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Effect of dairy effluent on the biomass, transpiration, and elemental composition of *Salix kinuyanagi* Kimura

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ABSTRACT

The land treatment of Dairy Effluent (DE), comprising urine and faeces is common practice, yet can lead to nutrient imbalances in plants and soils. We aimed to determine the growth, transpiration, and elemental composition of *Salix kinuyanagi* Kimura (Clone No. PN 386) as affected by DE application. DE was applied for 15 weeks to eighteen 122 dm^3 lysimeters, either bare or planted with *S. kinuyanagi*, at N application rates of 0–558 kg ha^{-1} over three months. DE application increased biomass and transpiration. Chlorosis, possibly caused by excess Cl, appeared in the highest treatment. DE application increased foliar concentrations ofN, P, K, Cl, and the foliar N:S ratio to above 15, a level indicative of S deficiency. Concentrations of essential trace elements were unaffected. Trees receiving the N equivalent of 279 kg ha^{-1} removed similar amounts of N and K as were applied in the DE. All DE treatments added more Cl than the plants removed. Soil chloride accumulation may be harmful in drier climes. Future work should include a field trial to determine the long-term sustainability of DE application to willows, and the potential use of willows as animal fodder.

1. Introduction

Dairy production, and the housing of cattle during winter, results in large volumes of nutrient-rich dairy effluent (DE), comprising urine, faeces and possibly chemicals used to cleanse the milking or stabling area [1]. Typically, DE contains high concentrations of ammonium-N, P, K, dissolved organic carbon (DOC) and pathogens, such as *Enterococcus faecalis* [2]. Disposal of the untreated DE into waterways is illegal in most countries because the contents may present a human health risk and can induce eutrophication of rivers and lakes [1]. Disposal of the DE in the conventional sewerage system or installing an onsite aerobic or anaerobic treatment ponds can be expensive and may not be effective. Therefore, the application of DE onto land, normally pasture, either raw or after preliminary treatment [3], as an N-rich fertiliser has become commonplace [4]. DE application to pasture significantly enhances plant growth [5]. However, the long-term application

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of DE results in the accumulation of K in the topsoil and reduces the concentrations of Ca and Mg in pasture, which may possibly lead to deficiencies of these elements in grazing animals [5].

DE may also be applied to trees. Roygard et al. [6,7] demonstrated, using a lysimeter study, that the willow clone *Salix kinuyanagi* Kimura, could maintain nitrate concentrations in the drainage water below the New Zealand Drinking Water Standard of 11.3 g NO_3–N m^{-3} when irrigated with DE containing the N equivalent of 870 kg ha^{-1} over two years, more than twice the rate permitted by law. Numerous studies in North America and Northern Europe showed that sewage sludge and other effluents could be applied to willow plantations for biomass production [8–12]. Although willows can mitigate nitrate leaching from DE application, there is a lack of information on effect of various DE application rates on the growth and transpiration of willow.

Potentially, willow biomass produced in the treatment of DE may be fed to stock. Willows are used extensively in New Zealand as supplementary stock fodder during times of drought [13]. Douglas et al. [14] demonstrated that both foliage and small twigs of *S. kinuyanagi* are palatable for cattle. Robinson et al. [15] showed that *S. kinuyanagi* accumulated high concentrations of the essential animal nutrients Co, Fe, Mn, and Zn relative to pasture. The willow leaf Zn concentrations were >140 mg kg^{-1}, some seven times higher than that of pasture growing in the same soil.

The high concentration of DOC in DE may enhance trace element solubility in soils [16]. However, trace elements that are complexed with DE may be unavailable for plant uptake [17]. The application of DE to soil may therefore reduce the uptake of beneficial trace elements by willows. Accumulation of cations, such as K, in the root zone may compete with trace elements for binding sites on soil particles and in the plants' root system, thereby changing plant trace element concentrations.

