Original article

Type of constructed wetlands influence nutrient removal and nitrous oxide emissions from greenhouse wastewater

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Summary

In the current study, three constructed wetlands (CWs) were tested as a sustainable method of treating highly ion charged greenhouse wastewater before disposal. Because of their anaerobic conditions, it was hypothesized that free water surface flow (FWS) and horizontal-subsurface flow (HSS) CWs would be more efficient at removing NO_3^- and SO_4^{2-} from greenhouse wastewater than the vertical-flow (VSS) CW, but that FWS and HSS would emit more greenhouse gases. To test this hypothesis and propose the most sustainable CW for the greenhouse industry, this study compared three types of CWs (FWS, HSS and VSS) for their nutrient removal performance and nitrous oxide (N_2O) emissions. The experiment was conducted in a greenhouse and consisted of 36 wetland units (12 replicates) of 0.8 m^3 operated with reconstituted greenhouse wastewater enriched with sucrose (C:N ratio of 2.9) at a 10-day hydraulic retention time, corresponding to the effluent loading rate coming from commercial greenhouse vegetable crops. The CWs were filled with water (FWS), gravel (HSS), or sand (VSS) and planted with *Eichhornia crassipes* (FWS) or *Typha latifolia* (HSS, VSS), two macrophytes largely used to treat wastewaters heavily loaded in nutriment. Results showed that HSS performed better than the FWS and VSS at reducing pollutants from the greenhouse wastewater, with 45% total N load removed. Although 59% of the NO_3-N load was removed in the FWS and HSS, a high accumulation of NO_2^- (1.28 g N m^{-2} d^{-1}) occurred in FWS. The removal of ammonium (NH_4-N) (~26%) loadings was similar in all CWs. Only 4% of the SO_4-S load was removed in the FWS and HSS, and no SO_4-S reduction was observed in VSS. Mean cumulative N_2O emissions were 7 and 59 times higher in FWS (1.59 g m^{-2} d^{-1}) than in HSS and VSS, respectively. Although VSS emitted less N_2O than the other CWs tested in this study, HSS was the best option in terms of reducing CO_2 emissions and nutrient pollutants from greenhouse wastewater before disposal.

Keywords

greenhouse gases, nitrate, phosphate, sulfate, *Typha latifolia*, *Eichhornia crassipes*

Significance of this study

What is already known on this subject?
- Constructed wetland (CW) designs commonly used to treat wastewater are free water surface flow (FWS), horizontal-subsurface flow (HSS), and vertical-subsurface flow (VSS). However, it is not clear which CW system is the best sustainable option to treat greenhouse effluents, very rich in nutrients and low in dissolved organic carbon.

What are the new findings?
- The performance of three well-proven CW designs was compared within a robust experimental design. HSS performed better than FWS and VSS at reducing pollutants from the greenhouse effluent, while FWS showed a high accumulation of NO_2^-. N_2O emissions were 7 and 59 times higher in FWS than in HSS and VSS.

What is the expected impact on horticulture?
- The use of HSS constitutes a sustainable option to treat greenhouse effluents before disposal, while FWS causes important environmental burden in terms of N_2O emissions. However, sufficient organic carbon should be provided to maintain a complete denitrification and reduction of sulfate.

Introduction

Greenhouse wastewater is rich in nutrients (e.g., NO_3^-, PO_4^{3-}, SO_4^{2-}, Na^+, Cl^-), as a result of intensive use of synthetic fertilizers in the irrigation water (e.g., 200–300 mg N L^{-1}, 50 mg P L^{-1}, and 200 mg S L^{-1}), but contains little dissolved organic carbon (DOC; ~10 mg L^{-1}) (Gruyer et al., 2013a; Park et al., 2008; Prystay and Lo, 2001). The high SO_4^{2-} concentration found in greenhouse wastewater is caused by a substantial use of potassium and magnesium sulfates as low sulfate content fertilizers are not always available or too expensive for producers. Therefore, when closed growing systems are used to recycle drained water in commercial greenhouses, preferential plant ion uptake leads to the buildup of SO_4^{2-}, resulting in an unbalanced nutrient solution (Dorais et al., 2001) (Table S1). Consequently, most growers using a closed system need to discharge around 20% of their unbalanced nutrient solution into the environment. For open growing systems, which are still in place in several countries, including Canada, pollutant ions such as SO_4^{2-}, NO_3^- and PO_4^{3-} are

released in the environment, which constitute important environmental burdens (Prystay and Lo, 2001). Like sulphur-containing fertilizers, Na^+ which is mainly related to the use of fertilizers such as $NaNO_3$ or to the poor quality of the irrigation water also constitutes a major concern owing to their accumulation in the recycled nutrient solution which might reach damaging levels to crops (Rozema et al., 2014). Although Na^+ management is a key issue for some countries, the focus of this study is rather on the removal of NO_3^-, PO_4^{3-} and SO_4^{2-} from greenhouse wastewater before releasing in the environment in order to reduce the environmental footprint of the greenhouse industry.

Technologies such as reverse osmosis and membrane filtration are available to remove undesirable ions, but the operating costs of those technologies are high. Over the last 25 years, constructed wetlands (CWs) have been considered a green, low-energy technique for wastewater treatment (Lee et al., 2009). The use of CWs may offer a sustainable and practical option for removing environmentally harmful nutrients (White, 2013) for open and closed growing systems and may replace the use of lagoons usually used to store the greenhouse effluent. It was also shown that CWs can constitute an efficient, safe and low-cost alternative to control pathogen infestations of greenhouse effluents compared to UV and thermic disinfection (Gruyer et al., 2013b), limiting risk associated to disease propagation when treated water is reused in the crop (Dorais et al., 2001).

Constructed wetlands are designed to take advantage of several processes involved in nutrient removal (Kadlec and Wallace, 2009). Sedimentation, precipitation, adsorption, and assimilation by plants contribute to CW performance, but transformation by microorganisms remains the key process, especially for nitrogen and sulfate. Soluble inorganic nutrients are removed via aerobic processes such as nitrification of NH_4^+ [$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$] and anaerobic processes such as denitrification of NO_3^- [$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$] and reduction of SO_4^{2-} [$SO_4^{2-} \rightarrow H_2S$] (Reddy and DeLaune, 2008; Saeed and Sun, 2012). These processes are affected by the CW redox potential and the availability of electron donors. When the wastewater is low in labile organic carbon (C), such as greenhouse effluent, diverse sources of carbon are supplied to sustain anaerobic processes such as denitrification and SO_4^{2-} reduction in CWs (Gagnon et al., 2010; Gruyer et al., 2013a; Park et al., 2008; Prystay and Lo, 2001).

The CW designs most commonly used to treat wastewater are free water surface flow (FWS), horizontal-subsurface flow (HSS), and vertical-subsurface flow (VSS) CWs (Lee et al., 2009; Vymazal, 2008a, b). The FWS are similar to wastewater ponds or lagoons found on commercial greenhouse facilities collecting run-off nutrient water with wastewater flowing shallowly over saturated soil substrate (Vymazal, 2008b). FWS offer a low construction cost and provide an anaerobic environment for denitrification (Lee et al., 2009; Vymazal, 2008b) and SO_4^{2-} reduction (Postgate, 1984). *Eichhornia crassipes* is used in FWS (Wu et al., 2015), providing a large root area for microbial attachment and increasing denitrification potential when grown in lagoons filled with eutrophic wastewater (Gao et al., 2012; Yi et al., 2014).

