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Side-by-side comparison of 15 pilot-scale conventional and intensified subsurface flow wetlands for treatment of domestic wastewater

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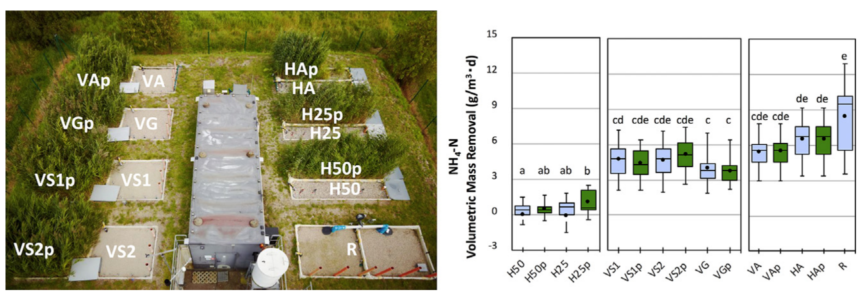
HIGHLIGHTS

- 15 pilot-scale treatment wetlands were compared side-by-side in an outdoor study.
- Pollutant removal rates increase with design complexity (HF < VF < Intensified).
- Plants had only minor effects on removal rates over the first two growing seasons.
- Volumetric mass removal rates should be used as an indicator of treatment efficacy.

GRAPHICAL ABSTRACT



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ABSTRACT

This study reports a systematic assessment of treatment efficacy for 15 pilot-scale subsurface flow constructed wetlands of different designs for $CBOD_5$, TSS, TOC, TN, NH_4-N, NO_3-N, NO_2-N, and *E. coli* over the course of one year in an outdoor study to evaluate the effects of design and plants. The systems consisted of a range of designs: horizontal flow (HF) with 50 and 25 cm depth, unsaturated vertical flow (VF) with sand or fine gravel, and intensified systems (horizontal and saturated vertical flow with aeration, and reciprocating fill and drain). Each system was built in duplicate: one was planted with *Phragmites* and one was left unplanted (with the exception of the reciprocating system, of which there was only one and it was unplanted). All systems were fed with the same primary-treated domestic wastewater. Effluent concentrations, areal and volumetric mass removal rates, and percent mass removal for the 15 systems are discussed. HF wetlands removed $CBOD_5$, TSS, TN, NH_4-N and *E. coli* by 73–83%, 93–95%, 17–41%, 0–27% and 1.5 log units, respectively. Unsaturated VF and aerated VF wetlands removed $CBOD_5$, TSS, TN, NH_4-N and *E. coli* by 69–99%, 76–99%, 17–40%, 69–99% and 0.9–2.4 log units, respectively. The aerated HF and reciprocating systems removed $CBOD_5$, TSS, TN, NH_4-N and *E. coli* by 99%, 99%, 43–70%, 94–99% and 3.0–3.8 log units, respectively. The aerated HF and reciprocating systems achieved the highest TN removal rate of all of the designs. Design complexity clearly enhanced treatment efficacy (HF < VF < Intensified, $p < 0.001$) during the first two years of plant growth while the presence of plants had minor effects on TN and NH_4-N removal in the shallow HF design only.

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1. Introduction

Subsurface-flow treatment wetlands are commonly used for the treatment of domestic wastewater. They are often implemented in regions where decentralized wastewater treatment is the most cost-effective option. Compared to conventional wastewater treatment technologies, treatment wetlands offer many advantages: they are low-cost, simple to operate, and can be constructed out of locally available materials (Dotro et al., 2017). These factors have resulted in the widespread use and implementation of treatment wetlands across the globe (Vymazal and Kröpfelová, 2008).

Conventional horizontal flow (HF) wetlands have long been proven to provide adequate treatment of domestic wastewater (Kadlec and Knight, 1996). However, discharge standards for treated wastewater are becoming ever more stringent, often requiring advanced removal of nutrients (Brix and Arias, 2005; ÖNORM, 2009; DWA, 2017). As such, there is growing interest in improving the treatment capacity of constructed wetland systems. Because subsurface oxygen limitation is one of the main rate-limiting factors in HF wetlands (Brix and Schierup, 1990), there is gaining interest in alternative and intensified wetland designs (Nivala et al., 2013b). In fact, many research studies have investigated alternative treatment wetland design configurations, including technology variations such as tidal flow wetlands (Austin, 2006), reciprocating wetlands (Behrends et al., 2001), aerated wetlands (Wallace et al., 2008; Murphy et al., 2016), vertical flow (VF) wetlands with an intermittent loading regime (Schwager and Boller, 1997), recirculating wetlands (Arias et al., 2005; Al-Zreiqat et al., 2018) or horizontal flow wetlands with a shallow bed depth (Aguirre et al., 2005). However, the objectives of these studies tend to focus on a single aspect (e.g., BOD removal, nitrification capacity, or solids accumulation) of a specific design (and often, of a single treatment system). Results from such studies are extremely valuable in furthering the understanding of specific treatment mechanisms of a particular design. However, due to differences in design, climate, and wastewater characteristics, it is difficult to compare the absolute performance of any one particular system against that of another. On the other hand, numerous studies have investigated different wetland designs in laboratory-scale experiments using artificial wastewater (Maltais-Landry et al., 2009; Jia et al., 2010; Fan et al., 2013; Liu et al., 2013), which eliminates the influence of environmental factors but limits the transfer of knowledge to real-world scenarios. A comprehensive side-by-side comparison of the most common treatment wetland designs in pilot-scale that enables practical knowledge transfer to engineering practice has not yet been reported in the literature.

This study compares the treatment efficacy of 15 pilot-scale subsurface flow constructed wetlands of different designs (with and without plants) treating real domestic wastewater in an outdoor experiment over the course of one year. The objective of the study was to quantify the effect of system design and plant presence on treatment efficacy. To investigate the influence of design on treatment efficacy, eight commonly used wetland designs were included (conventional HF and VF wetland designs as well as intensified designs). Seven of the designs were operated in planted (*Phragmites australis*) and unplanted pairs to determine whether the use of plants influences treatment efficacy. Areal and volumetric mass removal rates of $CBOD_5$, TSS, TOC, TN, NH_4-N, NO_3-N, NO_2-N, and *E. coli* are discussed to assess treatment efficacy for the removal of organic carbon, nitrogen and pathogens.

This work supplements the existing body of knowledge on conventional HF and VF wetland performance and expands beyond current scientific knowledge to include the first side-by-side analysis of planted and unplanted, conventional and intensified treatment wetland designs.

2. Materials and methods

2.1. Site and system description

The pilot-scale experiments were carried out at a research facility in Langenreichenbach, Germany shown in Fig. 1. The research facility is equipped with an onsite weather station that measures meteorological data on a 10-minute basis. The research facility receives raw wastewater from the sewer line to the adjacent municipal wastewater treatment plant servicing the nearby villages (population equivalent: 16,000 inhabitants). The raw wastewater is treated primarily in a two-chamber septic tank (nominal hydraulic retention time (nHRT) of 2.0 days) before being distributed to the individual pilot-scale systems. The following subsurface wetland designs were included: horizontal flow with 50 or 25 cm saturated bed depth (H50 and H25), unsaturated vertical flow with sand (VS) or gravel (VG) as media, aerated saturated vertical down-flow (VA), aerated horizontal flow (HA) and a reciprocating two-cell (R) design (Table 1). Except for the reciprocating system, all designs were built in planted (*Phragmites australis*) and unplanted pairs. Further details concerning the research facility are given in Nivala et al. (2013a).

Inflow to each treatment system was measured by an electromagnetic flow meter, outflow by recording the number of times a calibrated vessel filled and emptied each day. A programmable logic control (PLC) system was used to control flow measurement and system operation (Nivala et al., 2013b). All systems were built in 2009 and started operation in June 2010. The systems were planted in September 2009 at a density of five plants per square meter. Monitoring of water quality parameters begun in August 2010.

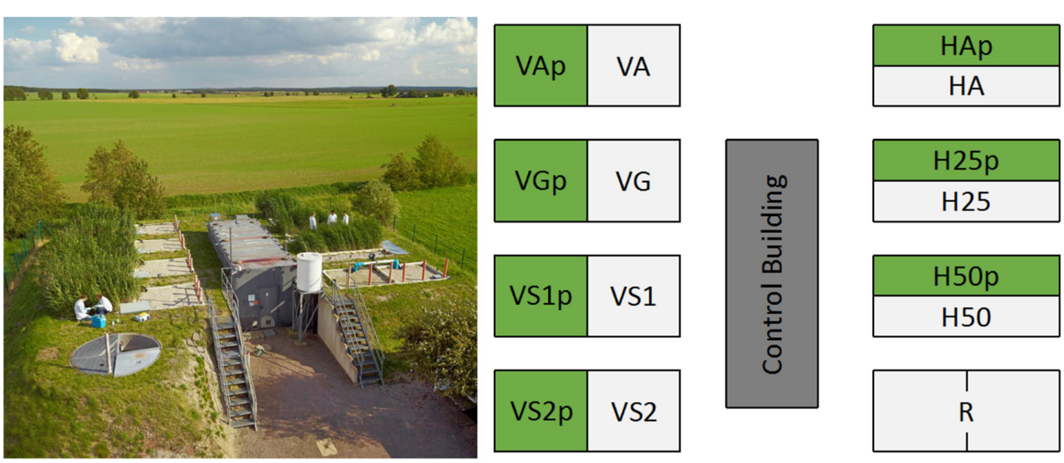

Fig. 1. The research platform in Langenreichenbach, Germany. Photo (left) and plan layout of the various systems (right). Photo credit André Künzelmann/UFZ.

Table 1
Design and operational details of the 15 treatment systems at the research platform in Langenreichenbach during the 12-month period of this study (October 2010–September 2011).

System abbreviation[a]	System type	Effective depth[b] (cm)	Saturation status	Filter media	Surface area (m^2)	Design flow (m^3/day)
Horizontal flow (HF)						
H25, H25p	HF	25	Saturated	8–16 mm gravel	5.6	0.10
H50, H50p	HF	50	Saturated	8–16 mm gravel	5.6	0.20
Vertical flow (VF)						
VS1[c], VS1p[c]	VF	85	Unsaturated	1–3 mm sand	6.2	0.60
VS2[d], VS2p[d]	VF	85	Unsaturated	1–3 mm sand	6.2	0.60
VG[c], VGp[c]	VF	85	Unsaturated	4–8 mm gravel	6.2	0.60
Intensified						
VA, VAp	VF + aeration	85	Saturated	8–16 mm gravel	6.2	0.60
HA, HAp	HF + aeration	100	Saturated	8–16 mm gravel	5.6	0.75
R	Reciprocating	95	Alternating	8–16 mm gravel	13.2	2.0

[a] Systems planted with *Phragmites australis* are denoted with "p" in the system abbreviation.
[b] Effective depth refers to the depth of the media involved in treatment. Depth of media not involved in treatment (such as the fill above distribution shields in a vertical flow bed, or the layer of dry gravel in a saturated bed) was not considered.
[c] Systems were loaded once every hour.
[d] Systems were loaded once every 2 h.

