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# Does the combination of two plant species improve removal efficiency in treatment wetlands?

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# ABSTRACT

We explored the effect of combining two plant species with complementary traits (*Phragmites australis* and *Phalaris arundinacea*), planted sequentially, on the performance of treatment wetlands (TWs). We performed a year-long experiment in mesocosm-scale TWs, aiming to answer the following question: will the combined removal efficiency of the two species equal the average efficiency of the separate monocultures, or will it outperform both monocultures, thus supporting the hypothesis that plant diversity improves pollutant removal in TWs? Root and shoot density and morphology particular to each plant species influenced the redox conditions of the rhizosphere; *Phragmites* rhizosphere oxidizing conditions enhanced nitrification and ammonification processes, while possibly limiting denitrification rate. On the other hand, *Phalaris* reducing conditions seemed to limit nitrification and enhance denitrification and sulfate reduction. Our results revealed that *Phragmites* was equal to or more efficient in removal than *Phalaris* for all pollutants except for nitrate. We found no evidence that combining both species would improve treatment efficiency for any pollutant taken individually, the best monoculture being always as efficient as or more efficient in removal than the combination of two plant species. However, combining both plant species may represent the best tradeoff between overall high pollutant removal and low nitrate level in the effluent.

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# 1. Introduction

Treatment wetlands (TWs) are simplified ecosystems designed for the purpose of wastewater treatment. Their removal efficiency is determined by the biological and physicochemical processes that take place between wastewater and the main components of TWs-substrate, plants and microbial communities. Plants play an important role in TW performance (Brix et al., 2002; Kadlec and Wallace, 2009). They uptake and store nutrients, and enhance microbial mediated processes by increasing the attachment surface area, supplying oxygen to the rhizosphere and providing organic carbon through root exudates (Coleman et al., 2001; Bais et al., 2006; Kadlec and Wallace, 2009; Vymazal, 2011). Given this influential role, the identity and number of plant species in a TW may have an impact on its treatment efficiency. It is generally assumed that plant species should be selected for TWs based on fast growth rate; rapid establishment, usually by clonal propagation; large biomass with a well-developed belowground system and good tol-

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http://dx.doi.org/10.1016/j.ecoleng.2016.02.047 0925-8574/© 2016 Elsevier B.V. All rights reserved. erance of TW conditions. While a wide variety of species possess these traits, in reality, macrophyte species selection for TWs mostly follows established practices and commonly considers only a limited number of species, and TWs are predominantly planted with a single species (Brisson and Chazarenc, 2009; Vymazal, 2011).

It has been hypothesized that combining different plant species in TWs can improve treatment efficiency by means of functional complementarity (Coleman et al., 2001; Fraser et al., 2004; Picard et al., 2005; Zhang et al., 2010; Zhu et al., 2010; Liang et al., 2011). Plant diversity in TWs may increase tolerance to changing conditions as well as stability in biogeochemical process (Eviner and Chapin, 2003). Furthermore, differences in seasonal plant activity, root affinity for microorganism colonization and ability to take up nutrients and organic compounds could result in temporal and spatial compensation, which might improve TW removal efficiency (Coleman et al., 2001; Allen et al., 2002; Zhang et al., 2010; Liang et al., 2011). However, only a few well-replicated experiments have measured the advantages of combining plant species, often with contradictory conclusions. For example, findings by Coleman et al. (2001), Fraser et al. (2004) and Picard et al. (2005) did not support the hypothesis that polycultures would be more efficient than monocultures for nutrient removal, while Zhu et al. (2010) and



Fig. 1. Schematic representation of the different treatments and the experimental systems (Top). Photo of the experimental set-up. Greenhouse, Montreal Botanical Garden. May 2013 (Bottom).

Zhang et al. (2010) found a positive correlation between nitrogen removal and number of plant species in TWs.

