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Fertilization of SRC Willow, II: Leaching and Element Balances

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Abstract Short rotation coppice (SRC) willow is an emerging cropping system in focus for production of biomass for energy. To increase production, the willow is commonly fertilized, but studies have shown differing effects of fertilization on biomass production, ranging from almost no response to considerable positive effects. Focus has also been on replacing mineral fertilizer with organic waste products, such as manure and sludge. However, the effect on biomass production and environmental impact of various dosage and types of fertilizer is not well described. Therefore we studied the environmental impacts of different doses of mineral fertilizer, manure and sewage sludge in a commercially grown SRC willow stand. We examined macro nutrient and heavy metal leaching rates and calculated element balances to evaluate the environmental impact. Growth responses were reported in a former paper (Sevel et al. "Fertilization of SRC Willow, I: Biomass Production Response" Bioenergy Research, 2013). Nitrogen leaching was generally low, between 1 and 7 kg N ha^{-1} year^{-1} when doses of up to 120 kg N ha^{-1} year^{-1} were applied. Higher doses of 240 and 360 kg N ha^{-1} as single applications caused leaching of 66 and 99 kg N ha^{-1} year^{-1}, respectively, indicating N saturation of the system. Previous intensive farming including high doses of fertilizer may be responsible for a high soil N status and the high N leaching rates. However, moderate fertilization input could not compensate P and K exports with the biomass harvest. No elevated leaching of heavy metals was observed for any fertilization treatments and more cadmium than applied with the fertilizer was removed with the biomass from the system.

Keywords COUP water model · Dose–response · Heavy metals · Leaching · Nitrate · *Salix*

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Introduction

Short rotation coppice (SRC) willow is an emerging cropping system in focus for production of biomass for energy. Management of SRC willow resemble conventional agriculture in many ways with focus on genetic material, soil preparation, fertilization, weed and pest control, and fertilization to secure a high biomass production [1–5]. Fertilization has shown varying effects on biomass production ranging from almost no response to considerable positive effects [6–14]. Presumably, the nitrogen status of the soil is crucial [6]. Only limited attention has been paid to the importance of the other macro elements like P and K [15, 16].

Contrary to conventional farming, low nitrate leaching is generally observed in well-established and moderately fertilized SRC willow [14, 17–19]. Rapid juvenile growth, long growing season and the continuously present root system is presumed to explain the relative low leaching. Leaching after harvesting have also been found to be low [11], whereas significant leaching has been reported in the establishment phase [11, 14, 19, 20]. However, a few studies report elevated nitrate leaching following application of large doses of fertilizers [15]. The varying results may be explained by differences in nutrient status of the soil, availability of nutrients in the fertilizer, and the growth rate of the willows [9, 21–24].

Willow is also known to take up the most mobile fractions of heavy metals from the soil and retain them in the biomass. Several studies have examined this ability in relation to phytoremediation purposes [25–28]. In particular enhanced uptake of cadmium (Cd) and zinc (Zn) has been reported [29, 30].

The accumulation of heavy metals combined with the high N uptake, have led to the promotion of SRC willow stands as a multifunctional and environmentally friendly system where organic waste products, such as sewage sludge or waste water, may be applied in large quantities [31–34]. In these systems nutrients in the waste products are utilized, costs for handling the waste products and fertilization cost are reduced, and the capability to take up and store problematic elements such as heavy metals from the sludge and soil is utilized [35, 36].

However, fertilization in well-established SRC willow stands with shoots that may be up to 8 meter tall provides a technical challenge. This may call for nutrient application methods where the fertilizer for a full rotation is applied as a one-time dose, for example right after harvest when conventional farm machinery for fertilization can operate in the field. The effect of such potentially high doses of fertilizers is not well known.

To study the dose–response effects from different fertilization treatments we established a fertilization experiment in a commercially grown SRC willow stand and tested different fertilization doses from 60 kg N annually and up to 360 kg N applied as a single dose in a two year rotation. The experiment included six fertilization treatments with conventional NPK fertilizer, two treatments with dairy cattle manure and two treatments with sewage sludge. We also included treatments comparing the effects of annual versus one time application of the same dose. Growth responses of the fertilization treatments are reported by Sevel et al. [6]. In this paper we reported leaching of macro elements and heavy metals. Furthermore, we evaluated the environmental impact by use of the element balance approach where inputs by deposition and fertilization and outputs by leaching and harvest were used to describe whether elements were accumulated or lost from the soil. We tested the following hypotheses: 1) irrespective of fertilizer type, 120 kg N ha^{-1} $year^{-1}$ can be applied without increase in N leaching; 2) higher N doses increase N leaching significantly; 3) high doses of manure and sludge can be applied without increased leaching of heavy metals.

Materials and Methods

Site Conditions, Experimental Design and Fertilization

The study was established in 2009 in an existing SRC willow plantation planted in 2006 at the commercial farm *Nordic Biomass* in the northern part of Denmark (EUREF89, UTM zone 32N, N: 552400, E: 6369025). The plantation was established with the clone *Tordis*, a high performing commercial cross-breed between the two species *Salix viminalis* and *Salix schwerinii* ((*S. viminalis* × *S. schwerinii*) × *S. viminalis*).The sandy soil was well drained and developed on marine Yoldia Sea sediments. However, the soil texture varied slightly across the experimental area with a higher clay content in the Bt and C horizons in the northern part compared to the southern part, see Sevel et al (2012, submitted to Bioenergy Research) for further details. The experiment was established with ten treatments repeated in three blocks (A, B and C). Each plot was 175 m^2 (20 m×8.75 m). To avoid edge effects, net plots of 60 m^2 (10 m×6 m) were established within each plot. A net plot included three double rows.

Three different types of fertilizers were applied: mineral fertilizer (NPK, 21-3-10), dairy cattle manure, and sewage sludge. The mineral fertilizer was spread by hand, sewage sludge using a wheel barrow and a shovel, and the manure using a commercial spreader. In all cases, care was taken to spread fertilizers evenly and to limit application to the specific plot. The N doses were based on multiplications (0.5, 1 or 1.5) of the legal Danish standard for N application of 120 kg N ha^{-1} $year^{-1}$ to SRC willow stands [2] (Tables 1, 2). Due to a technical failure in the application of manure to plot 6 in Block B ($Manure_{240}$), this plot received more manure than the target and was therefore not included in the dataset. Site conditions and experimental design are described further by Sevel et al. [6].

Sampling

Soil solution was collected by Super Quartz suction cups made of porous PTFE (polytetrafluorethylen, Teflon) mixed with silica flour (Prenart Equipment ApS). In each plot, three suction cups were installed in 100-cm soil depth and connected by PTFE tubes to a sampling bottle placed in an insulated box placed in the soil. All connecting tubes were dug into the soil at a depth of 20 cm to prevent influence from sun light and temperature fluctuations and to enable tillage and driving with machines in the field. The system of soil solution samplers was evacuated by applying a continuous low pressure of 400–500 mbar. Soil solution from the three samples was pooled into one sample (giving a total of 29 samples from each sampling occasion) for chemical analyses. Three PE funnels were placed 2 m above ground on a neighboring field, 50 m from the edge of the experimental site, for collection of

Table 1 Fertilization treatments (all doses in kilogramme per hectare). Application of other elements can be extracted from Tables 8 and 9

Treatment	Target amount N[a]	Applied amount					
		N		P		K	
		2009	2010	2009	2010	2009	2010
Control	0	0		0		0	
NPK_{60+60}	60	59	59	7	7	27	27
NPK_{120}	120	118		15		55	
$NPK_{120+120}$	120	118	118	15	15	110	110
NPK_{240}	240	235		30		220	
NPK_{360}	360	353		45		165	
$Manure_{120}$	120	121		26		91	
$Manure_{240}$	240	241		52		183	
$Sludge_{120}$+NPK_{120}	120	105	118	114	30	15	55
$Sludge_{240}$	240	210		259		31	

[a] Difference between target N dose and real N dose are because actual N concentration were lower than declared from the plant

precipitation. Precipitation samples were used for assessment of wet deposition. Samples from the tree funnels were pooled into one representative sample for chemical analyses.

Table 2 Concentrations of selected elements in the sewage sludge, dairy cattle manure and mineral fertilizer

Element	Sludge mg g^{-1} dw	Manure	NPK
Dry matter	154	78	
Total N	43.7	58.0	206
NH_3–N+NH_4–N	0.6	2.1	115
NO_3–N			91
Total P	53.9	12.5	26
Citrate-soluble P	30.5	6.6	
Water-soluble P	2.8	4.8	18
K	6.5	44.0	96
Ca	21.6	22.7	–
Mg	6.2	9.7	10
	μg g^{-1} dw		
Cu (1000)	105	100	5.6
Zn (4000)	697	448	0.0
Cd (0.8)	1.4	0.3	<0.05
Pb (120)	36.4	1.4	0.0
Ni (30)	15.1	5.8	9.0
Cr (100)	23.3	2.4	3.8

Values in brackets are heavy metal limits in sludge spread on agricultural soils, set by the Danish Ministry of the Environment [67]

All soil solution and precipitation samples were collected monthly from May 2009 to May 2011in polyethylene containers and immediately transported (within 24 h) to the laboratory and stored at 4 °C prior to analyses. During summer, samples were transported in a portable cooling box.

