Nitrogen removal by wetlands in a cold climate

Understanding interactions between macrophytes and microorganisms

Maidul I. Choudhury
Faculty of Natural Resources and Agricultural Sciences
Department of Aquatic Sciences and Assessment
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2018

Acta Universitatis agriculturae Sueciae
2018:48

Cover: Nitrogen removal in wetlands
(illustration: Maidul I. Choudhury)

ISSN 1652-6880
ISBN (print version) 978-91-7760-236-1
ISBN (electronic version) 978-91-7760-237-8

Print: SLU Service/Repro, Uppsala 2018

Nitrogen removal by wetlands in a cold climate: Understanding interactions between macrophytes and microorganisms

Abstract

Nitrogen (N), a fundamental component of living organisms, has become one of the main global concerns for human society due to the myriad of negative effects of excessive N on ecosystems. Anthropogenic activities such as agriculture, industrial production, urbanisation and mining are major sources of N to freshwaters. Semi-natural and constructed wetlands planted with macrophytes are now widely used in many parts of the world to remove N from water. However, the potential of constructed wetlands for N removal under cold climatic conditions is still not well studied. We also have limited understanding of how macrophyte species and growth form richness as well as functional trait diversity affect N-cycling in constructed wetlands by influencing plant N accumulation, plant associated denitrification and abundance of denitrifying bacterial communities.

In mesocosm experiments and *in situ* studies, I investigated how species and growth form (emerging and submerged macrophytes and bryophytes) richness as well as plant functional trait diversity of macrophytes affect N-cycling in wetlands. Moreover, I tested the applicability of constructed floating wetlands for improved N removal at the local scale in a cold climate.

My results highlight that macrophytes are important for both main N removal pathways, *viz*. uptake and denitrification. Moreover, bacterial denitrification gene abundance on roots and shoots of macrophytes were an important predictor of the denitrification potential of macrophytes. Species and growth form richness of macrophytes enhanced N removal in wetlands. Moreover, I identified complementarity and a selection effect as important diversity related mechanisms, explaining the total N removal from water including plant N accumulation. Functional traits of macrophytes affected N-cycling in wetlands through direct and indirect pathways. The application of constructed floating wetlands at the local scale is feasible in a cold climate with denitrification as the main N removal pathway in these wetland type. Further *in situ* studies with high numbers of species and growth forms are needed to generalize my findings. Future studies should also consider plant secondary metabolites to better understand the function of the macrophyte-denitrifier interplay.

Keywords: Aquatic plants, biodiversity, complementarity, denitrification, ecosystem function, functional trait, growth form, gene abundance, plant uptake, selection effect

Author's address: Maidul I. Choudhury, SLU, Department of Aquatic Sciences and Assessment, P.O. Box 7050, 750 07 Uppsala, Sweden

Kväverening i våtmarker i kallt klimat: Interaktioner mellan makrofyter och mikroorganismer

Sammanfattning

Kväve (N), en grundläggande beståndsdel i levande organismer, har blivit ett av de största globala problemen för det mänskliga samhället på grund av den myriad av negativa effekter som uppkommer i ekosystem med förhöjda N-nivåer. Antropogena aktiviteter så som jordbruk, industriell produktion, urbanisering och gruvdrift är stora källor till N i sötvatten. På många platser världen över använder man nu seminaturliga och anlagda våtmarker planterade med makrofyter för att avlägsna N från vattnet, men hur väl anlagda våtmarker fungerar för bortförsel av N i kalla klimat är fortfarande inte ordentligt undersökt. Kunskapen om hur makrofyternas artrikedom, mångfalden av växtformer och makrofyternas funktionella egenskaper påverkar växternas upptag av N, denitrifierande bakteriesamhällen och den associerade denitrifikationen i anlagda våtmarker är också dålig.

Genom mesokosmexperiment och *in situ*-studier undersökte jag hur artrikedom, växtformens (övervattensväxter, elodeider och mossor) mångfald liksom mångfalden av makrofyternas funktionella egenskaper påverkar N-cykeln i våtmarker. Jag undersökte också hur konstgjorda, flytande våtmarker fungerar för en ökad bortförsel av N på en lokal skala i ett kallt klimat.

Mina resultat visade att makrofyter är viktiga för de huvudsakliga borttagningsvägarna för N, dvs. upptag och denitrifikation. Resultaten visade också att antalet gener från olika denitrifierande bakterier på rötter och skott hos makrofyter var en viktig indikator för denitrifikationspotentialen hos makrofyterna. Artrikedom och mångfald av växtformer hos makrofyter ökade bortforsling av N i våtmarker. Jag upptäckte också att komplementaritet och en selektionseffekt är viktiga förklaringsmekanismer för variationer i den totala bortforslingen av N inklusive ackumulering av N i växterna. Funktionella egenskaper hos makrofyterna påverkade N-cykeln i våtmarker både direkt och indirekt. Det går att använda konstruerade flytande våtmarker på en lokal skala i kalla klimat och den huvudsakliga bortforslingen av N sker genom denitrifikation. Fler *in situ*-studier med stor artrikedom och en mångfald av växtformer behövs för att generalisera mina resultat. Framtida undersökningar borde också överväga att undersöka sekundära metaboliter hos makrofyter för en bättre förståelse av samspelet mellan växterna och denitrifierande bakterier.

Nyckelord: Akvatiska växter, biodiversitet, komplementaritet, denitrifikation, ekosystemfunktion, funktionella egenskaper, växtform, genabundans, växtupptag, selektionseffekt

Author's address: Maidul I. Choudhury, SLU, Department of Aquatic Sciences and Assessment, P.O. Box 7050, 750 07 Uppsala, Sweden

Dedication

First, to my mother,

Without her endless efforts and prayer, it would have been impossible for me to come to this stage of my life and career.

Second, to my lovely wife,

Thanks for being part of my life and for your continuous support……..

After four years of PhD studies, I imagine myself as a tiny sailing boat in the ocean of knowledge.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Hallin, S*., Hellman, M., Choudhury, M. I. & Ecke, F. (2015). Relative importance of plant uptake and plant associated denitrification for removal of nitrogen from mine drainage in sub-arctic wetlands. *Water Research*, 85, pp. 377-383.

II Choudhury, M. I.*, McKie, B. G., Hallin, S. & Ecke, F. (2018). Mixtures of macrophyte growth forms promote nitrogen cycling in wetlands. *Science of the Total Environment*, 635, pp. 1436-1443.

III Choudhury, M. I.*, Hallin, S., Hubalek, V., Juhanson, J., Frainer, A., McKie, B. G. & Ecke, F. (2018). Plant traits explain uptake of nitrogen and plant associated denitrification in wetlands (manuscript).

IV Choudhury, M. I.*, Hallin, S., Hellman. M, Segersten, J. McKie, B. G. & Ecke, F. (2018) Potential of constructed floating wetlands (CFWs) for nitrogen removal in a cold climate (manuscript).

Papers I-II are reproduced with the permission of the publishers.

* Corresponding author.

The contribution of Maidul I. Choudhury to the papers included in this thesis was as follows:

I Had the main responsibility for data handling, statistical analysis & participated in interpretation of results and writing with co-authors.

II Planned the study with co-authors. Had main responsibility for carrying out the experiment, laboratory work, data handling, statistical analysis, interpretation of results and writing.

III Planned the study with co-authors. Had the main responsibility for carrying out the experiment, laboratory work, data handling, statistical analysis, interpretation of results and writing.

IV Planned the study with co-authors. Contributed to experimental, field and laboratory work and had the main responsibility for data handling, statistical analysis, interpretation of results and writing.

List of tables

List of figures

Abbreviations

ANOVA	Analysis of variance
CFWs	Constructed floating wetlands
CWM	Community weighted mean
CWs	Constructed wetlands
DO	Dissolved oxygen
DW	Dry weight
EC	Electrical conductivity
FD	Functional diversity
FDis	Functional dispersion
FW	Fresh weight
GLM	Generalized linear model
N	Nitrogen
N_2	Nitrogen gas
NO_3^-	Nitrate
PDA	Potential denitrification activity
SEM	Structural equation modelling

1 Introduction

Nitrogen (N) is one of the fundamental components of living organisms and essential for almost all biochemical reactions (Zaehle, 2013; Horne & Goldman, 1994). In 1836, Jean-Baptiste Boussingault first realized that the effectiveness of fertilizers for plant growth depends on their N content, although Bernard Palissy first recognized the scientific connection between fertilizers and food production in the 16th century (Smil, 2001). Today, the global anthropogenic input of N on land, mainly through the use of fertilizer N in agriculture, combustion and agricultural biological nitrogen fixation, is estimated to be 210 Tg N yr^{-1} (Fowler *et al.*, 2013). Some anthropogenic nutrient pressures are not only intense in tropical and temperate regions but are also expanding to boreal and sub-arctic regions due to increasing industrialization and exploitation of natural resources (Bayley *et al.*, 2013; Schindler, 1998).

Massive nutrient inputs of especially N and phosphorus (P) into freshwater ecosystems, due to anthropogenic activities such as agriculture, industrial production, urbanization and mining, have become a major concern for human water security and degradation of global freshwater biodiversity (Bailey *et al.*, 2013; Vörösmarty *et al.*, 2010; Foley *et al.*, 2005). The land-wetland-river continuum retains 70% (101 Tg N yr^{-1}) of the anthropogenic reactive N added to the continental biosphere (Billen *et al.*, 2013) and wetlands, lakes and rivers can retain 64%, 34% and 2%, respectively, of the total nitrogen (TN) load (Saunders & Kalff, 2001). N released from point and non-point sources is one of the major nutrients causing degradation of freshwater ecosystems, with a high socio-economic burden (Dodds *et al.*, 2009; Pretty *et al.*, 2003; Carpenter *et al.*, 1998).

N released to freshwater systems from undetonated ammonium-nitrate (NH_4NO_3) based explosives used in mining or other blasting operations has become an emerging environmental issue (Herbert *et al.*, 2014; Bailey *et al.*, 2013). During blasting operations in underground mines, 15 - 19% of the N in

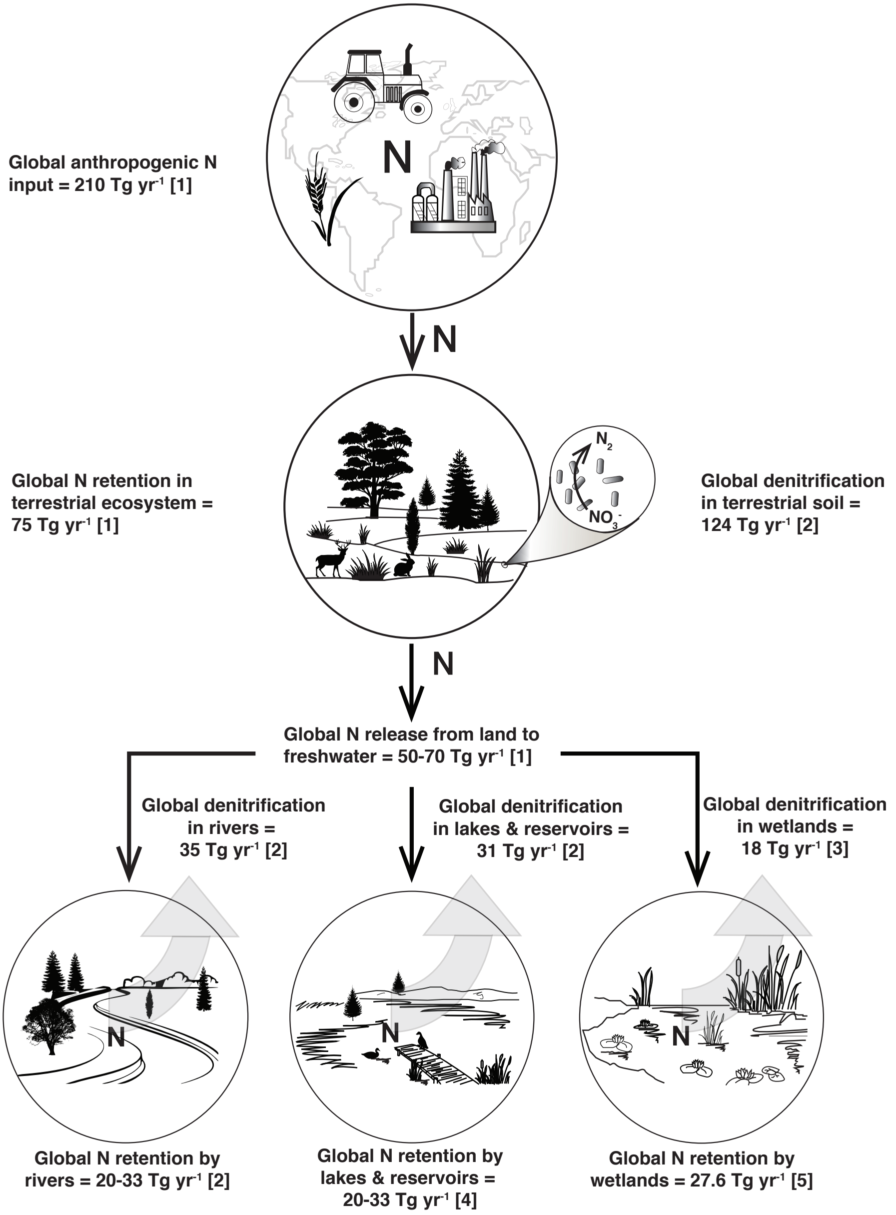


Figure 1. A simplified schematic diagram of nitrogen (N) cycling in terrestrial and freshwater ecosystems ([1] = Fowler *et al.,* 2013, [2] = Seitzinger *et al.,* 2006, [3] = Jordan *et al.*, 2011, [4] = Harrison *et al.,* 2009, [5] = adopted from Mitsch *et al.,* 2013).

explosives may remain undetonated (Forsberg & Åkerlund, 1999). Since NH_4NO_3 is highly soluble in water, it enters into the mine drainage and is eventually discharged to the environment in the form of nitrate (NO_3^-) and ammonium (NH_4^+). Usually Nitrate (NO_3^-) is the main N species in recipient systems impacted by undetonated ammonium-nitrate based explosives (Herbert *et al.*, 2014).

