DRAFT for Dr. Okello: Nutrient Enrichment Mesocosm Experiment on Napoleon Gulf Technical Report

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1.0 Introduction

Nutrient enrichment of water bodies by human activities, through non-point source pollution (eg. agriculture, livestock) and end of pipe effluent (eg. water treatment facilities), is understood to have long term detrimental effects to water quality and wildlife. When nutrients in the form of nitrogen and phosphorus compounds are in excess, a water body is deemed to be eutrophic. Algal mats or *blooms* can rapidly colonize and blanket the water's surface in nutrient-rich lakes. In the worst cases, entire lakes have been covered beneath thick algal blooms. Harmful algal blooms (HABs), those rich in cyanobacteria (eg. *Microcystis*, *Anabaena* spp.) for example, create negative health effects not only for aquatic wildlife but also humans, their livestock, and other animals exposed to these blooms and the waters they contaminate. Cyanobacteria out-compete other phytoplankton communities through a quick and efficiently soaking of excess nutrients, such as urea [$CO(NH_2)_2$] and build allelopathic compounds which are understood to poison their competitors. These compounds collectively described as cyanotoxins (eg. microcystin) are semi-persistent and vary in the potency of their toxic effects but research shows they are hepatotoxic, neurotoxic and cause contact dermatitis. Furthermore, as blooms decompose following episodes of high nutrient availability, they deplete dissolved oxygen resulting in hypoxia which can kill fish and other aquatic organisms not adapted to anoxic conditions. Through these effects' algal blooms and their associated environmental toxicants can destabilize ecosystems and bias lakes to certain species, which can further disrupt food webs and nutrient flows.

Some lakes are hypereutrophic due to constant input of nutrients by human activities and high degree of settlement along lakes. The economic incentive which capture fisheries in the African Great Lakes represent has created a situation where communities with productive fish are experiencing population growth through immigration following the development of the industrial fish trade in Uganda. Those who are drawn to the fish trade are often the most economically

disadvantaged and have few skills to ply other than subsistence agriculture. Land use change in the form of industry, subsistence and commercial agriculture, animal grazing and settlement, are deemed to be contributing to the eutrophication of water bodies in East Africa. Without infrastructure development to support positive livelihoods and good health, both people and the ecosystems they depend upon are at risk.

Algal blooms are expected to increase without cooperation between government agencies to create effective nutrient management strategies which are equitable to local communities to create sustainable outcomes. With the hope of demonstrating the effects of nutrient pollution and creating effective policy solutions, we sought to understand the outcomes for algal biomass and community composition due to nutrient enrichment by urea and other nitrogenous compounds in a tropical freshwater lake. Following the methodology of Finley *et al.* (2010) we conducted a 3600-litre mesocosm experiment within Napoleon Gulf in Jinja, Uganda. A discussion of the social implications for the ongoing use of these freshwater resources and their viability as a renewable resource follows.

2.0 Field Work / Experimental Design

Overview

A nutrient enrichment experiment was designed to measure in situ the impacts of different forms of nitrogen on cyanobacteria growth in Napoleon Gulf on Lake Victoria, Uganda. The experiment was made up of seven treatments and a control with three replicates for each. The treatments were Phosphorus (P), Urea, Ammonium (NH_4), Nitrate (NO_3), Urea and Phosphorus (Urea + P), Ammonium and Phosphorus (NH_4 + P) and Nitrate and Phosphorous (NO_3 + P). The experiment was comprised of 3 trials over the course of June, July and August. Due to unforeseeable issues within the duration of the trials, the mesocosm design and sampling technique varied. Therefore, the report only documents the findings from Trial 3 as Trials 1 and 2 were deemed unable (their results can be found in appendix X).

Objectives

1. Design and deploy a mesocosm experiment
2. Measure in-situ field conditions
3. Measure in-situ chl-a growth
4. Collect water samples for chl-a and nutrient analysis

Trial 1 Overview

Trial 1 ran from June 2nd to June 24th (22 days) to determine the initial growth curve of cyanobacteria in the mesocosm bags and troubleshoot flaws in the experimental design. Water quality measurements were taken every other day and the treatment nutrients were added to the experiment on Day 0, 7, 14 and 21. The growth curve was estimated by using in-situ measurements of chl-a collected by a YSI EXO2 multiparameter measured.

Mesocosm Units

The mesocosm bags were made two days before the deployment date of June 2nd using materials sourced from Jinja Central Market, NaFIRRI and Makerere University. The design of the mesocosm units were based off of a similar experiment conducted at NaFIRRI by Dr. Okello. The units were comprised of plastic sheets (177cm in width and 150cm in height) heat-sealed at the bottom, a plastic ring (X in dimeter) and an inflatable tube, seen in figure X. The mesocosm bags were filled with 150 L of lake water which was filtered through a XX mesh sieve on Day 0 at NaFIRRI Farm.

Nutrients

The nutrients were added to the experiment on Day 0, 7, 14 and 21, and stock solutions were prepared the night before to ensure associated treatments would receive 0.02 mg/L of phosphorous and 0.5 mg/L of nitrogen. The chemicals used for each treatment were Urea ($[NH_2]2CO$), Ammonium Chloride (NH_4Cl), Potassium Nitrate (KNO_3), and Potassium Phosphate Dibasic (K_2HPO_4). The concentrations for each treatment were 3.609 mg/L of KNO3, 1.072 mg/L of $[NH_2]2CO$, 1.909 mg/L of NH_4Cl, and 0.112 mg/L of K_2HPO_4, the calculations are outlined in section 6.X. The nutrients were added before field samples were collected, on the enrichment days of 0, 7, 14 and 21.

In Field Measurements

In-situ measures were taken every other day at approximately 11 am using a YSI EXO2 multiparameter and HACH probe. The YSI EXO2 multiparameter measured pH [-], temperature [°C], conductivity [µS/cm], specific conductivity [µS/cm], chlorophyll-a [µg/L] and turbidity [FNU]. Next, each replicate was stirred thoroughly before measurements were taken. The YSI EXO2 took measurements every second for approximately one minute per replicate and the probe was rinsed in lake water between treatments. The average for each measurement in the replicates was calculated after. The HACH probe was used to measured dissolved oxygen [mg/L] at a single value in each replicate.

Results

The data collected from Trial 1 was unusable due to the failure of the plastic structure of the mesocosm bags over the course of the trial. The trial 1 Chl-a measurements were used, to help identify when sampling for nutrient analysis for the sequential trials. After trial 1 it was determined that new plastic would be needed and the sidings of the mesocosm bags would need to be reinforced with duct tape. The data collected from Trial 1 is reported in Appendix VVV.

Trial 2

Trial 2 ran from June 27th to June 28th and the data was unusable due to multiple punctures in the mesocosm bags. The results from this experiment were not reported but the trial failures were used to improve the mesocosm design for trial 3.

Trial 3 Overview

Trial 3 ran from July 3rd to July 23rd (14 days) to measure how cyanobacteria growth responded to different sources of nutrients (P and N). Water quality measurements and samples were taken every day at approximately 10 am to measure field conditions and daily chl-a response. On Day 0, 3, 7 and 14 larger water samples were collected for further nutrient and chl-a analysis and the nutrients were added on days 0 and 7.

On Day 0 the water quality data (pH, temperature, conductivity, specific conductivity, chlorophyll-a and turbidity) was collected by using the YSI EXO2 multiparameter and the HACH probe was used to collect dissolved oxygen. On Day 1 the water quality data was collected with both the YSI EXO2 multiparameter and HACH probe for pH, millivolts, temperature, conductivity, dissolved oxygen and percent saturation for a correction factor. The rest of the trial used only the HACH probe, although Days 10 – 14 were measured in the lab from samples collected from each replicate, due to difficulty with the HACH probe.
50 mL samples were collected each day from the individual replicates after each replicate was stirred to ensure the water column was mixed. These samples were stored in a cooler and brought back to the lab for analysis with the trilogy, further described in section X.X. 1-liter samples were collected on days 0, 3, 7, and 14 that were stored in a cooler and brought back to the lab for nutrient and chl-a analysis, described in section X.X.

3.0 Laboratory Analysis

The following section describes the laboratory analysis completed for trial 3 of the Napoleon Gulf mesocosm experiment. The methods used were based on the standards for XXX.

6.1 *Objectives*

1. Preparation of stock solutions for each nutrient enrichment treatment day (Day 0 and 7) for trial 3
    a. Urea ($[NH_2]2CO$)
    b. Ammonium Chloride (NH_4Cl)

c. Potassium Nitrate (KNO_3)
d. Potassium Phosphate Dibasic (K_2HPO_4).

2. Analysis daily water samples with the Trilogy to measure for Chl-a RFU
3. Filtering trial 3 samples from Day 0, 3, 7 and 14 for High Performance Liquid Chromatography (HPLC), microcystins congeners (MC-Cong) and Chl-a extraction
4. Nutrients analysis for Day 0, 3, 7, and 14
    a. Soluble reactive phosphorous (SRP)
    b. Total dissolved phosphorous (TDP)
    c. Total dissolved nitrogen (TDN)
    d. Ammonium (NH_4)
    e. Nitrate (NO_3)

6.2 *Nutrient Solutions*

The experimental design for trial 3 consists of eight treatments (CTRL, P, Urea, NH_4, NO_3, Urea + P, NH_4 + P and NO_3 + P), each containing three replicates that receive nutrient enrichment on Day 0 and Day 7. Each replicate of Urea, NH_4, NO_3, Urea + P, NH_4 + P and NO_3 + P receives 0.5 mg/L of Nitrogen. Each replicate of P, Urea + P, NH_4 + P and NO_3 + P, receives 0.02 mg/ L of phosphorus. Stock solutions were made for Urea, Ammonium Chloride, Potassium Nitrate and Potassium Phosphate Dibasic, for each of the treatment enrichment days. It was decided that 100 mL of the stock solution for Urea, Ammonium Chloride and Potassium Nitrate would be added to each of the according treatments and replicates and 50 mL of Potassium Phosphate Dibasic to each treatment requiring a P molecule. This same method was competed for trial 1 (Day 0, 7 and 14), trial 2 (Day 0) and trial 3 (Day 0 and 7).

CTRL 1	P 1	Urea 1	NH_4 1	NO_3 1	Urea + P 1	NH_4 + P 1	NO_3 + P 1
CTRL 2	P 2	Urea 2	NH_4 2	NO_3 2	Urea + P 2	NH_4 + P 2	NO_3 + P2
CTRL 3	P 3	Urea 3	NH_4 3	NO_3 3	Urea + P 3	NH_4 + P 3	NO_3 + P 3

The following section outlines the calculations to determine the mass needed for each compound 600 mL of stock solution for each treatment.

