Function and biomass production of willow wetlands applied in the polishing phase of sewage treatment in cold climate conditions

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Abstract

Willow wetlands can offer a low-cost solution for recovery of nutrients contained in sewage water and simultaneously produce plant biomass, which can be used in energy production. Willow (*Salix* spp.) is considered an excellent crop for this purpose, due to its good nutrient uptake and biomass production. Although willow wetlands have been used in sewage treatment in e.g. Denmark, Sweden and southern Finland, their use in northern regions is challenging due to the detrimental effects cold climate conditions can have on plant survival rates and wastewater purification efficiency. In this study, a pilot constructed wetland in northern Finland receiving effluent from a small-scale wastewater treatment plant was investigated. Four willow varieties were planted (Gudrun, Karin, Klara and one local variety) and retention of nutrients in the wetland and willow plant survival rate, biomass production and nutrient uptake were evaluated. Good retention of nutrients (e.g. Tot.N 66-86% and Tot.P 30-87%) was achieved throughout the study period. After two growing seasons, the variety Gudrun showed the best survival rate and significantly higher biomass production (5.7 t/ha) than Karin, Klara and the local variety (1.7, 3.0 and 0.02 t/ha, respectively). Thus, willow wetlands are suitable systems for nutrient recovery from pre-treated wastewater in cold climate regions. However, the willow variety used should be chosen carefully, as there can be significant differences in survival rate and biomass production between varieties.

Keywords: willow tree; water purification; plant growth; nutrients; Nordic conditions

1. Introduction

Wetlands are used worldwide as cost-effective and sustainable water purification systems for a number of wastewater sources such as sewage, mining, urban runoff etc. In sewage treatment, wetlands are widely applied as primary, secondary or tertiary treatment methods in warmer climate regions and to a lesser extent in cold climate conditions (Kadlec and Wallace, 2009; Mander and Jenssen, 2002, 2003). Willow (*Salix* spp.) wetlands (also referred to as willow vegetation filter or willow beds) have been implemented in municipal wastewater treatment in e.g. Sweden (Börjesson and Berndes, 2006; Rastas Amofah et al., 2012) and Denmark (Centre of Recycling, 2010) in recent decades. In Sweden, willow plantations destined for energy production have also been established besides wastewater treatment plants (Dimitriou and Aronsson, 2005). Sewage water and sewage sludge can be used for fertilising the plantation area, which provides an alternative method for nutrient retention. Willow is considered an excellent crop for this purpose, due to its good nutrient uptake and biomass production (Rastas Amofah et al., 2012). Furthermore, willow cultivation and use for energy purposes is estimated to be CO_2-neutral, since the amount of CO_2 stored by the plants during growth equals the amount of CO_2 released during incineration (Perttu, 1999). In cold climate regions, willow wetlands can operate seasonally (only summer) (Börjesson and Berndes, 2006) or all year round (Rastas Amofah et al., 2012). In some cases, the wastewater can be stored in ponds during winter and directed to willow wetlands during the growing season (Börjesson and Berndes, 2006).

Mean biomass production in commercial willow cultivation areas in Sweden ranges between 6 and 12 t/ha/year (Dimitriou and Aronsson, 2005). Precipitation/irrigation rate, sum of heat, nutrient availability and macronutrient ratio are factors known to affect willow biomass production. An irrigation rate of 10-20 mm/day has been identified as an optimal range, taking into consideration nutrient uptake and soil saturation (Dimitriou and Aronsson, 2005). Excessive irrigation rates can cause saturated conditions within the soil and decrease willow growth (Dimitriou and Aronsson, 2005), while lower irrigation rates have been found to decrease willow yield (Börjesson and Berndes, 2006). The optimal sum of heat, i.e. accumulated daily mean temperature above 5°C, is reported to be >1100 °C-days (Perttu, 1980 in Perttu, 1999). It has also been found that extra nutrient availability can increase biomass production to some extent (Dimitriou and Aronsson, 2011; Hytönen and Saarsalmi 2009). According to Ericsson (1981), the optimal macronutrient ratio (nitrogen (N):potassium (K):phosphorus (P):magnesium (Mg):calcium (Ca):sulphur (S)) for willow is 100:72:14:8.5:7:9. Although good biomass production can be accomplished in high-latitude regions, a cold climate generally has detrimental effects on plant survival and biomass growth (Rastas Amofah et al., 2012). Some cold-resistant varieties of willow have been developed, and e.g. central and Southern Finland have developed and cultivated their own variety (Suomen energiapaju

Oy, 2009), but harsh climate conditions such as those prevailing in northern Sweden have been identified as unsuitable for short-rotation willow cultivation (Rosenqvist et al., 2000).

Satisfactory results regarding purification efficiency have been reported for willow wetlands treating sewage water in Denmark (Centre of Recycling, 2010), Sweden (Börjesson and Berndes, 2006) and southern Finland (Niemi, 2014). Under specific conditions, complete transpiration of inflow water has been observed, with the constituent nutrients used in biomass growth (Gregersen and Brix, 2001). However, there is a lack of information on wetland efficiency and functioning and on the most suitable willow varieties (survival rates) for high-latitude conditions, where e.g. the sum of heat is 800-900 °C-days. Moreover, little is known about the effect of extra nutrients from sewage irrigation on biomass growth under these conditions. Thus, this study examined the suitability, efficiency and functioning of willow treatment wetlands in cold climate regions. The objectives were to determine whether: i) Willow wetlands can enhance wastewater purification (nutrient removal) even in harsh cold climate conditions; ii) willow wetlands can decrease the outflow of wastewater due to evapotranspiration; and iii) willow biomass production is higher in wetland irrigated with sewage water (extra nutrients) than in a reference area with no sewage irrigation.

