TREATING MUNICIPAL WASTEWATER THROUGH A VEGETATION FILTER WITH A SHORT-ROTATION POPLAR SPECIES

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ABSTRACT

The performance of a vegetation filter using a short-rotation coppice of poplars was evaluated over a 3-year period in terms of pollutant removal capacity. The vegetation filter was designed for scattered and small populations with no storage facilities and a wastewater application constrained by the own production of effluent.. Wastewater effluent was pre-treated in an Imhoff tank and applied to the vegetation filter. The chemical compositions of drainage water and groundwater were regularly monitored. Surface soil samples at the beginning and the end of the study were also collected. The monitored chemical species in drainage water and groundwater were DOC, COD, N_T, NO_3-N, NH_4-N, P_T, PO_4-P, and other major ions. Electrical conductivity, organic matter content (%), NO_3-N, available P, cation exchange capacity and major cations were analysed for soil. The vegetation filter presented efficient removal of wastewater-originated pollutants. DOC and COD removal reached values of 85%. A correlated increase in soil organic matter content was detected (from 1.0% to almost 2.8%). A similar removal capacity was observed for P_T which is interpreted as due to plant uptake mechanisms and PO_4^{3-} precipitation in the presence of soil Ca^{2+}. Around 73% of N_T was removed. However due to the high applied N_T load, the average N_T concentration in drainage water was about 41.9 mg/L, higher than the admissible concentration limit. When considering N_T mass, about 10% of the cumulative applied N_T leached through the vadose zone. Groundwater quality was not affected by the vegetation filter operation.

Keywords: vegetation filters, wastewater reuse, vadose zone, pollutant removal, short rotation coppice.

1. INTRODUCTION

Nature-based wastewater purification systems have been reported as a feasible solution for small municipalities and scattered populations with limited access to sewage networks (Ortega et al., 2011). They came about as an alternative to conventional treatment systems given their advantages in term of robustness, low management and maintenance costs, and environmental benefits, primarily related with low sludge production. Specifically, these systems imply reduced operational, energy and chemical requirements if compared to conventional methods (Dimitriou and Aronsson, 2011).

Vegetation filters (VFs), a specific type of nature-based wastewater purification systems, involve the application of pre-treated and/or treated wastewater to a vegetated soil surface. Such a system relies on soil attenuation capacity and plant uptake to remove potential wastewater contaminants (i.e., nutrients). The use of fast-growing tree species with a high evapotranspiration rate, and the fact that their root systems show excellent tolerance to anaerobic conditions, enable the application of considerable amounts of wastewater (Herschbach et al., 2005; Persson and Lindroth, 1994). In Northern European climates, the most widely used tree species are willows (*Salix spp.*) whereas poplars (*Populus spp.*) or eucalyptus (*Eucalyptus spp.*) are mostly used in Southern climates (Dimitriou and Rosenqvist, 2011). Commonly, a VF is characterised by the low density of planted trees (300-500 plants/ha) and long cutting periods (12-17 years) (de Bustamante, 1990; Magesan and Wang, 2003; Sanz et al., 2014).

Nowadays, VF technologies are oriented more towards short-rotation coppice (SRC) applications to enhance the removal of contaminants from infiltrating wastewater (Dimitriou & Aronsson, 2011; Holm & Heinsoo, 2013). SRC refers to an intensive biomass production strategy based on fast-growing species that are able to resprout from stumps after being harvested at short intervals (Dimitriou and Rosenqvist, 2011). SRC involves intensive tree management, which is more similar to agriculture than forestry practices. The application of domestic wastewater to these plantations has been identified as an appealing method to produce biomass, increase wastewater treatment and nutrient recycling, and to reduce greenhouse emissions (Dimitriou and Aronsson, 2005; Tzanakakis et al., 2009)

This paper reports the results of a 3-year research (March 2011-May 2014) conducted in Southern Spain in which a VF, based on SRC of poplars (*Populus alba*), was applied to treat the wastewater produced by an office building with a very high nutrient concentration. We aimed to evaluate the pollutant removal capacity of a VF characterised by lack of wastewater storage facilities and highly variable application rates in volume and quality terms. In addition, possible effects on groundwater were also investigated.

2. METHODS

To assess the efficiency of the VF we (i) monitored the chemical composition of the drainage water collected by a lysimeter; (ii) controlled the chemical composition of groundwater in a nearby piezometer; (iii) evaluated the changes in soil properties between the beginning and the end of the study, and used them as support information to interpret the vadose zone processes. The VF's pollutant removal capacity was investigated by considering the average concentration in drainage water and groundwater. However for DOC, COD, N_T and P_T, which are usually the most problematic pollutants, a mass calculation-based approach in the vadose zone was also considered.

2.1 Site description and VF design

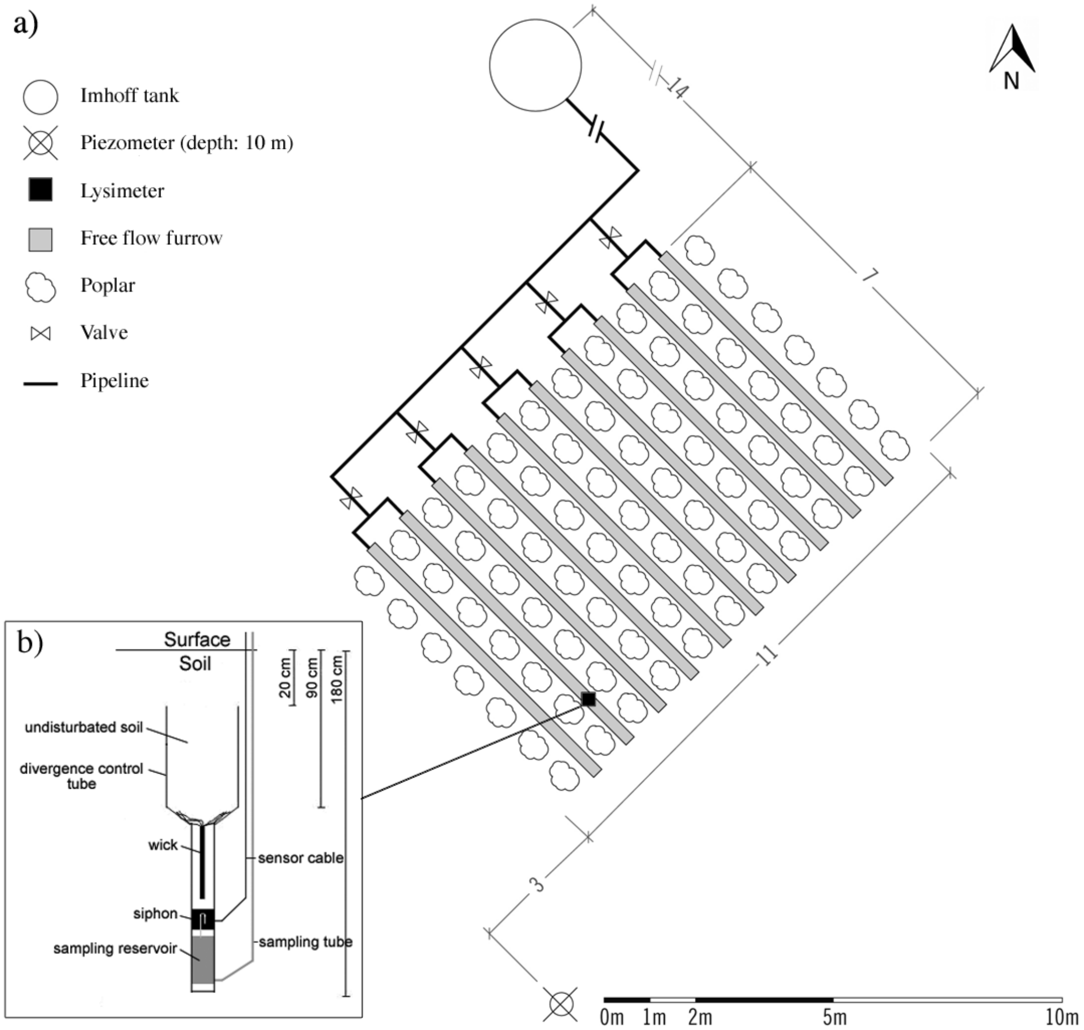
The study was carried out in South-East Spain at the R&D&I Centre of Carrión de los Céspedes (Seville) (37˚22´ N, 6˚19´ W). The VF was designed to treat effluent from an office building, with a capacity of 20 workers who produce an average wastewater volume of 0.5 m^3/day. The average annual rainfall and the reference crop evapotranspiration in the area are 543 mm and 1,448 mm, respectively. The average temperature is 17.4ºC (AEMET, 2011). Climate is characterised by scarce, but intense, rainy events, which take place mainly in spring and autumn, and also by a hot dry summer season.

