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RESEARCH

How Benthic Sediment Microbial Communities Respond to Glyphosate and Its Metabolite: a Microcosm Experiment

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Abstract
Glyphosate is the most commonly used agricultural herbicide in the world. In aquatic ecosystems, glyphosate often adsorbs to benthic substrates or is metabolized and degraded by microorganisms. The effects of glyphosate on microbial communities vary widely as microorganisms respond differently to exposure. To help understand the impacts of glyphosate on the sediment microbiome, we conducted a microcosm experiment examining the responses of benthic sediment microbial communities to herbicide treatments. Sediments from a prairie pothole wetland were collected, and 16S rRNA gene sequencing was used to analyze community composition 2-h and 14-days after a single treatment of low (0.07 ppm), medium (0.7 ppm), or high (7 ppm) glyphosate, aminomethylphosphonic acid (glyphosate metabolite), or a glyphosate-based commercial formula. We found no significant differences in microbial community composition across treatments, concentration levels, or day of sampling. These findings suggest that microbial species in the Prairie Pothole Region of North America may be tolerant to glyphosate exposure.

Keywords Glyphosate · AMPA · Sediments · Microorganisms · Wetlands · Microcosms

Introduction

Agrochemical contamination of aquatic ecosystems is an ongoing concern due to the direct and indirect risks to environmental health. Glyphosate (i.e., Roundup®) is a non-selective, systemic herbicide that has become the most commonly used herbicide in the world since the 1990s [1]. The substantial use of glyphosate has resulted in its widespread and frequent detection in surface waters and groundwater [2, 3], where benthic sediments often become sinks [4]. Microbial metabolism is the primary degradation mechanism of glyphosate [5], resulting in its metabolites, aminomethylphosphonic acid (AMPA), glyoxylate or phosphate, and sarcosine [6].

Glyphosate targets higher plants through the inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme in the shikimate pathway [7], but this pathway is also present in some microorganisms [8]. Approximately 80% of archaea and 50% of bacteria contain the class I glyphosate-sensitive EPSPS protein, whereas the class II–IV glyphosate-resistant EPSPS protein is less common [9]. Therefore, it was initially presumed that glyphosate may have inadvertent effects on microbial communities. Studies have reported that glyphosate can have a wide range of negative, positive, or neutral impacts on microbial community composition and function in terrestrial and aquatic ecosystems. Aquatic microbial communities have been shown to utilize glyphosate as a nutrient source resulting in increased activity, whereas in others, microbes are inhibited by toxicological effects. Pérez et al. [10] found that 6 mg L^{-1} and 12 mg L^{-1} Roundup® caused a significant decrease in average microphytoplankton and nanophytoplankton abundance. However, Lu et al. [11] found no community structure shifts from 2.5 mg L^{-1} glyphosate and found significantly enhanced gene expression in mechanisms potentially related to glyphosate tolerance. Several microbial

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species are known to exhibit glyphosate tolerance [6], in addition to species capable of using glyphosate directly as a nutrient source [12, 13]. These examples demonstrate the variety of impacts glyphosate can have on aquatic microbial communities.

In addition to the impacts of glyphosate, AMPA, glyphosate's main metabolite, can also have direct and indirect effects on aquatic ecosystems [14]. Similar to glyphosate, AMPA can be degraded by microorganisms, but it is more mobile and persistent [15–17]. It is also a weak phytotoxin [18] with additional concerns regarding its potential to bioaccumulate [19]. AMPA is highly dependent on the presence and concentration of glyphosate [20], where both compounds frequently co-occur in areas of high agricultural intensity [5, 21]. Glyphosate and AMPA are most often detected in surface soil [22] and are frequently transported to surface waters, including wetlands [2, 5], where they could impact benthic microorganisms.

The Prairie Pothole Region (PPR) is a large, complex landscape covered with shallow wetlands and prairies [23]. Glyphosate is extensively used on corn and soybean, which are the predominant crops surrounding this region [1, 2]. Consequently, wetlands in the PPR are subject to prolonged glyphosate contamination, where glyphosate has been reported as the most frequently detected and the highest detected herbicide in this region [24]. These wetlands are key ecosystems, providing many economic and ecological services such as waterfowl production, flood protection, and nutrient cycling. For example, biogeochemical processes including C turnover and sequestration, N and P capture, and remediation of agrochemicals are essential ecosystem functions [25, 26], where benthic sediment microbial communities play a fundamental role in these ecosystem processes.

