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Treatment of Combined Dairy and Domestic Wastewater with Constructed Wetland System in Sicily (Italy). Pollutant Removal Efficiency and Effect of Vegetation

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Abstract: Dairy wastewater (DWW) contains large amounts of mineral and organic compounds, which can accumulate in soil and water causing serious environmental pollution. A constructed wetland (CW) is a sustainable technology for the treatment of DWW in small-medium sized farms. This paper reports a two-year study on the performance of a pilot-scale horizontal subsurface flow system for DWW treatment in Sicily (Italy). The CW system covered a total surface area of 100 m^2 and treated approximately 6 m^3 per day of wastewater produced by a small dairy farm, subsequent to biological treatment. Removal efficiency (RE) of the system was calculated. The biomass production of two emergent macrophytes was determined and the effect of plant growth on organic pollutant RE was recorded. All DWW parameters showed significant differences between inlet and outlet. For BOD_5 and COD, RE values were 76.00% and 62.00%, respectively. RE for total nitrogen (50.70%) was lower than that of organic compounds. RE levels of microbiological parameters were found to be higher than 80.00%. Giant reed produced greater biomass than umbrella sedge. A seasonal variation in RE of organic pollutants was recorded due to plant growth rate Our findings highlight the efficient use of a CW system for DWW treatment in dairy-cattle farms.

Keywords: dairy wastewater; horizontal sub-surface flow system; plant growth; removal efficiency

1. Introduction

The dairy industry is the largest Italian food sector and represents more than 15% of the national food business [1]. Estimates by the Milk Market Observatory [2] indicate that Italy is one of the largest milk and dairy-product producers in the European Union with an annual average total milk yield of 11,721,375 t over the last ten years [3]. In Sicily (Italy), dairy products represent a significant proportion of the total value of agricultural outputs and include raw milk from buffalo, cow, goat and sheep, butter, yoghurt and different types of cheese. The dairy sector is, thus, widely present in this region and it is mostly composed of specialized small and medium farms. These farms consume extremely high amounts of water annually, mainly used in cleaning and cooling systems, technological systems, steam generators and fire protection systems.

Taking into consideration the relationship between water consumption and milk production, a survey carried out in 35 Italian dairies [4] estimated an average water consumption of 1 L of water per kilogram of raw milk produced by the dairy. During butter and cheese processing, however, water consumption varied between 3 and 30 L of water per kilogram of raw milk [5]. Due to this high water consumption, the dairy industry produces vast quantities of wastewater, which must then be treated, causing a series of negative impacts on the surrounding natural ecosystem. A number of authors [6,7] reported 1–10 L of wastewater generated per liter of processed milk.

Dairy wastewater (DWW) is characterized by high levels of detergents, fats, minerals, organic compounds, proteins and a wide range of pH values [7–11]. As stated by various authors [7,12,13], typical DWW characteristics include 1400–50,000 mg L^{-1} biochemical oxygen demand (BOD_5), 2000–90,000 mg L^{-1} chemical oxygen demand (COD,) 70–800 mg L^{-1} total suspended solids (TSS), 100–1400 mg L^{-1} total nitrogen (TN) and 25–450 mg L^{-1} total phosphorus (TP).

However, the composition of DWW is not stable over time as affected by various factors such as seasonality of dairy activities, different dairy products produced, operating and processing conditions of dairy products and wastewater management [14,15]. In particular, a critical component of DWW is whey. Whey constitutes 85%–95.00% of the milk volume, it contains fats, lactose, minerals, proteins, vitamins and is considered the greatest pollutant in DWW due to the high organic load and volume that is produced [10,12,16]. Literature, in fact, calculates the global production of whey to be over 100 billion kilograms per year [17,18].

Despite the fact that DWW can be re-used in the production of some horticultural and open field crops [9,19,20], its long-term application can have a negative impact on the characteristics of soil structure, causing a decrease in crop yield [21,22]. Furthermore, when DWW is directly discharged into water bodies, it negatively affects aquatic life, leading to eutrophication of the receiving waters [12,23]. It was reported that, globally, every year approximately 4–11 million tons of DWW are released into the environment, causing severe hazard to all biodiversity [24,25].

On the basis of that, a number of treatment systems must be applied. DWW is usually treated by physical-chemical methods, such as coagulation/flocculation and/or biological methods, which include processes such as activated sludge, aerated lagoons, anaerobic sludge blanket reactors, anaerobic filters, sequencing batch reactor, trickling filters, or others [10,15,26]. However, the use of conventional treatments can be problematic for a series of reasons, such as variability in certain hydraulic aspects, significant sludge production, high management costs and the need for specialized staff to manage wastewater operations [13].

In Sicily and other Mediterranean countries, small and medium dairy farms are isolated from conventional treatment plants and are often located close to areas with high agricultural and ecological importance, such as lakes, lagoons, ponds and open fields [27]. However, the technical and biological characteristics of a constructed wetland (CW) make it an ideal DWW treatment system for these farms. Constructed wetland systems (CWs) are easy to use and manage, they reduce operation and maintenance costs, they provide high levels of pollutant removal efficiency (RE), improve water quality and preserve the soil and aquatic environments [12,13,24,27]. CWs have been successfully used in the treatment of DWW in only a few Mediterranean countries, such as Italy [13,27–31] and Greece [12,32,33]. Among CWs, the horizontal sub-surface flow system (HSSFs) is considered one of the best performing systems for DWW treatment, achieving very high RE values for organic compounds and nutrients, mainly [34].

However, taking the characteristics of DWW into consideration, and, in particular, the high organic load, it is realistic to assume that in the medium and long term a significant amount of organic matter will accumulate in the substrate, contributing to its clogging and reducing the pollutant RE of the system [35]. Thus, a combination of HSSFs with an effective pretreatment system is fundamental in order to maintain the high treatment performance of the system.

Literature on DWW treatment using CWs is quite substantial, however, most studies tend to give greater importance to chemical and engineering aspects and limited attention to plant species. In HSSFs, plants contribute greatly to the treatment process, however, their action is significantly affected by air temperature, increasing the performance when air temperature stimulates vegetative growth. A novelty of this paper is, thus, the comparison of two underused plant species in CWs, *Arundo donax* L. and *Cyperus alternifolius* L., in terms of growth, biomass production and N uptake, and highlighting how the choice of vegetation can affect DWW treatment.

