

WETLAND
ECO-ENGINEERING
WITH FINE SEDIMENT



RÉMON SAALTINK

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Wetland eco-engineering with fine sediment

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CHAPTER

1

Introduction

Rémon Saaltink

“Homo sapiens, a physical ecosystem engineer par excellence”

Clive G. Jones

Human beings have been interacting with nature for millennia and, in doing so, have drastically changed landscapes. Agricultural land has expanded at the expense of forests and wetlands (Verhoeven, 2014), especially in the last thousand years (e.g., Klein Goldewijk et al., 2017). It is estimated that 80% of the original, pristine wetland area on the European continent has been lost due to anthropogenic activities (Spiers, 1999). This comes at a huge cost because wetlands are considered to deliver a suite of ecosystem services such as nutrient and contaminant retention, which maintains healthy soils and productive ecosystems (De Groot et al., 2002). The average economic value of wetlands based on these ecosystem services has been calculated to be US\$1578 per hectare per year, with excesses up to US\$8811 per hectare per year (Woodward and Wui, 2001). Clearly, these ecosystems should have high priority in restoration programs.

Apart from reclamation, other human activities have been damaging coastal wetlands to improve coastal protection (Temmerman et al., 2013). Traditionally, conventional engineering – i.e. hard structures such as sea-walls, rock revetments, and levees – damages both aquatic and terrestrial ecosystems and comes with ever-rising maintenance costs (Cheong et al., 2013; Temmerman et al., 2013). These undesired effects from conventional engineering result in a need for an alternative approach that minimizes these non-target impacts on ecosystems while at the same time creating opportunities for ecosystem restoration of wetlands and other nature areas (Matthews et al., 2011). An engineering concept that fits this ecosystem-based view is called ecological engineering: in this concept man does not build *against* nature, but *with* nature.

1.1 THEORETICAL BACKGROUND

Ecosystem engineers play an important role in ecological engineering designs. The term **ecosystem engineer** was first defined by Jones et al. (1994) and comprises

organisms that play a role in the creation, modification, and maintenance of ecosystems (Jones et al., 1994; Gutiérrez & Jones, 2008). A well-known example of an ecosystem engineer is the beaver (*Castor canadensis*), who creates large and long-persisting wetlands by altering the hydrology (Naiman et al. 1988). Numerous other examples exist and can be classified either as *allogenic* – i.e., organisms that change the environment by transforming living or non-living materials (such as the beaver) – or *autogenic* – i.e., organisms that change the environment with physical structures. Allogenic examples include meiofauna and macrofauna, which actively change physical, chemical, and biological properties of sediments as a consequence of bioturbation, pelleting, and biodeposition (Kristensen, 1991; Reichelt, 1991; Montserrat, 2011), and terrestrial earthworms, which alter hydrology and affect plant biodiversity (Thompson et al., 1993). Autogenic examples include submerged macrophytes, which promote sedimentation and wave attenuation (Carpenter and Lodge, 1986); terrestrial plants, which alter biogeochemical processes and soil formation (Ehrenfeld et al. 2005); and mussel beds, which protect sediments from erosion and other disturbances (Borsje et al., 2011). An organism can also be simultaneously autogenic and allogenic, such as a terrestrial plant that stabilizes the sediment by root growth binding (Jones et al., 1994, 1997).

Understanding how ecosystem engineers modify the environment is essential for predicting how other ecological processes will influence ecosystem functioning (Jones et al., 1997). This can either be through positive effects, for example by creating habitat and increasing biodiversity (Wright et al., 2002; Gilad et al., 2004), or through negative effects, for example by mutual exclusion via modification of shared habitat (Van Wesenbeeck et al., 2007). Jones et al. (1997) and Gutiérrez & Jones (2006) commented that feedback effects occur when the physical state change directly affects the engineer itself either positively or negatively. Gutiérrez et al. (2003) elaborated on these effects and discussed two types of feedback mechanisms: 1) *resource-mediated feedbacks* that occur as a consequence of changes in resource availability; and 2) *organism-mediated feedbacks* that occur as a consequence of the facilitation or inhibition of other organisms that respond to changes in resource availability.

Such effects and feedback mechanisms are essential in ecosystem engineering. **Ecosystem engineering** is the process of maintaining and creating habitats by making

use of ecosystem engineers (Jones et al., 1994). There are many concepts related to ecosystem engineering. These include patch dynamic theory, in which patches are created and maintained by the activity of organisms, for example by goat and rabbit grazing in arid ecosystems (Verwijmeren et al., 2014, 2016). Keystone species are also related to ecosystem engineering, especially when the appearance or disappearance of a keystone species affects ecosystem engineers at lower trophic levels. For example, the Lesser snow goose (*Anser caerulescens*) determines the structure and species composition of coastal plant communities (Kerbes et al., 1990). Another related concept, quite similar to ecosystem engineering, is **ecological engineering**. The field of ecological engineering was first mentioned in 1962 by Howard Odum, who applied it to systems where the energy supplied by society is small relative to the natural resources, but sufficient to produce large effects (Odum, 1962). Mitsch (1996) redefined the term ecological engineering as:

“The design of sustainable ecosystems that integrate human society with its natural environment for the benefit of both.”

Although ecosystem engineering and ecological engineering have many similarities – such as the usage of effects and feedback mechanisms initiated by organisms – the key difference is in the aim of the concepts (Figure 1.1). Whereas ecosystem engineering focuses on maintaining and creating habitat to enhance ecological value (Jones et al., 1994), ecological engineering aims to restore and develop ecosystems that also have human value (Mitsch, 1996). Therefore, ecological engineering also adds to ecosystem services, such as nutrient retention by wetlands (Odum and Odum 2003). Creating wetland, however, always requires an initial conventional engineering investment (Mitsch, 1998), for example by manually planting seedlings or shaping an initial hydrological regime. Despite this human investment, natural processes determine for a large part the final design of the ecosystem (i.e., self-design). Thus, ecosystem self-design is a key principle in ecological engineering projects (Mitsch, 2012).

In the past few decades, numerous eco-engineering projects have been started worldwide, like the Olentangy River Wetland Research Park in Ohio State, which aims to investigate how ecological engineering can create and restore wetland and how

much human intervention is required (Mitsch et al., 1998, 2012). Mitsch et al. (2012) found that artificially planting the wetland increased species richness compared to the unplanted wetland but reduced overall productivity. Wetland restoration on Poplar Island in the Chesapeake Bay, Maryland, is another example: it aimed to restore wetland and to investigate how dredged material can be used as building material (Derrick et al., 2007; Weller et al., 2007). This project demonstrated that dredged material can create valuable island ecosystems that improve the ecological value of the area. For example, the number of bird species increased to 144, including special status species such as the Bald eagle (*Haliaeetus leucocephalus*) (Derrick et al., 2007).

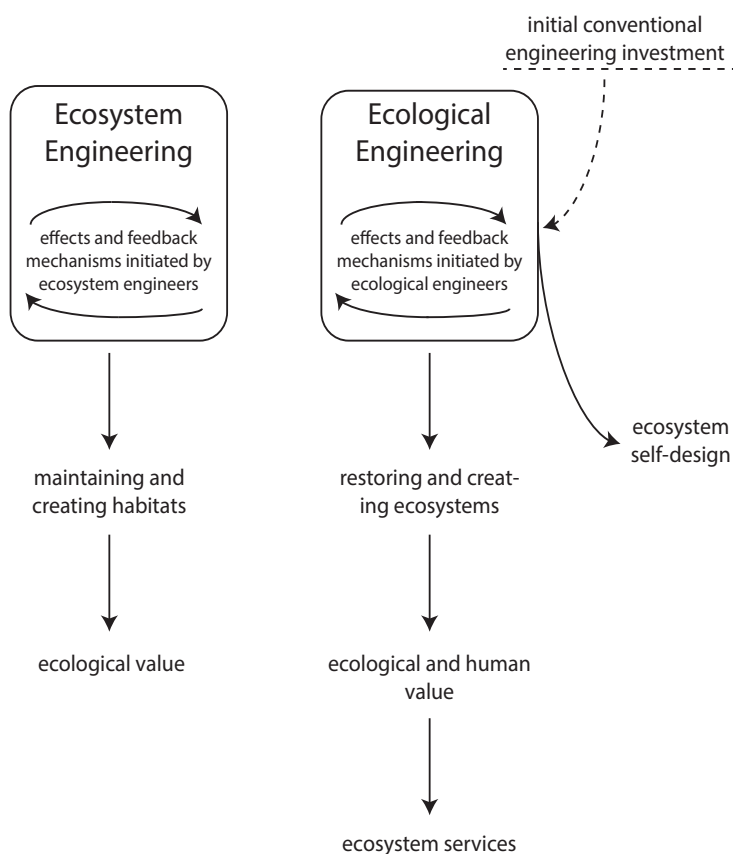


Figure 1.1. The similarities and differences between ecosystem engineering and ecological engineering. Dashed lines indicate human interference.

In the Netherlands, a program has been initiated by national authorities, dredging companies, engineering consultants, and research institutes that use the concept of ecological engineering to create additional benefits for nature and society. This program is called **Building with Nature**. Examples where this approach has been used include the construction of a large hook-shaped sand spit, also known as the Delfland Sand Engine, which functions as a mega-nourishment to combat coastal erosion (Temmerman, et al., 2013; Van Slobbe et al., 2013; De Boer et al. 2015) and the deposition of a sandy foreshore as a means to reinforce the Houtribdijk. In another innovative project within the Building with Nature program, fine sediment is being used to construct a large wetland in Markermeer, a lake in the Netherlands. This project will be explained in section 1.5.2 and will function as a case study. First, the relevance of fine sediment as building material will be explained, after which a brief history of the Markermeer area will be presented.

1.2 FROM SAND TO MUD AS BUILDING MATERIAL

Sand and gravel are dominantly used as building material for infrastructure, comprising 79% of total primary material input (Krausmann et al., 2017). Sand is widely used, especially in land reclamation (Sutherland et al., 2017). Most likely, land reclamation will increase in the future with the expansion of urban areas, especially near the coast (e.g., Small and Nichols, 2003). Sand as building material is mined from quarries, rivers, lakes, sea beds, and coasts and is rapidly becoming a scarce resource (Sutherland et al., 2017). Furthermore, human interference in sediment-rich ecosystems has resulted in shifts in sediment regimes (Van Eekelen et al., 2017). Examples include deepening of estuaries (Winterwerp et al., 2013) and damming of rivers (Vörösmarty et al., 2003). The latter example in particular has resulted in a reduction in sand as a resource. Thus, building with sand is becoming costly and sand demand will rise in the future as a result of rapid urbanization (Torres et al., 2017). Fine sediment (i.e., mud) as an alternative to sand is currently being explored in many projects (Sutherland et al., 2017; Van Eekelen et al., 2017). River delta regions are especially rich in fine sediment that is readily available for land reclamation (Jalowska et al., 2017; McKee et al., 2004). Strategies to overcome the challenges involved when

building with fine sediment (e.g., sediment consolidation, crest stability) still need to be developed. As the Marker Wadden will partly be constructed with fine sediment, useful insights will be gained, which will lead to new building strategies that can be implemented in other projects where fine sediment is used as building material.

1.3 HISTORY OF THE MARKERMEER AREA

Frequently occurring floods and storm surges have been damaging rural and urban areas in coastal zones of the Netherlands for centuries (Biggelaar et al., 2014; Van Geel et al., 1982/1983). The Zuiderzee region is one of those areas; it was a bay connected to the North Sea via the Wadden Sea, formed by erosion of peat layers in the central part of the Netherlands (Van Geel et al., 1982/1983). The earliest recorded storm surge in the Zuiderzee area is dated at 1170 AD. It was followed by storm surges in the years 1196, 1214, 1219, and 1248 (Gottschalk, 1971; Van Geel et al., 1982/1983). This indicates that floods occurred frequently in the coastal areas of the Zuiderzee.

Flood protection in this area began as far back as the eighth and ninth centuries, with the construction of dwelling mounds that enclosed low-lying silt marshes and peat fens (Verhoeven, 1992). More serious flood protection and land reclamation started around the thirteenth century (Van Veen, 1962; Van Koningsveld et al., 2008). In 1617, the Dutch engineer Hendrik Stevin was the first to propose a plan to seal off the Zuiderzee from the North Sea to prevent any more damage from the storm surges. Unfortunately, the technologies needed to execute such a vast project were not available at the time, but the proposal inspired engineers in the nineteenth century. In 1891, Cornelis Lely designed a plan known as the Zuiderzee reclamation works (Dutch: Zuiderzeewerken). Due to the vulnerability of the Dutch food supply during the First World War and a devastating storm surge in 1916 that caused extensive flooding, the Zuiderzee Act was passed by the Dutch parliament in 1918. For an extensive review of the Zuiderzeewerken, see Van Lier (1982). Figure 1.2 shows the original plan of Cornelis Lely.

The construction of the Afsluitdijk was completed in 1932 – two years after the North West polder (Wieringermeer) was reclaimed. The dike separated the Zuiderzee from the North Sea, after which the Zuiderzee became a freshwater lake known as

IJsselmeer. To meet the demand for food production, 220,000 hectares of land were gained by reclaiming large parts of lake IJsselmeer (Van Koningsveld et al., 2008). The original plan provided for five polders, four of which have been realized: the North West polder (Wieringermeer), North East Polder, Eastern Flevoland, and Southern Flevoland. The fifth polder has never been built, although preparations for land reclamation were made: in 1976, construction of the Houtribdijk was finalized between Eastern Flevoland and Enkhuizen (Noord-Holland). The lake area



Figure 1.2. Plan of the Zuiderzeewerken as made in 1891 by Cornelis Lely. The areas for land reclamation are outlined in orange (Collectie Nieuw Land; Zuiderzeecollectie).

south of this dam was named Markermeer (an area of c. 700 km²), while the area north of the dam remained IJsselmeer (an area of c. 1100 km²). The Markermeer was supposed to be reclaimed to form the fifth polder (the South West Polder, or Markerwaard) but after decades of dispute, the Dutch parliament finally made a decision in 2006 not to proceed with reclaiming this lake (VROM, 2006). Hence, the artificially created Markermeer was never intended to be a lake in the first place.

1.4 ECOLOGICAL STATUS OF MARKERMEER

At the moment, the ecological conditions in lake Markermeer are poor. In this section, first an explanation is given how the physical characteristics of the lake cause undesired abiotic conditions, after which the consequential environmental effects are discussed.

1.4.1 *Abiotic conditions*

The bottom of lake Markermeer is covered by a soft clay-rich layer. This layer is formed by physical weathering of the underlying Holocene sediments as well as bioturbation and may resuspend as a result of wind and wave action (Van Kessel et al. 2008; Vijverberg et al. 2011; De Lucas Pardo et al. 2013). The soft clay-rich layer accumulated after 1976, when sediment transport northward toward deeper gullies was blocked by the dam that separated Markermeer from IJsselmeer. This caused the suspended matter in the water column to increase to a concentration of 30 mg l⁻¹ (Van Kessel et al., 2008; Vijverberg et al., 2011). The suspended matter in the water column has a low settling velocity, keeping the lake turbid (Vlag, 1992; Eleveld, 2012). On a windy day, the amount of suspended matter in Markermeer can be as much as 0.4 Mton, which is almost half the amount of total sediment erosion/deposition per year (c. 1300 kg km⁻²) (Van Ledden et al., 2006). Before 1976, the suspended matter was transported to the deep underwater gullies of IJsselmeer and to the Wadden Sea and the North Sea, keeping suspended matter low in the lake (Zwart and Iedema, 2014). Since the lake's bed consists mostly of loamy and clayey deposits of marine origin (Zuiderzee deposits; Winkels, 1997) and as there is no longer an external supply of mineral sediments coming from rivers (Van Duin, 1992), the suspended matter in the water column primarily originates from these deposits. However, De

Lucas Pardo (2014) showed that only at very high wind speeds are the wind-induced waves strong enough to directly erode the Zuiderzee deposit and hence wave-induced erosion cannot explain the high amount of suspended matter measured in the water column. Instead, De Lucas Pardo et al. (2013) showed that bed erosion was caused by bioturbation of worms, mainly Tubificidae, which is producing a thick, soft, mud layer at the bottom of the lake.

Low nutrient availability in the water column is another characteristic that negatively impacts the lake's ecosystem. Currently, Markermeer is nutrient-limited, with low values of nitrate, ammonium, and phosphate (Noordhuis et al., 2014). The concentrations of nutrients in Markermeer are nearly exhausted in the growing season and currently limit algal growth (Noordhuis et al., 2014). Data from MWTL (National Water Monitoring Program) shows that concentrations of NO_3 , NH_4 , and PO_4 (orthophosphate) in Markermeer are significantly lower compared to those in lakes in the surrounding area (Table 1.1; see Figure 1.3 for the location of the lakes). By contrast, levels of sulfate and iron are significantly higher in Markermeer than in the other lakes except for Veluwemeer.

Table 1.1. Average concentrations of NO_3 , NH_4 , PO_4 , SO_4 , and Fe (aq) in mg l^{-1} for the lakes Markermeer, IJsselmeer, Veluwemeer, Eemmeer and Ketelmeer for the period 2008-2012. Significant differences ($p < 0.05$) with lake Markermeer for the same variable are indicated by an “*”. Data was retrieved from MWTL (National Water Monitoring Program).

	NO_3	NH_4	PO_4	SO_4	Fe (aq)
Markermeer	0.1	0.01	0.003	90	1.3
IJsselmeer	1.2*	0.03	0.009*	59*	0.5*
Veluwemeer	0.4*	0.07*	0.003	119*	0.3*
Eemmeer	1.1*	0.23*	0.086*	66*	0.6*
Ketelmeer	2.4*	0.11*	0.064*	57*	0.4*

Stony dikes and steep land – water gradients form a third characteristic that contribute to the degraded ecological state of the lake (Noordhuis, 2014; Zwart and Iedema, 2014). An essential habitat where aquatic plants and macrophytes can establish is missing. Littoral zones are desired, as they contribute significantly to the

overall productivity and metabolism in the lake and provide essential habitats for invertebrates and fish (Wetzel, 2001).

To summarize, the soft, clay-rich layer, the low nutrient availability and the absence of littoral zones form three undesired abiotic conditions that are responsible for the degraded ecological state of Markermeer.



Figure 1.3. Map showing the location of the lakes (Markermeer, IJsselmeer, Ketelmeer, Veluwemeer, Eemmeer, and Gooimeer).

1.4.2 Environmental effects

Ecological trends in the lake were studied in detail by the Dutch project “Autonome Neergaande Trendstudie” (ANT) (English: Autonomous Negative Trends; described in Noordhuis et al., 2014). That study pointed out that although primary productivity did not decrease, the composition of primary producers changed in favor of more nutrient-efficient species as a consequence of low nutrient availability. Moreover, high turbidity in the water column also affects primary producers by means of flocculation. Algae such as *Aphanotece* sp. form aggregates with suspended particles in the water, which on days with low wind speeds causes the algae to be deposited on the lake’s bed and hence removes them from the water column (De Lucas Pardo, 2014).

These changes in primary productivity have had cascading effects in the food web. After the transition toward more nutrient-efficient primary producers, the phytoplankton became less nutritious for secondary producers like mussels and zooplankton (Mandemakers, 2013; Noordhuis, 2014; Sarpe et al., 2014). In turn, a reduction in the nutritional value of secondary producers had consequences for fish and birds. For example, the Quagga mussel (*Dreissena burgensis*) – an invasive species – needs less nutrients in its diet to survive and is therefore currently replacing the Zebra mussel (*Dreissena polymorpha*) in the lake (Orlova et al., 2005; Matthews et al., 2014; Karatayev et al., 2015). This has caused negative trends in benthivorous birds, as muscle tissue of *D. burgensis* has a lower energetic value than *D. polymorpha* and is therefore generally avoided as food (Petrie and Knapton, 1999; Noordhuis et al., 2014).

Fish and piscivorous birds show negative trends as well, which can partly be related to changes in secondary productivity. For example, the decline of smelt (*Osmerus eperlanus*) coincided with the shift in species composition of phytoplankton, in turn lowering the nutritional value of zooplankton, the main food of smelt (Noordhuis, 2014). Because smelt is the main diet for piscivorous birds, and smelt has declined rapidly in the past decades, piscivorous birds have also declined. Furthermore, the lake does not provide essential habitats for fish to spawn (Noordhuis and Van Schie 2007) due to the absence of littoral zones with macrophytes and water plants (Zwart and Iedema, 2014).

1.5 RESTORATION PROGRAMS AND RESEARCH PROJECTS

Improving the conditions in the lake is necessary not only from an ecological perspective but also from a political perspective, as the Markermeer-IJmeer region has been designated as a Natura-2000 site (VNL, 2009). To counter these autonomous negative trends in the lake several programs and research projects were initiated by the government and private parties. The restoration programs are described briefly below, after which the Marker Wadden project is described. Last, a description is given of the research project. See Figure 1.4 for the position of the separate programs and research projects.

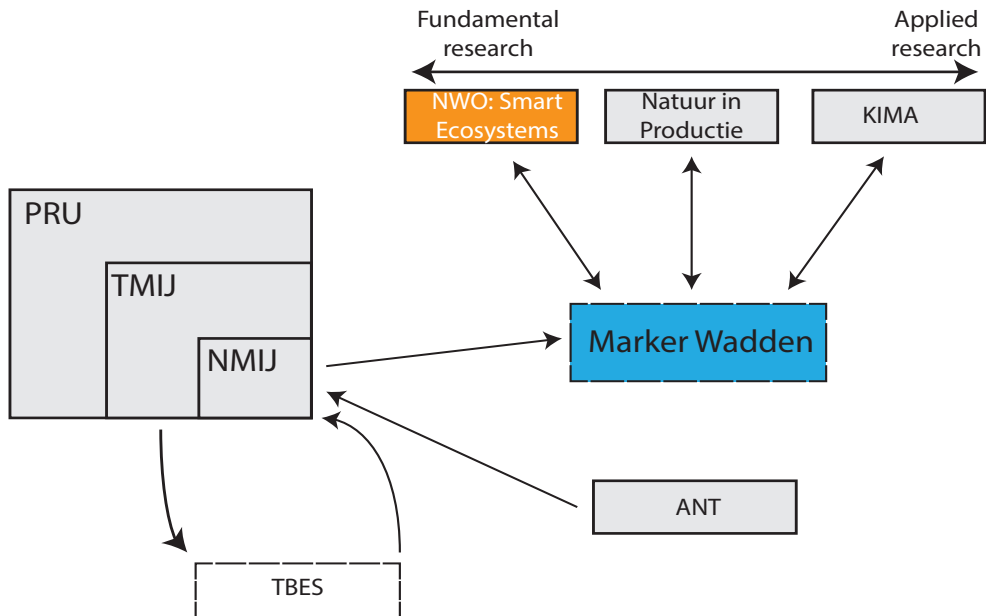


Figure 1.4. The position of the Marker Wadden project (blue) and the research project of this study (orange) within other related programs and projects. The programs, projects, and definitions include Programma Randstad Urgent (PRU), Toekomstagenda Markermeer-IJmeer (TMIJ), Natuurlijk(er) Markermeer-IJmeer (NMIJ), Toekomstbestendig Ecologisch Systeem (TBES), Kennis- en Innovatieprogramma Marker Wadden (KIMA), and Autonome Neergaande Trendstudie (ANT).

1.5.1 Restoration programs in Markermeer

In 2007, a program was commissioned by the government that aimed to determine which investments in nature restoration are most likely to achieve a robust ecosystem in Markermeer. This program was called Toekomstagenda Markermeer-IJmeer (TMIJ) and formed part of a larger program named Programma Randstad Urgent (PRU), a program encompassing 40 different projects to strengthen the international economic position of the Randstad (VROM, 2010). In 2008, TMIJ presented a development perspective for Markermeer in which the realization of a future resilient ecosystem plays a central role: the so-called Toekomstbestendig Ecologisch Systeem, abbreviated as TBES (Stuurgroep TMIJ, 2008). This perspective includes four requirements: 1) zones with clear water; 2) a gradual transition from clear to turbid water (i.e., a

turbidity gradient); 3) transition zones between land and water (i.e., littoral zones); 4) development of natural habitat on inner and outer dikes. This view was accepted by the government, which in addition stated that a strong ecological system is a precondition for further development of the Markermeer-IJmeer region (VROM, 2010). To test the perspective proposed by TMIJ, an applied research program was initiated by the Dutch Ministry of Infrastructure and Water Management (the former Ministry of Transport, Public Works, and Water Management). Called Natuurlijk(er) Markermeer-IJmeer (NMIJ), this research program was intended to determine the effectiveness and feasibility of several measures proposed by TMIJ and to contribute to the decision-making of PRU. Many measures were tested, including the construction of a large wetland in the lake (hereafter referred to as the NMIJ wetland). It was concluded that such a wetland would increase the ecological value in the lake, as it creates an extensive littoral zone, in turn broadening and strengthening the food pyramid in the lake (Haasnoot et al., 2009; Balkema et al., 2010; Dankers et al., 2015).

1.5.2 *Marker Wadden*

In 2012, the society for the preservation of nature monuments in the Netherlands (Dutch: Natuurmonumenten) launched a plan to construct a large wetland in the lake: the Marker Wadden (Verschoor and Rijdsdorp, 2012). Although somewhat similar to the NMIJ wetland proposal, it has some important differences, such as the method of construction (hydrological dredging for the Marker Wadden vs mechanical dredging for the NMIJ wetland) and the estimated cost of the first phase (c. €450 M for the Marker Wadden vs €900 M for the NMIJ-wetland) (Knoben, 2014). Because of the similarities between the NMIJ wetland and the Marker Wadden in terms of ecological effects – the Marker Wadden fulfilled the first three requirements of TBES proposed by TMIJ – the Marker Wadden project was integrated into the final recommendations by NMIJ.

In the project description, the Marker Wadden was defined as follows: a natural archipelago of islands spread across an area of about 10,000 ha in the northern part of Markermeer, between the cities of Lelystad and Enkhuizen (Verschoor and Rijdsdorp, 2012; see Figure 1.5 for the project design). It will consist of lagoons, mudflats, reed beds, tidal forests, and beaches. At the same time, the project aims to resolve the issues

in Markermeer described in Section 1.3 by creating zones with clear water, turbidity gradients, and littoral zones. The wetland should evolve in a self-regulating system once the optimal conditions to keep nature management to a minimum have been created. It is therefore necessary to identify and understand which ecological and other processes in the wetland are important for ecosystem development.

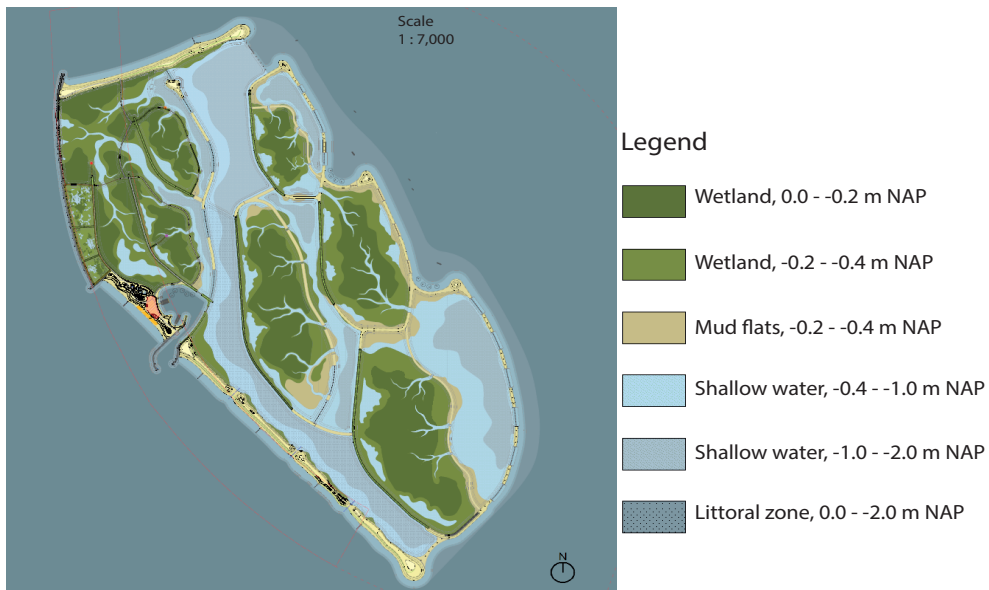


Figure 1.5. Design of the Marker Wadden. © Vista landschapsarchitectuur en stedenbouw.

The method of construction of the Marker Wadden is highly innovative, as fine sediment is used as building material for the wetland. Building with fine sediment has never been attempted before elsewhere in the world at this scale and might be a cost-efficient alternative to sand. However, to protect the wetland from waves and southwesterly winds, dams made of sand or stone have been constructed at the southwest and southeast edges. On the lee side of these dams, fines are hydraulically pumped in several layers to create natural land – water gradients (Boskalis, 2015). *Phragmites australis* is planted manually to promote ecosystem development on the islands (Boskalis, 2015).

By the end of 2018, five islands that cover c. 750 ha of wetland in total will be created. The dredged building material will amount to 30 million m³; it includes the soft clay-rich layer and the underlying Zuiderzee deposits, which apart from clay also consist of significant amounts of sand and peat. In September 2018, one island of c. 250 ha will open for visitors, with access to a boat landing stage, a sandy beach, birdwatching hides, a lookout tower, and hiking trails. Perpendicular to the island, a 10-meter deep gully and a pit 26 meters deep have been created to collect clay-rich material. It is currently being investigated if sufficient clay-rich material accumulates in gullies and pits to enable soft clay-rich material to be efficiently dredged to create future islands. The pace of realizing the remaining 9,000 ha depends on financial resources. The Marker Wadden project will probably remain work in progress for years to come.



Figure 1.6. Aerial photograph of the Marker Wadden in February 2018. © Natuurmonumenten/Straystone.

1.5.3 *Research project*

There are some innovative challenges involved when fine sediment is used as building material. To optimally decide on the building design of the wetland it is essential to

understand factors such as crest stability, consolidation, and soil formation in relation to this unique building material. Furthermore, insight into how to reduce nature management is desirable, and so is monitoring the effects of the wetland at a larger scale – i.e., the Markermeer as a whole. For these reasons, several research projects have been initiated that vary in size and scope.

In 2014, the research project “Smart Ecosystems” started, funded by the Netherlands Organization for Scientific Research (NWO) to Utrecht University, Delft University of Technology and the University of Amsterdam. The stakeholders involved are Boskalis, Van Oord, Deltares, RoyalHaskoningDHV, Natuurmonumenten, and Wageningen Environmental Research. This research project consists of three subprojects that focus on: 1) abiotic-biotic dynamics, 2) ecological and biogeochemical processes, and 3) integrating biophysics, governance, and ecosystem services. The overall aim is to identify and understand the non-linearities and feedbacks that occur in the transition from mud to a wetland system. This dissertation presents the findings of the second subproject within the NWO Smart Ecosystems research project.

1.6 THESIS SCOPE, RESEARCH OBJECTIVES, AND OUTLINE

As outlined in section 1.2, building with sand is becoming costlier and sand demand will rise in the future as a result of rapid urbanization (Torres et al., 2017). A potential alternative to sand is fine sediment, which is sufficiently available in many river delta regions (Jalowska et al., 2017; McKee et al., 2004). However, potential problems arise when fine sediments are used as building material for the wetland. Wetland plants must establish and the material needs to consolidate sufficiently fast to promote soil formation and prevent erosion by wind and wave action. In ecological engineering projects in particular, it is important to identify ecological processes and ecosystem engineers that interfere with consolidation, succession, and soil formation (e.g., Mitsch et al., 1998; Jones et al., 1994). At this moment, there is insufficient knowledge of the biogeochemical and wetland ecological mechanisms that control ecosystem development on artificial wetland built from fine sediment. To optimize the design of the eco-engineering project in lake Markermeer, knowledge about such mechanisms is essential.

The general aim of the research described in this thesis was to understand how biogeochemical and wetland ecological processes interact to enhance ecosystem development on wetlands built from soft clay-rich material (Figure 1.7). The results will be gathered in a toolbox for stakeholders involved in the construction and management of the Marker Wadden, including the findings of the other two subprojects. Hopefully, the knowledge generated from this subproject will be of help: 1) for improving the design of the eco-engineering project when the Marker Wadden is expanded with more islands, 2) when designing nature management practices for the Marker Wadden, and 3) for exporting the concept of this Building with Nature project to other countries.

Specifically, the objectives of the research described in this thesis were to determine:

1. Important biogeochemical processes in mud aeration and feedback mechanisms between plant and soil (Chapter 2),
2. The effects of indirect iron toxicity in terms of nutrient stoichiometry and biomass production for three wetland plant species (Chapter 3),
3. How the hydrological regime in relation to sediment type interferes with ecosystem development (Chapter 4),
4. To what extent *Phragmites australis* enhances the consolidation of the soft clay-rich layer (Chapter 5),
5. The influence of bioturbators on the nutrient availability in porewater and surface water in the littoral zone of the wetlands (Chapter 6).

A combination of greenhouse and laboratory experiments was conducted to address the research objectives mentioned above. In addition, some measured data from these experiments formed important input for subsequent analysis in a modeling environment. In **Chapter 2**, the geochemical composition of the building material of the wetlands is investigated in relation to plant development. The resulting knowledge formed a solid basis for follow-up experiments. This chapter describes biogeochemical plant – soil feedback processes that occur when oxidation, drying, and modification by *Phragmites australis* alter the biogeochemical conditions of Markermeer sediments, in turn affecting vegetation development. It also reports on how *P. australis* eco-engineers

its environment by expediting biogeochemical processes in the sediment. The iron-toxicity hypothesis posed in Chapter 2 was tested with a follow-up experiment described in **Chapter 3**. The follow-up experiment aimed to link initial plant development of three wetland species in iron-rich sediments to the concentration of nutrients, iron, and iron-bound phosphorus, as well as to the type of iron plaque formed on the roots. Furthermore, stoichiometric effects in both leaves and roots as a consequence of iron precipitation on roots were studied. Longer-term effects of plant development in the clay-rich sediments from Markermeer are described in **Chapter 4**. A two-year mesocosm experiment with *P. australis* and *Rumex maritimus* addressed the effects of periodic inundation and of sediment type on ecosystem functioning in terms of nutrient availability. Special attention was paid to optimal conditions for the development of *P. australis*, as this is the intended ecosystem engineer on the wetlands (Boskalis, 2015). **Chapter 5** shifts away from plant development and nutrient availability and focuses on consolidation. Enhancing consolidation is important to combat soil erosion and accelerate ecosystem development. This chapter presents the work of a joint experiment with Delft University of Technology. In a controlled column experiment we studied how *P. australis* acts as an ecosystem engineer that enhances drainage in soft clay-rich sediment, thereby promoting sediment consolidation. Finally, **Chapter 6** explores processes below water: the potential of bioturbating Tubificidae to alter biogeochemical processes by sediment aeration is investigated, especially in terms of nutrient availability in pore water and surface water.

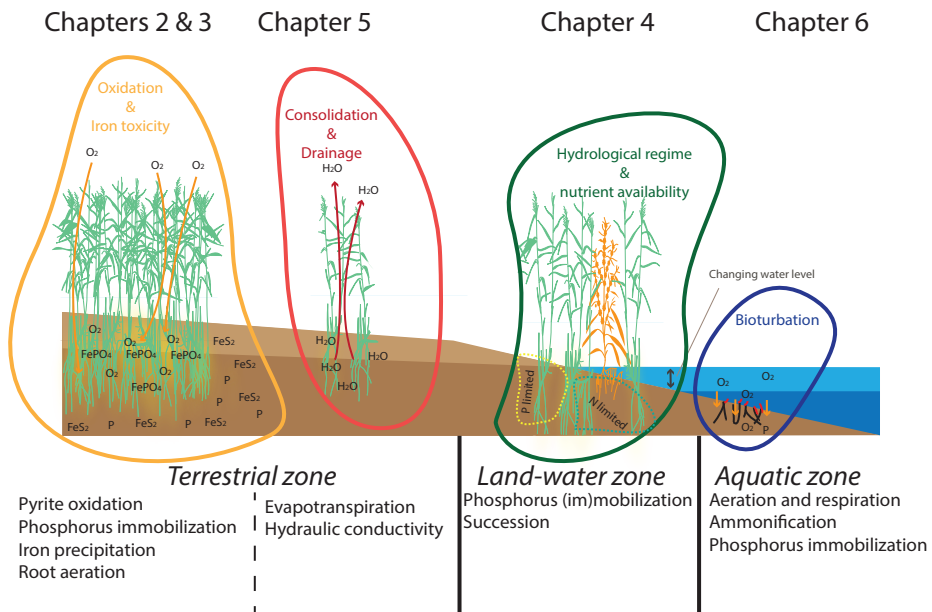


Figure 1.7. Different processes and several parts of a wetland system are covered in this thesis. Chapters 2 and 3 focus on effects of aeration and oxidation processes – including iron toxicity – initiated by plants (yellow: terrestrial zone), Chapter 4 focuses on hydrological regimes and their effects on nutrient availability (green: land – water zone), Chapter 5 on consolidation effects via enhanced drainage (red: terrestrial zone) and Chapter 6 focuses on aeration effects via bioturbating Tubificidae (blue: aquatic zone).

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CHAPTER

2

Wetland eco-engineering:
measuring and modeling
feedbacks of oxidation
processes between plants
and clay-rich material

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ABSTRACT

Interest is growing in using soft sediment as a foundation in eco-engineering projects. Wetland construction in the Dutch lake Markermeer is an example: here, dredging some of the clay-rich lake-bed sediment and using it to construct wetland will soon begin. Natural processes will be utilized during and after construction to accelerate ecosystem development. Knowing that plants can eco-engineer their environment via positive or negative biogeochemical plant – soil feedbacks, we conducted a 6-month greenhouse experiment to identify the key biogeochemical processes in the mud when *Phragmites australis* is used as an eco-engineering species. We applied inverse biogeochemical modeling to link observed changes in pore water composition to biogeochemical processes. Two months after transplantation we observed reduced plant growth and shriveling and yellowing of foliage. The N:P ratios of the plant tissue were low and these were affected not by hampered uptake of N, but by enhanced uptake of P. Subsequent analyses revealed high Fe concentrations in the leaves and roots. Sulfate concentrations rose drastically in our experiment due to pyrite oxidation; as reduction of sulfate will decouple Fe-P in reducing conditions, we argue that plant-induced iron toxicity hampered plant growth, forming a negative feedback loop, while simultaneously there was a positive feedback loop, as iron toxicity promotes P mobilization as a result of reduced conditions through root death, thereby stimulating plant growth and regeneration. Given these two feedback mechanisms, we propose the use of Fe-tolerant species rather than species that thrive in N-limited conditions. The results presented in this study demonstrate the importance of studying the biogeochemical properties of the situated sediment and the feedback mechanisms between plant and soil prior to finalizing the design of the eco-engineering project.

2.1 INTRODUCTION

Nowadays, natural processes are being used across the world to achieve fast ecosystem development while at the same time providing opportunities for developing hydraulic infrastructure, a concept called Building with Nature (BwN) (Temmerman et al., 2013). Though mostly focused on water safety and coastal protection (e.g., Borsje et al., 2011), BwN can also be applied to the management of fine sediments. A relevant application could be to use soft sediments as material for building freshwater wetlands. Here, vegetation can be used as an eco-engineer (Jones et al., 1994) to modify the environment (Lambers et al., 2009). When fine sediments are used for the construction of wetlands, however, the use of eco-engineers is anticipated to pose challenges in relation to crest stability, consolidation and soil formation.

In the Netherlands, a soft, clay-rich lake-bed sediment is causing serious turbidity problems in the Markermeer (an artificial lake of 691 km²): primary productivity is impeded and biodiversity in the lake is declining (Vijverberg et al., 2011; Noordhuis et al., 2014). Because the lake is shallow, wind-induced waves frequently induce high bed shear stress, which causes sediment to be resuspended (Vijverberg et al., 2011). To improve the ecological conditions in the lake, plans are underway to dredge some of the soft, clay-rich sediment and use it to construct approximately 10,000 ha of wetlands.

Plants produce root exudates, which influence soil formation by enhancing microbiological activity (Holtkamp et al., 2011), biological weathering and nutrient cycling (Taylor et al., 2009; Bradford et al., 2013). An example is the ability of plant roots to mobilize P by ligand exchange and dissolution of Fe-bound P (Fe-P) by citrate and oxalate excretion (Gerke et al., 2000). Plant roots may also enhance consolidation processes in substrate by increasing horizontal and vertical drainage (O'Kelly, 2006).

However, both negative and positive plant – soil feedbacks exist, in which the physical and chemical properties of the soil affect plant development and vice versa (Ehrenfeld et al., 2005). Therefore, when looking at soil formation, it is important to study the signs and strengths of these plant – soil feedback mechanisms. For example, nutrient conditions codetermine the type of plant community that develops (e.g., Olde Venterink, 2011), which in turn influences the nutrient conditions in the soil itself (Onipchenko et al., 2001). As feedback mechanisms differ between plant

species (Ehrenfeld et al., 2005), it is essential to determine which eco-engineer is most appropriate for accelerating ecosystem development in these sediments.

De Lucas Pardo (2014) found that the Markermeer mud deposits had a high water content (20 – 60% of fresh weight) and were largely anoxic, with oxygen present only in the top 2 mm. Therefore, when such mud is taken from the lake and spread out in contact with the air, biogeochemical plant – soil processes related to oxidation and drying of the top soil are expected to play a significant role. Two types of clay-rich deposits are the intended sediment for the wetland. Their composition is the product of a combination of historical and present-day factors. Prior to 1932, the year in which the dam cutting off the Zuiderzee from the North Sea was completed, this was a marine environment into which several rivers discharged, including a branch of the River Rhine (the River IJssel). Hence, a near-shore marine deposit underlies the present-day soft, clay-rich sediment. This soft, clay-rich layer is produced by bioturbation and physical weathering and continuously resuspends as a result of wave action (Van Kessel et al., 2008; De Lucas Pardo et al., 2013). This layer accumulated after 1976, when northward sediment transport was blocked by a second dam that separated Markermeer from IJsselmeer, thus allowing suspended matter to resettle on top of the marine deposit. We can therefore distinguish two layers: an upper disturbed mud layer prone to bioturbation and erosion and a relatively undisturbed layer below.

We set up an experiment to monitor the chemical composition of pore water to identify the biogeochemical plant – soil feedback processes that occur when oxidation, drying and modification by plants alter the biogeochemical conditions of these two sediment types, thus in turn affecting vegetation development. Our study has two subsidiary aims: to ascertain how *Phragmites australis* eco-engineers its environment by expediting biogeochemical processes in the deposits, and to simulate the geochemical differences between disturbed mud and undisturbed clay deposits and relate these to the processes identified from the pore water by using PHREEQC (Parkhurst and Appello, 2013) for inverse modeling. In addition, we altered the grain size of the disturbed mud deposit by adding inert sand to see how grain size distribution impacts pore water chemistry.

Changes in biogeochemical processes that are related to oxidation are expected to play a major role as *P. australis* is known for its high radial oxygen loss (Brix et

al., 1996; Dickopp et al., 2011; Smith and Luna, 2013). Oxidation of the sediment will decrease the concentration of phytotoxins typically found in waterlogged soils, such as iron, and therefore will have a positive effect on plant development. This will be more pronounced in undisturbed mud, which is largely anoxic, than in disturbed mud, of which the top layer is already oxidized and where bioturbation modified the sediment. The type of biogeochemical processes altered will depend on the intrinsic properties of the different sediment types, which will be examined in this study.

2.2 MATERIAL AND METHODS

2.2.1 Setup

A greenhouse experiment was conducted for 6 months at the test facility of Utrecht University. A basin of 4 m² (2 x 2 m) was filled with artificial rainwater and was refreshed every 2 weeks. At regular intervals, the chemistry of the water was checked to ensure that the water composition remained stable during the experiment. The artificial rainwater was made by adding 15 µmol NH₄(SO₄), 50 µmol NaNO₃ and 30 µmol NaCl to osmosis water. These values reflect the average rainwater composition in the Netherlands for the period 2012 – 2013 (LMRe, 2014).

The sediments used include the soft, clay-rich layer (Mud_{soft}) and the underlying, consolidated, Zuiderzee deposit (Clay). In principle, both sediments have the same origin and were collected in the same area. We also included a third sediment type (Mud_{sand}), as it is expected that Mud_{soft} will be too soft for constructing wetlands: a 1:1 mixture was made by mixing mud with Dorsilit® crystal silica sand (c. 99% SiO₂) which had been autoclaved for 1 h at 120 °C prior to mixing. The sand grains of this material are 0.3-0.8 mm in diameter, with D₅₀ being 0.57 mm. The Mud_{soft} and Clay sediments were collected by mechanical dredging in the southern part of the lake and were stored in airtight containers at 4 °C prior to the start of the experiment.

Plastic pots (diameter 10 cm, depth 18 cm) with a perforated base were filled to within 1 cm from the top with one of the three sediment types used ($t = 0$). In each pot, two soil moisture samplers (Rhizon Flex-5cm; Rhizosphere, Wageningen, the Netherlands) were installed horizontally at depths of 1 cm and 11 cm below the sediment surface (these depths are hereafter referred to as D_1 and D_{11}), their tips

reaching 5 cm from the pot wall. The pots stood in rows in the basin. The water level was maintained at 9 cm so that the sediment at D_{11} remained saturated, while the sediment at D_1 could oxidize and dry. Each sediment type had 13 replicates.

Reed seedlings (*Phragmites australis*) had been grown in nutrient-poor peat and when 35 – 40 days old (experimental time $t = 22$ days), a single reed seedling was planted per pot in eight of the replicates, leaving five replicates unplanted. Any other seedlings that germinated spontaneously in the pots were removed immediately.

2.2.2 Chemical analysis

Soil moisture at D_1 and D_{11} was collected from the moisture samplers on days 0, 3, 10, 22, 36, 64, 92, 134, and 174 from five of the pots per condition. The samples from the five replicates were pooled and chemically analyzed. Chloride, NH_4 , NO_2 , NO_3 , and SO_4 were determined using ion chromatography (IC); Ca, Fe, K, Mg, Na, P, Si, and Sr were determined with inductively coupled plasma optical emission spectrometry (ICP-OES), pH was determined by an ion-specific electrode, and alkalinity was measured by a classic titration method.

Table 2.1. List of steps used in the extraction procedure of phosphorus (based on Ruttenberg, 1992).

Step	Extractant	Separated P fraction
I	1M MgCl_2 , 30 min	Exchangeable or loosely sorbed P
II	A Citrate-dithionite-bicarbonate (CDB), 8 h	Easily reducible or reactive ferric Fe- P
	B 1M MgCl_2 , 30 min	
III	A Na acetate buffer (pH 4), 6 h	Amorphous apatite and carbonate P
	B 1M MgCl_2 , 30 min	
IV	1M HCl, 24 h	Crystalline apatite and other inorganic P
V	Ash at 550 °C, 2h; 1M HCl, 24 h	Organic P

Sediment samples were collected for each sediment type at $t = 0$ and were freeze-dried and stored anoxically prior to geochemical analysis. The major elements were determined using ICP-OES following an aqua regia destruction. Total S content was measured on an elemental CS analyzer and the mineralogical composition was determined with X-ray diffraction (XRD). A sequential extraction method based on Ruttenberg (1992) was applied to characterize solid P speciation. The method involves

five steps (Table 2.1), the first four of which were carried out anoxically. Loss on ignition (LOI) was determined by slowly heating to 1000 °C. LOI was also used as a proxy for organic matter content and total carbonates by calculating the weight loss between 105 and 550 °C for organic matter and the weight loss between 550 and 1000 °C for total carbonates (Howard, 1965). The cation exchange capacity (CEC) of the sediments was calculated from the organic matter content and the amounts and types of clay minerals present (Bauer and Velde, 2014).

Fifty seedlings of *P. australis* randomly chosen from the seedlings grown for the experiment were used to determine the initial tissue contents of Fe, K, P, and N. Their roots, shoots and leaves were separated and air-dried. The air-dried material was then ground and analyzed with total reflection X-ray fluorescence (TXRF) to determine tissue contents of Fe, K, and P. Nitrogen content was determined on an elemental CN analyzer. At the end of the experiment ($t = 174$), the plants in the pots were harvested and subjected to the same procedure to determine the tissue contents of Fe, K, P, and N.

2.2.3 Modeling of biogeochemical processes

To identify important biogeochemical processes during the incubation experiments, we modeled with PHREEQC (Parkhurst and Apello, 2013). PHREEQC modeling is frequently used in geochemical research focusing on issues of water quality: examples include investigating mineral weathering in a mountain river (Lecomte et al., 2005), deducing geochemical processes in groundwater (Belkhir et al., 2010) and investigating the interaction between two aquifers (Carucci et al., 2012). Here, we applied it to identify biogeochemical plant – soil processes during the oxidation and natural drying out of the soil.

The model approach is based on mass-balance equations of preselected mineral phases (reactants). The mineral phases can either precipitate (leave the solution) or dissolve (enter the solution), and these are expressed in mole transfers. As we only know the dynamics in concentrations of the pore water, we applied inverse modeling, in which all possible combinations of the mass-balance equations are accepted within a range of measured pore water concentrations $\pm 4\%$. We can simulate infiltration or evaporation rates from the pore water. Since in freshwater mud deposits, the

dissolution or precipitation of salts (e.g., NaCl) is negligible and can be ignored, the change in pore water Cl concentration was used to calculate the amount of water evaporated or infiltrated.

To enable the model to attribute some of the chemical changes to cation exchange processes we included an assemblage of exchangers (X): CaX_2 , FeX_2 , KX , MgX_2 , NaX , and NH_4X . The sum of this assemblage was defined as CEC calculated from the sediment composition. CEC is important, since it can buffer some of the biogeochemical processes in sediments by adsorption or desorption of cations.

We identified three time frames in our models: 1) oxidation and natural drying out of the soil before the seedlings were transplanted into the pots ($t = 0 - 22$ days); 2) initial stage of plant growth ($t = 22 - 64$ days); and 3) the stage at which roots started to influence pore water chemistry ($t = 64 - 176$ days). These time frames were identified by analyzing the chemical data that were collected. When concentrations at D_{11} in the planted condition started to deviate from the unplanted condition, this was seen as a sign that plant roots started to influence pore water chemistry.

Inverse modeling was applied for all combinations (sediment type, plant/no plant and depth) for each time frame. For every combination, several valid simulations were found, due to small differences in the amount of mole transfers attributed to the mineral phases. Here we present the plausible simulation with the least amount of mole transfers for each combination.

2.2.4 Statistical analysis

Statistical analysis was carried out using the programs R and SPSS. Differences in sediment, pore water and plant tissue concentrations between sediment treatments were determined using one-way ANOVA with a Tukey's honestly significant difference (HSD) post hoc test. No statistics could be applied to the mineralogical sediment composition (XRD analysis) due to the absence of replicates.

2.3 RESULTS AND DISCUSSION

First, the three sediment types will be compared in terms of certain geochemical and mineralogical elements. Next, the composition of the pore water will be introduced

and will be linked to biogeochemical processes by presenting and discussing the PHREEQC model simulations. Then, the plant response is presented and discussed in terms of biomass and plant tissue chemistry. Lastly, the implications for eco-engineering will be discussed.

2.3.1 A brief comparison between sediment types

Table 2.2 shows the geochemical composition of the disturbed Mud_{soft} and Mud_{sand} and undisturbed Clay sediments used in this study. The differences between Mud_{soft} and Mud_{sand} are solely attributable to the presence of inert Dorsilit®.

The total sediment concentrations of Al, Fe, Mg, Mn, Na, P, and Zn were significantly higher in Clay than in Mud_{soft} ($p < 0.05$). The quartz content was also higher in Clay, which suggests that there were more reactive minerals in this type of sediment.

Sequential P extraction revealed that the significant difference in total P consists of a significantly lower content of Fe-P in Mud_{soft} than in Clay (279 mg/kg versus 772 mg/kg; $p < 0.01$). The presence of Fe-P in the anoxic Clay sediment was unexpected, as in anoxic conditions Fe prefers to bind with S to form FeS₂. However, after exhaustion of S, precipitation of Fe(II) phosphates may occur (Jilbert and Slomp, 2013). Another possibility is that the reduction of crystalline Fe(III) is not complete in the anoxic sediment because kinetic processes are slow (Canavan et al., 2007). This is likely the case in Markermeer, given our strict anoxic procedures for storage and analysis of the samples. The exchangeable (or loosely sorbed) P was low in Mud_{soft} and Clay, indicating that only a small part of the total P found in the sediments was readily available for uptake. The other three P pools were fairly similar and did not differ significantly between the two types of sediment ($p = 0.42$ for Ca-bound P; $p = 0.11$ for detrital P; and $p = 0.94$ for organic P).

The mineralogical analysis (XRD) showed not only that the quartz content was lower in Mud_{soft} than in Clay (37% versus 48%) but that the amounts of calcite and pyrite did not differ between the two types of sediment (9% calcite and 0.6% pyrite). The amount of phyllosilicates (sum of illite, smectite, kaolinite, and chlorite) was higher in Mud_{soft} than in Clay: 43% versus 30%. This must also have caused the CEC

to be higher in Mud_{soft}, as the organic matter content did not differ much between the two (7.2% in Mud_{soft} and 6.8% in Clay).

Table 2.2. Geochemical and mineralogical composition of the sediment types used in this study. Significant differences between Mud_{soft} and Clay are indicated by * ($p < 0.05$).

		n per type	Clay		Mud _{soft}		Mud _{sand}	
	Unit		Mean	S.D.	Mean	S.D.	Mean	S.D.
Aqua regia/CS								
Al*	mg/kg	15	21989	4512	16593	3130	6394	2439
Ca	mg/kg	15	48031	3032	45635	6020	18877	3572
Fe*	mg/kg	15	27766	3764	20745	2987	7804	2281
K	mg/kg	15	5371	1262	4102	641	1723	742
Mg*	mg/kg	15	8041	1017	6636	906	2531	558
Mn*	mg/kg	15	710	166	577	160	238	62
Na*	mg/kg	15	992	379	526	158	219	64
P*	mg/kg	15	1186	217	649	169	259	56
S	mg/kg	15	5727	710	5586	698	3001	846
Sr	mg/kg	15	148	21	135	26	62	14
Ti	mg/kg	15	312	74	312	77	125	44
Zn*	mg/kg	15	159	58	110	29	43	18
Seq. P extraction								
Exchangeable P	mg/kg	15	14.3	6.81	11.9	3.50	5.9	1.79
Fe-bound P*	mg/kg	15	772	263	279	61.7	94.5	29.0
Ca-bound P	mg/kg	15	146	43.3	121	30.9	36.8	13.1
Detrital P	mg/kg	15	147	16.5	169	14.1	51.5	10.9
Organic P	mg/kg	15	99.6	20.0	117	25.1	47.7	8.38
XRD								
Quartz	%	1	48		37		n.a.	
Calcite	%	1	9		9		n.a.	
Pyrite	%	1	0.6		0.6		n.a.	
Illite	%	1	15		21		n.a.	
Smectite	%	1	11		14		n.a.	
Kaolinite	%	1	3		5		n.a.	
Chlorite	%	1	2		3		n.a.	
Other								
Organic matter	%	5	6.7	0.6	7.2	0.6	2.8	0.4
CEC (calculated)	meq/100g		30.0		37.2		12.4	

2.3.2 Pore water composition

Figure 2.1 presents time series for the pore water concentrations of the three macronutrients N, P, and K. The initial decrease in NH_4 and increase in NO_x at a depth D_1 for the planted conditions was most likely caused by nitrification as a result of oxidation (Figure 2.1a – f). At the end of the experiment, almost all dissolved inorganic nitrogen had been removed from the pore water in the pots with plants, whereas in the pots without plants, the NH_4 concentrations remained substantial. Furthermore, a high peak in NO_x was observed in Clay sediments at day 10 of the experiment. At a depth D_{11} , no large changes were found in general for NH_4 and NO_x .