Understanding the impacts of agrochemical contamination on microbial community structure is vital because shifts in microbial communities impact the whole ecosystem. This is especially important in a region with high agricultural intensity, like the PPR, because microorganisms are chronically exposed to chemical stressors like glyphosate. The objective of our research was to assess the responses of benthic sediment microbial communities in the PPR to glyphosate-based herbicide treatments. We hypothesized that (1) observed operational taxonomic units (OTUs) and microbial diversity would decrease at high herbicide concentration due to toxicological effects, and (2) microbial community composition would shift at low herbicide concentration in favor of species capable of metabolizing glyphosate.

Materials and Methods

Microcosm Preparation

Surface sediment (to a depth of ~10 cm) was collected in July 2019 and stored in a sterilized (90% ethanol) cooler from a wetland (47.0984758, −99.1018688) within the Cottonwood Lake Study Area (U.S. Fish and Wildlife Service managed Waterfowl Production Area) of the PPR located in Stutsman County, North Dakota. This wetland is composed of glacial till with approximately 26% organic matter content [Hu et al., unpublished]. The area has minimal agricultural influence, where native prairie grasslands and wetlands cover over 80% [27]. After collection, the sediment was stored refrigerated (~4 °C) until the initiation of the experiment. Thirty-two 5.7 L microcosms were prepared with the following contents: a 1 cm layer of homogenized sediment, 2.5 L of dechlorinated tap water, and covered with "no-see-um" mesh (Duluth Sport Nets, Duluth, MN). Microcosms were then stored in incubators at 20 °C with a 16:8 (light:dark) hour photoperiod and left to acclimate for one month prior to treatment. Over the entirety of the 6-week experiment, microcosms were monitored for water evaporation and were filled back to the 2.5 L volume with dechlorinated tap water when necessary.

Herbicide Treatment and Sediment Sampling

We conducted a factorial experiment with 3 herbicide treatments, ×3 concentration levels, ×3 replicates, and 5 controls ($n = 32$). Analysis was conducted at three timepoints (total $n = 32 \times 3 = 96$). Herbicide treatments consisted of analytical grade glyphosate (*N*-(phosphonomethyl)glycine; 98.1% purity) or AMPA (99% purity) purchased from Sigma-Aldrich (Saint Louis, Missouri) or 41% glyphosate concentrate (commercial formula), which contains glyphosate isopropylamine salt. All herbicide solutions were made using serial dilutions in HPLC water to reach a final glyphosate or AMPA concentration of 0.07 parts per million (ppm), 0.7 ppm, or 7 ppm. Our concentrations were chosen based on the U.S. maximum contaminant level (MCL) of glyphosate in drinking water (U.S. EPA 2015), which is equivalent to our medium concentration of 0.7 ppm (or 7 mg L^{-1}). Treatments were added to the water, lightly stirred to evenly distribute, and then allowed to settle for 2 h. A 50 mL sterile polypropylene corer (needle-less syringe) was used to collect approximately 20 g (wet weight) of sediment from each microcosm pre-treatment, 2 h post-treatment, and 2 weeks post-treatment (total $n = 96$). All samples were stored in sterile 50-mL polypropylene tubes at −80 °C immediately after sampling until analyses were performed.

Analyses: Herbicide Residues and Microbial 16S Sequencing

Samples were thawed to obtain subsamples for herbicide residue and microbial analyses, and they were immediately shipped or returned to −80 °C for their respective analyses.

Sediment subsamples (~10 g wet weight) were shipped frozen on dry ice to the Agriculture and Food Laboratory at the University of Guelph (accredited lab in Ontario, Canada) for glyphosate and AMPA residue analysis. Samples were homogenized to analyze (wet weight) a representative amount of each sediment sample using liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS) to quantify glyphosate and AMPA residues. Samples were extracted using a quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, which included acidified aqueous extraction (acetic acid in acetonitrile in the presence of anhydrous sodium acetate and magnesium sulfate) and solid phase extraction. Instrumentation analysis of sample extracts was conducted using SCIEX 5550 ESI-MS/MS with Agilent 1260 HPLC in positive mode with a cation guard column for chromatographic separation and 0.1% formic acid in water and acetonitrile as mobile phases A and B, respectively. The instrument limit of detection was 0.005 ppm, and the limit of quantification was 0.02 ppm for glyphosate and AMPA. Deuterium-labelled internal standards, matrix blanks, spikes, and calibration standards were analyzed for quality control (QC), identification, and quantification of both compounds.

