Science of the Total Environment 838 (2022) 156061

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of macrophyte species and biochar on the performance of treatment wetlands for the removal of glyphosate from agricultural runoff

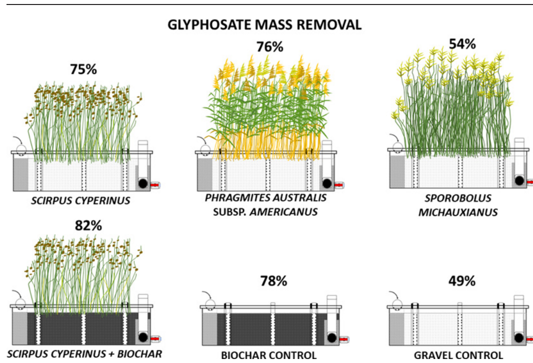
Olivier Boucher-Carrier [a], Jacques Brisson [a], Khalil Abas [a], Sung Vo Duy [b], Sébastien Sauvé [b], Margit Kõiv-Vainik [a,c,*]

[a] Institut de Recherche en Biologie Végétale, Département de Sciences Biologiques, Université de Montréal, 4101 Sherbrooke East, QC, H1X 2B2 Montréal, Québec, Canada
[b] Département de Chimie, Université de Montréal, Complexe des sciences, 1375 Avenue Thérèse-Lavoie-Roux, H2V 0B3 Montréal, Québec, Canada
[c] Department of Geography, Institute of Ecology and Earth Sciences, University of Tartu, 46 Vanemuise St., 51003 Tartu, Estonia

HIGHLIGHTS

- Hardwood biochar addition efficiently removed glyphosate in treatment wetlands.
- Planted systems showed high glyphosate removal efficiency.
- Plant species differed in their contribution to glyphosate removal.
- More AMPA was in the outflow of treatment wetlands with *Phragmites* and *Scirpus*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Paola Verlicchi

Keywords:
AMPA
Constructed wetland
Pesticide
Phytotechnology
Substrate enhancement
Subsurface flow

ABSTRACT

Glyphosate is the most widely used herbicide in the world, and consequently has polluted numerous water bodies through agricultural runoff. Treatment wetlands (TWs) have shown great promise for mitigating such pesticide contamination. The objectives of our study were to determine the effects of adding biochar to subsurface flow TW substrate, and to evaluate the performance of three North American macrophyte species (*Phragmites australis* subsp. *americanus*, *Scirpus cyperinus* and *Sporobolus michauxianus*) for removal of glyphosate. A synthetic agricultural runoff comprising 50 μg/L of glyphosate was applied to water-saturated TW mesocosms with mature vegetation during a 5.5-week period. Average removal efficiency, calculated on a mass balance basis, reached 78 and 82% for mesocosms with biochar (without and with plants, respectively), and 54 to 76% for those with macrophytes. *Sporobolus michauxianus* showed a lower evapotranspiration rate and less anoxic conditions in the lower part of the substrate, which resulted in lower overall removal performance. Aminomethylphosphonic acid (AMPA), the main toxic metabolite of glyphosate, was detected in all mesocosms, but at higher levels in planted ones. Results show that both the sorption capacity of biochar and the biodegradation processes associated with macrophytes contribute to glyphosate removal in TWs. Additionally, our results suggest that species selection is important to enhance favorable conditions and maximize removal of targeted pollutants.

* Corresponding author at: Institut de Recherche en Biologie Végétale, Département de Sciences Biologiques, Université de Montréal, 4101 Sherbrooke East, QC, H1X 2B2 Montréal, Québec, Canada.
E-mail address: margit.koiv.vainik@ut.ee (M. Kõiv-Vainik).

http://dx.doi.org/10.1016/j.scitotenv.2022.156061
Received 5 February 2022; Received in revised form 25 April 2022; Accepted 16 May 2022
Available online 20 May 2022

1. Introduction

The impact of pesticide application in widespread intensive cultivation methods has become an environmental issue all around the world (Alavanja et al., 2004; Brühl and Zaller, 2019; Sánchez-Bayo and Wyckhuys, 2019). Pesticides can enter waterbodies from agricultural fields through diffuse pathways, mostly through runoff (Bach and Huber, 2001; Blankenberg et al., 2008). Of all the pesticides in use, N-(phosphonomethyl) glycine, commonly known as glyphosate (GLP), is the most common herbicide worldwide (Benbrook, 2016; Hébert et al., 2019). It is considered toxic to birds, fish, aquatic invertebrates, fungi and microbial communities in soils and rivers (Battaglin et al., 2014; Giesy et al., 2000). Both GLP and its main metabolite, aminomethylphosphonic acid (AMPA), are highly water soluble (Annett et al., 2014; Grunewald et al., 2001), and have a high adsorption coefficient (Table A1; Sidoli et al., 2016; Sprankle et al., 1975; von Mérey et al., 2016). Glyphosate and AMPA have estimated half-lives of 2–91 days (Bergström et al., 2011; Giesy et al., 2000).

In addition to good management methods, the most common approach to prevent the spread of the pesticides through waterbodies is the addition of a vegetative barriers (Vymazal and Březinová, 2015; Zhang and Zhang, 2011). Among such barriers, constructed treatment wetlands (TWs) stand out as a well-documented type of system that can treat polluted runoff (Reichenberger et al., 2007; Schulz, 2004; Stehle et al., 2011; Vymazal and Březinová, 2015; Vymazal and Kröpfelová, 2008). The most important pollutant removal processes in TWs involve sedimentation, adsorption, hydrolysis, plant uptake and biochemical degradation by microorganisms (Vymazal and Březinová, 2015). In the case of free glyphosate, sedimentation and hydrolysis are negligible, and root assimilation is low (Briggs et al., 1982; Chen et al., 2007; la Cecilia and Maggi, 2018; Laitinen et al., 2007; Wagner et al., 2003). Consequently, in such cases, adsorption and microbial degradation should be enhanced in TWs to maximize glyphosate removal (Vymazal and Březinová, 2015).

Heterotrophic microorganisms can metabolize glyphosate and use it as a source of nitrogen, carbon and phosphates (Bergström et al., 2011; Borggaard and Gimsing, 2008; la Cecilia and Maggi, 2018). These metabolic processes are much slower when macrophytes are absent (Matamoros et al., 2007; Vallée et al., 2014; Vymazal and Březinová, 2015). Macrophytes create favorable conditions for microorganisms in the rhizosphere, through exudates and oxygen release from roots and rhizomes (Brix, 1987; Imfeld et al., 2013). Furthermore, the evapotranspiration (ET) rate in planted TWs can increase the retention time of pollutants, thereby increasing the time period during which these persistent molecules can be degraded (Beebe et al., 2014; Chazarenc et al., 2003). Removal efficiency for a particular pollutant may differ between plant species (Brisson and Chazarenc, 2009; Vallée et al., 2014). Plant resistance is an additional issue for herbicide removal, due to the potentially negative effect on plant growth, as reported for macrophytes in Bois et al. (2013). Finally, growing concerns about the threats of invasive macrophyte species increasingly suggest that using native species in TWs is preferable. Since comparing species performance in different contexts is problematic (Brisson and Chazarenc, 2009), using multiple native species in the same study constitutes the best approach to ensure selection of non-invasive species effective for removal of the targeted pollutants.

Retention of pesticides inside a TW can also be increased by the presence of sorption sites in the substrate. This is possible if the pollutant has a high soil organic carbon-water partition coefficient (K_{OC}), which is the case for glyphosate and AMPA (Stehle et al., 2011; Vymazal and Březinová, 2015). Accordingly, using biochar as a substrate enhancement could have a positive impact on pollutant removal in TWs (Kasak et al., 2018b) due to its important sorption ability (Lehmann, 2007) and potentially large surface area with a high density of negative charges on its surface (Liang et al., 2006). Previous studies have documented the removal of glyphosate in soil, through biochar application (Hagner et al., 2015a, 2013; Junqueira et al., 2020; Sharma and Lai, 2019), as well as from stormwater basins and raingardens (Bois et al., 2013; Imfeld et al., 2013; Yang et al., 2013). However, the efficiency of using biochar in TWs to treat agricultural runoff with a high concentration of glyphosate remains to be evaluated (Maillard et al., 2011). We conducted a mesocosm experiment with the objectives of: a) determining the effects of biochar that is added to TW substrate, and macrophytes, on removal efficiency of glyphosate from agricultural runoff, and b) comparing the efficiency of three species of macrophytes.

2. Materials and methods

2.1. Experimental setup

2.1.1. Design

The outdoor mesocosm experiment was conducted in the Montreal Botanical Garden (Canada), during the summer of 2020. Fourteen mesocosms were placed randomly in a posteriori block design to minimize the effect of abiotic conditions on species' results (Figs. 1; A1). Mesocosms were filled (depth 0.41 m) with inert granite gravel substrate. At the inflow and outflow zones of each mesocosm, two strips (0.2 m wide) of coarser gravel were placed so as to facilitate water flow (Fig. 1). The main gravel substrate of four mesocosms was enhanced with the addition of 15% by volume of biochar, which was mixed with the gravel. The inert substrate was chosen to isolate the roles of biochar and plants, and to minimize the effect of the media as an additional variable in the system. The biochar, made from hardwood composed of 25% beech, 25% birch and 50% maple as feedstock, was pyrolyzed at a maximum temperature of 350 °C in a Missouri oven. This type of biochar (i.e. a by-product of charcoal production) was chosen since it is the most available and cost effective for farmers. Furthermore, hardwood biochar is proven to reduce the leaching of GLP (Hagner et al., 2015b; Hall et al., 2018a).

