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Long-Term Monitoring of a Surface Flow Constructed Wetland Treating Agricultural Drainage Water in Northern Italy

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Abstract: Agricultural drainage water that has seeped into tile drainage systems can cause nitrogen and phosphorus pollution of the surface water bodies. Constructed wetlands (CWs) can help mitigate the effects of agricultural non-point sources of pollution and remove different pollutants from tile drainage water. In this study, hydrological and water quality data of a Northern Italian CW that has been treating agricultural drainage water since 2000 were considered to assess its ability to mitigate nitrogen and phosphorus pollution. The effects of such long-term operation on the nutrients and heavy metals that eventually accumulate in CW plants and sediments were also analysed. Since 2003, the CW has received different inflows with different nutrient loads due to several operation modes. However, on average, the outflow load has been 50% lower than the inflow one; thus, it can be said that the system has proved itself to be a viable option for tile drainage water treatment. It was found that the concentration of nitrogen and phosphorus in the plant tissues varied, whereas the nitrogen content of the soil increased more than 2.5 times. Heavy metals were found accumulated in the plant root systems and uniformly distributed throughout a 60 cm soil profile at levels suitable for private and public green areas, according to the Italian law

Keywords: agricultural drainage water; superficial flow constructed wetlands; nutrients; potentially toxic elements

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1. Introduction

Constructed wetlands (CWs) are man-made systems in which processes occurring in natural wetlands are applied for water treatment [1]. Compared to conventional wastewater treatment plants, CWs are less costly, easier to operate, and require less maintenance [2]. In addition to their general ability to improve wastewater quality, CWs are multifunctional systems that can offer different ecosystem services [3], including supporting habitat and biodiversity functions, together with recreational and socioeconomic services, including flood and drought control, water retention, and erosion prevention [4–6]. These systems can also be used as a part of water reuse schemes [7,8] and can have positive effects on carbon balance [9]. CWs are usually characterized by high evapotranspiration rates due to the presence of vegetation. In addition, the biomass produced can later be exploited for energy production [10,11]. CWs have been successfully used for treatment of many different types of wastewater [3,12,13], including agricultural drainage water. In general, CWs are more cost-effective for reducing non-point source pollution than other measures [14].

Agricultural drainage water contains different substances that can have negative effects on surface water bodies, and therefore its treatment is important in order to reduce environmental pollution [6]. For instance, this type of wastewater is a significant source of phosphorus in surface water bodies. Since it can be the limiting nutrient for plant growth, phosphorus removal is important in order to prevent eutrophication [15,16]. Even though loads from the point sources to surface water bodies have been reduced due to improvement in wastewater treatment, non-point source phosphorus pollution has increased because of intensified agricultural production [15]. Apart from phosphorus, another important surface water pollutant originating from agricultural non-point sources is nitrate, whose level has to be safely controlled and reduced according to the EU directive 91/676/EEC [17].

Since wetlands can reduce agricultural pollution [18], a broader implementation of CWs has been proposed as a measure for protecting aquatic systems [16,19]. The CW type that is usually applied for agricultural drainage water treatment is the surface flow constructed wetland (SFCW) [4], which has been reported to be able to remove nitrogen and phosphorus at relatively low cost [14]. Although there have been studies that discuss different aspects of this topic, not many of them present the general functioning of these systems over long periods of time.

Therefore, the aim of this study, which was carried out in northern Italy, was to review the results of long-term monitoring of a SFCW that had been in operation under a variety of conditions in terms of its ability to treat agricultural drainage water and the effect of such a long operational period on the CW soil and plant characteristics. The hypothesis of this research was that SFCWs can be considered as a long-term viable option for agricultural drainage water treatment.

2. Materials and Methods

The study was carried out on a non-waterproofed SFCW located on an experimental agricultural farm (44°34′22.2″ N, 11°31′44.9″ E) of the Canale Emiliano Romagnolo (CER) Land Reclamation Consortium near Budrio village in Emilia-Romagna region (Italy; Figure 1). According to the Köppen climate classification, the climate of the site is subhumid, with a mean annual rainfall of 771 mm and mean annual temperature of around 13.7 °C. The site receives most of its precipitation during spring and autumn. The coldest month is January (mean temperature 2.7 °C) and the warmest is July (mean temperature 24.3 °C). These precipitation and temperature data are 30-year normal values supplied by ARPAE (Regional Agency for Environmental Protection) from the nearest weather station [20]. However, the climate data used for the calculations in this paper were taken from a weather station managed by CER and located about 500 m from the SFCW.

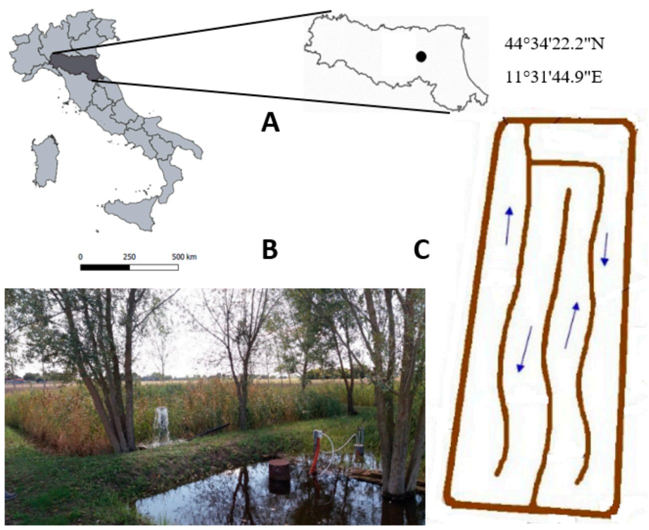


Figure 1. The location of the SFCW system in Emilia-Romagna region (Italy) (**A**), constructed wetland studied (**B**) and its hydraulic scheme (arrows show the direction of the water flow) (**C**).