2.2. Sampling and water quality analysis

Systems were sampled from October 2010–September 2011 on a rotating weekly basis as described in Nivala et al. (2013a). Water samples were collected from a tap installed prior to the outflow measuring device of each system. Water temperature, electrical conductivity (EC), oxidation reduction potential (ORP), and dissolved oxygen (DO) were measured in the onsite lab at the research facility (WTW Multi 350i Multimeter) as well as pH (WTW pH 96 meter). Remaining water quality parameters were analyzed within 24 h in a laboratory at the UFZ in Leipzig, Germany. Five-day carbonaceous biochemical oxygen demand ($CBOD_5$) was analyzed according to the DIN 38409 H52, using the WTW OxiTOP® system. Total organic carbon (TOC) was analyzed according to DIN EN 1484 using a Shimadzu TOC-VCSN device. Total nitrogen (TN) was analyzed according to DIN EN 12660, using a Shimadzu TNM-1 device. Ammonium, nitrate, and nitrite nitrogen were measured with an Eppendorf EPOS ANALYZER 5060 according to DIN 38406 E5, DIN 38405 D9, and DIN 38405 D10, respectively. Turbidity was measured according to DIN ISO EN 27027 using a Hach 2100AN Turbidimeter. *E. coli* was determined using the Colilert-18 Quanti-Tray™ method (IDEXX, USA) according to the manufacturer's specifications.

2.3. Data pre-processing

All steps were conducted in the statistic software environment *R (Version 3.1.2)* (R Core Team, 2018). Outliers were identified by visual inspections of time series plots and, if related to system malfunctions, obvious analytical errors, or heavy rain (defined as >10 mm of rain in the 48 h prior to sampling), excluded from further analysis. Laboratory results below the detection limit of an analytical procedure were set to its corresponding detection limit. This procedure may not be optimal as all censored values were set exactly to the same level, producing semi-continuous data which does not reflect reality. However, considering that the scale of the water quality parameters analyzed in this study is on the order of milligram per liter and that the applied statistical methods are robust for dealing with censored data, the effects of substitution at a decimal point can be neglected.

2.4. Treatment performance

Treatment performance was assessed on a mass reduction basis, which takes into account chemical loss and water fluxes in a wetland system (Eqs. (1), (2), and (3)), with inflow rate Q_{in} (m^3/day) and outflow rate Q_{out} (m^3/day), wetland area A (m^2), and wetland depth (effective depth = depth involved in treatment) h (m). Areal mass removal rates are reported in $g/m^2 \cdot day$ (where m^2 refers to the surface area of the bed, even if the system is HF and receives wastewater on the cross-sectional surface area that is perpendicular to the direction of flow) and volumetric removal rates are reported in $g/m^3 \cdot day$ (where m^3 refers to the physical volume of the wetland basin itself based on the dimensions length, width and depth).

$$\text{Percent Mass Removal} = 100 \times \left(1 - \frac{C_{out} \cdot Q_{out}}{C_{in} \cdot Q_{in}}\right) \tag{1}$$

$$\text{Area Mass Removal Rate} = \frac{(C_{in} \cdot Q_{in}) - (C_{out} \cdot Q_{out})}{A} \tag{2}$$

$$\text{Volumetric Mass Removal Rate} = \frac{(C_{in} \cdot Q_{in}) - (C_{out} \cdot Q_{out})}{A \cdot h} \tag{3}$$

2.5. Statistical analysis

Statistical hypothesis testing, including multiple comparison post-hoc tests, were used to compare the effluent concentrations, areal mass removal rates, and volumetric mass removal rates of the different wetlands with respect to the factors *design* and *plants*. Due to their non-reliance on a certain data distribution, generalized additive models (GAM) were set up for individual pollutant parameters to examine the presence of a statistically significant effect of the two factors. In addition to parametric terms which are used in linear statistical models (e.g. linear regression, analysis of variance), generalized additive models (GAMs) contain smooth terms which can fit non-linear data structures (e.g. seasonal influenced time-series data of wetland performance) without prior assumption of any functional relationship (Wood, 2006). This way, residual bias (e.g. auto-correlation, divergence from normality, heteroscedasticity) that often occurs when analyzing time-series data with linear models can be effectively reduced (Wood, 2006). Design and plants were used as parametric terms in the model; duration (representing the time of the experiment) and water temperature (representing the temperature of the water sample measured directly after sampling) were used as smoothing terms. All models were implemented using the *mgcv* (Mixed GAM Computation Vehicle (Wood, 2006)) package within the *R* software. If a significant effect was identified, post-hoc tests using the non-parametric pairwise Wilcoxon-rank-sum tests and parametric *t*-tests were applied to identify which experimental wetland systems were different from one another.

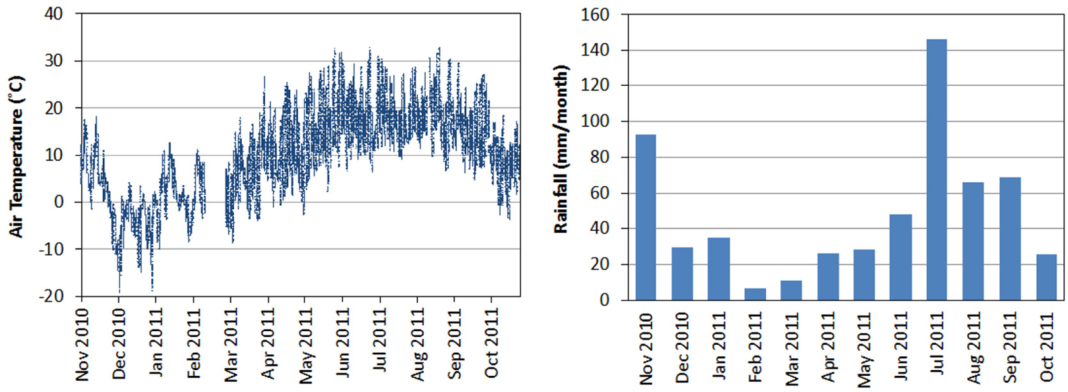

Fig. 2. Mean daily air temperature and monthly cumulative rainfall at the research platform in Langenreichenbach, Germany.

3. Results and discussion

3.1. Weather conditions

Average air temperature fluctuated from −5 °C to +20 °C; precipitation was higher in summer than in winter months (Fig. 2). Weather measurements, therefore, exhibited typical conditions of temperate climate in the northern hemisphere (Köppen, 2011) except the very low temperatures in December 2010 and January 2011 as well as unusually high precipitation in November 2010 and July 2011.

3.2. Hydraulic flow rates and pollutant concentrations

Influent wastewater quality (Table 2, Table 3) is within the range of "high strength" domestic wastewater (Metcalf and Eddy Inc., 2003). Inflow to the treatment systems, which was controlled by a Programmable Logic Control (PLC) system, was steady over the course of the study (within 5% of programmed inflow value) for all systems except for the reciprocating system, R. The inflow to R varied over the course of the study, starting at 1500 L/day for the first four months, and was then changed to 2256 L/day (96 L/h) for the remainder of the study. Mean annual effluent flow rates for the unplanted systems (H25, H50, VS1, VS2, VG, HA, VA, and R) were approximately equal to the inflow (Table 2). The planted systems (H25p, H50p, VS1p, VS2p, VGp, HAp and VAp) had lower and more variable mean outflow rates than their unplanted counterparts (Table 3), due to the higher evapotranspiration rates during the peak growing season. It is expected that more mature systems planted with *Phragmites australis* would exhibit greater evapotranspirative water losses. In general, hydraulic flow rates were stable enough for the wetland systems to develop a quasi–steady state, which is the basis to assess baseline performance.

Oxidation reduction potential (ORP) was generally negative in the effluent of the four horizontal flow systems, and positive in all of the other wetland systems (Table 2). Variation in ORP was greatest in H25p, which can likely be attributed to the presence of plants in the system and associated oxygen leakage via the roots. Of the four horizontal flow wetlands, the effluent DO concentration was highest in H25p, which can also likely be attributed to the presence of *Phragmites australis*. Several studies have shown that the presence of wetland macrophytes can have an influence over the observed ORP and DO concentrations within the rootzone of subsurface flow wetlands (Tanner et al., 1997; Bezbaruah and Zhang, 2004). Electrical conductivity was highest in the planted horizontal flow systems, H25p and H50p, because these two systems lost the largest percentage of flow to evapotranspiration (Table 3).

The 15 treatment systems removed the aforementioned pollutants to varying degrees. The horizontal flow systems H25, H25p, H50, and H50p achieved mean effluent $CBOD_5$ concentrations of 41–59 mg/L. These concentrations are higher than what would typically be expected for a horizontal flow wetland treating domestic wastewater (Vymazal and Kröpfelová, 2008) and is likely a result of the hydraulic residence time of approximately five days being insufficient considering the relatively high strength characteristics of the wastewater (mean influent $CBOD_5$ concentration 235 ± 76 mg/L). The poor treatment efficacy of

Table 2
Water quality data for the 15 treatment wetland systems over the 12-month period of record (POR) of the study. Mean and standard deviations are shown. Number of inlet-outlet pairs is listed as *n*.

