

Supplemental Information

Impact of beaver ponds on biogeochemistry of organic carbon and nitrogen along a fire-impacted stream

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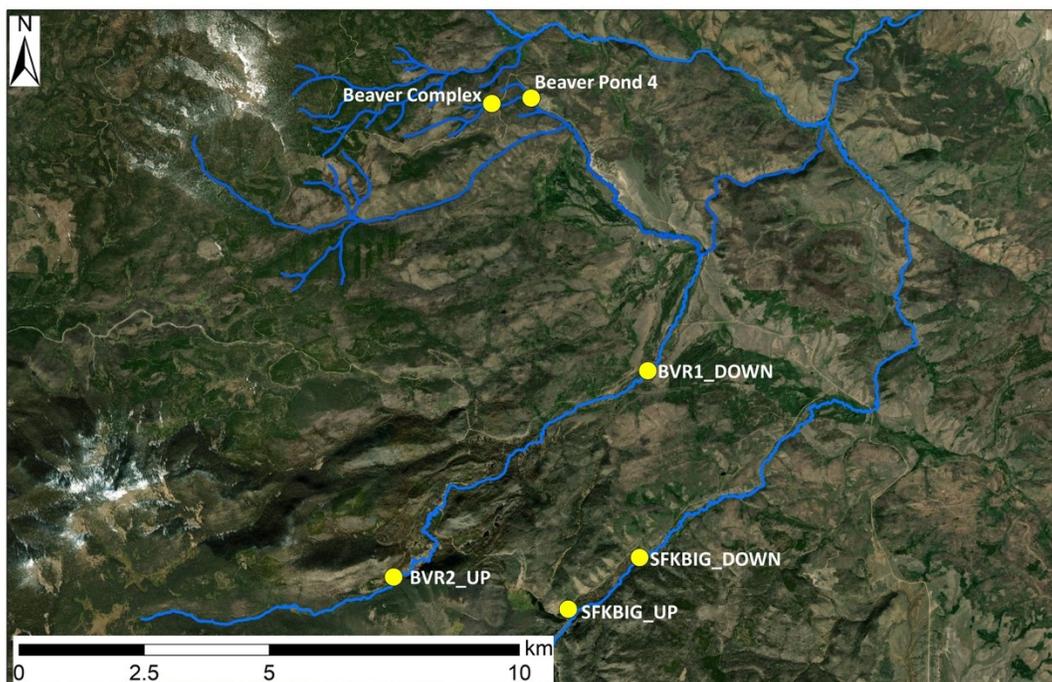


Figure S1: Sampling locations of adjacent beaver ponds outside the Ryan Fire burn scar near the Colorado-Wyoming border. Ponds were used as control ponds for chemical and microbial analyses. BVR1_DOWN = Control Pond 1, SFKBIG_DOWN = Control Pond 2, SFKBIG_UP = Control Pond 3.

Table S1: Average dissolved organic carbon (DOC), dissolved total nitrogen (DTN), and % dissolved organic nitrogen (%DON) for the monthly Ryan Fire water samples collected one-year post-fire (n = 5). Sampling locations located within the burned area are highlighted in gray.

Year	1	Unburned	Upstream	Beaver	Down-	Beaver Pond
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averages			Complex 1	stream	4
DOC (ppm)	4.27 ± 2.27	3.60 ± 0.100	5.59 ± 1.65	6.45 ± 1.84	7.01 ± 1.66
DTN (ppm)	0.16 ± 0.055	0.20 ± 0.076	0.34 ± 0.093	0.35 ± 0.098	0.370 ± 0.049
%DON	53 ± 30.	42 ± 19	70 ± 16	73 ± 17	75 ± 11
C:N	32 ± 22	20. ± 11	16 ± 3.7	18 ± 6.6	21 ± 7.0

Table S2: Results of F test and student's t test calculated to determine significance between sample means of DOC, DTN, %DON, and C:N.¹ All calculations at 95% confidence interval. Results of each test highlighted in blue.