A sharp decline in soluble P was visible at D_1 for all three sediments, probably because P precipitated with Fe(III) when oxygen penetrated the top layer (Figure 2.1g – i). However, in Clay this decline was preceded by an increase in P. After several weeks, a thin moss layer started to develop on top of the Mud_{soft} sediment, which probably prevented oxygen from penetrating and thereby increased the P concentrations (Figure 2.1g). Similar developments were observed for Mud_{sand} although here the moss layer developed much later. In Clay, no moss grew throughout the experiment.

Concentrations of K were higher than concentrations of N and P and increased in the first few weeks (Figure 2.1j – l). No difference was found between pots at D_{11} with or without plants. However, K was significantly higher at D_1 in the planted pots with Mud_{sand} ($p < 0.05$).

Although it may be important to study measured concentrations of nutrients in pore water in order to understand plant functioning, deriving biogeochemical processes from measured data is problematic as changes in pore water can be caused by multiple processes such as drying, dilution, dissolution and precipitation. Figure 2.2 reveals that the drying of soils at D_1 was probably an important factor because we observed an initial increase in Cl that indicated that Cl could not dissolve in the three sediments used (e.g., halite dissolution). Drying will have influenced other variables as well, such as sulfate (Figure 2.2d – f). Comparing the patterns of Cl and SO_4 suggests that the change in SO_4 concentrations at D_1 should be partly attributed to drying out

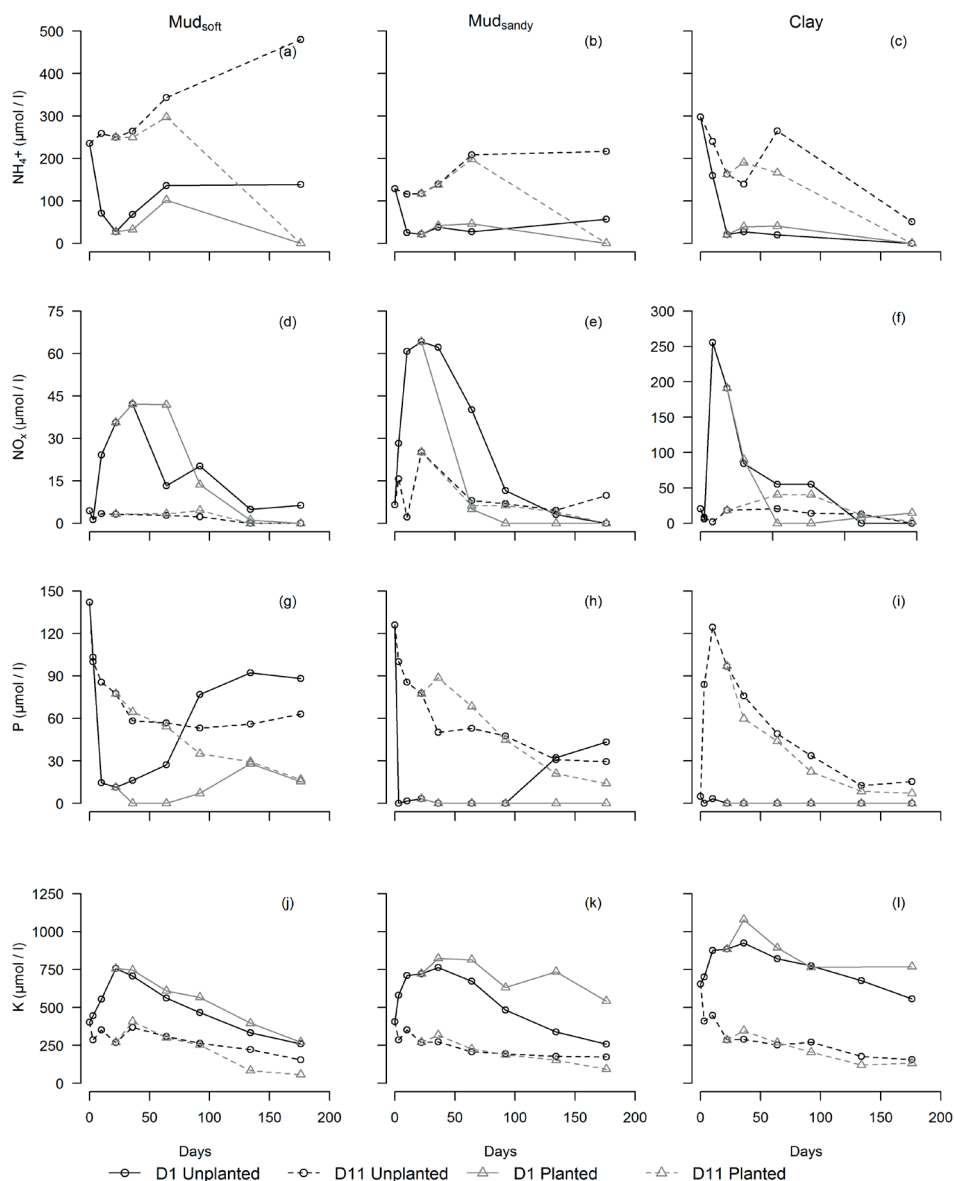


Figure 2.1. Time series of NH_4^+ (a–c), NO_x (d–f), P (g–i), and K (j–l) concentrations. Each column represents one sediment type: Mud_{soft} (a, d, g, j), $\text{Mud}_{\text{sandy}}$ (b, e, h, k), and Clay (c, f, i, l). The variable and the scale of the x-axis are the same for each row, except for the scale in f.

of soils and partly either to dissolution (e.g., pyrite oxidation) or to precipitation (e.g., gypsum formation). This highlights the need to use geochemical reaction models like PHREEQC to inversely derive biogeochemical processes from measured data

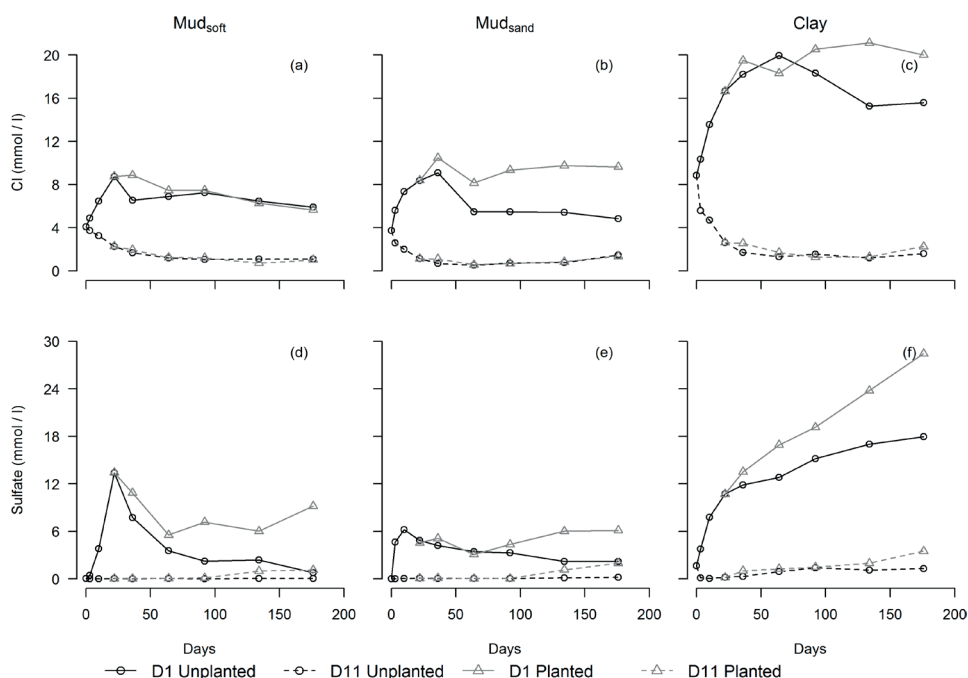


Figure 2.2. Time series of Cl (a–c) and SO_4 (d–f) concentrations. Each column represents one sediment type: Mud_{soft} (a, d), Mud_{sand} (b, e), and Clay (c, f). The variable and the scale of the x-axis are the same for each row.

2.3.3 Pore water processes (PHREEQC model simulations)

The main pore water processes modeled by PHREEQC are presented in Table 2.3. For clarity, only major reactants are included in this Table. Supplementary Tables A1 and A2 present mole transfers for all reactants used, as well as the number of valid simulations per combination found.

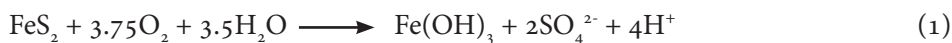
Table 2.3. Main pore water processes expressed in mole transfers ($\mu\text{mol l}^{-1} \text{ day}^{-1}$) as modeled by PHREEQC with pore water data retrieved at 1 cm and 11 cm below sediment surface (D_1 and D_{11} , respectively). Positive values indicate dissolution; negative values indicate precipitation. Cation exchange capacity (CEC) is the sum of Ca, Fe, K, Mg, Na and NH_4^+ .

Phase	Condition	Calcite		Gypsum		Fe(OH) ₃		Pyrite		ΣCEC		H ₂ O ($\times 10^3$)		O ₂	
		D ₁	D ₁₁	D ₁	D ₁₁	D ₁	D ₁₁	D ₁	D ₁₁	D ₁	D ₁₁	D ₁	D ₁₁	D ₁	D ₁₁
1. Oxidation t=0-22 days	Mud _{soft}	267	111	0.00	-72.5	-277	0.00	270	36.2	-31.3	20.2	-3364	0.00	1009	119
	Mud _{sand}	0.00	59.6	0.00	-40.7	-116	0.00	109	21.7	-4.99	7.92	-2591	0.00	432	69.5
	Clay	120	55.2	0.00	-53.4	-160	0.00	159	20.1	-91.4	14.0	-2364	0.00	659	61.9
2. Initial root development t=22-64 days	No plant	27.1	0.00	-236	0.00	0.95	-0.24	0.00	0.00	-23.1	1.43	0.00	0.00	2.62	0.00
	Plant	48.8	19.8	-208	-3.81	-10.0	-6.19	9.76	0.00	-7.63	1.43	0.00	0.00	45.5	0.00
	No plant	39.3	71.7	0.00	0.00	0.00	-41.2	0.21	0.00	1.90	1.46	380	0.00	0.00	0.00
	Plant	7.10	83.8	-83.4	0.00	0.00	-51.2	3.58	0.00	5.40	3.40	-996	0.00	0.00	0.00
	No plant	0.00	27.1	-32.1	0.00	-21.4	-25.0	21.2	0.00	0.01	-0.23	-286	0.00	41.9	0.00
3. Root influence t=64-176 days	Plant	36.9	16.2	0.00	0.00	-14.3	0.00	64.3	11.9	28.4	4.53	-6.67	0.00	186	40.5
	No plant	0.00	-3.21	-19.2	0.00	-1.34	-0.80	0.00	0.00	-1.07	-1.43	56.3	0.00	0.00	0.00
	Plant	25.8	0.00	0.00	0.00	-4.20	0.00	23.8	4.11	7.88	-4.65	49.1	0.00	83.6	13.6
	No plant	8.13	0.00	-7.59	0.00	-10.6	-1.34	0.00	0.00	-1.78	1.42	74.1	0.00	0.00	0.00
	Plant	0.00	0.00	-14.8	0.00	-13.3	-23.2	13.8	7.95	0.12	-10.6	-357	-652	44.7	32.6
	No plant	0.00	11.5	0.00	0.00	0.00	-13.8	33.3	0.00	23.9	0.36	134	0.00	113	0.00
	Plant	115	18.7	0.00	0.00	-58.5	-8.48	58.3	8.57	45.4	-5.73	0.00	-98.2	215	28.4

Phase 1: oxidation and drying (t = 0 – 22 days)

As discussed in section 3.2, initial drying of soils occurred at D_1 immediately after exposure to air. In the model, this is illustrated by high evaporation rates expressed as H_2O loss ($2300 - 3400 \text{ mmol l}^{-1} \text{ day}^{-1}$; Table 2.3). The model accounts for this loss by adjusting the solution fractions before calculating other mole transfers.

Exposure to air also leads to oxidation, more so at D_1 than at D_{11} (Table 2.3). The increase in measured sulfate is partly explained as pyrite oxidation ($109 - 270 \text{ } \mu\text{mol l}^{-1} \text{ day}^{-1}$ for D_1 and $20.1 - 36.2 \text{ } \mu\text{mol l}^{-1} \text{ day}^{-1}$ for D_{11} , respectively). Oxidation of pyrite also produces iron oxyhydroxides and protons, which in turn promotes the dissolution of calcite. The overall reactions are



followed by calcite dissolution



The mole transfers for pyrite and calcite presented in Table 2.3 indicate that not enough calcite is dissolved to buffer all H^+ produced by dissolution of pyrite. Indeed, a drop in pH was observed at the beginning of the experiment (not shown). However, the mineralogical composition presented in Table 2.2 shows that the amount of calcite (9%; 900 mmol) far exceeds that of pyrite (0.6%; 50 mmol). These numbers suggest that even if all pyrite were to be oxidized, enough calcite is present to buffer all H^+ produced (200 mmol). Note that for Mud_{sand} these values are lower due to mixing with Dorsilit®.

Some aeration occurred at D_{11} . The O_2 fluxes ranged between 61 and $119 \text{ } \mu\text{mol l}^{-1} \text{ day}^{-1}$, which resulted in small amounts of pyrite being oxidized ($20 - 36 \text{ } \mu\text{mol l}^{-1} \text{ day}^{-1}$). However, sulfate concentrations did not rise, as a result of subsequent precipitation with Ca to form gypsum ($53 - 73 \text{ } \mu\text{mol l}^{-1} \text{ day}^{-1}$). Furthermore, the CEC of the sediments buffered some processes in pore water chemistry by net adsorption of cations at D_1 and net desorption at D_{11} .

The processes described above occurred in all three sediments, although oxidation was higher in Mud_{soft} than in Mud_{sand} and Clay, probably because higher evaporation rates in Mud_{soft} enhanced oxidation and affected other reactants related to oxidation.

Phase 2: initial stage of plant growth (t = 22 – 64 days)

While the pore water compositions did not show clear differences between unplanted and planted conditions during the initial stage of plant growth, the inverse modeling provided clear evidence for differences at D_1 . However, chemical differences between unplanted and planted conditions for Mud_{sand} might simply be attributed to concentration/dilution due to H_2O loss/gain (-996 to $380 \text{ mmol l}^{-1} \text{ day}^{-1}$).

Overall, more pyrite was oxidized in the planted conditions, though the rates are much lower than in the first phase ($0 - 64.3 \text{ } \mu\text{mol l}^{-1} \text{ day}^{-1}$). This observation provides evidence that plants may enhance pyrite oxidation by radial oxygen loss (i.e., root aeration). Ferric oxide production on pyrite surfaces probably impeded further oxidation of pyrite, which is a common phenomenon in carbonate-buffered conditions (Nicholson et al., 1990). Indeed, the total pyrite that had oxidized after 64 days (6.3 mmol for Mud_{soft} , 2.5 mmol for Mud_{sand} and 6.2 mmol for Clay, calculated from the rates presented in Table 2.3) corresponds to a small fraction of total pyrite present (50 mmol).

Saturation with gypsum led to precipitation of SO_4 and Ca at D_1 . Table 2.3 shows that with the exception of Mud_{sand} , mole transfers were lower for planted conditions; the probable reason is that citric acid production by root tips retarded gypsum precipitation (Prisciandaro et al., 2005). This process was not relevant at D_{11} , as here aeration (and subsequent sulfate production) by plant roots was minor (in the case of Clay) or absent (in the case of Mud_{soft} and Mud_{sand}).

The thin moss layer that started to develop after several weeks in the unplanted condition on top of the Mud_{soft} sediment slowed down the aeration rate to $2.62 \text{ } \mu\text{mol l}^{-1} \text{ day}^{-1}$ and might be the reason for the moderate increase in P, which probably resulted from $\text{Fe}(\text{OH})_3$ dissolution ($0.95 \text{ } \mu\text{mol l}^{-1} \text{ day}^{-1}$) (Figure 2.1g, Table 2.3).

Phase 3: root influence (t = 64 – 176 days)

Phase 3 took place in the autumn, when temperatures were lower and therefore the soils did not dry out; hence there was a net gain in H_2O . The gain was less in planted conditions, due to the uptake of water by roots.

The fully grown plants continued to influence pore water chemistry at D_1 , but in the unplanted conditions the chemical changes were minor (Table 2.3). Radial oxygen loss continued the oxidation processes described in the previous sections. It should be noted that *P. australis* is known to have higher radial oxygen loss than other wetland species (Brix et al., 1996; Dickopp et al., 2011; Smith and Luna, 2013), so the aeration effect found in this study cannot be assumed to hold for other species.

In contrast to the previous phase, in phase 3 the influence of roots was clearly visible at D_{11} for all three sediments. All planted sediments showed increased aeration and subsequent oxidation of pyrite due to radial oxygen loss, with a notable difference between Mud_{soft} (lower) and Mud_{sand} (higher). This is somewhat surprising, as the belowground biomass was significantly higher in Mud_{soft} (section 3.4). It indicates that increasing the average grain size by adding sand enhanced aeration, even when root biomass production was low.

2.3.4 Plant response

Above- and belowground biomass was significantly higher in Mud_{soft} and Clay than in Mud_{sand} (Figure 2.3; $p < 0.02$). The difference between the two Mud sediments cannot be explained by nutrient concentrations in rainwater or light conditions in the greenhouse, as these were the same for the two sediments. As biomass production in Mud_{sand} was not limited by chemical or biological properties relative to Mud_{soft} , it seems likely that the reason for the lower biomass production in Mud_{sand} is a difference in physical properties. Voorhees et al. (1975) and Bengough and Mullins (1990) showed that so-called mechanical impedance (i.e., the resistance to penetration by the root tip) was higher in loamy sand than in clay, which was attributed to the higher bulk density of the loamy sand. Therefore, increasing the bulk density of Mud_{soft} by mixing with sand increased the mechanical impedance, and this might explain the lower biomass production we observed in Mud_{sand} .

Phragmites australis invested more in its root system than in its shoots and leaves for all sediments (Figure 2.3; $p < 0.01$). More investment in roots implies a limitation of N, P, and/or S (Ericsson, 1995; Shipley and Meziane, 2002). Figures 2.1a – i and 2d – f show that the N and P concentrations were indeed low in the planted conditions but that SO_4 was high, which rules out S limitation. During the experiment, we had

observed reduced plant growth and shriveling and yellowing of foliage 2 months after transplantation, which might have been caused by nutrient limitation.

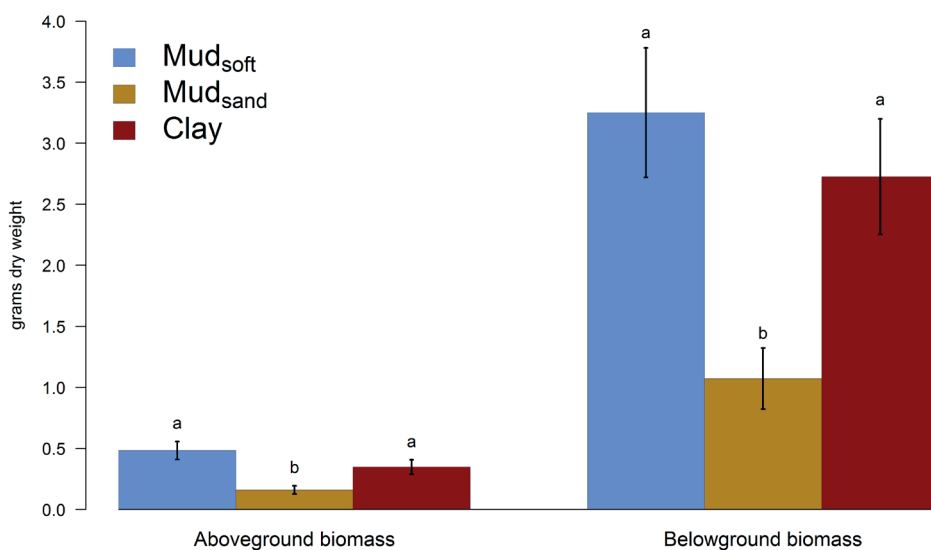


Figure 2.3. Above- and belowground biomass in grams dry weight, with error bars ($n = 5$). Significant differences between sediment types are indicated by different letters, and nonsignificant differences are indicated by the same letter.

Figure 2.4 shows the N, P and K contents as well as the N:P ratio for the roots of *P. australis* at the beginning and end of the experiment for the three sediment types. The N, P, and K contents in the roots increased in time, while the N:P ratio clearly decreased. The reduction in N:P ratio from 11 to 2 – 3 suggests N was the limiting nutrient as an N:P ratio of < 14 in plant tissue is indicative of N limitation (Koerselman and Meuleman, 1996). However, root N and P concentrations of *P. australis* should typically range between 0.64 and 1.04% for N and 0.06–0.13% for P (Wang et al., 2015). Figure 2.4 shows that the root N and P concentrations were above these values and that P was particularly high: they were higher by a factor of 5 to 10 (N: 1.14 – 1.63% and P: 0.52 – 0.62%). Hence, the concentrations of these nutrients in

the roots do not indicate that nutrient limitation is a likely cause of the reduced plant growth and shriveling and yellowing of foliage.

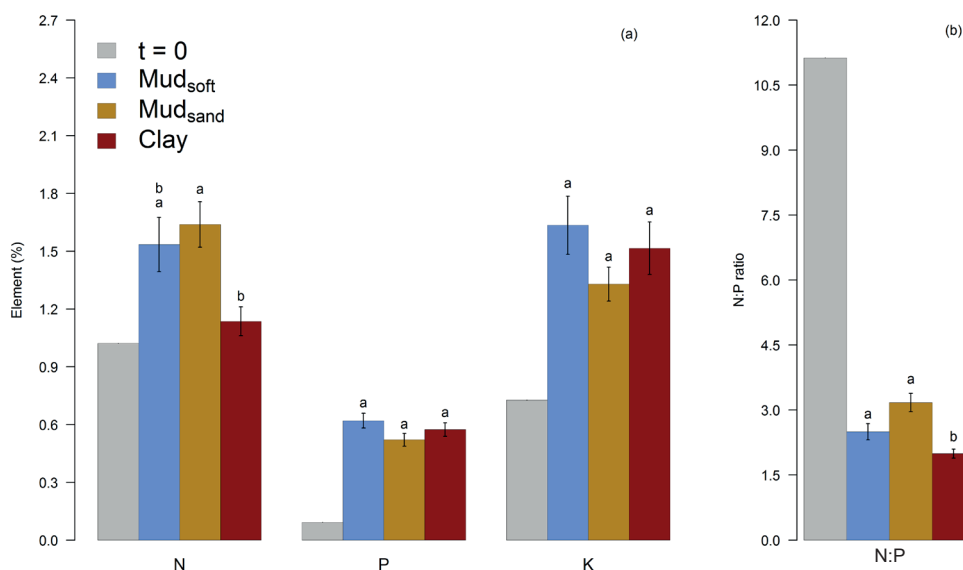


Figure 2.4. N, P, and K concentration in root tissue ($t = 176$) in percentage of dry weight (a) as well as the N:P ratio (b) with error bars when $n = 5$. Significant differences between sediment types are indicated by different letters, and nonsignificant differences are indicated by the same letter.

We hypothesize that coprecipitation of P with Fe on roots enhanced the concentrations of P in the plant roots (Snowden and Wheeler, 1995; Jørgenson et al., 2012). Snowden and Wheeler (1995) showed that this so-called iron plaque formation enhances the uptake of Fe and P. This may cause iron toxicity and is probably responsible for the elevated P concentrations in tissue and for the stunted growth and leaf decay we observed in the experiment. Note that the plant roots of *P. australis* initiate this process by oxidizing their environment and thereby enabling ferrous iron to oxidize into P-bearing ferric iron, which precipitates on roots.

The Fe concentration in the leaves and in the roots supports the “Fe-P coprecipitation hypothesis”: we measured an approximately 20-fold increase by comparison with the initial concentration in the seedlings (Figure 2.5). Furthermore, ferric oxide, a product of pyrite oxidation, precipitates on root surfaces (Jørgenson et al., 2012), and hence pyrite oxidation in sediments is directly linked to iron toxicity in plants.

Further evidence to support our hypothesis is provided by the results of the sequential phosphorus extraction conducted on the sediments: it revealed that the dominant P pool in the sediments is the Fe-P fraction (Table 2.2). Phosphorus coprecipitates with Fe on roots if it is bound to ferric oxides.

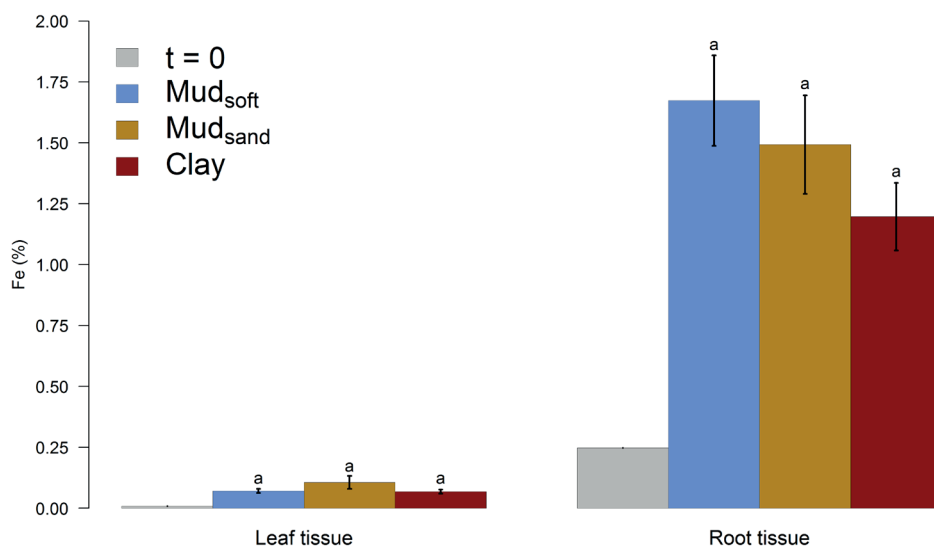


Figure 2.5. Fe concentration (% of dry weight) in leaf and root tissue with error bars when $n = 5$. Significant differences between sediment types are indicated by different letters, and nonsignificant differences are indicated by the same letter.

2.3.5 Implications for eco-engineering

Our results strongly point in the direction of iron toxicity as a major bottleneck prohibiting the healthy development of *P. australis*. Since the candidate material for the construction of the Markermeer wetland has high contents of Fe and Fe-P, we recommend using Fe-tolerant plant species as test species in the new wetland, rather than species optimized for growing in N-limited conditions.

Concomitantly with iron toxicity, a high Fe-P content in soil will trigger P mobilization if that soil is rewetted after having dried out and contains high amounts of SO_4 (Smolders and Roelofs, 1993; Lucassen et al., 2005). In some cases, this can result in elevated levels of sulfide, thereby promoting S toxicity in plants (Lamers et al., 1998; Van der Welle et al., 2007).

Figure 2.6 summarizes the important feedbacks and processes we expect play an important role in the clay-rich sediments. Following the feedback loops between plant and soil, we see a negative feedback loop that arises because plant roots induce aeration, which promotes iron toxicity, which decreases plant growth and results in plant death. Also, we see a positive feedback loop, as iron toxicity induces reduction processes as a result of root death, which leads to P mobilization and hence enhances plant growth and regeneration. Negative feedback loops diminish or buffer changes, whereas a positive feedback loop amplifies changes. So, a negative feedback loop normally stabilizes the system, in our case via the toxic effect of iron oxides on plants, but plant growth may increase due to the positive feedback loop via P mobilization. The relative strengths of these two feedback loops and the sensitivity of species to Fe toxicity determine the ultimate effect on vegetation development in wetlands built from these sediments.

As drying – rewetting cycles are likely to occur in these future wetlands and since the Fe-P concentrations in the situated sediment are high, these feedbacks might be an important factor influencing soil formation and ecosystem development. We therefore recommend studying the ultimate effects of the use of this material on ecosystem development by testing with various plant species and drying – rewetting cycles.

Not all environmental factors that potentially interfere with the processes and feedbacks described in this study could be taken into account with this experimental

design (e.g., wave action, wind). Therefore, we recommend carrying out experiments on the wetlands themselves once the crest has stabilized sufficiently.

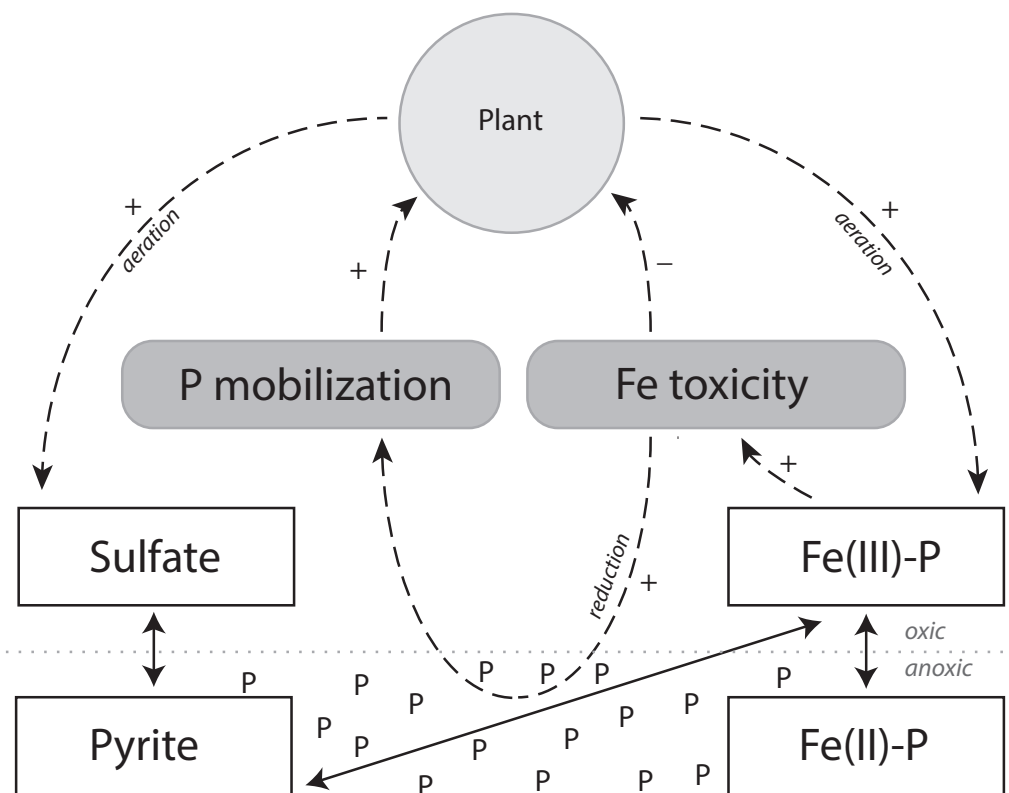


Figure 2.6. Most important biogeochemical processes and feedbacks identified in this study. + indicates positive feedback, - indicates negative feedback.

2.4 CONCLUSIONS

The results of this study show that plants expedite biogeochemical processes by oxidizing and modifying their environment, which in turn affects the growth conditions of the plants. In the mud deposits from Markermeer, the key processes influencing pore water chemistry are pyrite oxidation and associated calcite dissolution. The former is especially likely to be important as it is linked to iron toxicity and P mobilization and thus has the potential to initiate two feedback

mechanisms between plant and soil. We found strong indications of a negative feedback loop, where plant-induced iron toxicity is hampering plant growth, and a positive feedback loop, where iron toxicity promotes P mobilization, enhancing plant growth. The strength of these feedbacks and the balance between them will play an important role in regulating eco-engineering conditions for plants.

We found conclusive evidence that the low N:P ratio found in plant tissue was not caused by N limitation, as the ratio suggests, but probably results from enhanced P uptake as a result of coprecipitation with Fe on roots.

The magnitudes of the feedback mechanisms are expected to differ between the sediments used. The soft clay-rich layer has less Fe-P than the underlying clay layer, and therefore P mobilization is expected to be less in mud. However, when the mud is mixed with sand, the enhanced aeration due to the change in grain size composition results in higher oxidation rates, increasing the impact of the positive feedback mechanisms involving P mobilization and iron toxicity.

To study the effects of iron toxicity and P mobilization in greater detail, we recommend further testing with different plant species and drying – rewetting cycles. This is important because we expect these mechanisms to influence soil formation and ecosystem development in the created wetlands.

DATA AVAILABILITY

A dataset containing all relevant data acquired in this study is archived at DANS (Data Archiving and Networked Systems) and is accessible to all users (Saaltink et al., 2016).

ACKNOWLEDGEMENTS

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APPENDIX

Table A2.1. Pore water processes expressed in mole transfers ($\mu\text{mol l}^{-1} \text{ day}^{-1}$) as modeled by PHREEQC with pore water data retrieved at 1 cm below sediment surface. Positive values indicate dissolution, negative values indicate precipitation.

Reactant	Composition	Phase 1. Oxidation ($t=0-22$)			Phase 2. Initial root development ($t=22-64$)						Phase 3. Root influence ($t=64-176$)					
		No plant	No plant	No plant	No plant	Mud _{soft}	Mud _{sand}	Clay	No plant	Plant	No plant	Plant	No plant	Plant	No plant	Plant
Calcite	CaCO_3	267	0.00	120		27.1	48.8	39.3	7.1	0.00	36.9			25.8	8.13	0.00
Gypsum	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.00	0.00	0.00		-236	-208	0.00	-83.4	-32.1	0.00			0.00	-7.59	-14.8
Hydroxyapatite	$\text{Ca}_5(\text{PO}_4)_3(\text{OH})$	-5.00	-3.64	0.00		0.24	0.00	-0.02	-0.04	0.00	0.00			0.00	0.09	0.00
Chalcedony	SiO_2	-19.1	-15.5	-18.2		0.95	0.71	1.91	-3.37	-1.67	-2.14			0.00	0.54	1.43
$\text{Fe}(\text{OH})_3$ (a)	$\text{Fe}(\text{OH})_3$	-277	-116	-160		0.95	-10.0	0.00	0.00	-21.4	-14.3			-4.20	-10.6	-13.3
Pyrite	FeS_2	270	109	159		0.00	9.76	0.21	3.58	21.2	64.3			23.8	0.00	13.8
Rhodochrosite	MnCO_3	-11.8	-11.4	-2.27		2.86	1.19	1.23	0.34	-0.24	-0.24			-0.89	0.09	0.18
CEC	CaX_2	0.00	20.9	55.5		63.1	41.9	-9.11	0.00	0.00	0.00			0.00	-9.73	0.00
	FeX_2	0.00	0.00	0.00		0.00	0.00	-0.19	-4.11	0.00	-50.2			-19.8	11.7	0.00
	KX	-8.64	-5.00	-17.7		-4.76	0.00	3.78	-8.30	-6.19	0.00			-2.14	-2.77	-7.68
	MgX_2	31.4	-16.8	36.8		-39.8	-30.5	7.42	-1.35	0.00	21.7			12.0	0.00	0.00
	NaX	-20.9	0.00	-166		-46.4	-25.7	0.00	25.1	25.2	77.6			19.7	0.00	12.0
	NH_4^+X	-33.2	-4.09	0.00		4.76	6.67	0.00	-5.94	-19.0	-20.7			-1.88	-0.98	-4.20
H_2O (g)	$\text{H}_2\text{O} \times 10^3$	-3364	-2591	-2364		0.00	0.00	380	-996	-286	-6.67			49.1	74.1	-357
O_2 (g)	O_2	1009	432	659		2.62	45.5	0.00	0.00	41.9	186			83.6	0.00	44.7
CO_2 (g)	CO_2	-827	-532	-650		35.2	0.00	39.7	0.00	-55.5	-84.8			-33.1	0.00	44.6
No. models found		2	2	2	3	4	2	2	5	2	2	6	2	2	1	2

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CHAPTER

3

Plant-specific effects of iron-toxicity in wetlands

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ABSTRACT

Background and Aims: Understanding the potential effects of iron toxicity on plant development is important when constructing new wetland from iron-rich sediment. We aim to study plant species-specific effects of iron toxicity when grown in the iron-rich sediments of lake Markermeer (the Netherlands).

Methods: Using three sediment sources that varied in total Fe and Fe-P concentrations, we performed a greenhouse experiment to study the development of three wetland species that differ in their tolerance to iron and utilization capacity of Fe-P: *Rumex maritimus*, *Phragmites australis* and *Eupatorium cannabinum*.

Results: *Phragmites australis* was the only species that developed an epidermis-damaging iron plaque on its roots. Plaque formation mainly depended on the Fe(III) and Fe-P concentration of the sediment, which led to different nutrient imbalances in leaves. All three species showed reduced growth compared to the control substrate, which could not be linked to indirect Fe toxicity. In contrast, direct Fe toxicity following the uptake of Fe could not be excluded as a mechanism potentially explaining our results, and this result warrants further examination in longer-term experiments.

Conclusions: Our results highlight the importance of considering the Fe and Fe-P availability in sediments, as these properties may constrain plant performance and delay the development of pioneer ecosystems in wetland construction sites.

3.1 INTRODUCTION

The concept of ecological engineering is implemented globally nowadays and aims to use environmental technology that is tuned to ecosystem services (Mitsch 1998; Odum and Odum 2003; Temmerman et al. 2013). Often, plants are used as ecological engineers as they directly interact with the physical, chemical and biological components in the soil (Ehrenfeld et al. 2005), thereby potentially facilitating ecosystem development. Especially when constructing wetlands, the ability of plants to modify the biogeochemical conditions by radial oxygen loss (ROL) is important as it enables them to cope with the high concentration of phytotoxins typically found in these waterlogged soils (e.g. Fe^{2+} , Mn^{2+} , S^-) (Blom 1991; Lamers et al. 2012). Especially in constructed wetlands build from iron-rich sediments, this ability could be an important characteristic as optimal growing conditions are required for plants to fully operate as ecological engineers. Several studies showed that the iron availability in wetland soils is an important factor influencing plant species distribution, owing to the different strategies of plants to cope with excessive amounts of iron (Snowden and Wheeler 1995; Van der Welle et al. 2007; Geurts et al. 2009). High iron availability in soils can lead to *direct* iron toxicity or *indirect* iron toxicity in plants. *Direct* iron toxicity occurs when an excessive uptake of Fe damages cell structures, leading to reduced plant growth and injury to foliage (Wheeler et al. 1985; Laan et al. 1991; Ayeni et al. 2014) whereas *indirect* iron toxicity occurs when iron precipitates on roots, forming an iron plaque that acts as a barrier against iron (Snowden and Wheeler 1995; Tripathi et al. 2014). Although indirect iron toxicity is an exclusion mechanism that prevents the excessive uptake of iron – i.e., direct iron toxicity – it also inhibits nutrient uptake by damaging the epidermis surface of roots (Jørgenson et al. 2013). For example, Snowden and Wheeler (1995) found that increased iron concentrations in the soil induced a stronger decrease in shoot N concentrations than in root N concentrations, whereas the opposite was found for potassium (K). Hence, uptake and translocation mechanisms are affected in different ways depending on the type of nutrient.

The type of iron plaque that precipitates on plant roots also determines the plant response to indirect iron toxicity, especially with respect to phosphorus (P) uptake.

Iron can precipitate with P, forming a yellow-grey colored plaque, or without P, forming an ochreous colored plaque (Snowden and Wheeler 1995). Plants that favor co-precipitation of P induce a higher uptake of P but severely reduce translocation of P, leading to more phosphorus stress in the shoot (Snowden and Wheeler 1995; Xu et al. 2009). However, little is known about the relationship between the composition of iron in sediments and the type of iron plaque formed on the roots.

Studying how plants respond to direct and indirect iron toxicity is important if iron-rich sediments are used as a foundation for newly constructed wetlands. Sufficient plant growth is a prerequisite for vegetation to act as ecological engineers and speed up the development of these ecosystems. Wetland construction in lake Markermeer, an artificial lake in the Netherlands located northeast of Amsterdam, is an example. See Figure 3.1a for an artistic impression of these wetlands.

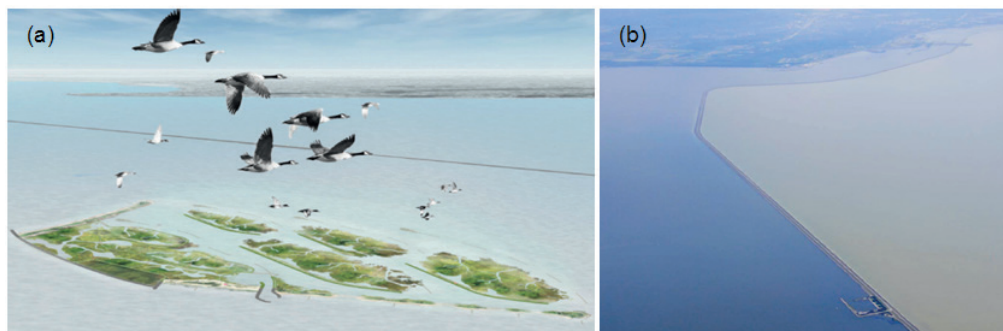


Figure 3.1. An artistic impression of the Marker Wadden design (a). The Marker Wadden should improve the ecological conditions in the lake by decreasing the present turbidity in Markermeer – right of embankment (b). © Vista landschapsarchitectuur en stedenbouw.

Nowadays, a soft, clay-rich layer is causing serious turbidity problems in lake Markermeer. The soft clay-rich layer is produced by bioturbation and physical weathering and continuously resuspends as a result of wave action (Van Kessel et al. 2008; Vijverberg et al. 2011; De Lucas Pardo et al. 2013). From an ecological point of view, removing the soft clay-rich layer is necessary as it is causing serious turbidity problems (Figure 3.1b) with severe consequences for the lake's biodiversity (Noordhuis et al. 2014). To improve the ecological conditions, it is planned to dredge a part of the soft clay-rich lake-bed sediment and use this as a building material in

constructing approximately 10,000 ha of wetland. In an earlier study, Saaltink et al. (2016) found that the soft, clay-rich layer contains significant amounts of pyrite (FeS_2) and iron-bound phosphorus and they hypothesized that the main bottleneck preventing prompt development of ecosystems within the newly constructed wetland sites could be a form of iron toxicity. High belowground production of pioneering vegetation is especially important when using vegetation as ecological engineers for building these wetlands from soft mud, as roots have stabilizing capabilities, reduce erosion, and will increase the consolidation process.

The aim of this study is to identify the effects of iron toxicity in plants when grown in the iron-rich sediments of lake Markermeer. Consequently, we formulated two subsidiary objectives to reach this aim: 1) determine the presence and composition of iron plaque on the plant roots and show that this plaque formation depends on the amount of iron and iron-bound phosphorus present in the sediments; 2) monitor concentrations of Fe, N, P, and K in leaves and roots and link changes in plant nutrient stoichiometry – such as changes in tissue N:P ratio due to altered uptake and translocation of N and/or P – to the presence and composition of iron plaque. We use three wetland species that commonly occur in the Markermeer lake area and that differ in their tolerance to iron. Moreover, we use three sediments from different locations in lake Markermeer that highly vary in their Fe, Fe-P and nutrient concentrations. We hypothesize that plant species with high radial oxygen loss will produce substantial amounts of iron plaque, whereas plant species with low radial oxygen loss will produce little or no iron plaque. We expect that more P is co-precipitated with Fe on roots in sediments that contain high amounts of Fe-P. Moreover, the presence of iron plaque as well as the type of iron-plaque is expected to induce changes in plant nutrient stoichiometry in plants. This study will enhance knowledge on plant–soil interactions and specifically how excessive iron influences vegetation performance in terms of plant growth, plaque formation and nutrient stoichiometry.

3.2 MATERIAL AND METHODS

3.2.1 Experimental design

A greenhouse experiment was conducted at the greenhouse test facility of Utrecht University in the period July – September 2015. We selected three plant species that differ in their ability to cope with iron toxicity: *Rumex maritimus* (Golden dock), *Phragmites australis* (Common reed) and *Eupatorium cannabinum* (Hemp-agrimony). All three species are commonly found in the Netherlands and usually grow on wet soils. *Rumex maritimus* is an annual, dicotyledonous species which can cope sufficiently with high iron concentrations in terms of growth despite observations of substantial leaf injury (Laan et al. 1991); *P. australis* is a perennial, monocotyledonous species that is moderately tolerant to Fe, showing marked signs of iron toxicity at high Fe concentrations (Snowden and Wheeler 1995); and *E. cannabinum* is a perennial, dicotyledonous species with low tolerance to high Fe concentrations, showing severe signs of iron toxicity (Snowden and Wheeler 1995). Plants were grown from seeds for c. 40 days on nutrient-poor turf soil before transplantation into the experimental sediments.

Lake bed sediment was collected by mechanical dredging at two points in Markermeer: the southern part (coordinates 52.3795°N; 5.0209°E, sampled in June 2014) and the northern part (coordinates 52.5462°N; 5.3878°E, sampled in March 2015). Fast changes in the geochemical composition due to seasonal effects are not expected as the buffer capacities of these sediments are large (Saaltink et al. 2016). From these sampling points, three sediment types were used: (1) the southern, well consolidated, Zuiderzee deposit of Holocene origin (10-50 cm depth), (2) the soft clay-rich layer which is found on top of this deposit (0-10 cm depth), and (3) also a soft clay-rich layer at the northern part (0-10 cm depth). Sediments were stored in air-tight containers at 4 °C prior to the start of the experiment. As a fourth sediment type, potting soil was used as a control treatment. As plant growth impairment by iron toxicity was not likely in the control treatment, it provided the reference needed to assess growth impairment in the Markermeer sediments. Pots (diameter 5 cm, depth 18 cm) with a perforated base were filled with one of the four sediment types. A batch of 30 pots were placed in small basins and filled with water to a level of 6 centimeters (12 centimeters below soil surface). The water was treated by reverse osmosis, after

which NaCl was dissolved to create a solution of $30 \times 10^{-6} \text{ mol l}^{-1}$. This value reflects the average NaCl concentration in rainwater in the Netherlands for the period 2012–2013 (LMRE 2013). One sediment type per basin was used to prevent any crossing-over between sediment conditions.

A seedling was planted in each pot after it was filled with sediment. For each sediment type – species combination, 15 pots were made to account for the number of harvests (3) and replicates (5). Seedlings from other plants that spontaneously emerged in the pots were removed immediately.

3.2.2 Data collection

Roots, stems and leaves were harvested, separated, air-dried and weighed prior to the start of the experiment and after 28, 56, and 84 days. In addition, flowers were harvested from *R. maritimus* and *E. cannabinum* after 56 and 84 days in the control sediment and in the sediment with an intermediate concentration of iron and after 84 days in the sediments with high and low concentrations of iron. *Phragmites australis* did not flower in any of the sediment treatments during the experiment. The air-dried material was then ground and analyzed using total reflection X-ray fluorescence (S2 Picofox, Bruker) to determine tissue contents of Fe, K, and P. Nitrogen was determined on a CN elemental analyzer (NA1500, Fisons Instruments). At the start of the experiment, the biomass of a single seedling was too low to enable chemical analysis. Therefore, seedlings were harvested and processed in sets of 10 per replicate. Part of a lateral root of the harvest after 84 days was randomly selected for micro X-ray fluorescence spectrometry (Orbis PC, Edax) to determine the elemental composition of the root surface.

Before the start of the experiment, soil samples were freeze-dried and stored in anoxic conditions prior to geochemical analysis in order to preserve the solid P and Fe speciation in the soil. A series of geochemical analyses was performed on the field samples of the sediments. The total concentrations of Fe, P, and K were determined using ICP-OES (Arcos, Spectro) following aqua regia destruction. Soil N was measured on a CN elemental analyzer (NA1500, Fisons Instruments). Sequential extraction methods were applied to characterize solid P speciation (Ruttenberg 1992) and solid Fe speciation (Poulton and Canfield 2005).

Table 3.1. List of steps used in the extraction procedure of phosphorus (based on Ruttenberg, 1992) and iron (based on Poulton and Canfield, 2005).

Ruttenberg, 1992			Poulton and Canfield, 2005		
Step	P-extractant	Separated P fraction	Step	Fe-extractant	Separated Fe fraction
I	1M MgCl ₂ , 30 min	Exchangeable or loosely sorbed P	I	1M Na acetate (pH 4.5), 24 h	Carbonate associated Fe, including siderite and ankerite
II			II	1M Hydroxylamine-HCL in 25% acetic acid (v/v), 24 h	Amorphous oxides, including ferrihydrite and lepidocrocite
A	Citrate-dithionite-bicarbonate (CDB), 8 h	Easily reducible or reactive ferric Fe- P	III	0.3M Dithionite. pH 4.8 with 0.35N acetic acid/0.2M Na citrate, 2 h	Crystalline oxides, including goethite and hematite
B	1M MgCl ₂ , 30 min		IV	0.2M NH ₄ -oxalate/0.17N oxalic acid, 2 h	Recalcitrant oxides, mostly magnetite
III			V	12N HCl, boil 1 minute	Poorly reactive sheet silicate Fe
A	Na acetate buffer (pH 4), 6 h	Amorphous apatite and carbonate P			
B	1M MgCl ₂ , 30 min				
IV	1N HCl, 24 h	Crystalline apatite and other inorganic P			
V	Ash at 550 °C, 2 h; 1N HCl, 24 h	Organic P			
Calculated					
			VI	Sediment Fe(II)	Σ (Step I + Pyrite)
			VII	Sediment Fe(III)	Σ (Step II + III)
			VIII	Root reactive Fe (RR-Fe)	Σ (Step I-III + Pyrite)
			IX	Unreactive iron	Total Fe – Σ (step I-V + Pyrite)

The steps involved in these methods are listed in Table 3.1. A distinction was made in sediment Fe(II), involving pyrite and carbonate-Fe, and sediment Fe(III), comprising Fe-oxides. The sum of the Fe(II) and Fe(III) fractions was defined as the root reactive iron (RR-Fe) fraction. Pyrite (FeS_2) was determined separately using X-ray diffraction (Qmineral, Heverlee, Belgium). All geochemical analyses were carried out for 5 replicates per sediment type, except for the XRD analysis (1 replicate per sediment type).

3.2.3 Statistical analyses

To identify the main effects and interaction effects of the fixed factors sediment type, plant species and time of harvest on the response variables N, P, K, and Fe concentrations in roots and leaves, we used univariate, general linear models that included these main effects and the species x sediment interaction effect. More specifically, we analyzed how species differed in the uptake of N, P, K, and Fe in general, and how uptake was mediated by sediment type. Moreover, as the sediment types differed in Fe concentration, we examined whether plant growth decreased with increasing Fe concentration in the sediment. In principle, this analysis could be performed with a two-way ANOVA model design. We observed, however, that elemental concentrations in the plant gradually declined over the course of the experiment. To correct for this effect, we therefore developed a general linear model that also included time of harvest as a fixed factor. Moreover, the previously reported variation in the plant species tolerance to iron toxicity (Laan et al. 1991; Snowden and Wheeler 1995) motivated the inclusion of a species x sediment interaction effect in the models. As these fixed factors are orthogonal, effect sizes were determined using type III sum of squares. Before each model-run, the dependent variable – i.e. tissue concentrations of N, P, K, and Fe – was tested for homogeneity of variances with a Levene's test ($p > 0.05$). When a dependent variable did not meet the assumption of homogeneity of variance, the log-transformed variable was used if this improved the homogeneity of variances as quantified by the Levene test statistic. We note, however, that a univariate general linear model is robust for departures from homogeneity of variance when the sample sizes are more or less equal (Box 1954). In contrast, alternative non-parametric procedures (such as the Kruskal-Wallis H test followed by

a Dunn's post hoc test) have very low power and associated high type II error rates, especially for our type of study design with relatively low replication within groups (e.g. Day and Quinn 1989). Therefore, when a dependent variable did not meet the assumption of homogeneity of variances after log-transformation, the output of the model could still be interpreted because the sample sizes between groups were equal in this experiment. Differences between groups were analyzed using least square difference tests ($p < 0.05$) following Webster (2007). The same post hoc test was used to assess geochemical differences between sediment types using the geochemical variables analysed on the field sediment samples. All statistical analyses were carried out in SPSS 22.0.0.1.

3.3 RESULTS

3.3.1 Iron and nutrient concentrations in the sediment types

Table 3.2 lists the composition of macronutrients and iron present in the four sediment types used in this study. These sediments differed significantly in chemical composition ($F(39, 12.6) = 62.05$, $p < .0005$; Wilk's $\Lambda < .0005$, partial $\eta^2 = 0.994$). The southern, well consolidated, Zuiderzee Holocene-deposit of Markermeer material contained the highest concentration of RR-Fe and Fe-P and is hereafter referred to as M_H , while the soft mud from the north of the lake contained the lowest concentration of RR-Fe and Fe-P and is hereafter referred to as M_L . Soft mud from the south (on top of the Zuiderzee deposit) had RR-Fe and Fe-P concentrations in between M_H and M_L (hereafter referred to as M_M)

The total phosphorus concentration ranged from 1191 mg kg^{-1} in M_H to 361 mg kg^{-1} in M_L . Iron-bound phosphorus was the dominant P fraction in M_H and M_M , whereas P in crystalline apatite was the dominant P fraction in M_L . The amount of N present in the Markermeer sediments ranged from 2833 mg kg^{-1} in M_M to 1611 mg kg^{-1} in M_L . The nitrogen concentrations between M_H and M_M did not differ significantly, but both were higher than M_L ($p < 0.01$). Potassium ranged from 4579 mg kg^{-1} in M_H to 2619 mg kg^{-1} in M_L , and all Markermeer sediments had significantly different K concentrations ($p < 0.05$).

Table 3.2. Sediment composition of the southern Zuiderzee deposit (M_H), the soft clay-rich layer on top of this deposit (M_M), the northern soft clay-rich layer (M_L) and the control sediment for the macronutrients N, P, and K as well as Fe. Average concentrations in mg kg^{-1} dry wt with standard deviations ($n = 5$). na signifies “not analysed”. Significant differences between sediment types are indicated by different letters ($p < 0.05$).