We investigated the effect of various levels of DE application on the growth, transpiration, and elemental concentrations of *S. kinuyanagi* (Clone No. PN 386), with a view to its use for the land treatment of DE combined with stock fodder production. Potentially, *S. kinuyanagi*, already proven to mitigate nitrate leaching when irrigated with dairy effluent, could provide a nutritious supplementary feed source that is rich in essential trace elements.

2. Materials and methods

We conducted a lysimeter experiment from September 2004 to February 2005 in a shade house at Plant and Food Research, Palmerston North, New Zealand (40°22′41.94′′S, 175°36′51.01″E, elevation 34 m). A meteorological station installed in the greenhouse recorded temperature, solar radiation, and relative humidity. Table 1 shows the average daily temperatures and potential evapotranspiration, which was calculated using the Penman–Monteith equation. In September, we set up eighteen 0.122 m^3 lysmeters. Each lysimeter was 1 m high and had diameters of 76 cm at the top and 65 cm at the base. The surface area of the soil was 4536 cm^2. Perforated drainage collection tubes (internal diameter 1.2 cm) were installed at the bottom of each lysimeter. The drainage was collected in 0.01 m^3 polythene jerry cans located in a trench below the level of the bottom of the lysimeters. The drainage collection tubes were covered with river gravel with an average particle diameter of 5 mm to a depth of 50 mm. We filled each lysimeter with 900 mm of loamy sand, repacked to a bulk density of 1500 kg m^{-3}. Table 2 gives the chemical properties of the soil. The sand, silt, and clay fractions were 0.84, 0.10, and 0.06 g g^{-1} respectively. The lysimeters were irrigated to field capacity, corresponding to a water content of 46%, and left for two weeks. DE was applied weakly on the surface of the lysimeters.

On the 1st of October (Day Of Experiment, DOE = 1), 12 of the lysimeters were planted with one 1 m unrooted willow rod each, inserted to a depth of 200 mm The poles had an average diameter of 32 mm and an average fresh weight of 540 g. Six of the lysimeters remained unplanted. Table 3 lists the treatments and the amounts of water, DE, and N that the lysimeters received. There were two control treatments (np0, p0), which received only water and four DE treatments (np0.5, p0.25, p0.5 and p1), with mixtures of water and DE. There were three replicates of each treatment distributed in a block design. Fig. 1 shows the experimental set up.

Table 1 – Average daily minimum and maximum temperatures (°C) and average daily potential evapotranspiration (mm) throughout the experiment.

	Minimum temp (°C)	Maximum temp (°C)	Potential evapotranspiration (mm)
September 04	7.4	16.8	2.3
October 04	9.8	19.2	2.5
November 04	11.9	23.6	4.4
December 04	11.5	22.2	3.6
January 05	14.6	26.3	4.5
February 05	20.3	31.7	6.7

Table 2 – Characteristics of the dairy effluent and soil used in the experiment. Concentrations are in g m^{-3} (dairy effluent) and mg kg^{-1} (soil). n.d. = not determined.

	Effluent (g m^{-3})	Soil (mg kg^{-1})
pH	7.8	5.7
Organic carbon	185	9000
Total N	110	475
NH_4–N	95	n.d.
NO_3–N	15	n.d.
P	24	287
S	2	41
K	168	10784
Ca	23	6332
Mg	14	2260
Na	8	3436
Fe	0.62	21729
Zn	0.11	40
Mn	0.68	332
B	<0.1	2.3
Co	n.d.	8.3
Mo	n.d.	1.8
Cl	75	71
Cu	<0.1	10.2

Table 3 – Description of control and Dairy Effluent (DE) treatments. Each treatment was replicated three times.

Label		Water added (m^3)	DE added (kg m^{-3})	Equivalent DE application rate (kg Nitrogen ha^{-1})
np0	no tree	0.121	0	0
np0.5	no tree	0.118	112000	279
p0	tree	0.307	0	0
p0.25	tree	0.331	56000	139
p0.5	tree	0.345	112000	278
p1	tree	0.343	224000	558

When we started the DE application, the willow rods had developed primary branches ca. 300 mm in length.