Sediment microbial communities were analyzed after the extraction of environmental DNA with the Qiagen DNeasy® PowerSoil® kit. Briefly, 0.25 g of sediment (wet weight) was lysed using PowerBead Tubes in a beadbeater (Biospec Mini-Beadbeater-24). Kit solutions (C1–C6) were added stepwise to purify, wash, and elute DNA into the 100 μL final volume. The microbial 16S rRNA gene was PCR amplified using universal primers, 27F and U1492R, for QC to verify suitability for sequencing. Sequencing library preparation and sequencing were performed according to the Oxford Nanopore Technologies 16S Barcoding Kit (SQK-RAB204) protocol and reagents. Briefly, amplicons were cleaned using AMPure XP bead cleanup, quantified via PicoGreen™ analysis (Quant-iT PicoGreen™ Kit, modified from Invitrogen's Quant-iT PicoGreen™ dsDNA Reagent and Kits protocol), and combined into a pooled sample to obtain a final DNA concentration of 50–100 ng. Pooled samples contained up to twelve uniquely barcoded samples for sequencing using an Oxford Nanopore minION™. Each library was run for approximately 4 h. Raw fast5 reads were basecalled and demultiplexed using Guppy v3.4. The EPI2ME 16S workflow (http://epi2me.nanoporetech.com., rev 2.1.0) was used for QC and initial characterization. For QIIME2 analysis, sequences were pre-processed using MetONTIIME [28], and QIIME2 was used to filter and analyze the resulting OTU table abundances. The SILVA v138 database was downloaded and utilized as a reference database for taxonomic identification [29, 30]. Taxonomy barplots, describing the composition of each sample at the desired taxonomic level, were visualized at QIIME2 view (https://view.qiime2.org/). Sequence tables were filtered to remove taxonomically unassigned sequences. A table of absolute OTU abundances was exported in BIOM format for further analysis in R.

Statistical Analysis

A feature table containing absolute abundances of family-level OTUs from QIIME2 was imported into R (version 4.0.2) using the R/biomformat package (1.24.0) [31]. Unassigned taxa and taxa that were present in $\leq$ 10% of samples were removed to minimize the risk of sequencing error carryover and due to low statistical power to analyze underrepresented OTUs, respectively. All subsequent analyses were conducted using the R/stats (3.6.2) and R/vegan (2.6–2) packages [32, 33]. Shannon's H diversity index was calculated using the "diversity" function, and observed OTUs were calculated using the "specnumber" function. Differences in diversity and observed OTUs between herbicide treatments, concentration levels, and day of sampling were analyzed with the Kruskal-Wallis rank sum test using the "kruskal.test" function. Lastly, the Bray-Curtis dissimilarity distance measures were calculated and the square root transformed for non-metric multidimensional scaling (NMDS) using the "metaMDS" function to display community structure.

Results

After unassigned and rare taxa were removed, a total of 430 OTUs were detected across sediment samples taken at three timepoints from 32 microcosms (n = 96). Relative abundances across herbicide treatments were similar (Fig. 1). The three most abundant families in all treatments were Hydrogenophilaceae in the Pseudomonadota (Gammaproteobacteria) phylum and Sulfurovaceae and Desulfobacteraceae, which are assigned to the Proteobacteria (Deltaproteobacteria) phylum. Prolixibacteraceae, a family within the Bacteroidota phylum thought to be associated with sediment, was only detected in the control and glyphosate treatments.

The Shannon H index and observed OTUs were similar among all treatments and controls over the entirety of the experiment (Figs. 2 and 3, Table 1). The Kruskal-Wallis tests showed no significant differences in alpha diversity metrics across herbicide treatments, concentration levels, or day of sampling (Table 2). A two-dimensional ordination solution was reached (stress = 0.1793708) using NMDS. Microbial communities were similar in multivariate space, which showed that there were no compositional differences across treatments (Fig. 4).

Both glyphosate and AMPA concentrations in sediments increased over time within each herbicide treatment but not