We explored the effect of combining two plant species with complementary traits, planted sequentially, on the performance of TWs. Based on the assumption that greater morphological or temporal dissimilarities in plant species may have a better chance of producing complementary interactions, we selected *Phragmites australis* (common reed) and *Phalaris arundinacea* (reed canarygrass) for this mesocosm-scale TW. These two species are commonly used for wastewater treatment (Vymazal, 2011). Both species are from the Poaceae family, and form dense, nearly monospecific stands. Their ability to concentrate nitrogen, phosphorus and trace metal in tissues is also comparable (Vymazal et al., 2007; Vymazal and Kröpfelovaí, 2008). However, prior studies comparing them in TWs revealed differences in seasonality and growth development that suggest they may show some complementarity in functions. While *Phalaris*' growing season begins early in spring, *Phragmites* starts growing later but continues until autumn (Vymazal and Kröpfelovaí, 2005). *Phalaris*' root system is very dense but superficial, while *Phragmites*' root zone is less dense but grows deeper with longer rhizomes (Gagnon et al., 2007). *Phragmites* has more aerenchymae than *Phalaris* (Bernard, 1999), and up to three times the cross-sectional gas space in its rhizomes (Coops et al., 1996). The greater capacity for internal gas transport in *Phragmites* may explain its higher tolerance to deep flooding when compared to *Phalaris* (Waring and Maricle, 2012). N<sub>2</sub> and N<sub>2</sub>O gas emissions also differ greatly between the two species (Augustin et al., 2001; Maltais-Landry et al., 2007). Root distribution and gas exchange capacity may in turn affect bacterial activity. In one microcosm experiment, bacterial density and aerobic respiration rate were significantly greater in the upper soil layer of a *Phalaris* monoculture compared to *Phragmites* (Gagnon et al., 2007).

We performed a year-long experiment in mesocosm-scale HSSF-TWs to evaluate whether planting *Phragmites* (X) and *Phalaris* (O) in four sequential combinations (*i.e.* XX, XO, OX, OO) affects wastewater treatment efficiency. We aimed to answer the following questions: given the removal efficiency of two macrophyte species in monoculture for a particular pollutant, how will the two species perform in combination? Will their combined removal efficiency equal the average efficiency of the separate monocultures, or will it outperform both monocultures, thus supporting the hypothesis that plant diversity improves pollutant removal?

# 2. Methods

# 2.1. Experimental set-up

The experiment was carried out in a controlled greenhouse at the Montreal Botanical Garden, Québec (Canada) (latitude: 45°33'43.00" N; longitude: 73°34'18.50" W). Sixteen mesocosmscale experimental constructed wetlands were set up and evaluated over eight sampling periods between July 2012 and July 2013. Working at mesocosm scale allows for replication, but it may affect plant development through increased exposure to lateral lighting or ramification of the rhizomes against the mesocosm wall (Busnardo et al., 1992). Also, because they are shallow, removal efficiency in mesocosms may overestimate removal efficiency compared to full-scale TW (Caselles-Osorio and García, 2007). Since all our treatments are subject to the same biases, relative differences in productivity or removal efficiency found between treatments remain qualitatively valid, but the quantitative results should not be directly extrapolated to full-scale applications. The temperature of the greenhouse ranged from 35 °C in summer to 5 °C in winter, with an average of 15 °C in autumn and spring. Each experimental system consisted of two coupled plastic basins (each measuring: L 70 cm W 51 cm H 36 cm) operating in series—hereafter referred to as "mesocosm 1" and "mesocosm 2". Each mesocosm was filled up to 2 cm from the mesocosm top with granitic river gravel  $(\emptyset = 10-15 \text{ mm})$ , resulting in a free water volume of 24 L. The experimental systems were planted according to each of the following four plant treatments: monocultures (same species in mesocosms 1 and 2) of *Phragmites* (XX) and *Phalaris* (OO); the combination of the two plant species, with Phragmites in mesocosm 1 and Phalaris in mesocosm 2 (XO); and Phalaris in mesocosm 1 with Phragmites in mesocosm 2 (OX) (Fig. 1). Each plant treatment was replicated four times following a randomized block design. The two species were planted in two distinct mesocosms connected in series, rather than mixing them in a single mesocosm, to prevent one macrophyte from invading the other's space, thus ensuring equal representation of both species throughout the entire experimental period. It also allowed us to monitor water quality at the frontier between the two species (passage from mesocosm 1 to mesocosm 2). Phalaris was planted from seeds and Phragmites from rhizomes collected from Îles-de-Boucherville National Park with permission from provincial authorities (Québec, Canada: 45°35′13.19″ N, 73°29′03.33″ W) in May 2009. For this experiment, we used the European Phragmites (P. australis subsp. australis) rather than the American native Phragmites subspecies (P. australis subsp. americanus).