The experimental plot, with a gentle surface slope directed towards south, is characterised by loamy soils (20.4% Clay, 46.8% Sand and 32.8% Silt) classified as Calcic Haploxeralf (de Bustamante, 2010) according to the USDA classification (Soil Survey Staff, 2010). Before beginning the wastewater application, soil pH, electrical conductivity (EC) and organic matter content (OM) were 8.3, 520 µS/cm and 1.0%, respectively. The soil infiltration capacity measured by a double ring infiltrometer test ranged between 6.5 and 8.0 mm/h. The water table depth varied from 1 m to 3 m. Prior to the investigation the area of the experimental plot was left fallow during the last decade.

The VF surface was calculated using the methodology proposed by de Bustamante et al. (2009), which estimates the VF extension through soil water balance. Considering the natural precipitation in the area, the wastewater volume to be applied and the volume of water evapotranspired, it is possible to quantify the VF surface by minimising run off or water stress periods. Daily and monthly climatic data were obtained from the Spanish Agroclimatic Information System for Irrigation (SIAR). Thus, information from the nearest agroclimatic station (Aznalcázar, Seville) was used to estimate the water requirements of plants. The crop coefficient (Kc) required to estimate the poplar monthly evapotranspiration was obtained from Urbano-Terrón (1992). The maximum daily water flow to be applied was estimated by taking into account the results obtained from the infiltration test. However after installing the VF, the number of workers in the office building unexpectedly lowered and resulted in an average wastewater production of about 0.25 m^3/d.

Given the objective to enhance nutrient pollutant uptake by maximising biomass production, an SRC-based management with 3-year harvesting cycles was chosen. Poplars were planted along 10 lines with a distance between them of 1 m, resulting in a plantation density of 10,000 plants/ha (Fig. 1a). Planting was performed from unrooted hardwood cuttings of about 1.5 m high. Spontaneous vegetation growth was not prevented. Before irrigation, wastewater was previously treated in an Imhoff tank (volume of 2.5 m^3). Subsequently, effluent was applied into free flow furrows of 30 x 20 cm, which were filled with gravel to avoid odours and direct contact (Fig. 1a). Irrigation lines were grouped into five blocks. The irrigation schedule was designed to apply effluent to each block once per week (5 working days per week). Devices that regulate wastewater irrigation rates were not used. Such a decision reflects the purpose of simulating wastewater alternative treatment for scattered small populations for which wastewater is not stored, but applied according to its production. The irrigation rate was monitored during the study by flowmeters.

Figure 1. Scheme of the VF and detail of the lysimeter device (annotated lengths in the main scheme are in meters).



The experimental plot was equipped with a Gee Passive Capillary Lysimeter with a surface area of 471.2 cm^2 and connected to an EM5b data-logger (Decagon Devices, Pullman, Washington, USA). The lysimeter comprises a divergence control tube where undisturbed soil is stored (Fig. 1b). The undisturbed soil was collected according to the lysimeter Operator manual (Decagon Service, 2003) by striking the divergence control tube with a sledgehammer. Until obtaining the tube completely filled, the soil around the portion of the divergence control tube pounded in the soil was dug away during each pounding step. The first 20 cm of the soil that could have been disturbed by the sampling procedure were removed. The lysimeter is connected to a wick, which is able to apply constant tension to soil, and maintains the flow rate within the lysimeter equivalent to the flow rate in the surrounding soil. The volume of drainage water is measured by a syphon and a 1-litre sampling container is used for storage. The lysimeter was used to monitor the volume of water leached from the vadose zone (at a depth of 90 cm) and to collect the leachate for the water chemical analysis. To assess the potential effects of wastewater irrigation on groundwater quality, a piezometer (filter at a depth of 10 m) located 4 m downgradient of the VF was also monitored.

2.2 Water samples analysis

To determine changes in the physico-chemical properties, water samples were collected every month from the Imhoff tank effluent and the piezometer, and after drainage events also in the lysimeter. In all, 116 leachate and wastewater samples were analysed according to the Standard Method for the Examination of Water and Wastewater (Eaton et al., 2005). Analyses were performed at the IMDEA Water (Madrid, Spain) and at the CENTA Foundation laboratories (Seville, Spain). When the analyses were not performed immediately after sampling, the samples were stored for no more than 30 days at -21°C. The measured parameters were: EC and pH (Crison Multimeter MM 41); chemical oxygen demand (COD – Merck Spectroquant TR420 and Spectroquant NOVA60 Spectrophotometer); dissolved organic carbon (DOC - Shimadzu TOC analyzer); the ions Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4-N, Cl^-, SO_4^{2-}, NO_3-N (Metrohm ion chromatography Advanced Compact IC with two-channel) and HCO_3^- (Metrohm Titrando 809). For N_T and P_T determination, measures were previously digested using the peroxodisulphate oxidation method (Ebina et al., 1983) and were subsequently analysed in the forms of NO_3-N and PO_4-P with a Brand+Luebbe Segmented Flow Analyser due to the high electrical conductivity derived from the extraction method.

2.3 Soil samples analysis

Surface soil samples (up to a depth of 20 cm) were collected in quadruplicate at the beginning and the end of the study. Prior to the analysis, samples were air-dried, gently crushed and passed through a 2-mm sieve. Soil pH and EC were measured in a soil-water suspension (1:2.5 and 1:5 soil-water ratio, respectively). OM was determined by the Walkley-Black method, consisting in potassium dichromate-sulphuric acid oxidation (Nelson and Sommers, 1982). Soil available P was extracted by the Olsen and Dean method (Olsen et al., 1954), determined by the ascorbic acid molybdate blue method (Murphy and Riley, 1962) and quantified using a spectrophotometer at 880 nm. NO_3-N was extracted by $CaSO_4$ (Griffin et al., 2009) and analysed by ionic chromatography. Cation Exchange Capacity (CEC) was determined by extraction with ammonium and sodium acetate solutions and the Exchangeable Bases by extraction with ammonium acetate. After soil extraction, exchangeable cation concentrations (Na^+, K^+, Mg^{2+}, Ca^{2+}) were analysed by ICP-MS at the IMDEA Water facilities.

