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Research Article

Crushed Glass as a Constructed Wetland Substrate: Invertebrate Community Responses to Simulated Wastewater Inputs

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Abstract

Constructed wetlands (CWs) are an increasingly common polishing step prior to the release of municipal wastewater treatment facility effluents, especially in smaller and more isolated communities. It is hypothesized that recycled crushed glass could be a suitable alternative matrix for CW construction. In comparison to commonly used substrates, recycled crushed glass has several advantages: it is less expensive, more environmentally friendly, and it can be transformed into various sizes to meet specific design requirements. The material is inert, transparent, has large pore spaces, and significant surface area. Components that impair receiving water quality (e.g., nutrients, pharmaceuticals, and pathogenic bacteria) could be reduced by enhancing light penetration, macrophytes for uptake and assimilation, surface area for microbes, and overall retention time. To explore the ability of crushed glass to support relevant biological communities, twelve outdoor mesocosms were established with and without emergent plants, and crushed glass was contrasted with a typical gravel base in triplicate. Specifically, we examined the response of the zooplankton community. After these systems were acclimated, they were treated with a single pulse of synthetic wastewater (e.g., nutrients, pharmaceuticals, and salts). Mesocosms exposed to the synthetic effluent developed a significantly ($p<0.05$) different invertebrate community response in total abundance when compared to the unexposed control treatment. There were no significant ($p>0.05$) differences among the mesocosms with crushed glass as a substrate (including controls) for all diversity indices, indicating that the addition of synthetic effluent and macrophytes had no significant impacts on the invertebrate community structure. Overall, recycled crushed glass was determined to be suitable matrix for zooplankton communities, with water quality and effective treatments being maintained relative to gravel systems. Though the treatments with a gravel substrate had greater total invertebrate abundance, it was found that the gravel treatments were significantly ($p<0.05$) less diverse (Shannon's index) and had less evenness than all other treatments with glass substrates. We recommend that future studies should explore the effectiveness of recycled crushed glass in CWs on a larger scale, as these results suggest that recycled crushed glass could be a viable surrogate for gravel in subsurface filtration processes.

Keywords: Constructed Wetlands, Pharmaceuticals, Alternative Substrate, Zooplankton, Substrate

1 Introduction

Constructed wetlands (CWs) are an increasingly common polishing step prior to the release of municipal effluents, especially in small communities. Constructed wetlands can be a cost-effective treatment option for the removal of contaminants and excess nutrients from both treated and untreated wastewater effluents[1, 2]. The systems make use of aerobic conditions, aquatic plants, and extended hydraulic residence times to promote degradation of pharmaceuticals and related contaminants[3]. Organic contaminants, such as pharmaceuticals and personal care products (PPCPs), are often not fully degraded or absorbed in the bodies of human users[4]. Consequently, these compounds are present in municipal wastewater facilities and surrounding surface waters[5]. PPCPs incorporate a wide variety of different groups, such as hormones, antibiotics, disinfectants, synthetic fragrances, and preservatives. Discharge from wastewater facilities is the primary source of PPCPs in surface waters, as the removal of PPCPs and their metabolites by these systems is often incomplete, resulting in a continuous discharge that can make these contaminants "pseudo-persistent"[6, 7, 8]. These low concentrations of PPCPs are unlikely to pose an acute risk to aquatic organisms; however, there is potential risk for chronic toxicity in non-target organisms downstream of effluent releases[9, 10, 11].

Constructed wetlands have been used as secondary or tertiary steps for wastewater treatment. In these systems, macrophytes have been utilized in several designs for the attenuation of PPCPs to improve overall removal efficiency[12]. Multiple plant species have been incorporated into these systems, including emergent, submergent, and free-floating plants. The most common species are *Phragmites australis, Typha spp., Typha angustifolia,* and *Typha latifolia*[13]. The role of macrophytes in constructed wetlands is typically to stabilize the substrate surface, uptake nutrients and contaminants, prevent channeled flow, insulate against freezing via litter production, and to shield algae from solar radiation[14]. Still, the value of their role in PPCP removal is unclear[15].

Constructed wetlands provide habitats for micro- and meso-fauna, and promote zooplankton grazing of remaining algal solids[16]. Zooplankton play a significant role in aquatic ecosystems, as they drive nutrient cycles via the consumption of primary producers (e.g., phytoplankton), and function as prey for planktivorous fish. Additionally, their community dynamics of growth, mortality, diversity, and distribution, structure the ecosystem through trophic interactions. Their intermediate trophic position in aquatic food webs makes them susceptible to bottom-up as well as top-down trophic cascades, as their biomass has been shown to increase with nutrient enrichment[17]. The most common zooplankton species in freshwater ecosystems are copepods, cladocerans, and rotifers[18]. They are highly sensitive to abiotic factors, such as temperature, dissolved oxygen, pH, salinity, turbidity, heavy metals, and contaminants (e.g., pesticides[18]).

Zooplankton exhibit a diverse range of life-history patterns, rates of reproduction, and life cycles. Furthermore, studies, such as the one conducted by Shurin et al.[19], have used the species richness of zooplankton to test their association with environmental variability. Therefore, zooplankton are effective indicators of aquatic ecosystem health. As a result, similar studies, such as the one conducted by Lobson et al.[20], have utilized the response of zooplankton communities in mesocosms to address indirect effects of a contaminant. Mesocosms have been commonly used to address concerns of non-target effects, as key variables can be manipulated to better observe both direct and indirect effects of a stressor on a lentic system[21].

Various substrate materials have been used in CWs, with the most popular being gravel, sand, and light-expanded clay aggregate[13, 22]. Dordio and Carvalho[22] found that light-expanded clay aggregate is a suitable substrate for agricultural wastewater treatment, as ionic contaminants are primarily adsorbed to substrates via electrostatic interactions. In gravel-bed CWs, wastewaters are treated via subsurface-flow through shallow channels, where the gravel provides surfaces for sorption and biofilm growth, physical support for macrophyte growth, and promotes the settling and filtration of suspended solids[23]. Recycled crushed glass represents a potential alternative substrate, but there is currently a lack of knowledge surrounding the performance of recycled crushed glass as a surrogate substrate material in CWs. There is an abundance of recycled crushed glass available in Canada, due to the immense quantities produced annually.

In comparison to the common silica sand substrate, crushed glass has several advantages: it is less expensive, more environmentally friendly (since it is a recycled material), and it can be transformed into various sizes to meet specific design requirements[24]. The material is inert, with significant surface area, and has large pore spaces. This means no risk of chemical contamination, a suitable matrix for microbial growth, and a large aerobic zone and support for root development of aquatic plants (e.g., cattail). This research is part of a larger study exploring the effectiveness of crushed glass as a substrate in CWs. Recycling of glass is at an impasse.

Few options exist at the moment for how this material can be made economically viable. It was hypothesized that recycled crushed glass could be a suitable matrix for constructed wetlands. The ability of recycled crushed glass to provide the same, if not better, nutrient and contamination removal as gravel will be examined. The objective of this study was to characterize the mesocosm zooplankton communities to determine the suitability of a glass substrate to support natural populations. These data can serve as the basis for further development of cost-effective and environmentally friendly wastewater treatment facilities based on recycled glass.

