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## Establishment and potential use of woody species in treatment wetlands

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### ABSTRACT

Plant species selection is an important criterion for improving treatment wetland performance. The aim of this work was to evaluate removal efficiency and potential uses of woody species in treatment wetlands during the establishment year. Plant development, removal efficiency and evapotranspiration rate of five woody species (*Salix interior*, *Salix miyabeana*, *Sambucus canadensis*, *Myrica gale*, *Acer saccharinum*) and four herbaceous taxa typically used in treatment wetlands (*Typha angustifolia*, *Phragmites australis australis*, *Phragmites australis americanus*, *Phalaris arundinacea*) were compared in a mesocosm-scale study during one growing season. Woody species showed significantly slower growth, but displayed several characteristics of interest for treatment wetland applications: good adaptation to wetlands conditions; high organic matter removal (76–88%); high nutrient accumulation in tissues and high evapotranspiration capacity. During the establishment year, herbaceous species showed greater biomass development (above- and below-ground parts), higher evapotranspiration rate ( $>3.84 \text{ L m}^{-2} \text{ d}^{-1}$  compared to  $<3.23 \text{ L m}^{-2} \text{ d}^{-1}$  for woody species) and overall pollutant removal efficiency. These characteristics confirm the high efficiency of treatment wetlands planted with herbaceous species even in the first growing season. However, given their greater potential biomass development, woody species could represent an excellent alternative for improving treatment wetlands long-term performance.

### KEYWORDS

Herbaceous species; plant selection; treatment wetlands; wastewater treatment; woody species

## Introduction

Treatment wetlands (TWs) represent an economical and ecological alternative to conventional technologies for the treatment of polluted water of various kinds. Plants are essential components of TW operation and performance (Brix 1997; Kadlec and Wallace 2008). The roots and rhizomes stimulate bacterial activity, have a filtering effect and help prevent clogging and erosion. The aboveground biomass better insulates the system and contributes to water lost by plant transpiration (Bahlo and Wach 1990; Brix 1997). Plant species selection can improve treatment efficiency of TWs because of possible differences in storage and nutrient uptake, oxygen transfer and evapotranspiration (ET) rate between species (Brisson and Chazarenc 2009; Shelef *et al.* 2013).

Some TWs are dominated by woody emergent macrophytes. These include bio-retention systems, large modified natural wetlands used for treatment and zero-discharge evapotranspirative TW (Fonder and Headley 2013). Yet, the vast majority of research and application in TWs has focused only on herbaceous plant species. Several wetland herbaceous macrophytes are ideal for TW because they establish and multiply easily, grow rapidly, have a dense root system and show good pollutant removal capacity. In a

survey of more than 600 TWs, Vymazal (2013) reports the use of 150 herbaceous macrophyte species but makes no mention of woody species. The herbaceous species most commonly used in TW are *Typha*, *Scirpus*, *Phragmites*, *Juncus*, and *Eleocharis* (Vymazal 2013). Since woody species have been tested much less frequently in this context, little is known about their performance in terms of pollutant removal efficiency compared to herbaceous species.

While there are practical reasons for favoring the use of herbaceous species, some woody species have characteristics that, in principle, should lead to high performance in TWs. This is particularly the case for willows (genus *Salix*) whose rapid development, large biomass, and capacity to achieve a considerable evapotranspiration rate make them ideal plants for biomass production and valorization (Labrecque and Teodorescu 2005; Guidi *et al.* 2013; Gizińska-Górna *et al.* 2016). Willows and poplars (genus *Populus*) have been successfully used in landfill leachate treatment (Duggan 2005; Białowiec *et al.* 2007; Justin *et al.* 2010) and have also been used occasionally in TWs (Perttu and Kowalik 1997). Their high evapotranspiration rate makes them ideal plants for zero-discharge TWs (Gregersen and Brix 2001; Frédette *et al.* 2019).

**Table 1.** Information on the plant species used in the experiment.

Code	Species name	Family	Origin
<b>Herbaceous</b>			
Phala	<i>Phalaris arundinacea</i>	Poaceae	N. America
PhragA	<i>Phragmites australis</i> subsp. <i>americanus</i>	Poaceae	N. America
PhragE	<i>Phragmites australis</i> subsp. <i>australis</i>	Poaceae	Eurasia
Typha	<i>Typha angustifolia</i>	Typhaceae	N. America
<b>Woody</b>			
Acer	<i>Acer saccharinum</i>	Aceraceae	N. America
Myrica	<i>Myrica gale</i>	Myricaceae	N. America
SalixI	<i>Salix interior</i>	Salicaceae	N. America
SalixM	<i>Salix miyabeana</i> 'SX67' <sup>a</sup>	Salicaceae	Asia
Sambu	<i>Sambucus canadensis</i>	Adoxaceae	N. America

<sup>a</sup>Cultivar.

Very few studies have compared wastewater treatment of herbaceous and woody species under the same conditions. Białowiec *et al.* (2012) found that *Phragmites australis* and *Salix viminalis* × *burjatica* were equally efficient in nitrogen and COD removal in TWs treating landfill leachate. Morgan *et al.* (2008) reported a better rate of denitrification of woody species (*Salix nigra* and *Hibiscus moscheutos*) compared to two herbaceous species (*Cyperus papyrus* and *Colocasia esculentus*) while the highest levels of ammonium removal were achieved when mesocosms were planted with a mix of herbaceous and woody species.

A better understanding of the differences between the two groups of species could improve plant selection for TW design. Important characteristics such as root morphology, and the ability to take up nutrients and to stock them in different plant parts, need to be studied to evaluate the important differences between the two groups of species in TW applications. Because woody species are generally slower to establish and take longer to reach maximum size, comparative studies must extend over several years. As a first step toward this general goal, in the present study, we focused on the differences in plant performance of several herbaceous and woody species during the establishment phase in a TW. More specifically, the objectives of this study were:

- to compare morphology and growth characteristics of woody and herbaceous species in TW conditions;
- to compare evapotranspiration rate and pollutant removal efficiency of woody and herbaceous species.

