frontiers

Supplementary Material

1 Supplementary Data

1.1 Detailed Physicochemical Characteristics of Experiments

Sample location influenced the physicochemical characteristics of the water used in the experiments. Exp1 water samples were collected from Chaleur Bay, into which the Restigouche River flows (Figure S1). Exp2 and Exp3 water samples were collected in the open Gulf. Exp1 had the lowest salinity samples, with Exp3 having the highest salinities, while the total salinity ranged from 26.92 – 28.04 (Figure S2). The *in situ* chl *a* concentrations were highest for the first 3 samples of Exp1, while the final sample for the last dilution was lower and more similar to concentrations measured across Exp2 and Exp3. *In situ* water temperatures followed the same pattern as those measured in the mesocosms (Figure S3a), although they were generally lower. The difference between the *in situ* temperatures (17.23 ± 1.27 °C) and the mesocosm temperatures (18.69 ± 1.10 °C) was likely driven by the air temperature, with no significant effect of treatment (Table S2).

Although there was a slick on the surface during I1, the experiments were started in the evening and this period occurred during the night so there was no measurable difference in light exposure among treatments (Table S2, Figure S3b). After D1, there was no slick in the oiled enclosures and there was no significant difference in the light at the bottom of the enclosures due to treatment. There was large variation in the light measured at any one time and over the course of each day. This variation may have been due to differences in cloud cover during the day and may also have been influenced by shadows cast by the ship as it moved in relation to the sun.

No additional nutrients were added to the experimental enclosures and the only additional carbon was from the Heidrun or Bunker C additions. NH_4^+, silicate and PO_4^{-3} showed no significant effects of treatment on concentrations during the experiments (Table S2); however, there were differences among experiments due to different nutrient concentrations across the Gulf (Figure S4a – c). The patterns over time varied depending on the parameter measured. Generally, NH_4^+ increased during the incubation periods and decreased with dilution, with the highest concentrations in Exp2. PO_4^{-3} was low in Exp2 and Exp3 and decreased over the course of Exp1. Most of the samples indicate P limitation in this system, with N dominated by NH_4^+. Silicate changed little over the course of Exp2 and Exp3, but decreased over time in Exp1 as the seawater used for dilution was collected further into the gulf. Neither TOC nor TIC showed any clear patterns over time (Figure S4d and e), although both were variable among enclosures. Although approximately 3600 mg of carbon was added to each oil amended enclosure, which would represent 300 mg L^{-1}, there was no significant increase in the TOC concentrations in the oil amended enclosures, indicating most of the oil remained on the surface.

1.2 Hydrocarbon Concentrations in Control Enclosures

TPH concentrations in samples from control enclosures were always below the reporting limit of 5×10^4 ng L^{-1}. A few samples did contain detectable PAHs, with most PAHs detected in the control enclosures being C1, C2 and C3 naphthalenes (36 samples). Twelve samples contained acenaphthene.

Based on the profile, one sample from 21 h may have been contaminated with exhaust from the vessel, but the source of the other PAHs is unclear..

