Appendix C

Blacklock Restoration: Phragmites Control and Revegetation Efficacy and Water Quality Results

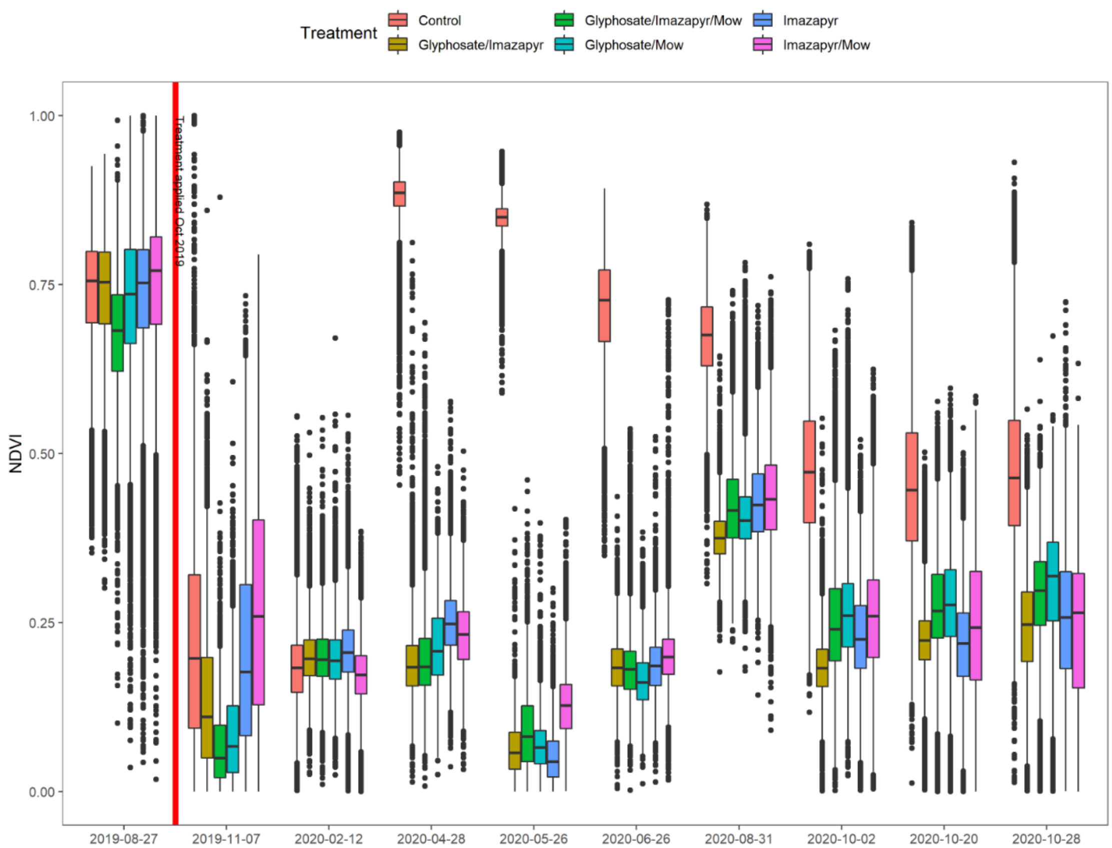
NDVI Efficacy Results

Results Summary

Analysis of NDVI values from 18 UAV imagery surveys between 2019 and 2022 indicates that all treatments are effective in significantly reducing *Phragmites* growth. NDVI values show strong seasonal trends for *Phragmites* growth in plots, with plants in senescence during late fall, winter, and early spring months (Figures 1, 2, 3, 4). Therefore, only UAV surveys flown when plants were not in senescence (late spring, summer, and early to mid-fall) should be considered to assess treatment efficacy. Glyphosate and imazapyr tank mix plots had significantly lower NDVI values than control plots within 10 seasons, imazapyr treatment plots within 6 seasons, tank mix/mowed plots within 7 seasons, glyphosate/mow plots within 8 seasons, and imazapyr mow plots within 3 seasons (Tables 1, 2, 3).

Methods

After confirming that datasets met appropriate test assumptions, one-way ANOVAs and Kruskal-Wallis H tests were ran to determine the effect of treatment on median NDVI values (a proxy for *Phragmites* health and growth). ANOVAs were ran with control as a factor to identify if median NDVI values were significantly different among treatment types within each survey date. If parametric assumptions were not met, data was analyzed using a non-parametric Kruskal-Wallis H test. If the test indicated that treatment type did have a significant effect, post hoc tests (Tukey's tests for parametric data Dunn's tests for nonparametric data) were run to examine pairwise comparisons among treatment types within each survey date.



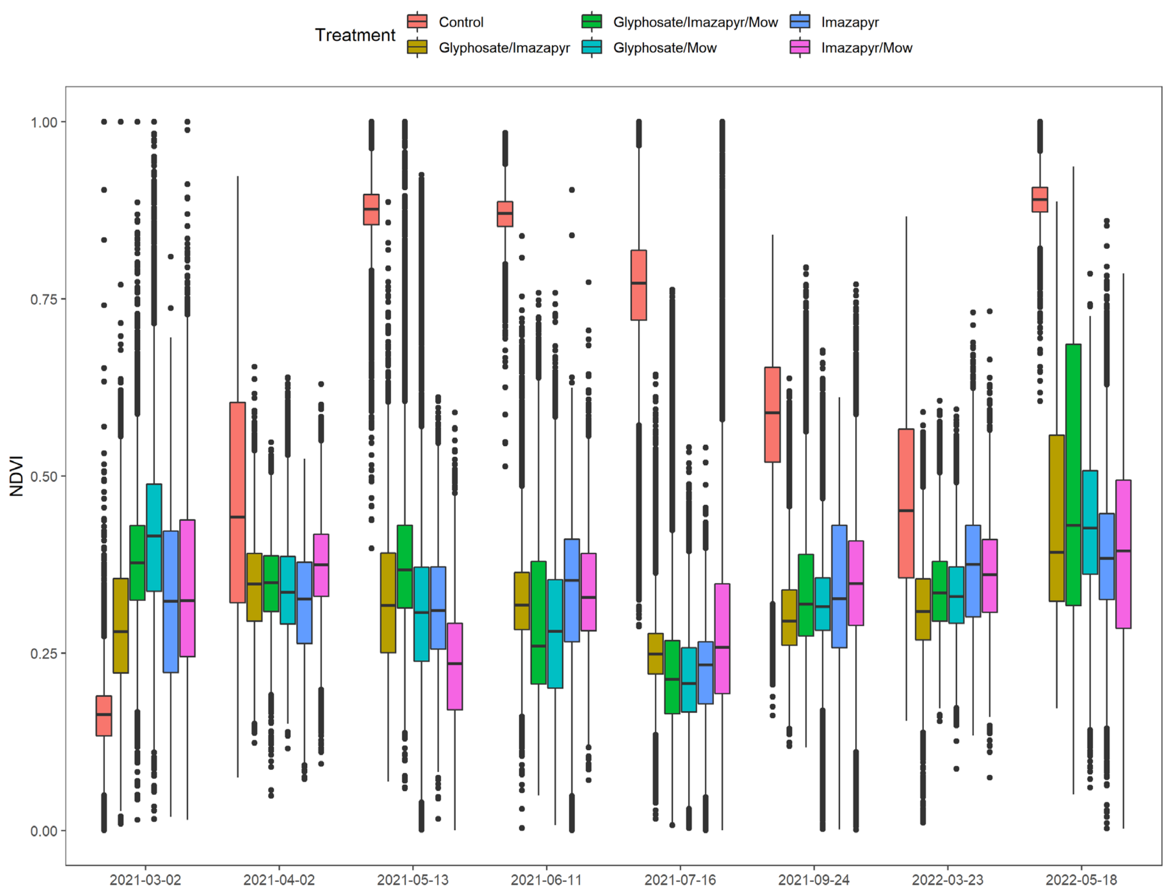


Figure 1. NDVI values among treatment and control plots from 2019-2021.