1.1 Nitrogen uptake and denitrification

In both terrestrial and aquatic systems, biotic NO_3^- cycling generally involves N uptake by primary producers (e.g. vascular plants, algae, phytoplankton) and microorganisms (e.g. decomposers) as well as denitrification, i.e. the reduction of nitrate or nitrite to N_2O or N_2 under oxygen limiting conditions by heterotrophic bacteria (Niu *et al.*, 2016; McClain *et al.*, 2003; Hodge *et al.*, 2000; Howard-Williams, 1985). Vegetation and soil-litter constitute 15% and 2%, respectively, of global total N added to terrestrial ecosystem by direct and indirect anthropogenic factors during 2001-2010 (Zaehle, 2013). The main N removal processes in terrestrial ecosystems are discharge of NO_3^- to aquatic systems and denitrification, which releases N_2 back to the atmosphere (Fowler *et al.*, 2013). Aquatic systems have an almost equal importance for N removal compared to terrestrial systems, as 20% of total global denitrification occurs in freshwater systems, e.g. ground water, rivers, lakes and wetlands, compared to 22% in terrestrial soils (Seitzinger *et al.*, 2006). Although the importance of aquatic vegetation (macrophytes) for supporting denitrifying organisms as well as denitrification is well appreciated, the role of macrophytes for overall N retention is usually overlooked since the magnitude of plant N uptake is found to be much lower than the denitrification in streams, lakes and wetlands (Saunders & Kalff, 2001).

1.2 Nitrogen cycling in wetlands

Wetlands are some of the most productive systems in the world. They are rich in biodiversity, provide shelter and food for many groups of organisms (Halls, 1997), perform many ecosystem functions and provide services to human society (Costanza *et al.*, 1997). One of the important functions performed by wetlands is nutrient cycling, especially N and P cycling (Vymazal, 2007).

Wetlands are usually fed with nutrient rich surface water from surroundings and sometimes also groundwater. Different transformation pathways of N occur in wetland soil and water, driven by physical and biological factors (Vymazal, 2007; Thullen *et al.*, 2005). Emerging and submerged macrophytes assimilate N from sediments through their root systems (Barko *et al.*, 1991; Sculthorpe, 1967). In contrast, submerged species, especially if non-rooted, can further fulfil their nutrient demand by uptake via their leaves from the water column (Madsen

& Cedergreen, 2002), similar to bryophytes that take up nutrients from water with their entire thallus surface. Macrophytes act as a temporary storage (sink) of N that is eventually remineralized as macrophytes decay.

N is also transformed in wetlands through denitrification (Figure 2) performed by bacteria and archaea, with the majority being heterotrophs that inhabit sediments and the plant rhizosphere (Hallin *et al.*, 2018). During denitrification, different enzymes take part in the conversion of NO_3^- to N_2, e.g. enzymes encoded by the genes *nirK* and *nirS* help to convert NO_2^- to N_2O or NO while those encoded by *nosZI* and *nosZII* convert N_2O to N_2 (Hallin *et al.*, 2018). Macrophytes significantly contribute to microbial activity in the rhizosphere by providing root surface for microbial growth, root exudates as an energy source in the form of carbon, as well as by creating aerobic conditions through root oxygen release (Wu *et al.*, 2017; Gagnon *et al.*, 2007). Such aerobic conditions trigger nitrification, i.e. oxidation of NH_3 to nitrite and nitrate, near the root zone. When the oxygen is depleted, denitrification by facultative anaerobic bacteria takes place (Horne & Goldman, 1994). Although sediments have been considered the most important wetland habitat for denitrification (Kjellin *et al.*, 2007; Whitmire & Hamilton, 2005), periphytic communities on roots and shoots of emerging and submerged macrophytes can significantly contribute to N removal through denitrification (Salvato *et al.*, 2012; Morgan *et al.*, 2008; Eriksson & Weisner, 1997). Moreover, denitrification rates are especially high

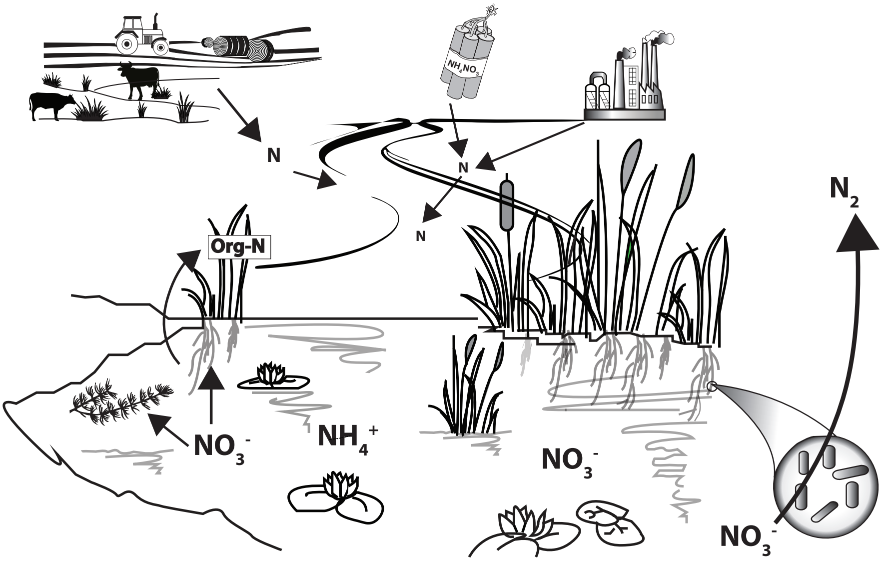


Figure 2. A simplified illustration of main nitrogen (N) removal pathways, i.e. plant uptake and denitrification in wetlands dominated by macrophytes.

in the rhizosphere or periphyton of macrophyte species compared to bare sediment (Kofoed *et al.*, 2012; Ruiz-Rueda *et al.*, 2009). Besides plant uptake and denitrification, other N transformations in wetlands are volatilization, ammonification, nitrate-ammonification, N_2 fixation, microbial uptake, ammonia adsorption, organic N burial and ANAMMOX (anaerobic ammonia oxidation) (Vymazal, 2007).

In the last decades, numerous studies have demonstrated the importance of wetlands for nutrient cycling (Vymazal, 2011) and humans have realized that the ecosystem processes related to nutrient cycling occurring in natural wetlands can be mimicked in constructed wetlands (CWs). Nowadays, semi-natural CWs and constructed floating wetlands (CFWs) planted with macrophytes are widely used for water treatment in many parts of the world (Pavlineri *et al.*, 2017; Zhang *et al.*, 2015; Brix, 1997). In contrast, there is a considerable research gap in understanding the N removal from surface water by natural and semi-natural wetlands, as well as CFWs dominated by macrophytes in boreal and sub-arctic regions, despite the increasing anthropogenic pressures associated with resource extraction in these regions (McDonald & Knox, 2014; Bayley *et al.*, 2013). Moreover, macrophytes used in free water surface CWs are mostly limited to emerging macrophytes such as *Phragmites* spp., *Typha* spp., *Juncus* spp., *Scirpus* spp. and *Eleocharis* spp. (Vymazal, 2013). However, typical wetland macrophyte species growing in temperate regions are rare or do not occur in the northern hemisphere or in cold climates. Therefore, we have only limited understanding of the potential of native, sub-arctic macrophytes for N removal by plant N accumulation and associated denitrification in cold climate wetlands and impacted by anthropogenic activities such as mining.

Despite the numerous ecosystem functions and services provided by wetlands (Costanza *et al.*, 1997), very little research has focused on understanding how macrophyte diversity affects key ecosystem functions, such as nutrient uptake and denitrification that underpin nutrient cycling in wetlands (Schultz *et al.*, 2012; Bouchard *et al.*, 2007; Engelhardt & Ritchie, 2001). Previous studies have mainly focused on diversity of emerging and/or submerged growth forms of macrophytes (Schultz *et al.*, 2012; Weisner & Thiere, 2010; Engelhardt & Ritchie, 2001), while the potential of bryophytes for N-cycling through plant uptake and denitrification is largely unknown despite their vast distribution in different biomes (Turetsky, 2003). It is largely acknowledged that complementarity, a positive interaction between species that increases a process rate among interacting species, is important for ecosystem functioning in diverse plant communities (Cardinale *et al.*, 2007). The effects of functional traits or characteristics of organisms on ecosystem functions (Díaz & Cabido, 2001), e.g. resource extraction from the environment (McGill *et al.*, 2006), are important for understanding the biodiversity-ecosystem functioning (B-EF) relationship. There is growing recognition that functional measures of diversity (FD) based on quantification of functionally important species traits are useful for explaining variation in ecosystem functions (Frainer *et al.*, 2018; Flynn *et al.*, 2011; Díaz & Cabido, 2001). For example, in biodiversity-ecosystem function studies, primary productivity (Naeem, 2002), carbon fluxes in ecosystems (Milcu *et al.*, 2014) and diversity of soil biota (Milcu *et al.*, 2013) have been better predicted by functional trait diversity than by species richness *per se*. However, the driving mechanisms linking specific traits to these functions remain poorly understood, hindering development of a predictive framework for ecosystem functioning based on species traits (Cadotte, 2017; Truchy *et al.*, 2015). In wetlands, the effect of macrophyte growth form richness, as well as functional diversity on N-cycling is not yet well explored. Moreover, how complementarity among different macrophyte species affects ecosystem functions such as denitrification, through influencing denitrifying bacterial communities, has to my knowledge not been tested.

2 Objectives and research questions

The overall objective of this thesis is to improve our understanding of N removal from surface water in semi-natural and constructed wetlands (CWs) through plant uptake and associated microorganisms in a cold climate. Since our understanding of the effect of macrophytes growth form richness as well as plant functional traits on ecosystem functions, especially plant N uptake and denitrification in wetlands, is still inadequate, this thesis experimentally addressed these knowledge gaps.

To address this goal, I investigated the following research questions:

- What is the relative contribution of N uptake *versu*s denitrification in the rhizosphere for N removal in wetlands in a cold climate? (Paper I, III, IV)
- Does macrophyte growth form richness affect plant N uptake and plant associated denitrification in wetlands? (Paper I, II, III)
- Which abiotic factors and plant traits explain N uptake and plant associated denitrification in wetlands? (Paper I, II, III)
- Is the application of constructed floating wetlands (CFWs) feasible for long term N removal at the local scale in a cold climate? (Paper IV)
- How can the results from paper I-IV be applied to construct operational wetlands in a cold climate?

3 Materials and methods

This thesis is based on a series of experiments conducted through mesocosm studies in a controlled environment and on field sampling *in situ*. Paper I includes both field sampling and a mesocosm study in the laboratory. Paper II & III are based on a greenhouse mesocosm experiment and Paper IV is based on a mesocosm and an *in situ* study. Throughout the experiments, I focused on how to improve our understanding of N removal from water in wetlands by plant N accumulation and macrophyte mediated denitrification, with a particular focus on cold climates. I investigated how macrophyte species and growth form combinations, plant functional traits and abiotic factors influence N-cycling in semi-natural, CWs and CFWs.

3.1 Study sites & macrophyte sampling (Paper I -IV)

My field study site was located in a mining area situated in Kiruna, Northern Sweden, located 145 km north of the Arctic Circle (67°51'N 20°13'E). The field studies were conducted on two occasions, *viz.* July-September 2012 (Paper I) and June 2014 - August 2016 (Paper IV). The active growth period for plants in this area is approximately 120 days (Raab & Vedin, 1995). The mean air temperature during the study period 2012 was 10.1 ºC (minimum 3.9 ºC and maximum 16.7 ºC) and the mean annual temperature in 2016 was -2 ºC, with maximum-minimum temperature range of 25.9 ºC to -2.1 ºC during summer, while in winter the temperature ranged from 5.9 ºC to -37 ºC (SMHI). The mesocosm studies were conducted in a growth chamber (Paper I) and in a greenhouse (Paper II & III), both located at the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden.

