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Evaluation of the performance and space requirement by three different hybrid constructed wetlands in a stack arrangement

This is the submitted version (pre peer-review, preprint) of the following publication:

*Published Version:*

Evaluation of the performance and space requirement by three different hybrid constructed wetlands in a stack arrangement / Maribel Zapater-Pereyra, Huma Ilyas, Stevo Lavrnić, J.A. van Bruggen, P.N.L. Lens. - In: ECOLOGICAL ENGINEERING. - ISSN 0925-8574. - ELETTRONICO. - 82:September 2015(2015), pp. 290-300. [10.1016/j.ecoleng.2015.04.097]

*Availability:*

This version is available at: <https://hdl.handle.net/11585/765927> since: 2024-02-05

*Published:*

DOI: <http://doi.org/10.1016/j.ecoleng.2015.04.097>

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(Article begins on next page)

Manuscript Number:

Title: EVALUATION OF THE PERFORMANCE AND THE SPACE NEEDED BY THREE DIFFERENT HYBRID CONSTRUCTED WETLANDS IN A STACK ARRANGEMENT

Article Type: Research Paper

Keywords: Constructed wetland, footprint, nutrients, organic matter

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**Abstract:** Lack of space for a wastewater treatment plant is a common problem in many areas, especially in highly dense cities. There is a large interest for developing green and compact technology that is capable of efficiently treating domestic wastewater. Constructed wetlands (CWs) are efficient natural systems; however they require large areas for an appropriate wastewater treatment. The aim of this study is the development of a compact CW design for the treatment of domestic wastewater, called the Duplex-CW: a hybrid system with a stacked design (vertical flow CW -VFCW - on top of a horizontal flow filter - HFF -) that allows keeping a large volume of media without increasing the required area. The performance of three different configurations of Duplex-CW, called Fill and drain, Stagnant batch and Free drain, was compared. The VFCWs operated differently with the intention of creating different oxygen conditions, whereas the HFFs were operated identically. The CWs were subjected to three different wastewater strengths, corresponding to designs of 7.9, 3.4 and 2.6 m<sup>2</sup> PE-1. The highest strength wastewater was treated with and without artificial aeration of the VFCW of each configuration. The contribution to the total removal of each compartment (VFCW and HFF), the effects of the use of artificial aeration, the solids accumulation, above- and below-ground biomass and the footprint requirements of the three configurations tested were determined. The Fill and Drain configuration performed better than the other two, being the VFCW compartment more active in the treatment than the HFF. It achieved an area of 2.6 m<sup>2</sup> PE-1 and it needed 2-3 times lower area than what a single VFCW would have needed to reach similar TN effluent concentrations. For other parameters (e.g. COD, TSS and TP), the Duplex-CW did not contributed to the footprint reduction.

The final published version of this article is available online at:

<https://doi.org/10.1016/j.ecoleng.2015.04.097>

Ecological Engineering Volume 82, September 2015, Pages 290-300

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To:  
The Editor  
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**Date**

October 08, 2014

**Subject**

Submission of research paper

Dear Editor,

We are herewith submitting our manuscript entitled **“EVALUATION OF THE PERFORMANCE AND THE SPACE NEEDED BY THREE DIFFERENT HYBRID CONSTRUCTED WETLANDS IN A STACK ARRANGEMENT”**, authored by M. Zapater-Pereyra, H. Ilyas, S. Lavrnić, J.J.A van Bruggen, P.N.L. Lens, for possible publication. The authors of this manuscript have agreed for the submission. The manuscript has been prepared according to the journal format.

This paper is an original submission that studies a novel hybrid constructed wetland (CW), called Duplex-CW, designed in a stack arrangement (two compartments, a vertical flow CW on top of a horizontal flow filter) for the purpose of reducing the footprint needed by CWs. This research has vastly studied three different Duplex-CW configurations treating three different wastewater strengths and has analyzed the performance of the total system and of each compartment. Also, this manuscript includes the application of artificial aeration and a detailed explanation of the (unusual) poor beneficial treatment effect. Insights in area reduction and comparison with other studies are as well included in this manuscript.

We are looking forward to hear from you.

Yours sincerely,

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# Evaluation of the performance and the space needed by three different hybrid constructed wetlands in a stack arrangement

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## ABSTRACT

Lack of space for a wastewater treatment plant is a common problem in many areas, especially in highly dense cities. There is a large interest for developing green and compact technology that is capable of efficiently treating domestic wastewater. Constructed wetlands (CWs) are efficient natural systems; however they require large areas for an appropriate wastewater treatment. The aim of this study is the development of a compact CW design for the treatment of domestic wastewater, called the Duplex-CW: a hybrid system with a stacked design (vertical flow CW - VFCW - on top of a horizontal flow filter - HFF -) that allows keeping a large volume of media without increasing the required area. The performance of three different configurations of Duplex-CW, called Fill and drain, Stagnant batch and Free drain, was compared. The VFCWs operated differently with the intention of creating different oxygen conditions, whereas the HFFs were operated identically. The CWs were subjected to three different wastewater strengths, corresponding to designs of 7.9, 3.4 and 2.6 m<sup>2</sup> PE<sup>-1</sup>. The highest strength wastewater was treated with and without artificial aeration of the VFCW of each configuration. The contribution to the total removal of each compartment (VFCW and HFF), the effects of the use of artificial aeration, the solids accumulation, above- and below-ground biomass and the footprint requirements of the three configurations tested were determined. The Fill and Drain configuration performed better than the other two, being the VFCW compartment more active in the treatment than the HFF. It achieved an area of 2.6 m<sup>2</sup> PE<sup>-1</sup> and it needed 2-3 times lower area than what a single VFCW would have needed to reach similar TN effluent concentrations. For other parameters (e.g. COD, TSS and TP), the Duplex-CW did not contributed to the footprint reduction.

## KEYWORDS

Constructed wetland, footprint, nutrients, organic matter

## 1. INTRODUCTION

Constructed wetlands (CWs) are engineered to mimic natural wetlands and efficiently remove a wide range of pollutants (mainly organic matter) from wastewater. In certain situations, their application is limited because a conventional CW design has the drawback of requiring large land areas to guarantee a good quality treatment (Kivaisi 2001; Ghosh and Gopal 2010; Foladori et al. 2013). This area can even be enlarged if different CW stages are necessary, e.g., a first stage that provides aerobic conditions focussing on organic matter removal/nitrification and a second stage that provides anoxic conditions targeting denitrification. Their space requirements can become a limiting factor in densely populated areas. For developing countries land availability is only partially important; operativity costs are the critical factor. However, if land availability is low, land cost increase and therefore, can become a critical factor as well (Von Sperling 1996). For that reason it is important to design CWs capable of appropriate wastewater treatment but assuring a smaller footprint.

Vertical flow CWs (VFCWs) are generally sized in Europe with  $1\text{--}3\text{ m}^2\text{ PE}^{-1}$  (population equivalent) and horizontal flow CWs (HFCWs) with  $5\text{ m}^2\text{ PE}^{-1}$  (Vymazal 2011). The design depends on factors such as effluent needs, ambient temperatures, technology combinations and use of energy. The area requirement of treatment ponds can vary between  $0.2$  and  $9.4\text{ m}^2\text{ PE}^{-1}$ , and are usually similar as the space taken by CWs (Von Sperling 1996; Mara 2006; Mburu et al. 2013). However, CW possess the aesthetic advantage (beneficial for green infrastructure within a city) and allow shorter retention times as compared to treatment ponds, though CWs costs are

more elevated than those needed for treatment ponds. If land area requirement is the main factor that decides the selection of a suitable wastewater treatment system, other technologies such as activated sludge ( $0.2\text{-}0.4\text{ m}^2\text{ PE}^{-1}$ ), trickling filters ( $0.3\text{-}0.7\text{ m}^2\text{ PE}^{-1}$ ) or upflow anaerobic sludge blanket reactors ( $0.05\text{-}0.4\text{ m}^2\text{ PE}^{-1}$ ) (von Sperling 1996; Mburu et al. 2013) can become the foremost option.

This study aimed to develop a novel CW setup, called Duplex-CW, which can be used when land availability is scarce. A Duplex-CW is a hybrid system that combines two compartments: a VFCW and a horizontal flow filter (HFF). Hybrid CWs can provide a better effluent quality but can even enlarge the land requirement (Foladori et al. 2012). However, placing the systems on top of each other (stack design) can considerably reduce the area needed and can enhance the efficiency of the system per unit of area. In the Duplex-CW, the VFCW is placed on top of the HFF and the wastewater path follows that sequence. In this study, three Duplex-CW configurations were tested, with different operation conditions (i.e. feeding regime and hydraulic retention time -HRT-) applied to the VFCWs in order to create different oxygen conditions, while the HFFs were operated similarly. The specific design considerations of the Duplex-CW are not defined and therefore the objectives of this research were: (i) to assess the differences among three different Duplex-CW configurations subjected to different domestic wastewater types, (ii) to select the most appropriate configuration for the Duplex-CW that can reduce the area requirements without deteriorating the effluent quality, (iii) to evaluate the need of (intermittent) artificial aeration in the Duplex-CW design and (iv) to compare the Duplex-CW land requirements with other CWs.

## 2. MATERIALS AND METHODS

### 2.1 Experimental set-up

Three laboratory scale Duplex-CWs, planted with *Phragmites australis*, were evaluated in this study. The support medium was fine sand (1-2 mm) and the drainage layer consisted of gravel (15-30 mm). Each Duplex-CW had a surface area of 0.24 m<sup>2</sup>, while the depths were 0.80 m (0.70 m of sand and 0.10 m of drainage layer) for the VFCW and 0.35 m (only sand) for the HFF (Fig. 1). To provide artificial (active) aeration to the VFCWs, perforated horizontal pipes were placed between the sand and gravel layer. The systems were operated in a greenhouse under controlled temperature (20-23 °C) and light intensity (85-100 μmol photons m<sup>-2</sup> sec<sup>-1</sup> for 16 h d<sup>-1</sup>).