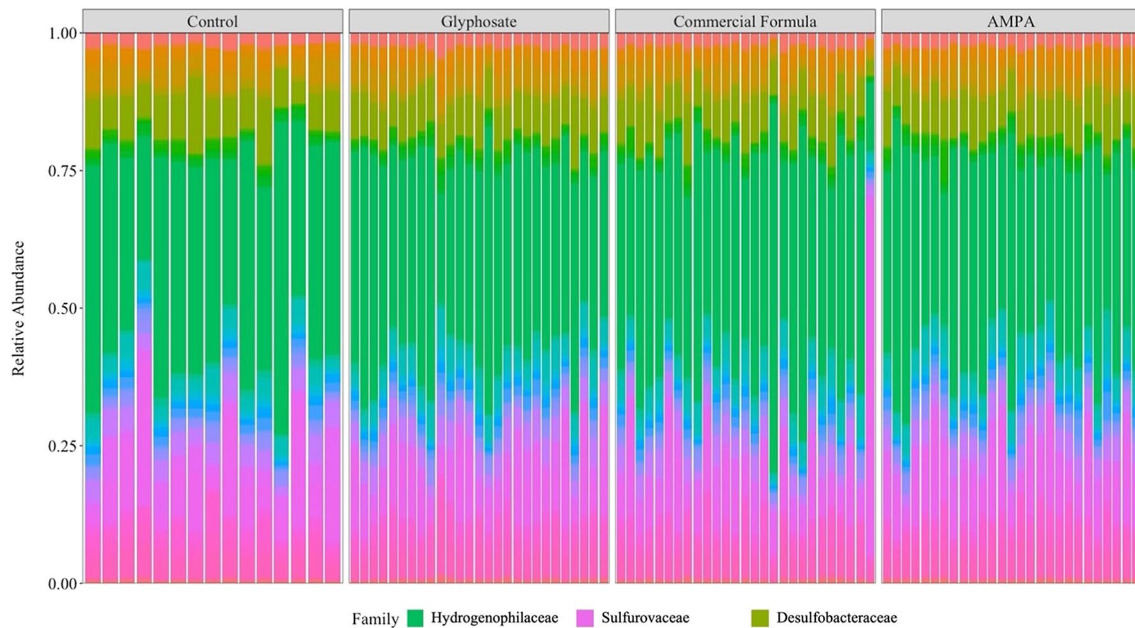

Fig. 1 Relative abundance of microbial families within microcosm sediments of each herbicide treatment at all sampling timepoints (n = 96); legend shows the top three most abundant families across all herbicide treatments (full legend can be found in Supplemental Fig. 1)

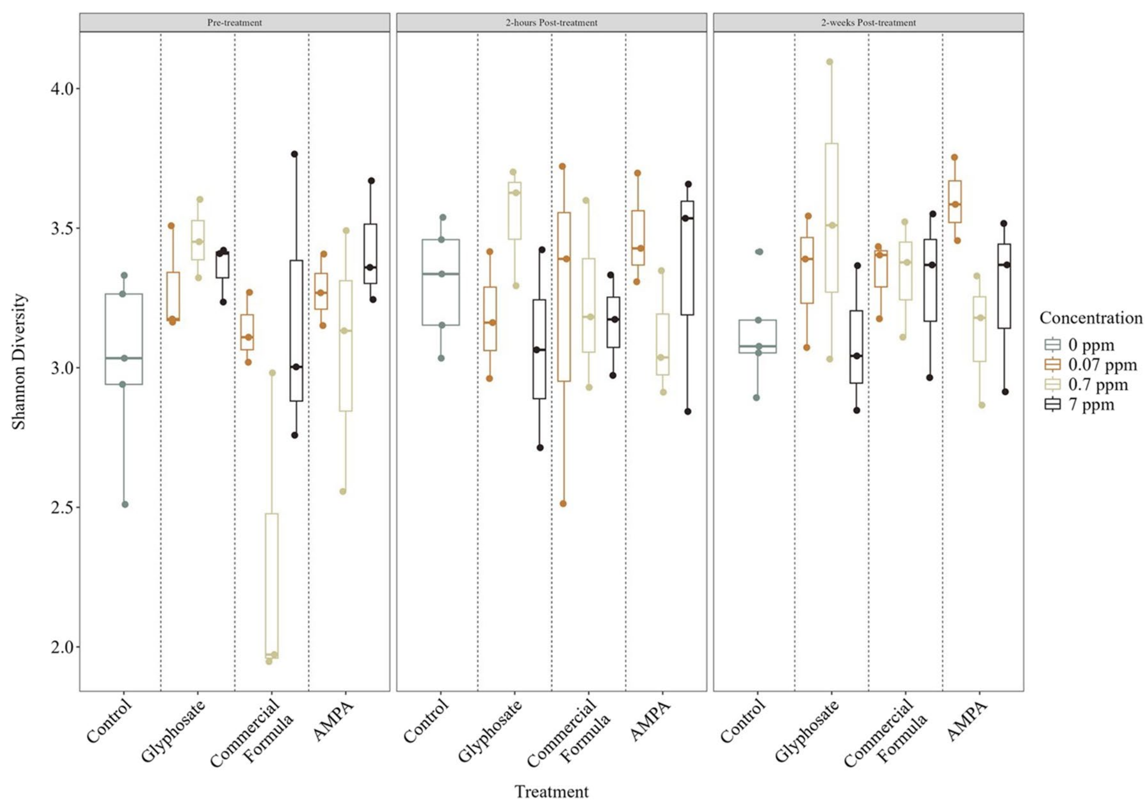

Fig. 2 Boxplots of Shannon's H diversity index quantified from microcosm sediments by herbicide treatments and concentration and paneled by day of sampling (control treatment, n = 5; herbicide treatments, n = 3). Boxplots represent: 25th quartile = bottom of the box; 75th quartile = top of the box; median = line across each box; minimum and maximum = whiskers; and data points = individual data points

