

2,4-D and Glyphosate Affect Aquatic Biofilm Accrual, Gross Primary Production, and Community Respiration

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Background

Herbicides

Very large quantities of herbicides are applied to agricultural land in Alberta, Canada. In 2008, 6100 tons of glyphosate and 840 tons of 2,4-dichlorophenoxyacetic acid (2,4-D) were sold in Alberta.¹ Both herbicides have been detected frequently in Alberta surface waters, at a range of concentrations²:

- 2,4-D: median measurable concentration 0.043 ppb; maximum concentration 439 ppb
- Glyphosate: median measurable concentration 0.3 ppb; maximum concentration 6.1 ppb

Herbicides and Aquatic Biofilms

There are a number of studies showing that 2,4-D and glyphosate are toxic towards algae (for example, ref. 3). Most of these are studies on single species of phytoplankton, in laboratory conditions.

Diffusing Substrates

Nutrient-diffusing substrates (NDSs) are an established technique to study the growth aquatic biofilms.⁴ NDSs consist of a porous substrate material fastened atop a plug of agar that contains N and P nutrients that diffuse through the agar and substrate, affecting the biofilm. Recently, the NDS technique has been modified to investigate the effects of low concentrations of pharmaceutical compounds on aquatic biofilms, *in situ*.⁵ This technique can be applied to study the effects of any water-soluble compound on biofilm growth.

Ecological Functions

The ecological functions of biofilms - gross primary production (GPP) and community respiration (CR) - can be measured by incubating the intact biofilms on the substrates in light/dark conditions. These measurements reveal how biofilms function at the community level as opposed to the species level. Aquatic biofilms underpin food webs and are thus important to aquatic ecosystems as a whole.



Figure 1. Rural pond, site of substrate deployment.

Methodology

Diffusing Substrates

70-mL plastic specimen jars, with a 25 mm hole in the lid. Jars were filled with 2% agar, amended with:

- 50 mM NaNO₃ and 3mM KH₂PO₄ (Redfield ratio)
- 15 mM, 9.0 mM, or 1.5 mM of glyphosate (isopropyl amine salt) or 2,4-D (99%)

Topped with either:

- GFC glass fibre filter (autotrophs)
- Cellulose filter paper (heterotrophs)

Deployment

- 22 days in a rural pond located on Athabasca University property, adjacent to cultivated land (Figure 1).
- Random array of diffusing substrates suspended approx. 15 cm below surface (Figure 2).
- n = 4 for each treatment

Gross Primary Production, Community Respiration

- Incubated in airtight 12-mL Labco Exetainers
- 30-min light/dark (GPP), 30-min dark (CR)
- DO measured before/after with Pyroscience Firesting DO meter (retractable needle probe)

Chlorophyll a

- After GPP measurements, samples were filtered on 0.2 µm filter
- Analyzed according to Standard Method (absorbance method, with phaeophytin correction)

Statistical Analysis:

One-way ANOVA with pairwise comparisons (Tukey HSD).



Figure 2. Random array of diffusing substrates suspended below surface. Arrays were tethered to a metal post.

Table 1. Water Quality Parameters for Pond

Parameter	Initial (Aug 16, 2012)	Final (Sep 7, 2012)
Temp (°C)	23.1 ± 0.5	15.6 ± 0.1
DO (mg/L)	13.52 ± 0.34	5.74 ± 0.04
pH	7.58 ± 0.08	7.25 ± 0.03
Turb (NTU)	2.78 ± 1.2	2.3 ± 0.7

Results

Effects of 2,4-D

For autotrophs, 2,4-D causes a reduction in GPP and biofilm accrual, as measured by chlorophyll a content (Figures 3 & 4).

For heterotrophs, it causes a reduction in CR (Figure 5). In all cases, the effect was only significant for the 15 mM 2,4-D.

