Nitrogen Removal in Created Wetlands

Considerations – Challenges – Possibilities

JOSEFIN E. NILSSON
Created wetlands in agricultural landscapes deliver a multitude of ecosystem services, one of which is the removal of nitrogen (N) from water to reduce eutrophication. Wetland N removal, primarily through denitrification, is influenced by various factors. For instance, macrophytes support denitrifying microorganisms and thus N removal, and the extent of N removal varies both spatially and temporally.

The overall aim of this thesis was to provide a broadened understanding of considerations, challenges, and possibilities associated with achieving high N removal in created wetlands. To fulfil this aim, and thereby address knowledge gaps concerning wetland N removal, this thesis evaluates how N removal is affected by wetland placement and design, planting and harvesting of vegetation, installation of floating wetlands, changing climatic conditions, and interactions with other ecosystem services. These assessments were done using a combination of experimental wetland studies, a field study of created wetlands, and a literature review, all presented in the five included papers.

The results highlight the rapid achievement of high N removal in wetlands planted with emergent vegetation, but also the diminishing effects of initial planting as wetland ecosystems approach maturity. Further, N removal is promoted in wetlands placed downstream of fertilised arable land to intercept as much runoff as possible, and in wetlands of elongated shape with maximised distance between inlet and outlet. Through such placement and design, created wetlands can maintain efficient N removal in spite of the anticipated increase in summer droughts. Additionally, multiple wetlands can jointly enhance landscape multifunctionality despite trade-offs between ecosystem services in individual wetlands. Even within a wetland, ecosystem services can synergistically interact. Although dependent on design, created wetlands can efficiently remove N and attenuate floods, without elevating greenhouse gas emissions. Moreover, specific wetland properties promote ecosystem services additional to N removal, making it possible to increase N removal by creating wetlands with other primary objectives. Lastly, N removal in heavily overgrown wetlands can be enhanced through macrophyte harvest, and floating wetlands offer further possibilities of improved N removal.

In conclusion, with an improved understanding of wetland N removal, future creation and restoration of wetlands in agricultural areas hold the potential to further contribute to mitigating eutrophication and its detrimental consequences.

Keywords: Ecosystem services, eutrophication, constructed wetlands, agricultural runoff, denitrification, wetland multifunctionality, climate change, nutrient retention, vegetation, hydrology, greenhouse gas emissions, biodiversity

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To my mother, my dog, and clowns
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Additional publications

In addition to the papers included in this thesis, the author contributed to the following report:

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Introduction

“Everything is water”, declared the ancient Greek philosopher Thales of Miletus to emphasise the vital significance of water for life (Rojcewicz, 2014). To this day, the recognition of water as essential to sustain all known life forms persists (Westall and Brack, 2018). Despite this knowledge, many aquatic ecosystems are threatened. Oceans, rivers, and lakes worldwide are facing increased anthropogenic stress from factors such as pollution and climate change, endangering the life of aquatic biota as well as humans (Miles, 2009; Best, 2019; Barouillet et al., 2022). Similarly, wetland ecosystems are in a state of global degradation and loss (Ramsar, 2015).

Numerous definitions of wetlands exist, but all share a common trait: the presence of water. For instance, the Ramsar Convention on Wetlands (Ramsar, 2007) described wetlands as “areas where water is the primary factor controlling the environment and the associated plant and animal life”. Wetlands can be naturally occurring, restored, or entirely man-made. Man-made wetlands are commonly referred to as constructed or created wetlands. In this thesis, the term “created wetland” specifically relates to man-made wetlands in agricultural landscapes, designed to closely resemble natural wetlands in appearance and ecological function.

Wetlands are among the most productive ecosystems in the world, and essential for the survival of numerous species of animals and plants (Ramsar, 2007). The significance of wetlands extends beyond their ecological value. Wetlands support human lives via their unique ability to deliver a multitude of ecosystem services (Davidson et al., 2019), defined by the Millennium Ecosystem Assessment (MEA, 2003) as “the benefits people obtain from ecosystems”. For instance, wetlands can alleviate the negative consequences of eutrophication, climate change, floods, and droughts (de Groot et al., 2002; Xu et al., 2020). However, vast areas of natural wetlands have been drained since the beginning of the 18th century (Davidson, 2014; Fluet-Chouinard et al., 2023) to accommodate increased agricultural production for a growing human population (van Asselen et al., 2013). Although slowed down, the decrease in global wetland area has continued over the past 50 years (Dixon et al., 2016; Davidson et al., 2018). In parts of the world, only 10% of the original wetland
area remains today (Junk et al., 2013). As a consequence, significant losses of crucial ecosystem services are observed across the globe (Ramsar, 2015).

In recent decades, there has been a notable increase in the recognition of the intrinsic values of wetlands (e.g., Davidson et al., 2019). Although land use conflicts related to wetlands are still common in parts of the world (Kingsford et al., 2016), great efforts have been undertaken to restore lost wetland areas, for example in Europe (Verhoeven, 2014). In many cases, the results are promising. Reports from around the world indicate successful delivery of ecosystem services through created wetlands (e.g., Jenkins et al., 2010; Blackwell and Pilgrim, 2011; Natuhara, 2013). In Sweden, for instance, extensive creation of wetlands has reduced nutrient transports from agricultural landscapes to the seas (Weisner et al., 2015), and thereby contributed to the mitigation of eutrophication.

Causes and consequences of eutrophication

Eutrophication is the process where a body of water, such as a lake, river, or coastal sea, becomes excessively enriched with nutrients, particularly nitrogen (N) and phosphorus (P). These increased nutrient levels in the water can have severe ecological consequences, including recurrent toxic algal blooms and hypoxia (low oxygen concentration in the water) (Diaz and Rosenberg, 2008; O’Neil et al., 2012), that harm both animals and humans. Hypoxia can result in the death of fish and other oxygen-dependent organisms, an effect that can reverberate through the food web and disrupt the whole ecosystem (Baird et al., 2004; Weinke and Biddanda, 2018). Additionally, toxic cyanobacterial blooms can contaminate drinking water supplies and pose an immediate threat to human health (Wu et al., 2021). Over time, eutrophication has become increasingly prevalent (Le Moal et al., 2019), and is now a global issue that affects numerous aquatic systems worldwide (Smith, 2003; Malone and Newton, 2020).

Nitrogen (N) is a key driver of eutrophication, especially in coastal and marine environments (Howarth and Marino, 2006; Elser et al., 2007). Agriculture is a major source of N inputs into aquatic systems, and therefore a significant contributor to eutrophication (Withers et al., 2014; Beusen et al., 2016). By conversion of atmospheric nitrogen gas (N₂) to bioavailable N through the industrial Haber-Bosch process, humans have amplified the global N cycle (Galloway et al., 2003). The amount of bioavailable N has increased tenfold since the latter half of the 19th century (Galloway et al., 2004) and now far exceeds the planetary boundary (Rockström et al., 2009). This industrially produced bioavailable N is used abundantly as fertiliser in agriculture (Carpenter et al., 1998). However, not all applied N is absorbed by crops.
Instead, variable portions that leach from agricultural soils are exported with rainwater (Goulding, 2000; Giordano et al., 2021) and increase the N concentration in downstream water bodies (Juston et al., 2016; Djodjic et al., 2021). To prevent further eutrophication, it is imperative to interrupt these N transports, for instance by creating wetlands.

Nitrogen removal in wetlands

Wetlands have been recognised for their capacity to remove N from agricultural runoff at least since the 1980s (Fleischer et al., 1987; Vymazal, 2022). Over the subsequent decades, the understanding of wetland function advanced remarkably (Kadlec and Wallace, 2009; Land et al., 2016). The creation or restoration of wetlands in agricultural areas is now widely considered an efficient measure to remove N and thus counteract eutrophication (e.g., Audet et al., 2020; Crumpton et al., 2020; Xia et al., 2020).