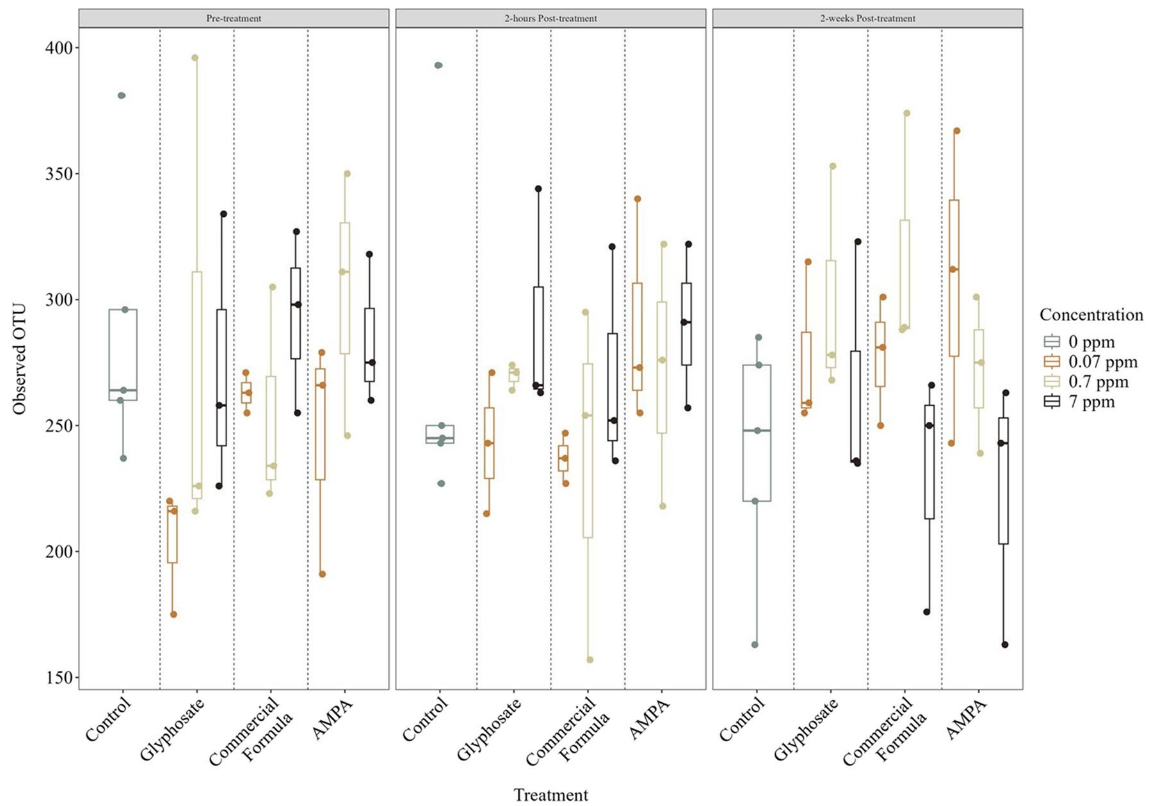

Fig. 3 Boxplots of observed OTUs quantified from microcosm sediments by herbicide treatments and concentration and paneled by day of sampling (control treatment, n = 5; herbicide treatments, n = 3). Boxplots represent: 25th quartile = bottom of the box; 75th quartile = top of the box; median = line across each box; minimum and maximum = whiskers; and data points = individual data points

Table 1 Average quantified species metrics ± standard deviation detected in microcosm sediments of each herbicide treatment over the entirety of the experiment

	Shannon diversity	Observed OTUs
Treatment		
Control	2.7 ± 0.24	266 ± 58
Glyphosate (purity = 98.1%)	2.9 ± 0.26	267 ± 50
Commercial formula	2.7 ± 0.41	264 ± 45
AMPA (purity = 99%)	2.9 ± 0.29	276 ± 47

