Which plants are needed for a French vertical-flow constructed wetland under a tropical climate?

R. Lombard Latune, O. Laporte-Daube, N. Fina, S. Peyrat, L. Pelus and P. Molle

ABSTRACT

Plants are essential in the functioning of constructed wetlands. When setting up systems in tropical areas, *Phragmites australis* is not always a good choice because of its invasiveness. In vertical-flow constructed wetlands (VFCWs) fed with raw wastewater, the main role of plants is their mechanical action, which helps prevent clogging of the deposited organic matter. Various species have already been used in some tropical climate studies, but generally not for such systems, and no attempt has been made to screen large numbers of alternative species. Here we describe a method to select species among a hundred studied, along with promising plants tested in batches, and at full scale. Species of the order Zingiberales showed good adaptation to the main stresses generated by VFCWs. They have long vegetative cycles, which may require weed growth control after plantation, but low harvesting frequency. Root systems with long rhizomes such as *Heliconia psittacorum* should take priority to ensure even growth and avoid clumps. To limit the phytosanitary risk with Musaceæ (banana tree), *Canna indica* or *Canna glauca* are preferable. Species of the genus *Cyperus* also demonstrate good adaptation, and could be of interest, especially when a high stem density is required (e.g. planted sludge drying beds).

Key words | anoxic stress, *Canna indica*, *Heliconia psittacorum*, hydric stress, tropical climate, vertical-flow constructed wetland

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INTRODUCTION

Macrophytes are essential components of constructed wetlands (CWs). Among their different roles (Brix 1997), the main one in vertical-flow constructed wetlands (VFCWs) fed with raw wastewater is their mechanical action, which helps prevent clogging of the deposited organic matter (Molle *et al.* 2006). To play this mechanical role, specific physical criteria have to be met, such as a high, even plant density, and sufficient height to enable wind to move the stems. To ensure rapid growth, plants also have to tolerate the vertical filter conditions (high organic feeding, drought during rest periods, etc.). *Phragmites australis* is mainly used in northern latitudes, but is not always suitable for tropical climates because of its invasive or potentially invasive behaviour, or because it is not native. Only a few studies have focused on species choice (Brisson & Chazarenc 2009), and more often on removal efficiency due to nutrient uptake (Tanner 1996; Stottmeister *et al.* 2003). The adaptation of French VFCWs thus required a specific study on plant choice. The method developed addresses two main issues: (i) the broad variety of plants in the tropics and (ii) the distance of the territories from the research centre. A specific method allowing the screening of many alternative species from the literature at full-scale evaluation was needed. It had to be simple enough to be within the capabilities of non-specialists (local technicians), and accurate enough to differentiate plants.

The study was conducted from plant criteria analysis, comparison of batch tests for specific environmental conditions, and full-scale monitoring. The objective was to select plants robust to anoxic or water stress conditions and able to compete successfully with weeds.

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METHODS

Criteria selection

Our first step was to define selection criteria and decide on potential plants to be used. This was done based on a literature review of CW experience in tropical climates (Kantawanichkul *et al.* 2009; Zhang *et al.* 2010, 2015; Calheiros *et al.* 2015; Molle *et al.* 2015), observations in natural local wetlands, and knowledge of local botanists from four French institutes (Conservatoire Botanique National des Mascarins of Mayotte, Herbier of Guyana, Martinique Environmental Office, and Conservatoire botanique of French Antilles).

Fourteen criteria were defined. Species had to be:

- native or already present in the territory (1), based on flora index (Fournet 2002a, 2002b; Funk *et al.* 2007; CBNM 2011, 2012), with no invasive risk (2),
- resistant to both anoxic stress (3) resulting from high organic loads, and water stress (4) between successive feeding periods,
- display fast (5) and even (6) growth (to avoid clumps) to over 1 m height, and have stem diameters in the range 0.5–3 cm (7) to maximize mechanical action and facilitate harvesting,
- perennial, non-woody (8) and with no thorns in aerial parts or sap toxicity (9),
- rhizome-bearing (10) to ensure good recovery after extracting sludge,
- tolerant to the particular growing conditions imposed by CWs, such as direct light (11) or draining substratum made of sand or gravel (12),
- free of water retention on aerial parts (13) that could enhance mosquito propagation, and
- of low fertility, to limit seed propagation (14).

