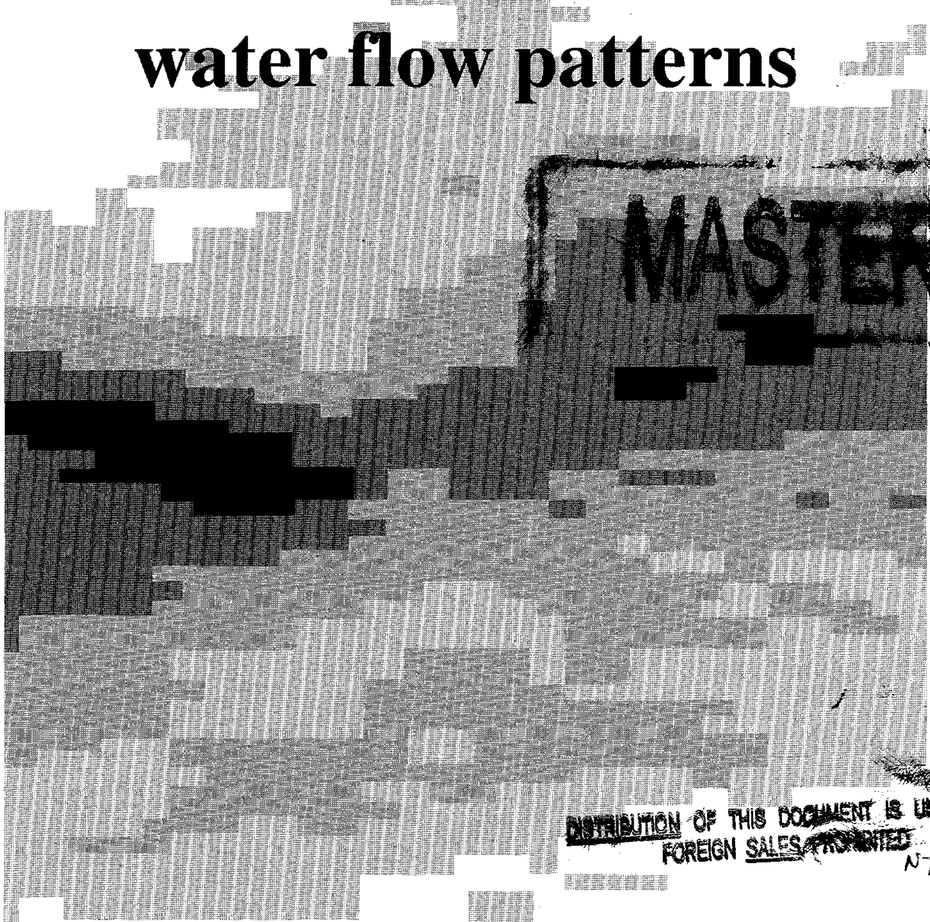
Nitrogen transformations in wetlands: Effects of water flow patterns



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Abstract

In this thesis, I have studied nitrogen turnover processes in watermeadows. A watermeadow is a wetland where water infiltrates through the soil of a grassland field. It is hypothesized that infiltration of water through the soil matrix promotes nutrient transformations compared to surface flow of water, by increasing the contact between water, nutrients, soil organic matter and bacteria. I have studied how the balance between nitrogen removal (denitrification, assimilative uptake, adsorption) and release (mineralization, desorption) processes are affected by water flow characteristics.

Mass balance studies and direct denitrification measurements at two field sites showed that, although denitrification was high, net nitrogen removal in the watermeadows was poor. This was due to release of ammonium and dissolved organic nitrogen (DON) from the soils.

In laboratory studies, using ^{15}N isotope techniques, I have shown that nitrogen turnover is considerably affected by hydrological conditions and by soil type. Infiltration increased virtually all the nitrogen processes, due to deeper penetration of nitrate and oxygen, and extended zones of turnover processes. On the contrary, soils and sediments with surface waterflow, diffusion is the main transfer mechanism. The relation between release and removal processes sometimes resulted in shifts towards net nitrogen production. This occurred in infiltration treatments when ammonium efflux was high in relation to denitrification. It was concluded that ammonium and DON was of soil origin and hence not a product of dissimilatory nitrate reduction to ammonium. Both denitrification potential and mineralization rates were higher in peaty than in sandy soil.

Vertical or horizontal subsurface flow is substantial in many wetland types, such as riparian zones, tidal salt marshes, fens, root-zone systems and watermeadows. Moreover, any environment where aquatic and terrestrial ecosystems meet, and where water level fluctuates, will possess some of the nutrient transformation features investigated in this thesis.

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Denitrification, nitrification, mineralization, nitrogen, wetland, surface flow, subsurface flow, infiltration, hydrology

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Torbjörn Davidsson

Dissertation
Lund 1997

A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part costitutes the formal thesis, which summarizes the accompanying papers. These have either already been published or are manuscript at various stages (in press, submitted or in ms).

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I Leonardson, L., Bengtsson, L., Davidsson, T., Persson, T. and Emanuelsson, U. 1994. Nitrogen retention in artificially flooded meadows. Ambio 23:332-341.

II Davidsson, T. E. and Leonardson, L. In press. Seasonal dynamics of denitrification activity in two water meadows. Hydrobiologia.

III Davidsson, T. E. and Leonardson, L. 1996. Effects of nitrate and organic carbon additions on denitrification in two artificially flooded soils. Ecol. Eng. 7:139-149.

IV Davidsson T. E., Leonardson, L. G. and Balkhag, P. In press. Small-scale variation in denitrification, nitrate, dissolved organic carbon and nitrous oxide in a flooded wetland soil. Verh. Internat. Verein. Limnol. 26:xxx-xxx

V Stepanauskas, R., Davidsson, T. E. and Leonardson, L. 1996. Nitrogen transformations in wetland soil cores measured by ^{15}N isotope pairing and dilution at four infiltration rates. Appl. Environ. Microbiol. 62:2345-2351.

VI Davidsson, T. E., Stepanauskas, R. and Leonardson, L. 1997. Vertical patterns of nitrogen transformations during infiltration in two wetland soils. Appl. Environ. Microbiol. 63:3648-3656.

VII Davidsson, T. E. Manuscript. Effects of flow direction on nitrogen transformations in two wetland soils. Submitted.

Contents

Introduction

Wetlands and hydrology

Construction of wetlands for improvement of water quality has been an expanding area of engineering and research during the last decades. Both natural and constructed wetlands have proven to efficient in treating low to heavily polluted water with respect to nitrogen, phosphorus, suspended solids and agricultural and industrial chemicals (Middlebrooks 1995, Kadlec and Ley 1994). Wetlands have been constructed for treatment of municipal wastewater (Bouwer 1985, Brix and Schierup 1989, Yamaguchi et al. 1990, Middlebrooks 1995), industrial and agricultural wastewater (White 1995, Tanner at al. 1995), urban stormwater and non point source pollution (Kadlec and Ley 1994, Raisin and Mitchell 1995, Pavel et al. 1996). Moreover, wetlands are nowadays appreciated for their ecological functions in the modern monotonous agricultural landscape.