The DE used for this experiment was collected from an effluent storage pond on Massey University's No. 4 dairy unit in early November. Table 2 shows the composition of the effluent. Effluent treatments began on the 15th of November (DOE = 46). The DE along with extra water were applied daily at 8 am, to achieve 100–500 m^3 of drainage per day. The control treatments were irrigated similarly with water. Fig. 2A and Fig. 2B show the cumulative volume of water and DE applied to each treatment. Drainage (Fig. 2C) was collected weekly and weighed. In the p0 treatment at DOE = 99, we increased the irrigation by 500 m^3 per day in response to falling drainage volumes that were approaching our minimum threshold of 700 m^3 per week. This caused a sharp increase in drainage volumes for this treatment (Fig. 2C). We did not determine the chemical composition of the drainage solutions.

An estimation of evapotranspiration was calculated by subtracting the drainage volume from the irrigation volume. An estimation of transpiration was calculated by subtracting the evaporation of the unplanted lysimeters from the evapotranspiration of the planted lysimeters.

On the 14th of February, the aboveground portions of the willows were excised, the new shoots separated from the original poles, and the leaves separated from the stems. Plant materials were dried in an oven at 80 °C until a constant weight was obtained. The samples were weighed, ground using a Tecator - Cyclotek 1093 sample mill, and stored for analyses. For each lysimeter, ca. 100 g soil samples were taken from three depths: 25–30 cm, 30–60 cm and 60–80 cm. The samples were dried at 105 °C for 72 h, and then sieved to < 2 mm using a nylon sieve.

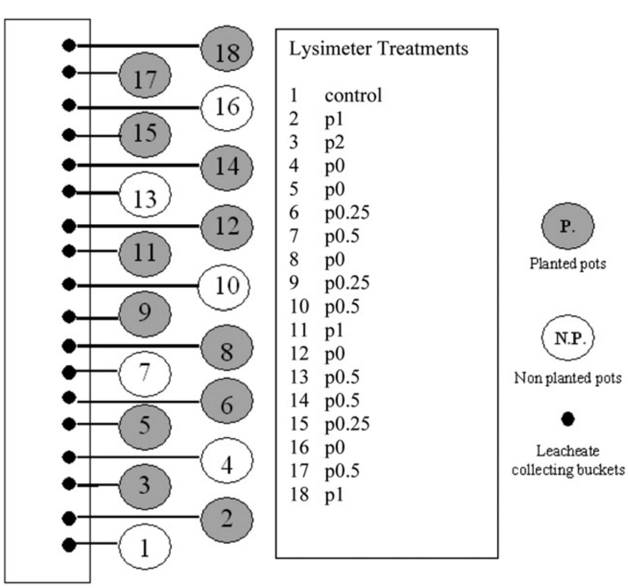

Fig. 1 – Scheme of the experimental set up.

2.1. *Elemental determination*

Four grams of ground material were mixed with 0.9 g of wax and pressed into tablets under a force of 21 GPa. The total element concentrations of the tablets were determined at ETH Zürich, Switzerland, using a Spectro X-lab 2000 X-Ray Fluorescence (XRF) spectrometer. For quality assurance, we analysed 4 Wageningen soil standards, 958, 998, 989, and 951 [18] as well as a certified plant reference material (poplar leaves NCS DC73350, China National Analysis Center for Iron and Steel, Beijing, China). The average recoveries (our measured concentration as a percentage of the certified reference concentration) for soils were: Ca 113%, Cu 117%, Fe 106%, K 100%, Mg 89%, Mn 112%, P 154%, S 193%, Zn 101%. For the plant standard, the recoveries were: Ca 96%, Cu 101%, Fe 98%, K 97%, Mg 67%, Mn 126%, P 82%, S 87%, Zn 99%.

The concentrations of cations in the DE were measured using a Varian ICP-OES. Total N in the DE, soil and leaf samples, as well as the DOC concentration in the DE was determined by a certified external laboratory (Laboratorio REI, s.r.l., via Spaggiari 110/A, 43100 Parma, Italy).