The predominant form of N in runoff from arable land is nitrate (NO₃⁻), originating from fertiliser application (Howarth et al., 1996; Lawniczak et al., 2016). This N is removed in wetlands mainly via denitrification: a microbial process where waterborne NO₃⁻ is converted to atmospheric N₂ under anoxic conditions (Xue et al., 1999; Kadlec, 2012). A lesser, but variable, portion of the NO₃⁻ is transformed to nitrous oxide (N₂O) instead of N₂ (Hernandez and Mitsch, 2007; Li et al., 2013). By converting bioavailable N back to N₂, denitrification reverses the Haber-Bosch process (Galloway et al., 2004). On a global scale, wetlands are increasingly recognised as crucial ecosystems for denitrification (Martínez-Espinosa et al., 2021).

There are, however, still knowledge gaps to be addressed concerning created wetlands and N removal. First, there is a lack of long-term studies on N removal in created wetlands (Vymazal, 2018). Second, further research is needed on how N removal can be combined with other wetland ecosystem services (Thorslund et al., 2017). Third, the effects on N removal of seasonally variable hydrology in created wetlands are not fully understood (Land et al., 2016), and fourth, the impacts of vegetation harvesting in wetlands are still unclear (Vymazal, 2017). The following sections explain the current understanding of how various factors influence the extent of denitrification and N removal in created wetlands.

The importance of wetland vegetation

Large aquatic plants that grow in wetlands, commonly referred to as macrophytes, are essential for N removal in created wetlands (Brix, 1997). While some N is removed through plant assimilation, this is typically a minor
removal pathway compared to denitrification (Bachand and Horne, 2000; Lin et al., 2002). In addition, decomposing vegetation will release assimilated N back into the water (Zhou et al., 2018). Denitrifying microorganisms are influenced by various environmental factors in wetlands (Faulwetter et al., 2009), and require for instance underwater surfaces for biofilm growth and bioavailable organic carbon (C) (Bastviken et al., 2003; Toet et al., 2003). Wetland vegetation, especially through the accumulation of plant litter, can provide these conditions and thus support N removal (Brix, 1997; Kadlec and Wallace, 2009).

Macrophytes may be categorised based on their growth form, and four groups can be distinguished: emergent macrophytes, submerged macrophytes, floating-leaved macrophytes, and free-floating macrophytes (Kadlec and Wallace, 2009; Lesiv et al., 2020). Although it is established that wetland vegetation promotes N removal (e.g., Alldred and Baines, 2016), there has been conflicting evidence regarding which of these vegetation types favour N removal the most. For instance, Eriksson and Weisner (1997) emphasised the importance of submerged macrophytes for denitrification, whereas Veraart et al. (2011) revealed higher denitrification rates associated with floating than with submerged vegetation. Furthermore, Weisner et al. (1994) suggested that a mixture of submerged and emergent vegetation would be most beneficial for N removal, but Weisner and Thiere (2010) showed the highest N removal in wetlands dominated by emergent vegetation. Planting macrophytes in wetlands has been suggested as a measure to increase vegetation cover and N removal (Lin et al., 2002). However, further examination is required to ascertain which type of vegetation ought to be planted in created wetlands to maximise N removal in both short-term and long-term perspectives.

Spatial and seasonal variations

Removal of N can vary greatly between wetlands located in different parts of an agricultural landscape. Absolute N removal (mass removal per area) is positively correlated to the N concentration, hydraulic loading rate (HLR, water volume entering the wetland per area and time), and N load reaching the wetland (Land et al., 2016; Weisner et al., 2016). Therefore, absolute N removal is promoted in wetlands located downstream of arable land where they can receive as much runoff as possible (Zedler, 2003; Tomer et al., 2013). Relative N removal (mass removal in proportion to N load), however, is negatively correlated to the HLR and N load (Fisher and Acreman, 2004; Crumpton et al., 2020). Therefore, achieving high absolute N removal comes at the expense of relative N removal, and vice versa (Kadlec, 2005). By maximising the amount of N removed, eutrophication effects in downstream water bodies can be reduced (Smith, 2003; Jeppesen et al., 2011). Hence, areas with high N loads should be prioritised for wetland creation or restoration.
In temperate climates, N removal also varies with season. Denitrification is temperature-dependent and promoted by higher temperatures (Bachand and Horne, 2000; Hernandez and Mitsch, 2007). Therefore, N removal generally peaks during the warm summers (Spieles and Mitsch, 2000; Bastviken et al., 2009). Seasonal variations in N removal are further influenced by the vegetation growth-decomposition cycle (Kadlec and Reddy, 2001), as well as seasonal hydrological patterns (Tanner and Kadlec, 2013). Thus, wetland N removal may be considerably influenced by climatic changes.

**Design, maintenance, and improvements**

The size of a wetland can significantly affect its N removal. A large wetland area ($A_w$) relative to the catchment area ($A_c$) generally corresponds to low HLR and N load, because runoff from the catchment is distributed across a more extensive wetland surface. Consequently, an increased $A_w:A_c$ ratio typically reduces the absolute N removal in individual wetlands (Tanner and Kadlec, 2013). However, a lowered HLR promotes a longer hydraulic residence time (HRT, an estimation of how long incoming water stays in the wetland), which favours relative N removal (Kadlec, 2012; Wu et al., 2015). Therefore, relative N removal is generally improved by a higher $A_w:A_c$ ratio (Vymazal, 2017; Audet et al., 2020). In addition, an increased $A_w:A_c$ ratio results in more kilograms of N removed from the catchment (Arheimer and Wittgren, 1994), even though the absolute N removal stated per wetland area may be low in individual wetlands.

Wetland N removal can be enhanced by appropriate wetland design. Hydraulic efficiency is a measure of water dispersion and degree of plug flow in the wetland (Persson et al., 1999). High hydraulic efficiency promotes N removal (Kadlec, 2005; Wörman and Kronnäs, 2005) by promoting contact of the through-flowing water with biofilms containing denitrifying microorganisms. The hydraulic efficiency of a wetland generally increases with a higher wetland length-to-width ratio (Persson and Wittgren, 2003) and decreases with increased water depth (Holland et al., 2004; Liu et al., 2016). Deep water can also hinder the development of emergent macrophytes (e.g., Coops et al., 1996; Vretare et al., 2001), making shallow wetlands more suitable for N removal.

Wetlands can continue to remove N for many years after creation (Audet et al., 2020), however, the removal rate may eventually decline (Braskerud, 2002). Both vegetation density and the amount of accumulated organic matter increase in wetlands over time (Craft, 1996; Kadlec and Wallace, 2009). Eventually, this can lead to channelised water flow and compromised hydraulic performance of the wetland (Jenkins and Greenway, 2005; Keefe et al., 2010). To avoid these effects, regular maintenance may be necessary (Bodin et al.,
Macrophyte harvest has been suggested to improve denitrification (Yang et al., 2016), and, additionally, to remove N stored in plant material (Asaeda et al., 2000). However, the removal of wetland vegetation has also been linked to reduced N removal (Kadlec, 2008). Therefore, the impact of harvesting on wetland N removal requires further investigation.

In created wetlands that lack the necessary vegetation for high N removal, for instance due to recent macrophyte harvest, N removal can potentially be improved through the implementation of floating wetlands. Floating wetlands, also commonly referred to as floating beds or mats (Shen et al., 2021), are buoyant structures that support emergent macrophytes with roots extending into the water column (Headley and Tanner, 2012). These root systems directly absorb N from the wetland water below the floating wetland and facilitate denitrification by providing extensive surfaces for microbial biofilm development (Shahid et al., 2018; Choudhury et al., 2019) and by supplying denitrifiers with organic C (Wu et al., 2017). So far, floating wetlands have mainly been tested in mesocosms (Pavlineri et al., 2017), and further studies on in situ applications are needed.

Additional ecosystem services

Interest in the concept of wetland multifunctionality, where multiple ecosystem services are integrated in wetlands, has recently grown (Acreman et al., 2011; Baylan and Karadeniz, 2018). However, interactions between different ecosystem services may be difficult to evaluate due to their complexity (Bennett et al., 2009; Boughton et al., 2019). Attempts to combine several ecosystem services within individual wetlands can result in synergistic, additive, as well as antagonistic effects, and there is a general lack of knowledge surrounding the many possible outcomes.