2.4 Statistical analysis

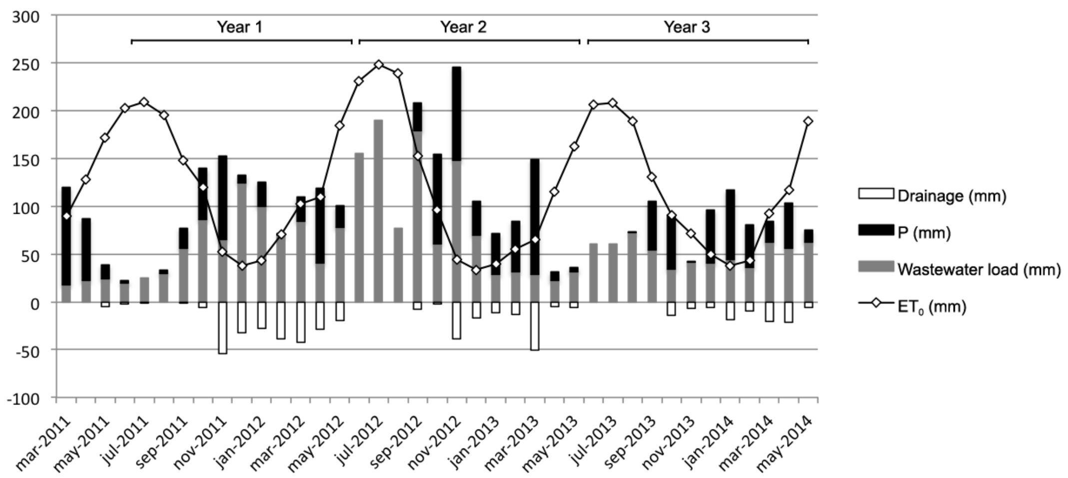
The differences in the chemical composition of the water collected from the effluent, leachate and groundwater among the three years were evaluated using a one-way ANOVA and Tukey´s test in the case of normal distributed data. When data did not present a normal distribution and homogeneity of variance, a Kruskall-Wallis test was applied. The Kolmogorov-Smirnov test was used to evaluate if data was well-modelled by a normal distribution and Levene test was used to verify homogeneity of variance. The significance level was set at $p \leq 0.05$. Calculations were carried out using the software Minitab (version 17) and Statgraphics (version Centurion XVI). Statistical analyses were not considered for soil chemical data due to the limited number of samples.

3 RESULTS

3.1 Effluent

As previously described, the effluent applied to the VF came from an isolated office building. The employed hydraulic load depended on the building's wastewater production and it did not always meet the water requirements of the plantation. According to the amount of applied wastewater, precipitation and potential evapotranspiration (ET_0), each year of the study was characterised by a water deficit period which occurred during dry seasons (spring-summer) (Fig. 2). Throughout the remaining study period, moderate water excess conditions were periodically recorded (Fig. 2). As specified by the Operator´s manual of the lysimeter (Decagon Service, 2003), we decided to set the equilibration period to the first three months of the study (from March to May 2011). One drainage period (from October to May) ocurred during each year of the study (in total 3 periods of drainage). Therefore, the annual data presented in this work refers to the period from June to May in agreement with the end of the drainage period (Fig. 2). The annual irrigated wastewater load was 778 mm, 1,022 mm and 625 mm for the first, second and third year, respectively (Fig. 2). Drainage water was approximately 15% of the hydraulic load (precipitation and wastewater) on the VF surface, mostly restricted to the drainage period (Fig. 2).

Figure 2. Temporal variation of the applied wastewater load, precipitation (P), drainage water and reference crop evapotranspiration (ET_0).



Owing to the water consumption pattern in such a building in which most of the water is used for toilet flushing and hand washing, the pollution load was remarkably high if compared to a typical domestic wastewater effluent (Table 1). This holds especially true for nutrients such as N_T and P_T, whose average wastewater concentrations were 154.9 and 16.1 mg/L, respectively. NH_4-N was the dominant N_T species (94%), which reflected the anaerobic conditions in the Imhoff tank. Org-N and org-P represented 6% and 23% of N_T and P_T, respectively. No significant differences were detected in the concentration of phosphorus compounds among the three years whereas in the case of nitrogen compounds, only the second year presented a concentration significantly higher than the others (Table 1).

The applied wastewater presented an average concentration of COD and DOC of 269.6 mg/L and 88.0 mg/L, respectively (Table 1). Their concentrations in the first year resulted to be significantly higher than concentrations measured in the following years. The average effluent pH was 7.7, whereas EC was slightly high with a value of 2,046.9 µS/cm (Table 1). However, no effects on tree growth were believed to occur given the high salt tolerance capacity of the poplars (Chen and Polle, 2010). Since wastewater showed a Sodium Adsorption Ratio (SAR) of 2.6, no decrease, or only a slight decrease, in the soil infiltration rate was expected. As suggested by Oster and Schroer (1978), when the SAR is higher than 2, infiltration rate could decrease. This phenomenon is due to the loss of the soil structure as a consequence of the Na^+ contained in the WW.

Table 1. Reference and effluent chemical composition. Mean values with standard deviations and applied loads for each parameter during the entire study period. The average values and applied loads for each year are also shown. Means with different letters were statistically different at $p < 0.05$.

	Reference (Metcalf & Eddy, 2003)	3-year study		1st Year		2nd Year		3rd Year	
		Mean	Load (kg/ha)	Mean	Load (kg/ha)	Mean	Load (kg/ha)	Mean	Load (kg/ha)
pH	-	7.7 ± 0.04	-	7.6 ± 0.04a	-	7.7 ± 0.1a	-	7.7 ± 0.1a	-
EC (μS/cm)	-	2,046.9 ± 72.0	-	1,933.2 ± 108.8ab	-	2,216.3 ± 143.1a	-	1,930.0 ± 55.9b	-
DOC (mg/L)	260.0	88.0 ± 7.2	476.5	113.9 ± 10.7a	692.9	58.8 ± 5.2b	271.4	62.4 ± 9.8b	346.3
COD (mg/L)	800.0	269.6 ± 22.0	1,458.9	360.6 ± 32.7a	2192.9	202.3 ± 21.7b	932.5	157.6 ± 27.5b	873.9
N_T (mg/L)	70.0	154.9 ± 7.8	838.4	156.7 ± 10.2a	952.7	176.9 ± 12.3b	815.5	146.1 ± 6.3a	810.4
NO_3-N (mg/L)	0.0	0.4 ± 0.3	2.3	0.1 ± 0.04a	0.4	1.2 ± 1.0b	5.6	0.3 ± 0.1b	1.5
NH_4-N (mg/L)	45.0	145.8 ± 7.2	789.3	146.4 ± 9.6a	890.0	164.2 ± 11.2b	756.9	142.5 ± 4.8a	790.2
P_T (mg/L)	12.0	16.1 ± 0.9	87.4	16.7 ± 1.3a	101.4	17.7 ± 1.8a	81.7	14.5 ± 0.6a	80.6
PO_4-P (mg/L)	8.0	12.4 ± 0.7	66.9	12.8 ± 1.0a	78.0	13.6 ± 1.4a	62.9	10.9 ± 0.6a	60.4
Cl^- (mg/L)	-	164.2 ± 7.9	888.9	156.5 ± 8.5a	951.6	156.6 ± 8.7a	721.3	146.7 ± 5.3a	813.6
$SO4^{2-}$ (mg/L)	-	41.4 ± 6.1	224.1	36.2 ± 9.3a	220.2	37.5 ± 5.5a	172.9	33.6 ± 3.0a	186.4
HCO^{3-} (mg/L)	-	831.0 ± 31.5	4,497.4	815.3 ± 51.2a	4,957.7	933.8 ± 56.3b	4305.0	772.7 ± 17.6a	4,284.9
Na^+ (mg/L)	-	100.3 ± 4.5	542.6	96.9 ± 5.2a	589.2	93.4 ± 5.3a	430.8	91.1 ± 3.6a	505.4
K+ (mg/L)	-	51.8 ± 2.3	280.6	51.3 ± 3.1a	311.9	56.9 ± 3.3a	262.1	50.4 ± 2.2a	279.3
Mg^{2+} (mg/L)	-	16.0 ± 0.8	86.8	14.2 ± 0.7a	86.4	16.7 ± 0.7b	77.1	16.7 ± 0.6b	92.9
Ca^{2+} (mg/L)	-	51.0 ± 5.8	276.1	45.3 ± 1.0a	275.4	51.3 ± 2.2b	236.6	36.8 ± 2.8c	204.0
SAR	-	2.6 ± 0.1	-	2.6 ± 0.1a	-	2.3 ± 0.1a	-	2.6 ± 0.1a	-

3.2 Contaminant removal capacity of the VF

To evaluate the effectiveness of the VF to remove wastewater contaminants, the chemical composition of the Imhoff tank effluent was compared with that of the leachate collected by the lysimeter in terms of concentrations. In Table 2, the average values of the leachate and the related removal percentages are reported separately for the three years of study. The corresponding values averaged throughout the research period are also presented. Some of the monitored parameters and chemical species (EC, Cl^-, SO_4^{2-}, Na^+, Mg^{2+} and Ca^{2+}) presented negative removal percentages; i.e., the values in the leachate were higher than that in the effluent (Table 2).