2 Methods

2.1 Experimental Design

This study occurred at the Prairie Wetland Research Facility (PWRF) at the University of Manitoba, Winnipeg, MB (49°48'35.9"N, 97°07'33.0"W). An array of twelve individual wetland mesocosms were installed at the PWRF. Each mesocosm consisted of a flat-bottomed, circular, low-density polyethylene tank (2.7m in diameter × 0.72m in height; $3.49m^3$ in volume), with no outflows or inflows. The two substrate materials assessed were recycled crushed glass and gravel. One of four treatments were randomly assigned, in triplicate, to each mesocosm (described in detail in Figure 1). The addition of synthetic wastewater and selected pharmaceuticals occurred to all treatments (excluding control treatments) in a single pulse application on August 14th, 2018.

2.2 Preparation of Mesocosms

Each mesocosm received approximately a 30-cm layer of recycled crushed glass or gravel. Gravel with a diameter ranging from 1.5-2.5cm was added to match the average approximate size of the crushed glass material. Tap water from the City of Winnipeg was used to fill the tanks to a volume of approximately 2400L. Floating debris from the recycled crushed glass (e.g., plastic caps, labels, corks, etc.) was removed from the tanks upon filling. Macrophytes (*Typha spp.*) were collected from Oak Hammock Marsh, MB (50°11′15″N, 97°7′30″W) on July 19th, 2018. Macrophytes were planted in the Gravel and Plant-Glass treatments, at a density of 5-10 plants per square metre, for a total of 25 plants per tank. The macrophytes were acclimated in the system for 26 days prior to the start of the experiment.

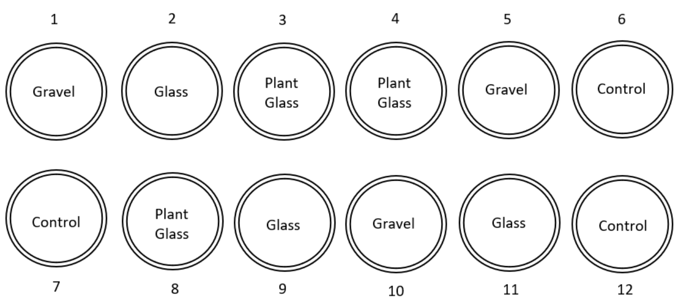

Figure 1: *Layout of randomly assigned treatments in twelve mesocosms. The treatments consist of: Control (crushed glass as substrate, unplanted), Gravel (gravel as substrate, planted with* Typha spp.*, addition of pharmaceuticals and synthetic wastewater), Plant Glass (crushed glass as a substrate, planted with* Typha spp.*, addition of pharmaceuticals and synthetic wastewater), and Glass (crushed glass as substrate, unplanted, addition of pharmaceuticals and synthetic wastewater).*

Zooplankton and benthic invertebrates were also collected from Oak Hammock Marsh using drag nets and kick nets near shore (35-µm and 73-µm mesh). Invertebrates were collected and introduced into the system at 26 and 18 days prior to the start of the experiment (July 19th and July 27th, 2018, respectively). Water containing collected organisms was added to each tank in equal volumes, and the mesocosms remained uncovered to allow for natural aerial colonization by insects throughout the duration of the experiment. Amphibians and fish were not included in the mesocosms, as a result of potential confounding effects on invertebrate-specific assessments.

2.3 Water Quality Monitoring

General water quality parameters – including dissolved oxygen (DO), chlorophyll-a, pH, temperature, oxidation-reduction potential (ORP), and conductivity – were measured every weekday morning, and once a week in the afternoon, using a YSI 6600 V2 Sonde. The concentration of DO provides an indication of the consumption and production rates of organic matter in the systems. Primary production by phytoplankton can be represented by chlorophyll-a concentrations, as chlorophyll-a is an indicator for phytoplankton biomass[25]. The YSI measurements began 29 days prior to the start of the experiment (July 16th, 2018), and continued for 57 days thereafter (October 10th, 2018). Depths were measured bi-weekly in five different locations for each tank, and then averaged to account for evaporation and to monitor the water volume. Photosynthetically active radiation (PAR) was measured at mid-day once a week. A total of two litres of synthetic wastewater and the mixture of pharmaceuticals (concentrations ranging from 5-10µg/L) were added to the corresponding treatments after the acclimation period, marking the start of the experiment (August 14th, 2018).

2.4 Mesocosm Treatment

Synthetic wastewater was added to the treated mesocosms (excluding controls). The synthetic wastewater contains (per litre): 32g peptone, 19g Lab Lemco powder meat extract, 6.7g $(NH_4)_2SO_4$, 3g urea, 3g yeast extract, 2.9g K_2HPO_4, 2.3g KH_2PO_4, 0.27g $CaCl_2{\cdot}2H_2O$, and 0.2g $MgSO_4{\cdot}2H_2O$. One litre of secondary wastewater from Dunnottar, MB (50°27'16.9"N, 96°57'06.5"W), was added to the mesocosms to provide established microorganism colonies. Pharmaceuticals were selected based on their frequency of detection in common wastewater treatment facilities, the low likelihood of acutely affecting aquatic organisms, and their persistence in the environment. The selected pharmaceuticals included atenolol ({β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).

2.5 Zooplankton Sampling Protocol

Zooplankton samples were collected from each mesocosm using passive traps as to not disturb the system; traps were deployed for 24 hour periods. The passive trap consisted of a clear 1-L Mason glass jar with a 243-mL Nalgene polypropylene powder funnel attached via rubber bands and s-hooks (adapted from Murkin et al.[26], Sibley et al.[27]). Each trap was filled with control mesocosm water prior to deployment into the treated mesocosms. Three passive traps were deployed on the substrate surface in an array for each tank. Replicate traps were integrated into a single sample from each mesocosm, and stored in 120-mL French square bottles, with 5% sugar formalin and distilled water to preserve the samples for further analysis. Samples were taken on Days -7, -1, 0, 1, 7, 14, 21, 28, 42, and 56, for a total of 120 samples.

2.6 Zooplankton Enumeration and Identification

Samples were selected randomly (using a random number generator) for enumeration to avoid a potential counting bias. Prior to enumeration, zooplankton samples were adjusted by concentrating the sample volume of 120mL to a consistent volume of 50mL. To ensure an even distribution of organisms, samples were inverted several times and mixed thoroughly prior to subsampling. Subsamples (5mL) were transferred into a Bogorov zooplankton counting chamber via an air displacement pipette. Subsamples were analyzed using a dissecting microscope at four to five times magnification. A minimum of one 5mL subsample (10% of the total sample volume) was enumerated entirely for each mesocosm sample. For taxa that did not have an abundance of at least 40 individuals in the first subsample, an additional 5mL subsample was enumerated (adapted from USEPA (2016)). The key of Balcer et al.[28] was used to identify cladocerans to genus, copepods to order, ostracods to class, and rotifers to phylum.

2.7 Statistical Analysis

Diversity metrics, such as number of taxa, evenness, Shannon's diversity, and Simpson's diversity, were calculated along with total and individual taxon abundance for each mesocosm. A single factor ANOVA ($\alpha = 0.05$) was used to identify significant differences in Shannon's diversity index, Simpson's diversity index, number of taxa, and evenness among sampling days and treatments. If significant differences were present, a two-sample t-Test assuming unequal variances was used to identify significant differences between treatments. Principal response curve (PRC) analysis was conducted to compare the response of the zooplankton community between different treatments over the course of the study compared to the control mesocosms[29, 30]. PRC analysis was performed using the *vegan* package (Version 2.5-5) and *ggplot2* package in RStudio (Version 1.1.463)[31, 32, 33].