The aims of the study were: (i) to assess the pollutant RE of a pilot-scale HSSFs CW for treatment of DWW produced by a small dairy farm in Sicily, (ii) to assess the plant growth during the year and its effect on organic pollutant RE.

2. Materials and Methods

2.1. Test Site

Tests were conducted in the two years from 2019 to 2020 on a pilot HSSFs CW in Raffadali, a rural municipality in the West of Sicily (37°24′ N–1°05′ E, 446 m a.s.l.).

The system was used to treat DWW produced by a small dairy-cattle farm located in the surrounding area. The farm produced milk for cheese-making. In particular, the farm had two sheds and an average of 80 lactating cows. The production capacity of milk was approximately of 1600 L day^{-1} (Figure 1).

Figure 1. Cheese production in the dairy-cattle farm.

DWW used in the study was composed of wastewater from the holding area (following solid liquid separation), milking parlor and, thus, mixed with domestic wastewater produced by the staff of the dairy farm.

2.2. Description of the HSSFs CW

The HSSFs CW system was built in the year 2000 and located in an urban park (Figure 2). It included two separate, parallel units each 50 m long and 1 m wide, with a total surface area of 100 m^2. The floor and walls of the units were made of concrete. The units were lined with sheets of ethylene and vinyl-acetate and were designed in order to receive a total of 6 m^3 of wastewater per day. Filter bed depth was 0.50 m with a water depth of 0.30 m and a 2% slope. The substrate was made of evenly-sized 30 mm silica quartz river gravel (Si 30.02%; Al 5.11%; Fe 6.10%; Ca 2.65%; Mg 1.05%) with a porosity of 35–40%.

Figure 2. A view of the pilot-scale horizontal sub-surface flow systems (HSSFs) constructed wetland CW.

The two units were separately planted with giant reed (*Arundo donax* L.) and umbrella sedge (*Cyperus alternifolius* L.). The information on propagation techniques of the two species and plant density were described by the authors in a previous study [36].

The layout of the system for the wastewater treatment is shown in Figure 3.

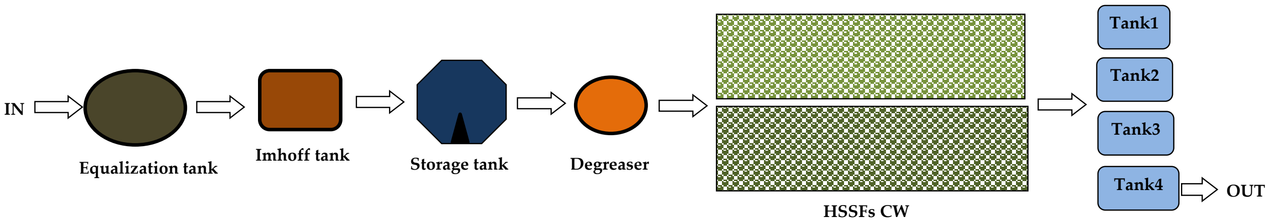


Figure 3. Layout of the system for treatment of combined dairy and domestic wastewater.

On the dairy farm, the DWW, mixed with domestic wastewater, was fed into an equalization tank and, subsequently, treated by two Imhoff septic tanks in order to remove TSS and organic matter. The pre-treated DWW from the dairy farm was then collected in a 15.00 m^3 storage tank at the HSSFs CW. The tank was equipped with a submerged electric pump to feed water into the CWs units, and with a liter gauge and outlet valve for periodic cleaning of solid sediments.

Initially, the wastewater was fed into a static degreaser to separate fats, soaps and food wastes and, subsequently, pumped through a 1.00 m wide perforated polyvinylchloride pipe into the two HSSFs CW units. The homogeneous distribution of wastewater in each unit was ensured through a timer-controlled pumping system. In each unit, the pipe was placed 10.00 cm from the surface of the substrate. Treated dairy wastewater (TDWW) was collected using a perforated drainage pipe system, placed at the bottom of the filter bed and then conducted downhill into a system of four interconnected tanks of 5.00 m^3 each.

The two units were tested using a hydraulic loading rate (HLR) of 6.00 cm day^{-1} and hydraulic retention time (HRT) of 8.30 days. Finally, TDWW was generally discharged into the soil using a subsurface irrigation system connected to the last of the four tanks. The subsurface irrigation system was designed taking into account the number of equivalent inhabitants and the physical characteristics of the soil.

2.3. Plant Measurements

Plant growth was determined by measuring the plant height, stem density and calculating the dry weight of the above-(leaves and stems) and below-ground plant parts (roots and rhizomes). The main morphological parameters were taken from March to November for each year.

Plant height was calculated fortnightly by measuring the maximum height of 10 plants, in good phytosanitary condition, selected randomly from the initial, the middle and the end sections of each unit. Leaf number per plant and root-system length were determined monthly by making a leaf count and measuring the root length of 10 plants selected randomly for each unit. Culm/stem density was calculated monthly on an area of 1.00 m^2 for each planted unit.

According to a previous study [37], four crop growth stages were identified: (a) initial stage: from greenup to the beginning of stem elongation; (b) crop development stage: from stem elongation to initial flowering; (c) mid-season stage: from flowering to initial canopy senescence; (d) late-season stage: from canopy senescence to plant harvest.

In November, the plants were cut back to a height of 50.00 cm above the gravel bed. Fresh above-ground and below-ground weights were determined on a representative sample of 10 plants from each unit. The above- and below-ground biomass dry weights were calculated by drying the collected plant material in an oven at 62.00 °C for 72 h. Nitrogen levels in the aboveground biomass parts of the plants were determined using a Carbon, Hydrogen, Nitrogen (CHN) elemental analyzer, in full compliance with plant biomass basic analysis standards. This process was repeated following the next cutting, after 12 months.

2.4. DWW Analysis

Wastewater samples were taken monthly from March to November, for both years, amounting to a total of 72 times (36 times per planted unit). The samples were collected at the inflow and outflow of each CW unit. 1.00 L of wastewater was collected from each of the two points at each sampling. The influent sample was taken close to the pipe while the effluent sample was collected at the mouth of the outflow pipe. Sampling always occurred at the same time, usually coinciding with milking procedure or other operations into the dairy farm.

