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Evaluation of long-term phosphorus uptake by *Schoenoplectus californicus* and *Phragmites australis* plants in pilot-scale constructed wetlands

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ABSTRACT

The aim of this study was to evaluate long-term phosphorus (P) retention in a pilot-scale system made of four horizontal subsurface flow (HSSF) constructed wetlands for wastewater treatment. Each wetland had an area of 4.5 m^2 and was operated for nearly 8 years (2833 days). Two wetlands with *Schoenoplectus californicus* (HSSF-Sch) and the other two with *Phragmites australis* (HSSF-Phr) were planted. The P removal efficiency was 18% for both types of HSSF wetlands. The primary factors that correlated with long-term P retention efficiency in HSSF were phosphorus loading rate (PLR), hydraulic loading rate (HLR) and dissolved oxygen (DO). Average biomass production of HSSF-Phr and HSSF-Sch was 4.8 and 12.1 kg dry weight (DW)/m^2, respectively. The P uptake by the plant increased over the years of operation from 1.8 gP/m2 to 7.1 gP/m2 for *Phragmites* and from 3.2 to 7.4 gP/m2 for *Schoenoplectus* over the same periods. Moreover, the warm season (S/Sm) was more efficient reaching 14% P uptake than the cold season (F/W) with 9%. These results suggest that both plants' P retention capacity in HSSF systems represents a sustainable treatment in the long term.

Novelty statement

Long-term (8 years) phosphorus uptake by *Schoenoplectus californicus and Phragmites australis* and retention in pilot-scale constructed wetlands are evaluated. *Schoenoplectus californicus* is an uncommon species that has been less studied for phosphorus uptake compared to *Phragmites australis*, a globally known species in constructed wetlands. Moreover, some studies evaluating the performance of constructed wetland systems for domestic wastewater treatment are usually limited in time (1–3 years). Therefore, this long-term study demonstrates that the plant plays an important role in phosphorus retention, especially the species *Schoenoplectus californicus*. So, the phosphorus uptake by plants can contribute between 9 and 14% of the phosphorus load of constructed wetland systems in early years of operation.

KEYWORDS

Constructed wetlands; phosphorus removal; *Phragmites australis;* plant uptake; *Schoenoplectus californicus*

Introduction

Constructed wetlands (CW) are a technology for natural, environmental and sustainable wastewater treatment. In terms of P treatment, they have shown removal efficiencies ranging from 10 to 80%, depending on the type of wetland, plant species or supporting media (Vymazal 2007; López *et al.* 2016; Jóźwiakowski *et al.* 2018; Maucieri *et al.* 2020). The P removal mechanisms occur through interactions between supporting media, plants, and microorganisms (Vera *et al.* 2013; Vymazal 2007). The plants play an important role in P reduction in HSSF through their effect on P transformations (Cheng *et al.* 2009). Key functions are associated with physicochemical effects such as oxygen release to the rhizosphere, regulation of hydraulic conditions, and stimulation of productivity, diversity, and microbial activity in the rhizosphere (Burgos *et al.* 2017; Leiva *et al.* 2018; Zheng *et al.* 2020).

The commonly used plants in CW are *Phragmites australis, Typha* spp. *and Scirpus* spp. (Vymazal 2020). To the contrary, *Schoenoplectus californicus* this is a common species in some parts of its range as along the Atlantic and Pacific coasts of the Americas from California to Chile (Macía and Balslev 2000). Similar to *Schoenoplectus californicus* is *Schoenoplectus validus* which is distributed in northern America and has been less widely used in CW (Greenway and Woolley 2001 ; Zhang *et al.* 2008). However, the effect of this plant species on P uptake in CW has not been thoroughly investigated.

The P uptake by plants has shown contradictory results on CW treatment efficiency. Lee *et al.* (2012) found that P uptake by plants was less than 1% in a six-cell surface flow CW planted with three species (*Phragmites australis, Miscanthus sacchariflorus, Typha orientalis*). Wu *et al.* (2013) treated river water in a microcosmic CW and demonstrated a P uptake of 4–22% by four types of plants

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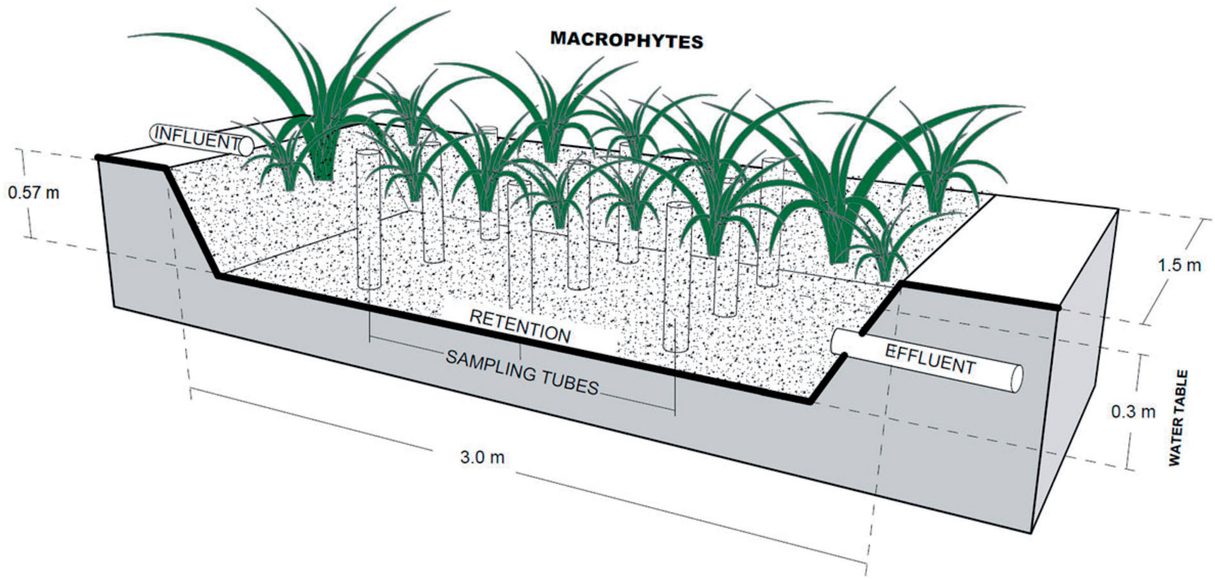

Figure 1. Overview of each pilot-scale HSSF showing dimensions, characteristics, and sampling tubes *in situ*.

(*Trema orientalis, Phragmites australis, Schoenoplectus validus, Iris pseudacorus*). In contrast, Leiva *et al.* (2018) reported 41% P uptake by plants for a HSSF planted with *Cyperus papyrus*. Therefore, P uptake capacity by plants is also influenced by factors such as CW configurations, plant type, and input P concentration.

The CW systems have shown finite retention capacity by plants and supporting media for long-term sustainable P removal (Wu *et al.* 2013). However, most studies evaluating the performance of CW systems for domestic wastewater treatment have been limited in time (1–3 years) (Arias *et al.* 2003; Bolton *et al.* 2019; Tondera *et al.* 2020). Therefore, they have not reached the maximum long-term nutrient treatment capacity, which precludes knowledge of the long-term performance of CWs. Notably, plants need to reach maturity before differences in treatment performance can be detected (Calheiros *et al.* 2007). For example, Zheng *et al.* (2020) showed that P removal in a free surface flow CW increased from about 9% in the first year to 42% in the sixth year. Furthermore, a study by Lv *et al.* (2017) showed that aeration and plants could influence long-term P removal and alter the substrate structure to extend the time of saturation by uptake.