	Control	M_L	M_M	M_H
Total Fe	2188 ^a (± 288)	14775 ^b (± 234)	21564 ^c (± 1502)	24822 ^d (± 749)
Fe: Pyrite	na	4700	2800	2800
Fe: Carbonates	3 ^a (± 4)	1867 ^b (± 155)	4249 ^c (± 582)	4562 ^c (± 136)
Fe: Amorphous oxides	816 ^a (± 149)	1159 ^b (± 136)	1770 ^c (± 50)	2281 ^d (± 191)
Fe: Crystalline oxides	1189 ^a (± 76)	1455 ^b (± 132)	2393 ^c (± 123)	3319 ^d (± 168)
Fe: Magnetite	111 ^a (± 28)	97 ^a (± 20)	193 ^{ab} (± 61)	257 ^b (± 113)
Fe: Poorly reactive sheet silicates	0 ^a (± 0)	3121 ^b (± 932)	5317 ^c (± 591)	7928 ^d (± 975)
Fe: Unreactive	69 ^a (-)	2376 ^b (-)	4842 ^d (-)	3675 ^c (-)
Root reactive iron (RR-Fe)	2008 (-)	9181 (-)	11213 (-)	12962 (-)
Sediment Fe(II)	3 (-)	6567 (-)	7049 (-)	7362 (-)
Sediment Fe(III)	2005 ^a (± 169)	2614 ^b (± 78)	4163 ^c (± 135)	5600 ^d (± 310)
Total P	599 ^a (± 66)	361 ^b (± 10)	937 ^c (± 54)	1191 ^d (± 45)
P: Exchangeable	269 ^a (± 5)	5 ^b (± 2)	11 ^c (± 2)	9 ^{bc} (± 1)
P: Fe-bound	240 ^a (± 28)	40 ^b (± 10)	564 ^c (± 92)	815 ^d (± 52)
P: Amorphous apatite	11 ^a (± 3)	89 ^b (± 18)	110 ^b (± 26)	140 ^c (± 12)
P: Crystalline apatite	0 ^a (± 0)	158 ^b (± 14)	135 ^b (± 15)	155 ^b (± 10)
P: Organic	108 ^a (± 22)	47 ^b (± 11)	82 ^a (± 25)	90 ^a (± 6)
Total N	13768 ^a (± 615)	1611 ^b (± 32)	2833 ^c (± 191)	2663 ^c (± 41)
Total K	1715 ^a (± 66)	2619 ^b (± 72)	3893 ^c (± 420)	4579 ^d (± 156)

Table 3.3. Average concentration ($\pm$ S.E.) of N, P, K, and Fe (% of dry wt) in leaves and roots ($n = 45$ for sediment type and $n = 60$ for plant species and time of harvest). Significant main effects of sediment type ($F(30, 467.4) = 20.62$, Wilk's $\Lambda = 0.084$, partial $\eta^2 = 0.562$), plant species ($F(20, 320) = 17.65$, Wilk's $\Lambda = 0.226$, partial $\eta^2 = 0.525$) and time of harvest ($F(20, 320) = 10.57$, Wilk's $\Lambda = 0.363$, partial $\eta^2 = 0.398$) are indicated by different letters along a row ($p < 0.05$). Sediment types include the southern Zuiderzee deposit (M_H), the soft clay-rich layer on top of this deposit (M_M), the northern soft clay-rich layer (M_L) and the control sediment (Cont.).

Element	Sediment type					Plant species				Time of harvest				
	Cont.	M _L	M _M	M _H	S.E.	R. mar.	P. aus.	E. can	S.E.	Day 28	Day 56	Day 84	S.E.	
N	Leaf	2.29 ^a	2.12 ^a	2.47 ^a	2.09 ^a	±0.07	1.91 ^a	2.96 ^b	1.83 ^a	±0.06	2.92 ^c	2.08 ^b	1.75 ^a	±0.06
	Root	1.23 ^b	0.77 ^a	0.89 ^a	0.80 ^a	±0.04	1.01 ^b	0.95 ^b	0.79 ^a	±0.04	1.31 ^b	0.81 ^a	0.67 ^a	±0.04
P	Leaf	0.26 ^b	0.19 ^a	0.30 ^{b,c}	0.37 ^c	±0.01	0.32 ^b	0.35 ^b	0.17 ^a	±0.01	0.41 ^c	0.28 ^b	0.16 ^a	±0.01
	Root	0.21 ^b	0.12 ^a	0.28 ^c	0.36 ^d	±0.01	0.28 ^b	0.28 ^b	0.17 ^a	±0.01	0.31 ^b	0.23 ^a	0.19 ^a	±0.01
K	Leaf	2.10 ^a	2.85 ^b	2.52 ^b	2.69 ^b	±0.11	2.93 ^b	3.16 ^b	1.51 ^a	±0.10	3.75 ^c	2.32 ^b	1.57 ^a	±0.09
	Root	1.40 ^a	2.06 ^{b,c}	1.77 ^{a,b}	2.19 ^c	±0.07	1.96 ^b	2.03 ^b	1.57 ^a	±0.07	2.85 ^b	1.42 ^a	1.32 ^a	±0.05
Fe	Leaf	0.03 ^a	0.09 ^b	0.07 ^b	0.08 ^b	±0.01	0.04 ^a	0.06 ^a	0.10 ^b	±0.00	0.06 ^a	0.08 ^b	0.05 ^a	±0.01
	Root	0.11 ^a	1.64 ^c	1.03 ^b	0.98 ^b	±0.06	0.48 ^a	1.70 ^b	0.60 ^a	±0.06	1.01 ^a	1.06 ^a	0.74 ^a	±0.07

3.3.2 Main effects on plant tissue composition

Table 3.3 shows how sediment type, plant species identity and time of harvest affected the average N, P, K, and Fe concentrations in leaves and roots. For the average N, P, K, and Fe concentrations in the leaves, no significant differences were observed between the Markermeer sediment types. Only the leaf P concentrations in M_H and M_M were significantly higher than in M_L . For the roots, more significant differences were found for the average concentration of P, K, and Fe, however, no differences were observed for root N. The root P concentration was significantly highest in M_H , followed by M_M and M_L . The root K concentration was also highest in M_H , but this was only significantly higher compared to M_M . The highest root Fe concentration was found in M_L .

Eupatorium cannabinum had lower N, P, and K concentrations in both the leaves and the roots, compared to *R. maritimus* and *P. australis* (Table 3.3). The latter two plants species were not significantly different, except for leaf N, where the average concentration in *P. australis* was significantly higher. Although nutrient concentrations did not show large differences, the Fe concentrations in both leaf and root showed clearly different patterns: The leaf Fe concentration was significantly highest in *E. cannabinum*, while the Fe concentration in roots was significantly highest in *P. australis*.

The leaf N, P, and K concentrations significantly decreased throughout the experiment: the highest concentrations were measured after 28 days and the lowest concentrations after 84 days (Table 3.3). Again, the Fe concentrations showed a contrasting pattern, both leaf and root Fe concentrations were more constant over time.

3.3.3 Root surface composition

Qualitative analysis of root surface composition showed that iron plaque was substantial on roots of *P. australis* (Figure 3.2). Some iron plaque was visible on roots of *R. maritimus*, while none could be discerned on roots of *E. cannabinum*.

For *P. australis*, iron plaque was absent on roots grown in the control sediment and interestingly, the composition of the plaque differed among the Markermeer sediment types (Figure 3.3). Potassium was more pronounced in iron plaque on roots

of *P. australis* grown in M_L as compared to the other two Markermeer sediments. Little co-precipitation of P occurred on roots of *P. australis* grown in M_M , while a substantial amount was precipitated on roots grown in M_H . In general, the higher the concentration of Fe(III) and Fe-P in the sediment, the more P co-precipitated with Fe on the roots.

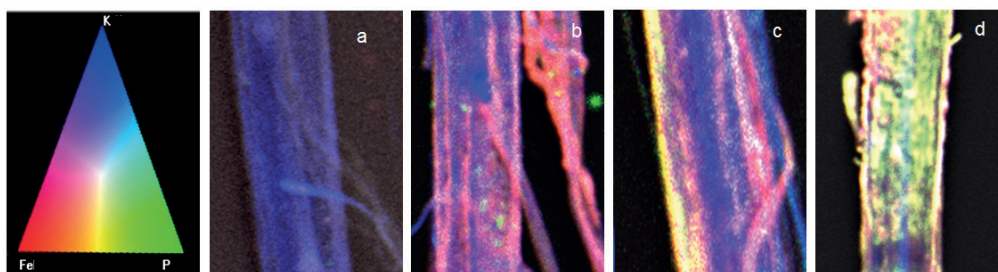


Figure 3.2. Surface composition in terms of Fe, K, and P on roots of *P. australis* in the control (a), M_L (b), M_M (c), and M_H (d) at $t = 84$ days. Colors indicate presence of Fe (red), K (blue), and P (green).

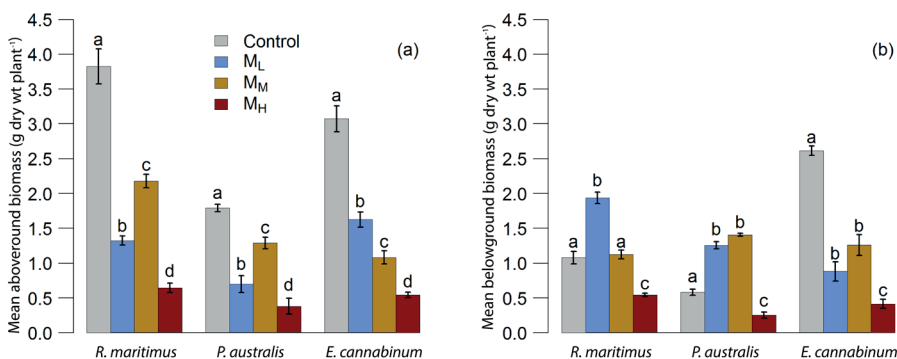


Figure 3.3. Aboveground (a) and belowground (b) biomass production (g dry wt) at $t = 84$. Error bars indicate S.E. Sediment types include the southern Zuiderzee deposit (M_H), the soft clay-rich layer on top of this deposit (M_M), the northern soft clay-rich layer (M_L) and the control sediment.

3.3.4 Biomass production

Aboveground biomass production was significantly lower in all three Markermeer sediments as compared to the control sediment (Figure 3.3a). Of the three Markermeer sediment types, aboveground biomass production of the iron-tolerant species *R. maritimus* and *P. australis* was highest in M_M , followed by M_L and M_H . For the iron-intolerant species *E. cannabinum* this was the other way around, the highest biomass was obtained in M_L , followed by M_M and M_H .

Root growth of *E. cannabinum* was reduced in all three Markermeer sediments compared to the control sediment, while for the other two plant species root growth was higher or similar to the control in M_M and M_L (Figure 3.3b). For *R. maritimus*, belowground biomass production was highest in M_L , whereas for *P. australis* root growth was highest in both M_M and M_L .

3.3.5 Species-specific effects of sediment type

Due to the presence of iron plaque on roots of *P. australis* (Figure 3.2), it is of particular interest to examine how the nutrient balance of this plant species changed relative to *R. maritimus* and *E. cannabinum* from M_L to M_M to M_H . These so-called sediment x species interaction effects are presented in Table 3.4, considering concentrations of N, P, K, and Fe as well as the N:P ratio in both leaves and roots.

Not many significant differences were found when looking at the root and leaf N and K concentrations (Table 3.4). However, the leaf P concentrations in *R. maritimus* and *E. cannabinum* were significantly lowest in M_L and highest in M_H , but did not differ significantly in *P. australis*. In contrast, all three plant species showed significantly lowest root P concentrations in M_L and significantly highest root P concentrations in M_H . As a result of these differences in leaf P and root P concentrations between the sediment types, significantly highest leaf N:P ratios for leaves and roots were found in M_L . However, this trend was not significant for the leaf N:P ratio in *P. australis*, as it kept its leaf P concentration relatively stable. These results indicate that sediment type influences the nutrient balance in *P. australis* in a different way compared to *R. maritimus* and *E. cannabinum* due to the presence of iron plaque on roots.

No clear species differences were found in leaf or root Fe when growing on the different sediment types (Table 3.4).

Table 3.4. Average concentration ($\pm$ S.E.) of N, P, K, and Fe (% of dry wt) as well as N:P ratio in leaves and roots of *R. maritimus*, *P. australis* and *E. cannabinum* grown in the four sediment types at $t = 28, 56$, and 84 ($n = 15$): the southern Zuiderzee deposit (M_H), the soft clay-rich layer on top of this deposit (M_M), the northern soft clay-rich layer (M_L) and the control sediment. For each plant species compartment (leaf or root), significant differences between sediment types are indicated by different letters ($p < 0.05$). For each sediment type, significant differences between (leaves or roots) plant species are indicated by different numbers ($p < 0.05$).

Element	Comp.	Plant	Control	M_L	M_M	M_H	S.E. Plant species
N	Leaf	<i>R. mar.</i>	1.59 ^{a;1}	1.85 ^{ab;1}	2.32 ^{c;1}	1.86 ^{ab;1}	± 0.07
		<i>P. aus.</i>	3.39 ^{a;2}	2.81 ^{a;2}	2.91 ^{a;2}	2.74 ^{a;2}	± 0.14
		<i>E. can.</i>	1.85 ^{a;1}	1.64 ^{a;1}	2.15 ^{b;1}	1.67 ^{a;1}	± 0.05
		S.E. Sediment	± 0.15	± 0.12	± 0.10	± 0.06	
	Root	<i>R. mar.</i>	1.23 ^{b;1}	0.87 ^{a;1}	1.04 ^{ab;1}	0.91 ^{a;2}	± 0.05
		<i>P. aus.</i>	1.83 ^{a;1}	0.73 ^{b;1}	0.81 ^{b;1}	0.82 ^{b;1;2}	± 0.09
		<i>E. can.</i>	0.98 ^{a;1}	0.70 ^{a;1}	0.80 ^{a;1}	0.67 ^{a;1}	± 0.08
		S.E. Sediment	± 0.16	± 0.04	± 0.05	± 0.04	
	Leaf	<i>R. mar.</i>	0.21 ^{a;1}	0.14 ^{a;1}	0.35 ^{b;2}	0.55 ^{c;3}	± 0.02
		<i>P. aus.</i>	0.40 ^{a;2}	0.30 ^{a;2}	0.36 ^{a;2}	0.35 ^{a;2}	± 0.02
		<i>E. can.</i>	0.18 ^{b;1}	0.11 ^{a;1}	0.18 ^{b;1}	0.20 ^{b;1}	± 0.01
		S.E. Sediment	± 0.03	± 0.01	± 0.02	± 0.02	
P	Root	<i>R. mar.</i>	0.25 ^{b;1}	0.13 ^{a;1}	0.30 ^{b;2}	0.41 ^{c;2}	± 0.02
		<i>P. aus.</i>	0.24 ^{b;1}	0.12 ^{a;1}	0.29 ^{b;2}	0.47 ^{c;2}	± 0.01
		<i>E. can.</i>	0.14 ^{a;1}	0.10 ^{a;1}	0.22 ^{b;1}	0.21 ^{b;1}	± 0.01
		S.E. Sediment	± 0.03	± 0.01	± 0.01	± 0.01	

Table 3.4. (continued).

Element	Comp.	Plant	Control	M _L	M _M	M _H	S.E. Plant species
N:P ratio	Leaf	R. mar.	9.1 ^{b;1}	12.4 ^{c;2}	8.0 ^{b;1}	3.8 ^{a;1}	±0.69
		P. aus.	9.6 ^{a;1}	10.4 ^{a;1}	8.3 ^{a;1}	9.5 ^{a;2}	±0.42
		E. can.	10.9 ^{b;1}	14.9 ^{c;2}	12.5 ^{b;2}	8.4 ^{a;2}	±0.38
		S.E. Sediment	±0.49	±0.89	±0.40	±0.49	
	Root	R. mar.	6.8 ^{b;1}	7.2 ^{b;2}	4.0 ^{a;2}	2.6 ^{a;1;2}	±0.32
		P. aus.	6.3 ^{d;1}	5.9 ^{c;1}	2.8 ^{b;1}	1.9 ^{a;1}	±0.14
		E. can.	5.4 ^{b;1}	8.1 ^{c;2}	3.9 ^{a;2}	3.2 ^{a;2}	±0.17
		S.E. Sediment	±0.41	±0.21	±0.21	±0.18	
	Leaf	R. mar.	1.10 ^{a;1}	3.88 ^{b;2}	3.15 ^{b;2}	3.53 ^{b;2}	±0.14
		P. aus.	3.53 ^{a;2}	3.30 ^{a;2}	2.98 ^{a;2}	2.82 ^{a;2}	±0.20
		E. can.	1.62 ^{a;1}	1.33 ^{a;1}	1.34 ^{a;1}	1.71 ^{a;1}	±0.08
		S.E. Sediment	±0.23	±0.13	±0.15	±0.14	
K	Root	R. mar.	1.06 ^{a;1}	2.66 ^{c;2}	1.74 ^{b;1}	2.36 ^{c;2}	±0.08
		P. aus.	2.21 ^{a;2}	1.60 ^{a;1}	1.94 ^{a;1}	2.38 ^{a;2}	±0.16
		E. can.	0.91 ^{a;1}	1.94 ^{b;1}	1.63 ^{b;1}	1.81 ^{b;1}	±0.10
		S.E. Sediment	±0.19	±0.13	±0.12	±0.07	
	Leaf	R. mar.	0.01 ^{a;1}	0.04 ^{b;1}	0.06 ^{c;1}	0.04 ^{b;1}	±0.00
		P. aus.	0.03 ^{a;2}	0.06 ^{b;1}	0.04 ^{a;1}	0.09 ^{b;2}	±0.01
		E. can.	0.04 ^{a;3}	0.16 ^{b;2}	0.10 ^{ab;2}	0.11 ^{ab;2}	±0.01
		S.E. Sediment	±0.00	±0.01	±0.01	±0.01	
	Root	R. mar.	0.09 ^{a;1}	0.85 ^{c;1}	0.48 ^{b;1}	0.51 ^{b;1}	±0.02
		P. aus.	0.21 ^{a;2}	3.26 ^{c;2}	1.68 ^{b;3}	1.65 ^{b;3}	±0.05
		E. can.	0.02 ^{a;1}	0.69 ^{b;1}	0.92 ^{b;2}	0.79 ^{b;2}	±0.05
		S.E. Sediment	±0.01	±0.04	±0.06	±0.05	

3.3.6 N:P ratio in roots vs. leaves

Figure 3.4 shows scatter plots of N, P, and the N:P ratio in both roots and leaves at experimental time $t = 28, 56$, and 84 days. The sediment types were treated separately, whereas the plant species and time of harvest were grouped together. No clear relations could be discerned between the N:P ratio of roots and leaves, which is especially evident in M_M and M_H (Figure 3.4a). The leaf N:P ratio shows that the majority of data points indicate N limitation, whereas some indicate N-P co-limitation or P limitation.

Figure 3.4b presents the total uptake of N and P and indicates that the relation between total N and P uptake did not differ at the three time-steps measured. This figure also shows that the total uptake of P was higher in M_H than in M_L , relative to the total uptake of N (and vice versa).

The N:P ratios of leaves relative to the concentrations of N in leaves and flowers did not show a trend for any of the three sediment types (Figure 3.4c). The same was observed when comparing the N:P ratios of roots with the concentrations of N in the roots (Figure 3.4e). As a consequence, when comparing the leaf and root N:P ratios with the P concentrations a negative trend was observed, with M_H having relatively low N:P ratios with high concentrations of P and M_L having relatively high N:P ratios with low concentrations of P (Figure 3.4d,f).

3.4 DISCUSSION

This experiment aimed to study the effects of iron toxicity in plants when grown in the iron-rich sediments of lake Markermeer. Therefore, we determined the presence and composition of iron plaque on the plant roots and measured the N, P, K, and Fe concentrations in both leaves and roots. The wetland species used in this study showed different responses to sediment type in terms of biomass production, iron plaque formation and N:P stoichiometry. In this section, we first discuss the presence and composition of iron plaque, after which we discuss the associated stoichiometric effects and its impacts on growth. Lastly, we briefly evaluate the implications for eco-engineering.

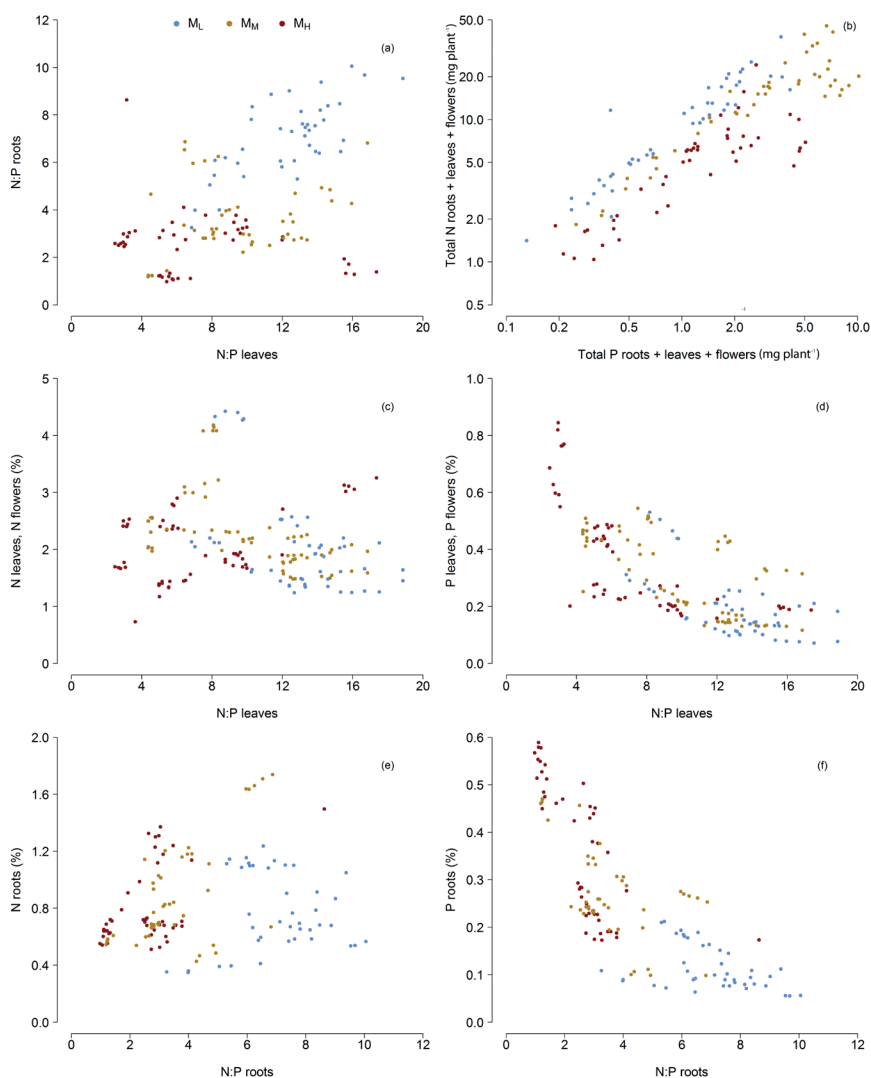


Figure 3.4. Scatter plots depicting the relation between N:P ratios in roots vs leaves (a), total content of N vs P in roots, leaves and flowers (b), N:P ratios in leaves vs concentration of N in leaves and flowers (c), N:P ratios in leaves vs P in leaves and flowers (d), N:P ratios in roots vs concentration of N in roots (e), N:P ratios in roots vs P in the roots (f). Sediment types include the southern Zuiderzee deposit (M_H), the soft clay-rich layer on top of this deposit (M_M), and the northern soft clay-rich layer (M_L).

3.4.1 Presence and composition of iron plaque

Analysis of root surface composition showed that only *P. australis* produced substantial amounts of iron plaque on all three Markermeer sediments. This plant species is known for its high radial oxygen loss compared to other wetland species (Brix et al. 1996; Dickopp et al. 2011; Tercero et al. 2015) and, as a consequence, causes iron to be precipitated on plant roots (St-Cyr and Crowder 1989). Iron plaque damages the epidermis surface of roots (Jørgenson et al. 2013), which could explain the high Fe concentrations in the roots in our experiment (Table 3.3). Moreover, the Fe-P concentration of the sediment determined the composition of the iron plaque on the plant roots of this species. Phosphorus co-precipitated with Fe in high Fe-P sediment, while K in iron plaque was observed in low Fe-P sediment. The presence of K is explained by leakage of this element from the root during drying. This is explained by Snowden and Wheeler (1995), who only observed K leakage during drying when phosphorus did not co-precipitate with Fe.

No substantial amounts of iron plaque were formed on the roots of *R. maritimus*, which was unexpected because earlier studies showed that radial oxygen loss of *R. maritimus* was high (Laan et al. 1989, 1990). However, when the diffusion path length of the root increases, radial oxygen loss can decrease ten-fold (Laan et al., 1989), which might explain the lack of iron plaque on *R. maritimus* in this study. The Fe-intolerant *E. cannabinum* did not produce any iron plaque on its roots which is in concordance with the findings of Snowden and Wheeler (1995).

3.4.2 Stoichiometric effects

Iron plaque induces a form of indirect iron toxicity by acting as a barrier against iron, which in turn has adverse effects on the nutrient uptake and translocation mechanisms in the plant (Snowden and Wheeler 1995; Xu et al. 2009; Tripathi et al. 2014). The Fe-P concentration of the sediments used are positively correlated to P concentrations in the roots and ranged from 40 mg kg⁻¹ in M_L to 815 mg kg⁻¹ in M_H (Table 3.2). However, the P concentrations in the leaves of *P. australis* did not follow the P concentrations in the roots, probably because the iron plaque produced on the roots inhibited translocation of P to the shoot. As a result, root N:P ratios were lower in individuals grown on high Fe-P sediment, whereas leaf N:P ratios were not differing

between individuals grown on high vs low Fe-P sediment. These results corroborate the findings of Snowden and Wheeler (1995) and Xu et al. (2009), who argued that plants that favor co-precipitation of P induce a higher uptake of P but severely reduce the translocation of P to the shoot.

Variation in leaf N concentrations was not large (interspecific and between-treatment average concentrations ranged between 1.59-3.39% dry wt), whereas leaf P concentrations varied more (0.11-0.55% dry wt). Nitrogen concentrations are well above the critical concentrations for N limitation (c. 1.4% cf De Wit et al. 1963) and leaf P concentrations are also above that for P limitation (c. 0.07% cf De Wit et al. 1963). N:P ratios of most of our leaf samples indicate slight to moderate N-limitation (< 14 cf Olde Venterink et al. 2011). Variation in N:P ratios in both leaves and roots was mainly caused by the variability in the concentration of P rather than N. This is because the homeostatic regulatory mechanisms of herbaceous plants result in relatively stable N concentrations (Güsewell 2004). In the current study, this was most evident in *R. maritimus*, an Fe-tolerant species that was, unlike the other two species, able to translocate more P to its leaves under Fe-P-rich conditions. For this species, iron plaque formed only in marginal amounts and did not reduce the translocation of P, which resulted in relatively low leaf N:P ratios ranging from 3.8 in M_H to 12.4 in M_L . These observations indicate that the sediment Fe-P fraction not only influences P concentrations in the roots, but also the P concentrations in the leaves. Moreover, no clear relationship could be discerned between the N:P ratio in leaves and the N:P ratio in roots for all three test species, especially at medium and high Fe-P rich conditions. These observations suggest that both inter- and intraspecific variation in iron plaque formation determines the uptake of P, but low plasticity (e.g., the ability of a species to increase or decrease the uptake according to its needs) in N uptake prevents Fe-tolerant species to profit from their more effective P acquisition as the intraspecific variation in N concentrations was low. Due to these stoichiometric effects, we argue that the leaf N:P ratios of plants grown in iron-rich sediments may not be a good proxy for discerning the type of nutrient limitation.

3.4.3 Impacts on growth

We found clear impacts of Fe-P sediments on biomass production for all three wetland species, which is partly related to iron plaque and its associated stoichiometric effects described in the previous sections. Compared with the control sediments, all species showed reduced aboveground growth, whereas *R. maritimus* and *P. australis* showed higher root production than the control in at least one sediment type (*R. maritimus* in M_L and *P. australis* in M_L and M_M). From a nutrient perspective, it makes sense that dry matter allocation is favored to roots when grown in sediment containing relatively low amounts of nutrients. However, the reduced aboveground biomass production in the nutrient-rich M_H suggests otherwise, as in high nutrient conditions dry matter allocation should be favored to the shoot (Ericson 1995; Shipley and Meziane 2002). In the high Fe-P sediment all plant species showed strong growth reduction, aboveground as well as belowground compared with the control sediments. Since only *P. australis* produced iron plaque, we may assume that indirect iron toxicity as a consequence of iron plaque was not the cause of reduced growth. Moreover, *R. maritimus* – although Fe tolerant and able to transport more P to its leaves when grown in high Fe-P sediment – appears to be as strongly limited in growth in high Fe-P sediment as the other two species. These observations do not support the hypothesis that indirect Fe toxicity in which iron plaque formation disrupts a balanced N and P acquisition is the major problem for our test plants. Our results also raise the question as to whether N and/or P are primary limiting factors for plant growth in these sediment types at all. The significantly raised Fe concentrations compared with the control sediments in the leaves of our three test species do indicate that direct Fe toxicity following the uptake of Fe cannot be excluded as a mechanism potentially explaining inhibited aboveground growth. Application of dolomite to these sediments could mitigate Fe-toxicity as it decreases the exchangeable soil Fe^{2+} (Suriyagoda et al. 2016).

3.4.4 Implications for eco-engineering

Initial high belowground biomass production is especially important when building wetland ecosystems from soft mud, as roots have the ability to stabilize sediments and reduce sediment degradation. This is also true for aboveground biomass as leaves

and stems dampen shear stress on the substrate by attenuating waves (Nepf 2012). Examples where plants are used successfully to stabilize sediment include tidal flats (Ganthy et al. 2011), riparian zones (Edmaier et al. 2011) and salt marsh creeks (Chen et al. 2012).

The results presented in this study suggest that of the iron-rich sediments collected in Markermeer, M_L is the best building material for constructing approximately 10,000 ha of wetland. It is foreseen by the building consortium to use wetland plants as eco-engineers to strengthen the soft mud and to speed up sediment consolidation and soil formation. We showed that based on the three commonly occurring wetland species in The Netherlands used in this study, *R. maritimus* is the best choice on the short-term because it has the potential to strengthen and stabilize the sediment at a faster pace than *P. australis* while at the same time having a fairly high aboveground biomass production.

Given the results presented in this study, we argue that the Fe(III) and Fe-P fraction in sediments should be explicitly considered when studying plant performance and nutrient dynamics in pioneer ecosystems. Especially in eco-engineering projects where wetlands are to be constructed by using iron-rich sediments and where plants are used as ecological engineers important soil characteristics should be measured and plant response should be monitored prior to deciding which building material and plant species to use.

3.5 CONCLUSIONS

This study shows that the production of iron plaque on plant roots is highly plant-species specific. Only *Phragmites australis* produced significant amounts of iron plaque, its composition mainly depending on the Fe(III) and iron-bound phosphorus concentration of the sediment. Plaque formation caused stoichiometric imbalances in leaves, suggesting an inhibited translocation of P to the leaves. Since all our test species showed inhibited aboveground growth, irrespective of iron plaque formation, we conclude that indirect iron toxicity and a disrupted N:P stoichiometry as a consequence of iron plaque was unlikely to be the cause of reduced growth. Thus, direct Fe toxicity following the uptake of Fe cannot be excluded as a mechanism

potentially explaining our results, but needs further examination in longer-term experiments. Our results stress the importance of considering the Fe(III) and Fe-P fraction in sediments, especially in eco-engineering projects where plants are used as ecological engineers for speeding up sediment consolidation and soil formation.

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CHAPTER

4

Vegetation growth and sediment dynamics in a created freshwater wetland

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ABSTRACT

Understanding how the hydrological regime in relation to sediment type interferes with ecosystem development is important when wetlands are created with soft muddy material. Especially when plants are used as ecological engineers to promote crest stability and soil formation. We carried out a two-year mesocosm experiment with sediments derived from the Dutch lake Markermeer to identify the effects of the hydrological regime and sediment type on ecosystem functioning in terms of nutrient availability. We measured plant productivity, plant nutrient stoichiometry, and concentrations of N, P, and K in shoots and roots of *Phragmites australis* and *Rumex maritimus* and monitored how the clay-rich sediment from lake Markermeer changed into a wetland soil. Plants grown on Markermeer sediments tend to be N limited when periodically inundated and P limited when not inundated at all. The P availability was determined by the hydrological regime, while the N availability was determined by both the hydrological regime and the sediment type. Ecosystem development on created wetlands can be manipulated by adequate management of the hydrological regime, as plant species respond differently to changes in nutrient availability. This should be considered in eco-engineering projects where plants are used as ecological engineers to fasten ecosystem development on wetlands that are to be created from clay-rich material.

4.1 INTRODUCTION

Wetlands are among the most valuable biomes on our planet providing important ecosystem services such as nutrient cycling, soil formation and wastewater treatment (Costanza et al., 1997; Zedler and Kercher, 2005). In the past 100 years, vast areas of wetlands have disappeared, but programs have been initiated to restore and create wetlands to compensate for this loss (e.g. Mitsch et al., 1998; Verhoeven, 2014). Although most created wetlands have been designed for wastewater treatment (Vymazal, 2011), they can also be designed to increase the ecological value of the ecosystem itself (e.g. Weller et al., 2007; Whigham et al., 2007; Stefanik and Mitsch, 2012). When designing such ecosystems, natural processes are oftentimes used to promote self-design, fasten ecosystem development and to replace conventional engineering structures (Mitsch, 1998). This concept is called ecological engineering and is implemented globally nowadays (Temmerman et al., 2013). However, to make ecological engineering projects successful, it is essential to understand how ecological, geomechanical and hydrological processes interact before making any decision on the design of such a project.

An important factor when creating wetlands is the hydrological regime as it determines the plant community and the speed of ecosystem development (Ernst, 1990; Seabloom et al., 1998; Bernhardt and Koch, 2002; Casanova and Brock, 2002). Periodical inundation often speeds up ecosystem development although the effectivity is unclear. For example, Peterson and Baldwin (2004) showed that flooding significantly decreased plant biodiversity in a freshwater wetland, but Brock et al. (1999) argued that when flooding regimes are managed correctly in terms of depth and duration of the flood, the number of habitat types increases, in turn positively affecting species richness. How wetland ecosystems respond to changes in the hydrological regime also depends on the geochemical composition, as it determines the biogeochemical processes that are induced when the water saturation gets altered (Speelman et al., 2007). For example, oxidation of pyrite can lead to severe acidification of soils, negatively impacting the environment by potential heavy metal release (Golez and Kyuma, 1997). On the other hand, if the sediment contains high amounts of iron-bound phosphorus, alternating water regimes can affect ecosystem

development by inducing P mobilization (Satawathanont et al., 1991; Portnoy, 1999; Lamers et al., 2012).

Understanding how the hydrological regime influences ecosystem development in relation to sediment type is important when the concept of ecological engineering is used. Oftentimes, fast initial plant growth is a prerequisite for vegetation to act as ecological engineers, especially when wetlands are created with soft muddy material. Wetland creation in lake Markermeer, a lake in the Netherlands located northeast of Amsterdam, is an example. In this lake, a part of the soft clay-rich lake-bed sediment is currently being dredged and used as a building material in creating approximately 10,000 ha of wetland.

Two distinct layers from the lake bed can be used as substrate for the wetlands: the relatively undisturbed consolidated near-shore Holocene marine deposits and the disturbed soft mud layer on top of these deposits. Saaltink et al. (2016, 2017) showed that these layers have a very distinct geochemical composition, also in terms of pyrite, iron-bound phosphorus and nutrients. The soft clay-rich layer is produced by bioturbation and physical weathering of the near-shore marine deposits and continuously resuspends because of wave action (Van Kessel et al., 2008; De Lucas Pardo et al., 2013).

To improve crest stability and promote soil formation on these artificial wetlands, it is important to understand how the hydrological regime could interfere with ecosystem development and what type of sediment functions best as building substrate. Because conducting small scale experiments can be an effective method to anticipate ecological engineering designs that might follow if implemented on a larger scale (Odum and Odum, 2003), we carried out a mesocosm experiment to identify the effects of the hydrological regime – i.e. periodical inundation vs no inundation – and the sediment type on ecosystem functioning in terms of nutrient availability. To reach this aim, we measure plant productivity, plant nutrient stoichiometry, and concentrations of N, P, and K in shoots and roots of *Phragmites australis* and *Rumex maritimus*. Subsequently, we monitor how the clay-rich sediment from lake Markermeer is changing into a wetland soil by regularly measuring geochemical variables of the sediment. We hypothesize that the hydrological regime especially influences the P availability as Markermeer sediments are rich in pyrite and Fe-P

(Saaltink et al., 2016), and alternating water regimes may result in P mobilization as reduction of sulfate decouples iron from phosphorus. We, therefore, expect that in sediments that are periodically inundated more P is available for plants than in sediments that receive no inundation. Since N is available in reduced (i.e., NH_4) as well as oxidized (i.e., NO_x) conditions, N availability is expected to be determined by the sediment type. This study will enhance knowledge on how the interplay between the hydrological regime and the sediment type influences ecosystem functioning by determining nutrient availability. Consequently, applications for management practices in ecological engineering projects can be extracted.

4.2 MATERIAL AND METHODS

4.2.1 Experimental mesocosms

A greenhouse experiment was conducted for 2 growing seasons at the greenhouse test facility of Utrecht University in the period April 2015 – November 2016. Mesocosms (diameter 30 cm, height 80 cm) were filled up to 50 cm with one sediment type from lake Markermeer. The sediment types were collected in March 2015 by mechanical dredging at a location (coordinates 52.5462°N; 5.3878°E) within the dredging area assigned for the collection of building material for the wetlands (Flach, 2014). The sediment types used are composed of the sediment from the soft, clay-rich layer (0-10 cm depth) and the underlying, consolidated, near-shore marine deposit of Holocene origin (10-50 cm depth); these sediments are hereafter referred to as mud and clay, respectively. Sediments were stored in air-tight containers at 4°C prior to the start of the experiment.

Two species were selected to be transplanted in the mesocosms: 1. *Rumex maritimus* (golden dock) – an annual or biennial, dicotyledonous plant species – was chosen because this plant was frequently found on the pilot wetland in lake Markermeer during monitoring studies (Dankers et al., 2015), and 2. *Phragmites australis* (common reed) – a perennial, monocotyledonous plant species – was selected because it is foreseen that this plant is used as eco-engineering species on the wetlands. Plants were grown from seeds for c. 40 days on nutrient-poor turf soil before transplantation into the mesocosms. Each mesocosm started with three seedlings of

R. maritimus and three seedlings of *P. australis* (i.e., six seedlings in total). Tinfoil was attached to the inner walls of the mesocosms to improve the light conditions for the seedlings.

Mesocosms were either periodically inundated or received weekly irrigation water. Following Güsewell et al. (2003) and Banach et al. (2009), the periodically inundated mesocosms were flooded for 7 weeks to a height of 10 cm above the sediment, after which the water was removed and the sediment could dry for two weeks. Drying is important for consolidating the soft building material so alternating dry and wet periods should be preferred over long-term inundation. The mesocosms that were not inundated received 17.3 mm water every week, which corresponds to a total water supply of 900 mm yr⁻¹. However, to prevent water stress in the plants grown in the non-inundated mesocosms an extra dose of water was added when temperatures in the greenhouse increased to 30°C or more (some weeks in July and August). For both hydrological conditions Markermeer water was used, thereby avoiding any impact on sediment geochemistry and plant function caused by chemical differences. Because lake Markermeer is nutrient limited with low values of nitrate, ammonium and phosphate (Noordhuis et al., 2014), nutrient concentrations in the water added to the mesocosms were low, averaged at 0.1 mg NO₃ l⁻¹, 0.01 mg NH₄ l⁻¹, and < 0.003 mg PO₄ l⁻¹ (Ministry of Infrastructure and the Environment, 2016).

Six mesocosms were used for each sediment – hydrology combination (24 in total). Seedlings from other plants that spontaneously emerged in the mesocosms were removed immediately.

4.2.2 Data collection

Aboveground biomass was clipped after each growing season (i.e., November 2015 and November 2016). At the end of the experiment, belowground biomass was measured for three mesocosms per condition (12 in total) by thoroughly washing and sieving the sediment. Plant material was separated per plant species, air-dried for 48 hours at 70°C, weighed, clipped and mixed prior to chemical analysis. Tissue was randomly selected and ground to determine contents of K and P using total reflection X-ray fluorescence (S2 Picofox, Bruker) and N using a CN analyzer (NA1500,

Fisons Instruments). Total uptake was calculated by multiplying biomass with tissue concentration.

The sediment samples were collected at the start of the experiment and were freeze-dried prior to geochemical analysis. Elemental contents of Al, Ca, Fe, K, Mg, Mn, Na, P, Sr, Ti, and Zn were determined using ICP-OES following aqua regia destruction. Sulfur contents were measured on an elemental CS analyzer (CS-300, LECO) and the N contents were determined on a CN analyzer (NA1500, Fisons Instruments). Quantitative bulk mineralogical compositions of the crystalline fraction of the sediments were determined by Rietveld refinement from the X-ray diffraction patterns (Scarlett & Madsen, 2006). Organic matter was determined by slowly heating to 550°C and then calculating the weight loss between 105 – 550°C (Howard, 1965). A sequential extraction method based on Ruttenberg (1992) was applied to characterize solid P speciation and to determine the content of iron oxides (Table 4.1). The extractable content of iron oxides was measured from the citrate-dithionite-bicarbonate (CDB) filtrate from the second step using ICP-OES. All geochemical analyses were carried out for 5 replicates per sediment type, except for the XRD analysis (1 replicate per sediment type).

Table 4.1. List of steps used in the extraction procedure of phosphorus (based on Ruttenberg, 1992).

Step	Extractant	Separated P fraction
I	1M MgCl ₂ , 30 min	Exchangeable or loosely sorbed P
II	A Citrate-dithionite-bicarbonate (CDB), 8 h	Easily reducible or reactive ferric Fe- P
	B 1M MgCl ₂ , 30 min	
III	A Na acetate buffer (pH 4), 6 h	Amorphous apatite and carbonate P
	B 1M MgCl ₂ , 30 min	
IV	1M HCl, 24 h	Crystalline apatite and other inorganic P
V	Ash at 550 °C, 2h; 1M HCl, 24 h	Organic P

In addition, sediment samples from the topsoil (0-2 cm) were collected in each mesocosm at $t = 6, 11$, and 18 months as in the topsoil highest geochemical effects caused by the vegetation are expected (Saaltink et al., 2016). These samples were freeze-dried and analyzed immediately in terms of solid P speciation, extractable iron oxides, N and organic matter contents.

At the end of the experiment, three replicate samples per mesocosm at 10, 20, and 30 cm depth were retrieved with a gouge (diameter 3 cm), and repeated for three mesocosms per experimental condition. The sediment samples at the same depth were pooled per mesocosm and chemically analyzed in terms of solid P speciation, extractable iron oxides, N and organic matter contents.

4.2.3 Statistical analyses

Statistical analysis was carried out using SPSS 22.0.0.1. Differences in biomass production, plant tissue concentrations and total uptake of N, P, and K as well as the N:P and N:K ratios between treatments were determined using univariate, general models with a least square difference (LSD) post hoc test ($p < 0.05$) following Webster (2007). From these GLMs, significant interaction effects between the sediment type and the hydrological regime were also identified. To correct for seasonal effects (season 1 vs. season 2), we included time as a fixed factor in our models when assessing significant differences between the treatments. These GLMs were carried out for *R. maritimus* and *P. australis* separately. Significant differences between *R. maritimus* and *P. australis* within treatments were determined using one-way ANOVA with LSD post hoc testing ($p < 0.05$). Differences in geochemical composition of mud and clay were assessed with a t-test ($p < 0.05$). No statistics could be applied to the mineralogical sediment composition (XRD analysis) due to absence of replicates, however.

4.3 RESULTS

4.3.1 Geochemical and mineralogical composition of lake Markermeer sediments

Table 4.2 presents the geochemical and mineralogical composition of mud and clay. All analyzed major elements (Al, Ca, Fe, K, Mg, Mn, Na, P, S, Sr, and Zn) except

for Ti, were significantly higher in mud than in clay ($p < 0.05$ as indicated with an asterisk in Table 4.2). Sequential P extraction revealed that the significantly higher values in total P in mud (361 mg kg^{-1}) than in clay (213 mg kg^{-1}) ($p < 0.001$) is attributed to significantly higher values for mud in Fe-P ($p < 0.01$), amorphous apatite and carbonate P (Ca-P) ($p < 0.01$) and organic P ($p < 0.001$); exchangeable P and crystalline apatite and other inorganic P (detrital P) did not differ significantly ($p = 0.10$ for exchangeable P; $p = 0.32$ for detrital P).

Relatedly, the two sediment types also differ with respect to their mineralogical composition. The quartz content was higher in clay, whereas the amounts of calcite and pyrite were higher in mud. In total, the amount of non-phyllsilicates (sum of quartz, plagioclase, alkali feldspar, calcite, dolomite, oxydes/hydroxydes and pyrite) was higher in clay (83.2%) than in mud (73.7%). As a consequence, the amount of phyllsilicates (sum of illite, smectite, kaolinite, and chlorite) was higher in mud than in clay: 26.3% vs 16.8%, i.e., the mud contains more swelling clay minerals.

4.3.2 Geochemical changes in the topsoil

Figure 4.1 presents the dynamics of the sediment concentrations of the topsoil (0-2 cm) of the iron oxides, phosphorus pools, N, and organic C during the experimental period for the four different treatments. Significant differences between time steps are presented in the Appendix (Table A4.1). Throughout the experiment, the concentrations of these solid compounds were higher in mud (dotted and solid grey lines) than in clay (dotted and solid black lines) ($p < 0.05$), except for detrital P ($p < 0.25$, Figure 4.1d). Interestingly, no significant changes were observed due to the different hydrological conditions, except for Ca-P, which was significantly higher in the non-inundated condition in mud ($p < 0.05$, dotted gray line).

Oxidation of the topsoil resulted in a linear increase of iron oxides regardless of the hydrological condition and sediment type (Figure 4.1a). A similar increase was observed for the Fe-P concentration in the first 10 months, after which it stabilized during the second growing season ($t = 10$ and 18 months) for the periodic inundated conditions (Figure 4.1b). The Fe-P content in the non-inundated conditions decreased significantly between month 10 and 18 ($p < 0.001$). In contrast to the Fe-P concentrations, Ca-P decreased in the first 10 months in mud, after which it increased

concentrations, Ca-P decreased in the first 10 months in mud, after which it increased significantly for both hydrological conditions ($p < 0.01$) (Figure 4.1c). These opposed temporal changes in Ca-P and Fe-P are true for mud but not for clay.

Table 4.2. Geochemical and mineralogical composition of clay and mud at the start of the experiment ($t = 0$). Average concentrations in dry wt with standard deviations ($n = 5$). Significant differences between clay and mud are indicated by an asterisk ($p < 0.05$).

	Unit	n	Clay	S.D.	Mud	S.D.
Aqua regia / CS / CN						
Al*	mg kg ⁻¹	5	4670	365	10398	219
Ca*	mg kg ⁻¹	5	25872	1661	48058	1288
Fe*	mg kg ⁻¹	5	6538	391	14766	234
K*	mg kg ⁻¹	5	1251	106	2619	72
Mg*	mg kg ⁻¹	5	2674	128	5106	135
Mn*	mg kg ⁻¹	5	161	9	360	10
N*	mg kg ⁻¹	5	617	62	1611	32
Na*	mg kg ⁻¹	5	49	13	97	34
P*	mg kg ⁻¹	5	213	7	361	10
S*	mg kg ⁻¹	5	1191	111	4513	187
Sr*	mg kg ⁻¹	5	70	5	113	3
Ti	mg kg ⁻¹	5	380	23	396	17
Zn*	mg kg ⁻¹	5	88	5	130	3
Seq. P extraction						
Exchangeable P	mg kg ⁻¹	5	3	1	5	2
Fe-bound P*	mg kg ⁻¹	5	14	2	40	10
Ca-bound P*	mg kg ⁻¹	5	42	8	89	18
Detrital P	mg kg ⁻¹	5	148	14	158	14
Organic P*	mg kg ⁻¹	5	16	1	47	11
Fe as oxides*	mg kg ⁻¹	5	508	40	851	39

Table 4.2. (continued).

	Unit	n	Clay	S.D.	Mud	S.D.
XRD						
Quartz	%	1	59.8		45.8	
Plagioclase	%	1	8.7		8.2	
Alkali feldspar	%	1	4.6		3.7	
Calcite	%	1	7.3		11.9	
Dolomite	%	1	2.8		2.8	
TiO₂	%	1	0.3		0.4	
Pyrite	%	1	0.4		1.0	
Phyllosilicates	%	1	16.8		26.3	
Other						
Organic matter*	%	5	2.6	0.2	4.0	0.1

Detrital P and organic P varied throughout the experiment, but the concentrations at $t = 18$ months did not differ significantly from the concentrations measured at the start of the experiment (Figure 4.1d,e), except for clay that was periodically inundated: the concentration of organic P was significantly higher at $t = 18$ months ($p < 0.05$). Despite the changes described above, the total P concentrations stayed more or less stable with only periodically inundated clay being significantly higher at the end of the experiment ($p < 0.01$) (Figure 4.1f). The concentration of N in clay was significantly higher at the end of both growing seasons ($t = 6$ and $t = 18$) compared to the start for periodically inundated clay ($p < 0.01$) (Figure 4.1g). No differences were observed for organic C (Figure 4.1h).

In general, the sediment type mainly affected changes in the geochemical composition of the topsoil, while the hydrological regime had only a marginal effect.

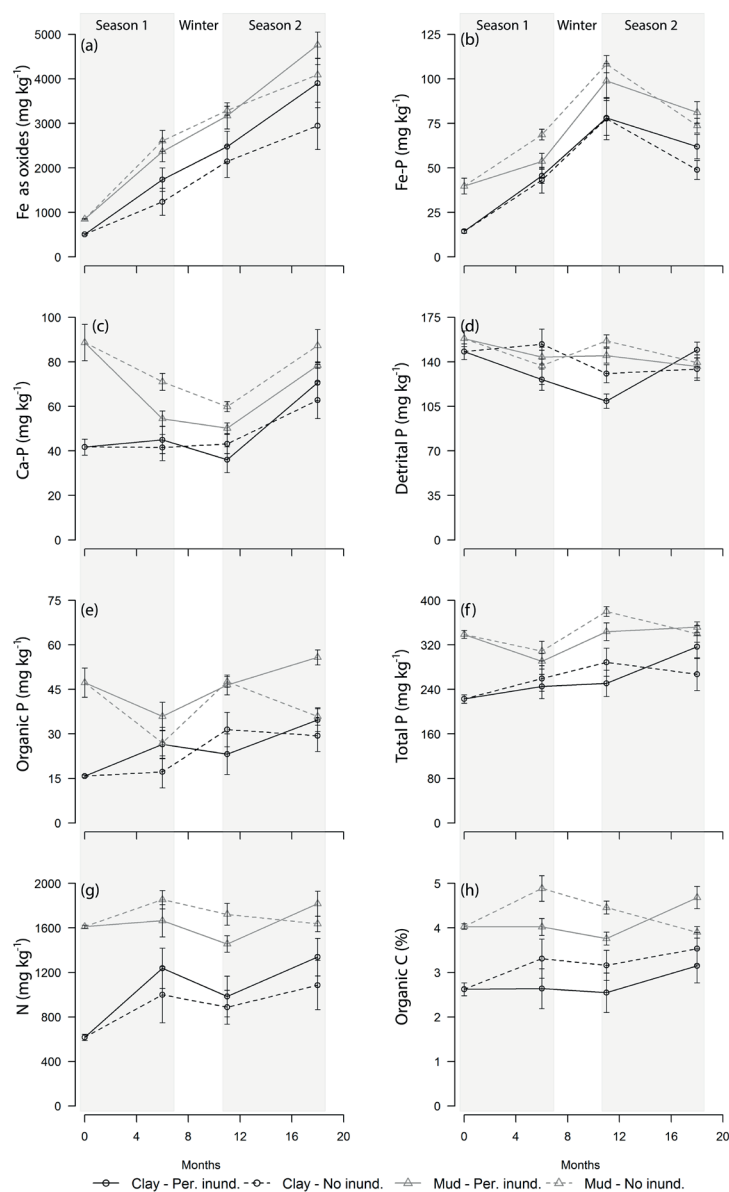


Figure 4.1. Time series of geochemical variables in the topsoil (0-2 cm) ($n = 6$): Fe as oxides (a), iron-bound P (Fe-P) (b), amorphous apatite and carbonate P (Ca-P) (c), crystalline apatite and other inorganic P (Detrital P) (d), Organic P (e), Total P (f) and N (g) in mg kg⁻¹ and organic C (h) in %. Error bars indicate S.E.

4.3.3 Sediment depth profile

Figure 4.2 presents a depth profile for the concentrations of Fe-P and iron after 18 months. Interesting is that no significant difference was observed between the hydrological conditions for the Fe-P concentration (Figure 4.2a). Mud had significantly higher concentrations throughout depth than clay ($p < 0.01$). In contrast, iron oxides were significantly higher in the sediments that were not inundated ($p < 0.05$ for both clay and mud) (Figure 4.2b), meaning that periodical inundation did not inhibit oxidation processes at the top soil, but clearly had an effect on oxidation processes at larger depth. In the periodically inundated sediments, concentrations of iron oxides decreased in mud from 3500 mg kg⁻¹ at the topsoil to 1900 mg kg⁻¹ at 30 cm depth and for clay from 4500 mg kg⁻¹ to 2200 mg kg⁻¹. Remarkably, the concentrations at 30 cm depth were higher than the initial concentrations as presented in Table 4.2 (508 mg kg⁻¹ in clay and 851 mg kg⁻¹ in mud).

4.3.4 Plant tissue composition

Table 4.3 shows how sediment type and the hydrological condition affected the average concentrations of N, P, and K and the N:P and N:K ratios in leaves and roots for *P. australis* and *R. maritimus*. Significant differences between the sediment types are indicated by orange rectangles and significant differences between the hydrological conditions are indicated by blue rectangles.

