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1 **Two-year performance of single-stage vertical flow treatment**
2 **wetlands planted with willows under cold-climate conditions**

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23 **Abstract**

24 Climate-related issues constitute an important obstacle for the development of treatment
25 wetland (TW) applications in regions with freezing winter temperatures. The aim of the
26 present study was to evaluate the efficiency of a new configuration of TWs based on the
27 vertical flow (VF) configuration. The proposed TWs system is planted with a willow
28 species (*Salix miyabeana* SX67) and including design adaptations for cold climate
29 operation. Two different flow modes for winter-time operation were proposed:
30 percolated and saturated with continuous artificial aeration. The pilot-scale systems
31 were tested with municipal wastewater, at an organic loading ranging from 5 to 20 g
32 CBOD₅ m⁻² d⁻¹.

33 The pilot TWs were successfully operated for 22 months despite freezing winter
34 temperatures reaching as low as -32 °C. Willow development was normal, with
35 evapotranspiration ranging from 19 to 23 mm/d in July 2017 for the pilot TWs at an
36 organic loading of 10 g CBOD₅ m⁻² d⁻¹. Organic matter removal efficiency was high for
37 all pilot TWs, with an average 91 % COD and 81 % TSS removal. Nitrification was
38 essentially complete during the summer period and remained high for pilot TWs
39 operated under percolating flow mode in winter but was lower for the saturated flow
40 mode, probably due to insufficient air supply. Our study confirms the successful
41 application of a modified version of VF TW in regions with freezing air temperatures.

42

43 **Keywords:** treatment wetland; cold climate; willow; artificial aeration; percolating
44 flow; saturated/percolating downflow

45 **1. Introduction**

46 The implementation of treatment wetland (TW) systems in regions with freezing winter
47 temperatures presents a challenge when year-round wetland loading is targeted. This is
48 particularly the case for northern regions with a long winter period and freezing
49 temperatures, including days with very low air temperature (less than -25 °C). Freezing
50 can damage the distribution system and the filtering bed. Furthermore, low temperatures
51 in a filtering bed reduce bacterial activity and thus affect treatment performance by
52 reducing organic matter removal and nitrogen transformation processes. The major
53 mechanisms of nitrogen removal in TWs - nitrification and denitrification - are critically
54 influenced by water temperature. The favourable range of temperatures for nitrification
55 in TWs is between 16 and 36 °C (Faulwetter et al., 2009). The best denitrification rate
56 has been found to be between 20 and 25 °C; below 5 °C, both mechanisms function at a
57 very low rate and proceed slowly (Brodrick et al., 1988; Werker et al., 2002; Vymazal,
58 2007; Saeed and Sun, 2012).

59 Climate-related risks have slowed the development of TW applications in climates with
60 long and freezing winter conditions. Several studies have shown that, under particular
61 conditions, TWs may be a suitable solution for treating wastewater in cold climate
62 (Jenssen et al., 1993; Jenssen et al., 2005; Smith et al., 2006; Wang et al., 2017). Most
63 successful cold climate applications have used horizontal sub-surface flow (HSSF)
64 TWs, which do not expose the piping system and water directly to air temperature
65 (Ouellet-Plamondon et al., 2006; Yates et al., 2016; Wang et al., 2017). However, HSSF
66 TWs present certain disadvantages, including low oxygenation, which affects removal
67 processes, and poses higher risk of clogging (Saeed and Sun 2012).

68 In comparison, the French vertical sub-surface flow (VSSF) design, usually planted
69 with *Phragmites australis*, shows a much higher organic matter and nitrification
70 removal capacity, and a lower risk of clogging (Molle et al., 2005; Wang et al., 2017).
71 This TW configuration is considered one of the most efficient water treatment
72 technologies and is widely used in Europe, particularly in France, to treat different types
73 of wastewater. However, standard French VSSF TW cannot be directly transposed to
74 climates with very low freezing temperatures. In addition to the risk of freezing,
75 *Phragmites australis australis* should not be used in regions where this plant species is
76 invasive, such as in North America (Albert et al., 2015; Mozdzer et al., 2013). To take
77 advantage of the benefits of VF TWs configuration in regions with long freezing winter
78 periods, three principal aspects should be modified: the filtering bed (composition;
79 depth), operation conditions (hydraulic and organic loading; flow mode; water level
80 fluctuation; type and frequency of effluent flow distribution; aeration) and species
81 planted. These modifications aim to favor a more homogeneous hydraulic distribution,
82 thus avoiding preferential flow patterns and enabling better temperature maintenance in
83 the filtering bed (Munoz et al., 2006); they also avert the risks involved in using an
84 invasive species. In the present study, we tested experimental systems with specific
85 design modifications that address the impact of a cold climate on these three aspects.
86 *First*, the systems were designed to be more compact, with a one-stage TW instead of
87 the two successive stages used in the French classical system. This configuration is
88 similar to a compact version of the VF TW, but without recirculation or classical
89 feeding and resting periods, to reduce cooling during winter and facilitate maintenance
90 of the TW system. The compact VF TWs were initially implemented to reduce the
91 overall footprint and could also reduce heat loss during the treatment process, while

92 maintaining a high pollutant removal rate (Prost-Boucle and Molle, 2012; Paing et al.,
93 2015).

94 *Second*, operating conditions were adapted to maintain a high treatment efficiency of
95 the system in winter by testing two downflow modes: percolated, and saturated with
96 artificial aeration. The aims of the forced aeration in the saturated beds were to increase
97 pollutant removal efficiency and to reduce risks of freezing by inducing water
98 movement. The surface of TWs was insulated with a mulch layer. Different flow modes
99 and water levels were tested to determine the best approach for providing increased
100 natural insulation with ice and snow.

101 *Third*, since the study was to take place in North America, we selected a willow (*Salix*
102 sp.) as a replacement for *Phragmites australis australis*. Willows (*Salix* sp.) are among
103 the most widely used woody plants for wastewater and soil treatment purposes (Perttu
104 and Kovalik, 1997; Kuzovkina and Volk, 2009; Grebenshchykova et al., 2020). Several
105 willow species have beneficial morphological and physiological characteristics for
106 treatment wetlands, such as rapid growth rate, high biomass production, deep root
107 system with high density, high evapotranspiration rate, high nutrient uptake and
108 resistance to chemical contaminants (Tharakan et al., 2005; Kuzovkina and Volk, 2009;
109 Frédette et al., 2019). Willows tolerate different types of extreme weather conditions
110 and habitat-related ecological stresses (Perttu and Kowalik, 1997; Verwijst, 2001;
111 Major et al., 2017). Due to these characteristics, willows are commonly used to treat
112 different types of wastewater: domestic wastewater (Gregersen and Brix, 2001;
113 Lachapelle-T et al., 2019), landfill leachate (Duggan, 2005; Białowiec et al., 2007;
114 Justin et al., 2010) and sewage sludge (Listosz et al., 2018).