Therefore, this study aims to evaluate P uptake by *Schoenoplectus californicus* and *Phragmites australis* in a general way in CW treatment. Taking into account the basis of in situ and operational parameters, the biomass production by each species, especially the adsorption and retention of P in the long term.

Materials and methods

Design parameters of HSSF pilot-scale system

The treatment system was located in Hualqui, Biobío Region, Chile (36°59′26.93″ S latitude and 72°56′47.23″ W longitude). The influent originated from a wastewater treatment plant in the rural community of 20,000 inhabitants. The wastewater was first subjected to a sand degreaser (630 L) as pretreatment, and then to a septic tank (1200 L) and a pumping tank (630 L) as primary treatment. The resulting wastewater was then stored in a 1000 L distribution tank, where it was distributed by gravity to four HSSF CW (López *et al.* 2016; Sepúlveda-Mardones *et al.* 2017; Leiva *et al.* 2018). Two HSSF wetlands were planted with *Phragmites australis* (HSSF-Phr; HSSF-Phr2) and the other two with *Schoenoplectus californicus* (HSSF-Sch1; HSSF-Sch2).

Figure 1 provides details of the design and characteristics of each pilot-scale HSSF CW. Each HSSF unit had an area of 4.5 m^2, a total volume of 1.28 m^3 and an average height of 0.57 m. The effective volume was 0.76 m^3 and the water table was at a depth of 0.3 m. The support medium used was 19–25 mm gravel with a porosity of 0.57% (Rojas *et al.* 2013; Vera *et al.* 2014). Each HSSF was divided into three zones with a sampling tube in the middle of each zone: Zone A (inlet zone) with the sampling tube 0.65 m from the inlet; Zone B (middle zone) with the sampling tube 1.4 m from the inlet; and Zone C (outlet zone) with the tube 2.25 m from the inlet. The gravel used in this study had the same characteristics as that described in Andrés *et al.* (2021), with a P adsorption capacity of 0.03–0.05 gP/kg.

Monitoring and operating conditions

The system was launched in July 2011. The stabilization time was 85 days during the winter season, and the HSSF ran from 2011 to 2019, for 2833 days in total Monitoring consisted of collecting samples by season (spring (S), summer (Sm), fall (F) and winter (W)) during the entire period of operation. The total number of samples was 74, which are heterogeneously distributed, with a minimum of 2 samples per season and a maximum of 6 samples. Due to the large number of samples, by zones (A, B, C) and by HSSF (HSSF-Phr1; HSSF-Phr2; HSSF-Sch1; HSSF-Sch2), the parameters are presented in periods from I to VIII for each

Table 1. Operating conditions for HSSF-Phr and HSSF-Sch during the different periods of operation.

Days of operation	Period	HLR [mm/d]	OLR [g/m^{2}d]	PLR [gP/m^{2}d]	HRT (d)
85–248	I	19.6 ± 0.0	3.6 ± 0.1	0.3 ± 0.0	8.7 ± 0.0
272–399	II	19.6 ± 0.0	3.6 ± 0.0	0.3 ± 0.0	8.7 ± 0.0
640–855	III	30.7 ± 0.0	4.6 ± 0.6	0.4 ± 0.0	5.6 ± 0.0
999–1286	IV	30.2 ± 0.6	4.6 ± 0.3	0.4 ± 0.0	5.7 ± 0.1
1330–1574	V	29.8 ± 0.0	5.3 ± 1.5	0.4 ± 0.0	5.7 ± 0.0
1707–1945	VI	22.3 ± 1.7	4.9 ± 0.1	0.3 ± 0.0	7.7 ± 0.6
1974–2285	VII	29.8 ± 0.0	6.1 ± 0.9	0.5 ± 0.0	5.7 ± 0.0
2650–2833	VIII	27.8 ± 0.6	3.0 ± 0.8	0.5 ± 0.1	6.2 ± 0.1

HLR: hydraulic loading rate; OLR: organic loading rate; PLR: Phosphorus loading rate; HRT: hydraulic retention time.

year, according to the days of operation elapsed. Each period represents an average of the fall-winter cold season (F/W) and the spring-summer warm season (S/Sm). However, in period II of the year 2012, samples were only collected during the cold season F/W. Moreover, in the year 2018 no samples were collected, so period VIII corresponds to the year 2019. During the periods of operation, average temperatures were 10.4 °C in F/W (March to September) and 15.1 °C in S/Sm (September to March). There were marked seasonal trends in rainfall, with higher average rainfall in F/W of 3.6 mm/d and a minimum of 0.6 mm/d in S/Sm. Evapotranspiration (ET) presented minima between 1 and 1.5 in winter and maxima of 4.0–4.8 mm/d in summer. The meteorological data were provided by the climate explorer of the Climate and Resistance Science Center (Climate and Resilience Center (CR2) 2020), Chile.

Table 1 shows the operating conditions for both HSSF-Phr and HSSF-Sch during the monitoring periods. However, the data are presented in periods because the values between the hot and cold seasons were similar. The hydraulic loading rate (HLR) varied between 19.6 and 30.7 mm/d, the organic loading rate (OLR) in the range of 2.4–6.7 gBOD_5/m^{2}d, the phosphorus loading rate (PLR) was 0.3–0.6 g/m^{2}d and the hydraulic retention time (HRT) varied between 5.6 and 8.7 days. As regards the inorganic load applied in the HSSFs expressed as ammonium and phosphate was 2.1 ± 0.8 gNH_4^+-N/m^{2}d and 0.3 ± 01 gPO_4^{-3}-P/m^{2}d, respectively. Water samples from influent and effluents were collected each season and period, and stored under refrigeration (4°C) until physicochemical analysis. In addition, for each HSSF, the following parameters were monitored on site: temperature (T), pH, oxidation reduction potential (ORP) and dissolved oxygen (DO).

Analytical methods

In situ parameters such as T, pH and ORP were measured through sampling tubes using OAKTON (PC650–480485) portable equipment. DO concentration was analyzed by a portable oxygen sensor (HANNA OXI 330i/set HI 9146-04). The influent samples were in triplicate and were filtered using a 0.45 μm pore size membrane. Physicochemical parameters were determined according to APHA, AWWA, WEF (2012). Chemical oxygen demand (COD) (colorimetric method, 5210-B) biological oxygen demand (BOD_5) (modified Winkler azide method, 5210-B), total suspended solids (TSS) and volatile suspended solids (VSS) (gravimetric method, 2540-D and 2540-E, respectively), phosphate phosphorus (PO_4^{-3}-P) (colorimetric method), total phosphorus (TP) and total nitrogen (TN) (Spectroquant-Nova 60, Merck kits). The term P is presented as TP value in the results of this paper.

Macrophyte analysis

Macrophyte analyses were performed in different seasons and periods. The period II was in spring after 482 days of operation, the period III in winter after 745 days, the period IV in summer after 1286 days, the period VI in summer after 1675 days, and in winter after 1842 days, the period VII in fall after 2080 days and in spring after 2284 days, the period VIII in winter after 2695 days and in summer after 2833 days. Therefore, no leaf analyses were performed in the initial period (I) or period V. Biomass analysis was not carried out in the initial period (I) or in period V, due to the reduced biomass. As for harvesting, 2 manual harvests were carried out in spring, the first in period IV and the second in 2018, which was not considered within the periods.