To ensure even distribution of the influent water to the inflow zone of all mesocosms, a PVC pipe perforated on the bottom was placed on top of the coarse gravel area at the inflow zone of each mesocosm (Fig. 1). To collect water at the outflow zone of each mesocosm, a similarly perforated PVC pipe was placed 5.9 cm from the bottom of the mesocosm, which was connected through the side of the mesocosm to an outflow pipe with a valve at the end. A vertical overflow pipe system was added to each outflow pipe to maintain the water level inside the mesocosm at a maximum of 0.39 m (Fig. 1) from the mesocosm bottom, 0.02 m under the substrate surface.

Inside each mesocosm, three diagonally arranged piezometers were placed vertically for sampling and monitoring (A, B and C). In the current experiment, only the B piezometer was used (Fig. 1B). Finally, 2 rhizotrons were added to each mesocosm to monitor root system growth. Other details of the experimental setup are provided in Table A2 and Fig. A2 of the Supplementary material. The setup was also used for an experiment on removal of the insecticide chlorantraniliprole in the summer of 2019 (Abas et al., 2022) and some of the results of the current experiment are compared with findings in that study.

2.1.2. Plant species

Of the 14 SSF TW mesocosms, nine were planted with three different species of macrophytes: *Phragmites australis* subsp. *americanus* (American Common Reed), *Scirpus cyperinus* (Woolgrass) and *Sporobolus michauxianus* (syn. = *Spartina pectinata*; Prairie cordgrass), each in three replicates. The three species were selected for their general characteristics: habitat preferences, rapid growth, high biomass production, large root system and resistance to pollutants (Abas et al., 2022; Boe et al., 2009; Vymazal, 2011; Wu et al., 2000). Three additional mesocosms with biochar added were planted with *Scirpus cyperinus*. Finally, two mesocosms were left unplanted, one with biochar added to the gravel substrate and one without. The macrophytes (11 individuals per mesocosm) were planted in the summer of 2018 in order to give the plants time to colonize the mesocosms and mature. By the time the present experiment was conducted in 2020, the mesocosms were fully colonized with mature plants (Fig. A3). For further simplicity, the different mesocosms are referred to hereafter as

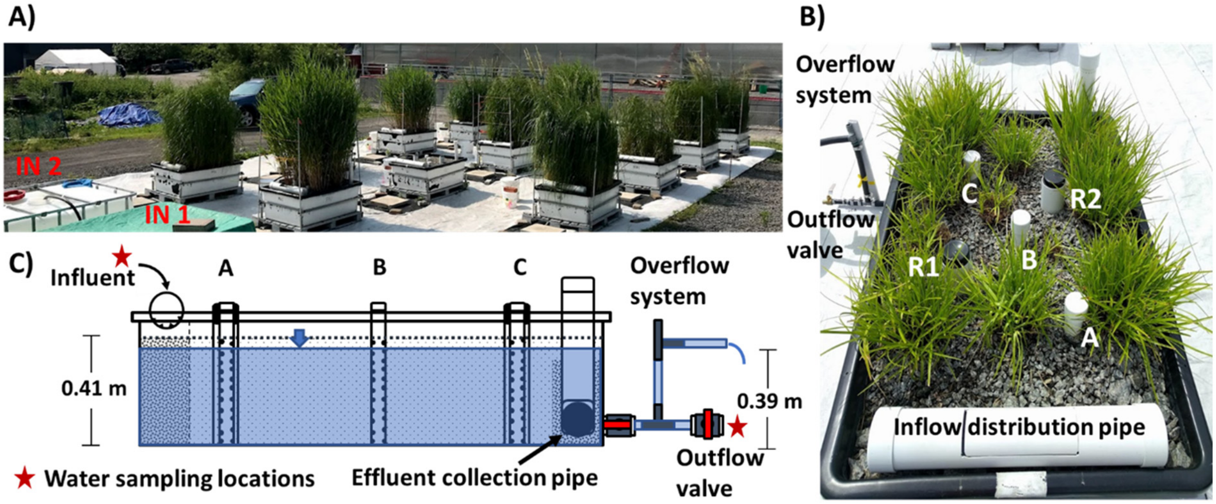


Fig. 1. A: View of the experimental setup: IN1 - inflow tank 1; IN2 - inflow tank 2. Each planted treatment had 3 replicates and the two unplanted only one; B: Photo of a mesocosm with location of influent distribution pipe and overflow system, vertical perforated sampling piezometers (A, B, C), and rhizotrons (R1 and R2); C: Schematic side view of the mesocosm design.

"unplanted", "unplanted with biochar", "Phragmites", "Sporobolus", "Scirpus" and "Scirpus with biochar".

2.2. Synthetic agricultural runoff

2.2.1. Influent composition

A synthetic runoff modified from a Hoagland solution was used as influent to the experimental mesocosms (see Table A3 for details). The solution was added to obtain theoretical concentrations of 40 mg/L for nitrogen, 6 mg/L for phosphorus, 50 mg/L for potassium, 27 mg/L for calcium, 5.8 mg/L for magnesium, 20.4 mg/L for sulfur, 60 mg/L for carbon and 0.69 mg/L for iron. This fertilizer solution was prepared at a concentration stronger than that of typical agricultural runoff (Kasak et al., 2018a; Kato et al., 2009), given that the mesocosm substrate was made of inert gravel, without organic matter or soil nutrients. Nitric acid was added to adjust the pH of the solution, prepared with tap water, to around 6.5, in order to render the nutrients more available to plants (Asao, 2012).

During the experiment with glyphosate, the pesticide was added in the influent using the product Factor® 540, to reach a concentration of 50 μg/L. This concentration was based on the highest concentration of pesticide pollution obtained in a recent survey of rivers in the province of Quebec (Giroux et al., 2019), and multiplying that figure by 10. This multiplication factor took into account that Giroux et al. (2019) surveyed pesticide concentrations in rivers receiving water from agricultural ditches, rather than in ditches themselves, where pesticide concentrations would no doubt be higher.

2.2.2. Influent application

The synthetic runoff, without glyphosate, was applied from June 22 to July 28, 2020, with 80 L per feeding and two feedings per week (Mondays and Thursdays), for a total of 10 applications (watering schematic presented as Fig. A4). The synthetic runoff with glyphosate added was applied from July 30 to September 7, for a total of 11 additional applications (see Fig. A4 for details). For each event, the synthetic solution was mixed in both influent tanks with a pump for 15 min. The mesocosms were watered in 4 rounds of 20 L each per mesocosm to better distribute the feeding among the 14 mesocosms in time. Each feeding event lasted 5 to 6 h. The theoretical hydraulic retention time (HRT) was 6.2 days, which is comparable to other similar studies (5 to 7 days) using SSF for the removal of organophosphate pesticides (Agudelo et al., 2012; Matamoros et al., 2007).

2.3. Monitoring

2.3.1. Plant health

Plant health was continuously monitored visually. Every month, 360° photos of the root systems were taken using the two rhizotrons and a root camera (CI-600 In-Situ Root Imager; CID Bio-Science). Finally, at the end of the season, the aboveground biomass was harvested and weighed the same day. A subset of the biomass was then dried in paper bags for a month at 35 °C and weighed again. For further methodological details, see also Abas et al. (2022).

2.3.2. Evapotranspiration

Every watering day (twice a week), the water level in the B piezometer of each mesocosm was noted before and after watering, to calculate the volume of water in the mesocosm using the pore factor measured at the beginning of the season (3.71 L/cm; Eq. (A1)). Water volume inside the mesocosms and precipitation from the previous application (Government of Canada, 2020) were used to estimate mesocosm daily ET rate (L) (evaporation only for unplanted mesocosms) between applications (Eq. (A2)).

2.3.3. Water sampling and analysis

Water samples were taken from the outflow pipe of each mesocosm on Mondays, prior to watering. This pipe is situated above the dead volume of the mesocosm and is also a part of the overflow system, making it the best location at which to sample this system (see Fig. A4). Outflow sample represents the concentration of pollutants that would be released into the environment after treatment in a SSF TW. Influent samples were taken by combining equal parts of the solution from each watering round. As the measured and calculated influent concentrations were very similar, we used the calculated concentration in subsequent calculations (Eq. (A3)). First, pH, oxidoreduction potential (ORP) (sensor HI7698194-1) and temperature were measured with a Hanna-HI98194 multiprobe (Hanna Instruments®). The multiprobe was used on site biweekly right after water samples were collected, from July 6 to September 7, for a total of 6 times. Samples of pollutants other than glyphosate and AMPA were collected in designated laboratory plastic bottles, placed in a cooler with ice packs for conservation and delivered to an accredited laboratory (Eurofins Environex, Longueuil, Canada) immediately after sampling. Water samples were collected weekly from July 3 to September 7, for a total of 6 sampling events. The glyphosate and AMPA samples were put in 30 mL amber glass bottles and frozen (−18 °C) weekly on the same dates.

Removal efficiency of pollutants other than GPL and AMPA in the mesocosm is provided as complementary information that shows the general performance of our mesocosms. Analyses of these pollutants were performed according to standard APHA and CEAEQ methods (CEAEQ, 2019; Rice et al., 2017) to evaluate: total suspended solids (TSS), orthophosphates (PO_4^{3-}), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and nitrates (NO_3^-). Only pH, ORP, TKN and nitrates results are presented in this article. For results of all other parameters, see Table A5.