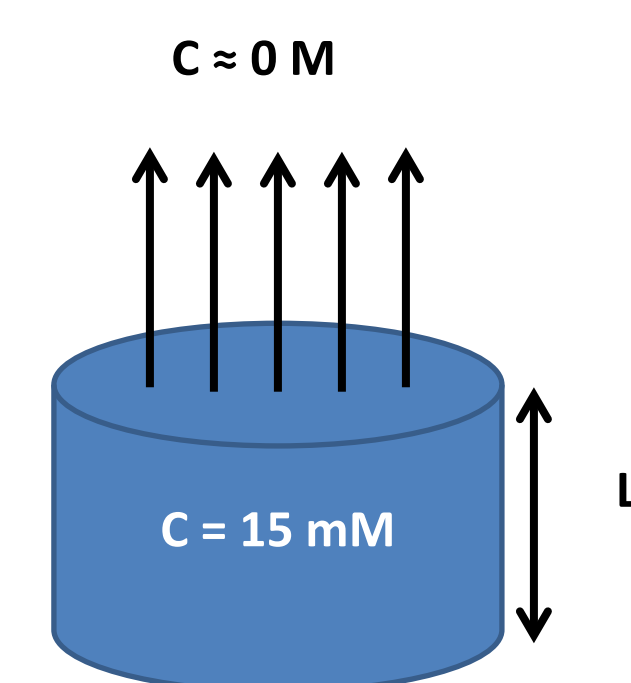
Effects of Glyphosate

For autotrophs, glyphosate causes a reduction in biofilm accrual and GPP (Figures 3 & 4).

The reduction is dose-dependent. For heterotrophs, it causes a reduction in CR (Figure 5).

- Glyphosate toxicity to immobilized algae is known.⁶ However, this study is the first to show toxicity towards an *in situ* biofilm, as opposed to a single species in the laboratory.

Release Rates from Diffusing Substrates



Fick's Law:

$$J = -D \frac{\partial c}{\partial x}$$

Solution for one-dimensional diffusion from a 'slab'(ref. 7):

$$\frac{M_t}{M_0} = 2 \left(\frac{Dt}{\pi L^2} \right)^{1/2}$$

Table 2. Calculated release rates for diffusing substrates. Based on one-dimensional diffusion from a slab, for 22 days, assuming $D = 5 \times 10^{-10} \text{ m}^2\text{s}^{-1}$.

	Conc. (mM)	Total released (mg)	Avg. release rate (µg/min/cm ²)
2,4-D	15	135	0.87
	9	80.8	0.52
	1.5	13.5	0.087
GPP	15	103	0.66
	9	61.8	0.40
	1.5	10.3	0.066

Implications

This study shows the effects of low concentrations of 2,4-D and glyphosate on the ecological functions of aquatic biofilms in a lentic system. Further work is needed to understand the link between the release rates from diffusing substrates and environmental concentrations, to understand the probable range of environmental concentrations that cause reductions in biofilm accrual, GPP, and CR.

