How benthic sediment microbial communities respond to glyphosate and its metabolite: A microcosm experiment

Christine Cornish[1], Peter Bergholz[2,3], Kaycie Schmidt[2], and Jon Sweetman[1,4]

[1]Department of Biological Sciences and [2]Department of Microbiological Sciences, North Dakota State University

1340 Administration Avenue, Fargo, ND 58105, United States

[3]Institute for Quantitative Health Sciences & Engineering, and Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI 48823

[4]Department of Ecosystem Science and Management, The Pennsylvania State University

457 ASI Building, University Park, PA 16802

Corresponding author: Christine Cornish, christine.cornish@ndsu.edu, (636) 485-0649

Acknowledgments

Thank you to the North Dakota Established Program to Stimulate Competitive Research for funding this research. Thank you to Dr. López-Martínez for providing support and usage of equipment. Additionally, thank you to the anonymous reviewers for their time and feedback of our manuscript.

Abstract

Glyphosate is the most commonly used agricultural herbicide in the world. In aquatic ecosystems, glyphosate adsorbs to benthic substrates or is metabolized and degraded by microorganisms. The effects of glyphosate on microbial communities varies 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 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. Our results showed no significant differences in microbial community composition between treatments. These findings suggest that microbial species in the Prairie Pothole Region of North America may be tolerant to glyphosate exposure.

Keywords: glyphosate, AMPA, sediments, benthic microorganisms, 16S sequencing, 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 (Cobb and Reade 2010; Annett et al. 2014; Benbrook 2016; Gomes et al. 2019). The substantial use of glyphosate has resulted in its widespread and frequent detection in surface waters and groundwater (Scribner et al. 2007; Byer et al. 2008; Coupe et al. 2012; Belden et al. 2012), where benthic sediments often become sinks (Warren et al. 2003). Specifically, in shallow wetlands, glyphosate rapidly dissipates from the water column and adsorbs to sediments (Degenhardt et al. 2012; Battaglin et al. 2014) or is biodegraded (Gill et al. 2017). Microbial metabolism is the primary degradation mechanism of glyphosate (Battaglin et al. 2014) via the glyphosate oxidoreductase and carbon (C)-phosphorus (P) lyase enzymes resulting in aminomethylphosphonic acid (AMPA) or phosphate and sarcosine, respectively (Zhan et al. 2018; Bhatt et al. 2021).

Glyphosate targets higher plants through the inhibition of the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme in the shikimate pathway (Cobb and Reade 2010; Hetrick and Blankinship 2015). The shikimate pathway is also present in some microorganisms (Cox 1995; Herrmann and Weaver 1999), thus 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. For example, (Druille et al. 2015) tested annual applications of 0.81 L ha^{-1} glyphosate, which is approximately 3.5 times less than the recommended field application rate, on diazotrophs (nitrogen fixing bacteria and archaea). They found that after the fourth year of

application, diazotroph densities were reduced 82%. On the contrary, (Zabaloy et al. 2012) found the relative abundance of soil bacteria increased approximately four times after one week in 150 mg kg^{-1} glyphosate treated microcosms compared to controls. (Busse et al. 2001) found no impact after 9 – 13 years repeated treatment of Roundup® at 3 kg [active ingredient] ha^{-1} (recommended field concentration) on soil microbial respiration and metabolism, or total bacterial count within the top 10 cm of soil. In addition to the variability of results within glyphosate-microbial research, there has been differential results within studies on the same species (Moorman 1986).

Research on the effects of glyphosate in aquatic systems is also conflicting where, in some instances, 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. (Vera et al. 2010) found 8 mg L^{-1} glyphosate (applied as Roundup®) in mesocosms inhibited periphyton colonization and caused diatom mortality, but also found 3.5 mg L^{-1} glyphosate (applied as Glifosato Atanor®) increased bacterioplankton and planktonic picocyanobacterial abundance in microcosms (Vera et al. 2012). (Lu et al. 2020) used 16S rRNA gene sequencing to assess the impact of 2.5 mg L^{-1} glyphosate on aquatic microbial communities. They found significantly enhanced gene expression in mechanisms potentially related to glyphosate tolerance, but did not find overall community structure shifts. (Pérez et al. 2007) found 6 mg L^{-1} and 12 mg L^{-1} Roundup® caused a significant decrease in average microphytoplankton and nanophytoplankton abundance. These examples demonstrate that in some instances, glyphosate can have a range of impacts on microbial communities within the same system.

In other instances glyphosate tolerance has been observed in several species, including *Anabaena variabilis* (Powell et al. 1991), *Staphylococcus aureus* (Priestman et al. 2005), *Pseudomonas* spp. (Zhao et al. 2015), *Achromobacter* spp. (Zhan et al. 2018), and *Ochrobactrum intermedium* (Firdous et al. 2018). In some cases, microbial species are able to use glyphosate directly as a nutrient source (Kools et al. 2005; Forlani et al. 2008; Shushkova et al. 2009). Previous research has suggested that the ability of microorganisms to metabolize glyphosate and/or use it as a P, C, or N source increases with previous or repeated exposure to glyphosate (Araújo et al. 2003; Nguyen et al. 2016; Guijarro et al. 2018; Tang et al. 2019).

In addition to the impacts of glyphosate, AMPA, glyphosate's main metabolite, can also have both direct and indirect effects on aquatic ecosystems (Grandcoin et al. 2017). Similar to glyphosate, AMPA strongly adsorbs to sediments and can be degraded by microorganisms (Blake and Pallett 2018), but is more mobile and persistent (Kjaer et al. 2005; Degenhardt et al. 2012; Imfeld et al. 2013). AMPA is highly dependent on the presence and concentration of glyphosate (Beecraft and Rooney 2021), where both compounds frequently co-occur in areas of high agricultural intensity (Battaglin et al. 2014; Malaj et al. 2020). In agricultural areas, glyphosate and AMPA are most often detected in surface soil (Okada et al. 2016), and frequently transported to surface waters, including wetlands (Coupe et al. 2012; Battaglin et al. 2014) where they can impact benthic microorganisms. AMPA is a known phytotoxin (Reddy et al. 2008) with additional concerns regarding its potential to bioaccumulate (Bai and Ogbourne 2016). However, there is a gap in the literature researching the effects of AMPA on aquatic microbial communities.