The *Phragmites* and *schoenoplectus* macrophytes were randomly considered by counting the number of individuals in a PVC quadrat of 0.0625 m^2 (0.25 × 0.25 m) (López *et al.* 2016) to calculate the density (individuals/m^2) by zone A, B and C (Each zone had an area of 1.5 m^2) (Leiva *et al.* 2018). Coverage was determined by measuring the surface area that was unplanted in each of the zones of the HSSF, and subsequently, the percentage of coverage was determined considering the total area. In addition, plant samples of aboveground biomass (stems) and belowground biomass (roots) were taken and a proximal analysis was performed to obtain the concentration of TP in plant tissues. For the total TP content in the plant, only the TP contained in the stem and root were considered. Following the protocol described by Sadzawka *et al.* (2007), the separated biomasses were powdered and analyzed for nutrient content. P content was determined by calcination (500 °C) and then by colorimetry (466 nm). P content in plant tissues was calculated using equation (1) (Lee *et al.* 2012):

$$\text{P content (g/kgDW)} = \text{mass P (g)/dry weight (kg)} \tag{1}$$

Mass balance

Mass balances were calculated in terms of TP. Equation (2) explains the procedure for calculating balances as follows:

$$(Ci \cdot Qi \cdot Do)/ \cdot A - Ci + r - Cp = (Ce \cdot Qe \cdot De)/A \tag{2}$$

where Ci = influent concentration of TP (g/m^2); Qi = input stream wastewater (L/d); Do = operation time (d); A = surface area (m^2); Ci + r=intake by microorganisms or retention in HSSF (g/m^2); Cp = plant uptake (g/m^2); Ce = effluent concentration of TP (g/m^2); and Qe = effluent output stream (L/d). This procedure was modified from Kadlec and Wallace (2009).

Table 2. Physicochemical characteristics of the wastewater influent for HSSF-Phr and HSSF-Sch during periods of operation.

		Average [mg/L] ± SD							
Parameters	Range [mg/L]	Period I	Period II	Period III	Period IV	Period V	Period VI	Period VII	Period VIII
BOD_5	48–348	187 ± 72	184 ± 58	149 ± 50	153 ± 64	179 ± 66	220 ± 85	204 ± 34	108 ± 41
COD	115–465	281 ± 69	324 ± 97	222 ± 101	297 ± 81	293 ± 73	303 ± 93	327 ± 77	212 ± 69
TSS	40–565	259 ± 126	408 ± 141	199 ± 85	177 ± 65	256 ± 92	179 ± 61	282 ± 105	68 ± 24
VSS	33–513	145 ± 100	346 ± 167	146 ± 77	150 ± 60	236 ± 74	211 ± 87	277 ± 117	103 ± 66
TP	10–21	15 ± 3.5	15 ± 1.7	14 ± 2.2	14 ± 1.3	13 ± 1.2	13 ± 2.3	16 ± 2.8	17 ± 1.5
PO_4^{-3}-P	5–17	7 ± 2.2	12 ± 1.8	12 ± 2.3	12 ± 1.5	12 ± 1.5	11 ± 2.3	11 ± 2.2	16 ± 1.3
TN	41–142	73 ± 22	91 ± 37	102 ± 26	99 ± 22	100 ± 21	94 ± 14	107 ± 22	84 ± 40

BOD_5: biological oxygen demand; COD: chemical oxygen demand; TSS: total suspended solids; VSS: volatile suspended solids; TP: total phosphorus; PO_4^{-3}-P: phosphate phosphorus; TN: total nitrogen; SD: standard deviation

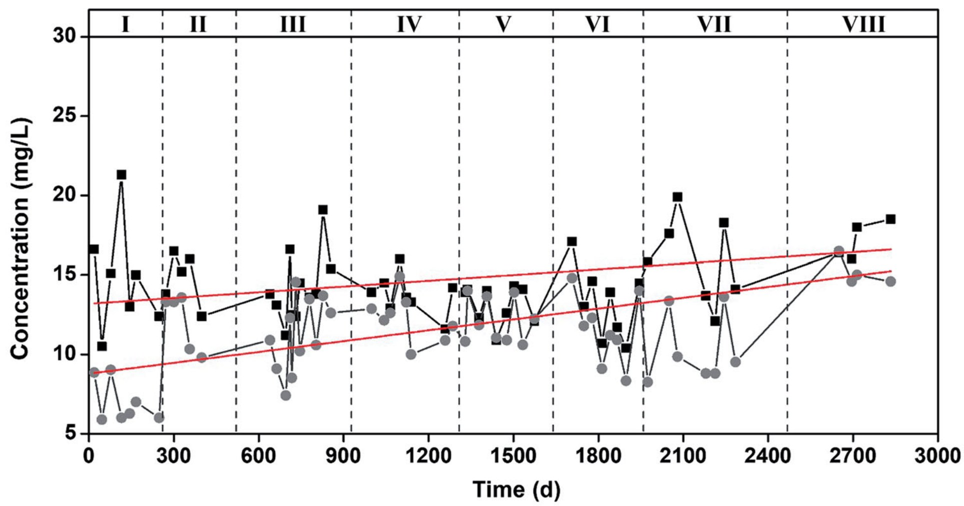

Figure 2. Average concentrations of TP (—■—) and PO_4^{-3}-P (—●—) in the influent during the periods of operation. The red line (—) shows the long-term trend.

Statistical analyses

Statistical analysis compared influent and effluent concentrations, removal efficiency and *in-situ* parameters during different seasons (F/W and S/Sm) and periods (I–VIII). First, the data were subjected to a test of normality (the Shapiro–Wilk test). The following tests were performed: (a) for data with a normal distribution, an ANOVA test, and (b) for data without a normal distribution, a Kruskal–Wallis test. To compare the differences between HSSFs, we used a *t*-test. Principal component analysis (PCA) was carried out with the first three principal components. Data was standardized and P removal efficiencies were correlated with the *in situ* and operational parameters. Then, the Fisher's Least Significant Difference (LSD) was performed to discriminate between periods and stations for P removal efficiency when the ANOVA results were significant. Statistical analyses were carried out using the statistical program Rstudio (Version 1.3.959) with a level of significance of $p = 0.05$.

Results and discussion

Influent characteristics and phosphorus removal efficiency

Table 2 shows the results of the physical-chemical characterization of the influent wastewater throughout the period of operation (2833 days). The influent showed mean BOD_5 concentrations in the range 48–348 mg/L. The mean COD influent concentration were in the range of 115–465 mg/L. The minimum biodegradability coefficient (BOD_5/COD) found in this study was 0.36, the maximum 0.92. These low ratios indicate that the organic matter was simple to degrade (Henze *et al.* 2002). No significant differences ($p > 0.05$) in mean BOD_5 and COD concentrations between seasons and period were observed. The ratio of organic carbon to P in the influent (COD/TP) varied from 11 to 26 mgCOD/mgTP, what is a low COD/TP ratio (10–20 mgCOD/mgTP) that is favorable to phosphate-accumulating organisms (PAO) and P removal (Oehmen *et al.* 2007).