The pH and electrical conductivity (EC) were determined directly on site using a portable Universal meter (Multiline WTW P4). Using Italian water analytical methods [38], TSS, BOD_5, COD, TN, ammonia nitrogen (NH_4-N), organic nitrogen (ON), TP and heavy metals (Cu, Ni, Pb and Zn) were determined. Total coliforms (TC), fecal streptococci (FS), Escherichia coli (E. coli) and Salmonella spp. levels were determined by membrane filter methods, based on standard methods for water testing [39]. RE of the HSSFs CW was based on pollutant concentrations and calculated in accordance with International Water Association [40]:

$$RE = \frac{C_i - C_0}{C_i}$$

where C_i and C_0 are the mean concentrations of the pollutants in the influent and effluent.

2.5. Weather Data

A weather station belonging to the agrometeorological information service of the Sicilian Government [41] was used to collect climate data. It was located close to the pilot HSSFs CW. The station was equipped with a MTX datalogger (model WST1800, Padova, Italy) and various climate sensors. In particular, a temperature sensor MTX (model TAM platinum PT100 thermo-resistance with anti-radiation screen) and a rainfall sensor MTX (model PPR with a tipping bucket rain gauge) provided data on daily minimum and maximum air temperatures and total 10-day rainfall data.

2.6. Statistical Analysis

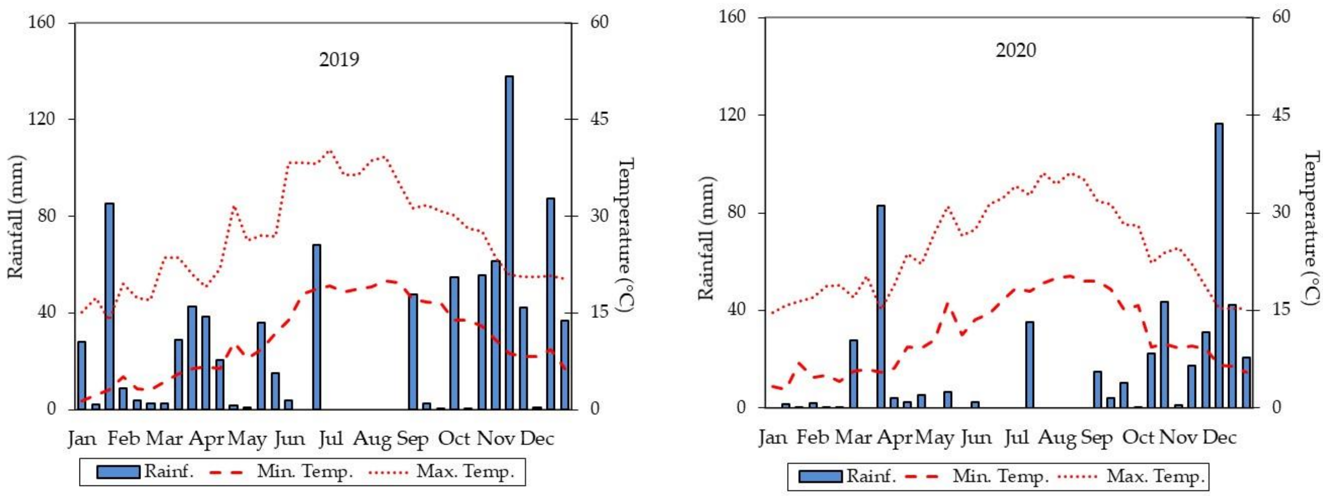
Statistical analyses were performed using the package MINITAB 17 for Windows. A paired *t*-test was used to compare the mean levels of each chemical and microbiological parameter at influent and outlet. A level of $p < 0.05$ was used for all comparisons. For DWW composition, all the representative values were presented using mean ± standard deviation calculations.

3. Results and Discussion

3.1. Rainfall and Air Temperature Trends in the HSSFs CW Area

According to the Köppen–Geiger climate classification, the study location is characterized by a warm temperate climate with hot-dry summers and rainfall not well distributed throughout the year [42]. With reference to time series 1982–2012, the annual average rainfall was approximately 650.00 mm, the average air temperature was 17.50 °C, the average maximum air temperature was 23.50 °C, and the average minimum air temperature was 11.20 °C.

Figure 4 shows air temperatures and total rainfall trends in 2019 and 2020.



(**a**) (**b**)

Figure 4. Rainfall and air temperature trends. Graph (**a**) refers to 2019 while graph (**b**) refers to 2020.

In both years, maximum and minimum air temperatures increased greatly from the beginning of April to the third 10-day period of August and decreased up to the end of December. The highest maximum air temperature (40.30 °C) was recorded in the first 10-day period of July 2019 and the lowest minimum air temperature (1.40 °C) was determined in the first 10-day period of January 2019. Annual rainfall ranged between 917 mm (2019) and 495 mm (2020). The highest rainfall levels (138 mm) occurred during the second 10-day period of November 2019. The distribution of rainy days during the seasons was quite different over the two years. The days of absence of rainfall were more in 2020 than in 2019 and concentrated also in the winter season. Particularly, in summer, average monthly rainfall was 22.70 mm in 2019) and 18.13 mm in 2020.

The highest treatment performance of HSSFs CW was recorded from April to August in both years when air temperatures positively affected plant growth and microbiological activities in the CW units. Furthermore, during the autumn months, we observed no significant decrease in plant activity due to mild air temperatures. The climate conditions allowed the two macrophytes to extend their vegetative cycle until the end of autumn, delaying senescence and their ability to remove pollutants from DWW.

3.2. Monitoring and Pollutants Removal Efficiency of the HSSFs CW

Tables 1 and 2 show the average influent and effluent concentrations of chemical and physical parameters, as well as RE percentages of the HSSFs CW.

Table 1. Variation (VA) of pH and EC in the planted units from March to November 2019/2020. For each planted unit, two-year average values (± standard deviation) are shown (n = 36).

Parameter	Influent	Effluent [1]	Effluent [2]	VA (%) [1]	VA (%) [2]	Discharge in Soil [3]	*t*-Test [4]
pH	7.95 ± 0.27	7.35 ± 0.18	7.30 ± 0.10	7.90	7.80	6–8	*
EC (mS cm^{-1})	4.45 ± 1.11	5.75 ± 0.98	5.43 ± 1.02	29.10	22.01	-	*

Notes: [1] Giant reed-planted unit; [2] umbrella sedge-planted unit. [3] Threshold values for Italian Decree 156/2006. [4] Significant (*) differences between influent and effluent values ($p < 0.05$).