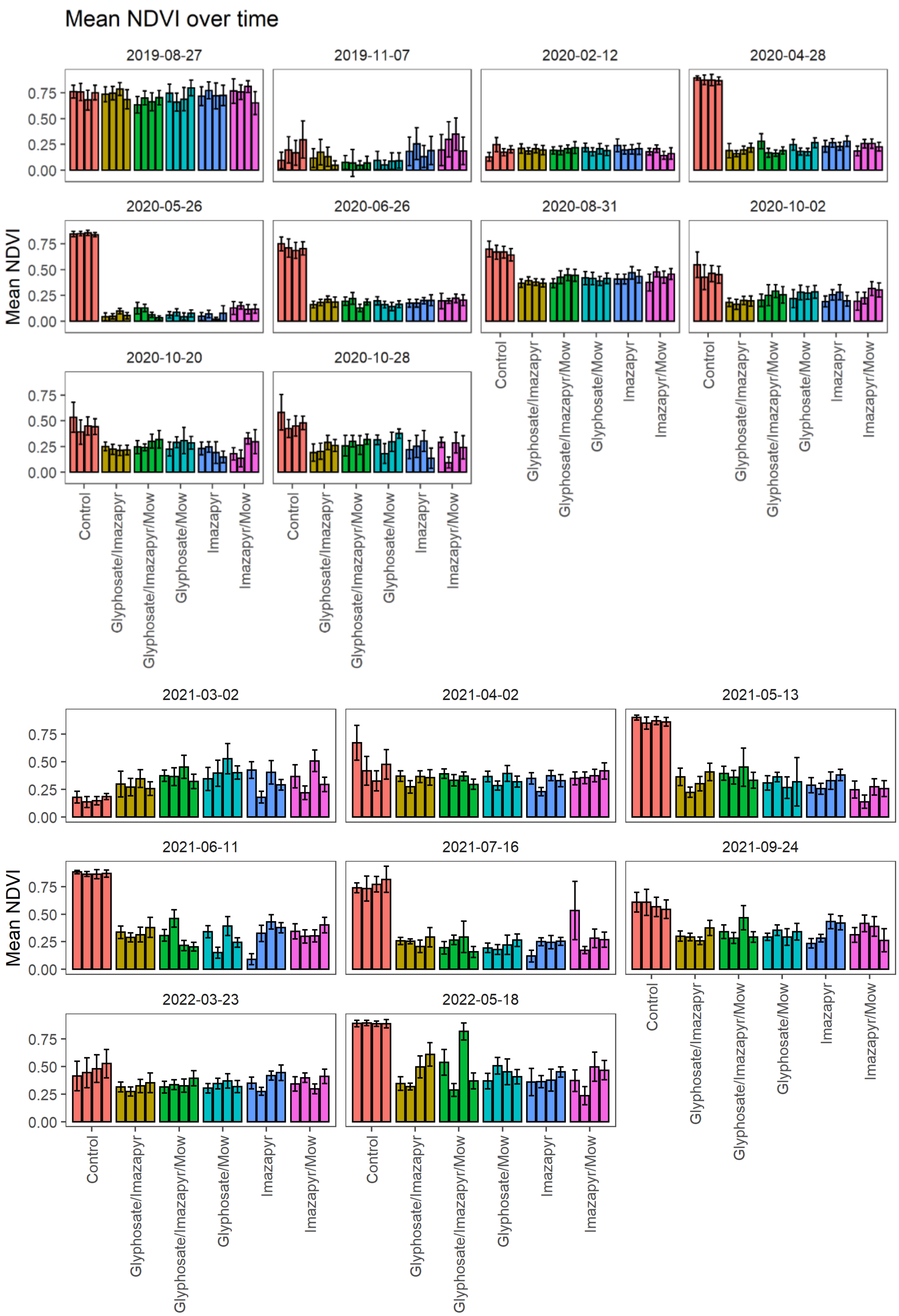


Figure 2. Mean NDVI values among treatment and control plots from 2019-2021.