Figure 2 shows the long-term trend of influent TP and PO_4^{-3}-P concentration. TP influent concentrations ranged from 10-21 mg/L, with no significant differences between periods and seasons ($p > 0.05$), while PO_4^{-3}-P presented a higher variation, between 5 and 17 mg/L, with significant differences between periods ($p < 0.05$). In period I, the average PO_4^{-3}-P concentration was 36% lower than the overall average (11 ± 2.9 mg/L). In contrast, in period VIII, PO_4^{-3}-P concentration increased by 42% compared to the overall average. The variation of PO_4^{-3}-P was also expressed in the TP/PO_4^{-3}-P ratio in the influent, with an overall average of 80% of TP, and a range of 50% (period I) to 95% (period VIII). Dzakpasu *et al.* (2015) and Yang *et al.* (2007) reported similar observations, where the range varied over the days of operation. In general, influent wastewater concentrations were similar to those found by Jóźwiakowski *et al.* (2020),

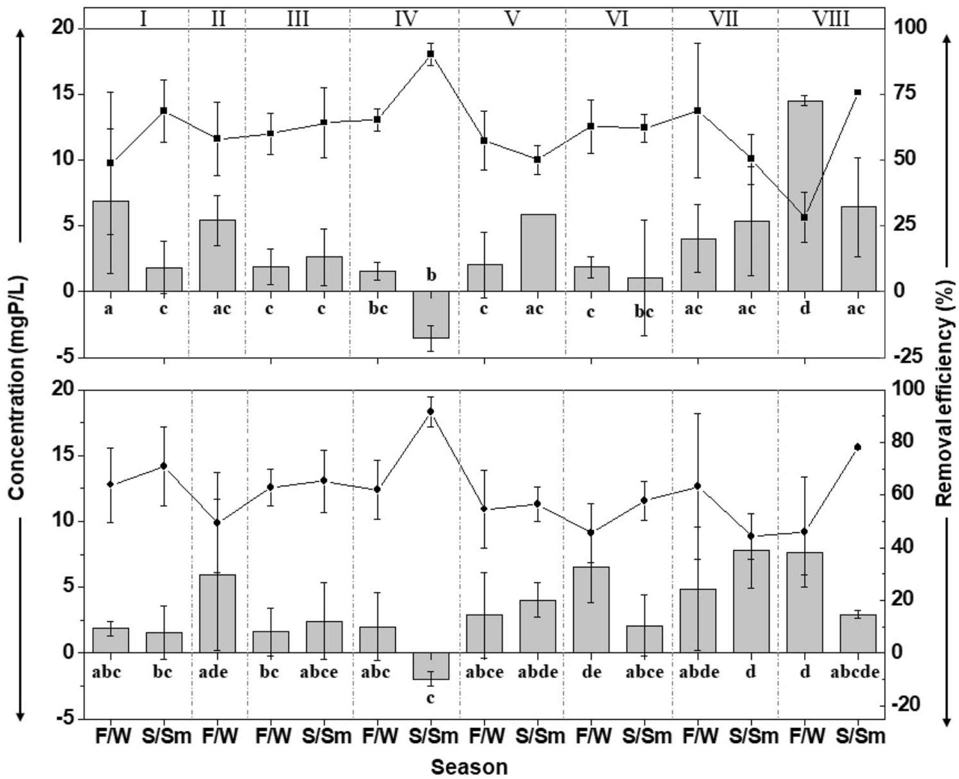

Figure 3. Average concentration (line) and removal efficiency (bar graph) for TP in effluent by season and monitored periods. (a) HSSF-Phr (—■—) and (b) HSSF-Sch (—●—). Different letters indicate significant differences in P retention efficiencies between periods and seasons.

between 5 and 42 mg/L for TP and Bolton *et al.* (2019) between 7 and 30 mg/L for PO_4^{-3}-P.

Figure 3 shows the average effluent concentrations and removal efficiency of P for both HSSF-Phr and HSSF-Sch, respectively, during the F/W and S/Sm seasons during the periods of operation. Effluent TP concentrations averaged 12.2 ± 3.1 mg/L for HSSF-Phr and 11.9 ± 3.2 mg/L for HSSF-Sch, with no significant differences between species. The P values measured in the effluent are still too high for the discharge of wastewater and thus avoid eutrophication of water. The required limits for P discharges from wastewater are between 1-2 mg/L in Europe, 0.5 mg/L in China, protected waters in Europe and the USA 0.05 and 0.01 mg/L, respectively. In Europe, according to Directive 91/271/EC, the requirements depend on the size of the wastewater treatment plant (WWTP) expressed in population equivalent (PE) and also by its sensitivity to eutrophication. Countries such as Italy, Ireland, Spain, Portugal and the United Kingdom, among others, only selected water areas as sensitive (Preisner *et al.* 2020). Other countries, such as Switzerland, require zero discharge, which makes it mandatory to recover P. In Latin American countries such as Chile, the allowable P discharge varies from 2 to 15 mg/L, depending on the dilution capacity of the receiving water body (Committee Report (AWWA) 1970; Zou and Wang 2016 ; WWAP (United Nations World Water Assessment Programme)) 2017). Therefore, in such treatment context, reuse of the treated wastewater by CWs should be encouraged.

Comparison of the two seasons in the HSSF shows that they differ by 14%, with a mean of 12.8 ± 3.2 mg/L in S/Sm and 11.2 ± 3.1 mg/L in F/W ($p < 0.05$). This difference can be attributed to the TP concentration in the effluent, which is accentuated by evapotranspiration in summer. In this case, high temperatures (>20 °C), reduce water solubility, which may be an influential factor in P removal efficiency. This was particularly well expressed in period IV, this period showing the highest TP concentrations in the effluent, with 14.5 ± 2.7 mg/L for HSSF-Phr and 14.2 ± 3.4 mg/L for HSSF-Sch. Overall it represented 21–23% above the annual mean, which was associated with the high temperatures in the S/Sm season. Also, in a study on the effect of seasons on a CW, Jóźwiakowski *et al.* (2020) found that, in spring, TP concentrations were 2.3–4 mg/L higher. Over the periods, no clear trend could be observed for any of the HSSFs.

In both HSSF systems, TP removal efficiency averaged 18%, with greater variation (−10 ± 5.0 to 61 ± 5.8%) for HSSF-Phr than HSSF-Sch (−10 ± 2.8 to 44 ± 0.0%). Therefore, no significant differences were observed between species and seasons. However, significant differences were observed between periods ($p < 0.05$). Generally, gravel based CW has demonstrated low TP removal efficiency, ranging from −40 to 40% (Vohla *et al.* 2011). The data obtained on removal efficiency were irregular, similar to the results reported by Mateus and Pinho (2010) and Shilton *et al.* (2006). In 14 years of operation, a CW studied by Jóźwiakowski *et al.* (2018) achieved highly varied removal of TP (mg/L), with a standard deviation of ±19.9 mg/L as in the present research (±18.5 mg/L).

In situ parameters of HSSF-Phr and HSSF-Sch

Figure 4 shows the behavior of the *in situ* parameters pH, T, ORP and DO for HSSF-Phr and HSSF-Sch. There were no