2.3.4. Glyphosate and AMPA analysis

Chemical analyses of glyphosate and AMPA were conducted as per Montiel-León et al. (2019), with some modifications. GFF-filtered water samples were amended with isotope-labelled internal standards (ILIS) and derivatized with FMOC-Cl (9-fluorenylmethyl chloroformate). The reacted samples were subsequently analyzed by on-line solid-phase extraction liquid chromatography heated electrospray ionization high-resolution mass spectrometry (Thermo Q-Exactive Orbitrap UHPLC-HRMS). The method detection limit was 5 ng/L. Further details regarding the derivatization and instrumental analysis are provided in Appendix A1. Matrix-matched internal calibration curves (5–1000 ng/L, $R^2 > 0.99$) were used for quantification. For samples exceeding the upper limit of quantification (uLOQ, 1000 ng/L) were diluted before ILIS addition and derivatization.

$$\% \, Mass \ removal = \frac{(M_{in} + M_{t1}) - M_{t2}}{M_{in} + M_{t1}} \quad (1)$$

M_{in}: Glyphosate mass in the influent.M_{t1}: Glyphosate mass in the mesocosm water before watering.M_{t2}: Glyphosate mass in the mesocosm water before subsequent watering.

Similarly, as ET, the pore factor was used to determine the water volume inside each mesocosm. This volume was then used to determine the mass of pollutants in each mesocosm. With volumes and concentrations, it was possible to calculate percentage of mass removal of the glyphosate per mesocosm (Eq. (1)). This removal is a partial removal as a portion of it is degraded into AMPA, which is also a pollutant.

Since the concentrations of glyphosate inside the mesocosms were only measured on Mondays to calculate the mass removal, the same concentrations were used for the preceding Thursdays, while the true concentration may have varied due to random changes (not systematically related to a particular day of the week), such as weather, and hence did not create any bias. Furthermore, the glyphosate concentration in the outflow from the mesocosms was considered to be the same as the concentration of the pesticides prior to watering (more details in Eq. (A3)).

2.4. Statistical analyses

All values are reported as an average ± the standard error of the mean unless otherwise specified. Comparison of the following parameters was tested statistically between treatments: dry biomass, glyphosate removal, AMPA mass, seasonal evapotranspiration, ORP, pH, NO_3^- mass and TKN mass. Unplanted mesocosms were included in graphs for qualitative comparison without statistical analysis, given the absence of replication.

R software (version 4.0.2, R Core Team) was used to perform statistical analyses. Linear mixed effects model (LMM) was applied to examine the significance of the interaction between treatment and time where suitable, adding the bloc effect as a random factor in the model. When the interaction was significant, separate analyses, also using LMM, were conducted for each sampling date. All models were assessed for normality and homogeneity of the variance by visual inspection of plots of residuals against fitted values. Variables that did not meet normality or heterogeneity assumptions were modified using the appropriate transformation (Log10 or square root). A repeated measurement ANOVA was used on the different LMM ($\alpha = 0.05$) to determine if treatment differences were statistically significant. When the overall ANOVA was significant, it was followed by a post-hoc Tukey's HSD test. Error outliers were removed from the dataset using boxplot for visualization (Aguinis et al., 2013), followed by the interquartile deviation method (Rousseeuw and Croux, 1993).

3. Results

3.1. Plant health

Plant density was high for all species (Table S2). The macrophytes grew well throughout the summer, with average shoot and flower heights in August of 187 ± 13 cm for Phragmites, 136 ± 18 cm for Scirpus and 169 ± 17 cm for Sporobolus, measurements that are consistent with findings for mature plants in nature (Rodríguez and Brisson, 2015; USDA, 2020). Root systems were well developed in all three species (e.g., Fig. A5). Roots and rhizomes were present from the top to the bottom of the substrate for Phragmites and Scirpus, but the lower portion of the substrate was not colonized by Sporobolus (Fig. A5).

The dry aboveground biomass weight per square meter (Fig. 2) of Sporobolus (average of 3.13 ± 0.41 kg/m^2) was significantly higher compared to Phragmites (average of 2.59 ± 0.13 kg/m^2) Scirpus (average of 2.38 ± 0.21 kg/m^2) and Scirpus with biochar (average of 2.1 ± 0.15 kg/m^2). The two latter mesocosms showed no significant differences between them. There was an important variance in the total biomass weight of Sporobolus replicates. Comparison of biomass weight to results of the 2019 experiment on chlorantraniliprole removal (Abas et al., 2022), showed similar patterns of weight relationships between the different treatments.

3.2. Evapotranspiration rate

Average evaporation rates in mesocosms that were unplanted and unplanted with biochar (0.6 and 0.5 cm/day respectively; Fig. 3) were much lower compared to the rates in planted systems. From the beginning of August, when glyphosate application began, there was a slight decrease in ET rates over time in planted mesocosms, which was also observed in the 2019 experiment (Abas et al., 2022). Sporobolus had a significantly lower average ET rate (1.4 cm/day) compared to other planted mesocosms (2.2 to 2.3 cm/day) throughout the season (Fig. 3).

3.3. Changes in water chemistry

The seasonal average of the TKN mass and pH value showed no significant differences between mesocosms (Table A6). However, Sporobolus (average of 2.3 mg) and Phragmites (average of 2.3 mg) mesocosms had significantly lower nitrate mass, when compared with Scirpus (average of 210 mg). Furthermore, Sporobolus had significantly lower ORP (average of −345 mV), from which we can deduce that anoxic conditions may have been present in those 3 mesocosms. In contrast, Phragmites had significantly higher ORP (average of 3.2 mV). Unplanted (average of 1450 mg) and unplanted with biochar (average of 1510 mg) had the highest nitrate mass per mesocosms.

3.4. Changes in glyphosate and AMPA concentration and mass

3.4.1. Pollutant concentrations and mass

Concentrations of glyphosate were lower (consistently below 29 μg/L) in all TWs 3.5 days after application than in the influent (50 μg/L; Fig. A6). Unplanted and Sporobolus had the highest concentration, with 25 and 28 μg/L of GLP after the last watering event. AMPA concentrations, on the other hand, were always higher in the TWs than in the influent (0.7 μg/L). Three treatments, Sporobolus, unplanted and unplanted with biochar, had the lowest maximum concentrations, with respectively 3.1, 3.6 and 3.6 μg/L (Fig. A6).

The average mass of glyphosate and AMPA in the mesocosms prior to the Monday application (hence 3.5 days after the previous watering), during the final 4 weeks of glyphosate application, is presented in Fig. 3. In all mesocosms, the average mass of GLP after 3.5 days of residence time

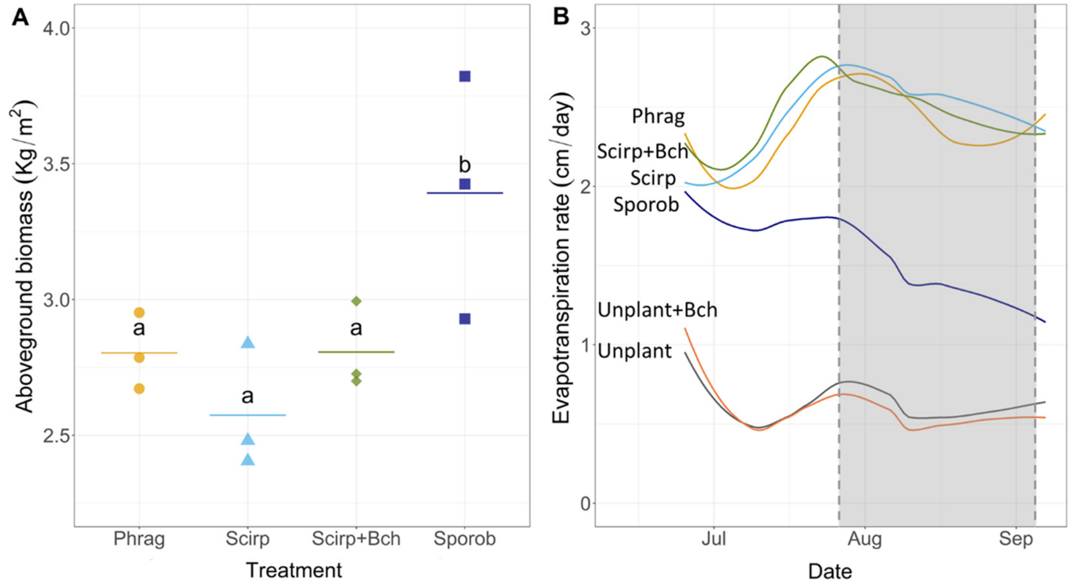


Fig. 2. A: Average aboveground biomass weight according to treatment at the end of the growing season. Groups a and b are significantly different ($p < 0.05$); B: average evapotranspiration and evaporation rates of the mesocosms monitored during the 2020 growing season The Loess smoothing method was used, with a span of 0.65. The grey frame indicates glyphosate application period. Abbreviations: Sporob = *Sporobolus michauxianus*; Phrag = *Phragmites australis* subsp. *americanus*; Scirp = *Scirpus cyperinus*; Scirp + bch = *Scirpus cyperinus* with biochar added.