Urea

$$N/Urea = \frac{14.0067\ (\mathrm{g}/mol) * 2}{60.6\ (\mathrm{g}/mol)}$$

$$N/Urea\ = 0.138538642\ [-]$$

$$C_{Urea} = \frac{0.5\ (mg/L)}{0.466423576\ (-)}$$

$$C_{Urea} = \ 1.071986978\ \ (mg\ /L)$$

$$M_{Urea} = \frac{1.071986978\ \ (mg\ /\ L\ ) *\ 150\ (L\ )}{1000\ (mg\ /\ g\ \ )}$$

$$M_{Urea} = \ 0.160798047\ \ [g] * 6\ [replicates]$$

$$M_{Urea} = \ 3.24819122\ [\mathrm{g}]$$

Therefore 3.248 g of Urea was measured in the lab and mixed into 600 mL of distilled water. Next, 100 mL of Urea stock solution was added in-situ to the replicates of Urea and Urea + P treatments.

N/NH_4Cl

$$N/\mathrm{NH4Cl} = \frac{14.0067\ (\mathrm{g}/mol) * 2}{53.491\ \ (\mathrm{g}/mol)}$$

$$N/\mathrm{NH4Cl}\ = 0.261851526\ [-]$$

$$C_{\mathrm{NH4Cl}} = \frac{0.5\ (mg/L)}{0.261851526\ (-)}$$

$$C_{\mathrm{NH4Cl}} = \ 1.909479035\ \ (mg\ /L)$$

$$M_{\mathrm{NH4Cl}} = \frac{1.909479035\ \ (mg\ /\ L\ ) *\ 150\ (L\ )}{1000\ (mg\ /\ g\ \ )}$$

$$M_{\mathrm{NH4Cl}} = \ 0.286421855\ [g] * 6\ [replicates]$$

$$M_{\mathrm{NH4Cl}} = \ 1.71853113\ [\mathrm{g}]$$

Therefore 1.719 g of NH_4Cl was measured in the lab and mixed into 600 mL of distilled water. Next, 100 mL of NH_4Cl stock solution was added in-situ to the replicates of NH_4 and NH_4 + P treatments.

KNO3

$$N/KNO3 = \frac{14.0067\ (\mathrm{g}/mol)}{101.1032\ \ (\mathrm{g}/mol)}$$

$$N/KNO3\ = 0.466423576\ [-]$$

$$C_{KNO3} = \frac{0.5\ (mg/L)}{0.138538642\ (-)}$$

$$C_{KNO3} = \ 3.609101359\ \ (mg\ /L)$$

$$M_{KNO3} = \frac{3.609101359\ \ (mg\ /\ L\ \ )*\ 150\ (L\ )}{1000\ (mg\ /\ g\ \ )}$$

$$M_{KNO3} = \ 0.541365204\ [g] * 6\ [replicates]$$

$$M_{KNO3} = \ 0.96478828\ [\mathrm{g}]$$

Therefore 0.965 g of KNO_3 was measured in the lab and mixed into 600 mL of distilled water. Next, 100 mL of KNO_3 stock solution was be added in-situ to the replicates of NO3 and NO3 + P treatments.

KNO3

$$P/\mathrm{K2HPO4} = \frac{30.973762\ (\mathrm{g/mol})}{174.2\ \ (\mathrm{g}/mol)}$$

$$P/\mathrm{K2HPO4}\ = 0.177805752\ [-]$$

$$C_{\mathrm{K2HPO4}} = \frac{0.02\ (mg/L)}{0.177805752\ (-)}$$

$$C_{\mathrm{K2HPO4}} = \ 0.1124823\ \ (mg\ /L)$$

$$M_{\mathrm{K2HPO4}} = \frac{0.1124823\ \ (mg\ /\ L\ \ )*\ 150\ (L\ )}{1000\ (mg\ /\ g\ \ )}$$

$$M_{\mathrm{K2HPO4}} = \ 0.016872345\ \ [g] * 12\ [replicates]$$

$$M_{\mathrm{K2HPO4}} = 0.20246814 \ [\mathrm{g}]$$

Therefore 0.202 g of K_2HPO_4 was measured in the lab and mixed into 600 mL of distilled water. Next, 50 mL of K_2HPO_4 stock solution was be added in-situ to the replicates of P, Urea + P, NH_4 + P and NO_3 + P.

Each of these calculations and measurements are completed before the enrichment day, which are Day 0 and 7 for trial 3.

6.4 Filtration

Objective

1 L samples were collected on Day 0, 3, 7 and 14 for further lab analysis. These samples were filtered through varying sizes of GF/F filters for chl-a extraction, HPLC and MC-Cong analysis. The chl-a extraction was completed at NaFIRRI but the HPLC and MC-Cong analysis will be completed at a later date in Canada.

Methods

250 mL of the unfiltered sample was measured into a graduated cylinder and transferred into the filtration system. The sample is then filtered through a 47 mm GF/F and collected for future HPLC analysis. The collected filters are stored in tinfoil in temperatures below 0 [C]. The filtered sample is discarded and the process is repeated for each replicated in the experiment. 250 mL of the unfiltered sample was measured into a graduated cylinder and transferred into the filtration system. The sample is then filtered through a 47 mm GF/F and collected for chl-a extraction, further described in section 6.X. The 50 mL of the filtered sample was collected for the nutrient analysis described in section 6.X. A 5 mL unfiltered sample is collected and stored in a Lugol solution.

For the 25 mm GF/F filtration a recorded amount of unfiltered sample was filtered, the amount varied depending on the observable particulates within the sample. The first set of 25 mm GF/F were stored in tinfoil at temperatures below 0 [C] for HPLC analysis, and the second set of 25 mm GF/F were stored in tinfoil at temperatures below 0 [C] for MC-Cong analysis.

6.6 Chl-a Extraction

Overview

The purpose of the chl-a extraction is to determine the concentration of chl-a in each treatment.

Method

The chl-a extraction uses the hot ethanol technique paired with the spectrophotometric method. The collected samples (chl-a extraction 47 mm GF/F) described in section, 6. X were stored in the Falcon tube at temperatures below [0]. The filtered samples contained 0.25 L of unfiltered solution, filtered through the 47 mm GF/F described in section 6.X. Each sample had 10 mL of 90% Ethanol added to the Falcon tube and preceded to incubate in a water bath at 78 [C] for 2 minutes. This becomes the volume of extract (Ve). After the required two minutes the samples were sonificated in a sonicator bath for 15 minutes. This process inactivates the chlorophyllase and accelerates the lyses of pigments. The samples are cooled to room temperature in a dark room for approximately 1 hour. After the cooling the falcon tube will be inverted and tapped on the table to ensure the filter moves out of solution. The filter will then be squeezed with forceps to facilitate extraction and the filter will be removed from the sample. The remaining solution will then be filtered through a 25 mm GF/F filter back into the 10 mL Falcon tube. At this point the extraction is completed and the sample will be run through the spectrophotometric machine at a 1 cm pathlength (d).

Each sample (3 replicates per 8 treatments and 24 total). A blank is measured using the 90 % Ethanol solution with one cuvette for all treatments. The sample will be poured into a cuvette (kept through the remainder of the trial) and the absorbance was measured at 665 nm (E_{665}). The machine was then switched to 750 nm (E_{750}) and re-calibrated using the same 90% Ethanol and the same sample was measured at 750 nm for absorbance to correct for turbidity. The sample then had X mL of 0.25 N hydrochloric acid and the machine was re-calibrated to measure this at 664 nm (E^a_{665}) and 750 nm (E^a_{750}). This process was repeated for each replicate. This process was completed for day 0, 3, 7 and 14. From this information the chl-a concentration ($C_{chl\text{-}a}$) was calculated using equation X. The concentration of chl-a was then displayed over time (days) and can be seen in section 6.X.

$$C_{chl-a} = [(E_{665} - E_{750}) - (E^{a}{}_{665} - E^{a}{}_{750})] * 2.43 * 12.19 * \left(\frac{Ve}{Vs * d}\right)$$

6.3 Trilogy Chl-a

Overview

The Trilogy Laboratory Fluorometer was used to measure relative fluorescence units (RFU) to determine the daily relative amount of chlorophyll-a in response to the nutrient enrichments over the course of trial 3.

Method

50 mL samples were collected from each replicate in the field every day (0 to 14). The samples were taken approximately at an arms-length depth, after each mesocosm unit was thoroughly mixed. The samples were stored in a cooler until returned to the lab the same day for analysis (within an hour of sampling). The 50 mL samples were inverted to ensure that the sample was mixed and uniform throughout. The sample was poured into a standard cuvette which was used for the remainder of trial 3. The samples were run through the Trilogy Laboratory Fluorometer using the In vivo chlorophyll-a blue module. The RFU were recorded and plotted against time [days] for each treatment seen in section X figure X. A blank was measured using tap water from NaFIRRI.

6.4 *Nutrient Analysis*

Objective

50 mL of the filtered samples (described in section 6.x) collected on Day 0, 3, 7 and 14 were used to measure the concentration of nutrients within the mesocosm units. The following nutrients were analyzed: soluble reactive phosphorous (SRP), total dissolved phosphorous (TDP), total dissolved nitrogen (TDN), ammonium (NH4) and Nitrate (NO3).

Methods

SRP Analysis

The SPR analysis follows the steps described in *The Chemical Analysis of Fresh Water* by Stainton, Capel and Armstrong (1977).

The first step in this process was to mix the reagent solutions. The first reagent (A) made was Acid Molybdate-antimony which was comprised of 100 mL of distilled water, 1.875 g of ammonium paramolybdate, 0.035 g of antimony potassium tartrate, 22 mL of sulphfuric acid, topped with 250 mL of DI H_2O. The second reagent, "ascorbic acid" (B) was made from 1 g of ascorbic acid and 40 mL of deionized water. The third reagent, "mixed molybdate" (C) was comprised of 4 of the following parts: reagent A with one part, reagent B with one part and 2 mL of reagent C is added to the 10 mL unfiltered samples for the SPR reaction.

10 mL standards were prepared at concentrations 0, 20, 40, 60, 80, 100, 200, 400, and 500 ug/L with 2 mL of mixed molybdate added.

After 5 minutes of the reagent being added, the standards and samples were run through the spectrophotometer at a wavelength of 885 nm and 1 cm pathlength. The measurements for the concentration standards produced a standard curve, shown in Figure X and equation [X] with an R^2 of 0.9999 was found. The absorbance measurements for the samples and the rearranged standard curve equation [B] were used to calculate the concentration of SRP in each treatment.