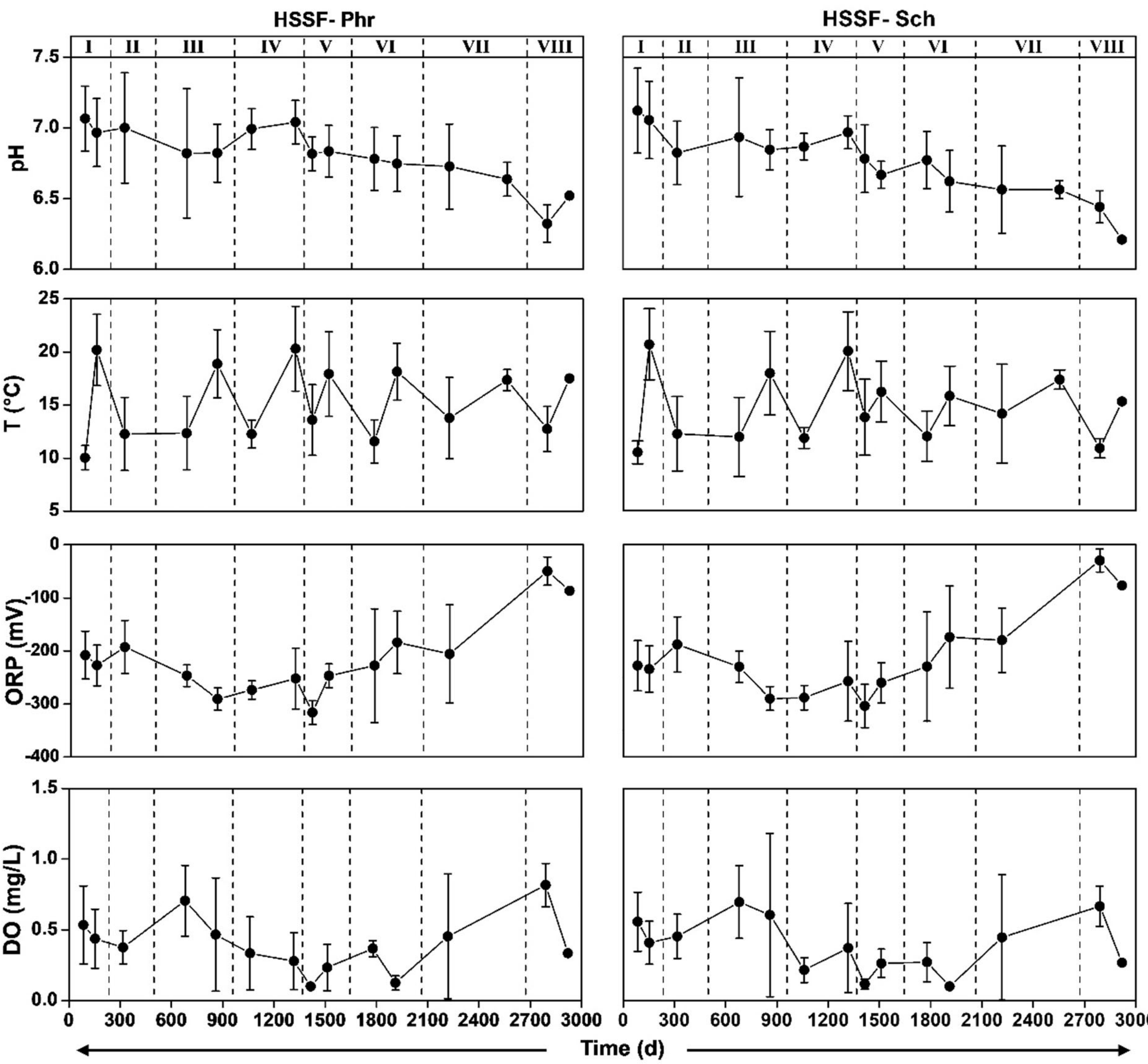

Figure 4. *In situ* parameters such as pH, temperature (T), oxide reduction potential (ORP) and dissolved oxygen (DO) of HSSF-Phr and HSSF-Sch in the different periods of operation.

significant differences between HSSF-Phr and HSSF-Sch for any *in situ* parameters measured. The pH values did not change significantly between F/W and S/Sm, averaging 6.9 ± 0.4 and 6.9 ± 0.3 respectively for both species. However, a significant effect occurred over the course of the experiment, with the initial pH value of 7.0 ± 0.3 decreasing to 6.4 ± 0.1 after 2833 days of operation. Similar results were obtained in a study by Hench *et al.* (2003), where the pH decreased from 7.05 to 6.58. This decrease in pH in CW may be due to the interaction between plants and microorganisms, to the mineralization of organic matter, to the release of ions (H^+) and to the production of organic substances that acidify the medium (Garcia *et al.* 2010; Shan *et al.* 2011). The temperature for both species showed a significant difference between seasons with a mean of 12.4 ± 3.3 °C for F/W and 19.0 ± 3.6 °C for S/Sm. It has been observed that increased temperature may benefit P assimilation by microorganisms. A study conducted in the same pilot-scale HSSF showed a higher growth of bacteria (38%) and archaea (50%) during the S/Sm season than in the F/W season. In addition, the species *Schoenoplectus californicus* showed a higher amount of bacteria (4–48%) and archaea (34–43%) than *Phragmites australis* (López *et al.* 2019). Differences were also observed between period I and period IV, where the difference between the seasons was greater, with 10.6 and 8.2 °C, respectively. However, for the rest of the periods, the difference between seasons was lower, with an average of 4.0 °C. This trend could be due to the development of the macrophytes, which provided a lower coverage during the stabilization period and an increasingly higher coverage over time, along with better thermal insulation (Vymazal and Krőpfelová 2005; Sepúlveda-Mardones *et al.* 2017).

The ORP fluctuated, with mean values from -316.6 ± 23 mV to -29.5 ± 21 mV on both HSSF systems while in operation. No significant differences were observed between seasons, but there were differences between periods of operation. The ORP values for periods III, IV, V were 33.2–58.5 for HSSF-Phr and 22.2–63.1 mV for HSSF-Sch, lower than their averages of -236.0 and -229.1 mV,

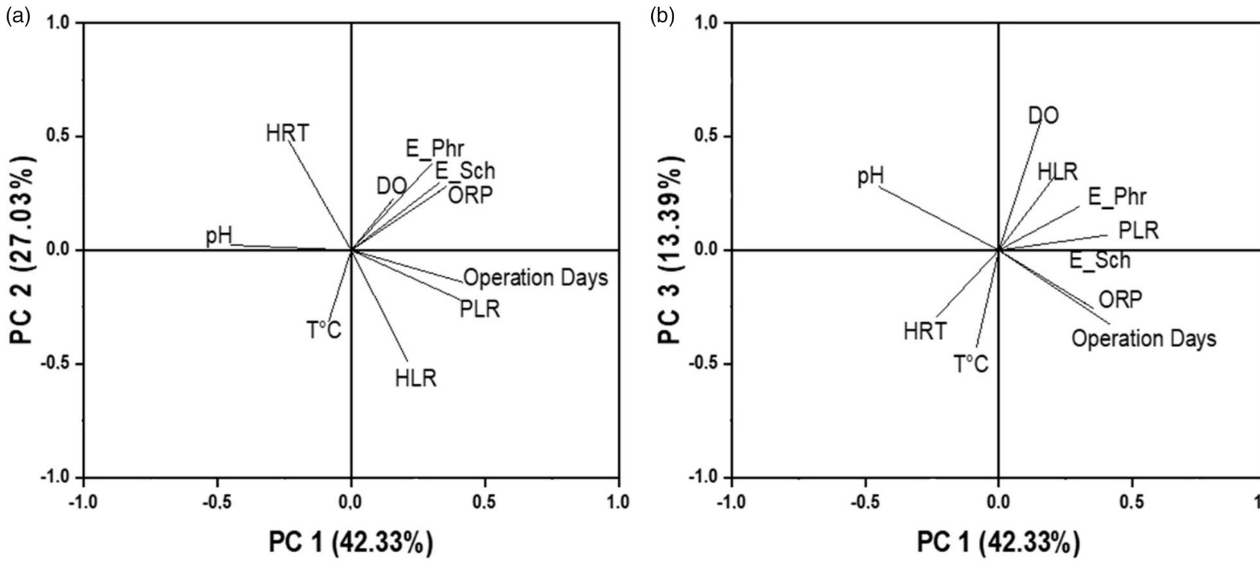

Figure 5. Principal component analysis (PCA) of operational and *in situ* parameters related to Phr (E_Phr) and Sch efficiency (E_Sch), explaining results of (a) principal components PC1 and PC2, (b) principal components PC1 and PC3.