The CW treats tile drainage water coming from a 12.5 ha experimental farm that grows different crops (e.g., fruit trees, vegetables, and cereals) throughout the year. The area of the SFCW represents around 3% of the total farm surface, and it is divided into four 8–10 m wide meanders that create a 470-m-long water course (Figure 1). The total capacity of the wetland is about 1500 m^3 when the water level reaches 0.4 m, which is the maximum water height below the discharge level. The whole farm area is drained to a main ditch from which water is conveyed into the CW by means of two pumps. The CW effluent is discharged into a canal, which collects excess water from the fields of other neighbouring farms.

2.1. Experimental Design

Since its construction in 2000, the CW has functioned continuously; however, its monitoring was done from 2003 to 2009, because of different projects conducted at the farm. In 2017, the monitoring started again, thanks to an Italian National Research Project (Green4Water) coordinated by the University of Bologna.

The operation of the SFCW depends mostly on the frequency and volume of precipitation. However, in the past its operation has sometimes been modified, and the system exposed to different feeding conditions, because of specific research requirements, as indicated in the following. During summer 2004, an unusually high inflow (around 3300 m^3) entered the system, since a specific experiment regarding nutrient removal was being conducted. Additionally, in 2007 and partly in 2008, the influent and effluent pumps were often turned off in order to allow plantation of willow and poplar as the farm participated in a project related to biomass production.

2.1.1. Water Balance and Quality

The system is equipped with two mechanical flow meters that record influent and effluent volumes every hour, and two automatic samplers that take influent and effluent water samples on the basis of flow from the beginning of the event (at specific inlet water volume values) and time (every 24 h), respectively. Since the functioning of the system depends mostly on the presence of precipitation, no general sampling schedule was established, and sometimes no samples were taken for a few weeks due to the lack of drainage water. The water level inside the CW is measured by a specific sensor. All the collected data are managed and recorded by a central control system. The precipitation height data are taken from the farm weather station, which is also equipped with a precipitation sampling unit.

Since August is generally the month with the lowest inflow volume, the hydrological year was defined as the period between 1st September and 31st August. TN and TP, as well as NO_3^- and NH_4^+ balances, were defined for six hydrological years (from 2003–2004 to 2008–2009). The SFCW dynamic water budget [1] can be expressed as:

$$Q_{in} + (P \times A) - Q_{out} - I - (ET \times A) = \frac{dV}{dt} \quad (1)$$

where:

Q_{in} = inflow rate ($m^3 \cdot d^{-1}$);
P = precipitation rate ($m \cdot d^{-1}$);
A = wetland top surface area (m^2);
Q_{out} = outflow rate ($m^3 \cdot d^{-1}$);
I = infiltration flow rate ($m^3 \cdot d^{-1}$);
ET = evapotranspiration rate ($m \cdot d^{-1}$);
V = water storage inside the SFCW (m^3);
t = time (d).

Over long averaging periods (Δt), the change in storage (ΔV) can be considered negligible [1]. Moreover, it was not possible to measure infiltration and evapotranspiration rates separately. Therefore, a simplified water balance over each hydrological year was calculated as:

$$Q_{in} + (P \times A) - Q_{out} = I + (ET \times A) \quad (2)$$

In Equation (2) the term ($I + (ET \times A)$) was considered as the overall water loss from the SFCW. Accordingly, the outflow/inflow ratio (R, %) was expressed as:

$$R = \frac{Q_{out}}{(P \times A) + Q_{in}} \times 100 \quad (3)$$

Equation (3) represents the percentage of influent water flowing out of the system through the outlet device, while the complementary percentage is assumed to be the amount of water jointly lost due to infiltration and evapotranspiration.

Water samples (i.e., precipitation and CW influent and effluent) were analysed for total nitrogen (TN), nitrate (NO_3^-), ammonium (NH_4^+), and total phosphorus (TP). TN was measured by the elemental analyser Shimadzu TNM-1 (Shimadzu, Kioto, Japan), and NO_3^- and NH_4^+ concentrations were determined by using a flow analyser (AA3, Bran Luebbe, Norderstedt, Germany). TP analysis was performed by using an inductively coupled plasma optical emission spectrometer ICP-OES which was equipped with a plasma source and an optical detector with a charge-coupled device CCD (SPECTRO Analytical Instruments GmbH & Co., Kleve, Germany). Water samples were analysed for TN after addition of 1% HNO_3 (>69% *v*/*v*), for trace analysis, Sigma-Aldrich, St. Louis, MO, USA). Before analysis, all samples were filtrated through Watman 42 filters (Merck KGaA, Darmstadt, Germany).

The samples taken and analysed for nutrient concentrations at inlet (C_{in}, mg·L^{-1}) and outlet (C_{out}, mg·L^{-1}) were multiplied by the related measured volume of water that flowed into (V_{in}, m^3) or out (V_{out}, m^3) of the system, respectively. Afterwards, all the inflow and outflow loads during one hydrological year were summed to calculate the mass of nutrients (kg·year^{-1}) entering and exiting the wetland yearly. On this basis, yearly mass retention rate (RR, %), over each hydrological year, was calculated as:

$$RR = \frac{\Sigma V_{in} \times C_{in} - \Sigma V_{out} \times C_{out}}{\Sigma V_{in} \times C_{in}} \times 100 \quad (4)$$

2.1.2. Vegetation

The SFCW has been continuously inhabited by different aquatic plant species, such as common reed (*Phragmites australis)* or cattail (*Typha latifolia*, *T. angustifolia*). In autumn 2006, after the removal of the above-ground biomass of the aquatic plants from the first two meanders, willow (*Salix alba*) and poplar (*Populus alba*) were planted, as requested by the project on biomass production. With the exception of this partial removal, the plant biomass has never been harvested.