As an example, in agricultural areas where intense rainfall events elevate N transports (Jiang et al., 2010; Vaughan et al., 2017), N removal can potentially be combined with flood attenuation (Jessop et al., 2015). Wetlands can mitigate floods by reducing flow rates and retaining water (Acreman and Holden, 2013; Wu et al., 2020). Enhancing water storage capacity by releasing water before a flood (Tang et al., 2020a) enables wetlands to retain N-rich floodwater for extended periods, which should promote N removal. However, the feasibility of combining these ecosystem services remains uncertain (Thorslund et al., 2017) and requires further investigation. Additionally, potential increases in greenhouse gas (GHG) emissions, especially N₂O resulting from fluctuating water levels (Hernandez and Mitsch, 2006; Jørgensen and Elberling, 2012), should be considered during such investigations.
Effects of a changing climate

Climate change can affect wetland N removal in several ways, for instance by altering hydrological patterns. Climate change intensifies the global water cycle, increasing both precipitation and evaporation (Huntington, 2006; Sohail et al., 2022). In the northern temperate zone, this may amplify seasonal variations in runoff and N transports by lowering summer transports and elevating winter transports (Pilling and Jones, 2002; Moore et al., 2008). In addition, climate change increases the likelihood of floods in many parts of the world (Forzieri et al., 2016; Kreibich et al., 2022), as well as the frequency of intense rainfall events during otherwise dry summers (Kendon et al., 2014; Rousi et al., 2021). In the near future, temperate wetlands may therefore receive a larger proportion of the annual N load during the colder months, when the potential for N removal is lowered, and during periods of intense precipitation. However, the impacts of climate change on wetland N removal need to be further examined.

Changes in global climate conditions, including rising water temperatures, are exacerbating eutrophication and its negative consequences (Rabalais et al., 2009). Concurrently, the increased eutrophication leads to elevated emissions of GHGs such as N₂O, methane (CH₄), and potentially carbon dioxide (CO₂) from aquatic systems, thus amplifying the effects of climate change (Moss et al., 2011; Meerhoff et al., 2022). Due to this positive feedback loop linking eutrophication and climate change, the already important role of created wetlands is becoming increasingly crucial.
Aims and objectives of this thesis

The objective of this thesis was to assess various aspects of wetland N removal, with the aim of providing a broadened understanding of considerations, challenges, and possibilities associated with achieving high N removal in created wetlands. Considerations involve evaluating the necessity of planting and suitability of different vegetation types (Paper I), as well as determining optimal wetland placement (Paper II) and design (Paper III). Challenges may arise due to trade-offs in the delivery of multiple ecosystem services by individual wetlands (Paper II) and as a result of altered flow patterns caused by climate change (Paper III). Possibilities for enhancing N removal are linked to synergistic effects among different ecosystem services (Paper II), specifically N removal and flood attenuation (Paper IV), and to macrophyte harvest as well as installation of floating wetlands (Paper V).
Material and methods

Field data was collected either in an experimental wetland facility (Paper I, IV, and V) or in created wetlands (Paper III), all located in southern Sweden (Figure 1). Paper II is a literature review for which no experimental data was collected. The methods used to conduct these studies are described below.

Figure 1. Map of southern Sweden zoomed in from a map of Europe, highlighting all the study sites included in this thesis. The location of the experimental wetland facility is represented by a black dot (Paper I, IV, and V), and white dots indicate the locations of the created wetlands (Paper III). Figure modified from Paper III.

Experimental wetland facility

Paper I, IV, and V are based on data obtained from an experimental wetland facility situated near Halmstad in south-western Sweden (Figure 1). This facility has been in operation since 2003, and consists of 18 replicated wetlands designed to simulate created wetlands. The wetlands in the facility are placed four meters apart, in two rows of nine (Figure 2a). All wetlands were constructed to be 40 m$^2$ at ground level, sloping down to a flat bottom of 12 m$^2$ (Figure 2b). Each wetland is equipped with an inlet pipe and an outlet pipe positioned on opposite short sides of the basin. Wetland depth is controlled by the height of the outlet pipe, and inlet flow rate is adjustable with a faucet on the inlet pipe. Incoming water to the facility is groundwater with high N concentration, almost exclusively in the form of NO$_3^-$, infiltrated from agricultural fields around the facility.
Figure 2. a) Schematic diagram of the experimental wetland facility. All water is taken from groundwater source A for distribution to inlet tanks B, C, and D. From B, C, and D, water is then fed to the six wetlands encircled by the same broken line. Wetlands are numbered from 1 to 18. In each wetland, the inlet pipe is represented by a line and the outlet pipe is represented by a dot, and the water surface is shown in blue. Figure modified from Paper I. b) Design of the wetlands in the experimental wetland facility. This illustration depicts a wetland with a water depth of 0.6 m, with blue water and dark grey inlet and outlet pipes. Numbers represent distances in meters. Figure modified from Paper IV.

Following the construction of the facility, six of the wetlands were planted with emergent vegetation (*Phragmites australis*, *Glyceria maxima*, and *Phalaris arundinacea*) while six were planted with submerged vegetation.
Elodea canadensis, Myriophyllum alterniflorum, and Ceratophyllum demersum). The remaining six wetlands were left unplanted for natural colonisation. After nearly two decades, all the experimental wetlands had become overgrown with emergent vegetation, mainly P. australis, and the bottom and sides of those initially planted with emergent vegetation were partly covered by a thick root mat. In 2020, above-ground plant biomass was removed from the wetlands to restore them to a less overgrown state.

Created wetlands

Paper III is based on data collected in created (as opposed to experimental) wetlands located on the western and eastern coasts of southern Sweden (Figure 1). In total, nine wetlands of varying size, shape, and catchment characteristics were included in this study (Figure 3).

Figure 3. a) Aerial photograph of one of the studied created wetlands, with a water surface area of 0.4 ha. Photographed by Magnus Danbolt, 2019-09-20. b) Ground-level photograph of the same wetland. Photographed 2019-05-20. Figure modified from Nilsson et al. (2021).
The three wetlands on the western coast were created between 1991 and 2002 to intercept agricultural runoff from catchments consisting almost exclusively (85–95%) of arable land. These wetlands had water surface areas of between 0.2 and 0.4 ha. The six wetlands on the eastern coast were also created mainly for nutrient removal (N and/or P), but either in agricultural catchments or in catchments predominantly covered by forests. The size range of these wetlands was 0.02 to 2.5 ha, and their creation took place between 2007 and 2011.

**Experiment designs**

**Paper I** is a long-term study on how initial planting of created wetlands affects N removal over time. The wetlands in the experimental wetland facility were divided into three treatments based on initial planting: emergent vegetation wetlands (EV), submerged vegetation wetlands (SV), and unplanted free development wetlands (FD). From the year the wetlands were created (2003, defined as year 0) to 11 years afterwards, water samples (for N analyses) and measurements (e.g., flow rates and water temperatures) were collected on average twice a month. Plant species composition and the proportion of emergent vegetation cover in the wetlands were determined annually.

**Paper III** compares N removal in created wetlands under different climate conditions. Due to spatial and temporal differences in precipitation, flow rates during summers to the wetlands in south-eastern Sweden (east wetlands, studied between 2017 and 2019) were significantly lower compared to the summer flows to the wetlands in south-western Sweden (west wetlands, studied between 2003 and 2006). East wetlands were used in this study to represent wetlands in a future temperate climate where summer droughts have become more common. Over 18 months to three years, automatic samplers collected measurements and flow-proportional water samples. In all wetlands, depth profile surveys were conducted, and emergent vegetation cover as well as hydraulic shape (explained in the results section) were estimated.

**Paper IV** tests if N removal can be synergistically combined with flood attenuation in wetlands. In this study, the wetlands in the experimental wetland facility were subjected to recurring flow and no-flow periods during May to September 2021. Half of the wetlands were intermittently flooded (high water storage capacity) and the other half were permanently flooded (low water storage capacity). The effects of increased water storage capacity on N removal and GHG emissions were tested in wetlands of different designs (shallow, deep, and large). Measurements and samples (for N and GHG analyses) were collected from the facility typically every second day during flow periods, and every fourth day during no-flow periods. Vegetation cover and species composition in the wetlands were estimated once during this study.
Paper V examines the possibility of increasing N removal in heavily overgrown wetlands by harvesting macrophytes and installing floating wetlands (woodchips-based floating beds, WFBs). Four treatments were compared: (1) harvest, (2) harvest and 5% of the water surface area covered with WFBs, (3) harvest and 20% cover of WFBs, and (4) unaltered control. The WFBs were constructed out of jute bags that were filled with fresh woodchips (Betula spp.) and planted with G. maxima and Filipendula ulmaria. Water samples and measurements were collected twice a month from June to September 2020. After the experiment, vegetation growth on the WFBs and denitrification gene abundance (nirS, nirK, nosZI, and nosZII), used as a proxy for biofilm denitrification potential, on plant roots and woodchips were measured.