Table 2. Chemical composition of the leachate collected by the lysimeter. Mean values and standard deviations for the entire period and for each year of the study. Percentages of removal are also reported. Means with different letters were statistically different at $p < 0.05$.

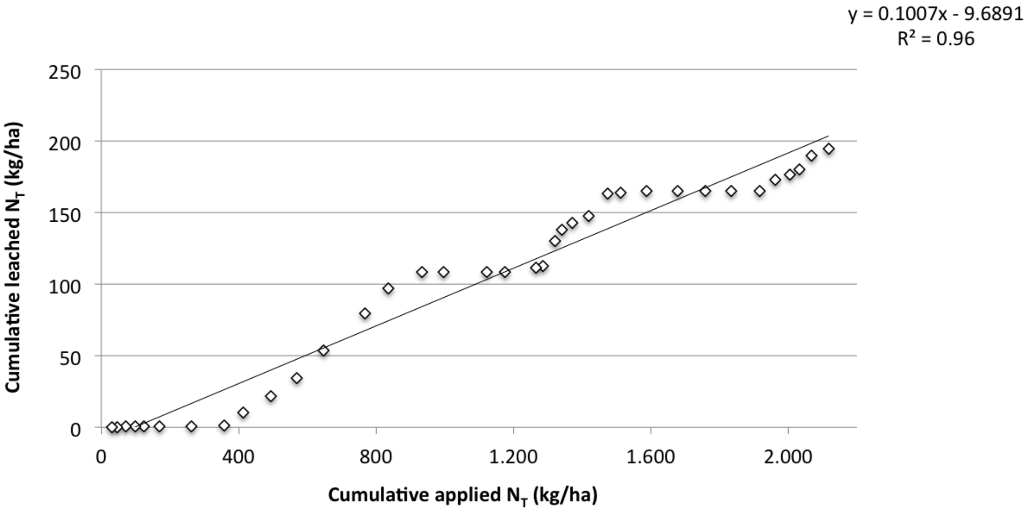
	3-year study		1st Year		2nd Year		3rd Year	
	Mean	% Removal	Mean	% Removal	Mean	% Removal	Mean	% Removal
pH	7.8 ± 0.1	-	7.8 ± 0.1a	-	7.8 ± 0.2a	-	7.7 ± 0.16a	-
EC (µS/cm)	2,821.3 ± 218.2	-37.8	3,557.9 ± 222.7a	-84.1	1,688.3 ± 90.3b	23.8	1,687.9 ± 74.33b	12.6
DOC (mg/L)	12.7 ± 0.7	85.6	12.2 ± 1.0a	89.3	14.5 ± 1.6a	75.4	12.6 ± 0.62a	79.9
COD (mg/L)	40.1 ± 3.0	85.1	41.7 ± 3.5a	88.5	48.7 ± 8.3a	75.9	28.3 ± 0.77b	82.0
N_T (mg/L)	41.9 ± 3.4	73.0	43.4 ± 4.3a	72.3	27.3 ± 5.4a	84.6	50.2 ± 3.09a	65.7
NO_3-N (mg/L)	40.1 ± 3.4	*	41.4 ± 4.4a	*	25.8 ± 4.8a	*	48.5 ± 3.15a	*
NH_4-N (mg/L)	0.6 ± 0.3	*	0.7 ± 0.4a	*	0.7 ± 0.7a	*	0.3 ± 0.28a	*
P_T (mg/L)	1.5 ± 0.3	90.7	1.8 ± 0.4a	89.5	1.9 ± 0.5a	89.5	0.5 ± 0.11a	96.8
PO_4-P (mg/L)	1.0 ± 0.2	*	1.2 ± 0.3a	*	1.3 ± 0.3a	*	0.3 ± 0.07a	*
Cl^- (mg/L)	497.2 ± 55.6	-202.7	693.9 ± 53.4a	-343.4	195.8 ± 7.9b	-25.2	193.6 ± 12.80b	-31.9
$SO4^{2-}$ (mg/L)	294.9 ± 33.6	-612.1	413.9 ± 32.5a	-1,043.1	126.6 ± 8.1b	-237.5	99.3 ± 5.86b	-195.4
HCO^{3-} (mg/L)	391.5 ± 11.7	52.9	417.7 ± 11.6a	48.8	405.2 ± 23.2a	56.6	356.0 ± 13.80b	53.9
Na^+ (mg/L)	235.1 ± 18.4	-134.5	297.3 ± 18.3a	-206.8	131.2 ± 1.9b	-40.4	146.4 ± 5.86b	-60.6
K+ (mg/L)	5.2 ± 0.5	90.0	5.4 ± 0.5a	89.4	7.1 ± 1.4a	87.5	2.8 ± 0.38b	94.5
Mg^{2+} (mg/L)	53.9 ± 4.6	-236.0	68.5 ± 4.9a	-381.9	30.2 ± 0.5b	-80.7	32.4 ± 1.22b	-93.2
Ca^{2+} (mg/L)	290.2 ± 22.7	-468.9	369.0 ± 22.9a	-714.9	174.5 ± 8.7b	-240.0	164.0 ± 8.30b	-345.8

*Transformed compound

The DOC and COD values in drainage water were relatively constant throughout the study with mean values of 12.7 and 40.1 mg/L, respectively. The average removal percentage calculated by taking into account the whole study period was about 85% for both parameters. However this value was higher during the first drainage period (more than 88%) and lower during the following periods (between 75% and 82%). The higher removal percentage corresponds to the first drainage period, when the concentration in the applied wastewater was lower.

Concerning nitrogen species, the mean concentrations of N_T, NO_3-N and NH_4-N in drainage water were 41.9, 40.1 and 0.6 mg/L, respectively (differences among years were not statistically significant). Due mainly to the nitrification processes which occurred in the vadose zone, NO_3-N was the dominant nitrogen species in the leachate (96%). The average N_T removal capacity of the VF, after taking into account the average concentrations measured during the entire experiment, reached a value of about 73% (Table 2). When the three years were considered separately, the removal percentage of the first and third year were lower (72 and 65%, respectively) than that obtained during the second year of the study (85%) This is related to the high concentration of N_T in the effluent measured during this year (Table 1). Concerning N_T mass, cumulative leached N_T was plotted against cumulative applied N_T (Fig. 3). Both values were calculated for each month. The resulting linear regression model (R^2= 0.96) can be used to estimate the future cumulative leached N_T when the applied N_T mass is known (Duan et al., 2010a). The slope of the regression model indicates that, during the study period, only 10% of cumulative applied N_T leached through the vadose zone confirming the VF's high removal capacity. The model has been also applied to P_T, DOC and COD and only about 5% of the cumulative applied load infiltrates towards the lysimeter (data not shown).

Figure 3. Cumulative applied N_T *vs.* cumulative leached N_T.



Other nutrients such as P_T or K^+ were almost fully removed by the VF, and their mean concentrations in drainage water were 1.5 and 5.2 mg/L, respectively. The soil-plant system's removal capacity for these two species was relatively constant during the study period with values generally above 89% (Table 2).