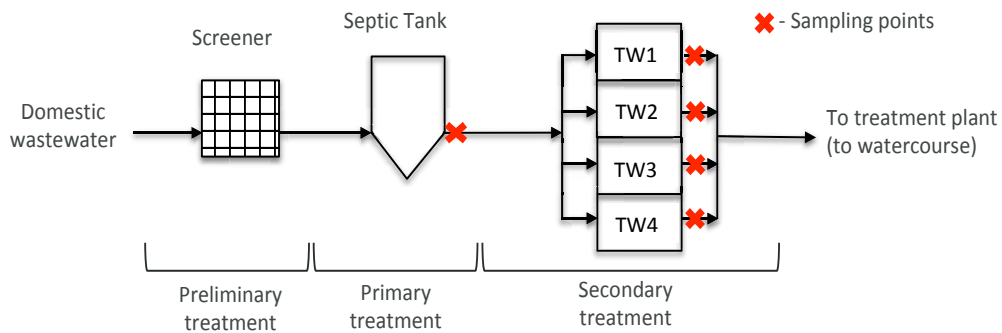
115 The objective of present study was (i) to evaluate the development of willows in TW
116 conditions in regions exposed to very low winter temperatures for several months per
117 year, (ii) to evaluate the performance of secondary treatment of the modified VF TWs
118 under these climatic conditions and (iii) to compare percolating and saturated downflow
119 modes during winter.

120 **2. Materials and Methods**

121 *2.1 Experimental design*

122 Four experimental pilot-scale units of single-stage VF TWs planted with willows were
123 set up at the water resource recovery facility (WRRF) of Saint-Roch-de-l'Achigan in
124 Quebec, Canada. The climate in this region is characterized by a cold period of five
125 months (from November to March) with an average temperature of -5.5 °C (extreme
126 minimum temperature of -36.4 °C in January 2009) and a warm period from April to
127 October with an average temperature of 14.4 °C (extreme maximum temperature of
128 36.1 °C in July 2018). The average annual precipitation is 1114 mm (all values were
129 calculated for a period of 10 last years from 2009 to 2018; National Climate Data and
130 Information Archive, 2018). Southern Quebec (Canada) is a representative example of
131 the climate-related need for an alternative sustainable solution for treatment of
132 wastewater from small-scale municipalities (Werker et al., 2002). In Quebec,
133 municipalities need to treat wastewater at least up to a secondary level (<BOD₅ 25
134 mg/L; TSS <25 mg/L, absence of acute toxicity; MDDELCC, 2010).

135 The pilot units were fed with primary settled municipal wastewater for 22 months, from
136 July 2016 to May 2018 (Figure 1). The primary treatment installation included three
137 septic tank compartments and a pumping tank to feed the TWs.



138

139 **Figure 1.** Schematic representation of the pilot treatment system used for
 140 experimentation with the position of sampling points.

141 Each pilot TW had a surface of 11.3 m^2 ($4.5 \text{ m} \times 2.5 \text{ m}$), and a total filtering bed depth
 142 of 1.2 m. The filling material was the same for all pilot units and was chosen to create a
 143 single stage filter providing a high physical filtration capacity. From top to bottom, each
 144 filtering bed contained three principal layers of 30 cm each of a mix of gravel 14-20
 145 mm and 20-40 mm; gravel 2.5-5 mm and a layer of sand (Table 1). Three additional
 146 layers of different sizes of gravel with a total thickness of 30 cm were added at the
 147 bottom for water drainage.

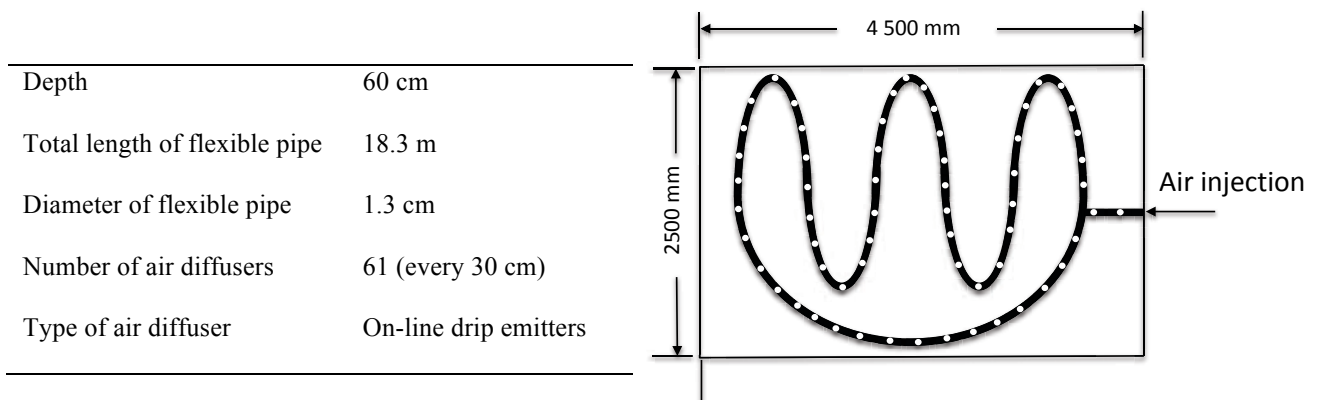
148 **Table 1.** Filtering bed composition (from top to bottom).

Thickness (cm)	Media type	Media size (mm)
30	Gravel	20 – 40 & 14 – 20
30	Gravel	2.5 – 5
30	Sand	$D_{10} = 0.34$
10	Gravel	2.5 – 5
5	Gravel	14 – 20
15	Gravel	20 – 40

149

150 Two parallel pipes 2.5 cm in diameter and 3.9 m in length were used to distribute the
 151 influent on top of each filter. Each pipe was perforated every 60 cm; the diameter of
 152 each perforation was 3.5 mm. Each distribution pipe was covered with a half round pipe
 153 of larger diameter (7.6 cm) in order to insulate the mulch from the influent. Three drains
 154 7.6 cm in diameter were installed at the bottom of the filter and connected with the
 155 effluent tank. Artificial aeration was installed at a depth of 60 cm in order to oxygenate
 156 the two principal gravel layers of the filtering bed without risking undesirable finer sand
 157 displacement in the deeper sand layer. It consisted of a flexible pipe 18.3 m in length
 158 and 1.3 cm in diameter that was perforated every 30 cm (Figure 2).

159



160 **Figure 2.** Characteristics and schematic plan view of the artificial air distribution
 161 system.