In the 2004–2009 period, vegetation was sampled once a year, approximately at the middle of each of the four meanders, for nutrient and heavy metal content evaluation. The samplings were performed at the end of October/beginning of November, so those results roughly correspond to the previous hydrological year, which ended in August, 2–3 months before the plant sampling.

Biomass dry weight, average height and number of shoots per m^2 were measured. Additionally, biomass samples were divided into below- and above-ground biomass, and were tested for TN and TP content, as well as for the presence of the semi-metal boron (B) and different heavy metals (cadmium (Cd), chrome (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni) and zinc (Zn)).

TN analysis of vegetation was performed by using a thermo-electron CHNS-O elemental analyser (Thermo Fisher Scientific, Waltham, MA, USA). TP and metals were measured using ICP-OES. Before elemental analysis, biomass samples were dissolved in a mixture of HNO_3 (>69% *v*/*v*, for trace analysis, Fluka, Sigma-Aldrich, St. Louis, MO, USA) and H_2O_2 (30% *v*/*v*, for trace analysis, VWR Prolabo Chemicals, Radnor, PA, USA) in a ratio of 4:1 (*v*:*v*) by microwave-assisted digestion (Start D, Micro-wave Digestion System, Milestone srl, Bergamo, Italy).

2.1.3. Soil

The first 15 cm of soil was sampled once a year (at the end of October/beginning of November) in the period 2004–2009, approximately at the middle of each of the four meanders. In July 2017, core soil samples (Figure 2) were taken up to 60 cm depth in order to see how the 17 years of CW operation had affected the soil composition along its profile. After manual removal of plant roots up to a diameter of ca. 1–2 mm, the samples were air-dried and sieved to 2 mm. Afterwards, they were tested for organic matter, TN and TP content, as well as for metal content. No data on metals and nutrients in the CW soil before 2004 is available as a control.

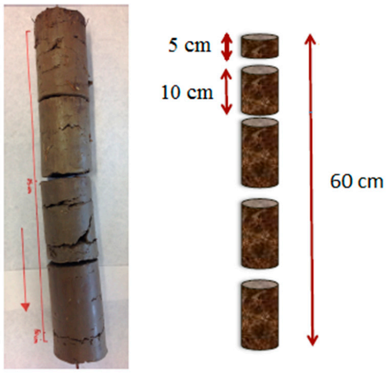


Figure 2. The soil core sampling and sectioning.

Total organic carbon (TOC) and TN in the soil samples were determined by using a thermo-electron CHNS-O elemental analyser (Thermo Fisher Scientific, Waltham, MA, USA). TP and metal concentrations were analysed by using ICP-OES, after dissolution of soil samples in a mixture of HCl (37% *v*/*v* for trace analysis, Sigma-Aldrich, St. Louis, MO, USA), HNO_3 (>69% *v*/*v*, for trace analysis, Fluka, Sigma-Aldrich, St. Louis, MO, USA), and H_2O_2 (30% *v*/*v*, for trace analysis, VWR Prolabo Chemicals, Radnor, PA, USA) in the ratio of 4:1:0.25 (*v*:*v*:*v*) by microwave-assisted digestion (Start D, Micro-wave Digestion System, Milestone, MD, USA).

2.2. Data Analysis

The correlation of inflow water volume and precipitation was checked with the Pearson Product Moment Correlation test. The water influent and effluent concentrations of different parameters considered were compared with Student's T-test, while the content of different compounds at different depths along the soil profile in 2017 were compared with ANOVA. Before those tests were performed, the data was checked for normality and equal variance, and if the assumptions were not met, the values were $\log_{10}$ transformed. In cases where the assumptions were not met even after the transformation, the Mann-Whitney U test and ANOVA on ranks were used. All analyses were performed using SigmaPlot 14 software (Systat Software, Inc, San Jose, CA, USA).

3. Results and Discussion

3.1. Simplified Water Balance

The water balance (cumulated yearly inflow and outflow volumes) and the outflow/inflow ratio (calculated as in Equation (3)) of the monitored CW are shown in Figure 3. With the exclusion of the 2006–2007 and 2007–2008 hydrological years, when the pumps were often turned off, the inflow was in the range 8400 to 19,500 $m^3 \cdot year^{-1}$, while the outflow was between 750 and 9500 $m^3 \cdot year^{-1}$. Such a high yearly variation between inflow and outflow of the system was due to precipitation differences during the years, as well as the specific functioning conditions required by the different research projects, as already explained in Section 2.1.1. On the other hand, the irregular water flow in the system and water loss can be better seen through the reported outflow/inflow ratio, which ranged between 9 and 65% (Figure 3). Since the SFCW was not waterproofed, such a high loss could mainly be attributed to the infiltration rate. However, it was also related to high evapotranspiration values, especially during the summer months, as already reported by Borin et al. for Northern Italy [21].

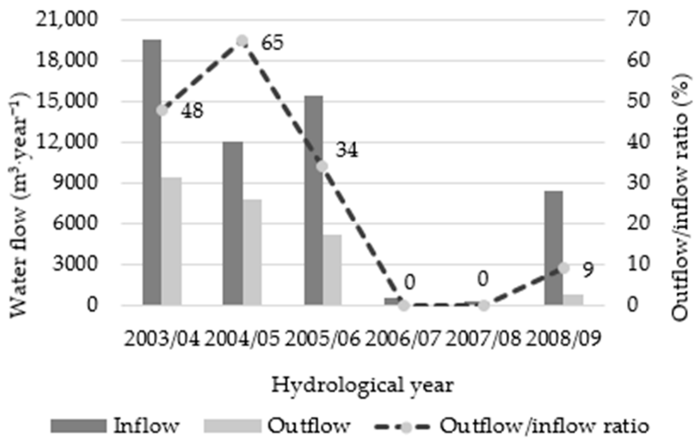


Figure 3. Water balance of the monitored SFCW.