Table 2. Main chemical and physical composition of the DWW from inlet to outlet of the HSSFs CW. Removal efficiency from March to November 2019/2020. For each planted unit, two-year average values (±standard deviation) are shown (n = 36).

Parameter	Influent	Effluent [1]	Effluent [2]	RE (%) [1]	RE (%) [2]	Discharge in Soil [3]	*t*-Test [4]
Color	P [5]	NP [6]	NP			-	
Odor	NU [7]	NU	NU			-	
Coarse matter	Present	Absent	Absent			Absent	
TSS (mg L^{-1})	147.11 ± 0.02	24.10 ± 3.35	25.89 ± 0.01	80.69	82.98	25	*
BOD_5 (mg L^{-1})	86.92 ± 6.88	19.22 ± 7.49	21.27 ± 7.79	78.02	75.61	20	*
COD (mg L^{-1})	215.29 ± 9.12	80.83 ± 8.53	84.12 ± 11.10	62.67	61.12	100	*
TN (mg L^{-1})	91.03 ± 3.43	43.73 ± 3.41	45.72 ± 2.41	51.84	49.68	15	*
N–NH_4 (mg L^{-1})	62.10 ± 3.45	34.12 ± 3.52	30.11 ± 3.23	45.05	51.51	-	*
ON (mg L^{-1})	24.12 ± 1.43	14.34 ± 1.27	13.26 ± 2.11	40.51	45.11	-	*
TP (mg L^{-1})	13.96 ± 0.55	8.40 ± 0.29	8.53 ± 0.28	39.86	38.88	2	*
Cu (mg L^{-1})	0.075 ± 0.001	0.042 ± 0.001	0.039 ± 0.001	44.11	48.31	0.10	*
Ni (mg L^{-1})	0.023 ± 0.001	0.015 ± 0.001	0.016 ± 0.001	35.17	31.03	0.20	*
Pb (mg L^{-1})	0.019 ± 0.001	0.013 ± 0.001	0.012 ± 0.001	31.57	36.84	0.10	*
Zn (mg L^{-1})	0.32 ± 0.002	0.14 ± 0.001	0.16 ± 0.001	56.25	50.33	0.50	*

Notes: [1] Giant reed-planted unit; [2] umbrella sedge-planted unit. [3] Threshold values for Italian Decree 156/2006. [4] Significant (*) differences between influent and effluent values ($p < 0.05$). [5] Perceptible; [6] not perceptible; [7] not unpleasant.

As reported by various authors [13–15,43], the levels of the main parameters in the study were different throughout the year and varied mainly due to seasonal changes in dairy activities.

Pre-treatment by the septic tank and degreaser provided effective treatment of the DWW due to biological, chemical and physical processes. At the inlet of the HSSFs CW, odors were not unpleasant, and no coarse matter was found in the DWW.

For pH measurements, literature [11,13,44] shows ranging between 3.5 and 11, depending on the dairy activities and the use of alkaline and acid cleaners. In our study, in both planted units, influent values were found to be slightly alkaline, significantly higher than effluent values. This was in agreement with the findings of other authors [24,27,28], who report pH values of the HSSFs CW effluent close to 7.0. As found by various authors [45,46], it is reasonable to assume that the decrease in pH values in the effluent are due to the production of carbon dioxide (CO_2) by the decomposition of plant residues, the removal of various components of the wastewaters in the root area and the nitrification of ammonia.

In the case of EC, influent values were significantly lower than effluent values. Furthermore, in the two planted units, the EC effluent values were found to be different, probably due to different evapotranspiration rates of the two emergent macrophytes. This physical process determined, in fact, high water consumption in the planted units and an increase in salt levels in the solution. The effect of evapotranspiration on EC levels of the CW effluent has been previously well explained by a number of authors [47–52].

TSS values were found to differ significantly between influent and effluent. TSS RE was found to be almost identical in the two planted units. These values were inside the range of those observed in HSSFs CW for the DWW treatment, which varied between 75% and 85% [13,24,27,40,44]. In Italy, some authors [28,30] reported TSS RE values of above 90%, however when using different pre-treatment systems and applying hybrid CWs. Previous studies investigated the reasons which could influence the TSS removal in a HSSFs and the majority of them agree with the fact that filtration and sedimentation processes contribute greatly to elimination of the TSS [53]. In our study, these physical processes carried out by substrate, plant roots and microorganisms improved the wastewater flow in the two planted units and, consequently, the treatment performance of the system.

BOD_5 and COD values showed significant differences between influent and effluent. BOD_5 RE varied from 78.02% (giant reed-unit) to 75.61% (umbrella sedge-unit). Similarly, COD RE varied from 62.67% (giant reed-unit) to 61.12% (umbrella sedge-unit). These values remained within limits consistent with findings of other authors concerning HSSFs. In Argentina, in a pilot-scale HSSFs CW located close to a dairy farm, the authors [24] found average RE values for BOD_5 and COD of 57.90% and 68.70%, respectively. In Vermont (USA), a series of integrated systems consisting of various combinations of HSSFs, and vertical sub-surface flow systems (VSSFs) were used to treat DWW, with a BOD_5 RE of 86–89.00% [54]. In Japan, multistage HSSF systems were designed to treat DWW under cold climate conditions. Researchers found high removal rates for COD RE (93–96.00%) [55]. In southern Europe, in various studies [12,27–29,31–33], the authors reported average values of BOD_5 RE ranging between 70% and 94% depending on various factors, such as the size of the CW and the wastewater pretreatment. In this study, at inlet of the HSSFs CW, the ratio between BOD_5 and COD was found to be 0.40, on average. As reported in literature, a ratio lower than 0.50 indicates low susceptibility of wastewater to biodegradation. In this study, due to the fact that the BOD_5/COD ratio was slightly lower than 0.50, it is reasonable to presume that most compounds in the DWW were easily biodegradable. The high average RE values of BOD_5 and COD can be explained by considering the role of plants, substrate and microorganisms in a CW and their interaction. Many authors [12,13,24,27,44,53] highlight, in fact, that filtration and sedimentation carried out by plants and substrate, together with microbiological degradation, are the main physical and chemical processes required for the elimination of organic matter in a CW. However, taking the functional and construction characteristics of HSSFs into consideration, it is not possible to conclude that the removal rate of organic compounds depends only on oxygen levels in the rhizosphere. As confirmed by previous studies [40,53], high RE values in a system can be explained by anaerobic biodegradation processes in the CW units.