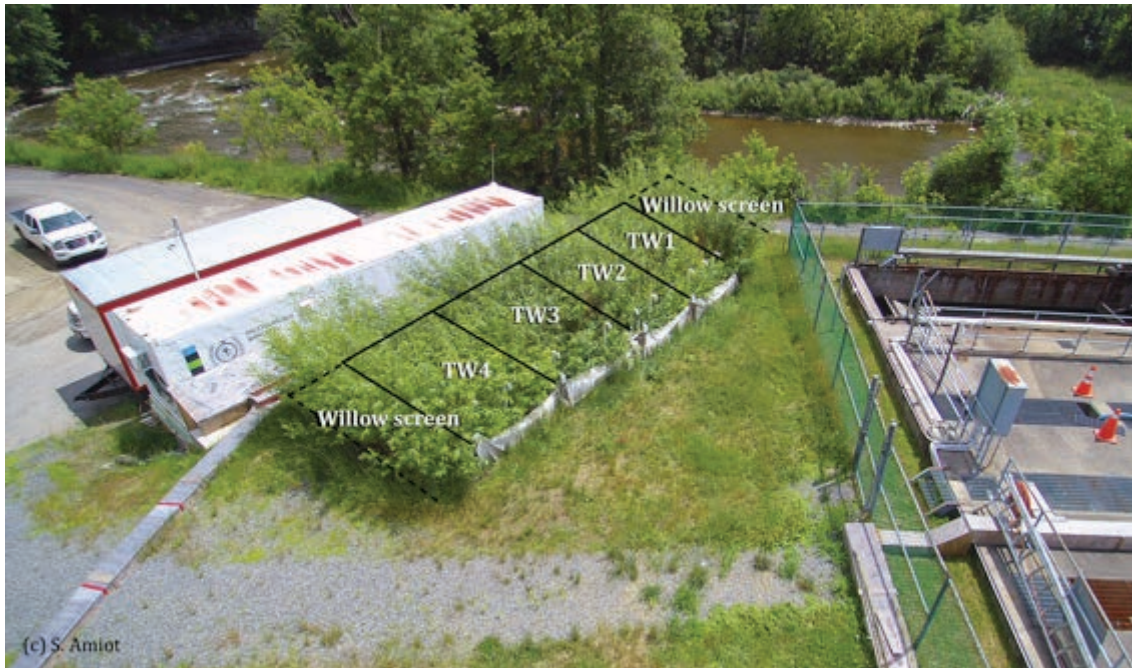
162 The flexible pipe was connected to a linear septic air pump Hiblow HP-200 (rated
 163 loading pressure: 20 kPa; airflow volume: 200 L/min) outside the filter zone. The air
 164 pump was insulated in a box to maintain a constant air temperature $> 3\text{ }^{\circ}\text{C}$ during
 165 winter.

166 2.2 *System insulation*

167 A mulch layer of 20 cm was added on top of each filter to insulate the bed and the
168 influent distribution pipe on the filter surface, as recommended by Wallace et al. (2001).
169 All external pipes between the septic tank and the distribution pipes on the filter
170 surfaces were surrounded by heating cables covered with aluminum foil. Insulation with
171 extruded polystyrene foam and plywood was added to protect the pipes from
172 precipitation and freezing temperatures in winter.

173 2.3 *Plant species*

174 A willow cultivar, *Salix miyabeana* “SX67”, was chosen for planting due to its
175 favorable characteristics such as high biomass productivity, high level of weed
176 competition and good resistance to diseases and parasites (Tharakan et al., 2005).
177 Although native to Asia, this cultivar developed at the University of Toronto does not
178 reproduce naturally by clonal propagation or seed germination, and thus should not
179 represent an invasion threat to surrounding native ecosystems (Labrecque and
180 Teodorescu, 2005; Kuzovkina and Volk, 2009). One-year-old willow plants grown from
181 cuttings were planted in each filter at a density of 4 plants/m² (45 plants/pilot unit) to
182 ensure uniform root distribution and complete surface coverage by the aboveground
183 biomass in the long-term. Two “willow screens” were planted on each side of the two
184 external pilot TWs to insure that all four TWs were similarly bordered on each of their
185 lateral sides by willows (Figure 3).



186

187 **Figure 3.** Aerial view of the experimental VF TW pilot systems.

188 *2.4 Operating conditions*

189 A percolating flow mode was used for all pilot units during the summer period. During
190 wintertime, two operation modes were tested: percolating for pilot units 1 and 4, and
191 saturated with artificial aeration for pilot units 2 and 3. Two types of saturated level
192 were tested to limit heat loss in the filtering beds: for the first winter, the high water
193 level was maintained 5-10 cm above the surface, while for the second winter the high
194 water level was maintained 5-10 cm below the mulch layer.

195 The organic loading rate was chosen to avoid organic overloading during the
196 wintertime. Thus, the TW footprint was lower than recommended for compact versions
197 of French TWs for temperate climates: $33 - 42 \text{ CBOD}_5 \text{ m}^{-2} \text{ d}^{-1}$ (raw wastewater) (Paing
198 et al., 2015) versus $5 - 20 \text{ CBOD}_5 \text{ m}^{-2} \text{ d}^{-1}$ (primary treated wastewater) recommended
199 for cold climate VF design by DWA (2006) and ÖNORM (2009). Two organic loadings
200 of 5 and $10 \text{ CBOD}_5 \text{ m}^{-2} \text{ d}^{-1}$ were tested from July 2016 to September 2017. From

201 September 2017 to May 2018, the organic loading was doubled to 10 and 20 CBOD₅ m⁻²
 202 d⁻¹ to test the limits of the TWs during cold temperatures in terms of organic loading
 203 (Table 2).

204 The feeding strategy for TWs was chosen to minimize heating loss in the filtering beds
 205 in winter using the natural heat of wastewater. The TWs were fed automatically with
 206 batches of 200 L each. The total number of batches applied per day was from 2 to 4
 207 during the first year (every 12 and 6 hours, respectively), and from 4 to 8 batches per
 208 day during the second year (every 6 and 3 hours, respectively).

209 **Table 2.** Operation conditions.

Pilot	Organic loading (g CBOD ₅ m ⁻² d ⁻¹)		Nb of batch events per 24 h		Flow mode	
	Year 1	Year 2 (winter 2)	Year 1	Year 2 (winter 2)	Winter	Summer
	TW1					percolating
TW3	10	20	4	8	saturated with aeration	percolating
TW2	5	10	2	4	saturated with aeration	
TW4					percolating	

210 The artificial aeration required for saturated flow mode was calculated according to the
 211 actual oxygen requirement (AOR) as follows (Kadlec and Wallace, 2008):

$$212 \quad AOR = COD_{B \text{ applied}} \times f_{CODB \text{ mineralized}} + (TKN \times 4.57) \times f_{TKN \text{ nitrified}} \quad (1)$$

213 where $COD_{B \text{ applied}}$ is the applied biodegradable COD (g/d), $f_{CODB \text{ mineralized}}$ the expected
 214 mineralized fraction of the applied COD (85 %), TKN is the TKN applied (mg N/L)

215 multiplied by the stoichiometric factor of 4.57, and $f_{TKN\ nitrified}$ is the theoretical nitrified
 216 fraction of the TKN applied (85 %).