During the plant establishment period, from spring 2009 to spring 2012, water level was maintained constant at 2 cm below the surface, and plants were fed with a 20:20:20 nutrient solution (percentage, by weight, of nitrogen–phosphorus–potassium:N–P–K) containing microelements. During this period, occasional dieback in some of the *Phragmites* mesocosms necessitated partial

replanting. Each fall, the aboveground portion of the plants was cut and removed. At the beginning of the experimental period, all units were densely colonized with mature macrophyte stands. Wooden sticks fixed to the corners of the mesocosms held strips of cloth that prevented plants from bending outside the surface area of the mesocosms (Fig. 1b). Following plant establishment, mesocosms were fed from April 2012 to the end of July 2013 with 15 Ld-<sup>1</sup> of diluted fish farm sludge amended with nutrients (urea:  $20.2 \text{ mg L}^{-1}$ and mono potassium phosphate:  $10.1 \text{ mg L}^{-1}$ ) at a hydraulic loading rate of  $42 \text{ Lm}^{-2} \text{ d}^{-1}$ . Average influent concentration (mg L<sup>-1</sup>) and (/) load (g m<sup>-2</sup> d<sup>-1</sup>) was TSS 363/11; COD 503/21; TP 37/1.5; TN 139/5.8.

# 2.2. Physico-chemical analyses

Wastewater treatment performance was monitored over eight sampling periods, three times in summer (July-August 2012, July 2013), twice in autumn (October-November 2012) and spring (April-May 2013) and once in winter (February 2013). Inflow and outflow samples as well as intermediate wastewater (outflow from mesocosm 1, which also corresponded to the inflow of mesocosm 2) were collected simultaneously, and the following parameters were measured according to Standard Methods (APHA, 2005): TSS, COD, TN, NH<sub>4</sub>–N, NO<sub>3</sub>–N, TP, S<sup>2–</sup>. The same day, samples were filtered and processed for inorganic dissolved N species and S<sup>2–</sup>. Samples for COD were kept refrigerated and analyzed the day after, and samples for TN and TP were kept frozen until processed for analysis. The outflow was collected in a container connected to the mesocosms, and its volume was measured daily during the sampling periods. Evapotranspiration was calculated as the difference between inflow and total outflow volume of the systems. Removal efficiencies were calculated based on a mass balance. The following parameters were measured in situ during each sampling period: pH and redox potential (Eh) (Oakton Ion Acorn series-pH/Ion C Meter Epoxy electrode), dissolved oxygen (Oakton DO 6 Acorn series), and electrical conductivity and temperature (YSI Environmental, EC 300). Measurements were taken in mesocosms 1 and 2 from interstitial water within pierced PVC tubes buried in the mesocosms. Eh, was measured using Oakton combination electrodes with Ag/AgCl reference cells and calibrated with Orion ORP Standard (+420 mV) to display Eh directly. The readings were taken 15 cm below the surface of the water and 10 min were allowed for stabilization at each measurement. For parameters such as Eh that may vary spatially in depth and from the vicinity of the root to the interstitial water, these measurements allow to compare overall patterns between treatments, but they are not an indicator of all the processes occurring in these heterogeneous environments.

# 2.3. Plant parameters

Root development was monitored visually following each sampling period by 360° belowground images captured with an *in situ* root imager (CI-600, CID Inc.) in transparent acrylic tubes buried in the mesocosms. Following the experimental period, at the end of July 2013, stem length and density were measured, and a portion of the plants (corresponding to 1/4 of the mesocosm area) was cut and dried to estimate aboveground dry biomass. Also, we used a hollow cylindrical drill (40 cm long/10 cm diameter) to extract a complete (34 cm) substrate core from the center of each mesocosm. Roots and rhizomes were separated from the gravel, dried and weighed to estimate belowground dry biomass. Leaf and root samples were collected from each mesocosm and analyzed for nutrient content at the Horticulture Research Center at Laval University (Québec, Canada).