The Prairie Pothole Region (PPR) is a large complex landscape covered with shallow wetlands and prairies (Dahl 2014). This region is surrounded by agriculture, primarily corn and

soybean croplands, which are the predominant crops glyphosate is used on (Benbrook, 2016; Coupe et al. 2012). 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 (McMurry et al. 2016). These wetlands are key ecosystems providing flood protection, habitat for biodiversity, and are critical breeding grounds for migratory waterfowl (Gleason et al. 2011). Additionally, they provide essential biogeochemical services including C turnover and sequestration, N and P capture, and remediation of agrochemicals (Holloway et al. 2011; Zeng and Arnold 2013; Dalcin Martins et al. 2017), where benthic sediment microbial communities play a fundamental role in these ecosystem processes (Hassan et al. 2005).

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 an area 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 from the PPR to glyphosate-based herbicide treatments. We hypothesized that 1) microbial diversity and richness would decrease with increasing herbicide concentrations due to toxicological effects, and 2) microbial community composition would shift at lower herbicide concentrations in favor of species capable of metabolizing glyphosate. Alternatively, microorganisms in this region may have evolved glyphosate-tolerance from prolonged exposure, thus there may be no change in microbial communities.

Materials and Methods

Microcosm preparation

Surface sediment (top 10 cm) was collected from a wetland (P1) located in the Cottonwood Lake Study Area (U.S. Fish and Wildlife service managed Waterfowl Production Area) of the PPR located in Stutsman County, North Dakota. The study area has been owned by the U.S. Fish and Wildlife Service since 1963 and used as a study site by the U.S. Geological Survey's Northern Prairie Wildlife Research Center since 1966 (Winter 2003). This area has minimal agricultural influence, where native prairie grasslands and wetlands cover over 80% (Winter 2003). After collection, sediment was stored refrigerated (~4 °C) until initiation of the experiment. Thirty-two 5.7 L microcosms were prepared with the following contents: 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 when necessary.

Herbicide treatment and sediment sampling

We conducted a 3 x 4 factorial experiment with three replicates and five controls (microcosm n = 32). Herbicide treatments consisted of analytical grade glyphosate (98.1% purity) or AMPA (99% purity), or 41% glyphosate concentrate (commercial formula) at concentrations 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 (US EPA 2015; Bai and Ogbourne 2016), 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 two hours. A 50 mL polypropylene corer (needle-less syringe) was used to collect sediment samples pre-treatment, two hours post-treatment, and two weeks post-treatment. All samples were stored in 50 mL polypropylene tubes at -80 °C immediately after sampling until analyses were performed.

Analyses: Herbicide residues and microbial 16S sequencing

Sediment subsamples were shipped frozen to the Agriculture and Food Laboratory at the University of Guelph (Ontario, Canada) for glyphosate and AMPA residue analysis. Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used with a limit of detection of 0.005 ppm and a limit of quantification of 0.02 ppm for both compounds.

Sediment microbial communities were analyzed after extraction of environmental DNA with the Qiagen DNeasy® PowerSoil® kit. Briefly, 0.25 g sediment (wet weight) was lysed using PowerBead Tubes in a bead-beater (Biospec Mini-beadbeater-24). Kit solutions (C1 – C6) were added stepwise to purify, wash, and elute DNA into 100 µL final volume. Microbial 16S rRNA gene was PCR amplified using 27F and U1492R primers for quality control to verify suitability for sequencing. Sequencing library preparation and sequencing were performed according to 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 pooled to obtain a final DNA concentration of 50 – 100 ng. Pooled samples contained up to twelve separately barcoded samples for sequencing using an Oxford Nanopore minION™. Each library was run for approximately four hours. Raw fast5 reads were basecalled and demultiplexed using Guppy v3.4. EPI2ME 16S workflow (https://epi2me.nanoporetech.com, rev 2.1.0) was used for QC and initial characterization. For

QIIME2 analysis, sequences were pre-processed using MetONTIIME (Maestri et al., 2019) and QIIME2 was used to filter and analyze the resulting operational taxonomic unit (OTU) table abundances (Bolyen et al., 2019). The SILVA v138 database was downloaded and utilized as a reference database for taxonomic identification (Pruesse et al. 2007; Quast et al. 2012). 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 OTU from QIIME2 was imported into R (version 4.0.2) using the R/biomformat package (1.24.0) (McMurdie and Paulson 2015). Unassigned taxa and OTUs that were present in ≤ 10% of samples were removed to minimize the risk of sequencing error carryover and due to low statistical power to analyze underrepresented OTU, respectively. All subsequent statistical analyses were conducted using the R/stats (3.6.2) and R/vegan (2.6-2) packages (Oksanen et al. 2017; R Core Team 2022). Shannon's diversity index was calculated using the "diversity" function and observed OTU (richness) was calculated using the "specnumber" function. Additionally, differences in diversity and richness between herbicide treatments, concentration levels, and time of sampling were analyzed with a Kruskal-Wallis Rank Sum test using the "kruskal.test" function. Lastly, non-metric multidimensional scaling (NMDS) was performed using the "metaMDS" function to display community structure. Square root transformed Bray-Curtis dissimilarity distance measures were used for the NMDS.

Results

Both glyphosate and AMPA concentrations in sediments increased over time within each herbicide treatment, but not in control treatments. Additionally, AMPA was detected in microcosms where glyphosate or commercial formula was added, confirming that glyphosate degradation did occur (Supplemental Table 1).