To reach our objectives, we conducted a mesocosm experiment comparing herbaceous and woody species under TW simulated conditions during the first growing season.

## Materials and methods

### Plant species selection

Four herbaceous taxa and five woody species were selected for the experiment (Table 1). We selected three of the herbaceous species most commonly used in TWs, as well as the North American subspecies of *Phragmites australis*. The Eurasian haplotype of *Phragmites australis*, sometimes referred to as the subspecies *P. australis australis*, is the species most commonly used in TWs (Vymazal 2011). Because of the invasive character of this taxon in North America,

one of the North American haplotypes of the species, sometimes referred to as *P. australis americanus* was also selected. In a mesocosm experiment, *P. australis americanus* was found to be as efficient as its Eurasian counterpart in pollutant removal, and possibly even more efficient in terms of phosphorus removal (Rodríguez and Brisson 2015). The two other species used, *Typha angustifolia* and *Phalaris arundinacea*, have also been shown to be highly efficient in TWs (Vymazal 2007). The latter has even better resistance to pathogen attacks than *P. australis*, which is an important criterion for species selection (Marchand *et al.* 2014). *Typha* is commonly used for multiple ecological purposes, including improvement of water quality, nutrient uptake, mercury accumulation and bioenergy feedstock harvesting (Verma and Suthar 2018). Moreover, this species increases oxygen uptake by litter, which can stimulate microbial activity and accelerate litter decomposition (Mason and Bryant 1975).

For woody species, we choose five species that met the following criteria: (1) wetland species or species that tolerate TW feeding conditions, (2) species with a high growth rate, and (3) species with resilience to different types of external disturbances (coppicing, high and low temperatures, etc.).

*Acer saccharinum*, *Sambucus canadensis*, and *Myrica gale* are woody species often present in natural wetlands in North America, and could, therefore, be potentially tolerant to TW feeding conditions. *Acer saccharinum* is a relatively fast-growing tree species with good potential for environmental adaptation (Peterson and Bazzaz 1984). It can also behave as a shrub when regularly cut back to the ground, as it then produces several new stems vegetatively, by basal resprouting. *Sambucus canadensis* and *M. gale* are both shrubs 1–3 m tall. *Sambucus canadensis* grows in sunny conditions and can adapt to wet soil. The roots of *M. gale* have nitrogen-fixing actinobacteria, and its original wetland habitat ranges from peatlands to swamps and shores of lakes and rivers (Bond 1951; Skene *et al.* 2000). Two willow species were also selected, *Salix interior* and *Salix miyabeana* (cultivar SX67) which are typically grown in short rotation and have been successfully tested for use in different phyto-technology applications, including water quality improvement (Gregersen and Brix 2001, Nissim *et al.* 2015), bioenergy (Labrecque and Teodorescu 2005; Amichev *et al.* 2014), erosion control (Wilkinson 1999) and other purposes (Kuzovkina and Volk 2009; Frédette *et al.* 2019).

### Experimental design

The mesocosm-scale experiment was conducted in a controlled greenhouse environment located at the Montreal Botanical Garden (Quebec, Canada) (latitude: 45° 33' 43.00'' N; longitude: 73° 34' 18.50'' W). The experimental set-up consisted of thirty 60 L column-shaped wetland mesocosms (37.0 cm diameter × 53.0 cm deep).

A drain tap was first installed 2–3 cm from the bottom of each mesocosm. The mesocosms were then filled with two layers: (1) the 30.0 cm top principal layer composed of 2.5–5 mm size gravel (Anthracite Black Granite) and (2) a 10.0 cm bottom drainage layer filled with 10–20 mm size

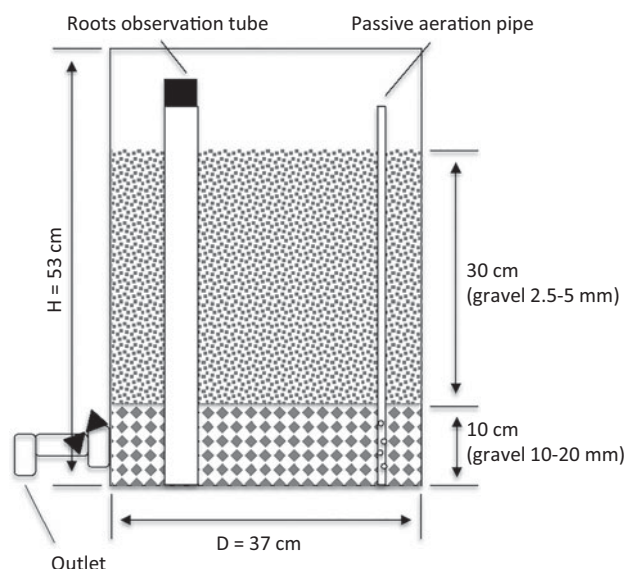


Figure 1. Cross-section view of a mesocosm.

gravel. A perforated PVC pipe ( $d = 2.54$  cm) for passive aeration of the deep layer of substrate and a transparent acrylic tube for root observation were also installed in each mesocosm (Figure 1). Each mesocosm was planted with one of the nine species and one treatment consisted of unplanted mesocosms. Each treatment was replicated 3 times, for a total of 30 mesocosms, and the treatments were randomly distributed within 3 blocks (Figure SM1).

One-year-old woody plants and herbaceous plants from rhizomes of similar size were transplanted in each mesocosm on July 5, 2017 (Table 1). At the beginning of the plant establishment period, the mesocosms were filled with tap water, to which 6 g of a nutrient solution with a ratio of N:P:K equal to 21:7:14 had been added, up to 1–3 cm below the surface. During the following two weeks, the water level was maintained with tap water without supplementary nutrient solution. At the end of this period, the solution was totally drained from the mesocosms and the experiment began.