Regarding TN, effluent values were significantly lower than influent values. TN RE values were recorded as being similar in both planted units. Moreover, these values were on average lower than those of TSS, BOD_5 and COD. Literature [40,53,56] remarks that, in a HSSFs CW, nitrification/denitrification and plant/microbial uptake represent the most frequent mechanisms for nitrogen removal. However, these processes depend greatly on the oxygen availability. In a HSSFs CW, the oxygen levels are usually low, and this condition can limit the ammonium nitrification process and explain the lower TN RE values recorded. Comparing our findings with those of other studies, both similarities and differences were found. In Lithuania, in a HSSFs vegetated with *Phragmites australis* for treatment of combined dairy and domestic wastewater, the authors [57] claimed that the system provided an average TN RE of 37–44.00%. In a review on various experiences from the Netherlands and Belgium [12], using CWs for DWW treatment, higher TN removal rates (>85–90.00%) than those of our study were reported. In Italy, assessing the HSSFs treatment performance for DWW, various authors [28,29] obtained TN RE values which were consistent with those in this study.

For TP, significant differences between influent and effluent concentrations were observed. Both planted units recorded similar TP RE average values, approximately 40%. Literature highlights that TP RE depends on several factors, such as the age of the HSSFs CW, the adsorption properties of the substrate, the gradual filling of the sorption sites over the years and the presence of under composed plant material around the substrate surface [58–60]. Furthermore, it may be related to plant uptake, as the macrophytes have different absorption and storage capacities [61]. However, it is important to highlight that, in a HSSFs CW, TP RE tends to decrease over the time and seems to be high when the plants are young, the root length density is low per unit of substrate volume and substrate adsorption is highly active [12,58–60]. Observing the low average value of TP RE recorded in this study, it was found to be in the range of 30.00–60.00% as shown by literature, largely due to the above-mentioned reasons. In Italy, in a study conducted on a dairy farm in the province of Reggio Emilia [28], a TP RE value of 60.00% was found, based on an average influent TP concentration of 12.80 mg L^{-1}; in another Italian study carried out in the Aosta Valley [29], TP RE was 40.00% with an average influent TP concentration of 10.00 mg L^{-1}. In Ireland, in an integrated CWs used to treat DWW, TP removal varied depending on the season (5%–84%), with lowest performance during the cold season [62].

Concerning heavy metals, significant differences between influent and effluent concentrations were found. In both planted units, RE values were, in general, acceptable. Our findings were in agreement with those obtained in a study carried out in Sicily using a HSSFs CW planted with *Phragmites australis* [27]. These results confirm the contribution of plants to the removal process and the importance of the interaction of plants, microorganisms and substrate in the CWs, as highlighted previously [63,64].

In the case of microbiological parameters, bacteria were always present in the effluent due to the fact that DWW was mixed with domestic wastewater. Domestic wastewater was found, in fact, to have the highest average TC, FC and E. coli levels (data not shown). It is worth noting that the levels of bacteria in combined dairy and domestic wastewater were not constant and varied over the time depending on dairy farming activities and practices.

Concerning the main results, in both planted units and for each microbiological parameter in the study, RE levels were found to be above 80.00% (Table 3). Significant differences were found between influent and effluent average values. Comparing these findings with those of other studies [24,27,28], many similarities were observed, despite different operating conditions at the CWs.

Table 3. Main microbiological composition of the DWW from inlet to outlet of the HSSFs CW. Removal efficiency from March to November 2019/2020. For each planted unit, two-year average values (± standard deviation) are shown (n = 36).

Parameter	Influent	Effluent [1]	Effluent [2]	RE [1]	RE [2]	Discharge in Soil [3]	*t*-Test [4]
TC (CFUs 100 mL^{-1})	3.97 ± 0.02 [5]	3.19 ± 0.11	3.21 ± 0.00	83.31	82.77	-	*
FS (CFUs 100 mL^{-1})	3.85 ± 0.01	3.04 ± 0.01	3.13 ± 0.01	84.32	81.17	-	*
Escherichia coli (CFUs 100 mL^{-1})	3.91 ± 0.01	3.01 ± 0.02	3.05 ± 0.02	87.44	86.48	≤3.69 [5]	*
Salmonella spp. (CFUs 100 mL^{-1})	Absent	Absent	Absent			-	

Notes: [1] Giant reed-planted unit; [2] umbrella sedge-planted unit. [3] Threshold values for Italian Decree 156/2006. [4] Significant (*) differences between influent and effluent values ($p < 0.05$). [5] The average concentration values are shown as units of Log_{10}.

The high pathogen RE can be explained considering all processes carried out by macrophytes and microorganisms in the substrate. A number of authors [40,53,65,66], in fact, maintain that typical processes in a CW, such as filtration and adsorption, chemical oxidation and sedimentation, are efficient for removal of microorganisms and that the more favorable the conditions for plant life and bacteria, the more effective the removal of pathogens.

Furthermore, as clearly explained in previous studies, the aerobic conditions in the root zone of a HSSFs CW permitted greater bacterial biofilm formation and promoted high pathogen RE [40,53].

In Italy, the discharge of treated wastewaters into the soil is regulated by Legislative Decree 156/2006. In this research study, average chemical and physical parameter results at the outlet of the HSSFs CW were not all within the legal limits of the Italian Decree. In particular, TN and TP concentration values did not meet the threshold values due to fact their removal was not high. Concerning the microbiological parameters, data recorded for *Escherichia coli* were not found to be within these legislative values. Reasons for this are varied and may be linked to the size of the two planted units, removal efficiency of the pretreatments and the seasonality of DWW. As pathogen removal is significantly affected by aerobic/anaerobic conditions in the substrate, a hybrid wetland, for example a combined HSSF-VSSF, could allow for better performance [32]. In fact, the two systems are characterized by diverse retention time and this could positively influence pathogen removal [36].

3.3. Plant Growth and Biomass Production

In both years, maximum plant growth was recorded during summer when the air temperatures were higher than those of other seasons.

Average plant heights ranged between 149.16 cm (giant reed) and 127.66 cm (umbrella sedge). In 2019, plant growth was more intense than in 2020 due to better climate conditions (Figure 5).