Internationally wetlands have been used for nutrient removal for at least 30 years (Kadlec and Knight 1996). The recognition of eutrophication of coastal waters, and the identification of nitrogen as the limiting nutrient in these areas, have increased the interest for wetland re-establishment in southern Sweden during the last decade (Granéli et al. 1986, 1990, Jansson et al. 1994). Agriculture has been shown to be responsible for a substantial part of the nitrogen exported to coastal areas, and this fact has called for measures within the catchments. At present, wetlands, ponds and buffer strips are being constructed and restored in several catchments, primarily to prevent dissolved nitrogen (mainly nitrate) transported by streams in the agricultural landscape, from being exported to the sea (Jansson et al. 1994).

There are several types of wetlands and several methods of classification. According to the Ramsar definition of wetlands (Ramsar Bureau 1990), a wetland can be anything from an ephemeral elevation of the groundwater table to a shallow lake or an estuary. Ponds, buffer strips, riparian zones, watermeadows, marshes and bogs are some wetland types, and they differ with respect to soil/sediment, hydrology, plant community etc.

Nutrient transformations in wetlands are both microbially and chemically mediated and have been shown to be greatly affected by hydrological conditions (Hill 1996). These conditions determine direction and rate of water movement. Depending on water flow paths, there are four main zones where denitrification and associated microbial processes can occur (Fig. 1): 1. at the soil/sediment water interface, as e.g. in ponds, 2. in groundwater flow paths within the soil, often referred to as aquiferes (Korom 1992), 3. in microbial communities associated with plant leaves, stems and roots, and 4. in shallow groundwater in biologically active soil, with either a lateral or vertical water movement component. The first case occurs when water flows over the soil surface, often referred to as Horton overland flow (Dunne and Leopold 1978), or over the sediment surface (Fig. 1a). In soils this occurs when supply rate exceeds infiltration capacity, e.g. at prolonged heavy rain. Transformation processes of compounds in the water will depend on diffusion across the soil/sediment surface. In the second case, water moves laterally within deeper layers of the soil, e.g. due to impermeable soil overlain by more permeable layers (Fig. 1b). Nutrient turnover depends on presence of reduced and oxidized compounds leached from surface soil layers. In the third case, the microbial community is supported by organic material from plants or associated periphytic communities, and influenced by the oxygen production and consumption in these environments (Fig. 1c). Microbial activity on plant surfaces can be important in e.g. ponds

(Eriksson and Weisner 1997). The fourth case is when water infiltrates through the active soil layer, on its way towards groundwater (Fig. 1d). Nutrient transformations will occur in the soil matrix and will be supported by organic material within the soil, and oxygen and nutrients in the water.

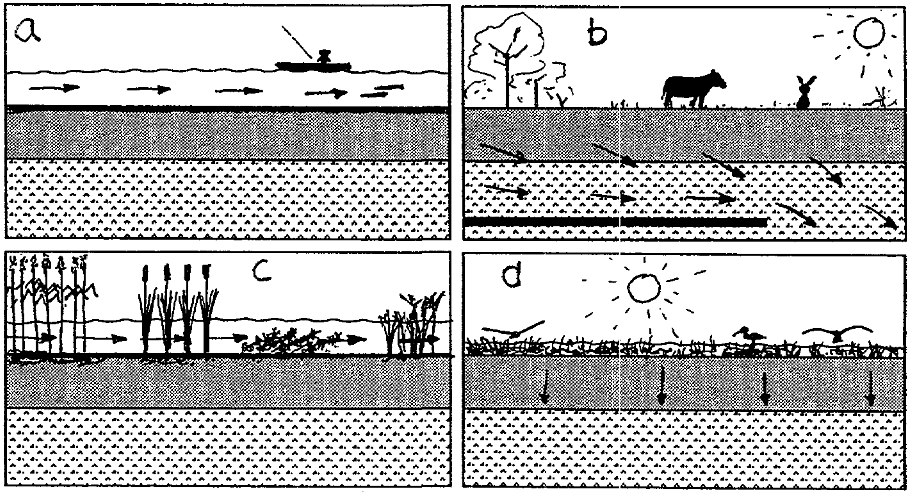

Figure 1. Four different zones where high microbial activity and nitrogen transformations can take place, depending on water flow direction and structures in the soil/water system. Arrows show water flow direction, shaded area show biological active soil/sediment, dotted area show mineral soil and black area show impermeable layer. a: impermeable soil/sediment overlain by water. b: water moving in an aquifer. c: water moving through stands of macrophytes and associated periphyton. d: water infiltrating through top soil layers.

In this thesis, I have studied a wetland type where water infiltrates through the uppermost soil layers - the flooded meadow, also called watermeadow. It is the infiltration feature that makes this wetland type very interesting, and it is hypothesized that infiltration promotes nutrient transformations by increasing the contact between water, nutrients, soil organic matter and bacteria. Subsurface flow is substantial in many wetland types, such as riparian zones, tidal salt marshes, fens, root-zone systems and watermeadows. Any soil that has high infiltration capacity, and sometimes gets waterlogged due to e.g. floods or heavy rain, will have conditions similar to those investigated in this thesis. Furthermore, any environment where aquatic and terrestrial ecosystems meet, and where water level fluctuates, will possess some of the nutrient transformation features investigated in this thesis. It has been shown that these occasionally inundated areas can make up a substantial part of a river catchment (Wetzel 1990).

Biogeochemical processes involved in wetland nitrogen removal

My main focus has been to study denitrification, since it is the main process removing nitrogen in wetlands. This is a bacterial process where nitrate is converted into nitrogen gas in a three step reduction series: $NO_3 \rightarrow NO_2 \rightarrow N_2O \rightarrow N_2$. The process occurs in soil and sediment, and is performed by heterotrophic, facultativly anaerobic bacteria (Fig. 2). These are decomposers which oxidize organic material, producing less complex compounds, CO_2 and water. In aerobic environments they use oxygen as an electron acceptor, but when oxygen is depleted, they switch to using nitrate. Nitrogen may be lost in the gaseous forms of N_2O and N_2, the latter being predominant in most anaerobic environments. Under certain conditions (e.g. intermediate O_2 or low pH), N_2O may be the terminal product released to the atmosphere (Firestone et al. 1980, Christensen et al. 1990, Davidson 1992).