The two SFCWs comparable to the system discussed in this study were located in the USA and had total volumes of 5400 and 1200 m^3 [19]. The outflow/inflow ratios of these systems were in the range 0–60%, indicating considerable water losses that were mostly attributed to infiltration. However, the operational variability of SFCW systems is very wide, and they were reported to function with higher outflow/inflow ratios [12,18] and different inflow rates [15,16]. Therefore, SFCWs can be considered to be flexible and adaptable to different hydrological conditions and loading rates. These characteristics will be discussed in the context of nutrient retention in the next section.

Of the six hydrological years considered, 2005–2006 was selected as representative of annual water flow in the system. As can be seen in Figure 4, the majority of inflow into the system took place during autumn (October and November) and early winter (December), when the greatest part of yearly precipitation also occurred. Although the precipitation that fell onto the farm area took a certain time to infiltrate and reach the SFCW, it was still significantly correlated with the inflow (r = 0.661), as shown with the Pearson Product Moment Correlation. The missing or very low outflow from the system in the period January–August 2006 is related to the low inflow. Since the maximum capacity of the SFCW studied was about 1500 m^3, inflows lower than the same value cannot usually cause considerable outflow (depending on the water level inside the SFCW). The water stored inside the system was later partly lost to infiltration and evapotranspiration.

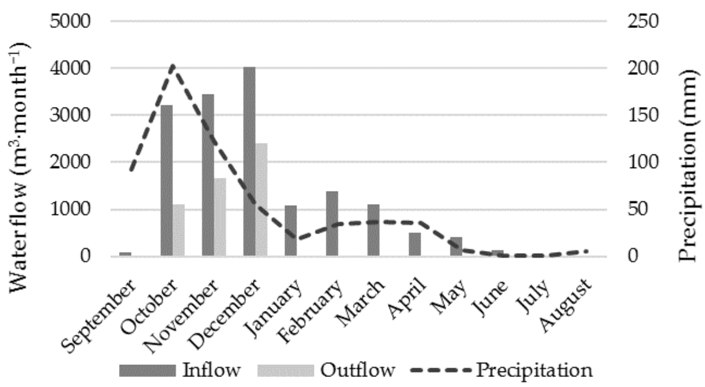


Figure 4. SFCW water flow and precipitation in the hydrological year 2005–2006.

3.2. Water Quality

Fertilisation was the main source of nitrogen that entered the farm (see Table S1 in supporting information). Since most of the applied TN (>80%) was not present in the drainage water, it can be inferred that in the field, the nutrient was uptaken by crops and retained by the soil components, although its release to the atmosphere in volatile forms cannot be ruled out.

Table 1 gives average influent and effluent concentrations of the measured parameters during the hydrological years considered. The TN influent concentration was in the range 13.7–21.9 mg·L^{-1}, if the hydrological years 2006–2007 and 2007–2008 (when the pumps were mostly turned off) are excluded. If the same two years are not considered, nitrate influent concentrations did not vary a lot. However, nitrate effluent concentration in 2005–2006 was considerably lower than in 2004–2005. On the other hand, both influent and effluent concentrations of ammonium and TP were low (<0.4 mg·L^{-1}). The significant differences ($p < 0.05$) between influent and effluent concentration were found only for TN and nitrate in the hydrological year 2005–2006 (Table 1). This might be connected to the high water loss during that year (Figure 3), which could be related to high infiltration and, therefore, nutrient loss. However, another factor that should be considered is increased residence time in the system, and thus the increased removal of pollutants.

Table 1. Average concentrations of different parameters (mg·L^{-1}) at the inlet and outlet of the monitored system (values are displayed as: mean ± st. error and number of samples in brackets).

Hydrological Year	Parameter	Total Nitrogen	Nitrate	Ammonium	Total Phosphorus
2003–2004	Inflow	21.9 ± 2.7 (34)	-	-	-
	Outflow	14.5 ± 2.4 (17)	-	-	-
	T test-*p* value	0.078	-	-	-
2004–2005	Inflow	16.5 ± 1.4 (27)	14.2 ± 1.4 (27)	0.1 ± 0.0 (27)	0.1 ± 0.0 (27)
	Outflow	13.4 ± 1.3 (33)	11.7 ± 1.5 (33)	0.3 ± 0.1 (33)	0.4 ± 0.1 (33)
	T test-*p* value	0.124	0.229	0.272	0.083
2005–2006	Inflow	13.7 ± 1.2 (17)	11.4 ± 1.1 (17)	0.1 ± 0.0 (17)	0.1 ± 0.0 (17)
	Outflow	8.2 ± 1.2 (11)	4.5 ± 0.8 (11)	0.1 ± 0.0 (11)	0.0 ± 0.0 (11)
	T test-*p* value	**0.004**	**<0.001**	0.226	0.353
2006–2007	Inflow	2.4 ± 0.6 (2)	0.8 ± 0.1 (2)	0.1 ± 0.0 (2)	0.2 ± 0.2 (2)
	Outflow	*	*	*	*
	T test-*p* value	-	-	-	-
2007–2008	Inflow	8.7 (1)	-	-	0.2 (1)
	Outflow	*	*	*	*
	T test-*p* value	-	-	-	-
2008–2009	Inflow	17.6 ± 2.1 (11)	-	-	-
	Outflow	11.2 ± 2.7 (10)	-	-	-
	T test-*p* value	0.071	-	-	-

Notes: T test *p* values show the statistical comparison (by T-test or Mann-Whitney U test) of the influent and effluents in the given year. Bolded values show significant difference. - Undetected. * No outflow.