Macrophyte species were collected from a semi-natural wetland (Paper I & IV) in the mining area, that receives slightly alkaline (pH 7.5 - 8.7) mine effluents with an N concentration of 18 - 28 mg NO_3-N L^{-1}. In Paper I, five macrophyte species were sampled at two occasions: at the end of July and the beginning of September, 2012. In Paper IV, six macrophyte species were collected in June 2014. In Paper II and III, twelve macrophyte species were collected from wetlands around Uppsala, Sweden (for details see Paper II, supplementary data).

3.2 Study design (Paper I-IV)

In Paper I, five macrophyte species: *Carex aquatilis* Wahlenb. (water sedge), *Carex rostrata* stokes (bottle sedge), *Eriophorum angustifolium* Honck. (common cottonsedge), *Equisetum fluviatile* L. (water horsetail), and the aquatic bryophyte *Drepanocladus fluitans* (Hedw.) Warnst (floating hook-moss) were cultivated in monocultures and *C. aquatilis* and *D. fluitans* were additionally cultivated in mixed cultures. Macrophytes were cultivated in 14 L plastic mesocosms filled with sediment and water collected from wetland in the mining area and grown in the growth chamber for 50 days at 10 ºC. Each species mesocosm was replicated five times. The plants were sampled *in situ* in July and September as well as after 50 days of experimental growth and the N content in above- and belowground biomass of the species was measured. Potential denitrification activity (PDA) on periphyton associated with macrophyte roots and shoots of bryophytes was measured according to the standard acetylene inhibition technique for the samples collected *in situ* in September. Abundance of bacterial denitrification genes on roots of macrophytes and shoots of bryophytes were measured by quantitative real-time PCR (qPCR) for both the samples collected *in situ* and at the end of mesocosm study. Finally, the dominant N removal pathways were measured by calculating the ratio between denitrification and plant uptake of the studied macrophytes.

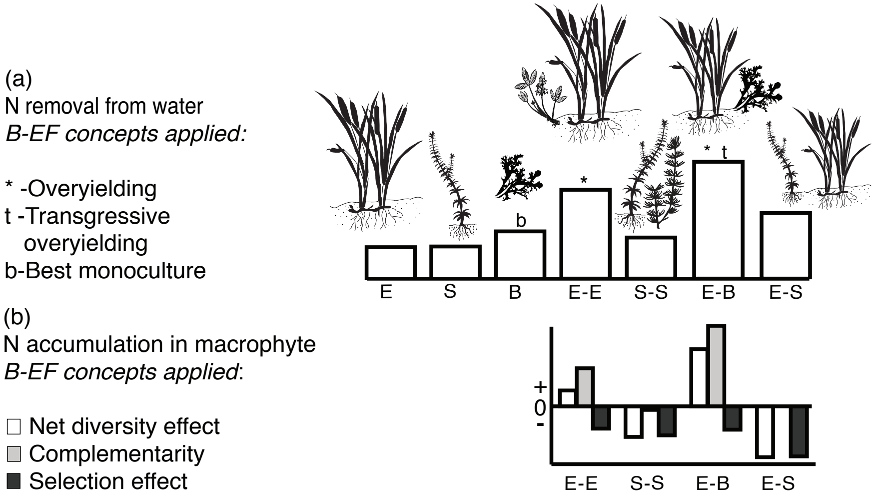
In Paper II & III, twelve macrophyte species were grown in wetland mesocosms in a greenhouse: *Menyanthes trifoliata* L. (bog bean), *Comarum palustre* L. (purple marshlocks), *C. rostrata*, *E. fluviatile*, *Phragmites australis* (Cav.) Steud. (common reed), *Elodea canadensis* Michx (Canadian water weed), *Myriophyllum alterniflorum* DC. (watermilfoil), *Hippuris vulgaris* L. (mare's tail), *Ceratophyllum demersum* L.(hornwort), *Leptodictyum riparium* (Hedw.) Warnst. (Kneiff's feather-moss), *Sphagnum fallax* (Klinggr.) (flat-topped bog-moss) and *Fontinalis antipyretica* Hedw. (greater water-moss). The selected species represent three growth forms: emerging and submerged macrophytes and bryophytes; they occur naturally in temperate, boreal and sub-arctic regions. The macrophyte species were planted in 15 L plastic mesocosms containing nutrient-amended water (20 mg NO_3-N L^{-1} and 0.01 mg PO_4-P L^{-1}), and sediment (sediment nitrogen content: 0.77 g N kg-dry weight^{-1}, collected from the clarification pond in the mining area). Nutrients were added three times during the experiment: in June, July and August. In total, 527 mg N was added to each mesocosm during the whole experimental period. The macrophyte species were allocated to the mesocosms in a nested design with three treatments: i) species number (single *vs.* two-species mixture), ii) growth form combination (nested within species number) and iii) species combination (nested within growth form and species number) (for details see Paper II). I measured N removal from water in wetland mesocosms during July, August and September (Paper II), total N removal for the whole experiment (Paper II & III), N accumulation in plant photosynthetic biomass (Paper II & III), PDA and abundance of bacterial denitrification genes on periphyton associated with macrophyte roots and shoots of bryophytes at the end of the experiment (Paper III), plant traits related to N accumulation, PDA and denitrification gene abundance (Paper III). In addition, I measured abiotic factors that affect N-cycling, *viz.* pH, electrical conductivity (EC) ($\mu S\ cm^{-1}$) and dissolved oxygen (DO) ($mg\ L^{-1}$) in the overlying water five times during the experiment. The experiment was conducted during 100 days from the middle of June to the end of September 2015, at a median temperature of 18 °C (interquartile range = 16 – 19 °C).

In Paper IV, I conducted the experiment in two types of systems: an outdoor mesocosm experiment was conducted in two plastic water tanks (volume: 3600 L) supplied with water from the clarification pond of the mining area (67°48'N 20°8'E) while a recipient experiment was conducted in Lake Mettä-Rakkurijärvi (67°48'N 20°8'E), which is the first natural recipient of mine effluents after passive treatment in the clarification pond. The NO_3-N concentration in the water of the clarification pond and recipient lake were 21.26 ± 1.35 (mean ± SD) $mg\ L^{-1}$ and $8.25 \pm 4.5\ mg\ L^{-1}$, respectively. I used six common macrophyte species that occur naturally in the Kiruna region: *C. palustre*, *E. fluviatile*, *C. rostrata*, *E. angustifolium*, *Filipendula ulmaria* (L.) Maxim. (meadowsweet) and *M. trifoliata*. Constructed floating wetlands (CFWs) planted with macrophytes were deployed in the water tanks and in the recipient lake. Macrophytes were grown in CFWs from June 2014 to August 2016. I measured plant standing biomass, N accumulation in aboveground biomass, PDA on periphyton associated with macrophyte roots and potential N removal pathways (plant uptake *vs.* denitrification) in CFWs at the end of the experiment.

3.3 Data evaluation and statistical analyses

I adopted different approaches for data evaluation. For example, in Paper I & IV, the ratio between denitrification and plant uptake for different macrophyte species combinations was used to determine potential N removal pathways in

Box 1. *Biodiversity-ecosystem function (B-EF) concepts applied to test the effect of macrophyte growth form richness on N removal from water and plant N accumulation in wetland mesocosms.*



Diversity effect: the contribution of a mixture of species to a measured ecosystem function that cannot be explained by summing the weighted individual contributions of the constituent species.

Selection effect: when an ecosystem function in a diverse plant community is affected by an individual species with highly influential traits relative to the measured ecosystem function. A selection effect can be either positive or negative for measured ecosystem function.

Complementarity effect: positive interactions among species in a diverse plant community that promote resource use efficiency or certain ecosystem functions.

Overyielding (OY): when a mixture of species performs a particular ecosystem function, e.g. nutrient removal from water significantly better than monocultures. Overyielding does not distinguish whether enhanced ecosystem functioning is due to selection effect or complementarity.

Transgressive overyielding (TOY): a mixture of species performs a particular ecosystem function significantly better than the most effective species in monocultures. TOY confirms the presence of complementarity on ecosystem functioning in a mixture of species.

In the diagram (a), it is not possible to say whether improved N removal in the E-E macrophyte species combination is due to complementarity between E and E or due to a selection effect of an individual species as it shows only OY. In contrast, the E-B combination showed OY along with TOY, which confirms the presence of complementarity between E and B in N removal from water.

Net diversity effect: it is measured by summing the differences between observed yield (or performance) of a mixture of species and their expected yield (or performance) across species relative to a measured ecosystem function (Loreau & Hector, 2001). This expected yield is calculated as the weighted average of the yields of the component species in monocultures. Net diversity effect can be further divided into two underlying components, complementarity and selection effect (diagram 2).

wetlands dominated by macrophytes. In Paper II, effects of macrophyte growth form richness on ecosystem functioning were measured differently for N removal from water and plant N accumulation (Box 1). In case of N removal from water, it was not possible to quantify the individual contribution of two species to N removal in a mixed culture. Therefore, I used the concept of overyielding and transgressive overyielding (McKie *et al.*, 2008; Cardinale *et al.*, 2006) to identify complementarity between different growth form combinations. On the other hand, it was possible to quantify the individual contribution of two species to plant N accumulation in mixed cultures. Therefore, I used Loreau and Hector (2001) additive partitioning to quantify the effect size of the net diversity effect, selection effect and complementarity on plant N accumulation. In Paper III, I used functional diversity (FD) of plant traits to explain N-cycling in wetland mesocosms through denitrification , abundance of denitrification genes and plant N accumulation. In this case, I used two indices of FD: community weighted mean (CWM) of each plant trait and functional dispersion (FDis) of multiple plant traits (Box 2) to test the effect of plant diversity on N removal from water in wetland mesocosms.

Box 2. *The approaches used in this thesis for quantifying functional diversity of plant traits important for ecosystem functioning in wetland mesocosms.*

Functional traits: morphological, physiological or phenological characteristics of an organism that are relevant to either its response to the environment and/or its effects on ecosystem function (Díaz & Cabido, 2001), e.g. resource extraction from the environment (McGill *et al.*, 2006).

Functional diversity (FD): a component of biodiversity that influences ecosystem functions (Tilman, 2001). In general, FD is the distribution of trait values in a community (Lavorel *et al.*, 2008). FD is measured by the trait values for the species that are present in an ecosystem that influence one or more aspects of the functioning of that ecosystem (Tilman, 2001).

There are different indices of measuring of FD. The two main indices are:

Community weighted mean (CWM): it can be defined as an aggregate values of plant traits in a community (Garnier *et al.*, 2004) and represents the expected functional trait value of a random community sample. In a species assemblage each trait has its own CWM value. The CWM for each trait is calculated by multiplying the mean of trait values in the community by the relative abundance of the species in the community carrying that trait value.

Functional dispersion (FDis): can be defined as the heterogeneity of trait values within a community (Lavorel *et al.*, 2008). High FDis indicates a high degree of niche differentiation in the community, thus lower competition for available resources. Enhanced ecosystem functioning might therefore be observed in the communities with high FDis due to more efficient resources use (Mason *et al.*, 2005).

Different statistical approaches were used for analysing my data, but the most important once were analysis of variance (ANOVA), mixed model analysis and structural equation modelling (SEM). Mixed model analysis can handle nested factors (with fixed and random effects) and these analyses are suitable for data where repeated measurements are drawn from the same objects over time. I used SEM to disentangle direct and indirect pathways that might explain variability in N removal from wetlands. SEM is a method for statistically evaluating a series of dependent relationships through the analysis of covariance (Grace & Pugesek, 1997) and allows partitioning of causal pathways in complex data (Grace *et al.*, 2010).

4 Results and discussion

My results highlight that macrophytes are important not only for plant N accumulation, but also for facilitating denitrification (Paper I, III and IV). Moreover, denitrification gene abundance on roots and shoots of macrophytes is an important predictor of their denitrification potential (Paper I- Figure 2; Paper III- Figure 2). Combinations of different macrophyte growth forms can potentially influence N-cycling in wetland systems (Paper I, II, III). The positive effects of macrophyte species and growth form richness on N-cycling in wetland mesocosms can be attributed to complementary interactions among species (i.e. "complementarity") and to the performance of single highly influential species (i.e. the "selection effect") (Paper II; see also Box 1). The diversity of plant functional traits (Box 2) further explained N accumulation in plant biomass, denitrification mediated by macrophytes, and denitrification gene abundance associated with macrophytes roots and shoots (Paper III). My study showed that plant functional traits directly and indirectly influence ecosystem functions that eventually affect ecosystem services (here, N removal from water), provided by wetlands (Paper III). Finally, I demonstrated the utility of constructed floating wetlands (CFWs), a recent development among constructed wetlands, to improve N removal at the local scale through plant N accumulation and plant associated denitrification (Paper IV).