For the duration of the experiment, a synthetic sewage solution based on the OCDE recipe (Guideline 2001) was produced twice a week in a refrigerated bulk tank. Mean influent concentration (in  $\text{mg L}^{-1}$ ) was COD  $283 \pm 13$ ; TKN  $49.6 \pm 3.5$ ; TP  $10.9 \pm 1.6$  with pH of 7.75 and conductivity of  $4310 \mu\text{S cm}^{-1}$ , representing a typical domestic effluent after primary settling in terms of nutrient content concentration. Each mesocosm was filled with this solution using a graduated cylinder. Twice a week, the mesocosms were emptied to provide a hydraulic residence time of 3.5 days. The effluent was then weighed to assess evapotranspiration and for mass balance analysis. Then, the mesocosms were immediately filled with new artificial wastewater. Evapotranspiration was calculated as the difference between influent and effluent from each mesocosm. The experimental period lasted from July to November 2017. Because the water level in the mesocosms was kept constant just below the surface of the substrate, our experiment more closely simulates conditions of a horizontal flow subsurface TW (HFSSF-TW) than those

of a surface flow (SF-TW) or vertical flow subsurface TW (VFSSF-TW).

The greenhouse conditions were controlled for each month of the experiment according to two principal parameters: temperature (by ventilation and heating) and light intensity (by high pressure sodium lamps).

During the experiment, the following greenhouse environmental conditions were recorded by sensors every 2 min: temperature, relative humidity, light intensity and periods of lamp activity. Daily temperature in greenhouse ranged from 10.2 to 25.9 °C (avg  $18.5 \pm 3.7$  °C), with relative humidity ranging between 39.4 and 82.8% ( $62.3 \pm 9.9\%$ ).

Treatment with insecticide solutions (Safer's End-All, 2%; Safer's End-All II, 5%; Trounce, 5%) was applied twice a week from August to October, to control an aphid infestation in the greenhouse.

### Water sample analyses

Water samples were collected weekly and stored at 4 °C from the influent tank and, after the first 3.5 days of retention, from the 30 mesocosms for 18 consecutive weeks, from July to November 2017. Laboratory analyses of chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorus (TP) were performed at Polytechnique Montreal according to Standard Methods (APHA 1998). Removal rates were calculated on mass basis considering the measured concentrations and influent volumetric flows.

### Harvest and plant analyses

Root development was monitored visually three times during the experiment (every 2 months) by 360° belowground images scanned by an In-Situ Root Imager (CI-600, CID Inc.) buried in the mesocosms in transparent acrylic tubes. One plant of each species was randomly selected for assessment of seasonal changes in root growth, and root length was estimated every two months by the root imager using RootSnap! Root Analysis Software, Version 1.3.2.25.

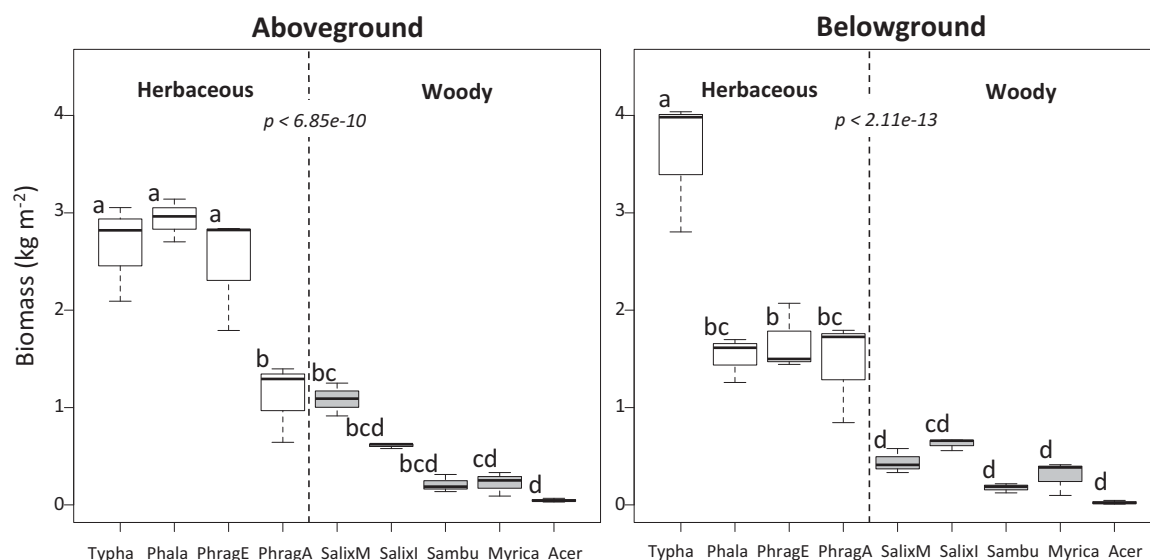
At the end of the experiment, all plants were harvested. After removing all sediment from the roots, the fresh plant parts – roots, shoots (only for woody species) and leaves – were weighed separately. This plant material was then oven-dried at 70 °C until a constant weight was reached and weighed again.

To estimate the total root length at the end of the experiment, a fresh root sample of one plant per species was weighed and scanned using WinRHIZO Software, then oven-dried and weighed again. From this procedure, the root length per gram of dry matter was calculated, to estimate the total root length for each plant.

The dry biomass of three replicates of each species was crushed and well mixed to create one sample of each species before the following analyses were performed:

- Total organic carbon using SC744 Analyser (Leco Corporation).





**Figure 2.** Final aboveground and belowground dry biomass ( $\text{kg m}^{-2}$ ) per species. Plants sharing the same letter are not significantly different.