As shown in Tables 1 and 2, nitrate was the most prevalent nitrogen species in the TN load in the influent to our CW. The retention rate (*RR*, %) of the TN load by the system varied during the six hydrological years monitored, but it never dropped below 47%. Comparing the data reported in Table 2 and Figure 3, it can be seen that the TN retention depended on the outflow/inflow ratio, rather than on the mass load of nitrogen that entered the CW. These findings are in accordance with Groh et al. [19], who concluded that nitrate removal mostly depends on the hydraulic loading, and who reported similar removal of TN. Retention rate of TP in the hydrological year 2005–2006 was 49%, which is higher than the 36% retention reported for a Swedish SFCW treating agricultural drainage water [12]. However, since the SFCW studied was not waterproofed, it is expected that some of the nutrients infiltrated through the ground, and therefore were not removed, but rather retained.

The lowest retention rate of TN, nitrate, ammonium, TP parameters in 2004–2005 can be explained by the highest outflow/inflow ratio, which consequently means shorter residence time. Similarly, in December 2005 (the month with the highest TN and NO_3^- load; data not shown, but included in Table 2: 2005–2006 nutrient balance), inflow and outflow volumes were comparable, thus suggesting a very short residence time. In addition, a similar hydrological situation repeated in March–April 2006 and during both months, TN and TP outflow loads were higher than the inflow ones (data not shown, but included in Table 2: 2005–2006 nutrient balance), thus suggesting that sediments containing these nutrients were partially flushed out.

Table 2. Nutrient balance of the monitored CW with the atmosphere input considered.

Balance	Parameter	2003–2004	2004–2005	2005–2006	2006–2007	2007–2008	2008–2009
TN	Input (kg·year^{-1})	313	216	219	6	12	180
	Output (kg·year^{-1})	125	115	39	0	0	11
	Retention rate (%)	60	47	82	100	100	94
NO_3^-	Input (kg·year^{-1})	-	182	180	0.5	-	-
	Output (kg·year^{-1})	-	99	21	*	*	-
	Retention rate (%)	-	45	88	100	-	-
NH_4^+	Input (kg·year^{-1})	-	1.1	1.3	0.1	-	-
	Output (kg·year^{-1})	-	1.1	0.4	*	*	-
	Retention rate (%)	-	0	71	100	-	-
TP	Input (kg·year^{-1})	-	1.2	1.4	0.1	0.1	-
	Output (kg·year^{-1})	-	2.1	0.7	0.0	0.0	-
	Retention rate (%)	-	-	49	100	100	-

Notes: - Undetected. *No outflow.

The formation of phosphorus species bound to the sediment components is one of the main mechanisms of TP removal from water in SFCWs [12]. It is likely that the rather high outflow/inflow ratio in 2004–2005 (Figure 3) mobilised the finest sediment components, thus explaining why the system acted as a TP source rather than a sink (Table 2). The hypothesised phosphorous dynamics can be confirmed by the observations reported in the following section.

3.2.1. Analysis of Single Events

Since six hydrological years is a long period of time, it can be useful to look at single events. Two of them (both 25 days long) were selected in order to stress the SFCW's efficiency in nutrient retention: the first one from 10th November to 5th December 2004 and the second one from 6th to 30th November 2005. As shown in Table 3, the system generally performed better during the latter one, despite the higher water flow.

Table 3. Water and nutrient balance during two different events.

Parameter	10th November–5th December 2004			6th–30th November 2005		
	Inflow	Outflow	Retention	Inflow	Outflow	Retention
Water volume (m^3)	1138	1086	-	2857	1223	-
TN (kg)	15.17	4.69	59%	41.63	10.20	75%
NO_3^- (kg)	11.52	1.64	85%	36.57	6.55	82%
NH_4^+ (kg)	0.06	0.57	-	0.13	0.07	45%
TP (kg)	0.03	0.57	-	0.12	0.07	43%

Note: - Undetected.

Water inflow and outflow during the first event were similar (1138 and 1086 m^3, respectively). On the contrary, during the second event the outflow (1223 m^3) was considerably lower than the inflow

(2857 m^3) (Table 3). This behaviour can be explained by the fact that, before the first event, the water level in the system was 35.7 cm, whereas before the second one, it was only 5 cm. Since the water level was close to its maximum value of 40 cm, the water already contained in the system at the beginning of the first event shortened the water retention time, thus causing lower compound retention rates. The sudden flush-out of the soil particles can explain the higher TP outflow than the inflow load [12].

3.3. CW Soil and Vegetation

3.3.1. Plant Development

The agronomic results of plant sampling are shown in Table 4. As already stated, plant species in the SFCW changed over the course of time due to the different conditions requested by some projects that the CER Land Reclamation Consortium participated in. The contained variation of plant biomass observed in the 2004–2006 period, ranging from 4.68 to 5.40 kg·dw·m^{-2}, has to be considered in the context of the equilibria among the different aquatic plant species inhabiting the system. In this period, TN and TP that entered the CW through agricultural drainage water enabled plant development. The observed above-ground biomass is in line with that reported by other studies, for example, [22] recorded 0.37–1.76 kg·dw·m^{-2} and [23] 1.2–1.4 kg·dw·m^{-2}.

Table 4. Agronomic results of plant sampling.

Plant Survey Parameter	2004	2005	2006	2007	2008	2009
Main species	P, T	P, T	P, T	P, T, W, L	P, T, W, L	P, T, W, L
Dry weight (kg·m^{-2})	5.32	5.40	4.68	3.02	7.03	5.63
above-ground	3.01	2.74	1.57	0.21	0.70	0.88
below-ground	2.31	2.66	3.11	2.81	6.33	4.75
above/below ground ratio	1.30	1.03	0.50	0.07	0.11	0.19
Average height (cm)	247	253	190	131	239	301
Shoots (number m^{-2})	223	171	210	73	105	57

Notes: P—*Phragmites*; T—*Typha*; W—Willow; L—Poplar.