Relative contribution of N uptake and denitrification to N removal

I found that N accumulation in plant biomass by emerging macrophytes in the studied sub-arctic wetlands impacted by mine effluents ranged between 1.1 - 30.3 mg N m^{-2} day^{-1} depending on species (Table 1). Even higher N accumulation rates (8 - 107 mg N m^{-2} day^{-1}) were observed for macrophytes grown in CFWs in the recipient of the mining area. In a subtropical wetland, N accumulation by a mixed community consisting of *Carex* spp. and *Juncus* spp.

Table 1. *Mean N accumulation in plant aboveground biomass and potential denitrification activity (PDA) associated with roots of emerging macrophytes studied in situ and mesocosms.*

Function	N uptake (mg N m^{-2} day^{-1})			
System	CW *in situ* [Ref.]	CW mesocosms [Ref.]	CFW *in situ* [Ref.]	CFW mesocosm [Ref.]
Species				
C. palustre	10.2 [1]	2.5 [2]	106.9 [4]	17.2 [4]
E. fluviatile	5.8 [1]	3.3 [3]	10.3 [4]	9.5 [4]
C. rostrata	30.3 [1]	5.2 [3]	23.0 [4]	21.7 [4]
C. aquatilis	14.9 [1]	5.9 [3]	-	-
M. trifoliata	1.1 [1]	5.3 [2]	8.0 [4]	7.5 [4]
E. angustifolium	-	2.3 [3]	17.5 [4]	14.9 [4]
F. ulmaria	-	-	105.5 [4]	24.7 [4]

Function	PDA (mg N_2O-N kg-DW^{-1} day^{-1})			
System	CW *in situ* [Ref.]	CW mesocosms [Ref.]	CFW *in situ* [Ref.]	CFW mesocosm [Ref.]
Species				
C. palustre	1578.77 [1]	498.35 [2]	596.75 [4]	763.49 [4]
E. fluviatile	1328.36 [1]	553.19 [2]	136.04 [4]	679.91 [4]
C. rostrata	2109.27 [1]	135.87 [2]	68.22 [4]	305.19 [4]
C. aquatilis	906.14 [1]	-	-	-
M. trifoliata	207.84 [1]	0.36 [2]	15.13 [4]	24.12 [4]
E. angustifolium	298.00 [3]	-	66.06 [4]	647.57 [4]
F. ulmaria	1237.35 [1]	-	194.77 [4]	507.63 [4]

CW = Constructed wetland; CFW = Constructed floating wetland ; [Ref.] = reference, [1] = unpublished data; [2] = Paper II; [3] = Paper I; [4] = Paper IV

was found to be 25.6 mg N m^{-2} day^{-1} (DeMeester & Richter, 2010). N accumulation by four *Carex* spp. (*C. diandra*, *C. rostrata*, *C. lasiocarpa* and *C. acutiformis*) in an eutrophic fen in the Netherlands was 14 - 28 mg N m^{-2} day^{-1} (Aerts *et al.*, 1992). Higher N accumulation was found for the graminoids *Glyceria maxima* (54.24 - 87.94 mg N m^{-2} day^{-1}) and *Phalaris arundinacea* (54.8 - 118.9 mg N m^{-2} day^{-1}) in wastewater treatment wetlands in Sweden (Wittgren & Mæhlum, 1997). Even though my studies were conducted in a cold climate, the observed N accumulation by the macrophytes is comparable with these literature data. However, low summer temperature and a short growing season are the main controlling factors for biomass production of macrophytes in sub-arctic regions (Solander, 1983), which might result in lower bulk N accumulation by macrophytes compared to temperate or tropical regions.

Denitrification is considered to be the dominating N removal pathway in natural and constructed wetlands (Bachand & Horne, 2000; Lund *et al.*, 2000; Howard-Williams, 1985) as well as in rivers and lakes (Saunders & Kalff, 2001). I found that emerging macrophytes could support denitrification resulting in a

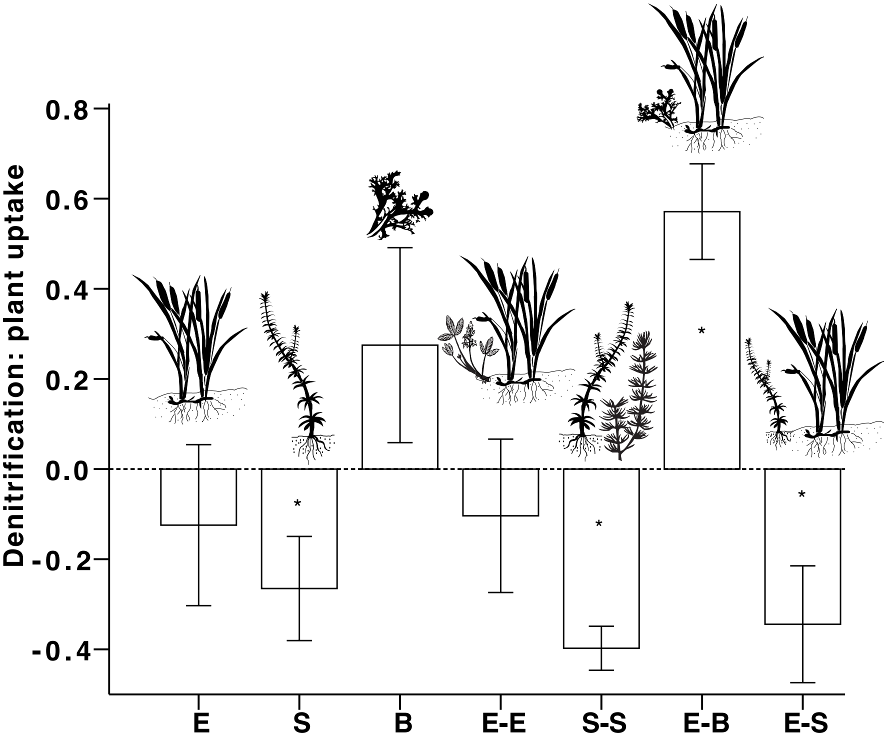


Figure 3. Potential N removal pathways calculated as the ratio between potential denitrification activity and plant N accumulation in different growth form combinations of macrophytes in wetland mesocosms. Values above 0 indicate denitrification as the main N removal pathway while values lower than 0 indicate plant accumulation as the main removal mechanism. Asterisks (*) inside the bars indicate a significant difference from 0 (E = emerging macrophytes, S = submerged macrophytes and B = bryophytes) (Paper III).

potential to remove 200 – 2100 mg N_2O-N kg-dw^{-1} day^{-1} and 24 - 763 mg N_2O-N kg-dw^{-1} day^{-1} in CW and CFWs, respectively, through denitrification (Table 1). This is comparable with the denitrification activity on the roots of *Cyperus papyrus* and *Hibiscus moscheutus*: 174 and 181 mg N_2O-N kg-dw^{-1} day^{-1}, respectively (Morgan *et al.*, 2008). Higher nitrate reductase activity was found for roots of the sedge *Cyperus laevigatus* and the forb *Canna indica*: 6230 and 3730 mg NO_2-N kg-dw^{-1} day^{-1}, respectively (Piwpuan *et al.*, 2013; Konnerup & Brix, 2010). I also found that the potential denitrification activity associated with macrophyte roots and shoots varied between the species and growth form combinations (Paper I; Paper III).

While investigating the N removal pathways of macrophytes in wetlands, contrasting results were observed for the studied species, supporting both plant uptake and denitrification as the main N removal pathways in wetland mesocosms (Paper III) and *in situ* (Paper I). However, denitrification was the main N removal pathway in the constructed floating wetlands (CFWs) (Paper IV). Potential N removal pathways varied among different macrophyte growth form combinations (Figure 3). Monocultures and emerging macrophytes showed importance of both plant uptake and denitrification (Figure 3; Paper I-Figure 2). Plant uptake was found to be the main N removal pathway for submerged macrophytes in my study, while bryophytes showed denitrification as a main N removal pathway (Figure 3; Paper I- Figure 2). In the mixtures of different macrophyte growth forms, emerging-submerged and submerged-submerged combinations of macrophytes showed plant uptake as a dominant pathway whereas the emerging-bryophyte combinations showed denitrification as the dominant pathway (Figure 3). However, species identity tended to influence the N removal pathways in wetland mesocosms in both monocultures and mixtures of growth forms (Figure 3-Paper III, Table S4-Paper III).

Macrophyte growth form richness affects N-cycling in wetlands

Different growth forms of macrophytes, e.g. emerging and submerged macrophytes as well as bryophytes, differ in their niche requirements and nutrient uptake strategies (Madsen & Cedergreen, 2002; Barko *et al.*, 1991; Sculthorpe, 1967). This highlights the potential of complementary resource use by macrophytes in nutrient cycling in freshwater ecosystems. However, the extent to which macrophyte species richness, and growth form richness in particular, affects variation in nutrient cycling in wetlands is poorly understood.

I found that N removal rates from water at the mesocosm level increased not only when species number increased from 1 to 2, but also when growth form richness increased (Paper II- Figure 2a & 3). This supports the idea that functional diversity of aquatic plants can elevate N removal from surface water, similar to what has been observed in terrestrial systems (Loreau & Hector, 2001; Tilman *et al.*, 1996). In my study, I found that combinations of emerging-bryophyte species were the best performing growth form combinations followed by emerging-emerging species. In contrast to my results, previous studies showed the importance of emerging and submerged macrophytes for nutrient retention in wetlands (Weisner & Thiere, 2010; Horppila & Nurminen, 2005; Engelhardt & Ritchie, 2001). However, these previous studies did not consider the combinations of different growth forms, and did not include bryophytes. As an underlying mechanism for differences in N removal from water among growth form combinations, I identified complementary resource use associated with the mixture of emerging and bryophyte species (Paper II- Figure 3).

Although emerging and submerged species are able to acquire nutrients from different strata, i.e. sediment and water column, I did not detect any positive diversity effect for the combinations of emerging-submerged or submerged-submerged species (Paper II- Figure 3). In contrast to my finding, in single-growth form mesocosms, Engelhardt and Ritchie (2001) found that richness of submerged macrophyte species increased nutrient retention in experimental mesocosms.

Co-cultivation of the sedge *C. aquatilis* and the bryophyte *D. fluitans* enhanced N content in aboveground biomass compared to their monocultures (Paper I), which indicates the presence of complementarity in N uptake between emerging macrophytes and bryophytes. The additive partitioning of the net diversity effect on plant N accumulation further confirmed the occurrence of complementarity between these two growth forms (Paper II- Figure 4). Niche differentiation between emerging macrophytes and bryophytes likely is one probable mechanism underpinning this positive effect of complementarity on overall N removal in wetland mesocosms. Because bryophytes can grow under and between the dominant vascular plants and competition with tracheophytes for light and nutrient are usually avoided (Bates, 1998; Grime *et al.*, 1990). They further take up nutrients through leaves (Glime, 2017), while sediment is the source of nutrients for emerging macrophytes. In contrast, I observed little or almost no complementarity between submerged-submerged growth form combinations (Paper II-Figure 4). This might be due to the strong interspecific competition for nutrients that prevents enhanced N accumulation. Further, the submerged species included in my study (*E. canadensis*, *C. demersum* and *H. vulgaris*) are known to have allelopathic effects (Grutters *et al.*, 2017; Gopal & Goel, 1993; Pip, 1992) that might impede the growth of other macrophytes and thus hamper N accumulation.

In addition to differences among growth forms regarding N removal from water and plant N accumulation, I also found significant differences in the potential denitrification activity (PDA) between the growth forms (mixed model, $F_{5,25} = 10.90$, $P < 0.000$), while no difference was observed between mixed and monocultures (mixed model, $F_{1,25} = 0.93$, $P > 0.05$). PDA was highest for emerging-bryophyte combinations and lowest for submerged-submerged species combinations. Moreover, mixtures of emerging-bryophyte growth forms showed higher PDA compared to their constituent monocultures (Tukey's pairwise comparison, $P < 0.05$). Although not significant, I found that co-occurrence of bryophytes and emerging macrophytes tended to increase *nirK* and *nirS* gene abundances on the roots of emerging macrophytes while bryophytes showed a contrasting response (Figure 4). This might explain the enhanced PDA in emerging-bryophyte combinations compared to their

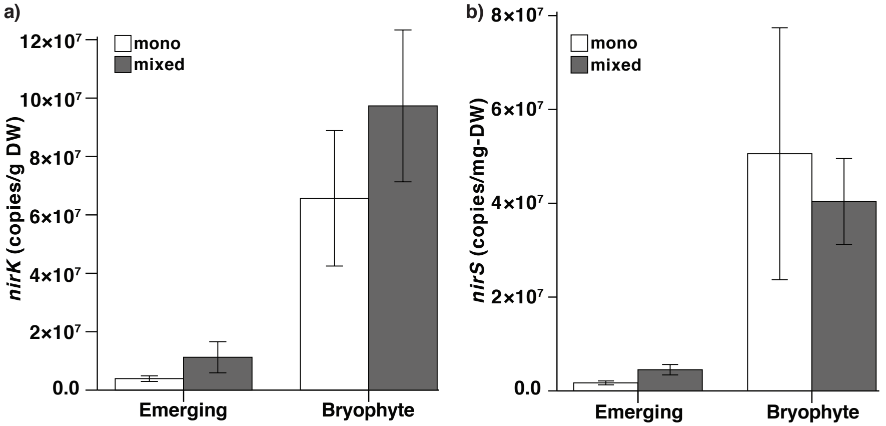


Figure 4. Bacterial denitrification gene abundance (mean ± 1 SE) a) *nirK* and b) *nirS* on the roots of emerging macrophytes and shoots of bryophytes (mono = monocultures, mixed = co-cultivation of emerging macrophytes and bryophytes).

monocultures (Figure 3, Paper III- Figure 3; Paper I). On the other hand, strong affinity for N and allelopathic potential of submerged species might exert strong interspecific competition with microbes for available nutrients, which in turn reduces denitrifying bacterial activity (de Vries & Bardgett, 2016; Moreau *et al.*, 2015; Hilt & Gross, 2008).