- Total phosphorus using automated Ion Analyzer QuikChem 8500 Series 2 FIA System according to QuickChem Method # 10-115-01- 1-C (Lachat Instruments).
- Total Kjeldahl nitrogen using automated Ion Analyzer QuikChem 8500 Series 2 FIA System according to QuickChem Method # 10-107-06- 2-D (Lachat Instruments).
- Lignin using NanoPhotometer P-Class P300 (Implen Inc.) according to the Standard Biomass Analytical Methods by the National Renewable Energy Laboratory (NREL/TP-510-42623).

### Statistical analyses

Comparison of the following parameters was tested statistically between treatments (non-planted control and mesocosms planted with different species): final dry mass, evapotranspiration rate, COD removal, TKN removal, and TP removal. An analysis of variance (ANOVA) was used for parameters with a normal distribution and Post Hoc comparison (Tukey's test) was carried out to specify the level of independent variables. Otherwise, the non-parametrical Kruskal–Wallis Post Hoc test was used. For all tests, the differences were deemed significant when  $p \leq 0.05$ . All analyses were performed using R 3.4.2 software (R Core Team 2013).

Other parameters were compared for tendencies without statistical analysis for lack of replication: root length development, root/shoot ratio, specific root length (SRL), TKN and TP concentration in plant tissues, total lignin, and total carbon content.

## Results

### Plant growth and biomass production

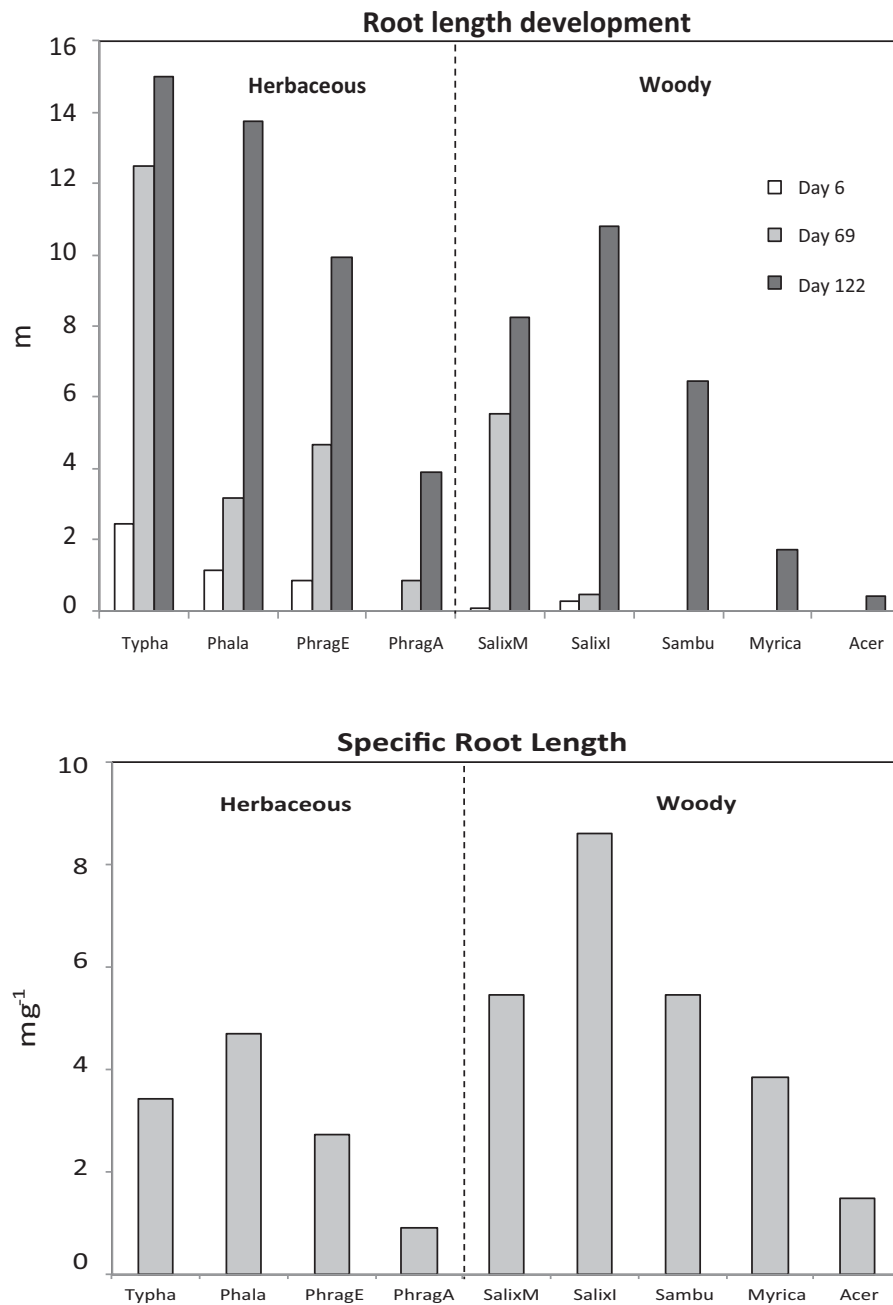
The plants from all species were healthy during the experiment, and by the end, almost all had covered the surface of mesocosms, exception for *A. saccharinum*, which grew very

little. As expected, there were large differences in plant size between species at the end of the experiment, the total dry aboveground biomass ranging from  $0.05$  to  $2.9 \text{ kg m}^{-2}$  (Figure 2). Aboveground biomass was clearly greater in herbaceous species, with three – *P. australis australis*, *T. angustifolia*, and *P. arundinacea* – above  $2.9 \text{ kg m}^{-2}$ . The lowest value among herbaceous species was observed in *P. australis americanus* –  $1.1 \text{ kg m}^{-2}$ . *Salix miyabeana* showed the highest value for aboveground biomass among woody species ( $1.1 \text{ kg m}^{-2}$ ), while all other woody species had biomass less than  $1 \text{ kg m}^{-2}$ . Belowground dry biomass mirrored the pattern aboveground, ranging from  $0.03$  to  $3.6 \text{ kg m}^{-2}$ , with herbaceous producing more than woody species. *Typha angustifolia* had the highest belowground biomass, followed by the two *Phragmites* species and *P. arundinacea*; all produced around  $1.6 \text{ kg m}^{-2}$ . All woody species produced less than  $1 \text{ kg m}^{-2}$  of root biomass.