2 Supplementary Figures and Tables

2.1 Supplementary Figures

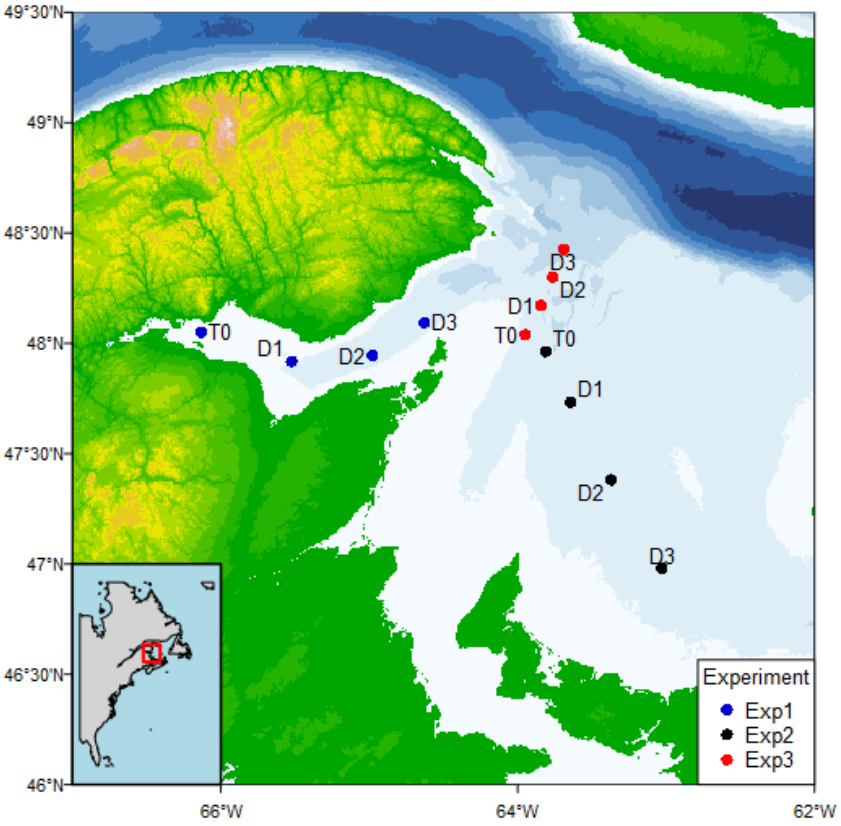

Figure S1. Map of sampling locations in the southern Gulf of St. Lawrence. The experiments are indicated by color with the labels indicating either the beginning of the experiment (T0) or each dilution (D1- D3). Shading in the ocean indicates depth. The inset map indicates the location the Gulf relative to the eastern coast of North America.

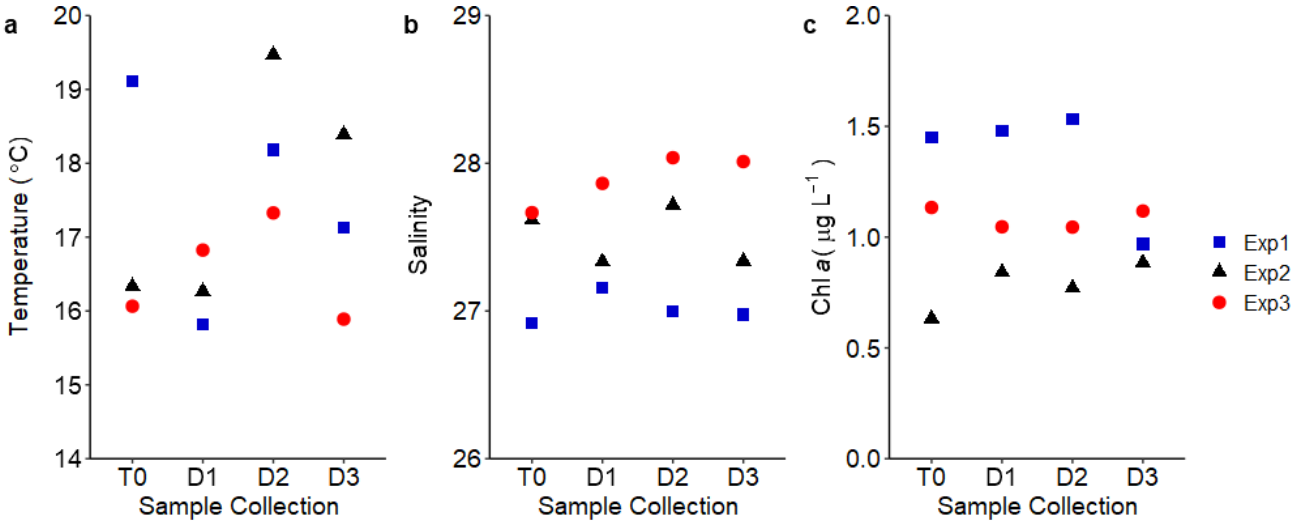

Figure S2. Temperature (a), salinity (b) and chl *a* concentrations (c) from the CTD data collected with the water samples. T0 samples were used to initiate the experiments with D1-D3 collected at 12, 24 and 36 h and used for dilutions. Samples were collected at the locations shown in Figure S1 with colors matching the experiments in the map.