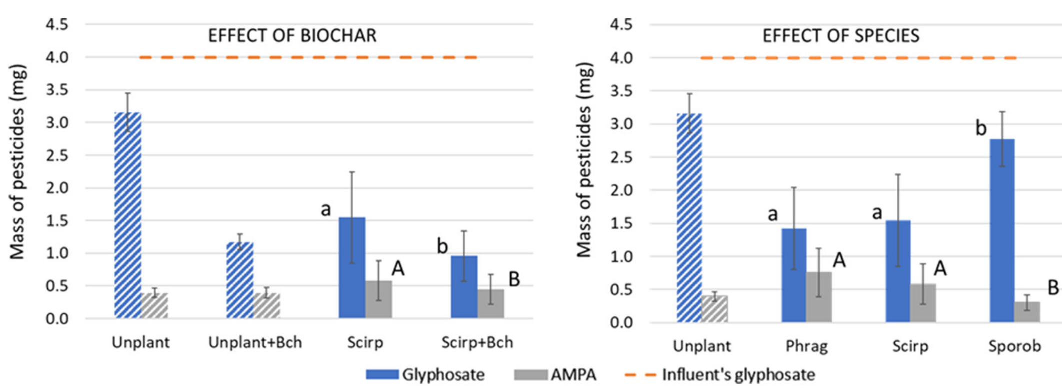


Fig. 3. Average mass of glyphosate and aminomethylphosphonic acid (AMPA) in the water of the mesocosms according to treatment prior to application on Mondays (3.5 days after previous watering) during the final 4 weeks of glyphosate application. Influent line refers to the mass added on Thursday during those weeks. Groups a and b differ significantly ($p < 0.05$) in glyphosate mass, while groups A and B differ significantly in AMPA mass. Unplanted, unplanted with biochar (hatched bars) and influent (dashed lines) are presented as references; no statistical analysis could be performed on them due to the lack of replicates. Abbreviations: Unplant = unplanted gravel control; Unplant + bch = unplanted with biochar added; Sporob = *Sporobolus michauxianus*; Phrag = *Phragmites australis* subsp. *americanus*; Scirp = *Scirpus cyperinus*; Scirp + bch = *Scirpus cyperinus* with biochar added.

for those last four sampling events was lower than the amount added by the influent (Fig. 3). The average mass of both GLP and AMPA in mesocosms of Scirpus with biochar (0.96 mg and 0.45 mg, respectively) were significantly lower than in mesocosms with Scirpus (1.55 mg and 0.58 mg, respectively). Scirpus with biochar (0.96 mg of GLP and 0.45 mg of AMPA) and unplanted with biochar (1.17 mg of GLP and 0.39 mg of AMPA) yielded similar results for average mass of both GLP and AMPA. The average GLP mass in the unplanted mesocosm with gravel (3.16 mg) was higher than that in unplanted with biochar (1.17 mg), while both of these treatments showed similar results for AMPA mass (0.40 mg and 0.39 mg respectively; Fig. 3). This demonstrates that biochar was effective in reducing glyphosate, but not in removing AMPA.

Comparing macrophyte species performance in terms of average mass of pollutants during the final 4 weeks of pesticide application (Fig. 3), Phragmites (1.42 mg) and Scirpus (1.55 mg) mesocosms showed no significant differences in average GLP mass, while the Sporobolus mesocosm had a significantly higher GLP mass (2.77 mg). Sporobolus also had a significantly lower average AMPA mass (0.12 mg), followed by Scirpus (0.30 mg) and Phragmites (0.36 mg), which had the highest. Like Sporobolus, the unplanted treatment had a high average GLP mass (3.16 mg) and a low average AMPA mass (0.40 mg).

3.4.2. Glyphosate removal and AMPA mass

At most sampling events throughout the application period, Scirpus with biochar and Scirpus showed no significant differences in percentage of glyphosate mass removed (average of 81.9% and 74.6%, respectively) or mass of AMPA present in the effluent (average of 327 μg and 416 μg, respectively; Fig. 4). However, the unplanted with biochar treatment (average of 78.4%) showed a much higher percentage of GLP removal compared to the unplanted one (average of 49.3%). On the other hand, the mass of AMPA measured in those two mesocosms was very similar (average of 290 μg and 301 μg, respectively). Among the planted mesocosms, at most sampling events, Phragmites and Scirpus were not significantly different regarding glyphosate mass removal (average of 75.5% and 74.6%,

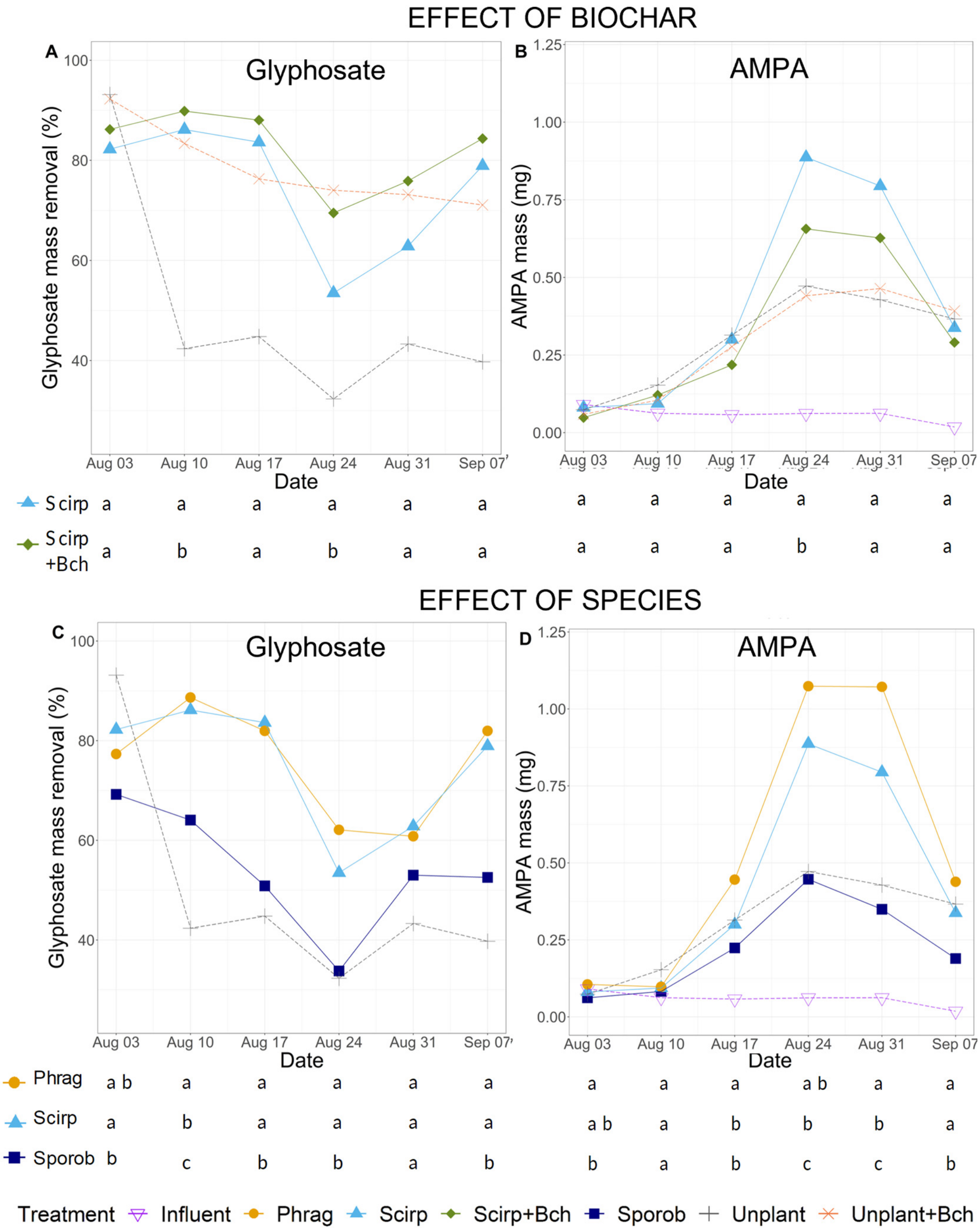


Fig. 4. Average glyphosate mass removal (%) and mass (mg) of aminomethylphosphonic acid (AMPA) in mesocosm water according to treatment, monitored on Mondays, 3.5 days after last watering. Graphs A and B represent the mesocosms with biochar, graphs C and D represent planted mesocosms. The table under each graph, to be interpreted vertically, shows groups (a, b, c) that are significantly different ($p < 0.05$) on a given day. Unplanted, unplanted with biochar and influent are presented as references (dashed lines); no statistical analysis could be performed on them due to the lack of replicates. Abbreviations: Unplant = unplanted gravel control; Unplant + bch = unplanted with biochar added; Sporob = *Sporobolus michauxianus*; Phrag = *Phragmites australis* subsp. *americanus*; Scirp = *Scirpus cyperinus*; Scirp + bch = *Scirpus cyperinus* with biochar added.

respectively) or AMPA mass (average of 539 μg and 416 μg, respectively; Fig. 4). In comparison, Sporobolus showed significantly lower GLP removal (average of 53.9%) at most sampling events. Results were mixed for Sporobolus mass of AMPA (average of 226 μg), as this treatment was not significantly different from Phragmites or Scirpus for the first three sampling events but was significantly lower at the last three samplings (Fig. 4).

4. Discussion

No visible changes in general plant health or appearance were observed during glyphosate applications. Results show that glyphosate removal was improved by the addition of biochar and the presence of macrophytes, and that the production of AMPA was higher in planted mesocosms. In our experiment, maximum average removal was reached in TWs that were both planted (with Scirpus) and with biochar added.

4.1. Effects of glyphosate on macrophyte health in treatment wetlands

In a microcosm stormwater experiment, Bois et al. (2013) showed that the presence of glyphosate and other herbicides (diuron and 3,4-dichloroaniline) in high concentration (50 mg/L) can have a negative effect on plant growth. In our experiment, application of an influent with a concentration of 50 μg/L of GLP for 5.5 weeks generated no noticeable change in density, biomass or health in any of the plant species. Similar results were found by Sesin et al. (2020), who exposed macrophytes to 41 μg/L of glyphosate in wetland water. Maillard et al. (2011) and Imfeld et al. (2013) treated agricultural runoff containing glyphosate in a stormwater wetland and also detected no negative effects on plants. All these findings concur with the documented low uptake of glyphosate by the root system (Lockhart et al., 1989; Wagner et al., 2003).