Table 2 Summary of the Kruskal-Wallis rank sum test on the effects of treatments on Shannon's diversity and observed OTU. χ^2, chi-square value; *df*, degrees of freedom; *P*, the *p*-value where alpha = 0.05

Quantified metric	Treatment	χ^2	df	*P*
Shannon diversity	Day of sampling	1.81	2	0.40
	Herbicide treatment	4.67	3	0.20
	Concentration	3.48	3	0.32
Observed OTU	Day of sampling	2.25	3	0.52
	Herbicide treatment	0.22	2	0.90
	Concentration	2.45	3	0.48

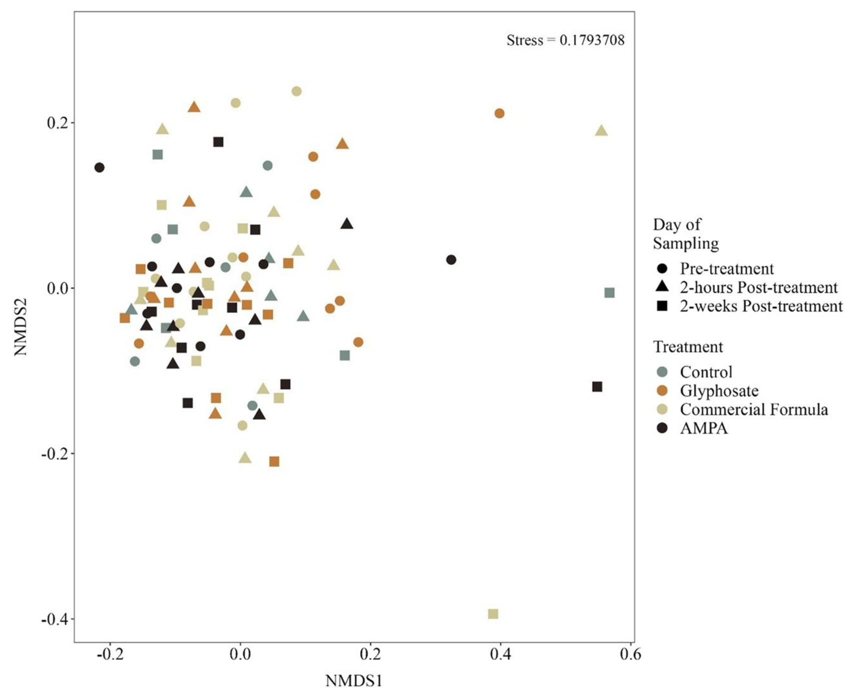

Fig. 4 NMDS plot of sediment microbial community structure across all samples ($n = 96$) where shapes represent the day of sampling and colors represent the herbicide treatment

Table 3 Average glyphosate concentration (ppm) ± standard deviation detected in microcosm sediments of each herbicide treatment and concentration at each sampling timepoint. Concentrations: $L = 0.07$ ppm, $M = 0.7$ ppm, and $H = 7$ ppm; *ND*, not detected (i.e., value of 0)

Treatment	Concentration	Pre-treatment	2-h post-treatment	2-weeks post-treatment
		Residues detected (ppm)[1]		
Control		< LOQ	ND	ND
Glyphosate (purity = 98.1%)	L	0.03 ± 0.04	ND	0.05 ± 0.08
	M	ND	0.03 ± 0.04	0.28 ± 0.1
	H	ND	0.67 ± 0.35	4.37 ± 0.35
Commercial formula	L	ND	ND	ND
	M	ND	ND	0.31 ± 0.36
	H	ND	0.41 ± 0.46	2.77 ± 1.30
AMPA (purity = 99%)	L	ND	< LOQ	ND
	M	ND	ND	ND
	H	< LOQ	ND	ND

[1]LOD = 0.005; LOQ = 0.02 ppm

Table 4 Average AMPA concentration (ppm) ± standard deviation detected in microcosm sediments of each herbicide treatment and concentration at each sampling timepoint. Concentrations: $L = 0.07$ ppm, $M = 0.7$ ppm, and $H = 7$ ppm; *ND*, not detected (i.e., value of 0)

Treatment	Concentration	Pre-treatment	2-h post-treatment	2-weeks post-treatment
		Residues detected (ppm)[1]		
Control		ND	ND	ND
Glyphosate (purity = 98.1%)	L	ND	ND	ND
	M	< LOQ	< LOQ	0.04 ± 0.02
	H	ND	0.05 ± 0.05	0.35 ± 0.15
Commercial formula	L	< LOQ	ND	< LOQ
	M	ND	ND	< LOQ
	H	ND	0.03 ± 0.02	0.19 ± 0.03
AMPA (purity = 99%)	L	< LOQ	< LOQ	0.04 ± 0.03
	M	ND	0.11 ± 0.04	0.48 ± 0.18
	H	ND	1.08 ± 0.57	6.07 ± 7.90

[1]LOD = 0.005 ppm; LOQ = 0.02 ppm

in control treatments (Tables 3 and 4). These demonstrated residues were dissipating out of the water column shortly after treatment and settling into the sediments. Additionally, AMPA was detected in microcosms where only glyphosate or commercial formula was added, confirming that glyphosate degradation did occur (Table 4).