< Insert Figure 1 >

The wastewater was applied intermittently, by means of a peristaltic pump, on top of the VFCW by means of a pipe manifold, twice per week (three batches of 13 L each day, batch interval of 6 h) corresponding to a hydraulic loading rate (HLR) of ~ 0.046 m<sup>3</sup> m<sup>-2</sup> d<sup>-1</sup>. The wastewater used was primary effluent from Harnaschpolder domestic wastewater treatment plant (Delft, The Netherlands) that was allowed to settle for approximately 2 h before its use. The physical and chemical characteristics of the settled wastewater are given in Table 1. This wastewater was applied during a 2-months start-up/adaptation period (previous to the experiments).

< Insert Table 1 >

The three configurations of the Duplex-CW were named fill and drain (Fill&D), stagnant batch (StagB) and free drain (FreeD), following the different functioning modes of their VFCWs (Fig. 2). In the Fill&D system, three batches of wastewater were added while the outlet valve was closed. After 1 d, the valve was opened and water drained into the HFF (Fig. 2A). In the StagB system, an elbow joint (17 cm height) was installed at the outlet of the VFCW to retain 1.25 batch (16.25 L) of wastewater (stagnant wastewater) (Fig. 2B). The time between two consecutive batches was ~ 6 h within a feeding day and 3-4 d between the last batch and the first batch of two consecutive feeding days, therefore the HRT in this configuration varied between 6 h and 4 d (Fig. 2B). In the FreeD system, the outlet (valve) of the VFCW was permanently open enabling the water to directly discharge to the HFF in ~ 1.5 h (Fig. 2C). The HFF of all configurations worked similarly and had a HRT of 3-4 d.

< Insert Figure 2 >

The variation in operational characteristics of each of the VFCW types were done with the intention of creating different oxygen conditions: (i) Fill&D, the resting period in between feeding days assured an aerobic bed that facilitated aerobic processes when the wastewater was introduced; (ii) StagB, the permanent saturated bottom layer (stagnant batch) and the unsaturated top layer kept within the VFCW, created both anoxic-anaerobic and aerobic zones, and (iii) FreeD, the wastewater trickling along the depth assured permanent aerobic conditions in the VFCW bed.

## **2.2 Experimental design**



Four experimental periods were tested in all Duplex-CWs (Table 2). In the first three periods, the performance of the Duplex-CW configurations was tested using three different domestic wastewater strengths for a total period of 21 weeks (Table 2). From here onwards, the wastewater types will be indicated with WW, WW<sup>+</sup> and WW<sup>++</sup> from low to high strength wastewater, respectively. The WW type consisted of the primary settled wastewater (~330 mg COD L<sup>-1</sup>, Table 1); for WW<sup>+</sup> and WW<sup>++</sup>, peptone was added to increase the strength and reach COD concentrations of ~600 (0.3 g peptone L<sup>-1</sup>) and ~800 (0.5 g peptone L<sup>-1</sup>) mg L<sup>-1</sup>, respectively.

< Insert Table 2 >

In the fourth experimental period, artificial aeration was applied to all VFCWs fed with WW<sup>++</sup>, for a period of 4 weeks (Table 2), in an intermittent mode that started at the moment of the first batch application and lasted for 24 h. The air flow was set to ~ 2 L min<sup>-1</sup> using an air flow meter (Key Instruments, USA). The results of this experiment (WW<sup>++</sup> with aeration, WW<sub>A</sub><sup>++</sup>) were compared with the results obtained with WW<sup>++</sup> (without aeration).

### **2.3 Sample collection and analytical methods**

Wastewater samples were collected weekly at the inlet and outlet of each compartment of the Duplex-CW during the four experimental periods and analyzed according to the procedure outlined in APHA (2005) for pH and dissolved oxygen (DO) by the electrometric methods, COD by the open reflux titrimetric method, TSS by the gravimetric method, TN and TP digestion by the persulfate method followed by measurements of NO<sub>3</sub><sup>-</sup>-N (ultraviolet spectrophotometric

screening method) and  $\text{PO}_4^{3-}\text{-P}$ , respectively. The  $\text{NH}_4^+\text{-N}$  concentration was measured by the dichloroisocyanurate method according to Dutch Standards (NEN 6472, 1983) and,  $\text{NO}_3^-\text{-N}$  by ion chromatography (ICS-1100, DIONEX<sup>TM</sup>, USA). The samples at the outlet of the VFCW were the same as the inlet of the HFF.

Organic matter compounds (i.e. humic-, fulvic- and protein-like) were analyzed by measuring the fluorescence excitation emission matrix (EEM) spectra of samples from the  $\text{WW}^+$ ,  $\text{WW}^{++}$  and  $\text{WW}_A^{++}$  periods (n=1) as described by Zapater-Pereyra et al. (2014) using MATLAB (version R2012b) to identify the compounds in contour maps as peaks of an EEM after correction of the intensities with the DOC dilution factor and after subtraction of the EEM of a blank (Milli-Q water).

When the experimental periods were finalized, the diffusion of oxygen in the VFCWs was quantified by monitoring the effluent DO and temperature of the anoxic water that was added. Fill&D and StagB CWs were emptied before the addition of anoxic water. The anoxic water was prepared in a container by mixing  $\text{CoCl}_2$  (33 mg) and  $\text{Na}_2\text{SO}_3$  (1.4 g) with demineralized water (20 L). The mixture was bubbled with nitrogen gas. The tap of the container was connected to the pipe manifold of the VFCW by means of butyl tubing, and the anoxic water was added. Samples were collected from the outlet of the VFCWs at time zero and at intervals of 10 min for a period of 2 h.

Above-ground biomass was harvested 3 times for each configuration (after sequential periods of 151, 98 and 105 d, n = 3). The dry (105 °C for at least 48 h) biomass content (weight) was

measured. After 435 d of operation (approximately 5.7 months after the  $WW_A^{++}$  experiment finished) the setups were dismantled to quantify the total below-ground dry biomass content (70 °C for at least 48 h,  $n = 1$ ). After the  $WW_A^{++}$  experiment finished, the systems received  $WW^{++}$  for 3.0 months and WW for 2.7 months.

When dismantling the systems, a large sand sample was taken at depths of 0-20, 21-40, 41-60 and 61-70 cm in the VFCWs and at the inlet, middle and outlet in the HFFs. Each sample was properly homogenized and 3 sub-samples (15 mL each  $\approx$  21 g dry weight,  $n = 3$ ) were collected to measure the accumulated solids on the sand. Briefly, each sand sub-sample was mixed with water and sonicated (Soniprep 150, MSE, UK) for 6 min at amplitude of 30 microns. The supernatant (containing the accumulated solids) was filtered with GF/C filters (Whatman<sup>TM</sup>, UK). The water addition, sonication and filtration were done three times per sub-sample, to assure the removal of all accumulated solids. All filters were dried at 105 °C for 24 h and the accumulated solids on each filter were calculated as in the TSS method. The sum of the solids on the three filters per subsample was reported as the total amount of accumulated solids.

## **2.4 Data analysis**

Analysis of variance (ANOVA) followed by Tukey Post Hoc Test for all pairwise multiple comparison ( $\alpha = 0.05$ ) were used to compare: (i) the differences between the WW types in each Duplex-CW configuration compartment per parameter measured, (ii) the differences between the studied configurations of Duplex-CW per parameter measured, (iii) the differences between the configurations' solid accumulation per compartment, (iv) the differences between configurations of Duplex-CW above-ground biomass nutrient concentration per parameter measured (TN and

TP) and (v) the differences between the configurations' above-ground biomass dry weight. Normality assumption and equal variance were tested using the Shapiro-Wilk and Levene Median Test, respectively. If assumptions were not met, values were  $\log_{10}$ -transformed. If the transformation was not useful to meet assumptions, ANOVA on ranks (Kruskal-Wallis) followed by the pairwise comparison using Dunn's Method was conducted. Comparison between the aerated and non-aerated Duplex-CWs was done by applying a two tailed Paired T-test ( $\alpha=0.05$ ). If the normality assumption was not met, the Wilcoxon Signed Rank test was performed. All tests were conducted using SigmaPlot 12.3 software.

### **3. RESULTS**

#### **3.1. Influence of different domestic wastewater strengths on the performance of the VFCW and HFF compartments**

WW. The influent WW had concentrations of  $330 \text{ mg L}^{-1}$  for COD,  $120 \text{ mg L}^{-1}$  for TSS,  $43 \text{ mg L}^{-1}$  for  $\text{NH}_4^+\text{-N}$ ,  $47 \text{ mg L}^{-1}$  for TN and  $9 \text{ mg L}^{-1}$  for TP (Table 1). All configurations tested reached similar removal efficiencies (Table 3) with final effluent concentrations of the same order of magnitude (Figs. 3 and 4). Only  $\text{NH}_4^+\text{-N}$  achieved a better removal in the Fill&D ( $6 \text{ mg L}^{-1}$ ) as compared to the other two configurations ( $12\text{-}13 \text{ mg L}^{-1}$ , Fig. 4).

In the Fill&D configuration, the major treatment location (from this point forward, the "major treatment location" refers to the compartment - VFCW or HFF - that provided most of the treatment) was given by the VFCW. The Fill&D VFCW contributed the most to the removal of all the other parameters (except for TN, Table 3) and to the production of  $\text{NO}_3^-\text{-N}$  (from 0 to  $17 \text{ mg L}^{-1}$ , Fig. 4A). The VFCW from the StagB configuration as well contributed the most to the

removal of almost all parameters, except for TP (Table 3). In this system there was no increment of  $\text{NO}_3^-$ -N in any compartment (Fig. 4B), despite that the  $\text{NH}_4^+$ -N and TN concentrations decreased (Figs. 4E and 4H). On the contrary, the HFF compartment was more active in the FreeD configuration (Table 3), except for  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N that were highly removed and produced, respectively, in the VFCW.