		Inflow	Outflow	Water temperature		Oxidation reduction potential		Electrical conductivity		pH		Dissolved oxygen	
		Q_i (L/day)	Q_o (L/day)	T (°C)	*n*	(mV)	*n*	μS/cm	*n*	–	*n*	(mg/L)	*n*
Influent[a]		–	–	13.1 ± 4.6	45	−140 ± 57	45	1549 ± 256	45	7.4 ± 0.2	45	0.4 ± 0.3	45
HF	H25	100 ± 4	97 ± 8	11.2 ± 6.7	33	−110 ± 63	33	1430 ± 227	33	7.8 ± 0.2	33	2.0 ± 1.3	33
	H25p	99 ± 5	78 ± 20	11.3 ± 6.7	33	−52 ± 137	33	1589 ± 344	33	7.5 ± 0.3	33	2.6 ± 1.3	33
	H50	200 ± 10	199 ± 10	11.4 ± 6.4	33	−154 ± 41	33	1430 ± 205	33	7.6 ± 0.2	33	1.1 ± 0.9	33
	H50p	200 ± 10	180 ± 21	11.3 ± 6.4	33	−153 ± 43	33	1536 ± 267	33	7.4 ± 0.2	33	1.3 ± 0.9	33
VF	VS1	603 ± 12	608 ± 10	12.0 ± 5.9	32	205 ± 35	32	1277 ± 203	32	6.7 ± 0.4	32	6.0 ± 1.6	32
	VS1p	605 ± 11	564 ± 36	11.8 ± 5.7	32	209 ± 28	32	1285 ± 182	32	6.6 ± 0.4	32	6.6 ± 1.5	32
	VS2	599 ± 1	600 ± 10	12.0 ± 6.0	32	209 ± 38	32	1288 ± 197	32	6.4 ± 0.4	32	4.8 ± 1.3	32
	VS2p	599 ± 2	574 ± 28	11.8 ± 5.7	32	209 ± 30	32	1293 ± 180	32	6.3 ± 0.5	32	5.6 ± 1.3	32
	VG	598 ± 2	596 ± 11	12.2 ± 5.8	32	183 ± 46	32	1312 ± 194	32	7.2 ± 0.2	32	4.6 ± 1.4	32
	VGp	598 ± 2	585 ± 15	12.2 ± 5.7	32	170 ± 56	32	1353 ± 209	32	7.2 ± 0.3	32	4.2 ± 1.6	32
Intensified	VA	597 ± 2	595 ± 10	13.0 ± 6.4	20	196 ± 38	20	1215 ± 121	20	6.9 ± 0.2	20	5.7 ± 2.0	20
	VAp	598 ± 2	575 ± 20	13.0 ± 6.3	20	195 ± 41	20	1242 ± 121	20	6.9 ± 0.3	20	6.3 ± 2.0	20
	HA	751 ± 39	747 ± 38	12.6 ± 7.0	20	183 ± 30	20	1197 ± 99	20	7.6 ± 0.2	20	8.3 ± 3.1	20
	HAp	751 ± 39	728 ± 46	12.7 ± 6.9	20	184 ± 32	20	1221 ± 105	20	7.5 ± 0.1	20	8.0 ± 2.5	20
	R	2013 ± 356	1998 ± 359	14.4 ± 5.7	35	177 ± 46	35	1202 ± 108	35	7.1 ± 0.2	35	3.6 ± 3.0	35

[a] The influent to all wetland systems is septic tank effluent.

Table 3
Hydraulic loading rate and effluent concentration for the 15 treatment wetland systems over the 12-month period of record (POR) of the study. Mean and standard deviations are shown. Number of inlet-outlet pairs is listed as *n*.

		Influent hydraulic loading rate[b]	$CBOD_5$		TSS		TOC		TN		NH_4-N		NO_3-N		NO_2-N		*E. coli*	
		($L/m^2 \cdot day$)	*C* (mg/L)	*n*	*C* (mg/L)	*n*	*C* (mg/L)	*n*	*C* (mg/L)	*n*	*C* (mg/L)	*n*	*C* (mg/L)	*n*	*C* (mg/L)	*n*	log_{10} (MPN/100 mL)	*n*
Influent[a]			235 ± 76	45	145 ± 64	45	146 ± 41	45	70 ± 18	45	49 ± 15	45	0.4 ± 0.3	45	0.04 ± 0.08	45	6.8 ± 0.3	45
HF	H25	18	45 ± 17	33	8 ± 2	33	32 ± 8	33	57 ± 12	33	53 ± 20	33	0.2 ± 0.2	33	0.03 ± 0.03	33	5.3 ± 0.4	33
	H25p	18	41 ± 19	33	7 ± 2	33	35 ± 9	33	50 ± 13	33	44 ± 15	33	0.2 ± 0.3	33	0.07 ± 0.15	33	5.2 ± 0.4	33
	H50	36	55 ± 19	33	8 ± 3	33	35 ± 10	33	58 ± 13	33	51 ± 16	33	0.2 ± 0.1	33	0.03 ± 0.05	33	5.5 ± 0.3	33
	H50p	36	59 ± 21	33	8 ± 3	33	38 ± 9	33	55 ± 12	33	48 ± 14	33	0.2 ± 0.1	33	0.05 ± 0.10	33	5.4 ± 0.2	33
VF	VS1	97	5 ± 4	32	6 ± 7	32	19 ± 6	32	55 ± 14	32	8 ± 8	32	41 ± 13	32	0.5 ± 0.5	32	5.0 ± 0.8	32
	VS1p	98	3 ± 3	32	2 ± 2	32	16 ± 4	32	51 ± 14	32	4 ± 5	32	43 ± 14	32	0.2 ± 0.2	32	4.4 ± 0.6	32
	VS2	97	5 ± 5	32	5 ± 4	32	19 ± 5	32	59 ± 13	32	8 ± 8	32	44 ± 13	32	0.3 ± 0.3	32	5.5 ± 0.3	32
	VS2p	97	4 ± 3	32	3 ± 2	32	17 ± 4	32	56 ± 13	32	4 ± 5	32	48 ± 16	32	0.1 ± 0.1	32	5.4 ± 0.2	32
	VG	96	20 ± 18	32	18 ± 15	32	28 ± 10	32	47 ± 11	32	15 ± 10	32	27 ± 8	32	0.6 ± 0.5	32	5.7 ± 0.5	32
	VGp	96	31 ± 29	32	39 ± 34	32	40 ± 21	32	49 ± 11	32	17 ± 12	32	25 ± 8	32	0.8 ± 0.9	32	5.9 ± 0.5	32
Intensified	VA	96	4 ± 3	20	9 ± 15	20	15 ± 2	20	40 ± 9	20	0.8 ± 0.7	20	35 ± 10	20	0.08 ± 0.08	20	4.6 ± 0.4	20
	VAp	96	5 ± 4	20	15 ± 15	20	18 ± 5	20	43 ± 9	20	0.5 ± 0.4	20	38 ± 12	20	0.06 ± 0.06	20	4.6 ± 0.5	20
	HA	107	2 ± 2	20	2 ± 2	20	12 ± 2	20	39 ± 7	20	0.3 ± 0.4	20	35 ± 12	20	0.04 ± 0.10	20	3.0 ± 0.5	20
	HAp	107	2 ± 2	20	2 ± 1	20	12 ± 2	20	38 ± 8	20	0.3 ± 0.4	20	35 ± 13	20	0.04 ± 0.06	20	3.3 ± 0.6	20
	R	153[c]	3 ± 3	35	2 ± 1	35	14 ± 2	35	19 ± 8	35	4 ± 3	35	12 ± 7	35	0.06 ± 0.04	35	3.8 ± 0.6	35

[a] The influent to all wetland systems is septic tank effluent.
[b] Hydraulic loading rate calculated on mean inflow rate and rounded to the nearest liter.
[c] Hydraulic loading rate calculated based on the total surface area of the two filters (13.2 m^2).

H50 and H50p due to the high organic content of the wastewater was also noted by a later study by Carranza-Diaz et al. (2014). The horizontal flow systems still provided good removal of TSS, with annual mean effluent concentrations < 10 mg/L. Despite the high strength of the influent wastewater, the vertical flow systems VS1, VS1p, VS2 and VS2p and intensified systems VA, VAp, HA, HAp, and R exhibited consistently low annual mean effluent $CBOD_5$ concentrations, below 5 mg/L. This is in line with the experience from other studies of vertical flow wetlands with sand as the main filter media (Weedon, 2003; Matamoros et al., 2007), aerated HF wetlands (Redmond et al., 2014) and fill-and-drain (reciprocating) systems (Leonard et al., 2003; Li et al., 2015; Wu et al., 2015). Mean effluent $CBOD_5$ concentrations for VG and VGp were higher; 20 mg/L and 31 mg/L, respectively; likely due to the coarse grain size of the material (4–8 mm), which resulted in a short retention time and poorer filtering effect in these systems (compared to sand-based vertical flow systems). The influence of plant roots in VGp could have also resulted in increased pathways for short-circuiting of the system, which is in agreement with the poorer treatment efficacy of this system for nearly all wastewater parameters, when compared to its unplanted counterpart VG. TOC effluent concentrations for the treatment systems followed similar trends as observed for $CBOD_5$. TSS removal in the sand-based vertical flow wetlands VS1, VS1p, VS2 and VS2p and the horizontal flow aerated wetlands HA and HAp was consistently good, with annual mean effluent TSS also below 10 mg/L. Good removal of TSS has been reported for other VF wetlands with sand media (Matamoros et al., 2007; Torrens et al., 2009) and aerated HF wetlands (Redmond et al., 2014). The effluent TSS concentrations of the aerated wetlands grouped according to flow direction (vertical or horizontal; Fig. 3). VA and VAp exhibited higher effluent TSS concentrations than HA, HAp or R, which is likely due to the well-mixed hydraulics of this wetland design (1.1–1.2 continuously-stirred tank reactors (CSTR) in series (Boog et al., 2014). Foladori et al. (2013) noticed similar effluent TSS concentrations of an aerated vertical flow constructed wetland despite using a finer main media (sand 1–6 mm). As a result of the hypothesis tests using GAMs, design turned out as significant factor ($p < 0.001$) for $CBOD_5$, TOC and TSS while plants were significant only for TOC ($p = 0.037$) (Fig. 4). Despite the low significance for plants in the GAMs, post-hoc tests did not describe significant differences between planted and unplanted system-pairs ($p > 0.05$), only between different designs. This indicates a predominance of design over plant presence, however, the statistical power of the post-hoc test was limited by the small sample size.

Similarly, none of the observed differences in annual mean effluent TN concentrations (Fig. 3) between any of the planted and unplanted pairs were significant ($p > 0.05$), despite plants turning out as overall significant factor in the GAMs ($p = 0.027$). However, design had a stronger influence on TN removal ($p < 0.001$). Total nitrogen removal (on a concentration basis) in the horizontal flow systems (H25, H25p, H50, and H50p) and sand-based vertical flow systems (VS1, VS1p, VS2, VS2p) was variable and somewhat limited, with the annual mean influent concentration reduced from 70 mg/L to effluent TN concentrations ranging between 50 and 59 mg/L. The relatively poor TN removal in the HF systems is to be expected, given the predominance of anoxic conditions and slow rates of oxygen transfer into the saturated substrate of these systems. This limits nitrification (Vymazal and Kröpfelová, 2008), as reflected by the relatively low DO concentrations and redox levels (Table 2). However, the poor TN removal is in contrast to other studies (Bayley et al., 2003; Davison et al., 2005; Headley et al., 2005; Tanner et al., 2012) which reported removals of 49–60% in several HF wetlands (0.4–1.0 m depth) receiving primary treated domestic sewage. However, all of these HF systems were operated with HRTs 8.9 and 16.1 days, compared to 5 days in the current study, demonstrating the possible effect of hydraulic loading rate on TN removal. In contrast to the HF systems, the limited TN removal in the unsaturated VF sand beds was due to the predominantly aerobic conditions, which promotes nitrification, but offers limited opportunities for the anoxic denitrification process to remove any nitrate that is generated. Annual mean effluent TN concentrations for the unsaturated gravel-based systems VG and VGp were slightly lower than their sand-based VF counterparts. This was possibly due to anoxic zones developing as a consequence of the poorer $CBOD_5$ removal, supporting some denitrification of the nitrate generated via nitrification—an effect also reported by Tanner et al. (2012). The observed differences in effluent TN concentrations were not significant ($p > 0.05$) for VG compared to VS1p or VGp compared to all of the HF beds and VS1, VS1p and VS2p. The aerated systems (VA, VAp, HA and HAp) had statistically similar ($p > 0.05$) effluent TN concentrations (between 38 and 43 mg/L), which were all significantly lower and less variable than that of the HF and sand-based VF beds ($p < 0.05$), with the exception of VAp which was not significantly different from VS1 and H25p. Similar effluent TN concentrations for aerated vertical flow wetlands were reported by Foladori et al. (2013) and for aerated horizontal flow wetlands by Uggetti et al. (2016). The lower effluent TN

