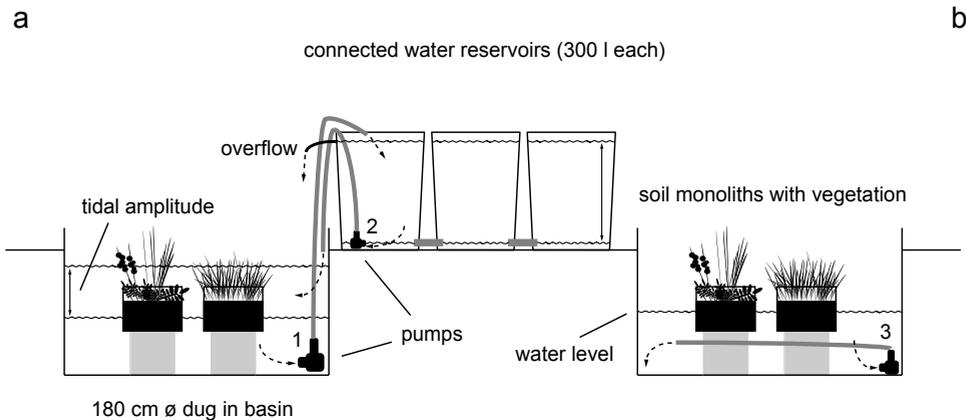
<sup>2)</sup> Soil moisture content (g water g dw soil<sup>-1</sup>)

<sup>3)</sup> Bulk density (g cm<sup>-3</sup>)

### Experimental set-up

The experiment was started in June 2004 and lasted until July 2005. The experimental design was full factorial with two factors: ‘salinity’, consisting of a fresh and oligohaline water type and ‘tide’, with a stagnant water level 10 cm below soil surface and a tidal version with levels fluctuating between 10 cm below and 10 cm above soil surface twice a day (Figure 2) These levels were based either on the present situation for former tidal flats in the Haringvliet estuary (stagnant) and levels in the tidal regime mimic possible post-restoration levels, when these areas are once more subjected to a tidal regime. In each experimental unit one soil monolith from the semi-natural grassland site and one of the agricultural grassland site was placed. Per treatment combination (stagnant-fresh, stagnant-oligohaline, tidal-fresh and tidal-oligohaline) four replicates were used in the experiment.

Sixteen water basins with a volume of 2500 L were used, which were partly dug into the ground to mitigate strong water temperature fluctuations by solar heating. Artificial water types were made by adding a sea salt mixture (Meersalz Professional, Wiegandt GmbH, Krefeld, Germany) to tap water, resulting in freshwater with a salinity of 0.1 and oligohaline water of salinity 3.0 (Table 2). A tidal movement was implemented in half of these basins by pumping app. 900 L of water (from total of 2100 L) to temporary storage basins over a period of six hours (ebb) and a reverse of this process by passive outflow in the six hours thereafter



**Figure 2.** Practical layout of the mesocosm experiment, with a tidal (a) and non-tidal (b) treatment. Per basins soil monoliths from both the semi-natural and agricultural grassland were present. Pump 1 in the tidal treatment is active during ebb; pump 2 initiates the (passive) flood. Pump 3 in the non-tidal treatment was working continuously to maintain a water current in these treatments as well. Dotted lines indicate direction of water movement.

(flood) (Figure 2). Occasionally, water had to be added to or removed from the basins during drought and rain periods, respectively, to maintain similar hydrological conditions. If salinity decreased below 3.0 in the oligohaline treatments (Multi 340i, WTW, Weilheim, Germany), sea salt was added to restore the original value. At two occasions, during the winter of 2004/2005, the tidal movement was stopped to prevent frost damage to the system.

**Table 2.** Composition of fresh and oligohaline water types at the start of the experiment (June 2004) with respect to several relevant cat- and anions. Concentrations were determined using a Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Spectro, Kleve, Germany).

Compound (mg l <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	Cl <sup>-</sup>	Na <sup>+</sup>	K <sup>+</sup>	SO <sub>4</sub> <sup>2- 1)</sup>	salinity <sup>2)</sup>
Freshwater type	4.2±0.3	0.03±0.01	97±2	61±1	4.8±0.1	59±1	0.1
Oligohaline type	4.4±0.2	0.04±0.01	1314±28	827±13	33.5±0.7	260±3	2.9

<sup>1)</sup> Based on total sulphur measurements by ICP-OES.

<sup>2)</sup> Measured with salinity electrode (Multi 340i, WTW, Weilheim, Germany)

### Sampling

Soil, water and vegetation samples were taken throughout the experiment. Soil sampling was restricted to four main sampling campaigns: 1. during collections of the monoliths (May and June 2004), 2. three weeks after the start of the experiment (July 2004), 3. at the end of the growing season (October 2004) and 4. at the end of the experiment (July 2005). During the last two sampling campaigns plant biomass samples were collected as well. Surface water was sampled weekly during the first two months, then monthly and towards the end of the experiment bi-monthly.

Soil samples were taken with a foil corer (ø 4 cm, length 10 cm) (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) to prevent disturbance of the soil cores. Soil cores for incubation purposes were collected with PVC tubes (ø 3 cm, length 12 cm). The holes in the monoliths, created by sampling, were filled by PVC tubes to keep the structure intact and prevent excess oxygen and water (with nutrients) from entering the soil. All soil samples were immediately put on ice and after transport to the laboratory stored at 4°C. Samples were processed as quickly as possible, but not later than one week after collection.

Surface water was collected with 100 ml polyethylene flasks and stored at  $-20^{\circ}\text{C}$  until further analysis. Aboveground biomass was sampled by cutting a surface of  $0.04\text{ m}^2$  at 1 cm above soil surface. The samples were stored at  $4^{\circ}\text{C}$  until analysis within two weeks.

#### *Chemical analyses*

Soil extractable concentrations of ammonium and nitrate were determined using a 0.4 M KCl-extraction (Houba et al. 1989). 10 g of fresh material was weighed, 100 ml of extraction solution was added and the sample was shaken for one hour at 100 rpm. The pH in the soil suspension was determined using a standard pH meter (WTW Measurement Systems, Ft. Myers, FL). After centrifuging (4 minutes 4000 rpm) the supernatant was filtered and stored at  $-20^{\circ}\text{C}$  until concentrations  $\text{N-NH}_4^+$  and  $\text{N-NO}_3^-$  were determined colourimetrically on a continuous flow analyser (SA-40, Skalar Analytical, Breda, the Netherlands).

Soil moisture content was determined gravimetrically after drying 20 g of fresh soil during 24 hours at  $105^{\circ}\text{C}$ . Soil organic matter content was determined by loss on ignition of dry ground soil at  $550^{\circ}\text{C}$  during 2.5 hours. Total soil nitrogen concentration was determined with a salicylic acid thiosulphate modification of the Kjeldahl digestion (Bremner & Mulvaney 1982). N concentration was determined colourimetrically on a continuous flow analyser (SA-40, Skalar Analytical, Breda, the Netherlands). The concentrations of  $\text{N-NH}_4^+$  and  $\text{N-NO}_3^-$  in the water samples were determined colourimetrically on an autoanalyser (Technicon AA II systems, Tarrytown, NY).

The aboveground biomass was sorted in living and senescent fractions, dried at  $70^{\circ}\text{C}$  for 48 hours and then weighed, than ground and stored until further analysis. Per sample 150 mg was used to determine nitrogen and phosphorus concentrations with an identical digestion method used to determine total N in the soil.

#### *Nitrogen conversion rate measurements*

Actual denitrification rates were measured using an intact core incubation method with acetylene inhibition in air-tight glass jars (Ryden et al. 1987). Acetylene inhibits the final step ( $\text{N}_2\text{O}$  to  $\text{N}_2$  conversion) in the chain of enzymatic reactions during denitrification (Yoshinari & Knowles 1976). The accumulation of  $\text{N}_2\text{O}$  can be measured in the headspace as an estimation of the actual denitrification rate in the soil. We used a slightly adapted version of the method of Hefting et al. (2003), focusing on the accumulation of  $\text{N}_2\text{O}$  after one hour of incubation compared to control measurements, as activity was mainly restricted to the first hour. Per soil monolith three soil cores were collected and wrapped in perforated aluminium foil. They were stored in 1.2 L glass jars under ambient atmospheric conditions for 16 hours at  $4^{\circ}\text{C}$  as direct analysis was not possible due to transport and time constraints. At the start of incubation, jars were flushed with nitrogen gas and sealed air-tight, amended with 100 ml acetone-free acetylene (app. 10% v/v) and incubated at average soil temperature ( $18^{\circ}\text{C}$ ). Gas samples were taken through air-lock septa 1 hour after the start of the incubation. Gas samples were analyzed by a gas chromatograph (Model 3300; Varian, Palo Alto, CA) equipped with an electron capture detector (ECD 63Ni) and Porapak Q columns (2-m-long packed columns; Alltech Associates, Deerfield, IL).

Potential denitrification rates (Denitrification Enzyme Activity - DEA) were determined at the end of the experiment (July 2005) to determine the size of the active denitrifying microbial community. Rates were measured in anoxic slurries with excess substrates added, composed of 10 g fresh wt soil and 10 ml of incubation medium. Nitrogen was added to the medium as  $\text{KNO}_3$  to a final concentration of  $10\text{ }\mu\text{g N-NO}_3^-$  per g fresh wt soil, while easily degradable carbon sources were added to a final concentration of 2 mg C-acetate and 2 mg C-glucose per g fresh wt soil. Glass infusion flasks (75 ml) containing 10 g of homogenised

soil per soil monolith were evacuated through air tight septa. Per flask, 7 ml acetylene was added plus 10 ml of incubation medium and finally  $N_2$  until ambient air pressure was restored. Samples were vortexed and incubated at 18°C. Gas samples were collected 4 and 8 hours after incubation started. The increase in  $N_2O$  concentration between the two sample moments was used to calculate DEA. Gas sample analyses and calculations were done similarly as for the actual denitrification measurements.

Net mineralisation rates were calculated by comparing the increase in 0.4 M KCl extractable  $N-NH_4^+$  and  $N-NO_3^-$  after a 14 day incubation at 18°C in the laboratory. Soil cores were incubated in PVC tubes, closed off with a PVC lid at the lower end and sealed with perforated paraffin film at the top to prevent drying out but facilitating gas exchange. After 14 days, soils were homogenised and extracted. Calculated mineralisation rates were corrected for N losses due to denitrification derived from the actual denitrification rate measurements.

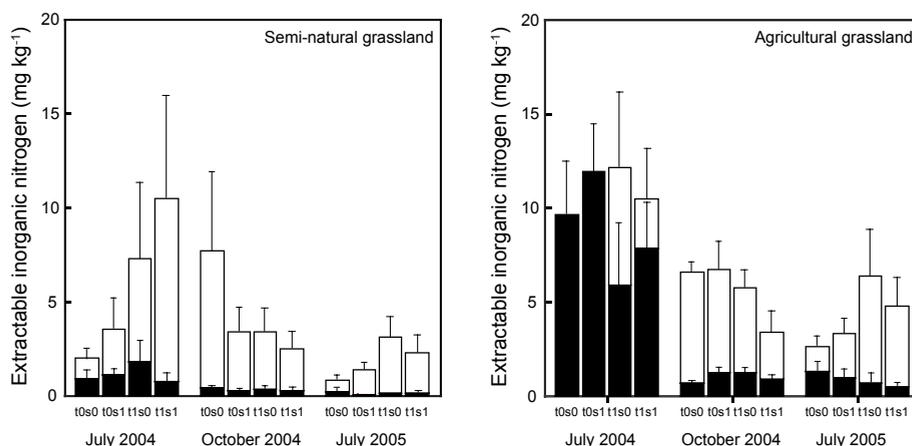
#### Data analysis and statistics

In all cases, statistical analyses of treatment effects were performed separately for soils from different origin. We chose this statistical approach as initial values of specific soil parameters were rather different (Table 1), which resulted in extremely heteroscedastic variables. Plant nutrient parameters met all conditions for performing ANOVA. Variances of soil nutrient parameters were in many cases heterogeneous as zero-values occurred, the most suitable solution to deal with this problem was determined for all tests individually; either transforming original data or performing non-parametric tests. The statistical software package SPSS 12.0.1 (SPSS Inc., Chicago, Ill.) was used for all statistical analyses.

## Results

#### Soil nitrogen parameters

Extractable inorganic nitrogen in the soil was initially higher in the agricultural grassland soils compared to the semi-natural grassland soils (Table 1). In general, this difference between soils persisted during the experiment (Rep Meas ANOVA,  $F=25.459$ ,  $p=0.000$ ) (Figure 3).



**Figure 2.** Concentrations of 0.4 M KCl extractable inorganic nitrogen, as summation of  $N-NO_3^-$  (black bars) and  $N-NH_4^+$  (white bars) during the experiment per treatment. Soil samples were collected in July, October 2004 and July 2005. Treatment codes indicate; t0s0: stagnant-fresh, t0s1: stagnant-oligohaline, t1s0: tidal-fresh and t1s1: tidal-oligohaline. Values are presented separately for the semi-natural (left) and agricultural grassland soils (right).

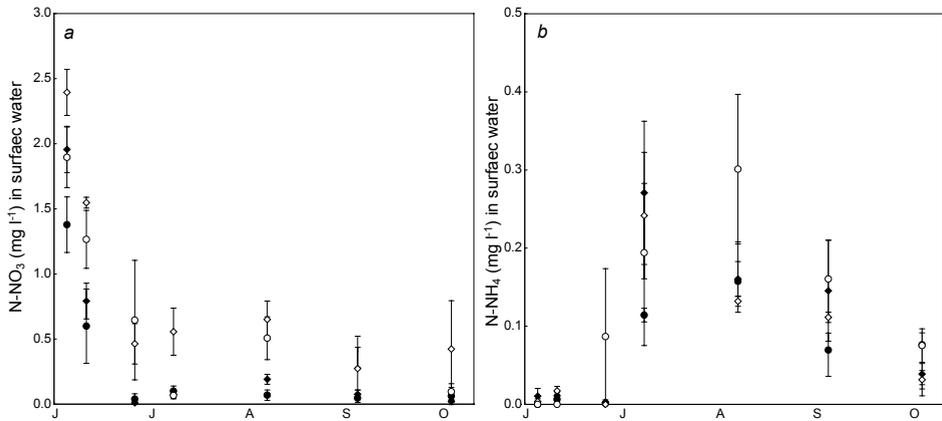
Treatment effects on available inorganic soil nitrogen concentrations were restricted to measurements at the end of the experiment (July 2005). A significant effect was only observed for semi-natural grassland soils (Ln transformed data, 2-way ANOVA,  $F=5.804$ ,  $p=0.033$ ), in which inorganic nitrogen was higher in tidal than stagnant treatments, whereas this effect was not-significant for the agricultural grassland soil (2-way ANOVA,  $F=4.154$ ,  $p=0.064$ ). The two soil types differed in the importance of the two inorganic nitrogen species ammonium and nitrate. Ammonium was the dominant species in the semi-natural grassland soil from the start and this did not change during the experiment, regardless of the treatments. In the agricultural grassland soil, nitrate was high while ammonium was almost absent before the experiment. The July 2004 measurements, three weeks after the onset of the experiment, revealed that in tidal treatments nitrate had decreased and ammonium had increased rapidly. From October 2004 onwards ammonium was the dominant N species in the agricultural soils too.

The concentration of total soil nitrogen (Kjeldahl; organic plus inorganic) was measured in July 2004 and July 2005 (Table 3). Total nitrogen concentrations were higher in the semi-natural grassland soils compared to agricultural soils at both times (T-Test,  $t=6.473$ ,  $p=0.000$  and  $t=3.053$ ,  $p=0.006$  respectively). No treatment effects within one sampling time were found, except in the tidal-fresh treatments, where total N was significantly lower in July 2005 compared to a year earlier for monoliths from the semi-natural grassland soils.