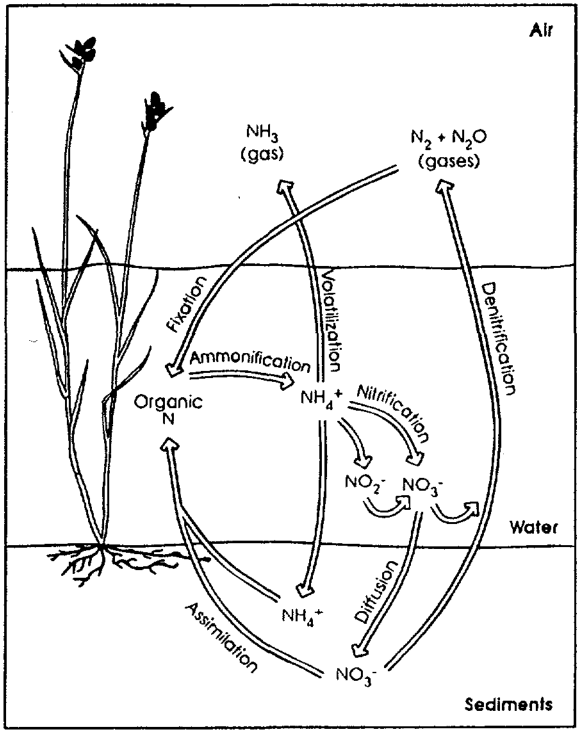

Figure 2. Simplified wetland nitrogen cycle. Redrawn from Kadlec and Knight (1996).

If ammonium is present in the environment, denitrification of nitrate produced by nitrification is an important process for wetland nitrogen removal, "coupled denitrification". Nitrification is an aerobic process carried out by chemoautotrophic bacteria, in which ammonium is oxidized to nitrate: $NH_4 \rightarrow N_2O \rightarrow NO_2 \rightarrow NO_3$ (Fig. 2). High production of N_2O during nitrification is a possible, though not well-studied, nitrogen removal process in wetlands.

Mineralization of organic material will cause a release of various fractions of nitrogen. In ammonification, ammonium is produced from particulate and dissolved organic material. (Fig. 2). The fate of ammonium depends on presence of oxygen and the cation exchange capacity (CEC) of the soil, i.e. it can be either nitrified, sorbed or exported. If exported, ammonification counteracts the removal of nitrogen. Sorption of ammonium to soil particles is therefore an important mechanism regulating net nitrogen loss from wetlands. During mineralization, dissolved organic nitrogen (DON) may be produced, consisting of amino acids, pyrimidines, purines and fulvic and humic acids (Kadlec and Knight 1996). Bioavailability of DON is an interesting issue that so far has been little studied.

Dissimilatory nitrate reduction to ammonium (DNRA) is an anaerobic microbial process where nitrate is reduced to ammonium. It is considered to produce more energy per amount of nitrate than denitrification, and occurs in strongly reduced environments (Buresh and Patrick 1978). It is considered to be an insignificant process in freshwater environments, but has not been thoroughly studied in wetlands (Tiedje et al. 1982).

Macrophyte, algal and microbial uptake of nitrate and ammonium plays an important role for short term removal of nitrogen (Fig. 2). However, to obtain a long-term net removal through such uptake, it is necessary that the biomass is harvested or accumulated in the wetland. Autotrophic organisms also play an important role in producing organic carbon, which can be used as an energy source by denitrifiers.

There are other biological, chemical and physical processes affecting nitrogen removal in wetlands that are not studied in this thesis (Fig. 2). Nitrogen fixation, i.e. assimilative uptake of nitrogen gas, followed by excretion or mineralization of the organic material is one proposed source of ammonium in wetlands (Valiela et al. 1978). Ammonia volatilisation is a chemical process where ammonia is produced from ammonium and released to the atmosphere, occurring at high temperature and pH (Kadlec and Knight 1996).

Questions

In this thesis, I compile the results from two experimental fields and laboratory studies on soil from three sites. Readers who want an overview of the field sites and some historical aspects of watermeadows should consult paper I, where some background information is provided. In paper II to VII the focus is on process rates, and the main questions are listed below. The questions have developed with time, and have been based on progressive findings.

Can watermeadows be used for nitrogen removal?
How high are the natural denitrification rates in watermeadows?
How do denitrification rates compare to mass balance estimates?
How do denitrification rates differ among sites and with season?
Which factors regulate denitrification activity?
What is the cause of, and how high is the spatial variability of denitrification?
What is the origin of exported ammonium?

What are the effects of infiltration rates on nitrogen turnover processes?
Where in soil the profile do nitrogen turnover processes occur?
How does water movement direction affect nitrogen turnover processes.

Methods to measure denitrification and other nitrogen turnover processes

Traditionally, there are three main methods to measure the magnitude of denitrification. The simplest is to measure nitrate concentration at the start and at the end of an experiment, or before and after water has passed through a defined system. The nitrate loss can in some cases be considered to represent denitrification. However, nitrate can be produced by nitrification, and consumed by uptake of plants and microbes, leading to either under- or overestimation of denitrification rates. This nitrate mass balance approach, along with mass balance of water and other nutrients is performed and described in the first papers of this thesis.

The acetylene blockage method is commonly used to measure denitrification in field experiments (Tiedje et al. 1989). It is based on the fact that acetylene C_2H_2 blocks the nitrous oxide reductase in the denitrification process, $NO_3 \rightarrow NO_2 \rightarrow N_2O \rightarrow N_2$, causing an accumulation of nitrous oxide (N_2O). This gas can easily be measured with a gas chromatograph equipped with an electron capture detector (ECD). I have used a soil core method to measure denitrification field rates, described in detail in papers II, III and IV. A combination of results from mass balance and acetylene inhibition methods gives a relatively good picture of denitrification rates and their magnitude in relation to other nitrogen processes (Papers I and II). The acetylene blockage technique has one major obstacle: acetylene also inhibits the nitrification process. If denitrification to a great extent is supported by nitrate from nitrification, the actual denitrification rate will be correspondingly underestimated. However, a high supply of external nitrate decreases the importance of denitrification coupled to nitrification. In my field experiments, nitrate concentration and supply rate were high and the uncoupled denitrification was considered to be the dominant of the two processes. Another problem connected with the use of core methods to estimate areal process rates, is that there often exists a considerable variability in nitrogen turnover rates in soils, both spatially and temporally. Hence, process estimates tend to have wide confidence intervals, and the scaling of rates from small core areas to hectares might cause under- or overestimations of actual rates.

To further investigate nitrogen turnover rates, I have used laboratory systems where water flows through soil cores (paper V, VI and VII). In these experiments, I have used a third method to study nitrogen turnover, ^{15}N isotope technique. This technique is very useful in laboratory experimental systems where inflow and outflow of water can be carefully controlled. By adding a nitrogen source containing the ^{15}N isotope, and monitoring concentrations and isotopic labelling of the nitrogen species in in- and outflowing water, rates of denitrification, nitrification, uptake and the origin of exported nitrogen can be determined. I have used 99.9% ^{15}N labelled nitrate as the only nitrogen source in inflowing water. Calculations of process rates have been made using estimates of concentrations and labelling, in a set of equations developed by Nishio et al. (1983), Nielsen (1992), Risgaard-Petersen et al. (1993) and Rysgaard et al. (1993).