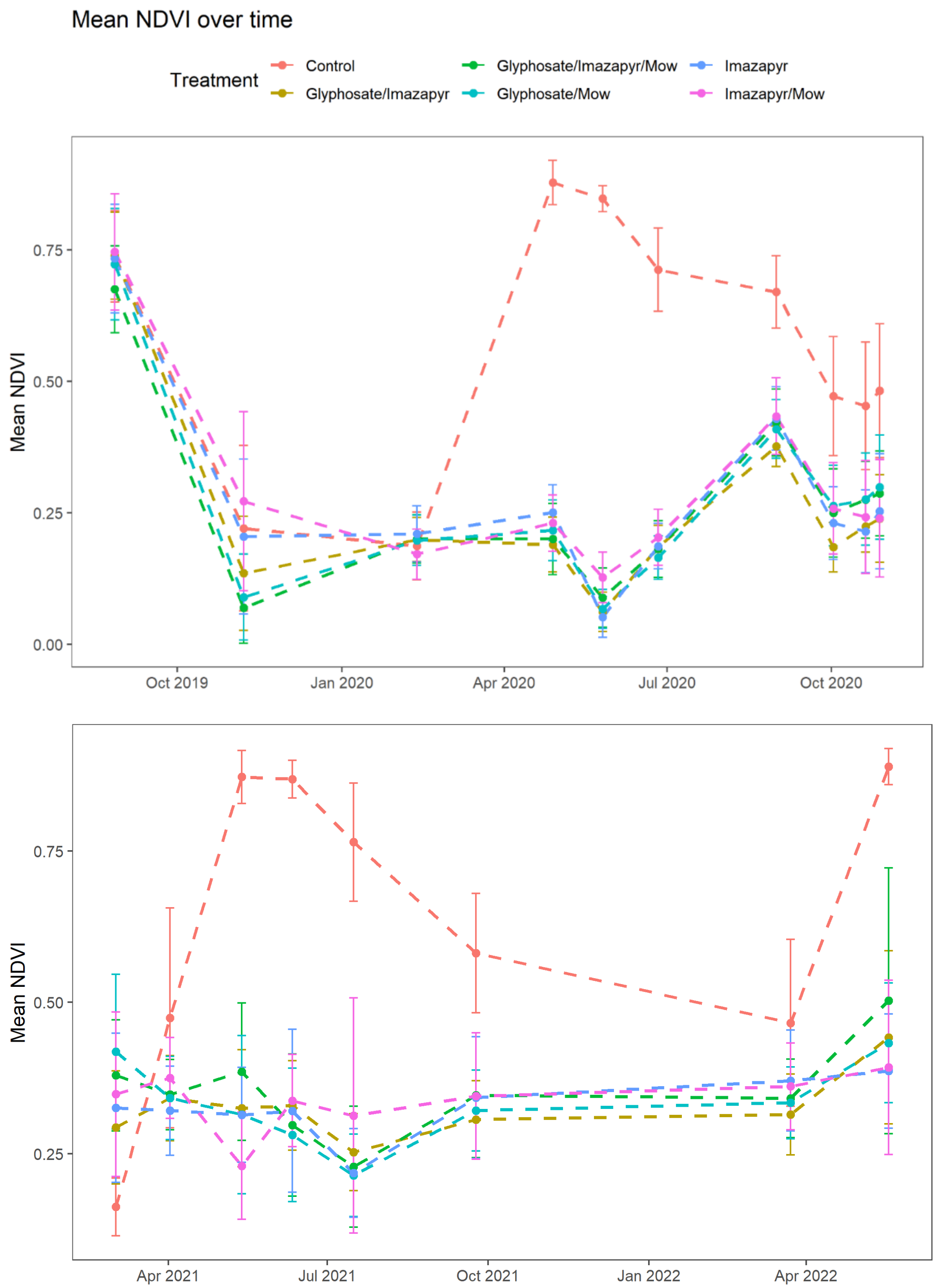


Figure 3. Mean NDVI values among treatment and control plots from 2019-2021.

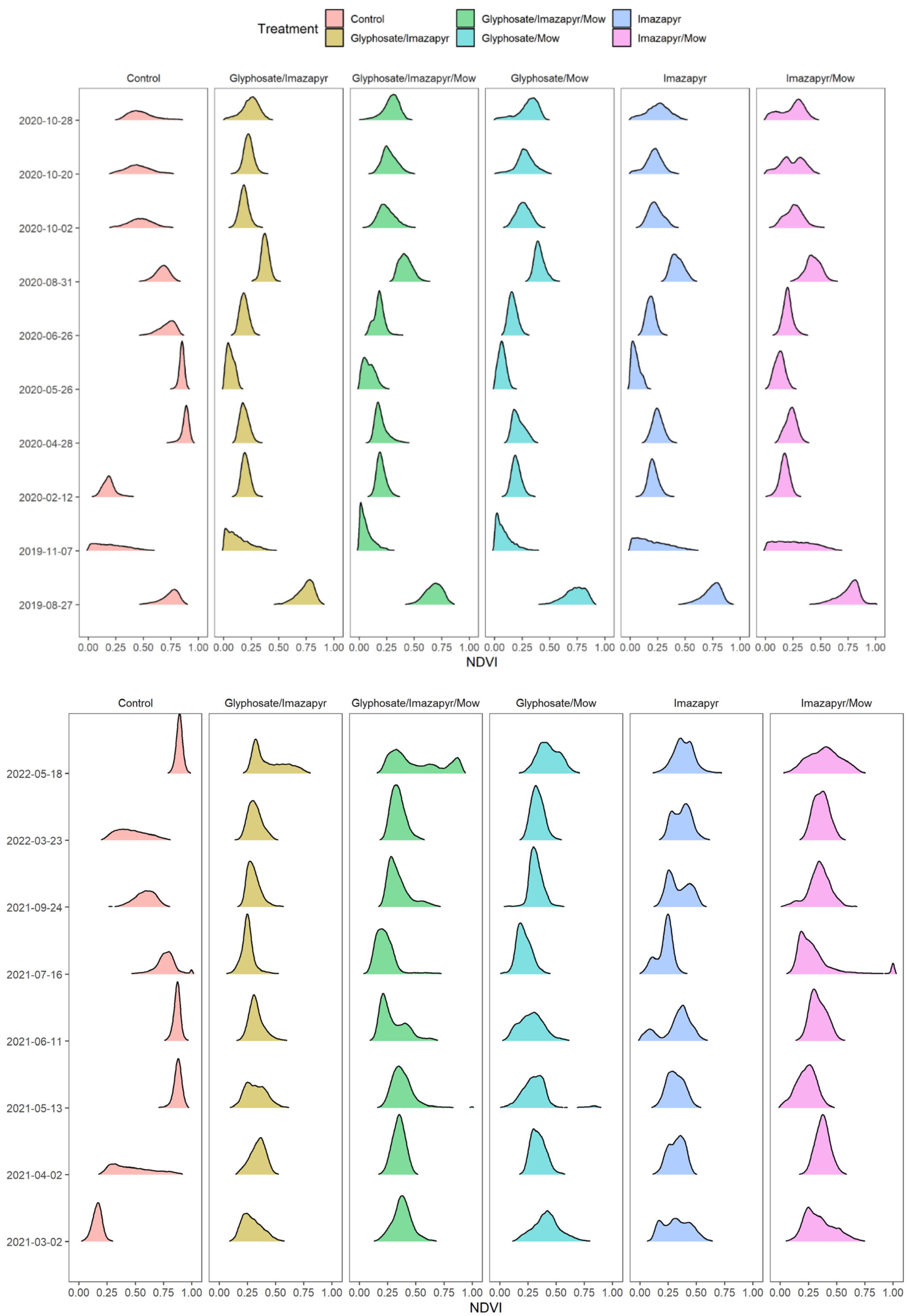


Figure 4. Density plots for treatment and control plot NDVI values from 2019-2021.


Figure 5. Aerial image of three treatment plots and a control plot with treatment boundaries indicated in blue. Imagery collected on 5/13/2021.

Table 1. Number of surveys that each treatment type had significantly lower NDVI values than control plots ($p<0.05$):

Glyphosate/Imazapyr	6
Imazapyr	4
Glyphosate/Imazapyr/Mow	5
Glyphosate/Mow	4
Imazapyr/Mow	3

Table 2. Number of surveys that each treatment type had marginally significantly lower NDVI values than control plots: ($0.05<p< 0.1$):

Glyphosate/Imazapyr	4
Imazapyr	2
Glyphosate/Imazapyr/Mow	2
Glyphosate/Mow	4
Imazapyr/Mow	0

Table 3. Results for all survey dates.

Date	**Comparison**	**P-value**	**Test**	**Notes**
8/7/19	No effect of treatment on NDVI	0.2258	1-way ANOVA	
11/7/19	Effect of treatment on NDVI	0.00594	Kruskall-Wallis	
	Control - Glyphosate/Imazapyr/Mow	0.04657249	Dunn's Test	
	Glyphosate/Imazapyr/Mow - Imazapyr	0.04661188	Dunn's Test	
	Glyphosate/Imazapyr/Mow - Imazapyr/Mow	0.01212173	Dunn's Test	
	Control - Glyphosate/Imazapyr	0.37521581	Dunn's Test	
	Control - Glyphosate/Mow	0.15398708	Dunn's Test	
	Control - Imazapyr	0.92034433	Dunn's Test	