2. Material and methods

2.1. Willow wetland construction and water distribution

The study site was Toranki wastewater treatment plant, which is located in the city of Kuusamo, northern Finland (68°58N, 29°11E), about 60 km south of the Arctic Circle. A pilot willow wetland (0.035 ha) was constructed within the Toranki wastewater treatment facility in 2015-2016 (Fig. 1). Groundwork was conducted in autumn 2015 and planting and installation of the wastewater distribution network and pumping system were carried out in early June 2016. The wetland consisted of three layers: sand, gravel and topsoil (the original excavated soil from which large rocks were removed). High-density polyethylene (HDPE) membrane acted as a bottom and side protection layer to prevent water infiltration. The wastewater distribution system was designed to ensure equal distribution of pumped wastewater volume throughout the wetland and a V-notch weir was installed in a measuring well at the outflow of the wetland (Fig. 2).

Willow development/growth were followed in 2016 and 2017 and purification efficiency were followed during three growing seasons (2016 -2018). Long-term average sum of heat (1981-2010) in the region is 800-900 °C-days (Finnish Meteorological Institute, 2019a). Although information regarding the sum of heat at the wetland location is not available for the operating period, in Finland the value was generally higher than the long-term average in 2016 and in 2018 and lower than average in 2017 (Finnish Meteorological Institute, 2019b). Long-term average precipitation (1981-2010) during the thermal growing season in the region is 280-320 mm (Finnish Meteorological Institute, 2019a).

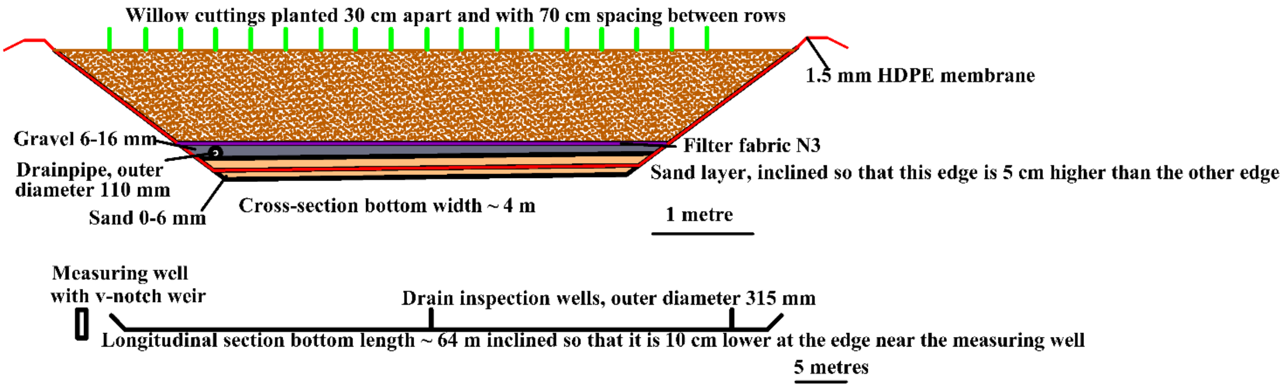

Fig 1. Cross-section view of the pilot-scale constructed willow wetland.

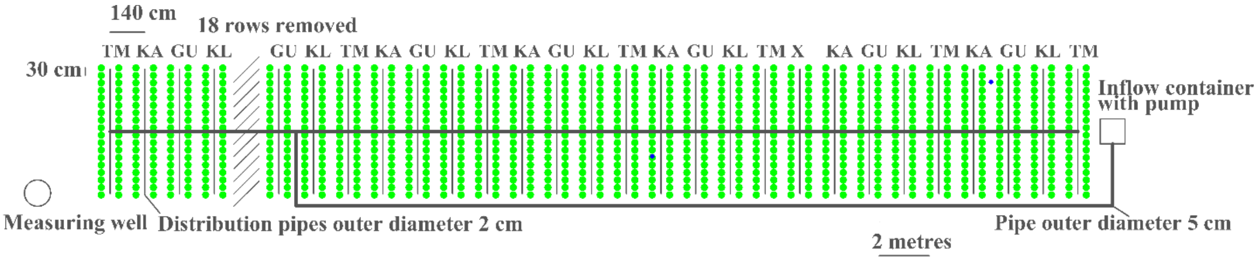

Fig 2. Schematic view from above of the pilot-scale constructed willow wetland. Water distribution system main pipeline (outer diameter 5 cm) and branch lines (outer diameter 2 cm, length 2.5 m, discharge vents diameter 3 mm and blocked ends). Placement of vents in lateral lines: lines 1-8 (from measuring well) five vents, lines 9-16 four vents, lines 17-32 three vents, lines 33-40 four vents and lines 41-46 five vents. The varieties (TM), Karin (KA), Gudrun (GU), and Klara (KL) were planted 2016 in sequential rows. In one separate row (row X in Fig. 2), six different hybrid varieties (Winter, K2242, T05, T06, LODEN and KOUV5) were planted as pot seedlings (six individuals/variety).