Compared to FWS, those with subsurface flow have an advantage under cold climate because treatment occurs at warmer temperatures below the CW surface (Werker et al., 2002). Under a moderate temperature (19°C), HSS were very efficient at removing heavy loads of NO_3^- (300 mg L^{-1}) and SO_4^{2-} (500 mg L^{-1}) from the greenhouse effluent by removing 95–98% of NO_3^- and 98% of SO_4^{2-} (Gruyer et al., 2013a).

Although VSS were reported to be less efficient than HSS to remove NO_3^- load (Lee et al., 2009), large reductions in NO_3^- (86%), PO_4^{3-} (100%), and SO_4^{2-} (63%) were observed when greenhouse wastewater at the commercial level was treated in serial VSS during summer, fall, and winter in Eastern Canada (Lévesque et al., 2011). In HSS, a gravel bed (5 to 20 mm) provides high hydraulic conductivity and poses low risks of clogging, whereas in VSS, sand (0.25 and 4 mm) provides good contact between wastewater and bacteria growing on the medium (Vymazal, 2008a).

Phragmite australis (common reed) largely used in CWs is tolerant of high salinity (Vymazal, 2013a), while *Typha latifolia* (cattail) was found to be more tolerant to high SO_4^{2-} concentrations present in greenhouse effluent (Lévesque et al., 2011). Maximum Na^+ uptake rates by macrophytes in wetland microcosms was estimated at 0.072 g m^{-2} day^{-1}, equivalent to less than 7% of the Na^+ removal from wastewater (Tanner, 1996). However, the macrophytes such as *T. latifolia* and *E. crassipes* were proven to have a good phytodesalinization potential of Na^+ in CWs environment (Rozema et al., 2014; Ajibade et al., 2013; Parwin and Paul, 2019).

Although removing nutrient pollutants from wastewater is essential in terms of disposed water quality, the emission of greenhouse gases (GHGs) such as N_2O and CH_4 from CWs should also be considered as they affect air quality. The production and emission of GHGs in CWs depend on redox potential and N and C contents, factors that are affected by the wastewater source and CW type (Kampschreur et al., 2009; Saeed and Sun, 2012). A meta-analysis of GHG losses from CW wastewater treatment systems concluded that N_2O emissions were similar between CW types but were highly variable within each type (FWS, 0.1 to 15.6 mg m^{-2} d^{-1}, average 3.1 mg m^{-2} d^{-1}; VSS, 0.1 to 10.2 mg m^{-2} d^{-1}, average 3.4 mg m^{-2} d^{-1}; HSS, 0.0 to 21.5 mg m^{-2} d^{-1}, average 5.8 mg m^{-2} d^{-1}) (Mander et al., 2014). Part of this variability was explained by the NO_3^- and labile C concentrations in the wastewater, which affected the end products of denitrification. The NO_3^- load of greenhouse wastewater (6.53–6.90 g N m^{-2} d^{-1}) (Gagnon et al., 2010; Prystay and Lo, 2001) is typically much higher than the effluent loads from other agricultural sources (1–203 mg N m^{-2} h^{-1}, equivalent to 0.02–4.87 g N m^{-2} d^{-1}) (Mander et al., 2014; Vymazal, 2007), and the impact of such NO_3^- concentrations on N_2O emissions from CWs is unknown (Kampschreur et al., 2009).

Even though the potential of CWs for treating various types of polluted water has been largely shown (Gao et al., 2012; Mander et al., 2014; Park et al., 2008; Saeed and Sun, 2012; Wu et al., 2014), it is still unclear which CW system is the best sustainable option for greenhouse effluents. According to the fertilizer commonly used by greenhouse tomato growers, high concentrations of NO_3^-, PO_4^{3-}, and SO_4^{2-} are found in crop effluents as shown by our one-year sampling at commercial greenhouses located in Eastern Canada (Table S1). The objective of the present study was to compare the performance of FWS, HSS, and VSS, with well-proven CW designs of published studied, for the removal of NO_3^-, PO_4^{3-}, and SO_4^{2-} found in greenhouse effluents and the reduction of N_2O emissions in order to reduce the environmental impact of the greenhouse industry when producers need to discharge their unbalanced nutrient solution into the environment. Specifically, a low-cost FWS planted with *E. crassipes* was compared with HSS and VSS planted with *T. latifolia* and filled with gravel and sand, respectively. Because of the anaerobic conditions in FWS and HSS, it was hypothesized that those CWs would be more efficient at removing NO_3^- and

SO_4^{2-} in greenhouse wastewater than VSS, but that FWS and HSS would emit more GHGs.

Materials and methods

Experimental set-up

A total of 36 CWs (height, 0.71 m; length, 1.44 m; width, 0.86 m) were established in December 2008 in a Venlo-type greenhouse (150 m²) located at Laval University (Quebec City, QC, Canada, 46°46'N, 71°16'W). The dimensions of CWs selected in this study were based according to the previous studies presented in the literature treating greenhouse wastewater (Gruyer et al., 2013a; Gagnon et al., 2010).

The greenhouse air temperature and vapor pressure deficit were maintained at 19.8 ± 0.7°C and 1.56 ± 0.08 kPa, respectively, during the experiment (March to October 2009), and the average temperature of the CWs was 22.1 ± 0.3°C. Three well-proven designs of CWs were compared (Figure 1): FWS, which was areas of open water planted with *E. crassipes* a free-floating plant; HSS, which was filled with river

FIGURE 1. Section view of the three constructed wetlands used in this study: free water surface flow (FWS), horizontal-subsurface flow (HSS), and vertical-subsurface flow (VSS).