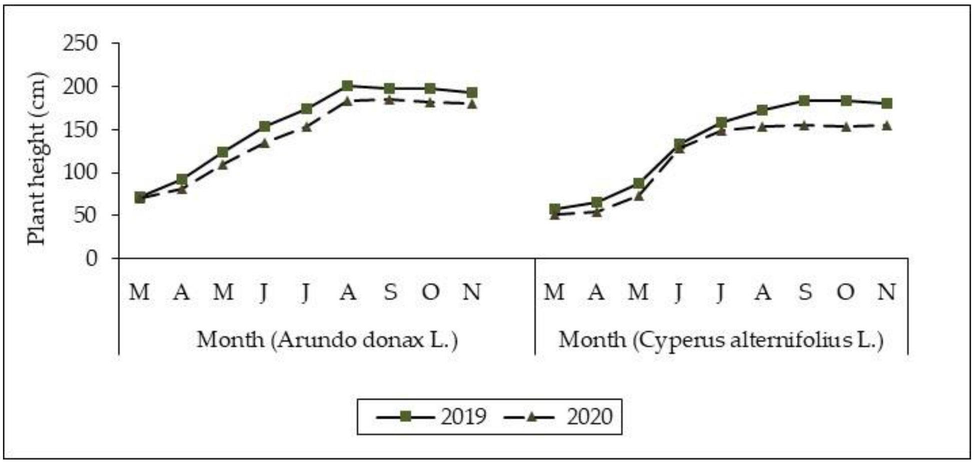


Figure 5. Plant height trend of giant reed and umbrella sedge (2019/2020).

During the two years, average culm/stem density, root diameter and root length were not similar for the two species, highlighting different morphological traits (Table 4).

Table 4. Morphological parameters of giant reed (CW_1) and umbrella sedge (CW_2) plants in the HSSFs CW. Two-year average values (±standard deviation) are shown *(n* = 18).

Parameter	CW_1	CW_2
Culm/stem density (n m^{-2})	22.02 ± 2.34	87.11 ± 3.55
Culm/stem height (cm)	149.16 ± 13.44	127.66 ± 12.67
Root diameter (cm)	41.10 ± 2.10	32.33 ± 3.31
Root length (cm)	30.03 ± 1.56	28.31 ± 2.03

In both planted units, culm/stem density decreased over the period despite the different air temperatures. This was probably due to a self-thinning process which is common in plant monocultures, as explained in a study aimed at comparing two emergent macrophytes in a CW [67]. The distribution of the root system was uniform in both planted units, however, the root length increased more in the giant reed-unit (Figure 6).

These findings confirmed the differences in terms of morphological parameters between the two macrophytes in the study, as found previously [36,68].

When observing the length of the growth stages, differences between the species were recorded over the two years (Figure 7).

The initial stage was found to be the shortest whilst crop development stage and mid-season stage were the longest, on average. For giant reed, mid-season stage occurred at mid-July and at the end of October. In the case of umbrella sedge, this stage was longer in 2020 than 2019 and occurred at beginning of July and at the end of October. As observed in previous studies [36,69], during late-season stage, leaf loss for giant reed was limited during both years and was found to be lower than for umbrella sedge. In 2019, late-season stage occurred between November and December. Plants were harvested when dormancy started (beginning of December) and nutrient-uptake capacity of the species decreased greatly.

(**a**) (**b**)

Figure 6. Root system of the two macrophytes. (**a**) refers to giant reed while (**b**) refers to umbrella sedge plants.

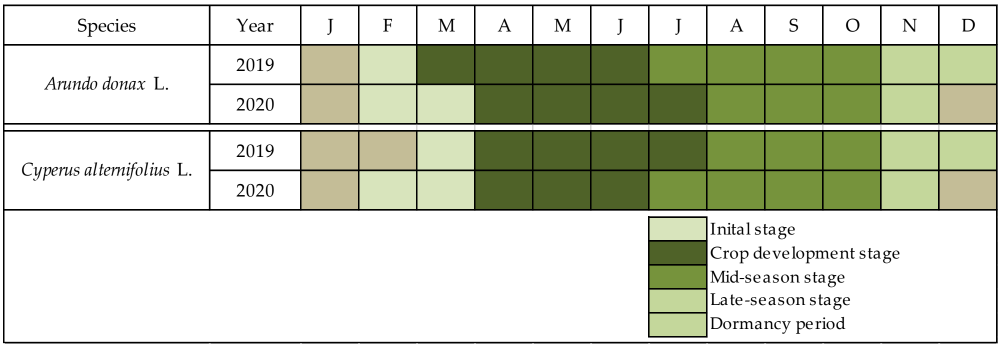


Figure 7. Duration of the main growth stages of giant reed and umbrella sedge.

Figure 8 shows average plant biomass and nitrogen content of the two macrophytes for the years 2019–2020.

In the study period, giant reed produced greater biomass than umbrella sedge and was confirmed as a plant with high biomass yield potential. Average dry matter for the above-ground parts of the giant reed was 42,400 g m^{-2} y^{-1}, and 63,000 g m^{-2} y^{-1} for the below-ground parts. Concerning umbrella sedge, average dry matter for the above-ground parts was 34,600 g m^{-2} y^{-1}, and 38,700 g m^{-2} y^{-1} for the below-ground parts.

The different levels of biomass production of the two species greatly affected their capacity to remove pollutants from DWW. As stated in a previous study [36], we can say that the greater the production of biomass, the greater the nutrient uptake by the plants. In fact, the higher average biomass levels of giant reed allowed the plants to uptake greater levels of nutrients with respect to umbrella sedge. The nutrients were then partly stored in the roots and partly translocated to stem and leaves in order to allow for vegetative growth. Average N levels in the above-ground parts were found to be 69.10 g m^{-2} y^{-1} for giant reed and 53.24 g m^{-2} y^{-1} for umbrella sedge. In contrast, average N content in the below-ground parts was 43.32 g m^{-2} y^{-1} for giant reed and 32.93 g m^{-2} y^{-1} for umbrella sedge.