A total of 430 microbial families were detected across all 96 samples. Relative abundances across treatments were not significantly different (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. Kruskal-Wallis tests showed no significant differences in alpha diversity (quantified by the Shannon H index) or observed OTU in herbicide treatments, concentration levels, and time of sampling (Table 1). Taxon alpha diversity were similar among all treatments and controls over the entirety of the experiment (Fig. 2 and Fig. 3). A two-dimensional ordination solution was reached (stress = 0.1793708) using NMDS. However, microbial communities were similar in multivariate space, which showed there were no significant compositional differences between herbicide treatments (Fig. 4).

Discussion

The current study used microcosms to examine the impact of glyphosate-based herbicide treatments on benthic sediment microbial communities from 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 (Degenhardt et al. 2012;

Battaglin et al. 2014). 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 (Gill et al. 2017). 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 have been reported in wetland sediments in the PPR (McMurry et al. 2016). Some research suggests that microbial responses are more dependent on previous glyphosate exposure history and application rates (Bai and Ogbourne 2016; Tang et al. 2019). The present experiment reflects conditions of acute glyphosate and AMPA exposure, rather than long-term exposure on the sediment community. Sediments used in our study were collected from a permanent wetland in the Cottonwood Lake Study Area, which is located near the Missouri Coteau in Stutsman County, North Dakota. This area is approximately 80% native prairie and wetlands (Winter 2003), and no routine pesticide use occurs 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 from wetlands in this area of the PPR. Additionally, the lack of effect on the microbial communities in our study may be a result of glyphosate- and AMPA-tolerance.

A lack of a response from microbial communities following glyphosate exposure has also been observed previously in the literature. For example, (Pesce et al. 2009) 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. 2012) conducted a six-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 represented by the relative abundances of functional microbial groups. (Muturi et al. 2017) 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. 2018) found no significant effects of a single glyphosate treatment at the recommended field rate (33.03 mg L^{-1}) on bacterial or archaeal evenness, richness, and composition after 60-days 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. 2020), for example, found increases in 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. 2008) 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. (Sura et al. 2012) 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. 2012) 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, whereas our results showed glyphosate can also have no effect. This discrepancy indicates that there are many underlying complexities on 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 EPSP enzyme and there are many glyphosate-tolerant microbial species listed in the literature (Powell et al. 1991; Padgette et al. 1995; Priestman et al. 2005; Zhao et al. 2015; Firdous et al. 2018; Zhan et al. 2018). Specifically, *Agrobacterium* sp. strain CP4 was the bacteria used for the original glyphosate-resistant EPSP gene in glyphosate-resistant crops (Padgette et al. 1995; Cerdeira and Duke 2006). Whereas, other species have evolved tolerance through mutations of the EPSP synthase, or metabolic or detoxifying processes (Hertel et al. 2021), which can be a result of prolonged or repeated glyphosate exposure. For example, (Tang et al. 2019) 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 high exposure history compared to low exposure history. Additionally, (Lane et al. 2012) 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*) (Cobb and Reade 2010;

Kryuchkova et al. 2014; Hernández-Alomia et al. 2022). 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.

Environmental variables also play a role in glyphosate's bioavailability and toxicity to microorganisms. Glyphosate is water soluble (National Center for Biotechnology Information 2022) allowing it to be easily transported into aquatic ecosystems. Initially, it was considered non-problematic because it rapidly dissipates out of the water column and adsorbs to sediments due to its affinity for oxides and organic matter content (Dion et al. 2001; Jacinthe et al. 2009). (Guijarro et al. 2018) found that glyphosate's sediment adsorption increased with higher clay content, and aluminum and iron. However, glyphosate's adsorption capacity, thus bioavailability, is also dependent on phosphate as they compete for sediment binding sites due to their structural similarities (de Jonge et al. 2001). (Guijarro et al. 2018) also found that glyphosate's sediment adsorption increased with lower phosphate. Temperature can also affect the persistence of glyphosate in the environment where higher temperatures facilitate degradation due to increased microbial activity (Helander et al. 2012; Muskus et al. 2022). The present study did not test for relationships between herbicide treatments and sediment characteristics, but the experiment was conducted using the same homogenized sediments across all treatments under controlled conditions (i.e., temperature and light). Therefore, environmental variables should not have had a confounding influence on our results.

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 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 concentration. Our results may be explained by 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 glyphosate's extensive use on aquatic ecosystems. Therefore, further research is still necessary to determine the full range of potential indirect or long-term effects of glyphosate on sediment microbial communities.

References

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

Araújo ASF, Monteiro RTR, Abarkeli RB (2003) Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52:799–804. https://doi.org/10.1016/S0045-6535(03)00266-2

Bai SH, Ogbourne SM (2016) Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. Environ Sci Pollut Res 23:18988–19001. https://doi.org/10.1007/s11356-016-7425-3

Battaglin WA, Meyer MT, Kuivila KM, Dietze JE (2014) Glyphosate and Its Degradation Product AMPA Occur Frequently and Widely in U.S. Soils, Surface Water, Groundwater, and Precipitation. JAWRA J Am Water Resour Assoc 50:275–290. https://doi.org/10.1111/jawr.12159

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

Belden JB, Hanson BR, McMurry ST, et al (2012) Assessment of the Effects of Farming and Conservation Programs on Pesticide Deposition in High Plains Wetlands. Environ Sci Technol 46:3424–3432. https://doi.org/10.1021/es300316q

Benbrook CM (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

Bhatt P, Joshi T, Bhatt K, et al (2021) Binding interaction of glyphosate with glyphosate oxidoreductase and C–P lyase: Molecular docking and molecular dynamics simulation studies. J Hazard Mater 409:124927. https://doi.org/10.1016/j.jhazmat.2020.124927

Blake R, Pallett K (2018) The Environmental Fate and Ecotoxicity of Glyphosate. Outlooks Pest Manag 29:266–269. https://doi.org/10.1564/v29_dec_08

Busse MD, Ratcliff AW, Shestak CJ, Powers RF (2001) Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biol Biochem 33:1777–1789. https://doi.org/10.1016/S0038-0717(01)00103-1