Variables	F test	Upstream	Beaver Complex 1	Downstream	Beaver Pond 4
DOC (ppm)	F _{calculated}	1.9	1.9	1.5	1.9
	F Critical one-tail	6.4	6.4	6.4	6.4
	Result	Equal	Equal	Equal	Equal
DTN (ppm)	F _{calculated}	1.9	2.8	2.3	1.8
	F Critical one-tail	6.4	6.4	6.4	6.4
	Result	Equal	Equal	Equal	Equal
%DON	F _{calculated}	2.5	3.5	3.1	7.1
	F Critical one-tail	6.4	6.4	6.4	6.4
	Result	Equal	Equal	Equal	Unequal
C:N	F _{calculated}	4.1	36	12	10.
	F Critical one-tail	6.4	6.4	6.4	6.4
	Result	Equal	Unequal	Unequal	Unequal
Variables	T test	Upstream	Beaver Complex 1	Downstream	Beaver Pond 4
DOC (ppm)	t Stat	0.60	1.1	1.7	2.2
	t Critical two-tail	2.3	2.3	2.3	2.3
	Result	Not significant	Not significant	Not significant	Not significant
DTN (ppm)	t Stat	1.0	3.8	4.7	4.4
	t Critical two-tail	2.3	2.3	2.3	2.3
	Result	Not significant	Significant	Significant	Significant
%DON	T Stat	0.68	1.1	1.3	1.5
	t Critical two-tail	2.3	2.3	2.3	2.3
	Result	Not significant	Not significant	Not significant	Not significant
C:N	t Stat	1.1	1.6	1.3	1.0
	t Critical two-tail	2.3	2.8	2.8	2.8
	Result	Not significant	Not significant	Not significant	Not significant

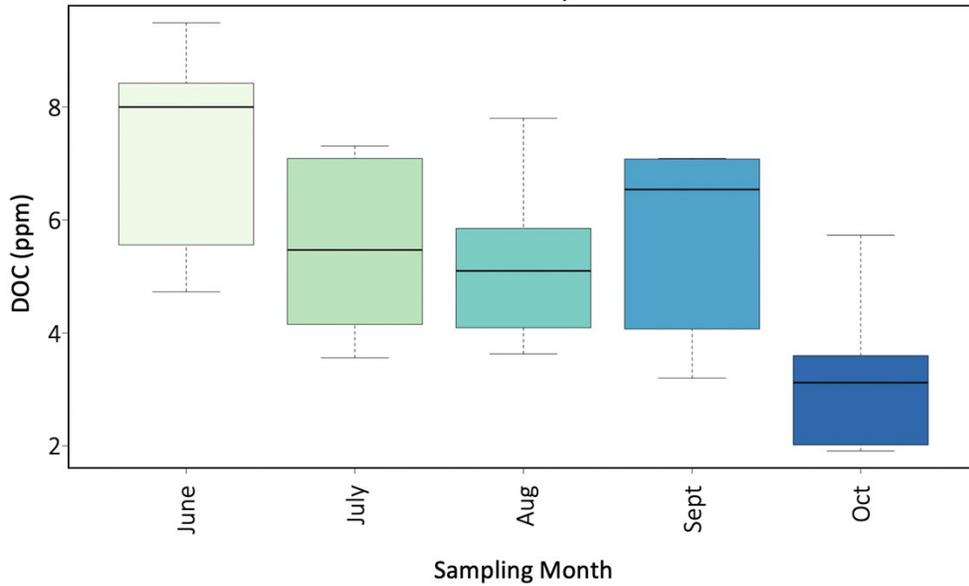


Figure S2: Monthly dissolved organic carbon (DOC) concentrations through the Ryan fire watershed. Each box plot displays the minimum, first quartile, median, third quartile, and maximum values for the dataset, consisting of data from five months of sampling one-year post-fire.