The uptake of N was significantly higher in non-inundated mud and clay for roots of *P. australis* ($p < 0.05$ for clay; $p < 0.01$ for mud) and for roots and shoots of *R. maritimus* grown in mud ($p < 0.001$). More generally, periodical inundation had a negative effect on N uptake although not always statistically significant. This effect was more pronounced in *R. maritimus* than in *P. australis*. The N concentrations were furthermore higher in plants grown in mud than in clay, which was significant for roots in *P. australis* ($p < 0.05$) and for roots and shoots in *R. maritimus* ($p < 0.001$ for roots and shoots) grown in the non-inundated sediment. This is in line with the finding that the N concentration in mud was significantly higher than in clay (Table 4.2, Figure 4.1c). So, both the hydrological regime and the sediment type affected the N tissue concentration for both species. Moreover, a significant ordinal interaction effect between the sediment type and the hydrological regime was found

for shoots in *R. maritimus* ($F(1, 44) = 13.205$, $p = .001$), further indicating that the effect of the hydrological regime depends on the sediment type (and vice versa).

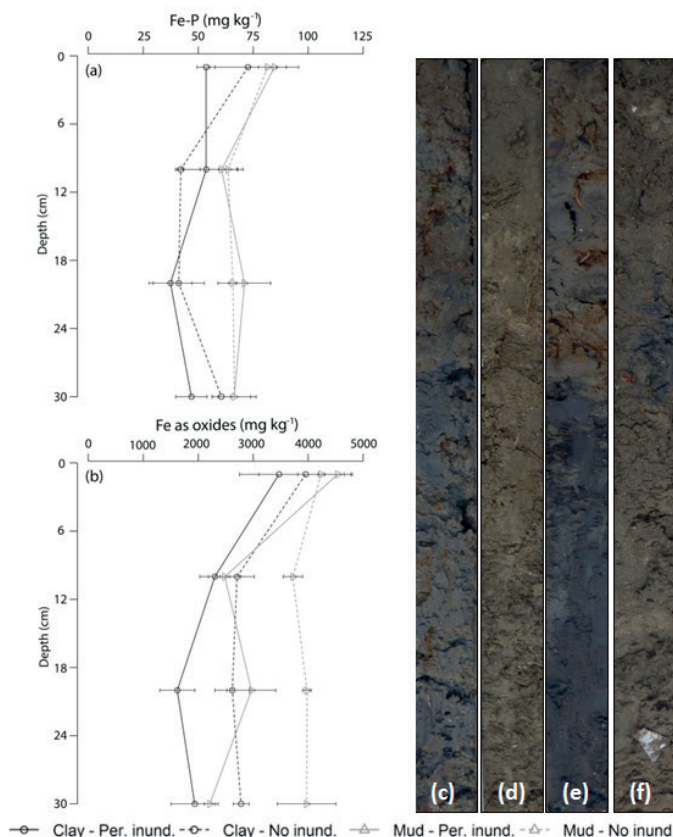


Figure 4.2. Sediment depth profile for iron-bound P (Fe-P) (a) and Fe as oxides (b) in mg kg⁻¹ ($\pm$ S.E.) at $t = 18$ months ($n = 9$). Random photographs of a sediment core in periodically inundated clay (c), non-inundated clay (d), periodically inundated mud (e) and non-inundated mud (f) of 30 cm depth.

In contrast to N, the average P concentrations were higher in plants grown in the periodically inundated sediments, meaning that wetter conditions enhance P uptake. This was significant for roots and shoots of *P. australis* ($p < 0.001$ for shoots; $p < 0.01$ for roots), although *R. maritimus* had highest P concentrations in the shoot. No clear differences were found between clay and mud, while total P and other P fractions

were significantly higher in mud (Table 4.2, Figure 4.1b,c,e,f). This suggests that the hydrological condition is driving P availability in these sediments.

As a result of these differences in plant nutrient concentrations between the hydrological conditions, the N:P ratios in roots and shoots of *P. australis* were significantly higher when grown in the non-inundated sediments ($p < 0.001$ for shoots grown in mud; $p < 0.01$ for shoots grown in clay and roots grown in mud; $p < 0.05$ for roots grown in clay). For *R. maritimus*, this was only significant for mud ($p < 0.001$ for shoots; $p < 0.05$ for roots). Furthermore, the N:P ratio was significantly higher in mud than in clay for shoots of *P. australis* ($p < 0.05$) and *R. maritimus* ($p < 0.001$) that were grown in the non-inundated sediment. For shoots in *R. maritimus*, a significant ordinal interaction effect was found between the sediment type and the hydrological regime ($F(1,44) = 8.010, p = .008$).

No significant differences were found for the K concentration in both leaves and roots of *P. australis* and *R. maritimus*. Hence, the differences observed in the N:K ratio in roots and shoots were caused by N. Upon result, the N:K ratio was higher in non-inundated mud and clay, which was only significant for roots of *P. australis* ($p < 0.05$) and for shoots and roots of *R. maritimus* grown in mud ($p < 0.01$ for shoots; $p < 0.05$ for roots). All these results show that periodical inundation had a negative impact on the uptake of N and a positive impact on the uptake of P. Although differences in sediment P concentrations were large, the sediment type had only an effect on the uptake of N.

4.3.5 Biomass production

Figure 4.3 presents biomass production per treatment for both plant species. Overall, shoot production was higher in mud than in clay for both seasons ($p < 0.01$). Interesting is that no significant differences in overall shoot production were found between the hydrological conditions in both seasons (Figure 4.3a,b). However, in the periodically inundated treatments, shoot biomass of *R. maritimus* decreased significantly in season 2 ($p < 0.05$ for clay; $p < 0.01$ for mud). Moreover, in the sediments that were periodically inundated, *P. australis* produced significantly more aboveground biomass than *R. maritimus* in season 2 ($p < 0.001$ for clay; $p < 0.01$ for mud).

Table 4.3. Average concentration ($\pm$ S.E.) of N, P, and K (% of dry wt) as well as N:P and N:K ratio in shoots ($n = 12$; season 1 and season 2) and roots ($n = 3$; season 2) of *P. australis* and *R. maritimus* for the sediment types (Clay and Mud) and the hydrological regimes (periodical inundation = Per. inund.; no inundation = No inund.). A significant difference ($p < 0.05$) between mud and clay is indicated by orange rectangles (i.e., horizontal configuration) and a significant difference ($p < 0.05$) between periodical inundation and no inundation is indicated by blue rectangles (i.e., vertical configuration).

		Plant species	<i>P. australis</i>			<i>R. maritimus</i>		
Element		Hydrology	Clay	Mud	S.E. sed.	Clay	Mud	S.E. sed.
N	Shoot	Per. inund.	1.44	1.54	±0.06	1.15	1.12	±0.08
		No inund.	1.62	1.67	±0.07	1.40	1.90	±0.15
	S.E. hydrology		±0.06	±0.07		±0.08	±0.17	
	Root	Per. inund.	0.53	0.67	±0.05	0.57	0.64	±0.02
		No inund.	0.88	1.20	±0.11	0.91	2.13	±0.37
	S.E. hydrology		±0.10	±0.13		±0.09	±0.41	
P	Shoot	Per. inund.	0.13	0.14	±0.01	0.25	0.25	±0.02
		No inund.	0.09	0.07	±0.01	0.21	0.18	±0.02
	S.E. hydrology		±0.01	±0.01		±0.02	±0.02	
	Root	Per. inund.	0.12	0.14	±0.01	0.08	0.13	±0.02
		No inund.	0.05	0.06	±0.01	0.14	0.08	±0.03
	S.E. hydrology		±0.02	±0.02		±0.04	±0.02	
K	Shoot	Per. inund.	1.66	1.49	±0.05	2.44	2.51	±0.16
		No inund.	1.65	1.52	±0.07	2.60	3.16	±0.28
	S.E. hydrology		±0.06	±0.06		±0.27	±0.21	
	Root	Per. inund.	1.42	1.73	±0.15	1.17	1.62	±0.14
		No inund.	1.43	1.49	±0.25	1.04	1.04	±0.34
	S.E. hydrology		±0.19	±0.21		±0.33	±0.31	

Table 4.3. (continued).

		Plant species	<i>P. australis</i>			<i>R. maritimus</i>		
Element		Hydrology	Clay	Mud	S.E. sed.	Clay	Mud	S.E. sed.
N:P ratio	Shoot	Per. inund.	11.1	11.8	±0.52	5.0	5.1	±0.52
		No inund.	19.9	28.1	±2.29	8.0	14.0	±1.72
		S.E. hydrology	±1.50	±2.50		±0.74	±1.84	
	Root	Per. inund.	4.6	4.7	±0.27	7.1	5.1	±0.76
		No inund.	18.1	24.8	±3.00	13.0	28.2	±5.76
		S.E. hydrology	±3.18	±5.10		±6.16	±5.84	
N:K ratio	Shoot	Per. inund.	0.88	1.07	±0.05	0.51	0.46	±0.05
		No inund.	1.01	1.12	±0.05	0.66	0.85	±0.07
		S.E. hydrology	±0.05	±0.05		±0.06	±0.07	
	Root	Per. inund.	0.39	0.40	±0.05	0.48	0.40	±0.03
		No inund.	0.71	0.90	±0.13	1.64	2.95	±0.63
		S.E. hydrology	±0.11	±0.15		±0.75	±0.66	

In contrast to shoot production, no significant difference between the two sediment types was found in root production. However, root production was higher in the sediments that were periodically inundated (Figure 4.3c), but this was only significant for clay ($p < 0.05$). *Phragmites australis* produced more belowground biomass than *R. maritimus* in all four conditions. Overall, the sediment type mainly influenced shoot biomass production, while the hydrological regime had an impact on root biomass production. These effects on biomass production were mainly caused by a decrease in shoot biomass of *R. maritimus* and an increase in root biomass of *P. australis* in the periodically inundated treatments in season 2.

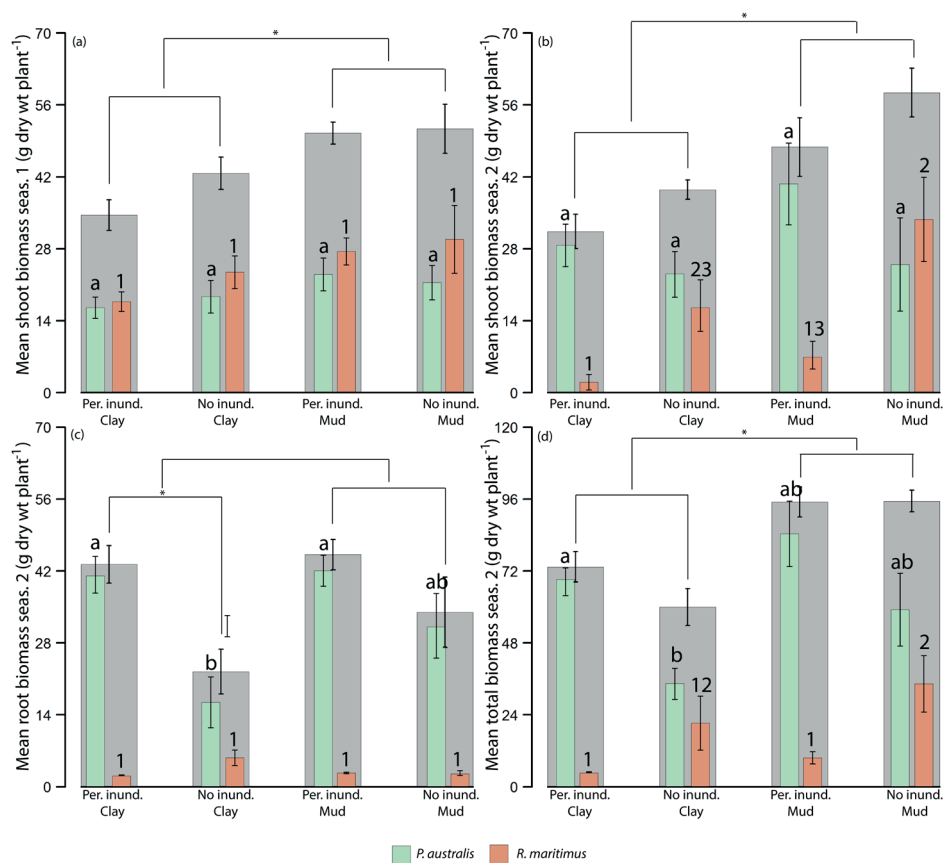


Figure 4.3. Shoot biomass production in season 1 ($n = 6$; $t = 6$ months) (a) and season 2 ($n = 6$; $t = 18$ months) (b), as well as root biomass production (c) and total biomass production (d) in season 2 ($n = 3$; $t = 18$ months) (g dry wt plant⁻¹) in all four conditions (Clay vs Mud and periodical inundation vs no inundation) separated for *P. australis* (green bars) and *R. maritimus* (orange bars) as well as the aggregate value of the two species (gray bars). Error bars indicate S.E. Significant differences between aggregates are indicated by an asterisk. Species specific significant differences between treatments are indicated by different letters (*P. australis*) and different numbers (*R. maritimus*) ($p < 0.05$).

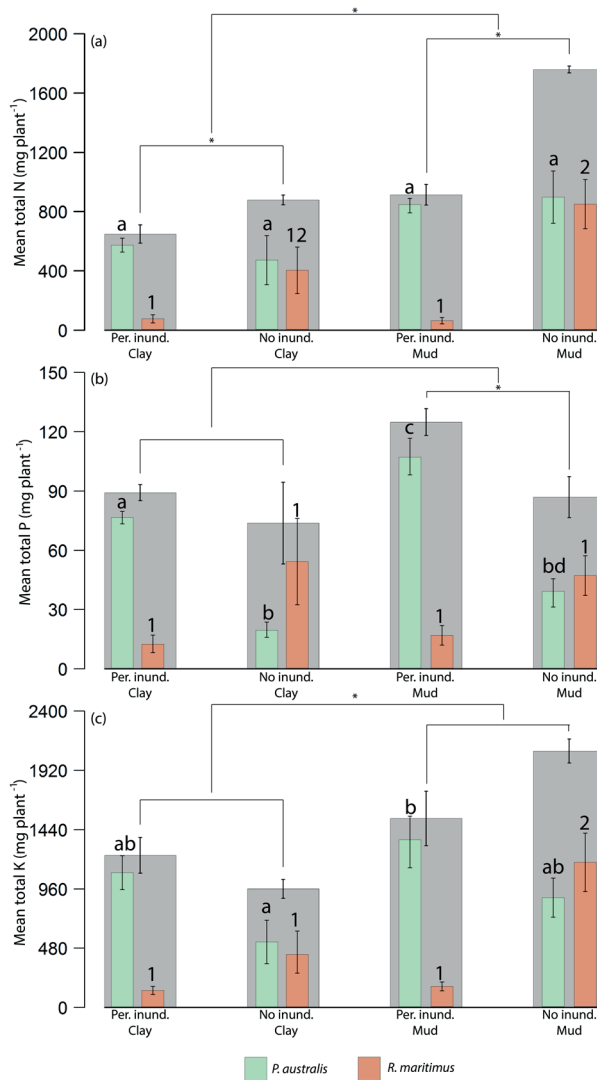


Figure 4.4. Total uptake of N (a), P (b), and K (c) in season 2 (mg plant⁻¹) in all four conditions (Clay vs Mud and periodical inundation vs no inundation) separated for *P. australis* (green bars) and *R. maritimus* (orange bars) as well as the aggregate value of the two species (gray bars). Error bars indicate S.E. Significant differences between aggregates are indicated by an asterisk. Species specific significant differences between treatments are indicated by different letters (*P. australis*) and different numbers (*R. maritimus*) ($p < 0.05$).

4.3.6 Nutrient uptake

Figure 4.4 presents total uptake of N, P, and K per treatment for both plant species. Figure 4.4a shows that significantly more N was incorporated in the plants that were grown in mud than in clay ($p < 0.05$), probably due to the higher N concentrations in this sediment type (Table 4.2, Figure 4.1g). Moreover, uptake of N was significantly lower in the periodically inundated sediments ($p < 0.001$ for mud; $p < 0.05$ for clay), which was caused by reduced uptake of *R. maritimus* compared to the control treatment. No significant difference in N uptake was found for *P. australis* between all treatments. In concordance with the plant P concentrations presented in Table 4.3, no significant difference was found between the two sediment types for the uptake of P in general. However, a significant difference was found in P uptake of *P. australis* in the periodically inundated treatments ($p < 0.05$) (Figure 4.4b). Moreover, the amount of P taken up by *P. australis* was higher in the periodically inundated sediments ($p < 0.01$ for clay and $p < 0.001$ for mud). The uptake of K was significantly higher in mud than in clay ($p < 0.01$) due to increased uptake of K in *R. maritimus* grown in mud ($p < 0.05$). No overall significant differences were found between the hydrological conditions. To summarize, the hydrological regime influenced the uptake of N in *R. maritimus* and P in *P. australis* and the sediment type influenced the uptake of N and K in *R. maritimus*.

4.4 DISCUSSION

4.4.1 Plant effects

The results show that plant nutrient-acquisition was influenced by both the hydrological regime and the sediment type. *Rumex maritimus* showed hampered uptake of N when both mud and clay were periodically inundated (Table 4.3; Figure 4.4a). Likely, this is the case because *P. australis* has high radial oxygen loss (Brix et al., 1996; Dickopp et al., 2011; Tercero et al., 2015), which sustains an oxygenated zone around the roots under waterlogged conditions. This is because such a zone promotes nitrification, and plants tend to perform better when NO_3^- is present as an N source (Falkengren-Grerup, 1995). Significantly more N was taken up in mud, as mud contained twice the amount of N compared with clay at the start of the experiment

(Table 4.2). Shoot N values of *R. maritimus* were below the critical threshold for N limitation in the periodically inundated treatment only (c. 1.4%; cf De Wit et al., 1963). Shoot N values of *P. australis* did not indicate N limitation in any treatment and might explain why this species outcompetes *R. maritimus* in the periodically inundated treatment.

In contrast to N-acquisition, root and shoot P concentrations of *P. australis* were significantly higher in the periodically inundated sediments. In roots and shoots of *R. maritimus*, no significant effect of periodical inundation could be discerned, which implies that decreased oxygen concentration in the periodically inundated treatment does not have a negative impact on the uptake of P. *Phragmites australis* clearly outcompetes *R. maritimus* in the second season in the periodically inundated treatments (Figure 4.3b). Combined with the finding that *P. australis* is more efficient than *R. maritimus* in P acquisition in the periodically inundated treatments (Figure 4.4) this is convincing evidence for concluding that in wet conditions *P. australis* is winning competition because it more effectively acquires essential nutrients.

Two mechanisms might explain this positive effect. First, a decrease in oxygen concentration in the sediment can also be an advantage when grown in iron-rich sediments as it reduces iron plaque formation on roots. Iron plaque induces a form of indirect iron toxicity by acting as a barrier against iron, which in turn has adverse effects on the nutrient uptake and translocation mechanisms in the plant (Snowden and Wheeler, 1995; Xu et al., 2009; Tripathi et al., 2014). Furthermore, Saaltink et al. (2017a) showed that iron plaque formation primarily affected the uptake of P, not N. They also showed that *P. australis* produced substantial amounts of iron plaque in aerated conditions, while little iron plaque formation was discerned on roots of *R. maritimus*. This also explains why we observed a positive effect of inundation on nutrient uptake in *P. australis* only. However, it must be noted that the shoot P concentrations in *R. maritimus* were above that of P limitation (c. 0.07% cf De Wit et al., 1963). Second, as the sediment contains high amounts of pyrite and iron-bound phosphorus, alternating water regimes can induce P mobilization (Satawathanont et al., 1991; Portnoy, 1999). This is because in reducing conditions, reduction of sulfate leads to P mobilization as Fe(II) favors sulfur over phosphorus in mineral precipitation. These two mechanisms might explain why periodical inundation

has a negative effect on the uptake of N but not on P. Although mud appeared to be more nutritious in terms of P, no significant difference in P tissue concentration could be discerned between the two sediment types. Overall, P availability in our experiments, apparently is determined by the hydrological regime, while N availability is determined by both the hydrological regime and the sediment type.

As a consequence of the inhibited uptake of N, the shoot N:P ratios of *R. maritimus* were low when grown in the periodically inundated sediments and were indicative of N limitation, while the shoot N:P ratios of *P. australis* indicated only moderate N limitation (c. < 14 cf Koerselman and Meuleman, 1996). However, when *P. australis* was grown in the non-inundated sediments, shoot N:P ratios were high and suggested P limitation (c. > 16 cf Koerselman and Meuleman, 1996). These results show that plants grown on Markermeer sediments tend to be N limited when periodically inundated and P limited when not inundated at all.

The aforementioned differences in the nutrient acquisition by plants between treatments affected biomass production: the sediment type dictated changes in the production of shoots, while the hydrological regime influenced the production of roots.

4.4.2 Sediment effects

In contrast to plant nutrient-acquisition, the hydrological regime did not determine changes in the geochemical composition that occurred at the top layer of 2 cm. It is likely that raised belowground biomass production of *P. australis* enhanced aeration in the periodically inundated treatments due to increased radial oxygen loss (Reddy et al., 1990), which negated the effect of inundation which normally reduces conditions. It must be noted that the rate at which iron oxides increase is constant throughout the experiment (Figure 4.1a), implying that absence of plants between growing seasons did not hamper the rate of oxidation. This might have occurred by Venturi-induced convection of gases through rhizomes of *P. australis* – i.e. clipped and dead culms still function as air tubes. Armstrong et al. (1991) showed that this mechanism raises oxygen concentrations in the soil during winter.

Plants do not only have the capacity to increase oxygen levels in the sediment, we also found indications that they alter the distribution of the phosphate pools in the

sediment. During soil development these wetland sediments can either have elevated Fe-P concentrations by transformation from dissolved Ca-P in season 1 or elevated Ca-P concentrations by transformation from Fe-P in season 2. The increase of Ca-P between month 10 and 18 suggest elevated concentrations of dissolved phosphate in the porewater (Wang et al., 2011). In our experiment, the source of dissolved phosphate comes from Fe-P (Figure 4.1b). This surprising result may be attributed to the ability of plants to dissolve iron oxides by H^+ and OH^- excretion by roots leading to mobilization of P (Bertrand et al., 1999). Moreover, Moorby et al. (1988) reported that H^+ release by roots is stimulated when P becomes deficient. This might be a reason why Fe-P dissolved only in the second season.

4.4.3 Implications for eco-engineering

The findings presented in this study can be implemented in eco-engineering projects where wetlands are to be created from clay-rich material. First, alternating the hydrological regime can enhance P availability for plants, especially in sediments with substantial amounts of Fe-P. Therefore, management of the hydrological regime opens possibilities for manipulating ecosystem development on the wetland, as the species assemblage of the future wetland and the succession that occurs is a response to changes in nutrient availability (e.g. Bedford et al., 1999). In our study, periodical inundation led to a relatively fast colonization of the target species *P. australis*. Second, the concentration of nutrients in the sediment influences aboveground biomass production. Fast shoot growth is especially preferred in lake wetlands build with soft mud as leaves and stems dampen shear stress on the substrate by attenuating waves (Nepf, 2012). In our study, muddy bed sediment contained higher concentration of nutrients than the consolidated clay deposit. To optimize the design of the eco-engineering project in lake Markermeer, we therefore suggest using mud for highest shoot production and to manage the hydrological regime to steer the P availability. After initial consolidation and crest stabilization of the sediment on the wetland, controlling the hydrological regime can also be used to promote biodiversity, as P availability is an important determinant for species richness (Olde Venterink et al., 2003). The main findings of this study are schematically summarized in Figure 4.5.

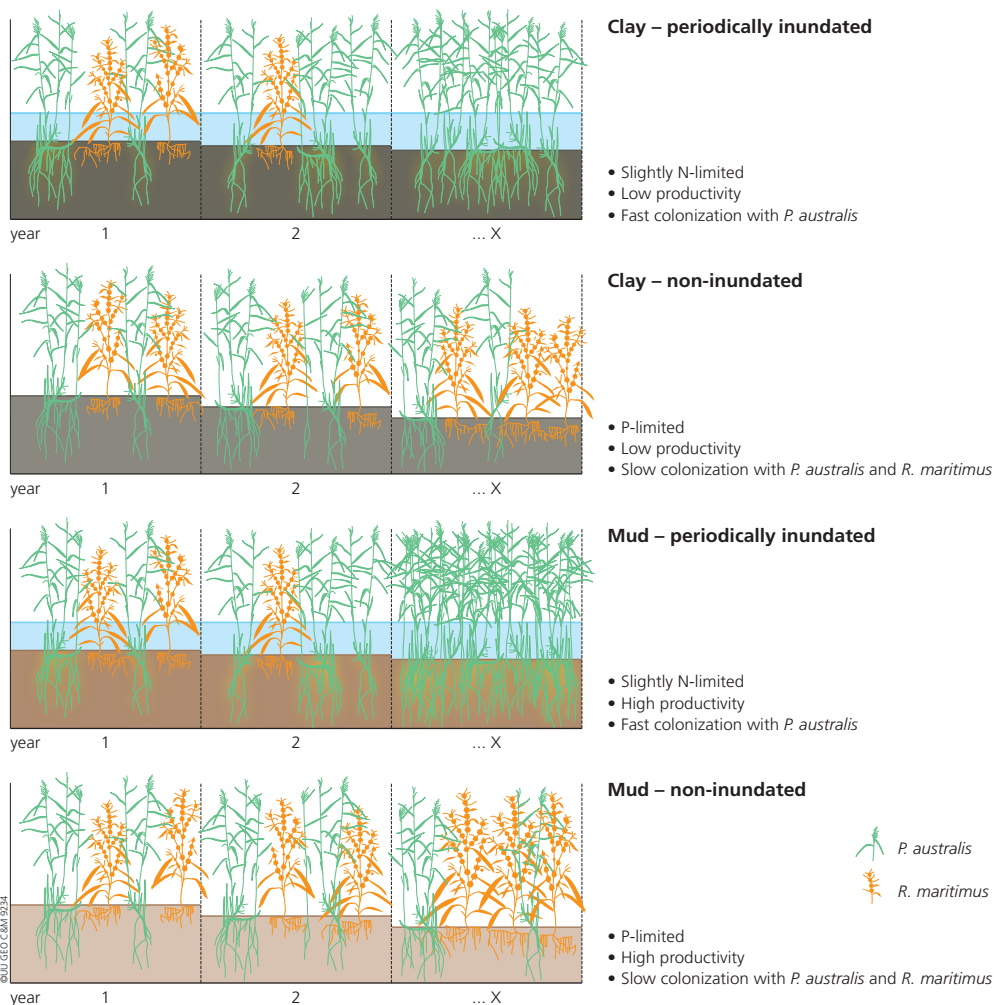


Figure 5. Schematic summary of main effects on ecosystem functioning based on each treatment used in this study.

4.5 CONCLUSIONS

The results of this study show that the hydrological regime and the sediment type influenced ecosystem functioning by determining nutrient availability. Periodical

inundation had a positive effect on the P availability in the sediment, especially for aerenchymatous species such as *P. australis*. However, *R. maritimus* showed hampered uptake of N when periodically inundated. Two mechanisms might explain why periodical inundation had a positive effect on P availability: 1) reduction of iron plaque formation on roots of *P. australis*; and 2) induced P mobilization by reduction of sulfate. In general, the results show that the P availability is determined by the hydrological regime, while the N availability is determined by both the hydrological regime and the sediment type. Despite these effects on nutrient availability no significant changes were discerned in total aboveground biomass production for *P. australis*, although root biomass was significantly higher in periodically inundated sediments. The sediment type in the mesocosms influenced plants to some extent, especially because of differences in sediment N concentrations. Furthermore, we found indications that plants alter the distribution of the phosphate pools in the sediment, probably by solubilizing Fe-P, thereby promoting Ca-P nucleation. These findings can be implemented in eco-engineering projects where plants are used as ecological engineers to fasten ecosystem development on wetlands that are to be created from clay-rich material.

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DATA ACCESSIBILITY

A dataset containing all relevant data acquired in this study is archived at DANS Data Archiving and Networked Systems) and is accessible to all users (Saaltink et al., 2017b).

APPENDIX

Table A4.1. Time series of geochemical variables in the topsoil (0-2 cm) ($n = 6$), with standard errors. Significant differences between time steps are indicated by different letters (horizontal configuration) ($p < 0.05$).

Element	Unit	Sediment	Hydrology	Month 0	Month 6		Month 11		Month 18		
Fe as oxides	mg kg ⁻¹	Clay	Per. inund.	508 ^a	±17.8	1738 ^b	±264	2482 ^b	±343	3905 ^c	±552
			No inund.	508 ^a	±17.8	1240 ^b	±303	2144 ^c	±359	2947 ^c	±533
		Mud	Per. inund.	851 ^a	±17.2	2369 ^b	±228	3171 ^c	±292	4760 ^d	±292
			No inund.	851 ^a	±17.2	2608 ^b	±234	3288 ^c	±97.3	4089 ^d	±231
Fe-P	mg kg ⁻¹	Clay	Per. inund.	14.4 ^a	±1.02	45.6 ^b	±4.28	78.1 ^c	±9.78	62.0 ^{bc}	±6.87
			No inund.	14.4 ^a	±1.02	43.2 ^b	±7.24	77.7 ^c	±11.8	48.9 ^b	±5.31
		Mud	Per. inund.	39.8 ^a	±4.44	53.7 ^a	±4.54	98.9 ^b	±9.73	81.2 ^b	±6.07
			No inund.	39.8 ^a	±4.44	68.7 ^b	±3.03	108.3 ^c	±4.83	73.8 ^b	±4.15
Ca-P	mg kg ⁻¹	Clay	Per. inund.	41.7 ^a	±3.64	45.0 ^a	±6.13	36.0 ^a	±5.79	70.7 ^b	±8.10
			No inund.	41.7 ^a	±3.64	41.5 ^a	±5.92	43.1 ^a	±4.29	62.8 ^b	±8.27
		Mud	Per. inund.	88.7 ^a	±8.13	54.5 ^b	±3.50	50.2 ^b	±2.44	78.5 ^a	±1.20
			No inund.	88.7 ^a	±8.13	71.0 ^{ab}	±3.78	59.9 ^b	±2.26	87.3 ^a	±7.27
Detrital P	mg kg ⁻¹	Clay	Per. inund.	148 ^a	±6.09	126 ^{ab}	±8.35	109 ^b	±5.61	149 ^a	±6.24
			No inund.	148 ^a	±6.09	154 ^a	±11.8	131 ^a	±7.06	134 ^a	±8.82
		Mud	Per. inund.	158 ^a	±6.22	144 ^a	±5.43	145 ^a	±5.82	136 ^a	±9.03
			No inund.	158 ^a	±6.22	137 ^a	±14.6	157 ^a	±4.77	140 ^a	±3.43

Table A4.1. (continued).

Element	Unit	Sediment	Hydrology	Month 0	Month 6	Month 11	Month 18
Organic P	mg kg ⁻¹	Clay	Per. inund.	15.8 ^a ±0.41	26.5 ^a ±4.79	23.2 ^a ±6.85	34.7 ^a ±3.81
			No inund.	15.8 ^a ±0.41	17.2 ^a ±5.38	31.5 ^a ±5.78	29.4 ^a ±5.31
		Mud	Per. inund.	47.3 ^{ab} ±4.90	35.9 ^a ±4.82	46.5 ^{ab} ±3.34	55.8 ^b ±2.54
			No inund.	47.3 ^a ±4.90	27.0 ^b ±5.20	47.6 ^a ±1.83	35.9 ^{ab} ±2.93
Total P	mg kg ⁻¹	Clay	Per. inund.	223 ^a ±7.43	245 ^{ab} ±21.8	251 ^{ab} ±23.6	317 ^b ±21.2
			No inund.	223 ^a ±7.43	260 ^a ±23.1	289 ^a ±25.2	267 ^a ±29.5
		Mud	Per. inund.	339 ^{ab} ±7.09	291 ^c ±14.0	344 ^b ±15.8	352 ^b ±9.64
			No inund.	339 ^{ab} ±7.09	309 ^a ±17.8	380 ^b ±8.90	340 ^{ab} ±14.8
N	mg kg ⁻¹	Clay	Per. inund.	617 ^a ±27.9	1237 ^b ±181	983 ^{ab} ±183	1338 ^b ±169
			No inund.	617 ^a ±27.9	1000 ^a ±251	887 ^a ±152	1086 ^a ±220
		Mud	Per. inund.	1611 ^a ±14.3	1664 ^a ±145	1455 ^a ±73.8	1817 ^b ±112
			No inund.	1611 ^a ±14.3	1854 ^a ±82.2	1722 ^a ±98.5	1637 ^a ±69.7
Organic C	%	Clay	Per. inund.	2.62 ^a ±0.14	2.64 ^a ±0.45	2.54 ^a ±0.44	3.15 ^a ±0.38
			No inund.	2.62 ^a ±0.14	3.31 ^a ±0.44	3.16 ^a ±0.34	3.53 ^a ±0.36
		Mud	Per. inund.	4.03 ^{ab} ±0.06	4.02 ^a ±0.19	3.76 ^a ±0.15	4.68 ^b ±0.25
			No inund.	4.03 ^{ab} ±0.06	4.89 ^a ±0.29	4.46 ^{ab} ±0.14	3.90 ^b ±0.13

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CHAPTER

5

Consolidation and drainage of soft cohesive sediment with or without *Phragmites australis* as an ecological engineer

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ABSTRACT

Conventional drainage techniques are often used to speed up consolidation of fine sediment. These techniques are relatively expensive, are invasive and often degrade the natural value of the system. This paper focusses on exploring alternative approaches that use natural processes, rather than technological solutions, to speed up the sediment consolidation process. In a controlled column experiment, we studied how *Phragmites australis* can act as an ecological engineer that enhances drainage in soft cohesive sediment, thereby promoting sediment consolidation. We measured the dynamics of pore pressures at 10 cm depth intervals during a 129-day period in a column with and without plants, where the water level was fixed. Water loss via evaporation was measured using Mariotte bottles and the photosynthetic processes – including plant transpiration – were measured with a LICOR photosynthesis system. The results show that several natural processes initiated by *P. australis* interfere with the physical processes involved in sediment drainage and consolidation. *Phragmites australis* effectively altered the pore pressure gradient via water extraction, especially between 17 and 37 cm below the water table. In this zone, daily cycles in pore pressures were observed which could directly be linked to the diurnal cycle of stomatal gas exchange. On average, water loss via evaporation and transpiration of leaves of *P. australis* amounted to 3.9 mm day^{-1} , whereas evaporation of bare soil amounted on average to 0.6 mm day^{-1} . Moreover, the depth-averaged hydraulic conductivity increased on average by 40% in presence of *P. australis*. The results presented in this study provide crucial information needed for predictive modelling of plants as ecological engineers to speed up soil forming processes in constructed wetlands build with soft cohesive sediment.

5.1 INTRODUCTION

Cohesive sediment is progressively being used for land reclamation (e.g., Poplar Island in the Chesapeake Bay, USA; Derrick et al. 2007) and construction of wetlands with soft sediment have become increasingly important worldwide (e.g., Mitsch et al. 1998; Saaltink et al. 2018, Barciela Rial et al. to be submitted). Conventional drainage is often used to speed up the consolidation process when building with soft cohesive sediments (Humphrey and Holtz 1986). The most common methods to drain these sediments include the installment of prefabricated vertical strip drains, horizontal vacuum drains and sand drains (Holtz 1987; Cognon et al. 1994; Li and Rowe, 2002). Without drainage, the cohesive sediment deposits consolidate primarily via self-weight consolidation (e.g., Gibson et al. 1967; Been and Sills 1981; Winterwerp and van Kesteren 2004). Artificial drainage is necessary because of the low bearing capacity and the low hydraulic conductivity of soft cohesive sediments. Hence, drainage is important when building with soft sediment as drainage effectively increases the erosion threshold, vane strength, and overall stability (Fagherazzi and Furbish 2001; Chen et al. 2012). However, conventional drainage techniques are relatively expensive, are invasive and may degrade the natural value of the system. Our current research focusses on exploring alternative approaches which use natural processes, rather than technological solutions, to speed up the sediment consolidation process. We refer to the concept of ecological engineering, which aims to fit environmental technology with ecosystem services (Odum and Odum 2003). This approach foresees the use of ecological engineers that speed up processes like sediment stability, soil formation, consolidation, and soil drainage (Jones et al. 1994).

Plants are excellent examples of ecological engineers as they directly interact with the physical and chemical components in the sediment (Angers and Caron 1998; Ehrenfeld et al. 2005). In cohesive sediments, plants are known to 1) increase the erosion threshold via roots and thereby stabilize the sediment (Waldron and Dakessian 1982; Friend et al. 2003; Reubens et al. 2007), 2) promote soil formation by oxidizing the sediment and by altering and initiating biogeochemical processes (Visser et al. 2000; Saaltink et al. 2016), 3) compress clay particles in the vicinity of roots, which promotes consolidation (Doriotz et al. 1993), and 4) change the soil hydraulic properties and soil moisture content by modifying the soil pore configuration (Angers

and Caron 1998; Kodešová et al. 2006; Gerke and Kuchenbuch 2007). Therefore, plant roots can be considered as a cost-effective alternative to conventional vertical drainage to speed up sediment consolidation and stability.

During vegetative development, the increase in transpirational water loss is sustained by an increase in water uptake by roots, which is mainly done by increasing the root surface area (Suku et al. 2014). As roots elongate, the zone in soils where water is most actively being taken up, changes as roots are more porous near their tips (Sanderson, 1983; Zwieniecki et al. 2003). Hence, the part of the sediment that is drained by plant roots is expected to change over time (Gerke and Kuchenbuch 2007). The soil response to suction induced by vegetation has been reported in many field studies (e.g., Liam et al. 1996; Smethurst et al. 2006; Leung 2015).

Although in principle the hydraulic function of a plant root resembles that of a porous pipe (Zwieniecki et al. 2003), little is known about the potential effect of living plant roots on the consolidation process in soft cohesive sediments, especially due to the nonlinear behavior of water distribution during vegetative development. A better understanding of how and to what extent plant roots drain cohesive sediments is essential to successfully deploy plants as eco-engineers when soft cohesive sediment is used for constructing wetlands.

In the Netherlands, a project started where soft cohesive sediment is used to construct a large wetland in Lake Markermeer. This should become a dynamic and biodiverse wetland system of 10,000 ha, while the concept of ecological engineering is used to speed up ecosystem development. To combat soil erosion and speed up ecosystem development, *Phragmites australis* (common reed) will be used as the ecological engineer to enhance the consolidation process and to improve sediment stability.

In controlled column experiments, we studied in a mechanistic way how and to what extent *P. australis* can increase drainage in soft cohesive sediment, thereby promoting consolidation: vegetated columns were deployed as well as a control column without vegetation. This study isolates the effects of plant roots thereby enhancing the understanding of the important plant-soil interactions in terms of consolidation by means of vertical drainage. The suitability of this species as an ecological engineer to speed up the consolidation process on newly constructed

wetland can be assessed accordingly and be applied to wetland construction with soft material worldwide.

5.2 MATERIAL AND METHODS

5.2.1 *Experimental set-up*

Consolidation experiments were conducted in perspex (methyl methacrylate) columns (inner diameter 10 cm, height 120 cm) in the fluid mechanics laboratory of Delft University in the period November 2016 – August 2017. To allow the control of boundary conditions, a hollow stainless-steel pipe (outer diameter 2 cm, inner diameter 1 cm) was fixed in the middle of each perspex column (see also Barciela Rial et al., 2015). This stainless-steel pipe contained Vyon 3.2D filters in its wall to control the water table at 77 cm above the column base while preventing sediment leaking into the pipe. We refer to this pipe as a drainage pipe. To induce the constant water table at the desired level in the perspex columns, the drainage pipe was connected to another column which contained Markermeer water at a fixed level of 77 cm. Water flowing from such a water column to the drainage pipe (i.e., because of water loss via plant transpiration and/or evaporation) was replenished from a Mariotte bottle containing Markermeer water. Figure 5.1 presents a sketch of this setup.

In total, six perspex columns were used in two experimental series (Table 5.1). Four columns with plants were harvested in experimental run 1 to determine root and shoot variables, while two columns were left intact and used for pore pressure measurements in experimental run 1 and 2. One of the two columns for pore pressure measurements was left unplanted (control column), the other column was planted with reed (vegetated column). Experimental run 1 was conducted in the laboratory hall from November 2016 till February 2017, with temperatures fluctuating between 15 °C and 20 °C, averaging at 17.3 °C. The average relative humidity was 72%, fluctuating between 50% and 80%. Because the pressure sensors are very sensitive to small changes in temperature, and because marginal changes in the water table were recorded, it was decided to repeat the experiment with only the sensor columns. The root and shoot parameters obtained in experimental run 1 were used for further analysis. Experimental run 2 took place from March 2017 till August 2017 in the

climate room ($t = 129$ days). The environmental conditions in the climate room were kept constant at the average conditions measured in the laboratory hall. The grow light in both experimental runs was installed at the same height. Because the variation in temperature and humidity in the laboratory hall was marginal, we could link the morphological root and shoot traits measured in the first experimental run to the changes in pore pressure of the sediment in the second experimental run. Hence, the vegetation development data used in this paper was obtained from experimental run 1, and the pressure sensor data was obtained from experimental run 2.

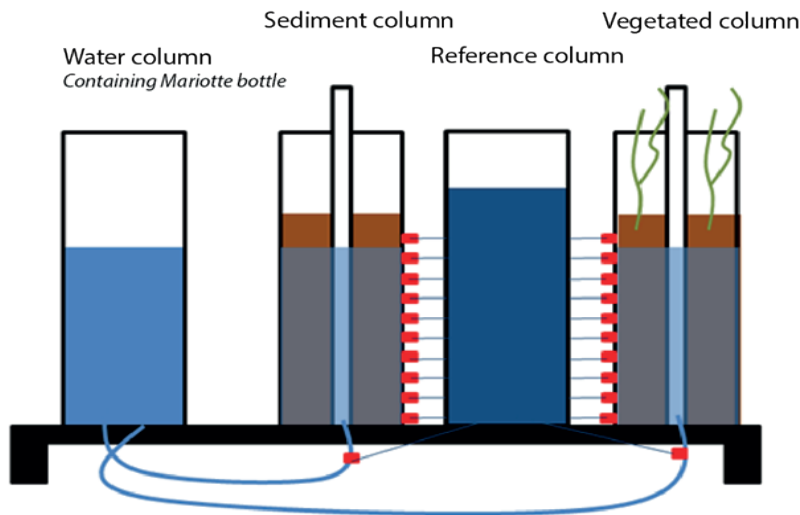


Figure 5.1. Experimental set-up of the columns and location of the sensors.

The columns used for pore pressure measurements were installed with 0.5 Pa Honeywell differential pressure sensors at 0.4, 10.4, 20.4, 30.4, 40.4, 50.4, 60.4, 70.4, and 80.4 cm from the base of the column. All the sensors were provided with a degassing system to avoid inaccuracy induced by air. Furthermore, a filter was installed at each connection point of the sensor to measure pore water pressure, instead of total pressure. To increase the accuracy by measuring differential pressures, all the pressure sensors were connected to a reference column filled with a constant water level, thus measuring relative over-pressures. Negative pore water pressures are

defined as suction. The calibration procedure of the pressure sensors is presented in the Appendix.

Table 5.1. Description of the two experimental runs that are part of this study.

Experimental runs		
<u>Experimental run 1</u>	November 2016 – February 2017	
Condition	<i>Set-up:</i> Laboratory hall; <i>Average temperature:</i> 17.3 °C; <i>Relative humidity:</i> 50-80%; <i>Photon flux density (light):</i> 300 $\mu\text{mol s m}^{-2}$; <i>Water level:</i> fluctuating around 77 cm from the base of the column.	
Column	Variables	Used in this study
1. Harvest column	Shoot: leaf surface area, biomass Root: surface area, length, biomass, rooting depth	Yes
2. Harvest column		Yes
3. Harvest column		Yes
4. Harvest column		Yes
5. Vegetated column	Pore pressure, evapotranspiration (Mariotte bottle), transpiration (Li-Cor)	No
6. Control column	Pore pressure, evaporation (Mariotte bottle).	No
<u>Experimental run 2</u>	March – August 2017	
Condition	<i>Set-up:</i> Climate room; <i>Fixed temperature:</i> 17.3 °C; <i>Relative humidity:</i> 50-80%; <i>Photon flux density (light):</i> 300 $\mu\text{mol s m}^{-2}$; <i>Water level:</i> stabilized at 77 cm from the base of the column.	
Column	Variables	Used in this study
1. Vegetated column	Pore pressure, evapotranspiration (Mariotte bottle), transpiration (Li-Cor)	Yes
2. Control column	Pore pressure, evaporation (Mariotte bottle).	Yes

The experimental perspex columns were filled with mud from Lake Markermeer, collected by hydraulic dredging (coordinates 52.54622°N; 5.38783°E). The sediment

was thoroughly mixed before adding it to the columns. The bulk density of the suspension was about 1260 kg m^{-3} , the gravimetric water content was 66.7% (water mass / total mass) and the initial concentration of solids was 425 g l^{-1} . The specific solids density was 2580 kg m^{-3} . The sediment was placed in the columns and remixed. After remixing, the suspension height was 118 cm for all columns.

The sediment could settle and consolidate for 14 days, after which the sediment interfaces were lowered to 92.5 cm for the control column and 92.3 cm for the vegetated column. Because this 2 mm difference between the columns is likely the result of irregularities of the bed surface at the measurement location, the consolidation rates in the two columns were considered the same, thus showing reproducibility of the consolidation experiments. Before the start of the experiment, the pore water that squeezed out during consolidation was removed from above the sediment without disturbing the consolidating sediment (time = 0 days). The bulk density of the sediment at $t=0$ was 1332 kg m^{-3} for both columns.

At $t = 0$ days, three shoots (size 2 cm) of *Phragmites australis* (common reed) were transplanted into the vegetated column and the harvest columns (Table 5.1). A grow light (Spectrabox Gold) with a photon flux density of $300 \mu\text{mol s}^{-2}$ was installed at a height of 123 cm above the sediment interface. The climate room was surrounded by a white film to maximize irradiance from the grow light. A ventilator blew constantly within the area to ensure air circulation.

5.2.2 Data collection

Pore pressure data from the sensors were transferred to a PC by using an analogue-digital converter and stored every second using DasyLab. Unfortunately, some data gaps occurred due to connection problems of the sensors to the computer. From the 129 experimental days, pore pressure data were recorded for 69 days. Data gaps are evenly distributed, as can be seen in Supplementary Figure A5.4. Hence, the quality and the resolution of the data were sufficient to capture temporal changes in pore pressure due to plant transpiration.

From the pore pressure data and the water losses, the hydraulic conductivity (k) in both columns can be calculated. As the horizontal spatial scales are much smaller than the vertical scales, drainage takes place preliminary in the horizontal plane (i.e.,

via the drainage pipe in the middle of the column). Thus, the continuity equation on cylindrical coordinates was solved (Barciela Rial, *in prep*) accounting for radial pore water flow towards the drainage pipe:

$$k(z) = \frac{\rho g Q_0}{2\pi \Delta P(z) H} \left(-\frac{1}{2} + \frac{R^2}{2r_o^2} + \ln\left(\frac{R}{r_o}\right) \right) \quad (1)$$

where k is the hydraulic conductivity in m s^{-1} , ρ is the density of water [kg m^{-3}], g is the acceleration of gravity [m s^{-2}], ΔP is the pressure difference in Pa, Q_0 is the measured flow in $\text{m}^3 \text{s}^{-1}$, R is the radius of the column wall, r_o is the radius of the drainage pipe, and H is the drainage length.

At experimental time $t = 40, 71, 88$, and 102 days, a column was harvested in experimental run 1 to measure root and shoot parameters. Above-ground biomass was cut off, after which the photosynthetic area was determined immediately. Plant tissue was air-dried at 70°C for 48 h to determine its dry weight. The leaf per mass area (LMA) could then be calculated. Samples of 5 cm sediment were serrated from the column, after which the roots were sieved from the sediment. The root surface area and the root length in each sample were determined with SmartRoot in ImageJ (Lobet et al. 2011). The dry weight mass of the roots was determined per sample after drying, following the same procedure as the aboveground biomass.

Plant transpiration and photosynthetic activity were measured on three leaves per plant per column using the Li-Cor portable photosynthesis system (LiCor 6400) at experimental time $t = 41, 61, 81$, and 97 days. Conditions within the Li-Cor chamber were kept constant: the ambient CO_2 concentration was kept at 450 ppm , the temperature in the chamber was set to 17.3°C , the relative humidity was maintained at 60% and the light intensity in the chamber was set to 1500 PAR .

5.2.3 Environmental conditions

Photosynthetic parameters of *P. australis* were determined with the statistical package R (Duursma 2015) to check whether plants remained healthy and were adapted to the low-light conditions in the climate room. As can be seen from Table 5.2, photosynthesis rates are realistic, with a maximum rate of Rubisco carboxylase

activity (V_{cmax}) varying between 115 and 39.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a maximum rate of photosynthetic electron transport (J_{max}) varying between 161 and 72.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Both variables decrease in time, which indicates a decrease in effectiveness when the leaves of *P. australis* mature (i.e. photosynthesis and transpiration decreases per unit leaf area). More detailed information on photosynthetic parameters is presented in Supplementary Figure A5.5 and A5.6. Figure A5.5 shows net photosynthetic rates of *P. australis* versus calculated leaf interior CO_2 concentrations, while Figure A5.6 shows net CO_2 assimilation rates versus light intensity.

Table 5.2. Photosynthetic parameters of *P. australis* at 61, 81 and 97 days. The maximum rate of Rubisco carboxylase activity (V_{cmax}), the maximum rate of photosynthetic electron transport (J_{max}) and the respiration rate (R_d) are shown ($\pm$ S.E.) as well as the light compensation point (Γ^*). All values are in $\mu\text{mol m}^{-2} \text{s}^{-1}$.

	Day 61		Day 81		Day 97	
V_{cmax}	115	± 8.72	59.21	± 3.50	39.8	± 1.20
J_{max}	161	± 6.17	108	± 4.84	72.9	± 1.89
R_d	1.67	± 0.68	3.99	± 0.55	0.46	± 0.18
Γ^*	28.79	-	28.84	-	28.63	-

5.3 RESULTS

5.3.1 Plant development and water loss

Table 5.3 shows that leaf area and leaf biomass increased in the first months to 406 cm^2 and 1.48 g at day 88 after which leaves started to wilt and leaf area and leaf biomass decreased to 263 cm^2 and 1.00 g at the end of the experiment. The plant roots proliferated throughout the column and reached the lowest part at the end of the experiment (84 cm). The length, the area and the biomass of the roots increased with time. Because the plants in the harvest columns did not grow at the same speed, we corrected the root area per depth interval for leaf area as measured right before harvest. The corrected root area as calculated from the four harvests is presented in Figure 5.2a, clearly showing that the root area at each depth interval increased with

time. A peak is observed from the first harvest (40 days) in the top 5 cm (1.65 cm^2 per unit leaf area). This is because plants invest more in their root system than in aboveground biomass after transplantation. At 40 days, root biomass increased to 1.22 g, while leaf biomass increased to only 0.17 g (Table 5.3).

Table 5.3. Plant characteristics at 40, 71, 88 and 102 days as measured from harvested columns. Root length, root area, root biomass, and root volume are expressed per cm^3 column volume.

		40 days	71 days	88 days	102 days
Leaf area	cm^2	48.8	189	406	263
Leaf biomass	gr	0.17	0.67	1.48	1.00
Leaf mass per area (LMA)	g m^2	342	354	365	382
Stem biomass	gr	0.43	1.46	2.13	2.42
Max. rooting depth	cm	18	48	68	80
Root length	cm cm^{-3}	0.26	0.36	0.60	0.59
Root area	$\text{cm}^2 \text{ cm}^{-3}$	0.07	0.18	0.33	0.29
Root biomass	mg cm^{-3}	0.90	0.80	1.30	1.07
Root volume	$\text{mm}^3 \text{ cm}^{-3}$	0.42	5.5	16	15
Shoot:Root ratio		0.49	0.74	0.54	0.53

Evaporation led to water loss in the control column, while both evaporation and plant transpiration led to water loss in the vegetated column. Figure 5.2b presents evaporation rates during the experiment for the control column only. Small variations in time are observed, but all evaporation rates fall in between 0.3 and 0.7 mm day^{-1} , averaging at 0.6 mm day^{-1} . For the vegetated column, it was difficult to distinguish these two mechanisms of water loss from the sediment. Although we are aware that plants alter evaporation to a minor extent via transpiration, we used the average evaporation of 0.6 mm day^{-1} from the control column to calculate evapotranspiration in the vegetated column (i.e., when measuring leaf transpiration, we added 0.6 mm to determine evapotranspiration). The evapotranspiration data are presented in Figure 5.2c. It clearly shows that increasing the leaf area led to a

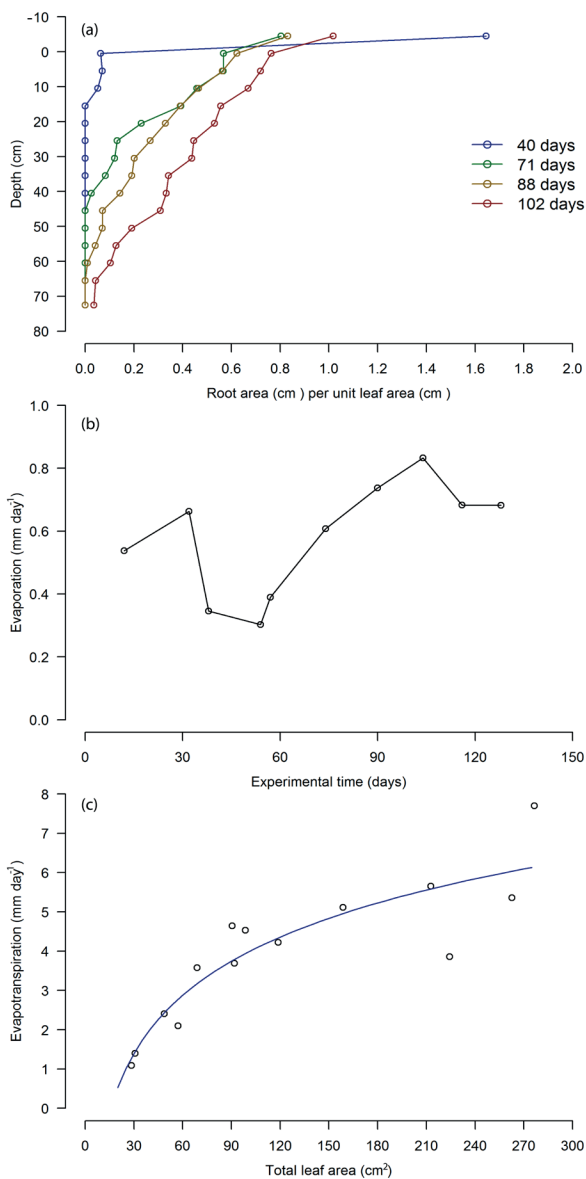


Figure 5.2. Root surface area per unit leaf area (cm²) across depth at four different time steps (a), evaporation rates in the control (mm day⁻¹) (b) and evapotranspiration (mm day⁻¹) as a function of total leaf area (cm²) (c). Evapotranspiration rates measured in experimental run 1 and 2 are combined.

non-linear increase in water loss via evapotranspiration. The lowest measured value of 1.4 mm day^{-1} corresponds to a total leaf area of 31 cm^2 . At a leaf area of 276 cm^2 , the highest evaporation rate was found (7.7 mm day^{-1}). Evapotranspiration rates do not scale linearly with leaf area, as when leaves mature, they become less effective in terms of photosynthetic capacity (Table 5.2). The average evapotranspiration rate of 3.9 mm day^{-1} found in this study closely coincides with the average evapotranspiration value of 3.7 mm day^{-1} measured in reedbeds in the Teesmouth Estuary in England during the growing season (Fermor et al. 2001). Similar rates were measured in the Biebrza wetlands in Poland, averaging between 3.0 and 3.5 in the summer months (Siedlecki et al. 2016).

