A Perspective on How Glyphosate and 2,4-D May Impact Climate Change

Christine M Cornish[1*] and Jon N Sweetman[2]

[1]North Dakota State University, Biological Sciences Department, Environmental and Conservation Sciences Program, Fargo, ND, USA

[2]The Pennsylvania State University, Department of Ecosystem Science and Management, University Park, PA, USA

*** Correspondence:**
Christine M Cornish
christine.cornish@ndsu.edu

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Abstract

An increase in herbicide use is occurring due to a growing population and herbicide-resistant crops in agriculture, which has resulted in more herbicide tolerant target species. Glyphosate and 2,4-Dichlorophenoxyacetic acid (2,4-D) are two of the most commonly used herbicides worldwide and are more recently being used in combination in pre-mixed commercial formulas. Subsequently, herbicide contamination of wetlands will increase subjecting microorganisms to multiple chemical stressors. Methane is a potent greenhouse gas naturally emitted from wetlands, but herbicides may disrupt biogeochemical processes leading to an unbalanced methane cycle. This perspective examined the potential effects of herbicides on climate change using glyphosate and 2,4-D as a case study. We highlighted previous research on glyphosate-derived nutrient enrichment and 2,4-D inhibition of methane oxidation. We also explained how the concurrent effects of these herbicides could alter microbial communities leading to increased methane production in wetlands. Our perspective elucidates the potential ecosystem-level implications of herbicides in wetlands, in addition to stating the importance for research on the combined effects of herbicides.

1 Introduction

Climate change is an ongoing global concern as greenhouse gas (GHG) emissions continue to increase (IPCC, 2021). Methane (CH_4) is the second most abundant GHG, after carbon dioxide (CO_2), but is about 25 times more potent (Islam et al., 2018; EPA, 2023). Wetlands are a large natural source of CH_4 as they play a significant role in carbon (C) sequestration and cycling (Andresen et al., 2017). It has recently been suggested that GHG emissions from freshwater ecosystems may be impacted by agrochemical use (Stehle and Schulz, 2015), which has substantially increased over the past 30 years due to the introduction of herbicide-resistant crops. Herbicide-resistant crops now comprise more than 90% of crops in the United States (USDA, 2022) and are increasingly used worldwide (Peterson et al., 2018). This has unexpectedly led to an increase in herbicide use (Bai and Ogbourne, 2016; Coupe and Capel, 2016), and subsequently weeds are chronically exposed to herbicide residues resulting in the development of herbicide tolerance (Heap and Duke, 2018). For example, the introduction of Roundup Ready® (i.e., glyphosate-resistant) crops resulted in a 15-fold increase in glyphosate use (Coupe and Capel, 2016; Hébert et al., 2019), and consequently, approximately 41 weed species worldwide have developed glyphosate tolerance (Jeschke and Teten, 2018). Ongoing climatic change is projected to result in intensified herbicide use (Delcour et al., 2015), creating a positive feedback loop if increasing herbicide use results in further CH_4 emissions. Temperature increases can also enhance toxicity and alter biodegradation processes of herbicides (Noyes et al., 2009; Koleva and Schneider, 2010; Matzrafi, 2019). Subsequently, as wetlands are subjected to a combination of more severe stressors, wetland biota, specifically microorganisms may be detrimentally affected. Pre-mixed herbicides containing multiple active ingredients (i.e., multiple modes of action), such as glyphosate and 2,4-Dichlorophenoxyacetic acid (2,4-D), are also now more commonly used to combat target species that have developed tolerance to single active ingredient herbicides (Freydier and Lundgren, 2016; Schütte et al., 2017). In this article we will present a case study on how glyphosate and 2,4-D accumulation in wetlands could impact climate change by focusing on their potential synergistic effects on microbial communities. Specifically, understanding their impacts on CH_4 dynamics is critical to understanding the role of wetlands in contributing to climate change.