Three willow (Salix ssp.) varieties were planted in 2016: *Salix gmelinii* (Gudrun (GU)), ((*Salix schwerinii*× *S. viminalis)*×*S. viminalis)*×*S. viminalis* (Karin (KA)) and ((*Salix* hybrid×*S. viminalis)*×*S. viminalis)*×*(S. viminalis*×*(S. schwerinii*×*S.viminalis*)) (Klara (KL)) (396 individuals/variety), and a local variety (probably *Salix phylicifolia*, referred to as TM; 432 individuals). Gudrun, Karin and Klara cuttings (length 18-20 cm, diameter 1-1.5 cm) were harvested from a willow plantation in Alavieska, Finland, in March 2016 and kept in cold storage until planting (-2 °C). Cuttings of the local variety (length 20-30 cm, diameter a few millimetres) were harvested near Toranki wastewater treatment plant and kept outdoors in cold temperatures (snow). Small diameter is a common characteristic of first-year shoots in areas where willow has not been cut previously. Before planting, all cuttings were placed in a water container for at least one day. The cuttings were planted at 30 cm spacing within rows and 70 cm spacing between rows (planting density 47,000 plants/ha). An extra row (row X, see Fig. 2) was planted as pot seedlings of six different hybrid varieties (Winter, K2242, T05, T06, LODEN and KOUV5; six individuals/variety) to assess survival rate under prevailing conditions, but biomass was not measured due to the small number of individuals/variety. Manual weeding was performed during the first and partly also during the second summer. Replicate willow plantations (about 10 individuals/variety) were established in a reference area about 5 m outside the wetland. Due to the poor survival rate observed for the local variety, at the beginning of 2018 all local variety plants were replaced by 120 cm cuttings of the ((*Salix schwerinii* Amgunskaja×*S. viminalis* Orm*)*×*(S. viminalis Rot 7*×*S. viminalis L 78013))*×*S. gmelinii* (Winter (WI)) hybrid variety. 147 dead trees of other varieties (KA and KL) were also replaced. In total, 543 winter cuttings were planted at 40 cm depth.

The wetland received water from the outflow of the wastewater treatment plant (effectively acting as a polishing phase in the treatment chain). The water was extracted from a pipe which directs treatment plant effluent to a lake. Water was pumped during 10 min (2016-2017) from an inspection well to a 1 m^3 container located in the wetland (Fig. 2). From the container, the water (about 930 L) was distributed to the wetland surface over a 3-minute pumping period. In 2018, water pumping time of the water from the pipe to the distribution container was increased to 11 min and the amount of water distributed to the wetland was increased to 1000 L/pumping cycle. The amount of water fed to the wetland daily was increased throughout the study period, with two pumping cycles/day between 10 and 28 June 2016, four cycles/day between 28 June and 12 October 2016, six cycles/day between 6 June and 29 September 2017 and 8 pumping cycles/day between 8 June and 10 September 2018. These pumping cycles resulted in total hydraulic loads of about 1860 L/d (5 mm/d), 3720 L/d (11 mm/d), 5580 L/d (16 mm/d) and 8000 L/d (23 mm/d), respectively.

2.2 Monitoring and evaluation of wetland hydraulic and hydrological parameters, purification efficiency and load

Outflow from the pilot wetland was determined based on water level data collected during the summers of 2017 and 2018. Water level in the V-notch weir was measured continuously using a Solinst Levellogger 3001 Gold F15/M5 sensor with accuracy ±0.3 cm. For calibration, a Solinst Barologger Edge sensor with accuracy ± 0.05 kPa was used. Manual water level measurements were also conducted as a backup measure. Rainfall in the study site area was measured using a tipping bucket rain gauge and TruTrack GP-HR logger. The water level inside the wetland was monitored with the help of four groundwater pipes. The decision to increase the daily amount of water pumped to the wetland from four times/day in 2016 to six times/day during summer 2017 and subsequently to eight times/day during summer 2018 was made based on groundwater level data. Water level measured in the groundwater monitoring pipes varied between 0-12 cm during the monitored summers (from bottom of pipes/wetland upwards). Despite the increased hydraulic load, no measurable groundwater level or layer was observed within the wetland area.

Water residence time was determined in August 2017 via an experiment in which 20 kg of sodium chloride (NaCl) were dissolved in 930 L of water and pumped into the wetland in an approximately 3-min pulse. Electric conductivity sensors (HOBO-loggers) were installed in the outflow well to monitor changes in the ionic strength of the outflowing water due to salt concentration. A detailed description of determination of mean residence time using the conservative tracer method can be found in Postila et al. (2015). Tracer yield (%) was calculated as the ratio between the mass of tracer added to the mass of tracer discharged in outflow water.

Five inflow and five outflow water samples were collected in 2016 and 2017 while four samples were collected in 2018 (at 2- to 6-week intervals) during the wetland operating periods (sampling periods 11 July-12 October 2016, 28 June-27 September 2017 and 26 June-10 September 2018). These water samples were analysed by an accredited laboratory for: total nitrogen (Tot.N), sum of nitrate and nitrite nitrogen (NO_{2+3}-N), ammonia nitrogen (NH_4-N), total phosphorus (Tot.P) and phosphate phosphorus (PO_4-P). On four occasions (June and September 2017 and two separate days in August 2018, the samples were also analysed for other constituents, such as K, Ca and Mg (SFS-EN ISO 11885:2009 and ICP-MS method). Concentration-based purification efficiency, R_c (%), was calculated based on mean inflow and outflow concentrations in individual operating periods as:

$$R_c= (C_{in}-C_{out})/C_{in}*100\% \quad (1)$$

where C_{in} and C_{out} are mean concentration (mg/L) in inflow and outflow, respectively, during one operating period.