The soil water content was measured monthly using the time domain reflectometry (TDR) technique [37]. TDR probes were installed vertically in 0–50-, 0–100- and 0–150-cm soil depths with three replicates in the control, NPK_{120} and NPK_{360} plots giving 81 measuring points in total. The three treatments were selected to test fertilization effects on the seepage water flux. Soil water measurements were made approximately monthly during the two growing seasons.

Biomass was sampled from each plot as described in Sevel et al. [6]. Samples were dried a 50 °C before chemical analysis.

Chemical Analyses

Soil Solution and Deposition

Samples were analyzed within 1 week from sampling for electrical conductivity and pH (Radiometer: CDM 83 and PHM 85, respectively), ammonium (NH_4–N) by continuous flow colorimetric (Perkin Elmer, FIAS 300, Perkin Elmer UV/VIS Spectrometer, Lambda 2), and chloride (Cl), nitrate (NO_3) and sulfate (SO_4) by high-performance liquid chromatography (Shimadzu LC-10AD, SCL-10Avp, CDD-10Avp). The concentrations of aluminum (Al), calcium (Ca), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P) and iron (Fe) were determined by ICP-OES (Perkin Elmer, Optima 3000). Soil solution samples from control, $NPK_{360,}$ $Manure_{240}$ and $Sludge_{240}$ were further analyzed by ICP-MS (Agilent 7500 C, Santa Clara, CA, USA) for cadmium (Cd), copper (Cu), chromium (Cr), zinc (Zn), lead (Pb) and nickel (Ni). Samples were acidified by adding 1.85 ml 65 % HNO_3 to 100 ml sample giving a concentration of 0.4 M nitric acid and shaken for 2 days in order to minimize adsorption to the container walls.

Willow Biomass

The dried biomass samples were ground first in a mill (Retsch SM 2000) and thereafter in an agate mortar. Carbon (C) and nitrogen (N) were analyzed by the Dumas method (Thermo Flash 2000) and P, K, Ca, Mg, Cd, Cu, Cr, Zn, Pb and Ni were determined in a microwave digest (70 % HNO_3 and 15 % H_2O_2) by ICP-MS (Agilent 7500 C, Santa Clara, CA, USA).

Water Flux Modeling

Seepage water flux at 100-cm soil depth was estimated by the CoupModel [38, 39]. The model estimates daily seepage water and transpiration fluxes based on information on soil properties, plant cover and climatic variables, and has been applied in many studies including willow plantations [38, 40, 41]. No clear treatment effects were found in the measured soil water contents. However, due to soil texture differences across the experimental field it was decided to set up two parameterizations (model I and II) by the use of pedotransfer functions [42]. Plant parameters include leaf area index (LAI), crop height and root depth. Maximum LAI was set to 4 and 6 in first and second growing season, respectively, based on measurements of LAI in a nearby comparable willow field. Height was estimated to develop from 0 to 7 m during the two growing seasons. The root density profile was assumed to decrease exponentially with depth to a maximum depth of 1.6 m based on observations in the two soil pits. The parameterization of the potential evapotranspiration was based on Persson [39] and Grib et al [43]. Precipitation was measured at a nearby climate station and global radiation, relative air humidity, air temperature, and wind speed were obtained from a grid-based dataset (40×40 km) of interpolated climate data from the Danish Meteorological Institute. Precipitation was corrected with the factor 1.15 to account for aerodynamic and wetness losses [44, 45]. Calibration of the soil models (model I and II) was done using the maximum soil water content (TDR values) measured in plots close to pit I and II, respectively, to identify the field capacity. This was assumed to be reached during early spring conditions. Outputs from the modeling were daily seepage water fluxes at various depths and evapotranspiration. Based on the comparison between the measured water content by TDR and the modeled water content of the two models (I and II) it was decided that all plots in block A were best described by model I while the plots from blocks B and C were best described by model II (Fig. 1a, b). The performance of the model setup was evaluated by the Cl balance. The modeled daily seepage fluxes were summed to obtain cumulated seepage representing the periods between water collections from the suction cups. The length of these intervals varied between 15 and 44 days.

Data Validation, Calculations and Statistics

Concentrations of macro elements in soil solution and deposition were validated by calculating the cation–anion balances for each sample. However, due to the high pH in the soil solution (between 7 and 8, data not shown) the concentration of the not-measured HCO_3^- would be considerable and the sum of measured cations exceeds the sum of measured anions. The balances were therefore only used to detect outliers. Outliers were removed if measured conductivity and calculated ion strength also indicated mistakes.

Limit of detection (LOD) was used in flux-calculations when concentrations were below LOD. Element fluxes (in kilogramme per hectare) for each collection period were calculated by multiplying the measured element concentrations with the modeled seepage water flux for the same collection period. Fluxes were summed to estimate the total annual

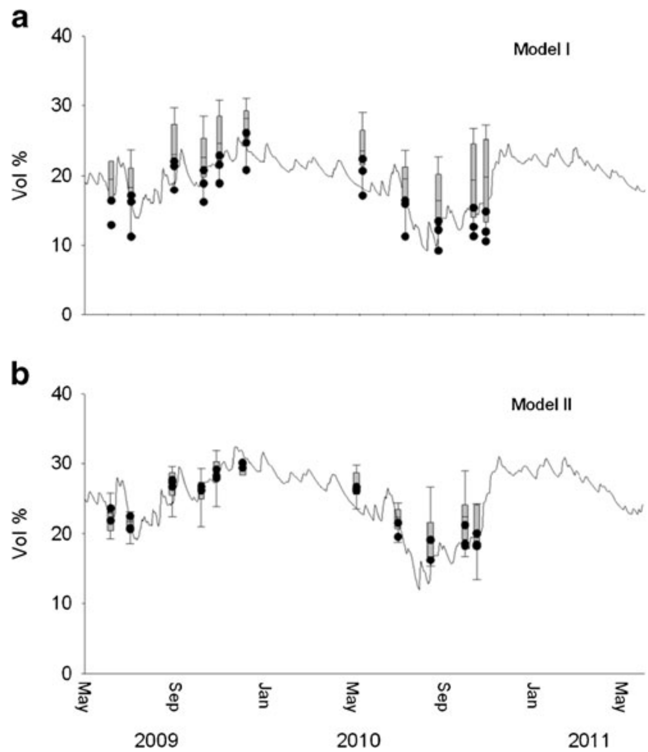

Fig. 1 Measured soil water content for model calibration (*filled circle*) and modeled soil water content (*line*). *Box plots* show all other measured soil water contents. Model I is used for block A, and model II is used for block B and C

leaching flux. During a long frost period with snow cover, no sampling could be done (58 out of totally 696 samples). However, in these periods no (or only negligible) seepage took place. Furthermore, we also missed 180 samples due to a defect pump. To get a complete data set and avoid underestimation, missing values were estimated by linear interpolation.

Total element contents in the harvested biomass were estimated by multiplying the biomass concentrations and the harvested amount of biomass from Sevel et al. [6].

The effects of the different fertilization treatments on the soil water concentrations (C) were analyzed using a mixed effects model, in which a first order autoregressive correlation was assumed between observations (PROC MIXED, [46]):

$$C_{i,j(k)} = N\left(\mu_{ij}, V\right), \text{where}$$
$$\mu_{ij} = \mu + \alpha\left(Treatment_j\right) + \beta(Date_i) + \gamma\left(Treatment_j, Date_i\right), \text{and}$$
$$V_{i_1,i_2} = \begin{cases} 0, \text{ if } j_1 \neq j_2 \text{ and } i_1 \neq i_2 \\ \nu^2 + \tau^2\rho^{|i_1-i_2|}, \text{ if } j_1 = j_2 \text{ and } i_1 \neq i_2 \\ \nu^2 + \tau^2 + \sigma^2, \text{ if } j_1 = j_2 \text{ and } i_1 = i_2 \end{cases}$$

$C_{i,\ j(k)}$ is the nutrient concentration of the ith measurement within the jth plot nested within the kth block. In the covariance structure ν^2 is the within plot covariance, τ^2 is the spatial covariance, ρ is correlation coefficient and σ^2 is the residual variance. The total variance of a single observation is $\nu^2+\tau^2+\sigma^2$.

Further, we analyzed the effect of the different fertilization treatments on the element concentrations in the biomass and the different fluxes and balances estimated using a generalized linear model:

$$X_{jk} = \alpha + \beta[\text{treatment}] + \gamma[\text{block}_k] + \varepsilon_{jk},$$

where X_{jk} is the response variable (element concentration or the flux) of the jth plot and kth block, α and β are model parameters and $\varepsilon_{jk} \approx N(0,\sigma^2)$ is the residual. Duncan's multiple range test was used for comparison of mean values. In this analysis, residuals were found to be non-normal, and we could not find any general transformation mitigating this problem. Results from the statistical analysis should therefore be interpreted with caution.