2 Case Study

Glyphosate and 2,4-D are two of the most commonly used herbicides and are also used in pre-mixed formulas such as Enlist Duo® (1:0.95 glyphosate:2,4-D) and Landmaster™ II (1:0.83 glyphosate:2,4-D) (Benbrook, 2016; Zuanazzi et al., 2020; EPA, 2022). Extensive use of glyphosate and 2,4-D are cause for environmental concern within aquatic ecosystems where herbicides are substantial contributors to wetland pollution (Casado et al., 2019). As wetlands are often located in the lowest drainage points of agricultural fields, they can serve as critical sinks for herbicide residues transported through spray drift, runoff, groundwater leaching, and wind and sediment erosion (Annett et al., 2014; Bento et al., 2017). Subsequently, this can result in bioavailable residues, where microorganisms are subjected to a “pesticide cocktail” (Aparicio et al., 2013; Islam et al., 2018). Microorganisms, such as prokaryotes and algae, are important contributors to wetland ecosystem functioning, including mediating biogeochemical cycling (Sun et al., 2013; Baker et al., 2020). The C biogeochemical processes of methanogens (i.e., CH_4 producers) and methanotrophs (i.e., CH_4 consumers), in addition to plant and algal-mediated transport, play a critical role in the global CH_4

budget of wetlands. Agrochemical contributions to increased GHG emissions (Stehle and Schulz, 2015) may be explained by an unbalanced CH_4 cycle via impacts to CH_4-associated microorganisms. The range of effects of glyphosate and 2,4-D on wetland microorganisms can vary including direct toxicity, nutrient enrichment, and alterations to metabolic and catabolic processes.

2.1 Glyphosate

Glyphosate's potential impacts on wetland microorganisms can be detrimental or advantageous to microbial species. While glyphosate's mode of action was developed to target the shikimate pathway in higher plants (Hetrick and Blankinship, 2015), many archaea and bacteria also utilize this pathway resulting in non-target effects (Herrmann and Weaver, 1999). Despite the potential negative impacts on microorganisms, in many instances increased growth, respiration, and enhanced metabolism in wetland microbial communities have been observed as a result of glyphosate biodegradation (Vera et al., 2012; Lu et al., 2020) and linked with the use of glyphosate as a nutritive source (Saxton et al., 2011; Wang et al., 2016). Due to glyphosate's chemical structure, its degradation often contributes substantial amounts of phosphorus (P), which has been found to be favored and more rapidly utilized by microorganisms compared to other sources of soil P (Hébert et al., 2019; Sun et al., 2019). Specifically, stimulated cyanobacterial growth and cyanotoxin production has been recorded from glyphosate-derived P enrichment (Vera et al., 2010; Qiu et al., 2013; Zhang et al., 2016; Hernández-García and Martínez-Jerónimo, 2020; Wang et al., 2021; Lin et al., 2023). Glyphosate degradation was found to be positively correlated with total P concentrations in surface waters (Carles et al., 2019). Glyphosate additions to aquatic ecosystems can contribute to water quality issues, such as eutrophication, which has been demonstrated to be an important driver of CH_4 emissions (Sepulveda-Jauregui et al., 2018; Beaulieu et al., 2019; Yang et al., 2019; Bertolet et al., 2020). In addition, glyphosate-derived P loading into wetlands could stimulate algal biomass leading to eutrophic conditions, which could then increase CH_4 production (Carles et al., 2019). Ultimately, increased glyphosate use could shift microbial community dynamics towards copiotrophs and algae, altering important C biogeochemical processes and resulting in an indirect increase in CH_4 production in wetlands (Figure 1, panels A and B).

2.2 2,4-D

Despite 2,4-D being the first synthetic herbicide, compared to glyphosate, relatively little research has been conducted on its effects on aquatic microorganisms (Donald et al., 2018; Malaj et al., 2020). However, similar to glyphosate, 2,4-D can have a variety of impacts on wetland microbial communities. It targets broadleaf plants through mimicking the plant growth hormone, indol-3-yl-acetic acid (IAA or auxin), resulting in plant overgrowth (Cobb and Reade, 2010), but auxin synthesis and usage in microorganisms is also well known making them vulnerable non-target organisms (Spaepen and Vanderleyden, 2011). In addition, 2,4-D is a widespread environmental contaminant frequently detected in aquatic ecosystems (Malaj et al., 2020), and its use has increased in recent decades with the development of herbicide-resistant crops, where its use will likely continue to increase in the future (Freydier and Lundgren, 2016). Consequently, wetland microorganisms could be highly susceptible to its toxic effects with limited capacity to degrade it. Previous research has found some species use 2,4-D as a C source, whereas other species are toxicologically inhibited (Benndorf et al., 2004; Zabaloy et al., 2008; Sachu et al., 2022). Research in microcosms has also found that increased 2,4-D concentrations resulted in inhibition of CH_4 oxidation, decreases in CH_4 removal time, and increased CH_4 emissions (Syamsul Arif et al., 1996; Kumaraswamy et al., 1997; Top et al., 1999). Where studies from Top et al. (1999) and Seghers et al. (2003) suggested decreases in CH_4 removal could be due to 2,4-D inhibition of methanotroph-mediated oxidative metabolism. Research on the effects of 2,4-D on CH_4 oxidation is extremely limited, however these studies do indicate that 2,4-D loading into wetlands could potentially alter the CH_4 cycle by suppressing the removal of CH_4 via the food web, resulting in greater concentrations within the water column and higher emissions (Figure 1, panels C and D).