Wetland sites investigated in the thesis

Vombs Ängar watermeadows. These old watermeadows were used for hay production during the second half of the 19th century up to the first half of the 20th century. The soil is sandy with an organic top layer, the present vegetation consists of grass species, and the area is now grazed by cattle. During flooding, water from the adjacent River Klingavälsån was led in through channels and distributed over the soil surface at a rate equivalent to a precipitation between 70 and 205 mm day^{-1}. Total nitrogen concentration in the river water was typically around 3 mg N l^{-1}, nitrate being the dominant fraction (ca 70%). The water moved as overland flow and was subsequently infiltrated. Ten percent of the water left the watermeadows as surface water and 90% as groundwater. Flooding periods were interrupted by drying-up periods, a procedure also used in the recent flooding experiments. The denitrification experiments were carried out during the years 1991-1993, and the area was divided into two sub areas with different flooding regimes. The Vomb West area (VW) was flooded for 11 days and drained for 3 days, and the East area (VE) was flooded for 7 days and drained for 7 days. The mass balance study was performed in 1992 by colleagues at the Department of Water Resources Engineering, Lund University. A more detailed site description is presented in paper I. This field site is investigated in papers I, II, III and IV. The soil is used in laboratory studies in papers V, VI and VII.

Isgrannatorp watermeadow. This is a recently constructed watermeadow, where water from River Vinneån was supplied by a pump and perforated hoses at a rate of between 50 and 60 mm day^{-1}, i.e. substantially lower rates than at Vombs Ängar. Here, 15% of the water left the experimental area as surface water and 85% as groundwater. Total nitrogen concentrations in river water were usually over 5 mg N l^{-1}, with nitrate concentrations above 4 mg N l^{-1}. The soil consists of marsh peat with high organic content and high hydraulic conductivity, and different grass species are the predominant vegetation. The meadow was flooded and drained in cycles of 7+7 days from September/October to May/June. The flooding experiments were conducted between 1991 and 1993 and the mass balance study was performed in 1992. Today the flooding has been ceased due to poor nitrogen removal performance. For a more detailed site description, see paper I. This field site is investigated in papers I, II and III. The soil is used in laboratory studies in paper VI.

Flyinge. Wetlands in the river valley of River Kävlingån near Flyinge are presently being restored as shallow ponds. The soil is sandy, but there are small areas of well decomposed marsh peat in the wetland. Soil collected from this construction site was used in the laboratory experiment presented in paper VII

Quantification of denitrification in field experiments (Papers I and II)

At Vombs Ängar, mass balance studies showed that net nitrogen removal was poor, and that the nitrate removal was counteracted by a considerable export of ammonium via groundwater flow. The direct measurements of denitrification, using the acetylene blockage technique, showed that the rates were higher when the soil was flooded than after draining. During draining, the rates were similar to those of a reference site with similar soil, situated near the watermeadow but where flooding was not applied. The site with the 7+7-day regime (VE) had significantly higher denitrification (ca. 50%) than the site with the 11+3-

day regime (VW). Annual denitrification rates were substantially higher than nitrate removal rates measured by mass balance methods (430-460 and 90-180 kg N ha^{-1} $year^{-1}$, respectively). One proposed explanation for this discrepancy is that nitrification of mineralized ammonium was high during drying-up periods of the sandy soil (Paper II).

At Isgrannatorp, nitrate removal efficiency was very high, but was counteracted by an equally large export of ammonium and DON. At this site denitrification estimates and nitrate removal rates agreed very well (220 and 225 kg N ha^{-1} $year^{-1}$, respectively). Contrary to Vombs Ängar, the soil at Isgrannatorp stayed wet during drying up periods, and nitrification of mineralized ammonium was probably low. Denitrification was higher during flooding than during draining, but the Isgrannatorp reference site (in the same area, similar soil, no flooding) had higher denitrification rates than the drained watermeadow. Although the soil in Isgrannatorp had higher organic content and was exposed to higher nitrate concentrations than the soil in Vombs Ängar, the denitrification activity was generally lower. Since we have shown that nitrate availability regulates denitrification, we ascribe this fact to the lower water (and thereby nitrate) load at Isgrannatorp. For each soil, there were very small differences in denitrification rates between 1991 and 1992. We have proposed two explanations for the high mineralization rates causing release of DON and ammonium: 1. mineralization was enhanced by the alternating wetting and drying regimes, and 2. when converting a terrestrial system into a wetland, mineralization can be high in an initial state due to the drastic changes in environmental conditions of a system loaded with nutrients.

Seasonal variation of denitrification reflects the variation of its regulating factors (Paper II). In both wetlands there was a positive correlation between denitrification rates and nitrate concentration, but a negative correlation between denitrification rate and temperature. Hence, we found higher denitrification rates during colder periods which is contrary to most findings reported in the literature. However, the nitrogen concentrations in streams are higher during the non-growing season in southern Sweden, due to lack of crops and the occurrence of frequent rains in combination with non-frozen ground. Hence, there is a seasonal effect impacting nitrate concentration in water, and a temperature effect influencing denitrification activity. Results from our field studies indicate that the seasonal effect is stronger. Competition for nitrate between plants and denitrification bacteria is an additional explanation for the low denitrification rates in spring.

Regulating factors and spatial variation of denitrification (Papers III and IV)

Denitrifying bacteria are heterotrophic organisms i.e. they use organic material as an energy and carbon source. Therefore, wetland nitrogen removal efficiency depends on the amount of organic carbon available in the wetland being enough to support denitrification of the nitrate load. In field experiments (Paper III), we investigated whether nitrate or organic carbon limited denitrification. We added nitrate alone, organic carbon alone and a combination of both, to soil cores taken at Vombs Ängar and Isgrannatorp. In the majority of cases nitrate stimulated denitrification, whereas organic carbon seldom had such effect. This indicates that there is an excess of bioavailable organic carbon in the watermeadow soils investigated here. A simple calculation based on stoichiometry reveals that to denitrify 500 kg N ha^{-1} $year^{-1}$, it takes a net production of 536 kg C ha^{-1} $year^{-1}$, which can be compared to an estimated average primary production in wetlands of 11250 kg C ha^{-1} $year^{-1}$ (Mitsch 1994). Moreover, aquatic systems generally have high allochthonous inputs that can support heterotrophic organisms with organic carbon (Wetzel 1983), and in many wetlands there is also a large pool of accumulated organic material. In our study, enhancement of denitrification due to nitrate additions was most pronounced during spring. We suggest that this was due to either competition for nitrate between plants and denitrifiers, or the generally lower nitrate concentrations in floodwater during spring, both causing a deficiency of NO_3 for the denitrifying bacteria. Other studies have shown that organic carbon often is in excess in wetlands, and that nitrate concentration is the main regulating parameter for denitrification (Westerman and Ahring 1987, Merrill and Zak 1992). This is contrary to the situation in drier soils, where denitrification often takes place in hot-spots with accumulated organic material (Parkin 1987, Christensen et al. 1990). It has been shown, however, that it is the anaerobiosis, driven by oxygen consumption in these hot spots, that enhances denitrification. In paper IV, we investigated small scale spatial patterns in denitrification and it's proposed regulating factors. It was shown that denitrification and pore water nitrate concentration patterns were fairly well correlated, whereas denitrification and pore water dissolved organic carbon (DOC) concentration were not. Furthermore, in two of the three (two 1x1 m and one 0.7x2 m) areas that were investigated, nitrate concentration and denitrification were higher than in the third area, whereas DOC was similar. This is another indication nitrate and not organic carbon regulated denitrification in this soil. Moreover, on molar basis, DOC concentrations were 20-160 times higher than nitrate concentrations, which means that even if only a small proportion of the dissolved organic carbon had been available to denitrifiers, this would have been enough to fuel denitrification of the nitrate present.