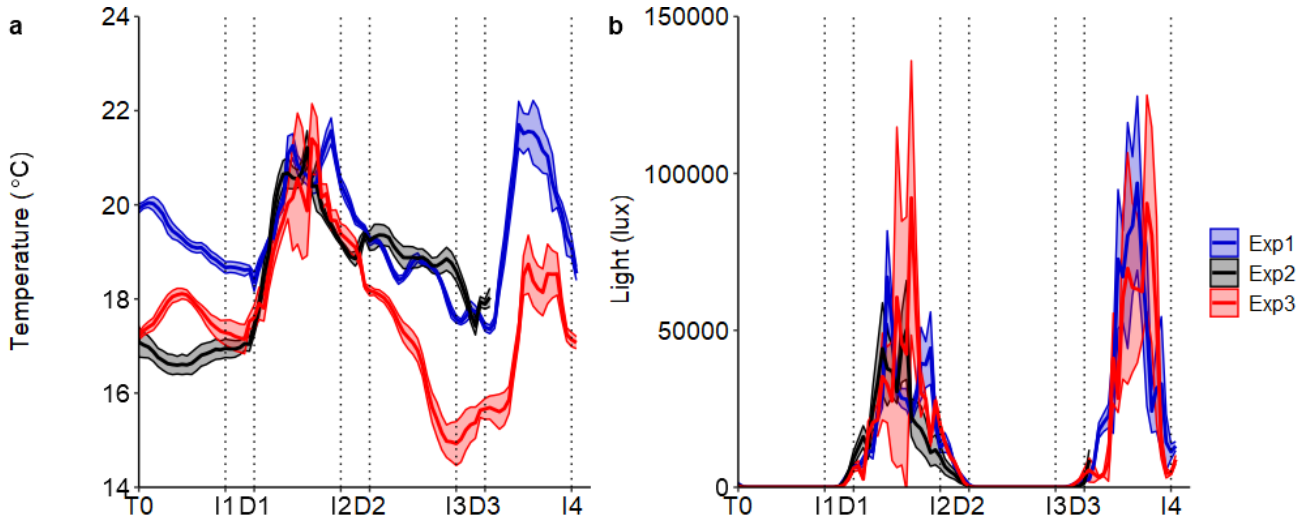

Figure S3. Average temperature (a) and incident light (b) within the enclosures over the course of the three experiments. The shading represents the standard deviation of the 9 enclosures pooled across treatments for each experiment as there was no significant effect of treatment on either temperature or light x-axis labels indicate the sampling period as described in Figure 1. Vertical dashed lines indicate sampling times corresponding to the end of an incubation (I1-4) or a dilution (D1-3). Colors follow those in Figure S1.

