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Nitrogen Retention in Vegetation Filters of Short-Rotation Willow Coppice

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SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract
Irrigation of short-rotation willow coppice (SRWC) is a potentially efficient way of treating various types of wastewaters. In this thesis the nitrogen retention capacity in such cropping systems (vegetation filters) is assessed both within season and for several years and rotations. In addition, the retention and potential leaching of viruses in such systems are assessed. The experimental work was carried out in two types of lysimeters and in experimental fields.

Nitrogen leaching loads from wastewater irrigated willow vegetation filters can be high or very high during the establishment phase (i.e. the year of planting), and thus, during establishment, neither wastewater nor commercial fertilizers should be applied to the crop. However, once established, nitrogen leaching loads from willow vegetation filters are low or very low, enabling high inputs of nitrogen-rich wastewater. Within reasonable limits, nitrogen leaching loads are independent on irrigation rates and thus dosing of wastewater should be based of nitrogen loads. The nitrogen retention in a willow vegetation filter (up to in the order of 200 kg N/ha·yr) is due to plant uptake and incorporation into woody tissue (including harvestable shoots), and to a build-up of the pool of soil organic matter. In addition, gaseous nitrogen losses (primarily due to denitrification) are probably substantial.

Preferential flow of water in cracks and fissures can facilitate a rapid transport of viruses applied to a structured clay soil, and within a few hours viruses might reach the groundwater. However, in a sandy, non-structured soil, viruses are efficiently retained in the soil mainly as a result of strong electrostatic interaction between viruses and soil colloids.

Keywords: willow, *Salix viminalis*, coppice, nitrate leaching, nitrogen retention, groundwater quality, lysimeter, virus, bacteriophages.

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Contents

Appendix

Papers I-IV

This thesis is based mainly on the following papers, which are referred to by their Roman numerals:

I Aronsson, P.G., Bergström, L.F. & Elowsson, S.N.E. 2000. Long-term influence of intensively cultured short-rotation willow coppice on nitrogen concentrations in groundwater. *Journal of Environmental Management*; 58:135-145.

II Aronsson, P.G. & Bergström, L.F. XXXX. Nitrate leaching from lysimeter-grown short-rotation willow coppice in relation to N-application, irrigation and soil type. (Submitted to *Biomass and Bioenergy*).

III Aronsson, P.G. XXXX. Dynamics of nitrate leaching and ^{15}N turnover in intensively fertilized and irrigated basket willow grown in lysimeters. (Submitted to *Biomass and Bioenergy*).

IV Carlander, A., Aronsson, P., Allestam, G., Stenström, T.A. & Perttu, K. 2000. Transport and retention of bacteriophages in two types of willow-cropped lysimeters. *J. Environ. Sci. Health*, 35(8): 1477 - 1492 (In Press).

Introduction

Background

Wastewaters from households and industries and leachate water from landfills and other polluted sites pose substantial environmental problems worldwide. When released into watercourses, and eventually to the sea, phosphorus and nitrogen contribute to an enhanced growth of plants, algae and bacteria, which may negatively affect flora and fauna composition and result in oxygen deficiency during decomposition. Bacteria, viruses, and other pathogens, as well as toxins produced during bacterial growth, may also lead to deterioration of both drinking water and recreational waters. In addition, decomposition of the organic matter and nitrification of the ammonium nitrogen in wastewaters directly consume oxygen, and the nitrification is an acidifying process. These problems have been recognized for decades and have been partly solved by the use of wastewater treatment plants, or by using more "natural" treatment systems such as constructed wetlands or lagoons (Wittgren & Hasselgren, 1992; Finnson et al., 1994; Obarska-Pempkowiak, 1994; Vymazal, 1996). In the Baltic region, improved wastewater treatment has been on the political agenda for more than 10 years as a means of improving the water quality of the Baltic Sea (Anon, 1997). In this context, nitrogen has been the element of most concern (Enell & Fejes, 1995), although it has been debated to what extent anthropogenic nitrogen contributes to the growth of algae in the Baltic Sea (Stal et al., 1999; Bianchi et al., 2000). As a result, Swedish wastewater treatment plants have encountered new and more rigorous demands for nitrogen removal efficiency (Anon, 1988; 1991; 1997). The conventional way of achieving this is to enhance the denitrification in the treatment plants, which usually requires substantial investments (Balmér & Mattsson, 1993).

Parallel to the efforts for improving the wastewater treatment, the need for utilizing and recycling of the plant nutrients in the wastewater has been recognized. The use of sludge and wastewater for fertilization or irrigation of crops is by no means new. It has been in practice in many parts of the world for a long time (Rose, 1986; Bartone, 1991). In Poland, for example, large plantations of poplars and willows have been used as recipients for wastewater since the 19th Century (Kowalik & Randerson, 1994). Wastewater irrigation of both arable land and forests has also been in practice for decades in USA (Crites, 1984), where substantial research efforts have been put into evaluating its possible environmental impact (e.g. Iskandar, 1981; Crites, 1984). In Sweden, wastewater irrigation of arable crops (using wastewater stored in lagoons for hygienization) has also been in practice for some time (Wittgren & Hasselgren, 1992; Stenström, 1996; Geber, 2000), but during recent decades, the focus has been on a new crop that is seemingly well suited for disposal and recycling of wastewater; i.e. fast-growing short-rotation willow coppice (SRWC) grown for energy purposes.

In Sweden, SRWC is a commercial crop grown on approximately 15 000 ha of farmland. When establishing a SRWC crop, each hectare is planted with approximately 15 000-18 000 cuttings of *Salix viminalis*, *S. dasyclados*, or *S. viminalis x schwerinii* in a double row system. Farmers are advised to fertilize the crop at rates corresponding to, on average, 80 kg N/ha·yr in order to enhance growth and profitability (Ledin et al., 1994), and every three to five years, during wintertime, the crop is harvested. The plants resprout after harvest, and the economic life span of a plantation is estimated to be around 25 years.

The use of SRWC for wastewater treatment is potentially profitable from an economic point of view (Rosenqvist et al., 1997), but the profitability is very sensitive to the nitrogen treatment efficiency of the system, which affects the area requirement. Another problem is that little is known about the nitrogen turnover, in and leaching from, intensively irrigated and fertilized SRWC, and the nitrogen treatment efficiency under various conditions has not been possible to predict. From the point of view of society, the potential environmental hazards of new treatment concepts must also be evaluated. Contamination of groundwater by nitrate or pathogens is one such hazard. Domestic wastewater always contains viruses and other pathogens and during irrigation these might rapidly percolate through the soil and reach the saturated zone. In this context, viruses are of special interest due to the potentially extremely high numbers present in wastewater (Rose, 1986; Stenström, 1996), the rapid transport in soil and in the saturated zone (Schaub & Sorber, 1977; Yates & Yates, 1988; McKay et al., 1993*a*; Sinton et al., 1997), and the usually low dose needed for infection (Rose, 1986).

Objectives

The main objective of the work presented in this thesis was to assess the nitrogen retention capacity of willow vegetation filters used for wastewater treatment. The specific aims were to: i) determine the long-term performance of short-rotation willow coppice (SRWC) in retaining high loads of nitrogen, ii) determine the nitrate nitrogen leaching loads from SRWC in relation to soil type, irrigation rate, nitrogen application rate, and shoot growth rate, iii) determine the seasonal dynamics of nitrogen retention in an intensively irrigated and fertilized SRWC. An additional objective of the work was to iv) quantify the transport and retention of a virus in two soils cropped with SRWC.

Definitions

In this thesis, the term willow vegetation filter is used for a short-rotation willow coppice (SRWC) crop that is used for re-use and treatment of wastewaters (Perttu, 1993). In contrast to conventional soil beds or sand filters mainly used for treatment of septic-tank effluents (Pell, 1991) a willow vegetation filter comprises both the soil matrix and the willow plants. In addition, a willow vegetation filter is off-loaded from plant nutrients at harvest, an important feature distinguishing it from most types of natural or constructed wetlands used for wastewater treatment. In a conventional wastewater treatment plant, the nitrogen treatment efficiency is equal to the difference between the amounts of nitrogen in incoming wastewater and in treated wastewater released from the treatment plant. Analogous to this, the "nitrogen retention" in a willow vegetation filter can be defined as the difference between the nitrogen input through wastewater irrigation and the nitrogen leaching. Consequently, the "plant-induced nitrogen retention" can be defined as the nitrogen retention that is due to the presence of the plants.