Root/shoot ratio, which reflects biomass allocation strategies, was highly variable, with no apparent difference between herbaceous and woody species. Two herbaceous species (*T. angustifolia* and *P. americanus*) and one woody species (*S. canadensis*) showed a ratio above 1.2 (greater allocation to belowground biomass), while four species (*P. arundinacea*, *P. australis*, *S. miyabeana*, *A. saccharinum*) had a ratio less than 0.7 (greater allocation to aboveground biomass). Two species, *S. interior* and *M. gale*, had a ratio between 0.7 and 1.2 (roughly equal biomass allocation to roots and shoots).

Analyses of images scanned by the root imager at the beginning, middle and end of the experiment showed different temporal patterns of root development (Figure 3). A majority of species showed greater root development during the second half of the growing season, while *T. arundinacea* and *S. interior* were more active during the first half of the season. *P. australis* showed uniform root growth throughout the experiment.

A specific root length (SRL), calculated after analyses performed using WinRHIZO Software at the end of the experiment, showed the smallest values for *P. americanus* and *A.*



**Figure 3.** Above: Root length (m) development during the experiment via images from the transparent acrylic tubes. Below: specific root length (SRL) ( $\text{m g}^{-1}$ ) at the end of the experiment.

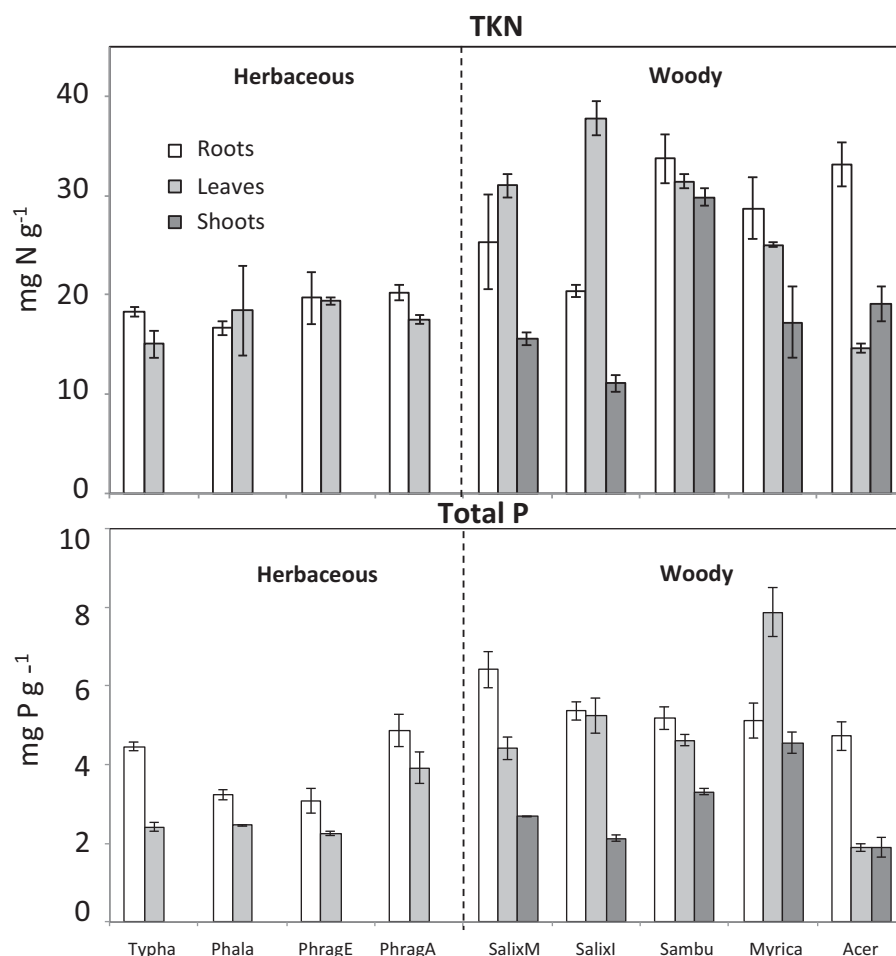
*saccharinum* (0.9 and  $1.5 \text{ m g}^{-1}$ , respectively) (Figure 3). The values for *T. angustifolia*, *P. australis* and *M. gale* ranged from 2.7 to  $3.8 \text{ m g}^{-1}$ . The root length of *S. interior* and *S. canadensis* was around  $5.5 \text{ m g}^{-1}$ . *Salix miyabeana* had the highest root length per g of dry matter ( $8.6 \text{ m g}^{-1}$ ).

#### Nutrient and lignin content in plant tissues

There was no apparent difference in mean TKN concentration in aboveground tissues between herbaceous and woody species, but variation was much greater in the latter than in the former. For herbaceous species, TKN concentration ranged from 15 to  $20 \text{ mg N g}^{-1}$ , with no important differences between belowground and aboveground parts (Figure 4).