gravel (ø 1.27 cm; 40% porosity) with a 10-cm section at the inlet filled with coarse gravel (ø 1.905 cm; 48% porosity) and were planted with *T. latifolia*; and VSS CWs, which was filled with 15 cm of coarse gravel (ø 1.905 cm; 48% porosity) and 45 cm of sand (ø 0.5–1 mm; 32.5% porosity) separated by a geotextile and were planted with *T. latifolia*. The *T. latifolia* rhizomes were planted at the same time of the establishment of CWs in December 2008 and were spaced 15 cm apart. The *E. crassipes* were previously grown in a pond and five free-floating plants were transferred in the each FWS at the establishment of CWs. The CWs were placed in three random blocks, each containing four randomly placed CWs per treatment for a total of 12 experimental units per block and 12 replicates per treatment.
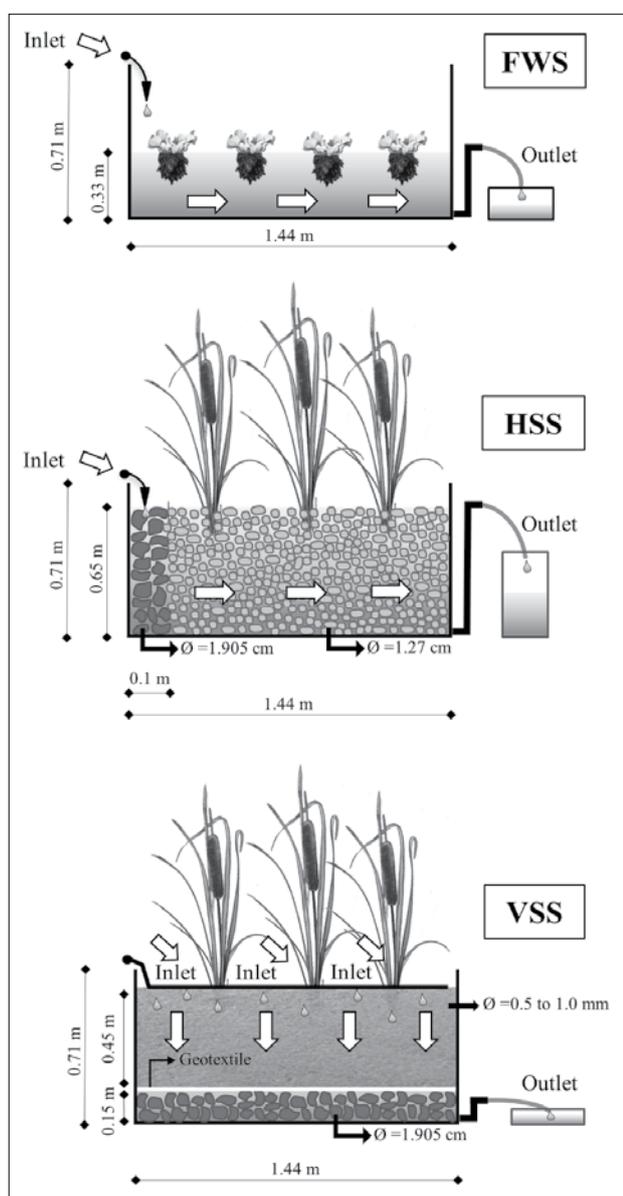
To simulate commercial greenhouse-irrigation management (batch feeding system), seven irrigation events (15 min per irrigation) were applied during the day. Wastewater was distributed to each unit using pipes (5/3 mm) connected to angled-arrow drippers (FWS and HSS) or drip tape (VSS) for a total of 30 L m⁻² d⁻¹ (FWS), 26 L m⁻² d⁻¹ (HSS), or 22 L m⁻² d⁻¹ (VSS), corresponding to a commonly used irrigation frequency for greenhouse vegetable crops. This inlet loading was different for each CW in order to ensure the same hydraulic retention time, 10 d, for all treatments. This hydraulic retention time was chosen because it was shown to be efficient for treating greenhouse wastewater (Prystay and Lo, 2001) and was representative of the commercial volume of wastewater that has to be treated. Hydrolytic pumps connected to timers-controlled wastewater addition, and the number of drippers or drip tapes controlled the volume added to each type of CW.

Based on average analyses of wastewater sampled for one year in commercial tomato greenhouses located in Eastern Canada, reconstituted greenhouse wastewater was made (Table S1). No sodium fertilizer, such as NaNO₃ was used as nutrient for tomato growth. Therefore, this element was not considered in the reconstituted greenhouse wastewater of this study. Because greenhouse effluents are low in organic C to carry out heterotrophic denitrification, sucrose was added at the inlet of each CW to maintain a C:N ratio of 2.9 (i.e., 400 mg sucrose L⁻¹:139.1 mg total N L⁻¹), which falls within the reported optimal range for N removal efficiency (Misiti et al., 2011; Zhao et al., 2010).

Chemical and physical analyses

Every week, inlet and outlet samples (24-h composite samples of 50 mL) were collected for each of the 36 CWs. Concentrations of NO_3^-, NO_2^-, SO_4^{2-}, and Cl^- were determined using the Dionex ICS-2000 ion chromatography system (IonPac AS18 column and AG18 pre-column; Sunnyvale, CA, United States), and PO_4^{3-} concentration was measured spectrophotometrically (Hitachi model U-1100 UV/vis w/cell; Tokyo, Japan) as described by Murphy and Riley (1962). Every two weeks, NH_4^+, K^+, and Ca^{2+} were determined using Cole Parmer specific electrodes (Montreal, QC, Canada), and Mg^{2+} was determined by atomic absorption spectrometry (Perkin Elmer model 3300; Waltham, MA, United States). Total nitrogen (TN) was calculated as the sum of the three N forms measured (NO_3^-, NO_2^-, and NH_4^+).

Starting in the third week of the experiment, chemical oxygen demand (COD) and biochemical oxygen demand (BOD₅) were determined every two weeks, as described in APHA (2005). Both BOD₅ and COD were measured in the inlets before entering CWs and in the outlets. The temperature in each CW was measured at a 10-cm depth using a thermo-

couple, and the values were averaged each hour (CR10X data logger; Campbell Scientific, Logan, UT, United States). On every sampling date, inlet and outlet volumes of water were measured for each CW unit. To get an accurate estimation of performance for the three types of CWs, inlet or outlet nutrient loading per day (g m^{-2} d^{-1}) were calculated from the raw data by using the nutrient concentrations and the total daily volumes of water measured at the CW inlet and outlet according the following equations:

$$\text{Inlet loading (g m}^{-2}\text{ d}^{-1}) = \left(\frac{\text{Inlet concentration (mg L}^{-1})}{1.000 \text{ mg g}^{-1}}\right) \times \left(\frac{\text{Inlet volume per day (L d}^{-1})}{\text{Surface area of CW (m}^2)}\right)$$

$$\text{Outlet loading (g m}^{-2}\text{ d}^{-1}) = \left(\frac{\text{Outlet concentration (mg L}^{-1})}{1.000 \text{ mg g}^{-1}}\right) \times \left(\frac{\text{Outlet volume per day (L d}^{-1})}{\text{Surface area of CW (m}^2)}\right)$$

The percentage of loading removal was calculated with the values of inlet and outlet loading according the following equation:

$$\text{Loading removal (\%)} = \left[1 - \left(\frac{\text{Outlet loading (g m}^{-2}\text{ d}^{-1})}{\text{Inlet loading (g m}^{-2}\text{ d}^{-1})}\right)\right] \times 100$$

Cumulative NO_3^- and NO_2^- exposure, an integrated measure of nutrients in each CW over time and representing the exposure of the CW microbial population to nitrogen (Burton et al., 2008), was calculated using a linear interpolation of the variation in nutrient (NO_3-N and NO_2-N) loading between the outlet and inlet of CWs among sampling dates.

Measurements of gas emissions

Starting in the third week of the experiment, emissions of N_2O, CO_2, and CH_4 from the CWs were measured every two weeks, before the first daily irrigation, using non-flow-through non-steady-state chambers (Rochette and Bertrand, 2008). Briefly, a clear acrylic frame (height, 0.14 m; width, 0.15 m; length, 1.33 m) was inserted in each CW to a depth of 4 cm under the water in FWS (maintained with cables), 10 cm under the gravel surface in HSS, and 8 cm under the sand surface in VSS, to ensure good airtightness, and was left in place during the experiment. To avoid errors in gas measurement, plant leaves present inside the frame were removed on the day before gas sampling. At the time of measurement, an insulated and vented 15-cm-high chamber was tightly attached to the top of the frame. Air samples (20 mL) were taken through a rubber septum using a syringe at 0, 12, 24, and 36 min after chamber deployment and transferred into pre-evacuated vials (12-mL Exetainer vials; Labco, High Wycombe, BUX, England). Gas concentrations were determined within 7 d with a gas chromatograph equipped with an electron capture detector (Model 3800; Varian Inc., Walnut Creek, CA, United States) and a headspace autoinjector (Combi PAL; CTC Analytics, Zurich, Switzerland). Gas emissions were calculated according to the equation of N_2O-N flux (Rochette and Bertrand, 2008). Flux measurements were performed on four randomly selected replicates for each type of CW, and cumulative gas emissions were calculated by linear interpolation between sampling dates.