Byer JD, Struger J, Klawunn P, et al (2008) Low Cost Monitoring of Glyphosate in Surface Waters Using the ELISA Method: An Evaluation. Environ Sci Technol 42:6052–6057. https://doi.org/10.1021/es8005207

Cerdeira AL, Duke SO (2006) The Current Status and Environmental Impacts of Glyphosate-Resistant Crops: A Review. J Environ Qual 35:1633–1658. https://doi.org/10.2134/jeq2005.0378

Cobb AH, Reade JPH (2010) Herbicides and Plant Physiology: Cobb/Herbicides and Plant Physiology. Wiley-Blackwell, Oxford, UK

Coupe RH, Kalkhoff SJ, Capel PD, Gregoire C (2012) Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. Pest Manag Sci 68:16–30. https://doi.org/10.1002/ps.2212

Cox C (1995) Glyphosate Fact Sheets: Part 1 and Part 2. J Pestic Reform 15:

Dahl TE (2014) Status and trends of prairie wetlands in the United States 1997 to 2009. U.S. Department of the Interior, Fish and Wildlife Service, Ecological Services, Washington, D.C.

Dalcin Martins P, Hoyt DW, Bansal S, et al (2017) Abundant carbon substrates drive extremely high sulfate reduction rates and methane fluxes in Prairie Pothole Wetlands. Glob Change Biol 23:3107–3120. https://doi.org/10.1111/gcb.13633

de Jonge H, de Jonge LW, Jacobsen OH, et al (2001) Glyphosate sorption in soils of different pH and phosphorus content. Soil Sci 166:230–238. https://doi.org/10.1097/00010694-200104000-00002

Degenhardt D, Humphries D, Cessna AJ, et al (2012) Dissipation of glyphosate and aminomethylphosphonic acid in water and sediment of two Canadian prairie wetlands. J Environ Sci Health Part B 47:631–639. https://doi.org/10.1080/03601234.2012.668459

Dennis PG, Kukulies T, Forstner C, et al (2018) The effects of glyphosate, glufosinate, paraquat and paraquat-diquat on soil microbial activity and bacterial, archaeal and nematode diversity. Sci Rep 8:2119. https://doi.org/10.1038/s41598-018-20589-6

Dion HM, Harsh JB, Hill Jr. HH (2001) Competitive sorption between glyphosate and inorganic phosphate on clay minerals and low organic matter soils. J Radioanal Nucl Chem 249:385–390. https://doi.org/10.1023/A:1013222704311

Druille M, Cabello MN, García Parisi PA, et al (2015) Glyphosate vulnerability explains changes in root-symbionts propagules viability in pampean grasslands. Agric Ecosyst Environ 202:48–55. https://doi.org/10.1016/j.agee.2014.12.017

Firdous S, Iqbal S, Anwar S, Jabeen H (2018) Identification and analysis of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene from glyphosate-resistant *Ochrobactrum intermedium* Sq20: Identification of EPSPS from *Ochrobactrum intermedium* Sq20. Pest Manag Sci 74:1184–1196. https://doi.org/10.1002/ps.4624

Forlani G, Pavan M, Gramek M, et al (2008) Biochemical Bases for a Widespread Tolerance of Cyanobacteria to the Phosphonate Herbicide Glyphosate. Plant Cell Physiol 49:443–456. https://doi.org/10.1093/pcp/pcn021

Gill JPK, Sethi N, Mohan A (2017) Analysis of the glyphosate herbicide in water, soil and food using derivatising agents. Environ Chem Lett 15:85–100. https://doi.org/10.1007/s10311-016-0585-z

Gleason RA, Euliss NH, Tangen BA, et al (2011) USDA conservation program and practice effects on wetland ecosystem services in the Prairie Pothole Region. Ecol Appl 21:S65–S81. https://doi.org/10.1890/09-0216.1

Gomes MP, Tavares DS, Richardi VS, et al (2019) Enrofloxacin and Roundup® interactive effects on the aquatic macrophyte Elodea canadensis physiology. Environ Pollut 249:453–462. https://doi.org/10.1016/j.envpol.2019.03.026

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

Guijarro KH, Aparicio V, De Gerónimo E, et al (2018) Soil microbial communities and glyphosate decay in soils with different herbicide application history. Sci Total Environ 634:974–982. https://doi.org/10.1016/j.scitotenv.2018.03.393

Hassan RM, Scholes RJ, Ash N, Millennium Ecosystem Assessment (Program) (eds) (2005) Ecosystems and human well-being: current state and trends: findings of the Condition and Trends Working Group of the Millennium Ecosystem Assessment. Island Press, Washington, DC

Helander M, Saloniemi I, Saikkonen K (2012) Glyphosate in northern ecosystems. Trends Plant Sci 17:569–574. https://doi.org/10.1016/j.tplants.2012.05.008

Hernández-Alomia F, Ballesteros I, Castillejo P (2022) Bioremediation potential of glyphosate-degrading microorganisms in eutrophicated Ecuadorian water bodies. Saudi J Biol Sci 29:1550–1558. https://doi.org/10.1016/j.sjbs.2021.11.013

Herrmann KM, Weaver LM (1999) THE SHIKIMATE PATHWAY. 32

Hertel R, Gibhardt J, Martienssen M, et al (2021) Molecular mechanisms underlying glyphosate resistance in bacteria. Environ Microbiol 23:2891–2905. https://doi.org/10.1111/1462-2920.15534

Hetrick J, Blankinship A (2015) Preliminary Ecological Risk Assessment in Support of the Registration Review of Glyphosate and Its Salts. Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency

Holloway JM, Goldhaber MB, Mills CT (2011) Carbon and nitrogen biogeochemistry of a Prairie Pothole wetland, Stutsman County, North Dakota, USA. Appl Geochem 26:S44–S47. https://doi.org/10.1016/j.apgeochem.2011.03.025

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

Jacinthe P-A, Vidon P, Tedesco LP (2009) Effects of vegetation and glyphosate on denitrification in constructed wetlands. Indiana University Purdue University Indianapolis, Berlin, Germany