Discussion

The current study used microcosms to examine the impact of glyphosate-based herbicide treatments on benthic sediment microbial communities in a prairie pothole wetland. Both glyphosate and AMPA were detected in microcosm sediments shortly following treatment, indicating that residues rapidly dissipate out of the water column [5, 16]. AMPA was also detected in sediments of microcosms that only received glyphosate (i.e., glyphosate or commercial formula treatment) but no direct AMPA treatment, indicating that glyphosate degradation occurred [34]. In our experimental microcosms, we found that microbial community diversity and composition were not significantly affected by glyphosate, AMPA, or commercial formula at any concentration, which was contrary to our original hypotheses. Our highest treatment level (7 ppm) was a magnitude higher than the U.S. EPA's MCL for drinking water, and approximately 41 times higher than the average concentration that has been reported in wetland sediments in the PPR [24]. Some research suggests that microbial responses are more dependent on previous glyphosate exposure history and application rates [19, 35]. The present experiment reflects conditions of acute glyphosate and AMPA exposure rather than long-term exposure in the sediment community. Sediments used in our study were collected from a wetland in North Dakota with no routine pesticide usage in the immediate catchment. Thus, agricultural inputs within this area are minimal to none compared to other areas of the PPR. However, we found no differences between treatments and controls, suggesting that glyphosate-based herbicides may not have adverse effects on sediment microbial communities in wetlands in this area of the PPR. This observed lack of effect may be the result of glyphosate- and AMPA-based selection pressure.

A lack of response from microbial communities following glyphosate exposure has also been observed previously in the literature. For example, Pesce et al. [36] exposed natural spring- and summer-collected riverine microbial communities to 10 μg L^{-1} glyphosate and found no effect on bacterial activity or diversity. Lane et al. [37] conducted a 6-month soil incubation experiment with monthly glyphosate treatments at 59 μg g^{-1} and 118 μg g^{-1} and found no significant glyphosate effect on community structure, as represented by the relative abundances of functional microbial groups. Muturi et al. [38] used 20 mg L^{-1} glyphosate treatment in microcosms and found no differences in water microbial diversity or richness after 3 and 7 days. Dennis et al. [39] found no significant effects of a single glyphosate treatment at the recommended field application rate (33.03 mg L^{-1}) on bacterial or archaeal evenness, richness, and composition after 60 days of incubation. These studies in addition to the current study represented a wide range of glyphosate concentrations, which all showed that glyphosate does not always have direct toxic effects on microbial communities. Presumably, this may be due to the presence of glyphosate-tolerant microbes.

Several studies have, however, shown shifts in microbial communities after either short- or long-term glyphosate exposure. Lu et al. [11], for example, found increases in the Shannon and Simpson diversity of lacustrine microbial communities, in addition to differences in community structure 10- and 15-days post-treatment of 2.5 mg L^{-1} glyphosate. Widenfalk et al. [40] also found that an environmentally relevant glyphosate concentration (150 μg kg^{-1} dry weight) caused significant shifts in lake sediment bacterial community composition in treated microcosms relative to controls after 31 days. In mesocosms, Pérez et al. [10] found that Roundup® treatment significantly decreased phytoplankton and periphyton abundance but increased picocyanobacterial abundance and primary production. Sura et al. [41] found pelagic and biofilm bacterial production in outdoor mesocosms was significantly inhibited by 225 μg L^{-1} glyphosate compared to controls. Microbial communities in our study and Sura et al. [41] were both collected from wetlands within the PPR; however, our results were not consistent, potentially due to differences in sediment- versus water-associated communities, land use within our collection site's watersheds, or incubation versus outdoor experimental design. These studies all showed that glyphosate can have various negative and positive effects on microbial communities, which may be partially attributed to heterotrophic versus autotrophic species, whereas our results showed that glyphosate can also have no effect. This discrepancy indicates that there are many underlying complexities in the effects of glyphosate at the microbial level, which may be more related to molecular mechanisms and/or environmental variables.