**Table 3** Changes in total (organic and inorganic) nitrogen in the soil ( $\text{mg g}^{-1}$ ) during the experiment as affected by tidal and salinity treatments for the semi-natural and agricultural grassland separately. The significance of change over the year within a treatment was tested using an independent samples T-test. ANOVA analyses of soil nitrogen differences within a sampling campaign between treatments did not yield any significant differences. Values are  $\pm$  s.e.m.,  $n=4$

Semi-natural grassland	total soil N concentration ( $\text{mg g}^{-1}$ )			
	<i>Tide0-Salt0</i>	<i>Tide0-Salt1</i>	<i>Tide1-Salt0</i>	<i>Tide1-Salt1</i>
July 2004	$3.84 \pm 0.25$	$3.82 \pm 0.26$	$5.35 \pm 0.63$	$4.37 \pm 0.72$
July 2005	$2.60 \pm 0.58$	$4.61 \pm 0.74$	$3.12 \pm 0.80$	$3.77 \pm 0.64$
p-value T-test (2-tailed)	0.123 <sup>n.s.</sup>	0.483 <sup>n.s.</sup>	0.014*	0.328 <sup>n.s.</sup>
Agricultural grassland	total soil N concentration ( $\text{mg g}^{-1}$ )			
	<i>Tide0-Salt0</i>	<i>Tide0-Salt1</i>	<i>Tide1-Salt0</i>	<i>Tide1-Salt1</i>
July 2004	$3.12 \pm 0.21$	$3.49 \pm 0.19$	$3.46 \pm 0.20$	$3.12 \pm 0.15$
July 2005	$2.39 \pm 0.61$	$2.30 \pm 0.43$	$2.25 \pm 0.27$	$2.10 \pm 0.31$
p-value T-test	0.345 <sup>n.s.</sup>	0.139 <sup>n.s.</sup>	0.081 <sup>n.s.</sup>	0.068 <sup>n.s.</sup>

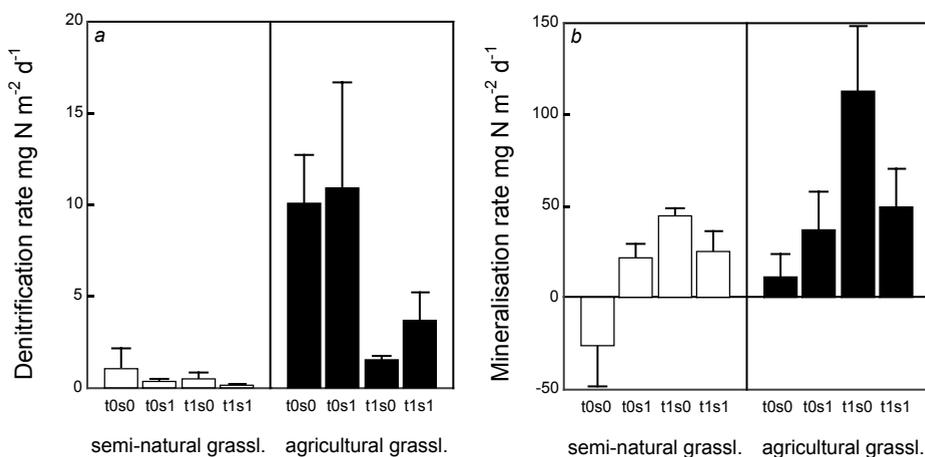
Nitrate concentrations in the surface water dropped within one month after the start of the experiment from values of  $1.5 - 2.5 \text{ mg N l}^{-1}$  to approximately  $0.5 \text{ mg N l}^{-1}$  (Figure 4a) in all treatments. In July 2004, an effect of tide was present with higher nitrate concentrations in tidal treatments (squared root transformed data, ANOVA,  $F=9.550$ ,  $p=0.009$ ). In October 2004 however, the effect was no longer present (squared root transformed data, ANOVA,  $F=2.614$ ,  $p=0.132$ ). Ammonium was low in the surface water at the start of the experiment; only from mid July onwards was ammonium found in all basins (Figure 4b). During the growing season, ammonium in the surface water generally increased until the end of August, but decreased from September onwards to below detection limits ( $0.1 \text{ mg N l}^{-1}$ ). No treatment effects on ammonium concentration in the surface water were present at any of the sampling times.



**Figure 4.** Concentrations of  $\text{N-NO}_3^-$  (a) and  $\text{N-NH}_4^+$  (b) in the water compartments of the mesocosm during the experiment from June 2004 to October 2004. Tidal treatments are indicated by open (tidal movement present) and closed markers (stagnant), salinity conditions are indicated by diamonds (freshwater) and circles (oligohaline). Error bars indicate 1.0 s.e.m.,  $n=4$ .

#### *Actual nitrogen conversion rates*

Three weeks after the start of the experiment (July 2004), no treatment effects on either denitrification or mineralisation rate were detected for both soil types (not shown). The measurements during October 2004 revealed that the tidal treatment had reduced actual denitrification rates in the agricultural soil compared to stagnant treatments, whereas still no effects were present for the semi-natural grassland soil (Figure 5a, Table 4). In addition, treatment effects of tide were present for mineralisation rates, with higher rates in tidal treatments compared to stagnant treatments in both semi-natural and agricultural grassland soils (Figure 5b, Table 4). No direct effects of the salinity treatments were present; only an interaction effect was found for mineralisation rates in the semi-natural soils, related to



**Figure 5.** Soil nitrogen conversion rates (measurements: October 2004); denitrification (a) and mineralisation (b) rates per treatment. Treatment codes indicate; t0s0: stagnant-fresh, t0s1: stagnant-oligohaline, t1s0: tidal-fresh and t1s1: tidal-oligohaline. Values are presented separately for the semi-natural (white bars) and agricultural grasslands (black bars). Refer to Table 4 for statistical testing of possible treatment effects. Error bars indicate 1.0 s.e.m.,  $n=4$ .

**Table 3.** Results of two-way ANOVAs concerning soil nitrogen conversion rates and quantitative biomass parameters at the end of the first growing season (October 2004). Statistical testing was performed within soil type for effects of the experimental factors 'tide' (stagnant vs. tidal movement) and 'salinity' (fresh vs. oligohaline). Significant effects are indicated with \*  $p < 0.05$ ,  $n=4$  (Figure 5a-b and 7a-d).

	Tide		Salinity		Tide x Salinity	
	F	p-value	F	p-value	F	p-value
<i>Semi-natural grassland</i>						
Soil nitrogen conversion rates						
Ln denitrification ( $\text{mg N m}^{-2} \text{d}^{-1}$ )	0.286	0.602	0.661	0.432	0.000	0.983
Mineralisation ( $\text{mg N m}^{-2} \text{d}^{-1}$ )	8.265	0.014*	1.211	0.293	6.648	0.024*
Quantitative biomass parameters						
Aboveground biomass ( $\text{g dw m}^{-2}$ )	0.513	0.487	7.512	0.018*	1.983	0.184
N standing stock ( $\text{g N m}^{-2}$ )	0.035	0.855	3.964	0.070	0.205	0.659
Ln N standing stock	0.502	0.823	5.052	0.044*	0.077	0.787
N:P ratio	0.246	0.629	0.772	0.379	0.120	0.735
Nitrogen concentration ( $\text{mg g}^{-1}$ )	0.048	0.830	0.334	0.547	1.106	0.314
<i>Agricultural grassland</i>						
Soil nitrogen conversion rates						
Ln denitrification ( $\text{mg N m}^{-2} \text{d}^{-1}$ )	8.521	0.013*	0.185	0.675	0.684	0.424
Mineralisation ( $\text{mg N m}^{-2} \text{d}^{-1}$ )	4.826	0.048*	0.533	0.479	2.933	0.112
Quantitative biomass parameters						
Aboveground biomass ( $\text{g dw m}^{-2}$ )	1.252	0.285	3.807	0.075	1.192	0.296
N standing stock ( $\text{g N m}^{-2}$ )	0.447	0.516	1.796	0.205	0.383	0.548
N:P ratio	0.947	0.343	2.727	0.125	0.628	0.443
Nitrogen concentration ( $\text{mg g}^{-1}$ )	4.297	0.060	1.407	0.258	1.059	0.324

negative values (i.e. nitrogen immobilisation) for the stagnant-fresh treatment combination. Furthermore, overall nitrogen conversion rates was higher in the agricultural grassland soils compared to the semi-natural grassland soils, especially denitrification rates were almost one magnitude higher.

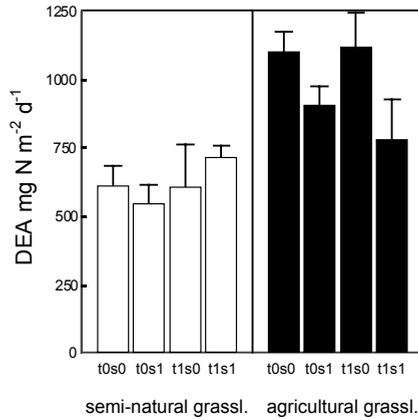
#### *Potential denitrification rates*

Potential denitrification rates were on average lower in the semi-natural grassland soils,  $609 \pm 168$  compared to  $974 \pm 542$   $\text{mg N m}^{-2} \text{d}^{-1}$  for the agricultural grassland (T-test  $p=0.000$ , Figure 6). A treatment effect was present for salinity, with DEA values lower in oligohaline treatments for the agricultural soil only (Table 5).

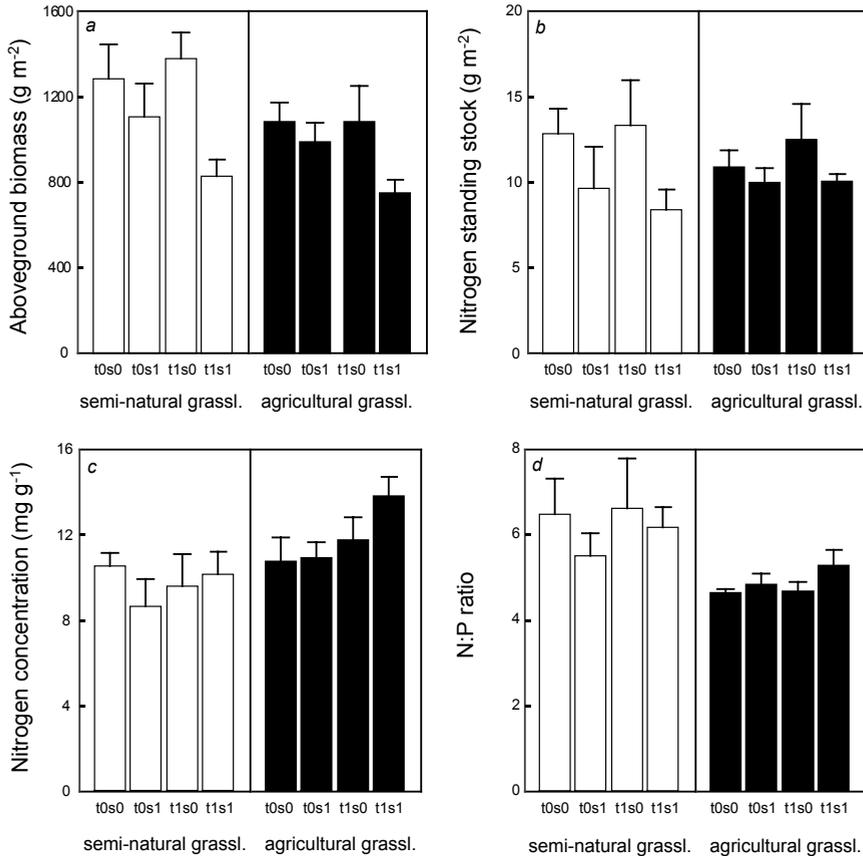
A stepwise regression analysis was also performed on potential denitrification rates incorporating several soil parameters (moisture content, pH, organic matter content, total nitrogen and soil nitrate content) as possible explanatory factors. The data were grouped by soil origin for this analysis, regardless the tidal or salinity treatments of the soil monoliths. In the semi-natural grassland soil, no model could be deduced for DEA. However, a significant model ( $p=0.037$ ) was present in the agricultural grassland soil, with soil nitrate concentrations as the only significant independent variable for DEA in the regression model.

#### *Vegetation parameters*

At the end of the growing season (October 2004), standing biomass was not significantly different between semi-natural and agricultural grassland monoliths (T-test,  $T=1.715$ ,  $p=0.097$ ) (Figure 7a). In the semi-natural grasslands, biomass was significantly less in



**Figure 6.** Potential denitrification rates (DEA) per treatment (measurements: June 2005). Treatment codes indicate; t0s0: stagnant-fresh, t0s1: stagnant-oligohaline, t1s0: tidal-fresh and t1s1: tidal-oligohaline. Values are presented separately for the semi-natural (white bars) and agricultural grasslands (black bars) Refer to Table 5 for statistical testing of possible treatment effects. Error bars indicate 1.0 s.e.m., n=4.



**Figure 7.** Quantitative vegetation variables per treatment (October 2004). Values of peak standing biomass (a), nitrogen standing stock (b), nitrogen concentration in living biomass (c) and N:P ratio in living biomass (d). Treatment codes indicate; t0s0: stagnant-fresh, t0s1: stagnant-oligohaline, t1s0: tidal-fresh and t1s1: tidal-oligohaline. Values are presented separately for the semi-natural (white bars) and agricultural grasslands (black bars) Refer to Table 4 for statistical testing of possible treatment effects. Error bars indicate 1.0 s.e.m., n=4.

oligohaline treatments compared to freshwater treatments (Table 4). No effect of tide on biomass was detected. Standing biomass on the agricultural grassland monoliths soils was not affected by either tide or salinity treatments. Nitrogen standing stock was not significantly different between soil monoliths from different origin at the end of the growing season (T-test,  $T=0.164$   $p=0.871$ ) (Figure 7b). Ln transformed data did reveal a negative effect of salinity on N standing stock on the semi-natural grassland monoliths related to biomass differences, while this effect was absent for the agricultural grassland vegetation. No treatment effects of tide were found in both vegetation types. Nitrogen concentrations and N:P ratios in living tissue were different between vegetation from different origin (Figure 7c-d), with highest N concentrations in the agricultural grassland vegetation (T-test,  $T=2.766$ ,  $p=0.01$ ) but lowest N:P ratios (T-test,  $T=3.358$ ,  $p=0.003$ ). No treatment effects on tissue nutrients were found for both vegetation types.

## Discussion

Two main important points can be derived from this study: firstly, tidal treatment enhanced mineralisation rates as expected whereas denitrification was either lower or not affected, which was not foreseen. Secondly, oligohaline treatments had virtually no effect on soil nitrogen conversion rates and soil nitrogen concentrations, whereas it did have a negative effect on productivity of the vegetation of the semi-natural grassland only.

### *Mesocosm application considerations*

Mesocosm experiments are designed to mimic a selected part of existing ecosystems to study processes at a small scale more closely (e.g. Odum 1984; Carpenter 1996). In our case, the experiment was used to study nitrogen processes in two soil-vegetation types. The starting point was comparable to field conditions, but a divergence from the actual situation in the field occurred over time, concerning mostly the input and output of inorganic nitrogen, but also contents of organic matter and associated organic nitrogen (and phosphorus). Surface water nitrate concentrations decreased rapidly over time (Figure 4a), most likely by denitrification or consumption by plants and algae. In the field situation, nitrate would be replenished continuously by either river water input (e.g. Jordan et al. 1983; Howarth et al. 1996; Verhoeven et al. 2006) or possibly also by nitrogen-enriched groundwater (Flindt et al. 1999; Tappin 2002). The overall depletion of nitrogen in the mesocosms was also visible in the decrease in organic nitrogen over one year (Table 3, T-test all measurements combined,  $t=3.952$ ,  $p=0.000$ ) and the significantly lower nitrogen concentrations in plant tissue at the end of the experiment (not shown) compared to concentrations at the end of the first growing season (October 2004). Therefore we restricted ourselves predominantly to results from analysis in the first period of the mesocosm experiment (until October 2004).