5.3.2 Sediment height

Figure 5.3 presents the sediment height over time. Both sediment columns had almost identical sediment heights during the experiment, ranging from 92.5 cm at the beginning of the experiment down to 85.1 cm at the end. Since root volume increased up to 16 mm^3 per cm^3 sediment volume in the vegetated column (Table 5.3), the volume once occupied by water is being replaced by roots. Additional drainage by roots did not influence sediment height, as the water table in the experiment was

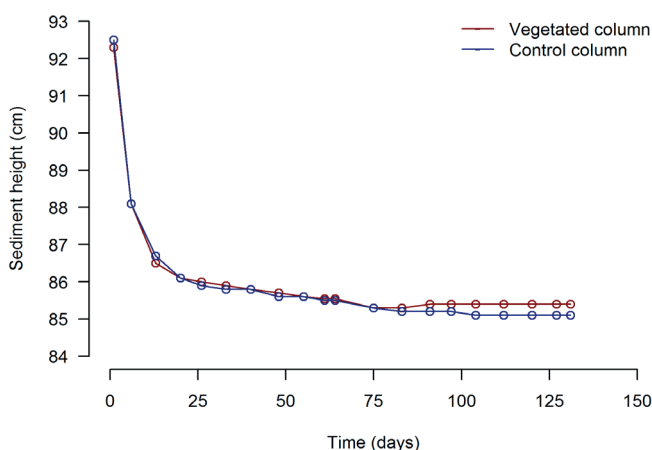


Figure 5.3. Sediment height (cm) during the experiment for the vegetated column (red) and for the control column (blue).

fixed. Thus, no consolidation effect by the vegetation is found (i.e., reduced sediment height compared to the control column), probably because we fixed the water level to 77 cm.

5.3.3 Pore pressure gradients

We selected three phases based on the successive stages of consolidation and drainage in the experiment as well as on the sediment height presented in Figure 5.3. For the first phase, we selected data of the timesteps $t = 0$ and $t = 1$ days, which represents fast initial consolidation. As we lack pore pressure data from $t = 2$ till $t = 11$ days (Figure A5.4), we used pore pressure data of the period $t = 12-40$ days for the second phase. This phase represents slow sediment consolidation with little influence of plant transpiration (i.e., plant roots started to grow but did not have a big impact on pore pressure). After 40 days, the effects of plant transpiration on pore pressure data increased. Therefore, pore pressure data of the period $t = 41-129$ is used for the third phase.

Figure 5.4 shows the measured pore water pressure relative to the water table in the sediment column for all three phases, as well as the excess pore water pressure (i.e., pore water pressure minus hydrostatic pressure) for the control column and vegetated column. For phases 2 and 3, the pore pressure in the control column increases slower with depth compared to the hydrostatic pressure in the first 17 cm, after which it increases down to 67 cm below the water table (Figure 5.4a). From 67 to 77 cm, a small decrease in pressure is observed (pressure decreased from 6.1 kPa at 67 cm to 5.8 kPa at 77 cm at $t = 129$ days). In contrast, the pore pressure increases faster with depth than the hydrostatic pressure down to 7 cm below the water table in phase 3 of the vegetated column (Figure 5.4d). In this phase, the pore pressure decreases remarkably from 1.2 kPa at 7 cm to -0.9 kPa at 27 cm, peaking at -1.7 kPa at $t = 74$ days. This reduction in pore pressure is likely caused by water uptake by plant roots. This is because total root area increased through time (Table 5.3), thereby increasing water uptake from the sediment.

An excess in pore pressure is observed in phases 2 and 3 down to 17 cm below the water table in the control column, where the excess in pore pressure above the water table is caused by capillary rise (Figure 5.4b). The pore pressure becomes lower

than the hydrostatic pressure at larger depth. Hence, from a depth larger than 17 cm, suction occurs and water from the drainage pipe flows into the sediment. Because of the sharp reduction in pore pressure in the vegetated column between 17 and 37 cm below the water table, high negative excess pressures are observed, averaging at -3.4 kPa relative to the hydrostatic pressure in phase 3 (Figure 5.4e). At the top (0-12 cm), the pore pressures are larger than the hydrostatic pressure and a positive excess pore water pressure exists, suggesting that at the less consolidated top layer of the sediment, self-weight consolidation is still the dominant process.

These results show that plants altered pore pressure especially between 17 and 37 cm below the water table by water extraction via roots. The negative pore pressures at these depths suggest that suction of water is an important process during consolidation in presence of plants. A twofold negative excess pore water pressure was observed in presence of plants compared to the control column. The pore water pressure profile is led by evapotranspiration and not by self-weight consolidation: to transport sufficient water to the roots, the excess pore pressure decreased at the height of the active root parts.

5.3.4 *Daily cycles in pore pressure*

Water is taken up by plant roots to compensate for water loss via leaf transpiration. Plants transpire especially during photosynthesis, when stomata are open for gas exchange. Hence, it is expected that pore pressures within the sediment follow a daily cycle in the presence of plant roots. Figure 5.5 shows pore pressures during a 6-day period. In the control column, no large difference in pore pressure is observed between day and night (Figure 5.5a). However, large variation is observed in the vegetated column, especially between 27 and 37 cm below the water table (Figure 5.5b). These results suggest that during the day plants effectively lower pore water pressure at the point where the roots are extracting most of the water (27 cm below the water table). During the night, the pore water pressures increased relative to day-time. This suggests that the dominant flow of water at night occurs from the drainage pipe into the sediment to compensate for the water losses during the day. A reverse cycle is visible in the vegetated column at 7 cm below the water table,

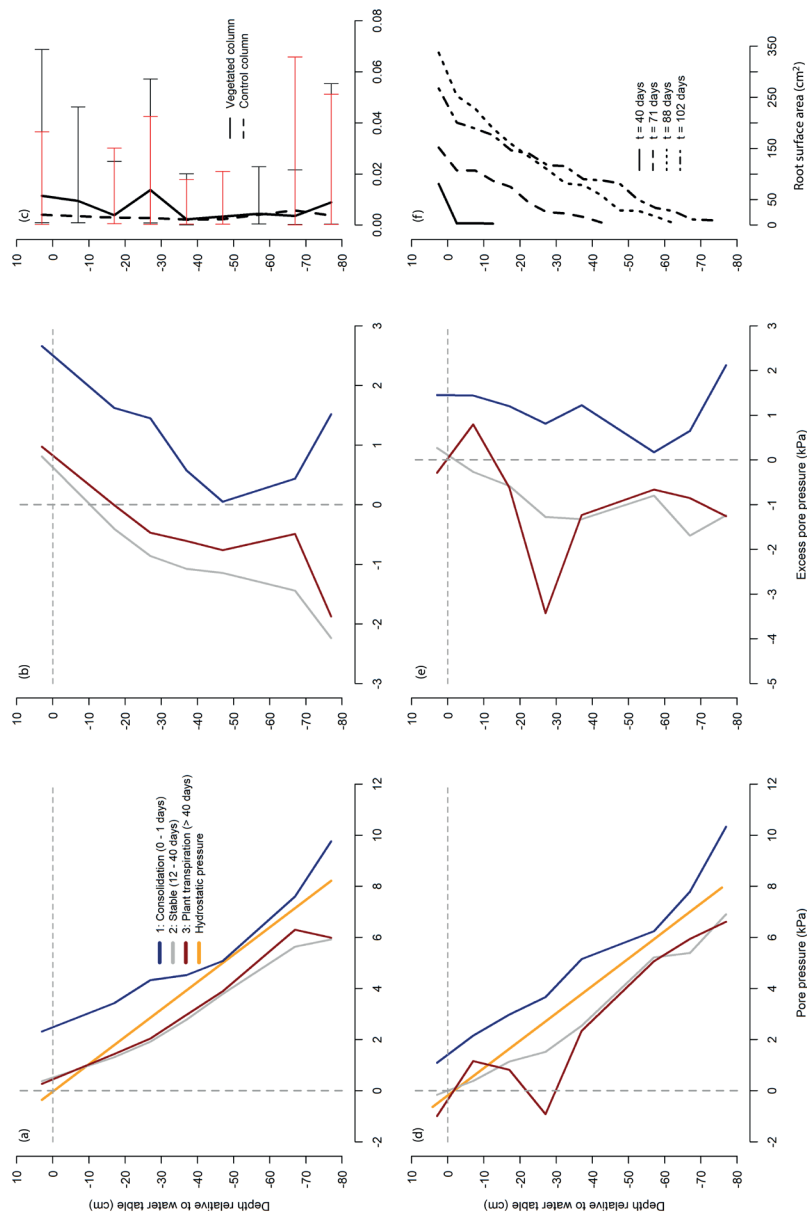


Figure 4. Pore pressure (kPa) relative to the water column in the control column (a) and vegetated column (d) as well as excess pore pressure (pore pressure minus hydrostatic pressure) in the control column (b) and the vegetated column (e). Pore pressures per depth are averaged for each phase. Average errors (kPa) for each pressure sensor are shown ($\pm$ the minimum and maximum error during the experiment) for the control column and vegetated column (c). The errors include the accuracy of the sensors (6.9 E⁻³ kPa) and the measured hourly variations (red is for the control column and black is for the vegetated column). Root surface area is presented from experiment 1 (f). Note that these leaf surface areas are from four individual plants ($n = 1$).

indicating that during the night, water flow from the drainage pipe decreased pressure values, likely because of a decreased water table due to plant drainage during the day.

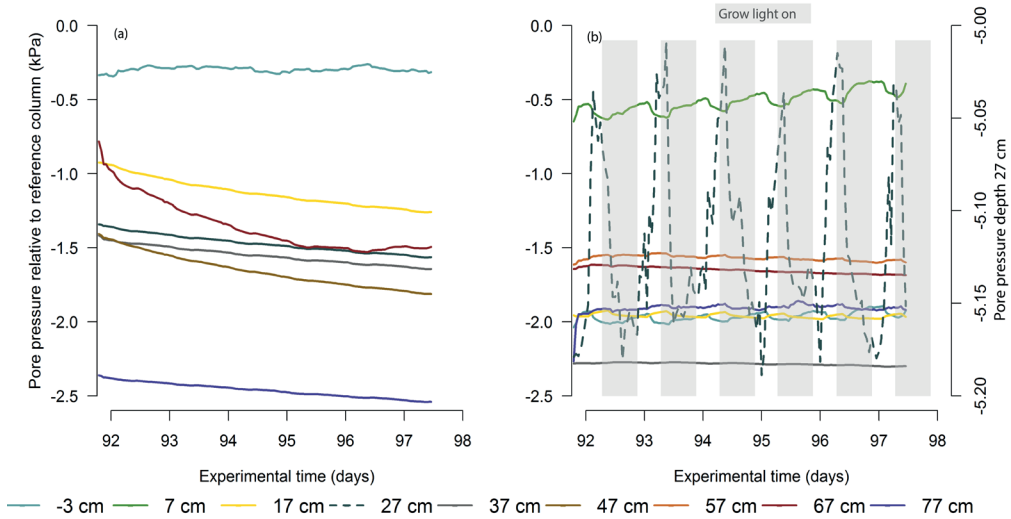


Figure 5.5. Hourly time series ($t = 92$ – 98 days) of pore pressure for the control column (a) and vegetated column (b).

5.3.5 Hydraulic conductivity

The measured water fluxes for the experimental columns are presented in Figure 5.6 and were used for calculating the hydraulic conductivity using Equation (1). The theoretical evaporation rate of 0.6 mm day^{-1} is defined as the average of the evaporation rates presented in Figure 5.2b. The vegetated column reached the evaporation rate after 30 days, after which the water flux increased via plant transpiration up to 6.2 mm day^{-1} . The flow in the vegetated column decreased at the end of the experiment due to maturing of the leaves of *P. australis* (Table 5.2).

Figure 5.7 shows the calculated hydraulic conductivity profiles of the vegetated column and the planted column. In supplementary Figure A5.7, the depth-averaged hydraulic conductivity of the control column and vegetated column for the duration of the experiment are presented. In the first two days of the experiment (phase 1), the hydraulic conductivity started relatively high

on average ($5.5 \times 10^{-8} \text{ m s}^{-1}$ for the vegetated column and $3.3 \times 10^{-8} \text{ m s}^{-1}$ for the control column). The hydraulic conductivity rapidly decreased due to the vertical drainage of water caused by self-weight consolidation in phase 2 to $0.8 \times 10^{-10} \text{ m s}^{-1}$ on average in the control column and to $7.0 \times 10^{-10} \text{ m s}^{-1}$ on average in the vegetated column. This is in line with Figure 5.3, that shows that sediment height in both columns is declining rapidly in the first 15 days. The difference in the initial hydraulic conductivities between the control column and the vegetated column might be caused by small disturbances induced when transplanting the reed seedlings at $t = 0$ days. The hydraulic conductivities in both columns stabilized on average to $2.0 \times 10^{-9} \text{ m s}^{-1}$ in the control column and $8.1 \times 10^{-9} \text{ m s}^{-1}$ in the vegetated column. In phase 3, the hydraulic conductivity in the vegetated

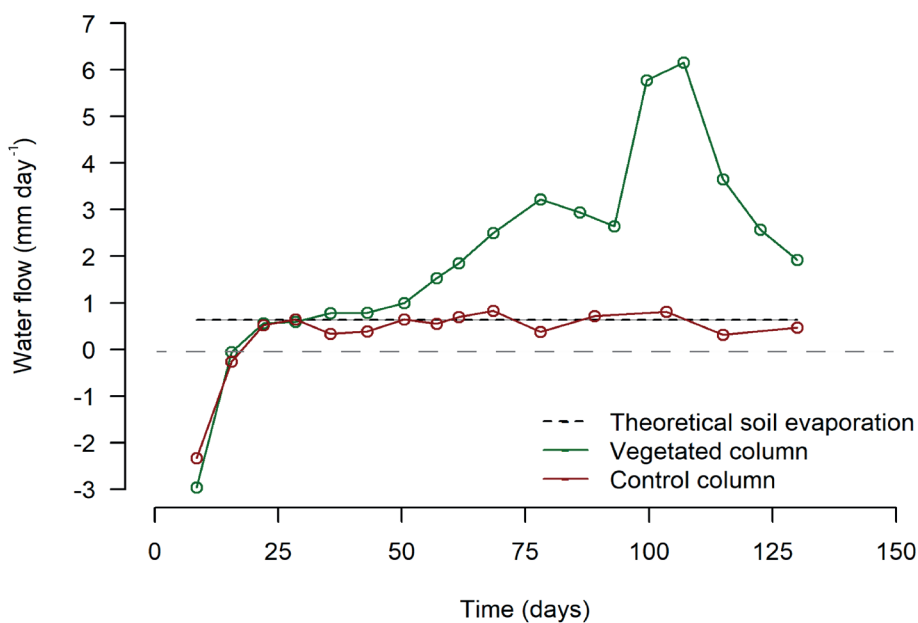


Figure 5.6. Water flows measured during the experiment for the control column (consolidation and evaporation) and the vegetated column (consolidation, evaporation and plant transpiration) compared to the theoretical soil evaporation rate. Negative values indicate consolidation (dewatering via the drainage pipe) and positive values indicate evaporation (and transpiration as well).

column diverged from the control column averaging at $1.2 \times 10^{-9} \text{ m s}^{-1}$, while the hydraulic conductivity in the control column averaged at $0.8 \times 10^{-9} \text{ m s}^{-1}$. Thus, in the phase when plants became active, the hydraulic conductivity increased with a factor 1.4 compared to the control column due to enhanced drainage via transpiration.

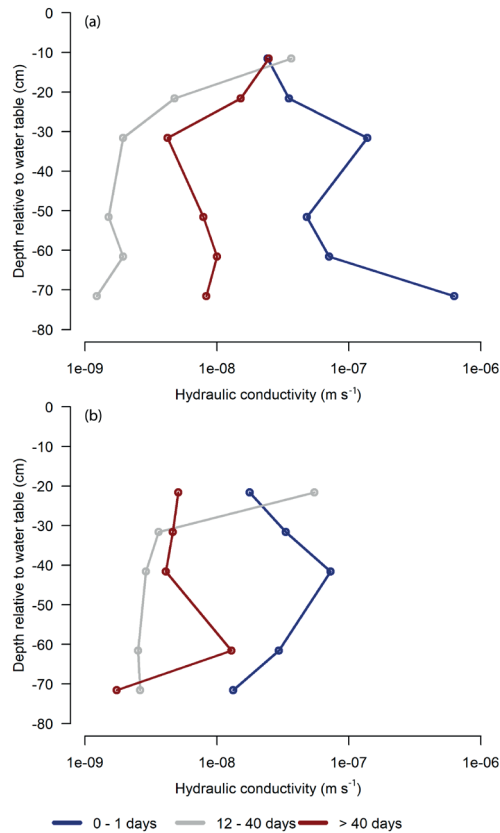


Figure 5.7. Conductivity profiles (m s^{-1}) for the vegetated column (a) and the control column (b). Profiles are averaged for three different time phases: 1) fast consolidation phase (0-1 days), 2) stable phase (12-40 days), and 3) plant transpiration phase (> 40 days).

5.4 DISCUSSION

5.4.1 Altered pore pressure gradients

The results of this study showed that *P. australis* effectively alters the pore pressure gradient in soft cohesive sediments. After self-weight consolidation (i.e., positive excess pore water pressures), pore pressure dropped below the hydrostatic pressure. This occurred in both the control column and the vegetated column. For the control column, this indicates that the water flux induced by evaporation is higher than the self-weight consolidation flux. The shape of the pressure depth profile (Figure 5.4) is comparable with typical profiles of bare silty soils (e.g., Blight 2003). For the vegetated column, the sharp drop in pore pressure between 17 and 37 cm below the water table suggests that the water flux induced by evapotranspiration is higher than evaporation alone. In the soil layer where plant roots extracted water, we found pressures up to four times higher than in the control column. Similar impacts of plants have been found by Leung et al. (2014, 2015). They showed that the air entry value (i.e., the pressure point after which air recedes into the soil pores) increased four times in presence of Ivy trees (*Schefflera heptaphylla*) compared to bare soil.

The part in the column where roots extracted the water did not shift downwards during the experiment: pore pressure was reduced remarkably between 17 and 37 cm below the water table. This was unexpected as roots of *P. australis* penetrated deeper sediment layers in time and water uptake is largely restricted to the region near the root tip (Kramer and Boyer 1995). The fact that pore pressure below 37 cm was relatively unaffected even though root area increased in deeper sediment layers (Figure 5.2a), suggests that the changing sediment physical properties were limiting water extraction to a depth of 17-37 cm below the water table. According to Zhuang et al. (2001), root hydraulic characteristics co-determine where water is taken up and this depends on the pattern by which the different parts of the root contribute to the overall water transport. These root characteristics were not measured in this experiment and it is, therefore, hard to explain why the part where water was extracted did not shift downwards in the column through time. Because of the daily cycles present at 27 cm below the water table, we are confident to link the observed reduction in pore pressure at this depth to water loss by root extraction (Figure 5.5b). Moreover, we measured an average water loss via evapotranspiration of 3.9 mm day^{-1}

in the vegetated column, whereas water loss via evaporation in the control column amounted on average to 0.6 mm day^{-1} . Although pore pressure was restoring during the night, the reduction in pore pressure during the day was larger than the increase during the night. This – together with the fact that root area kept on increasing in the zone of water extraction – explains why pore pressures decreased with time. During the night, the effect of recovery of the water table is observed at 7 cm below the water table in the vegetated column (Figure 5.5b). Water flow from the drainage pipe decreased suction values at 7 cm during the night because of a decreased water table due to plant drainage during the day.

5.4.2 Increased hydraulic conductivity

The results of this study showed that *P. australis* increased the hydraulic conductivity in the sediment by 40% compared to bare soil. An important mechanism by which plants increase the permeability in sediments involves the development of a macropore system, also called rhizopores, of which the main driver is root growth (Ghestem et al. 2011). In our case, these macropores represent live or decaying roots of *P. australis* (i.e., root channels). Especially in cohesive sediments, these root channels are the dominant flow paths of water (Perillo et al. 1999) and can contribute to 70-100% of total macropore space in the top 8 cm of the sediment (Noguchi et al., 1997; Newman et al., 2004). However, already a low fraction of macropores of total porosity increase the flux density of saturated soil (Beven and Germann, 1982). This is especially relevant in constructed wetlands where fast initial consolidation is important. In our experiment, we found that the hydraulic conductivity increased only to a limited extent, despite the increasing root area. Similar observations were reported by Vergani and Graf (2016), who observed stagnation in the increase of sediment permeability due to root proliferation when root length densities approached 0.1 cm cm^{-3} . This can be explained by two opposing processes taking place when roots proliferate in the sediment: 1) the contact area of water in root channels increases with increasing root density; at low root densities this accelerates water flow through the soil, and 2) the film thickness of water inside the root channels is decreasing with increasing root densities, decelerating water flow (Lange et al. 2009). Hence, a stagnant point is reached when the film thickness of the water becomes too thin to promote water

flow. Another reason is that photosynthesis and transpiration decrease per unit leaf area as leaves mature. This was also found for leaves of *P. australis* in our experiment (Table 5.2). The observed stagnation of the increase in hydraulic conductivity is, therefore, likely caused by a combination of a reduced photosynthetic capacity of the leaves and a reduction in film thickness.

Another important mechanism by which plants increase the hydraulic conductivity in sediments is via improved aggregate stability of the sediment. Both root exudation and particle enmeshment by roots result in physical and chemical interactions influencing the aggregation process (Bronick and Lal 2005). Overall, this process increases sediment porosity which promotes overall permeability of the sediment. Like the development of macropores, this mechanism also increases with increasing root density (Rillig et al. 2002). However, this mechanism is only relevant when root densities are higher than 2 cm cm^{-3} (Vergani and Graf 2016). In our experiment, root densities did not pass 0.60 cm cm^{-3} over the rooting length of 80 cm at $t = 102$ days (Table 5.3), suggesting that soil aggregate stability did not enhance the hydraulic conductivity of the sediment.

5.4.3 Comparison with field conditions and upscaling

The photosynthetic parameters measured during the experiment showed that *P. australis* behaved as expected from field conditions; the leaves were optimized to the low-light conditions in the experimental facility. Hence, the set-up of the experiment did not affect stomatal gas exchange and data from this experiment can be translated to field conditions. The average evapotranspiration of 3.9 mm day^{-1} indeed closely coincides with average evapotranspiration rates found in wetlands (e.g., Fermor et al. 2001; Siedlecki et al. 2016). Therefore, the data acquired from this experiment can be used to model the speed of drainage and consolidation in constructed wetlands build with soft, clay-rich material. From this model, the difference between mudflats transplanted with and without *P. australis* should become immediately visible. However, some complex variables were not taken into account in our experiment that will influence drainage and consolidation behavior in the field. These include the topography and the depth of the water table, which will probably be highly heterogeneous. Moreover, if vegetation develops in patches this will also

result in non-uniform plant–soil interactions. Furthermore, the higher the actual evapotranspiration of the plant species, the faster the suction recovery after a rainfall event for the same root biomass (Gaerg et al. 2015). Apart from the drainage effect, vegetation also induces biogeochemical processes (Saaltink et al. 2016), which induce pedogenic processes that accelerate the maturation or ripening of the soil (e.g. Pons and Zonneveld 1965; Barciela Rial et al. 2018). Despite these complexities, upscaling the presented results in a predictive plant–soil model will provide useful insights for the implementation of ecological engineers, such as *P. australis*, to speed up soil forming processes.

5.5 CONCLUSIONS

The results presented in this study identified how ecological engineers interfere with the physical processes involved in sediment drainage and consolidation. *Phragmites australis* effectively altered the pore pressure gradient in the soft, clay-rich sediment. In our experimental set-up, this is the case for the first 44 cm of the sediment (37 cm below the water table). In this zone, daily cycles in pore pressures were observed which could directly be linked to the diurnal cycle of stomatal gas exchange. On average, water loss via evaporation and transpiration of leaves of *P. australis* amounted to 3.9 mm day⁻¹, whereas evaporation of bare soil amounted on average to 0.6 mm day⁻¹. Moreover, the depth-averaged hydraulic conductivity increased on average by 40% in presence of *P. australis*. These findings highlight the feature of this plant to act as an eco-engineer to fasten drainage, in turn promoting consolidation and ripening. The data acquired from this experiment provide crucial information needed for predictive modelling of plants as ecological engineers to speed up soil forming processes in the newly constructed wetlands in the Netherlands and elsewhere.

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APPENDIX

Calibration procedure

Before the start of the experiment, all pore pressure sensors were thoroughly calibrated. The reference column and the two sediment columns were filled with water up to 88 cm prior to calibration (Figure A5.1). At this point, the relative pressure difference between each sediment column and the reference water column is zero and the output of all pore pressure sensors was set to 0.000 mV. Then, the water level in the reference column was increased/decreased with increments of 2 cm, while the water level in both sediment columns stayed at 88 cm. The output in mV was recorded at five relative water levels: -2 cm, 2 cm, 4 cm, 6 cm, and 8 cm (Figure A5.1). Calibration curves were determined for each pore pressure sensor. Figure 2 and 3 present these calibration curves for the control column and the vegetated column respectively.

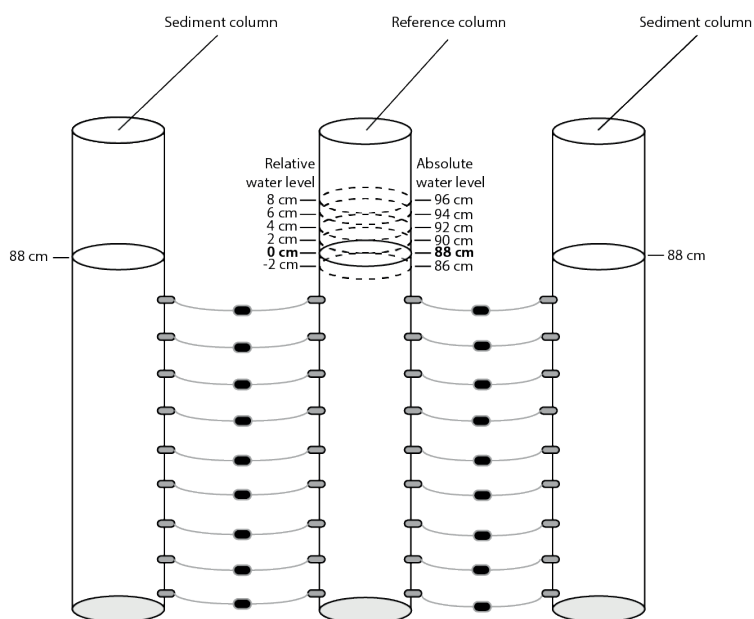


Figure A5.1. Schematic overview of the calibration set-up. All pore pressure sensors are calibrated based on six different water levels relative to the water level in the sediment column: -2, 0, 2, 4, 6, and 8 cm.

When converting the output of the pore pressure sensors in mV to kPa, we assumed a water density of $998.774 \text{ kg m}^{-3}$, corresponding to the constant lab temperature of 17.4°C . Furthermore, we worked with a gravity acceleration of 9.8125 m s^{-2} , corresponding to the latitude of the location of the laboratory (i.e., 52°N). This means that 0.01 m of water results in a pressure increase of $0.01 \text{ m} \times 998.774 \text{ kg m}^{-3} \times 9.8125 \text{ m s}^{-2} = 0.098004 \text{ kPa}$.

Using the equations of the calibration lines in Figure A5.2 and A5.3, we calculated the relative difference in pressure between the reference column and the sediment column for each pore pressure sensor: $\text{pressure (kPa)} = 0.098004 (a \text{ mV} + b - 87 \text{ cm})$. Because the water level in the reference column was fixed at 87 cm during the experiment, changes in kPa are directly related to changes in pore pressure in the sediment columns.

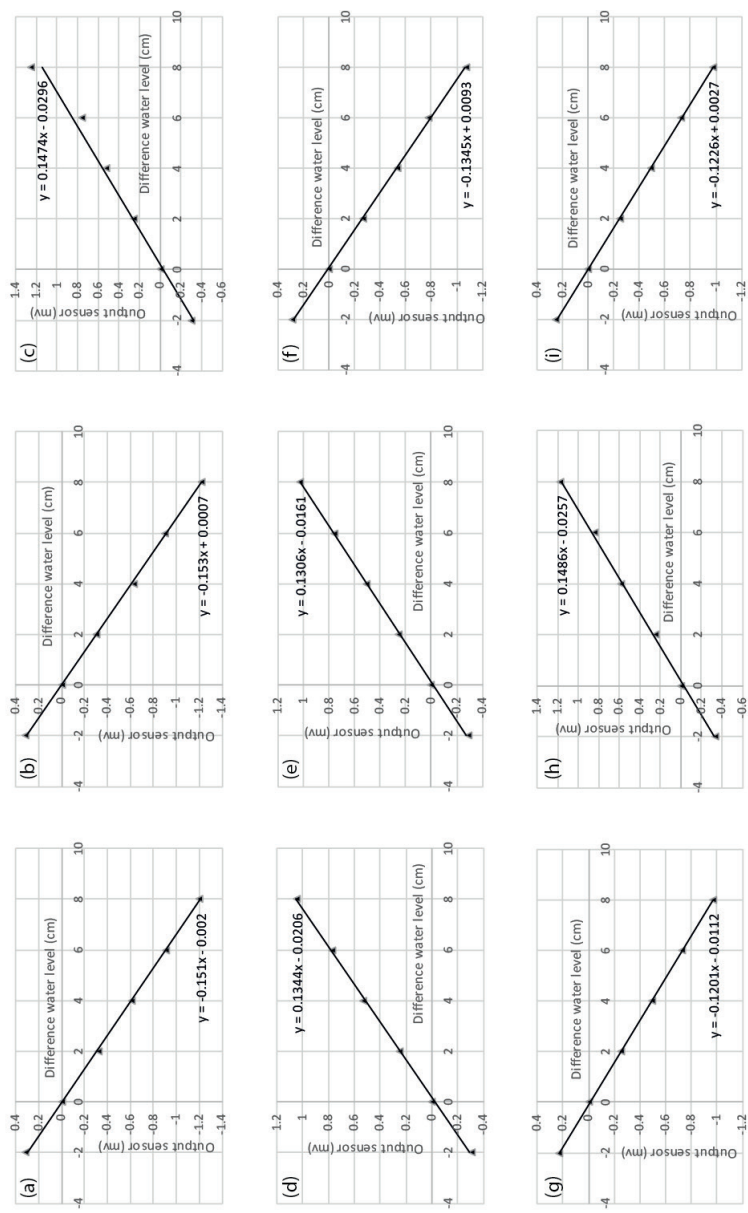


Figure A5.2. Calibration lines of all pore pressure sensors attached between the reference column and the control column: 4 cm depth (a), 14 cm depth (b), 24 cm depth (c), 34 cm depth (d), 44 cm depth (e), 54 cm depth (f), 64 cm depth (g), 74 cm depth (h), and 84 cm depth (i).

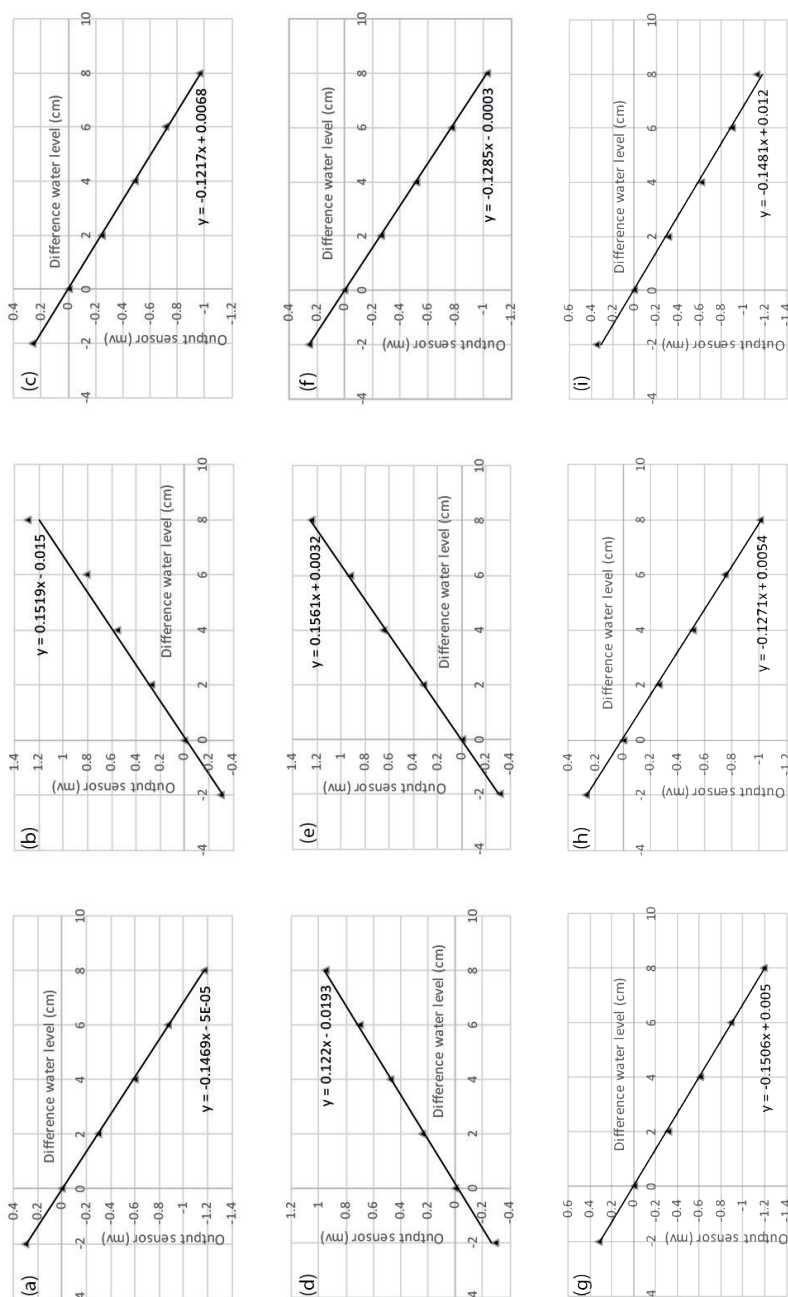


Figure A5-3. Calibration lines of all pore pressure sensors attached between the reference column and the vegetated column: 4 cm depth (a), 14 cm depth (b), 24 cm depth (c), 34 cm depth (d), 44 cm depth (e), 54 cm depth (f), 64 cm depth (g), 74 cm depth (h), and 84 cm depth (i).

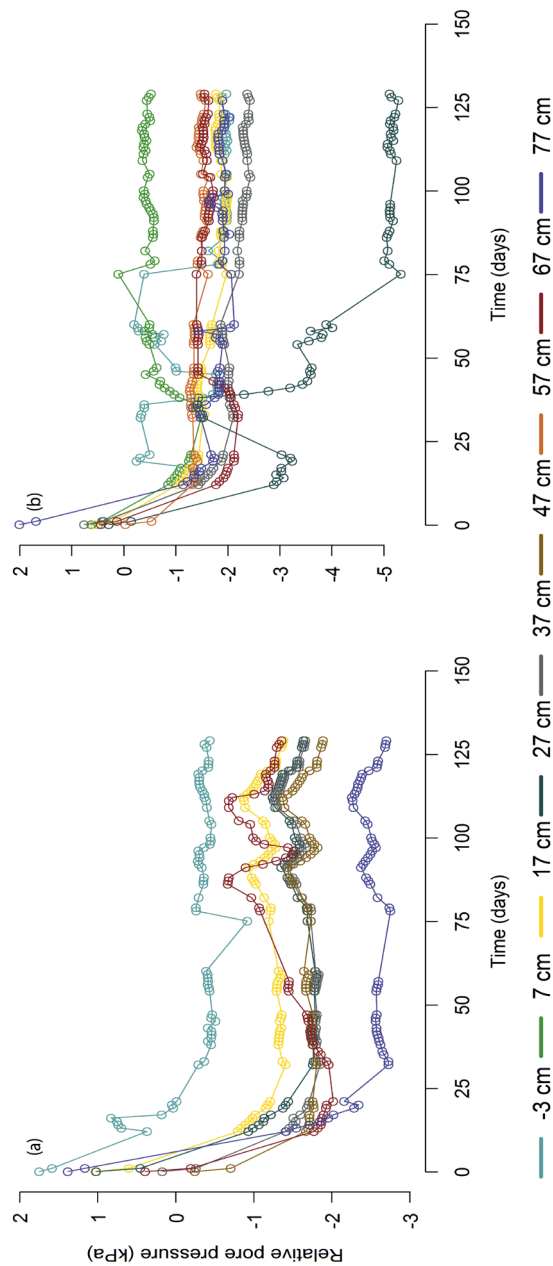


Figure A5.4. Relative pore pressure (kPa) relative to reference column at all depths of the control column (a) and vegetated column (b) for the duration of the experiment.

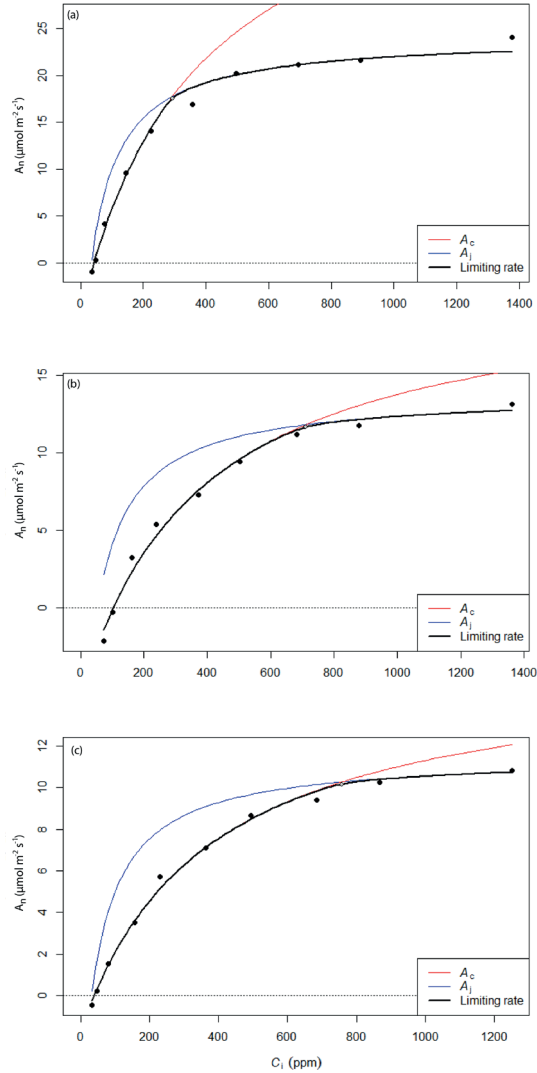


Figure A5.5. Net photosynthetic rates of *P. australis* (A_n) versus calculated leaf interior CO_2 concentrations (C_i) (ppm) at experimental time $t = 61$ days (a), $t = 81$ days (b) and $t = 97$ days (c). The black line represents the fitted model of photosynthesis based on the rate of Rubisco carboxylase activity (A_c) and the rate of photosynthetic electron transport (A_j). Black dots are actual measurements.

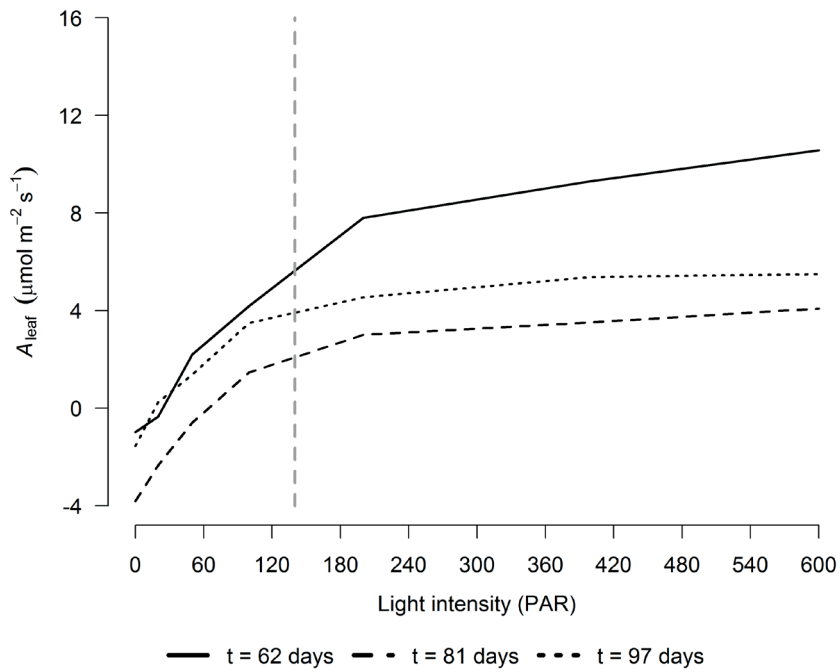


Figure A5.6. Net CO₂ assimilation rates ($\mu\text{mol m}^{-2} \text{s}^{-1}$) versus light intensity (PAR) at 61, 81 and 97 after the start of the experiment. The vertical grey dotted line represents the light condition during the experiment (140 PAR).

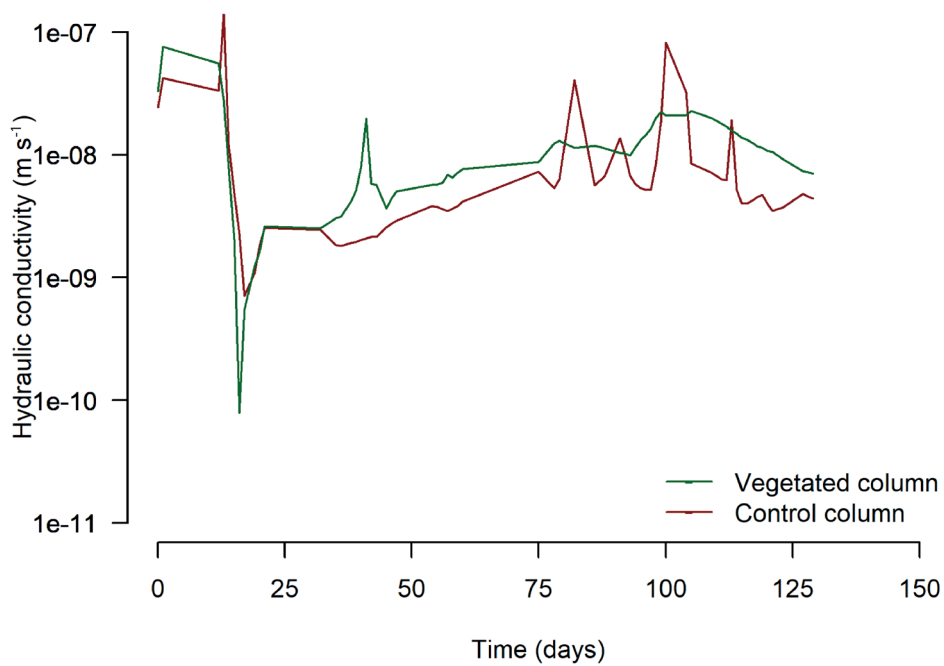


Figure A5.7. Depth-averaged conductivity (m s^{-1}) for the control column (red) and the vegetated column (green).

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CHAPTER

6

Respiration and aeration by bioturbating Tubificidae alter biogeochemical processes in aquatic sediment

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ABSTRACT

This study investigates the potential of bioturbating Tubificidae to alter biogeochemical processes by sediment aeration in order to enhance ecosystem development in eco-engineering projects. We introduced Tubificidae in three different densities in clay-rich sediment from lake Markermeer (The Netherlands): 5,000, 15,000 and 30,000 worms m^{-2} . Redox potential, nutrients and major elements were measured from the water column and porewater at different depths. Phase and redox transfers were chemically modelled and oxygen concentrations in bioturbated sediments for each density were mathematically predicted. The measured results of this experiment showed that Tubificidae oxygenated the upper 15 mm of the sediment. This resulted in decomposition of sedimentary organic matter with an associated six-fold increase in NH_4 and NO_x concentrations in the porewater and the water column. However, phosphorus concentrations were declining in the upper 16 mm, likely as a result of immobilization by pyrite oxidation and production of iron oxides. These bioturbation effects were highest in the treatment with an intermediate density of Tubificidae (15,000 worms m^{-2}) as aeration effects in the treatment with the highest density of Tubificidae (30,000 worms m^{-2}) was impeded by high respiration rates. Furthermore, with a two dimensional diffusion model, simulated effects of respiration and aeration on the oxygen concentration in the sediment suggest that the bioturbation effect is strongest at a density of 12,000 worms m^{-2} . In ecological engineering projects where fast ecosystem development is important, introducing Tubificidae to aquatic sediments to optimal densities might enhance initial ecosystem development due to improved availability of nitrogen as nutrient.

6.1 INTRODUCTION

Bioturbation in aquatic sediments play an important role in regulating geochemical cycling in lakes, rivers and estuaries (Mermillod-Blondin and Rosenberg 2006; Volkenborn et al. 2007). For example, processes like denitrification, nitrification and iron-sulfur cycling are governed by bioturbators occupying many lake sediments (e.g., Svensson and Leonardson 1996; Svensson et al. 2001; Lagauzère et al. 2011). Moreover, bioturbation also affects sediment porosity and shear strength, which enhance resuspension of sediment particles (Krantzberg 1985; Sanford 2006). Thus, enhanced turbidity levels in lakes can be associated with bioturbation activity in the sediment (Krantzberg 1985; De Lucas Pardo et al. 2013).

The bed sediment of lake Markermeer – a shallow artificial lake of c. 700 km² in the Netherlands – is occupied by bioturbating macroinvertebrates (Van Riel et al. 2018). These bioturbators formed the soft clay-rich layer that is now covering the bottom of the lake and may resuspend as a result of wind and wave action (Van Kessel et al. 2008; Vijverberg et al. 2011; De Lucas Pardo et al. 2013). The upper few millimeters to centimeters of this layer are in a dynamic equilibrium with the water column, unfavorably affecting the lakes turbidity. Furthermore, a decrease in phosphorus and nitrogen availability reduces primary productivity and adds to a decline in biodiversity (Noordhuis et al. 2014). To improve the ecological conditions in the lake, an innovative project was initiated in which part of the soft clay-rich bed will be dredged and used as building material for approximately 10,000 ha of new wetland within the lake (currently under construction). However, lake Markermeer is nutrient limited with low values of nitrate, ammonium and phosphate in the water column (Noordhuis et al. 2014; Ministry of Infrastructure and the Environment 2016). Hence, ecological development in the littoral zone of these wetlands may be hampered due to limited availability of nutrients.

To minimize total expenditure when building these wetlands, ecological processes will be used to speed up ecosystem development. This method is called ecological engineering and aims to use environmental technology that is tuned to ecosystem services (Mitsch 1998; Odum and Odum 2003; Temmerman et al. 2013). Because the building material (i.e., the soft clay-rich layer) is rich in nitrogen and iron-bound phosphorus (Saaltink et al. 2017), aquatic macroinvertebrates might be introduced to

these sediments to actively alter processes in the sediment-water interface and make nutrients available to stimulate plant growth. For example, filter feeders, like *Dreissena burgensis* and *Dreissena polymorpha*, are known to increase phosphorus levels in the water column by mobilizing phosphorus from sediment particles (Turner 2010; Ruginis et al. 2014).

Introducing bioturbating invertebrates to sediments can also be a fruitful method to alter nutrient conditions in the soil and water column (Hansen and Kristensen 1997). The soft clay-rich layer in Markermeer contains about 12,800 invertebrates m^{-2} , of which the Annelida take in about 40% (c. 5,000 individuals m^{-2}) (Van Riel et al. 2018). From these annelids, c. 3,900 individuals m^{-2} belong to the subclass Oligochaeta. Bioturbation implies the physical displacement of particles and water by macrofaunal reworking and ventilation (Kristensen et al. 2012). This process is also known as bio-mixing (i.e., solid particle transport) and bio-irrigation (i.e., solute transport). These types of bioturbation can have opposing effects (Van de Velde and Meysman 2016). Oligochaetes like Tubificidae dig tubes and feed with their head downwards, ingesting sediment and water, which pass vertically through the gut and are defaecated as faecal pellets on top of the sediment (Pelegri and Blackburn 1995; Martin et al. 2005). This type of bioturbation is called conveyor belt transport (Fisher et al. 1980). Additionally, the Tubificidae move through the sediment, hereby creating small tubes that extend in different directions in the sediment. Both types of behavior might be important for transporting nutrients from the sediments to the water column. In the following, when we use the term bioturbation, we refer in general to transportation of solutes.

Bioturbating oligochaetes increase the oxygen exchange over the sediment-water interface via irrigation of burrows, which leads to a downward movement of the aerobic zone in the sediment (Krantzberg 1985). These burrows produce a mosaic of oxic and anoxic interfaces as a result of aeration and respiration processes (Kristensen 2000). As many of the biogeochemical processes that occur in saturated sediments are governed by the availability of oxygen, bioturbation leads to changes in nutrient availability (Fillos and Swanson 1975; Callender and Hammond 1982; Vepraskas et al. 2001). Hansen and Kristensen (1997) found that worm activity accounted for up to 46% of the observed increase in nutrient concentrations, indicating enhanced

microbial activity in the sediment, which can accelerate the turn-over rate of nutrients. Schaller (2014) showed that apart from nutrients, Mg, Ca and Sr were also highly affected by bioturbation, whereas Al, Fe, Co, Cu, Mn, and Zn were affected only to a small extent. The above results demonstrate the importance of bioturbation in geochemical cycling in lake, river and marine sediments (Volkenborn et al. 2007; Vink 2009).

The overall effect of bioturbators on the biogeochemical processes depends on the characteristics of the sediment, environment, and benthic communities which are involved. To investigate the potential effect of bioturbating Tubificidae on the nutrient availability in the littoral zone to be constructed by the soft clay-rich sediment from lake Markermeer, we need to know how bioturbation alters biogeochemical processes in the topsoil. Since the impact of bioturbation on biogeochemistry depends on bioturbation activity, we carried out a microcosm experiment with different densities of Tubificidae to identify the effects on biogeochemical processes in porewater. To reach this aim, we monitored porewater chemistry at different depths in the soft clay-rich layer as well as surface water. We hypothesize that bioturbation positively influences nitrogen exchange to the water column by changing the oxidation state of the sediment. However, we also expect that the altered oxidation state will lead to immobilization of phosphorus by binding with produced iron oxides, as sediments from lake Markermeer are rich in pyrite.

6.2 METHODS

6.2.1 *Experimental set-up*

This study was performed with an experimental technique (EU-patent nos. 1018200/02077121.8, October 2001, J. Vink, Rijkswaterstaat), which was introduced as Sediment Or Fauna Incubation Experiment, or SOFIE® (Vink 2002; www.sofie.nl). This sampling device consists of a circular two-compartment cell (190 mm radius, 200 mm height). We used two two-compartment SOFIE®-devices to allow for simultaneous testing of four treatments. Each compartment was filled with soft sediment from the northern part of lake Markermeer. See Table 6.1 for the sediment properties of the soft clay-rich layer used in this study. This sediment was stored in

dark air-tight containers at 4°C prior to the start of the experiment. Porewater probes, constructed from a 0.1 µm-permeable polyethersulfone polymer (X-flow Industries, Almelo, The Netherlands), were installed in gastight connectors at a depth of 1, 6, 11, 16, 21, 31, 41, and 51 mm below soil surface. In addition, one probe was installed 9 mm above the soil surface to extract a sample from the water column. The probes were positioned in a circular manner in such a way that no interference among probes would occur during sampling.

Table 6.1. Geochemical and mineralogical composition of the sediment used in this study, with average concentrations in dry wt with standard deviations ($n = 5$). Adapted from Saaltink et al. 2018.

Method	Unit	n	Sediment	S.D.
<i>Aqua regia / CS / CN</i>				
Al	mg kg ⁻¹	5	10398	219
Ca	mg kg ⁻¹	5	48058	1288
Fe	mg kg ⁻¹	5	14766	234
K	mg kg ⁻¹	5	2619	72
Mg	mg kg ⁻¹	5	5106	135
Mn	mg kg ⁻¹	5	360	10
N	mg kg ⁻¹	5	1611	32
Na	mg kg ⁻¹	5	97	34
P	mg kg ⁻¹	5	361	10
S	mg kg ⁻¹	5	4513	187
Sr	mg kg ⁻¹	5	113	3
Ti	mg kg ⁻¹	5	396	17
Zn	mg kg ⁻¹	5	130	3

Table 6.1. (continued).

Method	Unit	n	Sediment	S.D.
<i>Seq. P extraction</i>				
Exchangeable P	mg kg ⁻¹	5	5	2
Fe-bound P	mg kg ⁻¹	5	40	10
Ca-bound P	mg kg ⁻¹	5	89	18
Detrital P	mg kg ⁻¹	5	158	14
Organic P	mg kg ⁻¹	5	47	11
Fe as oxides	mg kg ⁻¹	5	851	39
<i>XRD</i>				
Quartz	%	1	45.8	
Plagioclase	%	1	8.2	
Alkali feldspar	%	1	3.7	
Calcite	%	1	11.9	
Dolomite	%	1	2.8	
TiO₂	%	1	0.4	
Pyrite	%	1	1.0	
Phyllosilicates	%	1	26.3	
<i>Other</i>				
Organic matter	%	5	4.0	0.1

The SOFIE®-devices were placed in a dark, climate-controlled chamber at 12°C and were incubated for 4 months to allow stabilization of chemical processes after sediment disturbance. Two-Ampere electric pulses were applied biweekly in each compartment in the first two months of the 4-month incubation period to clear the sediments of any macroinvertebrates. After about six weeks, no activity related to bioturbation could be identified in the cells. At the start of the experiment Tubificidae were added to three of the four compartments at different densities: 5,000, 15,000,

and 30,000 Tubificidae per m² surface (these treatments are hereafter referred to as T_{5k} , T_{15k} , and T_{30k}). These Tubificidae were bought in The Netherlands (EAN: 4038358100154) and most likely can be classified as *Limnodrilus spp.*, present in varying sizes. The fourth compartment contained no Tubificidae and thus functioned as our control treatment. Because Annelids in lake Markermeer occur at densities of c. 5,000 individual m⁻² (Van Riel et al. 2018), the treatments used in this study allow to analyze differences in chemical processes in the sediment with increasing densities of bioturbating annelids compared to the field situation.