Significant differences between plant biomass production and water use, as well as elemental concentrations in plants and soils were analysed using ANOVA with Fisher's Least-Significant-Difference post-hoc test to compare means. The level of significance was 0.05. The statistical package used was Minitab® 16.

3. Results and discussion

3.1. *Tree water use and biomass production*

All trees grew throughout the experiment, although there were signs of chlorosis in the p1 treatment (Fig. 3). The p0.5 treatment produced the highest shoot biomass (Table 4). The roots of all trees in all the treatments had spread throughout the lysimeter. We did not quantify root mass or root distribution. Fig. 2D shows the cumulative evaporation (ΣE) for the unplanted lysimeters and the cumulative evapotranspiration (ΣET) from the planted lysimeters. The ΣET from the planted lysimeters was between 3.7 and 4.5 times that of the ΣE from the unplanted treatments. The p0.25, p0.5, and p1 treatments had significantly higher ΣET, than p0. There were no significant differences between the DE treatments. The cumulative transpiration (Table 4), as measured by subtracting the ΣE of unplanted treatment from those of the planted treatments, followed a similar pattern as ΣET. Leaf biomass as a percentage of total biomass (Table 4) was significantly higher in the p0.5 treatment. The higher proportion of leaf production upon DE application is advantageous because leaves are