Outline of experimental work

Long-term field trial in southwest Sweden (Paper I)

In 1988, a field trial was established at Långaveka in southwest Sweden (56°51'N, 12°36'E) in order to study the long-term influence of intensively cultured short-rotation willow coppice (SRWC) on nitrogen concentrations in groundwater. This study was part of a growth optimization trial, where different willow clones and other fast-growing tree species were grown under non-limiting conditions with regard to water and plant nutrients. Planting was carried out in spring 1988 (Year 1) and during the period 1989 to 1996, an average of 112 kg N/ha·yr was applied, either as solid fertilizer or in liquid form, together with 458 mm/yr irrigation water. The average precipitation during the study was 852 mm/yr. The SRWC was harvested in 1993 (Year 6), 1996 (Year 9) and 1999 (Year 12). In 1988, piezometers were installed in the field for measurements of the piezometric level in the saturated zone (i.e. practically the groundwater level), and for sampling of groundwater for chemical analyses. In Paper I, nitrogen concentrations in groundwater are presented for the period 1989-1997. In this thesis, measurements from 1998 and 1999 are also included.

Nitrate leaching and N-turnover in large (1200-l) lysimeters (Paper II)

At a lysimeter station in Uppsala, central Sweden, a lysimeter trial was started in 1997 in order to determine nitrate leaching from and nitrogen uptake by SRWC in relation to nitrogen application rate, irrigation rate, soil type, and shoot growth. In 1989, the lysimeters (surface area 1.0 m^2, volume 1200 l) were filled with a clay or a sand soil from nearby arable fields. In 1997, two cuttings of willow (*Salix viminalis*) were planted in each of 16 lysimeters, and by use of a drip irrigation system, water containing plant nutrients was applied during summer at rates corresponding to 3-6 mm/day and 110-244 kg N/ha·yr, respectively. Nitrate nitrogen (NO_3-N) leaching, drainage, and shoot growth were quantified for a three-year period after planting and ANOVA was performed in order to determine treatment effects on NO_3-N leaching and shoot growth.

Dynamics of nitrate leaching and ^{15}N uptake in small (68-l) lysimeters with undisturbed soil columns (Paper III)

In 1997, another lysimeter trial was started using lysimeters with undisturbed sand profiles (surface area 0.068 m^2, volume 68 l) in order to study the seasonal dynamics of nitrogen retention and the fate of fertilizer-N applied after the growing season. Willow cuttings were planted in eight lysimeters, and four lysimeters were uncropped and functioned as controls. Water containing plant nutrients was applied to all lysimeters daily during the first summer and most of the autumn. To four of the willow-cropped lysimeters, pulses of ^{15}N-labelled fertilizer were applied in autumn after leaf fall. The other four willow cropped lysimeters and two control lysimeters were fertilized and irrigated (fertigated) again during the following spring. NO_3-N leaching, transpiration, biomass productivity (leaves, shoots, stump and roots), plant-N-uptake and the ^{15}N-partitioning were determined.

Transport and retention of bacteriophages in two types of lysimeters (Paper IV)

The lysimeters described in Papers II and III were also used for a tracer study with bacteriophages (*Salmonella typhimurium* phage 28 B) in order to assess the risk of groundwater contamination by viruses as a result of wastewater irrigation. This bacteriophage study was performed parallel to the other experiments and in the same lysimeters. A pulse of bacteriophages was applied to eight large lysimeters (four clay and four sand) and six small lysimeters (four willow cropped and two controls) on two occasions (late autumn 1997 and early spring 1998). The drainage was sampled manually or by use of automatic samplers for

69-434 days, and the phage concentrations were determined using different plaque assays. The study was performed in close collaboration with the Swedish Institute for Infectious Disease Control, and is also part of the doctoral work by Anneli Carlander.

Sampling of drainage water in an old, intensively managed SRWC (unpublished data)

By use of suction cups soil water was sampled in field plots cropped to SRWC, which had been intensively irrigated and fertilized during a 12-year-period. This study has not been reported elsewhere and will only be presented briefly in this thesis. In spring 1997, seven suction cups were installed at 0.9-m depth in each of four 150-m^2 plots which had been planted with willow cuttings in 1984. The plots had been fertilized at rates corresponding to, on average, 104 kg N/ha·yr during the period 1985 to 1996. Vacuum bottles were connected to the suction cups and soil water samples were collected frequently from summer 1997 to summer 1999. The SRWC growing in the plots was irrigated daily during summer 1997 and 1998 with 6 mm of water containing plant nutrients, resulting in an accumulated annual load corresponding to 100 or 200 kg N/ha·yr.

Experimental considerations and potential limitations

Most of the research work was carried out using lysimeters filled with undisturbed (Papers III and IV) or disturbed (Papers II and IV) soil profiles. The use of lysimeters enabled a very good control of water fluxes in and out of the lysimeters, which in turn facilitated the establishment of budgets for water, plant nutrients and bacteriophages in the experiments. However, the water transport dynamics in lysimeters is known to be somewhat different from that in the field. The lysimeters were gravity-drained, which resulted in an artificial groundwater level at the bottom of the lysimeters. Under such circumstances the water tension might be reduced compared with that under field conditions, and there is also no support of water from underlying soil layers (Webster et al., 1993; Bergström et al., 1994; Bergström & Shirmohammadi, 1999). These factors might have affected the water dynamics somewhat and possibly also the accumulated water transport through the soil as compared with field conditions. When using lysimeters, there is also a risk that side-wall flow of water affects the time course of leaching of an element through the soil. Such side-wall flow has been found to be negligible in the small lysimeters used in Papers III and IV (Bergström et al., 1994; Bergström & Shirmohammadi, 1999), but might possibly have affected the results obtained from the large lysimeters in Papers II and IV. In addition, in the small lysimeters (mainly Paper III) the highly unrealistic relationship between the shoot size and the soil volume available for root growth and acquisition of water and plant nutrients was a possible source of misinterpretation. This was considered when presenting the results and drawing conclusions. Notwithstanding the potential sources of error presented, the overall relations and conclusions presented in the thesis are most likely valid for a field situation.

In the study using suction cups (unpublished data), the clay soil at the site complicated the sampling and in some of the suction cups water samples could only sometimes be obtained. In addition, in a structured soil it is difficult to know in detail which soil water fraction is being sampled (Webster et al., 1993). There is a risk that the sampled water originated from small pores which contributed little to the overall water transport through the soil.

Despite the overall objective of estimating nitrogen retention in willow vegetation filters used for wastewater treatment, no real wastewater was used in any of the experiments presented. Considering the highly variable composition of different types of wastewater, the possibility of isolating the specific effects of treatments, and the possibility of using the lysimeters for more than one study at a time, it was decided that artificial wastewater in the form of inorganic plant nutrients could be used instead of real wastewater. How this might have affected the results and the applicability of them is commented upon in a later section (page 25).

Potential fate of nitrogen applied to a SRWC

Nitrogen applied to a SRWC in the form of wastewater, sewage sludge or commercial fertilizer can be retained by the plants or by the soil, volatilized and lost to the atmosphere, or leached to drainage or groundwater. Plant uptake of nitrogen is one of the main processes responsible for nitrogen retention in a SRWC, and thus harvest of biomass is a way of off-loading nitrogen from the system. The amount of nitrogen removed from the site by harvest is dependent upon the shoot nitrogen concentration, which is higher in bark tissue than in the wood. Since the bark:wood ratio of willow plants decreases with shoot diameter and age (Nilsson & Ericsson, 1986; Ericsson et al., 1992; Verwijst, pers.comm.), the average shoot nitrogen concentrations usually decrease with time. Compared with other tree crops, the short time span between harvests of SRWC (three to five years) keeps the plants in a more or less continuous juvenile stage (Ericsson et al., 1992), and the bark:wood ratio is therefore relatively high.

After planting, the standing fine-root biomass of a SRWC crop increases, at least over the first three years (Rytter, 1997), but probably remains fairly constant thereafter (Ericsson et al., 1996). Contrary to the fine roots, the coarse-root biomass continues to increase for many years (Ericsson et al., 1996), although the rate of increase for willow plants is known only for the first three years after planting (Rytter, 1997). In a SRWC crop the non-harvestable stumps also comprise a substantial fraction of the above-ground biomass (Rytter, 1997). As for the coarse roots, the increase of the stump biomass certainly proceeds over the entire life span of the plantation, although the growth rate is known only for the first years after planting (Rytter, 1997). Thus, a fraction of the nitrogen taken up by the plants is long-term bound into non-harvestable biomass.