6.2.2 Sample analysis

Porewater and surface water were collected from the probes on days 0, 7, 14, and 28. Chloride, NO₂, NO₃, and SO₄ were determined using ion chromatography (IC); Ca, Fe, K, Mn, Na, Si and Sr were determined with Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), ammonium was determined with the phenol-hypochlorite method (Helder and De Vries 1979), PO₄ with the ammonium-heptamolybdate method (Kitson and Mellon 1944) and pH by an ion-specific electrode. The porewater volume extracted from the rhizons did not exceed 4 ml to minimize the disturbance of the internal water balance in the sediment. Redox potentials (Eh) were measured with a 1,6 x 40 mm needle sensor (RD-NP, Unisense), inserted through the sediments surface and 1 mm resolution profiles were made with a microprofiling system (Unisense).

The length of Tubificidae burrows at each depth were determined from pictures taken at day 7, 14, and 28. A specific section of the cell wall (3 cm wide, 10 cm long) was analysed from the photographs. We assume that the Tubificidae densities determined from these photographs are representative for the entire cell-compartment. Burrow length was calculated with SmartRoot in Image J (Lobet et al. 2011), which is especially designed for analyzing plant roots. As the contrast between burrow holes and undisturbed sediment was high enough for the software to make a distinction, we deemed this method suitable for determining the length of the burrows.

6.2.3 Statistical analysis

A robust factor analysis was used to determine main relationships between aqueous concentrations and soil properties within the three Tubificidae treatments. This method was chosen over a classical factor analysis because direct application of multivariate statistical analysis to compositional (closed) data might give a wrong impression (Filzmoser et al. 2009). Compositional variables from the dataset were first assessed with the isometric logratio transformation (ilr-transformation) (Egozcue et al. 2003). With this data, a robust estimation was obtained of the covariance matrix, after which the data was back transformed to the centered logratio (clr) space. This set of transformations was only done for the variables expressed in concentrations. Thus, depth and Eh were transformed with a normal logratio transformation. The transformed dataset was used for the parameter estimation in factor analysis. For a full description of this method, we refer to Filzmoser et al. (2009).

6.2.4 Modelling

Modeling phase transfers and redox transfers

We made use of the PHREEQC software (Parkhurst and Apello 2013) to identify chemical processes that occurred during the experiment. This software is based on mass-balance equations of preselected mineral phases (reactants). The mineral phases can either precipitate (leave the solution) or dissolve (enter the solution), expressed in mole transfers. Comparably, gases can either be consumed or released (leave the solution) or be taken up (enter the solution). Van der Grift et al. (2016) showed that iron hydroxyphosphates ($\text{FePO}_4(\text{OH})$) can precipitate upon oxidation in pH-neutral groundwater in presence of dissolved Fe(II) and PO_4 . To allow precipitation and dissolution of iron hydroxyphosphates, we added this phase to the PHREEQC database, using the solubility constant after Luedecke et al. (1989): $\text{Log } K[\text{Fe}_{2.5}\text{PO}_4(\text{OH})_{4.5}] = -96.7$. We applied inverse modeling between day 0 and 7, day 7 and 14 and day 14 and 28 in which all possible combinations of the mass-balance equations are accepted within a range of measured porewater concentrations $\pm 5\%$.

To enable the model to attribute some of the chemical changes to cation-exchange processes, we included an assemblage of exchangers (X): KX , MgX_2 , MnX_2 , NaX , and NH_4X . The sum of this assemblage was defined as the cation-exchange capacity

(CEC) calculated from the sediment composition, which was $30 \text{ meq } 100 \text{ g}^{-1}$ (Saaltink et al. 2016). The cation-exchange capacity is important to take into account, since it describes the buffering of some of the chemical processes in sediments by adsorption or desorption of cations. Likewise, redox transfers are modelled for Fe, N, O, and S, that can either oxidize or reduce.

Simulations explaining changes in water chemistry via phase and redox transfers were computed for 6, 21, and 51 mm depth for each treatment. For every situation, several valid simulations were found due to small differences in the amount of mole transfers attributed to the mineral phases. Here, we present the plausible simulation with the least amount of mole transfers.

Modeling aeration and respiration

An equilibrium 2D diffusion model was created to explain the combined effect of aeration and respiration by bioturbating Tubificidae. It is important to note that this equilibrium model is explaining diffusion and respiration processes in a general manner, ignoring that in situ many coefficients in this model would be affected by changing factors such as temperature of the water, wave action and even the species of Tubificidae. Moreover, the model could potentially underestimate the aeration potential by bioturbation, as it excludes two mechanisms: 1) peristaltic-like movements of Tubificidae that could in theory stimulate water movement in the tube; and 2) faecal pellet deposition that could result in passive irrigation of the tubes.

We schematize the role of Tubificidae in the sediment core as a burrow in the form of a cylinder. Through this burrow, oxygen can penetrate easily and is diffused into the sediment from the edge of this burrow, inducing an oxygen-rich cylinder. However, with increasing number of Tubificidae, the entire sediment surface becomes occupied and Tubificidae will move around, cross each other and curl together or fill the voids in between the oxygen-rich cylinders. Hence, the assumption that the number of Tubificidae scales linear with aeration is valid up to a critical number of Tubificidae. We assume that more Tubificidae will not increase the oxygen content in the sediment anymore. The aerated sediment volume as a function of the number of Tubificidae will roughly follow a smooth function, as shown in Figure 6.1b.

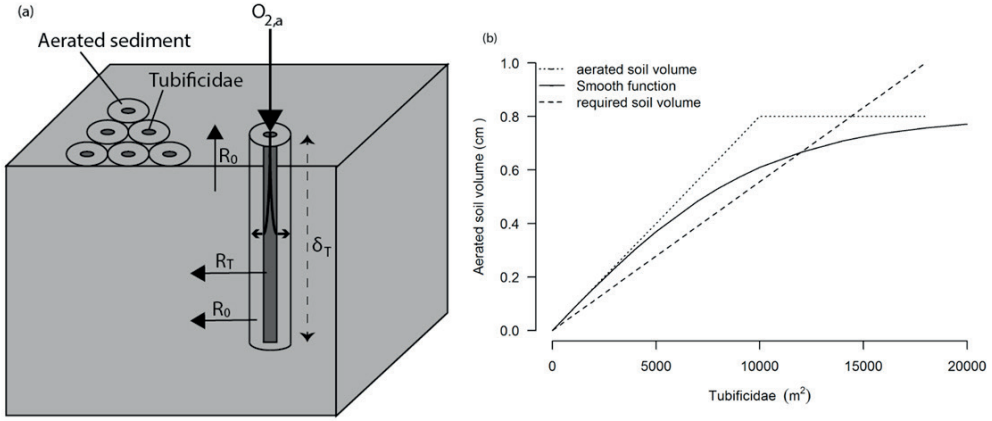


Figure 6.1. a) Conceptual overview of the aeration processes used in the model in which $O_{2,a}$ is the oxygen flow in the burrow into aerated sediment, R_0 is the soil respiration and R_T is the respiration rate per Tubificidae, (b) a diagram of the oxygen balance in the sediment: the aerated volume as a function of number of Tubificidae, its tangent hyperbolic approximation ($F(n)$), and the oxic sediment volume required for the respiration of n Tubificidae.

Furthermore, n Tubificidae consume nR_T amount of oxygen per unit time, where R_T is the respiration rate per Tubificidae. Note that nR_T and R_T reflect average values from literature; it is likely that these values change continuously in reality due to changes in activity, stress and water temperature. Depending on the aeration and respiration rate, the aeration and respiration curves can intersect. Likely, the oxygen concentration in the sediment initially increases with the number of Tubificidae and then decreases. We presume that the oxygen concentration yields the balance between respiration by n^* Tubificidae per burrow, and the flow of oxygen from the ambient water by diffusion ($O_{2,a}$). This diffusion is governed by the diffusion coefficient D_a of ambient water and the sediment (D_s). The abovementioned processes were included in a mathematical model, of which the final equilibrium equation reads:

$$\langle O_{2,s} \rangle = \frac{O_{2,a}}{4} - \frac{n^* R_T}{24 D_a} \delta_T^2 - \frac{R_0}{12 F(n) D_s} \delta_T^2 \quad (1)$$

This mathematical model is extensively explained in the Supplement. Equation (1) shows that there is an optimum in the mean oxygen concentration in the sediment $\langle O_{2,s} \rangle$ as a function of the number of Tubificidae n and their burrow function $F(n)$.

6.3 RESULTS

6.3.1 Experimental results

Effects of bioturbation on N, P, and Eh

Figure 6.2 presents depth profiles of all four treatments at experimental time $t = 28$ days. The length of the Tubificidae burrows (Figure 6.2a) decreased with depth for all three densities. The Tubificidae density effect on burrow length between T_{15k} and T_{30k} was only prevalent in the upper 20 mm, while the burrow length in T_{5k} was less than T_{15k} and T_{30k} down to 50 mm below sediment surface.

Marginal changes in PO_4 concentrations were found between the treatments (Figure 6.2b). As expected, PO_4 concentrations increased with increasing depth as presence of oxygen immobilizes phosphorus in iron-rich clay sediments. In the Tubificidae treatments, PO_4 was immobilized in the upper 11-16 mm, while P immobilization in the control occurred only in the upper 6 mm.

Nitrogen in the sediment was highly effected by bioturbation (Figure 6.2 c,d). Ammonium concentrations in T_{15k} and T_{30k} were higher than in the control for all depth intervals. As with PO_4 , NH_4 is expected to increase with increasing depth, but this is not the case for T_{30k} , where concentrations were dropping rapidly from 30 to 50 mm depth. Moreover, both T_{15k} and T_{30k} showed elevated NH_4 concentrations in the surface water (0.17 and 1.37 mg l⁻¹, respectively). In all Tubificidae treatments, elevated NO_x concentrations in the surface water were found (0.61 mg l⁻¹ for T_{5k} , 2.54 mg l⁻¹ for T_{15k} , and 18.3 mg l⁻¹ for T_{30k}) compared to the control (0.43 mg l⁻¹). Nitrate/Nitrite concentrations decreased rapidly with increasing depth and were almost zero below a depth of 20 mm.

The effect of bioturbation on the redox potential (Eh) is presented in Figure 6.3. Bioturbation resulted in a downward movement of the shift from oxidized to reduced sediment conditions, especially at a depth of 16 mm and lower. The largest shift was seen for T_{15k} , while T_{5k} and T_{30k} were more or less the same and were in between the

control treatment and T_{15k} . However, T_{30k} in the upper 16 mm decreased the Eh by approximately 30 mV compared to T_{5k} and T_{15k} , likely by high worm respiration.

These results clearly show that bioturbation by Tubificidae alters Eh and nitrogen concentration in both the porewater and surface water significantly, while only a marginal immobilizing effect was found for PO_4 .

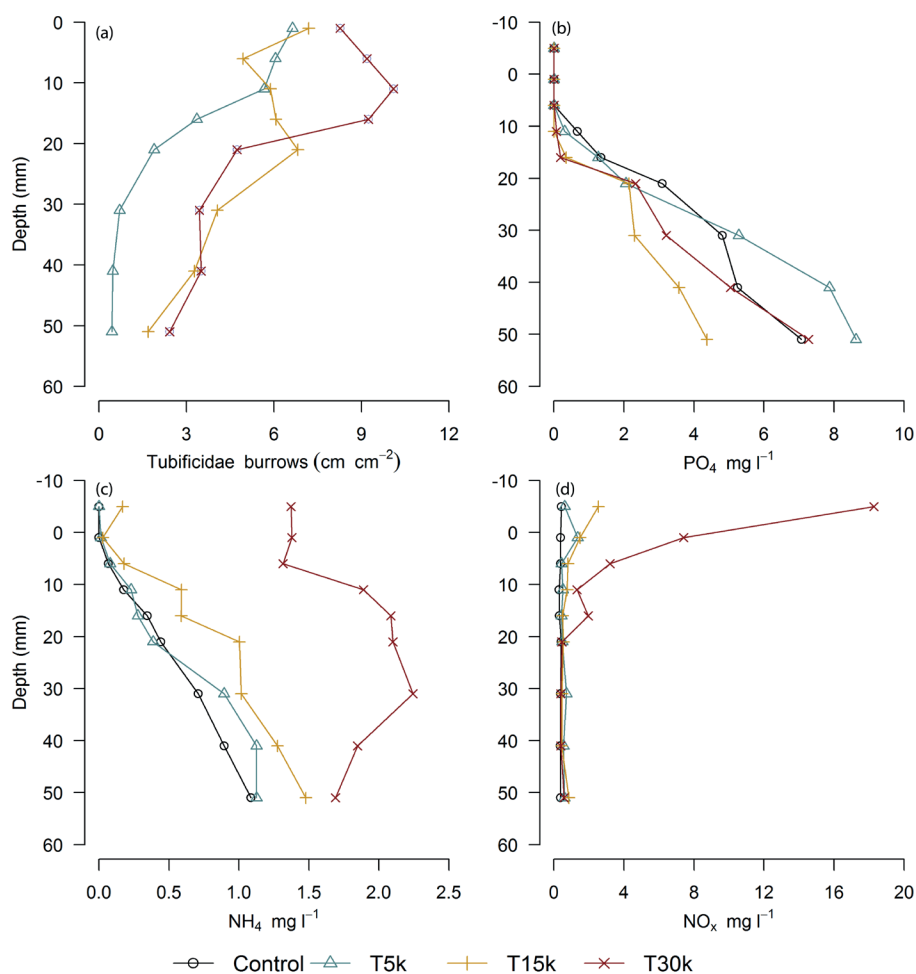


Figure 6.2. Measured depth profiles of Tubificidae burrows (cm) (a), PO_4 (b), NH_4 (c), and NO_x (d) in $mg\ l^{-1}$ for the control treatment as well as T_{5k} , T_{15k} , and T_{30k} .

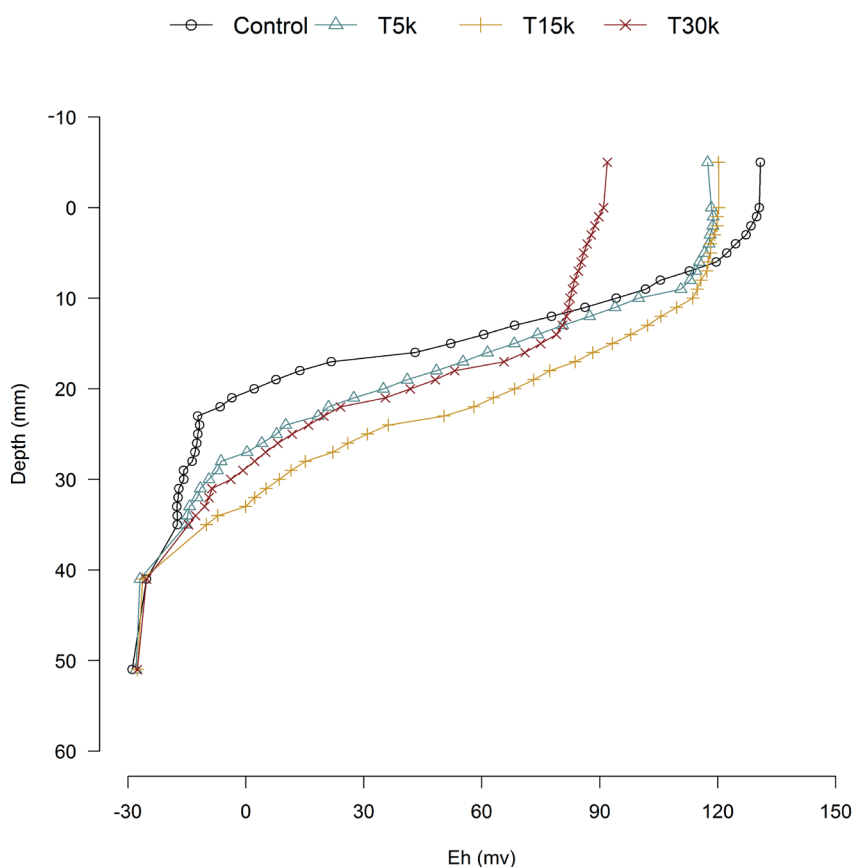


Figure 6.3. Measured depth profile of Eh (mv) for the control treatment as well as for T_{5k} , T_{15k} , and T_{30k} .

Aeration and respiration factors related to bioturbation

The summary output of the robust factor analysis for the three Tubificidae treatments is presented in Table 6.2. Both a respiration factor and an aeration factor by Tubificidae were identified from the relationship between Eh, burrow length and time. The respiration factor explained the largest part of the variance in the dataset (61.3% in T_{5k} and T_{15k} , 68.1% in T_{30k}) and is described by an inverse relationship between the burrow length and time with Eh; as burrow length increases through time, Eh decreases as a result of oxygen consumption. The second most important factor is

related to aeration by Tubificidae and is described by a positive relationship of time, burrow length and Eh. This aeration factor is largest for T_{15k} (27.4%), followed by T_{5k} (25.8%). However, this factor was absent in T_{30k} , most likely due to high oxygen consumption, as it is expected that total respiration scales linearly with the number of Tubificidae in the sediment. The results of this analysis suggest that the respiration effect was largest in T_{30k} , while the aeration effect was largest in T_{15k} , in line with the hypothesis of section 2.5.

Table 6.2. Output of robust factor analysis for the four treatments used in this study. A distinction is made between positive and negative loadings. Loading coefficients (r) between 0.5 and -0.5 are not shown.

Factor	% of explained variance	Sign. positive loadings	Sign. negative loadings	Interpretation
T_{5k}				
1	61.3	$r > 0.7$ Cl, Mg, Na, Sr, K, SO_4 , pH, Ca, Si, Fe, Eh	$r < -0.7$ Time	Respiration by Tubificidae
		$r > 0.5$ NO_x	$r < -0.5$ Burrows	
2	25.8	$r > 0.7$ Depth, PO_4 , NH_4	$r < -0.7$ Burrows	Aeration by
		$r > 0.5$ Mn, Fe	$r < -0.5$ Time, Eh	Tubificidae
3	4.4	$r > 0.7$	$r < -0.7$	
		$r > 0.5$ NO_x	$r < -0.5$ Mn	
T_{15k}				
1	61.3	$r > 0.7$ Cl, Na, Mg, SO_4 , Sr, K, Si, pH, Ca, Fe	$r < -0.7$ Time, Burrows	Respiration by Tubificidae
		$r > 0.5$ Eh	$r < -0.5$	
2	27.4	$r > 0.7$ Mn, NH_4 , PO_4 , Depth	$r < -0.7$ NO_x	Aeration by
		$r > 0.5$	$r < -0.5$ Burrows, Time, Eh	Tubificidae
3	5	$r > 0.7$	$r < -0.7$	
		$r > 0.5$ Eh	$r < -0.5$	

Table 6.2. (continued).

Factor	% of explained variance		Sign. positive loadings		Sign. negative loadings	Interpretation
T_{30k}						
1	68.1	$r > 0.7$	Cl, SO ₄ , Mg, Na, Sr, pH, K, Ca, Fe, Si, Mn	$r < -0.7$	Time, Burrows	Respiration by Tubificidae
		$r > 0.5$	Eh	$r < -0.5$		
2	20.6	$r > 0.7$	NO _x	$r < -0.7$	PO ₄ Depth	Depth effect; NO _x
		$r > 0.5$		$r < -0.5$	Mn	
3	6.1	$r > 0.7$		$r < -0.7$	NH ₄	
		$r > 0.5$		$r < -0.5$		

Table 6.3. Phase and redox changes expressed in mole transfers ($\mu\text{mol l}^{-1} \text{ day}^{-1}$) as modeled by PHREEQC. Positive values indicate dissolution for minerals and uptake for gases, negative values indicate precipitation for minerals and release for gases.

Treatment	Depth	Phase transfers					Redox transfers					
		Fe(OH) ₃	FePO ₄	CaCO ₃	CaPO ₄	FeS ₂	CO ₂	Fe	N	O	S	ΣRedox
Depth: 6 mm												
Control	Day 0-7		0.6		-0.3		-0.6		0.1		-0.1	0.0
	Day 7-14	-0.2			0.2			-0.2				-0.2
	Day 14-28				-0.4							
T _{5k}	Day 0-7	0.1										
	Day 7-14								0.3	1.3		1.7
	Day 14-28			26		25	-26		0.8	181	51	232
T _{15k}	Day 0-7		-9.1	32	2.6	22	-32	-23	2.0	173		152
	Day 7-14					0.4		0.2	-0.1	2.1	0.7	3.0
	Day 14-28		-5.1	17	1.7	13	-17	-13	-0.2	96	26	108
T _{30k}	Day 0-7		-2.3	23		5.3	-23	-3.9	16	102	11	125
	Day 7-14		0.1			-1.4		-0.4	1.0	-5.4	-2.8	-7.5
	Day 14-28	-1.5				2.1		-2.3	-4.0		4.3	-2.1

Table 6.3. (continued).

		Phase transfers					Redox transfers						
Treatment	Depth	Fe(OH) ₃	FePO ₄	CaCO ₃	CaPO ₄	FeS ₂	CO ₂	Fe	N	O	S	ΣRedox	
Depth: 21 mm													
Control	Day 0-7	-1.8	4.1	-1.3			-4.1	-4.2	-0.5			-4.8	
	Day 7-14												
	Day 14-28	-0.1		0.2				-0.2	0.1		-0.1	-0.3	
T _{5k}	Day 0-7	-8.2	28	3.3	22	-28	-21	0.9	167	44		191	
	Day 7-14	-3.4	12		8.5	-12	-8.5	-1.5	58	17		65	
	Day 14-28						0.3		1.3			1.7	
T _{15k}	Day 0-7	-0.9	12		5.1	-12	-2.3	4.6	57	10		68	
	Day 7-14												
	Day 14-28			0.2			-0.3	0.0				-0.4	
T _{30k}	Day 0-7		17		1.4	-17	0.1	15	70	2.7		88	
	Day 7-14			-1.5			0.1	-2.0	-7.9			-9.8	
	Day 14-28				-0.8			0.3	-4.4			-4.1	
Depth: 51 mm													
Control	Day 0-7												
	Day 7-14												
	Day 14-28			-0.4									
T _{5k}	Day 0-7												
	Day 7-14												
	Day 14-28							0.6	2.5			3.2	
T _{15k}	Day 0-7												
	Day 7-14						0.0	0.8	3.2			4.0	
	Day 14-28	-3.9	13		9.3	-13	-9.7	-2.1	61			50	
T _{30k}	Day 0-7												
	Day 7-14				1.1			0.9	11	2.2		14	
	Day 14-28							-0.6	-2.3			-2.9	

6.3.2 Modeled results

Effects of bioturbation on phase and redox transfers

Main phase and redox transfers at 6, 21, and 51 mm depth are depicted in Table 6.3. The models show that dissolution of calcite (CaCO_3) and pyrite (FeS_2) and precipitation of iron hydroxyphosphate (FePO_4) are important processes in the sediments with Tubificidae, suggesting that sediment aeration caused by bioturbation initiated these processes. The occurrence of these three processes together makes sense as oxidation of pyrite immobilizes phosphate by producing PO_4 -bearing Fe hydroxides, sulfate and protons, in turn promoting dissolution of calcite. This potentially affect bioturbation activity and speciation of chemical compounds. Dissolution and precipitation of apatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$) and iron(III) hydroxide ($\text{Fe}(\text{OH})_3$) were less important and these processes also occurred in the control (Table 6.3).

When analyzing respiration and aeration effects on bioturbation, modeling redox transfers can give valuable information. As expected, the net redox transfer in the control treatment is low at all three depths (Table 6.3). In the first week of the experiment, a positive net change in redox transfers was observed in all Tubificidae treatments (191 $\mu\text{mol electrons l}^{-1} \text{ day}^{-1}$ in T_{5k} at 21 mm depth; 152 $\mu\text{mol electrons l}^{-1} \text{ day}^{-1}$ in T_{15k} at 6 mm depth; 88 $\mu\text{mol electrons l}^{-1} \text{ day}^{-1}$ in T_{30k} at 21 mm depth). After the first week, the net redox change remained positive for T_{5k} and T_{15k} , and became negative for T_{30k} , except at 51 mm, where the net redox change became negative after two weeks.

The findings presented in Table 6.3 suggest that several chemical processes are altered by bioturbation. In line with Figure 6.3 and Table 6.2, inversely modeling redox transfers showed that there is a net oxidation effect for T_{5k} and T_{15k} , while a net reduction effect was found for T_{30k} .

Computed oxygen concentrations in bioturbated sediments

The results presented in sections 6.3.1-6.3.3 show that bioturbation results in aeration of the sediments. However, due to respiration by Tubificidae, the net aeration depends on the amount of Tubificidae per unit area. This was modeled, assuming that the relation between the number of Tubificidae and aerated soil volume follows a

Table 6.4. Input parameters to model the mean oxygen concentration $\langle O_{2,s} \text{ [mg l}^{-1}] \rangle$ in the sediment.

Parameter	Description	Value / Function	Source
$O_{2,a}$	Oxygen concentration in ambient water	11.5 mg l ⁻¹	Ministry of Infrastructure and the Environment (2017)
n	Number of Tubificidae	5k, 15k, 30k m ²	<i>From experiment</i>
R_T	Respiration rate per Tubificidae	0.0250 mg hr ⁻¹	Fowler and Goodnight, 1965; Lou et al., 2013
$F(n)$	Burrow function	0.80tanh(n) 1 cm: 8.35 cm 2 cm: 4.35 cm	<i>From experiment</i>
δ_T	Mean burrow length	3 cm: 2.74 cm 4 cm: 2.42 cm 5 cm: 1.52 cm	<i>From experiment</i>
d_T	Diameter of burrow	0.05 cm	Rogaar, 1979
δO	Thickness of oxic layer	0.2 cm	De Lucas Pardo, 2014
D_a	Diffusion coefficient ambient water	0.153 cm ² hr ⁻¹	Han and Bartels, 1996
D_s	Diffusion coefficient sediment	0.082 cm ² hr ⁻¹	Ullman and Aller, 1981; Iversen and Jørgensen, 1993
R_o	Respiration rate of sediment	0.0205 cm ² hr ⁻¹	From equation (2) in appendix

tangent-hyperbolic function. From the aerated soil volume for the three Tubificidae densities used in this experiment, this relation could be calibrated, yielding $V = 0.80 \tanh(n)$. With this function and the parameter input presented in Table 6.4, the oxygen concentration in the sediment for a given Tubificidae density was modeled using equation (1). Figure 6.4b presents the computed oxygen concentration for Tubificidae densities between 0 and 40,000 individuals m⁻², showing an optimum at 12,000 individuals m⁻² with an oxygen concentration of 0.85 mg l⁻¹ at 1 cm depth. The O₂ concentration quickly decreases with increasing depth, becoming zero below 3

cm depth. Moreover, these results show that there is an aeration effect at 1 cm depth with a Tubificidae density between 4,000 and 32,000 individuals m^{-2} . However, as already outlined, the model is simplified in such a way that some variables (e.g., water temperature and associated changes in oxygen concentration in ambient water) are kept constant, which do change in reality. In supplementary Figure 6.2, the effect of changing oxygen concentrations in ambient water – driven by seasonal changes in temperature – on the oxidation effect by Tubificidae is elaborated.

This model output explains the results presented in sections 6.3.1-6.3.3 by showing that T_{15k} is closest to the optimal aeration effect. It furthermore explains why reduced conditions are found in all treatments below a depth of 33 mm (Figure 6.3).

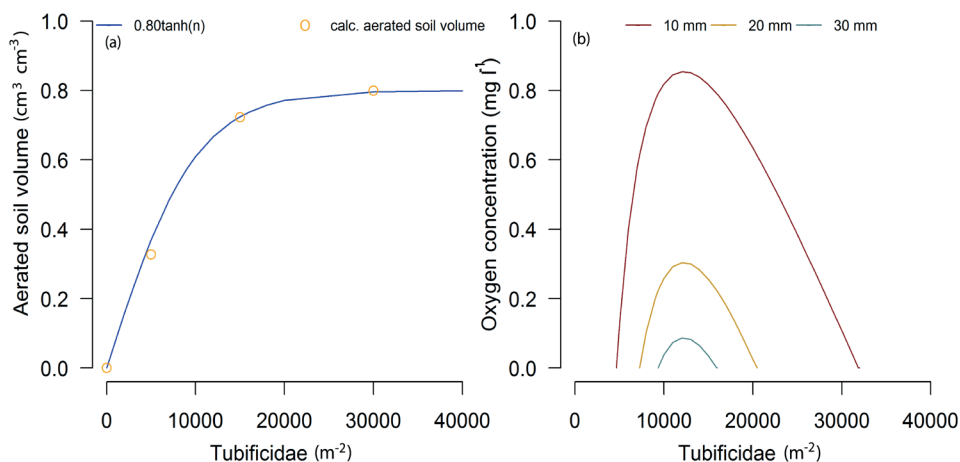


Figure 6.4. (a) Calculated average aerated soil volume (cm^3) for T_{5k} , T_{15k} , and T_{30k} (orange circles) and modeled aerated soil volume (cm^3) per number of Tubificidae (m^{-2}) (dark blue) (a). (b) The modeled burrow function corresponds to a tangent-hyperbolic function ($0.80 \tanh(n)$) and was used to model oxygen concentration in the sediment (mg l^{-1}) per number of Tubificidae (m^{-2}) for three different depths: 10 mm (red), 20 mm (yellow), and 30 mm (blue).

6.4 DISCUSSION

This study shows that bioturbation by Tubificidae effectively aerated the upper layer of the sediment. The largest aeration effect was discerned at a density of

15,000 worms m^{-2} . This becomes evident from our experiment (Figure 6.3), where a downward movement of the shift from oxidized to reduced sediment conditions is apparent at a depth of 16 mm and lower. Moreover, both the factor analysis and the modeled redox transfers suggest highest aeration effects in the T_{15k} treatment (Table 6.3 and 6.4). This conclusion is further supported by the model outcomes of the respiration and oxidation rates of bioturbating Tubificidae (Figure 6.4). That model predicts an optimum of 12,000 worms m^{-2} , which is close to the T_{15k} treatment in our experiment. Furthermore, the model showed that the effect of bioturbation on oxidation first increases with increasing densities and then, after the optimum density of 12,000 worms m^{-2} is exceeded, the aeration effect of Tubificidae diminishes as burrows start overlapping geometrically, while the respiration rate per worm stays the same. These opposing mechanisms explain why oxygen concentrations follows an optimum curve with increasing worm density. Likewise, these mechanisms also explain why Eh values decreased in the upper 16 mm in the T_{30k} treatment (Figure 6.3) and why no aeration effect was distinguished by factor analysis (Table 6.2). Tubificidae at a density of 30,000 worms m^{-2} required more oxygen than was made available by aeration. Note that although this density is apparently not suitable for optimizing oxidation in Markermeer sediments, these high densities do occur in reality, especially in organically enriched environments and waste water (e.g. Nogaro et al. 2009).

The aeration effect of bioturbating Tubificidae effectively altered biogeochemical processes in the upper layer of the sediment, thereby influencing nutrient availability. Both NH_4 and NO_x concentrations increased (Figure 6.2c,d). The accumulation of nitrate in the upper layer of the sediment is most likely a result of aerobic oxidation of an upward flux of NH_4 (Anschutz et al. 2010), i.e., the oxidation of oxygen into the sediment was large enough to promote nitrification. The difference in average NH_4 concentrations between T_{15k} (0.70 mg l^{-1}) and T_{30k} (1.77 mg l^{-1}) is remarkable and suggests enhanced ammonification. Since organic matter content in these sediments is low (Saaltink et al. 2018), ammonification suggests that the Tubificidae at a density of 30,000 worms m^{-2} required more oxygen than was made available by aeration. Moreover, decomposition of dead Tubificidae may occur at a fast rate in bioturbated sediments, as bioturbation enhances microbial activity (Hansen and Kristensen 1997), further explaining the increased NH_4 concentrations in the upper 50 mm of the

sediment in T_{30k} . Hence, treatments containing 5,000 and 15,000 worms m^{-2} effectively increased N availability in the sediment and in the water column. At a density of 30,000 worms m^{-2} , however, the increased N concentrations are likely caused by decomposition of the introduced Tubificidae themselves. Therefore, the largest effect of bioturbation on the N concentrations in the sediment and the water column of lake Markermeer was found for a Tubificidae density at 15,000 worms m^{-2} .

In contrast to N, a negative effect was found for P; bioturbation decreased P concentrations in the sediment (Figure 6.2b). Furthermore, no exchange of P with the water column was identified. This immobilization effect was mainly visible in the upper 16 mm of the sediment and is also explained by enhanced oxidation. The effect of P immobilization strongly depends on the geochemical composition of the sediment. For example, a positive upward flux of P to the water column was reported by Scicluna et al. (2015) and Zhu et al. (2016), especially after periods of hypoxia. Such P retention and release effects become especially important when significant amounts of iron-bound phosphorus are present in the sediment. As the sediment used in this experiment is rich in iron-bound phosphorus and pyrite (40 mg kg^{-1} for iron bound phosphorus and 10,000 mg kg^{-1} for pyrite; Table 6.1), it is not surprising, therefore, that the P concentration in the sediment decreased when oxygen concentrations increased. This is in concordance with the geochemical model simulations presented in this study (Table 6.3). Pyrite oxidation with associated calcite dissolution were identified as dominant geochemical processes induced by bioturbating Tubificidae. Oxidation of pyrite (FeS_2) is directly coupled to immobilization of P via precipitation of iron hydroxy phosphates (Table 6.3), which can occur upon aeration of pH-neutral and PO_4 rich groundwater (Griffioen, 2006; Van der Grift et al. 2016). Van de Velde and Meysman (2016) showed that bioturbation indeed improves the recycling of Fe and S between their reduced and oxidized states. From a sediment depth of 21 mm onwards, no clear effect on the P concentration was found between the treated and the control samples, with P concentrations at 51 mm ranging from 4 mg l^{-1} in T_{15k} to 9 mg l^{-1} in T_{5k} . This indicates that the oxidation effect of bioturbation below 21 mm did not have a distinct impact on geochemical processes associated with P availability.

Since the macrofauna density in lake Markermeer currently amounts to about 12,800 individuals m^{-2} (of which about 5,000 Annelida m^{-2}), colonizing the sediment

in the littoral zone of the created wetlands with about 7,000 Tubificidae m⁻² might enhance initial ecosystem development due to improved availability of N. However, when macroinvertebrates other than Annelida partly aerate the sediment, colonizing the sediment at a smaller density is preferred. Fast initial ecosystem development is crucial when wetlands are constructed with soft mud as aboveground plant biomass dampen erosive stresses on the substrate by attenuating waves (Nepf 2012). Although the outcome of this experiment provides valuable information for developing ecological engineering practices, a direct translation into practical measures to be implemented is difficult due to several limitations related to the set-up of the experiment. First, it must be noted that the precise effect of colonization by Tubificidae into the littoral zone is hard to predict, as in reality, hydrodynamic factors also determine sediment oxidation, such as wave action with associated resuspension and settling. However, Volkenborn et al. (2007) showed that the effect of bioturbation on geochemical processes in aquatic systems is even visible in physically dominated systems, such as the Wadden Sea. Second, the experimental sediment only contained Tubificidae, whereas in reality interactions with other macro invertebrates exist. How such interactions in sediment from lake Markermeer might influence nutrient availability remains unknown. Last, the oxidation effect of bioturbation partly depends on the oxygen concentration in the lake. These concentrations show seasonal variation from 8 mg l⁻¹ in summer to 14 mg l⁻¹ during winter (Ministry of Infrastructure and the Environment 2017). Thus, the oxidation effect of bioturbation is larger in winter and spring than in summer and autumn (Supplementary Figure 6.2). The aforementioned factors might explain the current density of Tubificidae present in lake Markermeer sediments (3,900 Tubificidae m⁻²). Despite these limitations, this study clearly showed significant effects of bioturbation by Tubificidae on sediment aeration and associated nutrient availability in the porewater and water column.

6.5 CONCLUSIONS

This study aimed to identify the effects of bioturbating Tubificidae on biogeochemical processes in porewater, especially processes related to the nutrient availability. The results of this experiment showed that Tubificidae promoted oxygen to penetrate the

upper layer of the sediment. This resulted in elevated concentrations of NH_4 and NO_x in both the porewater and the water column. In contrast, phosphorus concentrations were declining in the upper 16 mm as a result of immobilization due to iron hydroxy phosphate precipitation in response to pyrite oxidation. The observed bioturbation effects were highest in the treatment with an intermediate density of Tubificidae (15,000 worms m^{-2}). The modeled effects of aeration and respiration on the oxygen concentration in the sediment suggest that the bioturbation effect is strongest at a density of 12,000 worms m^{-2} . In ecological engineering projects where fast (aquatic) ecosystem development is important, inoculating aquatic sediments with Tubificidae to optimal densities might enhance initial ecosystem development due to enhanced availability of N. The precise effect will however be largely determined by the geochemical composition of the sediment.

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APPENDIX

Modeling aeration and respiration

The model starts from an initially anoxic sediment with a thin oxic layer on top, with thickness δ_o . The initial anoxic conditions within the sediment are the result of oxygen consumption from the oxidation of organic material. Through diffusion, oxygen from the atmosphere can penetrate into the anoxic sediment. The thickness of this oxic layer is described with a one-dimensional diffusion model:

$$\frac{\partial O_2}{\partial t} - D_s \frac{\partial^2 O_2}{\partial z^2} = -R_s \quad (1)$$

in which O_2 is the oxygen concentration in the oxic layer, D_s is the oxygen diffusion coefficient within the sediment, R_s is the rate of oxygen consumption in the soil without Tubificidae, and t and z are the time and vertical coordinate. The thickness of the oxic layer (δ_o) amounts to:

$$\delta_o \sim \sqrt{\frac{D_s}{R_s}} \quad (2)$$

We schematize the role of Tubificidae as a burrow in the form of a cylinder with diameter d_T and length δ_T (cm). See Figure A6.1a for a conceptual presentation of these variables. Through this burrow, oxygen can penetrate easily and is diffused into the soil from the edge of this burrow, inducing an oxygen-rich cylinder with length δ_T and diameter d_o . Here, we assume that this diffusion is similar to the diffusion length at the sediment surface, hence: $d_o = d_T + 2\delta_o$. In reality, the number, diameter and depths of the oxygen-rich cylinder created by Tubificidae is variable, depending on other factors such as food availability.

Now, we analyze a soil sample of unit area S and with thickness that equals the length of the burrow δ_T . If we initially assume that $\delta_o \ll \delta_T$, the equilibrium oxygen content in the sediment scales linearly with the number of Tubificidae n per unit area:

$$O_2 = n \frac{\pi(d_T + 2\delta_o)^2}{4S} O_{2,e} \quad (3)$$

However, with increasing time, the entire surface area S becomes occupied. At the densest packing, a fraction of $\pi/2\sqrt{3} \sim 0.91$ of the surface is occupied. More Tubificidae will move around, cross each other and curl together or fill the voids in between the oxygen-rich cylinders. Hence, (3) is valid up to a critical number of Tubificidae. More Tubificidae will not increase the oxygen content in the sediment anymore. The aerated sediment volume as a function of the number of Tubificidae will roughly follow a smooth function (Figure A6.1b).

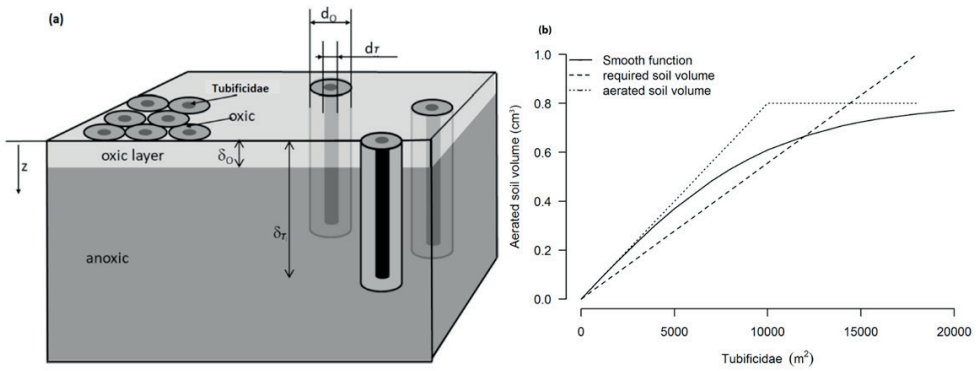


Figure A6.1. Conceptual overview of the sediment with thin oxalic layer on top of anoxic soil, and the effects of Tubificidae (a), as well as a diagram of the oxygen balance in the sediment (b): the aerated volume as a function of number of Tubificidae, and its tangent hyperbolic approximation, and the oxalic sediment volume required for the respiration of n Tubificidae.

Furthermore, n Tubificidae consume nR_T amount of oxygen per unit time, where R_T is the respiration rate per Tubificidae. Note that nR_T and R_T reflect average values from literature; it is likely that these values change continuously in reality due to changes in activity, stress and water temperature. Depending on the aeration and respiration rate, the aeration and respiration curves can intersect. Likely, the oxygen concentration in the sediment initially increases with the number of Tubificidae and then decreases.

For each aerated cylinder, we can write a diffusion equation, where we assume that $d_T \ll d_o$:

$$r \frac{\partial O_2}{\partial t} - D_s \left[\frac{\partial}{\partial r} r \frac{\partial O_2}{\partial r} + r \frac{\partial^2 O_2}{\partial z^2} \right] = -r R_s \quad (4)$$

in which r is the radial coordinate of the cylinder. For more Tubificidae, we find the following diffusion equation for a unit sediment volume:

$$\frac{\partial O_2}{\partial t} - D_s \left[\frac{1}{r} \frac{\partial}{\partial r} r \frac{\partial O_2}{\partial r} + r \frac{\partial^2 O_2}{\partial z^2} \right] = -R_s \quad (5)$$

For establishing the maximum oxygen concentration possible, we ignore the thin oxic layer at the soil surface. Hence, we ignore the term with the derivative to z . The general equilibrium solution to eq. (5) then reads:

$$O_2 = -\frac{R_s}{4D_s} r^2 + A \ln r + B \quad (6)$$

where A and B are integration constants, which follow from the boundary conditions without Tubificidae, assuming that oxygen enters the cylinders through the burrows in the sediment:

$$\begin{aligned} O_2 &= O_{2,b} \text{ at } r = 0 \\ O_2 &= 0 \text{ at } r = r_0 (= \frac{d_0}{2}) \end{aligned} \quad (7)$$

The oxygen concentration in the burrows $O_{2,b}$ should be equal to the oxygen concentration in the ambient water (i.e. $O_{2,e} = O_{2,b}$). However, Tubificidae in the burrows consume a part of the oxygen, so $O_{2,b} < O_{2,e}$. Therefore, we implicitly account for the respiration of Tubificidae which contribute to the aeration of the sediment. Substitution of eq. (7) into eq. (6) gives:

$$O_2(n) = O_{2,b} - \frac{R_s}{4D_s} r^2 \text{ for } r < r_0 \quad (8)$$

where $\sqrt{\frac{4D_s O_{2,e}}{R_s}}$

The average amount of oxygen in each cylinder $O_{2,cyl}$ reads:

$$\begin{aligned} \bar{O}_{2,cyl} &= \frac{1}{\pi r_0^2} \iint r O_2 dr d\theta = \frac{1}{\pi r_0^2} \iint_{2\pi r_0} \left[r O_{2,b} - \frac{R_s}{4D_s} r^3 \right] dr d\theta = \frac{1}{\pi r_0^2} \int_{2\pi} \left[\frac{1}{2} O_{2,b} r_0^2 \right. \\ &\quad \left. - \frac{R_s}{16D_s} r_0^4 \right] d\theta = O_{2,b} - \frac{R_s}{8D_s} r_0^2 \end{aligned} \quad (9)$$

The next step is assessing the oxygen concentration in the burrows $O_{2,b}$. We presume that the concentration yields the balance between respiration by n^* Tubificidae per burrow, and the flow of oxygen from the ambient water by diffusion. This diffusion is governed by the diffusion coefficient D_a of ambient water. Note that migration by Tubificidae may induce additional pumping, increasing D_a , possibly by two orders of magnitude. The diffusion coefficient in the sediment was calculated from D_a :

$$D_s = \frac{D_a}{\phi F} \quad (10)$$

where ϕ is the porosity of the sediment and F the formation factor. The formation factor can be calculated according to Iversen and Jørgensen (1993):

$$F = \frac{1}{\phi^m} \quad (11)$$

where m is a parameter and amounts to 2.75 in saturated clay-rich sediments (Ullman and Aller 1981).

The effective number of Tubificidae (n^*) in one burrow can be larger than one, owing to the fact that burrows may overlap geometrically, without adding further oxygen into the sediment by the mechanism described above. Thus $n^* = n/F(n)$. The oxygen balance in the burrow can then be described by another diffusion equation for $O_{2,b}$:

$$\frac{\partial O_{2,b}}{\partial t} - D_a \frac{\partial^2 O_{2,b}}{\partial z^2} = -n_* R_T \quad (12)$$

in which R_T is the respiration rate per Tubificidae. For equilibrium conditions, we obtain a parabolic oxygen distribution:

$$O_{2,b} = \frac{n_* R_T}{2D_a} z^2 + Pz + Q \quad (13)$$

The integration coefficients P and Q follow from the boundary conditions:

$$O_{2,b} = O_{2,a} \text{ at } z = 0 \quad (14)$$

$$O_{2,b} = 0 \text{ at } z = \delta_t$$

where $O_{2,a}$ is the oxygen concentration in the ambient water above the soil. Substitution yields:

$$O_{2,b} = \frac{n_* R_T}{2D_a} z^2 - \left(\frac{n_* R_T}{2D_a} \delta_t + \frac{O_{2,a}}{\delta_T} \right) z + O_{2,a} \quad (15)$$

Next, we assume that the mean oxygen concentration in the burrow is representative for the oxygen diffusion into the sediment, as described by (5). Thus integration of (15) over the burrow length δ_T and dividing by δ_T yields the mean oxygen concentration $\langle O_{2,b} \rangle$ in the burrow:

$$\langle O_{2,b} \rangle = \frac{n_* R_T}{6D_a} \delta_T^2 - \frac{n_* R_T}{4D_a} \delta_T^2 - \frac{O_{2,a}}{2} + O_{2,a} = \frac{O_{2,a}}{2} - \frac{n_* R_T}{12D_a} \delta_T^2 \quad (16)$$

Note that $\langle O_{2,b} \rangle$ cannot become negative. Finally, we assume that the number of Tubificidae per unit surface area is large, thus that we may treat their contribution to the oxygen balance of the sediment as a continuum. The oxygen balance for the sediment then becomes slightly different from (1):

$$\frac{\partial O_{2,s}}{\partial t} - F(n) D_s \frac{\partial^2 O_{2,s}}{\partial z^2} = -R_0 \quad (17)$$

with boundary conditions:

$$O_{2,b} = \langle O_{2,b} \rangle \text{ at } z = 0 \quad (18)$$

$$O_{2,b} = 0 \text{ at } z = \delta_T$$

Again, we determine the equilibrium solution from (17), using the boundary conditions (18). Then, we establish the mean oxygen concentration in the soil as a function of the number of Tubificidae, the length of the burrows and the two diffusion coefficients D_s and D_a . Integrating the equilibrium solution of (17) over the burrow length δ_T yields:

$$O_{2,s} = \frac{R_0}{2F(n)D_s} z^2 - \left(\frac{R_0}{2F(n)D_s} \delta_T - \frac{\langle O_{2,b} \rangle}{\delta_T} \right) z + \langle O_{2,b} \rangle \quad (19)$$

Integration over the burrow length δ_T and dividing by δ_T gives the mean oxygen concentration in the soil $\langle O_{2,s} \rangle$:

$$O_{2,s} = \frac{\langle O_{2,b} \rangle}{2} - \frac{R_0}{12F(n)D_s} \delta_T^2 \quad (20)$$

And substitution from (16) yields:

$$\langle O_{2,s} \rangle = \frac{O_{2,a}}{4} - \frac{n_* R_T}{24D_a} \delta_T^2 - \frac{R_0}{12F(n)D_s} \delta_T^2 = \frac{O_{2,a}}{4} - \frac{(2R_0 + nR_T)}{24F(n)D_a} \delta_T^2 \quad (21)$$

Equation (21) shows that there is an optimum in the mean oxygen concentration in the sediment $\langle O_{2,s} \rangle$ as a function of the number of Tubificidae n and their burrow function $F(n)$.

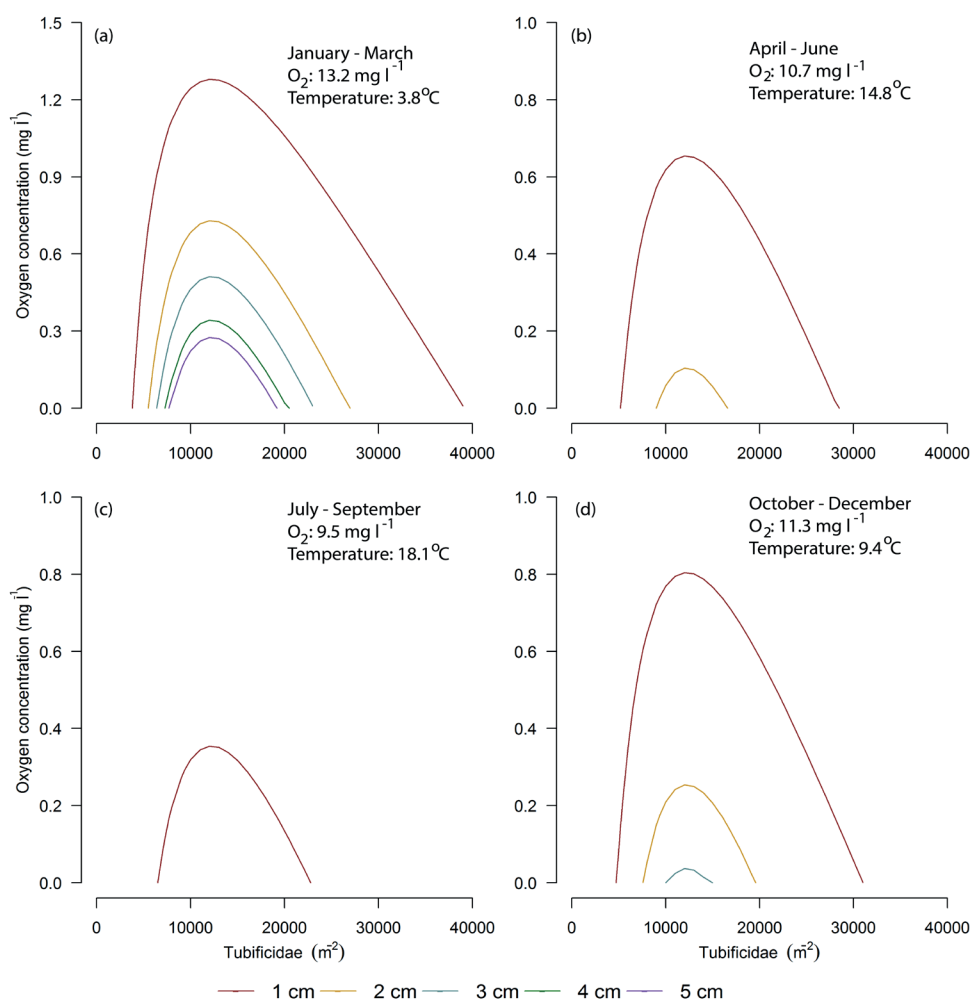


Figure A6.2. Modeled oxygen concentrations (mg l^{-1}) per number of Tubificidae (m^{-2}) in four three-month periods a year. The temperature and the oxygen concentration in the water column is an average of the three-month periods in the years 2008-2013 (Ministry of Infrastructure and the Environment 2017).

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CHAPTER

7

Synthesis

Rémon Saaltink

7.1 INTRODUCTION

The research presented in this thesis has highlighted biogeochemical, hydrological, and wetland ecological processes that interact and enhance ecosystem development on wetlands built on fine sediment. At the moment, coarse sand is widely used as building material for land reclamation (Sutherland et al., 2017) and as sand is becoming costly and sand demand will rise in the future as a result of rapid urbanization (Torres et al., 2017), fine sediment – available in large quantities in many river delta systems – may be used as an alternative (Jalowska et al., 2017; McKee et al., 2004). This study will help to overcome some of the challenges involved when using fine sediment as building material, as the experiments presented in Chapters 2-6 enabled me to determine:

1. Important biogeochemical processes in mud aeration and feedback mechanisms between plant and soil,
2. The effects of indirect iron toxicity in terms of nutrient stoichiometry and biomass production for three wetland plant species,
3. How the hydrological regime in relation to sediment type interferes with ecosystem development,
4. To what extent *Phragmites australis* enhances the consolidation of the soft clay-rich layer,
5. The influence of bioturbators on the nutrient availability in pore water and surface water in the littoral zone of the wetlands.

In this synthesis chapter, I will first summarize my findings. Subsequently, I will compare the geochemical composition of the sediments used in the experiments to the geochemical composition of the sediment in the basins of the Marker Wadden. Next, I will present relevant results from fieldwork and discuss why findings from greenhouse experiments and laboratory experiments provide useful information for large-scale projects. I will then present practical recommendations for the Marker Wadden project, especially for improving the design of the eco-engineering project when the Marker Wadden is expanded with more islands, and for efficient nature management practices for the existing islands. Suggestions for further research that may contribute to a better understanding of the biogeochemical and wetland–ecological interactions

in fine sediment are described. The chapter ends with a comment about ecological engineering and sustainability.

7.2 SUMMARY OF MAIN FINDINGS

The findings presented in Chapters 2-6 can be divided into four topics: 1) Plant–soil interactions in the terrestrial zone, 2) wetland–terrestrial processes influencing nutrient availability in the land–water zone, 3) effects of plants on sediment consolidation in the terrestrial zone, and 4) effects of bioturbation on nutrient availability in the aquatic zone. See Figure 6 in Chapter 1 for a conceptual overview of the relevant processes in this study.

7.2.1 Plant–soil interactions – terrestrial zone

In Chapter 2, I identified biogeochemical processes in the clay-rich material and studied the interactions between the sediment and *P. australis*. The aim and set-up of this experiment are summarized in Box 7.1. Key biogeochemical processes that influence pore water chemistry were found to be pyrite oxidation and associated calcite dissolution. *Phragmites australis* enhanced the oxidation process significantly by aerating its environment via radial oxygen loss (i.e., root aeration). The analysis of the mineralogical composition of the sediment used in this experiment showed that the amount of calcite (9%; 900 mmol kg⁻¹) far exceeds that of pyrite (0.6%; 50 mmol kg⁻¹). These figures suggest that even when complete pyrite oxidation to SO₄ and Fe-oxide occurs, enough calcite is present to buffer all H⁺ produced (200 mmol). The pH of the sediment was regularly checked in the experiments presented in Chapters 2 and 3. As expected, the pH remained stable, fluctuating around pH 7.6.