3 Results

3.1 Water Quality

Temporal trends of the measured water quality parameters can be found in the SI (Figures S1 to S4). Mean temperatures for the mesocosms displayed a general decline over time (Figure S1). Mean pH values remained relatively consistent throughout the duration of the study, except for a brief decline and rebound in all exposed treatments (excluding controls) following the addition of synthetic wastewater and pharmaceuticals on August 14th, 2018 (Figure S2). Similarly, mean DO concentration displayed a significant ($p<0.05$) decline and rebound in concentration in all exposed treatments (excluding controls) following the addition of synthetic wastewater and pharmaceuticals on August 14th, 2018 (Figure S3). The decline and rebound in DO was most pronounced in the Glass treatments (glass + effluent) (Figure S3). All treated mesocosms exhibited a brief increase in mean chlorophyll-a concentration following the addition of synthetic wastewater and pharmaceuticals relative to the controls (Figure S4). As with DO, the Glass treatments experienced the greatest increase in chlorophyll-a following the pulse exposure of synthetic effluent (Figure S4), and the Gravel treatments (gravel + effluent + plants) experienced the least increase in chlorophyll-a.

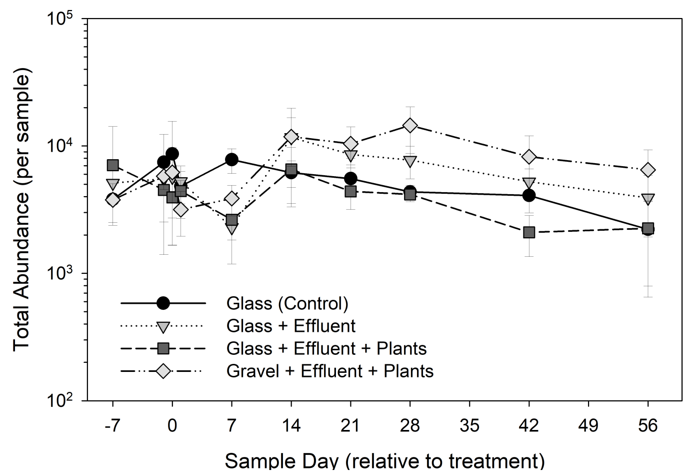

Figure 2: *Mean total invertebrate abundance following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). Error bars represent standard deviation.*

3.2 Invertebrate Abundance

Eleven zooplankton taxa were identified in the mesocosms (calanoid copepods, *Ceriodaphnia sp.*, chydorids, cyclopoid copepods, *Diaphanosoma sp.*, *Macrothrix sp.*, copepod nauplii, ostracods, rotifers, *Scapholeberis sp.* and *Simocephalus sp.* A total of two aquatic insect taxa were identified in mesocosms (*Chaoborus sp.* and Ephemeroptera larvae). The treatments with the greatest total abundance averaged across the three replicates for each treatment are in decreasing order as follows: Gravel (gravel substrate + effluent +

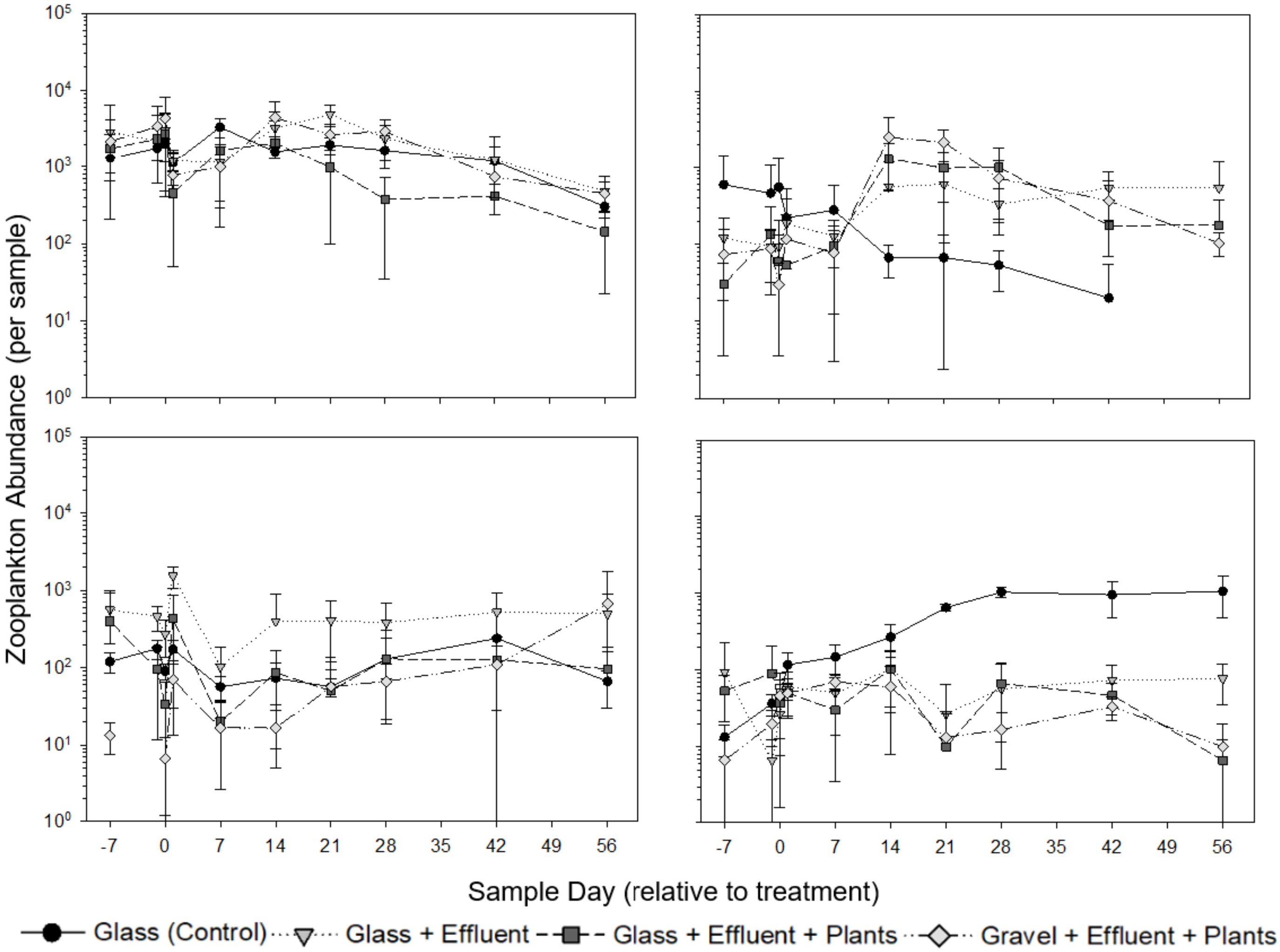

Figure 3: Ceriodaphnia sp. *(A),* Simocephalus sp. *(B), Chydorid (C), and Calanoid Copepod (D) abundances per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). Error bars represent standard deviation.*

plants), Glass (glass substrate + effluent), Control (glass substrate), and Plant Glass (glass substrate + effluent + plants) (Figure 2). The total invertebrate abundance declined for all treatments (including controls) on Day 1 relative to the pre-treatment abundance on Day -1, with only the exposed treatments (excluding controls) continuing to decline one week after the pulse exposure to synthetic effluent (Figure 2). The abundance in Control treatments declined from Day 7 through the remaining seven weeks of the study duration (Figure 2). All the exposed treatments experienced a rapid increase in total abundance from Day 7 to Day 14 (Figure 2) followed by a decline from Day 28 to Day 56 (Figure 2).