	Control - Imazapyr/Mow	0.53907966	Dunn's Test	
2/12/20	No Effect of treatment on NDVI	0.4531	1-way ANOVA	
4/28/20	Effect of treatment on NDVI	0.0184	Kruskall-Wallis	
	Control - Glyphosate/Imazapyr	0.01731075	Dunn's Test	
	Control - Glyphosate/Imazapyr/Mow	0.02798720	Dunn's Test	
	Control - Glyphosate/Mow	0.09386706	Dunn's Test	
	Control - Imazapyr/Mow	0.15136662	Dunn's Test	
5/26/20	Effect of treatment on NDVI	0.00832	Kruskall-Wallis	
	Control - Glyphosate/Imazapyr	0.02383304	Dunn's Test	
	Control - Glyphosate/Imazapyr/Mow	0.12109329	Dunn's Test	
	Control - Glyphosate/Mow	0.04661188	Dunn's Test	
	Control - Imazapyr	0.01450272	Dunn's Test	
	Imazapyr - Imazapyr/Mow	0.10427586	Dunn's Test	
6/26/20	Effect of treatment on NDVI	0.0256	Kruskall-Wallis	
	Control - Glyphosate/Imazapyr	0.10714216	Dunn's Test	
	Control - Glyphosate/Imazapyr/Mow	0.08197536	Dunn's Test	
	Control - Glyphosate/Mow	0.01212173	Dunn's Test	
	Control - Imazapyr	0.10427586	Dunn's Test	
	Control - Imazapyr/Mow	0.27399646	Dunn's Test	
8/31/20	Effect of treatment on NDVI	0.0175	Kruskall-Wallis	
	Control - Glyphosate/Imazapyr	0.005778467	Dunn's Test	
	Control - Glyphosate/Imazapyr/Mow	0.201822154	Dunn's Test	
	Control - Glyphosate/Mow	0.060368828	Dunn's Test	
	Control - Imazapyr	0.192940649	Dunn's Test	
	Control - Imazapyr/Mow	0.215374199	Dunn's Test	
10/2/20	Effect of treatment on NDVI	0.0139	Kruskall-Wallis	
	Control - Glyphosate/Imazapyr	0.003233992	Dunn's Test	
	Control - Glyphosate/Imazapyr/Mow	0.178644206	Dunn's Test	
	Control - Glyphosate/Mow	0.179651596	Dunn's Test	
	Control - Imazapyr	0.107142161	Dunn's Test	
	Control - Imazapyr/Mow	0.191910446	Dunn's Test	
10/20/20	Effect of treatment on NDVI	9.678e-05	1-way ANOVA	
	Glyphosate/Imazapyr-Control	0.0002337	Tukey's Test	
	Glyphosate/Imazapyr/Mow-Control	0.0039570	Tukey's Test	
	Glyphosate/Mow-Control	0.0058409	Tukey's Test	
	Imazapyr-Control	0.0000922	Tukey's Test	
	Imazapyr/Mow-Control	0.0005749	Tukey's Test	
10/28/20	Effect of treatment on NDVI	0.00119	1-way ANOVA	
	Glyphosate/Imazapyr-Control	0.0027126	Tukey's Test	
	Glyphosate/Imazapyr/Mow-Control	0.0200077	Tukey's Test	
	Glyphosate/Mow-Control	0.0244206	Tukey's Test	
	Imazapyr-Control	0.0017087	Tukey's Test	

	Imazapyr/Mow-Control	0.0022171	Tukey’s Test	
3/2/21	Effect of treatment on NDVI	0.004903	1-way ANOVA	
	Glyphosate/Imazapyr-Control	0.3329590	Tukey’s Test	Control is lower
	Glyphosate/Imazapyr/Mow-Control	0.0130750	Tukey’s Test	Control is lower
	Glyphosate/Mow-Control	0.0031344	Tukey’s Test	Control is lower
	Imazapyr-Control	0.0919948	Tukey’s Test	Control is lower
	Imazapyr/Mow-Control	0.0522417	Tukey’s Test	Control is lower
4/2/21	No effect of treatment on NDVI	0.407	Kruskal-Wallis	
5/13/21	Effect of treatment on NDVI	0.0108	Kruskal-Wallis	
	Control - Glyphosate/Imazapyr	0.083420685	Dunn’s Test	
	Control - Glyphosate/Imazapyr/Mow	0.363001817	Dunn’s Test	
	Control - Glyphosate/Mow	0.060368828	Dunn’s Test	
	Control - Imazapyr	0.107241100	Dunn’s Test	
	Control - Imazapyr/Mow	0.005778467	Dunn’s Test	
6/11/21	Effect of treatment on NDVI	0.0708	Kruskal-Wallis	
	Control - Glyphosate/Imazapyr	0.09386706	Dunn’s Test	
	Control - Glyphosate/Imazapyr/Mow	0.10400921	Dunn’s Test	
	Control - Glyphosate/Mow	0.06036883	Dunn’s Test	
	Control - Imazapyr	0.09466564	Dunn’s Test	
	Control - Imazapyr/Mow	0.11833206	Dunn’s Test	
7/16/21	Effect of treatment on NDVI	0.0289	Kruskal-Wallis	
	Control - Glyphosate/Imazapyr	0.24117581	Dunn’s Test	
	Control - Glyphosate/Imazapyr/Mow	0.05597440	Dunn’s Test	
	Control - Glyphosate/Mow	0.02555130	Dunn’s Test	
	Control - Imazapyr	0.03832695	Dunn’s Test	
	Control - Imazapyr/Mow	0.29682881	Dunn’s Test	
9/24/21	Effect of treatment on NDVI	0.0574	Kruskal-Wallis	
	Control - Glyphosate/Imazapyr	0.05597440	Dunn’s Test	
	Control - Glyphosate/Imazapyr/Mow	0.10714216	Dunn’s Test	
	Control - Glyphosate/Mow	0.06148152	Dunn’s Test	
	Control - Imazapyr	0.08197536	Dunn’s Test	
	Control - Imazapyr/Mow	0.19294065	Dunn’s Test	
3/23/22	Effect of treatment on NDVI	0.02208	1-way ANOVA	
	Glyphosate/Imazapyr-Control	0.0180294	Tukey’s Test	
	Glyphosate/Imazapyr/Mow-Control	0.0482098	Tukey’s Test	
	Glyphosate/Mow-Control	0.0440142	Tukey’s Test	
5/18/22	Effect of treatment on NDVI	0.0606	Kruskal-Wallis	
	Control - Glyphosate/Imazapyr	0.08197536	Dunn’s Test	
	Control - Imazapyr	0.04766609	Dunn’s Test	

Water Quality Results

Herbicide Detection Results

Of the 210 Imazapyr samples taken during 2019 through 2021 treatment events, 30 had positive detections (Table 4). Of these 3 detections, 15 were in plots that were treated on the same day of sampling (6 in Imazapyr plots and 9 in tank mix plots), 7 were in plots that were treated at least 24 hours prior to sampling (5 in imazapyr plots and 2 in tank mix plots), 4 were in control plots, and 2 each were in breach locations and reference locations. In addition, 5 of these detections were in 2019, 20 were in 2020, and 5 were in 2021.

No glyphosate or nonylphenol was detected during the 2019-2021 sampling events. With the maximum imazapyr concentration detected at 0.013 mg/L in plot 5b (treated with imazapyr) on the day of treatment, all detections were below the 11.2 mg/L instantaneous monitoring trigger set forth in the NPDES General Permit. In addition, all detections were below the LC50 values listed in the U.S. EPA's Ecotoxicity Database included in the General Permit, the lowest of which was 100 mg/L. The General Permit also states that imazapyr has no receiving water limitation due to "it's safe use in the environment and low toxicity to aquatic life as indicated in U.S. EPA's Ecotoxicity Database."

Table 4. Summary of herbicide detection results in water sample taken pre-and post-treatment during 2019-2021 treatment events.