< Insert Table 3 >

< Insert Figure 3 >

< Insert Figure 4 >

$\text{WW}^+$  and  $\text{WW}^{++}$ . The composition of the  $\text{WW}^+$  and  $\text{WW}^{++}$  differed from the original WW only for COD and TN, whereas  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations were similar (Figs. 3 and 4). Both  $\text{WW}^+$  and  $\text{WW}^{++}$  contained, for most of the cases, the three investigated organic matter compounds (humic-, fulvic- and protein-like), and their peak intensities were higher in the  $\text{WW}^{++}$  (Table 4).

< Insert Table 4 >

For all configurations tested, the increase in wastewater strength deteriorated the COD,  $\text{NH}_4^+$ -N, TN and TP effluent quality of each compartment, while this was not so evident for the  $\text{NO}_3^-$ -N and TSS concentrations. Statistical comparison (per compartment, per configuration) showed significant differences between the three wastewater types for the majority of the tested parameters (Figs. 3 and 4).

Despite the increment in the wastewater strength, the location of the majority of the treatment provided by the Fill&D Duplex-CW remained identical to that during the treatment of WW (in the VFCW). However, the treatment location reversed in a few cases for the StagB ( $WW^{++}$ ,  $NH_4^+-N$  and TP) and FreeD ( $WW^+$ , TP;  $WW^{++}$ , COD and TP) systems as compared to that encountered when treating WW (Table 3).

In the Fill&D configuration, the TSS effluent concentration remained similar for all wastewater types and at each compartment (Fig. 3D), while for the other parameters some variations (in at least one compartment) were observed. Briefly, the COD effluent concentration and the organic compounds removal per compartment were affected mainly when treating  $WW^{++}$  (Figs. 3A and 4D, Table 4). During the  $WW^+$  period, the fulvic-like and protein-like compounds were completely removed (100%) after the treatment of only the VFCW compartment. Humic-like compounds were never fully removed from  $WW^+$  and  $WW^{++}$ .

The nutrients removal by the Fill&D system was also affected by the increment in the wastewater strength. The TP concentration in the VFCW effluent maintained constant in the three wastewater types tested, but the HFF effluent concentration increased with the increase in strength despite the unchanged influent TP concentration (Fig. 4J). For  $NH_4^+-N$  and TN (Figs. 4D and 4G), the higher the wastewater strength the higher the VFCW and HFF effluent concentration. For  $NO_3^--N$  (Fig. 4A), the same occurred after the VFCW compartment and once in the HFF, this parameter strongly decreased. Moreover, the HFF removed further  $NO_3^--N$  and TN (from the VFCW effluent concentration) but not  $NH_4^+-N$ .

269

270 The use of  $WW^+$  and  $WW^{++}$  in the StagB configuration highly affected its performance when  
271 removing TSS,  $NH_4^+$ -N and TN (Table 3) finding effluent concentrations of more than double as  
272 compared to that when treating WW (Figs. 3 and 4). The overall removal efficiency of COD was  
273 not affected (Table 3), but the effluent concentrations of each compartment highly increased. TP  
274 and  $NO_3^-$ -N remained unaffected ( $p>0.05$ , Fig. 4B). Similar to the Fill&D configuration, the peak  
275 intensity of the humic-like compounds were not totally removed (up to 64%). However, with this  
276 system both fulvic- and protein-like compounds were 100% removed in both cases ( $WW^+$  and  
277  $WW^{++}$ ). The major treatment location remained identical during the  $WW^+$  period as compared to  
278 the WW period. However, during the  $WW^{++}$  period, the  $NH_4^+$ -N and TP treatment location  
279 reversed to the HFF and VFCW, respectively (Table 3).

280

281 The removal of all parameters shown in Table 3 greatly declined in the FreeD configuration. Its  
282 final effluent concentrations using  $WW^+$  and  $WW^{++}$  were higher than those from the StagB and  
283 Fill&D Duplex-CW for COD, TSS and TP. The  $NH_4^+$ -N and TN concentrations were higher than  
284 those from the Fill&D, but similar to those from the StagB. The  $NO_3^-$ -N final effluent  
285 concentration was similar in the three configurations and the behaviour in the system was similar  
286 to that in the Fill&D configuration (Fig. 4C). This configuration was only able to completely  
287 remove the protein-like organic compounds when using  $WW^+$ . The other organic compounds in  
288 both types of wastewater were not totally removed. The major treatment location shifted in many  
289 cases from the HFF to the VFCW, except for  $NH_4^+$ -N (the VFCW) and TN (the HFF) that  
290 remained unchanged despite the higher wastewater strengths.

291

### 3.2. Effect of artificial aeration on the treatment of WW<sup>++</sup>

For almost all the cases, the use of artificial aeration did not provide statistical differences at a certain compartment ( $p > 0.05$ , Figs. 3 and 4, presence of \* symbol indicates significant difference). No statistics were conducted for the organic matter compounds due to the sample size ( $n=1$ ), however it is visible that the use of artificial aeration enhanced the removal of the organic matter compounds in all configurations investigated (Table 4). The protein-like compounds were the only observed peak in all Duplex-CW configurations that was consistently reduced to 100% when aeration was provided (Table 4). In contrast, when aeration was not applied, the total removal of protein-like compounds did not always occur. For all cases, humic-like compounds showed higher peak intensity as compared to the peaks of other compounds, despite the presence or absence of artificial aeration.

No variations were observed in the major treatment location for almost all cases. In other words, contaminants that were removed mainly in the VFCW or HFF during the WW<sup>++</sup> period, were also removed in that compartment during the WW<sub>A</sub><sup>++</sup> period, except for NH<sub>4</sub><sup>+</sup>-N in the StagB and TSS in the FreeD configuration (Table 3).

### 3.3. Nutrient uptake, solids accumulation on the sand and plant biomass

The accumulated solids on the sand (expressed per volume of sand in Fig. 5 and by the total mass in Table 5) of the Fill&D HFF was lower from that in the VFCW. In the StagB and FreeD systems, it was evident that the HFF showed higher solids accumulation per volume of sand (2-8 mg<sub>solids</sub> mL<sup>-1</sup><sub>sand</sub>) than that on the VFCW (1-4 mg<sub>solids</sub> mL<sup>-1</sup><sub>sand</sub>) (Fig. 5). In terms of total mass, the VFCW and HFF from the StagB had a similar solids accumulation (353 and 325 g,



respectively) while for the FreeD, the solids accumulated in the HFF (454 g) were much higher than those accumulated in the VFCW (305 g) (Table 5). There were significant differences ( $p < 0.05$ ) among the VFCW and as well among the HFF compartments of the 3 systems, with the Fill&D system being significantly different than the FreeD setup in both compartments and the StagB setup in only the HFF (Fig. 5). The solids accumulation rate of the Fill&D HFF compartment was less than half than in all other compartments (Table 5).

< Insert Figure 5 >

< Insert Table 5 >

For each configuration, the amount of accumulated solids expected to be on the sand was calculated using the amount of TSS in and out each compartment and was compared to that measured (Table 5). For the VFCW compartments, the calculated accumulated solids were in the same order of magnitude to the values measured. On the contrary, the measured values for HFFs were much higher (3-7 times more) than those provided by the TSS load (Table 5).

The above-ground biomass and nutrient (nitrogen and phosphorus) uptake was similar for all configurations tested ( $p > 0.05$ , Table 6). The average contribution for nutrient uptake was between 3-5% for TN and 4-5% for TP of the influent. Below-ground biomass and nutrient uptake showed slight differences among the 3 configurations, however values were in the same order of magnitude (Table 6). The nutrient uptake (from the influent) contribution by below-ground biomass was 0.4-1.0%.

< Insert Table 6 >

### 3.4. VFCW oxygen diffusion experiment

After the addition of the anoxic water ( $\text{DO}_{\text{mean}} = 0.9 \text{ mg L}^{-1}$ ) to the 3 VFCWs, the DO levels started to increase to  $> 5 \text{ mg L}^{-1}$ . The FreeD reached its maximum DO value in 10 min and then maintained at similar levels for 1.5 h, while the Fill&D and StagB VFCWs required 20 min to reach the maximum DO value and immediately decreased back to levels of  $\sim 3 \text{ mgO}_2 \text{ L}^{-1}$  (Fig. 6).

< Insert Figure 6 >

## 4. DISCUSSION

### 4.1. Wastewater strength

Enriching the wastewater with peptone highly increased the initial COD concentration (from  $\sim 330$  to  $\sim 600\text{-}800 \text{ mg L}^{-1}$ ) but did not create additional solids (Fig. 3). The similar TSS and COD effluent concentration trend, per configuration, suggested that the added dissolved organic matter was degraded first (easily biodegradable) and the particulate organic matter remained (more resistant to biodegradation) in the Duplex-CWs. The relatively high DO concentration in the effluent of each compartment ( $> 2 \text{ mg L}^{-1}$ , Fig. 3) during the WW period suggested that the remaining oxic environment in the systems could be capable of treating more polluted wastewater. But, treating enriched wastewater ( $\text{COD} > 600 \text{ mg L}^{-1}$ ,  $\text{TN} > 80 \text{ mg L}^{-1}$ ) lowered the water DO to anoxic levels incapable to provide aerobic degradation as during the WW period

(Figs. 3A, 3B and 3C). This resulted in the deterioration of the COD concentration with the increase of strength, as aerobic COD degradation is more important in CWs than anaerobic degradation.

As the COD removal consumed the DO available (Fig. 3), higher  $\text{NH}_4^+\text{-N}$  effluent concentrations were found in all configurations when treating  $\text{WW}^+$  and  $\text{WW}^{++}$ , as compared to the WW period (Figs. 4D vs. 4E and 4F). Moreover, the use of peptone also affected the  $\text{NH}_4^+\text{-N}$  removal. Peptone is a rich source of organic nitrogen, hence, it increased the TN influent concentration but not the inorganic nitrogen concentration (Fig. 4). It should be noted that in common domestic wastewater, 35-40% of TN is organic nitrogen (Metcalf and Eddy 2003) and in our study we used values in the same order of magnitude (37% for  $\text{WW}^+$  and 50% for  $\text{WW}^{++}$ ). This organic nitrogen was quickly and completely (100%) converted to  $\text{NH}_4^+\text{-N}$  by ammonification (Vymazal 2007), thus increasing the load of  $\text{NH}_4^+\text{-N}$  to the systems. Ammonification was indirectly verified since the sum of inorganic nitrogen effluent concentrations was similar to that of TN for each compartment.