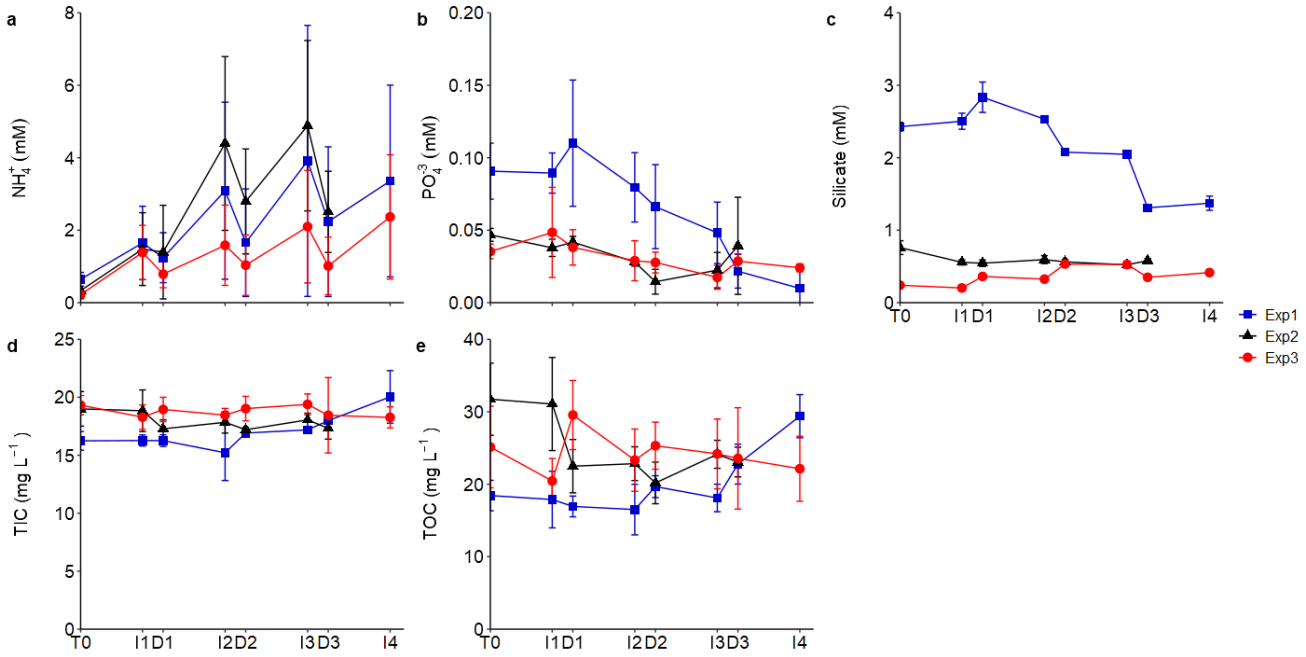

Figure S4. Average concentration of inorganic nutrients (a – c) and carbon (d –e) in the three experiments. There was no significant treatment effect for any of these parameters and all treatments have been pooled across each experiment. Error bars are standard deviations based on n=9. Note the differences in the y-axes. x-axis labels indicate the sampling period as described in Figure 1.

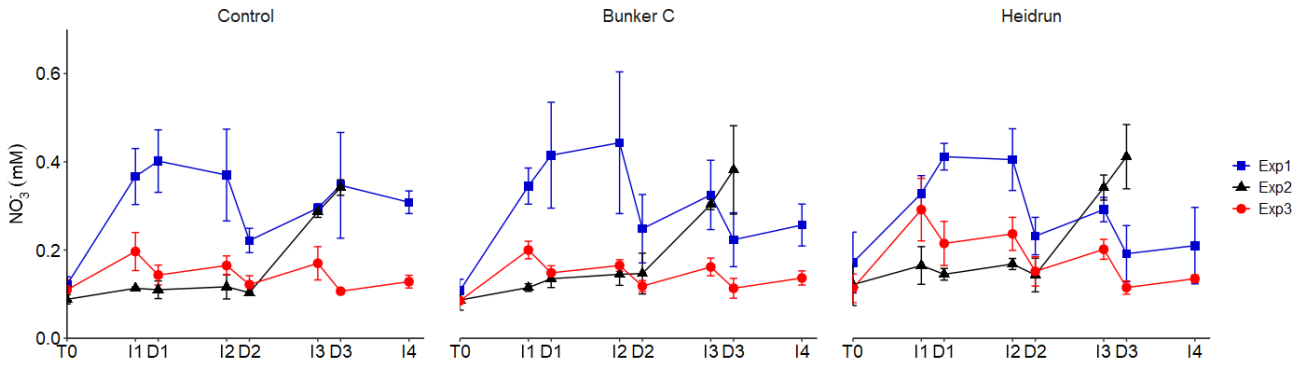

Figure S4. Average concentration of $NO3^-$ separated by treatment for the three experiments. Overall, concentrations with Heidrun were higher compared to control and Bunker C. Error bars are standard deviations based on n=3. x-axis labels indicate the sampling period as described in Figure 1.