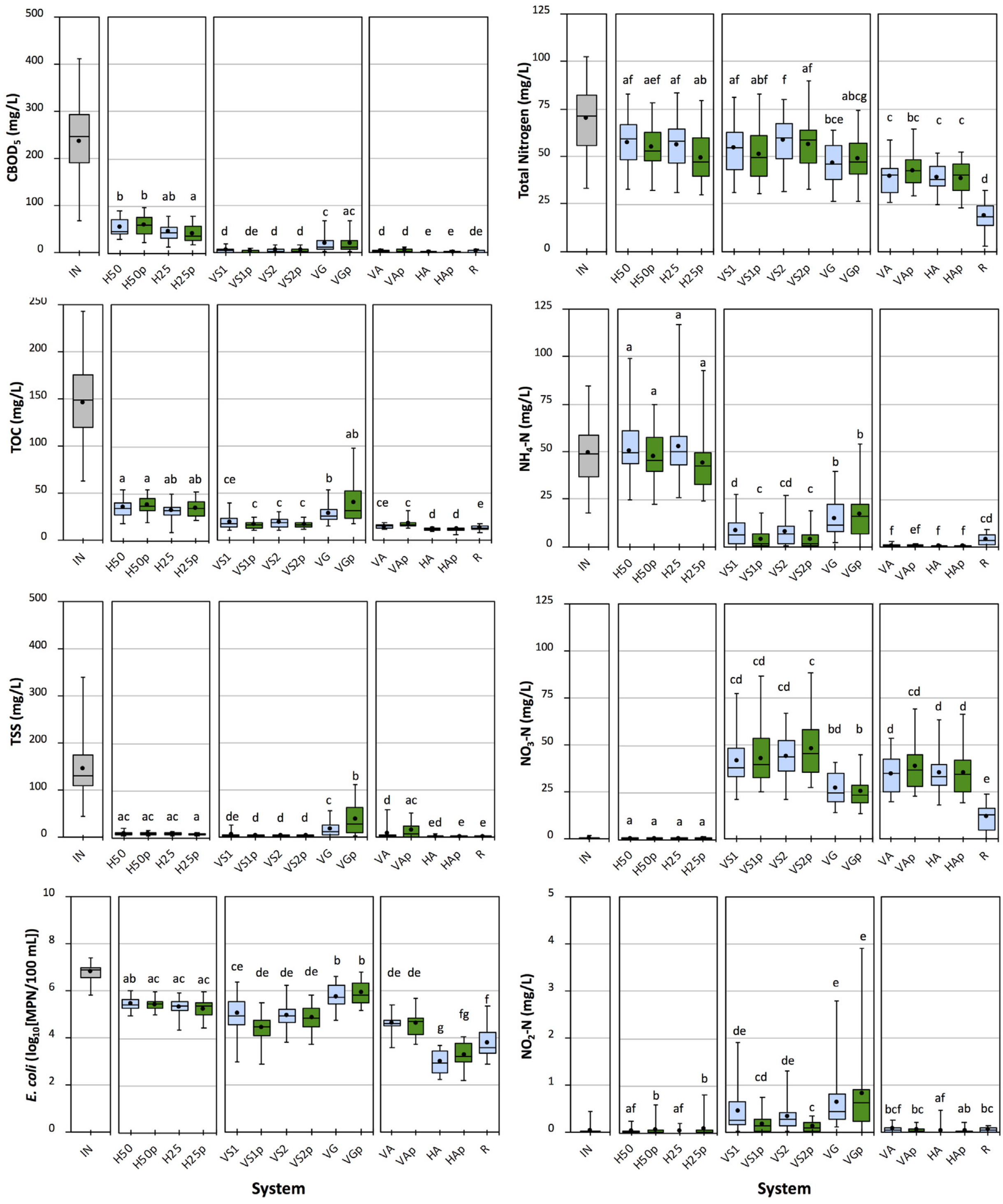

Fig. 3. Box plots of inlet and outlet concentrations for the 15 treatment wetland systems showing the mean (dot), median (line), first and third quartiles (box), and minimum and maximum values (whiskers). Letters indicate statistically significant differences ($p < 0.05$); systems with the same letters belong to the same group.

concentrations achieved by the aerated wetlands indicates the presence of some anoxic zones allowing for some denitrification to occur amongst the predominantly aerobic substrate. Interestingly, although the gravel-based VF beds (VG and VGp) displayed higher annual mean effluent TN concentrations than the aerated systems, these differences were not significant ($p > 0.05$).

The reciprocating system R had the lowest TN concentrations of all 15 systems (19 mg/L) which was statistically distinguishable from all other systems ($p < 0.001$, Fig. 3). The good TN removal for the reciprocating system is likely due to the alternating oxic and anoxic conditions created by the filling and draining phases (described in detail in Nivala et al., 2013a) promoting sequential nitrification-denitrification within a single treatment system. Other studies have shown that reciprocating systems provide suitable conditions for effective TN removal (Behrends et al., 2001; Leonard et al., 2003; Wu et al., 2015). When examining the difference between the planted and unplanted versions of each design variant, the planted HF wetlands and the sand-based VF wetlands had slightly lower annual mean effluent TN concentrations than their unplanted counterparts, possibly due to the added removal pathway of plant uptake.

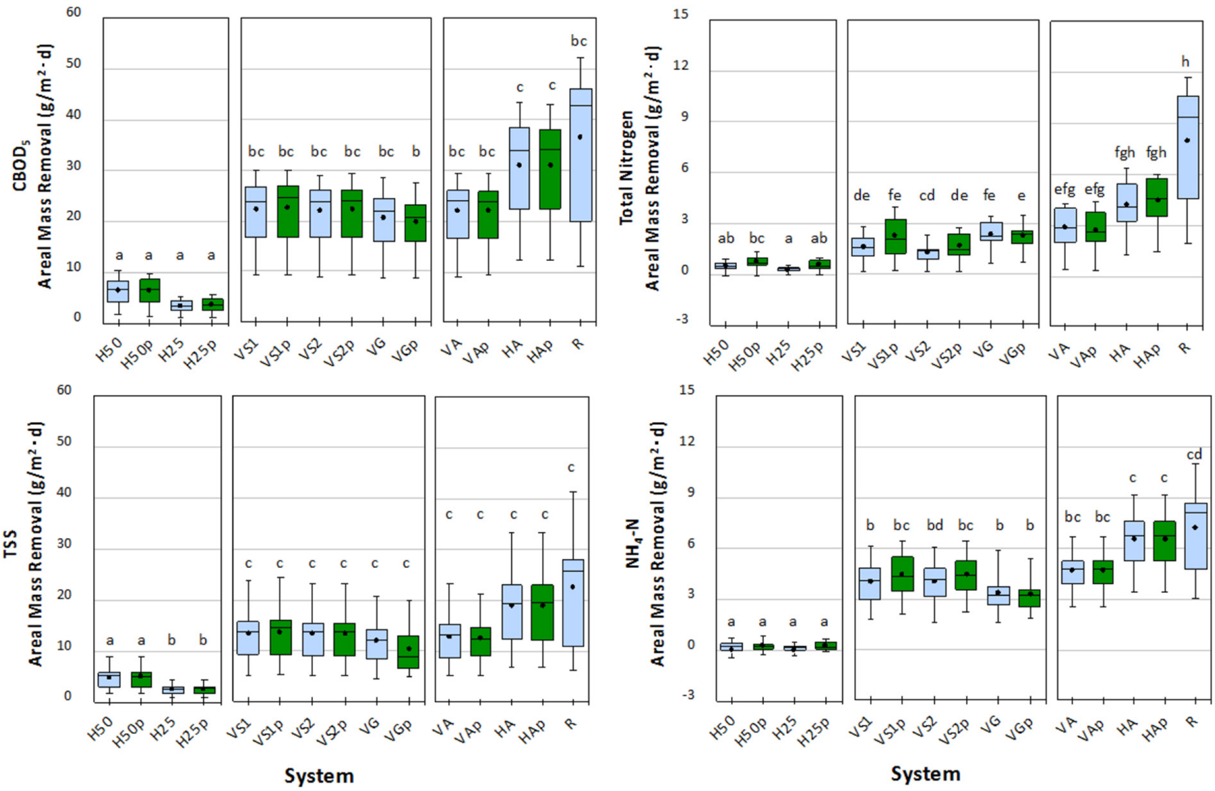

Fig. 4. Box plots of areal mass removal rates. Letters indicate statistically significant differences ($p < 0.05$); systems with the same letters belong to the same group.

On a concentration basis, ammonium nitrogen removal in the four horizontal flow systems H25, H25p, H50, and H50p was negligible, with effluent concentrations similar to the influent (44–53 mg/L) and showing high variability (Fig. 3). Studies which included intermediate sampling within HF wetlands with relatively long HRTs (10–16 days) showed that NH_4 removal occurs at a very slow rate until the BOD concentration is reduced to below at least 20 mg/L, allowing nitrifying bacteria to gain access to some of the oxygen that slowly diffuses into the bed (Bayley et al., 2003; Headley et al., 2005; Tanner et al., 2012). At the HRT of 5 days in the current study, the HF systems were only capable of reducing the $CBOD_5$ concentrations to 41–59 mg/L at the outlet, meaning there was not enough excess DO to support nitrification. The shallow planted HF wetland (H25p) had the lowest mean effluent NH_4-N concentration (44 mg/L) of the HF beds, followed by the 50 cm deep planted system (H50p), likely due either to plant uptake of NH_4-N or enhanced nitrification as a result of radial oxygen release from the roots of the *Phragmites australis*. However, none of the observed differences in effluent NH_4-N concentration amongst the HF systems were significant ($p > 0.05$), partly due to the high variability in effluent concentrations. The sand-based VF systems in this study had mean annual effluent ammonium concentrations between 4 and 8 mg/L, removing nearly all NH_4-N in the warm months and less in the cold months (data not shown). Comparing VS1 against VS2, and VS1p against VS2p, it can be seen that the influent dosing frequency (VS1 and VS1p received 24 hourly doses per day while VS2 and VS2p received 12 bi-hourly doses per day) had no significant effect on the effluent NH_4-N concentrations ($p > 0.05$), indicating that both loading regimes are acceptable from a nitrification perspective. The presence of *Phragmites australis* had a stronger influence on effluent NH_4-N concentrations, with VS1p and VS2p having slightly lower effluent concentrations than their unplanted counterparts (Fig. 3), either due to plant uptake or improved nitrification in the rootzone. However, the post-hoc test assessed that differences were only significant for VS1 and VS1p ($p < 0.05$), while the differences in effluent NH_4-N concentrations of VS2 and VS2p were not significant ($p > 0.05$). VG and VGp produced significantly higher annual mean effluent NH_4-N concentrations (15 and 17 mg/L respectively) than the sand-based VF systems ($p < 0.05$), indicating that coarse sand is a preferable filter media to fine gravel for VF wetlands designed to nitrify and remove NH_4-N. However, in locations where suitably graded sand is not available, fine gravel may be acceptable, providing the loading rate is reduced accordingly to compensate for the reduced nitrification efficiency. This was demonstrated in the studies of Al-Zreiqat et al. (2018) and Tanner et al. (2012) in which VF systems with fine gravel (4–8 mm and 5 mm respectively) achieved almost complete removal of NH_4-N.