Of the thirteen invertebrate taxa that were identified, four distinct trends in abundance were observed. Abundant taxa, such as *Ceriodaphnia sp.*, experienced a decline in abundance for all treatments (including controls) on Day 1 (Figure 3A). The exposed treatments displayed a relatively rapid increase in *Ceriodaphnia sp.* abundance from Day 1 to Day 7 (Figure 3A). From Day 21 to Day 56, all exposed treatments exhibited a decline in *Ceriodaphnia sp.* abundance (Figure 3A). The abundance of *Ceriodaphnia sp.* in the Control treatments declined steadily from Day 7 through Day 56 (Figure 3A).

Less abundant taxa, such as chydorids, experienced an increase in abundance for all exposed treatments on Day 1, which then was followed by a steep decline on Day 7 (Figure 3C). The Glass treatments had the greatest abundance of chydorids and remained relatively consistent from Day 14 to Day 56 (Figure 3C). The Gravel treatments displayed an increase in chydorid abundance from Day 14 to Day 56

(Figure 3C). The chydorid abundance in the Control mesocosms remained relatively consistent throughout the duration of the study (Figure 3C). Taxa that experienced significantly ($p<0.05$) different abundances in the exposed treatments when compared to the unexposed control treatments include *Simocephalus sp.* and *calanoid copepods*.

The abundance of *Simocephalus sp.* increased rapidly on Day 14 for all exposed treatments and declined steadily until Day 56 (Figure 3B). The Control mesocosms experienced a steady decline in *Simocephalus sp.* abundance throughout the duration of the study (Figure 3B). The abundance of calanoid copepods increased steadily throughout the entire study duration in the Control treatments (Figure 3D). The exposed treatments experienced a steep decline on Day 21, and rebound on Day 28, with less of a rebound in the Gravel treatments (Figure 3D). Additionally, *Diaphanosoma sp.* experienced a unique response in abundance when compared to the other taxa (Figure 4).

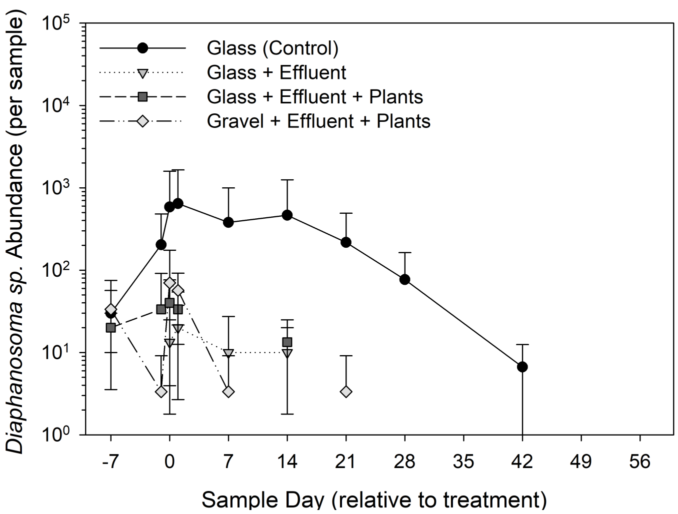

Figure 4: Diaphanosoma sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). Error bars represent standard deviation.*

The abundance of *Diaphanosoma sp.* was rare for all exposed treatments, with none being found after Day 21 (Figure 4). The exposed treatments experienced a decline in abundance from Day 0 to Day 7 (Figure 4). *Diaphanosoma sp.* was present in all Control treatments until Day 42, with a steady decline in abundance occurring from Day 1 through Day 42 (Figure 4).

The principal response curve (PRC) analysis revealed that 30.3% of the constrained variance is explained by the first PRC axis in Figure 5 relative to the control treatment. Conditional variance (i.e. time) accounted for 21.0% of the total variance, with the total constrained variance (i.e. treatment, interacting with time) accounting for an additional 34.8% of the total variance. Species scores were used in development of the PRC's community response (C_{dt}) from the study start date (Day -7, August 7th, 2018) to Day 56 (October 9th, 2018). Scores associated with the first axis explained 30.3% of the constrained variance in Figure 5. The permutation test for first constrained eigenvalue (first axis) resulted in an F value of 19.076 and a p value of 0.004.

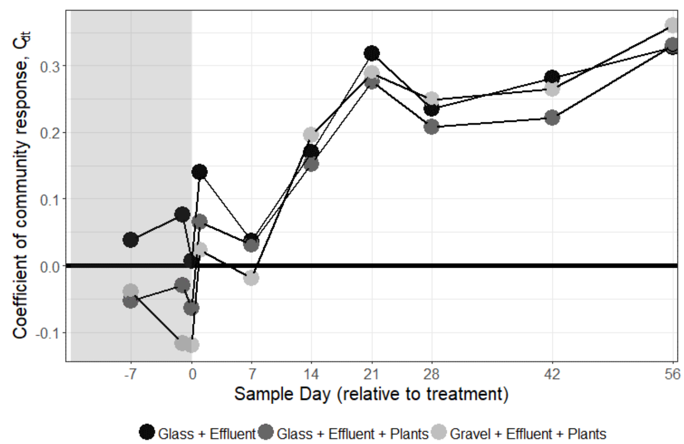

Figure 5: *Principal response curve (PRC) with species scores (b_k) showing the invertebrate community response (C_{dt}) following a single pulse exposure to synthetic effluent in outdoor mesocosms relative to the control treatment from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The black horizontal line ($C_{dt} = 0$) represents the control treatment and is used as the basis in determining response coefficients for each treatment relative to the control. The shaded region represents the pre-treatment response prior to the single pulse exposure on Day 0 (August 14th, 2018). 30.3% of the constrained variance is explained by the first axis, with conditional variance (i.e. time) accounting for 21.0% of the total variance, and the total constrained variance (i.e. treatment, interacting with time) accounts for an additional 34.8% of the total variance. Permutation test for first constrained eigenvalue (first axis) resulted in an F value of 19.076 and a p value of 0.004.*

A sustained difference in total zooplankton abundance in all treatments relative to the control is observed in the PRC. The PRC demonstrates that there is a significantly different ($p<0.05$) community response in the exposed treatments when compared to the controls (Figure 5), which can be shown in the contrasting abundance trends in Figures 3B and 3D. The main taxa driving the change observed include *Simocephalus sp.* and calanoid copepods; the former taxa decreased in abundance in the Control relative to the treatments, whereas calanoid copepod abundance increased in the Control only, as represented by opposite species scores (b_k) determined following redundancy analysis and PRC development. Additionally, *Diaphanosoma sp.* also increases in the Control, distinguished by its high negative species score (Figures 4 and 5).