Fig. 2. Example of root development of *Phalaris* and *Phragmites*. 360° belowground pictures (total substrate depth, 34 cm) captured each season with an *in situ* root scanner (CI-600, CID Inc.).

# 2.4. Data analysis

The comparison of removal performance and plant parameters among treatments was performed by one-way ANOVA analyses, after assessments of normality and homoscedasticity had been verified. Further differences between treatments were established with a post-hoc Tukey test at p < 0.05. Statistical analyses were performed using JMP software (JMP<sup>®</sup>, Version 6 for Mac. SAS Institute Inc.). One-way ANOVA tests were performed separately for mesocosms 1 and 2. In mesocosms 1, the performance of each species (*Phragmites* (X) and *Phalaris* (O); n = 16) was compared independently, while in mesocosms 2, each combination of the two species, arranged in series, was compared (XX, OO, XO, OX; n = 16). ANOVA analyses were performed for each sampling period (8), as well as for overall year-round mean removal efficiency.

# 3. Results

# 3.1. Plant parameters

We observed seasonal differences in species phenology. To summarize the parallels between *Phalaris/Phragmites* species-timing respectively: sprouting (early March/mid-March), inflorescence (May/August), maximum height reached (August/October) and senescence (October/late November). *Phragmites* and *Phalaris* showed different above and belowground biomass distributions. *Phragmites* had taller stems at a lower density compared to *Phalaris*, which had thin, shorter stems at very high density (Table 1). However, average dry biomass was similar for both species. Greater belowground biomass was measured for *Phragmites* than for *Phalaris*, although the difference was not significant (Table 1). Regardless of plant species, more belowground biomass was measured in mesocosms 2 than in 1. Both species in mesocosm 2 seemed to produce more root biomass following a mesocosm 1 planted with *Phragmites*, but the difference was not significant. *Phalaris* showed higher nutrient content than *Phragmites*: foliar P content and roots N content were significantly higher in all mesocosms 2 of *Phalaris* monoculture (OO) (Table 1).

Belowground pictures showed differences between the species' root systems and their evolution over the course of each season (Fig. 2). Phalaris' root systems were characterized by a very high density of fine roots, particularly in the top layer of the mesocosms. In contrast, Phragmites had large rhizomes and less dense fine roots, equally dispersed across depths. In spring 2012, following the addition of the reconstituted wastewater inflow, mesocosms planted with *Phalaris* developed a black precipitate at the bottom layer, typical of sulfide production and sulfide iron (FeS) precipitation in anaerobic environments (Kadlec et al., 2000), which was not observed in mesocosms planted with Phragmites (Fig. 2). The concentration of the black precipitate, as qualitatively indicated by the color intensity of the pictures, decreased during winter and increased during summer (Fig. 2). For both Phalaris and Phragmites mesocosms, the pictures show a reddish color close to the tip of the roots, which is most likely due to oxidized forms of iron (Kadlec et al., 2000). The intensity of the reddish color varied according to both the season and the species; in general, it was more abundant during winter and in mesocosms planted with *Phragmites* (Fig. 2).

# Table 1

Plant parameters and *in situ* measurements. Different letters indicate significant differences between treatments. An ANOVA test was performed separately for each of the mesocosms 1 and 2, *n* = 16. Plant parameters were measured in July 2013. *In situ* parameters were measured at each sampling period (8), except for the redox potential, which was measured beginning in autumn 2012 (6).