Abiotic factors and plant traits explaining N-cycle in wetlands

N removal in wetlands is influenced by different abiotic and biotic factors such as temperature, dissolved oxygen (DO), pH, dissolved organic carbon (DOC), electrical conductivity (EC) and vegetation (Lee *et al.*, 2009; Bachand & Horne, 2000). I found that pH, DO and EC in the overlying water varied among different growth form combinations in the wetland mesocosms (Paper II). DO and EC together explained 30% of the variance (adjusted $R^2 = 0.303$, $P < 0.001$) of N removal for the entire experimental period in the wetland mesocosms (Paper II).

I used plant functional traits to explain the effect of plant diversity on N-cycling in wetlands (Figure 5; Paper III). The key ecosystem services that support human societies are derived from a set of functions regulated by multiple organism groups, and often linking across habitat and ecosystem boundaries

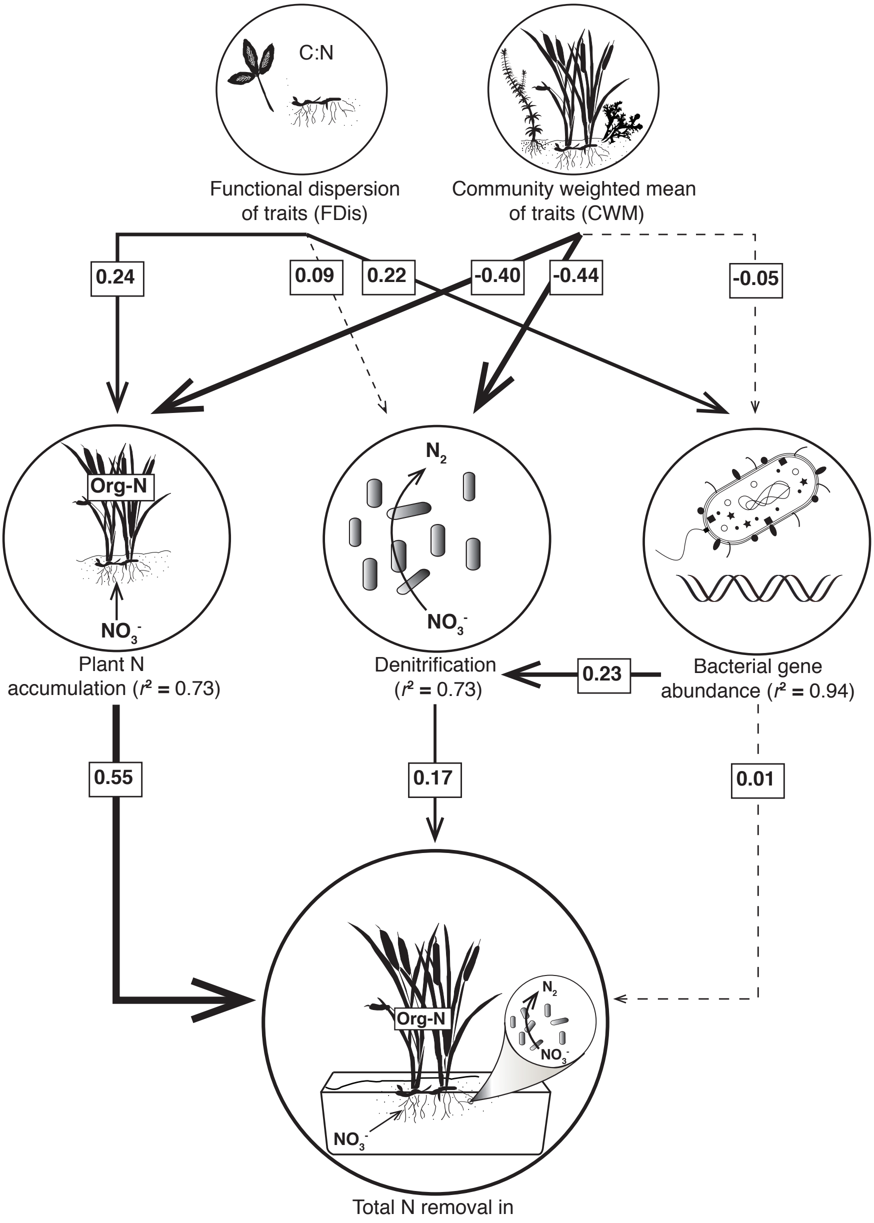


Figure 5. Structural equation modelling (SEM) of total nitrogen (N) removal from wetland mesocosms. Solid lines indicate significant ($P < 0.05$) positive and negative relationships while dashed lines indicate non-significant ($P > 0.05$) relationships. Thickness of the arrow lines indicates the strength of the respective relationship (standardised correlation coefficients). The variation explained in each response variable is denoted as r^2 in parenthesis (Org-N = organic N).

(Kareiva *et al.*, 2007; Kremen, 2005). By regulating the strength of these interactions and linkages, functional diversity of species traits can affect ecosystem services through influencing ecosystem properties relevant to for example, carbon, nutrient and water cycling (Díaz *et al.*, 2007). Accordingly, functional diversity has emerged as a strong predictor of ecosystem service provisions from natural and human-modified systems such as forests, grasslands, croplands, rivers and wetlands (Lavorel & Grigulis, 2012; Díaz *et al.*, 2011; de Bello *et al.*, 2010; Kremen, 2005).

Structural equation modelling (SEM) showed that functional dispersion (FDis) of macrophyte traits (Box 2) had a direct positive effect on plant N accumulation and denitrification gene abundance and an indirect effect on PDA by regulating denitrification gene abundance (Figure 5). In contrast, the community weighted mean (CWM) of plant traits significantly affected plant N accumulation and PDA but not denitrification gene abundance. I found that the latter significantly affected PDA. This supports my previous finding of the relationship between PDA and denitrification gene abundance (Paper I). However, gene abundance did not affect the overall N removal for the whole experimental period. Both plant N accumulation and PDA significantly affected total N removal by experimental mesocosms (Figure 5).

In case of CWM of combined macrophyte traits, macrophyte communities dominated by species with high Ellenberg N value, high root diameter and allelopathic potential had lower N accumulation rates and PDA while communities characterised by high end belowground biomass, high specific root surface area (SRSA) and high relative growth rate (RGR) tended to have higher N accumulation rates and PDA (Paper III-Figure 2 & 3). Generalised linear models showed that CWM of relative growth rate, specific leaf area and end aboveground biomass had a positive correlation with plant N accumulation and explained 62% of the total variation (Paper III- Table 1). On the other hand, 20% of the total variation in PDA was explained by the CWM of the specific root surface area, root diameter and plant C-strategy (Paper III- Table 1). Both root diameter and plant C- strategy showed a negative correlation with PDA while specific root surface area had a positive correlation.

N removal at the local scale in a cold climate

Wetlands play an important role in nutrient retention at the catchment scale (Verhoeven *et al.*, 2006; Whigham *et al.*, 1988). Catchments also have numerous small open water bodies such as ponds, small lakes, depressions etc. receiving nutrient rich surface water that also demands improved nutrient cycling at the local scale. In recent years, constructed floating wetlands (CFWs), planted with emerging macrophytes to optimise the N removal from surface water, have gained increased attention for nutrient removal in ponds, lakes and storm water reservoirs (Song *et al.*, 2014; Wang & Sample, 2014). Despite the evidence that wetlands in cold climates might be suitable sites for water treatment (Wang *et al.*, 2017; Hallin *et al.*, 2015; Tunçsiper *et al.*, 2015; Mæhlum & Stålnacke, 1999; Wittgren & Mæhlum, 1997), the application of CFWs in cold climates has to my knowledge not been tested.

My pilot study showed that establishment of macrophytes in CFWs is feasible, both in mesocosms and *in situ*, under cold climatic conditions (Paper IV). Standing plant biomass and bulk N accumulation differed among the studied species in CFWs. *C. palustre*, *F. ulmaria* and *C. rostrata* had higher standing biomass and bulk N accumulation compared to other studied species. These species are characterized by high canopy height, larger specific leaf area (SLA) (Grime *et al.*, 2007) and are typical for mesotrophic to eutrophic habitats (Fitter & Peat, 1994). The standing aboveground biomass of *C. rostrata* (2.2 kg-DW m^{-2}) in my study was higher than the biomass production by *C. rostrata* (25.2 g-DW m^{-2}) found in a boreal fen in southern Finland (Saarinen, 1996) and comparable to *C. rostrata* (1.2 kg-DW m^{-2}) grown *in situ* for two years in an eutrophic fen (Aerts *et al.*, 1992).

In general, potential denitrification activity (PDA) associated with macrophyte roots in CFWs was higher in mesocosms, receiving high amount of NO_3^-, compared to the recipient system with lower NO_3^- loading. Moreover, I found that PDA varied among the studied macrophytes. PDA in roots of *M. trifoliata* was barely detectable (Paper IV). A similar result was observed for this species in the wetland mesocosm experiment where no denitrification genes were detected on the root of this species (Paper III). *M. trifoliata* is known to have high allelopathy potential (Grutters *et al.*, 2017), which might inhibit the abundance and activity of root associated denitrifying bacteria. On the other hand, *C. rostrata*, *E. fluviatile* and *E. angustifolium* showed higher PDA in CFWs (Paper IV- Table S6) compared to that in the semi-natural wetland (Paper I- Figure 1). Comparing the magnitude of N accumulation and PDA, I found that denitrification was the main N removal pathway in CFWs, which highlights the potential of CFWs for improved N removal in freshwaters at the local scale.

5 Conclusion and future perspectives

My thesis shows that N removal in wetlands under cold climatic conditions is feasible by using native sub-arctic macrophyte species despite adverse environmental conditions (e.g. low temperature and short growing season) hindering the growth of macrophytes compared to temperate and tropical regions. Moreover, I found that growth form combinations can be a useful guide to select macrophyte species for enhanced N removal in constructed wetlands; a finding that might be applicable also to other climatic regions. The relative dominance of two potential N removal pathways, i.e. plant N accumulation and plant associated denitrification, depends on the studied systems (e.g. mesocosms or *in situ*) and on the combinations of macrophyte species. My studies further highlight the importance of trait diversity for N-cycling in wetlands, which is not well investigated for macrophytes. I also identified the underlying mechanisms, *viz.* complementarity and selection effect on N removal from water including plant N accumulation by different growth form combinations. Moreover, complementarity and trait dissimilarities between macrophytes can directly influence the microbial community, which in turn affects N removal in wetlands through denitrification. My studies also emphasized the importance of concomitant studies of both above- and belowground functions, for example of plant N accumulation and plant associated denitrification, to understand the role of macrophytes in N-cycling in wetlands. The current results are consistent with previous studies in different terrestrial systems, highlighting the impotence of maintaining biodiversity for proper ecosystem functioning also in wetlands. This thesis has broad ecological relevance for the restoration of wetland habitats and construction of new wetlands for enhanced nutrient removal by showing how selection of macrophyte species assemblages can optimize diversity of desired traits.

The current thesis was based on a series of mesocosm experiments and field studies. In order to quantify the actual N removal potential of different macrophyte species in wetlands it is also necessary to extrapolate my results to the wetland scale. This usually demands intensive and time-consuming field work with macrophyte sampling, vegetation mapping and estimation of above- and belowground plant biomass. Here, recent developments in high-resolution remote sensing with drones can potentially replace or at least complement field-based methods. Such studies would also have management implications by for example determining the appropriate size of CW. Further *in situ* studies with larger numbers of species and growth forms are also needed in order to generalise my findings to other macrophyte assemblages in boreal and sub-arctic regions. Future studies should also consider plant secondary metabolites to better understand macrophyte-denitrifier interactions. I demonstrated that the co-existence of emerging macrophytes and bryophytes, in contrast to combinations of submerged species, increased denitrification gene abundance and denitrification rates. However, the actual mechanisms regulating these relationships have still not been properly investigated. Understanding the molecular mechanisms via e.g. transcriptomic and proteomic analyses can provide valuable information regarding the function of the macrophyte-denitrifier interplay. Finally, in my CFWs experiment, I used floating wetlands made of non-biodegradable plastic materials that might raise environmental concerns. The construction of floating wetlands with more environment-friendly materials supporting macrophyte growth as well as denitrification should therefore be further investigated.