Laboratory analyses

Sampling and analyses were done in accordance with standard methods. Total nitrogen (Tot-N) and NO3-N concentrations in water samples from wetland inlets and outlets were measured in the laboratory at Halmstad University (Paper I, IV, V, and west samples from Paper III) or by the accredited commercial laboratory SYNLAB (east samples from Paper III). Concentrations of NO3-N were determined through flow injection analysis with spectrophotometric detection, and concentrations of Tot-N through flow injection analysis (Paper I, III, and V) or combustion and chemiluminescent detection (Paper IV). Headspace gas concentrations of N2O and CH4 were determined through gas chromatography in the laboratory at Aarhus University and converted to aqueous concentrations according to Henry’s law (Paper IV). The N content in harvested biomass was quantified in the laboratory at Halmstad University using combustion and gas chromatography (Paper V). Denitrification gene abundance was determined in the laboratory at Linnaeus University using real-time quantitative polymerase chain reaction (qPCR) (Paper V).

Calculations

In the experimental studies of this thesis, N removal was quantified and expressed as absolute N removal (Paper III, IV, and V), relative N removal (Paper III, IV, and V), the N removal rate coefficient k (Paper I), or the temperature-adjusted N removal rate coefficient ka (Paper V). Absolute N removal was calculated according to Eq. 1 and relative N removal according to Eq. 2. Calculations of k and ka were done according to Eq. 3 and Eq. 4, respectively (Kadlec, 2005).

\[
\text{Absolute N removal} = \frac{(C_{\text{in}} \times Q_{\text{in}}) - (C_{\text{out}} \times Q_{\text{out}})}{A} \quad \text{Eq. 1}
\]
Relative N removal \[= \frac{(C_{\text{in}} \times Q_{\text{in}}) - (C_{\text{out}} \times Q_{\text{out}})}{(C_{\text{in}} \times Q_{\text{in}})} \] Eq. 2

\[k = \frac{N \times Q_{\text{in}} \times \left( \frac{C_{\text{in}}}{C_{\text{out}}} \right)^{\frac{1}{N}} - 1}{A} \] Eq. 3

\[k_a = \frac{N \times Q_{\text{in}} \times \left( \frac{C_{\text{in}}}{C_{\text{out}}} \right)^{\frac{1}{N}} - 1}{A \times \theta(T - 20)} \] Eq. 4

In the equations above, \(C_{\text{in}}\) is the inlet N concentration, \(Q_{\text{in}}\) is the inlet flow rate, \(C_{\text{out}}\) is the outlet N concentration, \(Q_{\text{out}}\) is the outlet flow rate, \(A\) is the wetland surface area, \(N\) is the hydraulic efficiency parameter, \(\theta\) is the temperature coefficient, and \(T\) is the water temperature. Absolute N removal was expressed either in kg ha\(^{-1}\) yr\(^{-1}\) or g m\(^{-2}\) d\(^{-1}\), relative N removal in %, and \(k\) as well as \(k_a\) in m d\(^{-1}\). In Paper I and V, \(Q_{\text{in}}\) was equal to \(Q_{\text{out}}\), and in Paper IV, Eq. 1 was modified to account for changes in the N content of each wetland. For more information, see the respective paper.

Statistical analyses

The data collected in the experimental studies was evaluated with statistical analyses. Each studied wetland was treated as one individual experimental unit. The primary statistical method used in the studies conducted at the experimental wetland facility (Paper I, IV, and V) was analysis of variance (ANOVA). Differences among the means of more than two groups were assessed using one-way or two-way ANOVAs, while repeated measures ANOVAs were employed to analyse datasets containing multiple measurements obtained from the same wetland. Significant effects detected in the ANOVAs were complemented by Tukey HSD post hoc tests to identify specific differences in group means.

Independent samples t-tests were used to assess differences in means when only two groups were compared (Paper III, IV, and V). In addition, linear regression as well as analysis of covariance (ANCOVA) were used to evaluate changes in the dependent variable over time (Paper I), and relationships between variables were tested with Pearson correlation, linear regression, and multiple linear regression (Paper III). Statistical significance was determined at a significance level of \(p<0.05\).
Literature review

**Paper II** provides an overview of wetland multifunctionality, exploring potential trade-offs and synergies among ecosystem services at both individual wetland level and at the scale of whole landscapes with multiple wetlands (wetlandscapes). A three-step process, supported by a literature review, was used to identify wetland properties connected to four main objectives of wetland creation and restoration: (1) regulation of water flows and nutrient/pollutant loads, (2) climate change mitigation, (3) biodiversity conservation, and (4) cultural ecosystem services. The first step was a workshop with all co-authors held in October 2021. During this workshop, a preliminary list of important properties (biophysical, water, biological, and landscape properties) for the main objectives was compiled. This list was then transferred to a matrix linking properties with the objectives (as positive, negative, or variable impact). In the second step, the authors cross-checked each other’s assessments and added supporting references, and in the third step, the matrix was shared with colleagues within each field to uncover missing relationships and gather additional references. Finally, the matrix was used to identify potential trade-offs and synergies among the different objectives, and empirical studies examining such effects were reviewed. In this thesis, only aspects of wetland multifunctionality related to N removal are included.
Results and discussion

Considerations in wetland creation

*Should newly created wetlands be planted?*

The proportion of emergent vegetation cover increased with wetland age in *Paper I*. Vegetation cover was consistently higher in EV, where emergent vegetation had been planted, compared to the other treatments where submerged (SV) or no vegetation (FD) was planted. Emergent vegetation usually becomes established in wetlands within the first years after creation (Craft, 1996; Overbeek et al., 2020), as was observed in our study. One of the planted emergent species, *P. australis*, the most common macrophyte in European wetlands (Vymazal, 2013), thrived during this study. With its competitive advantage over other wetland plant species once settled (Paradis et al., 2014), and its ability to quickly spread to new environments (Packer et al., 2017), *P. australis* became the dominating species in EV and increasingly abundant over time in SV and FD. Similarly, *Typha latifolia* can rapidly colonise new habitats (Bansal et al., 2019). A few years after the wetlands were created, *T. latifolia* had spread from outside the facility and become established in FD, and this species was later established in SV as well. Four years after creation, EV were almost completely covered by emergent vegetation, whereas SV and FD only reached approximately 50% cover of emergent vegetation over the 12-year study duration. Submerged and floating-leaved species were more common in SV and FD than in EV. Thus, throughout the study, vegetation cover and species composition differed between treatments.

The wetlands in *Paper I* passed three distinct phases of N removal from the time of creation to 11 years later: initial phase, colonisation phase, and mature phase (Figure 4a). Initially, N removal was higher in planted (EV and SV) than in unplanted (FD) wetlands, which can be explained by FD lacking the necessary vegetation to support denitrification (Alldred and Baines, 2016). Once vegetation had established in FD, N removal in SV and FD never differed. During the colonisation phase, N removal in EV was generally higher than in the other treatments. While EV had relatively stable N removal throughout the study, there was a significant increase in N removal over time in SV and FD (Figure 4b). In the mature phase, N removal converged in all three treatments.
In agreement with other studies (e.g., Audet et al., 2021b), Paper I shows a positive effect of emergent vegetation on N removal. However, in older wetlands, N removal seemed to be determined by factors other than emergent vegetation cover. The variations in N removal were attributed to the process of wetland maturation. In addition to increased vegetation cover, sub-surface accumulation of plant material occurs in wetlands over time (Craft, 1996; Kadlec and Wallace, 2009). This organic matter provides additional bioavailable organic C and multiplies the surface area for biofilm growth, thus improving conditions for denitrification (Weisner et al., 1994; Brix, 1997). With higher biomass production and slower decomposition rates, emergent
vegetation can generate more plant litter than submerged or floating vegetation (Bachand and Horne, 2000; Overbeek et al., 2020). As a result, the maturation process may be hastened when highly productive emergent vegetation is the dominating vegetation type. Thus, by planting emergent vegetation in newly created wetlands, high N removal can be achieved quicker than when submerged or no vegetation is planted. However, in a long-term perspective, the effect of planting fades, as mature wetland ecosystems exhibit similar N removal regardless of initial planting.