217 Continuous air supply was provided at a flowrate of 18 (pilot unit 2) and 25 L/min (pilot
 218 unit 3) during the first winter experimentation period, and 28 (pilot unit 2) and 55 L
 219 O₂/min (pilot unit 3) during the second winter experimentation period. The first winter
 220 period lasted 91 days (from January 16 to April 17, 2017), and the second lasted 180
 221 days (from September 27, 2017 to April 23, 2018).

222 2.5 Monitoring

223 Grab samples were collected every two weeks at the end of the primary treatment
 224 (influent) before a batch event, and after completed treatment in each pilot unit
 225 (effluent) after a full batch event. There were no significant differences between the
 226 four tested seasons regarding the principal influent characteristics (Table 3).

227 **Table 3.** Influent characteristics.

Parameter	Units	Average	SD	Min	Max
Total COD	mg/L	264	103	114	647
TSS	mg/L	53	36	22	257
TKN	mg N/L	30.8	10.1	9.9	54.8
NH ₄	mg N/L	21.3	6.9	6.4	37.0
TP	mg P/L	4.9	1.3	1.5	7.7

228

229 The removal efficiency of total chemical oxygen demand (total COD), total suspended
 230 solids (TSS), total Kjeldahl nitrogen (TKN), ammonium-nitrogen (NH₄-N), nitrite and
 231 nitrate (NO_x-N), and total phosphorus (TP) was calculated with concentrations (mg/L)

232 as the difference between influent and effluent concentration and expressed in
233 percentage (%). Laboratory analyses were performed at the environmental engineering
234 laboratory of Polytechnique Montreal according to Standard Methods (APHA et al.,
235 2005). Dissolved oxygen (DO) was measured during the second winter by YSI 5000
236 Benchtop Dissolved Oxygen Meter in each TW effluent, immediately after sampling.
237 The average daily evapotranspiration rate was estimated in July 2017, using the water
238 balance method, as the difference between total inflow (sum of total influent and
239 precipitation) and total outflow, for every day of the month. A Stratus Precision Rain
240 Gauge measured rainfall twice a day at the treatment plant zone, providing precise
241 rainfall at the site for evapotranspiration rate calculations. Air temperature, humidity
242 and wind parameters during the experiment were provided by the L'Assomption
243 meteorological station (#7014160 (Environment Canada) 45°48'34" N, 73°26'05" O, 21
244 m), located 13 km from the WRRF.

245 *2.6 Statistical Analyses*

246 The statistical relationships between the TWs pilot units and between seasons were
247 tested using the non-parametric Kruskal-Wallis test. When significant, post-hoc
248 comparisons (Pairwise Wilcoxon test) were conducted to determine the difference
249 between groups. Differences were deemed significant at $p < 0.05$. All analyses were
250 performed using R 3.4.2 software.

251 **3. Results**

252 During the investigation period, the average temperature of the coldest month (January
253 2018) was -11.7 ± 8.0 °C, and the average temperature of the hottest month (July 2016)
254 was 20.7 ± 2.6 °C. The minimum temperature recorded on January 2, 2018 was -

255 32.5 °C and the maximum temperature recorded was 33.3 °C, on August 8, 2016
256 (National Climate Data and Information Archive, 2018).

257 *3.1 Cold climate system adaptation*

258 During the first winter, the TWs with lower organic loadings (5 g CBOD₅ m⁻² d⁻¹)
259 received two batches of wastewater of 200 L per 24 h (every 12 hours). This low
260 frequency resulted in the partial freezing of the influent distribution pipes during the
261 coldest periods. An increased frequency during second winter with batch feedings of
262 200 L every 6 hours was sufficient to maintain the functionality of the system during
263 cold period without supplementary maintenance.

264 During the first winter, for TWs operated in saturated flow mode with the water level
265 just above the surface, a partial ice layer formed that was too thin to reduce surface heat
266 loss sufficiently. As a result, the snow layer was unstable throughout that winter, with
267 frequent snow melting events. In the second winter, the water layer was kept below the
268 surface, which allowed a layer of snow more than one meter thick to form and persist
269 for most of that season, resulting in improved natural insulation of the distribution pipes
270 and the filtering beds.

271 *3.2 Willow adaptation*

272 Willows planted in TWs showed good development and a fast growth rate. The very
273 dense plantation resulted in the mortality of some willows located in the center of the
274 pilots. Also, after the second winter, die-back was observed in the top parts of many
275 willows, particularly those located at the edge of the experimental set-up. The same
276 response was noted at the margin of large willow plantation located in the region. The

277 willows affected resprouted back, and no negative effects were noted on treatment
 278 performances.

279 The average daily ET rate, measured during July 2017 for pilot units with a lower
 280 organic loading (TW2 and TW4), ranged from 10 to 14 mm/d. For pilot units under
 281 maximal organic loading (TW1 and TW3), ET ranged from 19 to 23 mm/d (Table 4).

282 **Table 4.** Average daily evapotranspiration rate for July 2017 obtained in the present
 283 experiment compared to results obtained by Frédette et al. (2019) and by Chazarenc et
 284 al. (2010) for TWs operated in the same area (Montreal region).

Source	Pilot ID	Species planted	Average ET (mm/d)	HLR ⁴ (L m ⁻² d ⁻¹)
Present study ¹	TW1	<i>Salix miyabeana</i> SX67	18.7 ± 2.9	73.2
	TW2		10.0 ± 2.4	37.8
	TW3		23.1 ± 3.2	73.2
	TW4		14.0 ± 3.2	37.9
Frédette et al., 2019 ²		<i>Salix miyabeana</i> SX67	28.7 ± 17.2	55.5
Chazarenc et al., 2010 ³		<i>Phragmites australis</i> <i>australis</i>	16.4; 16.7	30.0

285 *Note:* ¹: VF TWs, size: 11.2 m², plant density of 4 plants/m², July 2017; ²: HF TWs,
 286 size: 48 m², plant density of 2.3 plants/m²; July 2017; ³: HF TWs, size: 1 m², plant
 287 density: NA, July – August 2001; ⁴: for study 1: HLR = total influent (without
 288 precipitation).

289 The average ET obtained during the pilot scale experiment under maximal loading is
 290 within the range of those obtained from a pilot-scale treatment wetlands study by
 291 Frédette et al. (2019) conducted in the same geographical region (Table 4). Our results
 292 are also comparable to those of Chazarenc et al. (2010), obtained from a mesocosm-

293 scale study using the species most commonly used in TWs, *Phragmites australis*
294 *australis* (Table 4).