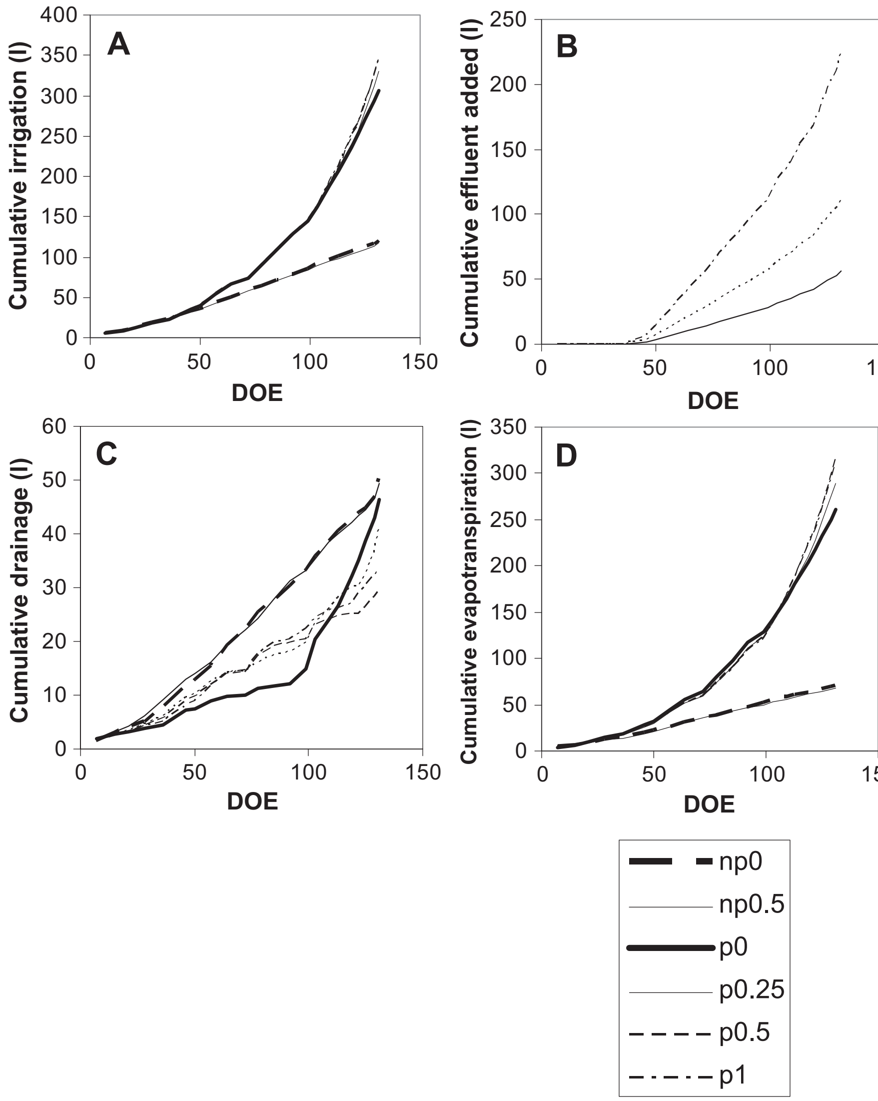

Fig. 2 – Cumulative irrigation (2A), dairy effluent addition (2B), drainage (2C) and evapotranspiration (2D), determined by water balance (assuming constant water storage), of the lysimeters throughout the experiment. DOE = Day of experiment.

Fig. 3 – Example of chlorotic leaves indicating the effects of increasing DE application rates: leaves 1–2 from treatment p0.25, 3–4 treatment p0.5, 5–6 treatment p1.

more palatable than stems to livestock, and have higher concentrations of essential elements (Table 5). The water use efficiency (WUE), calculated by the dividing the total above-ground biomass (kg) by the total water use (m^3), was significantly higher in the p0.5 treatment compared to the other treatments.

3.2. *Effect of DE addition on the elemental composition of plants and soils*

Table 5 shows the elemental concentrations in the leaves and stems of the willows at the end of the experiment. Plants in lysimeters receiving DE had significantly higher N, P, K and Cl concentrations compared to the control plants. There were no consistent increases or decreases among the trace elements or the macronutrients, Mg and Ca. This indicates that the application of DE is unlikely to affect the uptake of essential trace elements in animal nutrition, such as Zn, Cu and Fe.

Table 4 – Aboveground biomass production, total transpiration, and water use efficiency (WUE) of the willows. Values in brackets are the standard error of the mean. Means in the same row labelled with the same letter are not significantly different. The transpiration was calculated by subtracting the total evaporation from the unplanted lysimeters from the total evapotranspiration (Fig. 2D).

Treatment	p0	p0.25	p0.5	p1
Total dry shoot biomass (g)	376 (39)a	462 (21)ab	581 (3)c	536 (39)bc
% of total biomass as leaves	42 (2)a	52 (2)b	57 (1)c	54 (2)b
Transpiration (l)	191 (11)a	220 (10)b	246 (4)b	239 (7)b
WUE (kgm^{-3})	1.97 (0.17)a	2.10 (0.02)ab	2.37 (0.04)b	2.23 (0.13)ab

Leaf N concentrations of the plants in this study were higher than corresponding values reported by Weih and Nordh [19] for various willow clones in agricultural soil that received 20 and 150 kg ha^{-1} of Nitrogen. However, levels were within the same range as those of *Salix viminalis* grown on arable land amended with 150–300 kg ha^{-1} of Nitrogen in a study conducted by Jung et al. [20]. Leaf phosphorous concentrations were below 1.8 g kg^{-1}, a value reported by van den Burg [21] as sufficient for willow growth, although our values were comparable with those (ca 1–2 g kg^{-1}) reported by von Fircks et al. [22] for healthy specimens of *Salix dasyclados* near the end of the growing season. Potassium concentrations were within the range (10–15 g kg^{-1}) that Dimitiou et al. [23] reported for five willow clones grown on arable land in central Sweden.

Table 5 – Elemental concentrations (g kg^{-1}, or $^{+}$mg kg^{-1} dry matter) of the willow shoots. Values in brackets are the standard errors of the means. Means in the same row labelled with the same letter are not significantly different. *Mo and Co were below the detection limits (<1 and <3 mg kg^{-1}) in all samples.