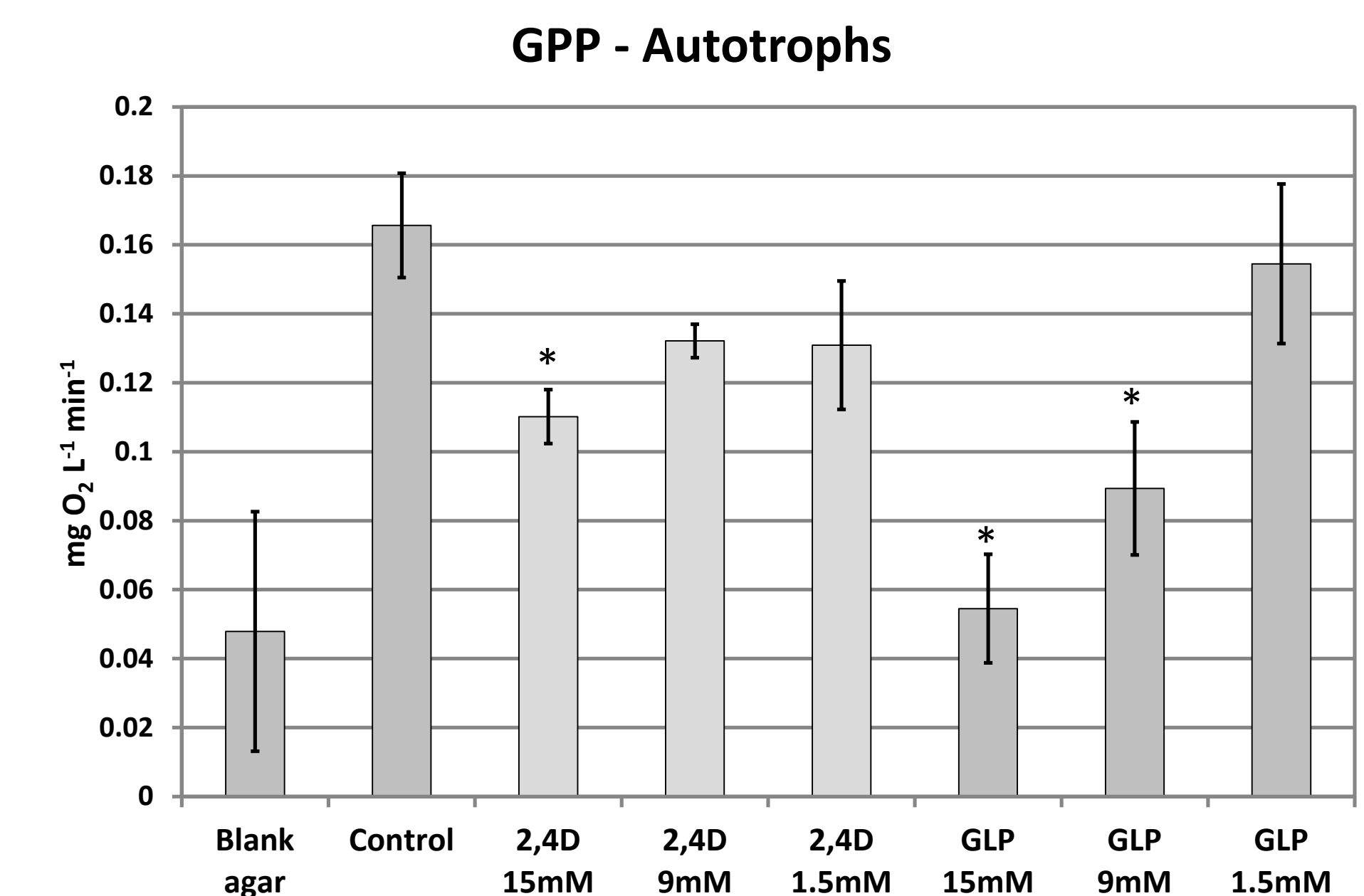


Figure 3. Gross Primary Production of biofilms grown on glass fibre diffusing substrates. *Indicates a significant difference relative to the control group. For 2,4-D: 15 mM ($p < 0.0269$). For glyphosate (GLP): 15 mM ($p < 0.0039$), 9 mM ($p < 0.0319$).