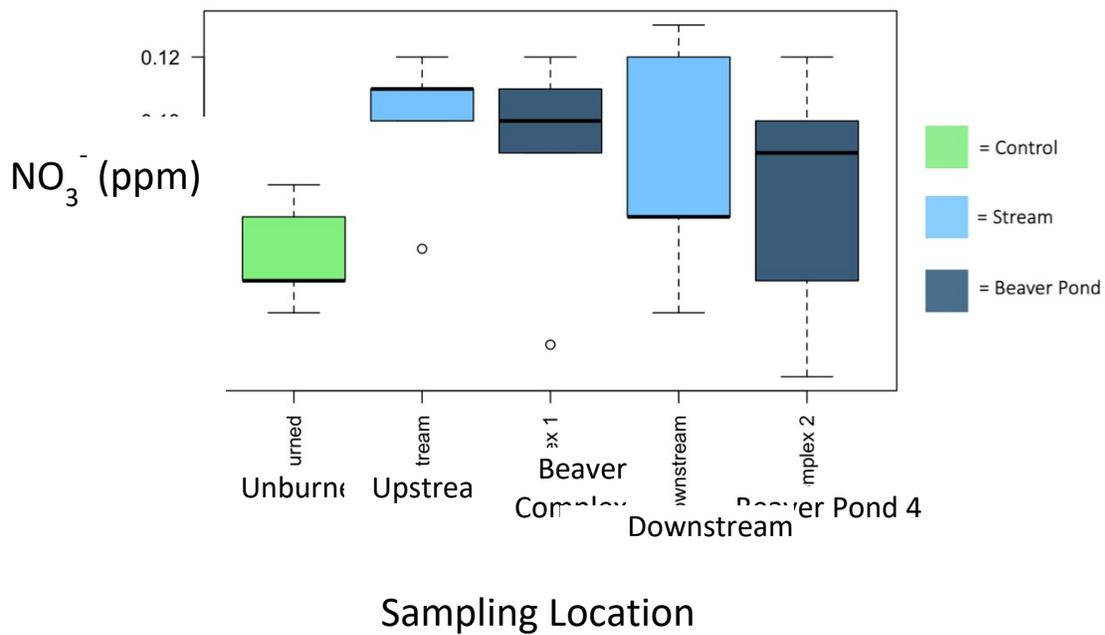


Figure S3: Nitrate (NO_3^-) concentrations through the Ryan fire watershed. Location is plotted on x-axis going from upstream to downstream (left to right) and concentration is plotted on the y-axis. Each box plot displays the minimum, first quartile, median, third quartile, and maximum values for the dataset, consisting of data from five months of sampling one-year post-fire.