		Mesocosms 1		Mesocosms 2			
		Phragmites X	Phalaris O	Phragmites XX	Phragmites OX	Phalaris OO	Phalaris XO
Stem density	stems m <sup>-2</sup>	663 <sup>v</sup>	1762 <sup>w</sup>	825 <sup>bc</sup>	750 <sup>c</sup>	1675 <sup>a</sup>	1425 <sup>ab</sup>
Stem length	m	2.2 <sup>v</sup>	1.6 <sup>w</sup>	2.3 <sup>a</sup>	2.0 <sup>a</sup>	1.7 <sup>b</sup>	1.7 <sup>b</sup>
Dry biomass							
Aboveground	g m <sup>-2</sup>	2900	2707	2942 <sup>a</sup>	2707 <sup>a</sup>	2506 <sup>ab</sup>	1965 <sup>b</sup>
Belowground	g m <sup>-2</sup>	339	263	375	347	267	310
Nitrogen content	-						
Foliar	%	3.38	3.62	3.14	3.46	3.34	3.03
Root	%	1.47 <sup>v</sup>	2.05 <sup>w</sup>	0.85 <sup>b</sup>	0.98 <sup>b</sup>	2.04 <sup>a</sup>	1.31 <sup>ab</sup>
Phosphorus content							
Foliar	%	0.25 <sup>v</sup>	0.50 <sup>w</sup>	0.27 <sup>a</sup>	0.29 <sup>a</sup>	0.46 <sup>b</sup>	0.45 <sup>b</sup>
Root	%	0.45	0.49	0.35 <sup>b</sup>	0.36 <sup>b</sup>	0.62 <sup>a</sup>	0.55 <sup>ab</sup>
In situ measurements							
Evapotranspiration	$ m mmd^{-1}$	3.3	2.4	4.2	3.3	3.0	2.6
Dissolved oxygen	$mgL^{-1}$	1.56	1.51	2.19 <sup>a</sup>	1.94 <sup>ab</sup>	1.68 <sup>b</sup>	1.70 <sup>ab</sup>
Redox potential	mV	-119.9	-130.3	50.8 <sup>a</sup>	44.0 <sup>a</sup>	-7.5 <sup>b</sup>	- 2.7 <sup>b</sup>
Electrical conductivity	mS cm <sup>-1</sup>	0.65	0.63	0.48	0.5	0.57	0.52
рН		6.40 <sup>v</sup>	6.75 <sup>w</sup>	6.12	6.16	6.3	6.17



**Fig. 3.** Seasonal variation of redox potential (mV) measured in mesocosms 2 for the different treatments (XX, XO, OO, OX; X for *Phragmites* and O for *Phalaris*). ANOVA test performed separately each season, *n* = 16.

### 3.2. 3.2. In situ measurements

Electrical conductivity ranged from 0.48 to 0.65 mS cm<sup>-1</sup> and pH from 6.1 to 6.7; in both cases higher values were measured in mesocosms 1, probably related to the higher organic and nutrient loads (Table 1). Evapotranspiration ranged from 2.4 to 4.2 mm d<sup>-1</sup> and was not significantly different between plant treatments, although *Phragmites* showed a slight tendency toward an evapotranspiration rate higher than *Phalaris*' (Table 1).

Dissolved oxygen (DO) and redox potential (Eh) increased from mesocosms 1–2 in all treatments, and plant species combination had an effect in mesocosms 2, but not in mesocosms 1 (Table 1). *Phragmites* monoculture (XX) showed significantly higher DO concentrations than *Phalaris* monoculture (OO) (Table 1).

Eh values in mesocosms 1 were negative and low, regardless of species, which is characteristic of strongly reducing conditions (Table 1). In mesocosms 2, *Phragmites* mesocosms (XX, OX) had positive Eh values all year round, while *Phalaris* (OO, XO) showed positive Eh values in autumn and winter, but negative Eh values in spring and summer (Fig. 3).