### *Comparison of semi-natural and agricultural grassland soils*

The total soil nitrogen concentrations found in this study, on average  $4.3 \text{ mg g}^{-1}$  for semi-natural grassland soils and  $3.4 \text{ mg g}^{-1}$  for agricultural grasslands soils, are comparable with values found for grassland soils in earlier studies; i.e. up to  $3.75 \text{ mg g}^{-1}$  for 50 year old *Lolium perenne* L.-grasslands (Accoe et al. 2004) and  $2.95 \text{ mg g}^{-1}$  for a grazed floodplain grassland (Van Oorschot et al. 2000). Lowest total nitrogen concentrations were found in the soils from the agricultural grassland. Although one might expect those to be higher due to decades of fertilisation and manure applications (Schott 2001; Edmeades 2003), the higher organic matter content in the semi-natural grassland soil apparently has resulted in higher organic (total)

nitrogen concentrations (Chapter 5; Morse et al. 2004). Nevertheless, the effects of agricultural use are clearly visible in the availability of inorganic nitrogen in the soil, which is highest in the agricultural grasslands soils throughout the experiment (Figure 3). In addition, biogeochemical process rates involving nitrogen conversions - mineralisation and denitrification - were also higher in these monoliths (Figure 5). Values up to  $10 \text{ mg N m}^{-2} \text{ d}^{-1}$  for denitrification found in the agricultural grassland soils were equal to the highest levels measured in a study of managed grassland soils in the Netherlands (De Klein & Van Logtestijn 1994) or in chronically nitrate loaded grass-vegetated riparian buffer zones (Hefting & de Klein 1998). They were well within the range of 1 and  $25 \text{ mg N m}^{-2} \text{ d}^{-1}$  documented for Dutch floodplain grasslands (Verhoeven et al. 1998). Higher mineralisation rates will lead to faster turnover of plant litter and soil organic matter and are therefore most likely also responsible for the difference in soil organic matter content between the semi-natural and agricultural grassland soils (Bridgman et al. 1998; Haag & Kaupenjohann 2001).

Ammonium concentrations were similar across both soil types, apart from higher ammonium concentrations in the semi-natural grassland soil during tidal conditions in the first month of the experiment. This difference was most likely caused by the initially higher soil moisture content of these soils, promoting dissimilatory nitrate reduction (Bonin et al. 1998), meanwhile suppressing nitrification due to oxygen shortage (Phillips 1999). During the experimental period, soil moisture levels in both soil types became more alike and similar to the original values of the semi-natural grassland soil (not shown), resulting in relatively high ammonium concentrations compared to nitrate. Nitrate concentrations remained higher in the agricultural grassland soil until the end of the experiment due to higher mineralisation and nitrification rates (Van Oorschot 1994; Phillips 1999). Differences in soil available nitrogen were reflected in standing biomass nitrogen concentrations, which were higher in the agricultural grassland vegetation.

Not only actual denitrification rates, but also potential denitrification (DEA) rates were highest in the agricultural grassland soil. This suggests that the active population of denitrifiers is larger in these soils, probably as a consequence of higher availability of nitrate or electron donors (carbon). Furthermore, from the stepwise regression analyses it can be deduced that, within this mesocosm experiment, there is an overall control of potential DEA by soil nitrate concentrations with respect to the agricultural grassland soils. This is in line with earlier studies (e.g. Reddy & Patrick Jr. 1984; Groffman & Tiedje 1989; Schipper et al. 1993). Additional analyses revealed that indeed in both soil types nitrate and/or carbon addition increased DEA (not shown).

#### *Effects of tide*

We expected nitrogen conversion rates to be higher in soils subjected to a tidal water regime. Indeed, mineralisation rates were higher in the tidal treatments across both grassland soil types, probably as a result of (temporarily) oxic conditions in these treatments (Flindt et al. 1999). Denitrification rates however, were not positively affected by tide, even lower denitrification rates were measured for tidally treated agricultural grassland soil monoliths in October 2004 compared to the stagnant treatments. If the actual denitrification rates would have been slowed down by the depletion of the nitrate pool in October 2004, we would have expected to see higher denitrification rates during the July 2004 measurements in the tidally treated soil monoliths from the agricultural grassland soils. However, no such treatment effects were present at the time (not shown). Therefore, we have to consider an alternative explanation; the tidal treatment did not enhance a coupling of nitrification / denitrification processes (Patrick Jr. & Reddy 1976), but it led to a more oxic soil compartment, and was therefore less favourable for denitrification (Olde Venterink et al. 2003a). This explanation is

supported by the fact that nitrate concentrations, although depleting rapidly as well, remained somewhat higher in the tidal basins compared to the stagnant treatments in July 2004. On the other hand, the faster replacement of nitrate by ammonium in the soil in the tidally treated agricultural grassland monoliths compared to stagnant treatments does not support the alternative explanation directly. It can be seen as an argument in favour of the original hypothesis as it suggests an overall lower oxygen availability in the tidal treatments, with accumulation of ammonium as a consequence (e.g. Waring & Bremner 1964; Buresh & Patrick Jr. 1981; Bowden 1986). However, the apparent lower activity of ammonia-oxidising bacteria in more oxygen-rich tidal treatments could be subscribed to the fact that they are poorer competitors for oxygen compared to the relatively large population of aerobic decomposers in these soils (Bodelier & Laanbroek 1997). Although neither detailed redox nor soil moisture oxygen saturation data are available, the aforementioned arguments support the alternative hypothesis stating that a relatively more oxic condition is on average present in the tidal treatments.

The N:P ratios in aboveground living tissue ( $<<14$ ) and rather low nitrogen concentrations (ca.  $10 \text{ mg g}^{-1}$ ) suggest limitation of plant growth by nitrogen availability (Koerselman & Meuleman 1996; Güsewell et al. 2003). Therefore we would expect effects of enhanced nitrogen mineralisation rates on productivity, with higher plant biomass and N standing stock in tidal treatments. However, we did not find any effects of tidal treatment-combinations on vegetation parameters. This could be due to the fact that at the time of the start of the experiment, vegetation was already halfway the growing season. Nitrogen uptake occurs mainly in the first half of the growing season and this might obscure effects of N shortage (Greenwood et al. 1990). However, analysis of vegetation parameters on collected biomass samples from July 2005, one year after the start of the experiment did not reveal any significant effects either (not shown). This suggests that, although nitrogen is a probable candidate in limiting productivity, this element was not limiting plant growth in our experiment to the extent that we could measure it in biomass responses. Aboveground biomass values in our experiment were rather high, clearly above  $600 \text{ g m}^{-2}$ , which is often regarded as upper limit for nutrient availability controlled growth (Wassen et al. 2005).

### *Effects of salinity*

In contrast to our expectation, we did not find any effects of the oligohaline treatments on either soil nitrogen conversion rates or nitrogen availability. We hypothesised that especially in the agricultural grassland soil, microbial communities would specifically be adapted to freshwater conditions and therefore would perform less well under imposed oligohaline treatments (Coci et al. 2005). It is possible that the salinity used in the oligohaline treatments (salinity of 3) was too low to have an inhibiting effect on nitrogen conversion rates. Supporting evidence on these matters is scarce as experimentally imposed salinity changes are rare; Rysgaard et al. (1999) documented a large reduction in denitrification activity in estuarine sediments when salinity was raised from 0 to 10. The experimental difference in salinity in their setup is unfortunately too large to be helpful in either supporting or refuting our assumption.

Although no effects on actual nitrogen conversion rates were found, we were able to detect a salinity effect for potential denitrification measurements (DEA). Lower rates were present under oligohaline conditions for the agricultural grassland soil only. This supports our hypothesis, that the agricultural soil would have more difficulties to adapt to changing salinity, as no legacy of sea influence was present. We were not able to detect this by the actual measurements, as either nitrate or carbon was limiting nitrogen conversion processes strongly and thereby obscuring the salinity effect.

In addition, we also expected a negative effect of oligohaline conditions on vegetation performance (biomass and nutrient uptake). Significantly lower biomass was indeed found for the semi-natural grassland vegetation under oligohaline conditions (non-significant trend for the agricultural grassland vegetation -  $p=0.075$ ). Nitrogen in plant standing stock was also lower on oligohaline treated soil monoliths from the semi-natural grassland. Although the main effect was as expected, we anticipated the largest (negative) salinity effects on the agricultural grassland. This vegetation was expected to be less adapted to oligohaline conditions, which was not confirmed by our data. Therefore it is likely that the imposed treatment with a salinity of 3 was still rather low and inflicted only minor stress on both the semi-natural and agricultural grassland vegetation.

#### *Consequences for estuarine rehabilitation*

The main conclusion of this experimental study, addressing the possible effects of renewed tide and salinity in the closed-off Haringvliet estuary, is that effects on actual soil nitrogen processes and shore grassland vegetation were rather limited within the time frame of the experiment. We only found effects by an imposed tidal regime. In addition, no large differences in response to the tidal and salinity treatments were found for two studied grasslands soils although they differed strongly in soil characteristics (Table 1).

The implication of this finding is that the nitrogen dynamics of semi-natural grasslands in the former estuary still function in a way similar to the original, oligohaline situation with tides. Therefore, rehabilitation planning will not change the nitrogen dynamics of these systems

We are confident to say that if the rehabilitation measures in the former Haringvliet estuary are carried out as planned, drastic changes in soil nitrogen processes and consequently availability of nitrogen for plant growth, but also vegetation composition, even in agricultural grasslands, will not occur. A more gradual change over the years is expected when dominant plant species in present situation will be replaced by species more characteristic for oligohaline tidal conditions, although this might take decades (Piesschaert et al. 2005).

Our findings may apply to ecological restoration of estuarine systems in general, as far as vegetated sites cut off from tidal action are concerned. We did not detect large changes for the Haringvliet estuary in our experiment, therefore we expect that in comparable estuarine rehabilitation projects, measures will not lead to an increase in nitrogen availability for the vegetation. Consequently, effects on plant productivity and vegetation (composition) will be relatively small. However, this only holds as long as restoration leads to a moderate increase in salinity only and if the present vegetation is able to survive the renewed tidal movement.

#### **Acknowledgements**

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*Sunset over the Steenmaard, a rehabilitated floodplain along the river Rhine during high discharge in winter 2002. (Photo: Martijn Antbeunnisse)*

# Chapter 6

## Restoration of floodplain vegetation and the importance of nitrogen: a synthesis

### Introduction

#### *River floodplain degradation in the last century*

River regulation and agricultural use of river floodplains in Western-Europe have had a large impact on their biodiversity and other ecological values (Hohensinner et al. 2004). Hydrodynamic gradients disappeared due to the construction of dikes and other flood defence measures and rivers became heavily loaded with nutrients and pollutants (Admiraal et al. 1993; Kooistra et al. 2004). Also the tidal, estuarine parts of river systems have been altered severely. In addition to increasing eutrophication and pollution occurring in the non-tidal floodplains, the characteristic tidal and salinity gradients were highly altered (Storm et al. 2005; Smits et al. 2006). In the Netherlands, parts of the Rhine-Meuse estuary were closed-off for enhanced sea defence with detrimental consequences for the presence of these characteristic estuarine gradients (Nienhuis et al. 1994; Smit et al. 1997).

*River floodplain rehabilitation since 1970*

In recent decades rehabilitation efforts have been undertaken to re-instate at least part of the ecological values of lowland rivers and their floodplains that used to exist until the 1940's (Boon 1998). The nutrient and pollution load of the river water and sediment of the largest western European rivers became very severe until 1970 and decreased thereafter (Wolff 1978; Cals et al. 1998). Since 1995, rehabilitation has not only been aiming at improving the chemical quality of water and sediment, but also at restoring natural hydrology and geomorphologic processes (Nienhuis et al. 1998; Schropp & Bakker 1998; Buijse et al. 2002). In the Netherlands, such restoration projects are being carried out within the Space for Rivers programme, which combines flood defence measures with floodplain restoration (Nienhuis & Leuven 2001; Hooijer et al. 2004; Pfister et al. 2004). In tidal river systems and estuaries rehabilitation efforts concern the reintroduction of tidal movement and seawater incursion, thereby renewing gradients of tidal range and salinity (e.g. Chapter 5; Smit et al. 1997; Tanner et al. 2002; Smits et al. 2006).

Restoration of characteristic floodplain vegetation types with accompanying faunal diversity is a main goal of river floodplain rehabilitation projects. Target vegetation types for non-tidal floodplains are soft- and hardwood forests, riverine grasslands and sedge marshes, whereas well-developed zones of helophyte species are aimed for in tidal systems (Bal et al. 2001; Nienhuis 2006). However, in many cases, the ecological targets of rehabilitation with respect to development of species-rich vegetation types have not been met so far; plant communities composed of only a small number of common species tend to dominate rehabilitated floodplains instead of the desired target vegetation types (Pedroli et al. 2002; Lamers et al. 2006).

*Floodplain restoration and possible constraints for vegetation development*

Constraints for the development of target vegetation in restored floodplain areas could be caused by new physical, chemical or even biological conditions created by the rehabilitation measures. Re-instatement of a more natural hydrological regime with a higher flooding frequency of the floodplain will strongly affect the existing vegetation. The locally occurring species, including those present in the seed bank, are probably not adapted to these altered flood regimes (Van Eck et al. 2004; Leyer 2005). In addition to hydrological constraints, possible chemical bottlenecks can be identified as well, for instance the presence or accumulation of toxic substances in the soil. The floodplains have been enriched with heavy metals (Cals et al. 1998), which can have a negative impact on the performance of certain species (Oetken et al. 2005). Furthermore, the accumulation of toxic substances is also a realistic danger; sulphate concentrations are high in Western European rivers (Chapter 2), which can lead to the formation of free sulphide under anoxic conditions. Floodplains have been also highly enriched with nutrients, due to sediment deposition and intensive agricultural use within the floodplain which led to an extra input by intensive fertiliser and manure applications (Lamers et al. 2006). Enhanced availability of nitrogen or phosphorus, depending on the type of nutrient limitation, gives fast-growing species a competitive advantage, thereby reducing the diversity of the plant communities (Van Oorschot 1994).

A major question in this research was whether the possible hypertrophic conditions of the rehabilitated floodplains acted as a major constraint for development of target vegetation types. I investigated how plant growth was controlled in herbaceous floodplain communities, i.e. by nutrient availability in the soil, and whether this was different for impacted and more natural river systems as well as for non-tidal and tidal reaches. In addition, other possible bottlenecks also received attention in this study.

## Environmental factors controlling plant growth and community composition of herbaceous vegetation in riverine floodplains

The possible constraints hampering development of herbaceous species-rich vegetation types after floodplain rehabilitation have been introduced in Chapter 1, namely: altered hydrology, enriched nutrient conditions in the floodplain soil, accumulation of toxicants and the absence of target species or a (nearby) source for dispersal. In impacted estuarine reaches of rivers, the absence of natural salinity gradients is an additional possible constraint. The next step in unravelling what specific constraint or set of constraints is preventing the ecological success of the floodplain restoration is to gain understanding how the altered environmental factors are affecting plant growth and thereby the structure and diversity of plant communities in rehabilitated floodplains.

### *Nutrient availability*

The Ancient Egyptians already recognised that floodplain systems are highly productive. Their advanced agricultural practice had its focal point in the floodplains of the river Nile and was controlled by the yearly over bank flooding of the river, which provided nutrient-rich deposits; people and their Kings heavily depended on them (Stanley et al. 2003). Similarly, in temperate regions in Europe, floodplains have also often been used for agriculture and still are. Floodplains in contact with the river channel are mostly in use as pastures. These riverine grasslands have received a large extra input of nutrients by fertilisation and manure application, which has eutroficated the already nutrient-rich floodplain soils (Admiraal et al. 1993; Lamers et al. 2006). As a consequence, almost all systems will be exceeding the intermediate levels of biomass production, known to be sustaining the highest levels of plant-diversity in herbaceous vegetation (Grime 1979). In a number of studies herbaceous floodplain vegetation has been found to be limited in growth by nitrogen (Van Oorschot et al. 1997; Ogden et al. 2002) especially in more natural floodplains which are not in intensive agricultural use (Joyce 2001). Therefore the availability of nitrogen is most likely a very important factor controlling plant community structure and diversity (Güsewell 2005). The research presented here shows that plant growth is still N-limited in the least disturbed floodplain habitats studied, whereas neither nitrogen nor phosphorus limits plant growth in floodplains with a history of agricultural use and/or deposition of nutrient-rich sediments (Chapter 2).