The organic compounds introduced by the use of peptone were protein-like compounds (Table 4), as peptone is made up of chains of amino acids. Hence, changes in the influent concentration of other compounds (carbohydrates and fats) are solely the quality fluctuations of the wastewater. Fulvic- and protein-like organic compounds from  $\text{WW}^+$  were completely removed (100%) in all configurations, while from  $\text{WW}^{++}$ , only the StagB configuration (after the HFF) was able to completely remove both organic compound peaks (Table 4). None could remove humic-like compounds further than 64% (Table 4). It has been reported that the hydrophobicity

of humic substances enhances their resistance to biodegradation (i.e. recalcitrant) (Dignac et al. 2000), while fulvic- (Saar and Weber 1982) and protein-like (Nam and Amy 2008) organic compounds are strongly hydrophilic, thus more prone to degrade easily (i.e. labile). Furthermore, their molecular weight also plays a role in their removal. According to Imai et al. (2002), the molecular weight of a hydrophilic fraction of humic substances is by far smaller than that of the hydrophobic fraction. For instance, Dignac et al. (2000) observed that proteins were the second most removed compound after lipids from wastewater treated in an activated sludge system.

In this study, the StagB was the most anoxic configuration ( $< 1 \text{ mg L}^{-1}$  in both compartments for WW<sup>+</sup> and WW<sup>++</sup>, Fig. 3) and had the lowest oxygen diffusion (Fig. 6). Nevertheless, it showed a better treatment performance of all organic compounds suggesting that adsorption was the main removal mechanism of organic matter removal in this configuration, and plausibly in the others as well.

Overall, the Fill&D system was the less affected configuration by the increment in TN and COD due to the longer HRT in the VFCW (Ghosh and Gopal 2010; Weerakoon et al. 2013). This provided more time for nitrification, as generally the nitrification rate is much slower than the denitrification rate (Verhoeven and Meuleman 1999), and thus more time for aerobic treatment processes as compared to the other systems. Nevertheless, during the first 3 wastewater periods, the effluent concentrations and removal efficiencies of all Duplex-CW configurations are comparable to the conventional hybrid CWs (VF-HF sequence) treating similar wastewater strengths, overviewed in Vymazal (2013). However, the increment in the strength of the wastewater in this study did deteriorate the effluent quality for the majority of the cases.

## **4.2. Contribution of the VFCW and HFF compartments to pollutant removal**

The VFCW compartment of the Fill&D and StagB provided the majority of the treatment for organic matter and solids. The  $\text{NH}_4^+$ -N concentrations in all systems were mainly removed in the VFCW compartment and, for all wastewater periods, Fill&D showed lower  $\text{NH}_4^+$ -N concentrations than the other two systems. The Fill&D and StagB had a longer HRT ( $> 3\text{h}$ ) as compared to the FreeD system ( $\sim 1.5\text{h}$ ). Longer HRTs usually result in better pollutant removal efficiencies (Ghosh and Gopal 2010; Weerakoon et al. 2013). The FreeD system had the higher DO concentration (in all wastewater periods, Fig. 3) and the best water passive oxygenation (Fig. 6). The continuous water movement/drainage towards the HFF (drain causes suction of fresh air) and the long resting periods reoxygenate this compartment fast. But this compartment was highly affected by the short HRT. The  $\sim 1.5\text{h}$  was not enough to provide an appropriate treatment and therefore, biodegradation occurred in the HFF first aerobically (as it came from the VFCW) and then anaerobic-anoxically (when oxygen was depleted) (Fig. 3C). Literature reports experiences with HRTs of above 10 d for an effective treatment (Kadlec and Wallace 2009; Ghosh and Gopal 2010). Organic matter removal ( $\text{BOD}_5$ ) is critical below 1 d HRT and improves until 7.5 d (Reed and Brown 1995 cited in Weerakoon et al. 2013). The FreeD was in the critical range.

The combination of saturated and unsaturated zones in the StagB VFCW contributed to the simultaneous production and elimination of  $\text{NO}_3^-$ -N within the same compartment. In other words, it contributed to the TN removal without a clear need for the use of a HFF. The HFF compartment was mainly necessary after the more aerobic VFCWs (Fill&D and FreeD). The

absence of a saturated layer in the VFCW of the Fill&D and FreeD did not provide sufficient anoxic conditions for denitrification.

The compartment contribution is as well highly influenced by their position in the Duplex-CW design. As the VFCW is the first compartment, it possesses more chances for a higher removal of pollutants. Probably, if the design would have started with a HFF, that would have been the compartment providing the major treatment. However, the design was intended to have a VFCW at the beginning not only for nitrification but, as it is fed intermittently, it would give more chances (in the resting periods) for the mineralization of any accumulated solids due to the aerobic environment as well (Kadlec and Wallace 2009; Molle 2014).

It is important to notice that despite one compartment provided the majority of its removal (for a certain parameter at a certain configuration), the use of the two compartments is still recommended. Both compartments support each other and mainly the second functions as a buffer when the first compartment cannot cope with the pollutant (e.g. due to a sudden wastewater strength increment) (Foladori et al. 2012). This effect is not only visualized in the parameters concentration (Figs. 3 and 4), but in the solids accumulation as well (Fig. 5, Table 5). It is clear that due to the short HRT in the FreeD VFCW, physical processes are mainly removing the solids and the organic matter (Foladori et al. 2012) thus no time for biodegradation and a high load of pollutants was transferred to the HFF.

Hence, it is important to maintain both compartments in the Duplex-CW design. However, for practical reasons, a Duplex-CW should aim for a HFF supporting the VFCW treatment rather

than providing the major treatment. This is recommended as its position in the Duplex-CW is at a complex location (bottom) to provide maintenance if needed. Therefore, high solids accumulation in the HFF media should be avoided, putting the FreeD Duplex-CW in disadvantage compared to the other systems.

### 4.3. Aeration

A close look at the effluent concentration of all parameters suggests that the use of aeration for the treatment of WW<sup>++</sup> improved the water quality (Figs. 3 and 4, Table 4). However, the few statistical differences suggest that the use of artificial aeration did not provide extra benefits in the Duplex-CWs (Figs. 3 and 4, \* symbol). Many studies have claimed that the use of artificial aeration enhances the removal of many pollutants namely COD, NH<sub>4</sub><sup>+</sup>-N and TN (e.g. Dong et al. 2012; Hu et al. 2012; Fan et al. 2013; Foladori et al. 2013; Zapater-Pereyra et al. 2014). However, some studies are conducted in HFCWs where the aeration effect probably plays a major role as oxygen transfer in HFCWs is lower than that in VFCWs (e.g. Fan et al. 2013; Zapater-Pereyra et al. 2014). Others (e.g. Dong et al. 2012) mention the benefits of aeration (without supporting it with statistics) when the actual concentration differences (between aerated and non aerated) are not tremendously different.

For "gently" artificially aerated systems, Kadlec and Wallace (2009) referred to an oxygen delivery of 50-100 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>. In this study, the oxygen demand to treat the WW<sup>++</sup> was 30 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>. The average oxygen transfer rate of 22 VFCWs given by Kadlec and Wallace (2009) is 3.5-24.7 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>. In other words, the oxygen demand of the Duplex-CWs when treating WW<sup>++</sup> was very close to the oxygen transfer rate provided by a non aerated VFCWs. Thus,

aeration was not extremely necessary. Delivering  $100 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  to the Duplex-CWs would imply that the Duplex-CW systems could cope with  $\sim 4000 \text{ mg COD L}^{-1}$  ( $186 \text{ g BOD}_5 \text{ m}^{-2} \text{ d}^{-1}$ ). It should not be immediately assumed, with this calculation, that adding more air to the Duplex-CW would treat completely any concentration of organic matter added. But mainly, this relates to an under loaded system yet not capable to fully trigger the benefits of artificial aeration. For instance, in the case of nitrogen, Hu et al. (2012) mention that artificial aeration "when dealing with high strength wastewater, artificial aeration seems to be the only option to achieve complete nitrification".

Although it was not possible to monitor the accumulation of solids with and without aeration in this study, it is important to note that aerobic conditions enhance faster mineralization of accumulated organic matter in the media (Kadlec and Wallace, 2009). Therefore, benefits of aeration should not only be considered "at the moment" (effluent quality), but as well as a long-term benefit (less solid accumulation and clogging). Furthermore, Kadlec and Wallace (2009) pointed out that from the economic perspective, the use of artificial aeration is worth only when the aeration cost is lower than the reduction in capital cost (e.g. aerated CWs occupy less area, thus lower construction costs than non aerated CWs). In that regard, intermittent aeration, as used in this study (24 h from the feeding time), it is more recommended than continuous aeration (Dong et al. 2012).

#### **4.4. Solids accumulation on the sand**

The approximately TSS loading rate applied was  $5.1 \text{ g TSS m}^{-2} \text{ d}^{-1}$  and the solids accumulation rates for the VFCWs ( $1.1\text{-}1.5 \text{ kg m}^{-2} \text{ y}^{-1}$ ) and HFFs ( $0.5\text{-}1.7 \text{ kg m}^{-2} \text{ y}^{-1}$ ) (Table 5) were within the



lower range reported in other studies ( $0.6\text{-}14.3\text{ kg m}^{-2}\text{ y}^{-1}$ ; e.g. Caselles-Osorio et al. 2007) and lower than Chazarenc et al. (2009) ( $7.2\text{-}13.2\text{ kg m}^{-2}\text{ y}^{-1}$ ). However, those systems were operated over 2 years and the Duplex-CWs in this study for only 1.1 years.

The operational characteristics of the configurations were reflected in the solids accumulated. The short retention time in the FreeD VFCW, as compared to the other configurations investigated, was not enough to allow appropriate sedimentation and filtration of the TSS, hence the solids were transferred to the HFF in a higher amount than in the other configurations.