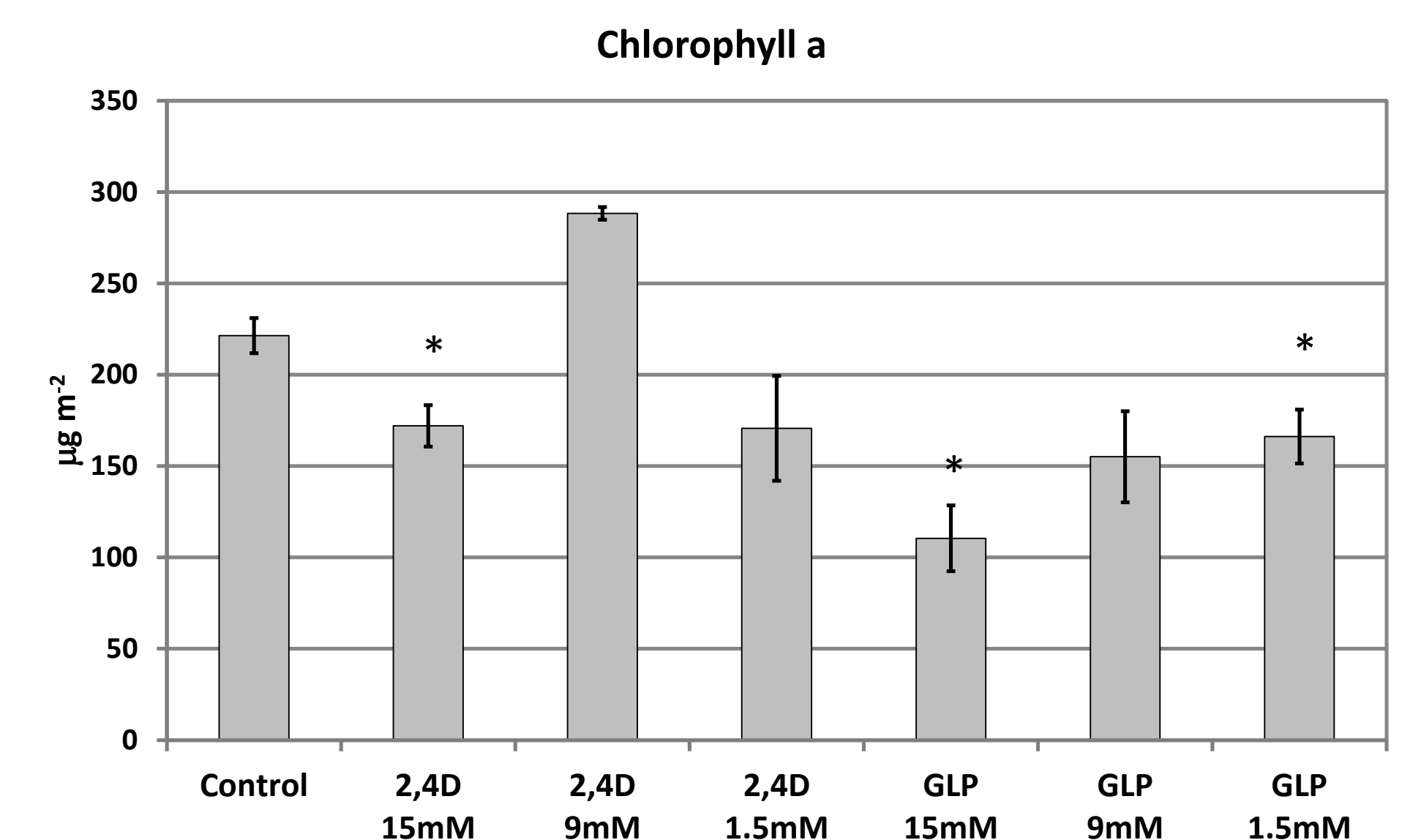


Figure 4. Chlorophyll a extracted from biofilms. *Indicates significant Difference relative to the control group. For 2,4-D: 15 mM ($p < 0.0251$). For glyphosate (GLP), 15 mM ($p < 0.0019$), 1.5 mM ($p < 0.0234$). The 9 mM dose was only significantly different to the control at the 90% level ($p < 0.0683$).