Results

Elements in Soil Solution and Precipitation

NO_3 concentrations in the soil solution differed significantly between treatments ($P<0.0001$), dates ($P<0.0001$) and between different treatments at different dates ($P<0.0001$). For the control, NPK_{60+60}, NPK_{120}, $Manure_{120}$, $Manure_{240}$ and $Sludge_{240}$ concentrations varied between 0.1 and 3.5 mg NO_3–N L^{-1}. High concentrations were measured for the NPK_{240} and NPK_{360} treatments with peak concentrations in November 2009 (49 mg NO_3–N L^{-1}) and October 2009 (101 mg NO_3–N L^{-1}) 6–7 months after fertilization (Fig. 2). In the second growing season, NO_3 concentrations decreased markedly for both treatments to average concentrations of 3.0 and 7.1 mg NO_3–N L^{-1} and peaks of 5.7 and 15.2 mg NO_3–N L^{-1}, respectively. For the annual fertilizer applications ($NPK_{120+120}$ and $Sludge_{120}$+NPK_{120}) we observed a small

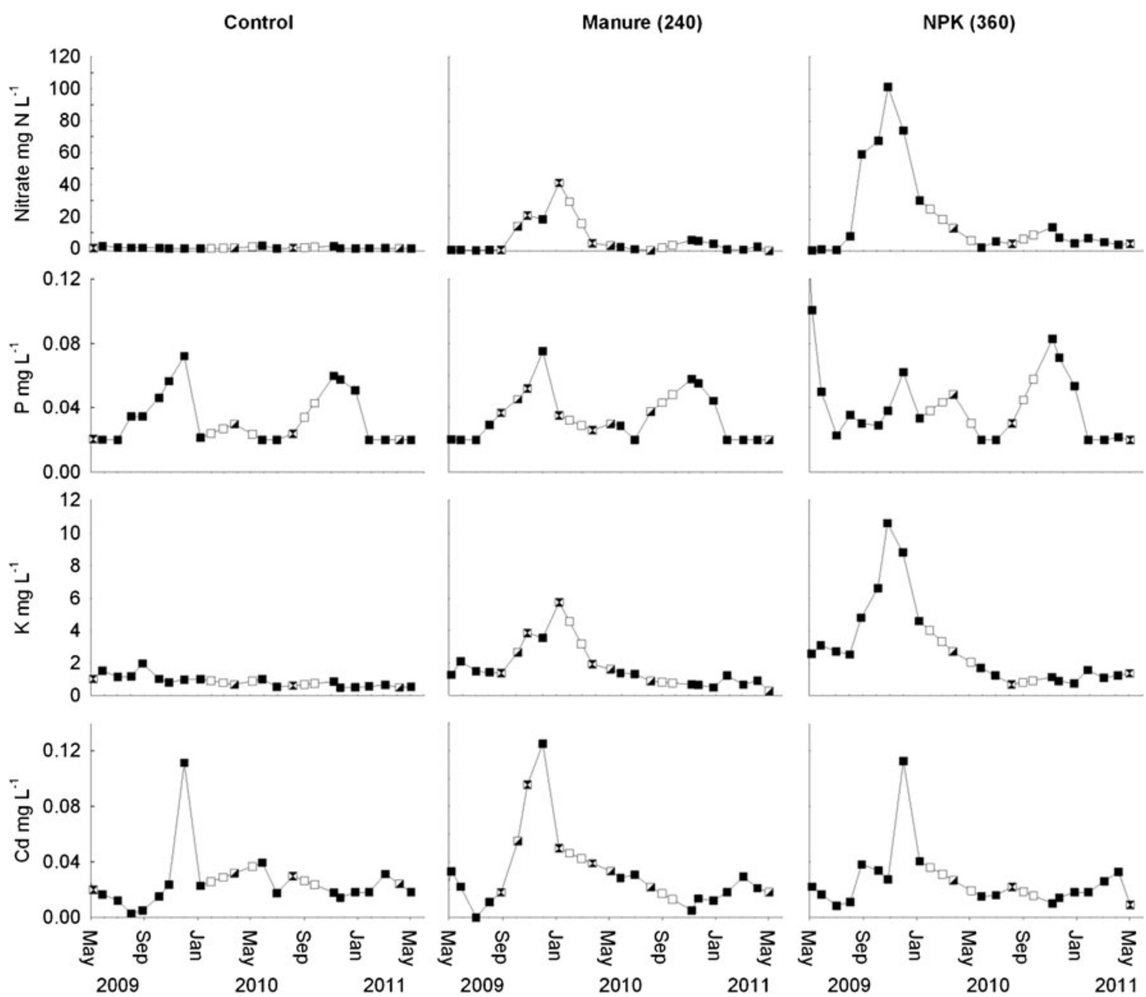

Fig. 2 Concentrations of N (nitrate), P, K and Cd for control, 240 and 360 treatments over the 2 years. Different degrees of square filling illustrate the use of interpolated results due to missing samples. *Filled squares* illustrate measured values from all three blocks, *hour-glass filled squares* illustrate measured values from two blocks and interpolation from the third block, *diagonally filled squares* illustrate measured values from one block and interpolated values from two blocks, and *open squares* illustrate interpolation values from all three blocks. See text for further explanation

increase in peak NO_3 concentrations from the first to the second growing season from 0.7 to 12.7 and from 0.9 to 7.9 mg L^{-1}, respectively (Table 3).

Calcium and Mg concentrations followed a similar pattern as observed for NO_3 with significant differences between the different fertilizer treatments ($P=0.0067$ for Ca and $P=0.0360$ for Mg), different dates ($P<0.0001$ for both Ca and Mg) and among different treatments at different dates ($P<0.0001$ for both Ca and Mg) with elevated concentrations for the NPK_{240} and NPK_{360} treatments (Table 3).

NH_4, P and K concentrations differed significantly between different dates ($P<0.0001$ for NH_4, P and K) and also between different treatments at different dates ($P<0.0001$ for NH_4, $P=0.0420$ for P and $P<0.0001$ for K). However, the main treatment effects were not significant ($P=0.1208$ for NH_4–N, $P=0.4760$ for P and $P=0.3443$ for K) and no differences for P and NH_4 concentrations between treatments were observed with P concentrations well below 0.1 and NH_4 concentrations below 0.04 mg L^{-1}. Although not significant a slight increase in K concentrations for the NPK_{240} and NPK_{360} treatments in November 2009 and October 2009, similar to NO_3 concentrations was observed.

The soil solution concentrations of Cu, Ni and Pb differed significantly between the different treatments, dates and their interactions (P varied from <0.0001 to 0.0233). Cu, Ni and Pb concentrations for the control and NPK_{360} treatments were in the range of 1.0–5.0, 0.5–4.0 and 0.2–2.0 μg L^{-1}, respectively. Higher Cu (up to 5.2 μg L^{-1}) and Ni (up to 2.5 μg L^{-1}) concentrations were observed for the $Sludge_{240}$ treatment and higher Pb concentrations (up to 2.9 μg L^{-1}) were observed for the $Manure_{240}$ treatment.

For Zn, Cd and Cr no differences between different treatments were found ($P=0.2394$, 0.9338, 0.3009). Significant differences between dates and between different treatments for different dates were found ($P<0.0001$) except for the interaction effect for Cd ($P=0.1130$). Concentrations of Zn and Cr were in the range of 0.5–6.0 and 0.2–0.8 ug L^{-1}, respectively, for all treatments. Cadmium concentrations were below 0.04 μg L^{-1} for all treatments except for a peak in October and November 2009 to around 0.1 μg L^{-1}.

Average, minimum and maximum concentrations of all elements in the soil solution are presented in Tables 3 and 4 and average concentrations in the precipitation are presented in Table 5.

Elements in Biomass

The concentration of N in the harvested biomass ranged between 4.0 and 6.1 mg N g^{-1} with the highest concentration in the NPK_{360} treatment and the lowest in the control treatment ($P=0.0499$, Table 6). Similarly P, Mg, Zn concentrations were significantly influenced by the treatments ($P=0.0012$, 0.0082, 0.0341 respectively). However, no effects of the treatments were observed for C, K, Ca, Cd, Cu, Cr, Ni and Pb in the biomass (Tables 6 and 7).

Fluxes and Balances

Seepage Water

A higher water-holding capacity was estimated in the northern part of the experimental area (model II) than in the southern part (model I), resulting in slightly higher modeled leaching rates in the southern part (Fig. 3).

Element Leaching

Generally, N (NO_3+NH_4) leaching was low, between 1 and 7 kg N ha^{-1} $year^{-1}$, from all treatments with the exception of the NPK_{240} and NPK_{360} treatments where leaching was 66 and 99 kg N ha^{-1} $year^{-1}$ respectively (Table 8). Leaching of N from the control plots was 1 kg N ha^{-1} $year^{-1}$. Phosphorus leaching was also low (0.1–0.2 kg P ha^{-1} $year^{-1}$) with no differences between treatments. K, Ca and Mg showed elevated leaching from the NPK_{240} and NPK_{360} treatments, similar to the observed leaching of N.

No differences in leaching rates from the different treatments were seen for Cd and Zn whereas significant differences for Cr, Cu, Ni and Pb were found (Table 9).