Among the hundred species studied, 25 plants were shortlisted. Some were awaiting validation for particular criteria (stress conditions, edaphic needs, etc.). As full-scale comparison of all 25 species was not possible, intermediate batch test experiments were carried out.

Batch tests

The objective was to assess the anoxic and water stress of the plants in substratum conditions close to those in a VFCW. Twenty species were compared in Mayotte (Indian Ocean) and Martinique (Caribbean Sea). To link results obtained in the different locations, two species were tested in both experimental sites.

Each 3-month-old plant was placed with its clod in a 12-litre pot. The pots were filled with 2/6 gravel (20 cm) and a 3–5 cm layer of compost on the top. A 1-month adaptation period with daily watering was allowed before the tests began.

For each test (control, anoxic stress, permanent water stress, and periodic water stress), five plants of each species were used as replicates. After 1 month adaptation to daily irrigation with treated wastewater, the tests consisted of:

- control: pots were irrigated daily as in the adaptation phase,
- anoxic stress: pots were sealed and regularly filled with raw wastewater to maintain 3 cm ponding and ensure redox conditions below 150 mV (standard hydrogen electrode),
- permanent water stress: pots were not irrigated,
- periodic water stress: pots were irrigated daily with treated wastewater for 1 week and rested for the same period.

For 3 months two observations a week were made to determine four categories for plant health and behaviour:

- osmotic potential based on plant establishment, leaf withering and death,
- photosynthetic potential based on chlorosis, leaf marbling, and necrosis,
- vegetative reproduction (sprout emergence and growth),
- sexual reproduction (flowering).

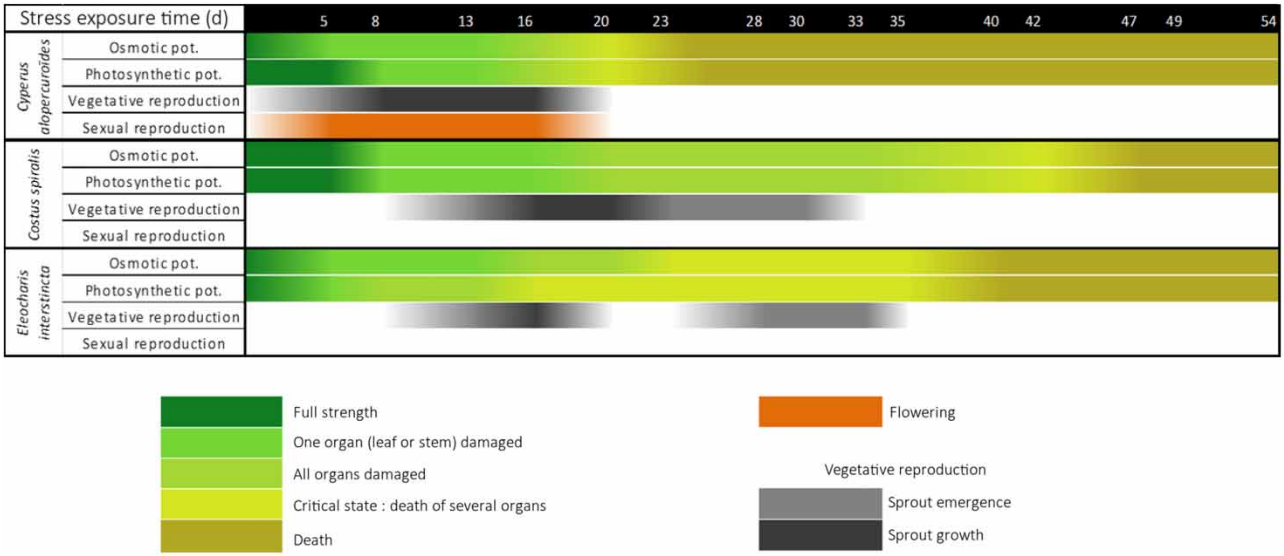
Results are presented in a chronological table for each plant and condition (Table 1). Time of appearance of a behaviour is used here for further discussion.

Full-scale experimentation

Full-scale tests were designed to observe plant growth and dynamics in real conditions including competition with weeds. The impacts of initial plant density and applied load were also studied. The test was done on a 480 population equivalent (PE) French VFCW of 0.8 $m^2 \cdot PE^{-1}$ with a 30 cm 3/6 mm gravel filtration layer in French Guyana. Plants initially present on the VFCW in operation since 2012 were removed before the experiment. Loads and performances of these treatment systems were studied and are presented in Molle *et al.* (2015). A replica experimentation was set up on Martinique island on a young filter. There was no difference except for *Heliconia psittacorum*.