Kjaer J, Olsen P, Ullum M, Grant R (2005) Leaching of Glyphosate and Amino-Methylphosphonic Acid from Danish Agricultural Field Sites. J Environ Qual 34:608–620. https://doi.org/10.2134/jeq2005.0608

Kools SAE, van Roovert M, van Gestel CAM, van Straalen NM (2005) Glyphosate degradation as a soil health indicator for heavy metal polluted soils. Soil Biol Biochem 37:1303–1307. https://doi.org/10.1016/j.soilbio.2004.11.026

Kryuchkova YV, Burygin GL, Gogoleva NE, et al (2014) Isolation and characterization of a glyphosate-degrading rhizosphere strain, Enterobacter cloacae K7. Microbiol Res 169:99–105. https://doi.org/10.1016/j.micres.2013.03.002

Lane M, Lorenz N, Saxena J, et al (2012) The effect of glyphosate on soil microbial activity, microbial community structure, and soil potassium. Pedobiologia 55:335–342. https://doi.org/10.1016/j.pedobi.2012.08.001

Lu T, Xu N, Zhang Q, et al (2020) Understanding the influence of glyphosate on the structure and function of freshwater microbial community in a microcosm. Environ Pollut 260:114012. https://doi.org/10.1016/j.envpol.2020.114012

Malaj E, Liber K, Morrissey CA (2020) Spatial distribution of agricultural pesticide use and predicted wetland exposure in the Canadian Prairie Pothole Region. Sci Total Environ 718:134765. https://doi.org/10.1016/j.scitotenv.2019.134765

McMurdie PJ, Paulson JN (2015) biomformat: An interface package for the BIOM file format

McMurry ST, Belden JB, Smith LM, et al (2016) Land use effects on pesticides in sediments of prairie pothole wetlands in North and South Dakota. Sci Total Environ 565:682–689. https://doi.org/10.1016/j.scitotenv.2016.04.209

Moorman TB (1986) Effects of Herbicides on the Survival of *Rhizobium japonicum* Strains. Weed Sci 34:628–633. https://doi.org/10.1017/S0043174500067564

Muskus AM, Miltner A, Hamer U, Nowak KM (2022) Microbial community composition and glyphosate degraders of two soils under the influence of temperature, total organic carbon and pH. Environ Pollut 297:118790. https://doi.org/10.1016/j.envpol.2022.118790

Muturi EJ, Donthu RK, Fields CJ, et al (2017) Effect of pesticides on microbial communities in container aquatic habitats. Sci Rep 7:44565. https://doi.org/10.1038/srep44565

Nguyen DB, Rose MT, Rose TJ, et al (2016) Impact of glyphosate on soil microbial biomass and respiration: A meta-analysis. Soil Biol Biochem 92:50–57. https://doi.org/10.1016/j.soilbio.2015.09.014

Okada E, Costa JL, Bedmar F (2016) Adsorption and mobility of glyphosate in different soils under no-till and conventional tillage. Geoderma 263:78–85. https://doi.org/10.1016/j.geoderma.2015.09.009

Oksanen J, Simpson G, Blanchet F, et al (2017) Vegan: Community Ecology Package

Padgette SR, Kolacz KH, Delannay X, et al (1995) Development, Identification, and Characterization of a Glyphosate-Tolerant Soybean Line. Crop Sci 35:1451–1461. https://doi.org/10.2135/cropsci1995.0011183X003500050032x

Pérez GL, Torremorell A, Mugni H, et al (2007) Effects of the Herbicide Roundup on Freshwater Microbial Communities: A Mesocosm Study. Ecol Appl 17:2310–2322. https://doi.org/10.1890/07-0499.1

Pesce S, Batisson I, Bardot C, et al (2009) Response of spring and summer riverine microbial communities following glyphosate exposure. Ecotoxicol Environ Saf 72:1905–1912. https://doi.org/10.1016/j.ecoenv.2009.07.004

Powell HA, Kerbby NW, Rowell P (1991) Natural tolerance of cyanobacteria to the herbicide glyphosate. New Phytol 119:421–426. https://doi.org/10.1111/j.1469-8137.1991.tb00042.x

Priestman MA, Funke T, Singh IM, et al (2005) 5-Enolpyruvylshikimate-3-phosphate synthase from *Staphylococcus aureus* is insensitive to glyphosate. FEBS Lett 579:728–732. https://doi.org/10.1016/j.febslet.2004.12.057

Pruesse E, Quast C, Knittel K, et al (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:7188–7196. https://doi.org/10.1093/nar/gkm864

Quast C, Pruesse E, Yilmaz P, et al (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219

R Core Team (2022) R: A language and environment for statistical computing

Reddy KN, Rimando AM, Duke SO, Nandula VK (2008) Aminomethylphosphonic Acid Accumulation in Plant Species Treated with Glyphosate. J Agric Food Chem 56:2125–2130. https://doi.org/10.1021/jf072954f

Scribner E, Battaglin W, Gilliom R, Meyer M (2007) Concentrations of glyphosate, its degradation product, aminomethylphosphonic acid, and glufosinate in ground- and surface-water, rainfall, and soil samples collected in the United States, 2001-06

Shushkova TV, Vasilieva GK, Ermakova IT, Leontievsky AA (2009) Sorption and microbial degradation of glyphosate in soil suspensions. Appl Biochem Microbiol 45:599–603. https://doi.org/10.1134/S0003683809060040

Sura S, Waiser M, Tumber V, et al (2012) Effects of Glyphosate and Two Herbicide Mixtures on Microbial Communities in Prairie Wetland Ecosystems: A Mesocosm Approach. J Environ Qual 41:732–743. https://doi.org/10.2134/jeq2011.0376

Tang FHM, Jeffries TC, Vervoort RW, et al (2019) Microcosm experiments and kinetic modeling of glyphosate biodegradation in soils and sediments. Sci Total Environ 658:105–115. https://doi.org/10.1016/j.scitotenv.2018.12.179

