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A perspective on how glyphosate and 2,4-D in wetlands may impact climate change

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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 exposure of 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. We review the impacts of these herbicides on aquatic microbial communities from glyphosate-derived nutrient enrichment and 2,4-D inhibition of methane oxidation, and examine how these altered metabolic processes may lead to increased methane production in wetlands. The response of wetland ecosystems to herbicide contamination will vary across regions, in part due to the complexity of microbial communities, however, this perspective gives a glimpse into the potential global implications of continuing herbicide use on wetlands and demonstrates the importance for research on ecosystem-level co-stressors.

KEYWORDS

glyphosate, 2,4-D, wetlands, methane, climate change

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), and it has recently been suggested that agrochemicals may impact GHG emissions from freshwater ecosystems (Stehle and Schulz, 2015). In particular, herbicide use has substantially increased over the past 30 years due to the introduction and rapid adoption of herbicide-resistant crops worldwide (Bai and Ogbourne, 2016; Coupe and Capel, 2016; Peterson et al., 2018). As a result, target plants have developed herbicide-resistance and the use of pre-mixed formulas that contain multiple active ingredients (i.e., multiple modes of action) has become more common (Freydier and Lundgren, 2016; Schütte et al., 2017). Herbicide use is also projected to increase due to ongoing climatic change (Delcour et al., 2015), where higher temperatures can enhance toxicity and alter biodegradation processes (Noyes et al., 2009; Koleva and Schneider, 2010; Matzrafi, 2019). Subsequently, wetland biota are subjected to combinations of more severe physical and chemical stressors.

Glyphosate and 2,4-D are two of the most commonly used herbicides globally, 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). Their extensive use is cause for environmental concern within aquatic ecosystems as herbicides are already substantial contributors to wetland pollution (Casado et al., 2019). Wetlands are often located in the lowest drainage points of agriculture fields, where they can serve as critical sinks for herbicides transported through spray drift, runoff, groundwater leaching, and wind and sediment erosion (Annett et al., 2014; Bento et al., 2017). However, their fate is dependent on many landscape- and ecosystem-level components such as, precipitation patterns, herbicide application dates, surrounding land use, and plant and animal composition. For example, glyphosate and 2,4-D are highly water soluble, thus a rainfall shortly after application would rapidly transport substantial amounts of these herbicides into nearby wetlands (Bertuzzo et al., 2013). These agrochemicals often persist in sediments of temperate and northern climates (Helander et al., 2012; Mierzejewska and Urbaniak, 2022), can bioaccumulate in organisms such as biofilms (Beecraft and Rooney, 2021), and can be transported between habitats via emerging insects (Roodt et al., 2023). Herbicides are frequently detected within aquatic ecosystems around the world, where they have even been found in protected conservation areas (Wolfram et al., 2023).

Consequently, “pesticide cocktails” can effect microorganisms that are important contributors to wetland biogeochemical cycling and overall ecosystem function (Aparicio et al., 2013; Sun et al., 2013; Islam et al., 2018; Baker et al., 2020). Microorganisms are sensitive to disturbances (Sun et al., 2013), thus as both herbicide use and climate change intensifies it is critical to assess the potential effects of herbicides on GHG emissions. 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, which may be impacted by glyphosate and 2,4-D. In this article we highlight previous research on glyphosate-derived nutrient enrichment and 2,4-D inhibited CH_4 oxidation to suggest that herbicides entering wetlands could alter CH_4 production via synergistic effects on microbial communities and consequently impact climate change.

1.1 Glyphosate

Glyphosate’s impacts on wetland microorganisms are often dose- and species-dependent (Bai and Ogbourne, 2016), therefore it can be detrimental or advantageous to different 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). 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 (Figures 1A, B).

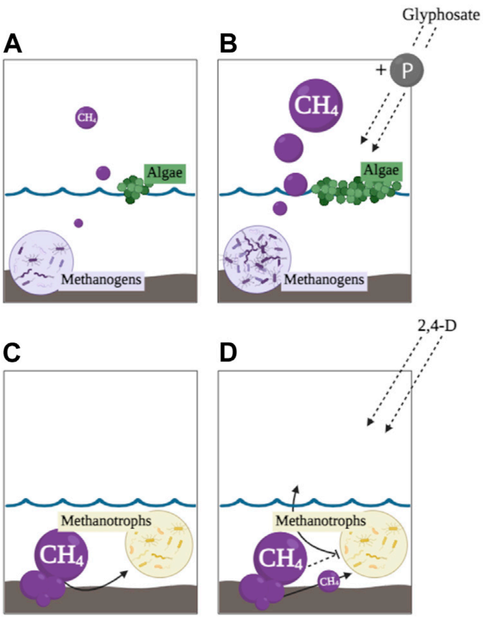

FIGURE 1
Conceptual diagrams of a wetland with methanogens, methanotrophs, and algae. **(A)** represents balanced CH_4 production; **(B)** represents glyphosate contamination stimulating methanogens and algae causing higher CH_4 emissions; **(C)** represents balanced CH_4 oxidation; **(D)** represents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

1.2 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 can be applied as an aquatic herbicide resulting in species being exposed to higher concentrations compared to terrestrial transport (Mierzejewska and Urbaniak, 2022). While 2,4-D is already a widespread environmental contaminant frequently detected in aquatic ecosystems (Malaj et al., 2020) and its use has also increased in recent decades with the development of herbicide-resistant crops, 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. (2005) 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 (Figures 1C, D).

1.3 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. (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. (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 h. 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. (2015) researched the effects of a herbicide mixture including glyphosate, 2,4-D, 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 2).

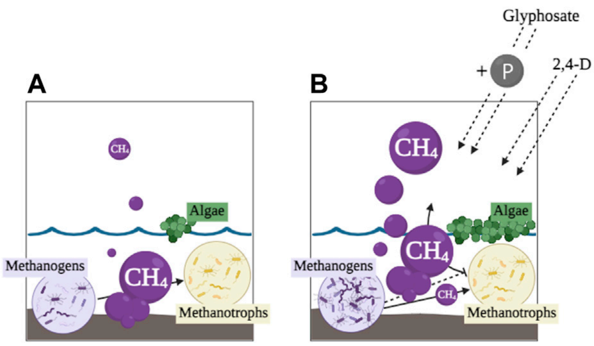

FIGURE 2
Conceptual diagrams of a wetland with methanogens, methanotrophs, and algae. **(A)** represents a balanced CH_4 cycle, where algae and methanogens produce CH_4, methanotrophs oxidize CH_4; **(B)** represents an unbalanced CH_4 cycle where glyphosate and 2,4-D contamination stimulate methanogens and algae and inhibit CH_4 oxidation, respectively, and thus result in higher CH_4 emissions.

2 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-h after exposure, but then inhibited growth after 48-h 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 (2001) found phytoplankton were significantly more sensitive to atrazine after 1 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. (2008) saw an increase in a 2,4-D degrading population in soils for approximately 1 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. (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.

3 Conclusion

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 herbicides 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.

3.1 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.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

CC: Writing–original draft. JS: Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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