Element Export in Harvested Biomass

Between 35–61 kg N ha^{-1} $year^{-1}$, 9–12 kg P ha^{-1} $year^{-1}$, 30–40 kg K ha^{-1} $year^{-1}$, 26–35 kg Ca ha^{-1} $year^{-1}$ and 4–5 kg Mg ha^{-1} $year^{-1}$ were exported with harvested willow shoots (Table 8) with no significant differences between treatments. Cadmium exports ranged between 5.9 and 9.8 g Cd ha^{-1} $year^{-1}$ and were not affected by the different treatments (Table 9). Similarly, there were no effects of treatment on the export of the other heavy metals (Cu, Cr, Zn, Ni and Pb).

Element Balances

During the 2-year rotation, N input (deposition plus fertilization) exceeded output (leaching plus harvesting) except for the control treatment (Table 8). The positive balances indicate that N was accumulated in the system contrary to the non-fertilized control plots where N was removed from the system. No difference in the balance was observed between application of 120 kg N ha^{-1} as a single dose in the first year and 120 kg N ha^{-1} as annual applications of 60 kg N ha^{-1}. Contrary, a significantly lower surplus between the application of 240 kg N ha^{-1} as a single dose and annual applications of 120 kg N ha^{-1} was observed as a result of higher leaching loss after the single application. For P, negative balances were estimated from the control, NPK_{60+60} and NPK_{120} treatments

Table 3 Average, minimum and, maximum concentrations in soil solution (monthly collected from May 2009 to May 2010 and from May 2010 to May 2011)

Element	Treatment	mg L^{-1}					
		2009/2010			2010/2011		
		Avg.	Min.	Max.	Avg.	Min.	Max.
NO_3–N $_{(LOD=0.1)}$	Control	0.5	<0.1	1.6	0.6	<0.1	1.7
	NPK_{60+60}	0.3	<0.1	1.2	1.7	<0.1	3.5
	NPK_{120}	0.5	0.3	0.9	0.3	<0.1	0.4
	$NPK_{120+120}$	0.2	<0.1	0.7	4.8	<0.1	12.7
	NPK_{240}	22.2	1.3	48.9	3.0	1.9	5.7
	NPK_{360}	34.6	1.2	101.4	7.1	2.7	15.2
	$Manure_{120}$	0.3	<0.1	1.0	0.6	<0.1	2.4
	$Manure_{240}$	0.1	<0.1	0.4	0.3	<0.1	0.6
	$Sludge_{120}$+NPK_{120}	0.4	<0.1	0.9	3.1	<0.1	7.9
	$Sludge_{240}$	1.0	0.5	2.5	1.6	0.6	2.7
NH_4–N $_{(LOD=0.05)}$	Control	0.14	<0.05	0.62	0.12	<0.05	0.44
	NPK_{60+60}	0.06	<0.05	0.09	0.06	<0.05	0.09
	NPK_{120}	0.09	<0.05	0.21	0.07	<0.05	0.16
	$NPK_{120+120}$	0.13	0.06	0.37	0.23	<0.05	1.75
	NPK_{240}	0.11	0.06	0.20	0.08	<0.05	0.21
	NPK_{360}	0.06	<0.05	0.08	0.06	<0.05	0.18
	$Manure_{120}$	0.07	<0.05	0.21	0.05	<0.05	0.06
	$Manure_{240}$	0.05	<0.05	0.06	0.06	<0.05	0.15
	$Sludge_{120}$+NPK_{120}	0.06	<0.05	0.07	0.09	<0.05	0.18
	$Sludge_{240}$	0.27	0.09	1.17	0.13	<0.05	0.24
P $_{(LOD=0.02)}$	Control	0.03	0.02	0.07	0.03	0.02	0.06
	NPK_{60+60}	0.03	0.02	0.07	0.03	0.02	0.06
	NPK_{120}	0.04	0.02	0.08	0.04	0.02	0.08
	$NPK_{120+120}$	0.03	0.02	0.07	0.03	0.02	0.06
	NPK_{240}	0.04	0.02	0.07	0.04	0.02	0.06
	NPK_{360}	0.04	0.02	0.06	0.04	0.02	0.08
	$Manure_{120}$	0.03	0.02	0.07	0.04	0.02	0.09
	$Manure_{240}$	0.04	0.02	0.08	0.03	0.02	0.06
	$Sludge_{120}$+NPK_{120}	0.04	0.02	0.07	0.03	0.02	0.06
	$Sludge_{240}$	0.05	0.02	0.09	0.04	0.02	0.09
K $_{(LOD=0.04)}$	Control	1.1	0.7	1.9	0.6	0.5	1.0
	NPK_{60+60}	1.3	0.8	2.2	1.0	0.6	1.8
	NPK_{120}	1.4	1.1	1.8	0.7	0.3	1.0
	$NPK_{120+120}$	1.2	0.9	2.3	1.0	0.7	1.2
	NPK_{240}	3.3	2.4	4.4	1.5	1.2	2.0
	NPK_{360}	4.7	2.1	10.6	1.1	0.7	1.7
	$Manure_{120}$	1.6	1.2	2.2	1.2	0.4	1.5
	$Manure_{240}$	1.5	1.2	1.7	0.8	0.3	1.2
	$Sludge_{120}$+NPK_{120}	1.3	0.8	1.9	0.9	0.4	1.3
	$Sludge_{240}$	1.9	1.3	2.8	1.6	1.1	2.5
Ca $_{(LOD=0.02)}$	Control	26	23	34	21	14	26
	NPK_{60+60}	26	20	33	23	18	31
	NPK_{120}	31	22	44	22	18	25
	$NPK_{120+120}$	32	27	37	35	23	52
	NPK_{240}	81	37	128	33	26	38
	NPK_{360}	87	21	217	28	21	41
	$Manure_{120}$	35	23	48	24	20	27
	$Manure_{240}$	36	27	48	28	22	32
	$Sludge_{120}$+NPK_{120}	29	24	35	29	22	45
	$Sludge_{240}$	24	19	30	21	15	29
Mg $_{(LOD=0.03)}$	Control	2.0	1.4	2.5	1.6	0.9	2.0
	NPK_{60+60}	2.1	1.4	2.6	1.9	1.5	2.6
	NPK_{120}	2.3	1.6	3.4	1.8	1.6	2.6
	$NPK_{120+120}$	2.5	2.0	2.9	2.5	1.9	3.4
	NPK_{240}	5.3	2.6	8.8	2.3	1.8	2.6
	NPK_{360}	6.9	2.0	18.9	2.4	1.8	3.7
	$Manure_{120}$	2.6	1.6	3.9	1.6	1.4	1.9
	$Manure_{240}$	2.4	1.6	3.5	2.0	1.4	2.4
	$Sludge_{120}$+NPK_{120}	2.5	2.1	3.1	2.6	2.0	4.1
	$Sludge_{240}$	2.1	1.6	2.5	1.7	1.1	2.6

LOD limit of detection

Table 4 Average, minimum and, maximum heavy metal concentrations in soil solution (monthly collected from May 2009 to May 2010 and from May 2010 to May 2011)

Element	Treatment	$\mu g\ L^{-1}$					
		2009/2010			2010/2011		
		Avg.	Min.	Max.	Avg.	Min.	Max.
Cd $_{(LOD=0.015)a}$	Control	0.028	<0.015	0.111	0.023	<0.015	0.039
	NPK_{360}	0.033	<0.015	0.113	0.018	<0.015	0.033
	$Manure_{240}$	0.050	<0.015	0.126	0.016	<0.015	0.035
	$Sludge_{240}$	0.057	<0.015	0.144	0.016	<0.015	0.032
Cr $_{(LOD=0.02)}$	Control	0.36	0.19	0.56	0.29	0.18	0.50
	NPK_{360}	0.44	0.21	1.14	0.33	0.22	0.50
	$Manure_{240}$	0.48	0.26	0.61	0.25	0.15	0.61
	$Sludge_{240}$	0.56	0.28	0.77	0.39	0.29	0.51
Cu $_{(LOD=0.02)}$	Control	3.4	2.4	5.3	3.3	2.4	4.1
	NPK_{360}	2.7	0.9	5.1	3.7	1.9	6.9
	$Manure_{240}$	3.8	2.8	5.8	3.0	2.1	4.1
	$Sludge_{240}$	5.2	3.4	9.1	3.8	2.7	4.9
Ni $_{(LOD=0.04)}$	Control	2.0	0.7	8.3	0.8	0.6	1.0
	NPK_{360}	1.2	0.5	5.1	0.8	0.4	1.1
	$Manure_{240}$	1.9	0.8	6.6	1.0	0.5	3.2
	$Sludge_{240}$	2.5	1.8	6.5	1.2	0.9	2.0
Pb $_{(LOD=0.009)}$	Control	0.61	0.18	1.69	0.61	0.31	1.95
	NPK_{360}	0.34	0.12	0.51	0.62	0.21	1.10
	$Manure_{240}$	0.97	0.30	2.89	0.54	0.19	2.86
	$Sludge_{240}$	0.80	0.39	1.30	0.60	0.23	1.32
Zn $_{(LOD=0.08)}$	Control	1.7	0.5	3.2	1.4	0.5	3.2
	NPK_{360}	1.7	1.0	2.1	2.3	0.7	6.2
	$Manure_{240}$	1.7	1.1	2.6	1.1	0.6	2.5
	$Sludge_{240}$	2.9	1.6	4.4	2.0	0.8	5.0

LOD limit of detection

[a] LOD was 0.015, 0.018 and 0.033 $\mu g\ L^{-1}$ for the three different analyzes which samples were analyzed within

and positive balances were only achieved when applying high doses ($NPK_{120+120}$, NPK_{240} or NPK_{360}). Contrary to the N balance, no difference in the P balance was observed between the single and the annual applications. The two sludge treatments resulted in high positive P balances.