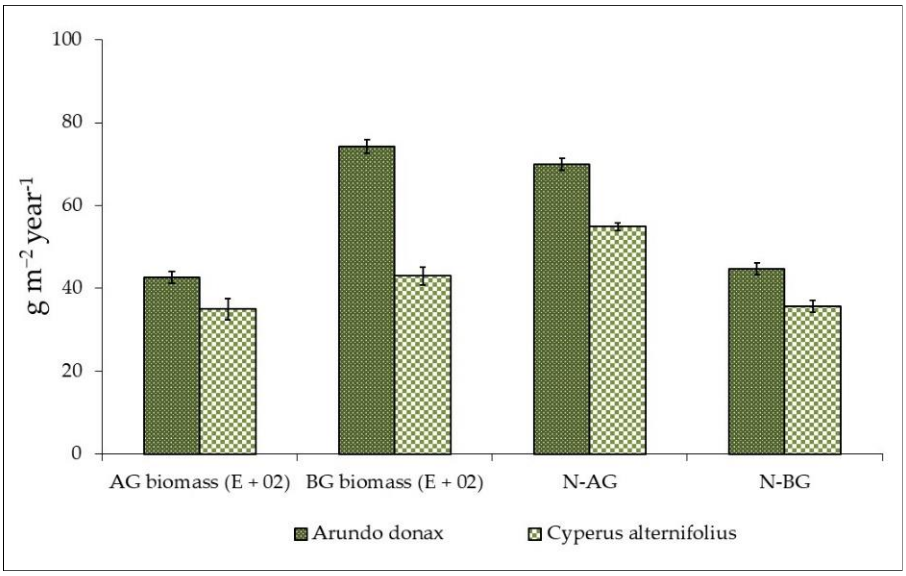


Figure 8. Above-ground (AG) and below-ground (BG) biomass and nitrogen content (N) of giant reed and umbrella sedge. Bars indicate standard error of the means.

Observing the results, both species accumulated more N in the aerial parts than roots and this was consistent with other studies [24,69,70]. These results demonstrate that plants show good potential for N uptake from DWW in a CW, despite the fact that their N removal performance is generally found to be lower than that of microorganism removal and ranged from 0.50% to 40.00% of the TN removal [40,71,72]. However, it is worth noting that greater or lesser ability of plants to produce high biomass yield and remove nutrients from wastewaters depends heavily on various factors, such as morphological characteristics of plants, plant age, growing season, environmental conditions, CW configuration, type of wastewater and loading ranges [73,74].

When comparing the two species in this study, giant reed performed better than umbrella sedge in terms of N uptake due to greater plant biomass production over the two years and probably better adaptation to the environmental conditions of the HSSFs CW area. The high performance of giant reed was also confirmed by previous studies [75–77] conducted under different climate conditions, which highlight the fact that giant reed is one of the most high-yielding biomass species [78,79], despite being relatively underutilized in CWs [79]. On the contrary, the performance of umbrella sedge was much lower than that obtained in tropical and subtropical areas [80–82], where this species is commonly used in CWs.

In this study, heavy metal content in the plant biomass was not determined due to lower average levels in DWW.

3.4. *Effect of Plant Growth on BOD_5 and COD RE*

In Figure 9, the average BOD_5 and COD concentrations at different dates in the two planted units are shown.

Observing the trend of BOD_5 and COD concentration values at the outlet of the two planted units in both years, it is possible to note that the lowest values were obtained during summer months while the highest values were found during autumn and the beginning of spring.

Seasonal variations in RE of organic pollutants contained in DWW was recorded in the HSSFs CW. This phenomenon is due to a number of factors, however, the effect of vegetation on pollutant RE seems to be one the most important. Vegetation, in fact, affects organic pollutant RE in a CW due to plant growth which differs during the seasons, depending on environmental conditions.

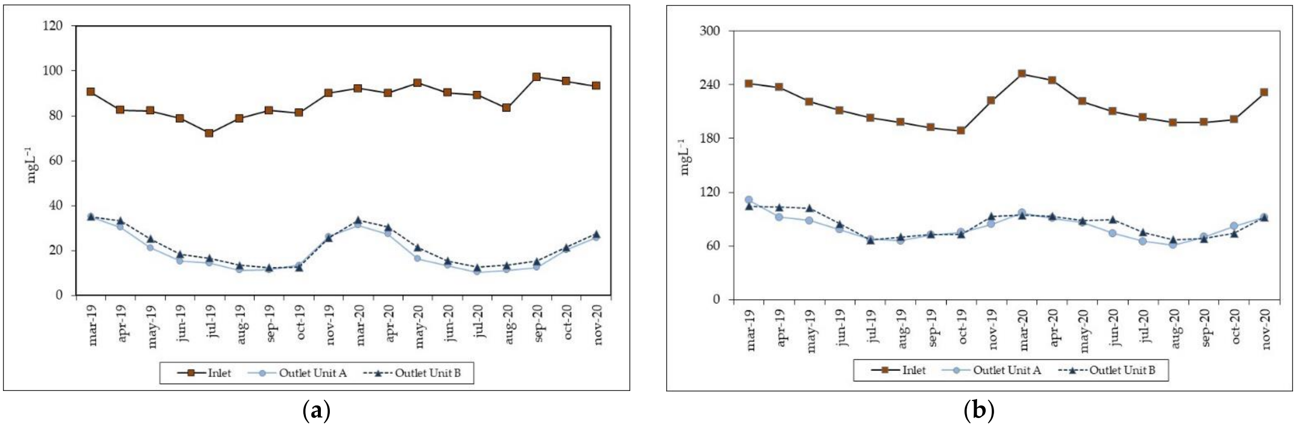


Figure 9. Times series charts for BOD_5 and COD removal with influent and effluent concentrations in the two planted units; (**a**) refers to BOD_5 while (**b**) refers to COD.

In both planted units, the correlations (Figure 10) between plant growth and organic pollutants RE were positive and allow to say that as plant growth increases, the removal efficiency of organic pollutant also increases.