Vera MS, Di Fiori E, Lagomarsino L, et al (2012) Direct and indirect effects of the glyphosate formulation Glifosato Atanor® on freshwater microbial communities. Ecotoxicology 21:1805–1816. https://doi.org/10.1007/s10646-012-0915-2

Vera MS, Lagomarsino L, Sylvester M, et al (2010) New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. Ecotoxicology 19:710–721. https://doi.org/10.1007/s10646-009-0446-7

Warren N, Allan IJ, Carter JE, et al (2003) Pesticides and other micro-organic contaminants in freshwater sedimentary environments—a review. Appl Geochem 18:159–194. https://doi.org/10.1016/S0883-2927(02)00159-2

Widenfalk A, Bertilsson S, Sundh I, Goedkoop W (2008) Effects of pesticides on community composition and activity of sediment microbes – responses at various levels of microbial community organization. Environ Pollut 152:576–584. https://doi.org/10.1016/j.envpol.2007.07.003

Winter TC (ed) (2003) Hydrological, chemical, and biological characteristics of a prairie pothole wetland complex under highly variable climate conditions: the Cottonwood Lake area, east-central North Dakota. U.S. Dept. of the Interior, U.S. Geological Survey : U.S. Geological Survey, Information Services [distributor], Denver, CO

Zabaloy MC, Gómez E, Garland JL, Gómez MA (2012) Assessment of microbial community function and structure in soil microcosms exposed to glyphosate. Appl Soil Ecol 61:333–339. https://doi.org/10.1016/j.apsoil.2011.12.004

Zeng T, Arnold WA (2013) Pesticide Photolysis in Prairie Potholes: Probing Photosensitized Processes. Environ Sci Technol 47:6735–6745. https://doi.org/10.1021/es3030808

Zhan H, Feng Y, Fan X, Chen S (2018) Recent advances in glyphosate biodegradation. Appl Microbiol Biotechnol 102:5033–5043. https://doi.org/10.1007/s00253-018-9035-0

Zhao H, Tao K, Zhu J, et al (2015) Bioremediation potential of glyphosate-degrading *Pseudomonas* spp. strains isolated from contaminated soil. J Gen Appl Microbiol 61:165–170. https://doi.org/10.2323/jgam.61.165

Statements and Declarations

Funding

This work was supported by a STEM Research and Education Seed Grant from North Dakota Established Program to Stimulate Competitive Research (FAR0032100).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

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.

Table 1 Summary of Kruskal-Wallis Rank Sum test on the effects of each treatment on Shannon diversity and observed OTU. χ^2 = Chi-square value, df = degrees of freedom, and *P* = the p-value where the significance level is 0.05

Quantified Metric	**Variable**	χ^2	**df**	***P***
Shannon Diversity	Day of Sampling	1.81	2	0.40
	Herbicide Treatment	4.67	3	0.20
	Treatment Level	3.48	3	0.32
Observed OTU	Day of Sampling	2.25	3	0.52
	Herbicide Treatment	0.22	2	0.90
	Treatment Level	2.45	3	0.48

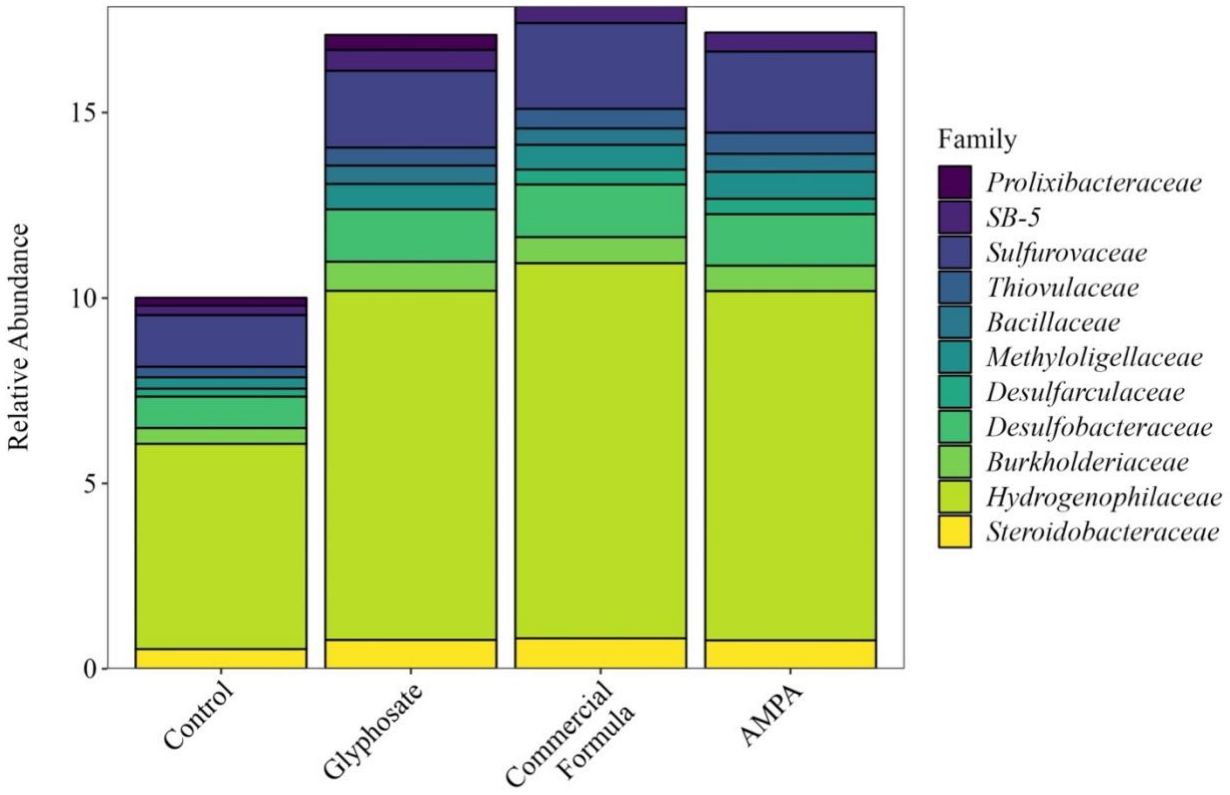

Fig. 1 Relative abundance of the ten most abundant microbial families within each herbicide treatment over the entirety of the experiment