[X] $y = 0.0006x - 0.0013$

[B] $C_{SRP} = \frac{Abs+0.0013}{0.0006}$

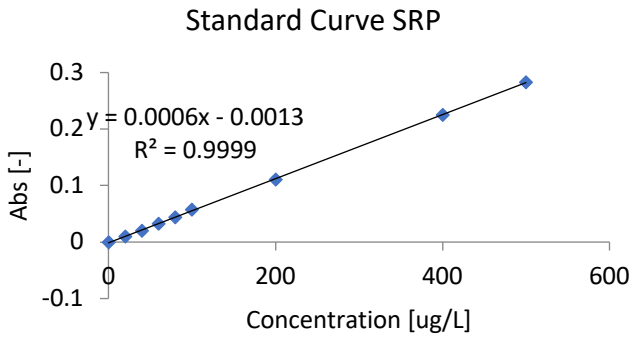

Figure X. The standard curve measured for SPR analysis

TDP Analysis

The TDP analysis follows the steps described in *The Chemical Analysis of Fresh Water* by Stainton, Capel and Armstrong (1977)

The first step is the persulphate digestion to hydrolyse phosphate ions. This was completed by transferring 50 mL of the unfiltered samples into pyrex destruction bottles (50-100 mL) with 2 g of potassium persulphate added. The destruction bottles were then loaded into the autoclave and boiled at 130 C for 1 hour. This process was repeated with 25 mL of the blank and dilution series (standard curve) using 1 g of potassium persulphate. These were then set to cool. The second step, was to measure 25 mL of the digested samples into clean plastic vials. Next, 0.25 mL of 4M sodium hydroxide was added, this process raises the pH for colour development during the addition of mixed molybdate which was described in the SRP analysis.

10 mL standards were prepared at concentrations 0, 20, 40, 60, 80, 100, 200, 400, and 500 ug/L with 2 mL of mixed molybdate added.

The reagent needed 5 minutes to react before standards and samples were run through the spectrophotometer at a wavelength of 885 nm and 1 cm pathlength. The measurements for the concentration standards produced a standard curve, shown in Figure W and equation [V] with an R^2 of 0.9991. The absorbance measurements for the samples and the rearranged standard curve equation [B], were used to calculate the concentration of TDP in each treatment.

[W] $y = 0.001x + 0.0158$

[V] $C_{TDP} = \frac{Abs+0.0158}{0.001}$

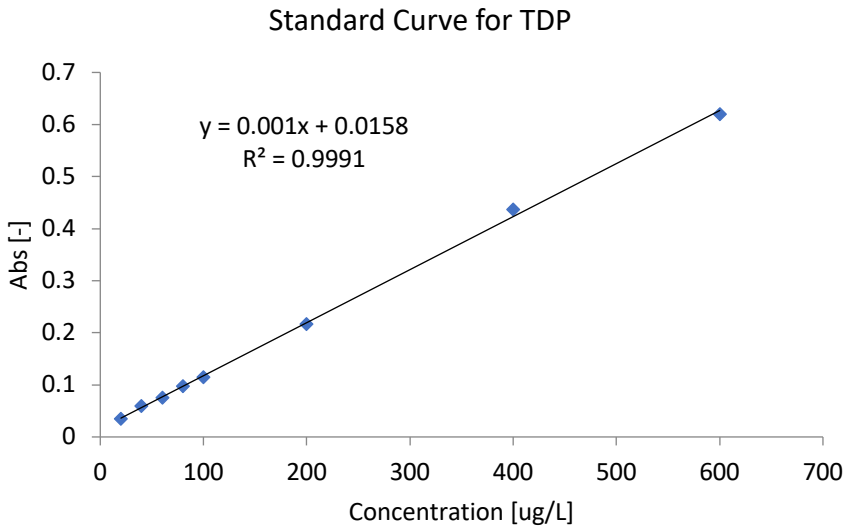

Figure X. Standard curve calculated for TDP

TDN Analysis

The TDN analysis follows the steps described in *The Chemical Analysis of Fresh Water* by Stainton, Capel and Armstrong (1977).

The first step is the persulphate digestion to hydrolyse phosphate ions, described in the TDP section. CHECK ABOUT FILTERED SAMPLE The second step was to transfer 25 mL of digested sample and digested dilution series (standard curves) into 50 mL vials. Next, 1.5 mL of 4M sodium hydroxide was added, followed by 3 mL of buffer solution (ammonium chloride, sodium tetraborate and EDTA).

10 mL standards were prepared at concentrations 0, 20, 40, 60, 80, 100, 200, 400, and 500 ug/Lwith 2 mL of mixed molybdate added.

The reagent needed 5 minutes to react before the standards and samples were run through the spectrophotometer at a wavelength of 640 nm and 1 cm pathlength. The measurements for the concentration standards produced a standard curve, shown in Figure X and equation [X] with an R^2 of 0.9976. The absorbance measurements for the samples and the rearranged standard curve equation [BB] were used to calculate the concentration of TDN in each treatment.

[X] $y = 0.001x - 0.0042$

[BB] $C_{TDN} = \frac{Abs+0.0042}{0.001}$

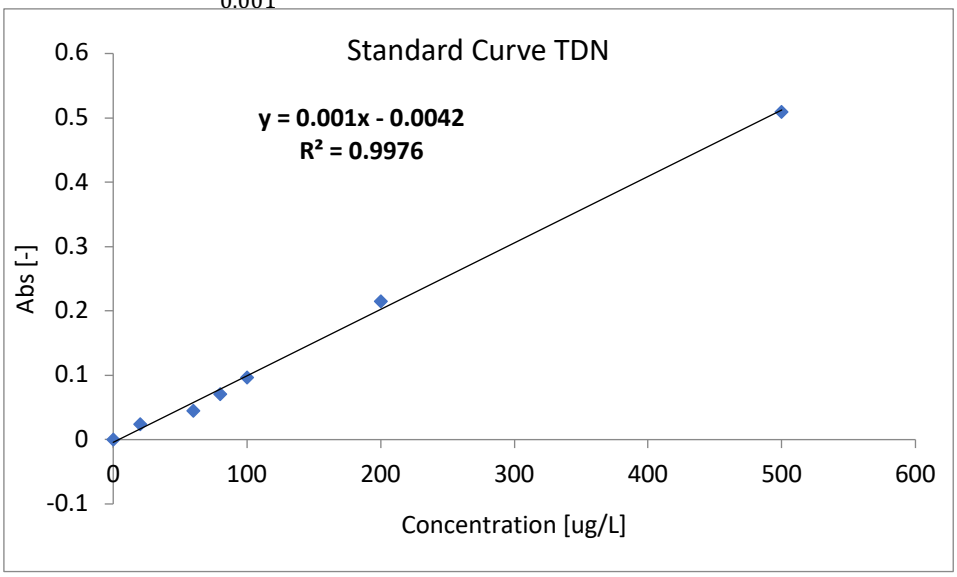

Figure X. The standard curve calculated for TDN

NH4 Analysis

The Ammonia-Nitrogen analysis follows the steps described in *The Chemical Analysis of Fresh Water* by Stainton, Capel and Armstrong (1977).

The first step was to mix the reagent solutions for the NH4 analysis. The first reagent was Alkaline Stock Solution which was made from 50 g of sodium citrate and 2.5 g of sodium hydroxide dissolved in deionized water to produce a 250 mL solution. Next, the oxidizing reagent was mixed and consisted of 4 parts of reagent, 1 plus 1 part of hypochlorite stock. The third reagent was sodium nitroprusside which was made from 1.5 g of sodium nitroprusside mixed with 150 mL of distilled deionized water. The final reagent was Phenol with 15 g of phenol mixed with 95 % ethanol to produce 150 mL of solution. The 10 mL filtered samples and standards had 0.4 mL of reagent 4 mixed with 0.4 mL of reagent 3 and had 1.0 mL of the oxidizing reagent.

The 10 mL standards were prepared at concentrations of 0, 20, 40, 60, 80, 100, 200 and 400 ug/L.

After 1 hour the samples were run through the spectrophotometer at a wavelength of 640 nm and 1 cm pathlength. The measurements for the concentration standards produced a standard curve, [P] and R^2 of 0.9997. The absorbance measurements for the samples and the rearranged standard curve equation [V] were used to calculate the concentration of NH4 in each treatment.

[P] $y = 0.0012x + 0.0029$

[V] $C_{NH4} = \frac{Abs+0.0029}{0.0012}$

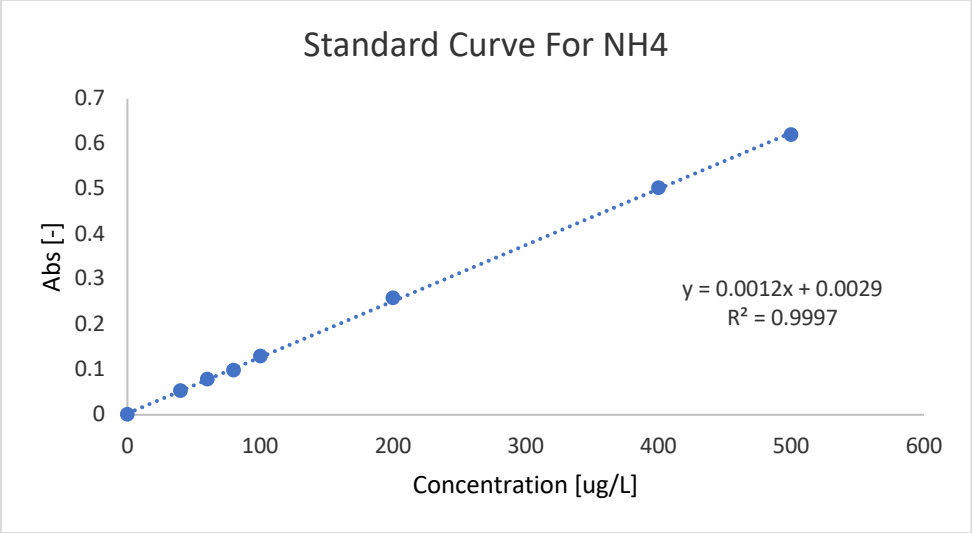

Figure X. The standard curve calculated for

NO3

The Ammonia-Nitrogen analysis follows the steps described in The Chemical Analysis of Fresh Water by Stainton, Capel and Armstrong (1977).

The first step was to make the buffer solution, using 100 g of ammonium, chloride, 20 g of sodium, tetra borate and 1 g EDTA dissolved to 1000 mL with distilled deionized water. The second reagent, sulphanilamide was made by mixing 5 g of sulphanilamide with 100 mL of concentration hydrochloric acid with up to 500 mL of distilled deionized water. The third reagent, N-1-naphthylrthylenediamine dihydrochloride (NNED), was made by mixing 0.5 of NNED dissolved in 500 mL of distilled deionized water and stored in a dark brown bottle. The fourth reagent, Cupric sulphate was made by mixing 20 g of cupric sulphate penta-hydrate

dissolved to 1000 mL of distilled deionized water. The final reagent was cadmium filings of 0.5 mm mesh size.