2.3 Pesticide cocktails: Glyphosate plus 2,4-D

The increased use of pre-mixed glyphosate and 2,4-D herbicides further exposes wetland microorganisms to combinations of chemical stressors, which could lead to unforeseen long-term effects. Research on the combined effects of pesticides has been conducted since the 1970’s, but the majority of the focus has been on the direct toxicological impacts to aquatic flora and fauna (Lichtenstein et al., 1973; Faust et al., 1994; Gardner and Grue, 1996; Hayes et al., 2006; Relyea, 2009; Moreira et al., 2020). These studies included compounds such as atrazine, chlorpyrifos, fipronil, etc., whereas research on the combined effects of glyphosate and 2,4-D is limited, especially at the aquatic microbial level. Additive and/or synergistic effects of glyphosate and 2,4-D have been found on fish and amphibian growth, fertilization, survival, and behavior (Carvalho et al., 2020; Pavan et al., 2021; Bernardi et al., 2022; Peluso et al., 2022), and zooplankton emergence (Portinho et al., 2018). Lozano et al. (Lozano et al., 2018) found additive impacts of glyphosate and 2,4-D on phytoplankton composition, abundance, and chlorophyll *a* after 7 days in microcosms, but also found an antagonist effect on total and live abundance of *Staurastrum* spp. In outdoor mesocosms Lozano et al. (Lozano et al., 2018) found a decrease in phytoplankton respiration and gross primary production from a high glyphosate (applied as Roundup Max®), low 2,4-D (applied as AsiMax 50®) treatment after 4 hours. Additionally, after 7 days in mesocosms with high glyphosate, an increase in primary production, chlorophyll *a*, and micro- and nanophytoplankton was observed (Lozano et al., 2020). Sura et al. (Sura et al., 2015) researched the effects of a herbicide mixture including glyphosate, 2,4-D, and MCPA, clopyralid, dicamba, dichlorprop, mecoprop, and bromoxynil on pelagic and benthic communities in nutrient-sufficient and nutrient-deficient wetlands. They found pelagic bacterial productivity significantly increased after treatment in the nutrient-sufficient wetland, but benthic bacterial productivity did not change, which suggests the stimulatory effect of these herbicides may be related to nutrient bioavailability. These results demonstrate the complexity of the direct effects of herbicide mixtures on aquatic microorganisms, but the potential indirect effects are still poorly understood. As pre-mixed glyphosate and 2,4-D herbicides become more common it is important to consider the extent of their effects on aquatic ecosystems. Glyphosate can easily be used as a nutrient source stimulating microbial activity, specifically algal communities, whereas 2,4-D may inhibit methanotrophic communities from oxidizing CH_4. As these compounds enter aquatic ecosystems their impacts on microorganisms may become synergistic and/or additive resulting in eutrophication and inhibition of methanotrophs from glyphosate and 2,4-D, respectively. Subsequently, eutrophic conditions and decreased CH_4 removal could cause increased CH_4 production via an unbalanced CH_4 cycle (Figure 3).