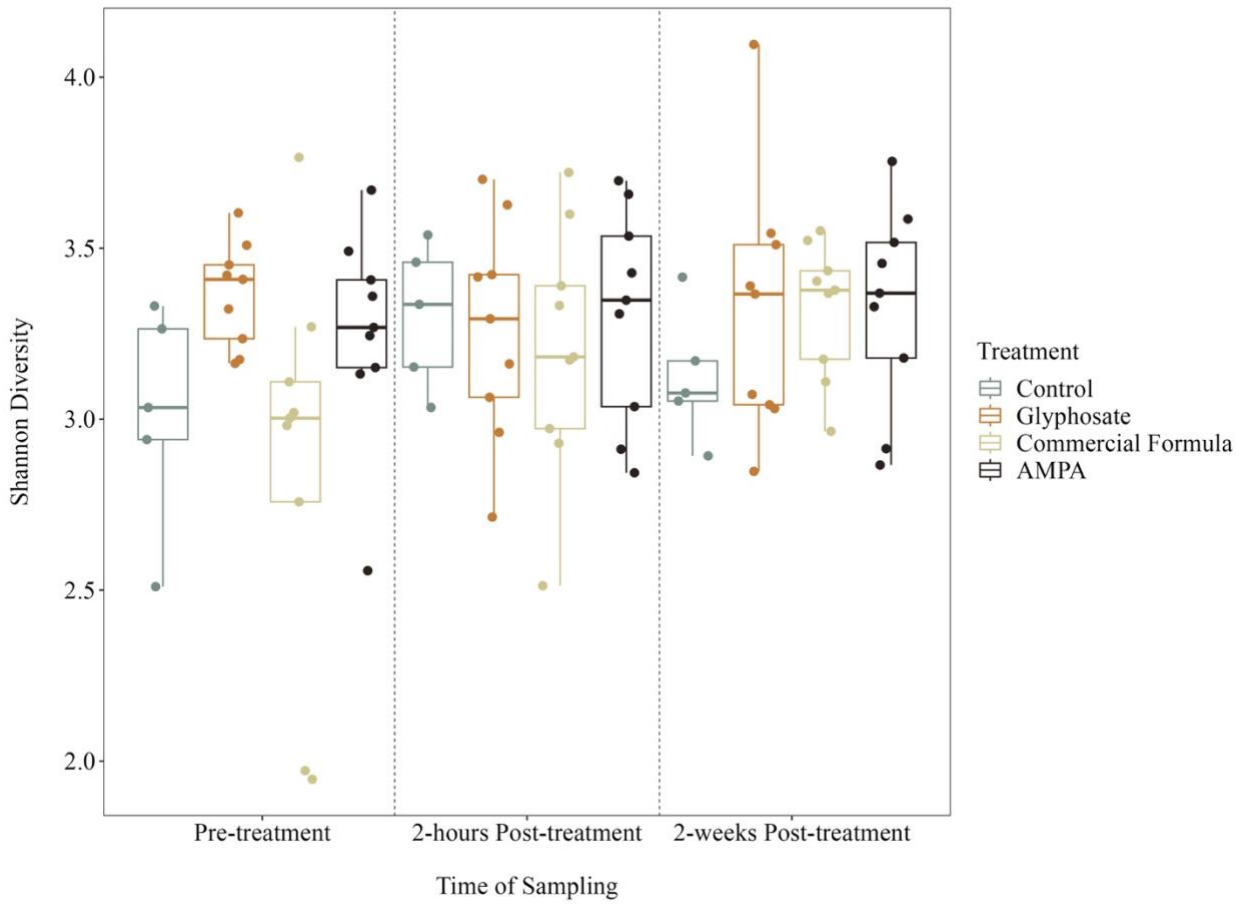

Fig. 2 Alpha diversity, represented by the Shannon H index, in microcosm sediments of each herbicide treatment at each sampling timepoint