References

Aerts, R., Decaluwe, H. & Konings, H. (1992). Seasonal allocation of biomass and nitrogen in 4 Carex species from mesotrophic and eutrophic fens as affected by nitrogen supply. *Journal of Ecology,* 80(4), pp. 653-664.

Bachand, P.A.M. & Horne, A.J. (2000). Denitrification in constructed free-water surface wetlands: II. Effects of vegetation and temperature. *Ecological Engineering,* 14(1-2), pp. 17-32.

Bailey, B.L., Smith, L.J.D., Blowes, D.W., Ptacek, C.J., Smith, L. & Sego, D.C. (2013). The Diavik Waste Rock Project: persistence of contaminants from blasting agents in waste rock effluent. *Applied Geochemistry,* 36, pp. 256-270.

Barko, J.W., Gunnison, D. & Carpenter, S.R. (1991). Sediment interactions with submersed macrophyte growth and community dynamics. *Aquatic Botany,* 41(1-3), pp. 41-65.

Bates, J.W. (1998). Is 'life-form' a useful concept in bryophyte ecology? *Oikos,* 82(2), pp. 223-237.

Bayley, S.E., Wong, A.S. & Thompson, J.E. (2013). Effects of agricultural encroachment and drought on wetlands and shallow lakes in the boreal transition zone of Canada. *Wetlands,* 33(1), pp. 17-28.

Billen, G., Garnier, J. & Lassaletta, L. (2013). The nitrogen cascade from agricultural soils to the sea: modelling nitrogen transfers at regional watershed and global scales. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 368(1621).

Bouchard, V., Frey, S.D., Gilbert, J.M. & Reed, S.E. (2007). Effects of macrophyte functional group richness on emergent freshwater wetland functions. *Ecology,* 88(11), pp. 2903-2914.

Brix, H. (1997). Do macrophytes play a role in constructed treatment wetlands? *Water Science and Technology,* 35(5), pp. 11-17.

Cadotte, M.W. (2017). Functional traits explain ecosystem function through opposing mechanisms. *Ecology Letters,* 20(8), pp. 989-996.

Cardinale, B.J., Srivastava, D.S., Duffy, J.E., Wright, J.P., Downing, A.L., Sankaran, M. & Jouseau, C. (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature,* 443(7114), pp. 989-992.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M. & Weis, J.J. (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences of the United States of America,* 104(46), pp. 18123-18128.

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N. & Smith, V.H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications,* 8(3), pp. 559-568.

Costanza, R., dArge, R., deGroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., ONeill, R.V., Paruelo, J., Raskin, R.G., Sutton, P. & vandenBelt, M. (1997). The value of the world's ecosystem services and natural capital. *Nature,* 387(6630), pp. 253-260.

de Bello, F., Lavorel, S., Díaz, S., Harrington, R., Cornelissen, J.H.C., Bardgett, R.D., Berg, M.P., Cipriotti, P., Feld, C.K., Hering, D., da Silva, P.M., Potts, S.G., Sandin, L., Sousa, J.P., Storkey, J., Wardle, D.A. & Harrison, P.A. (2010). Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation,* 19(10), pp. 2873-2893.

de Vries, F.T. & Bardgett, R.D. (2016). Plant community controls on short-term ecosystem nitrogen retention. *New Phytologist,* 210(3), pp. 861-874.

DeMeester, J.E. & Richter, D.D. (2010). Differences in wetland nitrogen cycling between the invasive grass *Microstegium vimineum* and a diverse plant community. *Ecological Applications,* 20(3), pp. 609-619.

Díaz, S. & Cabido, M. (2001). Vive la différence: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution,* 16(11), pp. 646-655.

Díaz, S., Lavorel, S., Chapin, F.S., Tecco, P.A., Gurvich, D.E. & Grigulis, K. (2007). Functional diversity – at the crossroads between ecosystem functioning and environmental filters. In: Canadell, J., Pataki, D.E. & Pitelka, L.F. (eds) *Terrestrial Ecosystem in a Changing World*. Berlin: Springer, pp. 79-91.

Díaz, S., Quétier, F., Cáceres, D.M., Trainor, S.F., Pérez-Harguindeguy, N., Bret-Harte, M.S., Finegan, B., Peña-Claros, M. & Poorter, L. (2011). Linking functional diversity and social actor strategies in a framework for interdisciplinary analysis of nature's benefits to society. *Proceedings of the National Academy of Sciences of the United States of America,* 108(3), pp. 895-902.

Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T. & Thornbrugh, D.J. (2009). Eutrophication of U.S. freshwaters: analysis of potential economic damages. *Environmental Science & Technology,* 43(1), pp. 12-19.

Engelhardt, K.A.M. & Ritchie, M.E. (2001). Effects of macrophyte species richness on wetland ecosystem functioning and services. *Nature,* 411(6838), pp. 687-689.

Eriksson, P.G. & Weisner, S.E.B. (1997). Nitrogen removal in a wastewater reservoir: the importance of denitrification by epiphytic biofilms on submersed vegetation. *Journal of Environmental Quality,* 26(3), pp. 905-910.

Fitter, A.H. & Peat, H.J. (1994). The ecological flora database. *Journal of Ecology,* 82(2), pp. 415-425. http://www.ecoflora.co.uk.

Flynn, D.F.B., Mirotchnick, N., Jain, M., Palmer, M.I. & Naeem, S. (2011). Functional and phylogenetic diversity as predictors of biodiversity-ecosystem-function relationships. *Ecology,* 92(8), pp. 1573-1581.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N. & Snyder, P.K. (2005). Global consequences of land use. *Science,* 309(5734), pp. 570-574.

Forsberg, H. & Åkerlund, H. (1999). *Kväve och sprängämnesrester I LKAB:s malm- gråbergs- och produktflöden*. Diss. Luleå: Luleå University of Technology, p. 122. In Swedish.

Fowler, D., Coyle, M., Skiba, U., Sutton, M.A., Cape, J.N., Reis, S., Sheppard, L.J., Jenkins, A., Grizzetti, B., Galloway, J.N., Vitousek, P., Leach, A., Bouwman, A.F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M. & Voss, M. (2013). The global nitrogen cycle in the twenty-first century. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 368(1621).

Frainer, A., Polvi, L.E., Jansson, R. & McKie, B.G. (2018). Enhanced ecosystem functioning following stream restoration: the roles of habitat heterogeneity and invertebrate species traits. *Journal of Applied Ecology,* 55(1), pp. 377-385.

Gagnon, V., Chazarenc, F., Comeau, Y. & Brisson, J. (2007). Influence of macrophyte species on microbial density and activity in constructed wetlands. *Water Science and Technology,* 56(3), pp. 249-254.

Garnier, E., Cortez, J., Billes, G., Navas, M.L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C. & Toussaint, J.P. (2004). Plant functional markers capture ecosystem properties during secondary succession. *Ecology,* 85(9), pp. 2630-2637.

Glime, J.M. (2017). Nutrient relations: uptake and location. Chapt. 8-4, in Bryophyte Ecology, 1 Physiological Ecology, pp. 8-4-3.

Gopal, B. & Goel, U. (1993). Competition and allelopathy in aquatic plant-communities. *Botanical Review,* 59(3), pp. 155-210.

Grace, J.B., Anderson, T.M., Olff, H. & Scheiner, S.M. (2010). On the specification of structural equation models for ecological systems. *Ecological Monographs,* 80(1), pp. 67-87.

Grace, J.B. & Pugesek, B.H. (1997). A structural equation model of plant species richness and its application to a coastal wetland. *American Naturalist,* 149(3), pp. 436-460.

Grime, J.P., Hodgson, J.G. & Hunt, R. (2007). *Comparative plant ecology: a functional approach to common British species (2nd ed)*. Colvend: Castlepoint Press.

Grime, J.P., Rincon, E.R. & Wickerson, B.E. (1990). Bryophytes and plant strategy theory. *Botanical Journal of the Linnean Society,* 104(1-3), pp. 175-186.

Grutters, B.M.C., Saccomanno, B., Gross, E.M., Van de Waal, D.B., van Donk, E. & Bakker, E.S. (2017). Growth strategy, phylogeny and stoichiometry determine the allelopathic potential of native and non-native plants. *Oikos,* 126(12), pp. 1770-1779.

Hallin, S., Hellman, M., Choudhury, M.I. & Ecke, F. (2015). Relative importance of plant uptake and plant associated denitrification for removal of nitrogen from mine drainage in sub-arctic wetlands. *Water Research,* 85, pp. 377-383.

Hallin, S., Philippot, L., Löffler, F.E., Sanford, R.A. & Jones, C.M. (2018). Genomics and ecology of novel N2O-reducing microorganisms. *Trends in Microbiology,* 26(1), pp. 43-55.

Halls, A.J.e. (1997). *Wetlands, Biodiversity and the Ramsar Convention: The role of the convention on wetlands in the conservation and wise use of biodiversity*. Gland, Switzerland: Ramsar Convention Bureau. available at https://www.ramsar.org/sites/default/files/documents/library/wetlands_biodiversity_and_the_ramsar_convention.pdf (accessed on 04-06-2018).

Harrison, J.A., Maranger, R.J., Alexander, R.B., Giblin, A.E., Jacinthe, P.A., Mayorga, E., Seitzinger, S.P., Sobota, D.J. & Wollheim, W.M. (2009). The regional and global significance of nitrogen removal in lakes and reservoirs. *Biogeochemistry,* 93(1-2), pp. 143-157.

Herbert, R.B., Winbjork, H., Hellman, M. & Hallin, S. (2014). Nitrogen removal and spatial distribution of denitrifier and anammox communities in a bioreactor for mine drainage treatment. *Water Research,* 66, pp. 350-360.

Hilt, S. & Gross, E.M. (2008). Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology,* 9(4), pp. 422-432.

Hodge, A., Robinson, D. & Fitter, A. (2000). Are microorganisms more effective than plants at competing for nitrogen? *Trends in Plant Science,* 5(7), pp. 304-308.

Horne, A.J. & Goldman, C.R. (1994). *Limnology*: McGraw-Hill, pp 134-150. Available from: https://books.google.se/books?id=D9L3EX_EZAsC.

Horppila, J. & Nurminen, L. (2005). Effects of different macrophyte growth forms on sediment and P resuspension in a shallow lake. *Hydrobiologia,* 545, pp. 167-175.

Howard-Williams, C. (1985). Cycling and retention of nitrogen and phosphorus in wetlands - a theoretical and applied perspective. *Freshwater Biology,* 15(4), pp. 391-431.

Jordan, S.J., Stoffer, J. & Nestlerode, J.A. (2011). Wetlands as sinks for reactive nitrogen at continental and global scales: a meta-analysis. *Ecosystems,* 14(1), pp. 144-155.

Kareiva, P., Watts, S., McDonald, R. & Boucher, T. (2007). Domesticated nature: shaping landscapes and ecosystems for human welfare. *Science,* 316(5833), pp. 1866-1869.

Kjellin, J., Hallin, S. & Worman, A. (2007). Spatial variations in denitrification activity in wetland sediments explained by hydrology and denitrifying community structure. *Water Research,* 41(20), pp. 4710-4720.

Kofoed, M.V.W., Stief, P., Hauzmayer, S., Schramm, A. & Herrmann, M. (2012). Higher nitrate-reducer diversity in macrophyte-colonized compared to unvegetated freshwater sediment. *Systematic and Applied Microbiology,* 35(7), pp. 465-472.

Konnerup, D. & Brix, H. (2010). Nitrogen nutrition of *Canna indica*: Effects of ammonium versus nitrate on growth, biomass allocation, photosynthesis, nitrate reductase activity and N uptake rates. *Aquatic Botany,* 92(2), pp. 142-148.

Kremen, C. (2005). Managing ecosystem services: what do we need to know about their ecology? *Ecology Letters,* 8(5), pp. 468-479.

Lavorel, S. & Grigulis, K. (2012). How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. *Journal of Ecology,* 100(1), pp. 128-140.

Lavorel, S., Grigulis, K., McIntyre, S., Williams, N.S.G., Garden, D., Dorrough, J., Berman, S., Quetier, F., Thebault, A. & Bonis, A. (2008). Assessing functional diversity in the field -methodology matters! *Functional Ecology,* 22(1), pp. 134-147.

Lee, C.G., Fletcher, T.D. & Sun, G.Z. (2009). Nitrogen removal in constructed wetland systems. *Engineering in Life Sciences,* 9(1), pp. 11-22.

Loreau, M. & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature,* 412(6842), pp. 72-76.

Lund, L.J., Horne, A.J. & Williams, A.E. (2000). Estimating denitrification in a large constructed wetland using stable nitrogen isotope ratios. *Ecological Engineering,* 14(1-2), pp. 67-76.

Madsen, T.V. & Cedergreen, N. (2002). Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology,* 47(2), pp. 283-291.

Mæhlum, T. & Stålnacke, P. (1999). Removal efficiency of three cold-climate constructed wetlands treating domestic wastewater: effects of temperature, seasons, loading rates and input concentrations. *Water Science and Technology,* 40(3), pp. 273-281.