Leaves and fine roots are the plant compartments with the highest nitrogen concentrations. A fraction of the leaf and root litter produced in a SRWC may enter the more or less stabile humus pool of organic matter. Leaf litter production is fairly easy to quantify, whereas fine-root litter is not. By use of minirhizotrons Rytter & Rytter (1998) estimated the fine-root turnover rate in a Swedish SRWC to be 4.8-8.1 times per year, which implies that the rate of decomposition of fine-root litter is important to consider when estimating the pathway of nitrogen in a SRWC crop. Due to the intensive competition between the plants in a SRWC, plant mortality may be high (Verwijst, 1996*a,b*). Therefore, a fraction of the coarse-root, stump and shoot biomass enters the litter pool annually. The decay rate of this "litter" is not known.

When measuring or modelling the nitrogen content of different organic pools in soils, the nitrogen incorporated into living microbial biomass is often distinguished from the other pools. Application of easily decomposable organic compounds (Foster et al., 1980; 1985), or phosphorus-rich residues or fertilizers (Carlyle et al., 1990; Nguluu et al., 1996) may trigger an enhanced activity or a net growth of the microbial biomass. Since microbes contain nitrogen in amounts corresponding to 1/5-1/8 of the microbial carbon (Paul & Clarke, 1989), a growing microbial biomass requires nitrogen and will compete with plants for mineral nitrogen (Foster et al., 1985; Johnson, 1992; Groffman et al., 1993; Jordan et al., 1997), which may in turn reduce plant growth. As a result of a reduced supply of energy (i.e. decomposable organic substances) or freezing (Groffmann & Tiedje, 1989*a*), the microbial population may collapse, which may in turn result in a flush of mineral nitrogen in the soil (Groffmann & Tiedje, 1989*a*; Mitchell et al., 1996). Thus, a large microbial biomass may temporarily retain substantial amounts of nitrogen, but also constitutes a large pool of potentially accessible nitrogen, which brings on an elevated risk for nitrogen leaching.

In addition to biological nitrogen immobilization, mineral nitrogen in the form of ammonium (NH_4) can be fixed to clay minerals in the soil matrix, from where it is very slowly available to exchange reactions (Paul & Clark, 1989; Johnson, 1992). The capacity of different soil substrates to adsorb NH_4 is highly variable and can be substantial, but the importance of this nitrogen immobilization in intensively fertilized SRWC is difficult to predict. One other potentially significant nitrogen retention process is non-biological reactions between mineral nitrogen and humus substances (Johnson, 1992; Currie et al., 1999), but fairly little is known about the significance of and the factors controlling this immobilization process.

Gaseous losses of nitrogen can be substantial in many cropping systems. Losses through denitrification constitute the most important pathway of gaseous nitrogen losses resulting from nitrogen in nitrous oxides (mainly NO_3) being converted to gaseous nitrogen in the form of N_2 or N_2O. Denitrification can be biological, based either on organic carbon or other oxidizing agents (Paul & Clark, 1989; van Bennekom et al., 1993), or chemical (not further discussed in this context). Biological denitrification is carried out by a large number of bacteria genera, which use organic carbon as an energy source and NO_3 for respiration in the absence of oxygen (Tiedje et al., 1989), which implies that denitrification only occurs under anoxic conditions. The denitrification rate is governed by the supply of NO_3 and decomposable organic matter (Gilbert et al., 1979; Seech & Beauchamp, 1988; Groffman & Tiedje, 1989*a*; Struwe & Kjøller, 1990). An adequate supply of easily decomposable organic matter as well as a high pH and high temperature usually promotes the complete denitrification to N_2 (Nômmik & Larsson, 1989; Weier et al., 1993). This is favourable since N_2O is a highly potent greenhouse gas (Bogren et al., 1998). However, addition of NO_3 might suppress the N_2 formation in favour of N_2O (Struwe & Kjøller, 1990). Like biological processes in general, denitrification is temperature dependent and has been considered to be low at soil temperatures below 4-5°C (Groffmann & Tiedje, 1989*a*).

Nitrogen can also be lost to the atmosphere in the form of easily volatilized ammonia (NH_3). When NH_4 is present in the soil, the relationship between NH_4 and NH_3 is governed by pH, and balances at pH 9.3. NH_3 volatilization can also occur directly from nitrogen-rich plant residues left on the soil surface (Whitehead et al., 1988; Whitehead & Lockyer, 1989; Janzen & McGinn, 1991), and this pathway of nitrogen might need consideration when estimating nitrogen budgets of SRWC systems. There is also a common suggestion that N_2O can be lost from the soil during nitrification (Paul & Clark, 1989), although the extent of such losses is hardly predictable.

Nitrogen that is either taken up by plants, immobilized in the soil, or volatilized will eventually be leached beyond the rooting zone. Nitrogen is leached from the soil usually in the form of NO_3 , which is a highly mobile ion. NH_4 is much less mobile due to its attachment to negatively charged colloid surfaces. In addition, in most arable soils pH is sufficiently high to enable a rapid nitrification of NH_4 to NO_3. Thus, leaching losses of nitrogen from arable land are often assumed to be equivalent to leaching losses of NO_3 (e.g. Webster et al., 1993). Sandy soils are usually regarded as being more prone to NO_3 leaching than clayey soils, a characteristic often explained by the lower water holding capacity and the more efficient washing out of NO_3 in a sandy soil (convective-dispersive flow behaviour) (Bergström, 1995; Bergström & Stenström, 1998; Hoffmann & Johnsson, 1999). In a clayey soil, the aggregate formation facilitates preferential flow of water enabling a rapid transport of NO_3, although the leaching loads are restricted by the rate of diffusion of NO_3 into and out of the aggregates (Bergström & Stenström, 1998). In addition, the high water holding capacity of clayey soils induces anoxic conditions, which is in turn favourable for denitrification (Groffmann & Tiedje, 1989*a*).

Fate of nitrogen applied to the experimental SRWC

Nitrogen retention

Plant uptake

Whole-season plant uptake

In the experiment using large (1200-l) lysimeters (Paper II), the estimated net nitrogen uptake by the willow plants during the year of plant establishment (i.e. Year 1) was low, and only 18 to 36 kg N/ha was incorporated into woody tissue (i.e. shoots, roots and stumps; Fig. 1). During the second growing season, the net amount of nitrogen incorporated into woody biomass was 64-149 kg N/ha and during the third 56-297 kg N/ha. Recalculated on the basis of biomass productivity, the net nitrogen uptake corresponded to 7.5 (clay soil) and 6.5 (sand soil) kg N per dry tonne of shoots produced during the second growing season, and 9.6 (clay) and 7.6 (sand) kg N per dry tonne shoots produced during the third growing season. The shoot nitrogen constituted the main fraction of this nitrogen, i.e. on average 49, 77, and 84% of the total net amount of nitrogen incorporated into woody tissue during Years 1 to 3, respectively.

The shoot nitrogen concentration, which is highly variable (Table 1), must be known if reliable nitrogen budgets are to be made. Typically, shoot nitrogen concentrations decrease with shoot age, and the average shoot nitrogen concentration at harvest of 30 conventionally managed SRWC of different willow clones and of varying age was 3.7 mg N/g d.w. (Table 1). However, the nitrogen concentrations of the three-year-old shoots of lysimeter-grown willow (Paper II) were approximately twice as high. This might have been due to storage of nitrogen in shoots, which is possible when nitrogen is available in excess of plant demand (Ericsson, 1984; Bollmark et al., 1999; Bollmark, 2000). Even higher nitrogen concentrations of willow shoots have been reported (Ericsson, 1984). In fact, the amount of nitrogen annually incorporated into the shoots of a SRWC crop can be much larger than that of other deciduous or evergreen trees (Ericsson, 1994; Miller, 1995).

Table 1. Examples of shoot nitrogen concentrations of willow clones of different ages, intensively (Paper II and III; Rytter, 1997; Nilsson & Ericsson, 1986; Elowsson, pers. comm.) or conventionally managed (Jug et al., 1999*a*; Vigre & Ledin, pers. comm.)