#### 3.3. Pollutant removal

All monocultures and polycultures of Phragmites and Phalaris showed high removal efficiency for all parameters tested (TSS, COD, TP, TN, NO<sub>3</sub>-N, NH<sub>4</sub>-N). TSS and COD removal was excellent for all treatments tested (85-95%) and there was no effect of plant species combination (Fig. 4). Physical processes including filtration, sedimentation and adsorption probably played a major role in organic and nutrient removal, given the high proportion of particulate matter from fish farm sludge in the inflow. Since seasonality did not seem to influence removal efficiency, results are shown only as the overall average. Comparing mesocosms 1, plants species had a significant effect on TP and TN removal, with Phragmites outperforming Phalaris (Fig. 4, Table SM1 of Supplementary material). TP removal showed the same pattern throughout the sampling year. Comparing mesocosms 2, the best combination for TP removal was Phragmites monoculture (XX). The combination Phalaris-Phragmites (OX) was more efficient at P removal than Phalaris monoculture (OO), although the difference was not significant (Fig. 4). Based on the year-round average, Phragmites was more efficient than Phalaris for TN removal in mesocosms. Overall, Phragmites monoculture (XX) was as efficient as the combination of both species (XO, OX) and more efficient than Phalaris monoculture (OO) (Fig. 4-TN). Phragmites outflow also had lower NH<sub>4</sub>-N and higher NO<sub>3</sub>-N than Phalaris, which might be the result of increased nitrification in Phragmites (Fig. 4-NH<sub>4</sub>-N, NO<sub>3</sub>-N). TN outflow concentrations increased in winter compared to other seasons, and experimental units planted with Phragmites in mesocosms 1 (XX, XO) were significantly more efficient than those planted with Phalaris (OO, OX) (Fig. 5).

Sulfide ( $S^{2-}$ ) outflow concentrations in spring and summer were significantly higher for *Phalaris* planted in mesocosms 2 (XX, OX) than for *Phragmites* (OO, XO) (Fig. 5). In winter, sulfide concentration was very low for all treatments, especially compared to autumn, and no difference was found between treatments (Fig. 5).

# 4. Discussion

Although we found differences in species traits that influenced the functioning of the wetland environment, in general, these differences did not seem to translate into more efficient nutrient removal when the species were combined. Overall, our results revealed that *Phragmites* was equal to or more efficient than *Phalaris* for pollutant removal except for NO<sub>3</sub>–N Therefore, combining both



**Fig. 4.** Year-round averages (8) of TSS, COD, TP, TN, NH<sub>4</sub>–N, NO<sub>3</sub>–N in gm<sup>-2</sup> d<sup>-1</sup>, measured at the outflow of mesocosms 1 and 2 (XX, XO, OO, OX; O for *Phalaris* and X for *Phragmites*). Average inflow in gm<sup>-2</sup> d<sup>-1</sup> was 11, 21, 1.5, 5.8, 0.8, and 0.04 respectively. Different letters indicate significant differences between treatments. The ANOVA test was performed separately for mesocosms 1 and 2 (M1, M2), n = 16.



**Fig. 5.** Seasonal variation of TN and  $S^{2-}$  (gm<sup>-2</sup> d<sup>-1</sup>) measured in mesocosms 2 for the different treatments (XX, XO, OO, OX; X for *Phragmites* and O for *Phalaris*). ANOVA test performed separately each season, n = 16.

plant species may represent the best tradeoff between overall high pollutant removal and low NO<sub>3</sub>–N level in the effluent.

Root and shoot density and morphology particular to each plant species influenced the oxidizing conditions of the rhizosphere. *Phragmites*' low density of large shoots and rhizomes evenly dispersed in the rhizosphere seemed to improve overall oxygen diffusion, in contrast to *Phalaris*, with its high density of fine shoots and superficial root system. Dissolved oxygen concentration was higher in *Phragmites* than in *Phalaris*, although the difference was significant only in mesocosms 2. Morphological differences between species could also have an impact on the flow distribution in the mesocosms (Wu et al., 2014).

Redox values measured in the mesocosms showed anoxic/anaerobic conditions, but through the experiment *Phragmites* showed higher redox potential than *Phalaris*. During spring and summer *Phalaris* showed negative Eh values (below – 100 mV) characteristic of strongly reducing environments. These results suggest an effect of plant species and season in the overall redox potential between treatments. Findings by Allen et al. (2002) and Stein et al. (2007) confirm that plant species selection in TWs is important due to its ability to influence rhizosphere redox conditions, which in turn have a strong impact on pollutant removal (Caselles-Osorio and García, 2007; Kadlec and Wallace, 2009). The influence of different plant species on redox potential in our experiment was more pronounced in mesocosms 2, which received lower organic load than mesocosms 1.