30 mL of filtered samples and standard curve samples were transferred into clean 50 mL plastic vials with 3 mL of buffer solution added. The samples and standards are then pasted through a reduction column packed with 5 g of cadmium that was washed with 2N HCl. Next, rinsed with deionized water and treated with 10 mL of cupric sulfate solutions. Samples and standards were delivered at a rate of 25 mL / 4 minutes.

Samples and standards went through the spectrophotometer at a wavelength of 543 nm and 1 cm pathlength. The measurements for the concentration standards produced a standard curve, [P] with an R^2 of 0.9984. The absorbance measurements for the samples and the rearranged standard curve equation [Q] were used to calculate the concentration of NO3 in each treatment.

$$[\mathrm{P}]\ y = 0.0018x - 0.0025$$

$$[Q]\ C_{NO3} = \frac{abs + 0.0025}{0.0018}$$

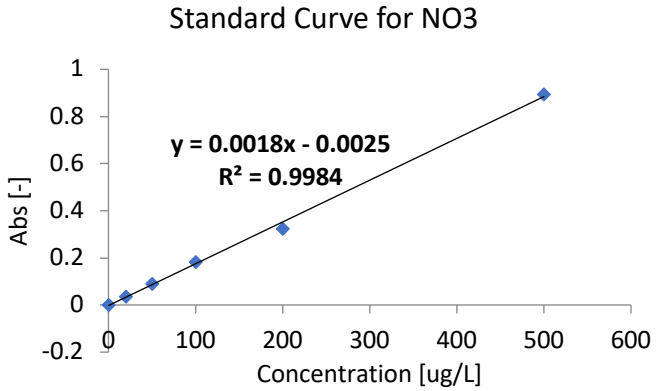

Figure X. The standard curve calculated for NO3

4.0 Results

The trend common to all of the chl-a (RFU) and nutrient concentration treatments was a chl-a peak, usually occurring 3 days after nutrient enrichment, with a corresponding depletion of said nutrient, followed by a crash in chl-a values which correlated with this reduced nutrient availability for phytoplankton (Fig. YY). The trend for chl-a (concentration) varied between treatments and is further discussed in the following sections.

Treatment Control

The **chl-a** RFU values for the **control treatment** follows the trend of other treatment's double sigmoid curve, at least in timing of peaks. As there was no nutrient enrichment for the control, we might say that it follows a "false" double sigmoid curve. We suggest that this might be correlated with weather conditions (i.e. sunny, warm) which are agreeable to algal communities. The RFU value started high, fell on day 1, rose again by day 3 and crashed by day 5. It then reaches its maximum on day 10. The values for the chl-a concentration analysis varied from the chl-a RFU trend. On Day 0 the initial chl-a concentration was measured to 17.77 (micro g L^{-1}), then dropped to 3.95 (micro g L^{-1}) on Day 3, then returned to 17.77 (micro g L^{-1}) on Day 7 and increased to 20.36 (micro g L^{-1}). The variation in between chl-a (RFU) and chl-a (micro g L^{-1}) suggests that the RFU measurements of the unfiltered sample may have measured other particulates within the sample. Another possibility is that a lab error occurred during the chl-a (micro g L^{-1}) analysis.

Peak mean concentrations of NH_4 in the control treatment occurred during day 3 (55 micro g L^{-1}) and values remained low with no correlation to chl-a peaks. Peak mean soluble reactive phosphorus (SRP) treatments occurred on day 3 (6.6 micro g L^{-1}) but saw a variability within 3 micro g L^{-1}. Total dissolved phosphorus (TDP) followed a fairly linear trend until day 14 which had marginally wider error bars and a peak mean value of 38.0 micro g L^{-1}. Total dissolved nitrogen (TDN) had a peak mean value (231 micro g L^{-1}) on day 14. Nitrate concentration remained stable throughout the trial period and had a peak mean value (36.6 micro g L^{-1}) on day 3.

Treatment P

The **chl-a** values of the **phosphate treatment** exhibited the "false" double sigmoid curve similar to the control treatment, starting at its peak RFU and having local peaks at days 3 and 10, with a crash on day 5. The chl-a concentration varied from the chl-a (RFU) trend. On Day 0 the initial chl-a concentration was measured to 18.56 (micro g L^{-1}), then dropped to 11.85 (micro g L^{-1}) on Day 3 and remained a similar value, 11.45 (micro g L^{-1}), on Day 7 and increased to 21.68 (micro g L^{-1}) on Day 14. This variation could be due to the reason suggested in the Treatment Control Discussion.

NH4 concentrations followed a curve similar to control with a peak mean concentration occurring on day 3 (71.5 micro g L^{-1}). SRP values declined from initial conditions on day 3, reaching similar levels of concentrations as day 14, and peak mean concentration on day 7 (33.3 micro g L^{-1}). TDP values started at ca. 25 micro g L^{-1}, and peaked on day 3, staying consistently high for the remainder of the trial. TDN values increased slightly throughout the trial, with peak mean concentration of 241.0 micro g L^{-1}) on day 14. Nitrate concentration remained stable throughout the trial period and had a peak mean value (33.6 micro g L^{-1}) on day 3.

Treatment Urea and Urea + P

Chl-a values for **Urea** and **Urea + P Treatments** followed the general double sigmoid curve. However, the urea treatment was shown to have much wider error bars than the Urea + P treatment. All three replicates of the Urea treatments started day 0 with high initial chl-a values which suggests there was a strong community presence of algae within the mesocosm bags for this treatment. There was anomalous data noted in the Urea 1 replicate. On day 3 chl-a peaked to 12857 RFU, more than double the highest peak following the first enrichment regimen. All treatments peaked by day 3 or 4 in the first week. The day 7 chl-a value for Urea 1 was noted to be lower than the daily treatment mean (3318.9 RFU, mean 4415.8 RFU) and following the Day 7 enrichment, Urea 1 demonstrated a slight chl-a increase, following population crash from which it does not recover, relative to the other two replicates. The high spike following the Day 3 anomaly and suppressed numbers following the second enrichment, suggest population overshoot and the swinging of low/high/low RFU values suggest continuing population perturbations. This population crash suppresses the visual effect of the double sigmoid curve of

the Urea Treatment, but when Urea 1 is removed, the double curve is more transparent. With that said, even with the omission of Urea 1 the curve for week 1 is not a smooth one and it is seen that there is still evidence of population overshoot as crash follows day 4 with some population recovery occurring before the second enrichment on Day 7. This suggests, that not all available nutrient was used up and there was competition for resources taking place. Following the population crash on Day 5, population pressures eased up, allowing for some recovery on Days 6 and 7. This also suggests that Urea is rapidly taken up by the algal communities which are found in the mesocosms. Little variability occurred in the Urea + P Treatment, with the exception of a slight population crash on Day 3 for Urea + P Replicate 3 and a population collapse on Day 5 for all treatments with a slight population recovery for Urea + P Replicate 2. The chl-a concentration for treatments Urea and Urea + P followed similar trends as the chl-a RFU measurements (Urea and Urea + P). With the varying concentrations of 20.14 (micro g L^{-1}), 35.94 (micro g L^{-1}), 15.79 (micro g L^{-1}), and 17.98 (micro g L^{-1}), respectively on Day 0, 3, 7 and 14 for Urea treatment and concentrations of 19.35 (micro g L^{-1}), 33.57 (micro g L^{-1}), 12.24 (micro g L^{-1}), and 17.74 (micro g L^{-1}), respectively on Day 0, 3, 7 and 14 for Urea + P treatment.

The mean NH_4 concentrations of the **Urea Treatment** started high (516 micro g L^{-1}) and reduced along a steep and smooth curve to near-zero concentrations by day 14. Mean SRP concentrations stayed relatively level throughout the trial, starting at 7.72 micro g L^{-1}, declining slightly, increasing to 5.50 micro g L^{-1} on day 7 and declining to 3.28 micro g L^{-1} by day 14. TDP concentrations for Urea Treatments showed wide variability between replicates with little discernable pattern in time for their lows and highs. The mean TDP started low, raised by day 3 and 7, and declined by day 14 with moderate elevated values for all 3 replicates. TDN concentration for Urea declined steadily for all 3 replicates with slight acceleration after day 7. NO_3 concentrations remained stable at around 30 micro g L^{-1} throughout, increasing slightly on Days 3 and 7 and reaching slightly reduced values by Day 14.

The mean NH_4 concentrations of the **Urea + P Treatment** followed the same steep and smooth decline to near zero-concentrations by day 14 noted in the Urea Treatment. Mean SRP concentrations resembled the Urea Treatment, with its the stable, but “low-high, low-high” trend. TDP concentrations showed similar variability to the Urea Treatments with little discernable

pattern in time for their lows and highs. TDN concentration for Urea + P declined steadily for all 3 replicates with slight acceleration after day 7 in a similar fashion to the Urea Treatment. Mean NO_3 concentrations started elevated (361.9 micro g L^{-1}) and declined in a steep but smooth curve, finishing to a uniform concentration of 25.8 micro g L^{-1} across all treatments.

Treatment NH_4 and NH_4 + P

NH_4 and **NH_4 + P Treatments** differed in their **chl-a responses**. Where NH_4 started at its peak value and experienced its local maxima on Days 3 and 10, NH_4 + P declined steadily following enrichment and only demonstrated the sigmoid curve following the second enrichment, reaching its peak by day 10. The cause of this decline is uncertain, but it is possible that the enrichment of this treatment biased the environment to some other, non-photosynthetic, component of the algal community. The chl-a concentrations varied from the RFU measurements and reported values of 20.54 (micro g L^{-1}), 15.40 (micro g L^{-1}), 19.35 (micro g L^{-1}) and 19.66 (micro g L^{-1}) for the NH4 treatment. The chl-a concentrations allowed the similar trend (decline on Day 3) as the chl-a RFU measurements for NH4 + P, with the corresponding values for Day 0, 3, 7 and 14 19.74 (micro g L^{-1}), 13.03 (micro g L^{-1}), 24.88 (micro g L^{-1}) and 17.18 (micro g L^{-1}).