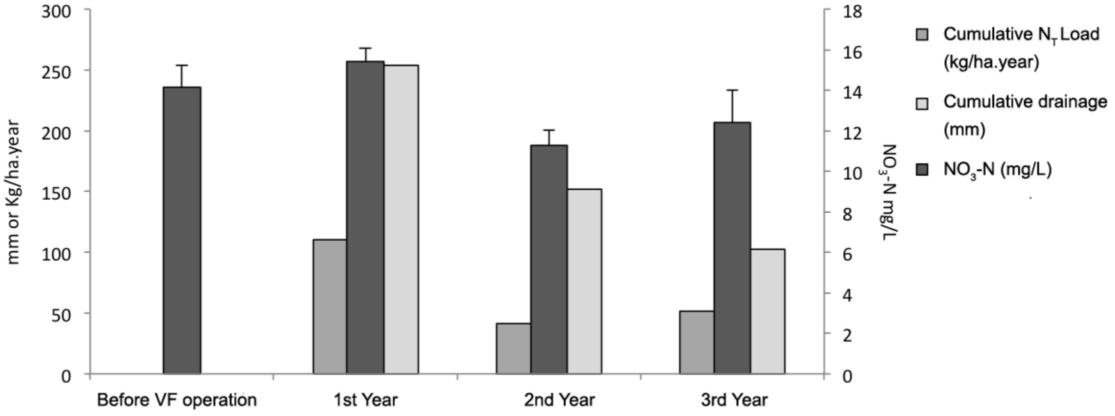
The EC variation in the drainage water was also evaluated (Table 2). A different trend of this parameter was observed between the first and the following years (significant statistical difference). During the first year, water showed an average EC that almost doubled that of the effluent (about 3,557.9 μS/cm). However, EC lowered to a mean value of 1,688.3 μS/cm and 1,687.9 μS/cm during the second and third years, respectively. The initial increase in EC is also reflected by the increased concentration of dissolved ions Cl^-, SO_4^{2-}, Na^+, Mg^{2+} and Ca^{2+} (significant statistical difference among first and following years).

3.3 Groundwater

The groundwater at the field site presented average pH and EC values of 7.6 and 4,100.7 μS/cm, respectively, and these values did not vary during the study period. The high EC values are related to the presence of dissolved salt and correlate well with the concentrations of Cl^-, SO_4^{2-} and Na^+. P_T was not detected in groundwater. The average DOC and COD were 5.9 and 21.0 mg/L, respectively.

The average N_T concentration in the groundwater at the field site before the VF operation was 14.1 mg/L (Fig. 4) and NO_3-N was the only detected nitrogen species. Therefore already before the wastewater application began, groundwater exhibited an NO_3-N concentration that was slightly above the legal limits (11.3 mg/L) (CEE, 1991b). In Figure 3, the amount of leached water (mm) and N_T leached loads (kg/ha.yr) calculated by the lysimeter for each year are also reported. Despite the wastewater application, the NO_3-N concentration in groundwater was higher (15.4 mg/L) than the background concentration only during the first year. In the following years, NO_3-N concentrations decreased to values of 11.3 and 12.5 mg/L, respectively (Fig. 4). However according to the results obtained by the statistical analysis, no significant differences were detected among the NO_3-N concentration before the VF operation and the 3-year application.

Figure 4. Mean NO_3-N concentration in groundwater (mg/L), cumulative drainage (mm) for each year and cumulative N_T leached load (kg/ha.yr) before the VF operation and during the first, second and third year. Standard deviations are also shown for NO_3-N concentration.



3.4 Chemical changes in soil properties

Differences in the soil chemical properties between the two soil sampling campaigns are shown in Table 3. Soil pH dropped by only 5% and showed a good buffering capacity despite the applied OM loads and plant activity. After the 3-year VF operation, the soil EC displayed almost a three-fold decrease in comparison to its initial value. The OM content in surface soil increased from 1.0% to 2.8% at the end of the study. Before the beginning of wastewater application the available P in soil was 14.1 mg/kg. After the 3-year study this parameter raised up to 43.8 mg/kg. Therefore, the final available P content increased by up to 210% if compared with the initial conditions. Nevertheless, NO_3-N decreased from 53.3 to 37.5 mg/kg. Soil CEC did not vary during the study.

Table 3. Average values and related standard deviations of chemical soil properties before and after the VF operation

	Sampling campaign	**Variation**

Properties	Beginning	End	(%)
pH	8.3 ± 0.1	7.9 ± 0.01	-4.8
EC (dS/m)	0.5 ± 0.03	0.2 ± 0.02	-65.3
Organic matter (%)	1.0 ± 0.1	2.8 ± 0.4	140.8
Available P (mg/kg)	14.1 ± 1.1	43.8 ± 7.3	209.8
NO_3^-N (mg/kg)	53.3 ± 2.1	37.5 ± 4.0	-29.6
B (mg/kg)	0.3 ± 0.04	0.5 ± 0.02	53.3
CEC (cmol/kg)	13.6 ± 0.3	14.5 ± 0.8	6.6
Na^+ (mg/kg)	108.3 ± 15.6	58.8 ± 4.5	-45.7
K^+ (mg/kg)	174.0 ± 19.0	84.3 ± 5.7	-51.5
Ca^{2+} (mg/kg)	2,196.7 ± 64.7	2,542.0 ± 167.3	15.7
Mg^{2+} (mg/kg)	188.6 ± 10.4	203.1 ± 38.0	8.8

4 DISCUSSION

Due to differences in plants species, effluent quality, hydraulic loads and adopted management, the comparison among results from different studies is anything but straightforward (Paranychianakis et al., 2006). However independently of the *modus operandi* of VFs, the experiences reported in the literature identify such a system as having a great potential for wastewater treatment. Thus, several articles published in the last decade have pointed out how poplars and other plants species together with soil and soil microbiota are able to reduce wastewater originated compound and enhance biomass production (Duan et al., 2010b; Justin et al., 2010; Tzanakakis et al., 2009; Tzanakakis et al., 2011; Dimitriou and Aronsson, 2011; Duan and Fedler, 2009; Heinsoo and Holm 2008 in Isebrands and Richardson Eds. 2014; Holm and Heinsoo, 2013). Results obtained by the present study confirm this outstanding capability.

Concerning the organic load, DOC and COD concentrations in the applied wastewater were far below those reported by Metcalf and Eddy (2003) for domestic wastewater. According to Ortega et al. (2011), the treatment through the Imhoff tank is able to remove about 20-30% of the total organic load. Therefore, the organic load was presumably reduced prior the entrance of the wastewater into the VF. The high removal percentage obtained along the 3-year VF operation (higher than 85%) ensured a COD concentrations below the limits established by Directive 91/271/EEC (CEE, 1991b) for discharges from urban wastewater treatment plants (125 mg/L or 75% of removal). Differences in the removal percentages among the years have been described in the previous section and according the statistical analysis (Tables 1 and 2), they seem to be related to changes in the quality (i.e. the DOC and COD concentrations) of the applied wastewater. However from the results obtained by other authors, the organic load is efficiently removed independently of the applied DOC and COD loads, plant species and soil types (Duan and Fedler 2010; Jonsson et al., 2004; Ou et al., 1997).

It should be noted that the reduction of DOC and COD in the drainage water reported here is accompanied by a substantial increase in OM soil content after a 3-year operation (Table 3). According to the classification proposed by Marañés et al. (1998), soil that is initially characterised by a low OM content (1.0%) becomes a soil with a high OM content (2.8%) at the end of the study. Similar soil OM accumulation results have been reported by Jueschke et al. (2008). These authors observed increased OM content in surface soil (0-20 cm) at three of the four sites irrigated with wastewater secondary effluent. According to Marschner & Kalbitz (2003), fine soil particles show delayed OM decomposition due to OM entrapment in micropores with a diameter under 0.2 µm. The small size of these micro-pores not only hampers the access of heterotrophic microorganisms to OM, but also limits the oxygen diffusion required for biodegradation.