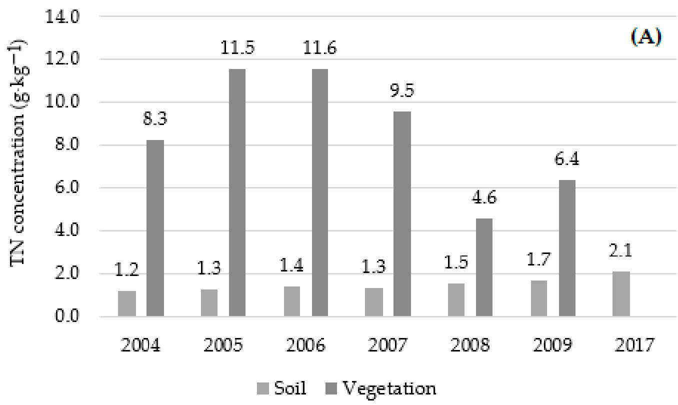
Over the years, there was a decrease in the ratio between above and below-ground biomass. While in 2004 it was 1.3, in 2009 it dropped to 0.2, meaning that below-ground biomass had 5 times greater weight than above-ground biomass. This observation is likely due to the fact that above-ground biomass was never harvested, so withered plants from the previous year blocked the sunlight for sprouting plants, took space and consequently limited development of new shoots [23].

The visible drop of all biomass parameters, including biomass, average height and number of shoots, in 2007 (Table 4) is mainly due to the removal of above-ground biomass from the first two meanders, as clearly indicated by the low value of the above-ground/below-ground biomass ratio, as well as the interruption of water inflow and nutrient input into the system to allow for the poplar and willow plantation/rooting. After 2007, the agronomic parameters indicated the establishment of new plant consortia.

3.3.2. Nutrients' Content of Biomass and Soil

Owing to the removal of above-ground biomass of aquatic plants from the first two meanders in autumn 2006, no nutrient distribution can be estimated between soil and vegetation. Nevertheless, some considerations on their occurrence are still possible.

The plant biomass sampled was analysed for its TN and TP content, as shown in Figure 5. In the 2004–2009 period, the content of both of these elements in the biomass show some fluctuations that could be related to the variation in inflow water (Figure 3) and nutrient loads (Table 2), as well as to the change in plant species that inhabited the SFCW. The plantation of willow and poplar trees in 2006 surely increased plant competition, thus modifying the equilibria among plant and microorganism species.



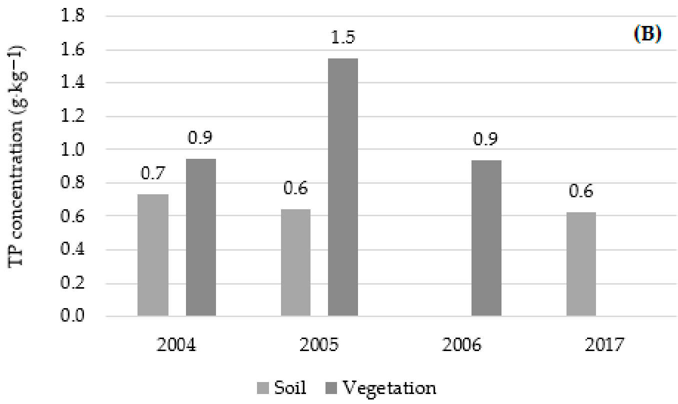


Figure 5. TN (A) and TP (B) content of the surface soil layer and vegetation in the SFCW.

Only partial TP data were available for the same period. Nevertheless, the content of plant biomass peaked in 2005 at 1.5 g·kg^{-1} (Figure 5). Similarly, the biomass TN content peaked in the 2005–2006 period at about 11.5 g·kg^{-1} (Figure 5). This value is a bit lower than the TN reported by [22] in a study where a SFCW received municipal wastewater that was more polluted than the agricultural drainage water used in this study.

TN and TP concentrations in the SFCW top soil are reported in Figure 5. Although no values for nutrients or for OMC (organic matter content) in CW soil are available from before 2004 to be considered as a reference, it is possible to observe that TN concentration slowly increased over the years, reaching a value of 2.1 g·kg^{-1} in 2017, almost twofold higher than that of 1.2 g·kg^{-1} measured in 2004. TP, on the other hand, did not accumulate in the top soil, as is evident by its quite constant level over the 13-year-long observation period. This can be explained by the low nutrient loads of the influent (Table 2) and the specific flush-out events already described in the Section 3.2.1.

As far as the OMC of the SFCW soil is concerned, an increase in the first 15 cm was observed since the beginning of the monitoring. While the OMC in 2004 was 19.6 g·kg^{-1}, it increased more than 2.5 times in 2017, reaching a value of 49.8 g·kg^{-1}. This positive trend suggests constant organic matter production and its sedimentation into the CW during the 14 years of the system's operation. The higher OMC increase in other SFCW soils reported in the literature is typical of applications of water containing higher amounts of organic matter and nutrients than those contained in our agricultural drainage water. For example, [24] reported a tenfold increase of TOC over a period of 5 years, but in this case several applications of slurry were made to the SFCW.

3.3.3. Boron and Heavy Metals in Biomass and Soil

Additional information on the state of the CW can be obtained by considering the metal content of the biomass and soil, as these elements, apart from being naturally contained in the soil in background levels, can enter the SFCW by water inflow and can be subsequently uptaken by plants or accumulated in soil. Cu and Zn are present in several plant products, either as active ingredients themselves or as counterions of organic products [25], so their inflow to the SFCW can be seasonal or constant, depending on their administration frequency. B is a plant micronutrient usually applied to crops as fertiliser [26]. Cd, Ni and Pb are considered potentially toxic elements (PTE) and can occur in wastewater and be accumulated in soil through different anthropogenic activities [27].

Figure 6 gives the content of these metals in the above- and below-ground biomass for the period 2004–2006, before the plantation of new trees in the SFCW. As a general trend, it is possible to observe that the metals were found to mostly have accumulated in the below-ground plant tissues, and only a small portion was transferred to the above-ground parts. These findings are in accordance with [22], which also reported accumulation of heavy metals in below-ground biomass.