Mason, N.W.H., Mouillot, D., Lee, W.G. & Wilson, J.B. (2005). Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos,* 111(1), pp. 112-118.

McClain, M.E., Boyer, E.W., Dent, C.L., Gergel, S.E., Grimm, N.B., Groffman, P.M., Hart, S.C., Harvey, J.W., Johnston, C.A., Mayorga, E., McDowell, W.H. & Pinay, G. (2003). Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems,* 6(4), pp. 301-312.

McDonald, R. & Knox, O.G.G. (2014). Cold region bioremediation of hydrocarbon contaminated soils: do we know enough? *Environmental Science & Technology,* 48(17), pp. 9980-9981.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution,* 21(4), pp. 178-185.

McKie, B.G., Woodward, G., Hladyz, S., Nistorescu, M., Preda, E., Popescu, C., Giller, P.S. & Malmqvist, B. (2008). Ecosystem functioning in stream assemblages from different regions: contrasting responses to variation in detritivore richness, evenness and density. *Journal of Animal Ecology,* 77(3), pp. 495-504.

Milcu, A., Allan, E., Roscher, C., Jenkins, T., Meyer, S.T., Flynn, D., Bessler, H., Buscot, F., Engels, C., Gubsch, M., Konig, S., Lipowsky, A., Loranger, J., Renker, C., Scherber, C., Schmid, B., Thebault, E., Wubet, T., Weisser, W.W., Scheu, S. & Eisenhauer, N. (2013). Functionally and phylogenetically diverse plant communities key to soil biota. *Ecology,* 94(8), pp. 1878-1885.

Milcu, A., Roscher, C., Gessler, A., Bachmann, D., Gockele, A., Guderle, M., Landais, D., Piel, C., Escape, C., Devidal, S., Ravel, O., Buchmann, N., Gleixner, G., Hildebrandt, A. & Roy, J. (2014). Functional diversity of leaf nitrogen concentrations drives grassland carbon fluxes. *Ecology Letters,* 17(4), pp. 435-444.

Mitsch, W.J., Bernal, B., Nahlik, A.M., Mander, U., Zhang, L., Anderson, C.J., Jorgensen, S.E. & Brix, H. (2013). Wetlands, carbon, and climate change. *Landscape Ecology,* 28(4), pp. 583-597.

Moreau, D., Pivato, B., Bru, D., Busset, H., Deau, F., Faivre, C., Matejicek, A., Strbik, F., Philippot, L. & Mougel, C. (2015). Plant traits related to nitrogen uptake influence plant-microbe competition. *Ecology,* 96(8), pp. 2300-2310.

Morgan, J.A., Martin, J.F. & Bouchard, V. (2008). Identifying plant species with root associated bacteria that promote nitrification and denitrification in ecological treatment systems. *Wetlands,* 28(1), pp. 220-231.

Naeem, S. (2002). Disentangling the impacts of diversity on ecosystem functioning in combinatorial experiments. *Ecology,* 83(10), pp. 2925-2935.

Niu, S.L., Classen, A.T., Dukes, J.S., Kardol, P., Liu, L.L., Luo, Y.Q., Rustad, L., Sun, J., Tang, J.W., Templer, P.H., Thomas, R.Q., Tian, D.S., Vicca, S., Wang, Y.P., Xia, J.Y. & Zaehle, S. (2016). Global patterns and substrate-based mechanisms of the terrestrial nitrogen cycle. *Ecology Letters,* 19(6), pp. 697-709.

Pavlineri, N., Skoulikidis, N.T. & Tsihrintzis, V.A. (2017). Constructed floating wetlands: a review of research, design, operation and management aspects, and data meta-analysis. *Chemical Engineering Journal,* 308, pp. 1120-1132.

Pip, E. (1992). Phenolic-compounds in macrophytes from the Lower Nelson River System, Canada. *Aquatic Botany,* 42(3), pp. 273-279.

Piwpuan, N., Zhai, X. & Brix, H. (2013). Nitrogen nutrition of *Cyperus laevigatus* and *Phormium tenax*: effects of ammonium versus nitrate on growth, nitrate reductase activity and N uptake kinetics. *Aquatic Botany,* 106, pp. 42-51.

Pretty, J.N., Mason, C.F., Nedwell, D.B., Hine, R.E., Leaf, S. & Dils, R. (2003). Environmental costs of freshwater eutrophication in England and Wales. *Environmental Science & Technology,* 37(2), pp. 201-208.

Raab, B. & Vedin, H.E. (1995). *Sveriges National Atlas- Klimat, sjöar och vatten- drag*: Höganäs.: Bokförlaget Bra Böcker.

Ruiz-Rueda, O., Hallin, S. & Baneras, L. (2009). Structure and function of denitrifying and nitrifying bacterial communities in relation to the plant species in a constructed wetland. *Fems Microbiology Ecology,* 67(2), pp. 308-319.

Saarinen, T. (1996). Biomass and production of two vascular plants in a boreal mesotrophic fen. *Canadian Journal of Botany-Revue Canadienne De Botanique,* 74(6), pp. 934-938.

Salvato, M., Borin, M., Doni, S., Macci, C., Ceccanti, B., Marinari, S. & Masciandaro, G. (2012). Wetland plants, micro-organisms and enzymatic activities interrelations in treating N polluted water. *Ecological Engineering,* 47, pp. 36-43.

Saunders, D.L. & Kalff, J. (2001). Nitrogen retention in wetlands, lakes and rivers. *Hydrobiologia,* 443(1-3), pp. 205-212.

Schindler, D.W. (1998). Sustaining aquatic ecosystems in boreal regions. *Conservation Ecology,* 2(2).

Schultz, R.E., Bouchard, V.L. & Frey, S.D. (2012). Overyielding and the role of complementary use of nitrogen in wetland plant communities. *Aquatic Botany,* 97(1), pp. 1-9.

Sculthorpe, C.D. (1967). *The biology of aquatic vascular plants*. London, pp172-435: Edward Arnold.

Seitzinger, S., Harrison, J.A., Bohlke, J.K., Bouwman, A.F., Lowrance, R., Peterson, B., Tobias, C. & Van Drecht, G. (2006). Denitrification across landscapes and waterscapes: a synthesis. *Ecological Applications,* 16(6), pp. 2064-2090.

SMHI, Swedish institute of hydrology and metrology http://www.smhi.se/klimatdata.

Smil, V. (2001). *Enriching the erath: Fritz Haber, Carl Bosch and the Transformation of World Food Production*. Cambridge, London: The MIT Press, p. 5.

Solander, D. (1983). Biomass and shoot production of *Carex rostrata* and *Equisetum fluviatile* in unfertilized and fertilized subarctic lakes. *Aquatic Botany,* 15(4), pp. 349-366.

Song, H.L., Li, X.N., Li, W. & Lu, X.W. (2014). Role of biologic components in a novel floating-bed combining *Ipomoea aquatic*, *Corbicula fluminea* and biofilm carrier media. *Frontiers of Environmental Science & Engineering,* 8(2), pp. 215-225.

Thullen, J.S., Sartoris, J.J. & Nelson, S.M. (2005). Managing vegetation in surface-flow wastewater-treatment wetlands for optimal treatment performance. *Ecological Engineering,* 25(5), pp. 583-593.

Tilman, D. (2001). Functional diversity. *Encyclopedia of Biodiversity,* 3, pp. 109-120.

Tilman, D., Wedin, D. & Knops, J. (1996). Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature,* 379(6567), pp. 718-720.

Truchy, A., Angeler, D.G., Sponseller, R.A., Johnson, R.K. & McKie, B.G. (2015). Linking biodiversity, ecosystem functioning and services, and ecological resilience: towards an integrative framework for improved management. *Advances in Ecological Research,* 53, pp. 55-96.

Tunçsiper, B., Drizo, A. & Twohig, E. (2015). Constructed wetlands as a potential management practice for cold climate dairy effluent treatment - VT, USA. *Catena,* 135, pp. 184-192.

Turetsky, M.R. (2003). The role of bryophytes in carbon and nitrogen cycling. *Bryologist,* 106(3), pp. 395-409.

Verhoeven, J.T.A., Arheimer, B., Yin, C.Q. & Hefting, M.M. (2006). Regional and global concerns over wetlands and water quality. *Trends in Ecology & Evolution,* 21(2), pp. 96-103.

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R. & Davies, P.M. (2010). Global threats to human water security and river biodiversity. *Nature,* 467(7315), pp. 555-561.

Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment,* 380(1-3), pp. 48-65.

Vymazal, J. (2011). Constructed wetlands for wastewater treatment: five decades of experience. *Environmental Science & Technology,* 45(1), pp. 61-69.

Vymazal, J. (2013). Emergent plants used in free water surface constructed wetlands: a review. *Ecological Engineering,* 61, pp. 582-592.

Wang, C.Y. & Sample, D.J. (2014). Assessment of the nutrient removal effectiveness of floating treatment wetlands applied to urban retention ponds. *Journal of Environmental Management,* 137, pp. 23-35.

Wang, M., Zhang, D.Q., Dong, J.W. & Tan, S.K. (2017). Constructed wetlands for wastewater treatment in cold climate - a review. *Journal of Environmental Sciences,* 57, pp. 293-311.

Weisner, S.E.B. & Thiere, G. (2010). Effects of vegetation state on biodiversity and nitrogen retention in created wetlands: a test of the biodiversity-ecosystem functioning hypothesis. *Freshwater Biology,* 55(2), pp. 387-396.

Whigham, D.F., Chitterling, C. & Palmer, B. (1988). Impacts of fresh-water wetlands on water-quality - a landscape perspective. *Environmental Management,* 12(5), pp. 663-671.

Whitmire, S.L. & Hamilton, S.K. (2005). Rapid removal of nitrate and sulfate in freshwater wetland sediments. *Journal of Environmental Quality,* 34(6), pp. 2062-2071.

Wittgren, H.B. & Mæhlum, T. (1997). Wastewater treatment wetlands in cold climates. *Water Science and Technology,* 35(5), pp. 45-53.

Wu, H.L., Wang, X.Z., He, X.J., Zhang, S.B., Liang, R.B. & Shen, J. (2017). Effects of root exudates on denitrifier gene abundance, community structure and activity in a micro-polluted constructed wetland. *Science of the Total Environment,* 598, pp. 697-703.

Zaehle, S. (2013). Terrestrial nitrogen - carbon cycle interactions at the global scale. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 368(1621).

Zhang, D.Q., Jinadasa, K.B.S.N., Gersberg, R.M., Liu, Y., Tan, S.K. & Ng, W.J. (2015). Application of constructed wetlands for wastewater treatment in tropical and subtropical regions (2000-2013). *Journal of Environmental Sciences,* 30, pp. 30-46.

Wetland vegetation improves water quality impacted by mining exploration

(Popular science summary)

Imagine you went to your summer house to spend an awesome vacation with your family, but you weren't able to swim in the lake close to your house because the water turned green with an algal bloom. You could not even fish in the lake since the fishes were dying. A bad and rotten smell came constantly from the lake and your vacation was completely ruined. Yes, this kind of situation can arise when water gets eutrophicated by the release of high levels of nutrients like nitrogen (N) and phosphorus (P).

Water pollution is of worldwide concern

Every day we are polluting our natural water bodies by different activities such as agriculture, urbanisation, industrialisation and other extensive land uses. Polluted water is not suitable for drinking, swimming, fishing, agriculture and other human uses. Every year around 842,000 people worldwide die only from diarrhoea, only, due to the use of unsafe water (World Health Organization WHO, February, 2018). Inorganic N concentrations in surface water should be in the range of 1-2.5 mg N L^{-1} to avoid eutrophication[1]. The release of nitrogen to freshwater bodies from undetonated ammonium-nitrate (NH_4NO_3) based explosives used in mining or other blasting operations has become an emerging environmental issue.

Mining can boost nitrogen input to water

Mining industries use NH_4NO_3 based explosives to blast rocks. Undetonated explosives can easily get dissolved in process water and reach surface water. The mining company LKAB, situated in northern Sweden, is facing problems with high

> *"Scientists are helping a mining company to reduce water pollution"*

levels of nitrogen in the wastewater from mining operations. The company is now collaborating with scientists from the Swedish University of Agricultural Sciences (SLU), Uppsala University and Luleå University of Technology, and is trying to improve water quality. The scientists are trying to increase our understanding of how wetland plants and associated microorganisms can help us to reduce levels of nitrogen in water.

Aquatic plants use nitrogen

Aquatic plants, including vascular plants and mosses, grow in different growth forms such as emerging, floating and submerged forms, in wetlands. Through their roots and shoots they take up nutrients such as nitrogen from the sediments and water. This nitrogen is stored temporarily in plant tissue and can be permanently removed from aquatic systems by harvesting the plants. Different plant species have different abilities for taking up nitrogen in wetlands[2]. Moreover, they increase nitrogen removal from water through a beneficiary relationship known as complementarity where interacting species help each other to perform a function like nutrient uptake[2]. Hence, diverse aquatic plant communities can play an important role in improving water quality by removing nitrogen from water.