For the VFCW compartments, the calculated accumulated solids (in g) were in the same order of magnitude of the values measured (Table 5), suggesting soil mineralization to occur, otherwise the values would have been much higher as the accumulated solids contain also biofilm, precipitates and plant litter (Kadlec and Wallace 2009).

On the contrary, the measured values for the HFF were much higher (3-7 times more) than that provided by the TSS load from the wastewater supply (Table 5). The HFF were not planted, therefore plant litter cannot contribute to the accumulated solids. Therefore, despite some literature mentioning that biofilms are not a relevant cause of solids accumulation (e.g. Langergraber et al. 2003), the biofilm seem to play an important role in solids accumulation in the HFF (Baveye et al. 1998; Thullner et al. 2002). Zhao et al. (2009) added glucose and starch to enrich the wastewater fed to different VFCWs as a source of respectively, soluble and particulate organic substrate. The glucose- and starch-fed systems were used to investigate the clogging process due to biofilm growth and to the combination of particle accumulation and

biofilm growth, respectively. Their glucose-fed systems had more accumulated organic matter as compared to the starch-fed systems, suggesting that biofilm growth governs the solids accumulation. Some studies mentioned that biomass occupy 3-8.5% of the initial soil pore volume (Seifert and Engesgaard 2007). Furthermore, in saturated soils (i.e. low DO environments) the biodegradation of organic matter is much lower than in aerobic environments while the biomass production (e.g. polysaccharides) continues. It could have also been that some solids accumulated in the VFCW were resuspended in the solution due to abrasion, e.g. caused by the artificial aeration (Zapater-Pereyra et al. 2014), that were then trapped in the HFF.

#### **4.5. Plant biomass and nutrient uptake**

It was expected that the difference in operation of the VFCWs would have an impact on the plants. However, the above- and below-ground biomass was similar for all configurations (Table 6). Engloner (2009) reviewed the contradictions between many studies about the optimal water depth for the optimal growth of *Phragmites australis*. Thus, it seems that the different water conditions in all VFCWs had the same influence in the plant growth. That can explain the similar nutrient uptake in all systems (Table 6).

The nutrients taken by the plants (0.4-5%), above- and below-ground, were a minor contribution to the nutrient removal in the Duplex-CW as commonly occurring in CWs (Kadlec and Wallace, 2009). Nutrient uptake by plants varies depending on the type of plant, climate and growing stage (Wu et al. 2013). An ample range of nutrient uptake (rates) has been reported by many authors: 77-218 mg N m<sup>-2</sup> d<sup>-1</sup> (Wu et al. 2013), 143-2304 mg N m<sup>-2</sup> d<sup>-1</sup> (Tanner et al. 2005), 1.4-44.4 mg P m<sup>-2</sup> d<sup>-1</sup> (as reviewed in García et al. 2010), 48.6 g N m<sup>-2</sup> and 28.91 g P m<sup>-2</sup> (in

*Pragmites australis*, About-Elela and Hellal 2012), 0.6-250 g N m<sup>-2</sup> and 0.01-45 g P m<sup>-2</sup> (as reviewed in Vymazal 2007), 583 g N m<sup>-2</sup> and 62 g P m<sup>-2</sup> (Lee et al. 2013). The nutrient uptake of above-ground biomass (41-67 g N m<sup>-2</sup> and 7-9 g P m<sup>-2</sup>, Table 6) and consequent removal rates (94-155 mg N m<sup>-2</sup> d<sup>-1</sup> and 17-21 mg P m<sup>-2</sup> d<sup>-1</sup>) were within the range of those reported values.

#### **4.6. Duplex-CW footprint reduction and design selection**

The application of WW, WW<sup>+</sup> and WW<sup>++</sup> resulted in a design of 7.9, 3.4 and 2.6 m<sup>2</sup> PE<sup>-1</sup> (Table 2), respectively. When comparing effluent quality from each configuration with the European disposal guidelines (35 mg L<sup>-1</sup> for TSS, 25 mg L<sup>-1</sup> for BOD, 125 mg L<sup>-1</sup> for COD, 15 mg L<sup>-1</sup> for TN and 2 mg L<sup>-1</sup> for TP), it is evident that none of the configurations tested in this study can treat TP to the recommended threshold discharge limits in any of the experimental periods (Steinel and Margane 2011). It is only the Fill&D system that met all the other parameters up to WW<sup>+</sup> (3.4 m<sup>2</sup> PE<sup>-1</sup>), when the other two configurations failed meeting the TN limit for even the WW period.

Foladori et al. (2013) used a VFCW with recirculation and aeration to reduce the area from 3.6 m<sup>2</sup> PE<sup>-1</sup> to 1.5 m<sup>2</sup> PE<sup>-1</sup>. They based their results in guidelines that only consider 125 mg L<sup>-1</sup> for COD, 35 mg L<sup>-1</sup> for TSS and 70% removal efficiency for TN. When applying these specifications to the Duplex-CWs tested, the Fill&D configuration will meet up to the WW<sup>++</sup> level (2.6 m<sup>2</sup> PE<sup>-1</sup>).

Based on this, it is clear that the area achieved by the CW is determined by the guidelines used. Therefore, the CW footprint reduction was also calculated using the first-order kinetic equation

(assuming no background concentration,  $C^* = 0 \text{ mg L}^{-1}$ ). The area obtained for each Duplex-CW configuration (using the final effluent concentration) was compared to that obtained for the VFCW alone (using the VFCW effluent concentration). In that regard, the Fill&D configuration almost did not save any area during the 4 experimental periods for the treatment of COD, TSS,  $\text{NH}_4^+$ -N and TP. However, for TN, the Duplex-CW needed 2-3 times less space than that needed to reach the same effluent concentration using only a VFCW. Similar to the area reduction for TN found by Zapater-Pereyra et al. (2014) when comparing a control HFCW with a hybrid HFCW. Thus, the use of the Fill&D Duplex-CW served to reduce the systems area only for TN.

Commonly, VFCWs are generally sized in Europe with  $1\text{-}3 \text{ m}^2 \text{ PE}^{-1}$  and HFCWs with  $5 \text{ m}^2 \text{ PE}^{-1}$  (Vymazal 2011) for the removal of organics and TSS, however that design is insufficient for nutrient removal (Babatunde et al. 2008). The Fill&D Duplex-CW area demand is included in the middle of this range. CWs like the French systems (e.g. Molle 2014) and that describe in Foladori et al. (2013) fit in the lower range ( $< 2 \text{ m}^2 \text{ PE}^{-1}$ ). Probably higher wastewater strength would have resulted in smaller Duplex-CW area, as in Foladori et al. (2013) (using  $74 \text{ g COD m}^{-2} \text{ d}^{-1}$ , double than in this study, reached the  $1.5 \text{ m}^2 \text{ PE}^{-1}$ ). For nutrients removal, areas of approximately  $15\text{-}30 \text{ m}^2 \text{ PE}^{-1}$  and  $40\text{-}70 \text{ m}^2 \text{ PE}^{-1}$  are suggested to be necessary to remove nitrogen ( $< 8 \text{ mg L}^{-1}$ ) and phosphorus ( $< 1.5 \text{ mg L}^{-1}$ ), respectively (Schierup et al. 1990, cited in Babatunde et al. 2008).

The operational conditions played a role in the performance, but not in the plant colonization, thus the benefits created by the plants (e.g. nutrient uptake) should not be taken as a reason for the configuration selection. Furthermore, the observed solid accumulation as well suggested that

the Fill&D configuration should be selected as the Duplex-CW as it had time for soil mineralization in the VFCW and not many solids reached the HFF.

## 5. CONCLUSION

- The Fill&D Duplex-CW performed better as compared to the StagB and FreeD systems due to the oxygen operational conditions and the HRT.
- The VFCW compartment contributed the most in the Duplex-CW overall treatment, since it was the first compartment in the Duplex-CW design and it showed high oxygen diffusion. The HFF contributed to further improve the VFCW treatment efficiency when needed.
- Artificial aeration improved effluent concentrations slightly, but not enough to show significant differences. Higher wastewater strength would have enhanced the benefit of artificial aeration.
- Biofilm growth had a major impact in the HFF solids accumulation. The solids provided by the wastewater generated almost all the solids in the VFCW.
- The Fill&D Duplex-CW needed 2-3 times lower area than what a single VFCW would have needed to reach similar TN effluent concentration. For other parameters (e.g. COD, TSS and TP), the Duplex-CW did not contributed to the footprint reduction. The area requirement achieved, with satisfactory effluent quality, was  $2.6-3.4 \text{ m}^2 \text{ PE}^{-1}$ , lower than common European design ( $5 \text{ m}^2 \text{ PE}^{-1}$ ), but still higher than many CWs. Higher wastewater strength would have resulted in smaller Duplex-CW area.
- The overall conclusion is that the preferred system to use is the Fill&D configuration. Both compartments (VFCW and HFF) are relevant in the design; however measures

should be taken to create a more anoxic HFF. Aeration is not needed in the design treating up to the tested wastewater strengths.

## **6. ACKNOWLEDGMENTS**

The authors would like to thank the assistance of the UNESCO-IHE lab staff and of Mr. Berthold Verkleij.

This study was financially supported by and carried out under the UNESCO–IHE Partnership Research Fund (UPaRF) project No. 32019417 NATSYS (Natural Systems for Wastewater Treatment and Reuse: Technology Adaptations and Implementation in Developing Countries), The Netherlands.