Role of water infiltration rates on nitrogen transformations (Paper V)

In order to further investigate the nitrogen turnover phenomena observed in the field experiments (Papers I and II), we designed a laboratory set-up, where ^{15}N-nitrate enriched water infiltrated through a soil matrix, held in gastight plastic tubes. In paper V, we investigated the effect on nitrogen transformations of four different infiltration rates, ranging from 72 to 638 mm day^{-1}, in homogenized soil from Vombs Ängar. Moreover, we compared the results from the field studies with those found for the corresponding infiltration rate in laboratory. It was concluded that higher infiltration rates caused higher mineralization and nitrification, causing an efflux of oxidized compounds. This was obviously an effect of high amounts of oxygen transported into the soil via the infiltrating water. Lower infiltration rates caused efflux of reduced compounds, i.e. ammonium export, but a larger fraction of the supplied nitrate was denitrified (97%). The rates of denitrification, however, did not differ between the three slowest infiltration rates, whereas the activity was lower in the treatment with highest infiltration rate. In the first case, the inhibiting effect of increased oxygen import with increasing flow rate was prabably counteracted by a stimulating effect of the increased import of nitrate. In the latter case, denitrification was probably inhibited by oxygen transported with water through the entire core. At one intermediate flow rate, nitrous oxide concentration markedly increased in the outflow water. This is thought to be a result of either an inhibition of nitrous oxide reductase due to presence of oxygen, or disturbances in the nitrification process, occurring in soil layers near the outlet. Net nitrogen removal was little affected by water flow rate, and denitrification losses were counteracted by release of reduced nitrogen, i.e ammonium and DON. Ammonification increased with increasing flow rate but a higher proportion of the ammonium was nitrified and net ammonium production decreased with higher flow rates. Low ^{15}N enrichment in effluent ammonium and DON excluded the importance of DNRA. It was also shown that the DON efflux increased with increasing infiltration rates. Simultaneously, the C/N ratio of dissolved organic matter decreased and the adsorbance ratio A_{250}/A_{365} increased, indicating a higher bioavailability of the dissolved organic matter (DeHaan 1972). The two lowest infiltration rates in the laboratory experiment corresponded to field infiltration rates between 70 and 205 mm day^{-1} (Paper I). Despite the large differences in conditions between laboratory and field experiments (absence/presence of plants, soil homogeneity/heterogeneity, constant/variable temperature etc.), the results agreed well (Paper V: Table 1).

Nitrogen transformation rate profiles in flooded soil (Paper VI)

In wetlands where water flows over a sediment/soil surface, the transfer of nitrogen species from water to soil and vice versa occurs mainly by diffusion (Patrick and Reddy 1976, Reddy et al. 1978). Nitrate in water diffuses into the soil, where denitrification is supported by soil organic carbon, and the nitrogen gas produced, diffuses back to the water. Ammonium released from the waterlogged soil diffuses across the oxygenated water soil interface, where it can be oxidized to nitrate by nitrification bacteria. This nitrate can either diffuse back into the soil and be denitrified, or diffuse up to the water. Good examples of concentration profiles and transformation rate profiles in sediments have been presented by Christensen et al. (1989). In infiltration soils however, water enters the soil by mass flow. In paper VI, we have investigated profiles of nitrogen concentration and process rates in 10-40 cm tall cores containing one peaty soil (Isgrannatorp) and one sandy soil (Vombs Ängar). Infiltration resulted in more extended concentration profiles than reported from studies where diffusion is the main transfer mechanism (Fig. 3). We found a substantial efflux of unlabelled ammonium from the soil, indicating that it was produced either by mineralization, nitrogen fixation or desorption, and not from DNRA. In the peaty soil the DON concentration increased with soil depth, whereas ammonium and DON concentrations in the sandy soil tended to level out after 10-20 cm. Contrary to diffusion-driven ammonium efflux from a sediment with an oxygenated surface layer, the ammonium produced during infiltration was released directly from a reduced soil layer, where it was not subjected to nitrification. The peaty soil had higher rates of both N-mineralization and denitrification than the sandy soil, but the balance between the processes resulted in the sandy soil being a sink for nitrogen, whereas the peaty soil was a source. In the sandy soil, net nitrogen removal occurred in cores 14 cm and longer, and in the longest core 40% was removed. In the peaty soil, release was equal to removal in the top 14 cm, but in the deeper layers release exceeded removal, leading to a 100% increase of total nitrogen in the effluent water from the longest core (38 cm). Net production of intermediates in the denitrification and nitrification processes (nitrite and nitrous oxide) corresponded to zones of low oxygen concentration, and accumulated at intermediate depths (5-15) cm. However, they were reduced further down, and efflux concentrations were low. As in paper V, the comparison between laboratory and field data revealed several similarities.

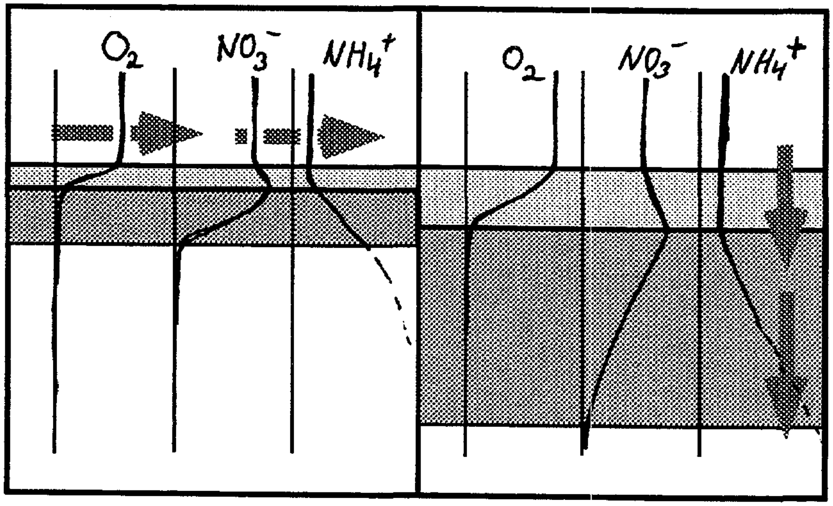

Figure 3. Schematic profiles of oxygen nitrate and ammonium in infiltration and surface flow wetlands. Shaded area represent the zone of high nutrient turnover, and arrows represent water flow direction.