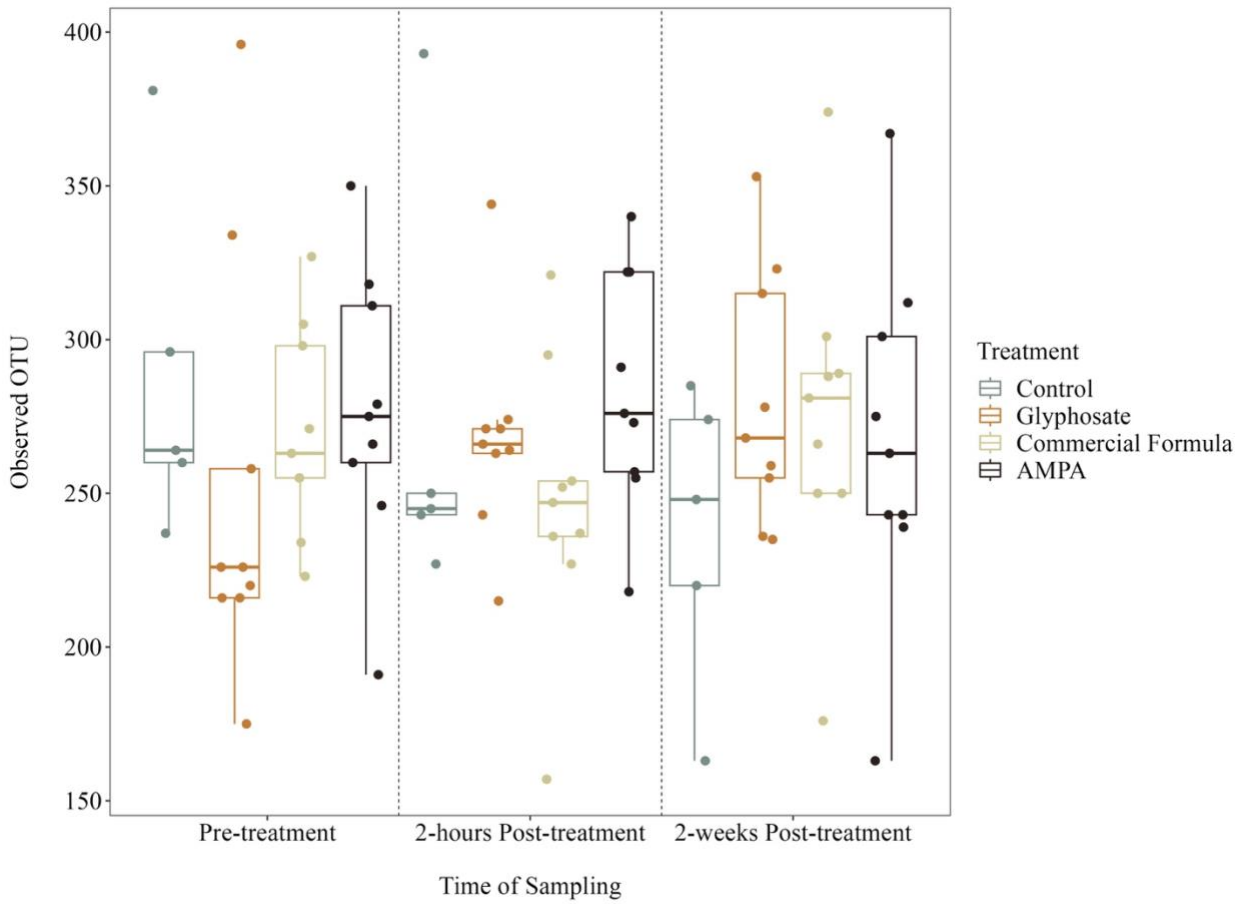

Fig. 3 Observed OTU in microcosm sediments of each herbicide treatment at each sampling timepoint

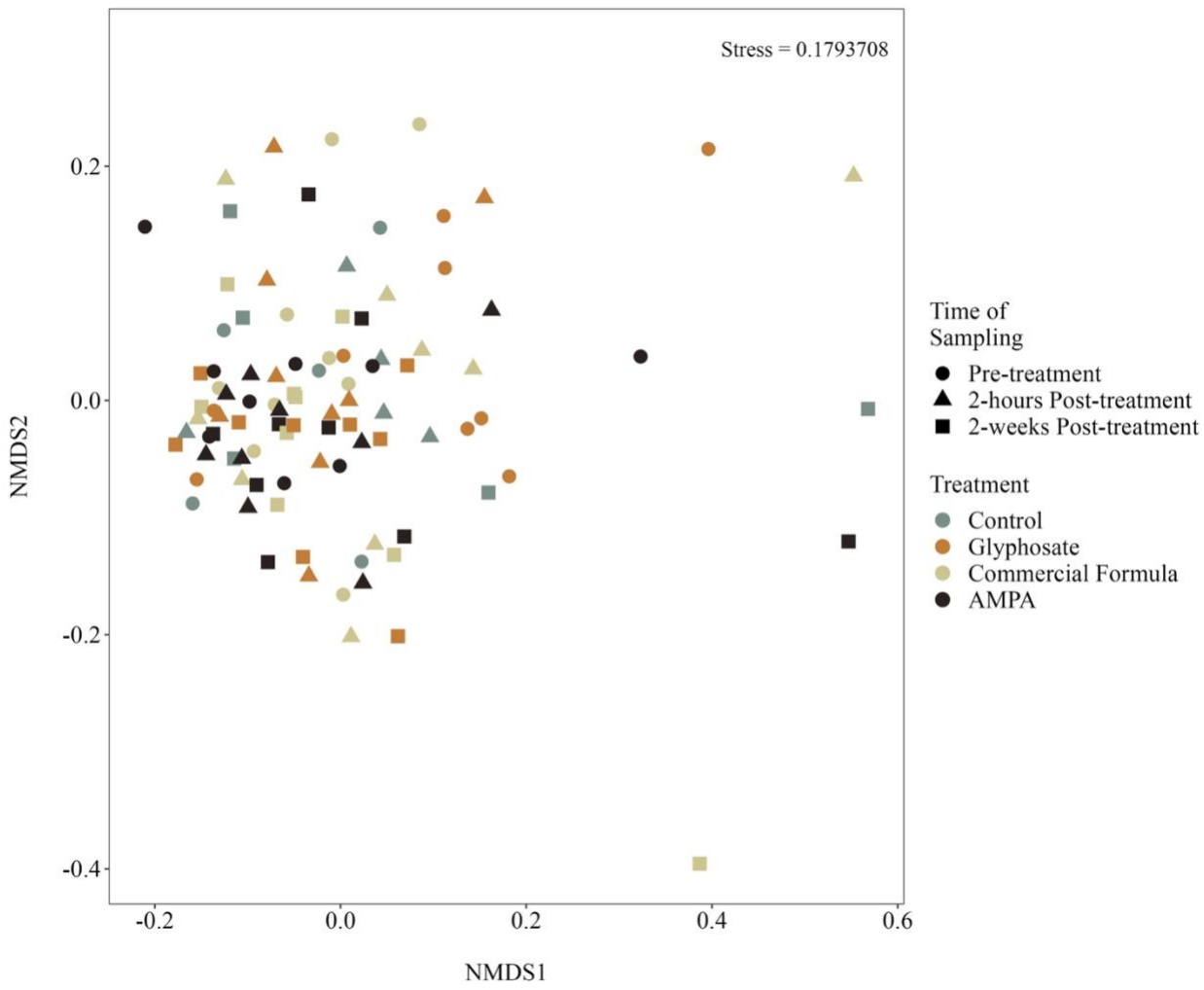

Fig. 4 NMDS plot of the microbial community structure across all sediment samples (*n* = 96) where shapes represent time of sampling and colors represent herbicide treatment