Species/clone	Shoot/root age (years)	soil type	N-conc. (mg N/g d.w.)	Reference
S. vim. 78183	1/1	sand	8.3	Paper III
S. vim. 78183	1/1	sand	8.5	Rytter, 1997
S. vim. 78183	1/1	clay	7.6	Rytter, 1997
S. vim.	1/1	silt	4.0	Nilsson & Ericsson, 1986
S. vim. 78183	1/2	sand	8.4	Elowsson, pers. comm.
S. vim. 78183	1/3	sand	6.6	Paper II
S. vim. 78183	1/3	clay	7.8	Paper II
S. vim. 78183	2/2	sand	5.6	Paper II
S. vim. 78183	2/2	clay	5.7	Paper II
S. vim. 78183	2/2	sand	5.3	Rytter, 1997
S. vim. 78183	2/2	clay	5.4	Rytter, 1997
S. vim.	2/2	silt	3.0	Nilsson & Ericsson, 1986
S. vim. 78183	2/3	sand	4.2	Elowsson, pers. comm.
S. vim. 78183	3/3	sand	5.8	Paper II
S. vim. 78183	3/3	clay	8.1	Paper II
S. vim. 78183	3/3	sand	3.4	Rytter, 1997
S. vim. 78183	3/3	clay	4.0	Rytter, 1997
S. vim. 78183	3/4	sand	5.0	Elowsson, pers. comm.
S. vim. 78183	3/8	sand	4.7	Elowsson, pers. comm.
S. sp. (n=8)	3/?	?	3.8	Vigre & Ledin, pers. comm.
S. vim. 78183	4/5	sand	3.7	Elowsson, pers. comm.
S. sp. (n=4)	4/?	?	3.8	Vigre & Ledin, pers. comm.
S. vim.	5/5	various	5.3-6.6	Jug et al., 1999*a*
S. vim.	5/10	various	4.1-5.9	Jug et al., 1999*a*
S. sp. (n=5)	5/?	?	3.6	Vigre & Ledin, pers. comm.
S. sp. (n=30)	3-7/?	?	3.7	Vigre & Ledin, pers. comm.

Late-autumn plant uptake

Pulses of ^{15}N-labelled nitrogen were applied in late autumn after leaf fall to four lysimeter-grown willow plants (Paper III). Most of this nitrogen was either lost through leaching (39%) or incorporated into the non-extractable pool of nitrogen in the soil (22%) within 9-10 days of application. However, during this period approximately 8% of the nitrogen was taken up by the plants, and almost all of this nitrogen was found in the fine roots (<2.0 mm diameter). The nutrient uptake capacity of willow roots during low-temperature conditions has not been quantified previously, but Rytter & Rytter (1998) detected fine-root growth of willow plants in mid-winter in a SRWC growing in central Sweden. The ability of willow plants to take up nitrogen both in late autumn and in early spring was also indicated by the dynamics of the NO_3-N leaching from the small lysimeters in Paper III (Fig. 2*a*). There was a marked discrepancy between NO_3-N leaching from the willow-cropped and the non-cropped lysimeters despite the inactivity of the above-ground plant parts. This indicates substantial nitrogen retention even during winter.

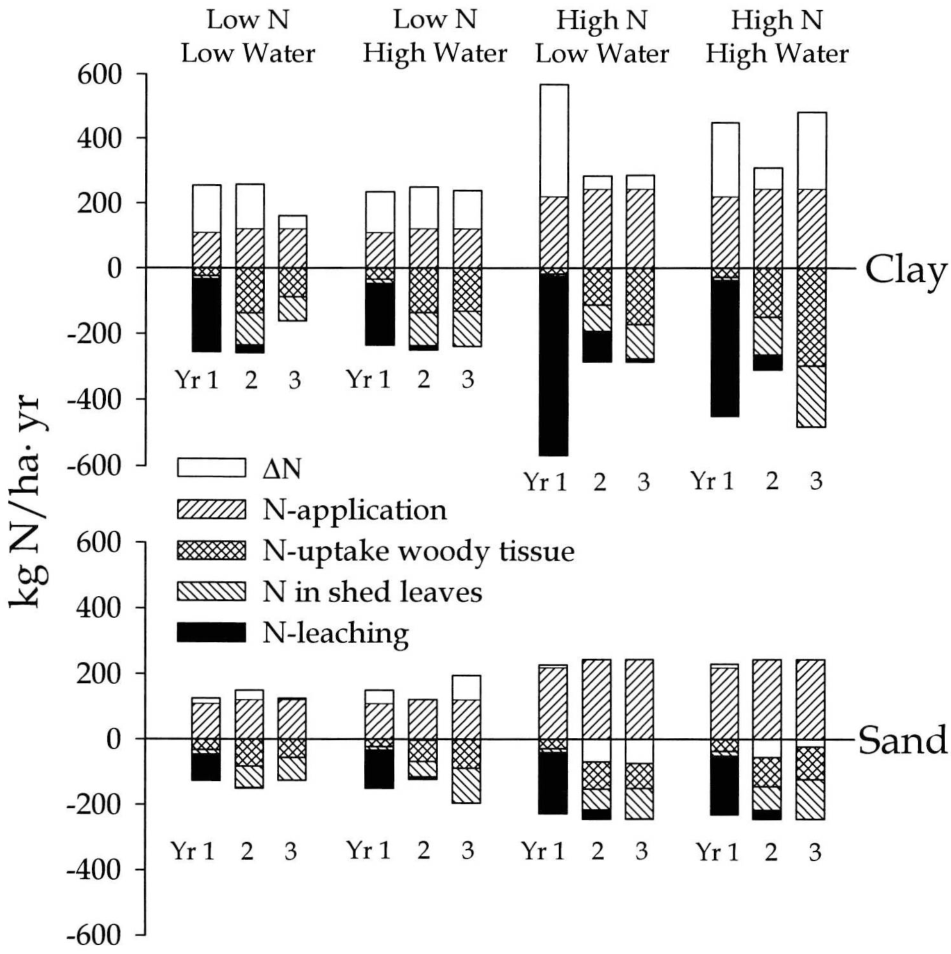

Fig. 1. Nitrogen balance in an intensively fertilized and irrigated SRWC during the three first years after planting (Paper II). ΔN represents the combined result of mineralization, immobilization, build-up of the soil pool of nitrogen and gaseous losses (e.g. denitrification). N-uptake in woody tissue includes the amount of nitrogen that is annually net incorporated into shoots, stumps and roots.

Immobilization in the soil

In the study of nitrate leaching and nitrogen retention in Paper II, the soil retention was not explicitly measured. However, in a parallel study (Granhall et al., pers. comm.), total organic carbon and microbial carbon at various soil depths in the lysimeters were measured. The results showed that there was probably no net build-up of the microbial biomass in the sand lysimeters during the first two years of the experiment, whereas there was a small net build-up in the clay lysimeters. In addition, there was no significant build-up of the organic pool of nitrogen in any of the soils. Thus, immobilization in the soil was probably not an important pathway of nitrogen in the lysimeter experiment. This is contradictory to what is usually the case after fertilization of conventional forests, where the nitrogen immobilization in the soil and litter horizon is often high or very high (Foster et al., 1985; Johnson, 1992; Aber et al., 1993; Christ et al., 1995; Magill et al., 1997), and can be in the order of 1000 kg N/ha during a few years of intensive fertilization. However, the opposite has also been found (e.g. Jordan et al., 1997), and in forest ecosystems that are subject to elevated long-term atmospheric nitrogen deposition, the soil nitrogen retention is usually low and frequently negative (Johnson, 1992).

During the year of plant establishment (Year 1), there was presumably a substantial nitrogen mineralization in the large clay lysimeters (Paper II). This was indicated in the nitrogen budget (Fig. 1) by the large values of ΔN, which expresses the combined effect of mineralization, immobilization, build-up of the soil pool of nitrogen and gaseous nitrogen losses. Unfortunately it was not possible to distinguish between nitrogen mineralized before and after planting, and the NO_3-N concentrations in drainage water (Fig. 2*b*) indicated that the large value of ΔN could be partly explained by the contribution of nitrogen that had been mineralized before planting.

In a SRWC, leaf litter does not to any significant degree accumulate on the soil surface (Johnson, 1989). Instead the litter is incorporated into the soil by soil fauna activity, which is highly stimulated by afforestation (Makeschin, 1994). The decomposition rate of willow leaves varies markedly and after four years Šlapokas (1991) recovered between 3 and 100% of the nitrogen from leaves buried in litter bags. The large differences were attributed to species characteristics and initial nitrogen concentrations. For *S. viminalis*, on average 42% of the leaf litter nitrogen remained after four years and was believed to have entered the more or less stabile humus pool. Accordingly, it can be assumed that about 40% of the nitrogen in willow leaf litter will be more or less permanently retained in soil.