Microbial analysis

Outlet samples (50 mL) were collected twice a month in each of the 36 CWs for the estimation of the microbial population using the most-probable-number technique. Bacto tryptic soy broth (BD, Mississauga, ON, Canada) was used to estimate aerobic microorganisms, whereas the growth medium for denitrifiers was prepared as described by Kern and Idler (1999). Water samples were subjected to tenfold serial dilution in 0.1 M $MgSO_4$, and a five-microtube most-probable-number series was used. Each 1.5-mL microtube containing 900 µL of culture medium received 100 µL of the diluted sample. The microtubes were incubated at 22°C for 24 h for aerobic microorganisms and for 14 d for denitrifiers. The growth of aerobic microorganisms was assessed visually by the presence of turbidity. The number of denitrifiers was assessed by determining the presence of NO_3^-, NO_2^-, or both by adding diphenylamine-sulfuric acid coloring reagent and recording the number of positive and negative denitrification reactions (McCarty et al., 2007). The total microbial enzymatic activity was measured by hydrolysis of fluorescein diacetate (FDA) according to the method of Adam and Duncan (2001).

Statistical analysis

The data were analyzed according to a randomized complete block design with 12 replicates and processed by Proc Mixed (repeated measures) in the SAS software package (v. 9.1, 2002–2003; SAS Institute Inc., Cary, NC, United States). Cumulative N_2O and CO_2 fluxes were normalized prior to analyze using natural logarithms and square root transformations, respectively. The three types of CWs were compared for pH and electrical conductivity, mineral concentrations, mineral loadings, cumulative gas emissions, and FDA hydrolysis. The differences between means were established by Tukey's test ($P \leq 0.05$). Pearson's product-moment correlation was performed to assess the linear relationship between two normally distributed interval variables.

Results and discussion

Temperature, electrical conductivity, and pH in each type of CW were constant during the experiment (data not shown), suggesting that the three months of adaptation prior to the start of this study had allowed the CWs to reach their steady state. The outlet pH (6.3) from VSS effluent was significantly lower than FWS (7.1) and HSS (7.0), and was similar to the inlet pH (6.2) from greenhouse wastewater (Table S1). These pH were suitable for most mechanisms of N removal to proceed, with the exception of NH_3 volatilization, being negligible at pH below 7.5 (Vymazal, 2007). Furthermore, nutrient removal performance of all CWs was stable during the 19-week experiment as shown in Figure 2 for N forms ($P > 0.05$). Therefore, nutrient removal analyses were performed for this time period.

Although *E. crassipes* were healthy at the establishment of the experiment and performs generally well in eutrophic water (Gao et al., 2012; Yi et al., 2014), the high NO_2^- concentrations measured in FWS affected their growth, causing them to wither and die after eight weeks. No plant disease was observed. Consequently, results for microbial abundance and activity, and for GHGs were analyzed and presented separately for the first 8 weeks (period 1) and for week -9 to -19 (period 2) as significant differences were observed for CO_2, FDA, COD and BDO_5 in FWS.

Nitrogen removal

The NO_3^- removal was higher ($P \leq 0.05$) in FWS and HSS than in VSS (Table 1). Because of their greater oxygen transfer capacity, VSS offer low conditions for denitrification (Vymazal, 2007), which explains the absence of NO_3^- removal from greenhouse wastewater. FWS and HSS removed 59% of
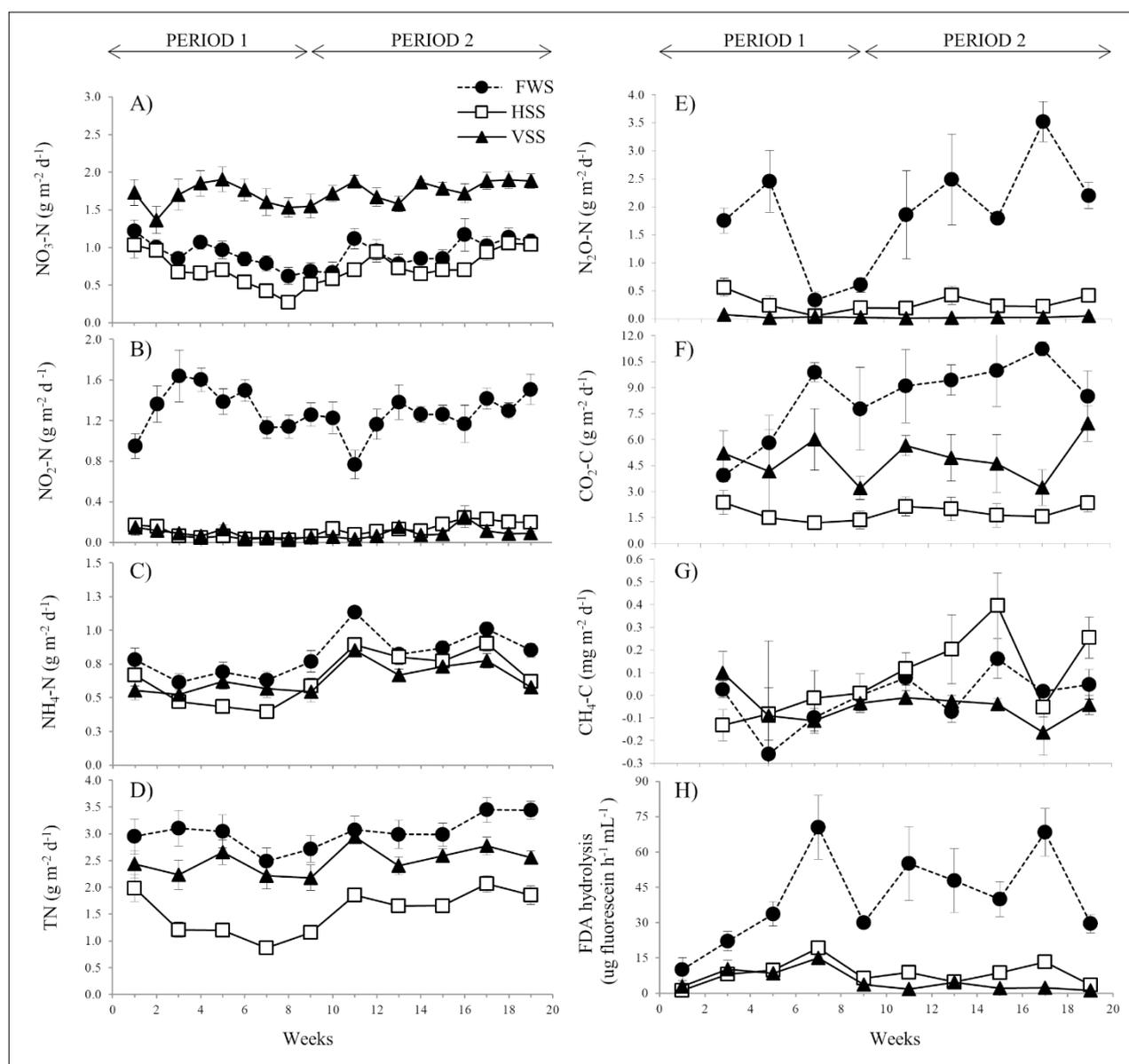
FIGURE 2. Effluent nitrogen (A, B, C, D), gas emissions (E, F, G), and microbial activity as reflected by fluorescein diacetate (FDA) hydrolysis (H) for the three constructed wetlands studied: free water surface flow (FWS), horizontal-subsurface flow (HSS), and vertical-subsurface flow (VSS). Data are means ± standard errors for outlet loadings of NO_3^-, NO_2^-, NH_4^+, and total nitrogen (TN) ($n = 12$), N_2O, CO_2, and CH_4 fluxes ($n = 4$), and FDA hydrolysis ($n = 6$).