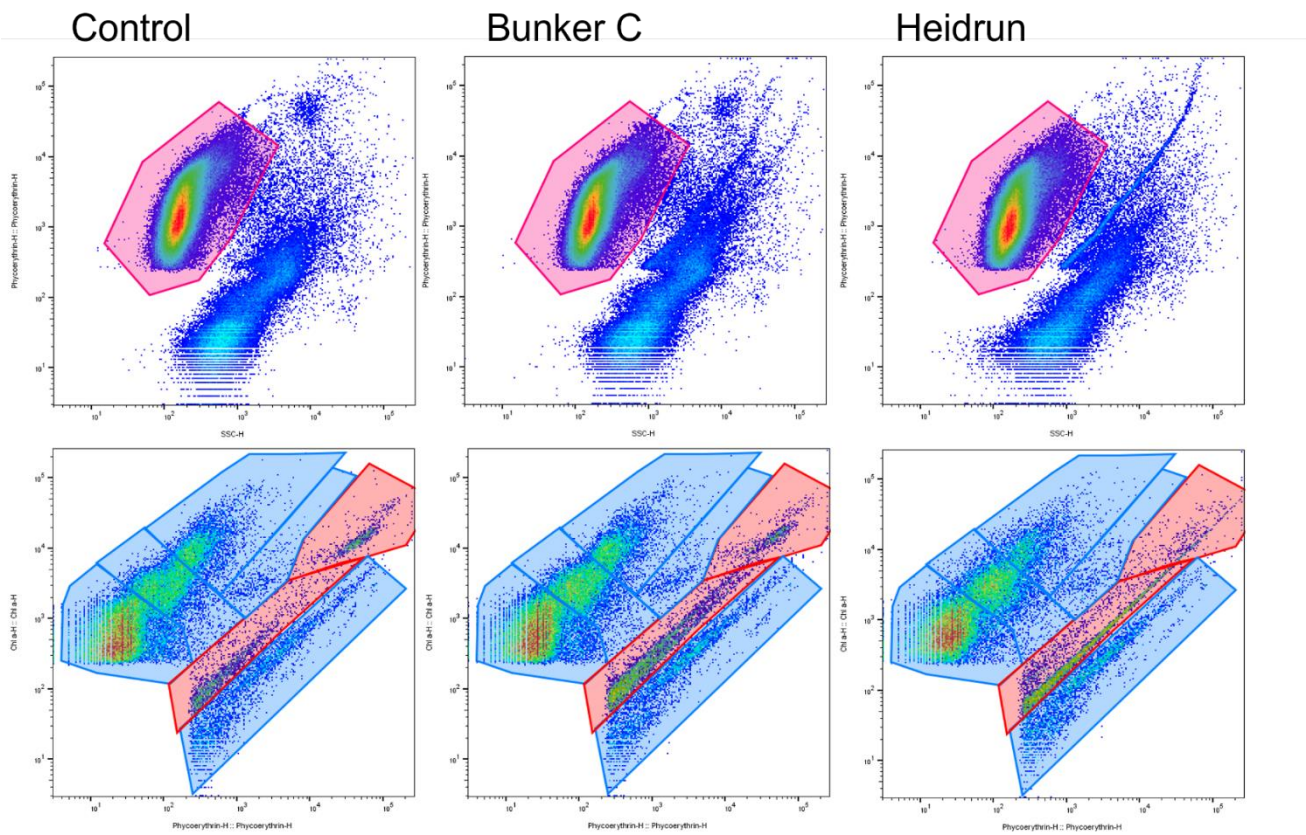

Figure S6. Dot plots of flow cytometry data from samples from Exp1, I1 from each of the three treatments. The top row represents a plot of phycoerythrin (y-axis) vs side scatter (x-axis). The pink highlighted region represents the *Synechococcus* cells and the blank round space was where the beads were removed. The bottom row represents chl *a* fluorescence (y-axis) vs phycoerythrin (x-axis). The red highlighted regions represent the two populations affect by oil droplets while the blue highlighted regions represent the populations combined as small phytoplankton in Figure 6.

Supplementary Tables

Table S1. GAMMs tested for each dependent variable (DV). ΔAIC values were used to select the most appropriate model. Time was included as a continuous variable with treatment a three-level factor (Control, Bunker C or Heidrun). The smoothing parameter estimation used the restricted maximum likelihood method (REML) and penalized cubic regression spline smooths (bs="cr"). Enclosure (B1 – B27) was nested in Experiment (Exp1, Exp2 and Exp3) with random effect splines (bs="re"). Interpretations are based on Alguero-Muniz *et al*. (2017) and Calderó-Pascual *et al*. (2021).

Model		Interpretation
1.	DV ~ s(Time, k=8, bs="cr") + s(Enclosure, by=Experiment, bs="re")	The DV varies over time **independent of treatment**, with no difference in the absolute values due to treatment. Individual mesocosms and experiments may have different initial starting values.
2.	DV ~ s(Time, k=8, bs="cr") + Treatment + s(Enclosure, by= Experiment, bs="re")	The DV varies over time **independent of treatment**, with **absolute values** varying depending on the treatment applied. Individual mesocosms and experiments may have different initial starting values.
3.	DV ~ s(Time, k=8, bs="cr") + s(Time, by=Treatment, k=8, bs="cr") + s(Enclosure, by= Experiment, bs="re")	The DV varies over time with **different patterns** depending on the treatment applied. Individual mesocosms and experiments may have different initial starting values.
4.	DV ~ s(Time, k=8, bs="cr") + Treatment + s(Time, by=Treatment, k=8, bs="cr") + s(Enclosure, by= Experiment, bs="re")	The DV varies over time with **different patterns and absolute values** depending on the treatment applied. Individual mesocosms and experiments may have different initial starting values.