Herbicide	Total samples taken (2019-2021)	Positive Detections (2019-2021)	Maximum Detection (mg/L)	Average Detection (mg/L)	NPDES Receiving Water Limit/Monitoring Trigger (mg/L)
Glyphosate	196	0	NA	NA	0.7
Imazapyr	210	30	0.013	0.00127	11.2

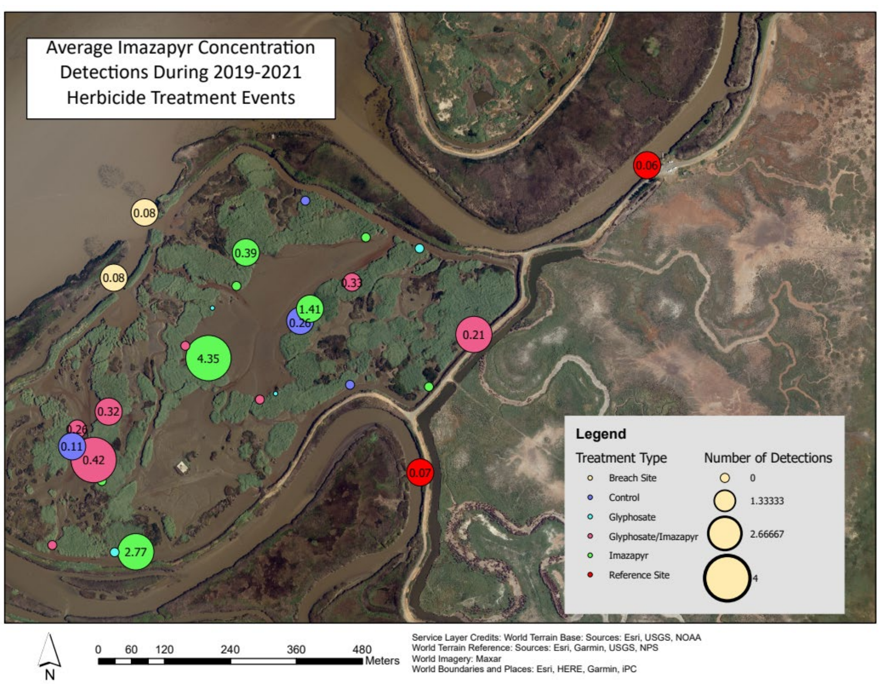


Figure 6. Water sampling locations in treatment plots, control plots, breached areas, and reference sites during 2019-2021 sampling events. The size of the circle represents the number of detections throughout sampling events (n=0-4) with the label being the average concentration (ug/L) of those detections. Small circles with no average concentration labeled did not have any herbicide detections during sampling.

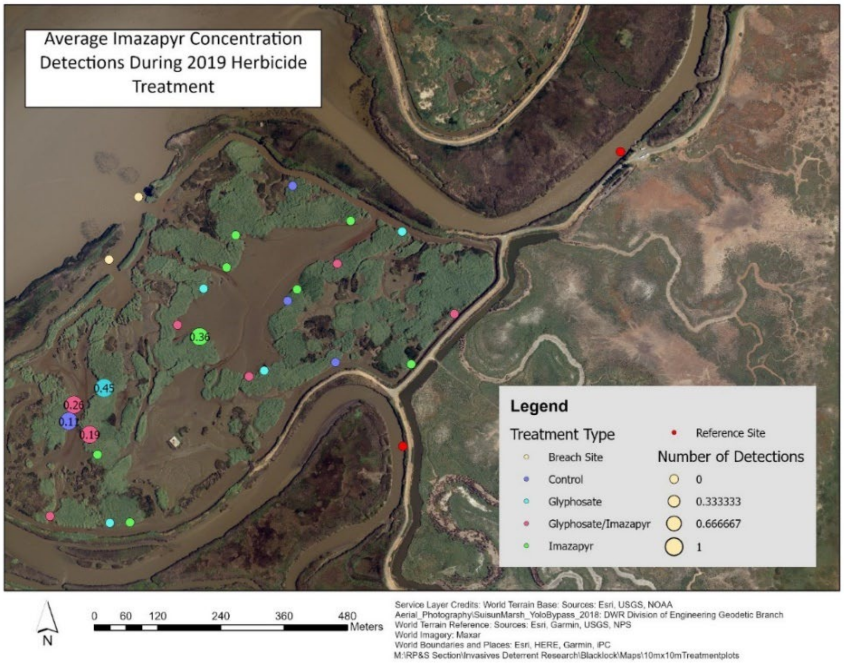


Figure 7. Water sampling locations in treatment plots, control plots, breached areas, and reference sites in 2019. The size of the circle represents the number of detections throughout sampling events (n=0-4) with the label being the average concentration (ug/L) of those detections. Small circles with no average concentration labeled did not have any herbicide detections during sampling.

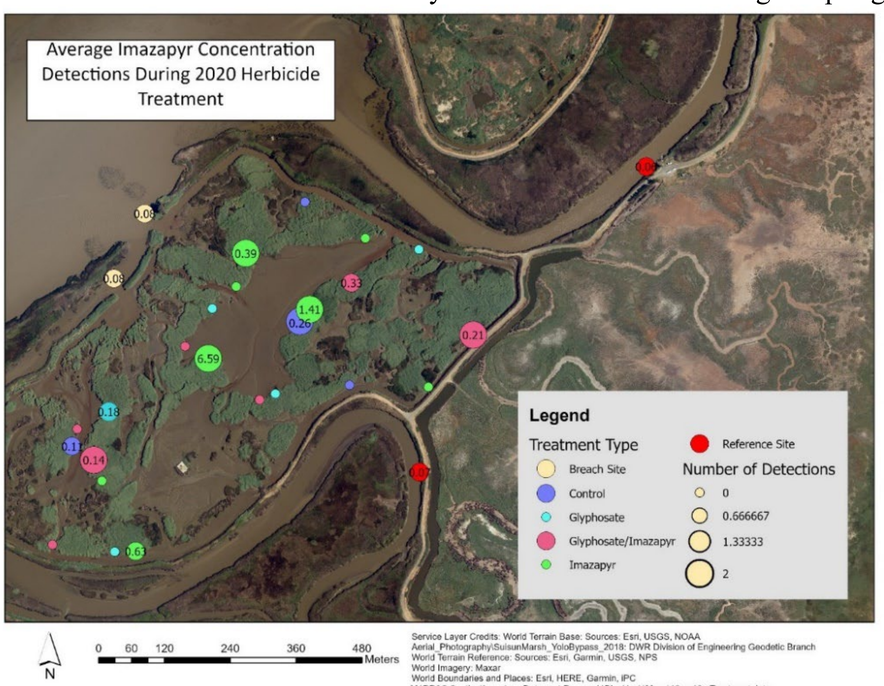


Figure 8. Water sampling locations in treatment plots, control plots, breached areas, and reference sites in 2020. The size of the circle represents the number of detections throughout sampling events (n=0-4) with the label being the average concentration (ug/L) of those detections. Small circles with no average concentration labeled did not have any herbicide detections during sampling.