The degree to which fine-root litter contributes to the formation of stable soil organic matter is poorly understood, but it is a well-known fact that growth and die-off of willow fine-roots proceed simultaneously throughout the year (Rytter & Rytter, 1998). However, the actual decay rate of willow fine-root litter (i.e. loss of dry weight and/or loss of mineral nutrients per unit of time) has not been quantified. Lõhmus & Ivask (1995) found that the "finest" (<1 mm) roots of Norway spruce (*Picea abies*) lost 39% of their dry weight but only 27% of their nitrogen during more than five years of decomposition. In the same study, the fine roots (<2 mm) lost 36% of their dry weight but actually gained nitrogen during two years of decomposition. Joslin & Henderson (1987) studied root turnover in a white oak (*Quercus alba*) stand and estimated the first-year decomposition of roots (<5 mm) in litterbags to be 30-35% of dry weight.

Root nitrogen concentrations are highly dependent upon root diameter (Cox et al., 1978). The fine-root nitrogen concentrations of young, laboratory-grown willow plants can vary between 6 (Bollmark et al., 1999) and 31.8 mg N/g d.w. (Ericsson, 1984), and Rytter (1997) reported fine-root nitrogen concentrations of 11.3-15.1 mg N/g d.w. of lysimeter-grown, 1-3-year-old willow plants. Adopting the results obtained by Rytter (1997) concerning biomass allocation and fine root turnover in a 2-3-year old Swedish SRWC grown on a clay soil and assuming an annual shoot production of 10 tonnes d.w./ha, the annual fine-root litter formation would be approximately 6.9 tonnes d.w./ha, and the annual leaf litter formation would be 2.3 tonnes d.w./ha. Furthermore, assuming a fine-root nitrogen concentration of 11.3 mg N/g d.w. and a leaf litter nitrogen concentration of 24.9 mg N/g d.w. (Rytter, 1997), these amounts would correspond to 78 kg N/ha·yr as fine-root litter and 57 kg N/ha·yr as leaf litter. From this it is obvious that the litter decomposition rate and the size of the litter fraction entering the stable humus pool to a large extent determine the long-term development of the pools of soil organic matter and nitrogen.

The results presented thus far definitely point to the possibility of a high nitrogen retention and improvement of site fertility by growing SRWC on arable land. However, the data available on this topic do not give a very clear picture. Both Alriksson (1998) and Jug et al. (1999*b*) found an accumulation of carbon and nitrogen in the top 5 or 10 cm of arable soils five to nine years after afforestation with willows and different species of *Populus*. In the deeper soil layers the situation was the opposite, and in all, Jug et al. (1999*b*) found that although the pool of organic carbon in the top soil increased somewhat, the pool of nitrogen could either increase or decrease. Thus, reliable predictions of the changes in the pools of soil organic carbon and nitrogen as a result of afforestation seem difficult to make. Considering also that these pools are constantly approaching states of equilibrium in relation to various interrelated biotic and abiotic factors, and that the initial state is due to a large degree to the preceding management including crop rotations, soil tillage practices, and fertilization regimes (including manuring), such predictions appear very difficult.

Gaseous losses

Neither denitrification nor other gaseous losses of nitrogen were directly measured in the experiments. However, indicative estimates of gaseous nitrogen losses (primarily through denitrification) could be made based on nitrogen budgets and ^{15}N recovery. In the small (68-l) lysimeters (Paper III), the nitrogen budget for the year of plant establishment shows that nitrogen retention in general was high, and that 19% of the plant-induced nitrogen retention was due to processes other than plant uptake. Of this nitrogen, a large portion was likely lost through denitrification. Recalculated on the basis of biomass production, these 19% would correspond to approximately 7 kg N per dry tonne of shoots produced. In addition, 32% of the ^{15}N-labelled nitrogen applied to the lysimeters in late autumn was not accounted for. The low temperature (0-6 °C) in the top soil during the study (Paper III) would hardly allow such high denitrification rates, but there is no alternative explanation, and, therefore, denitrification probably took place in the subsoil.

The nitrogen budget of the large lysimeters (Paper II) established for three consecutive years after planting also indicates that denitrification might have been substantial (Fig. 1) Since there was no substantial build-up of either the microbial or soil organic pool of nitrogen, and since some mineralization certainly must have occurred, denitrification might have been in the order of 70 kg N/ha·yr in the sand lysimeters during Year 3 (Fig. 1).

In studies where denitrification has actually been measured, highly variable results have been obtained. Groffmann & Tiedje (1989*b*) estimated the denitrification in natural forests to be less than 1 kg N/ha·yr on sandy soils but up to 40 kg N/ha·yr on poorly drained clayey soils. The low denitrification rates on the sandy soils were mainly due to the low NO_3 supply. On a conventionally cropped arable loamy soil, Svensson et al. (1991) estimated the denitrification to be in the range of 4 to 17 kg N/ha·yr. Denitrification has often been found to be higher in forest soils than in arable soils (Russell et al., 1993; Bragan et al., 1997), although, the opposite has also been reported (Jordan et al., 1997).

In the context of wastewater-irrigation, Schipper et al. (1989) estimated the nitrogen retention in a wastewater-irrigated *Pinus radiata* forest to be at least 800 kg N/ha·yr. The major part of this retention was assumed to be due to denitrification in a riparian forest down-hill from the spraying zone. Such extremely high figures sharply contrast with the denitrification losses of 2-3 kg N/ha·yr found by Jordan et al. (1997) and Barton et al. (1998) in wastewater-irrigated forest soils. A part of the discrepancy in denitrification rates between different studies might be explained by the different methodologies adopted for denitrification estimates, but still, as in any other arable or forest soil, the denitrification rates in wastewater-irrigated forest ecosystems seem very difficult to predict. In a willow vegetation filter, all prerequisites for high denitrification are met; presence of NO_3 from nitrification, access to metabolizable carbon through leaf and fine-root litter, and root exudates (Lambers, 1987), usually high soil pH, and probably also sufficiently anoxic conditions.

NO_3-N leaching

The general pattern of NO_3 leaching from SRWC seems clear. During the establishment phase, leaching can be moderate (Paper I; Fig. 2*a*, *c*) to very high (Paper II; Figs. 1 and 2*b*). However, once the SRWC is established, NO_3-N leaching is negligible (Papers I and II) despite high or very high nitrogen loads and irrigation rates. This is fully in line with the results obtained in Danish conventionally managed SRWC (Mortensen et al., 1998). In the long-term perspective, the results are contradictory. At the field trial in southwest Sweden

(Paper I), the NO_3-N concentrations in the groundwater remained at very low levels (typically below 1.0 mg N/l) during the 12 years (Fig. 2*c*), whereas NO_3-N concentrations in soil water sampled at 0.9-m depth in the Uppsala-field plots (Aronsson, unpublished data) were high (10-30 mg N/l) during Years 14 to 16 (Fig. 2*d*) indicating substantial nitrogen leaching losses. Soil conditions differed markedly between these two sites, and also between treatments, and the groundwater monitoring in Paper I might be more reliable than the suction-cup-sampling in the Uppsala-trial. However, if the suction-cup sampling reasonably well reflected the nitrogen concentrations in the soil water leaving the rooting zone, the results point to the risk of reaching a state of nitrogen saturation (e.g. Ågren & Bosatta, 1988; Binkley & Högberg, 1997), which is characterized by high NO_3-N leaching losses and a negligible or even negative nitrogen retention.

The prerequisites for reaching a state of nitrogen saturation are not fully known, but in wastewater-irrigated forest sites nitrogen-saturation-like conditions have been reached within a few years of irrigation (Hook & Kardos, 1978; Jordan et al., 1997; Kim & Burger, 1997). To what extent the vigorous growth of the SRWC and the export of nitrogen through frequent stem harvest can delay nitrogen saturation is not known. Considering the possibility of high denitrification losses, and the ability to take up and store nitrogen in (harvestable) stemwood, a speculative estimate would be that nitrogen saturation of a willow vegetation filter can at least be delayed compared with a conventional forest ecosystem receiving wastewater.

The irrigation rate clearly influenced the NO_3-N concentrations in drainage water from the large lysimeters (Paper II; Fig 2*b*), but did not influence NO_3-N leaching loads. This was unexpected since intensive irrigation or large precipitation surplus are generally believed to increase NO_3-N leaching loads (Bergström & Brink, 1986; Torstensson & Aronsson, 2000) The interpretation of this is that roots of willows are probably very efficient in filtrating NO_3-N out of water rapidly percolating through the rooting zone. The very low NO_3-N leaching loads during Year 3, including from lysimeters with plants being harvested after the 2nd growing season, indicate that NO_3-N leaching from SRWC in general is very low and much lower than from conventional arable crops (Hoffmann & Johnsson, 1999; Torstensson & Aronsson, 2000).