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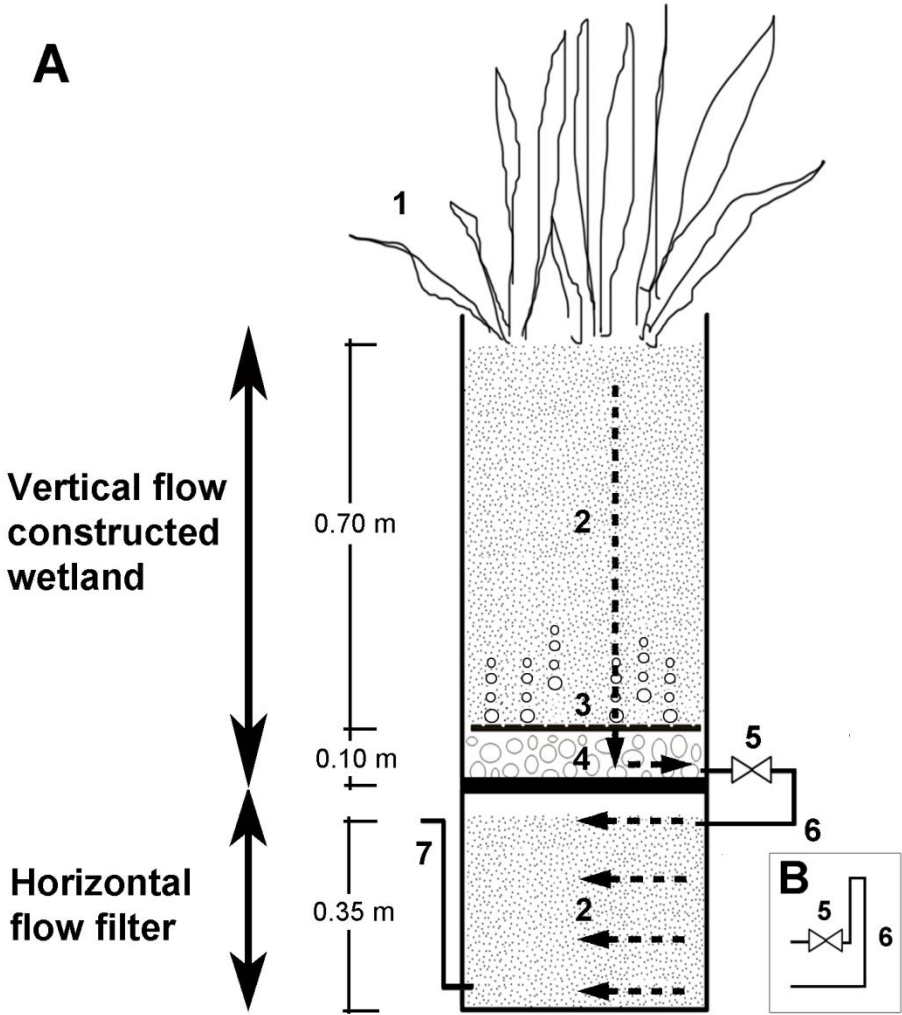
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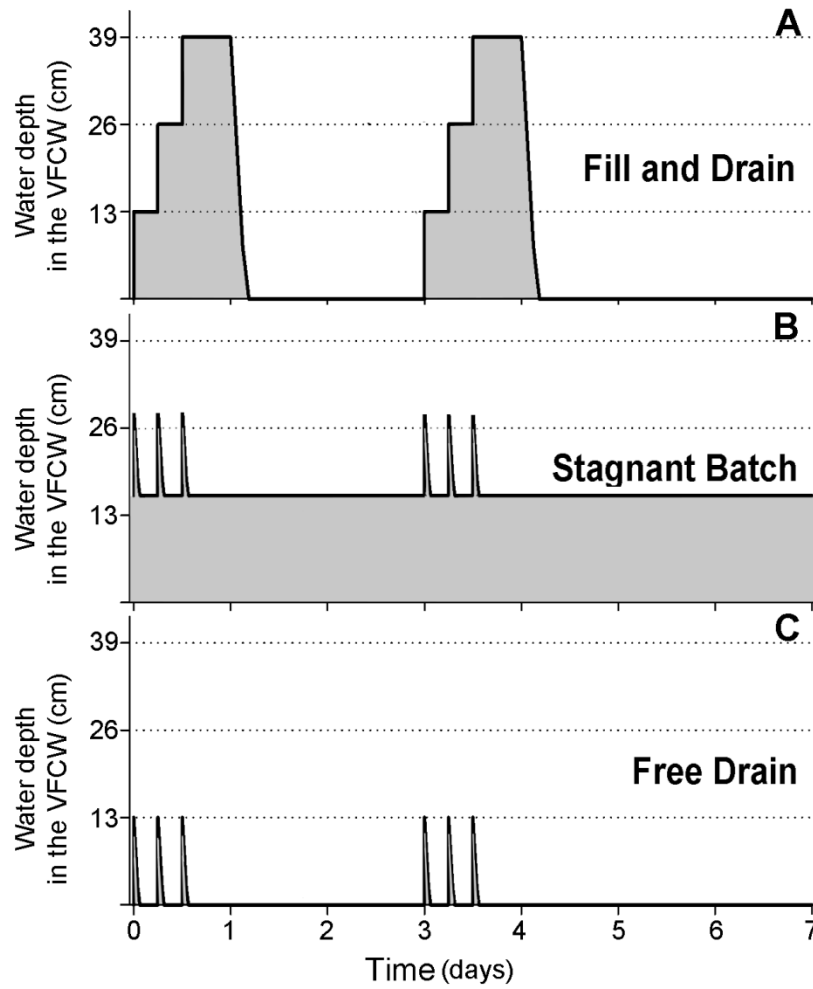
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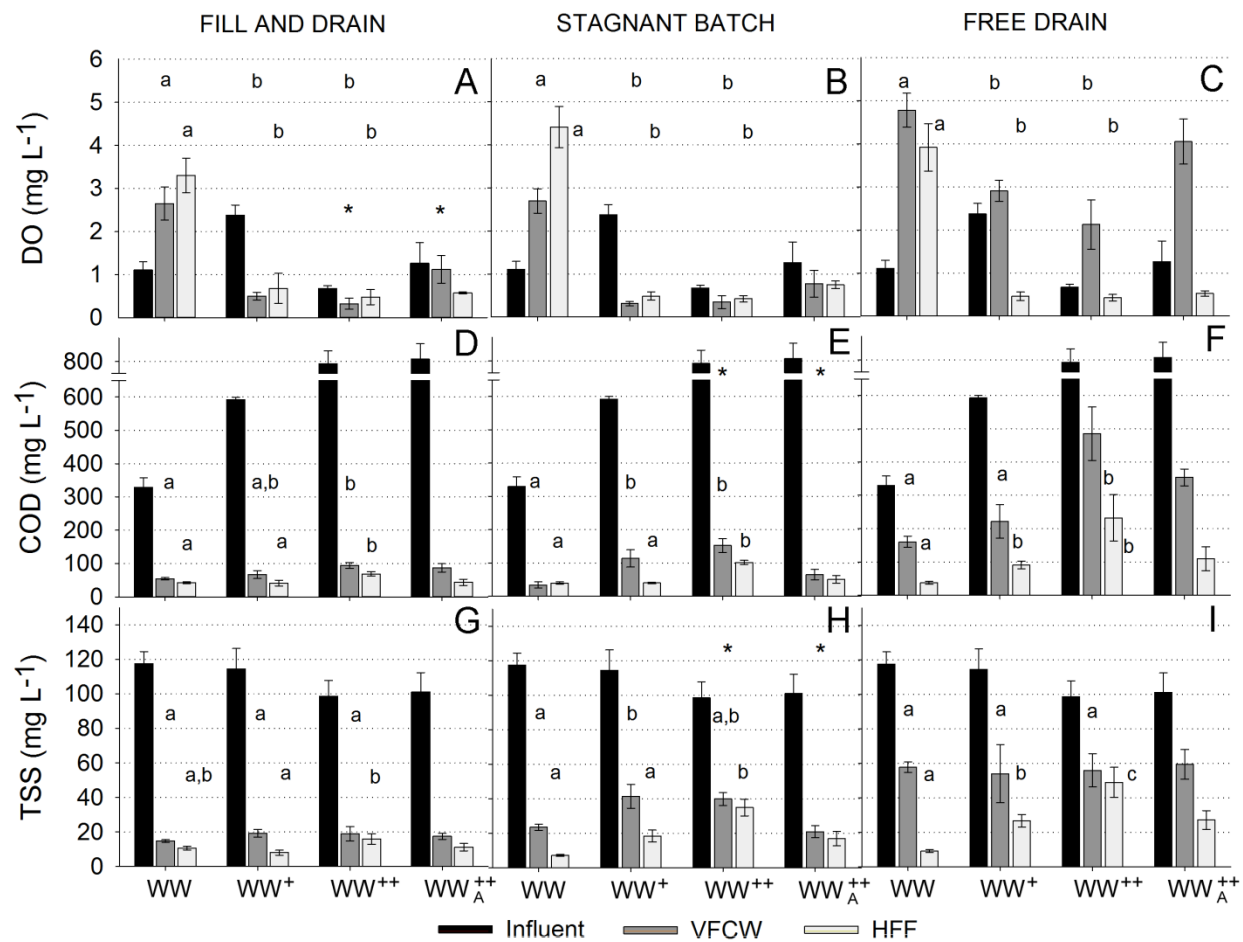


**Figure 1** Schematic representation of the Duplex-constructed wetland configurations (A) used in this study. 1- *Phragmites australis*, 2- Sand (support media), 3- Aeration pipe, 4- Gravel (drainage layer), 5- Valve, 6- Pipe connecting both compartments and 7- Outlet pipe. The dashed line shows the path of the wastewater in the system. The graph on the bottom-right (B) represents the modification (elbow), done for the "Stagnant batch" configuration