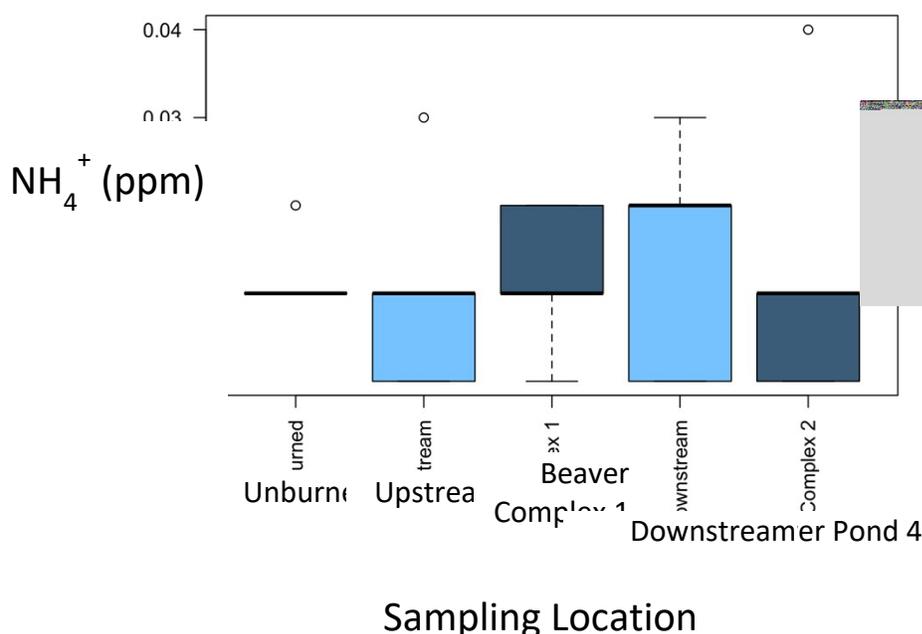


Figure S4: Ammonium (NH_4^+) concentrations through the Ryan fire watershed. Location is plotted on x-axis going from upstream to downstream (left to right) and concentration is plotted on the y-axis. Each box plot displays the minimum, first quartile, median, third quartile, and maximum values for the dataset, consisting of data from five months of sampling one-year post-fire.

Table S3: Abundance-weighted O/C ratios, modified aromaticity indices (AI_{mod}),^{2,3} and Nominal Oxidative State of Carbon (NOSC)⁴ for Ryan Fire formulas detected by -ESI FT-ICR MS.

-ESI	Unburned	Upstream	BP1	BP2	BP3	Downstream	BP4
O/C	0.494	0.514	0.526	0.512	0.509	0.517	0.501
AI_{mod}	0.35	0.38	0.40	0.38	0.37	0.35	0.37
NOSC	-0.058	-0.057	-0.019	-0.057	-0.056	-0.057	-0.095

Equation S1: Relationship between nominal oxidation state of carbon (NOSC) and change in Gibbs free energy for the oxidation of organic matter ($\Delta G^{\circ}_{\text{ox}}$) as described by LaRowe and Van Capellen.⁴

$$\Delta G^{\circ}_{\text{ox}} = 60.3 - 28.5 * \text{NOSC}$$

Table S4: Average molecular weight (MW; in daltons) and calculated C/N ratios and average N per formula for the Ryan Fire +ESI FT-ICR MS data.

+ESI	Unburned	Upstream	BP1	BP2	BP3	Downstream	BP4
MW	644	677	664	616	618	646	621
C/N	58.8	54.9	44.6	33.9	36.8	44.64	35.7
Average N	0.489	0.527	0.635	0.784	0.720	0.593	0.749