For both *Salix* species, TKN concentration was highest in the leaves (*S. interior* –  $31 \text{ mg N g}^{-1}$  and  $38 \text{ mg N g}^{-1}$  for *S. miyabeana*), intermediate in the roots (25 and  $20 \text{ mg N g}^{-1}$  respectively) and lowest in the stems ( $16$  and  $11 \text{ mg N g}^{-1}$ ). TKN concentration in *S. canadensis* was similar for roots, stems, and leaves ( $30$ – $34 \text{ mg N g}^{-1}$ ). Two woody species, *M. gale* and *A. saccharinum*, showed higher concentration in their roots ( $29$  and  $33 \text{ mg N g}^{-1}$ , respectively) than in leaves or stems.

Overall, TP concentration appeared to be higher in woody than in herbaceous species (Figure 4). There was little difference in TP concentration between herbaceous species, and concentration in roots was always higher than in shoots. The maximal concentration was found for *P. americanus*, with a root concentration of  $4.9 \text{ mg P g}^{-1}$  and a



**Figure 4.** TKN concentration ( $\text{mg N g}^{-1}$ ) and TP concentration ( $\text{mg P g}^{-1}$ ) in plant tissues measured at the end of the experiment. Error bars represent the standard deviation of 2 to 4 measurements per plant species.

shoot concentration of  $3.9 \text{ mg P g}^{-1}$ . The lowest TP concentration for all woody species tested was found in shoot parts (ranged from 2.1 to  $4.6 \text{ mg P g}^{-1}$ ) and the highest in roots (ranged from 4.7 to  $6.4 \text{ mg P g}^{-1}$ ), except for *M. gale*, which showed highest TP concentration in leaves –  $7.9 \text{ mg P g}^{-1}$ .

The total lignin content was similar for all herbaceous species, with the lowest values in roots (from 5.7 to 12.8%) and the highest in shoots (from 20.8 to 24.2%) (Figure 5). The percentage of lignin in root parts of woody species was at least double that in herbaceous roots (from 18.1 to 39.4%), with the highest content in *M. gale* roots. There were no important differences found between root and shoot content of lignin in woody species.

There was no difference in total carbon percentage between species (Figure 5). Total carbon was lower in roots (ranging from 38 to 46%) than in leaves and shoots (ranging from 46 to 50%).

### Evapotranspiration rate

The mean ET rate during the experiment ranged from 0.9 to  $10.1 \text{ L m}^{-2} \text{ d}^{-1}$  (Figure 6). The minimum was observed in unplanted mesocosms and in those planted with *A. saccharinum*, with no significant difference between these two treatments. The ET rate was significantly higher for all four

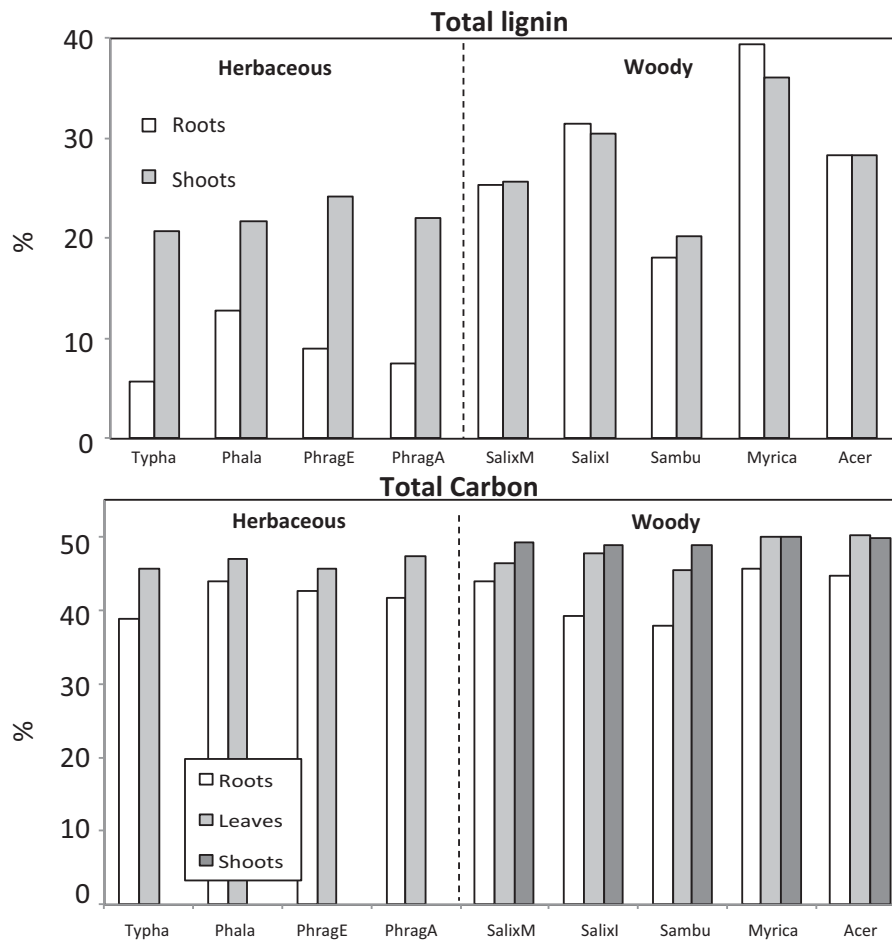
herbaceous ( $>3.84 \text{ L m}^{-2} \text{ d}^{-1}$ ) compared to woody species ( $<3.23 \text{ L m}^{-2} \text{ d}^{-1}$ ). The highest rate among the species tested was observed for *T. angustifolia*, *P. arundinacea*, and *Phragmites australis australis*, with mean values of 8.7, 8.8, and  $10.1 \text{ L m}^{-2} \text{ d}^{-1}$  respectively. Among woody species, the two *Salix* showed the highest ET rate with no significant difference between them.