Table 5 Average, minimum and, maximum concentrations in precipitation (monthly collected from May 2009 to May 2010 and from May 2010 to May 2011)

Element	$mg\ L^{-1}$					
	2009/2010			2010/2011		
	Avg.	Min.	Max.	Avg.	Min.	Max.
NO_3–N	0.7	0.3	1.1	1.0	0.1	4.0
NH_4–N	0.7	0.1	1.8	1.7	0.05	8.8
P	0.04	0.02	0.1	0.2	0.02	0.9
K	0.5	0.2	2.0	0.9	0.1	3.3
Ca	6.1	3.7	15.9	7.1	2.2	18.4
Mg	1.0	0.3	4.4	1.6	0.4	8.0
pH	6.6	4.7	7.3	6.7	6.2	7.1

The K balance showed the same pattern as the P balance for the NPK treatments with negative balances for the control, NPK_{60+60} and NPK_{120} treatments, and positive balances for the NPK_{240} and NPK_{360} treatments. A high positive K balance resulted from the $Manure_{240}$ contrary to the $Sludge_{240}$ treatment. In the combined treatment ($Sludge_{120}$+NPK_{120}) additional K was applied with the NPK fertilizer in the second year, resulting in a balance between input and output. The Ca balances were negative in all treatments and the Mg balances were negative for all NPK treatments except for the manure and sludge treatments.

The balances were negative for Cd, Cu, Cr, Zn, Ni and Pb for the control and NPK_{360} treatments indicating net export (Table 9). The $Manure_{240}$ and $Sludge_{240}$ treatments resulted in negative Cd balances contrary to balances of Cu, Cr, Zn, Ni and Pb which were positive or close to neutral. The negative heavy metal balances were caused by biomass export rather than leaching losses.

Table 6 Macro nutrient concentrations in the biomass, values in brackets are standard deviation

Treatment	mg g^{-1} dw					
	C	N	P	K	Ca	Mg
Control	490 (30)	4.0c (0.4)	1.14a (0.07)	3.6 (0.34)	3.2 (0.26)	0.50a (0.01)
NPK_{60+60}	500 (30)	4.7bc (0.4)	1.00bcd (0.04)	3.6 (0.31)	2.9 (0.19)	0.44abc (0.02)
NPK_{120}	500 (20)	4.3bc (0.6)	0.97cde (0.09)	3.0 (0.43)	2.9 (0.16)	0.43bc (0.01)
$NPK_{120+120}$	500 (20)	5.3abc (0.3)	0.94de (0.03)	3.0 (0.11)	2.7 (0.33)	0.38c (0.01)
NPK_{240}	490 (20)	4.6bc (1.0)	0.88e (0.07)	3.0 (0.09)	2.8 (0.46)	0.39c (0.04)
NPK_{360}	490 (20)	6.1a (0.6)	1.10bc (0.08)	3.5 (0.36)	3.4 (0.25)	0.47ab (0.02)
$Manure_{120}$	490 (20)	4.4bc (1.2)	0.95cde (0.01)	3.1 (0.18)	2.6 (0.35)	0.44abc (0.05)
$Manure_{240}$	500 (30)	5.1abc (0.6)	1.00bcd (0.01)	3.5 (0.53)	2.8 (0.28)	0.46ab (0.03)
$Sludge_{120}$+NPK_{120}	490 (20)	5.5ab (0.5)	1.07abc (0.06)	3.3 (0.12)	2.8 (0.36)	0.43bc (0.04)
$Sludge_{240}$	490 (30)	4.9abc (0.6)	0.94de (0.06)	3.0 (0.20)	2.8 (0.15)	0.43bc (0.05)

Lowercase letters indicate significant different concentrations between treatments

Discussion

Leaching

We generally measured low NO_3 concentrations (<3.5 mg NO_3–N L^{-1}) in the soil solution at 100-cm soil depth irrespectively of the different sources of N. The average concentrations in the seepage water (element leaching flux divided by the seepage water flux) used as an indicator of the potential environmental impact varied between 0.1 and 4.7 mg NO_3–N L^{-1}, almost at the same concentration level as in the soil solution and well below the Danish limit for drinking water (11.3 mg NO_3–N L^{-1}). Similar low concentrations have been reported from Sweden where concentrations of less than 0.5 mg N L^{-1} (NO_3+NH_4) were reported in groundwater 2 m below a long-term SRC willow fertilization experiment fertilized and irrigated annually with an average of 112 kg N ha^{-1} [17]. In the UK, NO_3 concentrations of around 2 mg NO_3–N L^{-1} in the seepage water (measured at 0.65 m depth) were reported during a 3-year period after application of 40, 60 and 100 kg N ha^{-1} in year 1, 2 and 3, respectively [19]. A Danish fertilization experiment showed less than 5 mg NO_3–N L^{-1} in the first rotation period except in the establishment year where concentrations up to 75–100 mg NO_3–N L^{-1} was found [14]. No increase in NO_3 leaching was seen in the second rotation after a single application of up to 280 kg N ha^{-1} as cattle manure [47]. However, after two consecutive years with repeated application of 280 kg N ha^{-1} an increase up to 10–20 mg NO_3–N L^{-1} was observed [47]. Our study supports the generally very low leaching rates from well-established SRC willow when doses lower than 240 kg N annually are applied.

We measured high NO_3 concentrations in the soil solution with peaks of 49 and 101 mg NO_3–N L^{-1} in the NPK_{240} and NPK_{360} treatments. The average concentrations in the seepage water were 30 and 44 mg NO_3–N L^{-1} in the NPK_{240} and NPK_{360} treatments, respectively, in the first year, but only 3 and 7 mg NO_3–N L^{-1} in the second year. High NO_3 leaching has mainly been reported in relation to the establishment phase

Table 7 Heavy metal concentrations in the biomass, values in brackets are standard deviation

Treatment	μg g^{-1} dw					
	Cd	Cr	Cu	Ni	Pb	Zn
Control	1.10 (0.55)	0.17 (0.07)	3.93 (0.15)	0.66 (0.23)	0.07 (0.03)	104a (15)
NPK_{60+60}	0.62 (0.07)	0.19 (0.12)	2.93 (0.59)	0.91 (0.78)	0.18 (0.14)	76abc (16)
NPK_{120}	0.57 (0.17)	0.19 (0.13)	3.42 (0.48)	0.31 (0.06)	1.37 (2.22)	77 abc (15)
$NPK_{120+120}$	0.58 (0.08)	0.14 (0.03)	2.41 (0.33)	0.31 (0.07)	0.10 (0.10)	66c (3)
NPK_{240}	0.57 (0.26)	0.11 (0.01)	2.38 (0.15)	0.30 (0.11)	0.08 (0.04)	65c (21)
NPK_{360}	0.61 (0.15)	0.22 (0.10)	2.10 (0.35)	0.69 (0.52)	0.22 (0.22)	63c (13)
$Manure_{120}$	0.55 (0.08)	0.19 (0.07)	3.29 (0.23)	0.42 (0.26)	0.10 (0.06)	88abc (10)
$Manure_{240}$	0.69 (0.33)	0.16 (0.09)	3.48 (0.09)	0.46 (0.37)	0.06 (0.01)	95ab (16)
$Sludge_{120}$+NPK_{120}	0.75 (0.13)	0.18 (0.10)	2.61 (0.46)	0.37 (0.06)	0.10 (0.07)	67bc (15)
$Sludge_{240}$	0.64 (0.09)	0.25 (0.22)	7.21 (7.33)	0.44 (0.20)	0.25 (0.28)	89abc (17)

Lowercase letters indicate significant different concentrations between treatments

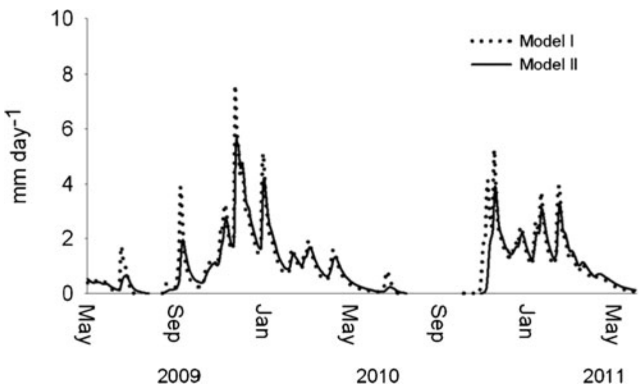

Fig. 3 Modeled seepage water flux

where a low plant uptake combined with high mineralization rates due to soil cultivation might cause leaching to the aquatic environment [11, 14, 19, 20].