Five of the most promising species were monitored over 1 year. Figure 1 shows the experimental set-up.

Table 1 | Example of a table of test batch observations (water stress in Martinique)



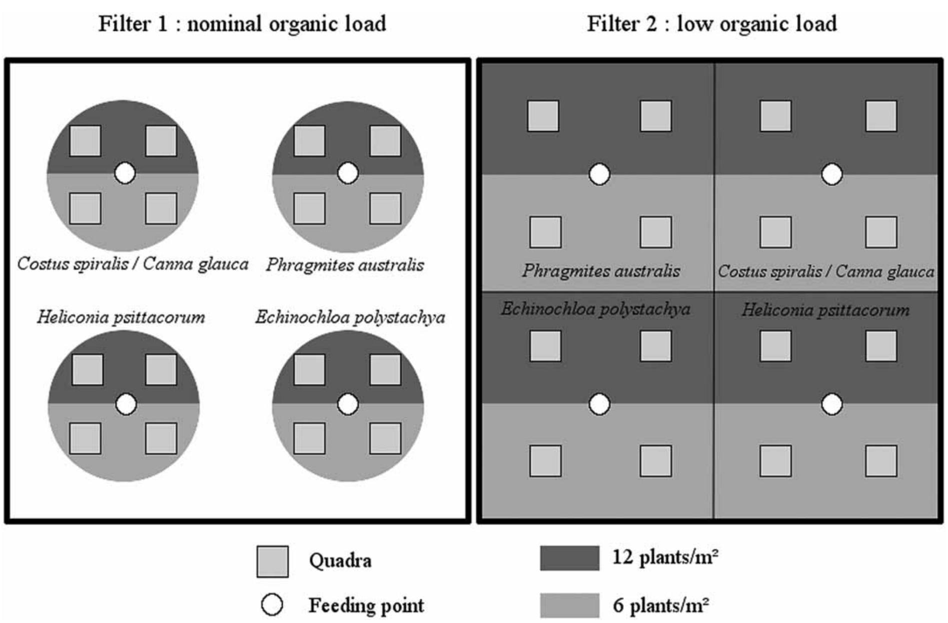


Figure 1 | Experimental configuration.

At each of the four feeding points, two zones of different plant density (6 and 12 plants per m^2) were set up. Four plants were initially planted (see Figure 1, *Phragmites australis* as reference). After 3 months, owing to the poor development of *Costus spiralis*, *Canna glauca* was planted. On one filter, a Plexiglas® separation was fitted (Filter 1) to reduce the infiltration zone to 16 m^2, instead of 48 m^2, in order to reach the nominal chemical oxygen demand (COD) load (300 $g.m^{-2}.d^{-1}$). Four 24 h flow composite sampling monitoring runs were performed during experimentation. Hydraulic loads applied were, respectively, 84 $cm.d^{-1}$ and 28 $cm.d^{-1}$ (225% and 75% of nominal load) and COD loads 231 $g.m^{-2}.d^{-1}$ and 78 $g.m^{-2}.d^{-1}$ (78% and 26% of nominal load) for filters 1 and 2, respectively.

Thirty-two quadrats (50 cm × 50 cm) were set up with duplicates for each condition. Monthly observations were monitored according to Pérez-Harguindeguy *et al.* (2013) for stem density and maximum heights. In parallel, used surface distribution ratio of weeds to plants was analysed. In order to describe vegetative cycles of species, all the physiological modifications (start and end of flowering period, senescence) were observed throughout the experimentation as in the batch tests. Lastly, the root systems of the different plants were described.

RESULTS AND DISCUSSION

Batch tests

Values extracted from the 51 observation tables (see example in Table 1) are shown in Table 2. They represent the time when plants reached a critical state, death (bold font), and the duration of active vegetative multiplication (sprout emergence and growth). Critical states correspond to overall manifestation of symptoms, on whole plant, that might affect mechanical action and growth capability.

Thysanolæna maxima and *Cyperus alternifolius* were used for site comparison. Responses to anoxic stresses in Mayotte and Martinique showed marked differences due to leaks in the Martinique test, which could not ensure permanent ponding. Conversely, water stress showed similar trends for both species. The small differences observed illustrate the uncertainties such tests and data processing may produce. The results were accordingly used to make some comparisons by group, representing the main trends observed during the tests.