There was a degree of within-treatment variability among the triplicate mesocosms, as invertebrate abundances were observed to change with time among the same treatments. External factors such as weather, fauna grazing from preferred mesocosms, or unevenly distributed invertebrates, may be attributed to the within-treatment variability among the triplicate mesocosms. The relative abundance and diversity of zooplankton present in the mesocosms are comparable to a similar study conducted at the Prairie Wetland Research Facility[20]. While the diversity of the taxa found in the mesocosms was lower than what has been observed in the field, the abundances of zooplankton present are representative of communities that have been observed in prairie wetlands[34]. Similar zooplankton densities have been found in samples from two separate mesocosm studies, both of which used activity traps similar to the passive traps used in this study[35, 36].

3.3 Invertebrate Diversity

The sample day and treatment with the greatest zooplankton diversity was the Glass treatment on Day 56, with evenness, Shannon's index, and Simpson's index values of 0.840, 2.014, and 6.646, respectively (Table 1). The sample day and treatment with the lowest diversity was the Gravel treatment on Day 42, with evenness, Shannon's index, and Simpson's index values of 0.382, 0.916, and 1.623, respectively (Table 1). It was determined by a single factor ANOVA and two-sample *t*-test (assuming unequal variances for Shannon's index), that the Gravel treatment was significantly ($p<0.05$) less diverse than all other treatments, including controls. The mean values for Shannon's diversity index of the Control, Gravel, Plant Glass, and Glass treatments were 1.67, 1.26, 1.68, and 1.66, respectively. The mean values for Simpson's diversity index of the Control, Gravel, Plant Glass, and Glass treatments were 3.99, 2.79, 4.16, and 3.98, respectively.

In terms of Shannon's and Simpson's diversity, none of the treatments were significantly ($p>0.05$) different from each other. The mean number of taxa of the Control, Gravel, Plant Glass, and Glass treatments were 12.5, 12.1, 12.2, and 12.1, respectively. The mean values for evenness of the Control, Gravel, Plant Glass, and Glass treatments were 0.662, 0.505, 0.673, and 0.668, respectively, with the Gravel treatment having significantly ($p<0.05$) lesser evenness than all other treatments, including controls. Though the Plant Glass treatment is not significantly ($p>0.05$) different from the Glass treatment, the greater mean values for the diversity metrics of the Plant Glass treatment is an indication that the presence of macrophytes may increase the diversity in the system.

4 Discussion

To explore the ability of crushed glass to support relevant biological communities in CWs, twelve outdoor mesocosms were established with and without emergent plants, and crushed glass was contrasted with a typical gravel base in triplicate. After these systems were acclimated, they were treated with a single pulse of synthetic wastewater. Mesocosms exposed to the synthetic effluent developed a significantly ($p<0.05$) different zooplankton community response in total abundance when compared to the unexposed control treatment. Four distinct trends in abundance were observed, with these trends being expressed most prevalently in taxa such as *Ceriodaphnia sp.*, chydorids, *Simocephalus sp.*, *Diaphanosoma sp.* and calanoid copepods. There were no significant ($p>0.05$) differences among the mesocosms with crushed glass as a substrate (including controls) for all diversity indices, indicating that the addition of synthetic effluent and macrophytes had no significant impacts on the invertebrate community structure. Though the Plant Glass treatment is not significantly different from the Glass treatment, the greater mean values for the diversity metrics of the Plant Glass treatment provides an indication that the presence of macrophytes may increase the diversity in the system. Overall, recycled crushed glass was determined to be suitable matrix for zooplankton communities in the context of CWs, with water quality and effective treatments being maintained relative to gravel systems.

Zooplankton abundance was predicted to decline in all treatments over time, as zooplankton are sensitive to temperature changes[37], and a declining trend in temperature was found throughout the duration of the study. This temperature-sensitive decline in abundance was reflected most prevalently in the Control treatments. Mean pH values remained relatively consistent throughout the experiment in each mesocosm, so pH was not likely a factor in differences observed among mesocosms and over time. Trophic interactions likely lead to a temporary increase in zooplankton abundance following the increase of primary production (phytoplankton) one week after the pulse exposure to synthetic wastewater and selected pharmaceuticals[38]. This response can be found in abundant taxa, such as *Ceriodaphnia sp.*, which experienced declines and rebounded following the single pulse exposure.

Table 1: *Summary of diversity indices across four treatments on sample Days -7, 0, 7, 14, 42, and 56. The four treatments are Control (glass), Glass (glass and effluent), Plant Glass (glass, effluent, and plants), and Gravel (gravel, effluent, and plants).*

Day	Treatment	Number of Taxa	Evenness	Shannon's Index	Simpson's Index
-7	Control	13	0.703	1.804	4.651
-7	Glass	11	0.641	1.537*	2.878*
-7	Plant Glass	13	0.530	1.359*	2.670*
-7	Gravel	13	0.530	1.265*	2.507*
0	Control	13	0.572	1.468	2.929
0	Glass	13	0.553	1.419	2.810
0	Plant Glass	13	0.472	1.211*	1.980*
0	Gravel	12	0.410	1.019*	1.909*
7	Control	13	0.622	1.594	3.467
7	Glass	13	0.635	1.629	3.306
7	Plant Glass	11	0.551	1.322*	2.324*
7	Gravel	13	0.602	1.545	3.545
14	Control	13	0.730	1.872	5.060
14	Glass	13	0.642	1.647	3.556*
14	Plant Glass	13	0.765	1.961	5.481
14	Gravel	12	0.623	1.547*	3.938*
42	Control	12	0.671	1.667	4.588
42	Glass	12	0.744	1.849	5.312
42	Plant Glass	12	0.810	2.013*	6.237*
42	Gravel	11	0.382*	0.916*	1.623*
56	Control	11	0.595	1.426	3.109
56	Glass	11	0.840*	2.014*	6.646*
56	Plant Glass	12	0.666	1.656	3.500
56	Gravel	11	0.487	1.168*	2.206*

*Asterisks indicate significant difference in zooplankton diversity of treatment relative to control treatment at specific sampling day ($p<0.05$).

Inversely, less abundant taxa, such as chydorids, experienced an increase and then decline following the single pulse exposure. This inverse relationship is likely due to competitive release[39], where the less abundant taxa can fill the niches of the more abundant taxa, possibly as a result of reduced pressure for resources. The cause for these relationships is likely a bottom-up trophic cascade, with nutrient addition being the primary driver, as the concentrations of pharmaceuticals used are not likely to pose an acute risk of toxicity to the organisms present[9, 10, 11]. In a similar study examining the effects of pharmaceutical mixtures on aquatic communities in outdoor microcosms, the authors indicate that organic enrichment may be contributing to the found effects of increased abundance and decreased diversity of zooplankton in the highest concentration treatment, resulting in trophic interactions with phytoplankton[40]. *Simocephalus sp.* had the most positive species score, as the taxon decreased in abundance in the Control relative to the exposed treatments.