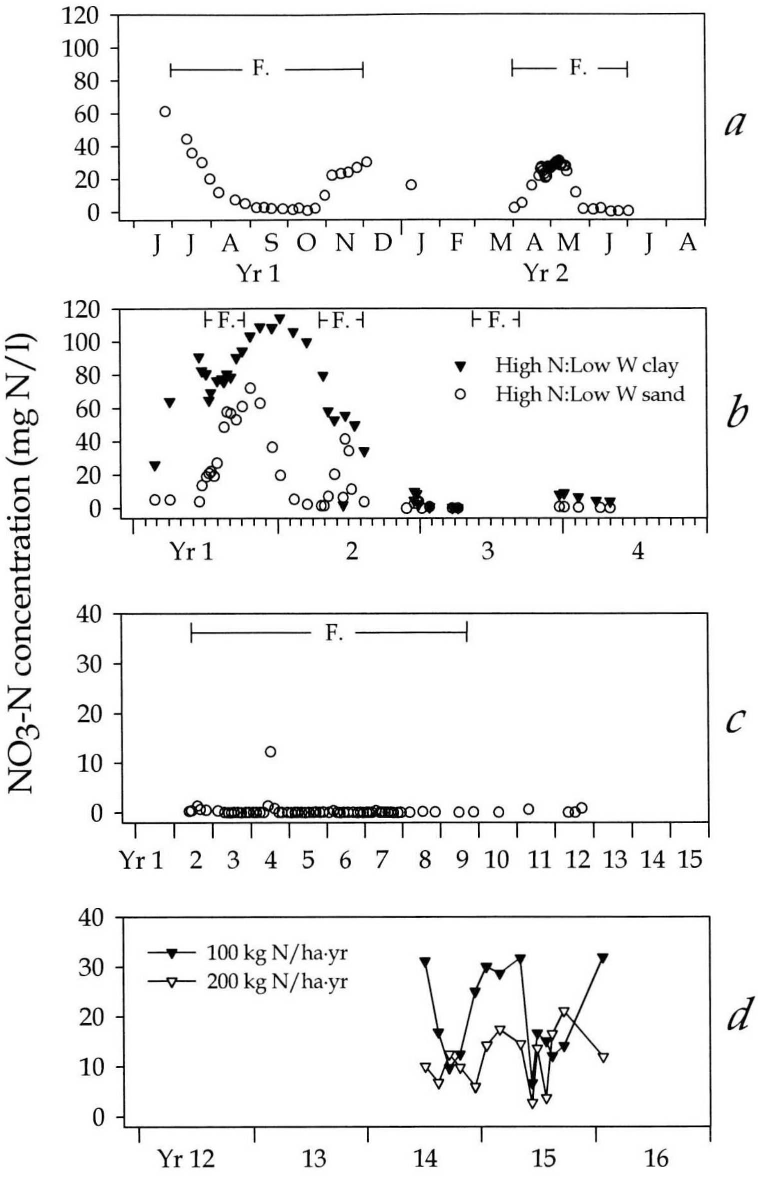

Fig. 2. NO_3-N concentrations in drainage water (*a, b*), groundwater at 2-m depth (*c*), and pore water at 0.9-m depth (*d*) sampled in intensively irrigated and fertilized (fertigated = F.) SRWC during different time periods, with Year 1 referring to the year of establishment (planting).

a: Paper III, Lysimeters E-H, N-concentration in irrigation water = 37 mg N/l (n=4),

b: Paper II, High N = 220-244 kg N/ha·yr, Low W = 3 mm water/day during summer (n=2),

c: Paper I, average N-application = 112 kg N/ha·yr, average irrigation rate = 458 mm/yr (n=4),

d: Aronsson, unpublished data, irrigation = 6 mm/day during summer, N-application = 100 or 200 kg N/ha·yr (n=2).

Applicability of results

If "real" wastewater had been used in the experiments instead of liquid and balanced plant nutrients, the results from the lysimeter experiments might have been somewhat different than those presented here. The main differences between "real" wastewater and artificial wastewater are that the former i) would probably have contained organic matter and organically bound nitrogen, ii) would possibly have had a higher NH_4:NO_3 ratio. If pre-treated wastewater is to be used, the NH_4:NO_3 ratio can vary substantially over the season (Jordan et al., 1997; Kim & Burger, 1997) and between treatment plants. Therefore, it is hardly possible to state which NH_4:NO_3 ratio is realistic and which is not. Thus, the low NH_4:NO_3 ratio in the artificial wastewater used in these experiments does not itself make the results less reliable or applicable. In addition, the low NH_4:NO_3 ratio implies that the NO_3-N leaching losses might have been higher than if "real" wastewater had been used. The lack of organic matter, on the other hand, should not be neglected in this context. If a substantial fraction of the total nitrogen had been organically bound, it can be assumed that NO_3-N leaching loads would have been lower, and that both denitrification and microbial immobilization would have been higher (e.g. Crites, 1984). In all, the use of artificial wastewater with a low NH_4:NO_3 ratio probably affected the results in such a way that the NO_3-N leaching losses were over-estimated, whereas the nitrogen retention was under-estimated.

Transport and retention of viruses in vegetation filters

Overview

In domestic wastewater a large number of species and strains of enteric bacteria, parasites and viruses may be present (Rose, 1986; Bitton, 1994). These organisms frequently contaminate drinking water as a result of leaking sewage pipes or inadequately located septic tank systems enabling the organisms to reach the groundwater (Keswick & Gerba, 1980; Stenström, 1996). Once they reach the groundwater they can survive for long periods and viruses can be transported several hundred metres (Schaub & Sorber, 1977; Yates & Yates, 1988; Sinton et al., 1997). Therefore, when using domestic wastewater for irrigation of crops, the risk of harmful microorganisms reaching the groundwater should be considered. Due to their much larger size, bacteria and parasites are in most cases efficiently filtered out in a soil or a constructed sand medium, whereas viruses have been found to be much more mobile in the same environments (Keswick & Gerba, 1980; Jansons et al., 1989; Bitton, 1994). Viruses are also of special interest because of the usually low dose needed for infection, often as low as 1-10 virus particles (Rose, 1986).

When applied on a soil surface, the depth and rate of percolation of viruses are mainly determined by their adsorption to the soil matrix (Bitton, 1994). The surface of a virus particle is usually charged, facilitating electrostatic interaction with clay colloids or minerals such as magnetite and haematite (Bitton, 1994). At low pH the surface is positively charged (cation binding to colloids), and at high pH it is negatively charged (anion binding). The isoelectric point (pH at which no net charge prevails) varies between virus species and strains (Gerba, 1984; Lipson & Stotzky, 1987; Alhajjar et al., 1988). Hydrophobic interaction between viruses and clay colloids is probably also of importance for virus adsorption. In addition, in a solution of high ionic strength viruses can also be adsorbed to colloid surfaces by van der Waals forces (Lance & Gerba, 1980; Gerba, 1984; Lipson & Stotzky, 1987). Accordingly, application of de-ionized water or rainwater may lead to resorption and deep penetration of viruses applied to a soil (Gerba, 1984). Since the number of binding sites in a soil matrix is highly dependent on the specific area of the soil, viruses are usually more efficiently adsorbed in clayey soils than in sandy soils (Keswick & Gerba, 1980; Jansons et al., 1989; Bitton, 1994). If present in the soil, humic acids (Bitton et al., 1976), proteins (Jin et al., 1997) or other organic substances (Schaub & Sorber, 1977; Gerba, 1984) might compete with viruses for the binding sites and thus reduce their adsorption or even facilitate resorption of viruses in the soil (Jin et al., 1997). Thus, virus adsorption can be poor in soils rich in organic matter (Schaub & Sorber, 1977; Keswick & Gerba, 1980).

In column studies it was found that non-saturated water flow promotes virus adsorption in the soil compared with saturated flow (Lance et al., 1982; Powelson et al., 1990; Powelson & Gerba, 1994; Poletika et al., 1995). This has been explained by the very strong forces acting at the air-water boundary retaining or even disrupting viruses (Bitton, 1980; Poletika et al., 1995). As a result, application of detergents reducing the surface tension in the soil matrix usually leads to a reduced adsorption or even resorption of viruses (Dizer et al., 1984). The concentration of virus, e.g. in wastewater applied to a soil surface, does not affect the maximum depth of penetration (Lance & Gerba, 1980; Lance et al., 1982), but a high infiltration rate promotes deep penetration in the soil (Poynter & Slade, 1977; Lance & Gerba, 1980; Lance et al., 1982). Low soil temperatures also reduce adsorption, although the mechanism behind this is not known (Poynter & Slade, 1977).