4.2. Evapotranspiration and root zone conditions

In August, ET began to decrease at varying rates in all the planted mesocosms (Fig. 1B). This is probably unrelated to the addition of glyphosate, since similar ET pattern was observed in the 2019 experiment, in which no herbicide was applied (Abas et al., 2022). Rather, it seems to be related to a natural decrease in ET rate at the end of the growing season. A similar pattern was observed by Pedescoll et al. (2013) and Milani et al. (2019) in constructed wetlands. In the present study, mesocosms planted with Sporobolus had the lowest ET and the root system did not colonize the lower portion of the TW, much like in the 2019 experiment (Abas et al., 2022). Still, Sporobolus produced significantly more biomass during the growing season than other species, perhaps due to early seasonal growth (Madakadze et al., 1998; Skinner et al., 2009).

According to Wu et al. (2000), the roots and rhizomes of Sporobolus have an important capacity to release oxygen in the substrate. The fact that its roots and rhizomes did not colonize the TW to its full depth may have created anoxic conditions in the part of the substrate from which they were absent (Brix, 1993; Vymazal, 2011). This could explain the low mass of nitrates in the Sporobolus mesocosms and the plant's lower oxidoreduction potential. Mass of TKN was similar in all planted treatments. In systems with low oxygen, microbial anaerobic respiration reduces nitrates, which in turn lowers the ORP. Pedescoll et al. (2013) found a positive relationship between evapotranspiration and ORP in TW, in which higher rates of ET by macrophytes were associated with higher ORP. Anaerobic conditions were also qualitatively indicated by the rotten egg smell of the effluent from Sporobolus mesocosms, which is characteristic of the presence of hydrogen sulphide (H_2S), created by the reduction of sulphate in anoxic conditions (Blodau et al., 2007; Vile et al., 2003).

4.3. Effects of biochar on glyphosate removal and AMPA mass

The unplanted mesocosm showed lower average glyphosate mass removal during the last four sampling events compared to the unplanted with biochar mesocosm. Thus, biochar alone had a positive effect on glyphosate reduction. The effect of biochar is still noticeable in the presence of macrophytes, as mesocosms 'Scirpus with biochar' had a slightly lower GLP mass than Scirpus alone. The important difference in GLP mass in the presence of biochar in the unplanted mesocosm concurs with findings in other studies on biochar capacity to remove pesticides and other organic pollutants (Kookana et al., 2011; Sun et al., 2012; Yu et al., 2006; Zheng et al., 2010). It also concurs with findings in studies showing that contaminants with high K_{OC}, like GLP and AMPA (Agriculture & environment research unit, 2007), are more adsorbed on substrate particles, biofilm and plant surfaces in TWs (Vymazal and Březinová, 2015). Biochar with high adsorption capacity is the result of its highly negative surface charge and charge density, coupled with its highly aromatic and porous structure (Liang et al., 2006; Zhang et al., 2013). The adsorption of molecules to its surface also lengthens their residence time. Glyphosate adsorption on biochar has been proven to be an effective process for removal of the pesticide in previous studies (Dissanayake Herath et al., 2019; Hagner et al., 2013; Hall et al., 2018b; Mayakaduwa et al., 2016).

In current study, the average pH value with biochar was over 7.0, and therefore, some inhibition of the GLP adsorption could have been present. Therefore, with optimal pH range we could see even higher adsorption and therefore better GLP removal efficiency.

Furthermore, phosphate is known to compete with the phosphonic radical of GLP for adsorbing sites (de Jonge et al., 2001; Hance, 1976). For instance, Hall et al. (2018b) concluded that, hardwood biochar could be an ineffective sorbent in soil with a high phosphate content. Glyphosate can also adsorb to iron and aluminium oxides (Ololade et al., 2014) which may have been present in the water from the substrate and in the iron of our fertilizer solution.

Very few studies have been conducted on the different mechanisms of adsorption of aqueous AMPA in the presence of biochar, but more have focused on its behavior in soil containing biochar. Sidoli et al. (2016) showed that AMPA adsorption was even more negatively affected by the presence of phosphate than GLP, competition with phosphate for adsorption sites being stronger with AMPA. In our study, all mesocosms produced more AMPA than what was applied with the influent. Contrary to GLP, the AMPA mass in the unplanted mesocosms, with or without biochar, was lower than in mesocosms with Scirpus. This may be due to the fact that the biotic metabolization of glyphosate into AMPA requires oxygen to take place (la Cecilia and Maggi, 2018) and that oxygen is lower in the absence of macrophytes (Brix, 1993; Vymazal, 2011).

4.4. Performance of macrophytes for glyphosate and AMPA removal

The most important pathway for the definitive elimination of pesticides is generally through degradation (Faulwetter et al., 2009; Wang et al., 2018). Still the metabolites that are formed through this process can also be toxic and persistent. Glyphosate degradation in TWs is carried out by the microorganisms in the substrate, mostly bacteria (Obojska et al., 1999; Ternan et al., 1998), which are denser in the root zone (i.e. in rhizosphere) (Brix, 1993; Faulwetter et al., 2009; la Cecilia et al., 2018; Vymazal, 2011).

Glyphosate can be used by microorganisms as a source of carbon, nitrogen and phosphorus (Obojska et al., 1999; Pipke and Amrhein, 1988; Ternan et al., 1998). The predominant pathway cleaves the C—N bond, which creates AMPA and glyoxylate (Balthazor and Hallas, 1986; Pipke and Amrhein, 1988; Sviridov et al., 2015). However, while microorganisms can readily use glyoxylate (Levering et al., 1984), AMPA is phytotoxic (Giesy et al., 2000; la Cecilia and Maggi, 2018) and more persistent than GLP (Grandcoin et al., 2017). Thus, GLP removal, as performed in our experiment, only partially reduces toxicity, since a certain portion of it is transformed into another toxic compound – AMPA. The biodegradation of AMPA into non-toxic compounds (methylamine, phosphate; Balthazor and Hallas, 1986; Sviridov et al., 2015) occurs at a slower rate than for glyphosate.

The presence of macrophytes in a TW is an important factor for achieving removal of glyphosate, since the plants support the growth of microbial communities through root exudates, and provide them with carbon, energy, and oxygen in the substrate (Brix, 1993; Faulwetter et al., 2009; Stottmeister et al., 2003; Vymazal, 2011). After a period during which the microorganisms adapt to the xenobiotics (Imfeld et al., 2013), they can rapidly break down glyphosate (Beecraft and Rooney, 2021; Sviridov et al., 2015). This time delay may explain the low removal rates at the two first sampling events in our study. The biofilm created by the microorganisms living in the rhizosphere can also adsorb and concentrate solutes present in the water (Battin et al., 2016), including pesticides (Rooney et al., 2020) and glyphosate (Beecraft and Rooney, 2021), thus enhancing their removal.

In this experiment, there was a difference in glyphosate average removal between species. Removal rate was higher for Scirpus (74.5%) and Phragmites (75.5%) than Sporobolus (54%) and unplanted (49%). These results are all lower than the 90% or higher removal rates in TWs, documented in the literature (Bois et al., 2013; Imfeld et al., 2013; Liang et al., 2020; Maillard et al., 2011; Yang et al., 2013). These previous experiments used soil or sediments in their TWs, which can increase glyphosate removal because greater microbial communities are associated to these materials or because these materials have an adsorption capacity, greater than that of gravel (Mann and Bavor, 1993; Yang et al., 2001).

A dense macrophyte cover should enhance removal (Faulwetter et al., 2009; Maillard et al., 2011). Yet, glyphosate removal and AMPA mass in our Sporobolus mesocosms were not noticeably different from the unplanted mesocosm without biochar. The poorer performance of Sporobolus in terms of GLP removal could also be attributable to its low ORP and lower ET rate during the experimental period compared to the other species (Fig. 4C) Higher ET rate should increase the portion of the substrate that has aerobic conditions, which are essential for the biodegradation of glyphosate (la Cecilia and Maggi, 2018). The low ET rate results in a lower retention time with the microbial communities. Had our experiment run longer, we might have been able to distinguish seasonal differences between species. However, our results show that species selection is important to ensure removal of GLP. Using multi-species combinations in TWs to maximize annual rates of pesticide removal could help identify which species perform best at which times during the year (Rodríguez and Brisson, 2015; Brisson et al., 2020).

Our experiment showed that planted systems (Scirpus or Phragmites) performed better than unplanted, and that unplanted with biochar performed better than unplanted without biochar. We can reasonably assume that planted systems mainly stimulated microbial degradation, while removal with biochar was due to sorption. While the percentage of GLP removed in our experiment was comparable between the two treatments, planted systems may have a longer beneficial effect since biodegradation is permanent, while a certain proportion of glyphosate can desorb from biochar due to competition with phosphate (Carlisle and Trevors, 1987; de Jonge et al., 2001; Hance, 1976; Sprankle et al., 1975). In our experiment, the maximum average removal was reached in TWs that were both planted (with Scirpus) and with biochar added (average of 82%), although the difference with Scirpus alone was not significant (average of 75%). This shows that the positive effect of biochar could be enhanced by the presence of macrophytes, as it was shown to enhance many macrophyte characteristics (Elad et al., 2011; Kasak et al., 2018b).