Due to the seasonal complementarity between Phalaris and *Phragmites*, it was expected that the longer period of plant activity would improve pollutant removal efficiency throughout a yearlong operation. Complementary seasonal activity between Phalaris and Phragmites was indeed observed over the course of the experiment. As documented by Vymazal and Kröpfelova (2005), Phalaris' growth period began and finished earlier than Phragmites'. However, this complementary growth period did not translate into a measurable greater removal efficiency. One possible explanation may be due to negligible plant uptake during the growing season compared to nutrient input load (Vymazal et al., 2007; Zhang et al., 2010). Also, bacterial activity in the rhizosphere, largely responsible for pollutant removal, may be only weakly related to seasonal plant activity, compared to other factors such as water content, temperature, oxygen level or redox potential (Stein et al., 2007 and Kadlec and Wallace, 2009). It is thus not surprising that pollutant removal in TWs is also high in winter, when the plants are dormant (Stein et al., 2007).

Differences according to plant species were also observed over the course of the seasons in the belowground biomass and accompanying root pictures of each mesocosm. Phalaris' rhizosphere presented a layer of black metal precipitate, characteristic of strongly reducing environments where the predominant electron acceptor is sulfate. Sulfate reducing bacteria (SRB) degrade organic matter to CO<sub>2</sub> and H<sub>2</sub>S, which can precipitate as metal FeS or MnS or exit the system (Kadlec et al., 2000). Phalaris mesocosms also showed high S<sup>2-</sup> outflow concentrations, confirming SRB activity. The black precipitate layer was more pronounced and larger during summer (2012), faded in autumn and particularly in winter, and regained prominence in summer (2013). During winter, root oxygen demand decreases due to plant dormancy, and water temperature decreases, increasing oxygen solubility (Kadlec and Wallace, 2009). As a result, more oxygen is available for aerobic microbial processes (Stein et al., 2007). This might explain the higher TN outflow concentrations in winter compared to the rest of the year by decreased denitrification rates. The higher oxygen level we found during winter may have been due to the effects of water temperature and plant dormancy or a consequence of plant harvest at the end of autumn. After harvest, the dead plant shoots enhance convective oxygen transport from the surface inducing

oxidative conditions in the rhizosphere (Kadlec and Wallace, 2009). Stein et al. (2007) showed that aerobic microbial activity increases during winter, as more favorable electron acceptors are available, while SRB activity is inhibited. The low S<sup>2–</sup> outflow concentrations and high redox values (ranging from +50 to 150 mV) we measured in *Phalaris* mesocosms during winter compared to the other seasons also suggest a decrease in SRB activity during this period.

*Phragmites*' rhizosphere, on the other hand, exhibited oxidizing conditions (Eh values ranging from +50 to 230 mV) over the course of the experiment. Only a slight amount of black precipitation was evident at the very bottom of *Phragmites* mesocosms during the summer. Radial oxygen loss was particularly evident in summer root pictures, in the reddish color of Fe and Mn oxides precipitate over the roots closer to the surface (Vymazal et al., 2007). Thus, our study confirms the conclusion by Allen et al. (2002) and Edwards et al. (2006) that *Phragmites* transfers more oxygen to the rhizosphere than *Phalaris*.

This difference between species might explain their dissimilar nutrient removal efficiency. For instance, *Phragmites* advantage over *Phalaris* for TP removal may be explained by the oxic conditions in *Phragmites* units that enhance phosphorus co-precipitation with iron (Vymazal and Kröpfelova, 2008). Plant nutrient uptake does not seem to explain this result, as both species produced similar amounts of biomass and *Phalaris* showed higher P content.