295 3.3 COD, CBOD₅ and TSS removal

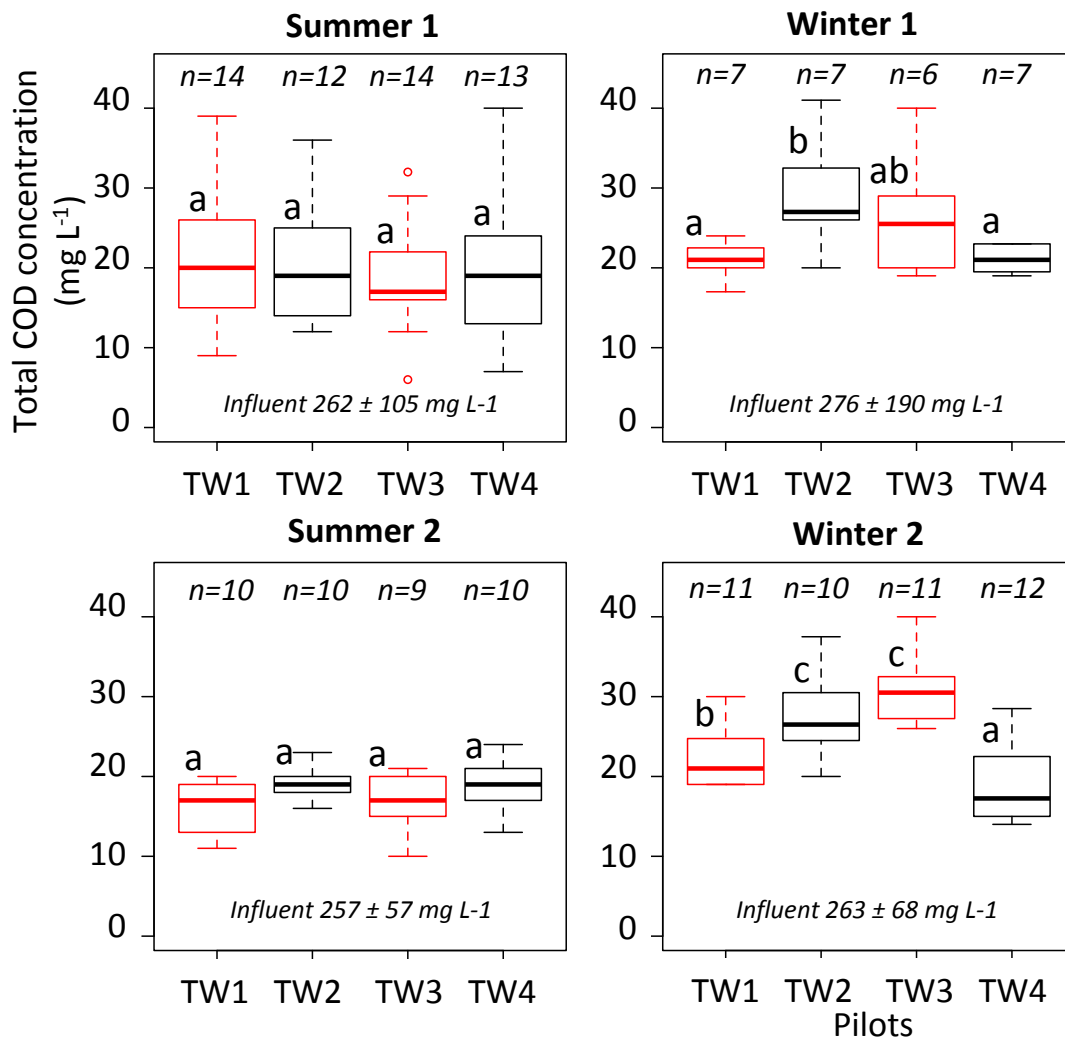
296 The average total COD removal was 91 ± 4 % during the experiment for all pilot units.
297 In summer, when all pilot units were operated in percolating flow mode, no significant
298 difference in total COD effluent concentration was observed between pilot units (Figure
299 4). In winter, the effluent concentration was significantly higher ($p = 0.026$) for pilot
300 units 2 and 3, which were operated on saturated flow mode. With increased organic
301 loading in winter 2 (Table 2) a significant increase ($p < 0.001$) in total COD
302 concentration was observed for TW1 with percolating flow as well. During the last
303 season, removal efficiency was lowest among all four seasons of the experiment.
304 During this season, the maximum removal efficiency was observed for TW1 and TW4
305 with 91 ± 3 % and 92 ± 3 % respectively. For TW2 and TW3 this efficiency was lower,
306 but still above 85 %.

307 The average effluent concentration for all tested periods was 21 ± 7 mg COD/L. Using a
308 typical secondary effluent BOD₅: COD ratio of 0.30 (Metcalf and Eddy-AECOM,
309 2014), the average BOD₅ was estimated as 6.4 ± 2.1 mg O₂/L, which is well under the
310 maximum of 25 mg O₂/L.

311 An average TSS removal of 85 ± 11 % was determined for all pilot units. No significant
312 differences were observed for effluent TSS concentration for all seasons except during
313 summer 2, during which significantly higher effluent TSS concentration ($p < 0.001$) was
314 reported for the two pilot units operated at the higher organic loading (TW1 and TW3).

315 The average effluent TSS concentration for all pilot units was 7.1 ± 5.7 mg TSS/L,

316 which is well under the discharge standards of 25 mg TSS/L required in the Province of
 317 Quebec.