Microorganisms can transform the nitrogen from water to the atmosphere

> *"Aquatic plants and bacteria help to remove nitrogen from water"*

Microorganisms such as bacteria living on the surface of roots and shoots of aquatic plants, as well as in sediments use nitrogen to get energy for their metabolic processes. They transform nitrogen compounds into nitrogen gas and help to transfer the bioavailable nitrogen in water to nitrogen gas, the dominating gas in the air. Aquatic plants provide surface area for bacteria to grow on as well as carbon to feed on. The abundance of bacteria living on plant surfaces varies among plant species and usually it is higher in moss species compared to vascular plants[3].

Scientists demonstrate the importance of aquatic plant diversity

Maidul Choudhury, Frauke Ecke, Sara Hallin and Brendan McKie together with other colleagues from SLU, found in their experiments that nitrogen removal from water increases when more aquatic plant species and growth forms are grown together. Moreover, different species have different nitrogen removal abilities at different time points during a growing season. For example, during July, emerging aquatic plants can remove higher amounts of nitrogen from water compared to submerged plants. In August this removal rate is higher for moss species. The research group also found that emerging plants and mosses showed positive interactions for nitrogen accumulation in plant biomass while the coexistence of two submerged species lowered nitrogen accumulation[2].

"Aquatic plant diversity is important for nitrogen removal from water"

Their experiments also revealed that the ability of bacteria to transform nitrogen is higher in moss species compared to emerging and submerged plants. Moreover, the presence of mosses increased bacterial activity on the roots of emerging plants[3,4]. The research group also showed that plant characteristics and properties influence both plant nitrogen uptake and bacterial activity[4].

Some aquatic plants enhance nitrogen removal in wetlands by plant uptake while others enhance it via influencing bacterial activity. Different growth form combinations as well as diversity of plant characteristics can be used as a potential tool to select suitable species for nitrogen removal from water[4]. By maintaining plant diversity in wetlands, we can reduce nutrient levels in water, which in the long run will help us to mitigate freshwater pollution.

References

[1]Camargo, J. A., & Alonso, Á. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environment International*, 32(6), pp. 831-849.

[2]Choudhury, M. I.; McKie, B. G.; Hallin S. & Ecke, F. (2018) Mixtures of macrophyte growth forms promote nitrogen cycling in wetlands. *Science of the Total Environment*, 635, pp. 1436–1443.

[3]Hallin, S., Hellman, M., Choudhury, M. I., & Ecke, F. (2015). Relative importance of plant uptake and plant associated denitrification for removal of nitrogen from mine drainage in sub-arctic wetlands. *Water Research*, 85, pp. 377-383.

[4]Choudhury, M. I.; Hallin S.; Hubalek, V.; Juhanson, J.; Frainer, A., McKie, B. G. & Ecke, F. (2018) Plant traits explain uptake of nitrogen and plant associated denitrification in wetlands (manuscript).

Våtmarksvegetation förbättrar vattenkvaliteten som påverkas av gruvdrift

(Populärvetenskaplig sammanfattning)

Tänk dig att du åker till sommarstugan för att ha en härlig semester med din familj, men att det inte gick att bada i sjön eftersom vattnet var grönt av alger. Det gick inte ens att fiska, det fanns inte längre någon fisk. Hela tiden kom en otäck, rutten lukt från vattnet och semestern var helt förstörd. Det här kan hända när stora mängder av näringsämnena fosfor och kväve orsakar övergödning av vattnet.

Förorenat vatten är en global angelägenhet

Varje dag förorenar vi våra naturliga vattendrag genom urbanisering, industriell aktivitet, jordbruk och andra markanvändningar. Förorenat vatten är otjänligt som dricksvatten, i jordbruk och för många av de aktiviteter vi vanligtvis förknippar med vatten, som att bada och fiska. Varje år dör 842 000 människor globalt bara från diarré på grund av användning av otjänligt vatten (Världshälsoorganisationen WHO, februari, 2018). För att undvika övergödning bör koncentrationen av oorganiska kväveföreningar i ytvattnet inte överstiga 1-2,5 mg N per liter[1]. Utsläpp av kväve från gruvdrift och andra områden där man använder kvävebaserade sprängämnen är ett växande miljöproblem eftersom det påverkar ytvattnets kvalitet.

Gruvdrift kan öka kväveutsläpp till vatten

> *"Forskare hjälper ett gruvföretag att minska vattenföroreningar"*

Gruvindustrin använder kvävebaserade sprängämnen i sin verksamhet. En del av dessa detonerar inte och de löses istället upp i processvattnet och når ytvattnet. Gruvbolaget LKAB i norra Sverige står inför problem med för höga halter av kväve i vattnet som släpps ut från gruvområdet. Företaget samarbetar nu med forskare från Sveriges lantbruksuniversitet (SLU), Uppsala universitet och Luleå tekniska universitet för att förbättra vattenkvaliteten. Målet är att öka förståelsen av hur våtmarker och de mikroorganismer som finns där kan bidra till att minska kvävehalten i vattnet.

Vattenväxter använder kväve

> *"Vattenväxter och bakterier hjälper till att avlägsna kväve från vatten"*

Vattenväxter, t ex kärlväxter och mossor, förekommer i olika växtformer; de kan vara övervattensväxter, vara rotade men inte nå över vattenytan eller växa fritt flytande i vattnet. De tar upp näringsämnen som kväve från sedimentet och ur vattnet genom sina rötter och skott. Kvävet lagras tillfälligt i växtens vävnader och om man skördar växterna avlägsnas det lagrade kvävet permanent från systemet. Olika växtarter har olika förmåga att ta upp kväve från våtmarker och de samverkar så att det sammanlagda kväveupptaget blir större jämförd med om arterna hade vuxit enskilt[2]. Ett växtsamhälle med en varierad artsammansättning kan alltså spela en viktig roll för att förbättra vattenkvaliteten genom kväveupptag.

Mikroorganismer kan omvandla kvävet från vattnet till kväve i atmosfären

Mikroorganismer som lever på ytan av vattenväxternas rötter och skott samt i sedimenten, använder kväve för att utvinna energi för sin ämnesomsättning. De omvandlar kväveföreningar till kvävgas, en stabil form av kväve som utgör största delen av luft. Vattenväxterna ger mikroorganismerna både en yta att växa på och det kol som behövs för att kunna omvandla kväveföreningarna i vattnet till kvävgas. Hur många mikroorganismer som lever på de olika arterna av vattenväxter varierar, det är vanligen fler på mossorna än på kärlväxter[3].

Forskare visar på vikten av diversitet i vattenväxtsamhällen

Forskarna Maidul Choudhury, Frauke Ecke, Sara Hallin och Brendan McKie visade, tillsammans med andra kollegor från SLU, att kvävehalten i vattnet minskade mer när flera arter av vattenväxter, med flera olika växtformer, fick växa tillsammans. Dessutom fann de att olika arter fungerar bäst under olika delar av säsongen. Till exempel avlägsnade övervattensväxterna mest kväve i juli, medan vattenmossorna var mer effektiva i augusti. Forskargruppen visade också att växternas upptag av kväve i vävnaderna var större när vattenmossor och övervattensväxter växte tillsammans, medan kväveupptaget var mindre om två rotade arter som enbart växer under vattenytan samodlades[2].

“Vattenväxthärdigheten är viktig för kväveavlägsnande från vatten”

Försöken visade också att de bakterier som fanns på mossan hade större förmåga att omvandla kvävet än vad bakterierna på växterna med övriga växtsätt hade. Dessutom gjorde mossan så att bakterierna på rötterna av övervattensväxter blev mer aktiva[3,4]. Forskargruppen visade också att växternas olika egenskaper kan påverka både upptaget av kväve i växten och bakteriernas aktivitet[4].

I en våtmark bidrar vissa vattenlevande växter till att minska kvävehalten genom att ta upp kvävet i vävnaderna medan andra arter bidrar genom att påverka aktiviteten hos bakterierna. Genom att kombinera växter med många olika egenskaper och växtsätt får man ett verktyg för att välja arter lämpliga för kväverening[4]. Genom att upprätthålla mångfalden av växter i våtmarker kan vi minska halterna av näringsämnen i vattnet, vilket på lång sikt kommer att hjälpa oss att handskas med förorenat sötvatten.

Referenslista

[1]Camargo, J. A., & Alonso, Á. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International*, 32(6), pp. 831-849.

[2]Choudhury, M. I.; McKie, B. G.; Hallin S. & Ecke, F. (2018) Mixtures of macrophyte growth forms promote nitrogen cycling in wetlands. *Science of the Total Environment*, 635, pp. 1436–1443.

[3]Hallin, S., Hellman, M., Choudhury, M. I., & Ecke, F. (2015). Relative importance of plant uptake and plant associated denitrification for removal of nitrogen from mine drainage in sub-arctic wetlands. *Water Research*, 85, pp. 377-383.

[4]Choudhury, M. I.; Hallin S.; Hubalek, V.; Juhanson, J.; Frainer, A., McKie, B. G. & Ecke, F. (2018) Plant traits explain uptake of nitrogen and plant associated denitrification in wetlands (manuscript).

Acknowledgements

This thesis is a product of continuous support from many people during the last four years and it's quite hard for me to acknowledge them in a just few sentences.

First, I like to thanks my supervisor **Frauke Ecke** for giving me such a great opportunity to start my scientific career as a PhD student and working with macrophytes. After completing my masters' studies and few research internships, I imagined myself as a new born kid in the world of research. During the last four years, you have taught me how to crawl and helped me to stand on my feet. This will definitely help me to walk in the world of academia in future. You taught me how to be an optimist, although I always think myself as a pessimist. You showed me how to appreciate even the tiny research ideas and how to look at all small details and accuracy.

Secondly, I want to thank my co-supervisors **Brendan G. McKie** and **Sara Hallin**. You thought me how to find a bigger picture of small things, how to critically evaluate all steps of research, from the planning an experiment to publishing a manuscript.

Next I would like to thanks **LKAB**, the mining company that funded most of this research project. I think this project is an example of successful collaboration between scientists and a private company, where we have achieved lots of experiences and win-win situations. This project highlights how corporate social responsibility can help to improve scientific understanding of the environmental problems emerging from the mining industry, and how to mitigate such problems through innovation and application of basic and applied sciences.

I would also like to thank all the people who helped me in the field, with laboratory works and providing language support for this thesis: particularly, **Joel Segersten**, **Maria Hellman**, Jaanis Juhanson, **Sophia Renes, Jenny Nilsson**. Special thanks to all current and graduated PhD students and other researchers: Sophia, Hannah, Jenny, Jasmina, Bonnie, Kristina, Amélie, Didier, Oded, Alex, André, Steffi, Wiebke, Vera, Mattias, Rikard, Jakob, Mr. Minh,

Lisa, Pianpian, Emma, Claudia, Sara, Malin, Jose, Salar, Elin, Joachim ***et al.*, (2014-2018)**. Although I have not joined most of the young researcher (YR) events or frisbee or brännboll, your sporty spirits always inspired me to get more energy for work.

Thanks, also, go to some people behind the scene: **Anders Hargeby**, who made me interested in aquatic ecology; **Dr. Liesbeth Bakker**, who introduced me to the world of lovely macrophytes; **Prof. Lars-Anders Hansson**, who provided me with opportunities to gain a lot of experience with macrophytes and mesocosms experiments, as well as valuable recommendation letters that greatly helped me to get this PhD position.

Finally, I am very grateful to my family, particularly my **Mother** for her endless supports and prayer. My lovely wife, **Momtahina Rista** who has continuously been supporting me from 2012 to until now, and hopefully for the rest of my life.

Personal reflection

PhD education is like a marathon that consists of courses, scientific experiments, field work, writing and publishing the manuscripts, attending in scientific conferences, preparing the thesis and finally defending the thesis in public. I think PhD studies not only prepare a student for a future career in science, but also help to think critically and take rational decisions both in their personal and societal life.

The whole PhD education process helps a student to question the current knowledge and views of society; think about a problem out of the box; to perform assigned tasks in a systematic manner within a limited time frame; draw conclusions based on facts rather than gut feelings and to resolve conflicts that might arise in interdisciplinary research due to different views on research ethics and philosophy in different disciplines and even between the PhD student and their supervisors.

Since my PhD project was interdisciplinary, it helped me to learn new research methods and techniques from other disciplines. It also helped me to see the wider applications of my research and generate new research ideas. In the process of my graduate studies, I learned how to plan and execute tasks, both in the field or in the office, how to handle unforeseen situations and how to convince my supervisors to support my research ideas. The most enjoyable part of my PhD was science communication: communicating my research findings with the scientific society as well as stakeholder, in my case the mining company. It's quite exiting to meet the scientists whose papers I read a lot and cited in my manuscripts. It's also very rewarding when I can see that my research has a social impact. My research findings can help to mitigate a real life problem. The professional language of scientists and other parts of the society are not always the same, and this is the main obstacle for science communication. In my PhD project I learned how to translate my research findings in a way that is meaningful and useful for the stakeholder. Finally, at the end of my graduate study I realised how little I know about science and nature!!