Where should wetlands be created?

In Paper II, the removal of nutrients, including N, is highlighted as a key objective for wetland creation and restoration. This review emphasises the importance of many wetland properties previously suggested to affect N removal in individual wetlands. For instance, the positive relationships between N removal and hydraulic efficiency, N concentration, and vegetation (e.g., Kadlec, 2005 and Paper I) are discussed.

Paper II additionally considers N removal at the scale of whole wetlandscapes. At this level, N removal may be more affected by the position of several wetlands in the landscape than by properties of individual wetlands. To increase the positive effect on N removal of a high $A_w:A_c$ ratio (Arheimer and Wittgren, 1994), wetlands need to be located where they can intercept substantial portions of the total N load (Quin et al., 2015; Thorslund et al., 2017). Transports of N from both active and legacy sources are generally greatest in catchments with a high proportion of agricultural land (Chen et al., 2021b). Strategic placement of wetlands to intercept as much agricultural runoff as possible from such catchments would enhance N removal at both the individual wetland and wetlandscapes level.

How should wetlands be designed?

Wetland shape is described in Paper III using a new concept: hydraulic shape. Hydraulic efficiency affects N removal (e.g., Wörman and Kronnäs, 2005) and is therefore an important aspect of N removal evaluations. However, conducting tracer studies and measuring hydraulic efficiency can be costly (Headley and Kadlec, 2007) and is not always feasible. We therefore saw a need for a more easily estimated indication of hydraulic performance. Hydraulic shape was defined as the ratio between the shortest travel distance for water from inlet to outlet and the square root of the water surface area. Thus, two factors influencing hydraulic efficiency, namely wetland length-to-width ratio and the relative positioning of wetland inlet and outlet (Persson and Wittgren, 2003; Sabokrouhiyeh et al., 2017), are reflected in hydraulic shape.

The design of created wetlands, including the wetlands in Paper III, does not always promote hydraulic efficiency (Wei et al., 2013; Laurent et al., 2015).
Our studied wetlands exhibited a range of hydraulic shapes. This range was reflected in the N removal, which was positively correlated to hydraulic shape. Absolute N removal was best explained (highest $R^2$) by a multiple regression model using N load and hydraulic shape as predictors, and relative N removal was best explained by emergent vegetation cover and hydraulic shape. The strong effect of hydraulic shape on N removal suggests that hydraulic shape may be a useful tool in planning wetland creation and evaluating wetland function. It further indicates that wetland design plays a fundamental role in explaining differences in annual N removal among created wetlands, and that N removal is promoted in wetlands of elongated shape with maximised distance between inlet and outlet.

Challenges in achieving high nitrogen removal

*What trade-offs occur between different ecosystem services?*

Certain trade-offs between N removal and other ecosystem services were identified in **Paper II**. For instance, the high concentrations and loads of N needed to sustain high N removal may entail elevated GHG emissions and reduced biological diversity in the wetland. Production of CH$_4$ can be stimulated in eutrophic systems (Beaulieu et al., 2019), and N$_2$O production generally rises with increasing N concentration (Moseman-Valtierra et al., 2011; Mander et al., 2014). Further, succession towards a less diverse vegetation state can be accelerated in N-rich wetlands (Khan and Ansari, 2005; Jessop et al., 2015). The highest N removal may be achieved in wetlands dominated by uniform emergent vegetation (Weisner and Thiere, 2010), where also the animal diversity is low (Jessop et al., 2015). Wetlands created to support biodiversity may lack properties associated with high N removal and, as a result, exhibit lower N removal compared to wetlands designed primarily to remove N (Weisner et al., 2016). These trade-offs may limit the possibility of multifunctionality in individual wetlands.

A key challenge for wetland multifunctionality presented in **Paper II** is thus the conflicting effects of certain properties on N removal and other ecosystem services. As previously suggested (Thorslund et al., 2017; Åhlén et al., 2020), we argue that maximising the delivery of ecosystem services requires a shift in focus from individual wetlands to whole wetlandscapes. Within these landscapes, multiple wetlands should be placed in different locations based on their main purpose to collectively promote N removal and various other ecosystem services (Figure 5, described in detail in the paper).
Will drier summers affect nitrogen removal?

The wetlands in Paper III, located in opposite coastal regions of southern Sweden (east and west), exhibited considerable differences in climatic conditions, especially during summers. In general, east wetlands were warmer...
during summers, colder during winters, and received less precipitation compared to the west wetlands. These disparities between the two wetland groups were expected, as the east region typically receives less than half of the normal annual precipitation in the west region (Persson et al., 2015a; b). In addition, the east region has experienced increasingly dry summers over time (Chen et al., 2021a), and the exceptionally dry summer of 2018 (Peters et al., 2020) fell within the sampling period for the east wetlands. Precipitation affected wetland hydrology. Water flow to east wetlands followed a distinct seasonal pattern, with HLRs approaching or reaching zero during summers. Thus, for extended periods, east wetlands received minimal inflow but still contained stagnant water. In contrast, HLRs to west wetlands were relatively stable across seasons, but variable from month to month. West wetlands did not experience any no-flow periods.

West wetlands in **Paper III** achieved maximum N removal during summers, and low N removal during winters, as can be expected under normal circumstances due to the temperature-dependency of denitrification (e.g., Hernandez and Mitsch, 2007). However, high N removal requires input of N-rich water. Hence, due to lack of inflow and N load, virtually no N was removed during summers in east wetlands. Instead, east wetlands displayed remarkably high N removal during winters, despite low temperatures. Thus, no-flow periods during summers seemingly created conditions that promoted winter N removal. Winter N removal was high enough to compensate for the loss of N removal during the dry eastern summers, and on an annual basis, there were no significant differences in absolute or relative N removal between the two wetland groups. Overall, annual N removal was determined by N load, vegetation cover, and hydraulic shape rather than climatic conditions.

The high winter N removal achieved by east wetlands in **Paper III** could potentially be explained by the periods of stagnant water. Stagnant water favours the development of anoxia (Mudge et al., 2007; Pardo and García, 2016), which in turn reduces the decomposition rate of organic matter (Brinson et al., 1981; Bastviken et al., 2004). In addition, the depletion of NO$_3^-$ in stagnant water (Kadlec and Wallace, 2009) prevents further denitrification that would otherwise consume organic matter. In this way, the breakdown of organic matter was likely hindered during summers in east wetlands. As a result, more organic matter, which promotes denitrification (e.g., Ballantine et al., 2014), may have been available in east wetlands throughout the rest of the year. Increased plant productivity during the warm summers (Asaeda et al., 2005; Eller et al., 2013) could be another explanation for high winter N removal in east wetlands. This study indicates that highly loaded created wetlands may remain effective for N removal even in a future climate with drier summers.
Possibilities to improve nitrogen removal

Is it possible to combine efficient nitrogen removal and flood attenuation?

Intermittently flooded wetlands in Paper IV demonstrated greater water retention compared to permanently flooded wetlands. In accordance with prior studies (Martinez-Martinez et al., 2014; Tang et al., 2020b), wetlands with the largest volume (relative to the hydraulic load) had the highest water buffering capacity. Water was mainly retained during the initial phase of the flow periods. This is a key aspect of flood mitigation, both to protect humans (Jonkman and Kelman, 2005) and to intercept peak N transports (Jiang et al., 2010). No significant differences were found in the aqueous concentrations of N₂O or CH₄ between permanently and intermittently flooded wetlands. Thus, a dynamic water storage did not seem to induce any elevation in GHG emissions.