Loading rate (L_1, kg/ha/d) to the wetland was determined as:

$$L_1 = ((C_{in}*f*Q_1)/A)*(1*10^{-6}) \quad (2)$$

where f is number of pumping cycles per day, Q_1 is pumped water volume (L) per cycle and A is wetland area (ha).

Total load (L_2, kg) to the wetland was determined based on mean inflow rate and pumped water volume in individual operating periods as:

$$L_2 = C_{in}*1*10^{-6}*Q_2 \quad (3)$$

where Q_2 is pumped water volume (L) to the wetland during one operational period.

Total outflowing load (L_3, kg) from the wetland was determined based on mean outflow rate and outflow water volume in individual operating periods as:

$$L_3 = C_{out}*1*10^{-6}*Q_3 \quad (4)$$

where Q_3 is outflow water volume (L) from the wetland during one operational period.

Removed load was calculated as L_2-L_3 and load based purification efficiency, R_L (%), was calculated based on mean inflow and outflow concentrations in individual operating periods as:

$$R_L = (L_2 - L_3)/L_2 * 100\% \quad (5)$$

Determination of L_3 and R_L (Eq. 4 and 5) were only possible for the years of 2017 and 2018 when discharge rate data was available.

2.3 Assessment of willow survival and biomass growth

Willow survival rates were checked in October 2016 and late September 2017. All plants were assessed and rated as alive, dead or inconclusive condition. Biomass growth was evaluated in autumn 2017 by measuring the diameter of individual shoots of all surviving willow plants at 10 cm above ground level, as described by Hytönen and Saarsalmi (2009). Five plants from each variety were then selected for harvesting (ranging from small to large sizes) and shoots were cut at around 1 cm above ground level. Plants were also harvested at around 1 cm above ground level from the nearby reference site, but due to the small number of plants per variety (about 10) and low survival rate, the number of harvested reference plants was five for GU, three for KL and one each for KA and TM. Stems and leaves of individual shoots were transported to the laboratory and dried (105 °C) separately to constant weight. Biomass was determined based on shoot diameter and dried weight of harvested plants (Hytönen and Saarsalmi 2009):

$$y = a * x^b \quad (6)$$

where y is dry mass (g), x is diameter at 10 cm height above ground level (mm) and a and b are constants for individual willow varieties. Constants a and b were fitted against the biomass measured from the harvested plants of each variety (Table 1) and the resulting equations were used to determine total biomass production by individual varieties.

Table 1. Above-ground biomass equations ($y=a*x^b$) for the individual willow varieties in the wetland and for GU and KL also in the reference area, where: y is dry mass (g), x is diameter at 10 cm above ground level (mm), a and b are constants and R^2 is coefficient of determination

Variety[a]	a	b	R^2
GU	0.0316	2.957	0.97
KA	0.0611	2.732	0.97
KL	0.1171	2.385	0.97
TM	0.0443	2.808	0.99
GU REF	0.0339	3.005	0.99
KL REF[b]	0.1351	2.211	0.999

[a] Varieties: GU = Gudrun, KA = Karin, KL = Klara, TM = local variety.
[b] Reference area (only 3 individuals)

2.4 Nutrient concentrations in willow shoots and wetland soil

Samples of willow shoots comprising around 10 g dry matter (DM) were analysed by an accredited laboratory for concentrations of P and different trace elements (e.g. K, Ca, Mg, sodium (Na)) using HNO_3/HCl wet incineration and ICP-OES (EPA3051a, SFS-EN ISO 11885). The N content was analysed in the same laboratory based on SFS-EN ISO 16948:2015. Based on the minimum mass of sample required for analysis (around 10 g or more), the nutrient content of different parts of the plants (leaves, stems) was analysed separately when possible or together when necessary. If one individual shoot did not produce enough biomass, then stems and leaves from different shoots on that plant were pooled and analysed together. The statistical significance ($p<0.01$) of the difference in nutrients concentrations between willow varieties was analysed by Kruskal-Wallis test utilizing the IBM SPSS Statistics 24 program.

Soil samples were collected from the surface layer of the wetland soil (depth 5-15 cm) at the beginning of June 2016 (four sampling points) and at the end of September 2017 (five sampling points, not the same as for 2016). Soil samples were analysed by an accredited laboratory for P, aluminium (Al), iron (Fe), Ca, K, Mg and manganese (Mn) using HNO_3/HCl wet incineration and ICP-OES (EPA3051a, SFS-EN ISO 11885). Nitrogen (Kjehldahl method, SFS-EN 13654-1) and soil organic content (SFS-EN 12879) were also analysed. The statistical significance ($p<0.01$) of the difference in soil nutrient concentrations between the years 2016 and 2017 was analysed by Mann-Whitney U-test utilizing the IBM SPSS Statistics 24 program.

3. Results

3.1. Hydrology, hydraulics and water purification efficiency

The amount of water discharging from the wetland (outflow) was higher than inflow and precipitation in June 2017 (Fig. 3a). During July and August 2017 and for most of summer 2018, outflow was mostly lower than inflow and precipitation volumes together (Fig. 3a and 3b). It is important to note the significant variation in outflow rates when the accuracy limit of the water level measurement sensor used in taken under consideration (thinner black lines in Figs. 3a and 3b). Based on the tracer experiment conducted in August 2017, the average water residence time in the wetland was about 7 days (low tracer yield of 13% but outflow approximately 60% of inflow volume (36-92% based on sensor accuracy)).