Effects of water movement direction on nitrogen transformations in flooded soil (Paper VII)

In paper VII, I further investigate the effect of water flow direction on nitrogen transformation processes. In laboratory experiments with two similar wetland soils (Flyinge and Vombs Ängar), water was made to flow either vertically through the soil matrix or horizontally over the surface. It was very clearly shown that infiltration of water increased the nitrogen turnover processes. Differences in ammonium production between the treatments were especially pronounced. The fact that ammonium had to diffuse axross an oxygenated sediment layer in the surface flow treatment, but was released from reduced soil layers in the surface flow treatment can explaine these large differences in net ammonium production, also discussed above. Despite the similarity of the two soils with respect to organic and nitrogen content, they behaved differently with respect to net nitrogen removal, again a consequence of the balance between release and removal processes. There was net nitrogen consumption in both treatments of Flyinge soil and in the surface flow treatment of Vomb soil. However, the Vomb soil infiltration treatment resulted in net nitrogen production, caused by high ammonium and DON production relative to denitrification. Differences in texture of the organic material in the soils was a probable cause of the differences in turnover rates. During sampling and preparation of the soil we observed a high degree of peat decomposition in Flyinge, and this might have resulted in a higher surface to volume ratio of the organic material. This prabably caused both the low hydraulic conductivity and the higher process rates in this soil. Production and consumption of intermediates in the denitrification and nitrification processes (NO_2 and N_2O) were highly variable. Nitrite production did not follow the general pattern of higher rates in infiltration treatment. This can be explained by the fact that the nitrous oxide and nitrite reduction enzymes have been shown to be sensitive to oxygen (Firestone et al. 1980). I compared field results from Vombs Ängar (Paper I) with the results obtained for the Vomb soil in this study (Paper VII: Fig. 3). It was shown that field observations of nitrogen in surface and groundwater outflow agreed fairly well to with the results from the laboratory. In both studies nitrate removal was higher in the infiltration treatment, whereas ammonium was either slowly produced (laboratory) or consumed (field) in the surface flow treatment. It was concluded that, compared to surface flow, infiltration promotes contact between soil organic matter, water, nutrients and bacteria, causing high nutrient transformation rates including both release and removal processes.

Summary and applied aspects on nitrogen performance in infiltration wetlands

In my studies infiltration of water through the soil in field and laboratory experiments led to high nitrogen turnover rates. I conclude that this was due to large contact areas between soil organic matter, water, nutrients and bacteria. Infiltration of nitrate rich water brings electron acceptors, both oxygen and nitrate, into the soil, which increases denitrification, organic carbon utilization and nitrogen mineralization in deeper soil layers. The oxidizing capacity of water containing high concentrations of nitrate is high. Oxygen respiration of e.g. glucose, based on one litre 100% oxygen saturated water at 15°C can produce 0.315 mmoles of CO_2. The same amount of carbon dioxide can be produced from 0.252 mmoles of nitrate, i.e. 3.53 mg NO_3-N in one litre. This indicates that the oxidizing capacity of water is substantially increased in agricultural streams. Contrary to oxygen, nitrate solubility is not temperature sensitive.

Both in the field and in the laboratory, ammonium and DON production were higher than usually reported for wetlands (c.f. paper VII). The ammonium produced can have different fates. If oxygen still is present in the soil profile, the ammonium produced during mineralization can be nitrified. However, mineralization in an anoxic environment in the soil results in net production of ammonium, which can have two fates in an infiltration water flow, either export to groundwater or adsorption to soil particles. The C/N ratio of the soil is important for the balance between denitrification and nitrogen mineralization rates. Stoichiometrically, organic matter oxidation using oxygen can be described by the simplified formula based on the Redfield C/N/P molar ratio of 106/16/1 (Kelly et al. 1982):

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 138O_2 \rightarrow 106CO_2 + 16HNO_3 + H_3PO_4 + 122H_2O$$

In this reaction, 1 mole of C in organic matter plus 1.3 mole of O_2, produces 1 mole of CO_2 and 0.15 mole of NO_3. It is assumed that nitrogen is produced as ammonium, and that the ammonium is nitrified. However, this calculation does not take into consideration the fact that mineralization produces other forms of N (dissolved and particulate organic nitrogen). During denitrification, when nitrate is used as an electron acceptor, nitrogen will be released as ammonium:

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 84.8NO_3 \rightarrow 106CO_2 + 16NH_3 + H_3PO_4 + 148.4H_2O$$

In this reaction, 1 mole of C in organic matter plus 0.8 mole of NO_3, produces 1 mole of CO_2 and 0.15 mole of NH_4. Assuming the Redfield C/N ratio of 6.6/1, mineralization using nitrate as electron acceptor would cause ammonium release corresponding to about 20% of the reduced nitrate. D'Angelo and Reddy (1994) reported a C/N/P ratio of 190/14/1 in a wetland floc sediment, and soil from Vombs Ängar had a C/N ratio of 9.8/1 (Paper V), values which would result in lower figures for nitrogen mineralization. Since there is a considerable net export of nitrogen from our systems, it is implied that they are not in balance with respect to nitrogen. Net ammonium and DON production at Isgrannatorp amounted to 280 kg ha^{-1} $year^{-1}$ and at Vomb to 90 kg ha^{-1} $year^{-1}$. There was also a considerable amount of nitrate produced in Vombs Ängar, as discussed above. Compared to areal nitrogen losses from farmland of 5 - 60 kg ha^{-1} $year^{-1}$, depending on crop, soil composition, fertilization and precipitation (Monitor 1983), these figures, and the figures obtained in the laboratory experiments, are remarkably high. Hence, it is reasonable to question if the release rates are sustainable. For how long time can nitrogen release from the soils used in our experiments proceed? If we assume that release rate is constant, the atmospheric input of nitrogen is negligable, and the amount of nitrogen in the soil corresponds to a 50 cm thick layer, with a nitrogen content equal to that in our laboratory experiments, we can make some speculations. Comparison of the amount of nitrogen present in Vomb and Isgrannatorp soil cores used in our laboratory experiments with the amount leached in the effluent during steady state, suggests that it would take between 3 and 10 years to deplete the soil nitrogen pool (Papers V, VI and VII). The same calculation for the field studies reveals that it takes considerably longer. Only considering ammonium and DON release, it would take one to two hundred years to deplete the nitrogen pool both in Vomb and Isgrannatorp. If the calculations also included in that some of the ammonium was nitrified and some was taken up by plants and harvested, the depletion time would be 30 to 40 years for Vombs Ängar, and still over 100 years for the peaty soil in Isgrannatorp. Based on these calculations, it is reasonable to consider the laboratory and field systems as non-steady-state systems, where easily degradable compounds have accumulated. I draw the conclusion that these disturbed systems, i.e. wetlands converted from agricultural land, can export considerable amounts of dissolved nitrogen initially. In the long run, however, the flux of nitrogen will decrease and approach a steady state condition, where nitrogen removal is higher than nitrogen release. In this perspective, construction of wetlands with infiltration or subsurface water flow might be favourable for nitrogen removal. Moreover, locating wetlands to sites with high C/N ratios in the soil organic matter would decrease the risks for export of ammonium and DON.