### *Hydrology*

In temperate river floodplain systems, floods occur predominantly in winter (Nilsson et al. 1991) and occasionally in summer (Sykora et al. 1988). Regular winter floods are less important in determining species distributions in floodplains than the occasional summer floods, as plants are more vulnerable for inundation during the growing season (Siebel & Blom 1998; Van Eck et al. 2006). It has been shown that summer flooding is always reflected in the presence of plants with adaptations promoting survival in flooded conditions (Klimesova 1994; Vervuren et al. 2003; Van Eck et al. 2005). Oxygen deficiency is probably the most important plant stressor during summer flooding (Crawford et al. 1996). In the mesocosm experiment treated in this thesis summer flooding had a negative impact on plant biomass on Dutch floodplain soils, while biomass remained unchanged on Polish soils. This result was probably related to the absence of summer flooding in the strongly regulated Dutch floodplains, while summer flooding is still common along Polish rivers (Chapter 3). In an additional experiment (see Box 1), the performance and survival after summer flooding of several plant species occurring in floodplains was investigated. The main result of this

## Box 1 - Plant survival after summer inundation

A greenhouse experiment was carried out in which five species, characteristic for either species-rich floodplain grasslands (*Carduus nutans* L., *Lamium maculatum* L. and *Centaurea jacea* L.) or less diverse, more eutrophic vegetation types (*Cirsium arvense* (L.) Scop. and *Urtica dioica* L.) were subjected to a summer inundation of 10 days with artificial river water (temperature >20°C., complete submergence). Plants were grown from seeds during five weeks, selected on similar initial weights, and individually transferred to columns with floodplain soil. Plant survival and performance, measured as biomass compared to biomass of an equal number of controls (both 6), were established immediately after the inundation and 18 days following the flooding event to assess recovery capabilities. Plant survival directly after the inundation was high, only two *C. nutans* plants died (Table 1); 18 days after the inundation also plants of *U. dioica* and *L. maculatum* had died. *C. nutans* was the only species which directly showed a lower performance, 18 days later, all submerged plants had lower performance compared to non-flooded controls, except *U. dioica*. The general conclusion is that summer flooding negatively affects all species tested in this experiment, and that most drastic effects were found for species characteristic for more species-rich floodplain vegetation.

**Table 1.** Survival of five floodplain species following a summer flood event and their relative performance. Performance is defined as the total biomass of inundated plants (above and belowground) to total biomass of non-inundated plants. Significant differences (2-tailed t-test) between biomass of control and inundated plants are indicated with \* if  $p < 0.05$ , \*\* if  $p < 0.01$  and <sup>n.s.</sup> if difference is not significant, +/- s.e.m, n=6.

Species	Survival (%) following inundation event		Relative performance after inundation	
	0 days	18 days	0 days	18 days
<i>Cirsium arvense</i>	100	100	70±30 <sup>n.s.</sup>	45±12*
<i>Urtica dioica</i>	100	66	106±19 <sup>n.s.</sup>	48±18 <sup>n.s.</sup>
<i>Carduus nutans</i>	66	17	28±14**	0±0**
<i>Lamium maculatum</i>	100	33	69±22 <sup>n.s.</sup>	5±4**
<i>Centaurea jacea</i>	100	100	91±18 <sup>n.s.</sup>	36±11**

experiment was that species characteristic for desired target vegetation types (Bal et al. 1995), suffered more from the summer flooding than more common species with broad ecological amplitudes.

In tidal floodplain systems, the hydrological dynamics are rather different with a tidal inundation almost twice a day and some gradual changes during a month from neap tide to spring tide. Therefore, species presence and community composition is strongly controlled by hydrology in tidal systems (Clevering & Van Gulik 1997; Coops et al. 1999). I demonstrated in a field study in Ireland and the Netherlands that tidal range was the most important factor in determining the distribution of plant communities (Chapter 4). The tides created zonations starting from ruderal vegetation types dominated by *Festuca rubra* L. and *Epilobium hirsutum* L. close to MHW (Mean High Water mark), followed by zones of helophyte species from *Phragmites australis* (Cav.) Steud. via *Bolboschoenus (Scirpus) maritimus* (L.) Palla to *Schoenoplectus spp.* and finally by stands of *Spartina anglica* C.E. Hubb and *Puccinellia maritima* (Huds.) Parl. closest to MLW (Mean Low Water mark).

### Dispersal

The species composition of plant communities is also affected by dispersal of seeds and other diaspores. These processes are very important in a restoration context, where changes in the vegetation are desired. In river systems, dispersal is often not considered as a bottleneck for (re)colonisation by plants as the river itself is an effective transporter of diaspores, albeit only downstream (Goodson et al. 2003). In a preliminary seed bank analysis in a rehabilitated floodplain of the river Rhine it was found that only common species are present in the soil, especially in soils which have been in agricultural use for decades (see Box 2). Although the

## Box 2 - A seedbank study of a partly rehabilitated floodplain along the Rhine

In a partly rehabilitated floodplain (Steenwaard, 51°58' N, 5°12' E) along the river Lek, a distributary of the river Rhine, the composition of the viable seed bank was investigated. We studied the seed bank of both the original floodplain and parts which had been lowered six years earlier. Topsoil samples (0-20 cm depth) were collected and analyzed for viable seeds during a 9 month germination trial (McDonald et al. 1996; Thompson et al. 1997). For each part of the floodplain 20 samples were analyzed. The summary in terms of presence of viable seeds and other propagules of plant species in the top layer is given in Table 1. Interestingly, a more diverse seed bank was found in the excavated parts, therefore we can conclude that recreating connectivity of the floodplain with the main river by excavation has been beneficial. As only a very limited number of species present in the original floodplain soil were missing in the lowered floodplain soil (*Betula spp.*, *Lysimachia vulgaris* L. and *Plantago major* L.), which are all common species, we can conclude that the rehabilitation measure of excavation in this floodplain was no constraint for vegetation development by removing species from the seed bank. These data only concern one single floodplain and extrapolation to other sites must be handled with care. However, Hölzel & Otte (2001) did not find an effect of increasing hydrological connectivity on seed bank composition in floodplains along the Upper Rhine, whereas flooding frequency had a positive effect on the quantity of viable seeds. From a study in the UK it was concluded that transport of seeds was highly related to transport of sediment (Goodson et al. 2003), suggesting that rehabilitation measures promoting flooding and deposition of new sediments would also promote the input of diaspores.

**Table 1.** Presence of viable propagules of floodplain species in excavated and original parts of the Steenwaard floodplain along the river Lek (n=20).

Species	Excavated floodplain 25	Original floodplain 14
<i>Atriplex prostrata</i>	x	
<i>Betula spp.</i>		x
<i>Bidens tripartita</i>	x	x
<i>Capsella bursa-pastoris</i>	x	x
<i>Cardamine hirsuta</i>	x	
<i>Cerastium sp.</i>	x	
<i>Chenopodium sp.</i>	x	
<i>Cirsium arvense</i>	x	x
<i>Conyza canadensis</i>	x	
<i>Epilobium spp.</i>	x	x
<i>Gnaphalium uliginosum</i>	x	
<i>Juncus bufonius</i>	x	
<i>Lycopus europaeus</i>	x	
<i>Lysimachia vulgaris</i>		x
<i>Lythrum salicaria</i>	x	
<i>Matricaria discoidea</i>	x	x
<i>Mentha aquatica</i>	x	
<i>Myosotis palustris</i>	x	
<i>Persicaria mitis</i>	x	
<i>Plantago major</i>		x
<i>Poa spp.</i>	x	x
<i>Ranunculus sceleratus</i>	x	x
<i>Rorippa palustris</i>	x	
<i>Rumex acetosa</i>	x	x
<i>Sonchus arvensis</i>	x	x
<i>Trifolium repens</i>	x	x
<i>Urtica dioica</i>	x	x
<i>Veronica anagallis-aquatica</i>	x	

species density was higher in parts with higher connectivity with the river channel, the number of target species found in the seed bank was very limited.

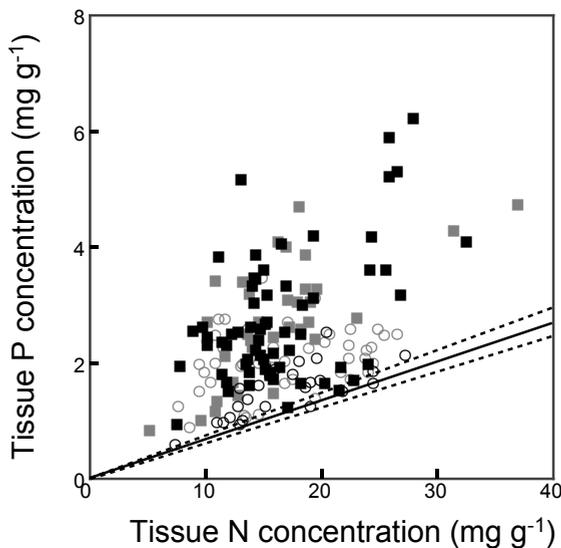
### *Salinity gradients*

In tidal floodplains, salinity is the second most important environmental factor influencing the occurrence of species and community composition (La Peyre et al. 2001; Wetzel et al. 2004). Species-specific adaptations to chloride toxicity determine the boundary conditions with respect to salt concentrations, above which species are not able to survive or out-competed by better adapted species (Hootsmans & Wiegman 1998; Parida & Das 2005). However, oligohaline conditions (salinity of 3), in a mesocosm experiment did not affect species composition in agricultural and semi-natural grassland vegetation in the first year, although overall vegetation performance (biomass production) was somewhat reduced by this treatment (Chapter 5).

## Shifting nutrient limitation along geographical and human impact gradients

### *Shifting nutrient limitation as possible constraint for development of species-rich floodplain vegetation*

Nutrient-enriched conditions in more impacted floodplains were identified as a likely candidate constraining the development of species-rich target vegetation types. The field studies carried out in both non-tidal lowland river floodplains (Chapter 2) and tidal reaches of river systems (Chapter 4) provide information whether there is a shift in type (or presence) of limitation of biomass production by either nitrogen or phosphorus availability along a gradient from more natural towards more impacted rivers systems.



**Figure 1.** Per floodplain, sample site (Chapter 2 and 4) plant tissue nitrogen concentration is plotted with accompanying phosphorus concentration. Impacted sites are represented by black and more natural floodplains by shaded symbols. Open symbols indicate tidal and closed symbols non-tidal floodplains. The critical N:P ratio of 15 (+/- 1) is given. The N:P ratio indicates nitrogen limitation for points above this line (i.e. ratio lower than 15) (Koerselman & Meuleman 1996; Güsewell 2004).

*Tissue N:P ratio: a useful indicator of plant growth limitation in this study?*

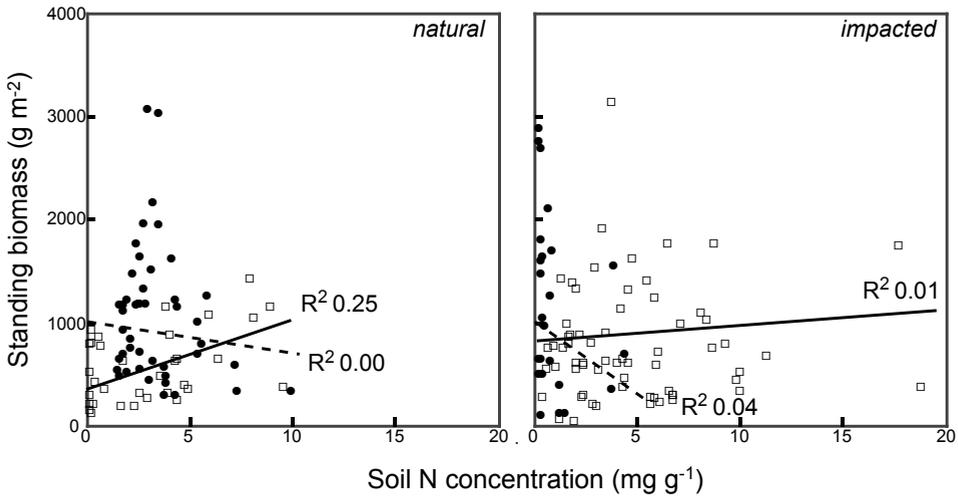
In multiple studies (e.g. Verhoeven et al. 1996; Boeye et al. 1997; Bedford et al. 1999; Willby et al. 2001; Wassen et al. 2005) the ratio between nitrogen and phosphorus in aboveground living biomass was proposed as an indicator of growth limitation by either N or P or both (Koerselman & Meuleman 1996). The N:P ratio is considered as a more robust indicator of limitation compared to the height of nutrient concentrations in plant tissue. Nutrient concentrations are highly variable between individual species and also throughout the year (Güsewell 2004).

An overall analysis with respect to nitrogen to phosphorus ratios in aboveground living biomass of all studied plots in the field surveys (Chapter 2 and 4) provides insight in whether nitrogen or phosphorus is limiting production on a specific point on the gradient continuum (Figure 1). The N:P ratio in tissue clearly indicates an absence of limitation of plant growth by phosphorus in all cases (N:P ratio < 15). All sites have N:P ratios indicating N limitation. It is, however, reasonable to assume that a number of sites were so nutrient-rich that neither N nor P availability limited plant growth, but rather self-shading or other density-dependent mechanisms. Although the existence of a critical N concentration in plant biomass indicating N limitation at the vegetation level has been severely doubted (Güsewell 2004), sites with very high N concentrations (right part of the diagram) are most probably not N-limited. Cases from sites with different characteristics - impacted and more natural as well as tidal and non-tidal conditions- are well-mixed along both axes in the figure, suggesting that there is no difference in type or presence of nutrient limitation between the groups. It, should, however, be realised that the plant species composition differed markedly between sites. This could have resulted in differences in N:P ratios, not caused by a dissimilarity in type or presence of nutrient limitation, but through intrinsic plant species-specific variation in N:P ratio (Güsewell 2004). Therefore I decided to perform an additional correlative analysis to detect possible differences in nutrient limitation.

*Nutrient dependent productivity*

In addition to the use of plant N and P concentrations I also directly compared productivity to the quantity and availability of nutrients in the soil for the non-tidal field sites (Chapter 2). This led to the conclusion that productivity is more dependent on nitrogen availability at natural sites than in impacted floodplains, which was not detected by the analysis of N:P ratio in aboveground biomass. Nevertheless, the importance of nitrogen is confirmed by earlier studies (Van Oorschot et al. 1997; Olde Venterink et al. 2001; Ogden et al. 2002). I repeated the analysis for all sites investigated, including those sampled in the tidal floodplain survey (Chapter 4). Total soil nitrogen concentrations was assumed to give a good prediction of nutrient availability throughout the growing season, instead of concentrations of inorganic nitrogen concentrations which are more dynamic and highly variable (Stenger et al. 2002). Above-ground peak biomass for the sites from both field surveys was correlated with total soil N (Figure 2) and soil P (not shown). Only one significant (linear) relation is found for nitrogen, regarding the more natural non-tidal floodplain sites, significant relations were absent for phosphorus.