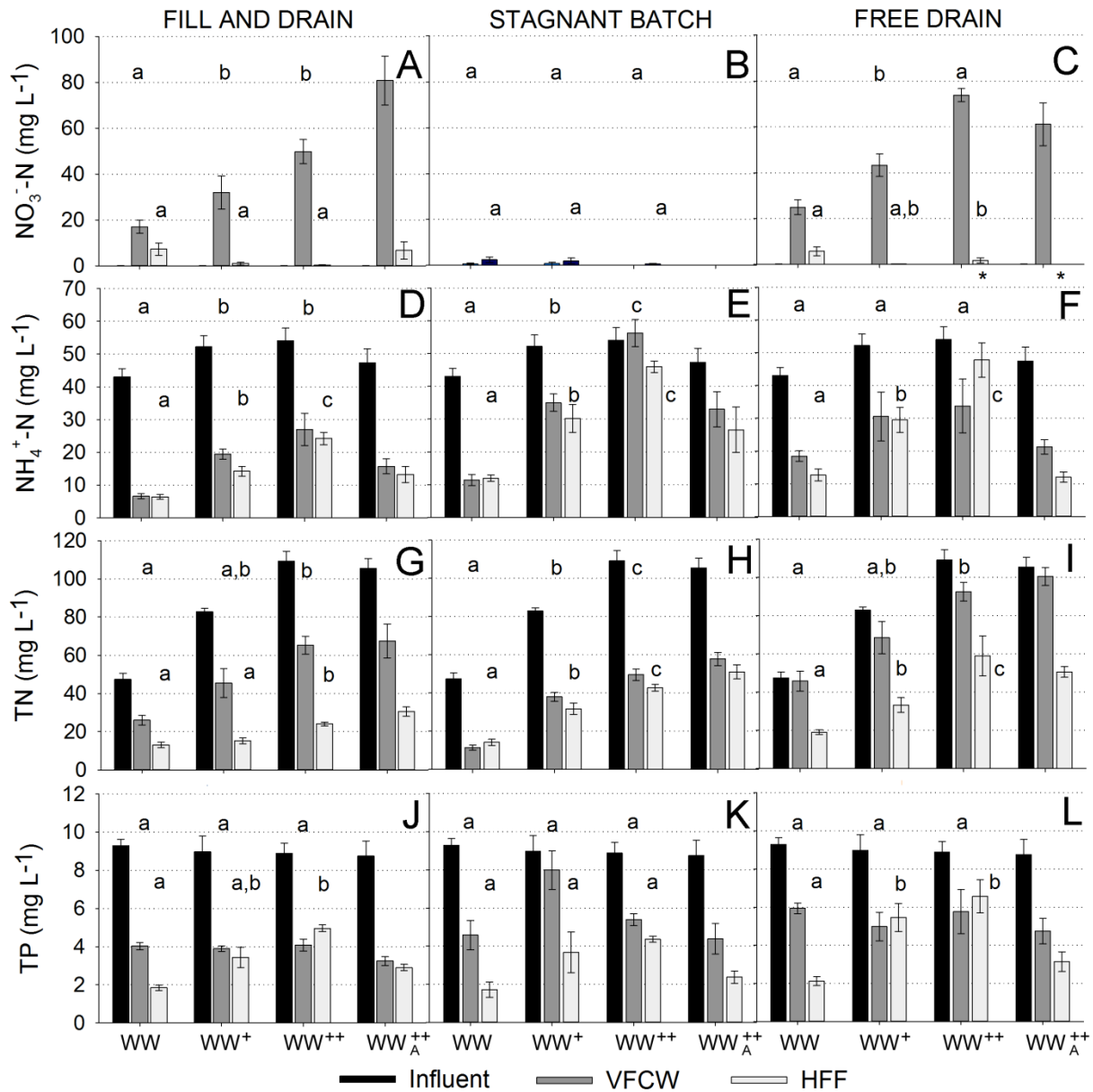
**Figure 2** Weekly hydraulic behaviour of the three Duplex-constructed wetland (CW) configurations used in this study. Each batch of wastewater contained 13 L and had a depth of 13 cm in the vertical flow CW (VFCW)

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**Figure 3** Mean ( $\pm$ SE) dissolved oxygen, organic matter and solids concentrations of influent and effluent of each Duplex-constructed wetland (CW) compartment (vertical flow - VFCW and horizontal flow filter - HFF) during each experimental period using different domestic wastewater (WW) strengths: WW; WW + 0.3 g peptone L<sup>-1</sup>, WW<sup>+</sup>; WW + 0.5 g peptone L<sup>-1</sup>, WW<sup>++</sup> and WW<sup>++</sup> with aeration, WW<sub>A</sub><sup>++</sup>

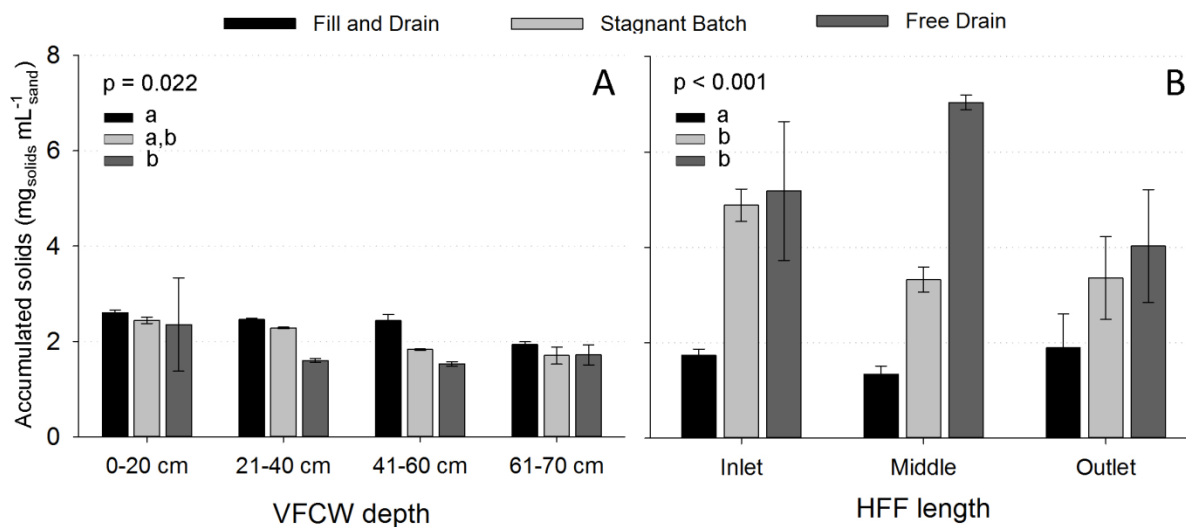
Statistics note: Letters a,b,c for a certain parameter at a certain compartment indicates significant differences within a particular Duplex-CW configuration for the WW, WW<sup>+</sup> and WW<sup>++</sup>. Upper and lower letters in each graph are the statistical results of VFCW and HFF, respectively. / The symbol \* displayed indicate statistical differences for a certain parameter at a certain compartment within a particular Duplex-CW configuration when artificial aeration was applied (WW<sub>A</sub><sup>++</sup>) or not (WW<sup>++</sup>).



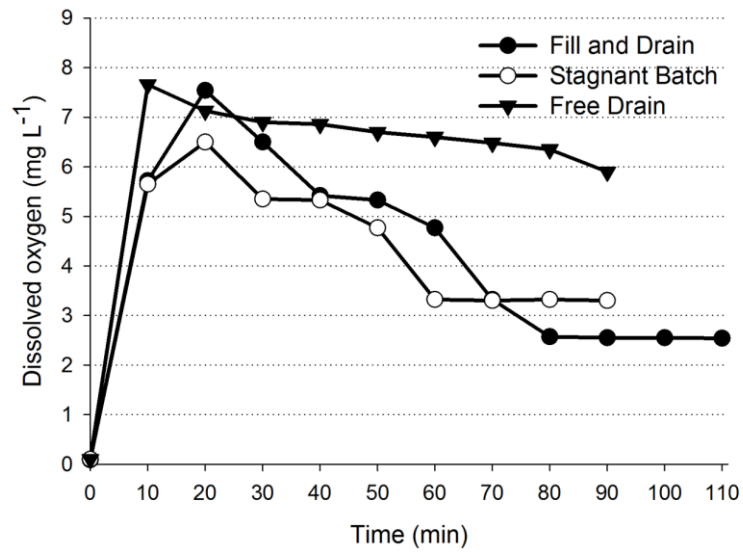
**Figure 4** Mean ( $\pm$ SE) nutrient concentrations of influent and effluent of each Duplex-constructed wetland (CW) compartment (vertical flow - VFCW and horizontal flow filter - HFF) during each experimental period using different domestic wastewater (WW) strengths: WW; WW + 0.3 g peptone L<sup>-1</sup>, WW<sup>+</sup>; WW + 0.5 g peptone L<sup>-1</sup>, WW<sup>++</sup> and WW<sup>++</sup> with aeration, WW<sup>++A</sup>

Statistics note: Letters a,b,c for a certain parameter at a certain compartment indicates significant differences within a particular Duplex-CW configuration for the WW, WW<sup>+</sup> and WW<sup>++</sup>. Upper and lower letters in each graph are the statistical results of VFCW and HFF, respectively. / The symbol \* displayed indicate statistical differences for a certain parameter at a certain compartment within a particular Duplex-CW configuration when artificial aeration was applied (WW<sup>++A</sup>) or not (WW<sup>++</sup>).