3 Pollution-Induced Community Tolerance (PICT)

Aquatic ecosystems are subjected to year-round herbicide contamination, where herbicide use differs across crop, season, habitat, and region. Microorganism structure and function can be impacted by herbicides, but toxicity is often dependent on the mode of action, concentration, and duration of exposure, as well as microbial species and environmental factors (DeLorenzo et al., 2001). For example, glyphosate stimulated *Chlorella vulgaris* growth 24-hours after exposure, but then inhibited growth after 48-hours at the same concentrations (i.e., hormesis) (Reno et al., 2014). In addition to the duration of exposure, the exposure to a different mode of action could also impact microorganisms by causing a community shift often appearing as changes in gene expression or diversity (Feld et al., 2015). Pollution-Induced Community Tolerance (PICT) refers to the response of a community to a pollutant, which results in an increased tolerance to that pollutant (Blanck, 2002). The use of PICT analysis is extensive in the toxicology literature, especially on phototrophic microorganisms, which are often more susceptible to herbicidal effects due to their similarities with target species (DeLorenzo et al., 2001; Larras et al., 2016). Bérard and Benninghoff (Bérard and Benninghoff, 2001) found phytoplankton were significantly more sensitive to atrazine after one day, but then significantly less sensitive after at least 11 days. Phototrophic biofilms were found to be increasingly more sensitive to diuron as contamination levels decreased 1-3 years after its ban in the European Union (Pesce et al., 2016). It has also been shown that selection pressure from multiple stressors can lead to more opportunistic species and higher tolerances (Rotter et al., 2013). Ultimately, PICT results suggest that more sensitive species are being replaced by less sensitive species creating a more tolerant community (Blanck, 2002). This has also been seen with both glyphosate and 2,4-D. Microbial communities from sediments with high glyphosate exposure were able to degrade glyphosate faster and had higher diversity compared to sediments with low to no previous exposure (Tang et al., 2019). Zabaloy et al. (Zabaloy et al., 2008) saw an increase in a 2,4-D degrading population in soils for approximately one month after treatment and found that agricultural soils had higher 2,4-D tolerance compared to reference soils via PICT analysis. In a study by de Lipthay et al. (de Lipthay et al., 2002) 2,4-D treatment induced transcription of the gene responsible for 2,4-D degradation (*tfdA*) which demonstrates a survival response from the microbial community. These studies demonstrate PICT can occur when communities are exposed to a herbicide, therefore contamination by an additional herbicide could further alter communities that have not been exposed before. It could be presumed that wetland microbial communities within a glyphosate-dominant region may substantially change when 2,4-D is introduced in combination with glyphosate and species are replaced. This potential shift would impact the biogeochemical functions of the community, subsequently altering herbicide degradation or metabolism.

4 Conclusions

Glyphosate and 2,4-D are frequently cited as having minimal to no environmental impacts (Peterson et al., 2016; Duke, 2020; Singh et al., 2020), however there is increasing evidence that their indirect effects may be of more substantial global concern. Wetlands naturally emit CH_4 via diffusion, ebullition (i.e., bubbles), and plant-mediated transport, and are the highest natural sources of CH_4 in the environment (Aben et al., 2017; Andresen et al., 2017), but emissions may be increasing due to agrochemical use adversely impacting CH_4 sink potential (Seghers et al., 2005). Glyphosate could stimulate microbial processes resulting in increased CH_4 production, in addition to 2,4-D inhibiting CH_4 oxidation further resulting in increased CH_4 production. Ultimately, this would lead to higher CH_4 production versus removal from freshwater creating elevated CH_4 in the atmosphere. Due to the widespread and extensive use of glyphosate and 2,4-D, these contaminants are frequently found in wetlands (Islam et al., 2018; Malaj et al., 2020). To our knowledge there has been no research investigating the combined impacts of glyphosate and 2,4-D on wetland microbial communities. The potential bottom-up effects of glyphosate and 2,4-D could be detrimental to a changing climate, thus improving our understanding of how these herbicides can impact GHG emissions is crucial.

4.1 Future Research

To investigate the effects of glyphosate and 2,4-D on CH_4 emissions from freshwater ecosystems, micro- or mesocosm experiments could be conducted. Experiments under controlled conditions could help determine how wetland microbial communities are affected by glyphosate and 2,4-D. Specifically, this research would give insight into the CH_4-related mechanisms that may be enhanced or disrupted in microorganisms. In addition to in-lab research, pesticide loading data could be incorporated into GHG models. These data are currently not included in estimations of CH_4 emissions from wetlands, but could be an important source of variation, and could be useful for future climate modeling. These potential impacts are crucial to research as herbicide use is only expected to increase over time, where chemical selection pressure on microbial communities could contribute to climate change.

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Conflict of Interest

This manuscript was written in the absence of commercial or financial conflicts of interest.

6 Author Contributions

Christine Cornish generated the first draft of the manuscript with revisions and comments from Jon Sweetman. Both authors have read and approved the submitted version.