Inversely, calanoid copepods and *Diaphanosoma sp.* had the most negative species scores, as both taxa increased in abundance in the Control relative to the exposed treatments. These four trends may be attributed to certain taxa being more resilient than others under the observed conditions[41], which may additionally confound our ability to observe treatment-specific impacts.

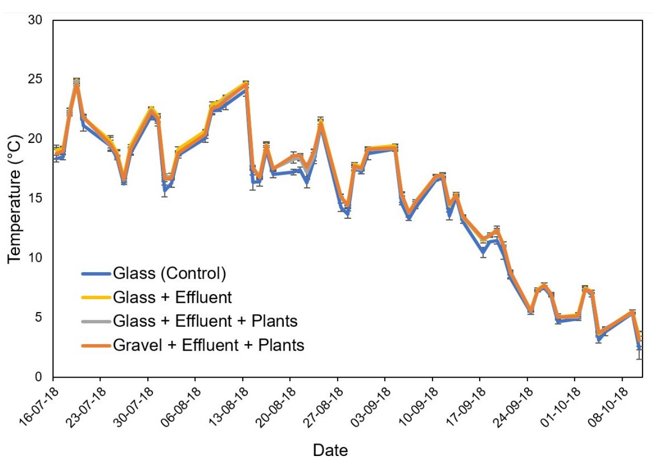

Figure S1. *Mean temperature (°C) of treated mesocosms at the Prairie Wetland Research Facility (PWRF). Temperature values were averaged across replicates (n=3) for each of the four treatments (control, gravel, plant glass, and glass). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16th, 2018 to October 10th, 2018. Error bars represent standard deviation.*

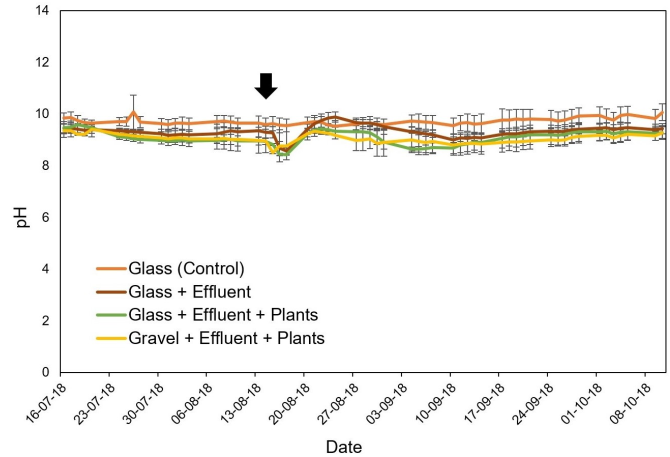

Figure S2. *Mean pH of treated mesocosms at the Prairie Wetland Research Facility (PWRF). pH values were averaged across replicates (n=3) for each of the four treatments (control, gravel, plant glass, and glass). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16th, 2018 to October 10th, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls). Error bars represent standard deviation.*

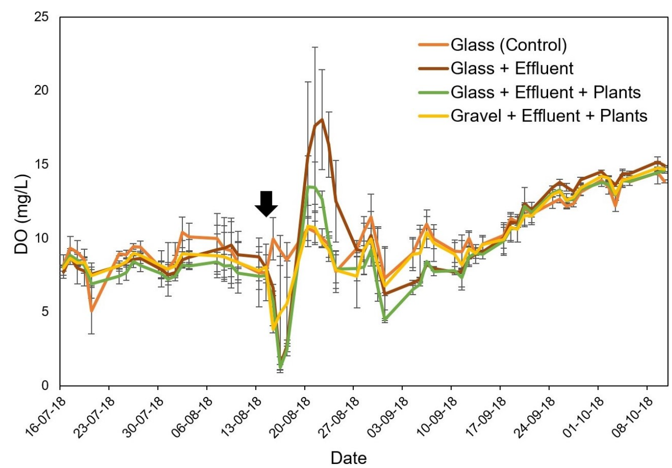

Figure S3. *Mean dissolved oxygen concentration (mg/L) of treated mesocosms at the Prairie Wetland Research Facility (PWRF). Dissolved oxygen values were averaged across replicates (n=3) for each of the four treatments (control, gravel, plant glass, and glass). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16th, 2018 to October 10th, 2018. The arrow indicates when the synthetic wastwater was added to the exposed treatments (excluding controls). Error bars represent standard deviation.*

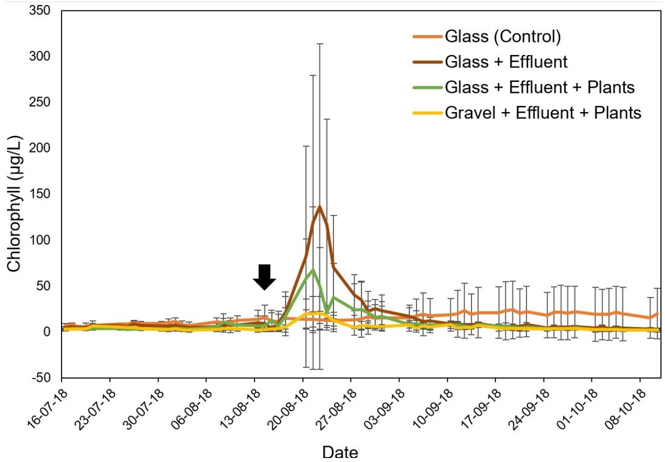

Figure S4. *Mean chlorophyll-a concentration (µg/L) of treated mesocosms at the Prairie Wetland Research Facility (PWRF). Chlorophyll values were averaged across replicates (n=3) for each of the four treatments (control, gravel, plant glass, and glass). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16th, 2018 to October 10th, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls). Error bars represent standard deviation.*

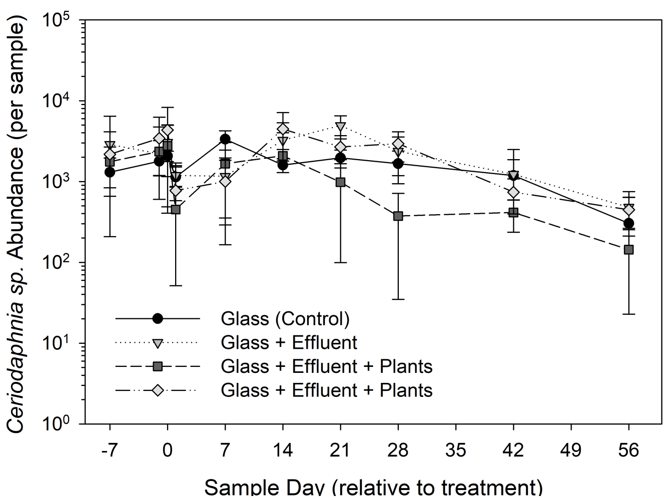

Figure S5. Ceriodaphnia sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (ß-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