During the experiment presented in Chapter 2, I observed stunted plant growth and shriveling and yellowing foliage. Analysis of plant tissue showed that this was not caused by nutrient limitation. Although the N:P ratios of plant tissue reduced from 11 to 2-3 – which suggests N limitation – the total tissue concentrations of *P. australis* made it clear that low N:P ratios were caused by enhanced uptake of P. I hypothesized that co-precipitation of P with Fe on roots enhanced the concentrations of P in the plant roots (Snowden and Wheeler, 1995; Jørgenson et al., 2012).

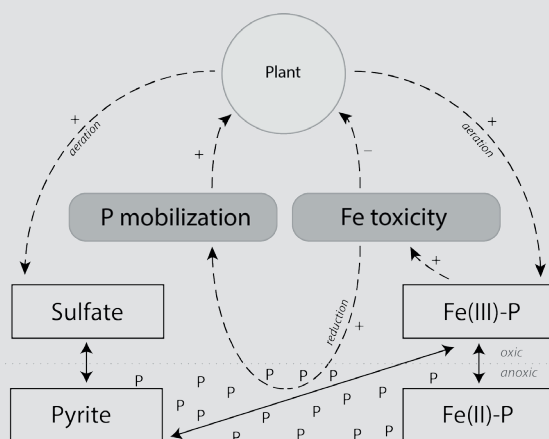
Box 7.1. Summarized aim and set-up of the experiment presented in Chapter 2.

Aim To identify biogeochemical plant–soil feedback processes that occur when oxidation, drying, and modification by plants alter the biogeochemical conditions in sediment from lake Markermeer.

Experimental set-up A greenhouse experiment was conducted for 24 weeks at the test facility of Utrecht University. The sediments used include the soft, clay-rich layer and the underlying, consolidated, Zuiderzee deposit. Each sediment type had 13 replicates. A single reed seedling (*P. australis*) was planted per pot in eight of the replicates, leaving five replicates unplanted. For 24 weeks, the quality of pore water, soil, and plants were measured and used to model biogeochemical processes in the PhreeqC program.



Key Figure



Most important biogeochemical processes and feedbacks identified from the experiment in Chapter 2. + indicates positive feedback, - indicates negative feedback. Saaltink et al., 2016.

The hypothesis is supported by the Fe concentration in the leaves, which increased approximately 20-fold compared to the initial concentration in the seedlings. Furthermore, ferric oxide, a product of pyrite oxidation, precipitated on root surfaces (Jørgenson et al., 2012). Figure 7.1 shows a root fragment of *P. australis* from our experiment that is covered with iron plaque.



Figure 7.1. Root fragment of *P. australis* retrieved from the experiment described in Chapter 2.

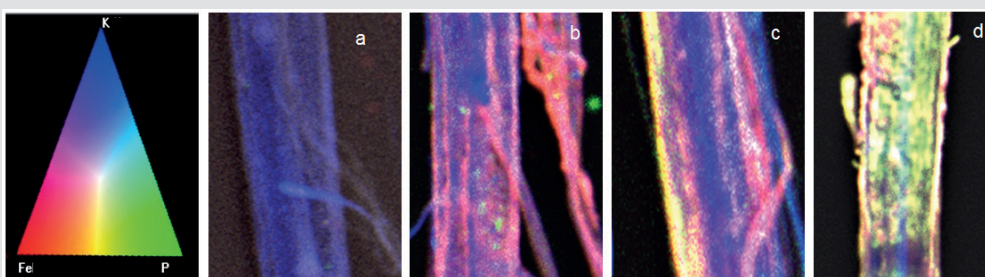
Several feedback mechanisms between the sediment and *P. australis* were identified based on the processes described above (see Key Figure in Box 7.1). One is a negative feedback loop that arises because plant roots induce aeration, which promotes iron toxicity, which in turn decreases plant growth and results in plant death. There is also a positive feedback loop, as iron toxicity induces reduction processes as a result of root death, which leads to P mobilization and hence enhances plant growth and regeneration.

The iron-toxicity hypothesis posed in Chapter 2 was tested with a follow-up experiment described in Chapter 3. The aim and set-up of this experiment are summarized in Box 7.2. Here, I will highlight three important observations from this experiment. First, a clear impact was found of iron-rich sediment on biomass production for all three species (see Figure 7.2 for an impression; biomass measurements are presented in Chapter 3). This effect was strongest in the sediment that contained highest contents of iron and iron-bound phosphorus, despite the fact that this sediment also contained the highest concentration of nutrients. Second, analysis of root surface composition showed that only *P. australis* produced substantial amounts of iron plaque. High radial oxygen loss in this plant

Box 7.2. Summarized aim and set-up of the experiment presented in Chapter 3.

Aim To study the effects of iron toxicity in plants when grown in the iron-rich sediments of lake Markermeer. The two sub-aims were: 1) to determine the presence and composition of iron plaque on the plant roots and show that this plaque formation depends on the amount of iron and iron-bound phosphorus present in the sediments, 2) to monitor contents of Fe, N, P, and K in leaves and roots and link changes in plant nutrient stoichiometry – such as changes in tissue N:P ratio due to altered uptake and translocation of N and/or P – to the presence and composition of iron plaque.

Experimental Set-up Three plant species were selected that differ in their tolerance to iron and are commonly found in the Markermeer area: *Rumex maritimus*, *Phragmites australis* and *Eupatorium cannabinum*. Seedlings were transplanted into three sediment types: (1) the southern, well consolidated, Zuiderzee deposit of Holocene origin (10-50 cm depth), (2) the soft clay-rich layer which is found on top of this deposit (0-10 cm depth), and (3) a soft clay-rich layer from the northern part of the lake (0-10 cm depth). Roots, stems, leaves, and flowers were harvested after 28, 56, and 84 days.



Key Figure

Surface composition in terms of Fe, K, and P on roots of *P. australis* in the control (a), low (b), medium (c) and high (d) contents of iron-bound phosphorus in the sediment. Colors indicate presence of Fe (red), K (blue), and P (green). Saaltink et al., 2017.

(Brix et al. 1996; Dickopp et al. 2011; Tercero et al. 2015) causes iron to be precipitated on plant roots (St-Cyr and Crowder 1989). Interestingly, the iron-bound phosphorus content (Fe-P) of the sediment determined the composition of the iron plaque on the plant roots: phosphorus co-precipitated with Fe in high Fe-P sediment. No substantial amounts of iron plaque were formed on the roots of *R. maritimus* and *E. cannabinum* did not produce any iron plaque on its roots. Third, plaque formation of *P. australis* caused stoichiometric imbalances in leaves, likely due to inhibited translocation of P to the leaves. This observation is in concordance with findings of Snowden and Wheeler (1995) and Xu et al. (2009), who showed that co-precipitation of P induces a higher uptake of P by the roots but severely reduces the translocation of P to the shoot. *Rumex maritimus*, considered an Fe-tolerant species, was able to translocate more P to its leaves under Fe-P rich conditions than *P. australis*.

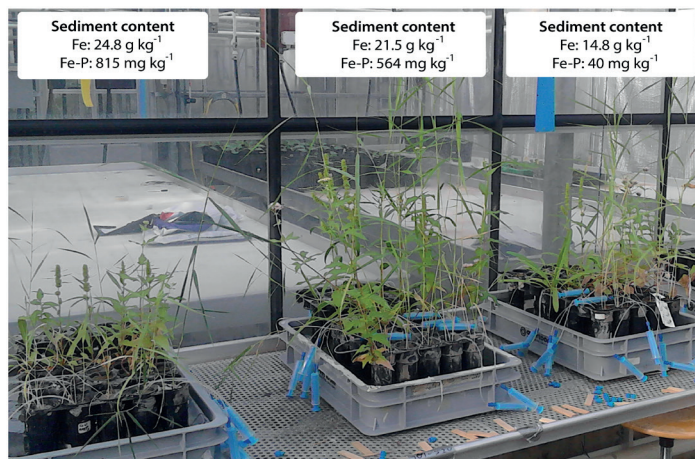


Figure 7.2. Aboveground biomass of three wetland plants grown in three different substrates from Markermeer differing in iron-bound phosphorus content in the sediment.

These observations do not support the hypothesis that indirect iron toxicity is a major problem in our experiments. However, the significantly raised iron concentrations of all three test species indicate that direct iron toxicity following the uptake of iron cannot be excluded as a possible explanatory mechanism of inhibited aboveground growth.

7.2.2 Wetland–terrestrial processes influencing nutrient availability – land–water zone

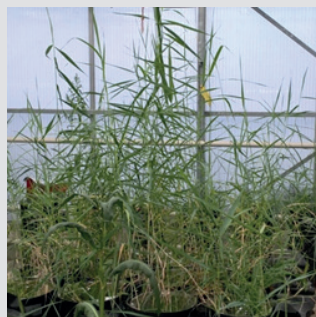
In Chapter 4, I studied how the hydrological regime could interfere with ecosystem development, especially in terms of nutrient availability. The aim and set-up of this experiment are summarized in Box 7.3. We found that periodic inundation resulted in N limitation of *R. maritimus*. This was not the case for *P. australis*, likely because this plant species has high radial oxygen loss (Brix et al., 1996; Dickop et al., 2011; Tercero et al., 2015), which sustains an oxygenated zone around the roots under waterlogged conditions. Periodic inundation furthermore led to enhanced P acquisition in *P. australis*, whereas for *R. maritimus* no significant effect could be discerned. The combined effects of periodic inundation on the N and P availability for both species led to the conclusion that in wet conditions *P. australis* is highly competitive because it keeps N available and increases the uptake of P. Two mechanisms might explain the positive effect of periodic inundation on the P acquisition for *P. australis*. The first is that decreased oxygen concentrations in the sediment can be an advantage when grown in iron-rich sediments, as they reduce iron plaque formation on roots. In Chapter 3, I showed that *P. australis* in particular produces iron plaque in oxic conditions and that this primarily affected the uptake of P. The second mechanism is possible because the sediment contains high amounts of pyrite and iron-bound phosphorus and therefore alternating water regimes can induce P mobilization (Satawathanont et al., 1991; Portnoy, 1999). This is because reduction of sulfate leads to P mobilization in reducing conditions, as Fe(II) favors sulfur over phosphorus in mineral precipitation.

Although the difference in total P content in the soft clay-rich layer (0–10 cm depth; 361 mg kg⁻¹) and the underlying Zuiderzee deposit (10–50 cm; 213 mg kg⁻¹) is significant, it did not affect the P tissue concentrations in either *R. maritimus* or *P. australis*. However, plants grown in the soft clay-rich layer had significantly higher N tissue concentrations compared with the concentrations in plants grown in the Zuiderzee deposit. To summarize, the results presented in Chapter 4 show that the P availability is determined by the hydrological regime, while the N availability is determined by both the hydrological regime and the sediment type.

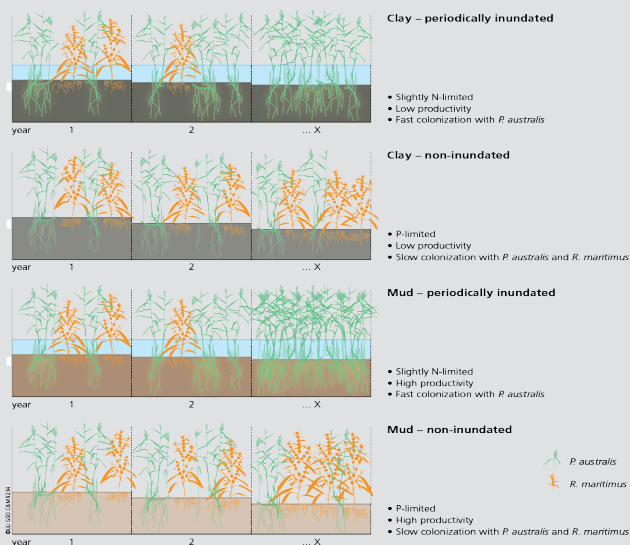
Box 7.3. Summarized aim and set-up of the experiment presented in Chapter 4.

Aim To understand how the hydrological regime interferes with ecosystem development and what type of sediment functions best as building substrate.

Experimental Set-up A greenhouse experiment was conducted for two growing seasons at the greenhouse test facility of Utrecht University in the period April 2015 – November 2016. Mesocosms were filled up to 50 cm with one sediment type from lake Markermeer: mud (0-10 cm) or clay (10-50 cm). Two species were selected to be transplanted in the mesocosms: *Rumex maritimus* and *Phragmites australis*. Mesocosms were either periodically inundated or received weekly irrigation water. Plant tissue and sediment geochemistry were monitored during the experiment.



Key Figure



Schematic summary of main effects on ecosystem functioning based on each treatment used in this study (Saaltink et al., 2018).

7.2.3 Effects of plants on sediment consolidation – terrestrial zone

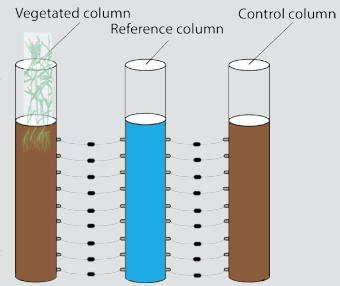
In Chapter 5, I studied how *P. australis* can act as an ecological engineer to enhance drainage in soft cohesive sediment, thereby promoting sediment consolidation. The aim and set-up of this experiment are summarized in Box 7.4. Because the water level in the experiment was fixed, the effects of evapotranspiration on pore pressure could precisely be seen but could not be directly linked to sediment consolidation. I explicitly assume that evapotranspiration will lead to sediment consolidation. This is because the hydraulic conductivity is low in cohesive sediments (Van den Akker and Soane, 2005) and evapotranspiration will lead to net water removal.

The results in Chapter 5 show that after an initial phase in which self-weight consolidation dominated (i.e., positive excess pore water pressures), pore pressure dropped below the hydrostatic pressure. *Phragmites australis* effectively altered the pore pressure gradient via water extraction, especially between 17 and 37 cm below the water table. In this zone, daily cycles in pore pressures which could directly be linked to the diurnal cycle of stomatal gas exchange were observed. Moreover, *P. australis* increased the hydraulic conductivity in the sediment by 40% compared to bare soil ($1.2 \times 10^{-9} \text{ m s}^{-1}$ for the vegetated column and $0.8 \times 10^{-9} \text{ m s}^{-1}$ for the control column). The average evapotranspiration rate of 3.95 mm day^{-1} closely coincides with average evapotranspiration rates in European wetland systems, such as the reedbeds in the Teesmouth Estuary in England (3.74 mm day^{-1} ; Fermor et al. 2001) and the Biebrza wetlands in Poland ($3.00\text{--}3.50 \text{ mm day}^{-1}$; Siedlecki et al. 2016). Therefore, the data acquired from this experiment can be used to model consolidation in artificial wetlands created on soft clay-rich material. The results presented in Chapter 5 can be used for developing a predictive plant–sediment model to provide additional insights into the usefulness of ecological engineers such as *P. australis* to enhance sediment consolidation.

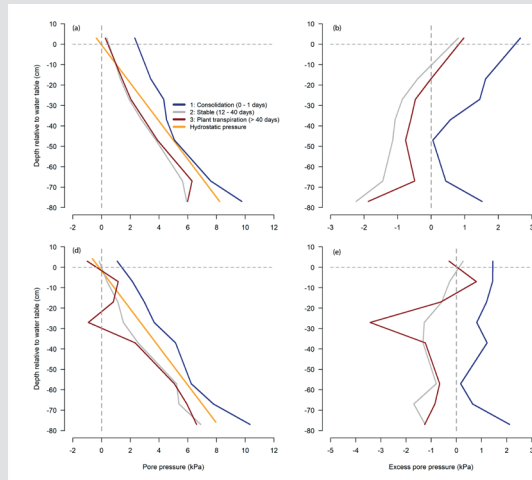
Box 7.4. Summarized aim and set-up of the experiment presented in Chapter 5.

Aim To study how and to what extent *P. australis* increases drainage in soft cohesive sediment, thereby promoting consolidation.

Experimental Set-up A consolidation experiment was conducted in Perspex (methyl methacrylate) columns. In total, six Perspex columns were used in two experiments. Four columns with plants were harvested in experiment 1 to determine root and shoot variables; in experiments 1 and 2 two columns were left intact and used for pore pressure measurements. One of the two columns for pore pressure measurements was left unplanted, the other was planted with *P. australis*. The columns used for pore pressure measurements were installed with differential pressure sensors at 0.4, 10.4, 20.4, 30.4, 40.4, 50.4, 60.4, 70.4, and 80.4 cm from the base of the column.



Key Figure



Pore pressure (kPa) relative to the water column in the control column (a) and vegetated column (d) as well as excess pore pressure (pore pressure minus hydrostatic pressure) in the control column (b) and the vegetated column (e).

7.2.4 Bioturbation – aquatic zone

In Chapter 6, I investigated the potential effect of bioturbating Tubificidae on the nutrient availability in the soft clay-rich sediment from lake Markermeer. In addition, I identified which biogeochemical processes become altered in the topsoil via aeration by bioturbating Tubificidae. The aim and set-up of this experiment are summarized in Box 7.5. Both the measured and modelled results showed that bioturbation by Tubificidae effectively aerated the upper layer of the sediment. The model presented in Chapter 6 predicts an optimum of 12,000 worms m^{-2} and showed that the effect of bioturbation on oxidation first increases with increasing densities and then, after the optimum density of 12,000 worms m^{-2} is exceeded, the aeration effect of Tubificidae diminishes as burrows start overlapping geometrically, while the respiration rate per worm stays the same. These opposing mechanisms explain why oxygen concentrations follow an optimum curve with increasing worm density. The aeration effect of bioturbating Tubificidae effectively altered biogeochemical processes in the upper layer of the sediment, thereby influencing nutrient availability. An increase was found for the NH_4 , and NO_x concentrations as a result of increased rates of ammonification and nitrification, whereas a decrease was found for the PO_4 concentrations as a result of P immobilization.

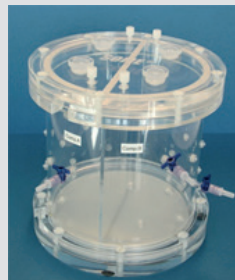
7.3 COMPARING THE GEOCHEMICAL COMPOSITION OF MARKERMEER SEDIMENTS

The geochemical composition of the sediment used in the experiments appears to be a major factor determining the geochemical processes described in Section 7.2. The sediment used in the experiments presented in Chapters 2 and 3 was collected in June 2014 in the southern part of the lake (coordinates 52.3795°N; 5.0209°E), whereas the sediment used in the experiments presented in Chapters 3, 4, and 6 was collected in March 2015 in the northern part of the lake (coordinates 52.5462°N; 5.3878°E). When the geochemical composition of the northern and southern sediments was properly analyzed, we were surprised by the heterogeneity in elemental contents. To assess the relevance of the findings to the Marker Wadden project, the geochemical composition of the sediment in the basins of the islands had to be determined and compared.

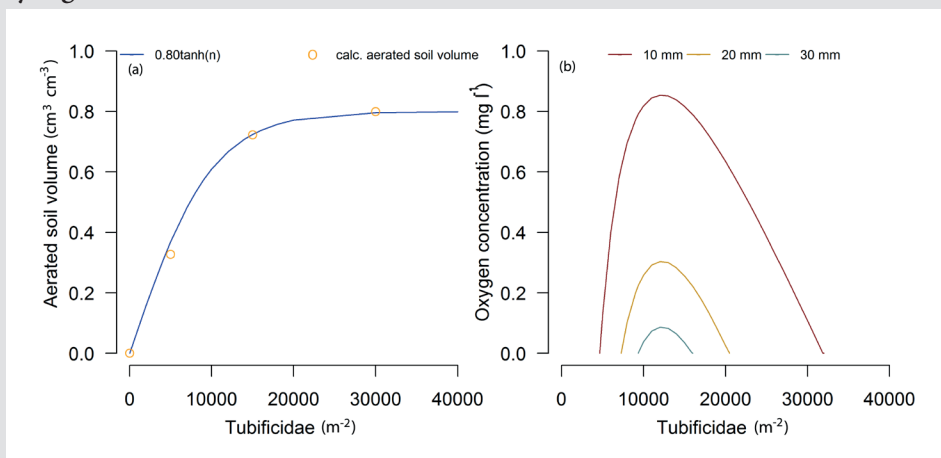
Box 7.5. Summarized aim and set-up of the experiment presented in Chapter 6.

Aim To investigate the potential effect of bioturbating Tubificidae on the nutrient availability in the soft clay-rich sediment from lake Markermeer.

Experimental Set-up This study was performed with an experimental technique introduced as Sediment Or Fauna Incubation Experiment, or SOFIE® (Vink, 2002). At the start of the experiment, Tubificidae were added to three of the four compartments at three densities: 5,000, 15,000, and 30,000 Tubificidae per m^2 surface. Pore water and surface water were collected from the probes on days 0, 7, 14, and 28 and chemically analyzed.



Key Figure



Calculated average aerated soil volume (cm^3) for 5,000 worms m^{-2} , 15,000 worms m^{-2} , and 30,000 worms m^{-2} (orange circles) and modeled aerated soil volume (cm^3) per number of Tubificidae (m^{-2}) (dark blue) (a). The modeled burrow function corresponds to a tangent-hyperbolic function ($0.80 \tanh(n)$) and was used to model oxygen concentration in the sediment (mg l^{-1}) per number of Tubificidae (m^{-2}) for three depths: 10 mm (red), 20 mm (yellow) and 30 mm (blue) (b).

In July 2017, therefore, 18 sediment samples were collected in the basins of the Marker Wadden by Marloes van den Akker (Radboud University Nijmegen, the Netherlands). The samples were analyzed in the laboratory facility of Utrecht University following the same procedure described in Saaltink et al. (2016, 2017).

Table 7.1 clearly shows that the geochemical composition of the Marker Wadden sediment is different from the sediment used in my experiments. Interesting differences include the content of iron, which in the Marker Wadden sediment is significantly higher than in the sediments used in my experiments (34.37 g kg^{-1} versus $6.54\text{--}27.77 \text{ g kg}^{-1}$). A remarkable significant difference was found for the content of sulfur: it was approximately three to four times higher in the Marker Wadden sediment than in the sediments used in my experiments. The mineralogical composition of the Marker Wadden sediment has not been assessed, so it remains unknown whether the high content of sulfur indicates elevated contents of pyrite. This might be important to know, especially because the content of calcium is significantly lower in Marker Wadden sediment (35.12 g kg^{-1}) than in the sediments collected from the south of the lake ($45.64\text{--}48.03 \text{ g kg}^{-1}$). The calcite content was not measured, but it is likely that most of the Ca content occurs as calcite, with the minority of the Ca content occurring as Al silicates or as exchangeable Ca. If calcite contents are low and pyrite contents are high, this might lead to a drop in pH when the sediment in the basins is oxidizing. In July 2017, the average pH measured in the basins was 7.4. This is almost the same as the average measured pH reported in Chapters 2 and 3 (pH 7.5).

Comparing the geochemical composition of the sediments used in the experiment to the sediment in the basins of the Marker Wadden underlies the importance of the potential effects of direct and indirect iron toxicity discerned in Chapters 2 and 3. These are highly relevant for the Marker Wadden as well, especially because the total iron contents are highest in the Marker Wadden sediment. Moreover, the Marker Wadden sediment is similar to the soft clay-rich sediment from the south in terms of the contents of iron-bound phosphorus, phosphorus and nitrogen. Therefore, iron plaque can be expected to form around the roots of *P. australis* with some co-precipitation of phosphorus (see Figure c in Box 7.2).

As the sediment in the basins of the Marker Wadden is rich in iron-bound phosphorus, the effects of periodic inundation discerned in Chapter 4 are relevant.

Periodic inundation can have a positive effect on the P acquisition of *P. australis* in the Marker Wadden sediment due to expected iron plaque formation in non-inundated conditions. However, the effect of periodic inundation on the acquisition of N in *R. maritimus* is questionable due to the large difference in total N content between the sediment used in the experiment (617–1611 mg kg⁻¹) and the Marker Wadden sediment (3270 mg kg⁻¹). Due to the expected positive effect on the P acquisition of *P. australis* in the Marker Wadden sediment, I still expect that *P. australis* outcompetes *R. maritimus*, as was concluded in Chapter 4. Furthermore, nutrient limitation may not be expected on the islands, as there the N, P, and K contents are high (3270 mg kg⁻¹ for N, 660 mg kg⁻¹ for P and 5491 mg kg⁻¹ for K).

Chapter 6 focused on the aquatic zone, where sediment aeration occurred via bioturbating Tubificidae. Although bioturbation is a significant aeration process, the concentration of oxygen entering the sediment is less than in terrestrial or wetland–terrestrial zones via radial oxygen loss by *P. australis*. For example, inverse modeling in PhreeqC showed that plants in terrestrial conditions aerated the mud with up to 215 μmol O₂ l⁻¹ day⁻¹ (Chapter 2, Table 3), whereas the Tubificidae in aquatic conditions aerated the mud with up to 91 μmol O₂ l⁻¹ day⁻¹ (Chapter 6, Table 3). The biogeochemical processes in both experiments are the same, despite the large difference in geochemical composition (Table 7.1). This presumes that the same biogeochemical processes are relevant in sediments from Markermeer with different geochemical compositions.

Chapter 5 focused on physical processes, rather than on biogeochemical processes. We did not assess the geochemical composition of the sediment used in the experiment presented in Chapter 5, but we know that the sediment was collected in the area from which material for building the Marker Wadden was extracted. We are aware that *P. australis* induces biogeochemical processes, in turn inducing pedogenic processes that accelerate the maturation or ripening of the soil (e.g. Pons and Zonneveld 1965; Barciela Rial et al. *to be submitted*). Thus, the geochemical composition can affect the consolidation behavior of the sediment. However, the drainage effect of plants measured in the experiment can still be translated into useful recommendations.

Table 7.1. The geochemical composition of the sediment collected in the basins of the Marker Wadden and the sediment types used in experiments 2 and 3. Significant differences between the sediments used in this study and the Marker Wadden are indicated by * ($p<0.05$).

	Unit	Marker Wadden		Chapters 3, 4, 6		Chapter 4		Chapters 2, 3		Chapters 2, 3			
		Mean	S.D.	Markermeer north 0-10cm n = 5	S.D.	Markermeer north 10-50cm n = 5	S.D.	Markermeer south 0-10cm n = 15	S.D.	Markermeer south 10-50cm n = 15	Mean	S.D.	
<i>Aqua regia</i>													
Al	g kg ⁻¹	36.37	14.00	10.40*	0.22	4.67*	0.36	16.59*	3.13	21.99*	4.51		
Ca	g kg ⁻¹	35.12	7.60	48.06*	1.29	25.87*	1.66	45.63*	6.02	48.03*	3.03		
Fe	g kg ⁻¹	34.37	8.92	14.77*	0.23	6.538*	0.39	20.75*	2.99	27.77*	3.76		
K	mg kg ⁻¹	5491	1549	2619*	72	1251*	106	4102*	641	5371	1262		
Mg	mg kg ⁻¹	8683	2085	5106*	135	2674*	128	6636*	906	8041	1017		
Mn	mg kg ⁻¹	882	294	360*	10	161*	9	577*	160	710	166		
N	mg kg ⁻¹	3270	813	1611*	32	617*	62	2833	191	2663*	41		
Na	mg kg ⁻¹	1763	524	97*	34	49*	13	526*	158	992*	379		
P	mg kg ⁻¹	660	193	361*	10	213*	7	649	169	1186*	217		
S	g kg ⁻¹	21.74	6.22	4.51*	0.19	1.19*	0.11	5.59*	0.70	5.73*	0.71		
Sr	mg kg ⁻¹	99	21	113*	3	70*	5	135*	26	148*	21		
Ti	mg kg ⁻¹	519	77	396*	17	380*	23	312*	77	312*	74		
Zn	mg kg ⁻¹	67	19	130*	3	88*	5	110*	29	159*	58		

Table 7.1. (continued).

Unit	Marker Wadden		Chapters 3, 4, 6		Chapter 4		Chapters 2, 3		Chapters 2, 3	
	Mean	S.D.	Markermeer north 0-10cm n = 5	S.D.	Markermeer north 10-50cm n = 5	S.D.	Markermeer south 0-10cm n = 15	S.D.	Markermeer south 10-50cm n = 15	S.D.
<i>Seq. P extraction</i>										
Exchangeable P	5	4	5	2	3*	1	12*	4	14*	7
Fe-bound P	291	155	40*	10	14*	2	279	62	772*	263
Ca-bound P	88	28	89	18	42*	8	121*	31	146*	43
Detrital P	111	22	158*	14	148*	14	169*	14	147*	17
Organic P	65	22	47*	11	16*	1	117*	25	100*	20
<i>Other</i>										
Organic matter	8.9	2.6	4.0*	0.1	2.6*	0.2	7.2*	0.6	6.7*	0.6

To summarize, the comparison of the sediments used in the experiments with the Marker Wadden sediment makes it clear that care must be taken when translating the results from Chapters 2-6 into practical recommendations for the Marker Wadden project. Despite the differences in terms of geochemical composition described in this section, the findings described are still relevant.

7.4 FROM MICROSCALE TO MACROSCALE: UPSCALING RESULTS TO FIELD CONDITIONS

In this section, I will first briefly discuss why findings from greenhouse experiments and laboratory experiments provide useful information for large-scale projects. Then, I will present data that we collected during two field trips to the NMIJ wetland, a pilot project in Markermeer. This data is compared with the results of the mesocosm experiment conducted in the greenhouse facility (Chapter 4).

7.4.1 Effectiveness of small-scale experiments

Predicting key wetland ecological processes in the field via small-scale experiments is difficult. Although relevant processes can be identified in microcosm and mesocosm experiments – like the experiments presented in Chapters 2-6 – the importance of those processes cannot be precisely assessed at a large scale (Roush, 1995; Pace, 2001; Odum and Odum, 2003). Not all abiotic and biotic factors are taken into account in controlled experiments. For example, factors like wind and wave action, interspecific competition between more than two species, and topography were not taken into account in this study. Nevertheless, small-scale experiments are effective in fine-tuning ecological engineering designs, as the main processes in the eco-engineered ecosystem can be anticipated (Odum and Odum, 2003). Moreover, conducting field research does not guarantee that all important biogeochemical and wetland ecological interactions will be identified, especially when the experiment lasts for only several years (Beyers and Odum, 1993; Kemp et al., 2001). Mitch and Wilson (1996) have argued that a created wetland ecosystem needs at least 15-20 years of maturation before the success of the eco-engineering design can be assessed, as the initial conditions of created ecosystems are often far from steady-state. To summarize, field

monitoring and field experiments should take place for much longer than is often the case, but in the meantime, small-scale and short-term experiments can be conducted to identify relevant processes that may improve ecological engineering designs.

7.4.2 Field research

To investigate the technical feasibility of constructing a large wetland with soft clay-rich material, a small 10 ha wetland has been constructed between the cities of Lelystad and Enkhuizen. This pilot project was part of the applied research program Natuurlijk(er) Markermeer IJmeer (NMIJ) (Dankers et al., 2015). Although this NMIJ wetland has some similarities with the Marker Wadden (e.g., islands in Markermeer constructed from soft clay-rich material), it has some important differences as well, like the method of construction (hydrological dredging for the Marker Wadden vs. mechanical dredging for the NMIJ wetland). Moreover, the building material for the NMIJ wetland was collected from the Amsterdam-Lemmer inland waterway with no additional mixing of Markermeer water (Wichman, 2015). Therefore, the initial sediment used in the NMIJ wetland is more consolidated than the initial sediment used for the Marker Wadden and in our experiments.

In 2014 and 2015, vegetation development on the NMIJ wetland was monitored (Van Kerkvoorde, 2015). In 2014, the dominant plant species were *R. maritimus*, *P. australis* and *Tephrosieris palustris* (Marsh fleawort). However, *P. australis* declined significantly in 2015, with presence of *R. maritimus* staying more or less the same. Van Kerkvoorde (2015) suggested that a difference in water level caused the shift in vegetation development. In 2015, the surrounding surface water level was higher due to consolidation of the sediment, which increased the amount of *R. maritimus*. This strengthens our conclusion from Chapter 4 that the hydrological regime is an important tool for controlling nutrient availability, which in turn influences species composition.

We complemented the observations reported in Van Kerkvoorde (2015) by collecting plant material in plots of 0.16 m² in August 2015 and July 2016. We only sampled monocultures. We harvested three plots of *P. australis* and three plots of *R. maritimus* in August 2015. *Phragmites australis* was no longer present in July 2016. Dominant species frequently found were *R. maritimus*, *Anthemis arvensis* (Corn

chamomile), and *Chamerion angustifolium* (Rosebay Willow-herb). The collected plant material was air-dried for 48 hours at 70 °C, weighed, clipped, and mixed prior to chemical analysis. Tissue was randomly selected and ground to determine contents of K and P using total reflection X-ray fluorescence (S2 Picofox, Bruker) and N using a CN analyzer (NA1500, Fisons Instruments). We also collected sediment samples and determined elemental contents of Al, Ca, Fe, K, Mg, Mn, Na, P, Sr, Ti, and Zn via ICP-OES following aqua regia destruction. We also performed a sequential extraction method based on Ruttenberg (1992) to characterize solid P speciation.

Table 7.2. Elemental concentrations (n = 1) and phosphorus fractions (n = 3) of the sediment (coordinates 52.6094°N; 5.4307°E) in the NMIJ wetland.

		Mean	S.D.
<i>Aqua regia</i>			
Al	g kg ⁻¹	10.48	-
Ca	g kg ⁻¹	44.63	-
Fe	g kg ⁻¹	15.42	-
K	mg kg ⁻¹	2647	-
Mg	mg kg ⁻¹	5526	-
Mn	mg kg ⁻¹	392	-
Na	mg kg ⁻¹	470	-
P	mg kg ⁻¹	411	-
S	mg kg ⁻¹	5935	-
Sr	mg kg ⁻¹	121	-
Ti	mg kg ⁻¹	300	-
Zn	mg kg ⁻¹	31	-
<i>Seq. P extraction</i>			
Exchangeable P	mg kg ⁻¹	12	7
Fe-bound P	mg kg ⁻¹	221	117
Ca-bound P	mg kg ⁻¹	83	12
Detrital P	mg kg ⁻¹	127	10
Organic P	mg kg ⁻¹	67	37

Table 7.2 presents the elemental concentrations of the sediment in the NMIJ wetland as well as the phosphorus fractions. Table 7.3 presents the biomass and tissue concentrations of plants collected in the NMIJ wetland, as well as the average biomass and tissue concentrations of the plants harvested in the mesocosm experiment presented in Chapter 4.

Table 7.3. Aboveground biomass (g m^{-2}) and plant tissue concentrations (%) with associated N:P ratios with standard errors. Plots of 0.16 m^2 were cleared of vegetation on the NMIJ wetland in 2015 and 2016.

Monoculture	n	Biomass	N	P	K	N:P
NMIJ wetland 2015						
<i>Phragmites australis</i>	3	1437 ¹⁸⁸	3.12 ^{0.33}	0.21	1.83 ^{0.02}	15.5 ^{0.35}
<i>Rumex maritimus</i>	3	873 ²⁰⁴	2.23 ^{0.31}	0.23	1.89 ^{0.04}	10.8 ^{0.32}
NMIJ wetland 2016						
<i>Chamerion angustifolium</i>	3	1040 ¹⁸⁸	2.72 ^{0.05}	0.27	2.92 ^{0.04}	10.7 ^{0.68}
<i>Anthemis arvensis</i>	3	860 ²⁴⁶	4.31 ^{0.02}	0.32	3.04 ^{0.06}	14.5 ^{0.17}
<i>Rumex maritimus</i>	4	1279 ¹¹⁵	3.20 ^{0.18}	0.33	3.06 ^{0.06}	10.9 ^{0.73}
Average values from the experiment presented in Chapter 4						
<i>Phragmites australis</i>	47	345 ²⁹	1.56 ^{0.05}	0.11	1.58 ^{0.01}	17.5 ^{0.04}
<i>Rumex maritimus</i>	42	311 ³⁰	1.55 ^{0.10}	0.22	2.70 ^{0.02}	9.3 ^{0.17}

Table 7.3 shows that the aboveground biomass production in the NMIJ wetland is higher than that of the plants grown in the mesocosm experiment (Chapter 4). This difference is explained by the initial conditions of the experiment. Only three seedlings of *P. australis* and *R. maritimus* were transplanted in the mesocosms, which had a surface area of 0.07 m², whereas the plants in the NMIJ wetland had already started to grow in 2014 and by 2015 and 2016 had formed dense stands. Another notable difference is observed in tissue N and P contents between plants grown on the NMIJ wetland (2.72-4.31% for N and 0.21-0.33% for P) and the plants grown in the mesocosm experiment (1.55-1.57% for N and 0.11-0.22% for P). Although the sediment N content of the NMIJ wetland is unknown, the high N tissue concentrations suggest that sufficient N in the sediment is available for plants. The tissue concentrations indicate that neither N nor P is limited: tissue N concentrations are above the critical threshold for N limitation (c. 1.40%; cf De Wit et al., 1963) and tissue P concentrations are above the critical threshold for P limitation (c. 0.07%; cf De Wit et al., 1963). This comparison also shows that the geochemical composition of the sediment type is an important factor influencing ecosystem development.

7.5 PRACTICAL RECOMMENDATIONS FOR THE MARKER WADDEN PROJECT

Although this thesis presents fundamental research that aims to elucidate how biogeochemical and wetland ecological processes interact to enhance ecosystem development on wetlands built from soft clay-rich material in general, the generated knowledge summarized in Section 7.2 is sufficiently robust for formulating some practical recommendations for the Marker Wadden project. Moreover, comparing the geochemical composition of the sediments used in the experiments with the geochemical composition of the Marker Wadden sediment made it clear that the findings reported in Section 7.2 are at least partly relevant. Here, I present five recommendations based on findings presented in the previous chapters. These recommendations may solve some of the issues that surfaced during the wetland construction phase, such as failure of *P. australis* to establish in the growing season of 2017, even though the species was planted in the basins, using hovercrafts. Moreover, these recommendations give relevant directions for correctly implementing the initial

plan to use soft clay-rich sediment as building material instead of the Holocene deposits used for the first 1,000 ha of wetlands.

Recommendation 1: *Study the biogeochemical properties – especially the amount of Fe(III) and iron-bound phosphorus – of the sediment and the feedback mechanisms between plant and soil, prior to finalizing the design of the future islands.* Chapters 2 and 3 pointed out that the geochemical composition of the sediment may have a large influence on the biogeochemical processes, in turn affecting ecosystem development. Moreover, Section 7.3 showed the heterogeneity in elemental contents of Markermeer sediments. Therefore, the biogeochemical properties of the situated sediment should be analysed prior to finalizing the design of the eco-engineering project.

Recommendation 2: *Use the soft clay-rich sediment as building material, instead of sediment excavated at greater depths.* It is currently being investigated if sufficient clay-rich material accumulates in gullies and pits for soft clay-rich material to be efficiently dredged for islands to be built in the future. If this is the case, it is expected that the geochemical composition of the building material is somewhat similar to the geochemical composition of the sediment collected in the north (at 0-10 cm depth). Deep sediment was used to create the first 1,000 ha of islands; this sediment contains higher concentrations of iron and sulfur and significantly lower concentrations of calcium (Table 7.1). Moreover, above and belowground biomass production of *P. australis* – the eco-engineering species on the islands – is high compared to biomass production of *P. australis* experimentally grown in the sediment that contained highest concentrations of iron and iron-bound phosphorus (Chapter 3). Initial high belowground biomass production is especially important when building terrestrial ecosystems from soft mud, as roots have the ability to stabilize sediments and reduce sediment degradation (Ganthy et al., 2011; Edmaier et al., 2012). This is also true for aboveground biomass, as leaves and stems reduce shear stress on the substrate by attenuating waves (Nepf, 2012).

Recommendation 3: *Apply periodic inundation to promote ecosystem development on the islands. Management of the hydrological regime opens possibilities for manipulating*

ecosystem development on the islands. The hydrological regime will determine the nutrient availability for plants to a large extent, in turn influencing the species assemblage of the future wetland and the succession that occurs (e.g., Bedford et al., 1999). Managing the hydrological regime is possible, as long as the ring dikes surrounding the individual islands are intact. Periodic inundation can be applied to promote fast colonization by the target species *P. australis*. In wet conditions *P. australis* outcompetes *R. maritimus* – a dominant plant species found on the pilot island of the Marker Wadden (Dankers et al., 2015) – because it acquires essential nutrients more effectively. This recommendation is further supported by the observation that on the NMIJ wetland *P. australis* declined from 2014 to 2015 and disappeared completely in 2016, likely as a result of a change in relative water level (Van Kerkvoorde, 2015; Section 7.4.2).

Recommendation 4: *Plant the basins with *P. australis*, in combination with managing the hydrological regime to promote fast colonization.* In Chapter 5, we showed that *P. australis* effectively removed water from the sediment via its roots. The overall stability of the sediment is expected to be improved significantly up to the depth at which roots are extracting the bulk of the water to compensate for leaf transpiration. In our experimental set-up, this was the case for the first 44 cm of the sediment. This highlights the plant's role as an eco-engineer to accelerate drainage, in turn promoting sediment consolidation and soil ripening. As conventional drainage techniques (e.g., prefabricated vertical strip drains) are relatively expensive, are invasive, and may degrade the natural value of the system, planting the basins with *P. australis* is recommended.

Recommendation 5: *Artificially colonize the sediment in the littoral zone to the optimum density of 12,000 bioturbators m⁻².* In Chapter 6 we showed that bioturbation effectively increases nitrogen concentrations in the pore water and surface water and that the optimal worm density is 12,000 worms m⁻². Since the macrofauna density in lake Markermeer currently amounts to about 5,000 Annelida m⁻², colonizing the sediment in the littoral zone of the created wetlands with about 7,000 Tubificidae m⁻² might enhance initial ecosystem development due to improved availability of

N. However, when macroinvertebrates other than Annelida are partly aerating the sediment, colonizing the sediment at a lower density is preferable. However, it must be noted that the precise effect of introducing Tubificidae into the littoral zone is hard to predict, as in reality, hydrodynamic factors such as wave action with associated resuspension and settling also determine sediment oxidation. These environmental factors were absent from the experiment presented in Chapter 6.

The abovementioned recommendations are largely in line with ecological engineering principles. Some initial conventional engineering investment is required – for example to plant the basins with *P. australis* and to manage the hydrological regime. These forms of human intervention steer natural processes, such as nutrient availability. However, the likelihood of the eco-engineering project succeeding may increase if much of the design is determined by the ecosystem itself (i.e., ecosystem self-design) (Mitsch, 2012). An example is multiple seeding, where seeds of several species are sown to enhance ecosystem development, but where the ecosystem itself determines which species ultimately establish. In the Marker Wadden project, seeds of a single plant species (i.e., *P. australis*) are sown in the basins. Although from the experiments presented in this study it is clear that *P. australis* is a suitable ecological engineer for the Marker Wadden project, it is known that in the longer term, introducing only one or several ecosystem engineers may be less successful (Mitsch and Wilson, 1996). I therefore recommend being aware of the benefits of ecosystem self-design when building additional islands in the future and would advocate sowing seed mixtures to improve the resiliency of the ecosystem.

7.6. SUGGESTIONS FOR FURTHER RESEARCH

The Marker Wadden consists of several lacustrine wetlands that have largely been built with soft clay-rich sediment. Building with fine sediment at this scale has never been attempted elsewhere in the world. As sand is becoming costly and sand demand will rise in the future (Torres et al., 2017), the Marker Wadden project is an important case study for optimizing ecological engineering designs that use fine sediment as an alternative building material. The uniqueness and the scientific and societal relevance

of the Marker Wadden project will no doubt lead to the wetlands becoming an important research arena in the future. Below, I present six suggestions for further research.

1. *Initial ecosystem development.* Fast ecosystem development is crucial when wetlands are built from soft clay-rich sediment, as the wetlands are susceptible to erosion by wind and wave action. At the moment, *P. australis* is planted in the basins to promote soil formation and ecosystem development. It is unknown to what extent the *P. australis* is contributing to ecosystem development in terms of plant diversity. To assess the suitability of *P. australis* as an ecological engineer in ecosystem development and restoration projects, it is advisable to monitor plant diversity in relation to soil formation in plots with and without planted *P. australis*. However, introducing only one or two ecosystem engineers may be less successful on longer time scales (Mitsch and Wilson, 1996). Alternatively, Odum and Odum (2003) recommend multiple seeding or multi-transplanting as a proper ecological engineering strategy. Strategies like these enable natural processes to become more important in determining the final design of the wetland, which also improves the resilience of the ecosystem (Odum and Odum 2003). Thus, the initial human investment in multiple seeding or multi-transplanting accelerates the development of resilient ecosystems. Therefore, I also suggest monitoring plant diversity and soil formation in plots where multiple seeding and/or multi-transplanting have been applied.

Establishment of pioneer vegetation in the growing season of 2018 has been monitored in the basins of the Marker Wadden where no multiple seeding or multi-transplanting has been carried out. The plant species that spontaneously emerge may be good candidates to include in the seed mixture. It was found that *Senecio vulgaris* (Groundsel), *Tephrosia palustris* (Marsh fleawort), and *Atriplex prostrata* (Spear-leaved orache) were abundantly present compared to other plant species in the basins of the Marker Wadden. *Rumex maritimus* (Golden dock), *Poa annua* (Annual meadow-grass), and *Rumex hydrolapathum* (Water dock) were occasionally present (De Bruin, 2018). Species that were rarely present include *Convolvulus arvensis* (Field bindweed), *Ranunculus sceleratus* (Celery-leaved buttercup), *Tussilago farfara* (Coltsfoot), *Carduus crispus* (Wetted thistle), *Rumex crispus* (Curly dock), *Persicaria*

maculosa (Redshank), and *Phragmites australis*. The knowledge generated from such monitoring studies may lead to improvements to ecological engineering strategies in projects where soft clay-rich sediment is used as building material for wetlands. The knowledge generated from such monitoring studies may lead to improvements to ecological engineering strategies in projects where soft clay-rich sediment is used as building material for wetlands.

2. *Wetland hydrology.* The success of a created wetland depends for a large part on the hydrology (Mitsch and Wilson, 1996). The importance of a suitable hydrology is pointed out by Erwin (1991), who judged 60% of the created wetlands in Florida to be a failure due to inappropriate water levels and/or flooding regimes. In Chapter 4, I showed how changing water levels promote colonization by *P. australis* and determine nutrient availability. Furthermore, Van Kerkvoorde (2015) suggested that a difference in water level caused a shift in vegetation development on the NMIJ wetland in Markermeer, favoring colonization by *R. maritimus* instead of by *P. australis*. Thus, incorporating a suitable wetland hydrology in the design of the Marker Wadden is likely a prerequisite for success. In the Marker Wadden project specifically, a ring dike was constructed around each island (Boskalis, 2015), to enable management of the wetland hydrology. In Section 7.5, I recommended manipulating the wetland hydrology to promote biodiversity and colonization of the eco-engineer *P. australis* as long as the ring dikes remain intact. However, these dikes will eventually be breached, to connect the islands to the Markermeer. Markermeer has a manipulated surface water level: it is kept high in summer and autumn (at -0.20 m below Dutch Ordnance Level (NAP)) and low in winter and spring (at -0.40 m below NAP) (Meijer et al., 2009). Creating wetlands that can cope with a non-natural surface water level is challenging, as this type of hydrology is often not beneficial for ecosystem development (Maarse et al., 2013). This calls for further research on ecological engineering designs that address the issue of how a successful wetland can be created in a system with a non-natural surface water level.

3. *Plant response in relation to sediment geochemistry.* In Section 7.3, I showed that the sediments from Markermeer are heterogeneous in terms of geochemical composition.

The standard deviations presented in Table 7.1 furthermore suggest that the sediment samples collected within the basins of the Marker Wadden are heterogeneous as well. It is likely that the intensity of the relevant processes identified in this study – such as pyrite oxidation with associated calcite dissolution and iron toxicity – may differ on a local scale. Therefore, I expect that the sediment geochemistry is affecting biomass production and biodiversity locally. Monitoring pore water chemistry, plant tissue concentrations, and plant diversity in plots with different soil compositions will lead to a better understanding of to what extent the biogeochemical properties of the sediment determine ecosystem development. In turn, effective project-specific ecological engineering strategies may be derived from microcosm and greenhouse experiments, especially when the building material for wetland creation is collected in geochemically heterogeneous environments.

4. *Bioturbation and mussel activity in relation to plant productivity.* In Chapter 6, I showed how bioturbation increased nitrogen concentrations in pore water and surface water. In oligotrophic systems like Markermeer (Noordhuis et al., 2014), additional input of available nitrogen may lead to enhanced productivity. As aboveground plant biomass dampens erosive stresses on the substrate by attenuating waves (Nepf, 2012), accelerating initial biomass production is important. However, no research has been conducted to test to what extent the additional input of nitrogen via bioturbation leads to increased biomass production. The relationship between bioturbation activity and biomass production of macrophytes or wetland plants such as *P. australis* could be tested in microcosm experiments. Given that the lake is especially limited in phosphorus (Noordhuis et al., 2014) and that in Chapter 6 I showed that bioturbation leads to reduced concentrations of phosphorus in the pore water, to significantly accelerate biomass production it may be important to also raise the phosphorus concentrations in the pore water and the surface water. Filter feeders like *Dreissena burgensis* and *Dreissena polymorpha* are known to increase phosphorus levels in the water column up to 40% by mobilizing phosphorus from suspended matter (Turner 2010; Ruginis et al. 2014). This process is especially relevant when iron-bound phosphorus is present in the sediments. That is because the gut of mussels is generally anoxic, thereby releasing phosphorus to the water column. As Markermeer

is rich in iron-bound phosphorus (14-772 mg kg⁻¹; Table 7.1) it may be relevant to also introduce mussels to the littoral zone of the islands to promote phosphorus availability. To summarize, testing the effects of mussels and bioturbation on plant and macrophyte biomass production by further enhancing the availability of nitrogen and phosphorus may improve ecological engineering techniques.

5. *Sediment consolidation via plant-mediated drainage.* In Chapter 5, I showed that *P. australis* removed water from the sediment via leaf transpiration. This led to pore pressure changes at depths between 17 and 37 cm below the water table. However, in our controlled microcosm experiment, important factors that influence drainage and consolidation behavior were not taken into account. As explained in Chapter 5, these factors include the topography and the depth of the water table. Next to the drainage effect, vegetation also induces biogeochemical processes which are among the pedogenic processes that accelerate the maturation or ripening of the sediment (e.g., Pons and Zonneveld 1965; Barciela Rial et al. 2018). At this moment, it is unknown how *P. australis* accelerates sediment consolidation in field conditions, especially in relation to pore pressure changes and sediment maturation. Measuring pressure heads in vegetated and non-vegetated plots in relation to physical sediment parameters like the dry bulk density may provide relevant information about the capabilities of *P. australis* – or plants in general – to effectively accelerate sediment consolidation.

6. *The influence of birds.* Increasing the number of birds and their diversity is often one of the objectives in ecological restoration programs. For example, one of the objectives of wetland restoration on Poplar Island in Chesapeake Bay, Maryland, was the resettlement of special status species such as the Bald eagle (*Haliaeetus leucocephalus*) (Derrick et al., 2007). Likewise, the core objective of the Marker Wadden project is to create a dynamic system rich in bird fauna (Verschoor and Rijdsdorp, 2012). In ecological engineering projects, birds can also act as ecological engineers, as they interfere with many biotic and abiotic processes. For example, Daborn et al. (1992) found that sediment cohesion is increased by birds, as bioturbation and grazing pressure on diatoms – which excrete extracellular polymeric substances that bind the sediment – decrease by predation on macrofauna. The

indirect influence of birds on the cohesion of clay particles may be an important factor on the islands of the Marker Wadden. Moreover, birds can function as biovectors, introducing large amounts of nutrients to ecosystems (e.g. Signa et al., 2012). These bird-derived nutrients may drive entire food web structures, which may result in either reduced or increased biodiversity (Vizzini et al., 2016). In fact, Greylag geese (*Anser anser*) and Black-headed gulls (*Chroicocephalus ridibundus*) in the Botshol Nature Reserve (The Netherlands) contribute 66% of the critical annual P load of the system (Rip et al., 2006). Moreover, grazing birds like geese can exert a strong grazing pressure on graminoid plants such as *P. australis* (Massé et al., 2001; Van den Wyngaert et al., 2003; Madsen et al., 2011), which may be an important factor determining plant development on the Marker Wadden. Therefore, I suggest studying how birds interfere in biotic and abiotic processes in ecological engineering projects where soft clay-rich sediment has been used as building material.

7.7. CONCLUDING COMMENT

At this moment, planetary boundaries are being crossed as a result of human activities (Steffen et al., 2015). Crossing such boundaries may have detrimental effects on the stable functioning of system Earth. Clearly, society is faced with serious sustainability challenges. Solving these challenges was not the aim of this study, but I would like to point out that ecological engineering should be embedded in the sustainability toolbox. Ecological engineering entails taking account of environmental protection, societal well-being, and economic development. These three topics, or pillars, are better known as the triple bottom line of sustainability. Projects that incorporate ecological engineering principles: 1) restore and create ecosystems, in turn improving the ecological value in the area, 2) provide ecosystem services, and 3) need less financial investment than conventional engineering projects (Mitsch, 1996; Odum and Odum, 2003; Temmerman et al., 2013). I strongly advocate applying ecological engineering as an alternative to conventional engineering when creating infrastructure. There are still many opportunities worldwide for ecological engineering, especially in light of current projections of population growth and urbanization. Cities can truly become seedbeds for innovation, possibly pushing

the field of ecological engineering forward. For example, conventional farming can partly be replaced by vertical farming in urban areas. Here, plant waste produced on higher floors provides food for livestock on lower floors. This form of urban farming, requires less space and increases crop production, while decreasing the amount of nutrients released to the environment. Furthermore, implementing ecological engineering techniques may overcome challenges when building infrastructure by using fine sediment as building material. Sand is rapidly becoming a scarce resource and sand mining is linked to many sustainability issues like biodiversity loss (Torres et al., 2017). With the research presented in this thesis I sincerely hope to have made a modest contribution to a better understanding of how fine sediment can be used in ecological engineering projects, thereby assisting the transition toward a sustainable world.

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Summary

Fine sediment – available in large quantities in many river delta's – may be used as an alternative to coarse sand for land reclamation. In this study, the Marker Wadden project functioned as a case for optimizing ecological engineering designs that use fine sediment as building material. The research presented in this thesis has highlighted (bio)geochemical, hydrological, and wetland ecological processes that interact and enhance ecosystem development on wetlands built on fine sediment. A combination of greenhouse and laboratory experiments were conducted. Some measured data from these experiments formed important input for subsequent analysis in a modeling environment. The findings presented in Chapters 2-6 can be divided into four topics: 1) Plant–soil interactions in the terrestrial zone, 2) wetland–terrestrial processes influencing nutrient availability in the land–water zone, 3) effects of plants on sediment consolidation in the terrestrial zone, and 4) effects of bioturbation on nutrient availability in the aquatic zone. The next sections give a summary of the results for these four topics. The last section summarizes the recommendations formulated for the Marker Wadden project.