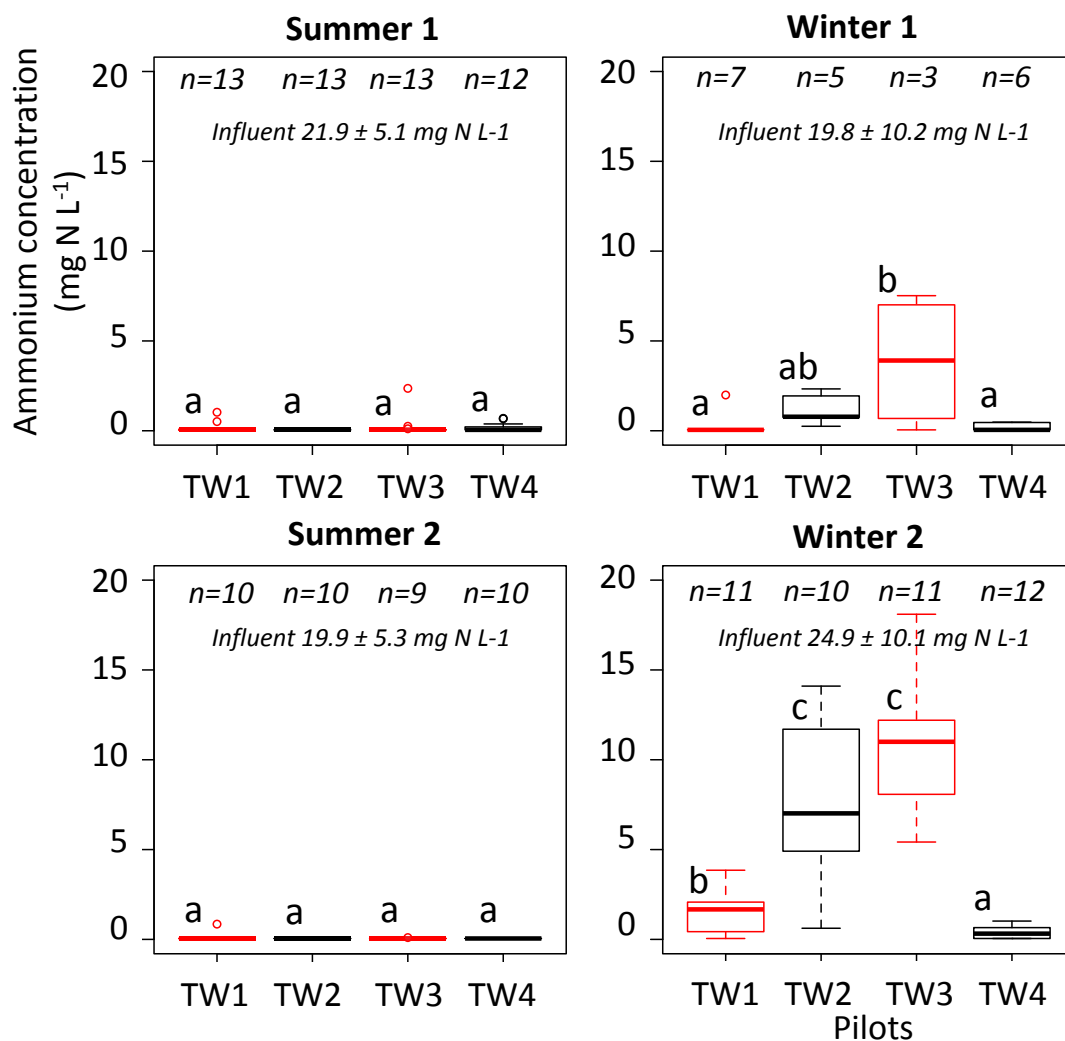
318

319 **Figure 4.** Effluent total COD concentration for summer and winter conditions for years
 320 1 and 2. Different letters above the boxplots indicate significant differences ($p < 0.05$)
 321 in total COD concentrations between the pilot units in the same season. Boxplots of the
 322 same colour indicate equal organic loadings (additional details provided in Table 2).

323

3.4 Nitrogen removal

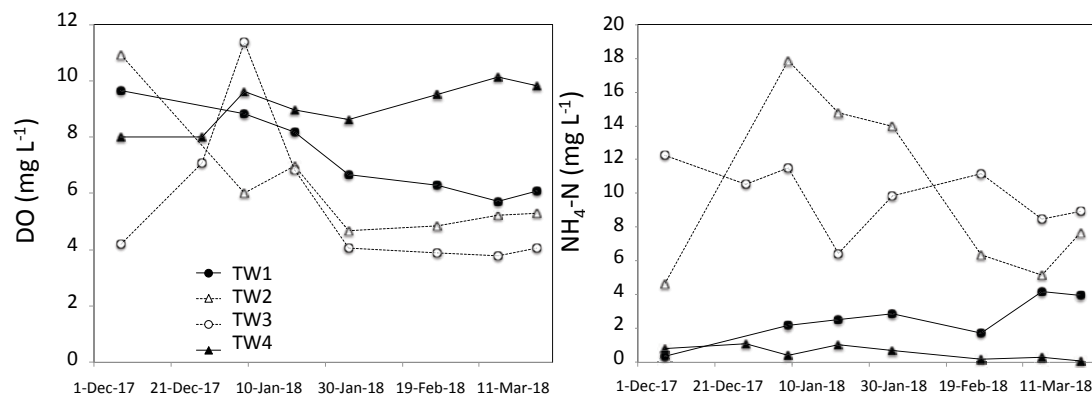
324 There were no significant differences between TWs in summer, with an almost
325 complete nitrification (average $\text{NH}_4\text{-N}$ for all pilot units was $0.12 \pm 0.30 \text{ mg N/L}$;
326 Figure 5).



327

328 **Figure 5.** Effluent ammonium-nitrogen concentration. Different letters above the
329 boxplots indicate significant differences ($p < 0.05$) in ammonium-nitrogen
330 concentrations between the pilot units in the same season. Boxplots of the same colour
331 indicate equal organic loadings (additional details in Table 2).

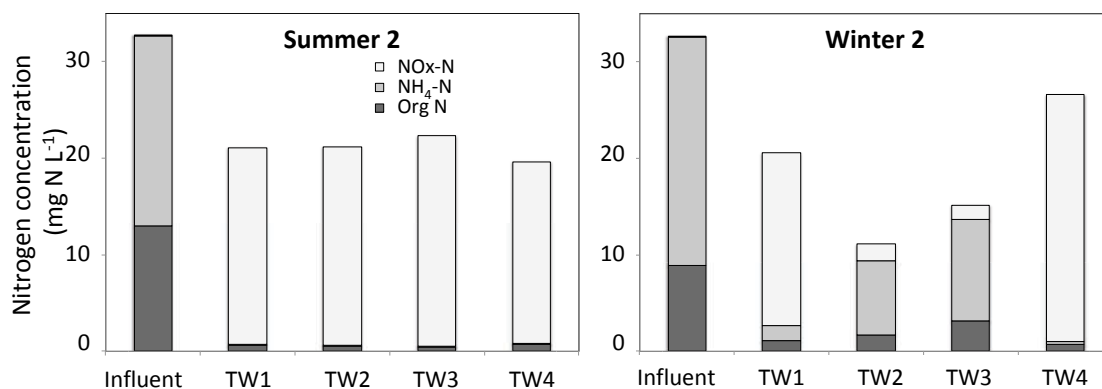
332 In winter, the ammonium-nitrogen concentration in saturated aerated flow mode (TW2
 333 and TW3) was significantly higher ($p = 0.03$) than in percolating flow mode (TW1 and
 334 TW4). This trend was even more noticeable during winter 2, with a higher ammonium-
 335 nitrogen concentration. The ammonium-nitrogen concentration in percolating flow
 336 mode in winter 2 was significantly higher ($p < 0.001$) for TW1, with an organic loading
 337 of $20 \text{ CBOD}_5 \text{ m}^{-2} \text{ d}^{-1}$, but lower than TWs operated in saturated flow mode (TW2 and
 338 TW3). The effluent DO concentrations measured during winter 2 indicated a lower DO
 339 concentration in saturated TWs (TW2 and TW3), as a result of the higher ammonium-
 340 nitrogen concentration (Figure 6).



341
 342 **Figure 6.** Dissolved oxygen concentration in effluent (*left*) and ammonium-nitrogen
 343 concentration in effluent (*right*) during winter 2.

344 Average nitrogen concentrations in the forms of nitrites + nitrates, ammonium-nitrogen
 345 and organic nitrogen are presented separately for the summer and winter seasons of the
 346 second year of the experiment, in Figure 7. In summer, all pilot units showed a similar
 347 concentration of each form of nitrogen with an average concentration of total nitrogen
 348 (TN) of $21 \pm 1 \text{ mg N/L}$ versus 33 mg N/L of TN in the influent. In winter, the effluent
 349 TN was lowest for TW2 (low organic loading + saturated flow mode), at 11 mg N/L .