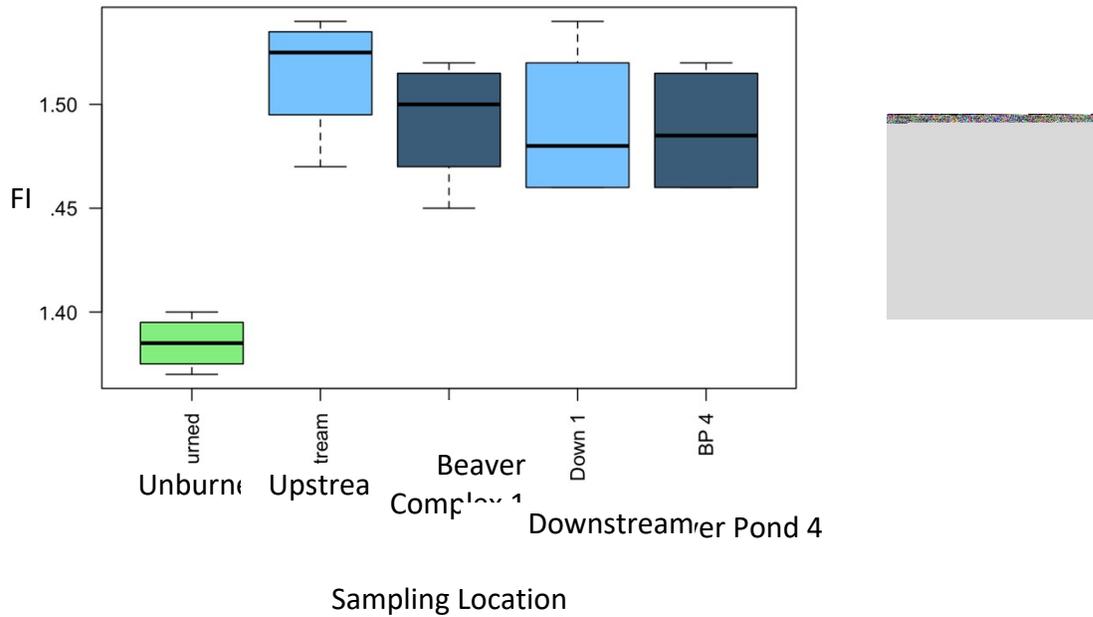


Figure S5: Fluorescence index⁵ through the Ryan fire watershed. Location is plotted on x-axis going from upstream to downstream (left to right) and concentration is plotted on the y-axis. Each box plot displays the minimum, first quartile, median, third quartile, and maximum values for the dataset, consisting of data from five months of sampling one-year post-fire.

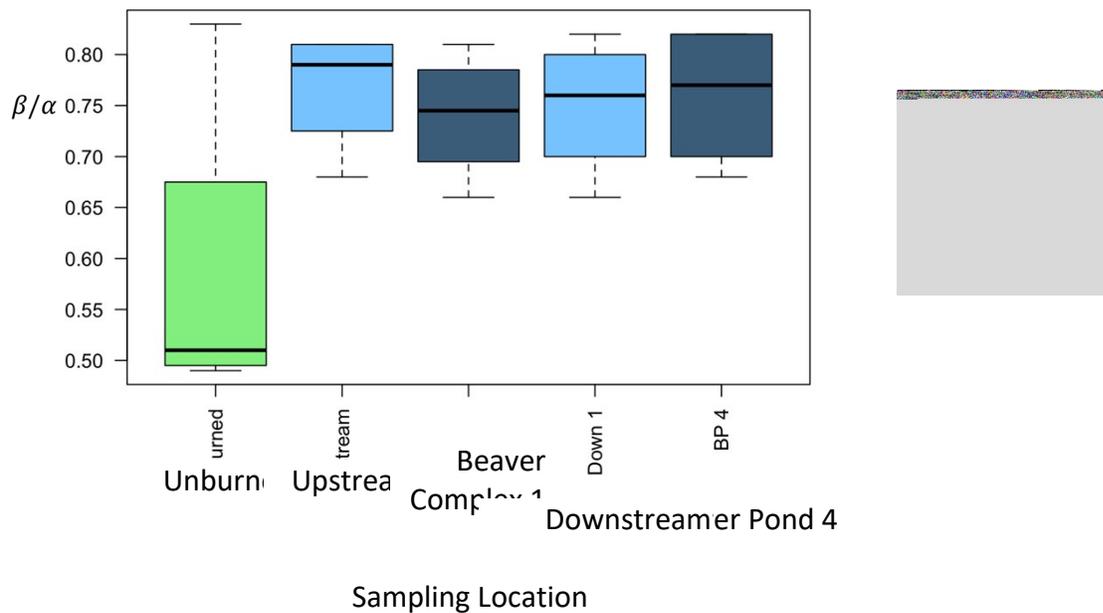


Figure S6: Freshness index⁶ through the Ryan Fire watershed. Location is plotted on x-axis going from upstream to downstream (left to right) and concentration is plotted on the y-axis. Each box plot displays the minimum, first quartile, median, third quartile, and maximum values for the dataset, consisting of data from five months of sampling one-year post-fire.