The absence of a relation between soil nitrogen and biomass in tidal floodplains, both more natural and impacted, suggests that the importance of nitrogen as a plant growth controlling factor is small in the estuarine floodplains investigated, notwithstanding the low N:P ratios in aboveground biomass. In other studies, salt marshes have been found to have N-limited plant growth (Van Wijnen & Bakker 1999; Sundareshwar et al. 2003; Tyler et al. 2003; Wigand et al. 2004). However, Morse et al (2004) did not find an increase in aboveground productivity by N or P additions in tidal fresh water marshes, while N:P ratios in the biomass



**Figure 2.** The relation between total soil nitrogen concentration and standing biomass at the end of the growing season is given for more natural river floodplains (left) and impacted floodplains (right) (Chapter 2 and 4). Filled circles indicate floodplains of tidal rivers and open squares depict non-tidal river floodplains. Linear regressions are plotted per floodplain type (dotted for tidal and straight lines for non-tidal respectively), a significant positive linear regression is only present for non-tidal sites in natural floodplains ( $p=0.002$ ).

suggested limitation by N, which corroborates our findings. The estuarine habitats sampled in this thesis are all close to channels or creeks and receive high sediment inputs, leading to absence of nitrogen limitation.

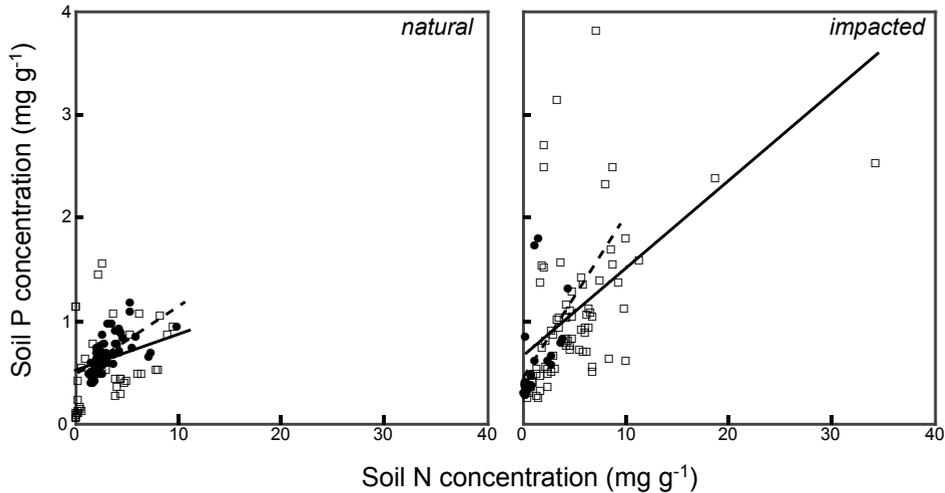
#### *Shifting nutrient limitation along gradients*

My initial hypothesis, in which I expected a shift from predominantly nitrogen limited floodplain plant communities in more natural systems towards disappearance of limitation in (highly) impacted systems, is supported by this overall analysis, for non-tidal river floodplains only. A similar relation was not found for the tidal river floodplains, both in more natural and impacted sites.

### **Biogeochemical processes related to nitrogen and phosphorus in floodplain soils**

#### *Eutrophication*

The soil nutrient concentrations measured during the field studies clearly showed eutrophication of the sediments, on average higher nitrogen and phosphorus concentrations were encountered in the impacted non-tidal river floodplains (Figure 3) (Chapter 2; Lamers et al. 2006). In tidal river systems this effect was not found (Chapter 4). The anthropogenic input there is only visible in the enhanced P concentrations, i.e. a steeper slope of the regression line in the impacted floodplain sediments (Figure 3). Furthermore, both in natural and in impacted floodplain soils, the regression equations are less steep for the non-tidal river floodplain soils. This is probably due to the more dynamic conditions in the tidal systems, with alternating oxic and anoxic soil conditions (Bowden et al. 1991) resulting in higher nitrogen mineralisation, nitrification and denitrification rates, eventually leading to higher N losses, compared to the non-tidal systems.



**Figure 3.** Relation between total (mostly organic) nitrogen and phosphorus in the soil for more natural river floodplains (left) and impacted floodplains (right) (Chapter 2 and 4). Filled circles indicate floodplains of tidal rivers and open squares depict non-tidal river floodplains. Linear regressions are plotted per floodplain type (dotted for tidal and straight lines for non-tidal respectively). Values of R-squared and p-value from testing for significance of positive linear relations are  $R^2$  0.13 -  $p$  0.001,  $R^2$  0.36 -  $p$  0.001,  $R^2$  0.27 -  $p$  0.001 and  $R^2$  0.29 -  $p$  0.001 for natural non-tidal, natural tidal, impacted non-tidal and impacted tidal floodplains respectively.

### Box 3 - Sedimentation; quantity and quality during a 21-day January flood in a floodplain along the Rhine

In the winter of 2002/2003 a number of sediment traps (0.5 x 0.5 m artificial grass mats) were placed in a frequently inundated grassland vegetation located in a floodplain (Steenwaard, 51°58' N, 5°12' E) along the river Lek, a distributary of the river Rhine (photo inset). The quantity of sediment was determined after an inundation period of 21-days, with a maximum submergence of 1.63 m. Hereto the sediment was washed off by a known amount of water, which was later analyzed for concentrations of  $N-NO_3^-$ ,  $N-NH_4^+$  and  $P-PO_4^{3-}$ . Furthermore, the sediment was extracted with a 0.1 M  $CaCl_2$ -solution for the determination of  $N-NO_3^-$  and  $N-NH_4^+$  and with an Olsen solution for the analysis of  $P-PO_4^{3-}$ , after drying for 24 hours at 105°C. See Chapter 5 for more detailed information on the chemical analysis. The total concentrations of inorganic nutrients were recalculated to  $g\ m^{-2}$  (Table 1). In addition, total N and P values were calculated using information from the field survey, carried out in the same floodplain (Chapter 2). Although direct measurements of total N and P were not performed, the calculation exercise done here stresses the fact that by far the larger part of nutrients deposited on the floodplain during sedimentation is in the organic form. These nutrients are not directly available for plant growth, but will at least partly become available over time during the growing season.

**Table 1.** Quantity and quality of sediment, deposited during a 21-day flood event in a floodplain along the River Rhine, values  $\pm$  s.e.m.,  $n=7$ , except total N and P values which were calculated from average bulk sediment deposition.

Bulk DW sediment ( $g\ m^{-2}$ )	485 $\pm$ 39
$N-NO_3^-$ ( $mg\ m^{-2}$ )	21 $\pm$ 3
$N-NH_4^+$ ( $mg\ m^{-2}$ )	100 $\pm$ 10
$P-PO_4^{3-}$ ( $mg\ m^{-2}$ )	3.6 $\pm$ 0.2
Total N ( $mg\ m^{-2}$ )	1600
Total P ( $mg\ m^{-2}$ )	460



*External nutrient sources*

Deposition of sediments with adsorbed inorganic nutrients and particulate organic nutrients is the largest source of nutrients in floodplain systems (Kronvang et al. 1998; Steiger et al. 2003; Van der Lee et al. 2004; Mahaney et al. 2005). In a small-scale sedimentation experiment in a floodplain along the river Rhine total N and P input of one flooding event was 1.6 and 0.46 g m<sup>-2</sup> respectively (see Box 3). These amounts are already in the same order of magnitude as the maximum aboveground standing nutrient stock for herbaceous vegetation in that specific floodplain (Chapter 2). In addition, dissolved inorganic and organic compounds in the flood water are a possible source of nutrients in floodplains (Olde Venterink et al. 2003b; Van der Lee et al. 2004). The external nutrient sources mentioned above naturally occur in river systems including rather pristine systems, albeit less intense. In highly impacted floodplains, there is an additional direct input of nutrients by agricultural or at least there has been in the past. Even in recent years in the Netherlands the input by agricultural has been more than one order of magnitude higher than the yearly input by sedimentation; 50 and 7.5 g m<sup>-2</sup> for N and P respectively (Lamers et al. 2006).

*Internal nutrient sources*

Although the input of nutrients by sedimentation may be large, they are mostly in organic forms, and therefore not directly available to the vegetation (Grootjans et al. 1985; Gale et al. 1992; Van Oorschot et al. 1998; Andersen 2004). The organic material has to be mineralised before plant uptake is possible. In an agricultural soil subjected to a tidal regime, nitrogen mineralisation rates were found to be as high as 0.13 g m<sup>-2</sup> d<sup>-1</sup> at the end of the growing season (Chapter 5). Probably values are highest at that time of the year, as the input of organic matter is high through litter fall and the relatively high temperature favours biogeochemical processes. Even if a yearly mineralisation average is considered to be 10 percent of these values, still 4.5 g N m<sup>-2</sup> is mineralised per year. Notwithstanding the uncertainties, this process can be considered as an important source of inorganic N, but also of P and other nutrients in floodplain systems (Zak & Grigal 1991; Van Oorschot 1994).

Phosphorus, when mineralised to inorganic phosphate, forms strong adsorptive complexes or chemical bindings with cations, especially under oxidised conditions (Zwolsman 1994; Coelho et al. 2004). During flooding, when the soil becomes anoxic and consequently more reduced, phosphate is mobilised, especially when sulphate concentrations are high (Koerselman et al. 1993; Beltman et al. 2000; Shenker et al. 2005). Under anoxic conditions, sulphate is reduced to sulphide (Laanbroek 1990), which will bind to iron, previously bound to phosphate and forms non-soluble FeS or FeS<sub>2</sub> (Smolders & Roelofs 1993; Lamers et al. 1998), resulting in higher dissolved phosphate concentrations (Chapter 3). Therefore, flooding can lead to high availability of phosphorus, so-called internal eutrophication, especially when flood water sulphate concentrations are high (Beltman et al. 2000; Lamers et al. 2001).

*Retention and removal of nutrients*

Many processes, leading to a higher availability of nutrients in floodplain soils have been discussed above. Biological, chemical and physical processes may also reduce the nutrient availability. A robust process of nutrient retention in all terrestrial ecosystems is the uptake of nitrogen and phosphorus by the vegetation (Van Oorschot 1994; Hefting et al. 2005). In the field studies in tidal and non-tidal floodplains (Chapter 2 and 4), values of N and P uptake by the vegetation showed an enormous range (Table 1). Especially in non-tidal more impacted floodplain systems, temporal retention can reach high values of up to 74 g N m<sup>-2</sup>. This value is one order of magnitude higher compared to average yearly input values by sedimentation and mineralisation and is in the same range as input values found under intensive agricultural

**Table 1.** Range of retention of nitrogen and phosphorus during the year, measured in peak standing biomass in floodplains in Poland, Ireland and the Netherlands. Floodplains are split in tidal and non-tidal and impacted and more natural (Chapter 2 and 4).

Uptake	g N m <sup>-2</sup> y <sup>-1</sup>		g P m <sup>-2</sup> y <sup>-1</sup>	
	<i>impacted</i>	<i>more natural</i>	<i>impacted</i>	<i>more natural</i>
non-tidal	1 - 74	2 - 21	0.3 - 6.0	0.3 - 3.2
tidal	2 - 66	6 - 69	0.2 - 5.0	0.5 - 7.0

regimes. However, retention by plant uptake is not a long-term sink, as especially in herbaceous plant communities all aboveground biomass dies off during winter and a major proportion of the nutrients is relocated to the soil compartment via litter fall. Therefore in systems in which no biomass is removed during the year - either through grazing, harvesting or transport by the river - retention of nutrients by plant uptake will not be a major player in removal of nutrients from the system (Van der Lee et al. 2004). On the other hand, storage of nutrients in senescent plant material and litter in the soil will have a buffering effect on the availability of nutrients, as nutrients are less mobile and not available for plant uptake in these form (Haycock & Pinay 1993; Hefting et al. 2005).

An important removal process of nitrogen is provided by bacteria involved in denitrification (Olde Venterink et al. 2003a; Van der Lee et al. 2004; Forshay & Stanley 2005; Gribsholt et al. 2005). Under anoxic conditions, denitrifiers use nitrate as an electron acceptor for breakdown of organic compounds. The products of this biogeochemical process are gaseous forms of nitrogen. Di-nitrogen gas, but also nitrous oxide (N<sub>2</sub>O) can be the end-product under certain conditions (Hefting et al. 2003). These gasses will escape to the atmosphere and the nitrogen will become unavailable for plant growth. When high amounts of nitrate are available, for instance by alternating oxic and anoxic conditions, stimulating both nitrification and denitrification respectively, high process rates can be maintained (Bowden 1986). Many studies have assessed the potential of denitrification in floodplains, and the rates measured show a large range; Olde Venterink et al. (2003a) and Spink et al. (1998) found values close to 1 g N m<sup>-2</sup> y<sup>-1</sup> for river floodplains, whereas Pinay (1993) and Seitzinger (1994) measured rates up to 10 g N m<sup>-2</sup> y<sup>-1</sup>. In the experiment in which soil monoliths were subjected to a tidal hydrological regime (Chapter 5), initial denitrification rates (not shown) were on average up to 20 g N m<sup>-2</sup> y<sup>-1</sup> and even higher maximum values were reached. Therefore, this process has the potential to be important as a major output in the nitrogen budget of floodplain soils and hence to limit the availability of nitrogen for plants. Recently, an additional pathway of nitrogen removal has been reported, i.e. the incomplete microbial oxidation of ammonium to gaseous nitrogen under oxygen-limited conditions by nitrifying bacteria. This latter process, called nitrifier denitrification can be rather substantial compared to denitrification activity and is independent on the presence of degradable carbon (Wrage et al. 2001; Ahn 2006).

Inorganic phosphorus entering a floodplain is not susceptible to permanent removal processes, as is the case with nitrogen. However, floodplain soils have in general a high binding capacity for phosphate, as iron, aluminium and calcium concentrations are often very high (Chapter 3; Lamers et al. 2006). Under more or less natural conditions, most of the phosphate will be bound to these cations and will only be marginally available for plant uptake. In conditions where the soil is highly enriched with phosphate, the binding sites will be all occupied and much higher concentrations of plant available phosphorus will occur (Lamers et al. 1998; Baldwin & Mitchell 2000).

## Identifying constraints and solutions for development of species-rich herbaceous vegetation types following ecological floodplain rehabilitation

### *Hydrology and connectivity*

Ecological rehabilitation of river floodplains is often aiming at restoring hydrological conditions, by removing summer levees and other water defence structures or lowering the floodplain itself (Buijse et al. 2002). This results in prolonged and more frequent flooding in winter, but also in inundations during intermediate discharges in summer. Flooding of vegetation during the growing season can have a tremendous impact on plant performance (see Box 1; Vervuren et al. 2003; Van Eck et al. 2004). Especially a community which has not been subjected to those conditions for a long can be heavily impacted by such an event (Chapter 3).

On the other hand, enhanced connectivity between the floodplain and the river channel also increases the possibility for diaspores and other propagules of target species to reach the floodplain (Bissels et al. 2004; Boedeltje et al. 2004). Therefore on the short term the enhanced connectivity might be a constraint, but in due time when new species have dispersed into the area, target vegetation might develop after all when the environmental conditions become suitable. Unfortunately, in the last century natural, species-rich vegetation types have disappeared from the floodplains of upstream river reaches (Burkart 2001), and the construction of dams hampered hydrochory thereby severely reducing the chance of establishment (Andersen 2004). A preliminary seed bank study already indicated that indeed the input of target species by the river was very limited in a rehabilitated floodplain with a renewed hydrologic connection with the river channel (see Box 2).

### *River water quality*

The concentrations of dissolved nutrients (N and P), but also sulphate and chloride in the river water are still enhanced in impacted river systems compared to historical or geographical references (Chapter 2 and 4) (Verhoeven et al. 2006). In one of the experiments, the impact of the quality of the flood water on vegetation and soil nutrient status was investigated (Chapter 3). It turned out that floodwater containing higher concentrations of nitrate, sulphate and chloride, mimicking Rhine water, had no different effect than cleaner water with lower concentrations of these three ions, mimicking Narew water, on soil nutrient status. Furthermore the actual exchange between surface water and soil during inundation (other than diffusion), is most likely to be rather limited, especially when the soil is water saturated. The extra input of nutrients through particulate matter and sediments transported and deposited by the river is much higher. At present the nutrient load of sediments deposited is probably lower than in the 1970's, but still enhanced due to the fact that large parts of the catchments of temperate European rivers are in agricultural use (Lamers et al. 2006)

### *Nutrient limitation*

The research in this thesis has shown that nutrient enrichment of floodplains has lifted the nitrogen limitation of plant growth, which has resulted in a loss of characteristic plant communities. The absence of N limitation is a major biogeochemical constraint for floodplain restoration. Therefore, restoration efforts should aim at counteracting eutrophication and recreating N-limited conditions in rehabilitated floodplain systems in addition to restoration of the hydrological dynamics.