During the experiment, the variations in ET rate were larger for three herbaceous species, *T. angustifolia*, *P. australis* and *P. arundinacea*, with the standard deviation ranging from 3.6 to  $1.7 \text{ L m}^{-2} \text{ d}^{-1}$  (SM2). The standard deviation for other species was relatively low ( $<1.3 \text{ L m}^{-2} \text{ d}^{-1}$ ), which was expected since the experiment lasted for a single growing season.

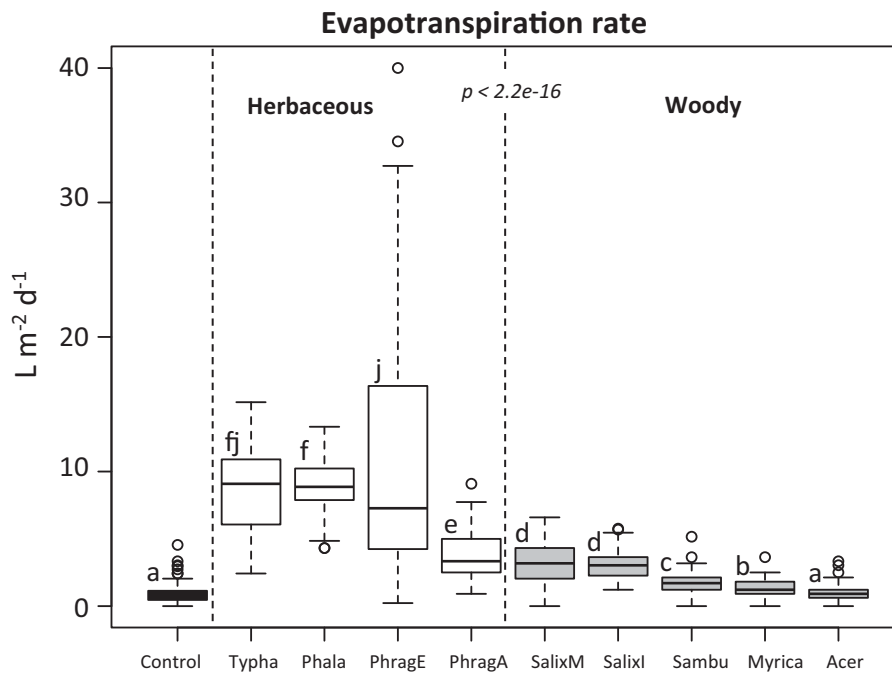
### Pollutant removal efficiency

Mean COD removal rate ranged from 76 to 88% for all mesocosms (Figure 7). The lowest COD removal efficiency was observed for unplanted mesocosms, and the highest for *T. angustifolia*. There was a significant difference between species, with herbaceous species having the highest efficiency.

Mean TKN removal was lowest for the unplanted mesocosms and those planted with *M. gale*, at 16 and 18%,

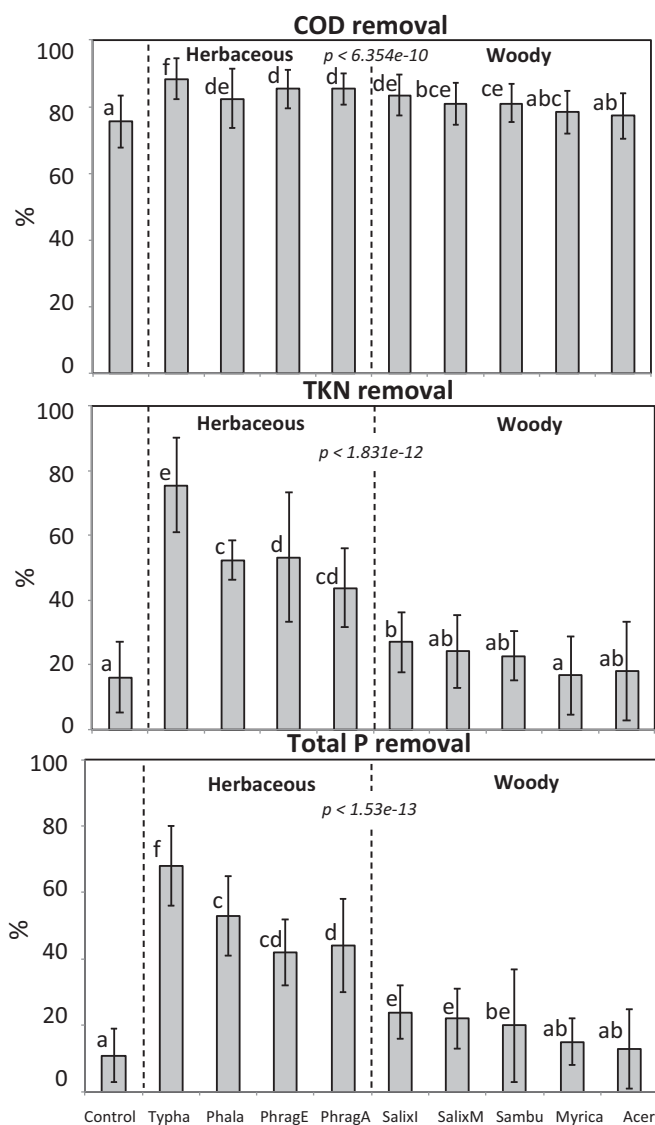


**Figure 5.** Percentage of total lignin (above) and total carbon (below) content in plant tissues at the end of the experiment.



**Figure 6.** Daily evapotranspiration rate ( $\text{L m}^{-2} \text{d}^{-1}$ ) during the experiment for all mesocosms. Each boxplot represents all evapotranspiration values measured (twice a week) for three replicates of the same species. Different letters above the boxplots indicate significant differences ( $p < 0.05$ ) between species. More details can be found on [Figure SM2](#).