the NO_3-N from the wastewater (Table 1), despite removal performance was higher ($P \leq 0.05$) in FWS (1.32 g N m^{-2} d^{-1}) than in HSS (1.06 g N m^{-2} d^{-1}). This difference is related to the fact that the inlet loading was higher in FWS (2.25 g N m^{-2} d^{-1}) than in HSS (1.76 g N m^{-2} d^{-1}) in order to ensure a same retention time of 10 d in all CW types (Table 1). Prystay and Lo (2001) reported lower values (13%–25%) for the treatment of greenhouse wastewater (50 mg N L^{-1}, 20 mg DOC L^{-1}) by HSS planted with *T. latifolia.* They attributed those low values to the low level of organic C. Indeed, higher removal rates (up to 72%) was reported for HSS when sucrose (726 mg sucrose L^{-1}) was added to greenhouse wastewater (228 mg NO_3-N L^{-1}) (Gagnon et al., 2010).

Although the NO_2^- inlet load was marginal in the CWs, the accumulation of NO_2^- was found to be very high in the FWS outlet loading (1.28 g N m^{-2} d^{-1}), while it was very low in HSS and VSS (<0.12 g N m^{-2} d^{-1}) (Table 1). The production of NO_2^- in CWs was also observed in other studies (Alleman, 1985; Kampschreur et al., 2009; Kim et al., 2008). They have

attributed this NO_2^- production to an incomplete nitrification because of the presence of high-free NH_3 inhibition, a lag in the growth of NO_2-oxidizing microbial populations at high pH levels (8.4 to 9.2), and slow NO_2^- oxidation under limiting O_2 availability. In our study, we observed little free NH_3 in the wastewater because of the slightly acidic conditions (Alleman, 1985). The absence of a typical high NO_2^- peak (Figure 2b) in FWS associated with a lag in microbial growth (Figure 2h) suggests that lower O_2 concentration would have resulted in nitrification-related NO_2^- production (Zhang et al., 2011). Lower O_2 availability in the FWS is in line with the high concentrations of COD and BOD_5 observed during the first 8-week experimental period (Figure S1) and with the high cumulative of CO_2 emissions, which was two to eight times greater in FWS than in the other CWs (Figure 2f; Table 2). Higher NO_2^- accumulation and CO_2 emissions in FWS than in HSS and VSS (Figures 2b, f; Table 2) were observed not only in week -9 to -19, when the dead *E. crassipes* biomass might have contributed to organic C inputs, but also in the first 8

TABLE 1. Mean inlet and outlet loadings for the three types of wetlands (free water surface flow, FWS; horizontal-subsurface flow, HSS; vertical-subsurface flow, VSS) adjusted to a hydraulic retention time of 10 days.

Parameters[1]	Inlet loading (g m^{-2} d^{-1})			Outlet loading (g m^{-2} d^{-1})			Δ inlet and outlet loading (g m^{-2} d^{-1})					
	FWS	HSS	VSS	FWS	HSS	VSS	FWS		HSS		VSS	
							Δ	Removal (%)[4]	Δ	Removal (%)	Δ	Removal (%)
NO$_3$-N	2.25 a[2]	1.76 b	1.72 b	0.93 c	0.73 c	1.73 b	1.32 A[3]	(59)	1.06 B	(59)	0.00 C	(NR)
NO$_2$-N	0.08 b	0.06 b	0.06 b	1.28 a	0.12 b	0.09 b	-1.21 B	(NR)	-0.05 A	(NR)	-0.03 A	(NR)
NH$_4$-N	1.10 a	0.88 b	0.83 b	0.80 b	0.64 c	0.63 c	0.28 A	(27)	0.25 A	(28)	0.19 B	(24)
TN	3.46 a	2.78 bc	2.61 bc	3.03 ab	1.52 d	2.48 c	0.42 B	(12)	1.29 A	(45)	0.15 C	(5)
PO$_4$-P	0.30 a	0.23 b	0.23 b	0.12 c	0.08 c	0.08 c	0.18 A	(60)	0.15 B	(65)	0.15 B	(64)
K$^+$	13.63 a	10.89 b	10.23 b	13.20 a	10.29 b	10.43 b	0.32 AB	(3)	0.75 A	(5)	-0.16 B	(NR)
SO$_4$-S	7.73 a	6.08 b	5.91 b	7.44 a	5.84 b	6.14 b	0.28 A	(4)	0.33 A	(4)	-0.19 B	(NR)
Ca^{2+}	5.82 a	4.66 b	4.41 bc	4.88 b	3.65 c	4.85 b	0.90 A	(16)	1.00 A	(22)	-0.45 B	(NR)
Mg^{2+}	4.24 a	4.43 a	4.03 a	3.19 b	2.53 c	2.61 bc	1.05 B	(25)	1.90 A	(43)	1.42 AB	(35)
Cl$^-$	2.07 a	1.64 b	1.58 b	1.91 a	1.17 c	1.40 bc	0.16 B	(8)	0.48 A	(28)	0.19 B	(12)

[1] The means were calculated during the 19 weeks of the experiment from $n=228$ (19 measurements with 12 replicates) for NO$_3$-N, NO$_2$-N, PO$_4$-P, SO$_4$-S, and Cl$^-$ and from $n=120$ (10 measurements with 12 replicates) for NH$_4$-N, total nitrogen (TN; sum of NO$_3^-$, NO$_2^-$, and NH$_4^+$), K$^+$, Ca^{2+}, and Mg^{2+}.
[2] In each row, means followed by different letters are significantly different according to Tukey's test ($P\leq0.05$).
[3] Δ, variation between inlet and outlet loading calculated from raw data.
[4] Loading removal (%) = [1 − (outlet loading/inlet loading)] × 100.
NR, no removal.

weeks, suggesting that *E. crassipes* was also a greater C source than *T. latifolia*, likely via root exudates and turnover. No significant difference between HSS and VSS was observed for NO$_2$-N accumulation (Table 1), while HSS had lower CO$_2$-C accumulation, but higher N$_2$O-N accumulation than VSS (Table 2).