5. Conclusion

Macrophytes had positive effect on GLP removal, and our results suggest that species selection is important to enhance favorable conditions and maximize removal of targeted pollutants. Biochar and the best performing macrophyte species showed comparable glyphosate removal capacity. AMPA was detected in all the systems, but to a lesser extent in Sporobolus and in both unplanted mesocosms, where glyphosate was less biodegraded. Planted systems with biochar added to the substrate may thus provide the best removal efficiency. Even with these promising results, implementing this type of TW as a full-size treatment for agricultural runoff presents a number of challenges, as multiple pesticides would simultaneously enter the system with possible combined effects. Sorption sites of the biochar will also eventually become saturated, resulting in a lower removal rate of pesticides by this system. Further full-scale study of this type of system is therefore needed, before it could be applied in an agricultural context.

CRediT authorship contribution statement

Olivier Boucher-Carrier: Investigation, Formal analysis, Writing - Original Draft, Visualization; **Jacques Brisson:** Conceptualization, Methodology, Validation, Resources, Writing - Review & Editing; Data Curation, Supervision, Project administration, Funding acquisition; **Khalil Abas:** Investigation, Writing - Review & Editing; **Sung Vo Duy:** Formal analysis, Writing - Review & Editing, **Sébastien Sauvé:** Resources, Writing - Review & Editing, Funding acquisition; **Margit Kõiv-Vainik:** Conceptualization, Methodology, Validation, Data Curation, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

Funding

This research was supported by the Traversy-Langlois fund for the protection of ecosystems and Estonian Research Council grant no. PUT1125. The funding sources were not involved in the research conducted or the preparation of the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Mary Céline Traversy and Raymond Langlois for making generous financial support available to the scientific community; Benoit Saint-Georges and Patrick Boivin for technical support and assistance with field work, Karen Grislis for linguistic revision of the manuscript and Stéphane Daigle and Jérémy Martel for statistical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156061.

References

Abas, K., Brisson, J., Amyot, M., Storck, V., Montiel-León, J.M., Vo Duy, S., Sauvé, S., Kõiv-Vainik, M., 2022. Effects of plants and biochar on the performance of treatment wetlands for removal of the pesticide chlorantraniliprole from agricultural runoff. Ecol. Eng. 175. https://doi.org/10.1016/j.ecoleng.2021.106477.

Agriculture & environment research unit, 2007. Pesticide Properties Database [WWW Document]. http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm (accessed 3.29.21).

Agudelo, R.M., Jaramillo, M.L., Peñuela, G., 2012. Comparison of the removal of chlorpyrifos and dissolved organic carbon in horizontal sub-surface and surface flow wetlands. Sci. Total Environ. 431, 271–277. https://doi.org/10.1016/j.scitotenv.2012.05.045.

Aguinis, H., Gottfredson, R.K., Joo, H., 2013. Best-practice recommendations for defining, identifying, and handling outliers. Organ. Res. Methods 16 (2), 270–301. https://doi.org/10.1177/1094428112470848.

Alavanja, M.C.R., Hoppin, J.A., Kamel, F., 2004. Health effects of chronic pesticide exposure: cancer and neurotoxicity. Annu. Rev. Public Health 25, 155–197. https://doi.org/10.1146/annurev.publhealth.25.101802.123020.

Annett, R., Habibi, H.R., Hontela, A., 2014. Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. J. Appl. Toxicol. 34, 458–479. https://doi.org/10.1002/jat.2997.

Asao, T., 2012. Hydroponics: A Standard Methodology for Plant Biological Researches. IntechOpen, London.

Bach, M., Huber, A., 2001. Input pathways and river load of pesticides in Germany – a national scale modeling assessment. Water Sci. Technol. 43 (5), 261–268.

Balthazor, T.M., Hallas, L.E., 1986. Glyphosate-degrading microorganisms from industrial activated sludge. Appl. Environ. Microbiol. 51 (2), 432–434. https://doi.org/10.1128/aem.51.2.432-434.

Battaglin, W.A., Meyer, M.T., Kuivila, K.M., Dietze, J.E., 2014. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. J. Am. Water Resour. Assoc. 50 (2), 275–290. https://doi.org/10.1111/jawr.12159.

Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., Packmann, A.I., 2016. The ecology and biogeochemistry of stream biofilms. Nat. Rev. Microbiol. 14 (4), 251–263. https://doi.org/10.1038/nrmicro.2016.15.

Beebe, D.A., Castle, J.W., Molz, F.J., Rodgers, J.H., 2014. Effects of evapotranspiration on treatment performance in constructed wetlands: experimental studies and modeling. Ecol. Eng. 71, 394–400. https://doi.org/10.1016/j.ecoleng.2014.07.052.

Beecraft, L., Rooney, R., 2021. Bioconcentration of glyphosate in wetland biofilms. Sci. Total Environ. 756, 143993. https://doi.org/10.1016/j.scitotenv.2020.143993.

Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. Environ. Sci. Eur. 28, 3. https://doi.org/10.1186/s12302-016-0070-0.

Bergström, L., Börjesson, E., Stenström, J., 2011. Laboratory and lysimeter studies of glyphosate and aminomethylphosphonic acid in a sand and a clay soil. J. Environ. Qual. 40, 98–108. https://doi.org/10.2134/jeq2010.0179.

Blankenberg, A.-G.B., Haarstad, K., Søvik, A.-K., 2008. Nitrogen retention in constructed wetland filters treating diffuse agriculture pollution. Desalination, 10th IWA International Specialized Conference on Diffuse Pollution and Sustainable Basin Management. 226, pp. 114–120. https://doi.org/10.1016/j.desal.2007.01.237.

Blodau, C., Mayer, B., Peiffer, S., Moore, T.R., 2007. Support for an anaerobic sulfur cycle in two Canadian peatland soils. J. Geophys. Res. Biogeosciences 112. https://doi.org/10.1029/2006JG000364.

Boe, A., Owens, V., Gonzalez-Hernandez, J., Stein, J., Lee, D.K., Koo, B.C., 2009. Morphology and biomass production of prairie cordgrass on marginal lands. GCB Bioenergy 1 (3), 240–250. https://doi.org/10.1111/j.1757-1707.2009.01018.x.

Bois, P., Huguenot, D., Jézéquel, K., Lollier, M., Cornu, J.Y., Lebeau, T., 2013. Herbicide mitigation in microcosms simulating stormwater basins subject to polluted water inputs. Water Res. 47, 1123–1135. https://doi.org/10.1016/j.watres.2012.11.029.

Borggaard, O.K., Gimsing, A.L., 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Manag. Sci. 64 (4), 441–456. https://doi.org/10.1002/ps.1512.

Briggs, G.G., Bromilow, R.H., Evans, A.A., 1982. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. Pestic. Sci. 13, 495–504. https://doi.org/10.1002/ps.2780130506.

Brisson, Jacques, Chazarenc, Florent, 2009. Maximizing pollutant removal in constructed wetlands: should we pay more attention to macrophyte species selection? Sci. Total Environ. 407 (13), 3923–3930. https://doi.org/10.1016/j.scitotenv.2008.05.047.

Brisson, Jacques, Rodriguez, Mariana, Martin, Charles A., Proulx, Raphaël, 2020. Plant diversity effect on water quality in wetlands: a meta-analysis based on experimental systems. Ecol. Appl. 30 (4). https://doi.org/10.1002/eap.2074.

Brix, H., 1987. Treatment of wastewater in the rhizosphere of wetland plants – the root-zone method. Water Sci. Technol. 19, 107–118. https://doi.org/10.2166/wst.1987.0193.

Brix, H., 1993. Macrophyte-mediated oxygen transfer in wetlands: transport mechanisms and rates. In: Moshiri, Gerald A. (Ed.), Constructed Wetlands for Water Quality Improvement, 1st CRC Press, pp. 391–398.

Brühl, C.A., Zaller, J.G., 2019. Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. Front. Environ. Sci. 7, 177. https://doi.org/10.3389/fenvs.2019.00177.

Carlisle, S.M., Trevors, J.T., 1987. Glyphosate in the Environment. 12. Univ. Guelph.

Centre d'expertise en analyse environnementale du Québec, 2019. Laboratory analyses [WWW Document]. Ministère Environ. Lutte Contre Chang. Clim. https://www.ceaeq.gouv.qc.ca/analyses/index_en.htm (accessed 4.27.21).

Chazarenc, F., Merlin, G., Gonthier, Y., 2003. Hydrodynamics of horizontal subsurface flow constructed wetlands. Ecol. Eng. 21, 165–173. https://doi.org/10.1016/j.ecoleng.2003.12.001.

Chen, Y., Wu, F., Lin, Y., Deng, N., Bazhin, N., Glebov, E., 2007. Photodegradation of glyphosate in the ferrioxalate system. J. Hazard. Mater. 148, 360–365. https://doi.org/10.1016/j.jhazmat.2007.02.044.

de Jonge, H., de Jonge, L.W., Jacobsen, O.H., Yamaguchi, T., Moldrup, P., 2001. Glyphosate sorption in soils of different pH and phosphorus content. Soil Sci. 166 (4), 230–238.

Dissanayake Herath, G.A., Poh, L.S., Ng, W.J., 2019. Statistical optimization of glyphosate adsorption by biochar and activated carbon with response surface methodology. Chemosphere 227, 533–540. https://doi.org/10.1016/j.chemosphere.2019.04.078.

Elad, Y., Cytryn, E., Harel, Y.M., Lew, B., Graber, E.R., 2011. The biochar effect: plant resistance to biotic stresses. Phytopathol. Mediterr. 50 (3), 335–349. https://doi.org/10.14601/Phytopathol_Mediterr-9807.

Faulwetter, J.L., Gagnon, V., Sundberg, C., Chazarenc, F., Burr, M.D., Brisson, J., Camper, A.K., Stein, O.R., 2009. Microbial processes influencing performance of treatment wetlands: a review. Ecol. Eng. 35 (6), 987–1004. https://doi.org/10.1016/j.ecoleng.2008.12.030.