respectively. This increase in ORP may be related to the increase in temperature between 15 and 16 °C in these periods. In contrast, in period VIII, there was an increase in average ORP values, with -54.6 ± 25.9 mV for HSSF-Phr and -38.9 ± 23.3 mV for HSSF-Sch. This behavior of the ORP can be attributed to the variations of the OLR during the period of operation, since these decreased from 4.9 g BOD_5/m^2d (period III, IV, V) to 3.0 $gBOD_5/m^2d$ (period VIII) (Leiva *et al.* 2018). It is also attributed to the decrease in pH to 6.3 ± 0.2, because of the pH decreases there is an increase in ORP (Shan *et al.* 2011). Likewise, DO concentrations showed values of 0.1 ± 0.0 to 0.8 ± 0.1 mg/L (average 0.4 ± 0.3) for both species. A significant difference was observed between periods. In period III, the mean of 0.67 ± 0.4 mg/L was higher than that in period I and II, and in period V, the mean decreased to 1.5 ± 0.1 mg/L DO. The highest DO values (0.6–0.7 mg/L) were achieved during the F/W season. Similarly, Oliver *et al.* (2017) higher DO concentration was measured in HSSF in winter, resulting in higher P retention during that season. Ilyas and Masih (2018), reported that aeration strategies could be responsible for P removal by enhancing DO levels to benefit precipitation and P uptake. In addition, DO concentrations determine the aerobic or anaerobic conditions of the wetland, thus to the associated microorganisms and in turn to the rhizosphere of plants (Ilyas and Masih 2018; López et al. 2019). These HSSF DO concentrations (<0.8 mg/L) and ORP values (−300 and −100 mV) indicate anaerobic conditions.

Influence of parameters on phosphorus removal

Some *in situ* or operational parameters, such as seasonal fluctuations (HLR), PLR and HRT, can affect long-term monitoring. Figure 5 shows the result of PCA of operational and *in situ* parameters related to Phr (E_Phr) and Sch efficiency (E_Sch). The first three principal components (PC1, PC2, PC3) explain 82.75% of the variation in the data. The 42.33% of variance is explained by PC1. This component is positively associated with PLR variables (0.40), operations days (0.42) and negatively with pH (−0.45). Oliver *et al.* (2017), reported a positive relationship between P mass removal efficiency and PLR. In this case, the PLR increased 0.2 g/m^2d over the days of operation. The 27.03% of variance is explained by PC2, which is positively associated with variables such as HRT (0.48) and negatively to HLR (−0.48). The removal efficiency of TP is reported to increase with increased HRT. Maximum removal efficiency values were achieved in period VIII for HSSF-Phr, with an average of $39.5 \pm 30.4\%$ and in period VII for HSSF-Sch with an average of $31.5 \pm 10.4\%$. The increase in period VIII is related to an increase of HRT to 6.2 days and a decrease of HLR to 27.8 mm/d.

Finally, PC3 represented *in situ* parameters with variables like DO (0.57) and temperature (−0.42), which explained less variation in the data (13.39%). DO and temperature can influence CW efficiency as they affect several P biogeochemical processes (Oliver *et al.* 2017). DO oscillated inversely with temperature, due to lower solubility and higher biochemical oxygen demand as temperature increases. Seasonal variation was observed with respect to these parameters. During the F/W season and higher DO concentration (0.6–0.7 mg/L), the average efficiency was higher at $22.5 \pm 18.5\%$ for HSSF-Phr and $21.6 \pm 13.2\%$ for HSSF-Sch, while at the S/Sm season lower DO concentration (0.1–0.3 mg/L), the efficiency averaged 13.1 ± 9.1 and $13.6 \pm 6.0\%$, respectively. Over the last years, the mean DO increased to 0.5 mg/L, hence the removal efficiency (30–40%). Therefore, DO may be an important parameter to consider in CW in the long term. Ilyas and Masih (2018) found a positive relationship between TP and PO_4^{-3}-P when increasing DO levels. They further proved that with redox manipulation and aeration strategies they could rejuvenate CW by recovering phosphorus removal processes. Despite the apparent differences observed, there were no

Table 3. Growth and biomass production characteristics in HSSF-Phr and HSSF-Sch during periods of operation.

		Density [individuals/m^2]		Coverage [%]		Total Biomass [kgDW/m^2]	
Period	Season	HSSF-Phr	HSSF-Sch	HSSF-Phr	HSSF-Sch	HSSF-Phr	HSSF-Sch
II	S/Sm	1200 ± 92	431 ± 367	80 ± 20	55 ± 33	1.9 ± 0.3	1.3 ± 1.2
III	F/W	3610 ± 2005	2279 ± 9	52 ± 32	81 ± 10	4.2 ± 2.2	4.1 ± 0.1
IV	S/Sm	981 ± 981	2366 ± 371	65 ± 38	86 ± 14	1.1 ± 0.4	5.3 ± 0.8
VI	S/Sm	1113 ± 206	2764 ± 28	52 ± 32	96 ± 7	1.4 ± 0.1	5.4 ± 0.2
	F/W	2815 ± 297	5472 ± 209	69 ± 27	100 ± 0	3.2 ± 1.0	8.9 ± 1.0
VII	F/W	4681 ± 506	5807 ± 347	92 ± 14	92 ± 13	2.5 ± 0.8	4.9 ± 0.8
	S/Sm	3372 ± 809	6970 ± 710	78 ± 16	98 ± 6	1.5 ± 1.1	6.0 ± 0.9
VIII	F/W	1255 ± 388	1760 ± 80	74 ± 19	100 ± 0	1.4 ± 1.0	3.0 ± 0.1

HSSF-Phr: units planted with *Phragmites australis*; HSSF-Sch: units planted with *Schoenoplectus californicus*. F/W: fall/winter, S/Sm: spring/summer, DW: dry weight.

significant seasonal differences, due to the high variability of the efficiencies in the seasons throughout the periods, which is evidenced by the standard deviations. The same pattern was observed in Lee *et al.* (2012), Oliver *et al.* (2017), and Jóźwiakowski *et al.* (2020).

Biomass production

Table 3 shows the growth characteristics and total biomass production of HSSF-Phr and HSSF-Sch during the periods of operation. Biomass production was significantly different between species, with a mean of 2.2 ± 1.1 kgDW/m^2 for HSSF-Phr and 4.9 ± 2.2 kgDW/m^2 for HSSF-Sch. The total biomass of HSSF-Sch was twice that of HSSF-Phr, with an average percentage of cover of 89 ± 15% compared to 70 ± 14%. In this research, the biomass production of HSSF-Phr was similar to that reported by the studies of Maucieri *et al.* (2020) and Liu *et al.* (2012), which is in the range of 1.6−5.7 kgDW/m^2, as reported by Vymazal and Krőpfelová (2005). As for HSSF harvesting or pruning, the first one took place in period IV, which had negative effects on the HSSFs, decreasing biomass, and consequently resulting in P release (−10% for both HSSFs) in the S/Sm season in that period. Frequent harvesting has been observed to reduce biomass by 27–32%, and may influence CW performance (Ingrao *et al.* 2020). On the other hand, harvesting strategies have been proposed to improve long-term performance in CW. Therefore, between 2 or 3 harvests may benefit plant productivity or environmental conditions for growth (Zheng *et al.* 2018).