**Figure 5** Mean ( $\pm$ SE) solids accumulation on the sand in the vertical flow constructed wetland (VFCW) and horizontal flow filter (HFF) of each duplex-CW configuration. The p-value in each graph (A and B) show the statistical significance among the three configurations and the a,b letter indicate their pairwise multiple comparison along the depth (A) and length (B)



**Figure 6** Dissolved oxygen concentrations as a function of operational time in the three VFCW compartments of the Duplex-CW configurations during the oxygen diffusion experiment

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**Table 1** Average composition of the primary settled domestic wastewater used in this study (n = 9)

Parameters	Unit	Mean ± Standard deviation
pH	-	7.0 ± 0.1
Electrical conductivity	µS cm <sup>-1</sup>	1271 ± 175.3
Dissolved oxygen	mg L <sup>-1</sup>	1.0 ± 0.6
Chemical oxygen demand	mg L <sup>-1</sup>	329 ± 87.2
Total suspended solids	mg L <sup>-1</sup>	118 ± 21
NH <sub>4</sub> <sup>+</sup> -N	mg L <sup>-1</sup>	43 ± 7.5
NO <sub>3</sub> <sup>-</sup> -N	mg L <sup>-1</sup>	0.1 ± 0.1
Total nitrogen	mg L <sup>-1</sup>	47 ± 9.5
Total phosphorus	mg L <sup>-1</sup>	9.0 ± 1.0

**Table 2** Characteristics of the influent used in the different periods of this study

Period	Experiment	Abbreviation	COD (mg L <sup>-1</sup> )	Organic loading rate (gCOD m <sup>-2</sup> d <sup>-1</sup> )	Experimental weeks	Area (m <sup>2</sup> PE <sup>-1</sup> )
1	Wastewater	WW	330	15	9	7.9
2	Wastewater + 0.3 g peptone L <sup>-1</sup>	WW <sup>+</sup>	600	27	4	3.4
3	Wastewater + 0.5 g peptone L <sup>-1</sup>	WW <sup>++</sup>	800	37	4	2.6
4	Wastewater + 0.5 g peptone L <sup>-1</sup> + aeration	WW <sub>A</sub> <sup>++</sup>	800	37	4	2.6

**Table 3** Removal efficiency and compartment where the majority of the treatment occurred in the Duplex-constructed wetland. Sample size of n=9 for the WW period and n=4 for each of the other periods

	WW			WW <sup>+</sup>			WW <sup>++</sup>			WW <sup>++</sup> <sub>A</sub>		
	% rem.	Major treatment location *	% rem.	Major treatment location *	% rem.	Major treatment location *	% rem.	Major treatment location *	% rem.	Major treatment location *	% rem.	Major treatment location *
<b>Fill and Drain</b>												
COD	87	VFCW <sub>69</sub>	93	VFCW <sub>65</sub>	91	VFCW <sub>70</sub>	95	VFCW <sub>70</sub>	95	VFCW <sub>61</sub>		
TSS	91	VFCW <sub>69</sub>	93	VFCW <sub>55</sub>	84	VFCW <sub>70</sub>	89	VFCW <sub>70</sub>	89	VFCW <sub>62</sub>		
NH <sub>4</sub> <sup>+</sup> -N	85	VFCW <sub>82</sub>	73	VFCW <sub>51</sub>	55	VFCW <sub>46</sub>	72	VFCW <sub>46</sub>	72	VFCW <sub>58</sub>		
Total Nitrogen	72	HFF <sub>38</sub>	82	HFF <sub>49</sub>	78	HFF <sub>48</sub>	71	HFF <sub>48</sub>	71	HFF <sub>43</sub>		
Total Phosphorus	80	VFCW <sub>42</sub>	61	VFCW <sub>50</sub>	44	VFCW	66	VFCW	66	VFCW <sub>59</sub>		
<b>Stagnant Batch</b>												
COD	88	VFCW	93	VFCW <sub>52</sub>	87	VFCW <sub>62</sub>	93	VFCW <sub>62</sub>	93	VFCW <sub>75</sub>		
TSS	94	VFCW <sub>50</sub>	84	VFCW <sub>45</sub>	64	VFCW <sub>53</sub>	83	VFCW <sub>53</sub>	83	VFCW <sub>67</sub>		
NH <sub>4</sub> <sup>+</sup> -N	72	VFCW	42	VFCW <sub>30</sub>	15	HFF	44	HFF	44	VFCW <sub>27</sub>		
Total Nitrogen	70	VFCW	61	VFCW <sub>47</sub>	61	VFCW <sub>49</sub>	52	VFCW <sub>49</sub>	52	VFCW <sub>41</sub>		
Total Phosphorus	81	HFF <sub>46</sub>	58	HFF <sub>46</sub>	52	VFCW <sub>33</sub>	74	VFCW <sub>33</sub>	74	VFCW <sub>39</sub>		
<b>Free Drain</b>												
COD	89	HFF <sub>53</sub>	85	VFCW <sub>44</sub>	71	HFF <sub>41</sub>	87	HFF <sub>41</sub>	87	HFF <sub>48</sub>		
TSS	92	HFF <sub>57</sub>	76	VFCW <sub>39</sub>	50	VFCW <sub>39</sub>	74	VFCW <sub>39</sub>	74	HFF <sub>42</sub>		
NH <sub>4</sub> <sup>+</sup> -N	71	VFCW <sub>46</sub>	43	VFCW <sub>40</sub>	12	VFCW	75	VFCW	75	VFCW <sub>42</sub>		
Total Nitrogen	60	HFF <sub>57</sub>	60	HFF <sub>45</sub>	46	HFF <sub>32</sub>	52	HFF <sub>32</sub>	52	HFF <sub>48</sub>		
Total Phosphorus	76	HFF <sub>48</sub>	39	VFCW	27	VFCW	63	VFCW	63	VFCW <sub>37</sub>		

\* Major treatment location refers to the compartment (vertical flow constructed wetland -VFCW- or horizontal flow filter -HFF-) that provided majority of the total removal.

The values shown as subscripts are the percentage removal that the mentioned compartment achieved from the total % removal. For example for Fill and Drain, WW strength, COD: VFCW<sub>69</sub> indicates that 69% of the total COD removed (87%) was removed by the VFCW, the remaining (18%) was removed by the HFF. Therefore the VFCW is the compartment providing the majority of the removal.

The subscript value was not indicated if the concentration of the VFCW and HFF effluent increased as compared to influent and VFCW concentration, respectively (in order to avoid confusions with negative values).

**Table 4** Peak intensity (% reduction of peak intensity from the influent) of humic-like, fulvic-like and protein-like organic matter compounds (n=1) per compartment (vertical flow constructed wetland - VFCW and horizontal flow filter - HFF) of each Duplex-CW when applying WW<sup>+</sup>, WW<sup>++</sup> and WW<sup>++A</sup>

	WW <sup>+</sup>			WW <sup>++</sup>			WW <sup>++A</sup>		
	Influent	VFCW	HFF	Influent	VFCW	HFF	Influent	VFCW	HFF
<b>Fill and Drain</b>									
Humic-like	8.3	4.3 (48)	3.5 (58)	10.9	6.8 (38)	4.9 (55)	14.6	6.6 (55)	5.5 (62)
Fulvic-like	10.2	0.0 (100)	0.0 (100)	12.2	5.1 (58)	3.5 (71)	13.9	5.0 (64)	4.0 (71)
Protein-like	3.3	0.0 (100)	0.0 (100)	21.5	8.1 (62)	5.3 (75)	14.1	6.2 (56)	0.0 (100)
<b>Stagnant Batch</b>									
Humic-like	9.2	4.6 (50)	3.3 (64)	12.5	11.5 (8)	4.9 (61)	16.8	6.7 (60)	4.7 (72)
Fulvic-like	ND	ND	ND	8.2	8.0 (2)	0.0 (100)	15.4	4.6 (70)	3.3 (79)
Protein-like	9.5	0.0 (100)	0.0 (100)	27.2	9.8 (64)	0.0 (100)	14.6	0.0 (100)	0.0 (100)
<b>Free Drain</b>									
Humic-like	6.9	5.2 (25)	3.6 (48)	11.3	8.6 (24)	7.9 (30)	15.9	7.0 (56)	4.1 (74)
Fulvic-like	ND	ND	ND	12.1	0.0 (100)	9.0 (26)	7.1	6.6 (7)	0.0 (100)
Protein-like	9.5	0.0 (100)	0.0 (100)	28.4	14.7 (48)	8.5 (70)	11.8	0.0 (100)	0.0 (100)

Excitation-Emission wavelength (λEx/Em): 240-260/ 410-450 nm for humic-like, 290-340/ 410-430 nm for fulvic-like and 270-280/ 300-350 nm for protein-like.  
 ND: Not detected

**Table 5** Amount of solids accumulated on the sand and solids accumulation rates in both compartments of the Duplex-constructed wetland configurations at the end of the study

	Calculated total accumulated solids* (g)	Measured total accumulated solids (g)	Measured total accumulated solids (kg m <sup>-2</sup> )	Measured total accumulated solids rates (kg m <sup>-2</sup> y <sup>-1</sup> )
	VFCW/HFF	VFCW/HFF	VFCW/HFF	VFCW/HFF
Fill and Drain	421/18	406/137	1.7/0.6	1.5/0.5
Stagnant Batch	357/54	353/325	1.5/1.4	1.3/1.2
Free Drain	240/145	305/454	1.3/1.9	1.1/1.7

\* Calculated values obtained from the total suspended solids applied with the wastewater considering the average removal values per experimental period per compartment

**Table 6** Above- and below-ground biomass quantification (dry weight) and nutrient uptake in the Duplex-constructed wetland configurations

	Dry weight (g)	Total nitrogen (mg g <sub>dry weight</sub> <sup>-1</sup> )	Total nitrogen (g m <sup>-2</sup> )	% rem.	Total phosphorus (mg g <sub>dry weight</sub> <sup>-1</sup> )	Total phosphorus (g m <sup>-2</sup> )	% rem.
Above-ground (Mean ± SE, n=3)							
Fill and Drain	1.6 ± 0.1 <sup>a*</sup>	23.2 ± 2.1 <sup>a</sup>	67	5	3.2 ± 0.1 <sup>a</sup>	9	5
Stagnant	1.3 ± 0.3 <sup>a*</sup>	24.1 ± 1.5 <sup>a</sup>	57	4	3.7 ± 0.3 <sup>a</sup>	9	5
Free Drain	1.0 ± 0.0 <sup>a*</sup>	22.5 ± 0.5 <sup>a</sup>	41	3	4.0 ± 0.3 <sup>a</sup>	7	4
Below-ground (n=1)							
Fill and Drain	106	11.2	4.9	0.4	2.5	1.1	0.6
Stagnant	93	14.9	5.8	0.4	3.1	1.2	0.7
Free Drain	96	14.9	6.0	0.4	4.7	1.9	1.0

\* Values are expressed in g d<sup>-1</sup> as each period between harvesting lasted slightly different.

Superscript (a,b) indicates statistical differences among the Duplex-CW configurations, per parameter.