Nitrogen can be removed from terrestrial ecosystem by harvesting the aboveground biomass and thereby removing N (Bakker 1989; Prach 1996). This could remove up to 74 g N m<sup>-2</sup> standing stock per year in the studied floodplain systems (Table 1), although less on

average. In comparison with total N in the upper 10 cm soil layer in those floodplain soils, this is only marginal, as values upto 3,500 g N m<sup>-2</sup> were found. In that specific case hay-making would take at least 50 years to remove the nitrogen. For soils highly enriched in nitrogen, complete removal of the richest layers should be considered (Schropp & Bakker 1998; Pedrolí et al. 2002). Excavating the topsoil is often already a measure taken in flood defence/rehabilitation projects, as it provides the river with more physical space and reinstates a more natural hydrological regime with a higher flood frequency. However, the costs of large-scale clay excavation are high. Projects on such a scale are only viable when a third party is involved, and the excavation can be combined with either sand or gravel mining or clay extraction for the local brick industries (Helmer 2000; Van der Meulen et al. 2006). Even then excavation is not possible in all cases as parts of floodplains are not only enriched in nutrients, but also in pollutants such as heavy metals, which could lead to these soils being classified as chemical waste (Leuven et al. 2005).

It is also possible to promote natural retention processes to remove nitrogen for instance by enhancing the denitrification rate. This could be done by (temporarily) altering the hydrology and implementin a slowly fluctuating water level, promoting both aerobic processes such as aerobic mineralisation an nitrification and anaerobic processes like denitrification (Bowden 1986). In addition conditions could be created in favour of development of dense helophyte or submerged macrophyte plant communities. These types of vegetation are known to enhance denitrification rates. Furthermore they provide a source of easy degradable carbon, which is an additional requirement for high denitrification rates (Eriksson & Weisner 1999; Olde Venterink et al. 2003a; Fear et al. 2005). However, high nitrate concentrations might favour the production of the greenhouse gas N<sub>2</sub>O instead of N<sub>2</sub> as end-product of denitrification (Hefting et al. 2003).

#### *Estuarine rehabilitation*

In estuarine, tidal systems, I found no evidence for extreme nutrient enrichment and associated shifts in nutrient limitation, so that N availability is not a major factor limiting plant productivity and thereby affecting the community composition. In these systems, the tidal range - coupled to elevation gradients - is the most dominant selecting environmental factor determining the plant species composition (Chapter 4). At the lower end of the tidal range, helophyte plant communities, often a target in restoration projects, benefit strongly from regular water level fluctuations (Coops et al. 1999). In addition, salinity gradients affect the composition of plant communities through selection of species with a higher chloride toxicity tolerance under more saline conditions (Wetzel et al. 2004). However, within the oligo- to mesohaline range, differences in salinity have little effect, so that restoration of the tidal fluctuations is the most essential here. In the more saline parts of the estuary, however, salinity gradients have a strong effect on species composition (Chapter 4 and 5). Therefore, restoring estuarine systems should involve creating conditions as natural as possible instead of only restoring a small tidal amplitude or a limited part of the salinity gradient (Storm et al. 2005)

### **General conclusions on rehabilitation chances**

The results of the studies presented in this thesis give more insight in biogeochemical processes related to nitrogen in temperate tidal and non-tidal floodplains. Furthermore, the research revealed that plant growth was controlled differently in impacted compared to more natural non-tidal floodplains. I concluded that recreating nitrogen-limited conditions will probably favour the development of species-rich vegetation types comprised of target species

and it will counteract the establishment of species-poor, highly productive plant communities. Hay-making and other conventional management methods are likely to not reduce sufficiently the available nitrogen concentrations in the soil, as deposition of new still enriched sediments and mineralisation of organic nutrients still continues. Large-scale surface lowering by extraction of clay or sand might be suitable to remove the most nutrient-rich soil layers and reset the floodplain systems to nitrogen-limited conditions. Additional measures will be still needed to prevent a renewed accumulation of nitrogen in the sediment.

Another important issue concerning floodplain rehabilitation and development of species-rich vegetation is the altered hydrology. The plant species still present in the floodplain, including those forming highly diverse, remnant plant populations, could suffer severely from restoration measures as they are most likely not adapted to prolonged inundation and different timing of flooding. Therefore it is necessary to investigate for every individual river restoration project whether the species, adapted to more natural hydrology, are still present in the area or capable to reach it through river flooding in due time. If this is not the case, additional measures, such as introduction of the species will be necessary to reach restoration targets with respect to the development of species-rich vegetation types.

To successfully rehabilitate estuarine, tidal floodplain systems it is important to recreate more natural hydrological regimes. A broad zonation of different helophyte plant communities is favoured by a larger tidal range, whereas the presence of a salinity gradient only becomes important if salinities are exceeding oligohaline conditions. The sediment quality is of lesser importance, as nutrients are not limiting plant growth either in natural or more impacted tidal floodplain systems. Furthermore, new hydrological conditions following restoration favour nitrogen removal processes. Therefore it is well possible to include highly enriched former agricultural soils in estuarine rehabilitation projects.

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*A side channel of the river Rhine near Nijmegen (Oude Waal) during high discharge in winter 2002-2003.  
(Photo: Martijn Antheunisse)*

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*Pilot field experiment in a floodplain of the river Rhine (Steenwaard). A summer flooding event was simulated by filling large PVC pipes. The pilot experiment did not yet result in a full-scale outdoor summer flooding experiment (Photo: Ronald van den Heuvel).*

# Summary

## **Floodplain rehabilitation and the importance of nitrogen dynamics for plant communities**

This thesis comprises a set of studies on the effects of floodplain rehabilitation on herbaceous plant communities and their development, with special reference to soil nitrogen state and biogeochemical processes. The research focused on restoration measures in non-tidal river floodplains, e.g. enhanced flooding by summer dike removal but also in the tidal, estuarine reaches of temperate river systems, e.g. restoration of tidal range gradients.

## **Floodplains of West European rivers and their vegetation**

Floodplains along the entire river continuum are characterised by high ecological values. They provide various functions for society such as food production, safeguarding the hinterland for flooding, water purification and nutrient retention. Furthermore, natural floodplains harbour highly diverse ecosystems with respect to plant communities and associated faunal assemblages. River regulation and agricultural use of floodplains in Western Europe have had a large impact on their biodiversity and other ecological values. Rivers and their floodplains have been heavily loaded with nutrients and pollutants, such as heavy metals and organic compounds. In addition, agricultural use of floodplain habitats has become very intensive locally with high fertiliser and manure applications. This has caused a decline in natural, species-rich floodplain plant communities. Furthermore, in the Netherlands, parts of the Rhine-Meuse estuary were closed-off for enhanced flood defence after the major coastal flooding disaster of 1953, with detrimental consequences for the ecological integrity of the originally tidal rivers (Chapter 1).

In recent decades rehabilitation efforts have been undertaken to re-instate at least part of the pre-degradation ecological values of lowland rivers and their floodplains. Since 1970 the nutrient and pollution load of the river water and sediment of the largest western European rivers has decreased. Nevertheless, for the Dutch situation, concentrations are still much higher compared to background values. Following the extreme discharge events in the winters of 1993 and 1995, the Dutch government launched the ‘Space for Rivers’ programme (*Ruimte voor de Rivier*). The strategy of only reinforcing and raising dikes was abandoned. Measures taken within this framework are aimed at a partial restoration of the connectivity between the main channel and its floodplains (Chapter 1). At the same time, many programmes are being initiated to recover the lost ecological values of estuaries and tidal river systems. These rehabilitation efforts concern the reintroduction of either tidal movement or seawater incursion or a combination of both (Chapter 5).

Establishment and development of diverse vegetation types are main goals of floodplain restoration projects. Although target vegetation types for floodplain restoration generally are

not restricted to herbaceous species, I specifically focused on grasslands and other herbaceous communities in this thesis. Herbaceous vegetation targets along a hydrology gradient are sedge marshes, species-rich wet grasslands and hayfields. In almost continuously inundated parts of the floodplain, valuable pioneer vegetation can develop during drawdown at low discharge (Chapter 2). Typical plant communities characteristic for tidal floodplains comprise helophyte (e.g. Common reed, *Phragmites australis* (Cav.) Steud.) stands forming a zonation along a flooding gradient. In addition, numerous species are adapted to a specific salt concentrations or large fluctuations in salinity and they occupy their own niche along the salinity gradient (Chapter 4 and 5). However, in many cases, rehabilitation measures have not led to the development of the targeted species-rich vegetation types, but instead to plant communities with a small number of common species.

### **Constraints for successful rehabilitation of plant communities**

Constraints for the development of target vegetation in restored floodplain areas are expected to be related to the new physical, chemical or even biological conditions created by the rehabilitation measures. Re-instatement of a more natural hydrological regime with a higher flooding frequency of the floodplain will strongly affect the existing vegetation. The locally occurring species are not adapted to these altered flood regimes. In the mesocosm experiment treated in this thesis, summer flooding had a negative impact on plant biomass on Dutch floodplain soils, while biomass remained unchanged on Polish soils. This result was probably related to the absence of summer flooding in the strongly regulated Dutch floodplains, while summer flooding is still common along Polish rivers (Chapter 3). In addition to hydrological constraints, geochemical bottlenecks might also exist, for instance the presence or accumulation of toxic substances in the soil. Floodplains have been also highly enriched with nutrients, due to sediment deposition and intensive agricultural use within the floodplain which led to an extra input by intensive fertiliser and manure applications (Chapter 2). Enhanced availability of nitrogen or phosphorus, depending on the type of nutrient limitation, gives fast-growing species a competitive advantage, thereby reducing the diversity of the plant communities.

The comparative field surveys carried out in Dutch and Polish floodplains shows that plant growth is still N-limited in the least disturbed floodplain habitats studied, whereas neither nitrogen nor phosphorus limits plant growth in floodplains with a history of agricultural use and/or deposition of nutrient-rich sediments (Chapter 2).

In tidal floodplain systems, the hydrological dynamics are rather different with a tidal inundation almost twice a day and some gradual changes during a month from neap tide to spring tide. Therefore, species presence and community composition is strongly controlled by hydrology in tidal systems. I demonstrated in a field study in Ireland and the Netherlands that tidal range was an important factor in determining the distribution of plant communities (Chapter 4). In tidal floodplains, salinity is another important environmental factor influencing the occurrence of species and community composition. However, oligohaline conditions in a mesocosm experiment did not affect species composition in agricultural and semi-natural grassland vegetation in the first year, although overall vegetation performance (biomass production) was somewhat reduced by this treatment (Chapter 5).

## Biogeochemical processes in floodplains and consequences for nitrogen availability

The soil nutrient concentrations measured during the field studies clearly showed eutrophication of the sediments, i.e., on average higher nitrogen and phosphorus concentrations were encountered in the impacted non-tidal river floodplains (Chapter 2), while in the tidal river systems only phosphorus concentrations were found to be higher. Due to the more dynamic conditions in the tidal systems, with alternating oxic and anoxic soil conditions and higher nitrogen conversion rates, higher N losses occur, so that the N concentrations remain comparable to those in the non-tidal systems.

Deposition of sediments with adsorbed inorganic nutrients and particulate organic nutrients is the largest source of nutrients in floodplain systems. In a small-scale sedimentation experiment in a floodplain along the river Rhine, total nutrient input by one flooding event was already in the same order of magnitude as the maximum aboveground standing nutrient stock for herbaceous vegetation in that specific floodplain (Chapter 6). In addition to sedimentation, dissolved inorganic and organic compounds in the flood water are a possible source of nutrients in floodplains. In highly impacted floodplains, there is an additional direct input of nutrients by agriculture or at least there has been in the past. Even in recent years in the Netherlands the annual nutrient input through agricultural activities has been more than one order of magnitude higher than the input by sedimentation.

Although large amounts of nutrients may be added to floodplain soils by sedimentation, the nutrients are mostly in organic forms, and therefore not directly available to the vegetation. The organic material has to be mineralised before plant uptake is possible. In an agricultural grassland soil subjected to a tidal regime, nitrogen mineralisation rates were estimated to be even higher than the input of nitrogen by sedimentation (Chapter 6). This process can be considered as an important source of inorganic N, but also of P and other nutrients in floodplain systems. Phosphorus, when mineralised to inorganic phosphate, forms strong adsorptive complexes or chemical bindings with cations, especially under oxidised conditions. During flooding, when the soil becomes anoxic and consequently more reduced, phosphate is mobilized (Chapter 3). Therefore, flooding can lead to high availability of phosphorus, so-called internal eutrophication, especially when floodwater sulphate concentrations are high.

Biological, chemical and physical processes may also reduce the nutrient availability. A robust process of nutrient retention in all terrestrial ecosystems is the uptake of nitrogen and phosphorus by the vegetation. In the field studies in tidal and non-tidal floodplains (Chapter 2 and 4), values of N and P uptake by the vegetation showed an enormous range, but especially in non-tidal more impacted floodplain systems, temporal retention can reach high values. This value is one order of magnitude higher compared to average yearly input values by sedimentation and is in the same range as input values found under intensive agricultural regimes (Chapter 6). However, retention by plant uptake is not a long-term sink, as especially in herbaceous plant communities all aboveground biomass dies off during winter and a major proportion of the nutrients is relocated to the soil compartment via litter fall. Therefore in systems in which no biomass is removed during the year - either through grazing, harvesting or transport by the river - retention of nutrients by plant uptake will not be a major player in removal of nutrients from the system.

An important removal process of nitrogen is provided by bacteria involved in denitrification. Under anoxic conditions, denitrifiers use nitrate as an electron acceptor for breakdown of organic compounds. The products of this biogeochemical process are gaseous forms of nitrogen. When high amounts of nitrate are available, for instance by alternating oxic and anoxic conditions, stimulating both nitrification and denitrification respectively, high process rates can be maintained (Chapter 5). This process has the potential to be important as

a major output in the nitrogen budget of floodplain soils and hence to limit the availability of nitrogen for plants.

## Conclusions and opportunities for rehabilitation

The results of the studies presented in this thesis give more insight in biogeochemical processes related to nitrogen in temperate tidal and non-tidal floodplains. Furthermore, the research revealed that plant growth was controlled differently in impacted compared to more natural non-tidal floodplains (Chapter 2). A major conclusion is that restoration of nitrogen-limited conditions will favour the development of species-rich vegetation types with a high presence of target species and it will counteract the establishment of species-poor, highly productive plant communities. Hay-making and other conventional management methods will probably not suffice to reduce the available nitrogen concentrations in the soil, as deposition of new still enriched sediments and mineralisation of organic nutrients still continues (Chapter 6). Large-scale surface lowering by extraction of clay or sand might be suitable to remove the most nutrient-rich soil layers and reset the floodplain systems to nitrogen-limited conditions. Additional measures, such as hay-making, will still be needed to prevent a renewed accumulation of nitrogen in the sediment.

Another important issue concerning floodplain rehabilitation and development of species-rich vegetation is the altered hydrology. The plant species still present in the floodplain in highly diverse communities could suffer severely from restoration measures as they are most likely not adapted to prolonged inundation and different timing of flooding (Chapter 3). Therefore in each river restoration project, it is necessary to investigate whether the species adapted to more natural hydrology are still present in the area or capable to reach it through river flooding in due time. If this is not the case, additional measures, such as introduction of the species will be necessary to reach restoration targets with respect to the development of species-rich vegetation types.