Some microorganisms naturally express a glyphosate-resistant form of the EPSPS enzyme of the shikimate pathway [9], and there are many glyphosate-tolerant microbial species listed in the literature [42, 43]. Specifically, *Agrobacterium* sp. strain CP4 was the bacteria used for the original glyphosate-resistant EPSPS gene in glyphosate-resistant crops [44], whereas other species have evolved tolerance through mutations of the EPSP synthase or metabolic or detoxifying processes [45], which can be a

result of prolonged or repeated glyphosate exposure. For example, Tang et al. [35] added 100 mg L^{-1} glyphosate to sediments with high, low, and no previous glyphosate exposure and found that microbes degraded glyphosate quicker in sediments with a high exposure history compared to sediments with a low exposure history. Additionally, Lane et al. [37] found significantly higher microbial respiration in soils after glyphosate treatment, specifically in soils with previous glyphosate exposure history. The sediments used in the present study were collected from an agriculturally undisturbed wetland located in an area with no known extensive glyphosate use (David Mushet, Research Wildlife Biologist, USGS Northern Prairie Wildlife Research Center, email communication June 21, 2022); thus, microbial communities should not have any prolonged exposure history. We did find multiple species across all treatments that are capable of glyphosate degradation and mineralization including *Cyanobiaceae*, *Enterobacteriaceae*, *Pseudomonadaceae*, and *Rhizobiaceae* (e.g., genus *Agrobacterium*). While our sediment collection site has no known history of glyphosate use within the immediate watershed, the majority of the PPR is in an agriculturally intensive area where glyphosate use is common. Therefore, microbial species from the regional species pool may have evolved tolerance despite our study wetland and its catchment having no existing agricultural pesticide use. On the contrary, microbial communities with and without a history of glyphosate exposure may not differ in glyphosate tolerance [46] potentially due to the presence of the glyphosate-resistant class II EPSPS protein naturally found in some prokaryotes [9]. Therefore, our findings may be the result of selection pressure, dispersal, or biochemical makeup.

Glyphosate is highly water-soluble, which also allows it to be easily transported into wetlands, where environmental variables can play a role in its bioavailability and toxicity to microorganisms. Glyphosate has an affinity for oxides, metal cations, and organic matter content, resulting in its rapid adsorption into sediments [47]. However, glyphosate and phosphate can compete for sediment binding sites due to their chemical structural similarities [48]. Thus, lower phosphate can increase glyphosate's sediment adsorption capacity [49], subsequently decreasing its bioavailability. Temperature can also affect glyphosate's environmental persistence, where higher temperatures facilitate degradation due to increased microbial activity [50, 51]. Sediments used in the present study were glacial till composed of approximately 26% organic matter content [Hu et al., unpublished]. Due to glyphosate's strong adsorption to organic matter, sediment microorganisms in our study may have had limited exposure to herbicide residues, thus preventing major toxicological effects. While the present study did not measure sediment physicochemical characteristics, homogenized sediments were used for all microcosms, and temperature and light conditions were controlled to help minimize variability.

Conclusion

We evaluated the responses of benthic sediment microbial communities to a single addition of low, medium, or high glyphosate-based herbicide treatment after 14 days of incubation. We expected to see toxicological-induced shifts potentially in favor of tolerant species; however, we found no differences in sediment microbial communities among treatments or concentrations after 2 weeks. Our results may be explained by the lower concentration of bioavailable glyphosate in the sediments compared to the actual concentrations added. Additionally, our results may suggest glyphosate tolerance in the benthic sediment microbial communities, but that is inconclusive without further investigation of the presence of the glyphosate-resistant EPSP gene within the microbiome. Our research suggests that in the PPR, the direct effects of glyphosate on sediment microorganisms may not be as severe as initially presumed. However, the literature continues to reveal new implications of the extensive use of glyphosate in aquatic ecosystems. Therefore, further research is still necessary to determine the full range of potential effects of glyphosate on sediment microbial communities.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-023-02296-6.

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Author Contribution All authors contributed to the study conception and design. Material preparation, data collection, and microbial analysis were performed by Christine Cornish and Kaycie Schmidt. The first draft of the manuscript was written by Christine Cornish, with additions from Jon Sweetman and Peter Bergholz. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

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Data Availability The data used in this study are available from the corresponding author upon reasonable request.

Declarations

Ethics Approval Not applicable.

Consent to Participate All authors have given consent to participate.

Consent for Publication All authors have given consent to publish.

Competing Interests The authors declare no competing interests.

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