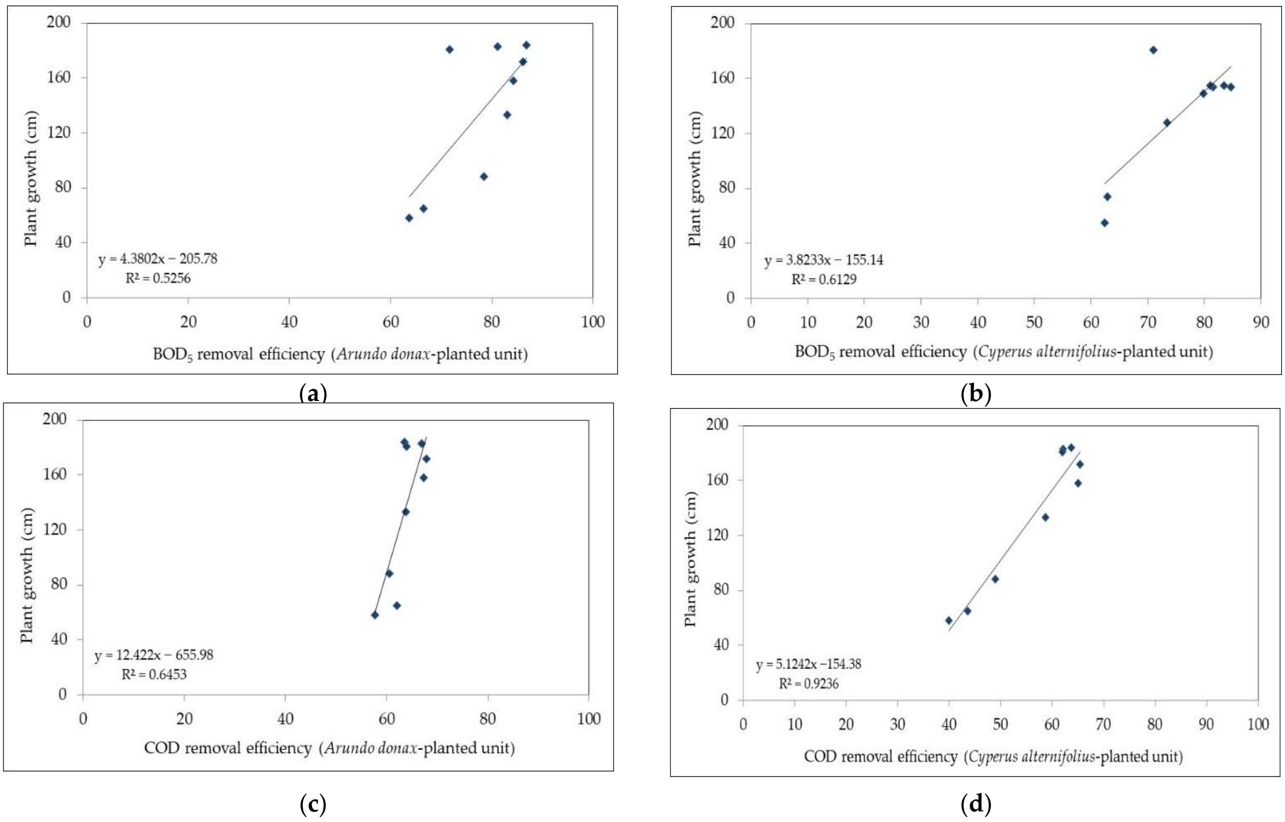


Figure 10. Correlations between plant growth and organic pollutant RE based on concentrations. (**a**) Refers to BOD_5 RE of giant reed-planted unit; (**b**) refers to BOD_5 RE of umbrella sedge-planted unit; (**c**) refers to COD RE of giant reed-planted unit; (**d**) refers to COD RE of umbrella sedge-planted unit.

This aspect can be explained by considering also the interaction between plant and microorganisms in the substrate. As well-known in literature [32,40,66], vegetation provides surface areas for microbial growth and transports oxygen from the leaves to the roots and from the roots to rhizosphere, where it is exploited by bacteria to carry out the oxidation of organic compounds. Thus, vegetation increases the dissolved oxygen concentration in the rhizosphere and makes a contribution to the degradation of organic compounds.

However, the release of oxygen by roots in terms of rate is not the same during the months, being high in spring and summer due to intense plant growth and low in winter due to senescence. Consequently, in spring/summer, when plants grow fast due to favorable climate conditions, the oxidation of organic compounds by aerobic microorganisms is usually documented to be higher than in other seasons because of a greater level of oxygen in the root zone

In the case of DWW, it contains easily biodegradable organic substances [32]. Therefore, we can assume that seasonal variations in RE of these substances can be expected if this type of wastewater is treated by HSSFs CW.

Our results were confirmed by a number of studies. In Japan, in a study [83] carried out in a hybrid CWs for milking parlor wastewater treatment, removal rates for TSS, COD, TN, total carbon and total coliform were found to increase during warm periods, however, the system also performed well during the cold period. In Vermont (USA), three hybrid CWs planted with *Schoenoplectus fluviatilis* were used to treat DWW showing higher performance during peak vegetation growth [54]. In Portugal, in a HSSFs CW planted with *Phragmites australis*, removal efficiencies exhibited seasonal trends for N and P compounds and higher N removal rates were recorded during the warm period due to more intense plant growth [84]. In China, the authors investigated how plants and air temperature affected CWs performance and found that removal efficiency of NH_4, NO_3, TN and TP decreased using polyculture systems and with the decline in temperature [85]. Similar results were obtained by other authors in China [86]. In Kentucky (USA), in 12 subsurface flow wetlands planted with various aquatic species and used for the treatment of domestic wastewater, it was observed that not only did the planted units perform better during the warmer months, but that a polyculture system provided more consistent treatment of various pollutants and was less susceptible to seasonal variation than a monoculture system [87].

The results of these studies confirm seasonal variations in RE of organic and mineral pollutants in CWs and highlight the significant effect of vegetation.

4. Conclusions

The results of this study confirm the efficiency of CWs for DWW treatment. The pilot HSSFs CW led to a significant improvement in the chemical and microbiological quality of combined dairy and domestic wastewater. In particular, RE values were high for BOD_5, COD, TSS and all microbiological parameters. This was also due to efficient pretreatment of the wastewater using biological technologies which were greatly efficient in removing organic compounds. On the other hand, TN, TP and *Escherichia coli* levels did not meet the threshold values in a constant manner required by Italian Legislative Decree 156/2006 concerning the discharge of treated wastewater into the soil. The role of vegetation was essential in DWW removal processes as plants affect the activity of microorganisms through the release of oxygen in the root zone. *Arundo donax* was more suitable for removal of nitrogen than *Cyperus alternifolius* and produced greater levels of plant biomass. Despite differing performances, both planted units showed seasonal variations in RE of organic pollutants, probably due to diverse intensity of plant growth over the course of the year. Thus, the removal of organic compounds was found to increase during warm periods and decrease in cold periods. This aspect should be taken into consideration as DWW treatment using CWs needs to be highly efficient throughout all seasons in order to prevent environmental pollution. It is possible to affirm that a combination of various CW systems and the use of a polyculture system with warm and cold-season species could lead to improvements in the treatment performance of DWW and to obtaining constantly high pollutant RE values. Further studies should be carried out focusing on the technical benefits of these solutions regarding the treatment of DWW on dairy farms.

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