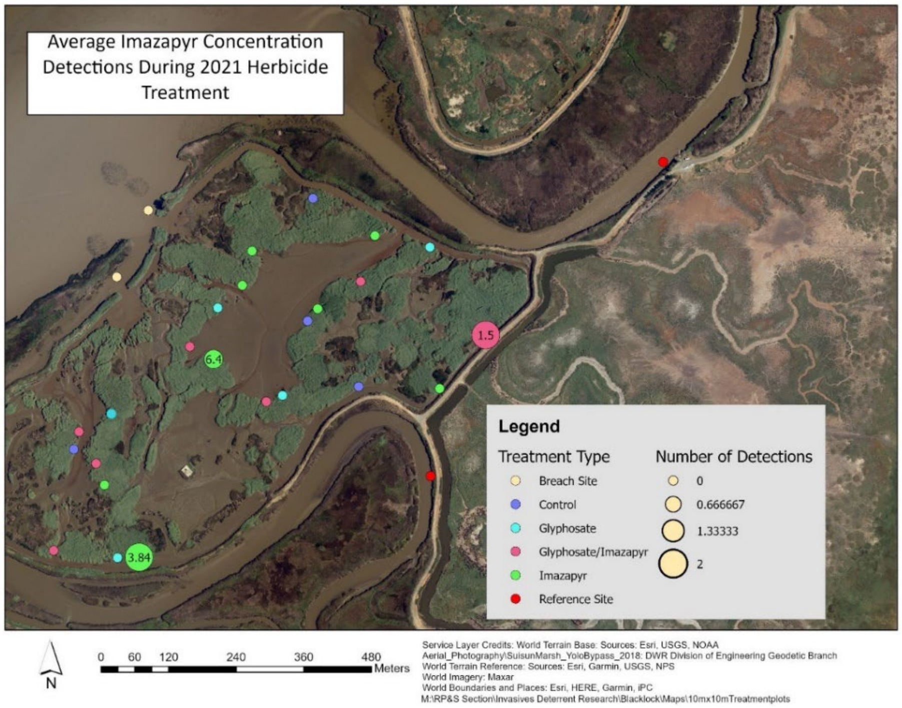


Figure 9. Water sampling locations in treatment plots, control plots, breached areas, and reference sites in 2021. The size of the circle represents the number of detections throughout sampling events (n=0-4) with the label being the average concentration (ug/L) of those detections. Small circles with no average concentration labeled did not have any herbicide detections during sampling.

Water Quality Parameters

Data were separated by samples collected pre-treatment, post-treatment but within 24 hours (same day as treatment), and more than 24 hours post-treatment.

To determine the effect of year on water quality parameters, one-way ANOVAs were first ran with year as a factor to identify if water quality parameters varied significantly among years. If year did not have a significant effect, water quality data from all three years were grouped together to increase sample size and test robustness. When year did have a significant effect and water quality parameters had to be analyzed within year, sample size was too small to run a valid ANOVA. If parametric assumptions were not met, data was analyzed using a non-parametric Kruskal-Wallis H test.

After confirming that datasets met appropriate test assumptions, one-way ANOVAs and Kruskal-Wallis H tests were ran with treatment as a factor to test differences in water quality parameters (Chlorophyll, turbidity, temperature, specific conductivity, DO, phycocyanin, pH) between herbicide and control plots.

No significant differences were found in water quality parameters between herbicide and control plots in pre- or post-treatment sampling in any of the three years (Tables 5, 6). In addition, water quality parameters did not exceed the NPDES San Francisco Basin Water Quality Limits described in the surface water objectives (Table 7). Herbicide treatments did not significantly impact water quality parameters during the Blacklock Phragmites Control Study.

Glyphosate Water Quality Parameters

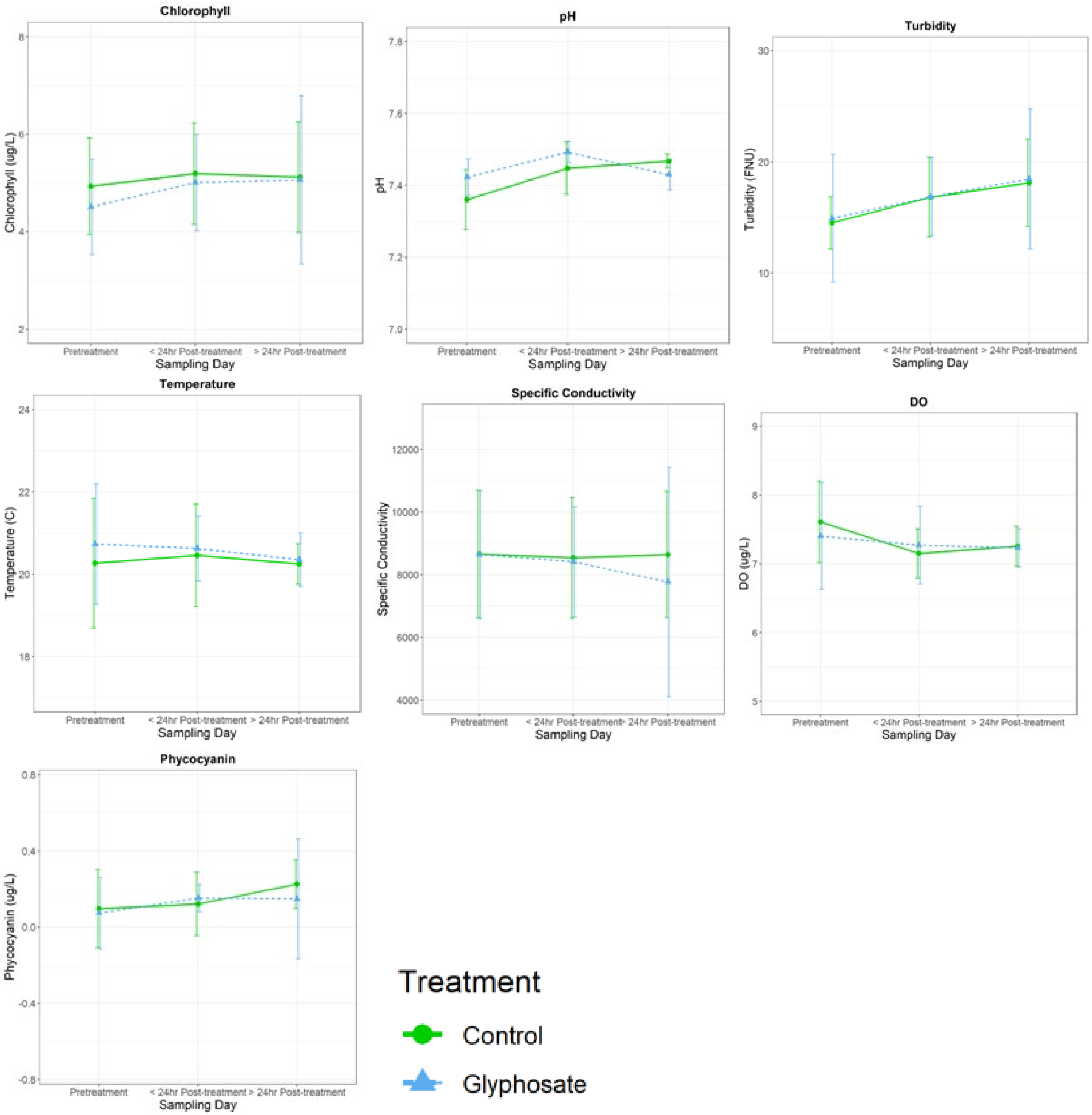


Figure 10. Water Quality Parameters for Glyphosate-treated plots during pre- and post-treatment sampling events.