The fraction of viruses that is not adsorbed in the soil has been found to be rapidly transported through the profile (McKay et al., 1993*a*; Sinton et al., 1997), and at velocities that may exceed the mean velocity of water by two orders of magnitude (McKay et al., 1993*a*), although the opposite also has been reported (Dizer et al., 1984; Powelson & Gerba, 1994). The often very rapid transport of viruses through the soil matrix can be explained by pore size exclusion; the viruses are excluded from the smallest pores due to their size (20-350 nm (Stenström, 1996)) and forced to move in larger pores, where the flow velocity is higher (McKay et al., 1993*a*). An alternative explanation is that viruses attach to colloids being rapidly transported in soil macropores (McKay et al., 1993*a,b*; Sinton et al., 1997).

Despite the extensive knowledge about factors influencing adsorption of viruses in soils, predictions of virus adsorption and transport in natural soils are still very difficult to make due to the complexity of soils and the interaction between different factors (Vilker, 1981; Jansons et al., 1989). Therefore, in order to assess the risk of virus contamination of groundwater following wastewater irrigation of SRWC, the transport and retention of a bacterial virus (bacteriophage) in two agricultural soils (clay and sand, respectively) cropped to SRWC was studied in lysimeters (Paper IV). The clay soil had a well developed structure down to at least 1-m depth, whereas the sand soil was practically structureless.

Transport and retention of bacteriophages in lysimeters

The accumulated transport of phages through the large (1200-l) clay lysimeters was more than two orders of magnitude larger than that through the large sand lysimeters (Paper IV). This contradicts the prevailing opinion that virus adsorption is more efficient in clayey soils than in sandy soils (Keswick & Gerba, 1980; Jansons et al., 1989; Bitton, 1994). The explanation for this is probably that the phages leached from the clay soil were mainly transported in large macropores in which neither electrostatic nor other adsorptive forces were sufficiently strong to retain the phages. Indeed, the very rapid breakthrough of phages in the clay soil (Fig. 3) must have been facilitated by macropore flow enabling phages reaching groundwater within a few hours after application despite very moderate irrigation rates (6 mm/day). In the sand soil, on the other hand, the phage breakthrough after roughly 0.5-1 pore volume of discharge indicates "piston-flow"-like transport of water and phages through the soil. Such conditions would allow for the full range of adsorptive forces of the soil to act. In the sandy soil the efficient virus retention resulted in a complete to a 6 $\log_{10}$ reduction in viruses. The markedly lower phage retention in the clay soil resulted in a 3 to 6 $\log_{10}$ reduction.

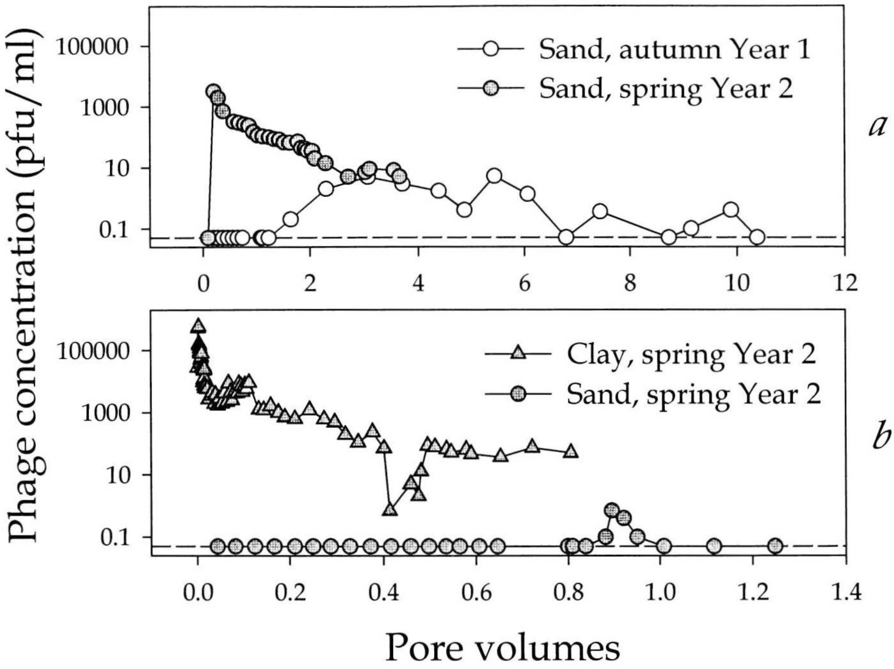

Fig. 3. Drainage water concentrations (plaque forming units per millilitre; pfu/ml) of bacteriophage *Salmonella typhimurium* phage 28B in relation to amounts of water discharged (expressed as pore volumes) from field lysimeters cropped with SRWC after pulse application of phages on soil surface. Reference lines represent non-detectable concentrations (from Paper IV).

a: Application on 8 October 1997 (autumn Year 1) and 21 April 1998 (spring Year 2), respectively to 68-l lysimeters followed by 44-mm daily irrigation. Phage concentration in pulse applied irrigation water=$3.4*10^7$ (autumn) and $2.4*10^8$ (spring) pfu/ml, respectively; total phage input=$1.1*10^{10}$ (autumn) and $3.9*10^{10}$ (spring) pfu, respectively

b: Application on 21 April 1998 (spring Year 2) to 1200-l lysimeters followed by 6-mm daily irrigation. Phage concentration in pulse applied irrigation water=$2.4*10^8$ pfu/ml; total phage input=$7.8*10^{11}$ pfu.

The transport and retention of phages in the small (68-l) lysimeters containing undisturbed monoliths of the same sandy, agricultural soil as the large sand lysimeters differed markedly from the pattern described above. The relative (accumulated) transport of phages was approximately 2-5 orders of magnitude larger than that from the large sand lysimeters (Paper IV). This might be explained by the much higher irrigation rates (44 mm/day) leading to more saturated-like flow conditions, which would reduce adsorption in soil (Lance et al., 1982; Powelson et al., 1990; Powelson & Gerba, 1994; Poletika et al., 1995). However, there was a drastic difference between the two application events as regards phage breakthrough (Fig. 3). After phage application in autumn Year 1, breakthrough was recorded after a discharge corresponding to 1.6-2.4 pore volumes, whereas after phage application in spring the following year, breakthrough was recorded after only 0.2 pore volumes.

After the spring application (Year 2) there was no clear difference as regards phage retention between non-cropped small lysimeters (controls) and those cropped with SRWC (Fig. 3). Unfortunately there were no control lysimeters at the autumn application (Year 1), so one can only speculate about the reason for the noticeable difference between years. It appears as if in autumn, the phages were retained by some strong adsorptive force, which was not active in spring the following year. Soil temperature and drainage conditions were similar during the two first weeks following phage application. However, at the autumn application, the willow plants were actively growing, whereas at the spring application no leaves had yet developed and plant growth had barely started (Paper III). Plant roots are known to leach carbohydrates to the soil (Lambers, 1987), which supports microbial activity. Such carbohydrates are easily degraded and the supply from the roots probably diminishes during winter dormancy. Thus, it could be assumed that the rhizosphere (soil-root environment) conditions differed between the two application events. How this in turn could have affected phage retention is difficult to judge. In a study of wastewater renovation in multi-species constructed wetlands, Karpischak et al. (1996) presented results indicating the possibility of plant composition affecting virus retention. The retention of coliphages (bacterial virus) in a duckweed (*Lemna* sp.) wetland was less than half the retention of enteric viruses in a multi-species (*Populus* sp., *Salix* sp., *Typha* sp., *Scirpus* sp. and *Arundo* sp.) wetland. Their findings do not directly support the speculations about rhizosphere conditions affecting virus retention, but point to such a possibility.

Conclusions

Nitrogen

It is tempting and important to try to estimate the nitrogen flows in a true field situation, where a willow vegetation filter is used for wastewater treatment. There are not enough data available from full-scale vegetation filter systems for making empirically based estimates. Therefore, an estimate must be based on interpolations and extrapolations of the experimental results presented in this thesis and elsewhere, experiences from research on arable crops, and pure speculation. Such an estimate was made for a Low and High intensity vegetation filter, respectively, for the two first rotations on a clay soil. The proportions of leaf- and fine-root litter nitrogen entering the non-easily degradable pool of soil organic matter ("humus") were assumed to be 40 and 50%, respectively. Typical Swedish conditions were adopted (e.g. Hoffmann et al., 2000). Plant tissue nitrogen concentrations were assumed to be the same as in Paper II, except for shoot nitrogen concentrations, which were assumed to be lower, 5 mg N/g d.w., during Year 3 and onwards, thus resulting in a conservative estimate of nitrogen uptake and removal by shoot harvest.