No significant seasonal differences were found, with a F/W coverage of 71% for HSSF-Phr and 93% for HSSF-Sch, and a S/Sm cover of 68 and 83%, respectively. In the S/Sm season of period IV, HSSF-Phr was affected by aphids, with a cover declining to 65 ± 38% and biomass decreasing to 1.1 ± 0.4 kgDW/m^2 in HSSF-Phr. Meanwhile, HSSF-Sch was not affected by aphid attack and biomass reached 5.3 ± 0.8 kgDW/m^2, representing a 27% increase compared to the previous period. Biomass production showed no correlation in P removal in both species (HSSF-Phr = 0.14; HSSF-Sch = 0.36). However, in the case of HSSF-Sch, when a biomass production of 8.9 kgDW/m^2 was reached, P removal efficiencies were higher than average with a range of 24.5–38.8%, during the period VI and VII. Regarding the relation between biomass production and P uptake, a moderate correlation was found with 0.42 for HSSF-Phr and 0.66 for HSSF-Sch. This behavior was shown for *Schoenoplectus* in period VI where the biomass production reached 8.9 kgDW/m^2 and an P uptake of 7.2 g/m^2. Vymazal (2020), indicated in a study of 4 HSSF in operation between 9 and 25 years that the high production of biomass (6.6 kgDW/m^2) is the main reason for P uptake.

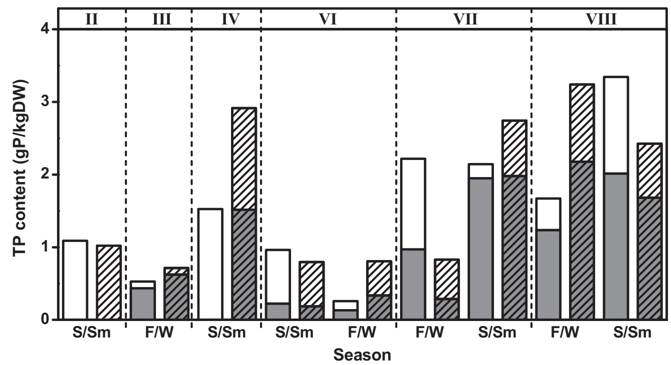

Figure 6. Distribution of TP content in different plant tissues, specifically in roots (■) and stems (□) for *P. australis* (without any line, left) and roots (▨) and stems (▨) for *S. californicus* (with line, right) in the HSSF system.

Plant phosphorus uptake

Figure 6 shows the TP contents in different plant tissues for both HSSF systems during the monitoring period. The total TP content of the dry plant biomass averaged 1.53 ± 0.95 gP/kgDW for *Phragmites* and 1.72 ± 0.8 gP/kgDW for *Schoenoplectus*, with no significant differences between species. The TP values in plant tissues were in the range of 0.2–4.0 gP/kgDW, as reported by some authors in the literature, varying according to the plant species, soil conditions, water availability, nutrient load and other factors (Malecki-Brown *et al.* 2010; Shan *et al.* 2011; Liu *et al.* 2012; Rycewicz-Borecki *et al.* 2017; Vincent *et al.* 2018). As for TP distribution in plant tissues, it was lower in stems than in roots, reaching 36% of total biomass for *Phragmites* and 48% for *Schoenoplectus*. The results found in this study, between 0.1 and 1.5 gP/kgDW for stems and 0.1–2.0 gP/kgDW for roots, are similar to those of Shan *et al.* (2011), who reported a TP content of 1.4 gP/kgDW in *Phragmites* stems and 1.8 gP/kgDW in its roots. However, the values for *Schoenoplectus* in this study, 0.1–1.4 gP/kgDW for stems and 0.2–2.2 gP/kgDW for roots, were lower than those of 2.1–3.2 and 1.7–1.7 gP/kgDW, respectively, found by Zhang

Table 4. Phosphorus mass balance for HSSF-Phr and HSSF-Sch according to periods of operation.

Period	Influent [gP/m^2]	Effluent [gP/m^2]		Retention [gP/m^2]		Plant uptake [gP/m^2]	
		HSSF-Phr	HSSF-Sch	HSSF-Phr	HSSF-Sch	HSSF-Phr	HSSF-Sch
I	31.8 ± 11.8	22.2 ± 9.5	25.1 ± 6.8	5.2 ± 2.2	2.4 ± 0.4	–	–
II	36.7 ± 0.0	27.6 ± 0.0	23.5 ± 0.0	21.7 ± 0.0	33.3 ± 0.0	1.8 ± 0.0	1.3 ± 0.0
III	39.7 ± 5.1	30.3 ± 8.7	31.4 ± 9.6	4.7 ± 0.4	3.6 ± 1.3	1.6 ± 0.0	3.0 ± 0.0
IV	55.0 ± 2.7	55.5 ± 12.4	54.9 ± 14.7	−7.1 ± 15.8	−6.5 ± 18.1	1.8 ± 0.0	5.2 ± 0.0
V	42.7 ± 19.4	32.3 ± 20.8	32.3 ± 17.6	6.1 ± 0.0	6.1 ± 3.2	–	–
VI	37.0 ± 10.1	34.9 ± 11.3	28.1 ± 4.4	3.0 ± 2.2	9.8 ± 9.1	1.9 ± 0.8	5.6 ± 2.2
VII	48.9 ± 24.2	25.8 ± 15.4	23.5 ± 14.7	8.0 ± 1.3	10.3 ± 2.1	4.5 ± 1.6	6.6 ± 3.7
VIII	41.1 ± 29.7	23.6 ± 24.4	26.3 ± 22.5	10.7 ± 2.1	8.0 ± 0.2	7.1 ± 1.3	7.4 ± 0.3

(–): no values; HSSF-Phr: units planted with *Phragmites australis*; HSSF-Sch: units planted with *Schoenoplectus californicus*.

et al. (2008). This may be because the concentration of nutrients in the plant gradually decreased and became diluted as biomass increased (Lee *et al.* 2012). Furthermore, these results differ from those of Zhang *et al.* (2008), who found that P content was higher in stems than roots. This difference may be attributable to the phase the plants were in. When plants go into dormancy, nutrients like N and P are translocated from the stems to the roots and rhizomes. In contrast, in growth phase, P content is concentrated in stems (Liu *et al.* 2012; Zheng *et al.* 2020).

The range of P uptake by plants was 1.3–7.9 g/m^2 for HSSF-Phr and 1.3–9.3 g/m^2 for HSSF-Sch during the monitoring period. These values are similar to those found by Vincent *et al.* (2018), with an average of 4.5 gP/m^2 for *Phragmites,* and by Greenway and Woolley (2001), with an average of 2.8 gP/m^2 for *Schoenoplectus*. Likewise, the maximum values of P uptake by roots were 4.8 gP/m^2 for *Phragmites* and 6.7 gP/m^2 for *Schoenoplectus* in this study. No significant differences were observed between F/W and S/Sm seasons. *Phragmites* averaged 3.9 ± 2.2 gP/m^2 for F/W and 3.2 ± 2.7 gP/m^2 for S/Sm, while for *Schoenoplectus,* the P uptakes in F/W and S/Sm were 5.4 ± 2.2 gP/m^2 and 5.4 ± 3.0 gP/m^2, respectively. In contrast, López *et al.* (2016) in the same HSSF but during the first three years of operation observed a significant difference where P uptake was higher in S/Sm than in F/W. From these contradictions it can be inferred that some parameters such as operation time are fundamental to CWs. Moreover, other parameters that can influence are the scale of the CW, the plant species, the region where it is built and it climatic conditions. During the last monitoring periods, an increase in P uptake was observed. The P uptake in *Phragmites* increased from an average of 1.8 gP/m^2 in the first three periods (II, III, IV) to 7.1 gP/m^2 in the last period (VIII), while *Schoenoplectus* increased from an average of 3.2–7.4 gP/m^2 in the same periods. These results are consistent with findings by Zheng *et al.* (2020) in a CW with *Phragmites*, where the uptake of P by plants also increased from 5.5 gP/m^2 in 2011 to 15.0 gP/m^2 in 2014. This suggests that plant uptake of P plays an important role in the long-term elimination of P from CWs. This highlights the important role of roots in creating suitable conditions for microbial activity, increasing the surface area of the substrate in the water, oxygenating the environment around the roots and facilitating the filtration and sedimentation of P (Kadlec and Wallace 2009).