A few studies report elevated leaching from well-established SRC willow. Cavanagh et al [15] investigated the effect of fertilization with pig slurry (four treatments of 148–590 kg N ha^{-1}) and mineral fertilizer (3 treatments of 100–300 kg N ha^{-1}) on biomass production and NO_3 leaching during the first 2 years. Due to incoherent data, they only presented data on leaching from the second year. They found elevated NO_3 leaching from slurry and mineral fertilizer (16–19 mg NO_3–N L^{-1}) compared to the control plots (0.5 mg NO_3–N L^{-1}). In a lysimeter study of different scenarios, Aronsson [31] and Dimitriou and Aronsson [48] reported concentrations of 80 to 100 mg NO_3–N L^{-1} immediately after the application of 360 kg N as waste water or mineral fertilizer. A month after application, the concentrations had declined to 0–1 mg NO_3–N L^{-1}. Aronsson [31] also reported elevated NO_3 concentrations in the seepage water (10–30 mg NO_3-N L^{-1}) from an established SRC willow field in Uppsala fertilized with 100–200 kg N ha^{-1} $year^{-1}$. Although he questioned the sampling method, he hypothesized that N saturation might have occurred.

The high leaching rates indicate that excess amounts of applied N could be retained neither in the soil nor as plant uptake, indicating N saturation [49, 50]. The absence of growth effects of doses higher than 60 kg N ha^{-1} annually in this experiment [6] support the notion that the system may be N saturated. Furthermore, a higher N concentration in the biomass in the NPK_{360} treatment was seen which could indicate a higher uptake and N saturation as reported by Ericsson [51] and Aronsson [31]. Nitrogen could also be stored, at least temporarily, by weeds. Weed cover was not systematically assessed in this study, but visual observations indicated a much higher intensity and vitality of weeds in the NPK_{240} and NPK_{360} treatments compared to the plots with lower N doses.

Soil C/N ratio has been reported as an effective indicator of the N mineralization with net release of N from soils with a C/N ratio less than 15 [52]. The C/N ratio of 11 in the top mineral soil in this study [6] indicates a possible net-release, probably caused by the former intensive agriculture practice.

We found a higher N leaching from the application of 240 kg N ha^{-1} as a single dose compared to annual doses of 120 kg N ha^{-1}during the 2-year rotation. The amount of N removed by harvest for the NPK_{240} and $NPK_{120+120}$ treatments were similar and no difference in the biomass production was found, indicating a better N use efficiency of annual application compared to a single dosage. N retained in stumps and roots was not estimated in this study although this fraction has been found to account for a considerable amount of the N retained in a SRC willow stand [31]. Hence, this fraction combined with the ability to build in the surplus of N in the soil organic matter probably explains the difference in leaching between the annual and single application of fertilizer.

Based on our findings and the reviewed literature in the above, it seems possible to apply up to 120 kg N ha^{-1} mineral fertilizer annually and up to 240 kg N ha^{-1} as a single dose of manure or sludge even on a well fertilized soil with a low C/N ration without increasing the risk of elevated N leaching. On soils with a high N status, single application of higher doses pose a risk of N leaching.

The concentrations of Cd, Cu, Cr, Zn, Ni and Pb in the soil solution were low and far below the limit for drinking water for all elements. This is in concordance with several studies reporting low mobility in near neutral soils [53–56]. Other factors such as soil organic matter content and texture may also be important for the mobility and thus the bioavailability [55].

Balances

We estimated balances by the differences in inputs by deposition and fertilization and outputs by leaching and harvest. The negative N balance for the control was caused by removal with harvested biomass. Contrary to this, all fertilized treatments resulted in positive balances, indicating an increase in the N storage in the system, presumably in the soil. Despite increased leaching of nitrate and harvesting from the high doses, the N pools in the system increased. Other studies report similar positive N balances and increases in soil organic nitrogen [11, 14, 57, 58]. Organic fertilizers such as manure and sludge contain a substantial fraction of organic bound N of which a part stays in relative stable compounds contributing to soil organic matter.

N might also be lost as gaseous compounds (N_2, NO, NO_2 or N_2O) through denitrification of NO_3 or volatilization of NH_3. We did not measure those fluxes and we might therefore

Table 8 Fluxes of inputs by deposition and fertilization, outputs by leaching and harvest, and the overall balance

Element	Treatment	Input		Output		Balance
		Deposition kg ha^{-1} $year^{-1}$	Fertilization	Leaching	Harvesting	
Total N (deposition and leaching: NO_3–N+NH_4–N)	Control	16	0	1b	35	−21e
	NPK_{60+60}	16	59	4 b	56	15ed
	NPK_{120}	16	59	2 b	50	23dec
	$NPK_{120+120}$	16	118	7 b	54	72ab
	NPK_{240}	16	118	66 a	47	21dec
	NPK_{360}	16	177	99 a	61	32bcd
	$Manure_{120}$	16	61	1 b	42	32bcd
	$Manure_{240}$	16	121	1 b	46	90a
	$Sludge_{120}$+NPK_{120}	16	112	8 b	57	63abc
	$Sludge_{240}$	16	105	5 b	50	66abc
P	Control	1.1	0	0.1	10	−9g
	NPK_{60+60}	1.1	8	0.1	12	−3f
	NPK_{120}	1.1	8	0.2	11	−3f
	$NPK_{120+120}$	1.1	15	0.1	10	6e
	NPK_{240}	1.1	15	0.1	9	7e
	NPK_{360}	1.1	23	0.1	11	13d
	$Manure_{120}$	1.1	13	0.1	9	5e
	$Manure_{240}$	1.1	26	0.2	10	17c
	$Sludge_{120}$+NPK_{120}	1.1	72	0.1	11	62b
	$Sludge_{240}$	1.1	130	0.2	10	121a
K	Control	5	0	3b	31	−29f
	NPK_{60+60}	5	28	4b	40	−11f
	NPK_{120}	5	28	4b	35	−7de
	$NPK_{120+120}$	5	55	3b	31	25c
	NPK_{240}	5	55	10ab	32	18c
	NPK_{360}	5	83	13a	34	40b
	$Manure_{120}$	5	46	5b	30	15c
	$Manure_{240}$	5	92	4b	34	57a
	$Sludge_{120}$+NPK_{120}	5	35	3b	34	2d
	$Sludge_{240}$	5	16	6b	31	−17ef
Ca	Control	55	0	85b	28	−58a
	NPK_{60+60}	55	[a]	94b	35	−74a
	NPK_{120}	55	[a]	102b	34	−80a
	$NPK_{120+120}$	55	[a]	118b	28	−91a
	NPK_{240}	55	[a]	252a	29	−226b
	NPK_{360}	55	[a]	265a	34	−244b
	$Manure_{120}$	55	24	116b	26	−63a
	$Manure_{240}$	55	47	122b	27	−47a
	$Sludge_{120}$+NPK_{120}	55	26	107b	29	−55a
	$Sludge_{240}$	55	52	82b	29	−3a
Mg	Control	8	0	6b	4	−2dc
	NPK_{60+60}	8	2	8b	5	−3dc
	NPK_{120}	8	3	8b	5	−2dc
	$NPK_{120+120}$	8	6	9b	4	1c
	NPK_{240}	8	6	18a	4	−9de
	NPK_{360}	8	9	24a	5	−12e
	$Manure_{120}$	8	10	9b	4	5bc
	$Manure_{240}$	8	20	9b	4	15a
	$Sludge_{120}$+NPK_{120}	8	11	10b	4	4bc
	$Sludge_{240}$	8	15	7b	4	12ab

Lowercase letters indicate significant difference between treatments

[a] No data

Table 9 Fluxes of inputs by deposition and fertilization, outputs by leaching and harvest, and the overall balance

Element	Treatment	Input	Output		Balance
		Fertilizer g ha^{-1} year^{-1}	Leaching	Harvest	
Cd	Control	0.0	0.1[a]	9.8	−9.9[a]
	NPK_{360}	0.1	0.1[a]	5.9	−6.0[a]
	$Manure_{240}$	0.7	0.2[a]	6.3	−5.8[a]
	$Sludge_{240}$	3.4	0.2[a]	6.6	−3.4[a]
Cu	Control	0	11[ab]	34	−45[b]
	NPK_{360}	5	98[a]	21	−25[b]
	$Manure_{240}$	208	11[ab]	34	163[a]
	$Sludge_{240}$	253	13[a]	71	169[a]
Cr	Control	0.0	1.0[b]	1.5	−2.5[c]
	NPK_{360}	3.3	1.4[ab]	2.3	−0.5[c]
	$Manure_{240}$	5.0	1.4[ab]	1.4	2.2[b]
	$Sludge_{240}$	56.0	1.6[a]	2.4	52.0[a]
Zn	Control	0	5.4[a]	895	−900[c]
	NPK_{360}	0	6.9[a]	641	−648[c]
	$Manure_{240}$	933	5.0[a]	901	−26[b]
	$Sludge_{240}$	1672	9.5[a]	910	753[a]
Ni	Control	0.0	3.6[ab]	5.6	−9.2[c]
	NPK_{360}	7.8	2.5[b]	7.4	−2.1[bc]
	$Manure_{240}$	12.2	4.4[ab]	4.0	3.7[b]
	$Sludge_{240}$	36.3	5.4[a]	4.4	26.5[a]
Pb	Control	0.0	1.8[ab]	0.6	−2.4[b]
	NPK_{360}	0.0	1.3[b]	2.4	−3.7[b]
	$Manure_{240}$	3.0	2.4[a]	0.6	0[b]
	$Sludge_{240}$	88.0	2.1[ab]	2.4	83.2[a]

Lowercase letters indicate significant difference between treatments

have overestimated the effect on N storage in the soil. However, usually they are of minor quantitative importance compared to the other fluxes [58, 59] except from manure or sludge.