Nitrite can also accumulate under conditions that prevent complete NO$_3^-$ reduction, as observed in Table 1. Such situations were reported in wastewater with high availability of organic C or incomplete anaerobic conditions where NO$_3^-$ is preferred to NO$_2^-$ as the electron acceptor (Alleman, 1985; Kelso et al., 1999; Zhang et al., 2014). Although denitrification is a dominant process in NO$_3^-$-rich environments with low C supplies, dissimilatory nitrate reduction to ammonium (DNRA) is a dominant process under high-C and NO$_3^-$-rich conditions (Reddy and DeLaune, 2008). In previous studies, it was suggested that the accumulation of NO$_2^-$ during the reduction of NO$_3^-$ from freshwater sediments rich in C originated predominantly from DNRA rather than from denitrification at sediment depths (Kelso et al., 1997, 1999). According to our study, the high amount of *E. crassipes* root detritus leaving more organic C substrates in FWS may have promoted an anaerobic environment for the DNRA process in those CWs and contributed to the accumulation of NO$_2^-$.

The removal of NH$_4^+$ varied from 24% for VSS to 27% and 28% for FWS and HSS, respectively (Table 1). These values are about half of the removal performance observed with CWs treating hydroponic wastewater (Gagnon et al., 2010) or greenhouse wastewater (Prystay and Lo, 2001). However, the NH$_4^+$ inlet loading in our study, corresponding to the one observed at the commercial level during one year, was 1.8 times higher than that used by those authors (~0.94 g N m^{-2} d^{-1} compared to 0.51 g N m^{-2} d^{-1}). The VSS was, however, less efficient at removing NH$_4^+$ (0.19 g N m^{-2} d^{-1}) than HSS (0.25 g N m^{-2} d^{-1}) and FWS (0.28 g N m^{-2} d^{-1}) (Table 1).

With respect to TN loading, the HSS were more efficient ($P\leq0.05$) at removing N from greenhouse wastewater than FWS and VSS (Figure 2d). In average, the HSS removed 45% of the three N forms measured (NO$_3^-$, NO$_2^-$, and NH$_4^+$), whereas the FWS removed 12%, and the VSS removed only 5% (Table 1).

Sulfate removal

Only 4% of the SO$_4^{2-}$ from the inlet loading (~6 g S m^{-2} d^{-1}) was removed in the FWS and HSS during the experiment, whereas VSS did not reduce any SO$_4^{2-}$ (Table 1). In contrast to the studies performed by Gruyer et al. (2013a) who observed 95–98% NO$_3^-$ and 98% SO$_4^{2-}$ removal from a reconstituted greenhouse wastewater (300 mg NO$_3^-$ L^{-1} and 500 mg SO$_4^{2-}$ L^{-1}) enriched with sucrose (2 g sucrose L^{-1}) in HSS, the anaerobic environment and the limited source of C in our three studied CWs were not sufficient to en-

TABLE 2. Mean cumulative fluxes of greenhouse gases from the three types of constructed wetlands (free water surface flow, FWS; horizontal-subsurface flow, HSS; vertical-subsurface flow, VSS) during two periods (period 1, first 8 weeks, $n = 36$; period 2, weeks 9 to 19, $n = 72$).

	Flux (mg m^{-2} d^{-1})		
	N_2O-N	CO_2-C	CH_4-C
Period 1[1]			
FWS	1266 a[2]	6763 b	<1 NS
HSS	191 b	1410 d	<1 NS
VSS	31 c	3116 c	<1 NS
Period 2[1]			
FWS	1906 a	9565 a	<1 NS
HSS	252 b	1283 d	<1 NS
VSS	22 c	4388 c	<1 NS

[1] The mean of cumulative output was calculated during period 1 and period 2 on the measurement dates indicated in Figure 2.

[2] In each column, means followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$).

NS, nonsignificant.

sure the SO_4^{2-} reduction by sulfate-reducing bacteria. Denitrification is a process that is energetically more favorable than SO_4^{2-} reduction, and SO_4^{2-} removal usually occurs after NO_3^- depletion (Whitmire and Hamilton, 2005). Consequently, the high remaining load of NO_3^- in our CWs (FWS, 0.93 g N m^{-2} d^{-1}; HSS, 0.73 g N m^{-2} d^{-1}; and VSS, 1.73 g N m^{-2} d^{-1}) (Table 1) prevented SO_4^{2-} reduction by sulfate-reducing bacteria.

Phosphate and others element removal

The efficiency of PO_4^{3-} removal during the experiment ranged from 60% to 65%, and the load removal was higher ($P \leq 0.05$) in FWS (0.18 g P m^{-2} d^{-1}) than in HSS and VSS (0.15 g P m^{-2} d^{-1}) (Table 1). Lower PO_4^{3-} removal efficiency (30%–40%) was obtained in HSS planted with *T. latifolia* when the PO_4^{3-} concentration was 2.7 times higher (32 mg P L^{-1}) than in our study (12 mg P L^{-1}) (Prystay and Lo, 2001), while Gagnon et al. (2010) observed a 30% PO_4^{3-} removal from hydroponic wastewater containing 57 mg L^{-1} of P. As filling materials such as sand and gravel, commonly used in VSS and HSS, provide very low capacity for P sorption and

precipitation (Vymazal, 2007), plant uptake and microbial immobilization may also have contributed for P removal from the wastewater in our study.

The removal of Mg^{2+}, K^+, Ca^{2+}, and Cl^- from wastewater is seldom reported, although their importance for the functioning of CWs (Kadlec and Wallace, 2009) and for plant nutrient balance when treated water is recycled as irrigation water of greenhouse crops. As detailed in Table 1, HSS performed better than VSS ($P \leq 0.05$) by removing 28% and 22% of Cl^- and Ca^{2+}, respectively, compared to 12% and 0%. A better performance of HSS compared with FWS was also observed for Cl^- and Mg^{2+} ($P \leq 0.05$).

Although the Na^+ content was negligible in effluents sampled for one year in commercial greenhouse facilities of our study, its concentration can accumulate in recirculating greenhouse nutrient solutions due to the irrigation water quality and the type of fertilizer used (Rozema et al., 2014). *Typha latifolia* used in two types of the investigated wetlands was proven to have a good phytodesalinization potential of Na^+ and Cl^- in microcosm and constructed wetland environment (Rozema et al., 2014). Indeed, after five weeks of growth, *T. latifolia* did take up (grams of each ion accumulated per m^2 of microcosm) up to 9.2 g m^{-2} of Na^+ and 27.2 g m^{-2} of Cl^- (equivalent to 12% Na^+ and 23% Cl^- of ions removed from greenhouse nutrient solution). On the other hand, *Eichhornia crassipes* was able to reduce by 70% the Na^+ content in domestic sewage having an initial concentration of 2.3 mg Na^+ L^{-1} (Ajibade et al., 2013), while the removal efficiency was 9% for kitchen wastewater having an initial concentration of 17 mg Na^+ L^{-1} (Parwin and Paul, 2019). The use of hybrid wetland systems combined with large areas of macrophytes having a high potential to accumulate Na^+ (e.g., *T. latifolia* up to 8.2 g Na^+ kg^{-1} dry weight accumulated in biomass, *Atriplex prostrate* up to 21 g Na^+ kg^{-1} dry weight and *Salicornia europaea* up to 38 g Na^+ kg^{-1} dry weight) could then be a sustainable approach to remove the Na^+ content occurring in the nutrient solution of closed growing systems (Morteau, 2016).