The quality and amount of organic material present, produced and imported to the wetland is crucial for the wetland's performance. Structure, porosity, water-holding capacity, degree of compostion and C/N ratio are some important soil properties that affect nutrient turnover. Moreover, there are probably high spatial and seasonal variations in build-up, degradation and quality of the organic material. Even in our controlled laboratory studies, we observed differing nitrogen performance of Vomb soil. In paper VI, the soil cores 14 cm and longer showed net nitrogen removal, whereas the 13 cm soil cores in paper VII showed net production.

My studies suggest that bacterial activity in waterlogged soil is limited by the amount of electron acceptors and not by reduced compounds. It is apparent that the nitrate concentration in water is an important factor for denitrification and nitrogen removal rates in both soils. The peaty soil at Isgrannatorp was highly efficient in nitrate removal, and this soil probably had a higher denitrification capacity than corresponded to the nitrate supply rates applied, both in the field and laboratory experiments. Paper IV also shows that nitrate disappeared quickly in the Isgrannatorp soil profile. Higher nitrate concentration in the supplied water or higher nitrate load would most likely increase denitrification rates, and would probably cause the system to shift towards a net nitrogen removal. Hence, if a wetland of a given size should have optimum nutrient removal it should be located to sites where nitrogen concentration in input water is high. This would allow for a high nutrient load relative to water load, which means a longer time of contact between nitrate, soil organic matter and bacteria. To increase nitrogen removal by increasing the load of water would only be benficial to a limited extent. In the wetland type investigated in this thesis, increased water load seemed to promote outflush of soil nitrogen, and denitrification would increase only where the soil has a much higher denitrifying potential. The latter might have been the case at Isgrannatorp peaty soil, where in essence all nitrate was removed (Paper I). However, increasing the water load at Vombs Ängar would probably not increase nitrate removal, and removal effiency (as percent of nitrate load) would certainly decrease.

DNRA has not been shown to be an important process in the watermeadows. Other studies have suggested that highly reduced conditions are required for DNRA to occur, e.g. deep organic sediment layers or glucose amended soil (Buresh and Patrick 1978, Tiedje et al. 1982). With the successive removal of nitrate in an increasingly reduced soil profile, the quantitative significance of DNRA is probably minor. Moreover, the exported ammonium in my experiments was only to a very small extent ^{15}N-labelled (Papers V and VI). This labelling could have been the result of direct transformation of ^{15}N-labelled nitrate to ammonium, but it might as well be due to other turnover processes, e.g. ^{15}N-nitrate assimilation followed by excretion or decomposition, as discussed in paper VI.

Intermediates in the nitrification and denitrification processes (NO_2 and N_2O) have been given attention due to their environmental impacts. Nitrite in small quantities is poisonous for infants, and nitrous oxide is involved in destruction of the ozone layer, and is also a greenhouse gas (Garrett 1991). Field measurements of nitrous oxide production in the watermeadows have shown that flooding did not increase the rates (Davidsson and Leonardson 1997). On the contrary, nitrous oxide was sometimes produced when the soil was drained and also at the reference sites. However, we only measured the production of the gas within the soil, and not the emissions. It is likely that some of the produced nitrous oxide was consumed in other microsites within the soil, without being released to the atmosphere. We concluded that flooding and draining i.e. alternating aerobic and anaerobic conditions promoted N_2O production. Results from the laboratory studies of this thesis confirm the finding that production of denitrification intermediates is favoured by intermediate oxygen concentrations. However, as is shown in paper VI, nitrate and nitrous oxide produced in some soil layers may be consumed in others, and in the field, they might never be exported from the system.

Proposed explanation for the poor nitrogen removal at Vombs Ängar and Isgrannatorp watermeadows

Although it was not the major aim of this thesis, I will propose some explanations for the poor nitrogen removal in the watermeadows at Vombs Ängar and Isgrannatorp.

Vombs Ängar

Excessive hydraulic load causing too short retention time.

The nitrate ions are transported through the soil too quickly to be denitrified.

Excessive hydraulic load promoting outflush of nitrogen from soil solution.

The dissolved compounds in the soil solution are constantly being washed out. Equilibria between ions adsorbed to particles is never reached, and desorption processes might increase.

Enhanced mineralization due to alternating flooding and draining.

Intermittent aerobic and anaerobic, and alternating wet and dry conditions have shown to increase the overall bacterial activity (Sørensen 1974).

Initial release effects due to conversion of a terrestrial system into a wetland.

If an agricultural soil loaded with nutrients from fertilization and atmospheric inputs is converted into an infiltration wetland, some of its pool of nutrients might initially be flushed out.

Too low nitrate concentration in the flood water.

Since nitrate has been shown to be an important regulator of denitrification, a higher nitrate concentration would increase the overall denitrification activity.

Isgrannatorp

Suboptimal hydraulic load causing insufficient nitrate to be infiltrated into the soil.

Since this soil had the potential to denitrify all the nitrate that was supplied, a higher load (of water as well as nitrate) would have increased the denitrification activity, and changed the balance towards a net nitrogen removing state.

Enhanced mineralization due to alternating flooding and draining.

Intermittent aerobic and anaerobic, i.e. alternating wet and dry conditions have shown to increase the overall bacterial activity.

Initial release effects due to conversion of a terrestrial system into a wetland.

If an agricultural soil loaded with nutrients from fertilization and atmospheric inputs, is converted into an infiltration wetland, some of this pool of nutrients might initially be flushed out.

Excessive and too nutrient rich organic material in the soil.

The organic peat, drained for several years, has subsided, and may have a high accumulation of nutrients in a state where degradation and leaking may be promoted by waterlogging.

Implication for choosing sites and design for wetlands

-It is beneficial to construct wetlands at sites where nitrate concentrations are high.

-Water infiltrating, or moving as shallow subsurface flow promotes high microbial process rates, which can cause either net removal or net release of nitrogen.

-Transforming agricultural land into wetlands may initially cause a net export of nitrogen, due to the drastic changes in environmental conditions of a system loaded with nutrients.

-Peat soils have a large pool of organic material that can support denitrifying bacteria, but can also export large quantities of of nitrogen.

-Soils with high C/N ratio produce less nitrogen during mineralization than soils with low C/N ratio.

In all cases, it would be advantageous to make investigations of the soil properties before constructing a wetland. Organic content and C/N ratio measurements and simple leaking experiments may add valuable information of the substrate's nirogen removal fuctions.