Even though high P doses, well above the Danish legislation limit of 90 kg P ha^{-1}, were applied over a 3-year period for the two sludge treatments (144 and 259 kg P ha^{-1}), neither elevated leaching nor any difference in P uptake by the biomass were observed, indicating a strong adsorption in the soil. Repeated application of high P doses may lead to accumulation in the soil and possibly saturation, increasing the risk of run-off or leaching, as reported by Dimitriou et al [60] and observed for many arable soils in Denmark [61]. In the control and low-dose plots, however, the balances were negative indicating a need also to consider P application from a nutritional viewpoint, at least in the long-term perspective.

Negative K balances were estimated for the control, the 120 kg N as NPK_{60+60} or NPK_{120} and the $Sludge_{240}$ treatments. Harvesting was the main responsible for the net export, and K application should also be considered to avoid K deficiency [62]. Negative Ca balances were estimated for all treatments mainly as a result of high leaching fluxes. Previous liming might be the reason for the high Ca status in the soil and leaching.

The negative balances for all heavy metals in the control and NPK_{360} treatment indicates a net removal from the soil

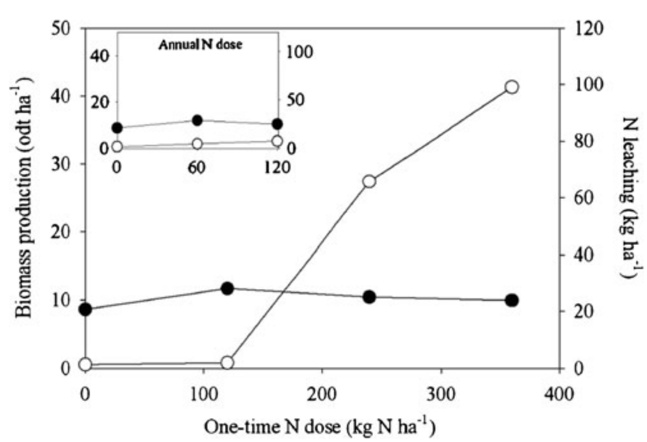

Fig. 4 Biomass production (*filled circles*) and N leaching (*empty circles*) responses of increasing N dose application (NPK). The *insert* shows annual application

primarily caused by biomass harvesting whereas leaching was negligible. Similar negative balances for Cd, Cu, Ni and Zn were also reported from a Swedish study of SRC willow fertilized with NPK [63]. Although varying results are reported in the literature, willow on moderately polluted agricultural soils fertilized with mineral fertilizer often results in negative balance with respect to plant available heavy metals such as Cd, Cu, Ni and Zn due to high up-take rates. A slightly different picture was observed from the $Manure_{240}$ and $Sludge_{240}$ treatments where higher inputs of Cr, Cu, Zn, Pb and Ni with the manure and sludge resulted in neutral balances or even small surpluses. However, Cd removal with biomass was still higher than the application in the manure and sludge treatments resulting in a release from the soil.

The high affinity for the heavy metals has inspired to use the phyto-remediative potential of SRC willow [27–29]. The potential seems to be most relevant for Cd, as several studies have reported high Cd uptake rates from polluted soils [26, 28, 30, 64]. Jensen et al [29] measured concentrations up to 27 mg Cd kg^{-1} in the biomass compared to 0.6–1.1 mg Cd kg^{-1} observed in the present study. From unpolluted soils, biomass concentrations are in the range between 0.5 and 5 mg Cd kg^{-1} [26, 63, 65–67]. The accumulation of Cr, Cu, Zn, Pb and Ni in the soil as indicated by the positive balances may however become a potential problem if accumulation continues and soil acidity increases which could lead to increased mobility and leaching.

Fertilization, Growth Response and Environmental Impacts

This study was conducted to investigate element leaching with focus on nitrate and heavy metal accumulation in the soil in relation to fertilization. However, the full picture should include the growth responses as well. The optimal N fertilization considering both growth and leaching may be annual applications of 60 kg N ha^{-1} but annual applications of up to 120 kg N ha^{-1} may be acceptable (Fig. 4). In the long-term perspective, attention should be paid to the P and K status as we observed substantial net exports mainly with the harvested biomass resulting in negative balances. Leaching of heavy metals was not being a problem in this study, even when high doses of sludge were applied. Long-term application of high doses of sludge could, however, be a problem if soil acidity increases as increased mobility and leaching might occur.

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References

1. Danfors B, Ledin S, Rosenqvist H (1997) Energiskogsodling: handledning för odlare. Jordbrukstekniska Institutet
2. Dansk Landbrugsrådgivning (2011) Dyrkningsvejledning, Pil (in Danish). Dansk Landbrugsrådgivning. https://www.landbrugsinfo.dk/planteavl/afgroeder/energiafgroeder/pil-energiskov/sider/startside.aspx. Accessed 10 Jan 2011
3. DEFRA (2004) Best practice guidelines for applicants to DEFRA's energy crops scheme—growing short rotation coppice. DEFRA Publication, Publication
4. Jordbruksverket (2012) Handbok för salixodlare (in Swedish). http://www2.jordbruksverket.se/webdav/files/SJV/trycksaker/Pdf_ovrigt/ovr250.pdf
5. Sennerby-Forsse L (1986) Energiskog—Handbok i praktisk odling (in Swedish). Avdelningen för energiskog, Institutionen för ekologi och miljövård
6. Sevel L, Nord-Larsen T, Ingerslev M, Jørgensen U, Raulund-Rasmussen K (2013) Fertilization of SRC Willow, I: Biomass Production Response. Bioenerg Res. doi:10.1007/s12155-013-9371-y
7. Adegbidi HG, Volk TA, White EH, Abrahamson LP, Briggs RD, Bickelhaupt DH (2001) Biomass and nutrient removal by willow clones in experimental bioenergy plantations in New York State. Biomass Bioenergy 20(6):399–411
8. Adegbidi HG, Briggs RD, Volk TA, White EH, Abrahamson LP (2003) Effect of organic amendments and slow-release nitrogen fertilizer on willow biomass production and soil chemical characteristics. Biomass Bioenergy 25(4):389–398
9. Alriksson B (1997) Influence of site factors on Salix growth with emphasis on nitrogen response under different soil conditions. Acta Universitatis agriculturae Sueciae, Silvestria, 46
10. Aronsson P, Rosenqvist H (2011) Gödslingsrekommendationer för salix 2011 (In Swedish). SLU, Institut för Växtproduktionsekologi, Rapport 23 marts 2011
11. Aronsson PG, Bergstrom LF (2001) Nitrate leaching from lysimeter-grown short-rotation willow coppice in relation to N-application, irrigation and soil type. Biomass Bioenergy 21(3):155–164
12. Kopp RF, Abrahamson LP, White EH, Volk TA, Nowak CA, Fillhart RC (2001) Willow biomass production during ten successive annual harvests. Biomass Bioenergy 20(1):1–7
13. Lærke P, Jørgensen U, Kjeldsen J (2010) Udbytte af pil fra 15 års forsøg (in Danish). Plantekongres 2010 Conference Proceedings, pp. 232–233
14. Mortensen J, Nielsen KH, Jorgensen U (1998) Nitrate leaching during establishment of willow (*Salix viminalis*) on two soil types and at two fertilization levels. Biomass Bioenergy 15(6):457–466
15. Cavanagh A, Gasser MO, Labrecque M (2011) Pig slurry as fertilizer on willow plantation. Biomass Bioenergy 35(10):4165–4173
16. Park BB, Yanai RD, Sahm JM, Lee DK, Abrahamson LP (2005) Wood ash effects on plant and soil in a willow bioenergy plantation. Biomass Bioenergy 28(4):355–365
17. Aronsson PG, Bergstrom LF, Elowson SNE (2000) Long-term influence of intensively cultured short-rotation Willow Coppice on nitrogen concentrations in groundwater. J Environ Manag 58 (2):135–145
18. Bergstrom L, Johansson R (1992) Influence of fertilized short-rotation forest plantations on nitrogen concentrations in groundwater. Soil Use Manag 8(1):36–40
19. Goodlass G, Green M, Hilton B, McDonough S (2007) Nitrate leaching from short-rotation coppice. Soil Use Manag 23(2):178–184
20. Dimitriou I, Aronsson P (2011) Wastewater and sewage sludge application to willows and poplars grown in lysimeters—plant response and treatment efficiency. Biomass Bioenergy 35(1):161–170
21. Hofmann-Schielle C, Jug A, Makeschin F, Rehfuess KE (1999) Short-rotation plantations of balsam poplars, aspen and willows on