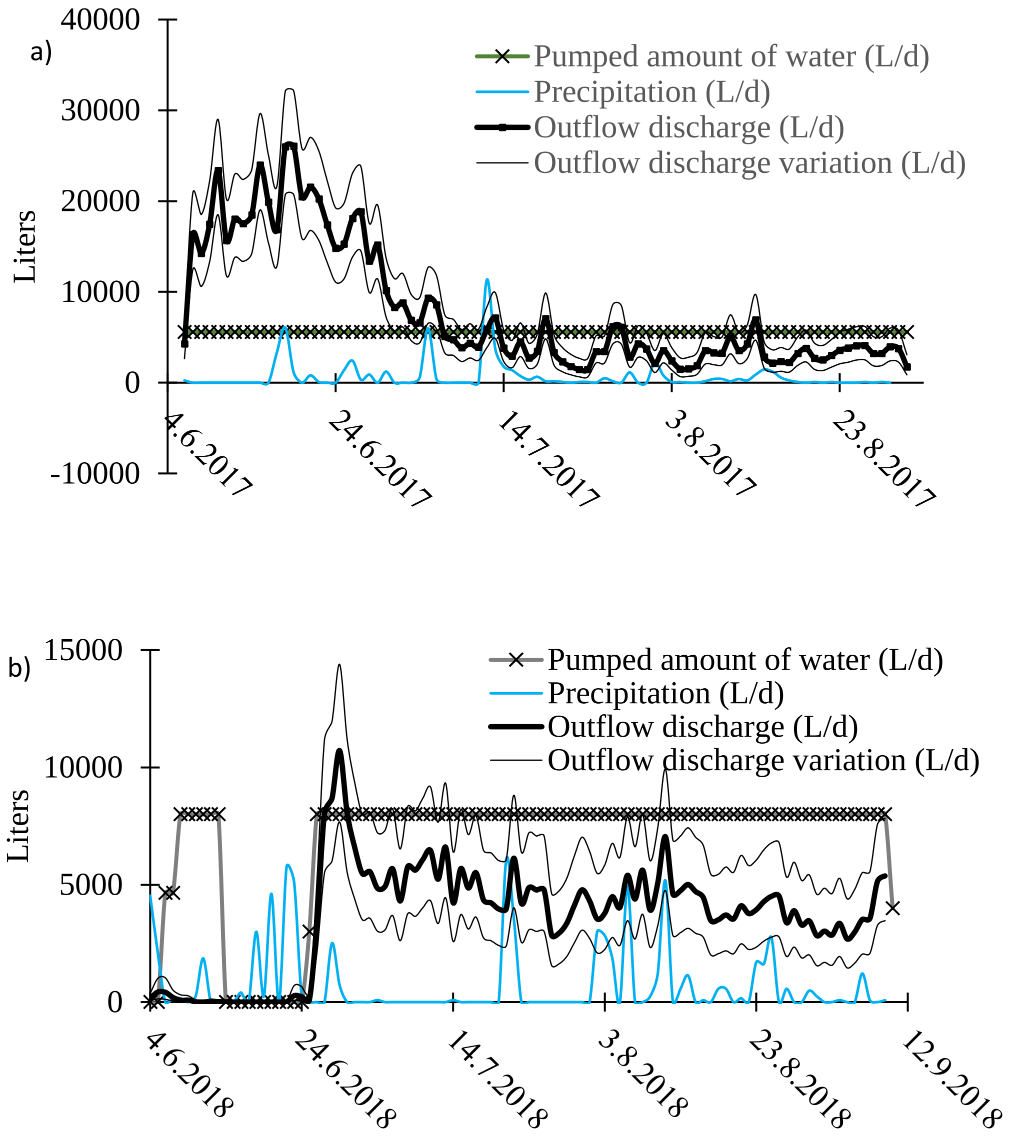

Fig. 3. Inflow (pumped water amount + precipitation, L/d) and outflow (discharge, L/d) from the wetland. Outflow water level measurement accuracy was ±0.3 cm, so outflow discharge variation (min-max, L/day) is also presented. a) 2017 and b) 2018.

Regarding removal of monitored substances, good retention of all N and P fractions was observed. Overall, purification efficiency (concentration-based) was higher in 2017 than 2016 and 2018, except for Tot.N (higher in 2016) and NO_{2+3}-N (Table 2). However, average inflow concentrations were also generally higher in 2017 than in 2016 and 2018, except for NO_{2+3}-N. In particular, the average inflow concentration of PO_4-P in 2018 was very low at 4 µg/L. This led to PO_4-P leaching from the wetland. When looking at load-based purification efficiency, higher Tot. N load retention was achieved in 2018 compared to 2017 while the opposite occurred for Tot. P (Table 2). Inflow concentration of the nutrients K, Ca and Mg was 25.4, 30.2 and 7.8 mg/L in 2017 and 30.4, 33.4 and 8.2 mg/L in 2018, respectively (based on two sampling campaigns per summer). The removal efficiency achieved for the K, Ca and Mg was 25%, -278% and -11%, in 2017 and 12%, -273% and -2% in 2018, respectively. The inflow water N:K:P:Mg:Ca ratio (average based on 4 sampling campaigns, two in 2017 and two in 2018) was 39.6:27.9:0.2:8.0:31.8. When setting N=100, the ratio was 100:70:0.4:20:80.