Nitrous oxide emissions and microbial activity

Daily N_2O fluxes in FWS varied ($P \leq 0.05$) during the 19-week experiment (300–3500 mg N m^{-2} d^{-1}; Figure 2e). The averaged mean cumulative N_2O emissions in FWS (~1586 mg N m^{-2} d^{-1}) were 7 to 59 times higher than in HSS (~222 mg N

TABLE 3. Mean populations of total aerobic and denitrifying microflora and fluorescein diacetate (FDA) hydrolysis measured at the outlets (period 1, first 8 weeks, $n = 30$; period 2, weeks 9 to 19, $n = 36$) of the three types of wetlands (free water surface flow, FWS; horizontal-subsurface flow, HSS; vertical-subsurface flow, VSS).

	Aerobic microflora[1]	Denitrifying microflora[1]	FDA hydrolysis[2]
	($\times 10^5$)	($\times 10^4$)	(µg fluorescein h^{-1} mL^{-1})
Period 1			
FWS	1.1 (0.3–3.7)	3.0 (0.9–9.9)	34.4 ± 5.6 a
HSS	0.2 (0.1–0.8)	0.5 (0.2–1.6)	9.6 ± 1.2 b
VSS	0.1 (0.0–0.5)	0.5 (0.2–1.7)	8.3 ± 1.3 b
Period 2			
FWS	2.1 (0.6–7.1)	8.3 (2.5–27.6)	45.0 ± 5.9 a
HSS	0.6 (0.2–1.9)	1.1 (0.3–3.8)	7.1 ± 0.9 b
VSS	0.5 (0.1–1.6)	0.9 (0.3–2.9)	2.2 ± 0.3 c

[1] For aerobic and denitrifying microflora, means are most probable number mL^{-1}, and values in parentheses represent the low and high confidence limits (95%).

[2] The means ± standard errors followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$).

m^{-2} d^{-1}) and in VSS (~27 mg N m^{-2} d^{-1}) (Table 2). Compared to daily N_2O fluxes by agricultural soils (0.2 to 540 mg N m^{-2} d^{-1}; Hénault et al., 2012), those by FWS were much higher, while the daily N_2O fluxes by HSS (100–600 mg N m^{-2} d^{-1}) and VSS (1 to 100 mg N m^{-2} d^{-1}) (Figure 2e) were similar to or lower than the agricultural soils. The daily N_2O flux is in line with greater microbial activity ($P \leq 0.05$) in FWS than in HSS and VSS (Figure 2h, Table 3). The population of aerobic microflora was 4 to 10 times higher (Table 3) and released substantially ($P \leq 0.05$) more CO_2 (Table 2) in FWS than in HSS or VSS, as indicated by the higher FDA hydrolysis ($P \leq 0.05$) observed in the FWS (Table 3). Several enzymes can hydrolyze FDA and are involved in the decomposition of many types of tissue (Adam and Duncan, 2001). The high FDA activity, microbial counts, and CO_2 emissions suggest the presence of substantial decomposition activity in the FWS throughout the experiment, indicating an additional C source for microbial activity. The negligible CH_4 production in our CWs (<1 mg C m^{-2} d^{-1}; Figure 2g; Table 2) indicates that the redox threshold below which methanogenesis occurs was not reached (Laanbroek, 2010), and microorganisms involved in the reduction of NO_3^- had consumed most of the C available.

In comparison with FWS, the low N_2O emissions (Figure 2e; Table 2) and the high efficiency at removing NO_y (NO_3-N + NO_2-N) loading in HSS (53%) than in FWS (5%) (Figures 2a, b; Table 1) show a greater denitrification potential in HSS. Contrary to HSS, the high redox potential in the VSS, as shown by the active nitrification, likely inhibited the activity of denitrifiers. This observation is in line with the high NO_3^- accumulation and low N_2O emissions in the VSS (Figures 2a, e), resulting mainly from the activity of strictly aerobic chemolithotrophic bacteria (Vymazal, 2007).

Although the O_2 concentration and COD:N ratio influence

N_2O emissions, the high concentration of NO_2^- is the most important operational parameter leading to N_2O emissions in wastewater treatment (Kampschreur et al., 2009; Kelso et al., 1997, 1999; Saeed and Sun, 2012; Zhang et al., 2014). Indeed, we observed a positive correlation ($r^2 = 0.870$; $P < 0.001$) between cumulative N_2O flux and NO_2^- exposure in our CWs (Figure 3). Similarly, a strong positive relationship between N_2O flux and NO_2^- exposure ($r^2 = 0.945$) was observed for VSS treating synthetic wastewater with different types of external C sources (Zhang et al., 2014). Under limited O_2 concentration, it was also reported that a NO_2^- concentration higher than 50 mg N L^{-1} results in increased N_2O production (Castro-Barros et al., 2016), which is in line with our FWS results where the outlet concentration of NO_2^- was ~57 mg N L^{-1}. As N_2O is produced predominantly via the incomplete denitrification of NO_3^- in CWs (Lind et al., 2013), the removal of NO_3^- in our CWs was also correlated ($r^2 = 0.483$; $P < 0.001$) with cumulative N_2O flux (Figure 3).

The sharp decrease in N_2O emissions and FDA hydrolysis at week -8 and -9 (Figures 2e, h) suggests that a change in microbial populations occurred in FWS. As *E. crassipes* were healthy at the beginning of the experiment, the long exposure to a high concentration of NO_2^- had a phytotoxic effect (Oke, 1966), resulting in gradual plant death, affecting microbial activity, such as denitrifying microflora (Table 3).