N_T removal capacity obtained by considering masses and concentration considerably differs (i.e., 90% and 74%, respectively). Although both values reflect the VF's effectiveness, better efficiency was calculated by the mass approach. This difference relates to the small drainage volume collected by the lysimeter. Obviously, better removal efficiency can also be estimated for other pollutants by taking into account masses instead of concentrations.

Even if the nutrient concentration in the wastewater applied in this study is much higher than those reported by Metcalf and Eddy (2003) for high strength urban wastewaters (Table 1), the soil-plant system was able to lower the N_T and P_T concentrations. Several studies report nutrient removal capacity of VF managed through SRC (Heinsoo and Holm, 2008 in Isebrands and Richardson, 2014; Holm and Heinsoo, 2013; Kuusemets et al. 2001 in Isebrands and Richardson, 2014). During our research, the cumulative loads of N_T and P_T were 838.4 kg/ha.yr and 87.4 kg/ha.yr respectively, which are notably higher than those applied in the cited studies. However Aronsson et al. (2010), i in their study on landfill leachate application to an SRC of willows in Sweden, report a satisfactorily N_T removal percentages up to 80% (based on mass calculation) when the supply of N_T was extremely high (varying between 720 and 2,160 kg/ha.yr). In our study even if a remarkably efficient N_T removal occurred under high application loads, the average N_T concentration in drainage water exceeded the limit value of 15 mg/L set by Directive 91/271/EEC (CEE, 1991b). However, the same Directive states that, according to the local situation, a minimum N_T reduction of 70-80% can also be applied as a regulatory criterion. In this case, the VF presented herein meets this part of the Directive regulation since average N_T removal was 73%.

N_T removal is controlled by mechanisms such as plant uptake, denitrification, soil storage by adsorption, bacterial immobilisation, ion exchange and ammonia volatilisation. Plant uptake is considered the primary N_T removal mechanism in a VF

(Tzanakakis et al., 2009). However, such a process depends highly on the plant species and adopted management (Barton et al., 2005). As reported by Tzanakakis et al. (2009) for a VF irrigated with domestic effluent, nutrient recovery by the biomass in an SCR poplar plantation is about 120 kg N_T /ha.yr. This value could be higher when spontaneous vegetation is taken into account. However since we did not regularly mow this vegetation, its nitrogen uptake is limited. The N_T removal reported in the present study is far beyond the nitrogen rates applied, so mechanisms other than nutrient uptake are expected to be involved in nitrogen removal.

The appearance of NO_3-N in drainage water during our study indicates that nitrification processes through the vadose zone are involved. Consequently, the produced NO_3-N can be further transformed by denitrification. As reported by Duan et al. (2010a), despite the wide variability, the denitrification process can play a key role in N_T removal. It can remove up to 80% of the N_T applied to a VF (EPA, 1981). In this sense, an increment in soil OM can expand the microbial population and activity by promoting nitrification and denitrification processes (de Miguel et al., 2013). Even if the field site conditions are prevalently aerobic as suggested by the nitrification processes, we cannot rule out that temporally anoxic conditions could have occurred immediately after applying the effluent. This could have facilitated NH_4-N sorption to soil clay particles and OM. Alternatively under temporal anoxic conditions, NH_4-N oxidation (Annamox) could have occurred as another process to remove N_T from the system (Sher et al., 2012). However, we consider that these processes are considerably less effective than nitrification and subsequent denitrification. As also described by several authors (Fenn and Kissel, 1975, 1973; Fenn and Miyamoto, 1981), ammonia volatilisation due to a reaction with calcium carbonate in soil, could have contributed to further N_T removal.

Nutrient uptake is an also important issue for P_T removal. Yet as with N_T, the highly applied load (about 87.4 kg P_T/ha.yr) substantially exceeds the nutrient recovery estimated by Tzanakakis et al. (2009) for poplars (17 kg P_T/ha.yr). Phosphate precipitation (PO_4^{3-}) in the presence of Ca^{2+}, which is relatively abundant in the experimental plot soil, might be the reason for the low P_T levels measured in the leachate (McGechan, 2002). This hypothesis is ratified by the increase in available P content in soil, whose value at the end of the study tripled that measured before wastewater was applied. Degens et al. (2000) stated how available P accumulation was 91%, mainly at a soil depth of 0-25 cm after 22 years of dairy effluent application. Falkiner and Polglase (1999) found that 97% of the applied P_T was recovered from soil at a depth of between 0 and 0.5 m. Neutral soil pH can increase P_T solubility, facilitating its transport to deeper soil horizons (Tzanakakis et al., 2011). In our research, the measured P_T concentrations in drainage water were below the legal limit of 2 mg/L (80% of reduction) established by Directive 91/271/EEC (CEE, 1991b).

Commonly, the EC in soil after wastewater application usually increases due to accumulation of salts (e.g. Duan et al., 2011). In the present study, the initial soil EC was about three times that measured at the end of the study. This may be interpreted as the result of soil salt lixiviation produced by irrigation, which is also reflected by an increased EC of the leachate during the first year. As already reported in the results section, this is related to the increase in the leachate of other dissolved ions concentrations (Cl^-, SO_4^{2-}, Na^+, Mg^{2+} and Ca^{2+}).

The groundwater monitored in the piezometer seems not to be affected by the VF operation. As expected for the relevant NO_3-N concentration in drainage water, one may consider that groundwater quality could have been degraded by leachate infiltration towards the saturated zone. However the background NO_3-N concentration in groundwater was already high, with levels above those set by groundwater legislation (CEE, 1991a). The high NO_3-N concentration in groundwater before the VF operation probably occurred as a result of the extensive, prolonged agricultural practices carried out in the areas surrounding the field site. Moreover, the lysimeter collecting drainage water was located at a depth of 90 cm. Therefore, the leachate had to travel a longer distance before reaching the groundwater surface which, in this area, seasonally fluctuates between 1 m and 3 m depths. Certainly while the leachate travelled from the lysimeter to groundwater, it underwent the previously described biotransformation processes that further reduced the NO_3-N concentration. Furthermore, any dissolved species undergo mechanical dispersion if they enter groundwater (due to variation in the groundwater velocity field) and molecular diffusion (due to concentration gradients), which also smoothes solute concentration peaks.

However, the N_T leaching may represent a serious concern when N_T application rates are, over the long-term, considerably higher than the crop requirements (Isebrands and Richardson, 2014). Although numerous studies have demonstrated that N_T leaching below VFs is negligible (e.g. Dimitriou and Aronsson, 2003), there are few case studies reporting N_T concentrations in the leachate above limits established by European and/or local directives (Aronsson et. al, 2010; de Bustamante et al., 1990; Perttu and Kowalik, 1997). It is a widely accepted criterion that when deciding wastewater irrigation rates, nitrogen plant requirements should be taken into account to avoid leaching of nitrogen compounds. Also soil physical and chemical attributes and microbiological community should be considered (Barton et al., 2005). However, the highly variable N_T

concentration in the wastewater renders arduous the calculation of wastewater irrigation rates based on this parameter.

The obtained removal percentages of N_T were more than satisfactory under the conditions of the experiment (high N_T loads). Considering that no affection on the groundwater quality was observed, the VF system could be a suitable wastewater treatment strategy for scattered small populations and isolated source of domestic wastewater. Our experience suggests that the low drainage volumes produced by such systems minimize the pollutant leached load and therefore the environmental risk associated with the pollutant spreading

5 CONCLUSION

According to the obtained results, the VF presented in the current study may be a feasible solution as an alternative wastewater treatment for scattered populations. The VF efficiently removed most wastewater-originated pollutants despite the high nutrient concentration and the variable hydraulic load, which did not always meet plant water requirements.