Table S2. Results of the GAMM analyses for each of the parameters measured. The best model, as defined by the simplest model with a $\Delta AIC \leq 2$ is indicated. For the best model, the adjusted R^2 and the % deviance explained is presented. Models and their interpretations are described in Table S1.

Parameter	Best Model	R^2	Deviance Explained	Parameter	Best Model	R^2	Deviance Explained
Physicochemical				**Microbial Abundances**			
Temperature	1	0.703	73.6	Prokaryote	1	0.532	56.1
Light	1	0.771	77.8	Chl *a*	3	0.796	82.6
NO_3^-	2	0.476	51.7	Eukaryotic phytoplankton	3	0.750	76.4
NH_4^+	1	0.869	88.9	*Synechococcus*	1	0.590	66.1
PO_4^{-3}	1	0.490	52.0	Oil and phytoplankton	3	0.811	92.6
Silicate	1	0.886	89.3				
TIC	1	0.243	29.9				
TOC	1	0.199	24.4				
Hydrocarbon Measurements				**Microbial Diversity**			
TPH	4	0.945	95.4	Prokaryote Chao1	1	0.602	64.6
ΣPAH	4	0.897	90.9	Microeukaryote Chao1	1	0.400	44.3
Σalkylated PAH	4	0.745	77.2	Prokaryote Shannon Diversity	3	0.594	65.3
Light Inhibition	4	0.851	87.0	Microeukaryote Shannon Diversity	1	0.538	57.2

Table S3 Percentage of the total PAHs (parent and alkylated homologs) identified as 2-ring PAHs at each sample time. Enclosures from all three experiments were pooled with the mean and standard deviation shown (n=9, except I4 n=6). From Table 1, 2-ring PAHs represented 21.62 and 63.28% of fresh Bunker C and Heidrun, respectively.

Time	Bunker C	Heidrun
T0	97.92 (3.09)	75.99 (22.26)
I1	68.26 (7.12)	74.10 (8.30)
D1	64.72 (12.13)	69.94 (8.99)
I2	26.44 (22.40)	39.80 (20.29)
D2	50.79 (37.89)	59.78 (40.93)
I3	87.81 (15.50)	97.94 (2.63)
D3	98.10 (2.92)	99.45 (1.64)
I4	100*	79.88 (34.86)

*when detected, not present in all enclosures

Table S4 Distances between the centroids for 0 h between each pair of experiments (bold). Along the diagonal the average distance between each sample and the experimental centroid is given (italics).

Prokaryotes			
	Exp1	Exp2	Exp3
Exp1	*0.0721*		
Exp2	**0.3176**	*0.0440*	
Exp3	**0.3515**	**0.2089**	*0.0458*
Microeukaryotes			
	Exp1	Exp2	Exp3
Exp1	*0.0932*		
Exp2	**0.6862**	*0.0708*	
Exp3	**0.6971**	**0.2192**	*0.0917*

Table S5. PERMANOVA analysis of prokaryote and microeukaryote community composition for each experiment using Adonis2 in the vegan package. Permutations were restricted using enclosure as the strata. Significant p-values are highlighted with bold.

	Exp1				Exp2				Exp3			
Prokaryote	**DF**	**R^2**	**F**	**P**	**DF**	**R^2**	**F**	**P**	**DF**	**R^2**	**F**	**P**
Treatment	2	0.067	3.263	**0.001**	2	0.037	2.549	**0.001**	2	0.060	3.499	**0.001**
Time	1	0.243	23.599	**0.001**	1	0.522	71.069	**0.001**	1	0.351	40.714	**0.001**
Treatment×Time	2	0.020	0.9769	0.217	2	0.228	1.556	0.199	2	0.020	1.173	0.259
Residual	65	0.670			57	0.418			66	0.569		
Total	70	1.000			62	1.000			71	1.000		
Microeukaryote	**DF**	**R^2**	**F**	**P**	**DF**	**R^2**	**F**	**P**	**DF**	**R^2**	**F**	**P**
Treatment	2	0.026	1.246	**0.001**	2	0.028	1.606	**0.001**	2	0.017	0.730	**0.001**
Time	1	0.299	29.217	**0.001**	1	0.460	52.046	**0.001**	1	0.190	15.964	**0.001**
Treatment×Time	2	0.010	0.479	0.902	2	0.009	0.511	0.840	2	0.009	0.393	0.958
Residual	65	0.666			57	0.503			66	0.784		
Total	70	1.000			62	1.000			71	1.000		