



## Effects of a herbicide mixture on primary and bacterial productivity in four prairie wetlands with varying salinities: An enclosure approach



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### HIGHLIGHTS

- Nutrients and salinity played an important role in the herbicide mixture toxicity.
- Primary productivity was stimulated in the nutrient-sufficient freshwater wetland.
- No stimulatory effect was observed in the nutrient-deficient saline wetlands.
- Biofilm communities were not affected by the herbicide mixture in all wetlands.
- Carbon utilization spectra indicated changes in the biofilm bacterial composition.

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### ABSTRACT

Wetlands in the Prairie pothole region of Saskatchewan and Manitoba serve an important role in providing wildlife habitat, water storage and water filtration. They display a wide range of water quality parameters such as salinity, nutrients and major ions with sulfate as the dominant ion for the most saline wetlands. The differences in these water quality parameters among wetlands are reflected in the composition of aquatic plant communities and their productivity. Interspersed within an intensely managed agricultural landscape where pesticides are commonly used, mixtures of herbicides are often detected in these wetlands as well as in rivers, and drinking water reservoirs. One freshwater and three wetlands of varying salinity in the St. Denis National Wildlife Area, Saskatchewan, Canada were selected to study the effects of a mixture of eight herbicides (2,4-D, MCPA, dicamba, clopyralid, bromoxynil, mecoprop, dichlorprop, and glyphosate) on wetland microbial communities using an outdoor enclosure approach. Six enclosures (three controls and three treatments) were installed in each wetland and the herbicide mixture added to the treatment enclosures. The concentration of each herbicide in the enclosure water was that which would have resulted from a direct overspray of a 0.5-m deep wetland at its recommended field application rate. After herbicide addition, primary and bacterial productivity, and algal biomass were measured in both planktonic and benthic communities over 28 days. The herbicide mixture had a stimulatory effect on primary productivity in the nutrient-sufficient freshwater wetland while no stimulatory effect was observed in the nutrient-deficient saline wetlands. The differences observed in the effects of the herbicide mixture appear to be related to the nutrient bioavailability in these wetlands.

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**Abbreviations:** PPR, Prairie pothole region; EEC, expected environmental concentration; PP, primary productivity; BP, bacterial productivity; Chl *a*, chlorophyll *a*; DOC, dissolved organic carbon; TP, total phosphorus; TDP, total dissolved phosphorus; TDN, total dissolved nitrogen.

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### 1. Introduction

The Prairie pothole region (PPR) across south central Canada and the north central United States contains more than four million wetlands (Mitsch and Gosselink, 2000). The total wetland area in North America, however, has declined substantially, mostly due to human activities such as drainage (Dahl, 2000). Prairie wetlands serve an important role in providing water filtration and storage, wildlife habitat, and carbon sequestration (Batt et al., 1989; Euliss et al., 2006). The food-webs in these wetlands consist of primary producers (free-living and

attached algae, submerged and emergent plants), bacteria, fungi, benthic invertebrates, emergent insects and higher trophic level mammals and migrating waterfowl. These aquatic ecosystems are key ecological features of the prairie region, supporting 50–80% of the North American waterfowl population and 50% of other migratory birds each year (Batt et al., 1989).

The glaciated plains of the North American continent have unique hydrological and hydrogeological characteristics due to the combination of the semiarid, cold climate, and the glacial deposits that underlay the area. The glacial deposits are a rich source of mineral nutrients for water bodies in the PPR. Nutrients trapped in those wetlands with closed drainage basins are recycled instead of being flushed out by surface runoff. Prairie wetlands located in lower landscape elevations are generally more saline due to underlying glacial deposits than those located at higher elevations (LaBaugh, 1989; Sloan, 1972). Wetlands and lakes of the northern Prairie region have sulfate as the dominant anion, especially in saline wetlands, originating from glacial deposits (Fritz et al., 1993; van der Valk, 1989). At the St. Denis National Wildlife Area, Saskatchewan, Canada (study site), specific conductivities of wetland water range from less than  $400 \mu\text{S cm}^{-1}$ , for shallow freshwater marshes, to over  $24,000 \mu\text{S cm}^{-1}$  for terminal saline wetlands, with sulfate as the dominant anion in saline wetlands (Driver and Peden, 1977). The difference in water quality parameters among wetlands, especially salinity, is reflected in the composition of their plant communities (Stewart and Kantrud, 1972).