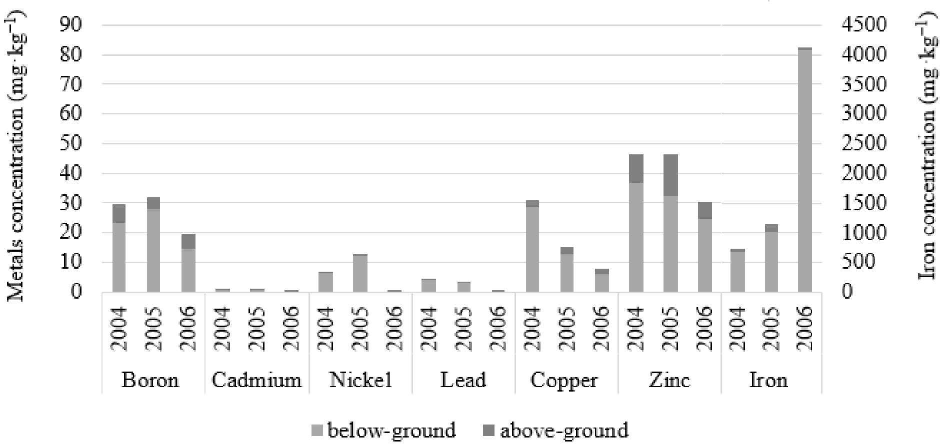


Figure 6. Heavy metal concentrations in below- and above-ground plant biomass.

Furthermore, high variation was observed among the amounts of heavy metals retained by plant tissues. The metal accumulated the most by plants was Fe, with a maximum concentration of more than 4000 mg·kg^{-1} in 2006. Cd was never higher than 0.8 mg·kg^{-1}, Cu ranged between 31 and 8 mg·kg^{-1}, while Zn was never less than 30 mg·kg^{-1} (Figure 6). Similar results were reported by [28] for *Phragmites australis* from different horizontal flow CWs. For example, slightly higher Zn levels and a few times higher Cu concentration in *Typha* were reported after a 15-day-long experiment [13]. As far as B is concerned, this nutrient was found to be well retained by plant root apparatus (27 mg·kg^{-1} on average) in the 2004–2006 period.

Some considerations can be drawn by comparing the concentrations of heavy metals in the biomass with their levels in the top soil (Table 5) for the period 2005–2006, when a complete set of data is available. In fact, even though the root system of aquatic plants can enter the soil deeper than 15 cm, the level of heavy metals in the top soil gives some indications as to their accumulation in the system and their potential bioavailability to plants.

Table 5. Concentration of heavy metals (mg·kg^{-1}) in below-ground biomass and the top soil layer.

Parameter	Biomass			Soil		
	2005	2006	Average	2005	2006	Average
Cadmium	0.4	0.0	0.2	0.0	0.0	0.0
Copper	12.7	6.1	9.4	35.5	32.0	33.8
Iron	1014.7	4086.2	2550.5	29.5	40.9	35.2
Lead	3.0	0.3	1.7	37.0	13.5	25.3
Nickel	12.2	0.2	6.2	54.5	54.0	54.3
Zinc	32.5	24.9	28.7	109.5	85.0	97.3

In general, the concentrations of heavy metals in the first 15 cm of the CW soil were on average lower than, but still in the range of, other studies, such as, for example, that reported by [29], who studied heavy metal removal using a horizontal flow CW from road runoff in Ireland.

In detail, in the 2005–2006 observation period, Cd was present at very low concentrations in both soil and biomass, whereas the average Pb level in the top soil of 25.3 $mg \cdot kg^{-1}$ and its relatively low concentration in the biomass (1.7 $mg \cdot kg^{-1}$, Table 5) are in accordance with the fact that it is a fairly immobile element [27], as well as its known low bioavailability to plants [30]. The other heavy metals, including Ni, Cu and Zn, are considered of medium bioavailability to plants in aerated soil [30]. In the system reported in this study, the average biomass concentrations of Ni, Cu, and Zn (6.2, 9.4 and 28.7 $mg \cdot kg^{-1}$, respectively) were lower than, but still in the range of, their levels in the top soil (54.3, 33.8 and 97.3 $mg \cdot kg^{-1}$, respectively), thus indicating moderate availability to plants.

The Fe level in the biomass (2550.5 $mg \cdot kg^{-1}$) was very high, and the top soil concentration of 35.2 $mg \cdot kg^{-1}$ cannot justify such a high accumulation at the roots. Specific root uptake mechanisms have to be considered for this metal, as already defined by [31], who have reported that aquatic plants may modify the rhizosphere by facilitating the formation of iron oxide plaques that immobilise and concentrate heavy metals.

Under conventional operational conditions, the aquatic plants of the system studied act as Fe accumulators and Cu, Pb, Ni and Zn bio-indicators. These findings could be of interest for further study on metal bioavailability for aquatic plants and phytoremediation mechanisms against PTE.

Finally, the actual SFCW state was monitored as well, in order to evaluate how the 17 years of operation had affected the distribution of nutrients and heavy metals along the soil profile (Table 6). As expected, the level of total organic carbon, TN, TP and B decreased from the top soil to the deeper layers, even though significant differences ($p < 0.05$) were shown only for the first two parameters (Table 6). This highlights the surface accumulation of nutrients and organic material produced by aquatic plants. The average pH value of the soil (measured in water) was found to be 8.51 $\pm$ 0.04 (20).