Table 2. Average inflow water quality (µg/L) and concentration-based purification efficiency (%) (five samples/year 2016 and 2017 and four samples/year 2018), loading rate (kg/ha/d) and total load to the study wetland (kg) during the 2016, 2017 and 2018 operating periods (five/four samples/year and water volume pumped) as well as the removed load in the study wetland (kg) and load-based purification efficiency (%) for 2017 and 2018.

Nutrient	2016				2017						2018					
	Inflow (mg/L)	Concentration based purification efficiency (%)	Load (kg/ha/d)	Load to the study wetland (kg)	Inflow (mg/L)	Concentration based purification efficiency (%)	Load (kg/ha/d)	Load to the study wetland (kg)	Removed load in the study wetland (kg)	Load based purification efficiency (%)	Inflow (mg/L)	Concentration based purification efficiency (%)	Load (kg/ha/d)	Load to the study wetland (kg)	Removed load in the study wetland (kg)	Load based purification efficiency (%)
Tot.N	31.2	86	3	13	39.6	78	6	25	19	76	39.0	66	9	26	21	82
NO$_{2+3}$-N	9.8	87	1	4	9.3	18	1	6	1	12	25.6	55	6	17	13	76
NH$_4$-N	8.5	78	1	4	27.3	95	4	18	17	95	13.8	87	3	9	9	93
Tot.P	0.16	58	0.02	0.07	0.24	87	0.04	0.15	0.13	86	0.055	30	0.01	0.04	0.02	63
PO$_4$-P	0.01	68	0.002	0.01	0.03	77	0.005	0.02	0.014	75	0.004	-329	0.001	0.002	-0.003	-129

3.2 Willow survival, biomass production and nutrient uptake

There were clear differences in survival rate between the different willow species, with GU showing the best survival. After the first summer (autumn 2016), 96% of individual GU plants were observed to be alive, while 86%, 75% and 44%, of KL, KA and TM plants were alive. In autumn 2017, the percentage of live plants observed was 95% for GU, 76% for KL, 63% for KA and 14% for the local variety (TM).

Total biomass production (stem and leaf) for the different varieties after two summers was: GU 5.7 t/ha, KA 1.7 t/ha, KL 3.0 t/ha and TM 0.02 t/ha. Based on the data obtained, stem biomass production was about 75% of total biomass in GU, 78% of total biomass in KL and KA, and 88% of total biomass in TM. Total biomass production in the reference area after two summers was 0.4 t/ha for GU and 0.1 t/ha for KL.

Overall, no clear correlation was identified between nutrient concentration in the harvested biomass and size of the willow plants. The average nutrient concentrations in shoots of all species were (per kg DM): 15 g N, 1.8 g P, 8.6 g K, 6.8 g Ca and 1.0 g Mg. The amount of nutrients (P and N) found in the biomass of different species (Table 3) varied slightly but the variations were found not statistically significant ($p<0.01$) and the variations observed were often smaller than possible variations due to determination accuracy of the analysis methods used (P: ±12%; N: ±15%). In general, plant survival rate and biomass production were the most visible aspects regarding the differences in nutrient uptake between the different varieties. The amount of nutrients used by the willow plants in the wetland for biomass growth was about 0.15 kg P and 1.3 kg N (Table 4). The amount of P used by the plants was little bit lower than the load received by the wetland with the inflowing treated sewage water (0.22 kg P; Table 2) in both study years, while the amount of N used was significantly smaller than the load received (38.8 kg N; Table 2).

Table 3. Average ± standard deviation (min-max) concentrations (mg/kg DM) of phosphorus (P) and nitrogen (N) in biomass fractions of the different willow varieties tested. GU = Gudrun, KA = Karin, KL = Klara, TM = local variety.

Variety	P in leaf[a] (mg/kg DM)	N in leaf[a] (mg/kg DM)	P in stem[a] (mg/kg DM)	N in stem[a] (mg/kg DM)	P in whole shoot[a] (mg/kg DM)	N in whole shoot[a] (mg/kg DM)	n[b]
GU	3458±278 (3140-3760)	31660 ±2037 (28700-33700)	1233±87 (1150-1350)	8873±606 (7900-9760)	1816±186 (1580-2092)	15193±1257 (13251-17189)	5/6/6[b]
KA	3215±n.a. (2920-3510)	31700±n.a. (31600-31800)	1405±n.a. (1010-1800)	9245±n.a. (9120-9370)	2010±561 (1467-2720)	15750±1507 (14405-17200)	2/2/4[b]
KL	2820±585 (2410-2490)	28600±6883 (20700-33300)	1110±338 (900-1500)	10240±1058 (9020-10900)	1506±457 (1238-2034)	14278±762 (13528-15051)	3/3/3[b]
TM	2730	23500	1440	10400	1631±n.a. (1622-1640)	13975±n.a. (12250-15700)	1/1/2[b]

[a] Amount of different individuals or shoots analysed per variety. If stem and leaf biomass from one shoot was sufficient (around 10 g DM), these fractions were analysed separately; otherwise they were analysed together. If one individual shoot did not produce enough biomass, then biomass from different shoots from same plant was analysed together.

[b] Number of leaf samples/stem samples/whole shoot samples

Table 4. Rate (kg/ha) and total (kg) uptake of phosphorus (P) and nitrogen (N) by the different willow varieties tested and total P and N uptake (kg) for all willows in the study wetland. GU = Gudrun, KA = Karin, KL = Klara, TM = local variety.