The role of plants in nitrogen removal is more complex as it involves several transformations and processes, and plant species may affect each process differently. Plant roots and rhizomes may affect sedimentation by increasing flow resistance and weakening convection (Wu et al., 2014). In our experiment, TSS removal was high and it occurred in the first mesocosms in all treatment combinations. We found no evidence that differences in root-rhizome morphology and abundance may have influenced TSS removal differently-thus particulate nitrogen removal-since there were no significant differences in removal between plant species at the outlet of mesocoms 1. Inorganic N species composed less than 15% of the TN in the inflow, but mineralization of both soluble organic N and some particulate nitrogen also produced ammonium. COD removal was similarly very high in the first mesocosms for both species, so that the remaining TN in the outflow of mesocosms 1 (inflow of mesocosms 2), was mainly inorganic N. NH<sub>3</sub> volatilization is considered minimal, given that the pH range was below 7 (Table 1). Some of the inorganic N is taken up by microbes, thus entering a cycle of nitrogen assimilation-production of particulate N-sedimentation or mineralization. Plant uptake is also responsible for some inorganic N-removal. Nitrogen in belowground tissue is eventually released at root and rhizome death. Nitrogen in aboveground plant tissue can be permanently removed from the TW if plants are harvested at the end of the growing season. However, this would account for less than 5% TN removal over a year in our mesocosms, a percentage that is similar to what is reported in the literature for horizontal subsurface flow TW (Vymazal et al., 2007; Kadlec and Wallace, 2009). Given the low concentrations of NH<sub>4</sub>-N and NO<sub>3</sub>-N in the outflow of our mesocosms, coupled nitrification and denitrification are most likely important processes in inorganic-N dynamics. In mesocosms 1, Phragmites outflow had lower NH<sub>4</sub>-N and higher NO<sub>3</sub>-N than Phalaris, which might be the result of increased nitrification in *Phragmites* (Fig. 4-NH<sub>4</sub>-N, NO<sub>3-</sub>N). The better oxic condition in *Phragmites* units (mesocosm 1 alone or XX monoculture) may be responsible for enhanced nitrification and ammonification, while possibly limiting the denitrification rate. Conversely, the more reducing conditions in Phalaris mesocosms (mesocosm 1 alone or OO monoculture) may result in lower nitrification but more complete denitrification, resulting in less overall TN removal but lower NO<sub>3</sub>–N level in the outflow.

When we compare monocultures to plant combination, our results show that plant combinations are intermediate between *Phragmites* and *Phalaris* monocultures for both TN and  $NH_4-N$ . As for  $NO_3-N$  in the outflow; the most effective treatments are those with *Phalaris* in the second mesocosm. Consequently, while *Phragmites* monoculture may be the best option if TN and  $NH_4-N$  removal are targeted, a plant combination composed of *Phragmites* followed by *Phalaris* offers a good compromise between high TN removal and low  $NO_3-N$  in the outflow.

Plant root systems provide mechanical support for microbial community attachment, secrete root exudates and transfer oxygen from aerial tissues into the rhizosphere (Weber and Legge, 2013). A microbial community level physiological profiling (CLPP) approach was used during the course of our study to evaluate whether the complimentary nature of *Phalaris* and *Phragmites* may lead to greater microbial functional diversity (Button et al., 2014). Plant species identity did have an influence on microbial activity. *Phalaris* planted in mesocosms 2 enhanced microbial richness and activity and increased the utilisation of specific carbon sources, compared to *Phragmites* (Button et al., 2014).

# 5. Conclusion

Plant species seemed to influence TW efficiency, Phragmites outperforming Phalaris. However, our results do not appear to support the hypothesis that a combination of plant species improves the performance of TWs. The best monoculture was as efficient as, or more efficient in removal than the combination of two plant species for any pollutant taken individually. The treatment efficiency of Phalaris was improved when it was combined with Phragmites, except with regard to NO3-N level in the outflow. Indeed, the combination Phragmites-Phalaris may represent the best tradeoff between high pollutant removal and low NO3-N level in the effluent. In our experiment, the TW experimental units were equally divided between the two species, but a different space allocation. such as a larger portion of Phragmites followed by Phalaris may further improve the overall performance. It has been documented that Phragmites populations tend to exclude Phalaris when growing together (Vymazal and Kröpfelova, 2005; Fu et al., 2011), therefore it is possible that additional investment in maintenance or design should be considered when combining the two species. Even when combining plant species in TWs does not represent an advantage in terms of pollutant removal compared to a monoculture of the best species, it may provide other benefits like higher resistance to environmental stress or diseases, improving aesthetic quality and better habitat quality (EPA, 2000; Kadlec and Wallace, 2009).

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# Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2016.02. 047.

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