Treatment	p0	p0.25	p0.5	p1
N leaves	24.1 (0.8)a		31.6 (3.1)bc	43.5 (0.4)c
P leaves	1.1 (0.1)a	1.4 (0.1)ab	1.4 (0.1)ab	1.5 (0.1)b
P stems	0.6 (0.0)a	0.8 (0.0)ab	0.9 (0.1)b	0.9 (0.1)b
S leaves	2.0 (0.1)a	1.8 (0.1)a	1.8 (0.1)a	1.7 (0.1)a
N:S leaves	12.0 (1.2)a	16.5 (1.3)ab	18.1 (2.1)b	25.7 (1.6)c
S stems	0.4 (0.0)a	0.4 (0.0)a	0.5 (0.1)a	0.4 (0.0)a
K leaves	11.6 (0.4)a	14.3 (0.2)b	14.8 (0.3)b	14.8 (1.1)b
K stems	3.6 (0.2)a	5.2 (0.4)ab	5.9 (0.8)b	5.6 (0.6)b
Ca leaves	11.2 (0.7)a	13.2 (1.0) a	11.6 (1.4)a	11.6 (1.0)a
Ca stems	6.6 (0.4)a	7.2 (1.0) a	7.6 (0.3)a	7.8 (0.5)a
Mg leaves	0.9 (0.0)a	0.9 (0.0)a	0.9 (0.1)a	1.0 (0.0)a
Mg stems	0.3 (0.0)a	0.3 (0.0)ab	0.4 (0.0)ab	0.3 (0.0)b
Cl leaves	1.6 (0.1)a	2.4 (0.2)b	2.8 (0.2)b	3.4 (0.1)c
Cl stems	0.1 (0.0)a	0.2 (0.0)ab	0.6 (0.2)ab	0.8 (0.3)b
$^{+}$Fe leaves	134 (2)a	123 (19)ab	85 (7)b	117 (12)ab
$^{+}$Fe stems	15 (1)a	17(3)ab	26 (3)b	16 (3)a
$^{+}$Mn leaves	55 (2)a	52 (4)a	48 (5)a	73 (7)b
$^{+}$Mn stems	22 (1)a	18 (1)b	19 (1)ab	18 (1)b
$^{+}$Zn leaves	36 (6)a	51 (9)a	50 (8)a	38 (1)a
$^{+}$Zn stems	40 (2)a	46 (4)a	42 (2)a	36 (4)a
$^{+}$Cu leaves	5.5 (0.2)a	6.5 (0.2)b	6.0 (0.4)a	7.7 (0.2)b
$^{+}$Cu stems	3.8 (0.3)a	5.0 (0.1)b	5.3 (0.5)ab	5.3 (0.5)b
$^{+}$B leaves	17 (6)a	35 (18)a	25 (3)a	25 (1)a

There was a significant increase the foliar N:S concentration ratio in the DE treatments. This ratio was >15 in all the DE treatments, which may indicate an S deficiency [24]. In all treatments, foliar S concentrations were at the lower end of the range of concentrations (1.6–9.1 g kg^{-1}) reported for healthy willow trees [23,25–27]. The decrease in plant S concentrations may not necessarily be caused by a reduction in S uptake by the roots. The higher biomass production of the treatments relative to the control implies that the shoot S concentration would decrease even if the root uptake remained constant.

There was a consistent increase in both the leaf and stem Cl concentration with increasing rates of DE application. Plant material from the p1 treatment contained twice the Cl concentration of the control (p0). In all plants, leaf Cl concentrations were an order of magnitude higher than 0.1 g kg^{-1}, a value reported by Salisbury et al. [28] as adequate for higher plants. Zalesny and Bauer [27] reported no symptoms of Cl toxicity in willows with foliar Cl concentrations up to 1.9 mg kg^{-1}, a concentration exceeded by all the DE treatments in this experiment. At the end of the experiment, one third of the leaves in plants under p1 regime were chlorotic, with symptoms resembling chloride toxicity (Fig. 3). Older leaves were the most affected, which is consistent with the toxicity symptoms of a xylem-transported element, such as Cl [28].