Removal of NH_4-N in the aerated wetlands VA, VAp, HA, and HAp was complete and stable, with year-round effluent concentrations below 1 mg/L. These results are supported by the DO concentrations shown in Table 2, which were consistently above 5 mg/L. Effluent winter water temperatures of the aerated systems reached as low as 1.4 °C (data not shown) and the mean air temperature reached −19 °C during this study, which is similar to that reported for a full-scale aerated HF treatment wetland which maintained successfully winter operation in Minnesota (Wallace and Nivala, 2005). The mean effluent NH_4-N concentrations of the aerated wetlands (<1 mg/L for VA, VAp, HA, and HAp) were highly significantly lower than those of the other treatment systems ($p < 0.001$, Fig. 3). It is worthwhile to note here that NH_4-N removal in these four aerated treatment wetlands did not vary much with water temperature, as evidenced by corresponding standard deviations of <1 mg/L. Almost complete NH_4-N removal under cold climate conditions by aerated horizontal flow wetlands was also reported by Redmond et al. (2014). NH_4-N removal in all other systems was influenced to some degree by water temperature, as evidenced by the relatively high standard deviations in Table 3. Based on the observed water quality data for the aerated systems, with almost complete removal of NH_4-N, $CBOD_5$ and elevated effluent DO concentrations of 5.7–8.3 mg/L, it is apparent that the $CBOD_5$ and NH_4 loading rates could be increased, or the aeration rate decreased, to some extent without compromising treatment efficacy. This was demonstrated by the study of Boog et al. (2014) in which the aeration rate on the VA wetland was reduced by a third from continuous (24 h/day) to intermittent aeration (8 h on; 4 h off), while still maintaining effective NH_4-N removal.

Effluent NO_3-N concentrations of the HF wetlands were consistently low due to a lack of nitrification and favorable conditions for denitrification (Table 2, Fig. 3). This is typical of HF wetlands (Bayley et al., 2003; Headley et al., 2005). The sand-based systems VS1, VS1p, VS2, and VS2p exhibited effluent NO_3-N concentrations in the range of 41–48 mg/L and were not significantly different from one another ($p > 0.05$), indicating that neither the dosing frequency or presence of plants had an effect on NO_3 concentrations. The NO_3-N produced was approximately equivalent to the NH_4-N removed, indicating that nitrification was the main process responsible for NH_4-N removal. The gravel-based VF beds (VG and VGp) had lower annual mean effluent NO_3-N concentrations than the sand-based systems, with all of these differences being significant for VGp, while the difference for VG was only statistically significant against VS2p ($p < 0.05$). Similarly, the reciprocating system R grouped separately from all other systems ($p < 0.001$). The aerated systems HA, HAp, VA, and VAp as well as the gravel-based unsaturated system VG exhibited effluent NO_3-N concentrations of approximately 27–38 mg/L, which were not significantly different from one another ($p > 0.05$) and were lower than the sand-based unsaturated systems. This reflects the presence of denitrification and subsequently higher TN removal in the aerated and gravel-based VF systems. The presence of plants did not have a statistically significant impact on effluent NO_3-N concentrations for any treatment wetland ($p > 0.05$). Effluent NO_2-N concentrations in the four conventional HF wetlands and the five intensified wetlands were consistently low (< 0.1 mg/L). Effluent NO_2-N concentrations > 0.1 mg/L in the unsaturated vertical flow wetlands were consistently observed, with annual mean effluent NO_2-N concentrations in VG and VGp of 0.6 and 0.8 mg/L, respectively.

E. coli removal in H25, H25p, H50, and H50p was on the order of 1.5 log units, which is in agreement with previous reports on HF wetlands treating septic tank effluent (Vymazal and Kröpfelová, 2008) and other studies on these same four treatment systems (Headley et al., 2013). Amongst the unsaturated vertical flow wetlands, the sand-based systems achieved significantly lower effluent *E. coli* concentrations than the gravel-based systems, which is in line with the findings of Tanner et al. (2012) and Morató et al. (2014). An influence of vegetation on *E. coli* removal could not be discerned, which was also the case for other unsaturated vertical flow systems examined by Torrens et al. (2009). The sand-based systems exhibited 1.3–2.4 $\log_{10}$ MPN/100 mL removal. *E. coli* removal in the vertical flow aerated wetlands VA and VAp was statistically similar to that of the unsaturated sand-based systems ($p > 0.05$). The system with the lowest effluent *E. coli* concentration was the horizontal flow aerated system HA, which exhibited 3.8 $\log_{10}$ *E. coli* removal. There was no influence of plants on *E. coli* removal in HA and HAp. The reciprocating system R had a mean effluent concentration of 3.8 $\log_{10}$ units of *E. coli*, which was significantly higher than HA, not significantly different from HAp and significantly lower than all of the other systems (at $p = 0.05$). The effluent *E. coli* concentrations for the intensified systems are also similar to those reported by Headley et al. (2013).

3.3. Mass removal rates

Both areal and volumetric mass removal rates, as well as percent mass removal, are presented in Table 5 for $CBOD_5$ and TSS, and Table 6 for TN and NH_4-N. These tables allow direct comparison of the 15 treatment systems in this study, showing the relative mass loadings and treatment efficacy in terms of the commonly accepted areal-based approach as well as on a volumetric basis. Placing the systems within this context allows insight into the relative merits of each design, not only within a subset of design variables (e.g., planted or unplanted; dosed once every hour or once every 2 h), but as well amongst different designs. It also accounts for changes in concentration that may occur due to differences in relative water loss rates, rather than pollutant degradation, that may occur across the various systems due to the presence of plants or relative differences in hydraulic loading rates. Figs. 4 and 5 show the areal and mass removal rates, respectively, and show the statistically significant groupings by post-hoc tests (Wilcoxon rank-sum tests) for a specific water quality parameter at a significance level of 0.05. The power of the statistical hypothesis tests is less for the removal rates than for effluent concentrations, as the number of data points (due to monthly averaging) is fewer for removal rates. Consequently, differences for one parameter may be statistically significant for the corresponding effluent concentration but not for the removal rate. Therefore, the statistical results for mass removal rates should be interpreted as rather conservative.

$CBOD_5$ removal rates for all systems are shown in Table 5. As for effluent concentrations, $CBOD_5$ rather depends on design ($p < 0.001$) then on plant presence ($p = 0.928$). On an areal basis, $CBOD_5$ removal rates generally increase with increasing hydraulic loading rate. The 50 cm deep HF systems (H50 and H50p) had higher $CBOD_5$ areal mass removal rates than the shallower HF beds (H25 and H25p), corresponding with the fact that the deeper systems received double the areal loading rate. However, these differences were not significant ($p > 0.05$). When compared on a volumetric basis, both H25 and H25p (25 cm saturated depth) have slightly higher $CBOD_5$ mass removal rates than H50 and H50p (50 cm saturated depth), although the differences were not statistically significant ($p > 0.05$). It is notable that on a volumetric mass removal basis, H25p performed better than the other three conventional HF systems for the removal of $CBOD_5$, which was also the case for NH_4-N and TN removal. However, these differences were not found to be significant ($p > 0.05$). $CBOD_5$ removal rates for these four passive HF systems were significantly lower than for the remaining systems on both an areal and volumetric basis ($p < 0.05$). $CBOD_5$ mass removal rates in the unsaturated vertical flow wetlands were significantly higher than that of the conventional HF wetlands, with >97% mass removal for the four unsaturated sand-based systems (VS1, VS1p, VS2, VS2p) and >88% for the two unsaturated gravel-based systems (VG, VGp). Intensified systems HA, HAp, VA, VAp, and R had the highest $CBOD_5$ removal rates and percent mass removal (>98%), but the removal rates (areal and volumetric) generally did not group separately from the unsaturated VF systems (Figs. 4 and 5). The lack of significant differences in the $CBOD_5$ removal rates across the VF and intensified systems may be partly due to the fact that these aerobic systems all reduced $CBOD_5$ down to outlet concentrations of 5 mg/L or less, which likely reflects the background $CBOD_5$ concentration for these systems. Consequently, these results present an under-estimation of the potential $CBOD_5$ mass removal rates for the VF and intensified systems, since the systems appear to have been under-loaded relative to their potential treatment capacity. Under higher organic loading conditions, differences in effluent concentrations and mass removal rates may emerge amongst systems of different design or vegetation status.