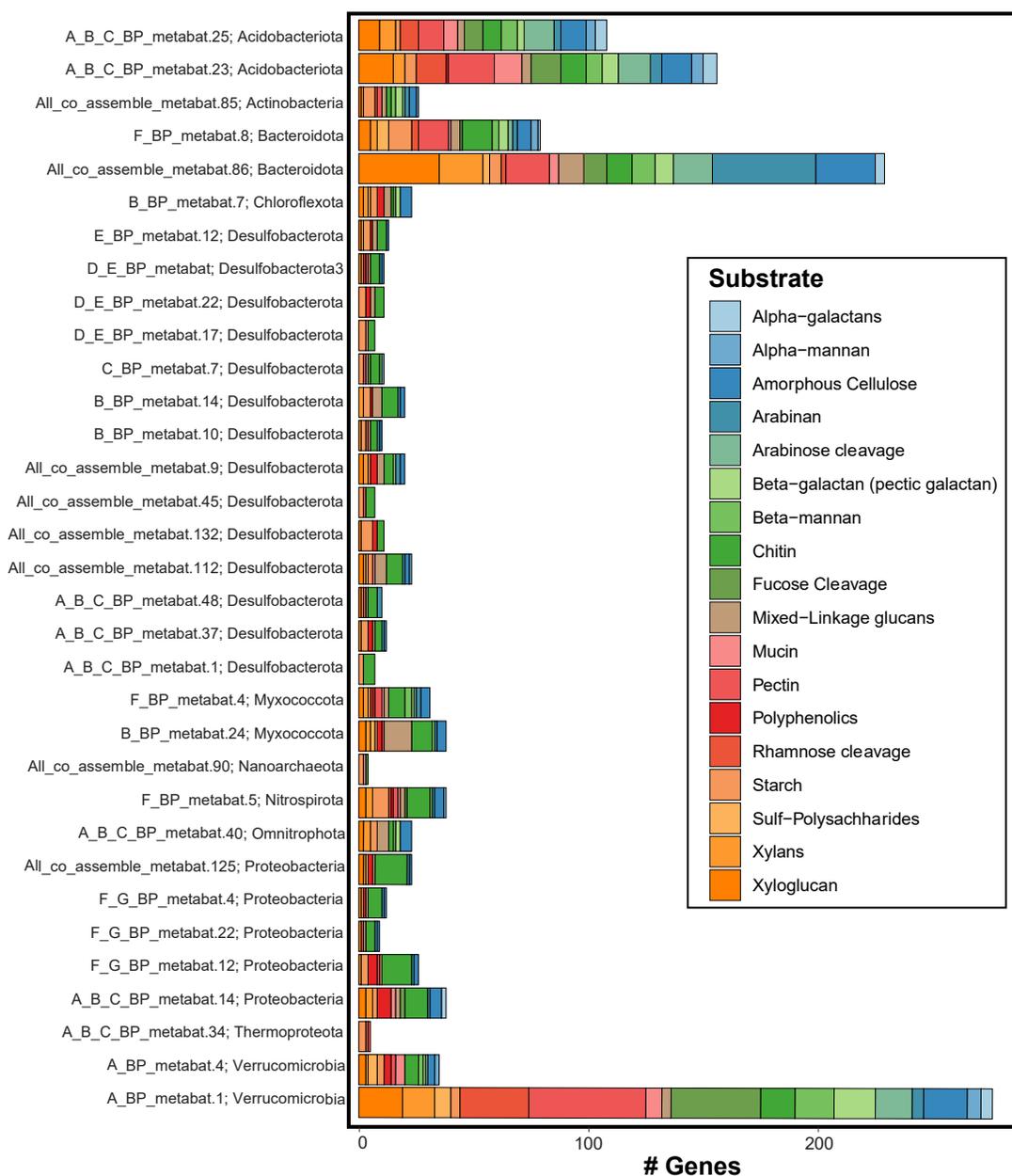


Figure S7: Metagenomics data collected from Ryan Fire-affected beaver pond sediments. The number of CAZymes encoded by each MAG (MAG name and phyla listed along y-axis), colored by the carbohydrate substrate upon which they act.