Mass balance

Table 4 shows the mass balance of P in HSSF-Phr and HSSF-Sch during monitoring periods with no significant difference between the two HSSF systems. The average annual load of P input was 31.8–55.0 gP/m^2, represented by the TP of influent. The highest P load was in period IV (55.0 ± 2.7 gP/m^2), and resulted in negative P removal efficiency. Some authors, such as Stefanakis and Tsihrintzis (2012) and Oliver *et al.* (2017), have shown a significant correlation between P load and P removal. Zhao and Piccone (2020) observed that, when annual P load was low, annual TP output in the effluent was low. As shown, P at effluent throughout the experiment was 22.2–55.5 gP/m^2 for both HSSF systems. Average P retention was 6.5 ± 8.0 gP/m^2 for HSSF-Phr and higher for HSSF-Sch, with an average of 8.4 ± 11.4 gP/m^2. This value is similar to those found by Shan *et al.* (2011) in a pilot scale HSSF planted with *Phragmites*, resulting in P removal of 8.35 gP/m^2. In addition, P uptake by the plant reached values of 1.3–7.4 gP/m^2. According to the literature, P concentration in plant tissue varies from 0.1 to 19 gP/m^2 depending on species and location, and also varies during the season (Vymazal 2007). As for the values expressed annually, the average P load was 78 ± 22 g/m^{2}year with a minimum of 37 g/m^{2}year and a maximum of 111 g/m^{2}year. The effluent from HSSF-Phr had a lower P load with 58 ± 25 g/m^{2}year, while HSSF-Sch with 62 ± 22 g/m^{2}year. The P removal load was 20 ± 16 g/m^{2}year and 21 ± 15 g/m^{2}year for HSSF-Phr and HSSF-Sch, respectively. These values are lower than those presented by Vymazal (2007) in full-scale HSSF with retention values of 45 g/m^{2}year as a function of an average input P load of 141 g/m^{2}year. However, our results were superior to those presented by Dzakpasu *et al.* (2015) treating domestic wastewater with a low P input load 16 g/m^{2}year, obtaining a P retention of 15 g/m^{2}year.

Figure 7 shows the proportion of influent TP through the different phosphorus removal pathways for the season F/W and S/Sm in (a) HSSF-Phr and (b) HSSF-Sch. From the total P entering the influent (100%), it is distributed into P that is uptaken by plants, P that is retained in the HSSF (media support and microbial uptake) and finally P rejected in the effluent. P retention by HSSF was 6.2–19.8%, plant uptake removed 7.8–13.5% of total P input, while P discharge to effluent was 71.9–78.2% of influent P. These results are consistent with P removal efficiency values, which were shown to be higher (8%) in the F/W season than in S/Sm. The effluents showed a lower P content in the F/W season, with

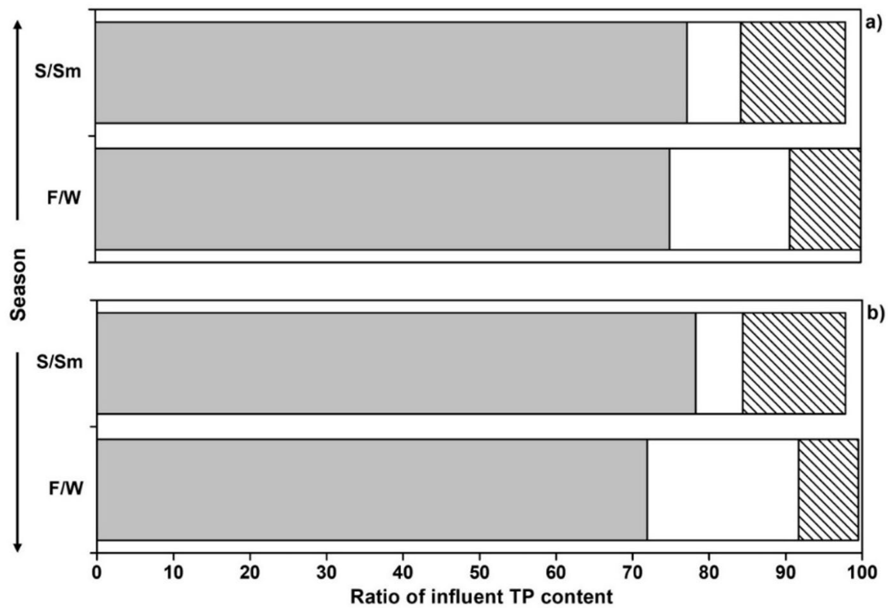

Figure 7. The proportion of influent TP removed by effluent (■), retention (□) and plant uptake (▨) for the F/W and S/Sm seasons in (a) HSSF-Phr and (b) HSSF-Sch.

75% for HSSF-Phr and 72% for HSSF-Sch. There was a small but not significant difference between the seasons in the uptake of P by plants, with 13% in both HSSF in S/Sm, but, in F/W, 9.3% for HSSF-Phr and 7.7% for HSSF-Sch. This distribution is explained by the translocation of the plants, where in cold seasons like F/W, the plants go into dormancy, while in S/Sm they resume their growth, and at that time there is a greater uptake of P due to the development of the plants (Zheng *et al.* 2020). Wu *et al.* (2013) reported plant uptake removed 4.8–22.3% of P input based on mass balance calculation, a rate comparable to that found in the present study. In general, P removal by plants has been reported to be low (<20%), but could be substantial for systems with lower P loads (10–20 gP/m^2). Accordingly, plants may be able to contribute to long-term sustainable P removal (Vymazal 2007).

Conclusions

The average TP removal efficiency during long-term operation (2833 days in total) was 18% for both HSSF. The F/W station obtained a higher efficiency of 22%, while the S/Sm station reached 13%. There were negative efficiencies of −10% in the S/Sm season of period IV which was due to high temperatures (<20°) and higher P input load. The main factors that positively correlated with long-term P retention efficiency in HSSF were PLR and DO, while HLR showed a negative correlation with efficiency. It is recommended that P input load be monitored and regulated according to seasonal flows (HLR). P retention stabilized during the last periods of operation (VI, VII, VIII), which was associated with P accumulation by plants. The species *Schoenoplectus californicus* showed a better performance in terms of biomass production and showed a positive correlation with P uptake, reaching 9.3 gP/m^2 in the last years. P content was higher in the root with 36% for HSSF-Phr and 48% for HSSF-Sch. The P retention in the HSSF varied between 6 and 20% with higher retention in the F/W season. On the other hand, in the S/Sm season, P adsorption by the plants was more significant, contributing 14% in P removal, which is attributed to its resumption in the growth stage. These results highlight the important role of plants in P removal, however, it is important to consider the right conditions to generate a sustainable long-term P treatment in HSSF systems.

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