Variety	P in willows (kg P/ha)	N in willows (kg N/ha)	P in study wetland willows (kg)	N in study wetland willows (kg)
GU	10.4	87.1	0.08	0.7
KA	3.4	26.9	0.03	0.2
KL	4.5	42.8	0.04	0.3
TM	0.03	0.26	0.0003	0.002
Total			0.15	1.3

3.3 Willow wetland soil characteristics

Based on analysis of dry soil samples after two summers of operation, the average organic content of the wetland soil was similar to that at start-up (4.5%), while the N content was 18% higher (initial 790 mg/kg) and the P content was 12% lower (initial 760 mg/kg). However, the difference was not statistically significant ($p<0.01$). The observed differences in nutrient concentration were mostly inside the determination limit of the methods used (N: ±15%; P: ±16%). In addition, there were no statistically significant differences in dry soil concentrations of e.g. Al, Fe, Ca, K, Mg and Mn. The average concentrations in samples collected in 2017 were (per kg dry soil): 8690 mg Al, 13,100 mg Fe, 4510 mg Ca, 1340 mg K, 4460 mg Mg and 170 mg Mn.

4 Discussion

4.1 Hydrology and hydraulics

Evapotranspiration in willow wetlands at lower latitudes in northern Europe can reduce outflow significantly (Dimitriou and Aronsson, 2011), or even completely under special (purposely designed) circumstances (Gregersen and Brix, 2001). It can be expected that, under the colder and wetter conditions at our study site in northern Finland, evaporation is lower than that reported for e.g. Southern Sweden, etc. (Dimitriou and Aronsson, 2011). Evapotranspiration measurements were not conducted during this study and due to the significant variations in the determined wetland outflow (caused by the accuracy limit of water level sensor used), accurate estimations of evapotranspiration values cannot be accomplished. Nevertheless, a general assessment of evapotranspiration values was conducted. Long-term average evaporation for the study site area (Kuusamo,1991-2010) is 37, 84, 81, 52 and 18 mm/month for May, June, July, August and September, respectively (Korhonen and Haavanlammi 2012). Based on information provided by Doody and Benyon (2011), willow tree average transpiration can vary from 1-6 mm/d/m^2. When the minimum transpiration reported value (1 mm/d/m^2) is added to the average evaporation values for the study site area for a particular month, evapotranspiration can be estimated for our pilot willow wetland as 39 and 29 m^3/month in July and August, respectively. If 6 mm/d/m^2 transpiration is selected then evapotranspiration would be 93 and 83 m^3/month in July and August, respectively. Inflow + precipitation rates to the pilot willow wetland was in average 54 m^3/month (-14-106m^3/month based on sensor accuracy) and 84 m^3/month (28-124 m^3/month) higher than outflow in July and August of 2017 respectively. While inflow + precipitation rates was in average 105 m^3/month (33-161 m^3/month) and 146 m^3/month (79-197 m^3/month) higher than outflow in July and August of 2018 respectively. It is good to note that in the study site area, July and August of 2018 were warmer than long-term average while July was also dryer but in August more precipitation than long-term average was observed (Finnish Meteorological Institute, 2019c). In general, the reduced outflow observed in the wetland in comparison to inflow + precipitation rates can largely be due to evapotranspiration. However, as evapotranspiration measurements were not conducted and uncertainties are attached to outflow measurements, it is probable that loss of water occurred due to other factors such as leakages caused by possible damage to the high-density polyethylene (HDPE) membrane. This could have been caused by e.g. the existence of point source load like some sharp stones being pressed against the membrane through the filter fabric by large soil mass. Or during earth work by heavy machinery, although measures were taken to prevent damage.

The water residence time, estimated using a conservative tracer experiment, was 7 days, which is quite high compared with residence times (1-2 days) reported for other types of wetland used in the polishing phase of sewage treatment in northern Finland (Ronkanen and Kløve, 2008). The tracer yield was only 13%, which may be partly due to the hydraulic conditions at the time of tracer experiment, where outflow volume was about 60% (36-92% based on sensor accuracy) of inflow, but also to uptake NaCl tracer by willow plants. The Na concentrations in willow plants harvested from the wetland (>400 mg/kg DM) were clearly higher than those in willow plants harvested from the reference area (under detection limit <40 mg/kg DM). In a previous study, Zupančič Justin and Zupančič (2009) found that a willow plantation (1.1 ha, with soil and grasses) removed around 166 mg/L from inflow with an average NaCl concentration of 961 mg/L during a two-year period (hydraulic load 3000-70000 mm/day). Thus, the residence time determined by the tracer experiment can be considered reliable, although the tracer yield was low.

4.2. Water purification efficiency and inflow water nutrient ratio

In general, good removal of N and P was accomplished in the pilot-scale willow wetland investigated in this study, as reported previously for this type of wetland (e.g. Dimitriou and Aronsson (2011) observed >90% removal of N and P). The average Tot.N (66-86%) and NH_4-N (78-95%) removal efficiencies obtained were higher than the 43% and 32%, respectively, observed by Rastas Amofah et al. (2012). The inflow concentration of Tot.N and NH_4-N was higher in that study (30-55 mg/L and 10-40 mg/L) than in the present study (31-40 mg/L and 8-27 mg/L), which could partly explain the lower removal rate observed. In general, concentration-based purification efficiency of Tot. N decreased from 2016 to 2018 in the studied wetland. However, when looking at load-based purification efficiency, higher Tot.N load retention was achieved in 2018 compared to 2017. One possible reason for the lower concentration-based purification efficiency in 2018 is that, evapotranspiration was higher in 2018 (lower outflow volume related the inflow compared to 2017) which could have caused the higher concentration found in outflow.