Imazapyr Water Quality Parameters

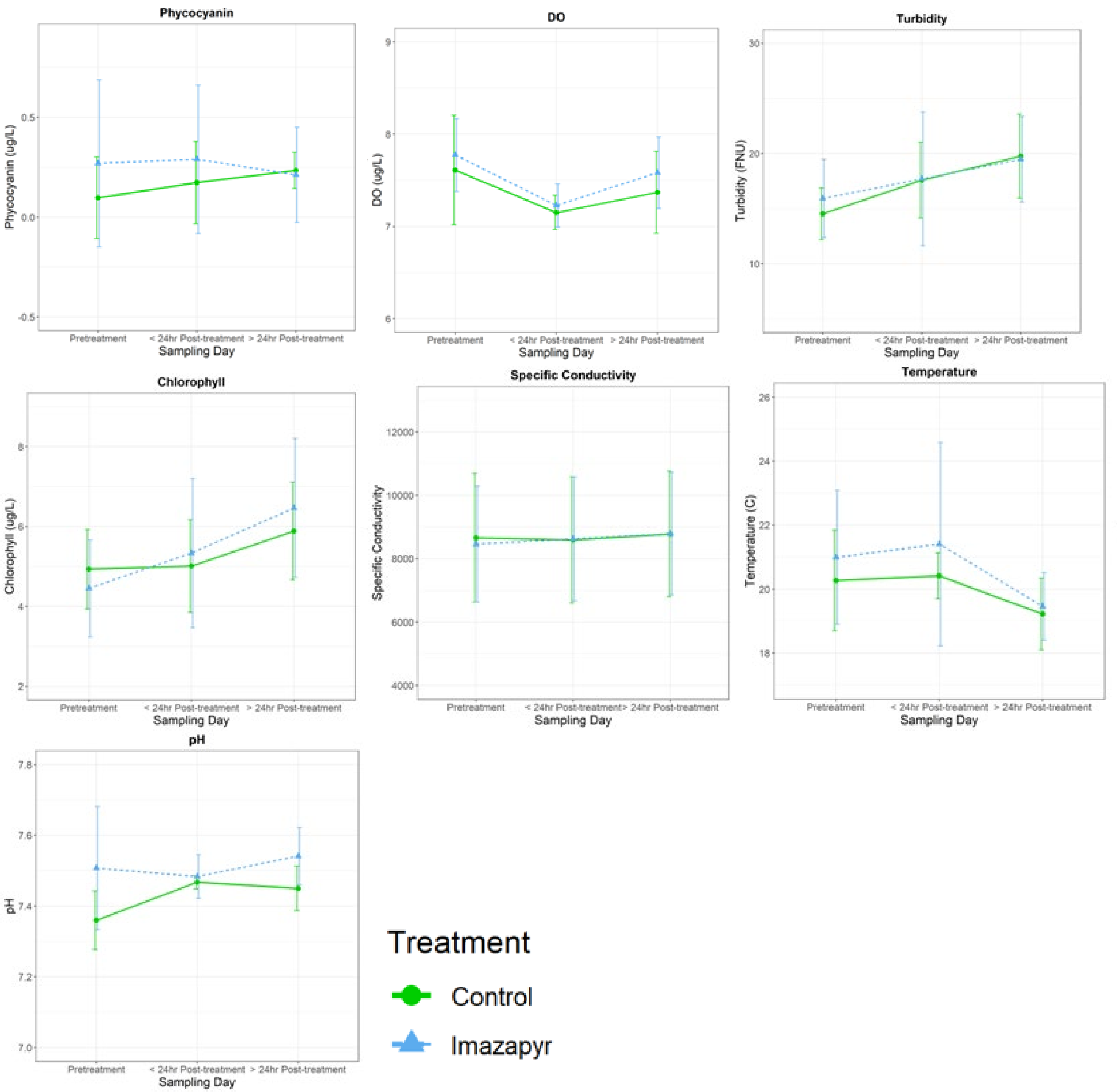


Figure 11. Water Quality Parameters for Imazapyr-treated plots during pre- and post-treatment sampling events.

Tank Mix Water Quality Parameters

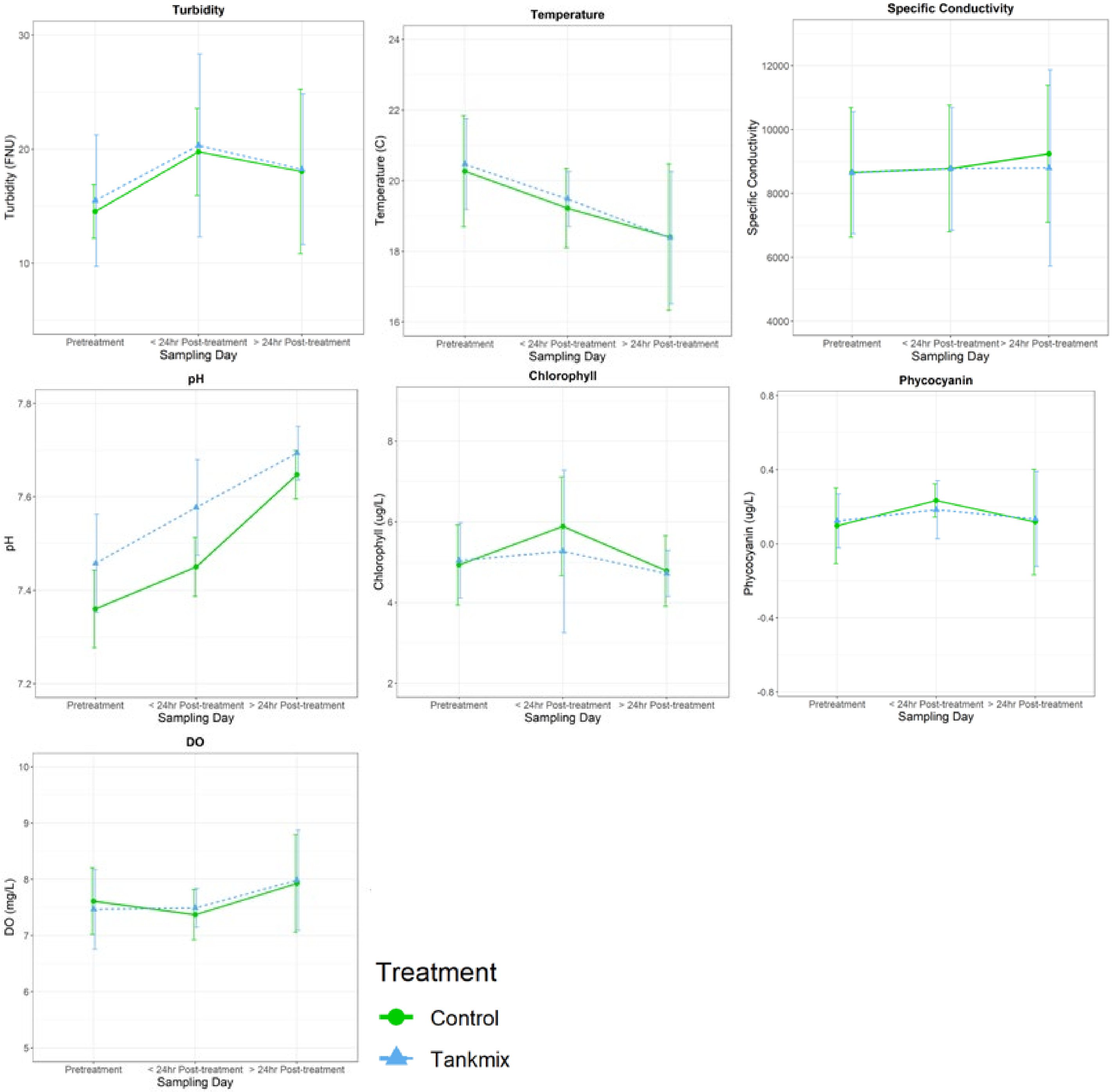


Figure 12. Water Quality Parameters for tank mix-treated plots during pre- and post-treatment sampling events.