All wetlands in Paper IV removed substantial amounts of N, but the N removal varied among wetlands of different design (shallow, deep, or large) and hydraulic type (intermittently or permanently flooded). Overall, wetlands with the lowest N load showed the lowest absolute N removal, but the highest relative N removal. Thus, the relationship between N load and N removal, previously shown by other authors (e.g., Vymazal, 2017) and in Paper II and III, was also apparent in this study. Among deep and large wetlands, higher N removal was achieved in permanently flooded than in intermittently flooded wetlands. This may be attributed to longer residence time in the permanently flooded wetlands. During no-flow periods, almost all N could be removed from the stagnant water in permanently flooded wetlands. In contrast, water was released from intermittently flooded wetlands while still containing N. However, among shallow wetlands, no differences in N removal were detected between the two hydraulic types.

The nearly complete emptying of intermittently flooded wetlands during no-flow periods in Paper IV may not have been ideal, as contact of wetland soil with the atmosphere can create unfavourable conditions for denitrification (Hunter et al., 2008; Song et al., 2010). Additionally, despite the large water storage capacity of an emptied wetland, dry soils might exacerbate flood risk (Acreman and Holden, 2013). Therefore, a more advantageous approach could be to partially empty wetlands before a flood event. Moreover, prolonging the retention of water in intermittently flooded wetlands before release could enhance N removal. Nevertheless, specific wetland designs allowed for a significant increase in water buffering capacity without compromising N removal or leading to elevated GHG emissions. Our findings, in line with Jessop et al. (2015), thus suggest that it is possible to combine efficient nitrogen removal and flood attenuation in created wetlands.
Which synergistic effects support wetland multifunctionality?

Several possible synergies and co-productions among wetland ecosystem services were identified in Paper II. For instance, wetland properties such as vegetation and high hydraulic efficiency can enhance the removal of N (e.g., Paper I and III) as well as P (Dierberg et al., 2005; Skinner, 2022). In addition, both absolute N removal and absolute P removal are promoted by high concentration and load of the respective nutrient, as well as high HLR (Land et al., 2016; Weisner et al., 2016). Increased P concentration can even improve N removal (Song et al., 2019), and high N removal can be achieved in wetlands created specifically to remove P (Nilsson et al., 2021). Because agricultural fertilisers are major sources of both nutrients (e.g., Beusen et al., 2016), the optimal wetland placement suggested in Figure 5 is the same regardless of targeted nutrient.

Paper II further elaborates on how specific properties can benefit multiple ecosystem services alongside N removal. Two examples are emergent vegetation and the total A_w in a landscape. An intermediate cover of emergent vegetation can support animal diversity by providing shelter from predation, nesting habitats, and food for various taxa (Cazzanelli et al., 2008; Ma et al., 2010; Shulse et al., 2012). Furthermore, emergent macrophytes play a crucial role in the accumulation of C and in making wetlands a net CO_2 sink (Moore and Hunt, 2012; Valach et al., 2021). Primary production and thereby C storage can increase with higher N concentration (Pacheco et al., 2014) that promotes N removal. Thus, as also identified by Jessop et al. (2015), C sequestration and N removal can be synergistically combined in wetlands. Moreover, an increase in the A_w:A_c ratio at the wetlandscape level enhances total N removal (Arheimer and Wittgren, 1994), while also leading to higher species richness of wetland taxa such as birds and amphibians (Dertien et al., 2020), and reduced downstream flooding (Tang et al., 2020b). Because of these synergies and co-productions, efforts to enhance the delivery of various wetland ecosystem services have the potential to simultaneously improve N removal.

Can macrophyte harvest and floating wetlands improve nitrogen removal?

During the experiment for Paper V, both G. maxima and F. ulmaria survived and grew on the woodchips-based floating beds (WFBs). Successful use of these species on floating wetlands has been shown before (van Oostrom, 1995; Choudhury et al., 2019), despite F. ulmaria not being categorised as a macrophyte. Relative biomass production was significantly higher for G. maxima than for F. ulmaria, and the average number of G. maxima shoots on the WFBs had increased threefold by the end of the experiment. In addition, G. maxima displayed significantly greater maximum root length and specific root surface area compared to F. ulmaria. Denitrification gene abundance, used as a proxy for biofilm denitrification potential (Faulwetter et al., 2009; Hallin et al.,
were higher on roots of *G. maxima* than on *F. ulmaria* roots. Thus, although *F. ulmaria* assimilated more N into plant biomass, *G. maxima* seemed to be overall more suitable for use on WFBs to promote N removal.

In **Paper V**, the removal of macrophytes from heavily overgrown wetlands resulted in significantly improved N removal (expressed as absolute and relative N removal, as well as the coefficient $k_a$). All manipulated treatments, i.e., wetlands where macrophytes were removed, showed overall higher N removal compared to the control wetlands. Treatment, sampling date, and their interaction affected N removal. Removal of N was generally the highest, and the difference between treatments was most prominent, through late June to early August (Figure 6). No significant differences in N removal were observed between harvested wetlands and wetlands with WFBs, or between wetlands with 5 or 20% cover of WFBs. Thus, the addition of WFBs did not further increase N removal in harvested wetlands. A boosting effect on N removal of macrophyte harvest, also observed in Yang et al. (2016) and Zheng et al. (2018), may be attributed to an increase in dissolved organic C (Hamilton and Frank, 2001), which promotes denitrification. This effect likely masked the impact of the WFBs on wetland N removal.

![Figure 6](image.png)

**Figure 6.** Relative nitrogen (N) removal in heavily overgrown wetlands (control), wetlands where macrophytes had been removed (harvest), and wetlands where 5–20% of the water surface area was covered by woodchips-based floating beds (WFBs) after macrophyte harvest (+5% WFBs and +20% WFBs). Columns represent average N removal in four replicate wetlands, and error bars represent standard error. Asterisks mark significant differences ($p<0.05$) between treatments (8/7, 13/7, and 10/8: higher in harvest, +5% WFBs, and +20% WFBs than in control; 17/8: higher in +20% WFBs than in control). Statistical tests were performed on transformed values. Figure modified from **Paper V**.
The WFBs used in **Paper V** hosted denitrification genes on both woodchips and plant roots, and the plants on the WFBs assimilated N. On certain sampling dates, N removal was higher in wetlands with WFBs compared to control wetlands, while N removal in harvested and control wetlands did not significantly differ (exemplified in Figure 6). Thus, despite the lack of overall effects, we identified several indicators of WFBs improving wetland N removal. The use of woodchips in the WFBs likely promoted N removal (Schipper et al., 2010; Audet et al., 2021a) by providing denitrifiers with bioavailable organic C and surfaces for biofilm growth. In agreement with previous studies (e.g., Headley and Tanner, 2012), we propose that the installation of WFBs offers a possibility to increase N removal in created wetlands. In addition, this study demonstrates that N removal can be enhanced in heavily overgrown wetlands through macrophyte harvest.
Conclusions and future perspectives

Throughout this thesis, the central theme has been considerations, challenges, and possibilities associated with achieving high N removal in created wetlands. By including studies on long-term effects of planting, interactions among multiple ecosystem services, seasonally variable hydrology, and effects of vegetation harvest as well as floating wetlands, this thesis contributes to filling several knowledge gaps in the context of wetland N removal. The following paragraphs summarise the conclusions drawn from the five included papers and highlight additional insights gained from this work.

Planting highly productive emergent vegetation is an effective method for accelerating ecosystem maturation and rapidly achieving high wetland N removal (Paper I). To maximise N removal in newly created wetlands, planting should therefore be considered. The choice of species to plant depends on the geographical location of the wetland. For instance, in North America, the invasive status of *P. australis* (e.g., Hazelton et al., 2014) necessitates the selection of alternative species. Over time, even unplanted wetlands can develop into fully functioning ecosystems with high N removal (Paper I). Planting should therefore be prioritised where there is an urgent need for high N removal, especially in wetlands where natural colonisation may be hindered.

Wetland placement and design also require consideration. Ideally, wetlands should be placed downstream of major N sources, such as fertilised arable land, and receive as much runoff as possible (Paper II). Optimising wetland placement not only enhances N removal but also increases the cost-effectiveness of wetland creation (Djodjic et al., 2022). A large part of the variation in N removal among created wetlands may be explained by hydraulic shape (Paper III). When planning wetland creation, an elongated wetland shape with maximised distance between inlet and outlet should be chosen to promote N removal. During wetland restoration projects, hydraulic efficiency can be improved by adding obstructions to distribute water flow (Su et al., 2009), which would also be reflected in improved hydraulic shape.