The VF fulfil with the EU legislation of wastewater discharge for COD, P_T and N_T percentage removals. Concerning concentrations, only N_T in the leachate was above legal limits.

Despite the high N_T loads, only 10% of the applied cumulative N_T mass leached through the vadose zone. Since the good removal efficiency of the VF cannot be explained by only the N_T plant uptake capacity, additional N_T removal mechanisms, such as denitrification, ammonia volatilisation and annamox, must have come into play.

P_T was also optimally removed, with a concentration in drainage water below 2 mg/L. Plant uptake processes are responsible mainly for P_T depletion. However, as reflected by the increase in soil available P, PO_4^{3-} could have been precipitated in the presence of naturally-occurring Ca^{2+} in soil.

The 3-year VF operation did not imply changes in the groundwater quality at the site. Despite the presence of NO_3-N in drainage water, groundwater seems no to be affected by an increase in the N_T concentration. Biotransformation processes and retardation effects could have further lowered NO_3-N concentrations during transport from the lysimeter location to the monitoring well.

6 ACKNOWLEDGEMENTS

Funding for this research is provided by the Subprogram of Non-Oriented Research REAGUA2 (CGL2012-39520-C03-01) and REAGUAM (CGL2009-13168-C03-0) and by the Subprogram CONSOLIDER-INGENIO (CSD2006-00044) of the Spanish Ministry of Science and Innovation. Special thanks go to Carolina Guillén Fuentes for assisting the chemical analyses. The authors also wish to thank the support of the Postgraduate Program of Hydrology and Water Resources Management (UAH-URJC). Finally, we would like to thank the reviewers for their helpful corrections.

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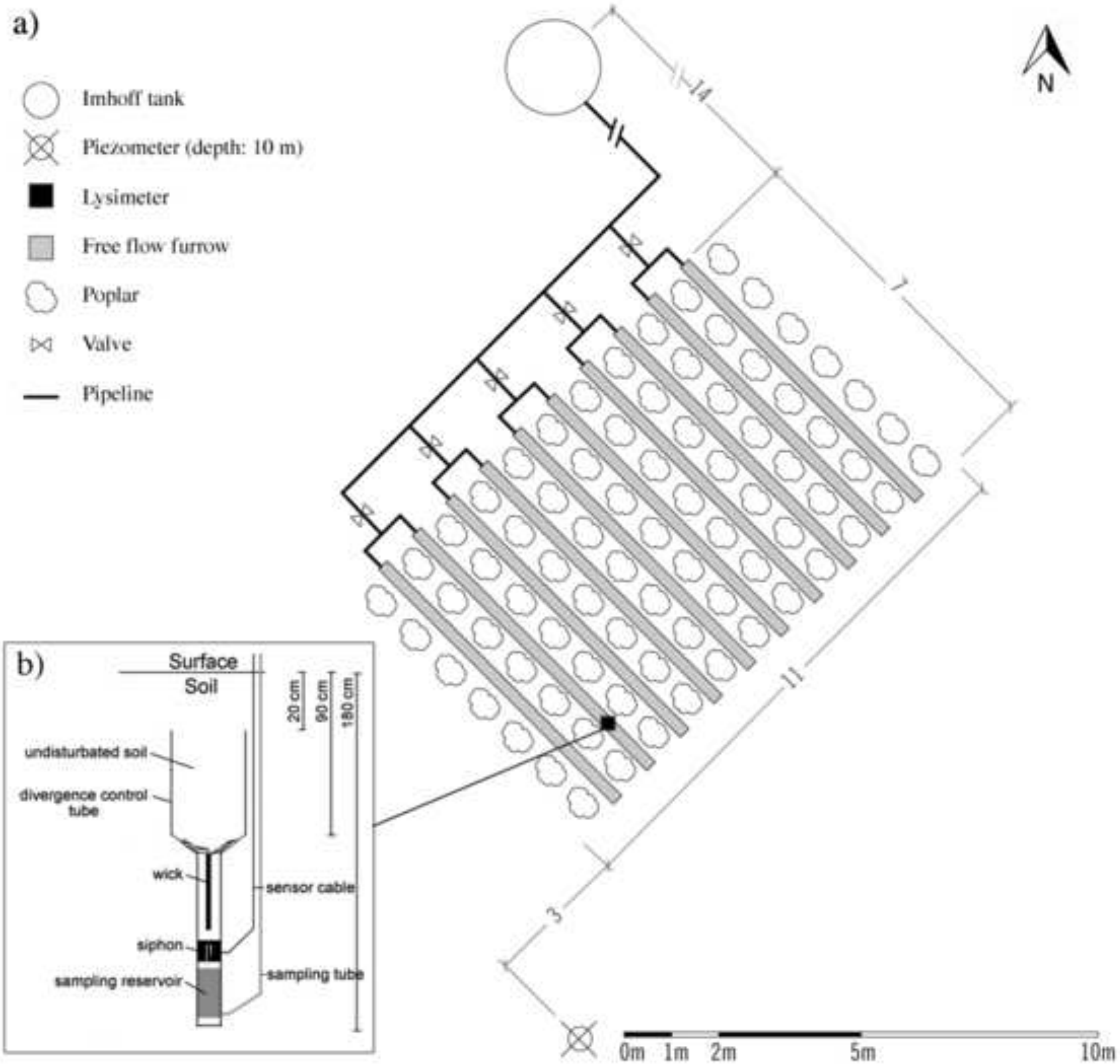
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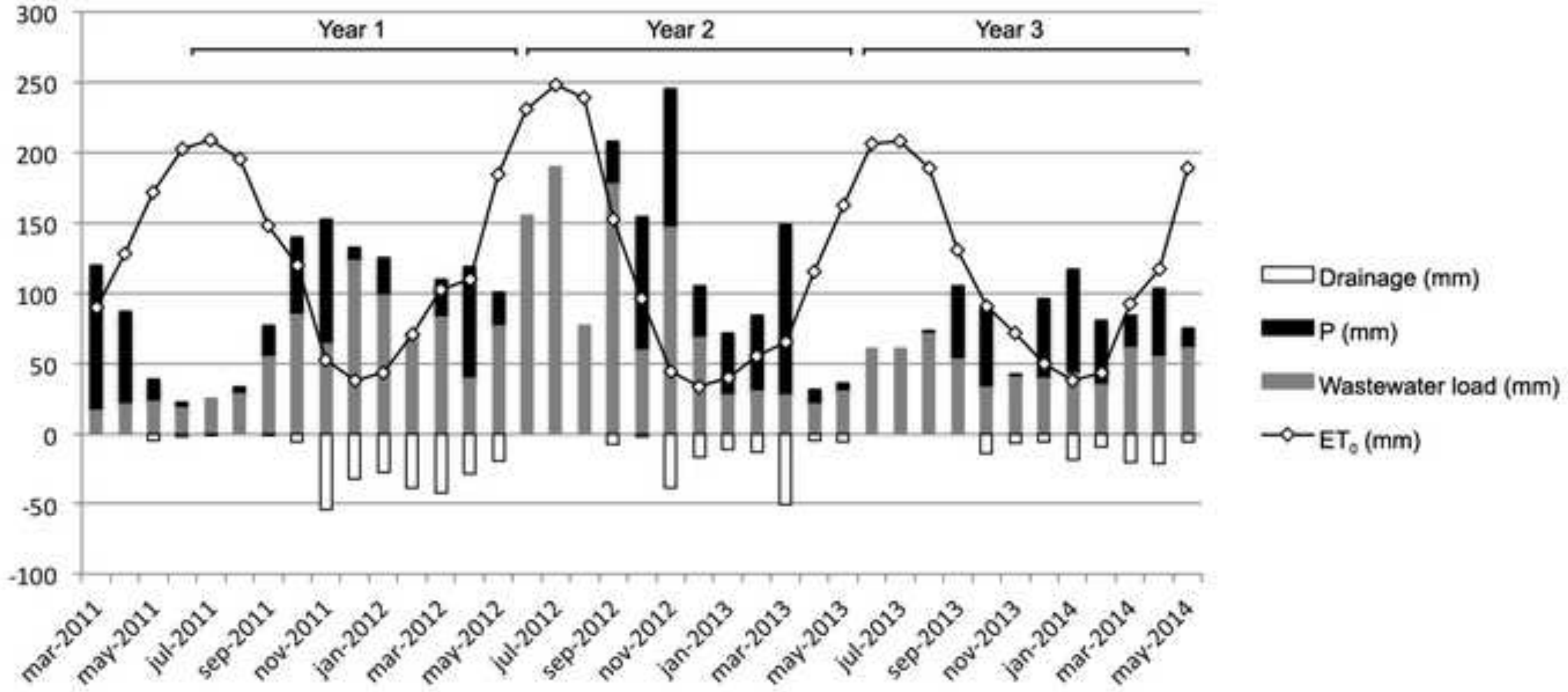