For their adaptation to stress, the plants were classified into three groups:

- Group 1 (*Clinogyne comorensis, Heliconia psittacorum, Canna indica, Costus speciosus* and *Costus spiralis*) did not reach a critical state over the experimentation for either stress, except for *Costus spiralis*, which started to show withering and chlorosis under water stress after 38 days.
- Group 2 (species from the *Cyperus* genus (*C. alternifolius, C. alopecuroides, C. articulatus, C. involucratus,* and *C. papyrus*), *Schoenoplectus litoralis, Eleocharis interstincta, Fuirena umbellata, Thysanolæna maxima* and *Brachiaria decumbens*) reached a critical state in 2 to 3 weeks under water stress, and died before the end of the experimentation. Under anoxic conditions, in most cases original stems died and plants stayed alive because of sprout growth. Within this group, *Thysanolæna maxima* was tested in a French system VFCW in a tropical climate (Molle *et al.* 2015), and was removed not because of stress sensitivity but owing to clumpy growth.
- Group 3 (*Alpinia purpurata, Curculigo angustifolia* and *Hyptis capitata*) did not cope with the growing conditions or the stresses, and died in 1 to 3 weeks. Even controls could often not grow, and died.

The batch tests allow the observation of plant responses to different stresses. The strategy used by plants to cope with stress combines tolerance (immediate physiological and biochemical responses, such as osmotic adjustment and stomatal closure) with stress avoidance mechanisms (long-term development and morphological trait modifications, such as decreased leafing) (Pagter *et al.* 2005). Our experimentations did not enable us to study plant tolerance, but showed adaptation to the stresses. After 3 days under anoxic stress, all the species had withered. Plants in the third group could not modify their morphology, and died. Species in the second group limited their leaf area by reducing the number of leaves; initial stems died, and plants stayed alive through sprout growth. The first group holds the most interest, because plants did not suffer excessively from the stresses. They displayed strong tolerance and/or adaptation mechanisms that did not interfere with their regular growth. These plants are of the tropical botanic order Zingiberales.

Outside stress adaptation, several species did not meet the criteria defined at the outset of the study, and so were discarded. *Cyperus articulatus*, *Schoenoplectus litoralis* and *Eleocharis interstincta* have a simple aerial part without leaves. *Fuirena umbellata* is a small (60 cm) delicate plant. *Brachiaria decumbens* is a tropical grass-like forage plant. These characteristics limit their mechanical action on the deposit layer.

After batch tests, *Cyperus* genus and Zingiberales remained alternatives to *Phragmites australis*.

Full-scale experimentation

To observe their behaviour in a real environment, by full-scale monitoring, three Zingiberales were studied (*Heliconia psittacorum*, *Canna glauca* and *Costus spiralis*) and two Poaceæ: *Echinochloa polystachya* (not available for batch test, but identified as an alternative of interest in

Table 2 | Batch test results, values extracted from the observations tables (see Table 1)

		Anoxic stress			**Permanent water stress**			**Periodic water stress**		
	Species	**Osmotic regulation**	**Photosynthetic potential**	**Days of active vegetative multiplication**	**Osmotic regulation**	**Photosynthetic potential**	**Days of active vegetative multiplication**	**Osmotic regulation**	**Photosynthetic potential**	**Days of active vegetative multiplication**
Mayotte (42 days)	*Thysanolæna maxima*	3–**15**	6–**15**	–	(3) 18–**26**	24–**26**	–			
	Cyperus alternifolius	(3)	5	36	18–**26**	18–**26**	–			
	Cyperus papyrus	(3)	–	33	24–**28**	24–**28**	–			
	Clinogyne comorensis	(3)	–	14	(3)	–	–			
	Curculigo angustifolia	3–**7**	5–**7**	–	3–**13**	9–**13**	–			
	Thysanolæna latifolia	3–**15**	6–**15**	–	(3) 18–**26**	24–**26**	–			
	Heliconia psittacorum	(3)	–	36	(3)	–	20		No data available	
	Alpinia purpurata	3–**7**	5–**7**	2	(3) 24–**26**	24–**26**	5			
	Costus speciosus	(3)	–	14	–	–	–			
	Canna indica	(3)	–	36	–	–	–			
	Schoenoplectus litoralis	(3)	–	28	(3) 28–**32**	28–**32**	13			
	Cyperus articulatus	(3)	–	38	(3) 21–**28**	24–**28**	15			
Martinique (55 days)	*Thysanolæna maxima*	47	47	34	10–**17**	13–**17**	10	47–**54**	–	10
	Cyperus alternifolius	–	–	46	20–**25**	16–**25**	18	–	–	48
	Cyperus involucratus	–	–	48	16–**22**	16–**22**	18	40–**54**	–	50
	Cyperus alopecuroides	47	–	38	18–**22**	18–**22**	18	–	–	39
	Hyptis capitata	45	49	36	6–**9**	6–**9**	4	(6–9) 36–**54**	8–**54**	36
	Fuirena umbellata	–	40	39	13–**25**	11–**25**	15	40–**54**	40–**54**	15
	Eleocharis interstincta	(5–10)	–	44	23–**36**	16–**36**	18	(13–18) (36–42)	–	35
	Brachiaria decumbens	42	45	24	11–**18**	13–**18**	–	(38–42)	–	18
	Costus spiralis	–	–	41	38–**42**	38–**42**	21	–	–	44