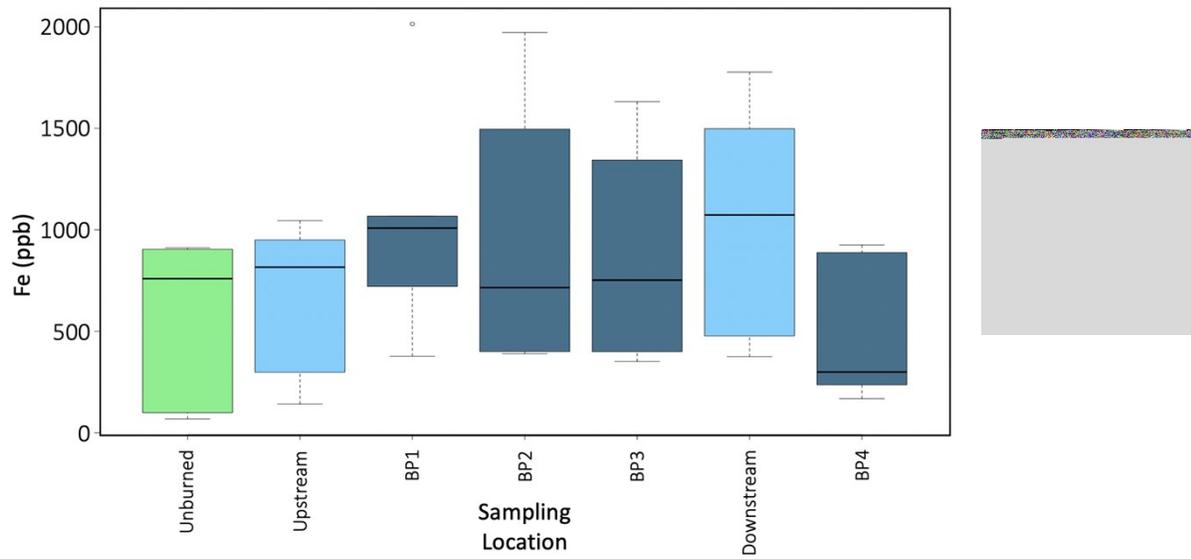


Figure S8: Iron (Fe) concentrations through the Ryan Fire watershed. Location is plotted on x-axis going from upstream to downstream (left to right) and concentration is plotted on the y-axis. Each box plot displays the minimum, first quartile, median, third quartile, and maximum values for the dataset, consisting of data from five months of sampling one-year post-fire.

References

- (1) Harris, D. C. *Exploring Chemical Analysis*, Fifth Edition.; Fiorillo, J., Murphy, B., Hadler, G. L., Simpson, J., Szczepanski, T., Eds.; W.H. Freeman and Company: New York, 2013.
- (2) Koch, B. P.; Dittmar, T. From Mass to Structure: An Aromaticity Index for High-Resolution Mass Data of Natural Organic Matter. *Rapid Communications in Mass Spectrometry* **2006**, *20* (5), 926–932. <https://doi.org/10.1002/rcm.2386>.
- (3) Koch, B. P.; Dittmar, T. Erratum: From Mass to Structure: An Aromaticity Index for High-Resolution Mass Data of Natural Organic Matter (Rapid Communications in Mass Spectrometry (2006) 20 (926-932) DOI: 10.1002/Rcm.2386). *Rapid Communications in Mass Spectrometry* **2016**, *30* (1), 250. <https://doi.org/10.1002/rcm.7433>.
- (4) LaRowe, D. E.; Van Cappellen, P. Degradation of Natural Organic Matter: A Thermodynamic Analysis. *Geochimica et Cosmochimica Acta* **2011**, *75* (8), 2030–2042. <https://doi.org/10.1016/j.gca.2011.01.020>.
- (5) McKnight, D. M.; Boyer, E. W.; Westerhoff, P. K.; Doran, P. T.; Kulbe, T.; Andersen, D. T. Spectrofluorometric Characterization of Dissolved Organic Matter for Indication of Precursor Organic Material and Aromaticity. *Limnology and Oceanography* **2001**, *46* (1), 38–48. <https://doi.org/10.4319/lo.2001.46.1.0038>.
- (6) Parlanti, E.; Woè Rz, K.; Geoëroy, L.; Lamotte, M. Dissolved Organic Matter Fluorescence Spectroscopy as a Tool to Estimate Biological Activity in a Coastal Zone Submitted to Anthropogenic Inputs. *Organic Geochemistry* **2000**, 1765–1781. [https://doi.org/10.1016/S0146-6380\(00\)00124-8](https://doi.org/10.1016/S0146-6380(00)00124-8).