Conclusions

Among the three types of CWs, with well-proven designs, HSS was the most efficient at removing N (1.29 g N m^{-2} d^{-1}), followed by FWS (0.42 g N m^{-2} d^{-1}), while VSS (0.15 g N m^{-2} d^{-1}) had poor performance. Although the FWS removed 59% of the NO_3-N from the inlet, they exhibited incomplete NO_3^- reduction, producing a very high concentration of NO_2^- in the
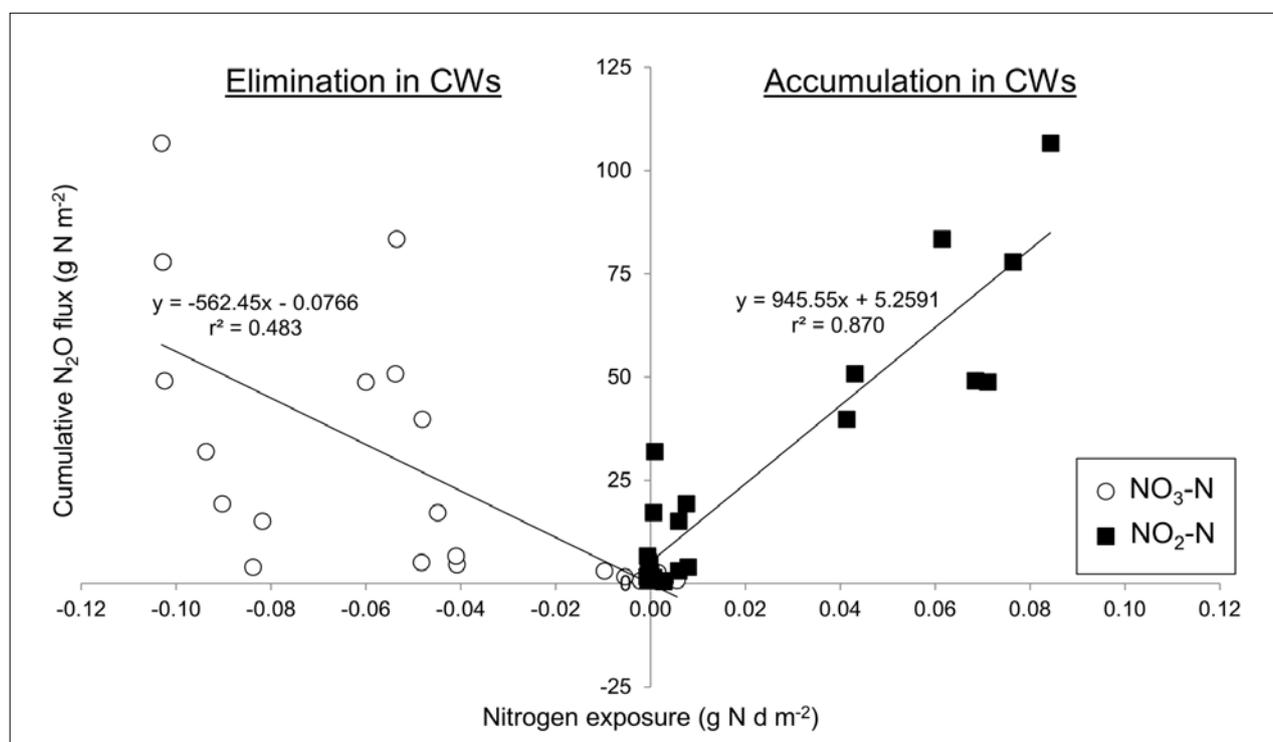
Figure 3. Relationship between N exposure forms and cumulative N_2O flux from the three constructed wetlands (CWs) studied: free water surface flow, horizontal-subsurface flow, and vertical-subsurface flow. Both NO_3-N and NO_2-N exposure are calculated as the sum of the daily mean of the loading variations between the outlet and inlet of the CWs throughout the experiment (19 weeks). The negative and positive values of NO_3-N and NO_2-N show the elimination and accumulation of these elements in the CWs, respectively.

outlet. In addition, the sustained high emissions of N_2O from this type of CW are a drawback in terms of increased global warming. Because denitrification and DNRA are both mechanisms involved in the reduction of NO_3^- in CWs, it is crucial while monitoring the elimination of NO_3^- to simultaneously monitor the accumulation of NO_2^- and the production of N_2O, in order to obtain a high wastewater treatment performance.

In contrast with previous studies (Misiti et al., 2011; Zhao et al., 2010), a C:N ratio of 2.9 was not high enough to ensure the complete removal of NO_3^- and, consequently, no substantial SO_4^{2-} load removal in the three CWs was observed. On the other hand, all studied CWs showed comparable P removal (average of 63% PO_4-P).

Although these commonly used CWs performed well for treating various types of wastewater, our study clearly showed that the best design for sustainable performance for the treatment of greenhouse wastewater highly loaded with nutrients was the HSS, confirming our hypothesis for NO_3^- removal, but not for SO_4^{2-} reduction due to a limitation of the carbon source. The HSS also had significantly lower NO_2^- accumulation and N_2O emissions than FWS, thus resulting in a lower environmental impact. Our study also showed that the use FWS or lagoons to store wastewater coming from the greenhouse crops, constitutes an important environmental burden in terms of N_2O emissions. Moreover, *E. crassipes* is not adapted to treat greenhouse wastewater rich in nutrients. Our previous study using three serial VSS CWs and treating greenhouse wastewater at the commercial scale shown a high efficiency to remove nitrate and phosphate from wastewater by up to 90% and 100%, respectively (Lévesque et al., 2011). So, although HSS has shown a good efficiency to remove NO_3^- from greenhouse wastewater with low N_2O emissions during treatment, the use of multiple and hybrid (VSS combined to HSS) CWs operated in series should be tested to improve the efficiency of removing nutrients, such as NO_3^- and SO_4^{2-} than single HSS or VSS CWs (Vymazal, 2013b).

In conclusion, this study has shown that the use of HSS constitutes a sustainable option to treat greenhouse effluents before disposal, while FWS causes important environmental burden in terms of N_2O emissions. However, sufficient organic carbon should be provided to maintain a complete denitrification and reduction of sulfate, and then reduce the environmental footprint related to the management of greenhouse effluents.

Acknowledgments

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SUPPLEMENTAL INFORMATION – TABLE S1. Mineral concentrations of greenhouse wastewater.

Parameters	Effluent[1]
NO_3-N (mg L^{-1})	91.8
NO_2-N (mg L^{-1})	3.2
NH_4-N (mg L^{-1})	44.1
PO_4-P (mg L^{-1})	12.1
K$^+$ (mg L^{-1})	541.6
SO_4-S (mg L^{-1})	313.1
Ca^{2+} (mg L^{-1})	234.3
Mg^{2+} (mg L^{-1})	134.1
Cl$^-$ (mg L^{-1})	83.1
EC (mS cm^{-1})[2]	5.2
pH	6.2

[1] Based on average analyses of wastewaters sampled for one year in commercial greenhouses located in Eastern Canada.

[2] Electrical conductivity (EC).
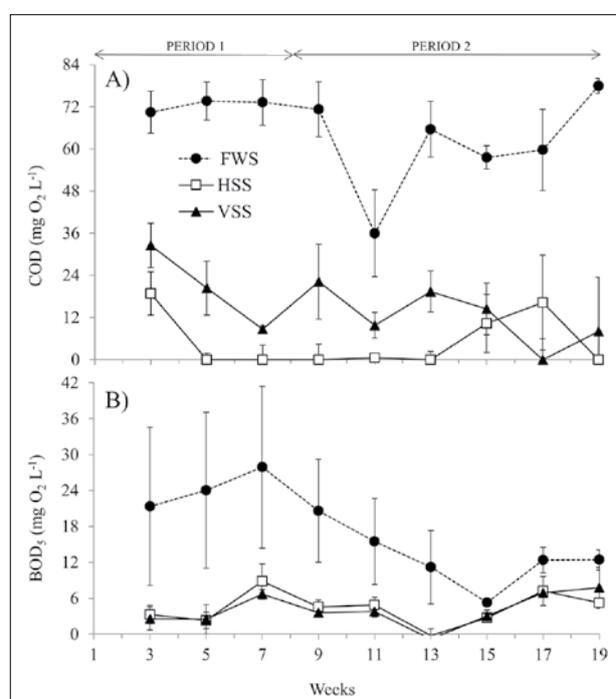
SUPPLEMENTAL INFORMATION – FIGURE S1. Chemical oxygen demand (COD) (a) and biochemical oxygen demand (BOD$_5$) (b) of the effluents of the three constructed wetlands (CWs) studied: free water surface flow (FWS), horizontal-subsurface flow (HSS), and vertical-subsurface flow (VSS). Data are means ± standard errors of outlet concentrations of COD and BOD$_5$ ($n = 6$) in the tested wetlands. The inlet concentrations before entering the CWs were 0.0 ± 1.7 mg COD L^{-1} and 3.6 ± 0.5 mg BOD$_5$ L^{-1}.