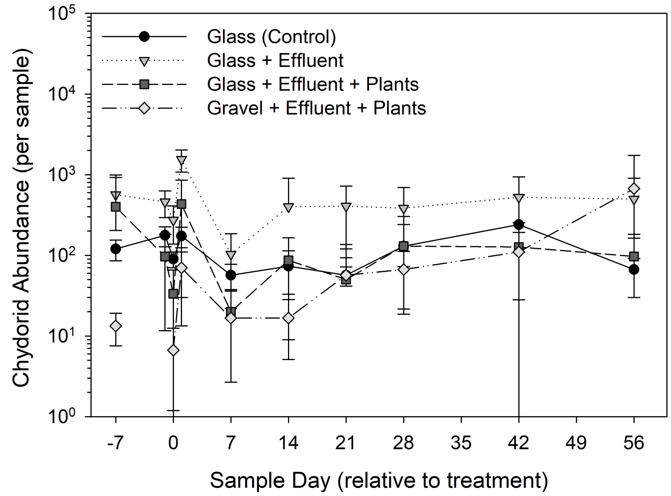

Figure S6. *Chydorid abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (ß-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

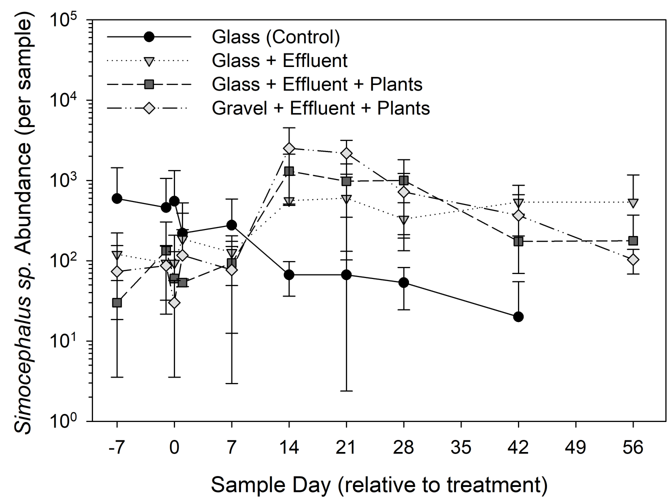

Figure S7. Simocephalus sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (ß-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

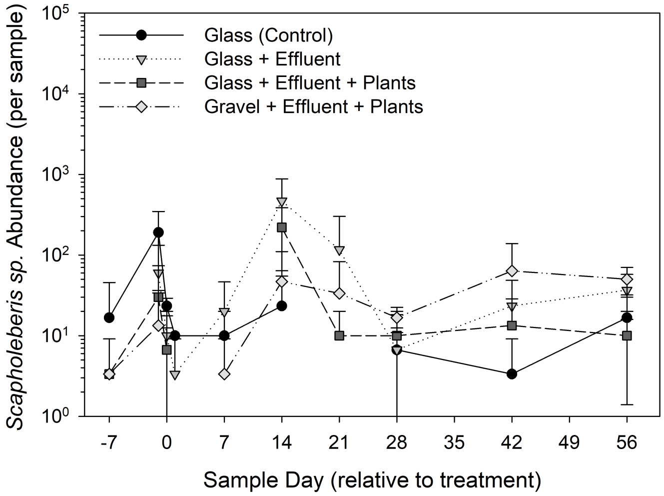

Figure S8. Scapholeberis sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

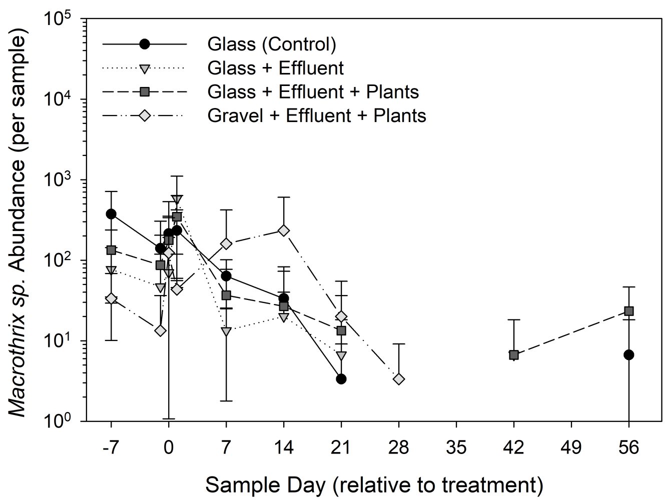

Figure S9. Macrothrix sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

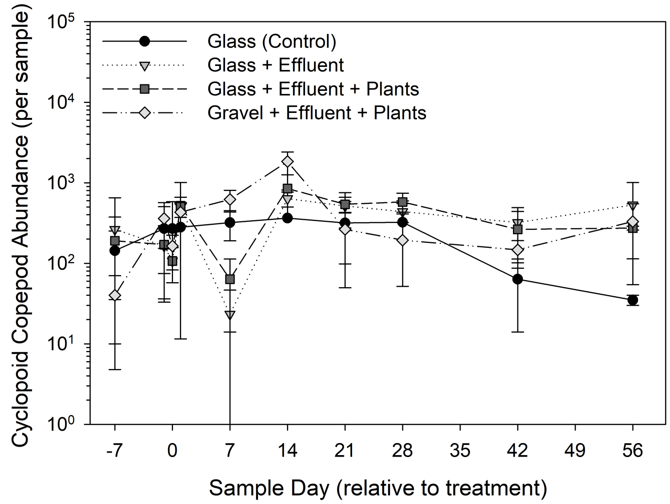

Figure S10. *Cyclopoid copepod abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

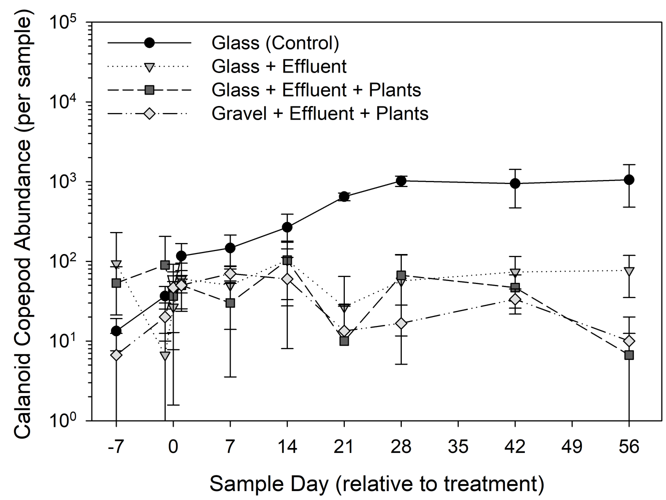

Figure S11. *Calanoid copepod abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

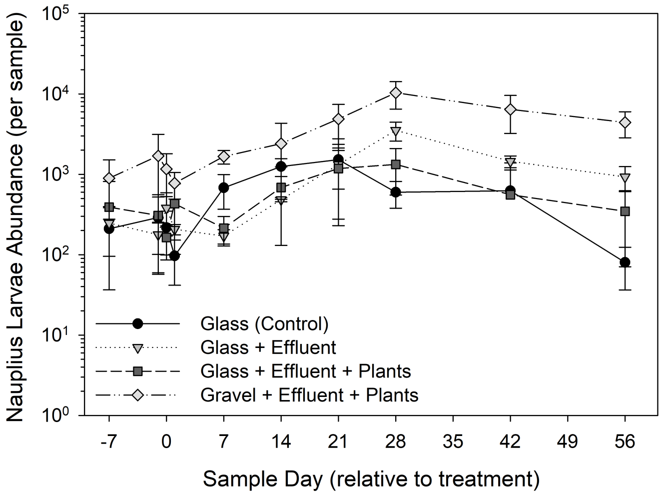

Figure S12. *Nauplius larvae abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (ß-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