Table 2. Prerequisites and assumptions for conclusive nitrogen budget estimates

		Yr 1	Yr 2	Yr 3	Yr 4-
Shoot production (tonnes d.w./ha·yr)	*Low intensity*	1	5	10	10
	High intensity	1	5	12	10
N-input by wastewater (kg N/ha·yr)	*Low intensity*	0	100	100	100
	High intensity	100	200	200	200
N-mineralization from humus[a] (kg N/ha·yr)	*Low intensity*	100	100	50	50
	High intensity	150	75	50	50

[a] humus corresponds to non-easily degradable organic matter

During the first rotation, nitrogen input through wastewater irrigation is much in excess of plant demand in both the Low and High intensity cases (Fig. 4). In addition, mineralization of humus results in a net contribution of nitrogen in the order of 40 kg N/ha·yr. Accordingly, leaching and gaseous losses of nitrogen are substantial during the first rotation. However, during the second rotation, plant growth is considerably higher resulting in a larger build-in and export of nitrogen, and there is a net build-up of humus nitrogen. Therefore, in the Low intensity case there is little excess nitrogen available for leaching or gaseous losses. However, in the High intensity case nitrogen input through wastewater is sufficiently high to promote substantial gaseous losses, whereas leaching losses are low. During the first rotation, the nitrogen retention (defined as nitrogen input through wastewater minus nitrogen leaching losses) is in the order of 50 and 110 kg N/ha·yr in the Low and High intensity case, respectively (Fig. 4). During the second (and probably also the third and fourth) rotation, the nitrogen retention is in the order of 100 and 190 kg N/ha·yr in the Low and High intensity case, respectively.

When deciding wastewater loads to a willow vegetation filter, it is important to consider the potentially high leaching losses during the establishment phase, especially in the High intensity case. Such losses should be avoided, and therefore wastewater loads should be low during the first two years, and preferably zero during the first year. By measuring shoot nitrogen concentrations and mineralization rate, it would be possible to adjust wastewater loads for increasing nitrogen retention or reducing leaching loads.

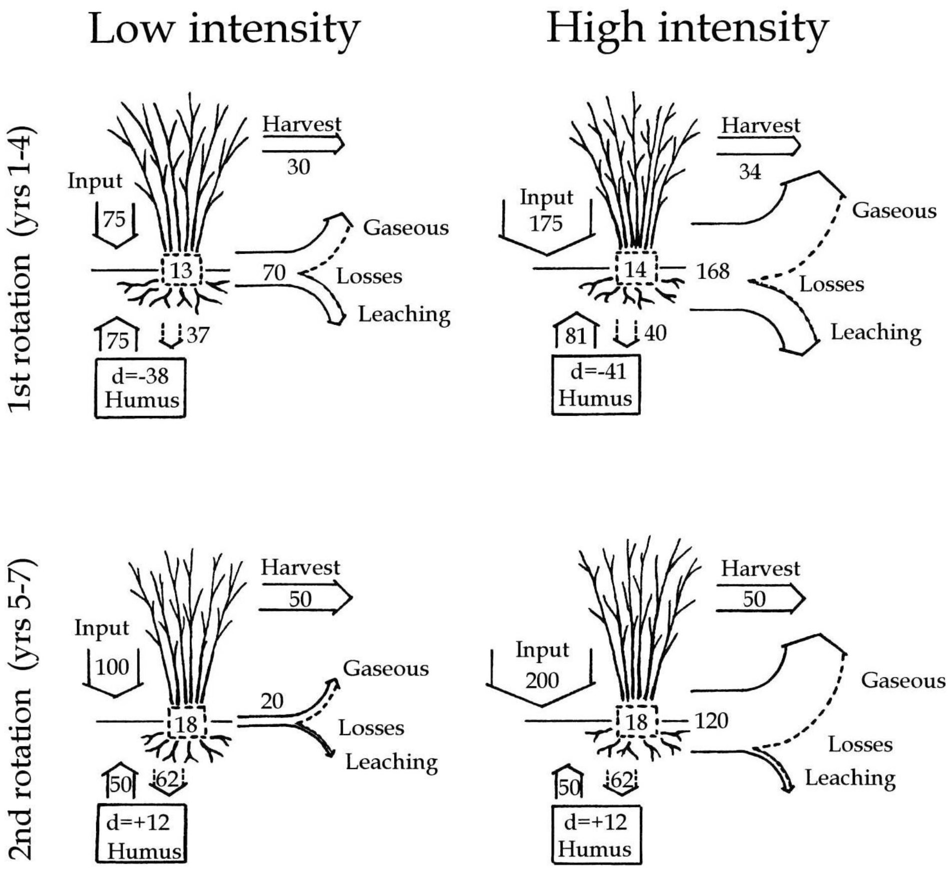

Fig. 4. Estimated annual flow of nitrogen (kg N/ha·yr) in a theoretical willow vegetation filter irrigated with wastewater at two rates (Low and High intensity). Dashed squares represent net nitrogen incorporation into non-harvestable woody tissue (i.e. roots and stump), and dashed arrows indicate a very high degree of uncertainty. Gaseous nitrogen losses are assumed to be mainly due to denitrification.

From the results presented in this thesis and elsewhere it is clear that nitrogen leaching loads from SRWC fertilized conventionally or through wastewater irrigation are low and much lower than from most conventional agricultural crops. However, during the year of plant establishment, nitrogen leaching loads can be substantial. The leaching loads are (at least within reasonable limits) independent of irrigation rates, and thus dosing of wastewater should be based on nitrogen rather than hydraulic load. High plant growth rates promote nitrogen retention and counteract leaching losses.

Based on the results from the small (68-l) lysimeters (Paper III), I conclude that the nitrogen retention in a SRWC proceeds after the growing season, partly due to root uptake and partly due to gaseous losses (probably mainly denitrification).

Thus, application of nitrogen, e.g. through wastewater, can proceed at low intensity in late autumn after leaf fall, as well as in early spring before bud break.

The results from the long-term study on groundwater quality in an intensively cultured SRWC (Paper I; Fig. 2*c*) suggest that a willow vegetation filter can be highly efficient in retaining high loads of nitrogen during several rotations. However, high soil nitrogen concentrations found in the 14-16-year-old SRWC (unpublished data; Fig 2*d*) point to the possibility of reaching a state of nitrogen saturation, leading to elevated nitrogen leaching losses. Since no relevant research has been carried out on nitrogen saturation phenomenon in ecosystems such as SRWC grown on arable soils, this finding cannot be validated and should therefore be treated with caution.

Virus

The results from the tracer study with bacteriophages (bacterial viruses) suggest that on a structured (clay) soil there is a significant risk that viruses rapidly and at high concentrations reach groundwater or tile drains. Therefore, wastewater irrigation should be avoided on structured soils in groundwater recharge areas if groundwater is used for human consumption. However, on a sandy, non-structured soil, viruses can be efficiently retained enabling safe wastewater irrigation.

Future application and research needs

There is one issue that needs particular attention in order to thoroughly evaluate the long-term efficiency and the possible negative environmental impact of willow vegetation filters for wastewater treatment; the denitrification. It is obvious that denitrification is a very important pathway of nitrogen in an intensively wastewater irrigated willow vegetation filter (Fig. 4). The problem is that denitrification might result both in fully harmless N_2 and in N_2O. Therefore, the denitrification rate and the N_2:N_2O ratio under various conditions as regards soil properties, wastewater loads and biomass productivity should be subject to further, process based studies.

The capacity or affinity of different willow clones to take up and store nitrogen in harvestable shoots varies considerably (Riddell-Black, pers. comm.). Thus, theoretically there could be opportunities to select or even breed clones with a high shoot allocation of nitrogen in order to maximize nitrogen retention and system sustainability.

As regards transport and retention in soils of viruses and other pathogens potentially present in wastewater, the possibility that the presence of plant roots affects the retention is a line that should be followed. If this holds true, it might also be of importance in drinking water supply systems where artificial groundwater recharge, e.g. in eskers, is being practised.

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Epilogue

At the medieval universities, disputations were important academic events. The respondents did not usually defend their own work, but instead, the theses were written by the professors, and preparing and performing the defence of the theses were important parts of the education. Such medieval pedagogics were kept in mind when deciding the outline of this thesis, and when writing it, which justified gormandizing in one's own and others' results.

At this stage, I realise that writing a thesis is indeed a very instructive process, worth repeating.

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