Plant–soil interactions – terrestrial zone

In Chapter 2, (bio)geochemical processes in the clay-rich material and interactions between the sediment and *Phragmites australis* were identified. Key (bio)geochemical processes that influence pore water chemistry were found to be pyrite oxidation and associated calcite dissolution. *Phragmites australis* enhanced the oxidation process significantly by aerating its environment via radial oxygen loss (i.e., root aeration). During the experiment presented in Chapter 2, stunted plant growth and shriveling and yellowing foliage were observed. Several feedback mechanisms between the sediment and *P. australis* were identified. One is a negative feedback loop that arises because plant roots induce aeration, which promotes iron toxicity, which in turn decreases plant growth and results in plant death. There is also a positive feedback loop, as iron toxicity induces reduction processes as a result of root death, which leads to P mobilization and hence enhances plant growth and regeneration.

The iron-toxicity hypothesis posed in Chapter 2 was tested with a follow-up experiment described in Chapter 3. In this experiment, a clear impact was found of iron-rich sediment on biomass production. Moreover, analysis of root surface

composition showed that *P. australis* produced substantial amounts of iron plaque. High radial oxygen loss in this plant causes iron to be precipitated on plant roots. Interestingly, the iron-bound phosphorus content (Fe-P) of the sediment determined the composition of the iron plaque on the plant roots: phosphorus co-precipitated with Fe in high Fe-P sediment. We also found that the plaque formation caused stoichiometric imbalances in the leaves, likely due to inhibited translocation of P to the leaves. These observations did not support the hypothesis formulated in Chapter 2 that indirect iron toxicity is a major problem in our experiments. However, the significantly raised iron concentrations in the leaves indicate that direct iron toxicity following the uptake of iron cannot be excluded as a possible explanatory mechanism of inhibited aboveground growth.

Wetland–terrestrial processes influencing nutrient availability – land–water zone

In Chapter 4, it was studied how the hydrological regime and the sediment type interferes with ecosystem development, especially in terms of nutrient availability. Periodic inundation resulted in N limitation of *R. maritimus*. This was not the case for *P. australis*, likely because this plant species has high radial oxygen loss, which sustains an oxygenated zone around the roots under waterlogged conditions. Periodic inundation furthermore led to enhanced P acquisition in *P. australis*, whereas for *R. maritimus* no significant effect could be discerned. The combined effects of periodic inundation on the N and P availability led to the conclusion that in wet conditions *P. australis* is highly competitive because it more effectively acquires essential nutrients.

Although the difference in total P content in the soft clay-rich layer and the underlying Zuiderzee deposit was significant, it did not affect the P tissue concentrations in either *R. maritimus* or *P. australis*. However, plants grown in the soft clay-rich layer had significantly higher N tissue concentrations compared with the concentrations in plants grown in the Zuiderzee deposit. To summarize, the results presented in Chapter 4 show that the P availability is determined by the hydrological regime, while the N availability is determined by both the hydrological regime and the sediment type.

Effects of plants on sediment consolidation – terrestrial zone

In Chapter 5, it was studied how *P. australis* can act as an ecological engineer to enhance drainage in soft cohesive sediment, thereby promoting sediment consolidation. The results in Chapter 5 show that after an initial phase in which self-weight consolidation dominated (i.e., positive excess pore water pressures), pore pressure dropped below the hydrostatic pressure. *Phragmites australis* effectively altered the pore pressure gradient via water extraction, especially between 17 and 37 cm below the water table. In this zone, daily cycles in pore pressures which could directly be linked to the diurnal cycle of stomatal gas exchange were observed. Moreover, *P. australis* increased the hydraulic conductivity in the sediment by 40% compared to bare soil. The results presented in Chapter 5 can be used for developing a predictive plant–sediment model to provide additional insights into the usefulness of ecological engineers such as *P. australis* to enhance sediment consolidation.

Bioturbation – aquatic zone

In Chapter 6, the potential effect of bioturbating Tubificidae on the nutrient availability in the soft clay-rich sediment from lake Markermeer was identified. In addition, the (bio)geochemical processes that became altered in the topsoil via aeration by bioturbating Tubificidae were studied. Both the measured and modelled results showed that bioturbation by Tubificidae effectively aerated the upper layer of the sediment. The model presented in Chapter 6 predicts an optimum of 12,000 worms m^{-2} and showed that the effect of bioturbation on oxidation first increases with increasing densities and then, after the optimum density of 12,000 worms m^{-2} is exceeded, the aeration effect of Tubificidae diminishes as burrows start overlapping geometrically, while the respiration rate per worm stays the same. These opposing mechanisms explain why oxygen concentrations follow an optimum curve with increasing worm density. Furthermore, an increase was found for the NH_4 and NO_x concentrations as a result of increased rates of ammonification and nitrification, whereas a decrease was found for the PO_4 concentrations as a result of P immobilization.

Practical recommendations for the Marker Wadden project

Although this thesis presents fundamental research that aims to elucidate how (bio)geochemical and wetland ecological processes interact to enhance ecosystem development on wetlands built from soft clay-rich material in general, the generated knowledge summarized is sufficiently robust for formulating five practical recommendations for the Marker Wadden project. These recommendations may solve some of the issues that surfaced during the wetland construction phase and give relevant directions for correctly implementing the initial plan to use soft clay-rich sediment as building material instead of the Holocene deposits used for the first 1,000 ha of wetlands.

Recommendation 1: Study the geochemical properties – especially the amount of Fe(III) and iron-bound phosphorus – of the sediment and the feedback mechanisms between plant and soil, prior to finalizing the design of the future islands.

Recommendation 2: Use the soft clay-rich sediment as building material, instead of sediment excavated at greater depths.

Recommendation 3: Apply periodic inundation as long as the ring dikes are closed to promote ecosystem development on the islands.

Recommendation 4: Plant the basins with *P. australis*, in combination with managing the hydrological regime to promote fast colonization.

Recommendation 5: Artificially colonize the sediment in the littoral zone to the optimum density of 12,000 bioturbators m⁻².

The abovementioned recommendations are largely in line with ecological engineering principles. However, when natural processes can determine a large part of the final design of the ecosystem (i.e., ecosystem self-design), resilience of the wetland may be improved, thereby increasing the likelihood of the eco-engineering project to succeed. Therefore, it is recommended to be aware of the benefits of ecosystem self-design when wetlands are constructed following ecological engineering principles.

Nederlandse samenvatting

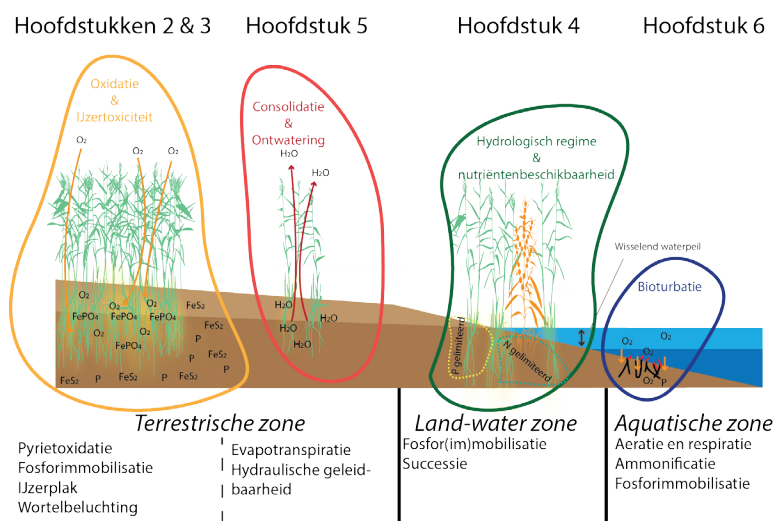
Kleirijk sediment wordt nog amper gebruikt als bouw materiaal voor landwinning, terwijl dit type sediment in overvloed aanwezig is in veel rivierdelta's. Bovendien is zand een schaars goed en zorgt de winning ervan voor veel milieuproblemen. Om het kleirijke materiaal als alternatief te kunnen gebruiken dienen de uitdagingen die daarmee gepaard gaan eerst te worden overwonnen. Zo moet ecosysteemontwikkeling snel op gang kunnen worden gebracht om erosie door wind en golfslag te voorkomen. Dit proefschrift richt zich op het Marker Wadden-project dat als casus dient voor kennisontwikkeling ten behoeve van het optimaliseren van bouwen met fijn sediment. Dit gebeurt in het kader van bouwen-met-natuur; een nieuwe manier van ontwerpen die natuurlijke processen benut waarbij technische en maatschappelijke doelen worden gediend. Het onderzoek richt zich op de (bio)geochemische, hydrologische en ecologische processen die ecosysteemontwikkeling versnellen in een waterrijk gebied, gebouwd van kleirijk sediment.

De experimenten die beschreven zijn in de hoofdstukken 2 t/m 6, zijn onder te verdelen in vier onderwerpen: 1) plant-bodem interacties in de terrestrische zone, 2) ecologische processen die de nutriëntenbeschikbaarheid beïnvloeden in de land-waterzone, 3) effecten van planten op de consolidatie van kleirijk sediment in de terrestrische zone, en 4) effecten van bioturbatie op de nutriëntenbeschikbaarheid in de aquatische zone. De uitkomsten van het onderzoek zullen aan de hand van deze vier onderwerpen worden samengevat. Zie Figuur 1 voor een conceptuele weergave van de relevante processen die beschreven zijn in de hoofdstukken 2 t/m 6. Aanbevelingen voor het Marker Wadden-project worden beschreven in de laatste paragraaf.

Plant-bodem interacties – terrestrische zone: Hoofdstuk 2 & 3

(Bio)geochemische processen in het kleirijke sediment en interacties tussen het sediment en *Phragmites australis* (riet), zijn geïdentificeerd in hoofdstuk 2. Box 1 geeft het doel en een beschrijving van dat experiment weer. Belangrijke (bio)geochemische processen die het poriewater in het kleirijke sediment beïnvloeden zijn pyrietoxidatie en de daarmee samenhangende oplossing van calciëet. *Phragmites australis* versnelde het oxidatieproces door het sediment te beluchten via de wortels. Analyse van de mineralogische samenstelling van het sediment toonde aan dat de hoeveelheid

calciet (9% ; 900 mmol kg^{-1}) veel groter is dan die van pyriet (0.6% ; 50 mmol kg^{-1}). Deze hoeveelheden suggereren dat zelfs wanneer volledige pyrietoxidatie tot SO_4 en Fe-oxide optreedt, voldoende calciet aanwezig is om al het geproduceerde H^+ (200 mmol) te bufferen. Dit werd bevestigd door pH metingen. In de experimenten is de pH van het sediment namelijk regelmatig gecontroleerd en zoals verwacht bleef de pH stabiel, fluctuerend rond pH 7.6.



Figuur 1. Processen en zones in een waterrijkgebied die zijn behandeld in de hoofdstukken 2 t/m 6.

In hoofdstuk 2 constateerden we dat *P. australis* in de loop van het experiment achterbleef in zijn groei en zagen we dat de bladeren vergeelden en verschrompelden. Tegelijkertijd zagen we dat de stikstof:fosfor ratio (N:P) daalde van 11 naar 3, hetgeen volgens diverse auteurs zou duiden op stikstoflimitatie. De analyse van het plantenweefsel toonde echter aan dat de gedaalde N:P ratio's verklaard kunnen worden door een verhoogde opname van fosfor (luxue consumptie). Analyse van het plantenweefsel sloot nutriëntenlimitatie uit als oorzaak van het vergelen en verschrompelen. We veronderstellen dat co-precipitatie van fosfor met ijzer op wortels de concentraties van P in de plantenwortels heeft verhoogd. Deze hypothese wordt ondersteund door de twintigvoudig-gestegen ijzerconcentraties in de bladeren.

Bovendien kunnen ijzer-oxiden – een product van pyrietoxidatie – neerslaan op het worteloppervlak. Figuur 2 toont een stukje wortel van *P. australis* afkomstig uit het experiment dat bedekt is met ijzerplak.



Figuur 2. Stukje wortel van *P. australis* afkomstig uit het experiment dat beschreven staat in hoofdstuk 2.

Op basis van de hierboven beschreven processen, zijn verschillende feedbackmechanismes tussen het sediment en *P. australis* geïdentificeerd (zie het hoofdfiguur in Box 1). Een daarvan is een negatieve feedbackloop die ontstaat doordat plantenwortels het sediment beluchten, wat ijzertoxiciteit bevordert. Omdat ijzertoxiciteit het sediment reduceert door afsterving van plantenwortels, is ook een positieve feedbackloop te verwachten. Dat komt omdat fosfor wordt gemobiliseerd in reducerende condities, wat de groei van planten bevordert.

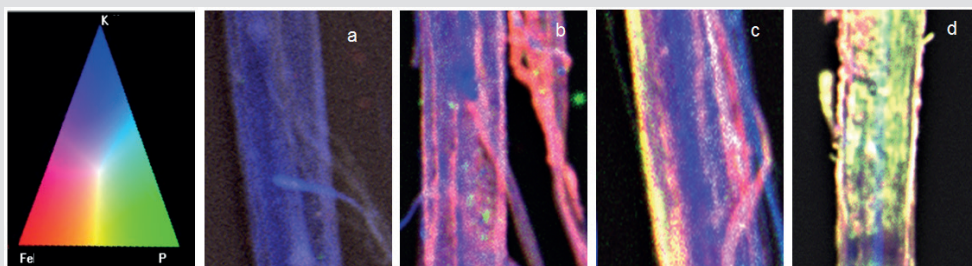
De ijzertoxiciteitshypothese opgesteld in hoofdstuk 2 werd getoetst in een vervollexperiment, beschreven in hoofdstuk 3. Box 2 geeft het doel en een beschrijving van dat experiment weer. De drie belangrijkste bevindingen zullen hier nu nader worden toegelicht. Ten eerste werd een duidelijk effect gevonden van het ijzerrijke sediment op de biomassaproductie van alle drie de planten (zie Figuur 3 voor een impressie). Dit effect was het sterkst in het sediment met de hoogste gehalten ijzer en ijzergebonden fosfor, niettegenstaande het feit dat dit type sediment ook het hoogste gehalte aan fosfor bevatte. Ten tweede bleek uit analyse van de worteloppervlaktesamenstelling dat alleen *P. australis* substantiële hoeveelheden ijzerplak produceerde. **Box 2.** Het doel en de opzet van het experiment zoals beschreven in hoofdstuk 3.

Doel Het bestuderen van de effecten van ijzertoxiciteit in planten die groeien in de ijzerrijke sedimenten van het Markermeer. De twee subdoelen zijn: 1) het bepalen van de aanwezigheid en de samenstelling van ijzerplak op de plantenwortels en achterhalen of de plakvorming afhangt van de ijzer- en ijzergebonden fosforgehaltes in het sediment, 2) het monitoren van de Fe, N, P en K-gehaltes in bladeren en wortels en het koppelen van veranderingen in de stoichiometrie van nutriënten - zoals veranderingen in de N:P ratio door veranderingen in opname en translocatie van N en/of P - aan de aanwezigheid en samenstelling van ijzerplak.

Experimentele opzet Voor dit experiment zijn drie plantensoorten geselecteerd die voorkomen in het Markermeergebied en die verschillen in hun tolerantie voor ijzer: *Rumex maritimus*, *Phragmites australis* en *Eupatorium cannabinum*. Zaaillingen van deze soorten zijn in drie sedimenttypen geplant: (1) de zuidelijkgelegen Zuiderzeeafzetting (10-50 cm diepte), (2) de zachte, kleirijke bodemlaag erbovenop (0- 10 cm diepte) en (3) de zachte, kleirijke bodemlaag afkomstig uit het noordelijke deel van het meer (0-10 cm diepte). Wortels, stengels, bladeren en bloemen werden geoogst na 28, 56 en 84 dagen.

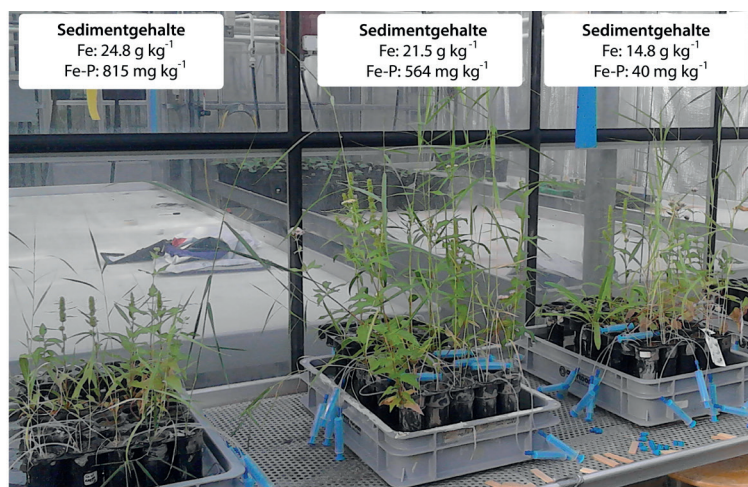
Hoofdfiguur

Oppervlaktecompositie van wortelen van *P. australis* in termen van Fe, K en P in de controle (a), lage (b), gemiddelde (c) en hoge (d) gehalten van ijzergebonden fosfor in het sediment.



Kleuren geven de aanwezigheid aan van Fe (rood), K (blauw) en P (groen). Saaltink et al., 2017.

Interessant is dat het ijzergebonden fosforgehalte (Fe-P) van het sediment, de chemische samenstelling van ijzerplak op de plantenwortels bepaalt: fosfor slaat samen met ijzer neer in sediment met een hoog gehalte aan Fe-P. Opvallend was dat geen substantiële hoeveelheden ijzerplak werden gevormd op de plantenwortels van *Rumex maritimus* en dat de wortels van *Eupatorium cannabinum* helemaal geen ijzerplak bevatten.



Figuur 3. Bovengrondse biomassa van de drie planten, die groeien in drie verschillende sedimenttypen uit het Markermeer. Deze sedimenttypen verschillen in ijzer- en ijzergebonden fosforgehaltes.

De derde belangrijke bevinding is dat ijzerplakvorming op wortels van *P. australis* stoichiometrische veranderingen veroorzaakte in de bladeren, waarschijnlijk door een geremde translocatie van fosfor naar de bovengrondse delen. *Rumex maritimus*, die beschouwd wordt als een ijzertolerante soort, was in Fe-P-rijk sediment in staat om meer fosfor naar de bladeren te transporteren dan *P. australis*.

De hypothese dat indirecte ijzertoxiciteit een probleem is voor plantengroei in deze drie sedimenttypen, werd dus niet bevestigd door bovenstaande waarnemingen. De significant verhoogde ijzerconcentraties in de bladeren van alle drie de testsoorten geven echter aan dat directe ijzertoxiciteit niet kan worden uitgesloten als een mogelijke verklaring voor de geremde bovengrondse biomassaproductie.

Ecologische processen die de nutriëntenbeschikbaarheid beïnvloeden – land-water zone: Hoofdstuk 4

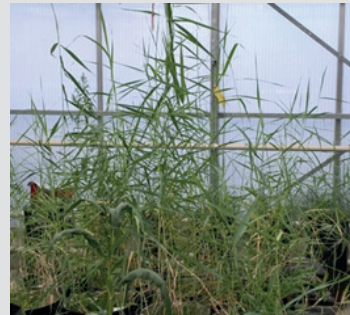
In hoofdstuk 4 hebben we onderzocht hoe het hydrologische regime ingrijpt op de ecosysteemontwikkeling, met name in termen van de nutriëntenbeschikbaarheid. Box 3 geeft het doel en een beschrijving van dat experiment weer. We vonden dat periodieke inundatie stikstoflimitatie in *R. maritimus* tot gevolg heeft. Dit effect werd niet gevonden voor *P. australis*, waarschijnlijk omdat deze soort in staat is om in zuurstofarme condities veel zuurstof af te geven via de wortels, en zo een zuurstofrijk laagje vormt rondom de wortels. Periodieke inundatie leidde verder tot een verhoogde fosforopname in *P. australis*, terwijl voor *R. maritimus* geen significant effect voor fosfor kon worden waargenomen. Deze effecten van periodieke inundatie op de stikstof- en fosforbeschikbaarheid voor beide soorten leidden ons tot de conclusie dat *P. australis* in natte omstandigheden concurrerend voordeel heeft ten opzichte van *R. maritimus*, omdat stikstof beschikbaar wordt gehouden terwijl de fosforopname wordt verhoogd. Het positieve effect van periodieke inundatie op de verhoogde fosforopname in *P. australis* kan door twee mechanismes worden verklaard. Allereerst kan een verlaagd zuurstofgehalte in het sediment een voordeel zijn wanneer planten groeien in ijzerrijk sediment. In hoofdstuk 3 is aangetoond dat juist *P. australis* ijzerplak vormt, wat met name de translocatie van P naar de bladeren remt. Daarnaast zorgen de hoge gehalten pyriet en ijzergebonden fosfor ervoor dat fosfor wordt gemobiliseerd wanneer het sediment wordt geïnundeerd. Dat komt omdat reductie van sulfaat leidt tot fosformobilisatie, aangezien Fe(II) sterker bindt met zwavel dan met fosfor.

Hoewel het verschil in totaal fosforgehalte groot is tussen de in dit experiment gebruikte zachte, kleirijke laag (0-10 cm diepte, 361 mg kg⁻¹) en de onderliggende Zuiderzeeafzetting (10-50 cm diepte, 213 mg kg⁻¹), heeft dit geen invloed gehad op het totaal fosforgehalte in het plantenweefsel van zowel *R. maritimus* als *P. australis*. Wel werd een effect van het sediment op de stikstofconcentratie in het plantenweefsel gevonden: planten die in de zachte, kleirijke laag groeiden, hadden hogere stikstofconcentraties dan planten die in de Zuiderzeeafzetting groeiden. Samenvattend laten de resultaten in hoofdstuk 4 zien dat de beschikbaarheid van fosfor wordt bepaald door het hydrologische regime, terwijl de beschikbaarheid van stikstof wordt bepaald door zowel het hydrologische regime als het sedimenttype.

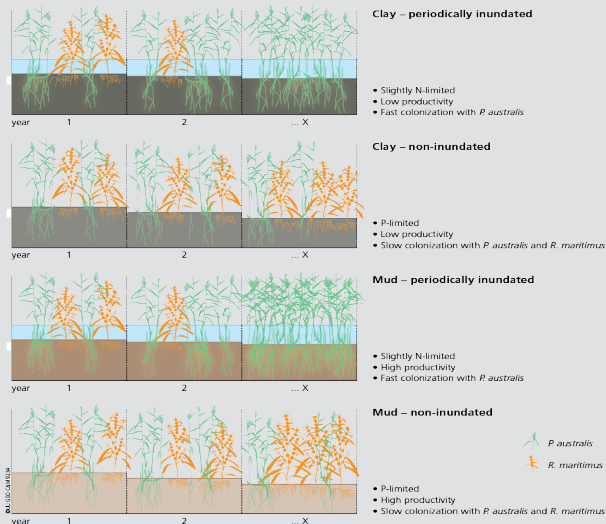
Box 3. Het doel en de opzet van het experiment zoals beschreven in hoofdstuk 4.

Doel Begrijpen hoe het hydrologische regime ingrijpt op de ecosystemontwikkeling.

Experimentele opzet Een kasexperiment werd gedurende twee groeiseizoenen uitgevoerd in de botanische tuin van de Universiteit Utrecht, waarbij twee sedimenttypen uit het Markermeer en twee plantensoorten werden gebruikt. Mesocosmossen ($\varnothing$ 0.3 m, diepte 0.5 m) werden gevuld met ofwel een zacht, kleirijk sediment (afkomstig van een diepte van 0-10 cm) ofwel een Zuiderzeeafzetting (afkomstig van een diepte van 10-50 cm). Twee plantensoorten werden geselecteerd en in de mesocosmossen geplant: *Rumex maritimus* en *Phragmites australis*. De mesocosmossen werden ofwel periodiek geïnundeerd ofwel wekelijks geïrrigeerd. Tijdens het experiment werd het plantenweefsel en de geochemische samenstelling van het sediment gemonitord.



Hoofdfiguur



Schematische samenvatting van de belangrijkste bevindingen van elke conditie die in dit onderzoek is getest (Saaltink et al., 2018).

Effecten van planten op de consolidatie van kleirijk sediment – terrestrische zone: Hoofdstuk 5

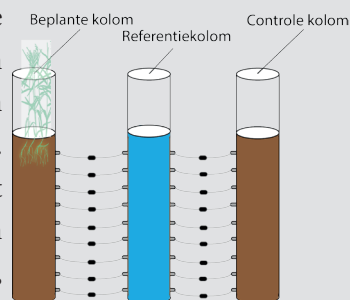
In hoofdstuk 5 hebben we onderzocht hoe en waar *P. australis* het zachte, kleirijke sediment draineert waarmee de consolidatie van het sediment wordt versneld. Box 4 geeft het doel en een beschrijving van dat experiment weer. Omdat het grondwaterniveau in het experiment op één niveau is vastgezet, konden we de effecten van evapo-transpiratie op de poriedruk in het sediment goed zien, maar daardoor konden we de verschillen in poriedruk niet rechtstreeks koppelen aan de consolidatie van het sediment. Echter, aannemende dat de hydraulische geleidbaarheid in kleirijke sedimenten laag is en evapo-transpiratie zal leiden tot netto verwijdering van water, nemen we aan dat een hogere evapo-transpiratie consolidatie van het sediment tot gevolg heeft.

De resultaten in hoofdstuk 5 laten zien dat na een initiële fase waarin de consolidatie bepaald werd door het (eigen) gewicht van het sediment (leidend tot een overdruk van het poriewater), de poriedruk daalde tot onder de hydrostatische druk. *Phragmites australis* veranderde de poriedrukgradiënt met name tussen 17 en 37 cm onder het grondwaterniveau. In deze zone werden dagelijkse cycli in poriedrukken waargenomen die direct gekoppeld konden worden aan de dag- en nachtcyclus van planten. Overdag staan de huidmondjes verder open dan 's nachts waardoor de gasuitwisseling toeneemt en de plant dus meer water kan verdampen. Bovendien verhoogde *P. australis* de hydraulische geleidbaarheid in het sediment met 40% ten opzichte van de conditie zonder plant ($1.2 \times 10^{-9} \text{ m s}^{-1}$ voor de begroeide kolom en $0.8 \times 10^{-9} \text{ m s}^{-1}$ voor de controle). De gemiddelde evapo-transpiratiewaarde van 3.95 mm dag^{-1} komt goed overeen met gemiddelde evapo-transpiratiewaarden gemeten in Europese waterrijke gebieden. We veronderstellen dan ook dat de resultaten van hoofdstuk 5 kunnen worden gebruikt om een plant-sedimentmodel te ontwikkelen dat aanvullende inzichten kan verschaffen in de mate waarin *P. australis* de consolidatie van kleirijk sediment kan versnellen.

Box 4. Het doel en de opzet van het experiment zoals beschreven in hoofdstuk 5.

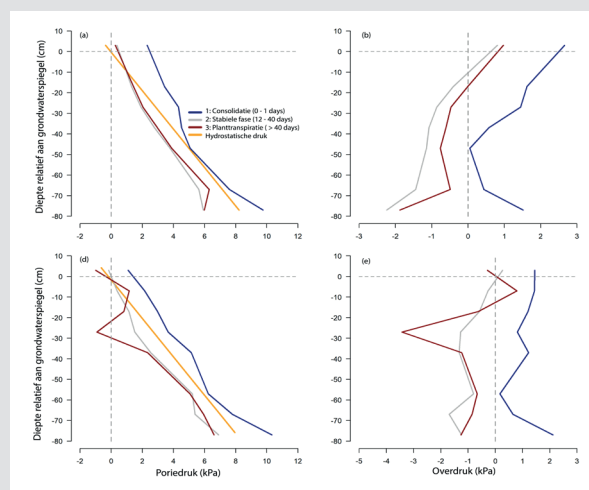
Doel Onderzoeken hoe en waar *P. australis* het zachte, kleirijke sediment draineert, om zo de consolidatie van het sediment te versnellen.

Experimentele opzet Twee opeenvolgende consolidatie-experimenten werden uitgevoerd in perspex kolommen. In beide experimenten werden twee kolommen gebruikt voor poriedrukmetingen. Een van de twee kolommen werd beplant met *P. australis*. In de kolommen werden druksensoren geïnstalleerd op 0.4, 10.4, 20.4, 30.4, 40.4, 50.4, 60.4, 70.4 en 80.4 cm vanaf de onderkant van de kolom.



Daarnaast werden in het eerste experiment vier kolommen met planten geoogst om plantenvariabelen te bepalen.

Hoofdfiguur



Poriedruk (kPa) ten opzichte van de waterkolom in de controlekolom (a) en begroeide kolom (d) evenals de overdruk (poriedruk minus hydrostatische druk) in de controlekolom (b) en de begroeide kolom (e).

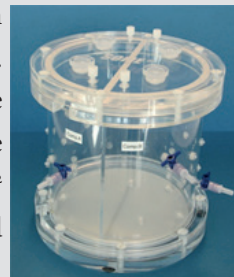
Effecten van bioturbatie op de nutriëntenbeschikbaarheid – aquatische zone:
Hoofdstuk 6

In hoofdstuk 6 bepaalden we het potentiële effect van bioturberende Tubificidae op de nutriëntenbeschikbaarheid in het kleirijke sediment. Daarnaast bestudeerden we op welke (bio)geochemische processen bioturberende wormen invloed hebben. Box 5 geeft het doel en een beschrijving van dat experiment weer. Zowel de gemeten als de gemodelleerde resultaten toonden aan dat bioturbatie door Tubificidae de bovenste laag van het sediment effectief beluchtte. Het model beschreven in hoofdstuk 6 voorspelt een optimum van 12.000 wormen m^{-2} . Dit oxiderende effect van bioturbatie neemt eerst toe met toenemende dichtheden Tubificidae en neemt vervolgens weer af wanneer het optimum van 12.000 wormen m^{-2} wordt overschreden. De reden hiervoor is zeer waarschijnlijk dat het beluchtingseffect van Tubificidae vermindert naarmate de gangenstelsels gaan overlappen, terwijl de respiratie per worm hetzelfde blijft. Deze tegengestelde mechanismes verklaren dus waarom de zuurstofconcentratie in het sediment als gevolg van beluchting door bioturberende Tubificidae een optimum heeft. Ook is gebleken dat het beluchtingseffect (bio)geochemische processen in de bovenste laag van het sediment veranderde, waardoor de nutriëntenbeschikbaarheid werd beïnvloed. De ammonium- en nitraatconcentraties in het poriewater namen toe als gevolg van verhoogde snelheden ammonificatie en nitrificatie. Een vermindering in de concentraties fosfaat werden waargenomen als gevolg van fosforimmobilisatie.

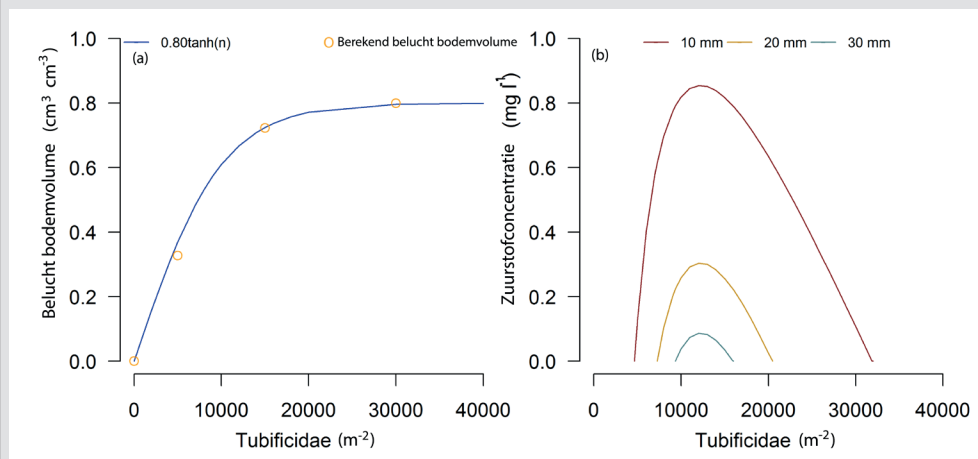
Box 5. Het doel en de opzet van het experiment zoals beschreven in hoofdstuk 6.

Doel Het bepalen van het potentiële effect van bioturberende Tubificidae op de nutriëntenbeschikbaarheid in het kleirijke sediment van het Markermeer.

Experimentele opzet Dit experiment maakte gebruik van SOFIE®-cellen (Sediment Or Fauna Incubation Experiment). Aan het begin van het experiment werden Tubificidae toegevoegd aan drie van de vier compartimenten in drie dichtheden: 5.000, 15.000 en 30.000 Tubificidae per m² oppervlak. Poriewater en oppervlaktewater werden bemonsterd op de dagen 0, 7, 14 en 28 en chemisch geanalyseerd.



Hoofdfiguur



Gemiddeld belucht sedimentvolume (cm³) berekend voor 5.000 wormen m⁻², 15.000 wormen m⁻², en 30.000 wormen m⁻² (oranje cirkels) en gemodelleerd belucht sedimentvolume (cm³) per Tubificidae (m⁻²) (donkerblauwe lijn) (a). De gemodelleerde gangenfunctie correspondeert met een tangens-hyperbolische functie ($0.80 \tanh(n)$) en werd gebruikt om de zuurstofconcentratie in het sediment (mg l⁻¹) per Tubificidae (m⁻²) voor drie diepten te modelleren: 10 mm (rood), 20 mm (geel) en 30 mm (blauw) (b).

Praktische aanbevelingen voor het Marker Wadden-project

Hoewel dit proefschrift fundamenteel van aard is en via experimenteel onderzoek beoogt te achterhalen hoe (bio)geochemische en ecologische processen op elkaar inspelen bij gebruik van zacht, kleirijk sediment, is de gegenereerde kennis voldoende toepasbaar om vijf praktische aanbevelingen voor het Marker Wadden-project te formuleren. Deze aanbevelingen stellen het bouwconsortium en natuurbeheerders van de Marker Wadden in staat om een aantal problemen op te lossen die tijdens de bouwfase aan het licht zijn gekomen en kunnen bovendien bijdragen aan een sterk ontwerp van eilanden die nog gebouwd moeten worden.

Aanbeveling 1: Bestudeer de geochemische eigenschappen – met name de Fe(III)- en ijzergebonden fosforgehaltes – van het sediment en de feedbackmechanismen tussen planten en sediment, voorafgaand aan het finale besluit over het ontwerp van toekomstige eilanden.

Aanbeveling 2: Gebruik het zachte, kleirijke sediment als bouw materiaal, in plaats van sediment dat op grotere dieptes moet worden gewonnen.

Aanbeveling 3: Pas periodieke inundatie toe zolang de ringdijken gesloten zijn, om ecosystemontwikkeling op de eilanden te bespoedigen.

Aanbeveling 4: Beplant de eilanden van de Marker Wadden met *P. australis*, in combinatie met een effectief beheer van het hydrologische regime, om kolonisatie van *P. australis* te bespoedigen.

Aanbeveling 5: Koloniseer het sediment in de oeverzone met bioturberende macrofauna tot de optimale dichtheid van 12.000 individuen m⁻².

Deze aanbevelingen zijn grotendeels in overeenstemming met bouwen-met-natuurprincipes. Daarnaast beveel ik aan om natuurlijke processen vooral ruimte te geven in het uiteindelijke ontwerp van het ecosysteem. De veerkracht van natuur is enorm. Er zijn vele voorbeelden te noemen waarbij verrassende ontwikkelingen plaatsvonden na herstelmaatregelen of andere ingrepen in het ecosysteem, die van te voren niet werden voorzien. Ik pleit derhalve voor het reserveren van ruimte – letterlijk en figuurlijk – voor verrassingen. Door mogelijkheden open te laten voor

deels onbekende processen kan de veerkracht van het ecosysteem worden verhoogd, waardoor de kans dat het bouwen-met-natuurproject slaagt, wordt vergroot.

Dankwoord

Curriculum vitae

List of publications

DANKWOORD

Telkens wanneer ik een nieuw proefschrift overhandigd kreeg, betrapte ik mezelf erop dat ik meteen doorbladerde naar het dankwoord. En velen met mij – met name collega's die nog moeten promoveren. Alsof wij, academici-in-wording, helemaal geen interesse hebben in het inhoudelijke verhaal van een directe collega. Van waar toch die aantrekkingskracht? Wat heeft dat woordje achteraf wat een syntheseshoofdstuk niet heeft? Mijn (niet-wetenschappelijk getoetste) theorie is – los van het feit dat het gewoon leuk is om je eigen naam in het dankwoord van een ander te lezen – dat het een bemoedigende werking heeft. Je beseft je dat jou eigen PhD-traject helemaal geen eenzame onderneming is, al lijkt dat op sommige momenten wel zo te zijn. Ook bij mij staat het ontegenzeggelijk vast dat veel mensen een directe of indirecte bijdrage hebben geleverd aan het eindproduct dat nu in uw handen ligt.

Allereerst wil ik Stefan Dekker bedanken, mijn dagelijks begeleider. Ik heb Stefan leren kennen als een zorgzame en betrouwbare supervisor die mij – maar ook zijn andere PhD's – nooit uit het oog verliest; hij is een mentor op wie je kunt bouwen. Het is niet voor niets dat we actief aan de slag zijn gegaan om hem te nomineren voor beste PhD-supervisor van Nederland. Natuurlijk waren we niet verrast dat hij daadwerkelijk werd genomineerd door de jury, wel vonden we het belachelijk dat hem niet de titel werd toegekend. Doorgestoken kaart, als je het mij vraagt! Stefan, zonder jou had ik het traject nooit zo smoothly kunnen doorlopen. Een diepe kniebuiging voor jou!

Jasper Griffioen vormt ook een zwaargewicht in deze dankbetuiging. Ik leerde Jasper al vroeg in mijn masterprogramma kennen – in februari 2012. Onze allereerste samenwerking werd meteen een groot succes. Het onderzoek dat we uitvoerden resulteerde in het mooie GEMAS-papertje dat nu ruim aan de leiding gaat wat betreft het aantal citaties (tenminste, wat mijn track-record betreft). Jasper is een ware wandelende encyclopedie, heeft gevoel voor humor en legt de lat iedere keer een stukje hoger. Bedankt Jasper, voor je uitmuntende begeleiding en het me laten zien hoe leuk (bio)geochemie kan zijn.

Aan het hoofd van de begeleiding stond Martin Wassen. Martin fungeerde als poortwachter van de inhoudelijke producties. Als een ware ecooloog in het veld

oversteeg hij het detailniveau en legde heel accuraat problemen bloot die iedereen over het hoofd had gezien. Martin is ook altijd in voor actie. Met veel elan overtuigde hij me ervan dat het de moeite loont om in een gammele kano met windkracht 5 het Markermeer op te peddelen om wat velddata te verzamelen. Het was een memorabele dag die ik me altijd zal blijven heugen! Martin, ik wil je bedanken voor de onmetelijke inzet van je scherpe geest en je enthousiasme.

Naast dit trio van begeleiding, zijn er een hoop knappe koppen die op een aantal momenten een flinke inhoudelijke bijdrage hebben geleverd aan dit onderzoek. Ik wil graag Maarten Eppinga bedanken, onze huis-statisticus. Hij heeft me haarfijn uitgelegd welke statistische methoden relevant zijn bij verschillende onderzoeken. Zijn bijdrage bij hoofdstuk 3 was zo groot, dat hij terecht coauteur is geworden van dat hoofdstuk. Ook wil ik Hugo de Boer bedanken, voor het begrijpelijk maken van een aantal belangrijke plant-fysiologische processen en het introduceren van de LiCOR. Door zijn bijdrage zijn de bevindingen in hoofdstuk 5 een stuk relevanter geworden! Met enige regelmaat heb ik kunnen leunen op de expertise binnen het projectteam. Met name hoofdstuk 6 getuigt van de goede samenwerking tussen experts uit verschillende disciplines. Zo hebben Han Winterwerp (TU Delft), Mariëlle van Riel (UvA), Piet Verdonschot (UvA) en Jos Vink (Deltares) allen een grote bijdrage geleverd aan het bioturbatie-experiment. Tijdens onze gezamenlijke projectvergaderingen gaven naast eerdergenoemden ook Thijs van Kessel (TU Delft), Ruurd Noordhuis (Deltares) en Harry Veld (Deltares) praktisch en inhoudelijk advies. Bedankt daarvoor! Ook een dank je wel aan Marloes van den Akker, voor het delen van bodemonsters van de Marker Wadden, het begeleiden van een aantal UU-studenten en voor het vrijhouden van een plekje op de boot naar de eilanden.

A special thanks to Maria Barciela Rial. Maria and I closely collaborated since her appointment at the TU in Delft. In 2015, we had a very ambitious idea to join forces in a shared experiment. That experiment was by far the most challenging part of the whole PhD and resulted in a valuable joint-chapter of our dissertations (Chapter 5). Maria, I admire your perseverance and determination. To stay on board despite all the things you've been through the past few years really shows your mental strength!

Mijn schriftelijk Engels heeft een flinke ontwikkeling ondergaan de afgelopen jaren. Toch blijft het een zwak punt. Ik wil Joy Burrough-Boennisch en Fulco

Teunissen bedanken voor het doorworstelen en editen van de tekst in de hoofdstukken 1, 2, 3 en 7. Zonder jullie kunde had deze tekst net zo gelezen als de hoofdstukken 4, 5 en 6!

Vrijwel bij alle experimenten die gepresenteerd zijn in dit proefschrift kwam een flinke dosis labwerk kijken. Dineke van de Meent en Thom Claessen hebben ongelooflijk veel advies gegeven (en kritiek geuit) op de chemische analyses die ik moest gaan uitvoeren. Peter Kraal en Nikki Dijkstra hebben mij met engelengeduld de gespecialiseerdere technieken aangeleerd, zoals de fosfor- en ijzerextractie. Bedankt daarvoor!

De afgelopen vier jaar heb ik ook de eer gehad om bachelor- en masterstudenten te begeleiden in hun onderzoek. Een aantal daarvan kozen er bewust voor om aan te haken bij mijn eigen onderzoek en in die zin hebben ook zij een bijdrage geleverd aan dit eindproduct. Ik wil Florentine Steijlen (TU Delft) bedanken voor haar bijdrage aan hoofdstuk 5 en Eldin Honingh (UU), voor zijn bijdrage aan hoofdstuk 6. Verder hebben Isabelle Trujillo Rocha, Jeroen de Bruin, Nathan Hayes en Maarten Steenhuis interessante experimenten uitgevoerd die meer inzicht hebben gegeven in de ecosysteemontwikkeling op de Marker Wadden. Fantastisch, jullie werk. Bedankt!

Ik wil Margien Bootsma bedanken voor de kans me verder te ontwikkelen in het onderwijs. Margien breidde mijn onderwijscomponent uit en fungeerde tevens als mijn BKO-coach. Ze heeft me de afgelopen twee jaar veel mogelijkheden gegeven om de onderwijsvaardigheden te ontwikkelen die nodig zijn voor de didactische bekwaamheid. Ook een dank je wel aan Jerry van Dijk, die mij regelmatig dingen heeft bijgeleerd over het vak. Zo heeft hij mij veel geleerd over toetsen en toetsanalyses en over het geven van hoorcolleges.

To me, Unnik 11.13A was the beating heart of the University, which attracted lots of interesting people. In the first year, there were Lennard and Noa, who on a regular basis brought some interesting – buzzing – stuff to the office. I remember one time, Lennard brought some empty egg cartons to cultivate (living!) food for his praying mantis. Apparently, he totally forgot the egg cartons, and, in a few weeks, it resulted in an office full of parasitic wasps, that massively hatched from the cultivated food (not so alive anymore). It took a while to get rid of those... A few years ago, I was very lucky to welcome the Coralita girls to 11.13A. Jetske and Elizabeth. They brought

lots of joy and chatter to the office. On a regular basis, Elizabeth brought delicious cookies and cakes. And I can tell you from experience, that is far better than parasitic wasps! I realize I wasn't their dreamed office mate for several reasons: 1) I did not decorate the wall in front of my desk, 2) I never brought cookies and cake myself, and 3) I did not take care of our plants. Especially the latter I am not so proud to confess as I successfully carried out many plant experiments.¹ Especially for Jetske, there is a fourth reason why I was not such a good office mate: I did not drink coffee. And because of that, poor Jetske had to accompany Frank on her own to the coffee machine every single morning of her PhD. Hopefully, I made it up a bit after my son was born, after which I was literally craving for coffee every morning. Jetske and Elizabeth, thanks for tolerating my shortcomings and for simply being wonderful office mates! A special thanks to Jetske, for the many nice conversations we've had and for decoding Martin's handwriting.

Outside 11.13A, life at University was equally well. Thank you, Iris, Ineke, Yasmina, Janneke, Maria, Floris K, Floris N, Brian, Mara, Jojanneke, Svenja, Gilian, Aat, Myrna, Feroz, John, Mart, Jiefei, Willem, Obbe, Walter, Judith, Bas, Annick and Leontien. You provided an excellent atmosphere full of respect and joy! Three years in a row, I joined Aat, Iris, Koen, Floris K., and Myrna to the small island Tiengemeten to let bachelor students practice fieldwork. Each day I spent on that island felt like a holiday. Thank you!

Iris, ik vond het een verademing om onze strubbelingen in én met het lab te kunnen delen. Fantastisch ook dat het mogelijk is gebleken een boeiend gesprek te onderhouden over pyrietjes. De zandmotor is allang niet meer het belangrijkste in je leven; geniet vooral van jouw Luuk tijdens het staartje van je PhD!

Regelmatige ontspanning houdt de geest scherp. Immers, om slimme dingen op te schrijven moet je je onbewuste aan het werk zetten en dat gaat nou eenmaal beter als je even niet inhoudelijk bezig bent. Buiten werktijden om mag ik graag een kaartje leggen met Kees, Arne en Erik. Buitengewoon goede vrienden. Er is dan niet alleen ruimte voor primitief gedrag, maar ook voor levensfilosofische discussies waar menig

¹ Jetske (rightfully) did not trust me for caring the office vegetation when they were off to the Caribbean and moved the plants to another office before she left.

wijsgeer een puntje aan kan zuigen. Ik hoop dat we die traditie lang in ere houden! De vriendschapsband met Max, Michiel, Susan, Danya, Bart en Marvin gaat ver terug. Vrinden, jullie zijn een stelletje idioten bij elkaar, helemaal zen en stuk voor stuk personen van wie ik veel leer. Ik hoop dat we nog jarenlang plezier met elkaar beleven! Sam, waar is die tijd gebleven dat we wekelijks in het Moortgat zaten te ouwehoeren? Onze drukke agenda's laten dit wekelijkse uitje niet meer toe, maar ik hoop van ganser harte dat we elkaar met enige regelmaat blijven zien. Lydia, bedankt voor het wegnemen van de keuzestress bij het kiezen van een goed speciaalbiertje. Jij weet altijd de juiste voor me te selecteren. We mogen onze dansschoenen dan allang hebben laten verstoffen, ik ben blij dat we elkaar nog steeds zien. Coco, superfijn dat je samen met Jany ons hele huis hebt gestyled. Heerlijk om thuis te komen in een knus en sfeervol huis! Je vocale kwaliteiten zijn werkelijk fenomenaal, dat ik daar nog maar lang van mag blijven genieten. Jane, ik hoop dat we gauw onze keel weer kunnen smeren met een rode, op houtgerijpte wijn. We babbelen zonder moeite een hele avond vol, waggelen naar huis en staan met hoofdpijn op. Heel bijzonder. Die activiteit zit er nu even niet meer in, maar, voor Pixel is alles de moeite waard!

Ik heb onwijs veel geluk met het gezin waarin ik ben opgegroeid. In het bijzonder wil ik mijn ouders bedanken, Karin en Erik, voor hun onvoorwaardelijke steun en zorg. Bedankt pa en ma, dat jullie altijd voor me klaar staan en me de bagage hebben meegegeven om succesvol te kunnen zijn. Zonder mijn vader had de inhoud van hoofdstuk 4 niet bestaan. Op een warme zomerdag ging het managen van het hydrologisch regime van mijn mesocosmossen finaal mis. Het water liep er aan alle kanten uit. Mijn vader heeft de hele zondag (tot ver na sluitingstijd van de Botanische Tuinen) – een dag voor hij met vakantie zou gaan – geholpen om de schade te repareren. Bedankt daarvoor! Mijn broer en zus, Marco en Nicol, ondanks dat we ieder ons eigen pad bewandelen, onderhouden we het contact. Heel fijn! Marco, ik hoop dat ik nog veel van al je (toekomstige) reizen kan meegenieten, en Nicol en Robbert, ik hoop nog veel te leren van jullie financiële handigheid!

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zijn stuk voor stuk inspirerende personen: of het nou gaat om ondernemen, kalmte en rust bewaren of je passie achternagaan – ook al wijzigt die soms – ik trek er wijze lessen uit.²

Lieve Tycho, je bent een fantastisch energiek kereltje, druk bezig om de wereld om je heen te ontdekken. Dat doen onderzoekers ook, al slapen die (meestal) 's nachts en ontdekken ze (doorgaans) overdag, en niet andersom, maar die ontdekking heb jij gelukkig al (bijna) gedaan. Het is een voorrecht dat ik de rest van mijn leven van je mag genieten.

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² Sorry, Jany, Coco en Erik, maar om jullie voor een tweede keer te bedanken is natuurlijk een beetje te gortig, de veren groeien niet op mijn rug!

CURRICULUM VITAE

Rémon Saaltink is geboren op 14 november 1988 in Arnhem. In 2007 behaalde hij zijn vwo-diploma op het Arentheem college, Thomas a Kempis. Tijdens zijn middelbareschooltijd haalde hij het meeste plezier uit biologie en het was om die reden dat hij ervoor koos dat vak te gaan studeren aan de universiteit. In 2010 behaalde hij zijn bachelor diploma biologie aan de Universiteit Utrecht. Tijdens zijn studententijd in Utrecht heeft hij de stad Arnhem nooit helemaal kunnen loslaten. Rémon was jarenlang actief bestuurslid van de Arnhem Band. De leerschool voor niet-academische vaardigheden. Voor hij aan zijn masteropleiding begon, deed hij vrijwilligerswerk in een tropisch regenwoud en in een kustdorpje in Ecuador. Tijdens zijn masterprogramma Sustainable Development deed hij samen met Jasper Griffioen onderzoek naar geogene en agrarische factoren die de geochemie verklaren van Europese bodems. Voor zijn afstudeeronderzoek ging hij naar Stockholm en bracht hij samen met Stefan Dekker en Ype van der Velde de mechanismes in kaart die de historische veranderingen in waterkwaliteit van de stroomgebieden van de Baltische Zee verklaren. In 2014 begon Rémon aan zijn promotieonderzoek naar biogeochemische en ecologische processen die ecosysteemontwikkeling op de Marker Wadden bespoedigen. Mede door de kundige begeleiding van Martin Wassen, Jasper Griffioen en Stefan Dekker ligt het eindproduct van dat onderzoek nu in uw handen. Na zijn promotieonderzoek is hij aan de slag gegaan als Docent Milieukunde aan de HAS hogeschool in 's-Hertogenbosch. Rémon woont in Arnhem samen met zijn vrouw Esther en zoon Tycho.

LIST OF PUBLICATIONS

Chapters of this dissertation

Hoofdstuk 2. Wetland eco-engineering: measuring and modeling feedbacks of oxidation processes between plants and clay-rich material

Rémon Saaltink, Stefan C. Dekker, Jasper Griffioen, Martin J. Wassen

Published in Biogeosciences (2016) 13:4945-4957

Hoofdstuk 3. Plant-specific effects of iron-toxicity in wetlands

Rémon Saaltink, Stefan C. Dekker, Maarten B. Eppinga, Jasper Griffioen, Martin J. Wassen

Published in Plant and Soil (2017) 416:83-96

Hoofdstuk 4. Vegetation growth and sediment dynamics in a created freshwater wetland

Rémon Saaltink, Stefan C. Dekker, Jasper Griffioen, Martin J. Wassen

Published in Ecological Engineering (2018) 111:11-21

Hoofdstuk 5. Consolidation and drainage of soft cohesive sediment with and without *Phragmites australis* as an ecological engineer

Rémon Saaltink, Maria Barciela Rial, Stefan C. Dekker, Thijs van Kessel, Hugo J. de Boer, Claire Chassagne, Jasper Griffioen, Martin J. Wassen, Johan C. Winterwerp

In preparation

Hoofdstuk 6. Respiration and aeration by bioturbating Tubificidae alter biogeochemical processes in aquatic sediment

Rémon Saaltink, Eldin Honingh, Stefan C. Dekker, Jasper Griffioen, Mariëlle C.

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Other publications

Saaltink RM, Y van der Velde, SC Dekker, SW Lyon, HE Dahlke (2014). Societal, land cover and climatic controls on river nutrient flows into the Baltic Sea. *Journal of Hydrology: Regional Studies* 1:44-56.

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The SENSE Research School declares that **Rémon Saaltink** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 44.4 EC, including the following activities:

SENSE PhD Courses

- o Environmental research in context (2015)
- o Research in context activity: 'Preparing and co-organizing the session "Ecosystem-based solutions to societal problems" at the Netherlands Annual Ecology Meeting (NAEM), on 9 February 2016'

Other PhD and Advanced MSc Courses

- o Presenting in English, Utrecht University (2015)
- o Structural Equation modelling, PE&RC, Wageningen University (2015/2016)
- o Maintaining Scientific Integrity in Present Day Academic Reality, Utrecht University (2015/2016)
- o Project Management for PhDs, Copernicus Institute Utrecht & Hertz (2016)
- o Research writing for Scientists, Utrecht University (2015)

Management and Didactic Skills Training

- o Supervising MSc student with thesis entitled 'Statistical Analysis of Markermeer mud deposits during incubation experiments' (2014)
- o Science blog writer at <http://geoblog.weebly.com/environmental-sciences-blog> (2014-2015)
- o Coordinating lunch lectures for Environmental Sciences, Utrecht University (2015-2016)
- o Obtained University Teaching Qualification (BKO) in 2017

Oral Presentations

- o *Marker Wadden: Implications for Boskalis*. Marker Wadden conference, 18 September 2014, Papendrecht, the Netherlands
- o *Marker Wadden: Using soft mud to create a dynamic wetland system*. Launch of the Academy of Ecosystem services, Utrecht University, 8 July 2015, Utrecht the Netherlands
- o *Wetland Eco-Engineering: Measuring and modelling feedbacks of oxidation processes between plants and clay-rich material*. Netherlands Annual Ecology Meeting (NAEM), 9 February 2016, Lunteren, The Netherlands

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