The pursuit of multifunctionality in individual wetlands may present challenges in achieving high N removal (Paper II). For instance, planting of emergent macrophytes to promote N removal may raise concerns for the
biodiversity in the wetland. One solution could be to plant several species to increase vegetation diversity (Williams and Ahn, 2015) while also improving N removal. Another solution would be a shift in focus, from multifunctionality at the individual wetland scale to multifunctional wetlandscapes (Paper II). In these wetlandscapes, several wetlands with different primary objectives can individually fulfill their specific purpose and collectively promote the delivery of various ecosystem services, including N removal.

Annual N removal may be influenced more by conditions that can be met with proper placement (N load) and design (hydraulic shape and emergent vegetation cover) than by the seasonality of the N load (Paper III). Dry summers might even promote N removal during colder seasons, potentially by increasing organic matter availability. The drawing of general conclusions is complicated by the vastly different hydrological responses to climate change on a global scale (e.g., van Vliet et al., 2013). Nevertheless, Paper III indicates that the drier summers awaiting Swedish wetlands may be a surmountable challenge in terms of annual N removal.

Efficient N removal can be combined with flood attenuation, without elevated GHG emissions, in wetlands of specific designs (Paper IV). In addition, N and P removal may be co-produced, and properties such as emergent vegetation and the total A_w in a catchment can benefit multiple ecosystem services (Paper II). Because of synergies and co-productions, wetlands created specifically to remove N can, for instance, increase landscape biodiversity (Strand and Weisner, 2013). Similarly, there are possibilities to improve N removal at a landscape scale by creating or restoring wetlands, even if N removal is not the primary objective of each individual wetland.

In heavily overgrown wetlands, N removal can be boosted by macrophyte harvest (Paper V). This opens up possibilities for additional use of wetlands, such as using the harvested biomass for biogas production (Roj-Rojewski et al., 2019), while simultaneously promoting N removal. There is further potential to improve N removal by adding WFBs to wetlands (Paper V). Macrophyte species that grow large root systems, such as G. maxima, are probably best suited for use on WFBs. In addition to their use in created wetlands, WFBs could be installed in deeper aquatic environments to promote N removal in areas where the growth of emergent macrophytes on wetland soil is hindered.

Several aspects of wetland N removal that require further investigations have been identified in this thesis. Additional research is necessary to fully understand the complex interactions between ecosystem services, and to reveal the mechanism behind the apparent boosting effect of dry summers on winter N removal. Furthermore, before implementing dynamic water storage in wetlands outside experimental testing, potential implications for ecosystem
services beyond flood attenuation and N removal should be assessed. In addition, to fully determine their potential, WFBs need further examination in experiments that extend beyond the initial effects of macrophyte harvest, and additional plant species should be tested for their suitability on WFBs.

In conclusion, wetlands, with their unique ability to deliver a multitude of ecosystem services, are important for both human well-being and for the environment. By incorporating the considerations, acknowledging the challenges, and actualising the possibilities presented in this thesis, high wetland N removal can be achieved. In effect, future creation and restoration of wetlands in agricultural areas have the potential to further contribute to mitigating eutrophication and protecting our essential water resources.
Summaries

Popular science summary

Water is essential for all living beings. However, despite its critical importance, many aquatic ecosystems worldwide are threatened by human interference. Among these ecosystems are wetlands – unique and highly productive environments necessary for the survival of numerous species of animals and plants. Wetlands also contribute to human well-being by providing various ecosystem services (benefits people obtain from ecosystems), including clean water and reduced flood risk. Globally, the extensive loss of wetland area over the past centuries, reaching up to 90% in certain regions, has led to significant declines in these crucial ecosystem services. Therefore, further efforts to create and restore wetlands are needed.

Eutrophication (excessive nutrient enrichment) is a global problem that affects numerous lakes, rivers, and coastal seas. Its detrimental effects, such as toxic algal blooms, endanger both animals and humans. The use of industrially produced fertilisers, resulting in elevated transports of nitrogen from arable land to sea, makes agriculture a major cause of eutrophication. Created wetlands (man-made wetlands in agricultural areas) can intercept these nitrogen transports by removing the nitrogen from the water. Nitrogen removal is primarily achieved through denitrification, a microbial process that converts dissolved nitrogen (in the form of nitrate) into nitrogen gas, which is naturally occurring in the atmosphere. In this way, wetlands contribute to the mitigation of eutrophication.

The extent of nitrogen removal in a wetland depends on many factors. For instance, plants support denitrifying microorganisms and are therefore indispensable in created wetlands. In addition, nitrogen removal is affected by wetland location and design, and varies seasonally. However, there are still knowledge gaps concerning wetland nitrogen removal over extended periods, the most suitable vegetation type for efficient nitrogen removal, and how removing plants from overgrown wetlands affects nitrogen removal. Additionally, further research is required to fully comprehend the complex interactions among different ecosystem services. Furthermore, climate change is altering hydrological patterns, as exemplified by increasingly dry summers in Europe. However, the implications of these changes for wetland nitrogen removal are
inadequately understood. A positive feedback effect between eutrophication and climate change underscores the escalating importance of created wetlands and the growing urgency to expand our knowledge on various aspects of wetland nitrogen removal.

The aim of this thesis was to provide a broadened understanding of considerations, challenges, and possibilities associated with achieving high nitrogen removal in created wetlands. Considerations include which vegetation type to plant, where to place wetlands, and how wetlands should be designed to promote N removal. Additionally, challenges arising from changing climatic conditions and trade-offs between ecosystem services are explored. Furthermore, synergistic effects that can occur among ecosystem services are highlighted. Lastly, the possibilities of enhancing nitrogen removal through plant harvest and installation of floating wetlands (floating platforms with emergent vegetation) are emphasised.

Five studies are included in this thesis. Field data was collected from either an experimental wetland facility or created wetlands, all located in southern Sweden. One of the studies is a literature review (an analysis of existing research), for which no new data was collected. **Paper I** is a long-term study on how planting of different types of vegetation in wetlands affects nitrogen removal over time (12 years). **Paper II** provides an overview of potential trade-offs and synergies among wetland ecosystem services (including nitrogen removal). **Paper III** compares nitrogen removal in created wetlands under different climate conditions (wet and dry summers). **Paper IV** tests if efficient nitrogen removal and reduced flood risk can be combined in the same wetland. **Paper V** evaluates how plant removal from heavily overgrown wetlands and addition of floating wetlands affect nitrogen removal.

The results of thesis show that planting emergent vegetation is an effective method to enhance nitrogen removal in newly created wetlands. Over time, however, high nitrogen removal is also achievable in unplanted wetlands, provided that vegetation can be established in them. Planting should therefore be prioritised when maximum nitrogen removal is needed rapidly, especially in isolated wetlands where natural colonisation of wetland plants may be hindered. This thesis further demonstrates that the ideal placement of wetlands is downstream of fertilised arable land, where as much agricultural runoff as possible can be intercepted. Finally, an elongated wetland shape, with maximised distance between inlet and outlet, is shown to promote nitrogen removal.

Because of trade-offs between ecosystem services, multifunctional wetlands might not be optimally placed or designed to promote high nitrogen removal. Thus, simultaneously achieving high nitrogen removal and promoting the
delivery of other ecosystem services in individual wetlands may be challenging. To maximise the delivery of ecosystem services, a shift in perspective from individual wetlands to entire landscapes of multiple wetlands is needed. Within such landscapes, several wetlands with different primary purposes can together promote both nitrogen removal and the delivery of other ecosystem services. Furthermore, this thesis shows that the challenge posed by altered seasonal patterns in water flow may be surmountable. The annual nitrogen removal in created wetlands can remain high despite dry summers, provided that the wetlands are properly placed, designed, and receive large quantities of nitrogen-rich water during winters.