All concentrations of NH_4 in the **NH_4 Treatment** followed the mean trend of starting low (46.1 micro g L^{-1}), reaching peak value on Day 7 (497.6 micro g L^{-1}) and reaching near-zero concentrations by day 14. SRP concentrations demonstrated the trend of a slightly elevated value (mean 9.39 micro g L^{-1}) which declined by Day 3 and spiked by Day 7 (mean 42.7 micro g L^{-1}) and a return concentration lower than background as recorded on Day 0. TDP concentrations varied across treatments and days but the mean followed a trend similar to the NH_4 Treatment SRP of peaking by Day 7. A negative value was recorded during the Day 14 measurement of NH_4 3 (-12 micro g L^{-1}) which showed a more greatly diminished mean value for Day 14, which stayed moderately elevated between the remaining replicates. TDN concentrations were shown to have doubled by Day 7, and return a similar, but slightly elevated level on Day 14. NO_3 concentrations remained stable across the trial and in general followed the trend of the mean, increasing slightly on Day 7 and declining to lower than initial concentrations by Day 14.
The concentrations of NH_4 in the **NH_4 + P Treatment** followed the same trend of the NH_4 Treatment staying low and spiking on Day 7, despite the gradually declining population curve. In

the second week, following along the predicted sigmoid growth curve, the concentration reaches a near-zero value by Day 14. SRP concentrations followed the trend set by the NH_4 Treatment of declining by Day 3, spiking albeit to a lesser degree (mean 25.5 micro g L^{-1}) and coming to a near-zero value by Day 14. TDP concentrations demonstrated no discernable pattern and a wide degree of variability, but the mean followed a trend which remained fairly stable before coming to a below-zero mean concentration for Day 14. This was the result of negative concentrations recorded during the Day 14 measurement of the NH_4 + P 1 and 2 Replicates (-14.5, -17.0 micro g L^{-1}). The TDP concentration of the NH_4 + P 3 Replicate started high and trended down for the full trial, finishing at 28 micro g L^{-1}. TDN concentrations resembled those of NH_4 Treatment, but by day 7 the mean concentrations skyrocketed to more than fourfold. The mean values of Day 14 then return to concentrations slightly elevated from Day 0. NO_3 concentrations followed the trend of the mean, increasing slightly on Day 7 and declining to lower than initial concentrations by Day 14.

Treatment NO_3 and NO_3 + P Treatments

The **chl-a response of NO_3** and **NO_3 + P Treatments** differed. Chl-a of the NO_3 Treatments was found to be at its maximum on Day 0 and dropped greatly by Day 1. It then climbed slowly to a local maximum on Day 4 before crashing on Day 5. The chl-a values then rise until day 7 which seemed to stall growth with a minimal increase on Day 8 and slight decrease on Day 9. By Days 10 and 11 the RFU reached values similar to Day 0 and slowly declined to value of 4903 RFU, just below mean of all days in the treatment. The NO_3 + P Treatment more closely followed the expected trend, peaking by Day 4 and declining until Day 7, after which there is another, lower peak by Day 10 and declining until Day 14. The measurements of chl-a concentration for the treatment NO3 varied from the chl-a (RFU) measurements. The concentrations were 19.75 (micro g L^{-1}) 17.77 (micro g L^{-1}). 24.49 (micro g L^{-1}) and 20.19 (micro g L^{-1}) for Day 0, 3, 7 and 14, which showed a decrease of concentration on Day 3. The chl-a concentrations for treatment NO3 + P, followed the same trend as chl-a (RFU) with the respective concentrations for Day 0, 3, 7 and 14, 20.54 (micro g L^{-1}) 30.80 (micro g L^{-1}) 19.75 (micro g L^{-1}) and 13.75 (micro g L^{-1}) Concentrations of NH_4 in the **NO_3 Treatment** were found to be relatively stable. There was a slight increase on Day 3, which remained stable at Day 7 which came to near-zero values by Day 14. SRP concentrations showed a slight decrease from Day 0 to Day 3, before spiking to 42.16

micro g L^{-1} on Day 7. The concentration returns to 4.9 28 micro g L^{-1} by Day 14. Concentrations of TDP demonstrated no discernable pattern and wide variability, except for on Day 3 which was the lowest concentration measured. TDN concentrations demonstrated a rising trend with a mean concentration of 879.3 28 micro g L^{-1} occurring on Day 14. Mean NO_3 concentrations of the NO_3 Treatment of Day 0 started elevated and decreased slightly by Day 3, it then rose to its peak value on Day 7 before coming to be depleted by Day 14 (30.8 micro g L^{-1}).

NH_4 in the **NO_3 + P Treatment** followed the trend of the NO_3 Treatment; a slight increase on Day 3, which remained stable at Day 7 which came to near-zero values by Day 14. SRP concentrations follow a similar trend to the NO_3, but with a much smaller peak on Day 7 (9.38 micro g L^{-1}). Concentration then returns to a near-zero value by Day 14. TDP showed a wide variability similar to the NO_3 Treatment with no discernable pattern, but the minimum occurred on Day 7. TDN concentrations differed from the NO_3 Treatment by peaking on Day 7 (544.3 micro g L^{-1}) and then declining by Day 14. NO_3 concentrations followed an expected trend with similar concentrations on Days 0 and 7, and a partial consumption by Day 3 and near total depletion by Day 14.

4.1 Chl-a (RFU) vs Nutrient (ug/L) graphs

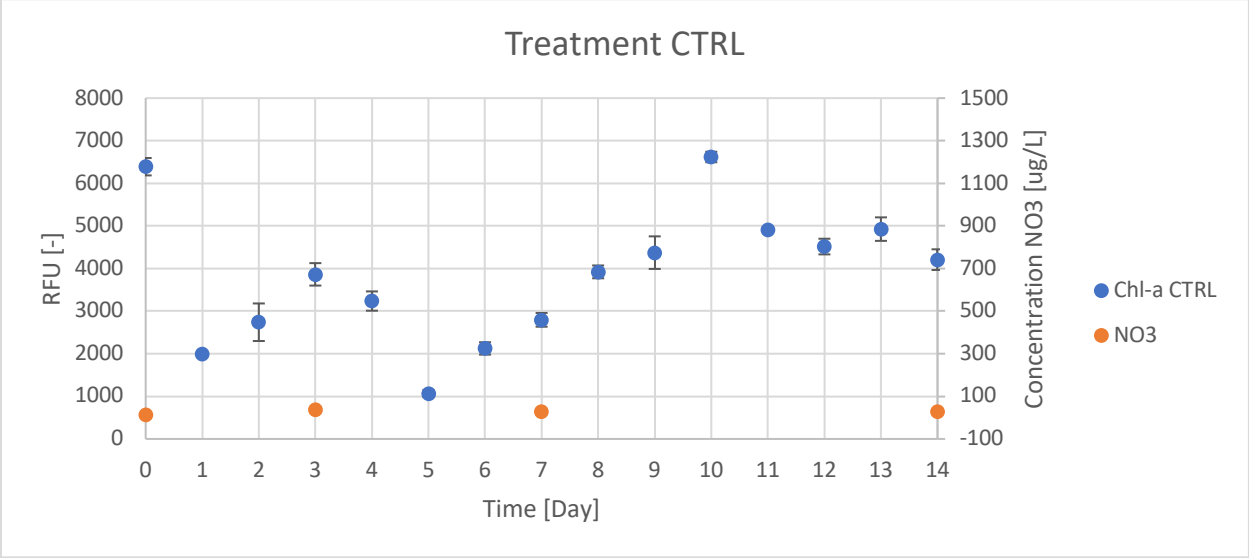

Figure X. The RFU of chl-a for average of CTRL treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

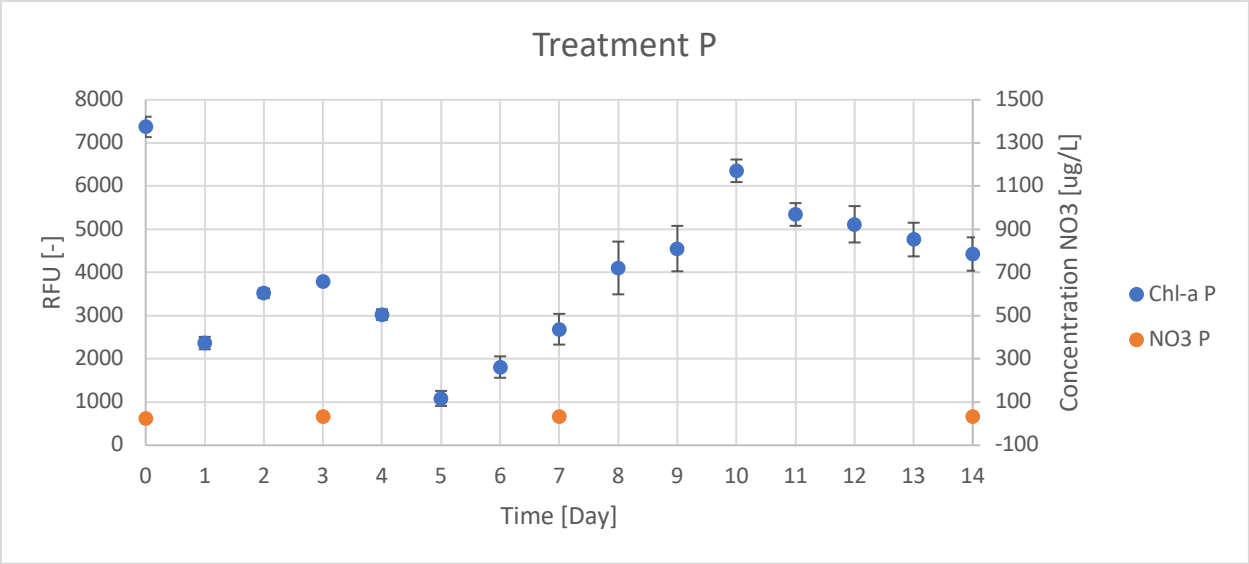

Figure X. The RFU of chl-a for average of P treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

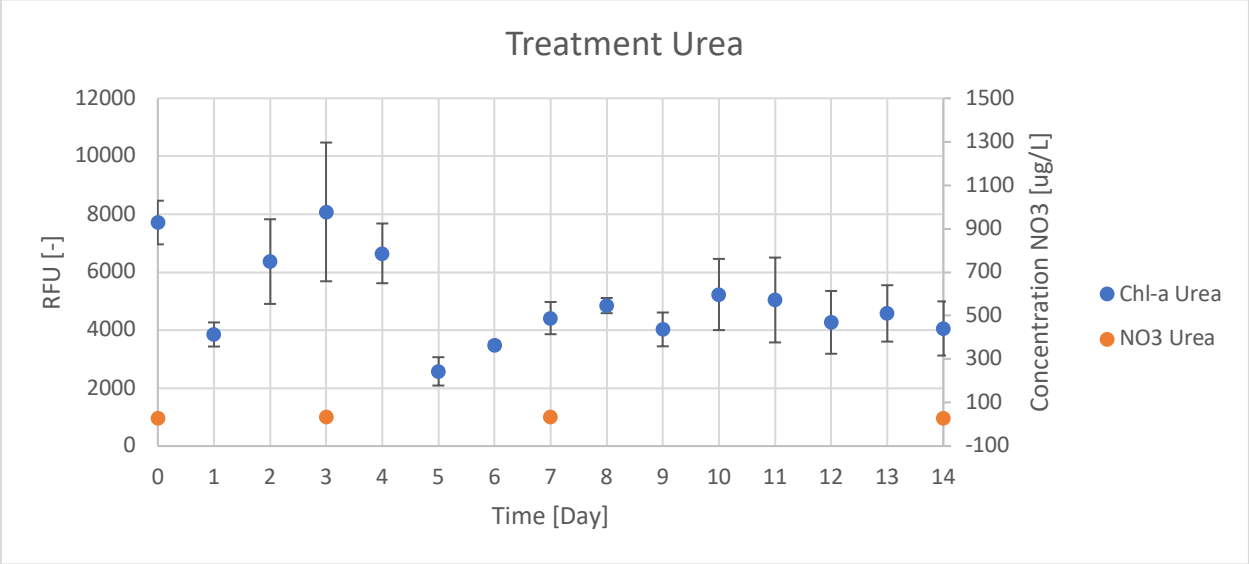

Figure X. The RFU of chl-a for average of Urea treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