To successfully rehabilitate estuarine, tidal floodplain systems it is important to restore more natural hydrological regimes with a higher tidal amplitude. A broad zonation of different helophyte plant communities is favoured by a larger tidal range, whereas the presence of a salinity gradient only becomes important if salinities are exceeding oligohaline conditions (Chapter 4). The sediment quality is of lesser importance, as nutrients are not limiting plant growth either in natural or more impacted tidal floodplain systems. Furthermore, new hydrological conditions following restoration favour nitrogen removal processes. Therefore it is well possible to include highly enriched former agricultural soils in estuarine rehabilitation projects (Chapter 5). In due time, more diverse grassland vegetation types comprising typical salt-tolerant species could develop on sites, at present occupied by agricultural *Lolium perenne* L.-grasslands.





*Sampling soil and vegetation in an isolated floodplain of the river Odra (Poland) during the field campaign in summer 2003 (Chapter 2) (Photo: Leon Lamers).*

# Samenvatting

## Ecologisch herstel van uiterwaarden en de invloed van de stikstofhuishouding op plantengemeenschappen

In dit proefschrift worden de effecten van veranderend rivierbeheer en daaraan gekoppelde natuurontwikkeling op karakteristieke kruidachtige plantgemeenschappen in de uiterwaarden beschreven. Met name de relatie tussen de soortensamenstelling van deze vegetaties en de beschikbaarheid van stikstof in de bodem en daarmee samenhangende biogeochemische processen staat centraal. Het eerste deel van het proefschrift beschrijft de effecten van restauratiemaatregelen in laaglandrivieren, waaronder de verhoogde inundatiekans door het verwijderen of verlagen van zomerdijken. In het tweede deel wordt het herstel van belangrijke milieugradiënten in getijderivieren en estuaria behandeld.

## De vegetatie in overstromingsvlakten van West-Europese rivieren

Overstromingsvlakten langs rivieren zijn ecologisch zeer waardevol, zowel landinwaarts als aan de monding van de rivier. Ecosystemen in uiterwaarden worden gekenmerkt door een hoge biodiversiteit van zowel flora als fauna. Naast hun hoge ecologische waarden hebben rivieren en uiterwaarden belangrijke economische functies, zoals de productie van voedsel, de bescherming van het achterland tegen overstroming en het zuiveren van water onder andere door retentie van nutriënten.

De normalisatie en gedeeltelijke kanalisatie van de West-Europese rivieren en de landbouw in de uiterwaarden in de 20<sup>e</sup> eeuw hebben een grote invloed gehad op de ecologische waarden en de biodiversiteit van deze systemen. Rivieren en uiterwaarden werden sterk belast met nutriënten en vervuilende stoffen zoals zware metalen en organische verbindingen. De druk van het agrarische gebruik van de uiterwaarden was lokaal zeer hoog, met hoge mest- en kunstmestgiften. Door al deze veranderingen verdwenen de halfnatuurlijke soortenrijke plantengemeenschappen uit de uiterwaarden. Ook de overstromingsvlakten in de estuaria aan de monding van de grote rivieren ontsprongen de dans niet. In Nederland werden verscheidene zee-armen afgesloten na de Watersnoodramp in 1953. De gevolgen hiervan voor de ecosystemen van het estuarium en de getijderivieren waren desastreus (Hoofdstuk 1).

Vanaf het begin van de jaren 70 heeft men zich de ingrijpende gevolgen van het intensieve gebruik van uiterwaarden en overstromingsvlakten gerealiseerd en wordt getracht een deel van de ecologische waarden te restaureren. De hoeveelheden nutriënten die met de rivier worden aangevoerd zijn weliswaar afgenomen, maar de concentraties fosfaat en met name stikstof zijn nog altijd veel hoger dan in natuurlijke riviersystemen. Na de extreem hoge waterstanden in de Rijn en Maas in de winters van 1993 en 1995 is de Nederlandse overheid een nieuwe weg ingeslagen met de beleidslijn 'Ruimte voor de Rivier'. De strategie van dijkverhoging om steeds hogere waterstanden het hoofd te bieden werd verlaten. Het huidige

beleid is gericht op het herstellen van de verbinding tussen de uiterwaarden (en komgronden) met het zomerbed om de rivier fysiek meer ruimte te geven. Ook in estuaria worden veel projecten gestart om de verloren gegane waardevolle ecosystemen te herstellen. In deze projecten staat het terugbrengen van de invloed van de zee centraal, waardoor een hogere getijslag en de ontwikkeling van zoutgradiënten in het water van de voormalige zee-armen mogelijk is (Hoofdstuk 5).

Een belangrijke doelstelling bij deze natuurherstel- en ontwikkelingsprojecten is het terugkrijgen van soortenrijke vegetatietypen in de overstromingsvlakten. Alhoewel doelvegetaties niet beperkt zijn tot kruidachtige planten, heb ik in dit onderzoek alleen graslanden en andere kruidachtige plantengemeenschappen bestudeerd. Zegge-moerassen en soortenrijke natte gras- en hooilanden vormen hierbij de belangrijkste doelvegetaties. In de bijna continu overstroomde delen van de uiterwaarden kunnen ook zeldzame pioniergemeenschappen voorkomen, die zich tijdens droogval in de zomer zeer snel kunnen ontwikkelen (Hoofdstuk 2).

Gezoneerde helofytengemeenschappen (met riet- en biezensoorten) vormen de doelvegetaties in estuaria. Andere karakteristieke doelvegetaties aldaar zijn plantengemeenschappen met soorten die zijn aangepast aan een specifieke zoutconcentratie of fluctuerende saliniteit van het overstromingswater (Hoofdstuk 4 en 5).

In veel herstelprojecten zijn de doelen wat betreft de ontwikkeling van vegetatietypen niet bereikt. In plaats daarvan domineren plantengemeenschappen met slechts enkele zeer algemene soorten de meeste van de heringerichte overstromingsvlakten en uiterwaarden.

### **Oorzaken van het uitblijven van de ontwikkeling van doelvegetaties**

Het uitblijven van ontwikkeling van doelvegetaties vindt zijn oorsprong onder meer in de verandering van de fysische, chemische en biologische toestand van uiterwaarden nadat natuurherstel heeft plaatsgevonden. Het versterken van de verbinding tussen de uiterwaarden en het zomerbed van de rivier leidt namelijk tot een grotere kans op inundatie, ook buiten het normale overstromingsseizoen. Dit beïnvloedt de daar aanwezige plantengemeenschappen sterk: de soorten waaruit de oorspronkelijke en soms ecologisch waardevolle vegetaties zijn samengesteld zijn namelijk niet aan de nieuwe situatie aangepast. In een mesocosmos-experiment waarbij plaggen met vegetatie werden blootgesteld aan een zomeroverstroming - inundatie in het groeiseizoen bij hoge temperaturen - werd de productie van vegetatie uit een Nederlandse uiterwaard langs de Rijn dan ook sterk gereduceerd, terwijl een systeem uit een Poolse overstromingsvlakte - waar dergelijke zomeroverstromingen nog wél plaats vinden - nauwelijks werd beïnvloed (Hoofdstuk 3).

Naast de veranderde hydrologie zijn er ook bodemchemische oorzaken aan te wijzen die de ontwikkeling van doelvegetaties belemmeren. Bodems van uiterwaarden zijn sterk ge-eutrofeerd door de depositie van nutriëntenrijke sedimenten en het agrarische gebruik (Hoofdstuk 2). De verhoogde beschikbaarheid van met name de nutriënten stikstof en fosfor geeft snelgroeiende plantensoorten een competitief voordeel, wat uiteindelijk leidt tot een verlaging van soortenrijkdom van de vegetaties. De vergelijkende veldonderzoeken, waarin zowel Nederlandse als Poolse uiterwaarden en overstromingsvlakten onderzocht zijn, hebben uitgewezen dat in de meest natuurlijke (Poolse) systemen de productie van de vegetatie beperkt wordt door de beschikbaarheid van stikstof in de bodem, terwijl in sterk beïnvloede (Nederlandse) systemen geen relatie meer te vinden is tussen de beschikbaarheid van nutriënten (stikstof en fosfor) en plantengroei. De oorzaak van het ontbreken van deze relatie ligt in het jarenlange intensieve agrarische gebruik van de Nederlandse uiterwaarden

(Hoofdstuk 2).

De hydrologie van overstromingsvlakten in estuaria verschilt sterk van die in de hierboven beschreven systemen. Inundatie is in deze systemen een zeer regelmatig terugkerend fenomeen door de getijdebeweging. Een vergelijkend veldonderzoek in Nederlandse en Ierse estuaria en getijderivieren liet duidelijk zien dat de getijdenbeweging zeer belangrijk is voor de soortensamenstelling van de plantengemeenschappen. Daarnaast is de zoutgradiënt een belangrijke milieufactoor in de verspreiding van deze soorten (Hoofdstuk 4). In een mesocosmosexperiment waarin de effecten van natuurherstel in het Haringvliet werden onderzocht bleek echter dat een verzilting van het oppervlaktewater van zoet naar licht-brak (3‰) geen effect had op de huidige oevervegetatie, althans niet binnen een jaar (Hoofdstuk 5).

### **Biogeochemische processen in uiterwaarden en de gevolgen voor de stikstofbeschikbaarheid**

De concentraties nutriënten in de bodem van uiterwaarden en overstromingsvlakten zijn in laaglandrivieren duidelijk hoger in meer beïnvloede systemen (Hoofdstuk 2). In estuaria en getijderivieren bleek de antropogene invloed alleen uit de relatief hoge fosforconcentratie ten opzichte van stikstof (Hoofdstuk 4). Getijdensystemen zijn meer dynamisch met een sterkere schommeling van de zuurstofconcentratie in de bodem en dit stimuleert onder andere bacteriële stikstofomzettingen. Uiteindelijk leidt dit tot een relatief hoger verlies van stikstof dan fosfor vergeleken met minder dynamische systemen zonder getij-invloed.

De grootste bron van voedingsstoffen in uiterwaarden is de aanvoer van sediment tijdens overstromingen in de vorm van geadsorbeerde anorganische en particuliere organische nutriënten. In een kleinschalig veldexperiment in een uiterwaard langs de Rijn, werd gevonden dat de hoeveelheid aangevoerde nutriënten tijdens een overstroming dezelfde orde van grootte had als de hoeveelheid die in een jaar door groei in bovengronds plantmateriaal werd opgenomen (Hoofdstuk 6). In sterk beïnvloede uiterwaardsystemen kan agrarisch gebruik een alternatieve bron van voedingsstoffen zijn of in het verleden geweest zijn. In Nederland is de gemiddelde aanvoer van nutriënten door agrarisch gebruik meer dan tien keer zo hoog als de jaarlijkse aanvoer van nutriënten door sedimentatie tijdens overstromingen.

Hoewel de aanvoer van nutriënten door sedimentatie aanzienlijk is, zijn deze voedingsstoffen niet direct beschikbaar voor opname door planten, omdat het overgrote deel in organische vorm is. Het organische materiaal moet eerst afgebroken en gemineraliseerd worden. In een landbouwbodem, onderhevig aan zeer dynamische hydrologische condities bleek de mineralisatiesnelheid erg hoog te zijn, zelfs hoger dan de aanvoer door sedimentatie (Hoofdstuk 6). Mineralisatie is dan ook een belangrijke bron van anorganische nutriënten zoals nitraat en ammonium, maar ook fosfaat en spore-elementen. Wanneer organisch fosfor gemineraliseerd wordt tot fosfaat, vormt het sterke adsorptie-bindingen met kationen, met name onder oxidische omstandigheden. Fosfaat wordt pas beschikbaar tijdens overstroming als de bodem anaeroob wordt en gereduceerd raakt. Inundatie leidt dan tot een verhoogde beschikbaarheid van fosfaat, zonder dat dit wordt aangevoerd met het rivierwater of meegevoerd sediment. Dit proces staat bekend als interne eutrofiëring (Hoofdstuk 3).

Er zijn ook biologische, chemische en fysische bodemprocessen die de beschikbaarheid van nutriënten verkleinen in de bodem. Een erg belangrijk retentieproces in terrestrische ecosystemen is de opname van stikstof en fosfor door de vegetatie. In de veldstudies uitgevoerd in overstromingsvlakten van laaglandrivieren en estuaria bleek dat de opname door de vegetatie enorme verschillen kan vertonen, maar dat vooral in sterk beïnvloede systemen

van laaglandrivieren de retentie hoge waarden kan bereiken (Hoofdstuk 2 en 4). De waarden zijn tien keer zo hoog als de aanvoer van voedingsstoffen door sedimentatie tijdens overstroming. Helaas is retentie door plantopname niet duurzaam, zeker niet in kruidachtige vegetaties die aan het eind van het groeiseizoen bovengronds compleet afsterven. Een groot deel van de voedingsstoffen komt dan via bladval weer terug in het systeem. Indien in deze systemen geen biomassa wordt verwijderd, door hooien of door de rivier zelf, zal de retentie door de vegetatie geen blijvend effect hebben op de reductie in voedingsstoffen in het systeem.

Denitrificatie is een bacterieel proces dat voor een groot deel verantwoordelijk is voor de verwijdering van stikstof. Onder zuurstofarme omstandigheden gebruiken deze bacteriën namelijk nitraat als alternatieve elektron-acceptor voor het afbreken van organische materiaal. Het eindproduct van deze omzetting zijn gasvormige stikstofproducten die kunnen ontsnappen naar de atmosfeer. Als nitraat in hoge concentraties aanwezig is, bijvoorbeeld door een continue afwisseling van zuurstofrijke en zuurstofarme condities in de bodem, waardoor zowel denitrificatie als nitrificatie (omzetting van ammonium naar nitraat) gestimuleerd worden, kunnen hoge omzettingssnelheden bereikt worden (Hoofdstuk 5). Denitrificatie kan in potentie voor een grote verwijdering van stikstof zorgen en daardoor de beschikbaarheid van deze voedingsstof voor plantengroei beperken.

### **Conclusies en mogelijkheden voor natuurherstel**

De resultaten van de verschillende studies die in dit proefschrift gepresenteerd zijn geven meer inzicht in de biogeochemische processen, met name wat betreft stikstof, in overstromingsvlakten van laaglandrivieren en estuaria in gematigde streken. Het onderzoek wees uit dat de productie van de vegetatie in natuurlijke en beïnvloede systemen niet door dezelfde factoren werd gereguleerd (Hoofdstuk 2). Het herstel van stikstof-gelimiteerde condities in beïnvloede uiterwaarden zal dan ook waarschijnlijk bijdragen aan de ontwikkeling van soortenrijke doelvegetaties en het tegengaan van verder verarmen van de plantengemeenschappen vanwege dominantie van enkele hoog-productieve soorten. Hooien en andere conventionele beheermethoden zijn niet voldoende om stikstof-limitatie te herstellen, zeker niet omdat de aanvoer van nutriëntenrijk slib door de rivier doorgaat (Hoofdstuk 6). Meer ingrijpende maatregelen zijn nodig, zoals het op grote schaal verwijderen van de ge-eutrofiëerde toplaag van de bodem. Zelfs in dat geval is aanvullend beheer nodig om hernieuwde accumulatie van voedingsstoffen tegen te gaan.

Een ander belangrijk aandachtspunt voor succesvol natuurherstel is de veranderde hydrologie. De plantengemeenschappen die nog aanwezig zijn in de uiterwaarden, waaronder soortenrijke relictvegetaties, hebben mogelijk veel te lijden van restauratiemaatregelen omdat ze niet aangepast zijn aan langdurige inundatie of inundatie buiten het standaard overstromingsseizoen (Hoofdstuk 3). Daarom is het noodzakelijk voor elk individueel natuurontwikkelingsproject te inventariseren of er doelsoorten aanwezig zijn die wel onder meer dynamische hydrologische omstandigheden kunnen groeien of dat deze soorten de mogelijkheid hebben om het gebied te bereiken. Als dit niet het geval is, zijn aanvullende maatregelen, zoals het (her)introduceren van deze soorten nodig, om tot een succesvolle ontwikkeling van soortenrijke doelvegetaties te komen.