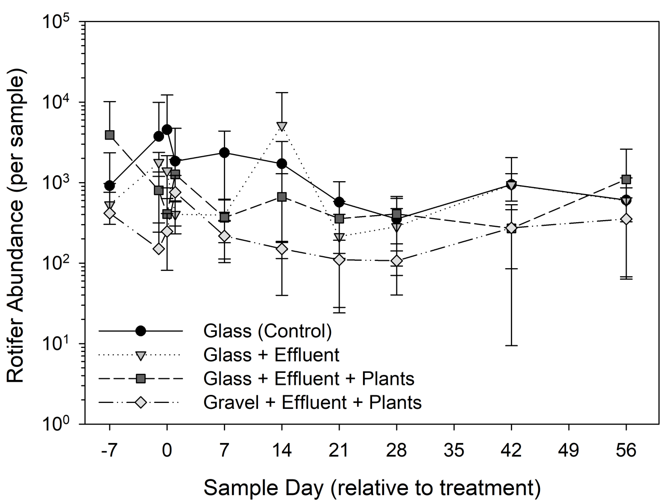

Figure S13. *Rotifer abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (ß-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

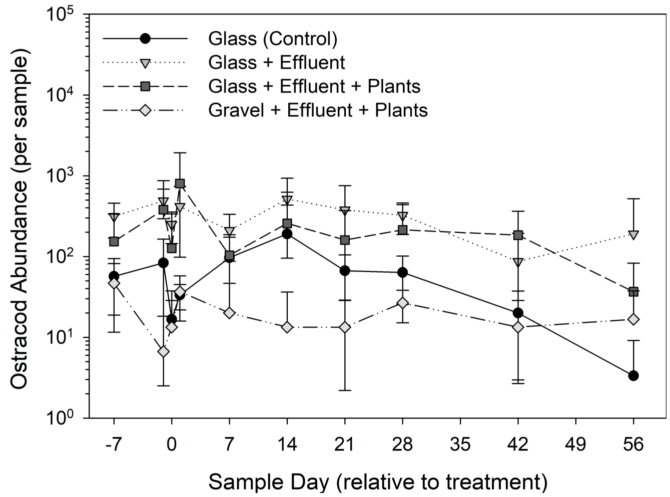

Figure S14. *Ostracod abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (ß-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

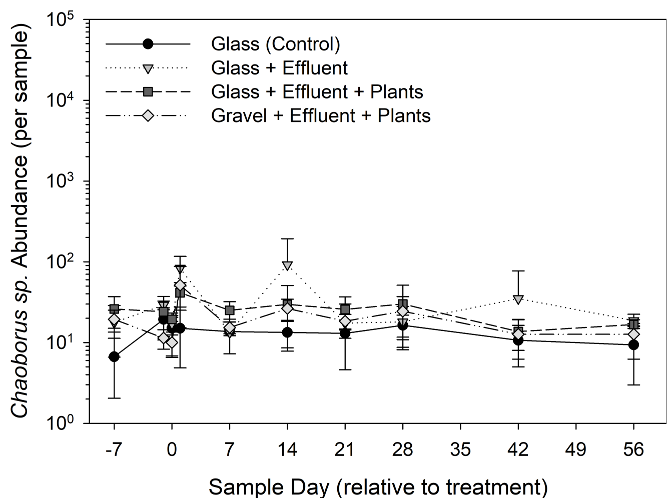

Figure S15. Chaoborus sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (ß-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

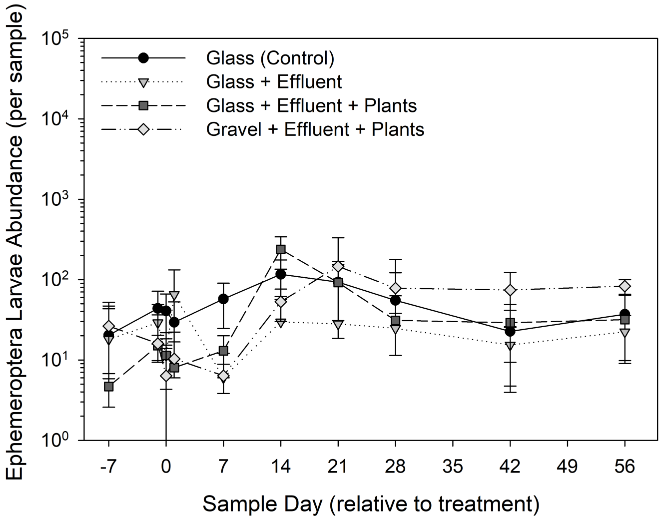

Figure S16. *Ephemeroptera larvae abundance per 50 mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

Systems with high levels of diversity are more likely to be resilient to structural changes in the community, with functional responses such as energy flow, biomass production, decay processes, and nutrient cycling being maintained through redundant roles in the ecosystem[42]. Using Shannon's diversity index, it was determined that the Gravel treatment is significantly less diverse than the other treatments. Additionally, the Gravel treatment was determined to have significantly less evenness than all other treatments. However, the Gravel treatment was not significantly different from other treatments when using Simpson's diversity index. In terms of diversity indices, Simpson's index is more sensitive to abundant species when compared to Shannon's index, which is likely resulting in the found differences in statistical significance[43]. In contrast, the Plant Glass treatment had the greatest mean values for Shannon's diversity index, Simpson's diversity index, and evenness, thereby supporting the hypothesis that recycled crushed glass can provide a matrix for natural populations comparable to, or an improvement, relative to gravel substrate.

There was a degree of within-treatment variability among the triplicate mesocosms. This variability may be confounding our ability to observe actual impacts from the treatments, as zooplankton abundance was observed to change with time among the same treatments. This within-treatment variability may be attributed to external factors such as weather, fauna grazing from preferred mesocosms, or unevenly distributed invertebrates. The natural variability among these systems could be reduced by allowing for the macrophytes and invertebrate communities to establish themselves for a longer duration prior to the exposure period. Biomass was not estimated in this study; however, it is worth investigating in similar future studies, as biomass estimates would help to provide more information on toxicity and grazing patterns, allowing for a clearer and more concise conclusion. Zooplankton are reported as number of organisms per cubic metre[44], which is a challenge for passive traps, since flow rate and volume of water filtered are required for the calculation. As a result, the abundance results in this study were reported as abundance per sample for each treatment. Future similar studies should try to incorporate a flowrate monitor into the experimental design.

5 Conclusion

In conclusion, recycled crushed glass is a suitable matrix for natural populations. There was no significant difference between the Control, Glass, and Plant Glass treatments for all diversity indices, indicating that the addition of synthetic effluent and macrophytes had no significant impacts on the invertebrate community structure. Though the effect of repeated pulsed exposures was not examined, it is unlikely that several repeated pulses, at much lower concentrations and environmentally relevant intervals between events would cause significant impacts on the zooplankton community dynamics when using recycled crushed glass as a substrate[20, 45]. Therefore, these results suggest that recycled crushed glass could be a viable surrogate for gravel in subsurface filtration processes, and further exploration is warranted.

6 Acknowledgements

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