Analyses among years

Table 5. Results of one-way ANOVAs among all three years of treatment for water quality parameters with sample time as a factor. Water quality parameters were only analyzed among years when year did not have a significant effect on that parameter.

Herbicide	Sample time	DO %	Chl (ug/L)	PC (RFU)	pH	Turbidity
Glyphosate X Control	*Pre*	F = 0.1934 p = 0.6668	F = 0.7419 P = 0.4036	*H=0.334 p=0.563	F = 1.6234 P = 0.2497	F = 0.0322 P = 0.8602
	Treatment day	H=1.10 P=0.293	F = 0.1272 P = 0.7267	H=0.0110 P=0.916	F = 1.31 P = 0.296	F = 3e-04 P = 0.9873
	Post	F = 0.0193 p = 0.8914	F = 0.0057 P = 0.941	H=0.0249 P=0.875	F = 2.6062 P = 0.1576	H=0.467 p=0.495
Imazapyr X Control	*Pre*	F= 1.0562 P = 0.3153	H=0.304 p=0.581	H=0.135 P=0.713	H=3.83 P=0.504	
	Treatment day	H=0.541 P=0.462	F = 0.201 P=0.6583	H=0.414 P=0.52	H=0.0658 P=0.798	
	Post	F=1.1872 P=0.2877	F=0.707 P=0.4095	F=0.497 P=0.481	F=3.8225 P=0.07907	
Tank Mix X Control	*Pre*	F=0.3148 P=0.5804	F=0.0695 P=0.7946	H=0.0600 P=0.806	F=2.5828 P=0.1391	
	Treatment day	F=0.184 P=0.668	F=0.6322 P=0.435	H=0.00844 P=0.927	F=5.0791 **P=0.04788***	
	Post	H=0.184 P=0.668	H=0.104 P=0.747	H=0.0150 P=0.903	F=1.8364 P=0.2052	

Analyses within year

Table 6. Results of one-way ANOVAs within the three years of treatment for water quality parameters with sample time as a factor. Water quality parameters were analyzed within year when year had a significant effect on water quality parameters.

Herbicide	Sample time	Year	SPC	Temperature	Turbidity
Glyphosate X Control	Pre	2019	F=0.0387 P=0.8505	F=1.5222 p=0.2634	
		2020	F=0.1499 P=0.712	F=0.5633 p=0.4813	
		2021	H=2.08 P=0.149	F=0.8865 0.3828	
	Treatment Day	2019	F=0.3281 P=0.6246	0.5 0.5528	
		2020	F=2.9359 P=0.2288	0.1 0.7818	
		2021	F=0.4892 P=0.5567	0.0092 0.9324	
	Post	2019	F=0.0027 P=0.9633	0.5294 0.5425	

		2020	F=9.9851 P=0.08724	H=0 P=1	
		2021	F=1.4776 P=0.3482	0.5765 0.527	
Imazapyr X Control	Pre	2019	F=0.4578 p= 0.514	H=1.85 P=0.174	0.7244 0.4146
		2020	F=0.0177 p=0.8969	H=2.34 P=0.126	0.2333 0.6395
		2021	F=0.0157 P=0.9027	H=0.588 P=0.443	0.4341 0.5249
	Treatment Day	2019	F=0.1545 P=0.7143	3.4386 0.1373	0.7272 0.4418
		2020	F=15.038 **P=0.01787**	H=1.38 P=0.24	0.1707 0.7007
		2021	H=0.214 P=0.643	0.9464 0.3857	1.4092 0.3009
	Post	2019	F=0.6113 P=0.4913	1.6277 0.2711	0.0213 0.8911
		2020	F=12.011 P=0.02568	0.8571 0.4069	0.1861 0.6884
		2021	F=1.4073 P=0.3012	0.3509 0.5855	0.3184 0.6027
Tank Mix X Control	Pre	2019	F=0.7831 P= 0.397	0.9046 0.364	H=0.721 P=0.396
		2020	F=0.023 P=0.8824	H=1.85 P=0.174	H=1.41 P=0.234
		2021	H=0.462 P=0.497	0.0579 0.8147	0.0482 0.8306
	Treatment Day	2019	F=0.0905 P=0.7785	1.7544 0.2559	H=0.214 P=0.643
		2020	F=8.2914 P=**0.04504**	0.03 0.871	0.1543 0.7145
		2021	F=0.7424 P=0.4375	H=0 P=1	0.1687 0.7023
	Post	2019	F=0.829 P=0.4141	0.0518 0.8311	2.531 0.1868
		2020	F=0.1306 P=0.7361	0.4596 0.535	0.1287 0.7379
		2021	F=2.7589 P=0.1721	0.3048 0.6103	0.0734 0.7998

San Francisco Basin Water Quality Limits

Table 7. NPDES San Francisco Basin Water Quality Limits (From Section 3.3. Objectives for Surface Waters

	Water Quality Objectives	Study Exceedances
Dissolved oxygen (Suisun Marsh objectives)	Acute objective: 3.8 mg/l minimum (daily average) Chronic objective: 5.0 mg/l minimum (30-day running average)	None
Chlorophyll	Waters shall not contain biostimulatory substances in concentrations that promote aquatic growths to the extent that such growths cause nuisance or adversely affect beneficial uses. Changes in chlorophyll a and associated phytoplankton communities follow complex dynamics that are sometimes associated with a discharge of biostimulatory substances. Irregular and extreme levels of chlorophyll a or phytoplankton blooms may indicate exceedance of this objective and require investigation	None
Temperature	The natural receiving water temperature of inland surface waters shall not be altered unless it can be demonstrated to the satisfaction of the Regional Board that such alteration in temperature does not adversely affect beneficial uses. The temperature of any cold or warm freshwater habitat shall not be increased by more than 5°F (2.8°C) above natural receiving water temperature	None
pH	The pH shall not be depressed below 6.5 nor raised above 8.5. This encompasses the pH range usually found in waters within the basin. Controllable water quality factors shall not cause changes greater than 0.5 units in normal ambient pH levels.	None, pH only recorded in 2019
Salinity	Controllable water quality factors shall not increase the total dissolved solids or salinity of waters of the state so as to adversely affect beneficial uses, particularly fish migration and estuarine habitat.	None
Turbidity	Waters shall be free of changes in turbidity that cause nuisance or adversely affect beneficial uses. Increases from normal background light penetration or turbidity relatable to waste discharge shall not be greater than 10 percent in areas where natural turbidity is greater than 50 NTU.	Only one measurement above 50 NTU (52.58), but this was not related to waste discharge.