With the exception of Cl, there were no significant differences in the elemental composition of the soils at the end of the experiment in any of the lysimeters (data not shown). Soil Cl concentrations (and standard errors) in the p0, p0.25, p0.5 and p1 treatments were 50(8), 70(21), 81(11), and 166(35) mg kg^{-1} respectively. This indicates that Cl accumulated in the DE treatments throughout the experiment. More chloride was added in the DE treatments than the plants accumulated in the shoots (Table 6). Except for a few soils with a high anion exchange capacity, chloride moves freely down the soil profile with percolating water [29]. In free-draining stands of DE-irrigated willows, excess chloride will leach below the root-zone when rainfall is greater than evapotranspiration. Therefore, Cl toxicity is more likely in arid climes.

Table 6 – Amounts (g) of N, K, and Cl added in the treatments and taken up into the willow shoots. Values in brackets are the standard errors of the means. Means in the same row labelled with the same letter are not significantly different. *Calculated using the leaves only.

Treatment	p0	p0.25	p0.5	p1
Cl added in effluent	0	4.2	8.4	16.8
Cl removed by plant	0.3 (0.0)a	0.6 (0.1)b	1.1 (0.1)bc	1.2 (0.1)c
Balance	−0.3	3.6	7.3	15.6
N added in effluent	0.0	6.2	12.3	24.6
N removed by plant*	3.8 (0.6)a	7.0 (0.5)b	10.4 (0.9)c	12.7 (1.4)c
Balance	−3.8	−0.8	1.9	11.9
K added in effluent	0.0	5.1	10.2	20.4
K removed by plant	2.6 (0.4)a	4.6 (0.3)bc	6.4 (0.1)c	5.2 (0.4)c
Balance	−2.6	0.5	3.8	14.7

3.3. *The amount of N, K, and Cl removed by the willows compared to that applied in the DE*

Table 6 shows the total mass of N and K removed by the trees compared to the total mass of N and K that was applied with the DE. For the p0.5 and p1 treatments, the application rates both elements exceeded their rates of removal by the trees. This indicates that effluent application rates equivalent to the p0.5 and p1 treatments may result in high levels of N leaching (not measured in this study) and the accumulation of K in the soil. In the p0.5 treatment, equivalent to an N application rate of 279 kg ha^{-1} yr^{-1}, the amounts of N and K extracted were similar to those applied. Nevertheless, leaching may occur in such a system due to preferential flow processes in the soil, which depend on rainfall distribution and soil type. Therefore, similar to pasture, the application rate of DE to willows should be determined with a series of field trials using recommended practices or, more efficiently, using simulations with mechanistic models such as SPASMO [30]. The acceptable rates of application are site-specific depending on local climatic and edaphic conditions.

Any leaching from a land treatment system could be mitigated if the drainage from the application area could be re-circulated onto the trees. Here, the maximum tolerable level of effluent application would be dependent on the tree health, rather than environmental concerns regarding the leaching. The long-term functioning of such systems may be susceptible to nutrient imbalances or the build up of solutes such as Cl and K. in the soil.

4. Conclusions

Potentially, dairy effluent could be irrigated onto stands of *S. kinuyanagi* to increase growth and produce nutritious biomass that could be fed back to the cows, thus resulting in increased nutrient cycling. The application of DE to *S. kinuyanagi* at rates up to the equivalent of 279 kg ha^{-1} of Nitrogen increased growth, transpiration, water use efficiency, and the percentage of biomass as leaves. There were no decreases in the shoot concentrations of animal-essential elements. The amounts of N and K removed by the plant were similar to those applied in the effluent. Soil Cl accumulation and consequent Cl toxicity may be problematic in areas where evapotranspiration exceeds rainfall.

The results of this three-month study do not necessarily indicate the long-term response of willows to DE application. Therefore, a long-term field trial is warranted to test the relative efficiencies of willows and pasture under various DE treatment regimens. The results of such a trial could be used to develop guidelines for the maximum allowable rates of DE application to stands of willow.

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