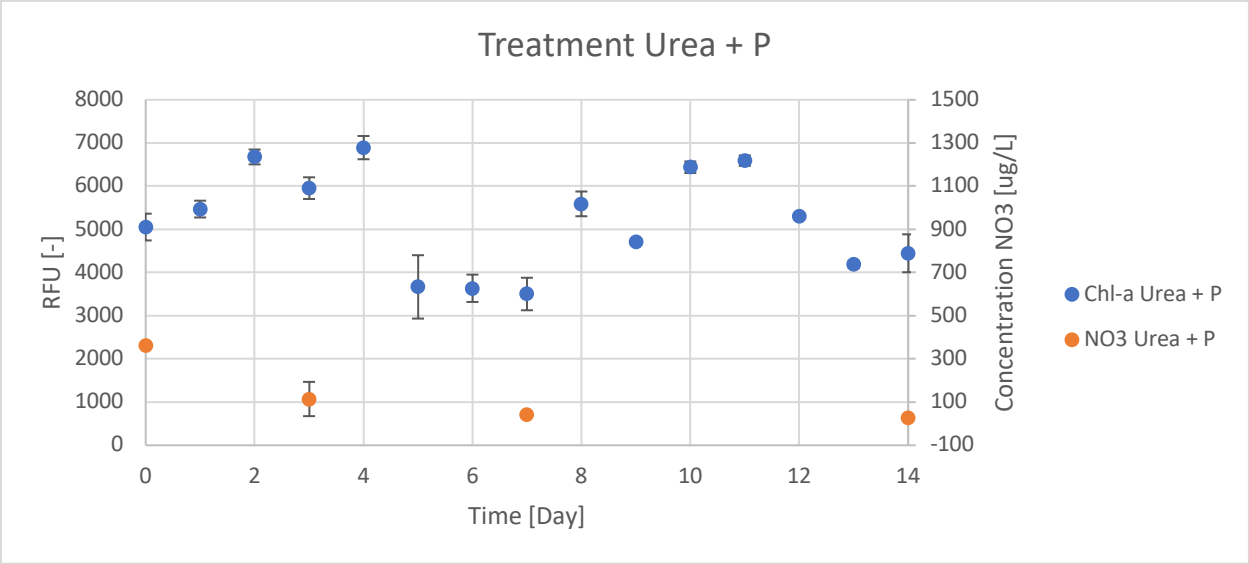

Figure X. The RFU of chl-a for average of Urea + P treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

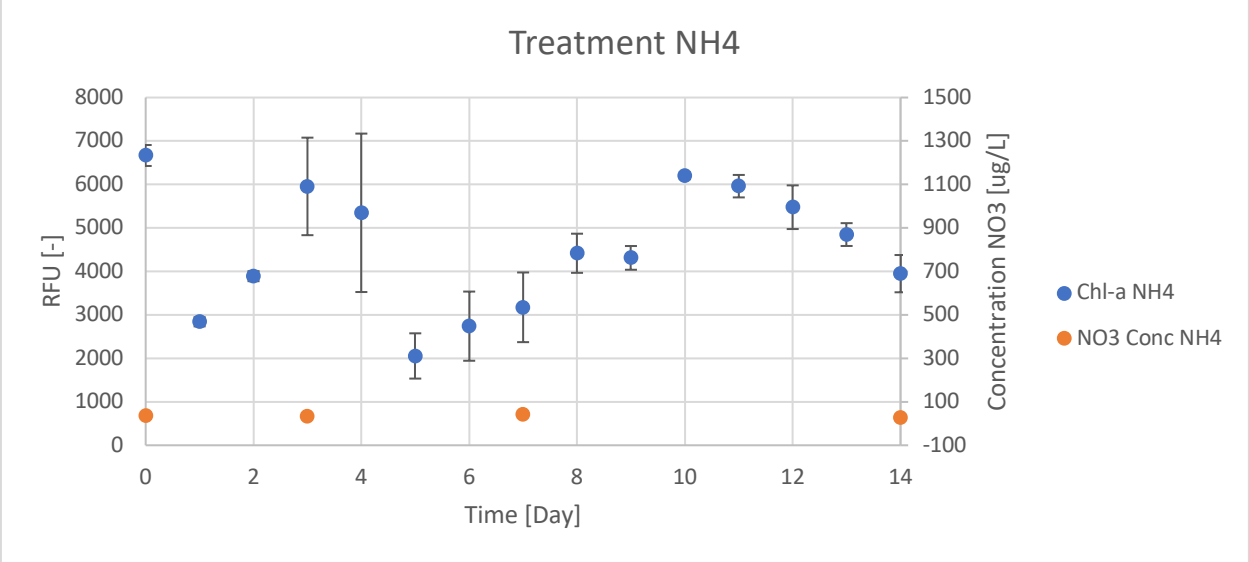

Figure X. The RFU of chl-a for average of NH4 treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

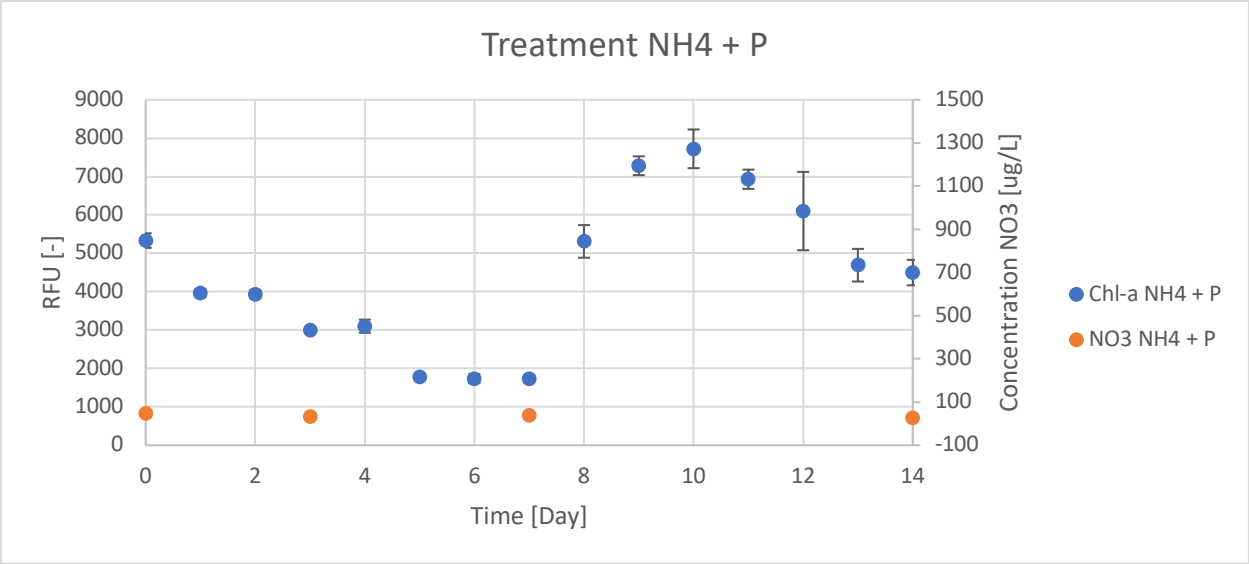

Figure X. The RFU of chl-a for average of NH4 + P treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

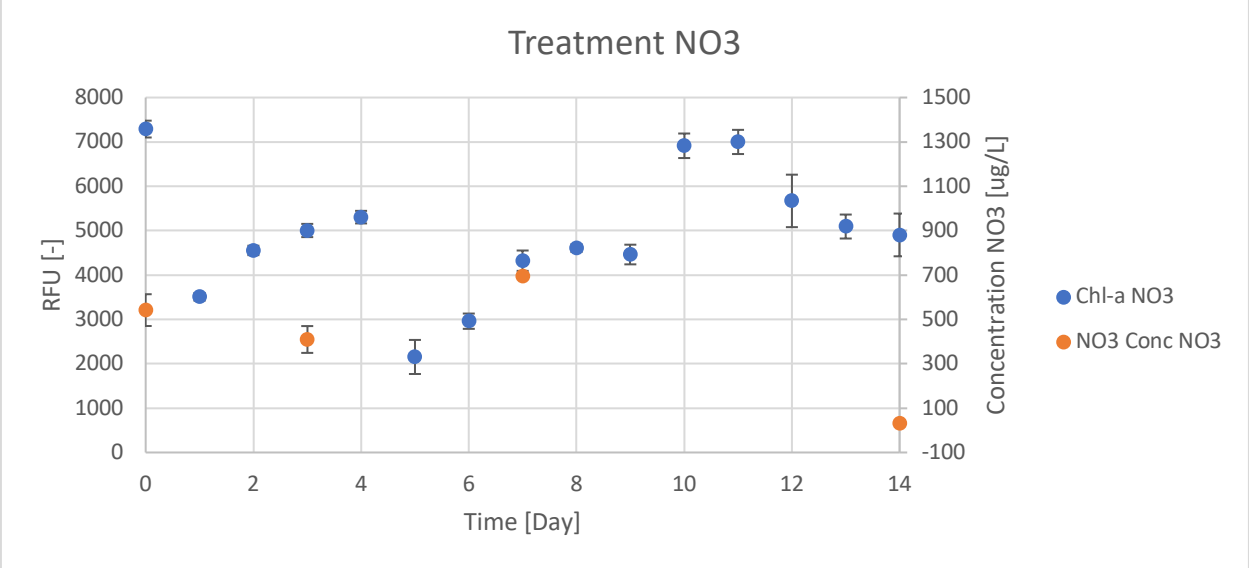

Figure X. The RFU of chl-a for average of NO3 treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