Higher removal of Tot.P and PO_4-P (30-87% and 69-82% (except summer 2018 when PO_4-P concentrations in outflow were higher than in inflow) was achieved in our study than in the study by Rastas Amofah et al. (2012) (23% and <10%). In the latter study, the inflow concentrations of P fractions (Tot.P 5-10 mg/L, PO_4-P 3-5 mg/L) were significantly higher than those observed in our wetland (Tot.P ≤0.5 mg/L, PO_4-P mostly <0.1 mg/L). In general, as reported in other studies (e.g. Postila et al., 2014), higher inflow P concentrations led to higher P removal efficiency. However, in contrary to results reported in other studies (e.g. Liu et al., 2018), higher inflow Tot. N concentrations/load did not lead to higher Tot.N removal in our pilot wetland. Leaching of Ca and Mg from the wetland occurred which account for the negative removal efficiencies reported and illustrate the high concentration of these nutrients in the wetland soil.

Based on the optimal inflow ratio of N:K:P:Mg:Ca (100:72:14:8.5:7) identified by Ericsson (1981), not enough P and too much Mg and Ca were fed to the wetland in this study (ratio 100:70:0.4:20:80). The very low P concentration in inflow at the study site was mostly due to the treatment processes applied at the wastewater treatment plant, for which retention of P in sewage water is better than N retention. In the wetland higher N retention than the amount used by the plants was observed. Other removal pathways such as uptake by weeds and nitrification-denitrification processes can account for the high N removal rates obtained.

4.3. Willow plant survival, biomass production and nutrient uptake

Willow plant survival rates varied significantly between the varieties grown, with GU showing the highest survival and TM the lowest. Probable reasons for the low survival rate of the local variety (TM) was that the cuttings used were thin, a common characteristic of first-year shoots in areas where willows have not been cut previously, and a number of planted cuttings were damaged at the end of the first summer during maintenance work. In addition, the extra nutrients fed to the wetland may had a detrimental effect on TM development. Furthermore, the TM rows were surrounded by KA and KL rows (see Fig. 2) and this order of plantation might have affected the survival rates of KA and KL, which were weaker than GU plants. The very low survival rate of TM meant that KA and KL had large empty spaces surrounding them with no support for growth (shoots of adjacent plants support each other). In the study by Rastas Amofah et al. (2012) biomass production was higher for KA than GU, so the lower survival rate and biomass production of KA observed in our study might not realistically describe differences in survival rates between KA and GU under similar conditions. Furthermore, it is important to note that although GU showed higher survival rates and biomass production, the use of monoculture wetlands (only one species) increases the risks related to issues such as rust and beetle attacks compared with polyculture plantations (Peacock et al., 2001).

The biomass production achieved by GU (5.7 t/ha) and KA (1.7 t/ha) in the pilot-scale wetland was substantially lower than that reported by Rastas Amofah et al. (2012), who found that the sum of first and second summer biomass production was 14.3 t/ha for GU and 15.7 t/ha for KA. It is important to note that Rastas Amofah et al. (2012) used a higher planting density (25 plants/m^2) than in our study (4.7 plants/m^2) and that they harvested after the first growing season. Planting density and harvesting are factors known to increase biomass production (Bullard et al., 2002). Higher biomass production was also reported by Hytönen and Saarsalmi (2009) who planted *Salix phylicifolia* and *Salix triandra* (plant density 4 individuals/m^2) in a fertilised peatland and found that biomass production over a 10-year period was 7.0-8.7 and 5.0-5.6 t/ha/year, respectively. However, the concentration of nutrients (N and P) found in willow stems in that study were about half those measured in willow plants at our study site.

Based on the results obtained, the extra nutrients contained in treated sewage water did not affect the nutrient concentrations in willow plants. However, the extra nutrients had a clear effect on biomass growth, with higher biomass production observed in the wetland area than in the untreated reference area (14-fold and 28-fold higher biomass production for varieties GU and KL, respectively). Beneficial effects of wastewater irrigation on biomass production have also been reported Khurelbaatar et al. (2017) and in a study in south-east Sweden by Börjesson and Berndes (2006), who achieved a 30-100% increase in average biomass yields compared with well-managed, rain-fed willow plantations established on good soils. The increased willow biomass was also noticed when sewage sludge was used as fertilizer (Urbaniak et al., 2017).

Conclusions

In general, good retention of nutrients was achieved in the pilot-scale constructed wetland examined in this study. After two growth seasons, the willow variety Gudrun showed higher survival rate and biomass production than the other varieties tested (Karin, Klara and a local variety). Thus, willow variety is an important factor affecting biomass production in wetlands area. The results confirmed that: willow wetlands can enhance recovery of nutrients from sewage water at high-latitude sites, i.e. in cold climate conditions; that wastewater volumes discharged from willow wetlands during summer (especially July and August) are lower than inflow volumes, due to evapotranspiration; and that biomass production in the wetland area is clearly higher than in untreated surrounding areas, partly due to the extra nutrients reaching the wetland with the wastewater.

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