Giesy, J.P., Dobson, S., Solomon, K.R., 2000. Ecotoxicological risk assessment for roundup® Herbicide. In: Ware, G.W. (Ed.), Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews. Springer, New York, NY, pp. 35–120.

Giroux, I., Auteuil-Potvin, F.d', Doussantousse, É., 2019. Québec (Province), Direction générale du suivi de l'état de l'environnement. Présence de pesticides dans l'eau au Québec: portrait et tendances dans les zones de maïs et de soya 2015 à 2017.

Government of Canada, 2020. Historical Climate Data [WWW Document]. Gov. Can. https://climate.weather.gc.ca/index_e.html (accessed 1.11.21).

Grandcoin, A., Piel, S., Baurès, E., 2017. AminoMethylPhosphonic acid (AMPA) in natural waters: its sources, behavior and environmental fate. Water Res. 117, 187–197. https://doi.org/10.1016/j.watres.2017.03.055.

Grunewald, K., Schmidt, W., Unger, C., Hanschmann, G., 2001. Behavior of glyphosate and aminomethylphosphonic acid (AMPA) in soils and water of reservoir Radeburg II catchment (Saxony/Germany). J. Plant Nutr. Soil Sci. 164, 65–70. https://doi.org/10.1002/1522-2624(200102)164:1<65::AID-JPLN65>3.0.CO;2-G.

Hagner, M., Penttinen, O.-P., Tiilikkala, K., Setälä, H., 2013. The effects of biochar, wood vinegar and plants on glyphosate leaching and degradation. Eur. J. Soil Biol. 58, 1–7. https://doi.org/10.1016/j.ejsobi.2013.05.002.

Hagner, M., Hallman, S., Jauhiainen, L., Kemppainen, R., Rämö, S., Tiilikkala, K., Setälä, H., 2015a. Birch (Betula spp.) wood biochar is a potential soil amendment to reduce glyphosate leaching in agricultural soils. J. Environ. Manag. 164, 46–52. https://doi.org/10.1016/j.jenvman.2015.08.039.

Hagner, M., Hallman, S., Jauhiainen, L., Kemppainen, R., Rämö, S., Tiilikkala, K., Setälä, H., 2015b. Birch (Betula spp.) wood biochar is a potential soil amendment to reduce glyphosate leaching in agricultural soils. J. Environ. Manag. 164, 46–52. https://doi.org/10.1016/j.jenvman.2015.08.039.

Hall, K.E., Spokas, K.A., Gamiz, B., Cox, L., Papiernik, S.K., Koskinen, W.C., 2018a. Glyphosate sorption/desorption on biochars – interactions of physical and chemical processes. Pest Manag. Sci. 74, 1206–1212. https://doi.org/10.1002/ps.4530.

Hall, K.E., Spokas, K.A., Gamiz, B., Cox, L., Papiernik, S.K., Koskinen, W.C., 2018b. Glyphosate sorption/desorption on biochars – interactions of physical and chemical processes. Pest Manag. Sci. 74, 1206–1212. https://doi.org/10.1002/ps.4530.

Hance, R.J., 1976. Adsorption of glyphosate by soils. Pestic. Sci. 7, 363–366. https://doi.org/10.1002/ps.2780070407.

Hébert, M.-P., Fugère, V., Gonzalez, A., 2019. The overlooked impact of rising glyphosate use on phosphorus loading in agricultural watersheds. Front. Ecol. Environ. 17, 48–56. https://doi.org/10.1002/fee.1985.

Imfeld, G., Lefrancq, M., Maillard, E., Payraudeau, S., 2013. Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland. Chemosphere 90, 1333–1339. https://doi.org/10.1016/j.chemosphere.2012.04.054.

Junqueira, L.V., Mendes, K.F., Sousa, R.N.de, Almeida, C.de S., Alonso, F.G., Tornisielo, V.L., 2020. Sorption-desorption isotherms and biodegradation of glyphosate in two tropical soils aged with eucalyptus biochar. Arch. Agron. Soil Sci. 66, 1651–1667. https://doi.org/10.1080/03650340.2019.1686139.

Kasak, K., Keit, K., Jaan, P., Ülo, M., 2018. Efficiency of a newly established in-stream constructed wetland treating diffuse agricultural pollution. Ecol. Eng. 119 (15), 1–7. https://doi.org/10.1016/j.ecoleng.2018.05.015.

Kasak, K., Truu, J., Ostonen, I., Sarjas, J., Oopkaup, K., Paiste, P., Kõiv-Vainik, M., Mander, Ü., Truu, M., 2018b. Biochar enhances plant growth and nutrient removal in horizontal subsurface flow constructed wetlands. Sci. Total Environ. 639, 67–74. https://doi.org/10.1016/j.scitotenv.2018.05.146.

Kato, T., Kuroda, H., Nakasone, H., 2009. Runoff characteristics of nutrients from an agricultural watershed with intensive livestock production. J. Hydrol. 368 (1–4), 79–87. https://doi.org/10.1016/j.jhydrol.2009.01.028.

Kookana, R.S., Sarmah, A.K., Zwieten, L.V., Krull, E., Singh, B., 2011. Biochar application to soil: agronomic and environmental benefits and unintended consequences. Adv. Agron. 112, 41. https://doi.org/10.1016/B978-0-12-385538-1.00003-2.

la Cecilia, D., Maggi, F., 2018. Analysis of glyphosate degradation in a soil microcosm. Environ. Pollut. 233, 201–207. https://doi.org/10.1016/j.envpol.2017.10.017.

la Cecilia, D., Tang, F.H.M., Coleman, N.V., Conoley, C., Vervoort, R.W., Maggi, F., 2018. Glyphosate dispersion, degradation, and aquifer contamination in vineyards and wheat fields in the Po Valley, Italy. Water Res. 146, 37–54. https://doi.org/10.1016/j.watres.2018.09.008.

Laitinen, P., Rämö, S., Siimes, K., 2007. Glyphosate translocation from plants to soil – does this constitute a significant proportion of residues in soil? Plant Soil 300, 51–60. https://doi.org/10.1007/s11104-007-9387-1.

Lehmann, J., 2007. Bio-energy in the black. Front. Ecol. Environ. 5, 381–387. https://doi.org/10.1890/1540-9295(2007)5[381:BITB]2.0.CO;2.

Levering, P.R., Dijkhuizen, L., Harder, W., 1984. Metabolic regulation in the facultative methylotroph arthrobacter P1. Growth on primary amines as carbon and energy sources. Arch. Microbiol. 139, 188–195. https://doi.org/10.1007/BF00401998.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizão, F.J., Petersen, J., Neves, E.G., 2006. Black carbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J. 70, 1719–1730. https://doi.org/10.2136/sssaj2005.0383.

Liang, Y., Wei, D., Hu, J., Zhang, J., Liu, Z., Li, A., Li, R., 2020. Glyphosate and nutrients removal from simulated agricultural runoff in a pilot pyrrhotite constructed wetland. Water Res. 168, 115154. https://doi.org/10.1016/j.watres.2019.115154.

Lockhart, W.L., Billeck, B.N., Baron, C.L., 1989. Bioassays with a floating aquatic plant (Lemna minor) for effects of sprayed and dissolved glyphosate. Hydrobiologia 188, 353–359. https://doi.org/10.1007/BF00027800.

Madakadze, I.C., Coulman, B.E., Mcelroy, A.R., Stewart, K.A., Smith, D.L., 1998. Evaluation of selected warm-season grasses for biomass production in areas with a short growing season. Bioresour. Technol. 65, 1–12. https://doi.org/10.1016/S0960-8524(98)00039-X.

Maillard, E., Payraudeau, S., Faivre, E., Grégoire, C., Gangloff, S., Imfeld, G., 2011. Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment. Sci. Total Environ. 409, 2317–2324. https://doi.org/10.1016/j.scitotenv.2011.01.057.

Mann, R.A., Bavor, H.J., 1993. Phosphorus removal in constructed wetlands using gravel and industrial waste substrata. Water Sci. Technol. 27, 107–113. https://doi.org/10.2166/wst.1993.0027.

Matamoros, V., Puigagut, J., García, J., Bayona, J.M., 2007. Behavior of selected priority organic pollutants in horizontal subsurface flow constructed wetlands: a preliminary screening. Chemosphere 69, 1374–1380. https://doi.org/10.1016/j.chemosphere.2007.05.012.

Mayakaduwa, S.S., Kumarathilaka, P., Herath, I., Ahmad, M., Al-Wabel, M., Ok, Y.S., Usman, A., Abduljabbar, A., Vithanage, M., 2016. Equilibrium and kinetic mechanisms of woody biochar on aqueous glyphosate removal. Chemosphere 144, 2516–2521. https://doi.org/10.1016/j.chemosphere.2015.07.080.

Milani, M., Marzo, A., Toscano, A., Consoli, S., Cirelli, G.L., Ventura, D., Barbagallo, S., 2019. Evapotranspiration from horizontal subsurface flow constructed wetlands planted with different perennial plant species. Water 11, 2159. https://doi.org/10.3390/w11102159.

Montiel-León, J.M., Munoz, G., Vo Duy, S., Do, D.T., Vaudreuil, M.-A., Goeury, K., Guillemette, F., Amyot, M., Sauvé, S., 2019. Widespread occurrence and spatial distribution of glyphosate, atrazine, and neonicotinoids pesticides in the St. Lawrence and tributary rivers. Environ. Pollut. 250, 29–39. https://doi.org/10.1016/j.envpol.2019.03.125.