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Sammanfattning

Våtmarker är en naturtyp som man finner i övergångszoner mellan land och vatten. Idag talas det mycket om våtmarkers positiva natur- och miljövärden i avrinningsområden i det moderna jordbrukslandskapet. Våtmarker ger omväxling i landskapet och är viktiga biotoper för växter och djur. I våtmarker ansamlas mycket dött växtmaterial och tillsammans med en god tillgång på vatten bildas betingelser för en hög mikrobiologisk aktivitet. Mikroorganismerna är viktiga komponenter i näringsväven i ett ekosystem och de är bl a inblandade i nedbrytning av organiskt material. I en bakteriell process som kallas denitrifikation övergår nitrat (NO_3^-) till kvävgas (N_2) som är den största beståndsdelen i vanlig luft, och denna process kan vara omfattande i just våtmarker (se Fig. 2, sid. 7). Nitrat är ett växtnäringsämne innehållande kväve som skapat problem med övergödning i vattnet i havsområden runt Sverige. Problemet är att kväve, som man tillför för att öka tillväxten på jordbruksmark, läcker ut i vattendrag och transporteras till havet. Genom att anlägga våtmarker i vattendragets avrinningsområde, tror man sig kunna minska kvävetransporten till havet. I en våtmark blir det ofta syrebrist i botten (som kan bestå av jord eller sediment). När det inträffar börjar bakterierna att respirera med hjälp av nitrat istället för med syre, d.v.s. denitrifiera, och detta är den viktigaste kväveborttagande processen i våtmarker. Man kan säga att våtmarken tack vare denitrifikation fungerar som ett kvävereningsverk. Det finns dock flera viktiga kväveomvandlingsprocesser som sker samtidigt. Vid nitrifikation, som är en process som sker i syrerika miljöer, övergår ammonium (NH_4^+) till nitrat (Fig. 2). Vid mineralizering bryts det organiska materialet i marken ned, varvid löst organiskt kväve och ammonium bildas. Då jonformiga kväveföreningar både kan produceras och konsumeras i en våtmark, är balansen mellan dessa båda processer av stor vikt för nettoreningsresultatet. I min doktorsavhandling har jag studerat våtmarker där vatten rör sig genom ytliga jordlager, och då främst en speciell typ kallad översilningsmarker. Jag har antagit att vattenrörelser genom jord gynnar hög mikrobiell aktivitet jämfört med om vattnet står stilla eller rör sig ovanför jord- eller sedimentytan. Orsaken är att kontaktytan mellan vatten, jord, näringsämnen och bakterier blir större. Det finns våtmarker där infiltration dominerar, t.ex. översilningsmarker, och våtmarker där ytflöde dominerar, t. ex. dammar, men ofta finns i en våtmark båda typer av flöden. Under tre års tid har jag har med en speciell metod (acetyleninhiberingsmetoden) mätt denitrifikationsaktiviteten i två översilningsmarker - Vombs Ängar, i Lunds Kommun och Isgrannatorp, i Kristianstad kommun. De uppmätta värdena har jag sedan jämfört med massbalanssiffror. Vid massbalansmätningar uppskattar man processerna i marken genom att analysera kväveföreningarna i in- och utgående vatten. Det har visat sig att trots att denitrifikationshastigheten har varit hög har våtmarkerna fungerat dåligt som kvävereningsverk. Detta har berott på att det har frigjorts andra

kväveföreningar i marken, nämligen löst organiskt kväve och ammonium. Dessa ämnen har transporterats till grundvattnet och nått vattendraget som ligger i anslutning till våtmarken. I båda översilningsmarkerna har ungefär lika mycket kväve bildats och tagits bort. I tre lab-experiment har jag undersökt vad som styr balansen mellan dessa processer. Jag har funnit att vattenrörelser genom den aktiva ytjorden ökar såväl denitrifikationshastighet som kvävemineralisering (produktion av ammonium och löst organiskt kväve) jämfört med om vatten rör sig ovanför jord/sedimentytan. Även infiltrationshastigheten påverkar vilka kväveprocesser som sker, men vad som är bra ur kvävereningssynpunkt är inte uppenbart. Ett försök visade att vid låga infiltrationshastigheter denitrifierades en större andel av nitratet men mycket ammonium läckte ut. Vid höga infiltrationshastigheter var skillnaden mellan ut och ingående vatten väldigt liten. Det är också troligt att vattenregimen som tillämpades på översilningsmarkerna med alternerande översvämning och upptorkning leder till ökad mineralisering och frisläppande av jordens kväve. Det är tydligt att flera av de hydrologiska parametrarna (vattenrörelser, infiltrationshastighet, vattenregimer) samtidigt påverkar både denitrifikation och produktion av kväve, och det är med dessa resultat svårt att ge några råd om anläggning av översilningsmarker eller andra typer av infiltrationsvåtmarker. Det står dock klart att en högre koncentration av nitrat i vattnet medför högre dentrifikationsaktivitet och högre reningseffektivitet för våtmarken. En hög nitratkoncentration möjliggör en hög nitratbelastning och samtidigt en låg vattenbelastning och därmed en längre uppehållstid för nitratjonerna i våtmarken. Om man räknar på kvävebalansen, dvs jämför hur mycket kväve som finns lagrat i marken med vad som frisläpps och tillförs, kommer man fram till att en nyanlagd våtmark inte kan läcka kväve i det långa loppet. Förmodligen är det läckage av ammonium och löst organiskt kväve vi har observerat i Vombs Ängar och Isgrannatorp ett initialt fenomen som uppstår när man omställer jordbruksmark som har varit ett terrestert system i många år, till en våtmark som belastas av stora mängder vatten. Stora förändringar i miljöbetingelser brukar generellt leda till störd näringsbalans i ett ekosystem. I detta perspektiv kan fastslås att det är viktigt att bevara de våtmarker som existerar idag och som är i balans med avseende på kväve.

Föreslagna orsaker till det dåliga kvävereningsresultat i översilningsmarkerna

Vombs Ängar

- För hög hydraulisk belastning vilket orsakade en för kort uppehållstid för vattnet och nitratjonerna i vattnet (här denitrifierades inte allt nitrat och vattentillförseln var hög).
- För hög hydraulisk belastning vilket medförde en utspolning av kväve från markens porvatten.
- Ökad nedbrytning av organiskt material p.g.a. alternerande översilning och upptorkning.
- Initial störningseffekt p.g.a. att man omför terrester mark med stort näringsförråd till en våtmark.
- För låg nitrathalt i vattnet.

Isgrannatorp

- För låg hydraulisk belastning medförde att för lite nitrat tillförs våtmarken (här försvann allt nitrat och vattentillförseln var låg).
- Ökad nedbrytning av organiskt material p.g.a. alternerande översilning och upptorkning.
- Initial störningseffekt p.g.a. att man omför terrester mark med stort näringsförråd till en våtmark.
- För stor och för näringsrik pool av organiskt material i jorden.

Vad man bör tänka på vid anläggande av våtmark

- Anläggning en våtmarker där nitratkoncentrationen är hög rekommenderas
- Vatten som infiltrerar eller rör sig som genom biologiskt aktivt jord gynnar hög mikrobiell aktivitet, vilket kan innebära både för och nackdelar ur ett kväveperspektiv
- Att omställa jordbruksmark till våtmarker kan initialt medföra en utlakning av kväveföreningar, därför att man inför en störning av ett system som under lång tid tillförts näringsämnen.
- Att anlägga våtmarker på jord som innehåller mycket torv kan medföra utlakning av kväve men också hög denitrifikationsaktivitet
- När den initiala urlakningseffekten avtagit torde infiltrationsvåtmarker vara effektiva ur kvävereningssynpunkt