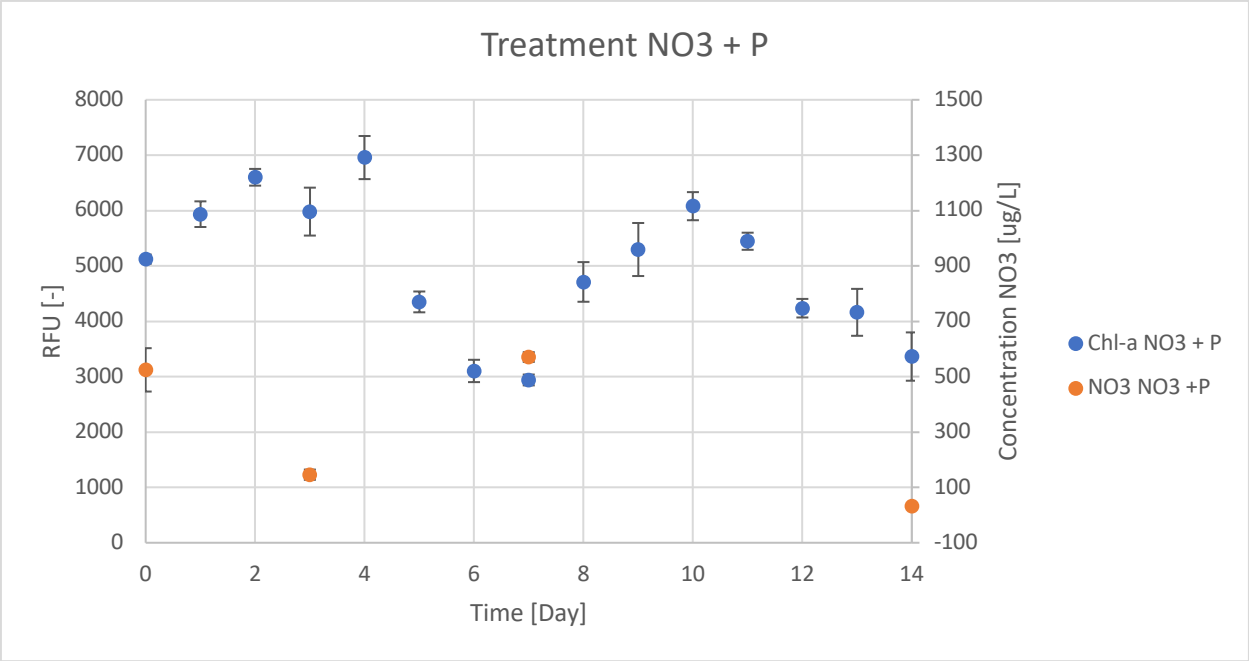

Figure X. The RFU of chl-a for average of NO3 + P treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

Chl-a (RFU) vs TDP

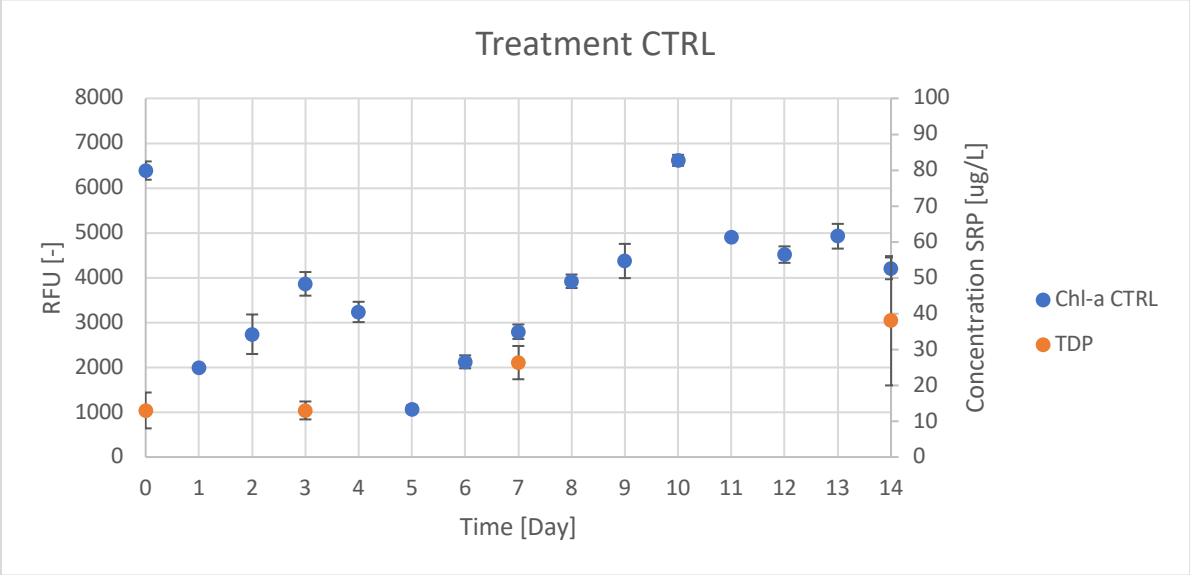

Figure X. The RFU of chl-a for average of CTRL treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

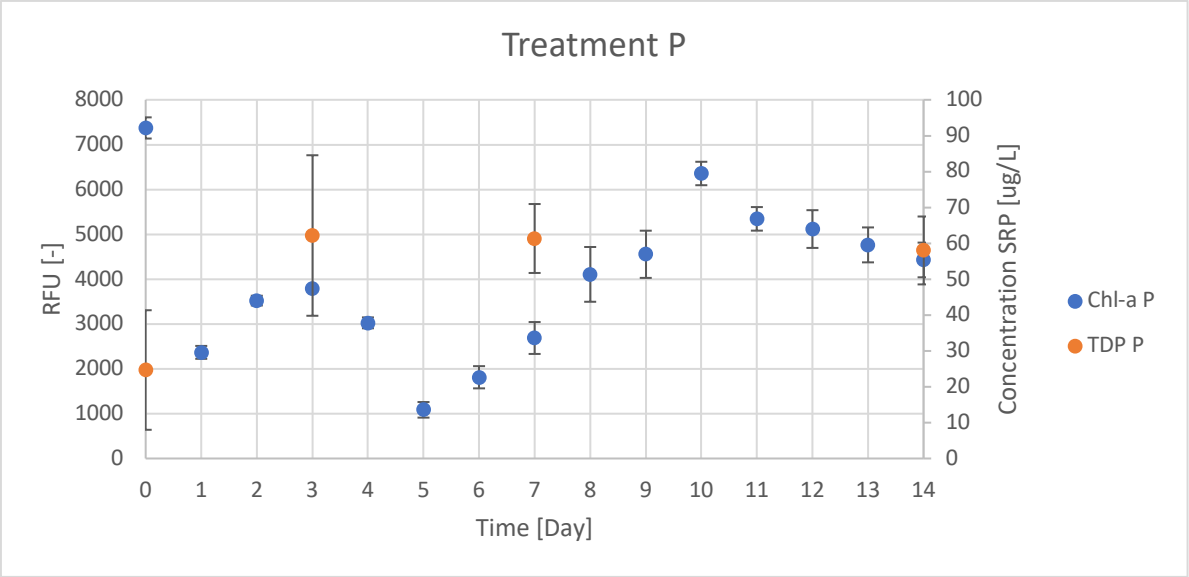

Figure X. The RFU of chl-a for average of P treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

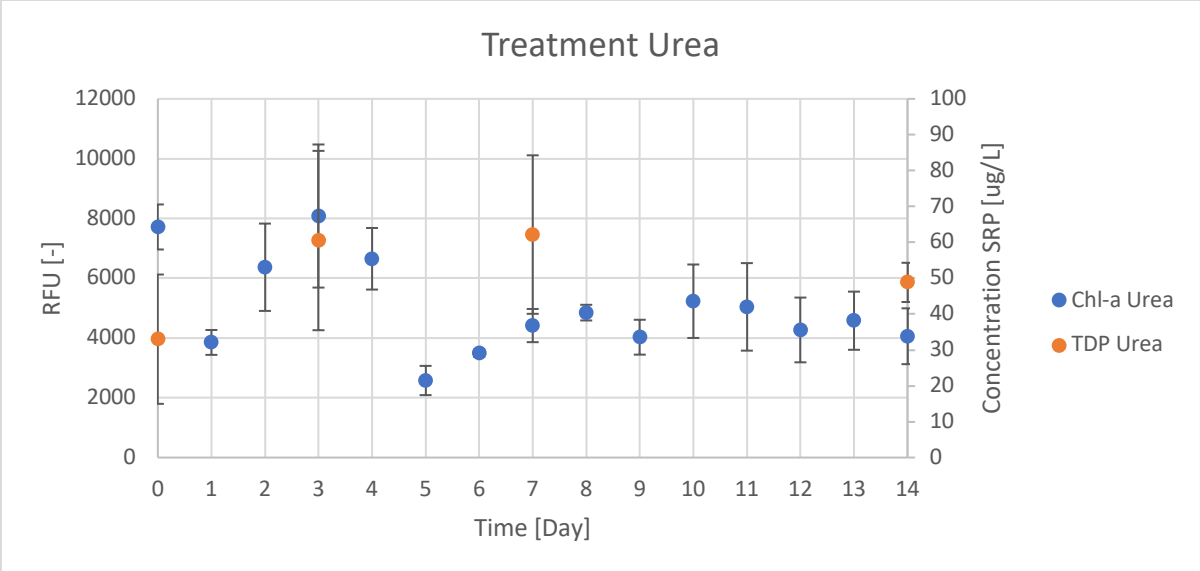

Figure X. The RFU of chl-a for average of Urea treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

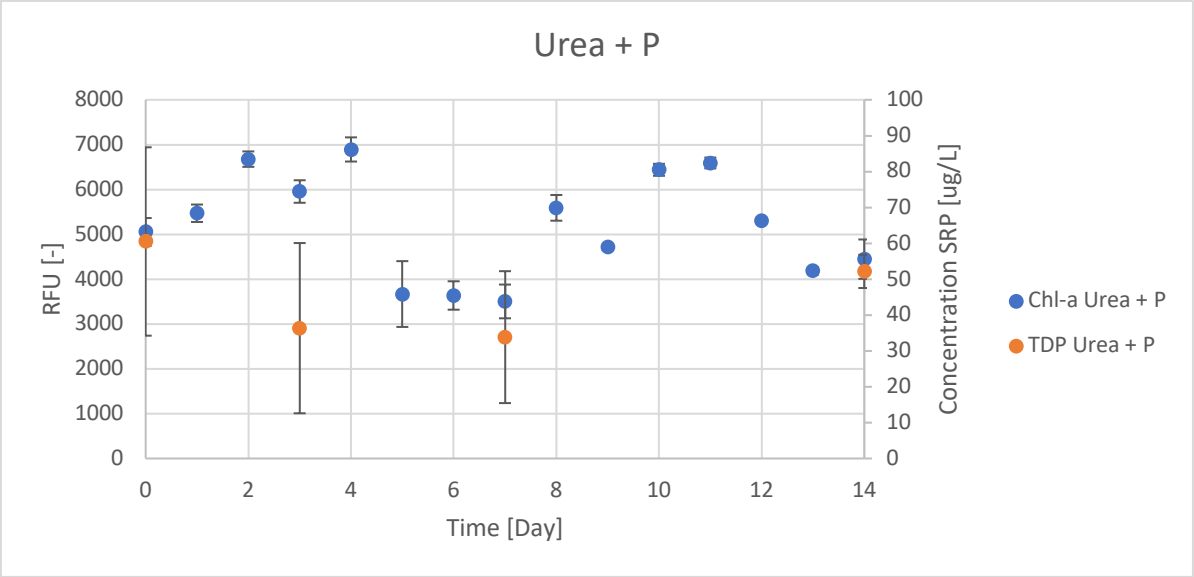

Figure X. The RFU of chl-a for average of Urea + P treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