350 The effluent TN concentration increased for TW3 (high organic loading + saturated
 351 flow mode), to a value of 15 mg N/L. The highest TN concentration was observed for
 352 TW1 (high organic loading + percolating flow mode) and for TW4 (low organic loading
 353 + percolating flow mode), at values of 21 and 27 mg N/L, respectively.

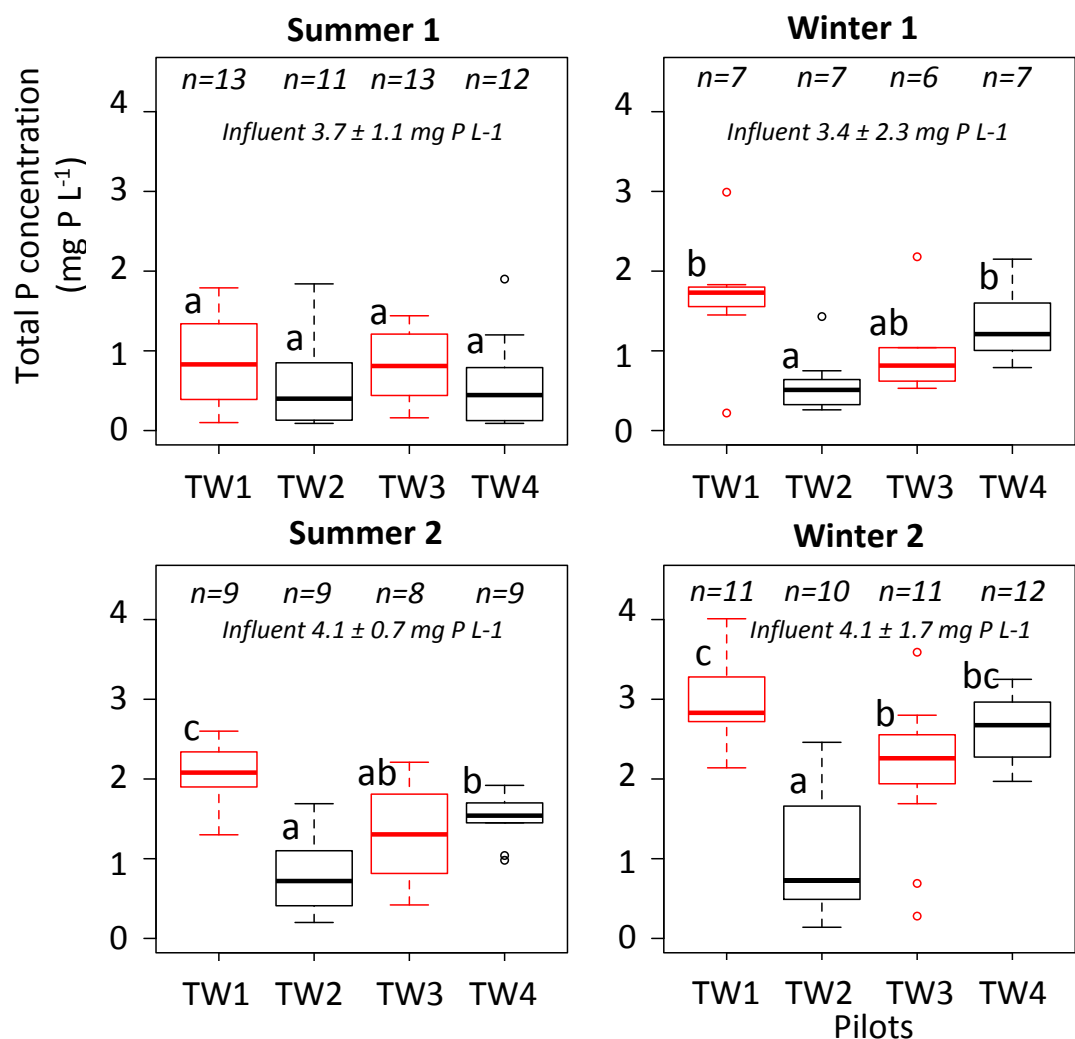


354

355 **Figure 7.** Average NO_x-N, NH₄-N and Org N concentration in influent and effluent of
 356 four tested pilot units during the second year of the experiment.

357 3.5 Phosphorus removal

358 During summer 1, there was no significant difference between effluent TP
 359 concentrations. The TP removal during this period was 78 ± 14 %. During winter 1, the
 360 TP removal of TW1 and TW4 (both operated in percolating flow mode) was
 361 significantly less efficient ($p = 0.02$) at 28 ± 40 % and 52 ± 17 %, respectively. Better
 362 TP removal was observed in TWs operated in saturated flow mode, with values of $81 \pm$
 363 8 % and 70 ± 9 % for TW2 and TW3, respectively. During the second year, the same
 364 trend was observed for each season, but at a higher effluent total P concentration
 365 (Figure 8). During summer 2, the TP removal efficiency decreased for all pilot units: 21
 366 ± 18 % for TW1, 71 ± 20 % for TW2, 39 ± 34 % for TW3 and 32 ± 18 % for TW4.



367

368 **Figure 8.** Effluent total phosphorus (TP) concentration for summer and winter, for
 369 years 1 and 2. Different letters above the boxplots indicate significant differences ($p <$
 370 0.05) in total P concentrations between the pilot units in the same season. Boxplots of
 371 the same colour indicate equal organic loadings (additional details in Table 2).

372 **4. Discussion**

373 The tested TW system was operated successfully during year-round wetland loading,
 374 even during extremely low air temperatures (-32 °C in January 2018). The type of
 375 wastewater distribution and feeding frequency of the TWs are important considerations

376 for cold period conditions. For this study, the distribution pipe was installed on the TW
377 surface under a mulch insulation layer, allowing for an appropriate balance between
378 pipe insulation and access in case of ice clogging. A proper TW feeding frequency
379 contributed to successful operation during winter. A minimum feeding frequency of
380 once per 8 hours at a hydraulic rate of 18 L/m² was efficient. The percolating flow mode
381 was maintained in winter without any modification. To minimize excessive surface heat
382 loss from the filtering bed in saturated flow mode, a water level 5 to 10 cm below the
383 TW surface is recommended. This configuration allowed a layer of snow more than one
384 meter thick to build up and persist for most of the winter, even though there were days
385 with positive temperatures, which should occur more frequently as a result of climate
386 change.