**Figure 7.** Percentage of removal efficiency for COD, TKN, and TP of all the mesocosms during the experiment.

respectively (Figure 7). Statistical analyses showed two distinct groups of species in terms of removal efficiency: lower efficiency for woody species (ranging from 17 to 27%); and higher efficiency for herbaceous species (ranging from 44 to 76%). The highest value was for the mesocosms planted with *T. angustifolia*. Standard deviation values ranged from 6% (for mesocosms planted with *P. arundinacea*) to 20% (for *P. australis australis*).

The pattern of TP removal was similar to that of TKN, with the lowest mean removal found in unplanted mesocosms (13%), a greater removal in herbaceous species (42–68%) compared to woody species (13–24%) and significant maximum removal in *T. angustifolia* (68%) (Figure 7).

## Discussion

The nutrient-rich, permanent water-saturated conditions in our experiment, analogous to those of an HFSSF-TW, promote anaerobic soil conditions that may be particularly stressful to root growth of woody species (Jackson and

Attwood 1996). Yet, except for *A. saccharinum*, woody species showed good adaptation to TW conditions, with a high percentage of surface coverage by aboveground plant parts, good root development, and acceptable pollutant removal, especially for *Salix* species. The ability to supply oxygen to the subsurface environment, which has been well documented for willows (Randerson *et al.* 2007, 2011; Williams *et al.* 2010) may contribute to the survival and growth of woody species in our experiment. In addition, this oxygen release may influence redox potential and promote bacterial processes responsible for nitrogen and organic matter removal (Randerson *et al.* 2011; Białowiec *et al.* 2012). However, during the first growing season, the herbaceous species developed more quickly and produced significantly more aboveground and belowground biomass than the woody species. ET rate was greater for herbaceous and *Salix* species. In terms of pollutant removal, herbaceous species were more efficient at nitrogen and phosphorus removal, although the relative nutrient content analyses performed at the end of the experiment showed that woody species stocked more nitrogen and phosphorus in their tissues. There was no difference in organic matter removal for the two types of plants. As expected, root lignin content was significantly higher in woody than in herbaceous species. Among the woody species tested, during the first growing season in TW conditions, establishment of *Salix* species was greatest and removal response highest.

The better performance of herbaceous species in term of pollutant removal and ET rate can be explained by significantly faster biomass development in the first growing season. This characteristic of herbaceous species is often one of the arguments in favor of selecting this type of species for use in TWs (Vymazal 2011).

Despite the difference in plant biomass, organic matter removal efficiency during the experiment was high and similar for the two types of species (COD removal from 76 to 88%). This confirms the high organic matter removal capacity of woody species in a long-term application. Nitrogen and phosphorus removal was less efficient in mesocosms with woody species due to the immature stage of the plants. Direct nutrient absorption by plants is not the most important function of plant species in TWs but the morphology of roots and rhizomes is directly related to the development of microorganisms attached to it. These microorganisms are the major mechanism of nutrient removal (Brix 1997; Listosz *et al.* 2018). Woody species prioritize root development in terms of length, with more associated tiny structures than herbaceous species (Figure 3). On the longer term, this morphological characteristic of woody species can be a benefit in TWs, because it may facilitate a more homogeneous root position for substrate filtration.

Lignin compound was found to be present in the roots of woody species tested (from 18 to 39%), as well as in roots of herbaceous species (from 6 to 13%). The proportion of lignin should increase in woody species over the years. The decomposition of plant species' (herbaceous and woody) roots in natural wetlands are slower than that of other plant parts (Verhoeven 1986; Mitsch and Gosselink 2007). A study

conducted by Chimner and Ewel (2005) in several natural wetlands showed that roots with a high concentration of lignin can be accumulated as peat. However, other factors contribute to the accumulation of extremely slow decomposition that results in peat formation: anaerobic conditions (poor drainage and high water level), poor substrate quality and low temperature are. These factors are uncommon in TW systems. If we consider the lifetime of TWs to be about 20–30 years, this aspect can facilitate physical filtration of suspended solids and prevent clogging (Brix 1997). It should be noted that shrubs' longer and harder roots raise concerns about possible damage to the subsurface flow TW membrane lining. Woody roots can puncture the membrane and allow wastewater to seep into groundwater (Kadlec and Wallace 2008). We found no documented failure due to membrane damage in TW wetlands planted with shrubs. However, as a precaution, a more resistant lining material or a deeper bed may be considered in TWs planted with woody species.

Shrubs and fast-growing tree species such as willow could be appropriate for TW applications due to their capacity to take on the role commonly played by herbaceous species in TWs. Despite their slower biomass development during the establishment phase, woody species could bring several important benefits to TWs over time, such as high ET rate (Beeson 2016; Frédette *et al.* 2019), high capacity to accumulate nutrients in tissues (present study) and biomass production for resource recovery purposes (Avellán and Gremillion 2019).

Further studies are necessary to better evaluate the possible benefits of using shrub species in TWs. In our case, a mesocosm-scale experiment was preferred because this type of unit can be installed in a well-controlled environment like a greenhouse, allowing replications and limiting possible confounding factors. Differences between herbaceous and woody species should be tested in full-scale TWs for validation. More importantly, our investigation was limited to a single growing season. Despite their slower development during this first season, several fast-growing woody species (*Salix* spp.) have the capacity to produce sufficient aboveground biomass to cover the planting surface and remove a high level of organic matter during this brief time frame. Over the long-term, shrub could potentially offer the same or an even higher level of pollutant removal capacity than herbaceous species, due to the quantity of biomass produced and potential to accumulate nutrients in tissues. Also, ET rate is likely to be higher for woody species in a long-term application, depending on the TW design and the maintenance requirements of vegetation. Since the benefits of using shrubs should increase with time, experiments with longer duration are necessary to compare pollutant removal and biomass production with herbaceous species. Nonetheless, our results based on one growing season confirm that the use of woody species in TWs may have been overlooked and deserve to be more often considered for TWs applications.

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