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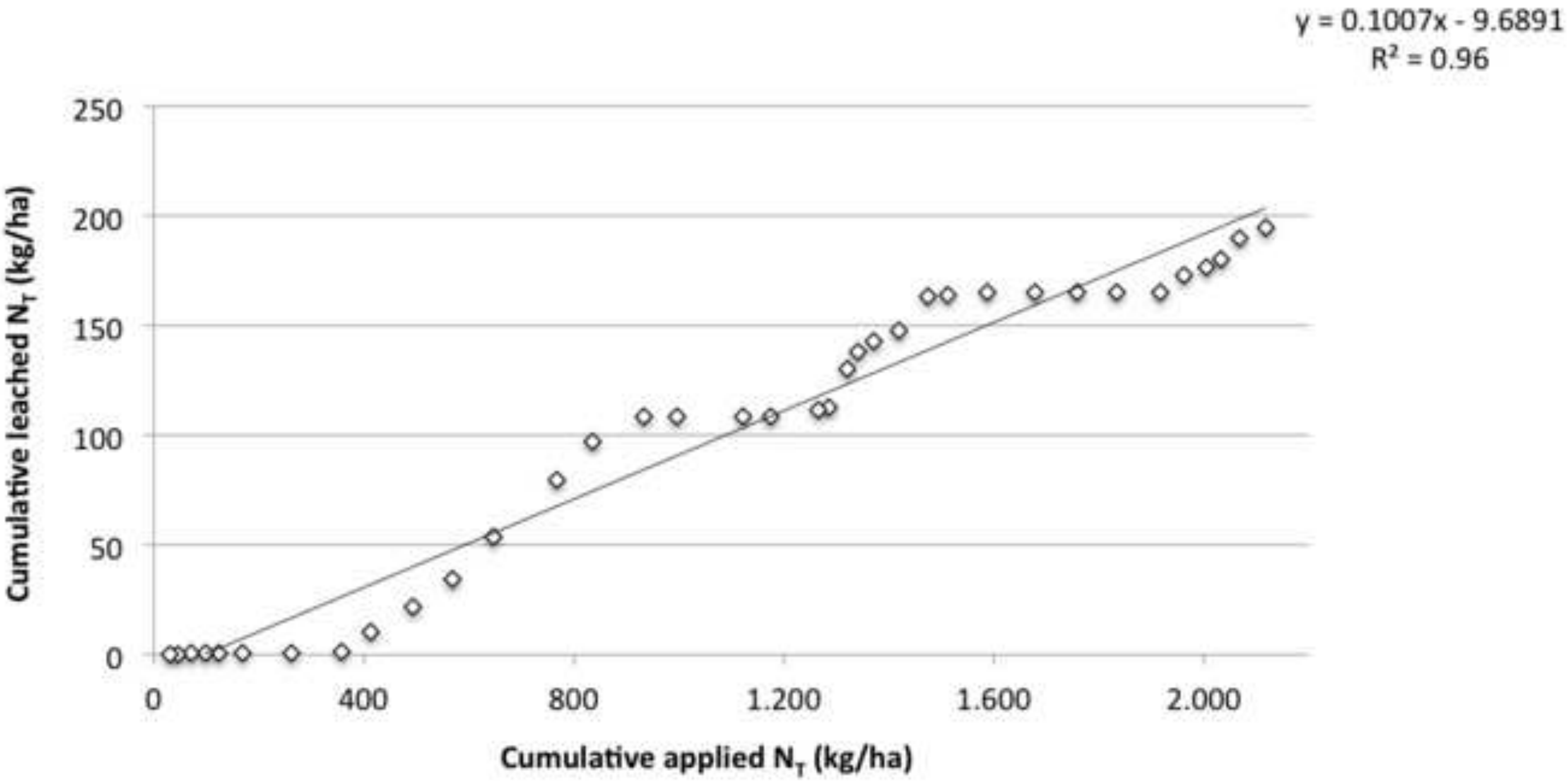

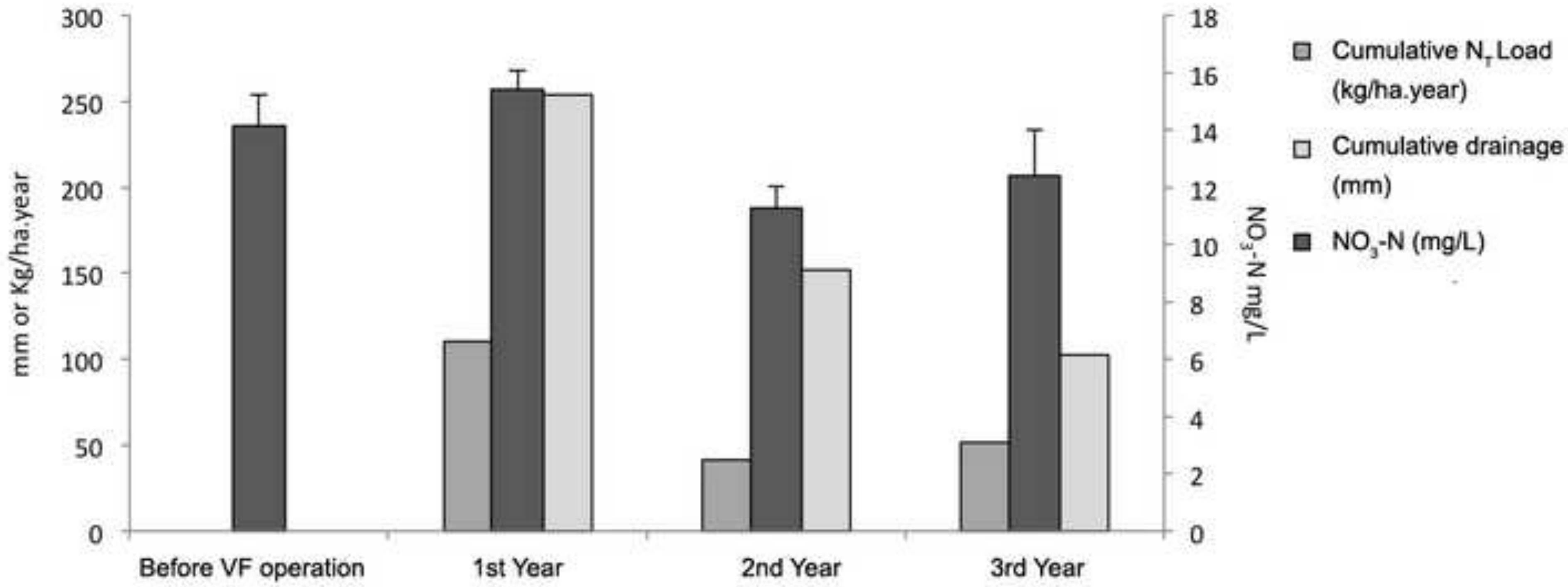
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