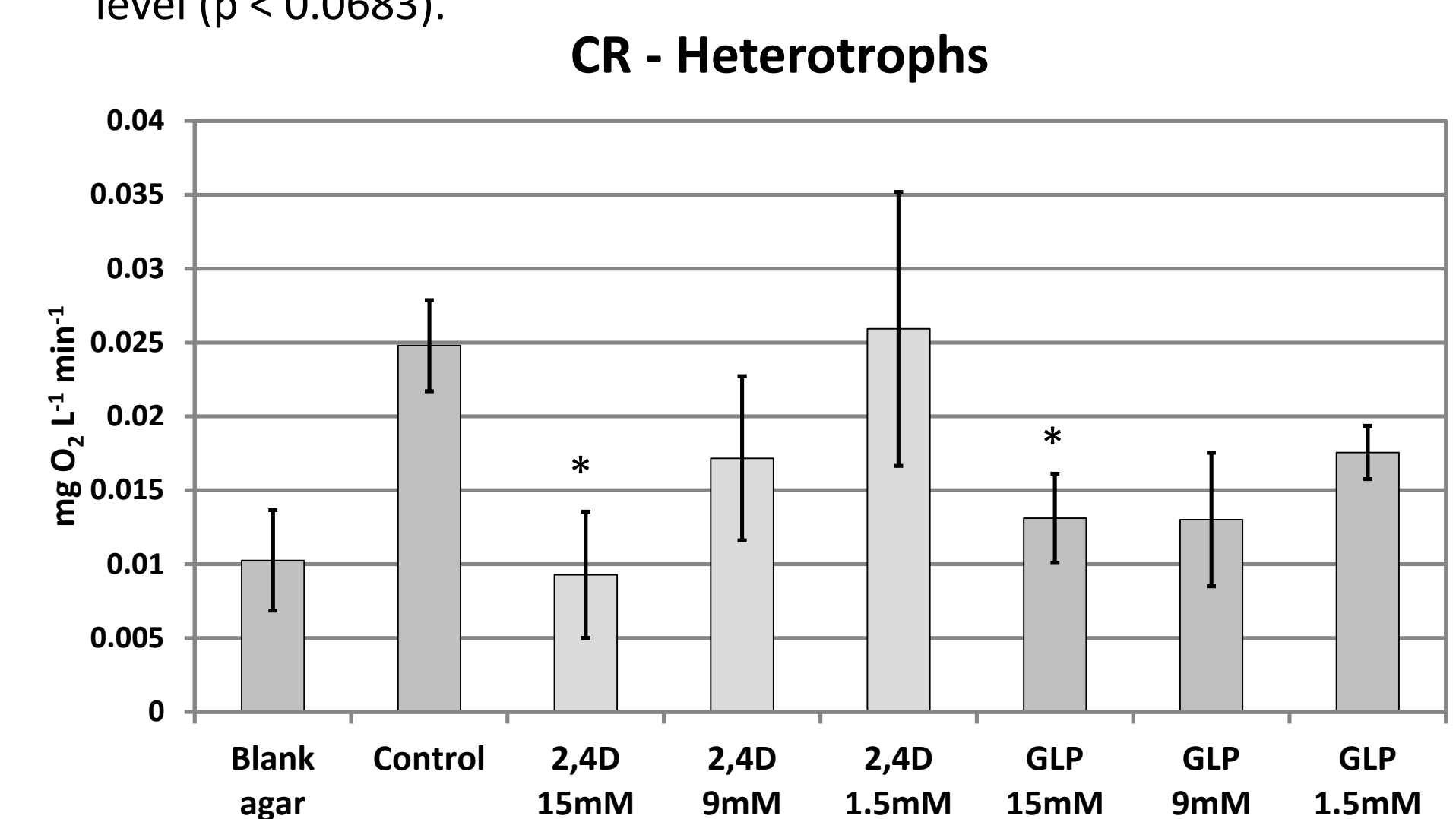


Figure 5. Community respiration of biofilms grown on cellulose substrates. *Indicates significant difference relative to the control group. For 2,4-D: 15 mM ($p < 0.0259$). For glyphosate (GLP): 15 mM ($p < 0.0352$).

References

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