Non-bold font corresponds to appearance of critical state. Bold represents plant death. Brackets mean the critical state was followed by recovery to a previous state of impact. Instead of time of appearance and end of sprout growth, the duration of active vegetative multiplication in days is given. Periodic water stress was not applied in Mayotte. Control plants remained strong over the tests, except for some species in the third group.

French Guyana) and *Phragmites australis* used as reference (native to Guyana coastal area). Observations of main physical growth characteristics are presented in Figures 2 and 3.

Poaceæ species showed fast growth. They reached their maximum height in 2–3 months (Figure 3). *Phragmites australis* had the highest density, over 350 stems·m^{-2} after 3 months (Figure 2). *Echinochloa polystachya* reached a hundred stems·m^{-2} after 1 month; counting then became more and more difficult owing to layering, and was stopped at the fourth month. *Phragmites australis* density decreased, and harvesting was carried out in the sixth month. *Echinochloa polystachya* was harvested at the seventh month. Plants recovered their maximal height within 1 month. Stem diameters stabilized after 1 month: 0.5 cm for *Phragmites australis* and 1 cm for *Echinochloa polystachya.* We did not observe any load or planting density impacts on Poaceæ growth.

Zingiberales species grew slowly. After 1 year of monitoring, density and average heights of *Heliconia psittacorum* were still increasing. Density reached 50 stems·m^{-2} after 10 months, while *Canna glauca* took 6 months to reach this value. The filter was 5 years old, and had long remained unplanted because of species experimentation. In contrast, *Heliconia psittacorum* growth on the young filter in Martinique reached 50 stems·m^{-2} after 3 months (Figure 2), and 200 stems·m^{-2} after 7 months. The competition with weeds on French Guyana filter was stronger as the filter was already mature. The applied load affected Zingiberales growth. Under nominal organic load and high hydraulic load, *Costus spiralis* totally disappeared because of rhizome rotting. *Heliconia psittacorum* died on half of their quadrats. After 4 months *Canna glauca* was 3 times smaller than under low loads. The high hydraulic load applied (225% of nominal load) led to permanent ponding, far removed from normal starting conditions.

Zingiberales stems were composed of leaf petioles, producing diameter increases as plants grew. At the end of monitoring, diameters were 2.5–3 cm.