In estuaria is het met name belangrijk om natuurlijke hydrologische regimes te herstellen. Een grote getijslag draagt bij aan een rijke zonerings van helofyten (Hoofdstuk 5). Een zoutgradiënt wordt eigenlijk pas belangrijk als de zoutconcentratie van het oppervlaktewater licht-brakke condities overstijgt. De kwaliteit van het sediment is minder belangrijk, omdat

nutriënten niet limiterend zijn voor de biomassaproductie. Daarnaast zal een meer dynamische hydrologie processen van stikstofverwijdering stimuleren, waardoor het ook mogelijk is om voormalige landbouwgronden te betrekken in estuariene herstelprojecten (Hoofdstuk 5).



*Sampling soil pore water of soil monoliths during inundation (Chapter 3) (Photo: Martijn Antbeunisse).*

## Tot besluit

Voor u ligt het product van vijf jaar onderzoek. In mijn eentje was het nooit gelukt het onderzoek tot een goed einde te brengen en dit boekje vol met interessante resultaten te krijgen. Veel mensen hebben een welkome bijdrage geleverd en ik wil hen op deze plaats hartelijk bedanken.

In de eerste plaats zijn daar mijn promotoren cq. begeleiders. Jos Verhoeven heeft mij vanaf het allereerste begin van mijn promotietraject (en ook daarvoor) de juiste richting gewezen, maar hij heeft mij gelukkig ook de ruimte gegeven om fouten te maken. Het enthousiasme van Jos voor het ecologisch (fundamentele) onderzoek heeft mij aangestoken en hoewel ik eerst niet zonder twijfel was heeft hij me het vak van onderzoeker enorm leren waarderen. De hulp van Jos was met name onmisbaar tijdens de fase waarin uitgebreide spreadsheets met gegevens in wetenschappelijke artikelen omgezet moesten worden. Mijn 2<sup>e</sup> promotor, Riks Laanbroek, was wel vanaf het begin van het project betrokken, maar pas in de laatste fase actief als mijn directe begeleider. Dankzij Riks heb ik veel geleerd van en over de microbiële kant van dit onderzoek en de mogelijkheden om deze te verenigen met de landschapsecologische kant.

Mijn (andere) collega's bij de leerstoelgroep Landschapsecologie hebben op alle mogelijke manieren geholpen; door het stellen van kritische vragen over niet-realiseerbare onderzoeksplannen, door te helpen bij veldwerk (vaak een dagje, maar soms ook weken achtereen tijdens buitenlandse monstercampagnes), door mee te helpen met het analyseren van eindeloze reeksen bodem- en watermonsters in het lab en last-but-not-least: door het creëren van een prima (werk?)sfeer. Dankjewel: Boudewijn Beltman (waarom draaien onze gesprekken toch altijd op Ierland uit?), Judith Sarneel, Merel Soons, Mariet Hefting (voor de fantastische hulp met de stikstofprocessen, mooi dat we de samenwerking een vervolg hebben kunnen geven!), Marcel Klaassen, Dennis Whigham, Nina Smits, Roland Bobbink, Maaïke Weijters, Edu Dorland, Victor Beumer, Alice Liu, Ronald van den Heuvel, Yuki Fuyita, Gerrit Heil, Gerrit Rouwenhorst, Sandra Robat, Paul van der Ven, Susan Sollie en Bas van de Riet (ik vind het heel tof dat jullie twee paranimf willen zijn!) en natuurlijk ook alle collega's die in de afgelopen vijf jaar een andere werkplek hebben gevonden: Isabel Van den Wyngaert, Hein Pijnappel, Mariken Verhoeven, Agnieszka Lawniczka (mijn zeer gewaardeerde, goedlachse kamergenote en fantastische tolk-op-afstand met de Poolse gendarmerie), Karin Tromp, Maurice Paulissen, Margje Voeten en Marloes Vermeer.

In de afgelopen vier jaar heeft een aantal studenten ervoor gekozen bij mij een afstudeeronderwerp of literatuurscriptie te doen. De samenwerking leidde in veel gevallen tot waardevolle (en/of onverwachte) resultaten - zie ook Hoofdstuk 6 in dit proefschrift - en tot veel inspirerende discussies. Dankjewel, ook voor de zeer welkome hulp bij mijn veld- en labwerk: Kim Renders, Ciska Blom, Sylvia Delvaux, Ronald van den Heuvel, Rutger Zeijpveld, Jordie Netten, Nienke van Geel en Angela Sigterman.

Het onderzoek dat in dit proefschrift beschreven wordt is een onderdeel van een groter onderzoeksproject naar natuurherstel in uiterwaarden (NWO-TRIAS programma). Ik wil mijn

collega-AIO's, Roos Loeb (RUN) en Marzia Miletto (NIOO-KNAW) heel hartelijk bedanken voor de goede samenwerking in het veld en bij de gezamenlijk uitgevoerde experimenten, het kritisch doorkijken van draft-versies van artikelen en de jammergenoeg spaarzame, doch zeer geslaagde diners. De hulp en inbreng van de andere consortium-leden - Leon Lamers, Paul Bodelier en Jan Roelofs - was ook zeer welkom bij het richting geven aan het onderzoek en het goed afstemmen van de verschillende deel-projecten.

De halfjaarlijkse presentaties van de voortgangrapportages voor de begeleidingscommissie (vanuit NWO) waren soms iets te tijdrovend in drukke veldwerkperiodes. Toch heeft juist deze steeds terugkerende verplichting ervoor gezorgd dat ik mijn promotie-onderzoek binnen afzienbare tijd heb kunnen afronden, door de continue controle op planning en richting van het onderzoek. Hartelijk dank Helenius Rogaar, Hugo Coops, Jan Streefkerk, Jos Vink, Gerard Muijzer, Jaap van der Waarde en Peter Doelman.

Binnen de Universiteit Utrecht zijn er behalve de directe collega's bij Landschapsecologie nog meer mensen onmisbaar geweest voor de totstandkoming van dit proefschrift; alle medewerkers van het beheercluster Went-gebouw, de Botanische Tuinen en Biostatistiek wil ik hier dan ook voor alle ondersteuning bedanken.

Het veldwerk en de experimenten waren niet mogelijk geweest zonder de toestemming van de eigenaren en beheerders van de vele terreinen waar ik gemonsterd heb of materiaal verzameld heb voor experimenten. Met name wil ik de medewerkers van Staatsbosbeheer - beheerseenheid Heuvelrug-Zuid bedanken voor het gemak waarmee we (steeds weer) toestemming kregen om te monstern en te experimenteren in de Steenwaard. Ook wil ik het Utrechts Landschap, Rijkswaterstaat, Vereniging Natuurmonumenten, de beheerders van het landschapspark Lomzyskiiego PK Doliny Narwi (Polen) en alle particulieren bedanken voor de verleende toegang tot hun gebieden (of men nu van onze komst op de hoogte was of niet).

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Als laatste wil ik Toos (mijn vriendinnetje) heel hartelijk bedanken. Ze is de afgelopen vier jaar een prima hulp geweest; in het veld (o.a. in Ierland) en ook bij labwerk. Daarnaast heeft ze altijd kritisch (doch wel rechtvaardig) naar voorlopige versies van mijn teksten gekeken. Bovenal heeft ze me regelmatig vanachter de PC gesleurd en naar buiten geschopt: want schrijven over uiterwaarden, dat kan natuurlijk niet als je er niet elke week even middenin staat!





*Sampling soil in the monoliths in the mesocosm experiment on estuarine restoration of Lake Haringvliet (described in Chapter 5)*

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# Publications

## Publications in international peer-reviewed journals

- Antheunisse, A.M., Loeb, R., Lamers, L.P.M. & Verhoeven, J.T.A. (2006) Regional differences in nutrient limitation in floodplains of selected European rivers: implications for rehabilitation of characteristic floodplain vegetation. *River Research & Applications* **22**: 1039-1055
- Antheunisse, A.M. & Verhoeven, J.T.A. (2006) Short-term response of soil nutrient dynamics and herbaceous riverine plant communities to summer inundation. *Wetlands*, accepted
- Antheunisse, A.M. & Verhoeven, J.T.A. (2006) Restoration of natural hydrological and salinity gradients in tidal rivers: consequences for helophyte vegetation. *Estuarine, Coastal and Shelf Science*, accepted
- Antheunisse, A.M., Loeb, R., Miletto, M., Lamers, L.P.M., Laanbroek, H.J. & Verhoeven, J.T.A. (2006) Response of nitrogen dynamics in semi-natural and agricultural grassland soils to experimental variation in tide and salinity. *Plant and Soil*, accepted
- Lamers, L.P.M., Loeb, R., Antheunisse, A.M., Miletto, M., Lucassen, E.C.H.E.T., Boxman, A.W., Smolders, A.J.P. & Roelofs, J.G.M. (2006) Biogeochemical constraints on the ecological rehabilitation of wetland vegetation in river floodplains. *Hydrobiologia*, **565**, 165-186.
- Loeb, R., Antheunisse, A.M., Miletto, M., Lamers, L.P.M., Bodelier, P.L.E., Laanbroek, H.J., Verhoeven, J.T.A. & Roelofs, J.G.M. (2006) Effects of restored salinity and tide on biogeochemical processes and vegetation in the Rhine-Meuse estuary; a mesocosm experiment. *Marine Ecology - Progress Series*, submitted

## Oral and poster presentations at national and international symposia

- Antheunisse,A.M., Loeb,R., Lamers,L.P.M. & Verhoeven,J.T.A. (2002) Biogeochemical constraints for sustainable development in floodplains in riverine regions - poster presentation, *BodemDiep Conference*, Zeist, the Netherlands
- Antheunisse,A.M., Loeb,R., Lamers,L.P.M. & Verhoeven,J.T.A. (2002) The effect of biogeochemical processes on the development of nature in floodplain areas: a field survey - poster presentation, *NCR days*, Nijmegen, the Netherlands (In: Proceedings NCR-days 2002, current themes in Dutch river research, Leuven,R.S.E.W., Van Os,A.G. & Nienhuis,P.H. (eds.))
- Antheunisse,A.M. (2003) Effects of water level fluctuations on biogeochemical mechanisms controlling the development of characteristic and species-rich plant communities in floodplain areas - poster presentation, *Verwey Ph.D. meeting*, Texel, the Netherlands
- Antheunisse,A.M. Loeb,R. & Miletto,M. (2003) Nutrients, water chemistry and sulphate-reducers in floodplain areas - oral presentation, *Soil & Water Conference*, Zeist, the Netherlands
- Antheunisse,A.M. (2003) Biogeochemical variables controlling the establishment and development of biodiverse riverine vegetation types - poster presentation, *8<sup>th</sup> International Symposium on Biogeochemistry of Wetlands*, Ghent, Belgium
- Antheunisse,A.M. (2004) The effect of summer flooding on soil geochemistry and vegetation - poster presentation, *Soil & Water Conference*, Zeist, the Netherlands
- Van den Heuvel,R. & Antheunisse,A.M. (2004) Increased nutrient availability following a summer flooding - poster presentation, *Soil & Water Conference*, Zeist, the Netherlands
- Antheunisse,A.M. (2004) The effect of summer flooding on soil geochemistry and vegetation: experimental comparison between a disturbed and a more pristine floodplain system - oral presentation, *The 7<sup>th</sup> Intecol International Wetlands Conference*, Utrecht, the Netherlands.
- Antheunisse,A.M. & Loeb,R. (2005) Bottlenecks for ecological rehabilitation of species-rich vegetation in river forelands. A comparative study in Dutch and Polish river systems - poster presentation, *Soil & Water Conference*, Zeist, the Netherlands
- Loeb,R., Antheunisse,A.M., Miletto,M., Verhoeven,J.T.A., Bodelier,P., Laanbroek,H.J., Lamers,L.P.M. & Roelofs,J.G.M. (2005) Effects of increased salinity and tide in the Haringvliet on biogeochemical processes; a mesocosm experiment - oral presentation, *Soil & Water Conference*, Zeist, the Netherlands
- Antheunisse,A.M., Loeb,R. & Miletto,M. (2005) Restoration of a closed-off estuary in the Netherlands, consequences for biogeochemistry and terrestrial vegetation - oral presentation, *World Ecological Restoration Conference*, Zaragoza, Spain

Antheunisse, A.M. & Verhoeven, J.T.A. (2006) Short-term response of soil nutrient dynamics and herbaceous riverine plant communities to summer inundation - oral presentation, 1<sup>st</sup> *European Annual Meeting of the Society of Wetland Scientists*, Bangor, United Kingdom



*The author during the 2004 field campaign in Ireland (Chapter 4), searching for suitable study sites along the shores of the tidal river Bandon (Photo: Rutger Zeijppveld).*

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## Curriculum Vitae

Martijn Antheunisse werd op 21 februari 1978 geboren in het Zeeuwse 's-Heer Abtskerke. De eerste stappen op weg naar het doctoraat werden gezet op het kleine dorpsschooltje OBS De Poel. Vanaf zijn 12<sup>e</sup> fietste hij elke dag naar Goes op-en-neer, waar hij gymnasium volgde aan SSG Het Goese Lyceum. Zijn examen-pakket bestond met name uit bèta-vakken, maar hij deed ook eindexamen in geschiedenis, want wat kan je met wetenschappelijke kennis zonder maatschappelijke en historische context? De liefde en interesse voor de natuur werd met de paplepel ingegoten en tijdens de middelbare school periode werd dit versterkt toen Martijn met de NJN (Nederlandse Jeugdbond voor Natuurstudie) in aanraking kwam. Eerst ging hij alleen mee met wekelijkse excursies, waarbij vooral de kust een onweerstaanbare aantrekking op Martijn had. Later ging hij ook mee op nationale en internationale zomerkampen en was hij steeds vaker excursieleider in plaats van deelnemer. Na verscheidene functies in het afdelingsbestuur, regiobestuur en de landelijke organisatie (waaronder jarenlang de redactie van de Sepia, het orgaan van de Strandwerkgroep) vervuld te hebben, nam Martijn uiteindelijk in 2000 zitting in het hoofdbestuur als secretaris.

Op dat moment was hij al behoorlijk gevorderd met zijn studie Biologie aan de Universiteit Utrecht (1996-2001). In de eerste drie jaar van de studie koos Martijn voor een ecologisch en botanisch accent wat betreft te volgen cursussen en collegeries, maar ook de maatschappelijke relevantie van biologie werd niet uit het oog verloren.

Hij kwam dan ook niet geheel toevallig terecht bij de leerstoelgroep Landschapsecologie voor zijn eerste afstudeeronderwerp. Hierin werd de invloed van sulfide op verlanding in laagveengebieden behandeld. Dit onderzoek werd gevolgd door een literatuurscriptie waarin de mogelijke successietrajecten van laagveenvegetaties tijdens verlanding werden onderzocht. Het tweede afstudeeronderwerp volgde Martijn buiten de universiteit, bij de groep ecosysteem studies van het NIOO-CEMO in Yerseke: Terug naar Zeeland en terug naar de zee, want in deze periode bestudeerde Martijn gegevens van vispopulaties in de Oosterschelde voor en na de aanleg van de stormvloedkering. In de laatste fase van zijn studie assisteerde Martijn bij vele cursussen voor 2<sup>e</sup> en 3<sup>e</sup>-jaars studenten, wat vaak veel leerzamer bleek dan het volgen van de cursus zelf.

Na het behalen van zijn doctoraal-diploma (2001) keerde Martijn terug naar Utrecht voor een promotieaanstelling, wederom bij de leerstoelgroep Landschapsecologie (2002-2006). Het onderzoek, onderdeel van een groter consortium, gefinancierd door NWO, was gericht op het ecologische herstel van uiterwaarden en de invloed van de stikstofhuishouding op plantengemeenschappen. In de loop van dit onderzoek verschoof de focus van uiterwaarden van benedenstroomse rivieren langzaam richting de monding, want de zee blijft roepen...

Vanaf mei 2006 werkt Martijn aan een onderzoeksproject van dezelfde leerstoelgroep naar de zuiveringspotentie van natte bufferstroken voor afstromend water van aangrenzende akkers en graslanden, in samenwerking met de STOWA, het LEI en verscheidene waterschappen.