One of the most important features of the PPR is that wetlands are interspersed within agricultural fields where pesticides are commonly used (Donald et al., 1999; Main et al., 2014; Waiser and Robarts, 1997). The pesticides are transported to the wetlands via spray drift, atmospheric deposition, surface runoff, and ground water flow (Grover et al., 1988; Waite et al., 1992). As a result, they are frequently detected in prairie surface waters across this region such as wetlands (Main et al., 2014; Donald et al., 1999, 2001; Waite et al., 2002, 2004), lakes (Donald and Syrgiannis, 1995), farm dugouts (Cessna and Elliott, 2004; Grover et al., 1997), and drinking water reservoirs (Donald et al., 2007). The seven herbicides most frequently detected in prairie drinking water reservoirs were: 2,4-D [(2,4-dichlorophenoxy)acetic acid], MCPA [(4-chloro-2-methylphenoxy)acetic acid], dicamba [3,6-dichloro-2-methoxybenzoic acid], clopyralid [3,6-dichloropyridine-2-carboxylic acid], dichlorprop [2-(2,4-dichlorophenoxy)propanoic acid], mecoprop [2-(4-chloro-2-methylphenoxy)propanoic acid], and bromoxynil [3,5-dibromo-4-hydroxybenzotriazole] (Donald et al., 2007). Glyphosate [N-(phosphonomethyl)glycine], a broad-spectrum, non-selective systemic herbicide, has also been detected in prairie wetlands (Messing et al., 2011). These herbicides, many of which were detected in prairie wetlands, lakes, and farm dugouts, are also among the most widely used in PPR for crop production (Byrtus, 2011; Ribo, 1986; Waiser and Holm, 2005). Glyphosate herbicide formulations account for about one-fifth of the total area of crop land treated with herbicides in the Province of Manitoba, which is also within the PPR region (Shymko et al., 2008). Six of the eight herbicides most frequently detected in the drinking water reservoirs are auxin-type compounds and are known to have bi-phasic behavior, i.e., inhibitory effects at higher concentrations and stimulatory effects at lower concentrations (Sura et al., 2012a, 2012b). However, whether these herbicides are present in wetlands singly or as mixtures, there is limited information regarding their toxic effects, especially on the microbial communities, and how they vary with wetland hydrology. In fact, DeLorenzo et al. (2001) opined that the influence of water quality on pesticide toxicity to aquatic microorganisms was a much under-researched area. Water quality parameters such as pH, salinity and nutrient concentrations may have an important influence on how pesticides affect wetland microbial communities. Consequently, the results presented here, comparing microbial community responses to herbicide mixtures under nutrient-enriched freshwater and lower-nutrient saline conditions, would represent a useful contribution to the existing literature (DeLorenzo et al., 2001).

The objective of this study was to use a multi-trophic outdoor enclosure system to mimic the wetland ecosystem and to investigate the effects of an environmentally relevant concentration of a herbicide mixture (MCPA, clopyralid, dicamba, dichlorprop, mecoprop, 2,4-D, bromoxynil, and glyphosate) on wetland microbial communities (algae and bacteria) from wetlands varying in salinity and nutrient concentration. In addition, we hypothesize that the herbicide mixture will have inhibitory effects on pelagic primary productivity because of herbicidal effects.

## 2. Materials and methods

### 2.1. Study site and design

Four wetlands, P109, P02, P50, and P67, situated in the St. Denis National Wildlife Area ( $52^{\circ}02' \text{ N } 106^{\circ}06' \text{ W}$ ), located 45 km east of Saskatoon, Saskatchewan, in the Prairie pothole region (PPR) of Saskatchewan were selected for study based on their relative salinities. In the order of their increasing specific conductivity, P109 was a freshwater wetland ( $\sim 500 \mu\text{S cm}^{-1}$ ) while P02 ( $\sim 3597 \mu\text{S cm}^{-1}$ ), P50 ( $\sim 3875 \mu\text{S cm}^{-1}$ ), and P67 ( $\sim 4535 \mu\text{S cm}^{-1}$ ) were saline. Mean depth, volume, area, and perimeter of these wetlands are listed in Table 1. Six enclosures were installed in each wetland in early May 2008 and the aquatic environment within the enclosures was allowed to acclimatize in situ for 6 weeks before the start of the experiment. Each enclosure was a circular plastic hollow tube, measuring 100 cm (diameter)  $\times$  160 cm (height) and open at both ends. Enclosures were pushed into the bottom sediments of each wetland, isolating part of the wetland water column and sediment. To ensure there was no exchange of water between the enclosures and the wetland, the lower end of each enclosure was pushed into the sediment approximately 25 to 30 cm. Six enclosures in each wetland were placed randomly in close proximity to each other to ensure similar conditions, such as water level, exposure to sunlight, vegetation cover, and sediment. Heights of the enclosures were adjusted by cutting off the excess so that only 35–40 cm was above the water surface level. Water depth was measured and volumes were calculated for all enclosures one day prior to herbicide treatment. Initial water volumes ranged from approximately 560 to 750 L. At the end of the 28-day study period, about 24% of water was lost in fresh water pond enclosures while 10–12% was lost in saline pond enclosures.

### 2.2. Herbicide treatment

A mixture of eight commercially formulated herbicides was used (Table 2). The target concentration or the expected environmental concentration (EEC) of each herbicide in the treatment enclosures was based on 100% direct overspray of herbicide at recommended field application rate on a 0.5-m deep wetland (Cessna et al., 2006). Assuming minimal herbicide concentrations in the wetland when the overspray occurs, the resulting EEC due to overspray should represent a maximum exposure scenario for aquatic flora and fauna. For example, glyphosate, at the recommended application rate of  $360 \text{ g ha}^{-1}$ , when applied to a

**Table 1**  
Mean depth, perimeter, area, mean volume and dominant vegetation for wetlands P109, P02, P50 and P67 at St. Denis National Wildlife Area, Saskatchewan, Canada.

Wetland	Max depth <sup>a,b</sup> (m)	Mean volume <sup>c</sup> (m <sup>3</sup> )	Area <sup>d</sup> (m <sup>2</sup> )	Perimeter <sup>d</sup> (m)
P109	1.13	3200	3700	260
P02	1.19	5600	5900	320
P50	1.80	53,100	52,700	950
P67	1.33	24,600	21,000	630

<sup>a</sup> Reported maximum depth and perimeter values are averages of measurements taken in May and July 2008.

<sup>b</sup> Data provided by Garth van der Kamp, Environment Canada.

<sup>c</sup> Mean volumes were calculated from depth measurements using the equation from Hayashi and van der Kamp (2000).

<sup>d</sup> Data provided by Mark Bidwell, Environment Canada.

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