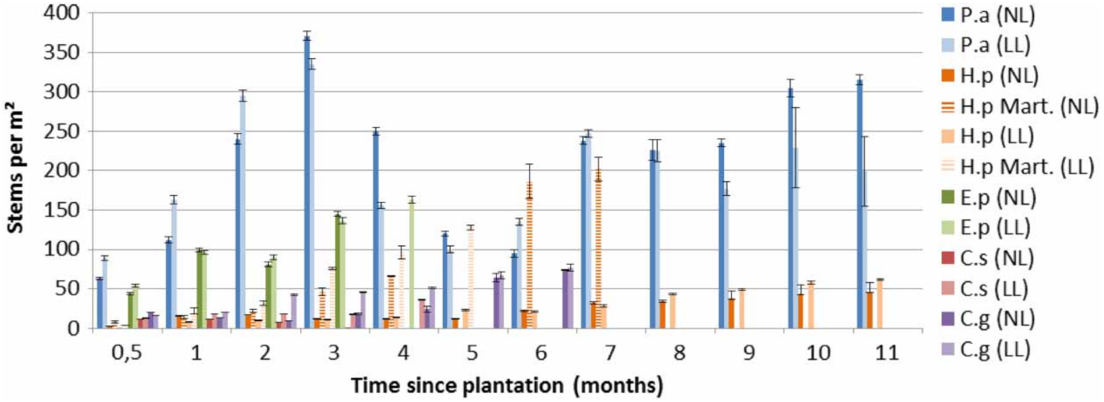


Figure 2 | Plant density in number of stems per square metre for nominal (NL) and low (LL) load. P.a: *Phragmites australis*, H.p: *Heliconia psittacorum*, E.p: *Echinochloa polystachya*, C.s: *Costus spiralis*, C.g: *Canna glauca*, Mart.: Martinique Island.

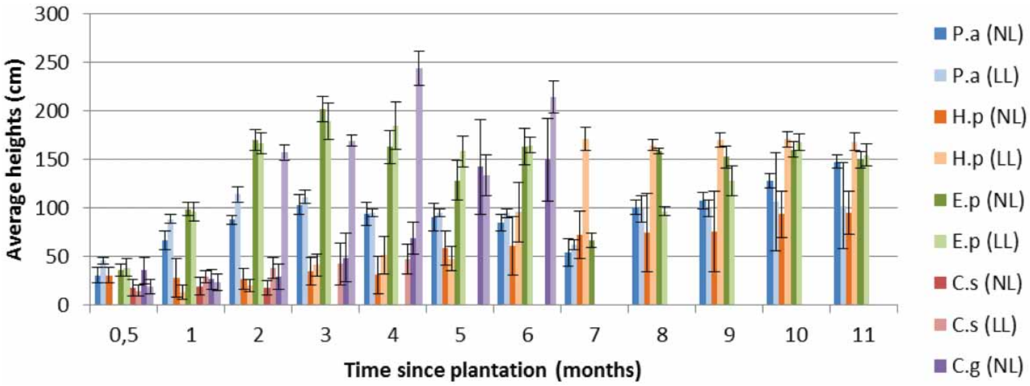


Figure 3 | Average maximum height for each species for nominal| (NL) and low load (LL). P.a: *Phragmites australis*, H.p: *Heliconia psittacorum*, E.p: *Echinochloa polystachya*, C.s: *Costus spiralis*, C.g: *Canna glauca*. P.a, E.p and C.g (LL) were harvested respectively at 6, 7 and 5 months.

There is no clear influence of plantation density (6 or 12 plants/m^2) on growth.

Comparison of growth observation with appearance of the different vegetative stages (Table 3) confirms that Poaceæ had a short vegetative cycle, lasting 4 months for *Phragmites australis* to flowering. The flowering period corresponded to the highest density observed, and senescence started with the decrease in density. Harvesting at the beginning of the sixth month started a new cycle. The density increased even though no flowering was observed. By contrast, a second flowering period was observed for *Echinochloa polystachya* 6 weeks after harvesting. As density monitoring was interrupted and senescence was not observed, its vegetative cycle could not be precisely defined.

The vegetative cycle of Zingiberales was longer than 1 year, and could not be determined as the plants were still flowering at the end of the monitoring period.

Regarding competition with weeds for soil occupation, Figure 4 shows that fast-growing Poaceæ prevailed in 2–3 months. Nevertheless, *Phragmites australis* seems unable to occupy the filters definitively.

Competition seemed initially harder for the Zingiberales. During the first 3 months of the monitoring, especially under low load conditions, maintenance took time to remove weeds from the filters for *Heliconia psittacorum* and *Costus spiralis*. Figure 4 shows no clear impact of this work, as finally after 6 months *Heliconia psittacorum* dominated without help. *Canna glauca* seemed more competitive.

Harvesting Poaceæ took longer than weed control under the low load condition. Concerning maintenance, it seems advantageous to have plants with long vegetative cycles, even though some weed control is required after plantation, rather than plants with short cycles that need harvesting twice a year.

Root system observations gave information on the spreading strategy of species. *Phragmites australis* has an extensive rhizome system. Vegetative multiplication is based on both stolons and sprout emission by rhizomes that guarantee fast colonization and avoid clumpy growth.

The root system of *Echinochloa polystachya* is fasciculated with small rhizomes. It spreads mainly by layering.