Obojska, A., Lejczak, B., Kubrak, M., 1999. Degradation of phosphonates by streptomycete isolates. Appl. Microbiol. Biotechnol. 51, 872–876. https://doi.org/10.1007/s002530051476.

Ololade, I.A., Oladoja, N.A., Oloye, F.F., Alomaja, F., Akerele, D.D., Iwaye, J., Aikpokpodion, P., 2014. Sorption of glyphosate on soil components: the roles of metal oxides and organic materials. Soil Sediment Contam. Int. J. 23, 571–585. https://doi.org/10.1080/15320383.2014.846900.

Pedescoll, A., Sidrach-Cardona, R., Sánchez, J.C., Bécares, E., 2013. Evapotranspiration affecting redox conditions in horizontal constructed wetlands under Mediterranean climate: influence of plant species. Ecol. Eng. 58, 335–343. https://doi.org/10.1016/j.ecoleng.2013.07.007.

Pipke, R., Amrhein, N., 1988. Isolation and characterization of a mutant of Arthrobacter sp. strain GLP-1 which utilizes the herbicide glyphosate as its sole source of phosphorus and nitrogen. Appl. Environ. Microbiol. 54 (11), 2868–2870. https://doi.org/10.1128/aem.54.11.2868-2870.1988.

Reichenberger, S., Bach, M., Skitschak, A., Frede, H.-G., 2007. Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; a review. Sci. Total Environ. 384, 1–35. https://doi.org/10.1016/j.scitotenv.2007.04.046.

Rice, E.W., Baird, R.B., Eaton, A.D., 2017. Standard Methods for the Examination of Water and Wastewater. 23rd. ed. Water Environment Federation, American Public Health Association, American Water Works Association.

Rodríguez, M., Brisson, J., 2015. Pollutant removal efficiency of native versus exotic common reed (Phragmites australis) in north american treatment wetlands. Ecol. Eng. 74, 364–370. https://doi.org/10.1016/j.ecoleng.2014.11.005.

Rooney, R.C., Davy, C., Gilbert, J., Prosser, R., Robichaud, C., Sheedy, C., 2020. Periphyton bioconcentrates pesticides downstream of catchment dominated by agricultural land use. Sci. Total Environ. 702, 134472. https://doi.org/10.1016/j.scitotenv.2019.134472.

Rousseeuw, P.J., Croux, C., 1993. Alternatives to the median absolute deviation. J. Am. Stat. Assoc. 88, 1273–1283. https://doi.org/10.1080/01621459.1993.10476408.

Sánchez-Bayo, F., Wyckhuys, K.A.G., 2019. Worldwide decline of the entomofauna: a review of its drivers. Biol. Conserv. 232, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020.

Schulz, R., 2004. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: a review. J. Environ. Qual. 33, 419–448. https://doi.org/10.2134/jeq2004.4190.

Sesin, V., Davy, C.M., Stevens, K.J., Hamp, R., Freeland, J.R., 2020. Glyphosate toxicity to native nontarget macrophytes following three different routes of incidental exposure. Integr. Environ. Assess. Manag. https://doi.org/10.1002/ieam.4350.

Sharma, A.D., Lai, D., 2019. Sorption of radiolabelled glyphosate on biochar aged in contrasting soils. J. Environ. Sci. Health Part B 54, 49–53. https://doi.org/10.1080/03601234.2018.1531658.

Sidoli, P., Baran, N., Angulo-Jaramillo, R., 2016. Glyphosate and AMPA adsorption in soils: laboratory experiments and pedotransfer rules. Environ. Sci. Pollut. Res. 23 (6), 5733–5742. https://doi.org/10.1007/s11356-015-5796-5.

Skinner, R.H., Zobel, R.W., van der Grinten, M., Skaradek, W., 2009. Evaluation of native warm-season grass cultivars for riparian zones. J. Soil Water Conserv. 64, 413–422. https://doi.org/10.2489/jswc.64.6.413.

Sprankle, P., Meggitt, W.F., Penner, D., 1975. Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Sci. 23 (3), 229–234. https://doi.org/10.1017/S0043174500052929.

Stehle, S., Elsaesser, D., Gregoire, C., Imfeld, G., Niehaus, E., Passeport, E., Payraudeau, S., Schäfer, R.B., Tournebize, J., Schulz, R., 2011. Pesticide risk mitigation by vegetated treatment systems: a meta-analysis. J. Environ. Qual. 40, 1068–1080. https://doi.org/10.2134/jeq2010.0510.

Stottmeister, U., Wießner, A., Kuschk, P., Kappelmeyer, U., Kästner, M., Bederski, O., Müller, R.A., Moormann, H., 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnol. Adv. 22, 93–117. https://doi.org/10.1016/j.biotechadv.2003.08.010 VI International Symposium on Environmental Biotechnology.

Sun, K., Gao, B., Ro, K.S., Novak, J.M., Wang, Z., Herbert, S., Xing, B., 2012. Assessment of herbicide sorption by biochars and organic matter associated with soil and sediment. Environ. Pollut. 163, 167–173. https://doi.org/10.1016/j.envpol.2011.12.015.

Sviridov, A.V., Shushkova, T.V., Ermakova, I.T., Ivanova, E.V., Epiktetov, D.O., Leontievsky, A.A., 2015. Microbial degradation of glyphosate herbicides (Review). Appl. Biochem. Microbiol. 51, 188–195. https://doi.org/10.1134/S0003683815020209.

Ternan, N.G., Mc Grath, J.W., Mc Mullan, G., Quinn, J.P., 1998. Review: organophosphonates: occurrence, synthesis and biodegradation by microorganisms. World J. Microbiol. Biotechnol. 14, 635–647. https://doi.org/10.1023/A:1008848401799.

United States Department of Agriculture - Natural Resources Conservation Service, 2020. PLANTS Database - USDA PLANTS [WWW Document]. (accessed 1.11.21) https://plants.sc.egov.usda.gov/java/.

Vallée, R., Dousset, S., Billet, D., Benoit, M., 2014. Sorption of selected pesticides on soils, sediment and straw from a constructed agricultural drainage ditch or pond. Environ. Sci. Pollut. Res. 21, 4895–4905. https://doi.org/10.1007/s11356-013-1840-5.

Vile, M.A., Bridgham, S.D., Wieder, R.K., Novák, M., 2003. Atmospheric sulfur deposition alters pathways of gaseous carbon production in peatlands. Glob. Biogeochem. Cycles 17. https://doi.org/10.1029/2002GB001966.

von Mérey, G., Manson, P.S., Mehrsheikh, A., Sutton, P., Levine, S.L., 2016. Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota. Environ. Toxicol. Chem. 35 (11), 11. https://doi.org/10.1002/etc.3438.

Vymazal, J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia 674, 133–156. https://doi.org/10.1007/s10750-011-0738-9.

Vymazal, J., Březinová, T., 2015. The use of constructed wetlands for removal of pesticides from agricultural runoff and drainage: a review. Environ. Int. 75, 11–20. https://doi.org/10.1016/j.envint.2014.10.026.

Vymazal, J., Kröpfelová, L., 2008. Wastewater treatment in constructed wetlands with horizontal sub-surface flow. Environmental Pollution14. Springer Science & Business Media, p. 566.

Wagner, R., Kogan, M., Parada, A.M., 2003. Phytotoxic activity of root absorbed glyphosate in corn seedlings (Zea mays L.). Weed Biol. Manag. 3, 228–232. https://doi.org/10.1046/j.1444-6162.2003.00110.x.

Wang, Y., Ren, B., Zhao, Y., English, A., Cannon, M., 2018. A comparison of alum sludge with peat for aqueous glyphosate removal for maximizing their value for practical use. Water Sci. Technol. 2017, 450–456. https://doi.org/10.2166/wst.2018.165.

Wu, M.-Y., Franz, E., Chen, S., 2000. Spartina pectinata: a candidate species for constructed treatment wetlands. Presented at the Wetlands & Remediation: An International Conference, pp. 133–139.

Yang, L., Chang, H.-T., Huang, M.-N.L., 2001. Nutrient removal in gravel- and soil-based wetland microcosms with and without vegetation. Ecol. Eng. 18, 91–105. https://doi.org/10.1016/S0925-8574(01)00068-4.

Yang, H., Dick, W.A., McCoy, E.L., Phelan, P.L., Grewal, P.S., 2013. Field evaluation of a new biphasic rain garden for stormwater flow management and pollutant removal. Ecol. Eng. 54, 22–31. https://doi.org/10.1016/j.ecoleng.2013.01.005.

Yu, X.-Y., Ying, G.-G., Kookana, R.S., 2006. Sorption and desorption behaviors of diuron in soils amended with charcoal. J. Agric. Food Chem. 54, 8545–8550. https://doi.org/10.1021/jf061354y.

Zhang, X., Zhang, M., 2011. Modeling effectiveness of agricultural BMPs to reduce sediment load and organophosphate pesticides in surface runoff. Sci. Total Environ. 409, 1949–1958. https://doi.org/10.1016/j.scitotenv.2011.02.012.

Zhang, P., Sun, H., Yu, L., Sun, T., 2013. Adsorption and catalytic hydrolysis of carbaryl and atrazine on pig manure-derived biochars: impact of structural properties of biochars. J. Hazard. Mater. 244–245, 217–224. https://doi.org/10.1016/j.jhazmat.2012.11.046.

Zheng, W., Guo, M., Chow, T., Bennett, D.N., Rajagopalan, N., 2010. Sorption properties of greenwaste biochar for two triazine pesticides. J. Hazard. Mater. 181, 121–126. https://doi.org/10.1016/j.jhazmat.2010.04.103.