Wetlands of specific designs can efficiently remove nitrogen while also reducing the risk of floods, all without causing a rise in greenhouse gas emissions. In addition, certain factors that promote nitrogen removal, such as emergent vegetation and the total area of wetlands in a catchment, also promote other ecosystem services. Thus, by creating or restoring wetlands for the delivery of these other ecosystem services, there is a possibility of simultaneously increasing nitrogen removal. Moreover, nitrogen removal in heavily overgrown wetlands can be enhanced through plant harvest, and there is potential to further improve nitrogen removal by installing floating wetlands.

To summarise, wetlands are important ecosystems for human well-being and for the environment. With a focus on wetland nitrogen removal, this thesis sheds light on important considerations to take into account, challenges to overcome, and possibilities to actualise. Certain aspects, such as the underlying processes causing interactions between ecosystem services, require further research. As the climate changes, the need for wetlands increases even more. Through improved understanding of wetland nitrogen removal, future wetlands may further contribute to mitigating eutrophication and thus protect our essential water resources.
Populärvetenskaplig sammanfattning


I vilken utsträckning kväve avskiljs i en våtmark beror på många faktorer. Exempelvis gynnas denitrifierande mikroorganismer av att det finns växter i våtmarken, vilket gör våtmarksväxter till en essentiell del av anlagda våtmarker. Dessutom påverkas kväveavskiljningen av våtmarkens placering och utformning, samt varierar med säsong. Det finns dock fortfarande kunskapsluckor, exempelvis vad gäller kväveavskiljningen över längre tidsperioder, vilken som är den mest lämpliga vegetationstypen, och hur kväveavskiljningen påverkas av att växter tas bort från övervuxna våtmarker. Vidare behövs ytterligare forskning för att fullt ut förstå de komplexa interaktionerna mellan olika ekosystemtjänster. Det är inte heller tillräckligt känt hur framtida klimatförändringar, till exempel allt torrare somrar i Europa, kommer att påverka kväveavskiljning i våtmarker. Eftersom klimatförändringar också förväntas förstärka de negativa effekterna av övergödning kommer betydelsen av anlagda våtmarker att öka i framtiden. Därmed finns det ett växande behov av att utvidga vår förståelse av olika aspekter av våtmarkers kväveavskiljning.
Syftet med denna avhandling var att ge en omfattande bild av överväganden, utmaningar och möjligheter som är förknippade med att uppnå hög kväveavskiljning i anlagda våtmarker. Till exempel handlar det om vikten av att överväga vilken vegetationstyp som ska planteras, var våtmarker bör placeras och hur de bör utformas. Dessutom undersöks de utmaningar som följer av förändrade klimatförhållanden samt att behöva kompromissa mellan olika ekosystemtjänster. Vidare belyses de potentiella synergieffekter som kan uppstå när olika ekosystemtjänster interagerar. Slutligen diskuteras även möjligheterna att förbättra kväveavskiljningen genom att skördta växter och installera flytande våtmarker, det vill säga flytande plattformar med övervattensvegetation såsom vass.

Fem studier är inkluderade i denna avhandling. Fältdata samlades in antingen från en experimentell våtmarksanläggning eller från anlagda våtmarker, alla belägna i södra Sverige. En av studierna är en litteraturöversikt (en analys av befintlig forskning), till vilken ingen originaldata samlades in. **Artikel I** är en långtidsstudie över hur plantering av olika typer av vegetation i våtmarker påverkar kväveavskiljning över tid (12 år). **Artikel II** ger en överblick över potentiella avvägningar och synergier mellan våtmarkers ekosystemtjänster (inklusive kväveavskiljning). **Artikel III** jämför kväveavskiljning i anlagda våtmarker under olika klimatförhållanden (blöta och torra somrar). **Artikel IV** testar om effektiv kväveavskiljning och minskad översvämningsrisk kan uppnås i samma våtmark. **Artikel V** utvärderar hur kväveavskiljningen påverkas när växter tas bort från kraftigt övervuxna våtmarker och flytande våtmarker installeras.


På grund av avvägningar mellan olika ekosystemtjänster så är så kallade multifunktionella våtmarker inte nödvändigtvis optimalt placerade eller utformade för att främja hög kväveavskiljning. Det kan alltså vara svårt att uppnå hög kväveavskiljning samtidigt som andra ekosystemtjänster främjas i enskilda våtmarker. För att maximera leveransen av ekosystemtjänster krävs ett perspektivskifte från enskilda våtmarker till hela landskap med flera
våtmarker. I sådana landskap kan flera våtmarker med olika huvudsyften tillsammans främja både kväveavskiljning och leverans av andra ekosystemtjänster. Denna avhandling visar dessutom att utmaningen med förändrade säsongsmönster i vattenflöden kan vara överkomlig. Den årliga kväveavskiljningen kan fortsätta vara hög trots torra somrar, förutsatt att våtmarkerna är korrekt placerade, utformade och tar emot stora mängder kväverikt vatten under vintrar.

Specifikt utformade våtmarker kan effektivt avskilja kväve samtidigt som de minskar översvämningsrisk, utan att orsaka ökade utsläpp av växthusgaser. Vissa faktorer som främjar kväveavskiljning, såsom övervattensvegetation och den totala våtmarksarean i ett avrinningsområde, främjar även andra ekosystemtjänster. Således finns det en möjlighet att kväveavskiljningen ökar genom att våtmarker anläggs eller restaureras med syftet att leverera dessa andra ekosystemtjänster. Kväveavskiljningen i kraftigt övervuxna våtmarker kan dessutom förbättras genom växtskörd, och det finns en möjlighet att ytterligare förbättra kväveavskiljningen genom att använda flytande våtmarker.

Acknowledgements

First of all, I would like to thank my main supervisor, Antonia Liess. Thank you for all the help, encouragement, and for believing in me from the very start. Your support throughout these years has been invaluable to me.

I would also like to express my gratitude to my co-supervisors, Stefan Weisner and Eva Lindström. Stefan, thank you for all the expertise, for the constructive criticism, and for trusting me with your data. Eva, thank you for joining the supervisory team and for your guidance in handling the practical aspects of being a PhD student at Uppsala University.

My thankfulness further extends to all of my co-authors: Per Magnus Ehde, thank you for your dedication in the field and laboratory, and for the lunch breaks we have shared. Joachim Audet, thank you for broadening my perspectives by adding greenhouse gas measurements to our experiment. Maidul Choudhury, thank you for wanting to conduct this experiment with us in Halmstad and for all your work with the manuscript. Samuel Hylander and Marc Hauber, thank you for your contributions in writing Paper V and for your assistance in the field. Furthermore, I wish to thank all who contributed to Paper II, with special recognition to Peter Hambäck for all the effort put into the writing of the paper and for inviting me to the workshop that led to its creation.

I additionally want to extend my thanks to those who assisted me with fieldwork and who conducted laboratory analyses, with special mention of Jasmin Borgert, Matyas Baan, Léa Velut, and Maëlys Bockhoff.

Furthermore, I want to express my appreciation for my colleagues at Halmstad University, and I especially want to thank Lars-Gunnar Franzén, Anna Hansson, Niklas Karlsson, Marie Magnheden, and Marie Mattsson for the company during lunches and coffee breaks.

I also want to thank everyone from Länsstyrelsen Kalmar län who I collaborated with, particularly Eva Hammarström and John-John Bertholdsson. Thank you for giving me the opportunity to work with your data and for the help along the way.
In addition, I would like to thank John Strand for the involvement in our Naturvårdsverket project, Peter Eklöv for all the assistance with ISPs and similar matters, those who provided feedback on various occasions such as my halftime seminar, and all the individuals I have had the pleasure of meeting and sharing experiences with at workshops, conferences, and university visits over the past five years.

To my friends: thank you. Special thanks to Angelica and Jessika for the time we have spent together, to Tomas for every beer we have shared, and to Micke, Elin, and Jimmy for all the adventures in Azeroth.

My gratitude further extends to my family, as well as to my partner. Thomas, you have helped me in more ways than I can describe or thank you enough for. You are my favourite person.

Lastly, I want to thank my friend and constant companion, who has stood by me through all the highs and lows, and who always makes me feel better. Thank you, Lennon, my beloved dog.
References


Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 2302

Editor: The Dean of the Faculty of Science and Technology

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