Table 3 | Appearance of the different vegetative stages.

time (months)	1	2	3	4	5	6	7	8	9	10	11
Phragmites australis						H					
Heliconia psittacorum											
Echinochloa polystachya							H				
Costus spiralis											
Canna glauca					H						

Light grey represents flowering periods, dark grey senescence. H means harvesting. *Canna glauca* under low load was harvested owing to proliferation of mice. Crossed cells refer to periods without data.

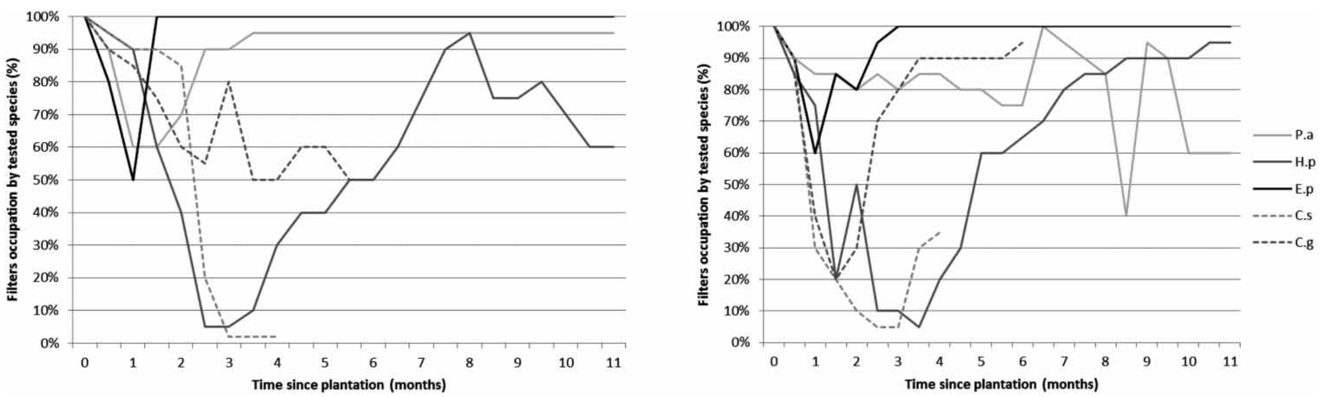


Figure 4 | Time course of filter occupation under high (left) and low (right) hydraulic loads. P.a: *Phragmites australis*, H.p: *Heliconia psittacorum*, E.p: *Echinochloa polystachya*, C.s: *Costus spiralis*, C.g: *Canna glauca*.

Stems and roots rapidly interlaced, preventing weed growth, but also hindering the required mechanical action.

Heliconia psittacorum has a deep root system, with a few long rhizomes. Vegetative multiplication is long and sparse, but even.

Costus spiralis has short thick bulbous rhizomes. They are sensitive to submersion, which favours rot. Plants grow in clumps and spread slowly.

Canna glauca shows a mixed root system with short rhizomes and fasciculated roots. It spreads by rhizomes and grows densely locally, but did not seem to form clumps.

CONCLUSION

To find substitution species to replace *Phragmites australis* for raw wastewater VFCWs under tropical climates, a broad review of tropical plants was undertaken. Among Zingiberales, Heliconiaceæ and Cannaceæ showed good adaptation to the main stresses generated by VFCWs. They have long vegetative cycles that may require weed growth control after plantation, but low harvesting frequency. Stem density and maximum height were still increasing at the end of the monitoring period, and would need to be evaluated. Root systems with long rhizomes like *Heliconia psittacorum* should take priority to ensure even growth and avoid clumps. Musaceæ (banana tree) are also Zingiberales; using *Heliconia psittacorum* close to banana tree plantations could increase phytosanitary risk (hosts for parasite propagation). According to the Martinique phytosanitary observatory, the risk is real for Heliconiaceæ but slight for Cannaceæ. In this case *Canna indica* or *Canna glauca* are preferable.

Zingiberales nevertheless showed low stem density, which might hinder the mechanical action. Consequently, it could be problematic to use them with systems with higher sludge deposit accumulation, such as sludge treatment wetlands. Species of the *Cyperus* genus demonstrated good adaptation to water and anoxic stresses and could be of interest. Their behaviour on full-scale systems has still to be studied for weed competition and even growth.

Other solutions may be found in the large Poaceæ family. The species studied here showed a short vegetative cycle, requiring high harvesting frequency to maximize density and control proliferation (even invasive behaviour). This may limit their interest unless they can be improved by breeding. Layering as a multiplication mechanism reduces mechanical action, and so species with this ability are to be avoided.

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