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9 Data Availability Statement

Not applicable.

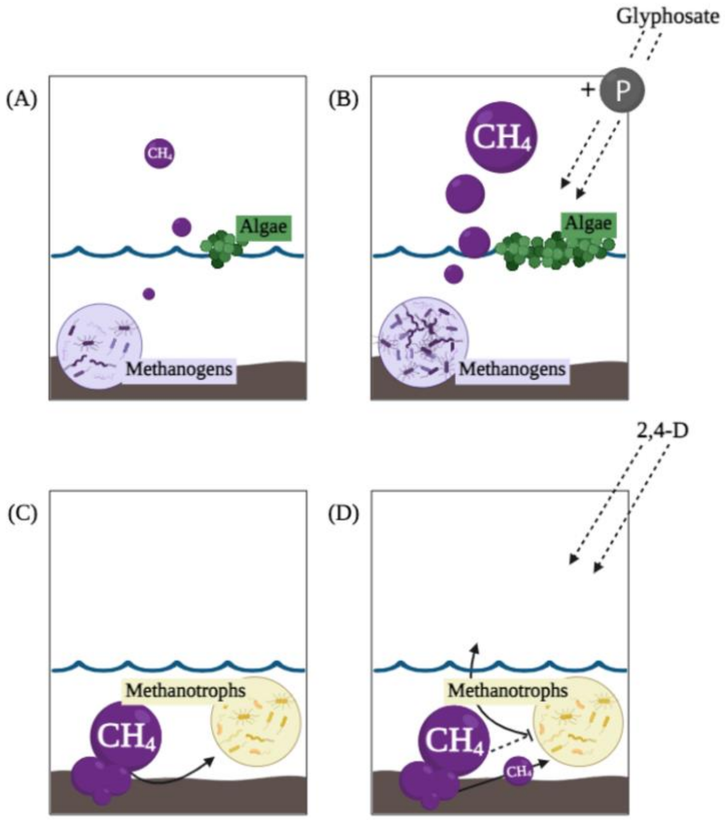

Figure 1. Conceptual diagrams of a wetland with methanogens, methanotrophs, and algae. (**A**) represents balanced CH_4 production; (**B**) represents glyphosate contamination stimulating methanogens and algae causing higher CH_4 emissions; (**C**) represents balanced CH_4 oxidation; (**D**) represents 2,4-D contamination inhibiting oxidation (i.e., removal) of CH_4 by methanotrophs causing higher CH_4 emissions.

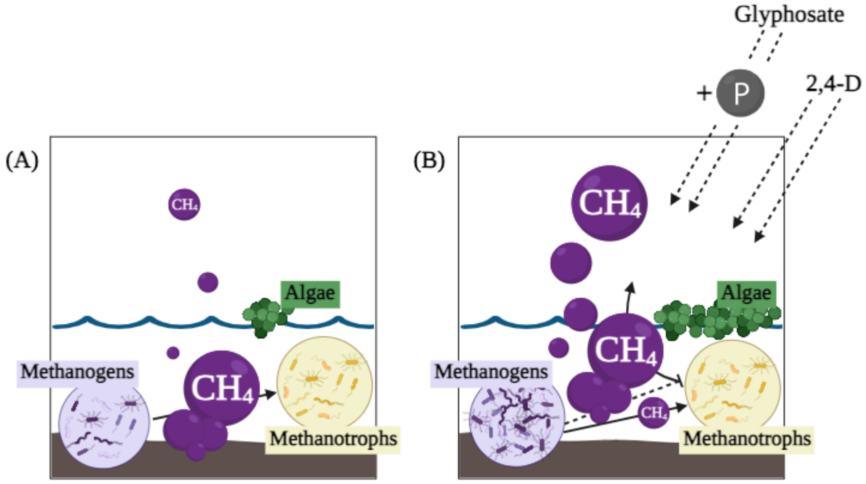

Figure 2. Conceptual diagrams of a wetland with methanogens, methanotrophs, and algae. Panel (**A**) represents a balanced CH_4 cycle, where algae and methanogens produce CH_4, methanotrophs oxidize CH_4; panel (**B**) represents an unbalanced CH_4 cycle where glyphosate and 2,4-D contamination

stimulate methanogens and algae and inhibit CH_4 oxidation, respectively, and thus result in higher CH_4 emissions.