387 Willow showed fast growth, and its high ET rates (ranging from 25.6 % to 36.9 % of
388 applied HLR during the measured period), is comparable to the ET rate of the common
389 *Phragmites australis*. A high ET rate increases HRT in TWs and helps to concentrate
390 the effluent. One of the important consequences of high ET in TWs is an improved
391 removal efficiency of TKN (He and Mankin, 2001; Chazarenc et al., 2010). Our results
392 confirm the successful use of willows in TW conditions in cold climate proposed by
393 previous studies (Khurelbaatar et al., 2017; Grebenshchykova et al, 2020). In winter,
394 when ET rate is null, the woody stems favor the accumulation of snow cover on the TW
395 surface to better insulate the filtering bed. During the experiment, the willows were not
396 coppiced. Coppicing of willows is recommended every 3-4 years for optimal willow
397 growth in plantations (Labrecque and Teodorescu, 2005; Tharakan et al., 2005). Future
398 studies should clarify the optimal stem density to avoid stems mortality and the best
399 strategy for willow biomass maintenance in TWs.

400 Treatment performance was better in percolating flow mode. During the investigation
401 period, COD and TSS removal were high ($> 85\%$) and were not affected by low
402 temperatures. Similar results were observed in previous studies (Smith et al., 2006;
403 Prost-Boucle and Molle, 2012; Tunçsiper et al., 2015). The total COD concentration
404 measured in TW effluents operated in a percolating flow mode (around 20 mg/L) likely
405 corresponded to the soluble non-biodegradable fraction of typical municipal effluent. It
406 can therefore be affirmed that the TWs in percolated flow mode completely removed
407 the biodegradable part of total COD (92 % of the total COD according to Metcalf and
408 Eddy, 2003). In saturated flow mode with aeration during wintertime, total COD
409 removal was significantly less efficient (although still high at $> 85\%$) than in
410 percolating flow mode (92 %).

411 In winter, nitrification was more efficient for TWs in percolating flow mode. With 20 g
412 $\text{CBOD}_5 \text{ m}^{-2} \text{ d}^{-1}$ of organic loading in winter, the nitrification in percolating flow
413 decreased due to the lower activity of nitrifying bacteria and lower oxygenation from
414 plant roots (Ouellet-Plamondon et al., 2006). In saturated flow mode, the lower
415 nitrification rate can be explained by a lack of oxygen, as confirmed by a low DO
416 correlated with a high ammonium-nitrogen concentration in effluent. This lower oxygen
417 concentration could be attributed to a deficient air distribution system (Figure 2). In
418 contrast, the higher denitrification observed in winter in saturated flow mode was most
419 likely due to the favourable conditions provided in the deep layers of the filtering bed
420 without artificial aeration (low oxygen concentration, slightly higher COD
421 concentration).

422 Phosphorus removal from domestic wastewater using a subsurface TW with a standard
423 neutral substrate is not efficient over the long-term. Previous studies showed that a low

424 temperature does not have a negative influence on TP removal (Wang et al., 2017).
425 Once short-term phosphorus surface retention has been saturated, the effluent
426 phosphorus concentration increases, and a typical long-term TP removal is around 10-
427 20 % (Smith et al., 2006; Kadlec and Wallace, 2008; Molle et al., 2012; Troesch et al.,
428 2014; Dotro et al., 2017). In the present experiment, this period was relatively short.
429 TW1, which was operated in percolating flow mode at a maximal organic loading,
430 showed a phosphorus removal efficiency of about 20 %. The combination of two
431 factors, a lower organic loading and a saturated flow mode in winter, allowed a slow
432 rate of phosphorus saturation of the filtering bed. However, the trend of decreasing
433 phosphorus removal held true for all TWs. When a low phosphorus concentration in
434 effluent is targeted, a special media with a high phosphorus adsorption capacity must be
435 used, or another treatment system must be installed downstream or upstream from the
436 TWs to provide efficient removal (Jenssen et al., 1993; Mæhlum and Stålnacke, 1999;
437 Vohla et al., 2011; Claveau-Mallet et al., 2013).

438 The present study suggests higher treatment performances of percolating flow mode in
439 winter. With organic loading above $20 \text{ g CBOD}_5 \text{ m}^{-2} \text{ d}^{-1}$, the pollutant removal
440 efficiency in percolating mode begins to decrease. The saturated flow mode with
441 artificial aeration during cold period showed less pollutant removal efficiency in our
442 study. However, we believe our aeration system was partly inefficient, so that this flow
443 mode with properly designed artificial aeration remains a potential solution for full-
444 scale application in regions with harsh winter conditions using an organic loading above
445 $20 \text{ g CBOD}_5 \text{ m}^{-2} \text{ d}^{-1}$. A possible reason for the low nitrification rate in saturated TWs
446 with artificial aeration during wintertime in this study was the creation of preferential
447 air flows to the surface, which would result in non-homogeneous aeration of the

448 filtering bed. Previous studies showed high nitrification in TW when artificial aeration
449 is properly configured (Ouellet-Plamondon, 2006; Fan et al., 2013). An aeration system
450 with proper air distribution pipe position (depth and density), and air emitter number
451 and spacing (Nivala et al. 2018) should be considered as a solution to improve
452 homogeneous distribution of air in filtering bed. As a consequence of these
453 modifications, the nitrification rate - the principal limiting process in TWs tested in this
454 study - should be improved. For continuous aeration, a wind-driven air pump can be
455 used to keep costs down and TW technology as extensive as possible (Boog et al.,
456 2016). Intermittent aeration, sometimes used in VF TWs, represents another interesting
457 approach because it improves conditions for nitrification and denitrification in the same
458 filtering bed (Dong et al., 2012; Wu et al., 2015). The potential of this type of aeration
459 needs to be tested under real cold climate conditions, due to the high risk of TW
460 freezing during periods without aeration.

461 We tested percolating flow and partial saturation of the bottom layer separately, but
462 combining them may represent a potential promising solution by providing favorable
463 conditions for total nitrogen removal. Silveira et al. (2015) showed a high performance
464 of such system using a raw domestic wastewater, under a tropical climate. A particular
465 adaptation strategy to protect from freezing would be necessary to apply this solution
466 under cold climate.

467 **Conclusions**

468 This study demonstrated a successful application of a modified version of VF TWs in
469 regions with freezing air temperatures for five months per year. *Salix miyabeana* was
470 well-adapted to TW conditions in this climate. Applying either of two flow modes

471 (percolating or saturated with artificial aeration) in cold period, the effluent
472 concentrations were under the permitted discharge requirements ($<BOD_5$ 25 mg/L;
473 $<TSS$ 25 mg/L). The tested design with percolated downflow mode can potentially
474 receive at least two times higher organic loading ($40 \text{ g CBOD}_5 \text{ m}^{-2} \text{ d}^{-1}$) and meet
475 discharge standards, as it makes it possible to maintain a higher temperature within the
476 TW system and thus better protection from freezing during winter. Properly designed
477 artificial aeration in a saturated flow mode can potentially increase the organic loading
478 capacity and may be essential for full-scale TWs in regions with very cold winter
479 temperatures. More studies are needed to find the best configuration of artificial
480 aeration system in extremely cold conditions.

481 A better understanding of the thermal regime inside the filtering bed is necessary to
482 determine the optimal size of TW (balance between a small footprint and an acceptable
483 risk of filtering bed cooling) for very cold climates.

484 **Declarations of interest**

485 None.

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