The trends observed for $CBOD_5$ removal rates were generally also observed for TSS removal in the 15 wetland treatment systems (Table 5). Similarly, design turned out to be significant ($p < 0.001$) while plants did not ($p > 0.05$) for both areal and volumetric mass removal rates (Fig. 5). One exception is that the areal TSS removal rates for H50 and H50p were significantly higher than those of H25 and H25p, although still significantly lower than all of the other systems (Fig. 4). TSS percent mass removal was generally high (>93%) for all systems; with the exception of VG (76.1%) and VGp (87.5%), which is likely due to the coarse size (4–8 mm) of gravel used in these two unsaturated systems resulting in a poorer filtering efficiency. The vertical flow aerated wetlands VA and VAp exhibited a lower TSS percent mass removal (93%) than the horizontal flow aerated wetlands (99%), which although not statistically different ($p > 0.05$), could be due to the difference in hydraulics of the treatment systems. Tracer testing of Boog et al. (2014) shows that VAp operated with an equivalent tanks-in-series (TIS) value of 1.1, indicating the system is hydraulically well-mixed; whereas HAp operated with an equivalent TIS of 4.2 (Boog, 2013). The near-

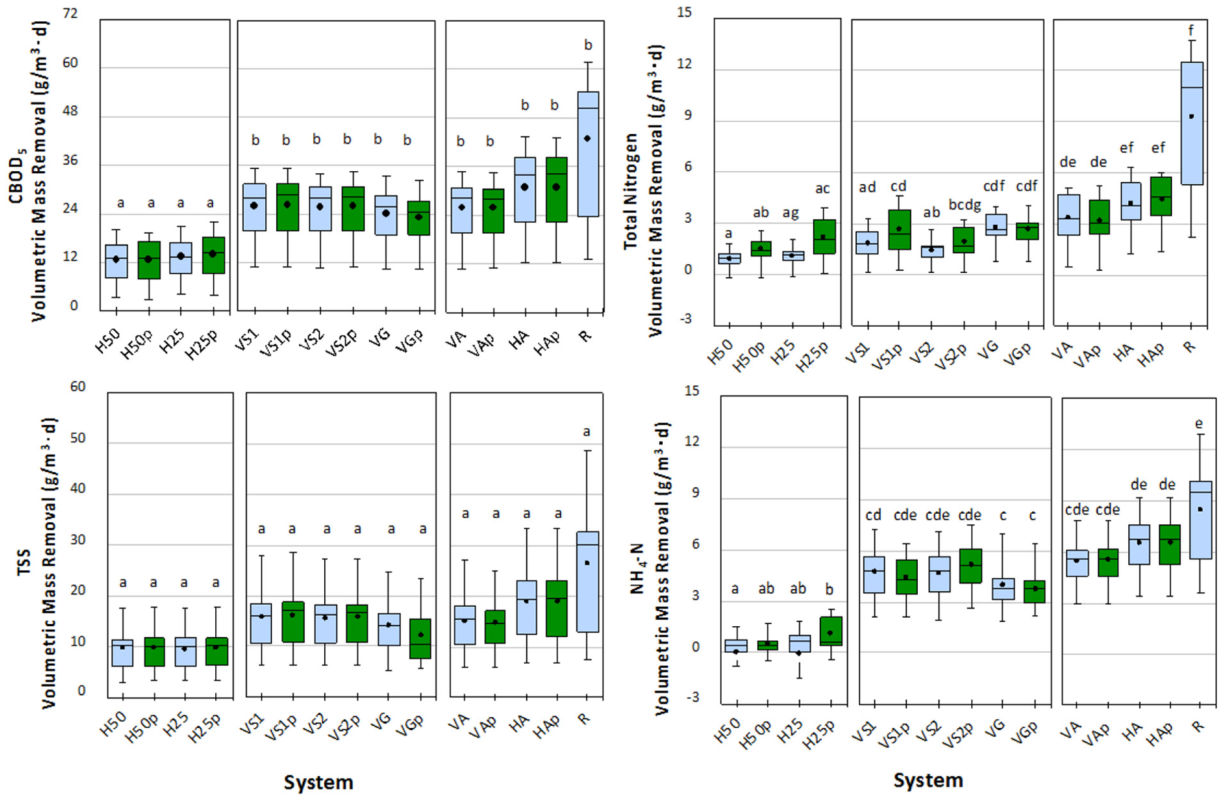

Fig. 5. Box plots of volumetric mass removal rates. Letters indicate statistically significant differences ($p < 0.05$); systems with the same letters belong to the same group.

complete mixing in VAp results in a small portion of wastewater having a very short retention time in the treatment system, which could result in higher effluent concentrations in VAp when compared to HAp (observed for $CBOD_5$, TSS, and *E. coli*; Table 3).

Overall, design ($p < 0.001$) was significant for areal and volumetric ammonia removal rates, while plants were significant only for volumetric removal rates ($p < 0.05$). Additionally, comparing individual design pairs using post-hoc tests did not yield clear-cut results. Annual mean areal and volumetric NH_4-N removal rates for the unplanted systems H25 and H50 were zero (or slightly negative), with negative calculated annual mean percent mass removals (Table 6). This apparent production of ammonium nitrogen is possibly due to the ammonification of organic nitrogen present in the wastewater, which could result in an internal NH_4-N load within the system. This phenomenon was also reported by Kadlec and Wallace (2009) using data from HF wetlands of Akratos and Tsihrintzis (2007). The presence of vegetation in H25p and H50p improved the ammonia removal, resulting in slightly positive mean annual areal and volumetric NH_4-N removal rates (Table 6) and annual mean mass removal of NH_4-N of 12.4% for H50p and 26.7% for H25p. This is in agreement with findings reported for HF wetlands in a recent review by Saeed and Sun (2017) and the general fact of ammonia uptake by plants. However, the effect of plants was not found to be statistically significant on an annual average basis. The only significant difference in NH_4-N removal amongst the conventional HF systems was the volumetric removal rate of H25p which was significantly higher than H50 ($p < 0.05$). Thus, the combination of plants and shallow bed depth improved the volumetric efficiency of NH_4-N removal in the HF systems, although the improvement is relatively small compared to adopting a different design approach altogether (e.g. VF or intensified). Furthermore, this improvement in volumetric efficiency did not translate into an improvement in areal efficiency, meaning the footprint of the shallow planted HF would still be the same as the other HF systems.

Annual mean areal and volumetric NH_4-N removal rates for the pairs of unsaturated vertical flow wetlands VS1, VS1p, VS2, VS2p, VG, and VGp were not statistically different from one another ($p > 0.05$), despite slightly higher annual mean NH_4-N mass removal in VS1p and VS2p (each 91.9%) compared to the unplanted systems VS1 and VS1p (83.0% and 83.4% mass removal, respectively) (Figs. 4 and 5). Interestingly, the NH_4-N mass removal rates of the saturated VF systems with aeration (VA and VAp) were not significantly different from the unsaturated VF systems, indicating that both design approaches are equally as effective for nitrification at the influent loading rates in this study. Annual mean NH_4-N mass removals for VG and VGp were somewhat lower (70.7% and 68.6%, respectively), likely due to the short retention time and lower surface area for the attached-growth nitrifying bacteria in the coarser filter media. Annual mean areal and volumetric NH_4-N removal rates for the intensified systems were the highest. These systems also exhibited annual NH_4-N mass removal of >99% (aerated systems) and >94% (reciprocating system). Such results were also reported by Liu et al. (2016). However, the statistical separation of the intensified and conventional VF systems was not clear-cut, with the areal mass removal rates of the planted sand-based VF systems (VS1p and VS2p) not being significantly different to the intensified systems. On a volumetric basis, the NH_4-N mass removal rate of the intensified systems and the conventional VF systems with sand media were not significantly different, with the exception of the unplanted VS1 and reciprocating system which were significantly different from one another.

For TN, both design ($p < 0.001$) and plants ($p < 0.05$) turned out as significant for areal and volumetric removal rates. However, the significance level for design was smaller and its effects on annual mean rates higher (Table 6). Differences within planted and unplanted design pairs were not significant. Annual mean areal and volumetric TN removal rates for the four conventional HF wetlands were greater than zero and were positively influenced by the presence of plants However, the only two HF systems that had statistically distinguishable TN mass removal rates were H25 and H50p on an areal basis (Fig. 4). Annual mean mass removal in H25 and H25p increased from 20.4% to 41.3%

with the presence of plants; for H50 and H50p this difference was still observed, but was smaller (17% and 27.4%, respectively).

In general, the TN mass removal rates of the conventional VF systems and the intensified systems were closely grouped statistically. However, there were numerous specific differences between individual systems; some of which are not easy to explain and may be due to random variation in the treatment efficacy of some of the systems (e.g. VS2 on an areal basis). Nevertheless, some interesting relationships are indicated by the statistical analyses. For example, the TN mass removal rates (areal and volumetric) were not significantly different between the aerated beds (HA, HAp, VA and VAp), indicating that neither flow direction nor plant presence had a significant effect on removal of TN in these systems ($p > 0.05$), at least not on an annual average basis during the first two years of plant growth and first year of water quality monitoring. Aerated wetlands are also reported to achieve high TN removal rates (Liu et al., 2016; Ilyas and Masih, 2017). The main reason is the high oxygen transfer rate that enables near-complete ammonia removal. At the same time, the redox gradients and coarse aeration grid in HF aerated wetlands allow the development of aerobic and anoxic zones that may favor multiple nitrogen removal pathways such as sequential nitrification-denitrification, simultaneous nitrification-denitrification, and aerobic nitrification. For example, Hou et al. (2018) reported simultaneous partial nitrification, anammox and denitrification (SNAD) in an intermittently aerated VF wetland. Aerobic denitrification has been reported by Coban et al. (2015) in a HF wetland treating contaminated groundwater. In aerated VF wetlands, the high degree of mixing distributes can distribute influent organic carbon rapidly throughout the entire system, which enhances heterotrophic denitrification. High TN removal is often reported from aerated VF wetlands (Foladori et al., 2013; Liu et al., 2016). As for NH_4-N removal, the TN mass removal rates of the aerated VF beds (VA and VAp) were not significantly different from the conventional VF systems, with the exception of VS2 which appears to be something of an anomaly. This indicates that both unsaturated intermittent loading of VF beds and actively pumping air into a saturated VF bed, may be considered equally effective means of removing nitrogen from domestic sewage in beds with a vertical flow configuration, at least on a mass removal basis under the conditions of this study. However, the adoption of active aeration in the design provides the benefit that the rate of oxygen transfer can be potentially adjusted either in design or operation, whereas the operator has little ability to control the rate of oxygen transfer into a conventional unsaturated VF system once it is built.

The reciprocating system had by far the highest annual mean TN mass removal rate of any of the systems studied, on both areal and volumetric bases, owing to its ability to accommodate both anoxic and oxic conditions within the one reactor through the alternating cycle of saturated and unsaturated conditions. On an areal basis, the TN removal rate of the reciprocating system (R) was significantly higher than that of the conventional VF systems and the aerated VF beds ($p > 0.05$), but statistically similar to the aerated HF beds (HA and HAp). Similarly, on a volumetric basis, the reciprocating system (R) had a significantly better TN removal rate than the conventional VF systems with sand media and the aerated VF systems ($p > 0.05$) but was not significantly different from the aerated HF beds or the conventional VFs with gravel media.