former arable land in the Federal Republic of Germany. I. Site–growth relationships. For Ecol Manag 121(1–2):41–55
22. Labrecque M, Teodorescu TI, Daigle S (1998) Early performance and nutrition of two willow species in short-rotation intensive culture fertilized with wastewater sludge and impact on the soil characteristics. Can J For Res Rev Can de Rech Forestiere 28(11):1621–1635
23. Labrecque M, Teodorescu TI (2001) Influence of plantation site and wastewater sludge fertilization on the performance and foliar nutrient status of two willow species grown under SRIC in southern Quebec (Canada). For Ecol Manag 150(3):223–239
24. Labrecque M, Teodorescu TI, Daigle S (1997) Biomass productivity and wood energy of Salix species after 2 years growth in SRIC fertilized with wastewater sludge. Biomass Bioenergy 12(6):409–417
25. Hammer D, Kayser A, Keller C (2003) Phytoextraction of Cd and Zn with *Salix viminalis* in field trials. Soil Use Manag 19(3):187–192
26. Landberg T, Greger M (1996) Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. Appl Geochem 11(1–2):175–180
27. Marmiroli M, Pietrini F, Maestri E, Zacchini M, Marmiroli N, Massacci A (2011) Growth, physiological and molecular traits in Salicaceae trees investigated for phytoremediation of heavy metals and organics. Tree Physiol 31(12):1319–1334
28. Pulford ID, Riddell-Black D, Stewart C (2002) Heavy metal uptake by willow clones from sewage sludge-treated soil: the potential for phytoremediation. Int J Phytoremediation 4(1):59–72
29. Jensen JK, Holm PE, Nejrup J, Larsen MB, Borggaard OK (2009) The potential of willow for remediation of heavy metal polluted calcareous urban soils. Environ Pollut 157(3):931–937
30. Pulford I, Dickinson N(2005) Phytoremediation technologies using trees. Trace elements in the environment. CRC Press, Bocca Raton, pp. 383–403
31. Aronsson P (2000) Nitrogen retention in vegetation filters of short-rotation willow coppice. Acta Univ Agric Sueciae Silvestria 161:1–39
32. Aronsson P, Perttu K (2001) Willow vegetation filters for wastewater treatment and soil remediation combined with biomass production. Forest Chron 77(2):293–299
33. Dimitriou I (2005) Performance and sustainability of short-rotation energy crops treated with municipal and industrial residues. Acta Univ Agric Sueciae 44
34. DEFRA (2010) Fertiliser Manual (RB209) 8th Edition. DEFRA Publication
35. Borjesson P, Berndes G (2006) The prospects for willow plantations for wastewater treatment in Sweden. Biomass Bioenergy 30(5):428–438
36. Dimitriou I, Rosenqvist H (2011) Sewage sludge and wastewater fertilisation of short rotation coppice (SRC) for increased bioenergy production—biological and economic potential. Biomass Bioenergy 35(2):835–842
37. Robinson DA, Jones SB, Wraith JM, Or D, Friedman SP (2003) A review of advances in dielectric and electrical conductivity measurement in soils using time domain reflectometry. Vadose Zone J 2(4):444–475
38. Jansson PE, Cienciala E, Grelle A, Kellner E, Lindahl A, Lundblad M (1999) Simulated evapotranspiration from the Norunda forest stand during the growing season of a dry year. Agric For Meteorol 98–9:621–628
39. Persson G (1995) Willow stand evapotranspiration simulated for Swedish soils. Agric Water Manag 28(4):271–293
40. Christiansen JR, Elberling B, Jansson PE (2006) Modelling water balance and nitrate leaching in temperate Norway spruce and beech forests located on the same soil type with the CoupModel. For Ecol Manag 237(1–3):545–556
41. Persson G, Lindroth A (1994) Simulating evaporation from short-rotation forest—variations within and between seasons. J Hydrol 156(1–4):21–45
42. Katterer T, Andren O, Jansson PE (2006) Pedotransfer functions for estimating plant available water and bulk density in Swedish agricultural soils. Acta Agric Scand Sect B Soil Plant Sci 56(4):263–276
43. Grip H, Halldin S, Lindroth A (1989) Water-use by intensively cultivated willow using estimated stomatal parameter values. Hydrol Process 3(1):51–63
44. Allerup P, Madsen H, Vejen F (1997) A comprehensive model for correcting point precipitation. Nord Hydrol 28(1):1–20
45. Michelson DB (2004) Systematic correction of precipitation gauge observations using analyzed meteorological variables. J Hydrol 290(3–4):161–177
46. SAS Institute Inc (2008) SAS® 9.2 Software. Copyright 2002–2008 by SAS Institute Inc., Cary, NC, USA
47. Jørgensen U (2005) How to reduce nitrate leaching by production of perennial energy crops. In 3rd International Nitrogen Conference, Nanjing, China, 2004 Edited by Zhu Z, Minami K, Xing G Science Press USA Inc : 2005: 513–518
48. Dimitriou L, Aronsson P (2004) Nitrogen leaching from short-rotation willow coppice after intensive irrigation with wastewater. Biomass Bioenergy 26(5):433–441
49. Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M et al (1998) Nitrogen saturation in temperate forest ecosystems—hypotheses revisited. Bioscience 48(11):921–934
50. Gundersen P, Schmidt IK, Raulund-Rasmussen K (2006) Leaching of nitrate from temperate forests—effects of air pollution and forest management. Environ Rev 14(1):1–57
51. Ericsson T (1994) Nutrient cycling in energy forest plantations. Biomass Bioenergy 6(1–2):115–121
52. Springob G, Kirchmann H (2003) Bulk soil C to N ratio as a simple measure of net N mineralization from stabilized soil organic matter in sandy arable soils. Soil Biol Biochem 35(4): 629–632
53. Andersen MK, Refsgaard A, Raulund-Rasmussen K, Strobel BW, Hansen HCB (2002) Content, distribution, and solubility of cadmium in arable and forest soils. Soil Sci Soc Am J 66(6): 1829–1835
54. Holm PE, Rootzen H, Borggaard OK, Moberg JP, Christensen TH (2003) Correlation of cadmium distribution coefficients to soil characteristics. J Environ Qual 32(1):138–145
55. Sauve S, Hendershot W, Allen HE (2000) Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. Environ Sci Technol 34(7):1125–1131
56. Sukreeyapongse O, Holm PE, Strobel BW, Panichsakpatana S, Magid J, Hansen HCB (2002) pH-dependent release of cadmium, copper, and lead from natural and sludge-amended soils. J Environ Qual 31(6):1901–1909
57. Grelle A, Aronsson P, Weslien P, Klemedtsson L, Lindroth A (2007) Large carbon-sink potential by Kyoto forests in Sweden—a case study on willow plantations. Tellus Ser B Chem Phys Meteorol 59(5):910–918
58. Hellebrand HJ, Strahle M, Scholz V, Kern J (2010) Soil carbon, soil nitrate, and soil emissions of nitrous oxide during cultivation of energy crops. Nutr Cycl Agroecosyst 87(2):175–186
59. Hellebrand HJ, Scholz V, Kern J (2008) Fertiliser induced nitrous oxide emissions during energy crop cultivation on loamy sand soils. Atmos Environ 42(36):8403–8411
60. Dimitriou I, Mola-Yudego B, Aronsson P (2012) Impact of willow short rotation coppice on water quality. Bioenergy Res 5(3):537–545
61. Kyllingsbaek A, Hansen JF (2007) Development in nutrient balances in Danish agriculture 1980–2004. Nutr Cycl Agroecosyst 79(3):267–280

62. Hasselgren K (1998) Use of municipal waste products in energy forestry: highlights from 15 years of experience. Biomass Bioenergy 15(1):71–74
63. Dimitriou I, Eriksson J, Adler A, Aronsson P, Verwijst T (2006) Fate of heavy metals after application of sewage sludge and wood-ash mixtures to short-rotation willow coppice. Environ Pollut 142(1): 160–169
64. Landberg T, Greger M (2002) Interclonal variation of heavy metal interactions in *Salix viminalis*. Environ Toxicol Chem 21(12):2669–2674
65. Eriksson J, Ledin S (1999) Changes in phytoavailability and concentration of cadmium in soil following long term *Salix* cropping. Water Air Soil Pollut 114(1–2):171–184
66. Klang-Westin E, Perttu K (2002) Effects of nutrient supply and soil cadmium concentration on cadmium removal by willow. Biomass Bioenergy 23(6):415–426
67. Mleczek M, Rutkowski P, Rissmann I, Kaczmarek Z, Golinski P, Szentner K et al (2010) Biomass productivity and phytoremediation potential of *Salix alba* and *Salix viminalis*. Biomass Bioenergy 34(9): 1410–1418