No visible accumulation of heavy metals can be seen along the vertical soil profile, and there were no significant differences among them (Table 6). Most probably, this is due to the fact that the root systems of aquatic plants can accumulate large amounts of heavy metals [32] and occupy large soil volumes. Moreover, when compared to their legal limits, as introduced by the Italian law [33], all of the heavy metals resulted at concentrations below the lowest admitted threshold (limit A: soil suitable for private and public green areas). In addition, the data for Cr, Ni, Zn, Cu, Pb, and Sn of CW soil still safely fall within the range of the local anthropic-natural available background data ($\leq$75, $\leq$120, $\leq$75; $\leq$60, and $\leq$50 $mg \cdot kg^{-1}$, respectively [34]), thus indicating that no important changes have affected the soil heavy metal content. Therefore, even after 17 years of functioning, the SFCW can still be considered to be a bio-filter with no visible accumulation of PTE in either the top or the deep soil layers (up to 60 cm depth), and with levels below the current legal limits.

Table 6. Concentrations of nutrients or heavy metals at different depths of the CW soil in 2017 (values are displayed as: mean ± std error and number of samples in brackets).

Parameter	0–5 cm	5–15 cm	15–30 cm	30–45 cm	45–60 cm	Limit A	Limit B
Total organic carbon ($g \cdot kg^{-1}$) *	41.5 ± 6.1 (4) [a]	16.3 ± 1.7 (4) [b]	10.2 ± 0.5 (4) [b,c]	8.4 ± 1.1 (4) [c]	7.2 ± 1.3 (4) [c]	-	-
Total nitrogen ($g \cdot kg^{-1}$) *	3.5 ± 0.4 (4) [a]	1.6 ± 0.1 (4) [b]	1.2 ± 0.0 (4) [b,c]	1.0 ± 0.1 (4) [b,c]	0.9 ± 0.2 (4) [c]	-	-
Total phosphorus ($g \cdot kg^{-1}$)	0.7 ± 0.0 (4)	0.6 ± 0.0 (4)	0.6 ± 0.1 (4)	0.5 ± 0.0 (4)	0.5 ± 0.1 (4)	-	-
Boron ($mg \cdot kg^{-1}$)	44.5 ± 0.6 (4)	45.3 ± 0.3 (4)	44.0 ± 0.4 (4)	42.3 ± 1.7 (4)	40.5 ± 2.1 (4)	-	-
Cadmium ($mg \cdot kg^{-1}$)	0.2 ± 0.0 (4)	0.2 ± 0.0 (4)	0.1 ± 0.0 (4)	0.2 ± 0.0 (4)	0.1 ± 0.0 (4)	2	15
Chrome ($mg \cdot kg^{-1}$)	67.9 ± 0.8 (4)	72.0 ± 0.6 (4)	71.6 ± 1.0 (4)	68.4 ± 1.5 (4)	66.4 ± 2.1 (4)	150	500
Copper ($mg \cdot kg^{-1}$)	36.9 ± 1.6 (4)	36.6 ± 1.4 (4)	38.9 ± 4.5 (4)	35.0 ± 3.5 (4)	32.2 ± 4.0 (4)	120	600
Iron ($g \cdot kg^{-1}$)	24.9 ± 0.5 (4)	26.5 ± 0.3 (4)	26.5 ± 0.1 (4)	25.9 ± 0.4 (4)	25.0 ± 0.7 (4)	-	-
Lead ($mg \cdot kg^{-1}$)	24.8 ± 2.6 (4)	23.8 ± 1.2 (4)	24.9 ± 2.0 (4)	22.9 ± 1.4 (4)	20.0 ± 1.1 (4)	100	1000
Nickel ($mg \cdot kg^{-1}$)	48.5 ± 0.7 (4)	51.2 ± 0.6 (4)	51.2 ± 0.2 (4)	49.5 ± 1.1 (4)	48.3 ± 1.3 (4)	120	500
Zinc ($mg \cdot kg^{-1}$)	78.3 ± 1.4 (4)	77.1 ± 1.6 (4)	77.0 ± 2.6 (4)	73.7 ± 2.7 (4)	70.0 ± 2.8 (4)	150	1500

Notes: Limit A–green areas, private and residential use; Limit B–commercial and industrial use (Italian D.Lgs. 152, 2006). * ANOVA test showed significant differences only for Total organic carbon and Total nitrogen. Letters for concentrations of these two parameters at different depths indicate significant ($p < 0.05$) difference (different letter) or not (the same letter).
- Undetected.

4. Conclusions

This study presents the main findings of monitoring performed over a long period of time (2003–2017) in the operation of a SFCW located at the farm of the CER Land Reclamation Consortium (Northern Italy), treating agricultural drainage water. Its functioning was particular, and it depended on the needs of the farm and specific research projects, and was therefore receiving different water volumes with different nutrient/pollutant loads throughout the years.

The retention of TN and TP, the two nutrients that are mostly responsible for the surface water bodies' pollution and eutrophication, was never below 47% and 49%, respectively. Since the SFCW received varied inflow loads over the years, it can be said that the system proved itself to be a viable option for tile drainage water treatment. As a general rule, TN and TP retention depended on their residence time in the system, rather than their inflow loads. Even though the loads of these nutrients varied a lot over the years, their analysis in the biomass and soil showed a certain accumulation.

In addition, heavy metals that entered the SFCW were mostly retained by the root system, thus acting as a biofilter for the collected agricultural drainage water and protecting the receiving water bodies. For several heavy metals, the distribution between biomass and soil made it possible to define their varying bioavailability to the aquatic plants inhabiting the system and showed their potential for the removal of these PTEs. Finally, and most importantly, after 17 years of functioning, the SFCW soil content of each of the heavy metals considered was found to be below the lowest limit imposed by the Italian law for soils of private and public green areas.

In light of these observations, it is possible to conclude that the monitored SFCW was able to adapt its performance and ecosystem services to different operational conditions over a long period of time, without losing its ability to improve the inflow water quality.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/10/5/644/s1, Table S1: Farm TN input and SFCW nitrogen balance (all the values are in kg).

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