3.4. Effect of design and plants on treatment efficacy

Overall, increasing design complexity clearly turned out as most important factor to reduce effluent concentrations and increase mass removal rates for all pollutants ($p < 0.001$). Treatment efficacy increased with increasing design complexity (HF < VF < Intensified). In contrast, the role of plants was less important (Table 4), indeed, it was even more difficult to identify. Removal of organic carbon and nitrogen in sub-surface flow treatment wetlands is mainly microbially induced (Kadlec and Wallace, 2009; Ilyas and Masih, 2017). In this context, associated microorganisms need constant access to pollutants and, except for nitrate removal, dissolved oxygen and favorable environmental conditions including sufficient contact time to unfold the full potential of the wetland technology. Therefore, the ability of a design to supply the aforementioned aspects defines its efficacy. Here, the main mechanisms to involved in the different designs were: 1) increased oxygen transfer into bulk water by using gravity-driven unsaturated flow in VF systems, mechanical aeration (HF aerated systems), and water level control (R system); 2) alteration of hydraulics by varying flow direction in aerated beds, using flow reciprocation (R system), and different media sizes (unsaturated VF systems) to facilitate access to pollutants (especially carbon for denitrification). The mentioned mechanisms and design factors are also reported in recent reviews as key driver of treatment efficacy for organic carbon and nitrogen in treatment wetlands (Wu et al., 2014; Liu et al., 2016; Ilyas and Masih, 2017).

Design intensification played also a major role for *E. coli* removal ($p < 0.001$). The intensified designs achieved lowest effluent *E. coli* concentrations (Fig. 3). According to Wu et al. (2016), the main mechanisms for pathogen removal in treatment wetlands are: die-off by starvation or predation, sedimentation, filtration and adsorption. Die-off by starvation and adsorption can be stimulated by decreasing concentration of organics and nutrients (Wu et al., 2016), which is a probable reason why unsaturated sand-based systems, aerated systems and the reciprocating system performed superior to the HF wetlands. Additionally, sedimentation is meant to increase with increasing TSS removal, which further explains why aerated horizontal flow systems perform well. Hydraulic retention time is also reported to increase pathogen removal, however, the studies cited by Wu et al. (2016) did not involve different designs. HRT governs pathogen removal within a certain design as shown by Headley et al. (2013), but not between designs as found this the current study (best *E. coli* removal was in HA, which had a lower HRT than the H50, which had the worst *E. coli* removal). Filtration capacity can be increased by using finer media (Morató et al., 2014), which was reflected in the increased removal in the sand-based (VS, VSp) vs. the gravel-based unsaturated systems (VG, VGp). Plants have been reported to increase pathogen removal to a certain amount in an HF wetland Wu et al. (2016), and hybrid wetland (García et al., 2013) to 0.5–1.0 log units. This is rather low compared to increased removal by design intensification such as aeration or flow reciprocation (3–4 log units). In this study, plants did not matter for pathogen removal ($p < 0.05$). Plants were significant only for effluent concentrations for TOC ($p = 0.037$), TN ($p = 0.027$), NH_4-N ($p < 0.001$) as well as volumetric mass removal rates for TN ($p = 0.001$) and NH4N ($p = 0.023$), and, TN areal mass removal rate ($p = 0.042$). The low significance levels are reflected in the low effects on average effluent concentrations (Fig. 3) and removal rates (Figs. 4 and 5) were compared to design. Plants effects were also confounded with design, however, this

Table 4
Significance of the hypothesis test factor *plants*. Additional information (e.g. df, F-scores, residual statistics) can be found in the Supplementary information.

Performance indicator	Parameter	p-Value*
		Plants
Effluent concentration	$CBOD_5$	0.804
Effluent concentration	TOC	0.037
Effluent concentration	TN	0.027
Effluent concentration	NH_4-N	<0.001
Effluent concentration	NO_3-N	0.423
Effluent concentration	*E. coli*	0.074
Effluent concentration	TSS	0.955
Volumetric mass removal rate	$CBOD_5$	0.928
Volumetric mass removal rate	TOC	0.938
Volumetric mass removal rate	TN	0.001
Volumetric mass removal rate	NH_4-N	0.023
Areal mass removal rate	$CBOD_5$	0.985
Areal mass removal rate	TOC	0.968
Areal mass removal rate	TN	0.042
Areal mass removal rate	NH_4-N	0.081

* p-Value of factor *Design* was <0.001 for all parameters.

Table 5
Annual mean influent areal and volumetric mass loadings, areal and volumetric removal rates, and percent mass removal for $CBOD_5$ and TSS. Annual values are calculated from monthly mean flow and concentration data.

		$CBOD_5$					TSS				
		Influent areal mass load ($g/m^2 \cdot day$)	Areal mass removal rate ($g/m^2 \cdot day$)	Influent volumetric mass load ($g/m^3 \cdot day$)	Volumetric mass removal rate ($g/m^3 \cdot day$)	Percent mass removal	Influent areal mass load ($g/m^2 \cdot day$)	Areal removal rate ($g/m^2 \cdot day$)	Influent volumetric mass load ($g/m^3 \cdot day$)	Volumetric removal rate ($g/m^3 \cdot day$)	Percent mass removal
HF	H25	4.1 ± 1.2	3.4 ± 1.2	16.4 ± 4.9	13.4 ± 5.0	79.5%	2.5 ± 1.0	2.4 ± 1.0	10.0 ± 4.1	9.5 ± 4.1	93.8%
	H25p	4.1 ± 1.2	3.5 ± 1.3	16.4 ± 4.9	14.0 ± 5.4	82.6%	2.5 ± 1.0	2.4 ± 1.1	10.0 ± 4.1	9.6 ± 4.2	94.8%
	H50	8.3 ± 2.5	6.3 ± 2.6	16.5 ± 5.0	12.6 ± 5.2	73.3%	5.0 ± 2.1	4.8 ± 2.1	10.1 ± 4.2	9.5 ± 4.1	93.4%
	H50p	8.3 ± 2.5	6.3 ± 2.7	16.5 ± 5.0	12.6 ± 5.3	72.9%	5.0 ± 2.1	4.8 ± 2.1	10.1 ± 4.2	9.6 ± 4.2	94.5%
VF	VS1	22.6 ± 6.4	22.1 ± 6.2	26.5 ± 7.5	26.0 ± 7.3	98.2%	13.7 ± 5.5	13.2 ± 6.2	16.2 ± 6.4	15.6 ± 6.2	96.6%
	VS1p	22.6 ± 6.4	22.4 ± 6.3	26.6 ± 7.5	26.3 ± 7.4	98.8%	13.8 ± 5.5	13.6 ± 6.4	16.2 ± 6.5	16.0 ± 6.4	98.8%
	VS2	22.4 ± 6.2	21.9 ± 6.0	26.3 ± 7.3	25.8 ± 7.1	97.8%	13.6 ± 5.3	13.2 ± 6.1	16.0 ± 6.2	15.5 ± 6.1	96.9%
	VS2p	22.4 ± 6.2	22.1 ± 6.1	26.3 ± 7.3	26.0 ± 7.1	98.6%	13.6 ± 5.3	13.2 ± 6.1	16.0 ± 6.2	15.7 ± 6.1	98.2%
	VG	22.3 ± 6.2	20.5 ± 6.0	26.3 ± 7.3	24.1 ± 7.0	91.4%	13.6 ± 5.3	11.9 ± 5.8	16.0 ± 6.2	14.0 ± 5.8	87.5%
	VGp	22.3 ± 6.2	19.7 ± 5.2	26.3 ± 7.3	23.1 ± 7.0	88.7%	13.6 ± 5.3	10.2 ± 5.6	16.0 ± 6.2	12.0 ± 5.6	76.1%
Intensified	VA	22.3 ± 6.2	22.0 ± 6.0	26.3 ± 7.3	25.9 ± 7.1	98.5%	13.6 ± 5.3	12.7 ± 6.2	16.0 ± 6.2	14.9 ± 6.2	93.6%
	VAp	22.3 ± 6.2	21.9 ± 6.0	26.3 ± 7.3	25.8 ± 7.0	98.4%	13.6 ± 5.3	12.5 ± 5.5	16.0 ± 6.2	14.7 ± 5.5	93.1%
	HA	31.1 ± 9.4	30.9 ± 9.3	31.1 ± 9.4	30.9 ± 9.3	99.5%	19.0 ± 7.9	18.7 ± 7.8	19.0 ± 7.9	18.7 ± 7.8	98.5%
	HAp	31.0 ± 9.4	30.9 ± 9.2	31.0 ± 9.4	30.9 ± 9.2	99.5%	18.9 ± 7.9	18.8 ± 7.8	18.9 ± 7.9	18.8 ± 7.8	99.0%
	R	36.8 ± 14.7	36.4 ± 14.4	43.2 ± 17.3	36.4 ± 14.4	99.1%	22.6 ± 11.2	22.6 ± 11.2	26.6 ± 13.1	26.4 ± 13.0	99.1%

was not statistically significant, probably, due to the low power of the post-hoc tests as a results of the small samples size. Statistical power could be increased by taking more samples and relinquish monthly averaging, or simply increase the monitoring period over two additional years.

In general, plants in subsurface flow treatment wetlands are reported to stabilize the surface of beds, facilitate physical filtration, mitigate clogging in vertical and horizontal flow systems, and supply dissolved oxygen into the subsurface zone (Brix, 1997). However, as engineering modifications provide other means, for example, of supplying DO, the relative contribution of the plants to the overall rate of pollutant degradation diminishes. For example, plant mediated oxygen transfer has been measured over a range of 0.014–12.0 g m^{-2} day^{-1} by Armstrong et al. (1990) and Ye et al. (2012), however, this is orders of magnitude lower than what can be provided by mechanical aeration (Nivala et al., 2013b; Boog et al., 2014). This is especially true for treatment of domestic wastewater, where nutrient loadings and subsequent oxygen demands are orders of magnitude higher than what is observed in natural environments and watercourses. Vymazal and Kröpfelová (2008) as well as Stottmeister (2003) considered plant nutrient uptake as of minor importance compared to microbial removal in treatment wetlands at comparable loads of nutrients. Wu et al. (2018) also argue that design intensification outcompetes the effect of plants. Other studies report no clear and consistent effect of plants on organic matter removal in VF wetlands (Matamoros et al., 2007; Torrens et al., 2009), passive HF wetlands (Akratos and Tsihrintzis, 2007) and aerated wetlands (Maltais-Landry et al., 2009). In contrast, Zhang et al. (2010) compared aerated and non-aerated HF pilot systems and found that planted beds consistently achieved better BOD removal than unplanted beds. It is also worth highlighting that the aerated HF systems of Zhang et al. (2010) received intermittent aeration at a relatively low rate (compared to the current study) as required to raise the DO to 0.6 mg/L before shutting off. Thus, the substantially higher aeration rates used in the current and other reported studies are much more likely to outweigh the influence that vegetation might have on organic matter removal. Plants contribute a smaller relative proportion of the overall removal in intensified systems due to higher influent pollutant loadings and removal mechanisms that are increased by mechanical methods (e.g., pumping of air or water in the system).

A more considerable effect of plants on treatment efficacy is evapotranspirative water loss; specifically observed in H25p. The significant loss of water to plant evapotranspiration is important to consider, especially for shallow HF wetlands such as H25p where up to 50% of the inflow can be lost to evapotranspiration during the summer months

Table 6
Annual mean influent areal and volumetric mass loadings, areal and volumetric removal rates, and percent mass removal for TN and NH_4-N. Annual values are calculated from monthly mean flow and concentration data.

		TN					NH_4-N				
		Influent areal mass load ($g/m^2 \cdot day$)	Areal mass removal rate ($g/m^2 \cdot day$)	Influent volumetric mass load ($g/m^3 \cdot day$)	Volumetric mass removal rate ($g/m^3 \cdot day$)	Percent mass removal	Influent areal mass load ($g/m^2 \cdot day$)	Areal mass removal rate ($g/m^2 \cdot day$)	Influent volumetric mass load ($g/m^3 \cdot day$)	Volumetric mass removal rate ($g/m^3 \cdot day$)	Percent mass removal
HF	H25	1.2 ± 0.3	0.3 ± 0.1	4.9 ± 1.1	1.1 ± 0.6	20.4%	0.9 ± 0.2	0.0 ± 0.2	3.4 ± 0.9	−0.1 ± 0.8	−3.7%
	H25p	1.2 ± 0.3	0.5 ± 0.3	4.9 ± 1.1	2.1 ± 1.2	41.3%	0.9 ± 0.2	0.3 ± 0.3	3.4 ± 0.9	1.1 ± 1.0	26.7%
	H50	2.5 ± 0.6	0.4 ± 0.3	4.9 ± 1.2	0.9 ± 0.6	17.0%	1.7 ± 0.5	0.0 ± 0.3	3.5 ± 1.0	0.0 ± 0.6	−2.2%
	H50p	2.5 ± 0.6	0.7 ± 0.4	4.9 ± 1.2	1.4 ± 0.8	27.4%	1.7 ± 0.5	0.2 ± 0.3	3.5 ± 1.0	0.5 ± 0.6	12.4%
VF	VS1	6.8 ± 1.5	1.5 ± 0.8	8.0 ± 1.7	1.8 ± 0.9	22.0%	4.7 ± 1.2	4.0 ± 1.3	5.6 ± 1.4	4.7 ± 1.5	83.0%
	VS1p	6.8 ± 1.5	2.2 ± 1.2	8.0 ± 1.7	2.6 ± 1.4	31.5%	4.7 ± 1.2	4.4 ± 1.4	5.6 ± 1.4	4.4 ± 1.4	91.9%
	VS2	6.7 ± 1.4	1.2 ± 0.6	7.9 ± 1.7	1.4 ± 0.7	17.1%	4.7 ± 1.2	4.0 ± 1.3	5.5 ± 1.4	4.7 ± 1.5	83.4%
	VS2p	6.7 ± 1.4	1.6 ± 0.8	7.9 ± 1.7	1.9 ± 0.9	23.3%	4.7 ± 1.2	4.4 ± 1.3	5.5 ± 1.4	5.1 ± 1.5	91.9%
	VG	6.7 ± 1.4	2.3 ± 0.8	7.9 ± 1.7	2.7 ± 0.8	33.6%	4.7 ± 1.2	3.3 ± 1.1	5.5 ± 1.4	3.9 ± 1.3	70.7%
	VGp	6.7 ± 1.4	2.2 ± 0.7	7.9 ± 1.7	2.6 ± 1.4	32.3%	4.7 ± 1.2	3.2 ± 1.0	5.5 ± 1.4	3.7 ± 1.2	68.6%
Intensified	VA	6.7 ± 1.4	2.8 ± 1.2	7.9 ± 1.7	3.3 ± 1.4	40.0%	4.7 ± 1.2	4.6 ± 1.2	5.5 ± 1.4	5.4 ± 1.4	98.5%
	VAp	6.7 ± 1.4	2.7 ± 1.2	7.9 ± 1.7	3.1 ± 1.7	37.9%	4.7 ± 1.2	4.6 ± 1.2	5.5 ± 1.4	5.5 ± 1.4	99.1%
	HA	9.3 ± 2.2	4.2 ± 1.7	9.3 ± 2.2	4.2 ± 1.7	43.2%	6.5 ± 1.8	6.5 ± 1.8	6.5 ± 1.8	6.5 ± 1.8	99.3%
	HAp	9.3 ± 2.2	4.4 ± 1.5	9.3 ± 2.2	4.4 ± 1.5	46.0%	6.5 ± 1.8	6.5 ± 1.8	6.5 ± 1.8	6.5 ± 1.8	99.1%
	R	10.8 ± 3.7	7.9 ± 3.5	12.8 ± 4.4	9.3 ± 4.1	69.5%	7.7 ± 2.9	7.1 ± 2.6	9.0 ± 3.4	8.4 ± 3.0	94.3%

(data not shown). This has repercussions for any effluent standards that are concentration-based. Even though the system may have good mass removal rates, the loss of water to evapotranspiration results in higher effluent pollutant concentrations and higher salt concentrations in the final effluent. In arid regions or in situations where low effluent pollutant concentrations and/or reuse in irrigation is a desired end-goal, unplanted and unsaturated VF wetlands may produce a more suitable effluent (e.g., lower pollutant concentrations and lower salt concentrations) than planted HF treatment wetlands (Al-Zreiqat et al., 2018).

3.5. Strengths and limits of this study

This study provides evidence for design complexity as a main factor governing treatment efficacy of organic carbon, nitrogen and pathogens as well as its predominance over plants. The influence of plants on treatment efficacy was minor and limited to nitrogen removal, however, mean effluent concentration and mean mass removal indicated that plant importance depended on design as well despite mostly insignificant post-hoc comparisons between planted and unplanted design pairs. This is a consequence of the low statistical power due to the limited number of samples and the monthly averaging. Statistical power could be increased by sampling at higher frequency, or without averaging on a monthly basis. Nevertheless, monthly averaging for hydraulic flow and water quality data was done to address the bias associated with contemporaneous inlet-outlet sampling (grab sampling) when computing mass removal rates. Grab sampling does not account for the delay of pollutant transport within in a wetland. To account for this, Kadlec and Wallace (2009) recommend averaging flow and water quality data over three to four times the nominal hydraulic retention time (nHRT). The range of nHRTs of this experiment (e.g. hours for unsaturated systems up to six days for saturated ones) translate this into an averaging period of 25 days at maximum; here, it was defined as a whole month for the sake of simplicity. Further research should investigate the trade-offs between sampling frequency and data averaging period to maximize the power of the applied statistics while minimizing any biases of transport delays.

A twelve-month monitoring period was chosen in order to minimize unnecessary bias in the data analysis, such that all four seasons are equally represented in the dataset. Additionally, the scale of the study definitely has practical relevance (design types, system size, real-world environmental conditions, real domestic wastewater) and allows a scale-up of results into engineering practice. This is another strength of this study considering that the percentage of pilot-scale studies in recent reviews is much less compared to lab-scale experiments (Wu et al., 2014; Liu et al., 2016; Ilyas and Masih, 2017). Such pilot-scale studies are, of course, intense in their financial and organizational requirements, which limit the sample size (and thus the statistical power) and the parameters monitored. Phosphorous was not monitored in this study for budgetary reasons. Moreover, for high influent phosphorus loads for subsurface flow constructed wetlands treating domestic wastewater, sustainable phosphorus removal cannot be expected unless a phosphorus-sorbing aggregate is used (Saeed and Sun, 2017).

Another strength of the study is that several indicators of treatment efficacy (effluent concentrations as well as areal, volumetric and percentage mass removal rates) were computed, which allows are more profound comparison of individual treatment systems. It also highlights short-comings of using areal-based mass removal rates as a basis of comparison. Wetland area governs many ecosystem processes such as gas diffusion, evapotranspiration and gravitational settling that contribute to pollutant removal in conventional subsurface flow treatment wetland designs (horizontal flow and vertical flow) (Kadlec and Wallace, 2009). In the scientific literature as well as in the wetland design process, mass removal rates are often normalized to the surface area of the wetland ($g/m^2 \cdot day$). It is important to note, however, that areal-based mass removal rates do *not* imply that the mass load is distributed evenly over the surface area of the wetland. In fact, the practice of reporting influent mass load and mass removal rates normalized to wetland area should be used with caution for horizontal flow wetlands, because the cross-sectional area receiving the influent wastewater is generally very small compared to the surface area of the entire treatment system. For horizontal flow wetlands, the cross-sectional area perpendicular to the flow is often the limiting factor in sizing for organic matter removal (e.g., COD or BOD) (Wallace and Knight, 2006; Kadlec and Wallace, 2009; DWA, 2017). Comparing mass removal on a volumetric basis provides insights that are not distinguishable on an areal basis, especially the effect of depth on treatment efficacy (or when comparing systems of different depths). Percent concentration reduction is often used in the literature (Kadlec and Wallace, 2009; Ilyas and Masih, 2017) especially for lab-scale studies, however, it was not included in this study because it does not reflect water fluxes in wetlands, which are known to affect treatment efficacy in outdoor wetland treatment systems.

4. Conclusions

This study investigated 12 months of water quality data for 15 pilot-scale treatment wetland systems of varying designs with and without plant presence, all receiving the same primary treated domestic wastewater. Treatment efficacy increased with increasing design complexity (HF < VF < Intensified), while the overall influence of plant-presence decreased. The influence of plants (*Phragmites australis*) on treatment efficacy was most profoundly observed in the HF wetlands. In the sand-based unsaturated VF systems, a positive influence of plants on treatment efficacy was still noticeable but was negligible for the intensified systems. When comparing the removal rates of the planted and unplanted variants during the first two years of vegetation growth, the applied statistical analysis found a significant effect of plants on removal of NH_4-N and TN only. Groupings can be formed according to design: HF wetlands generally achieve low rates of removal for $CBOD_5$, TOC, TSS, TN, NH_4-N, and *E. coli*. Sand- or gravel-based VF systems, and VF systems with aeration, achieve intermediate rates of removal for these same pollutants. HF systems with aeration and reciprocating systems achieve the highest removal rates of the wetland designs compared, under the specific loading conditions in this study. Higher loading rates for VF and intensified systems are possible in principle, but the long-term sustainability of increased pollutant loading is unknown due to potential concerns such as clogging. This study clearly highlights the importance of design on treatment efficacy. Intensified designs are capable of achieving high quality effluents that are able to comply with increasingly stringent discharge and re-use standards. Future studies should continue to focus on evaluating a range of different treatment wetland designs treating the same wastewater; however, for a longer time (on the order of years) so that the dynamics of treatment efficacy over time and ability to sustain treatment efficacy can be more precisely investigated and understood. Monitoring planted and unplanted systems over the long-term will also give deeper insights to the long-term influence of mature plant growth on the role of plants in treatment wetland systems (e.g., other functions such as providing insulation or preventing clogging) and the overall treatment efficacy of constructed wetland systems.

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Appendix A. Supplementary information

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.12.165.

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