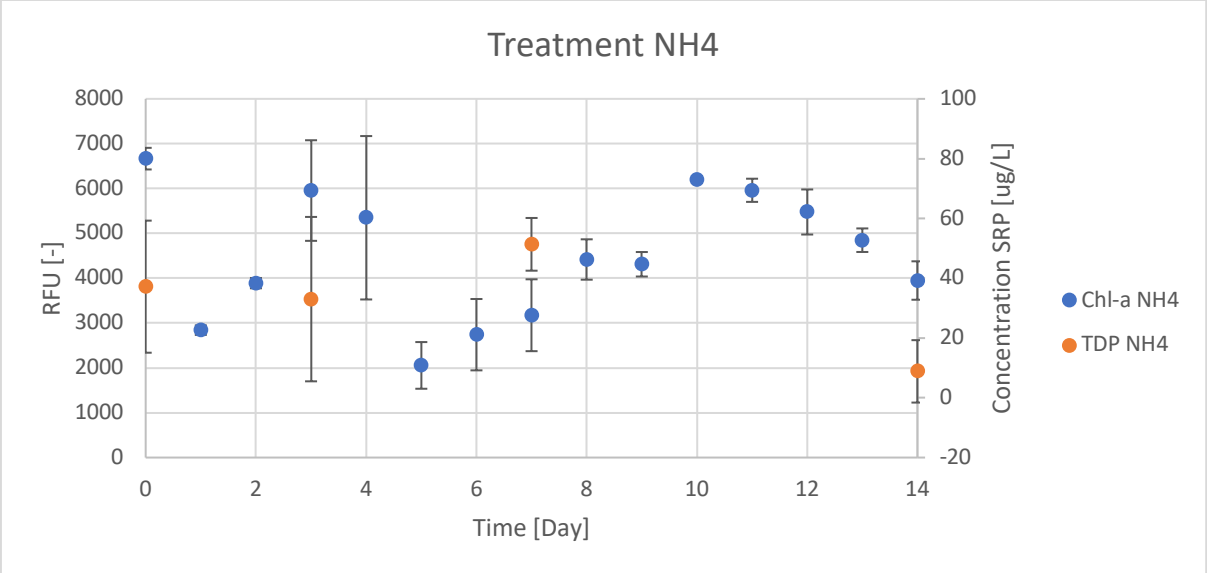

Figure X. The RFU of chl-a for average of NH4 treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

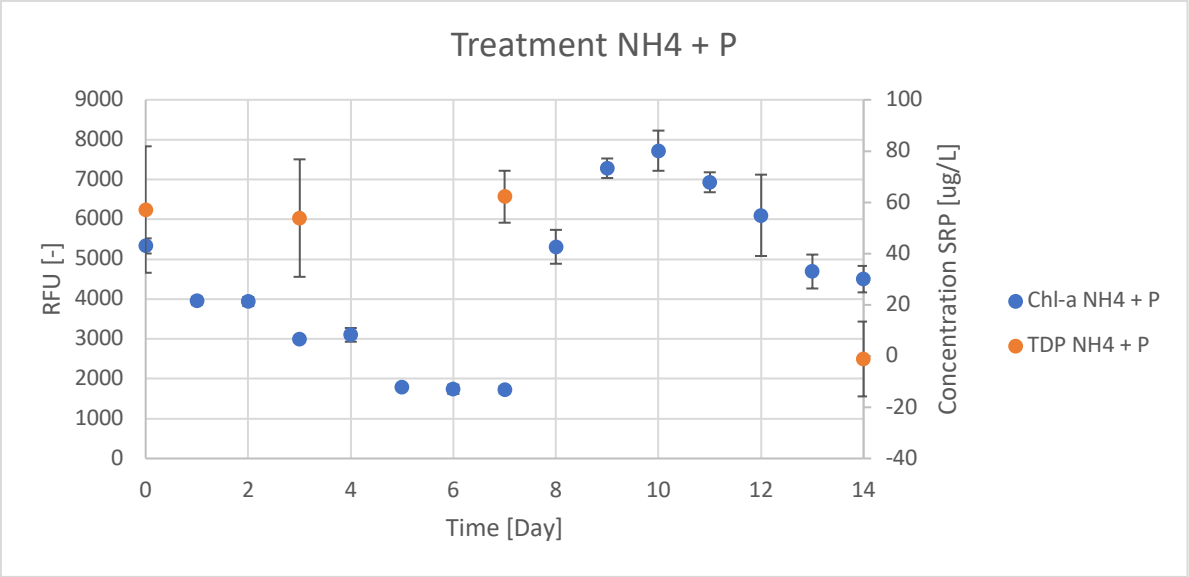

Figure X. The RFU of chl-a for average of NH4 + P treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

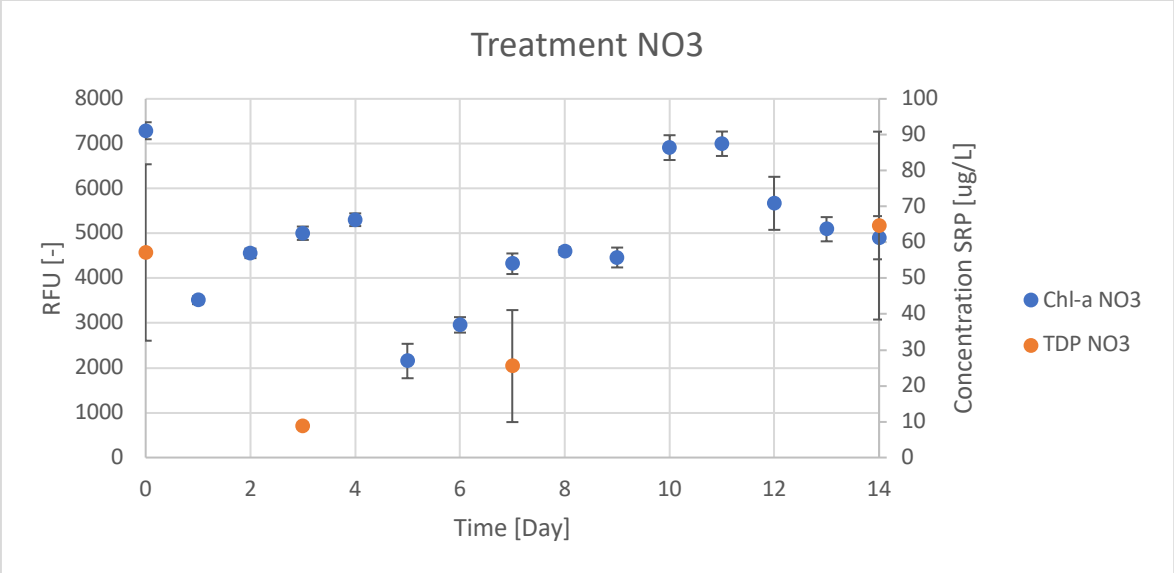

Figure X. The RFU of chl-a for average of NO3 treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

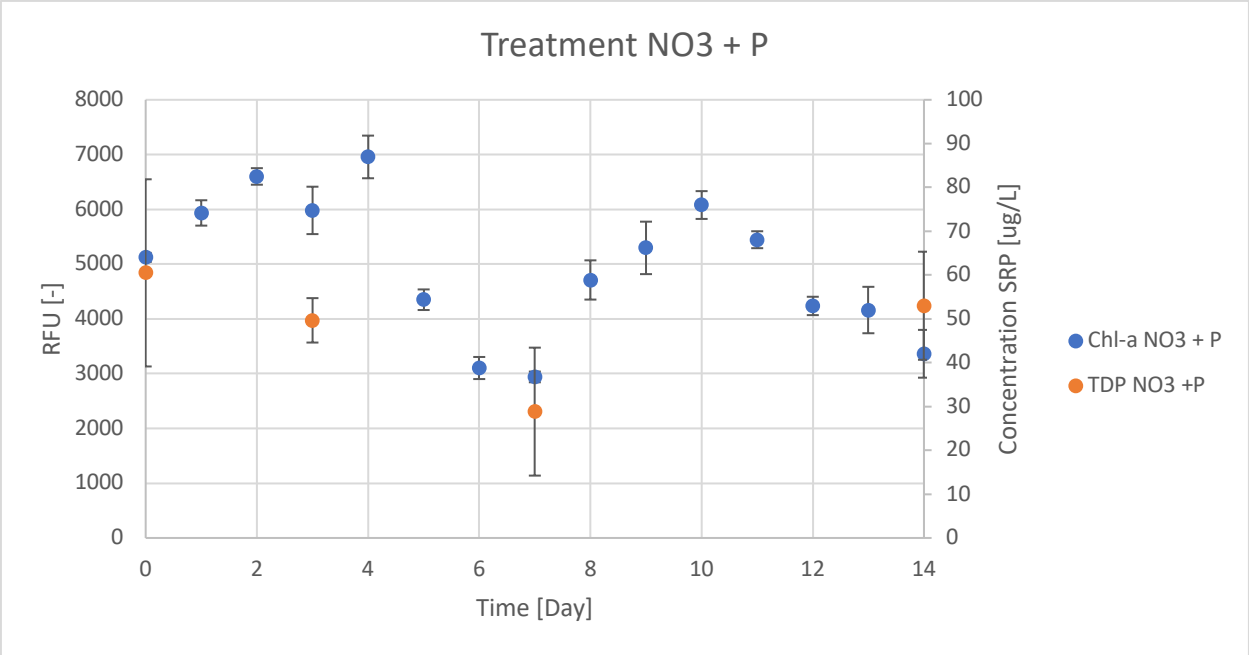

Figure X. The RFU of chl-a for average of NO3 + P treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

CHL-A VS SRP

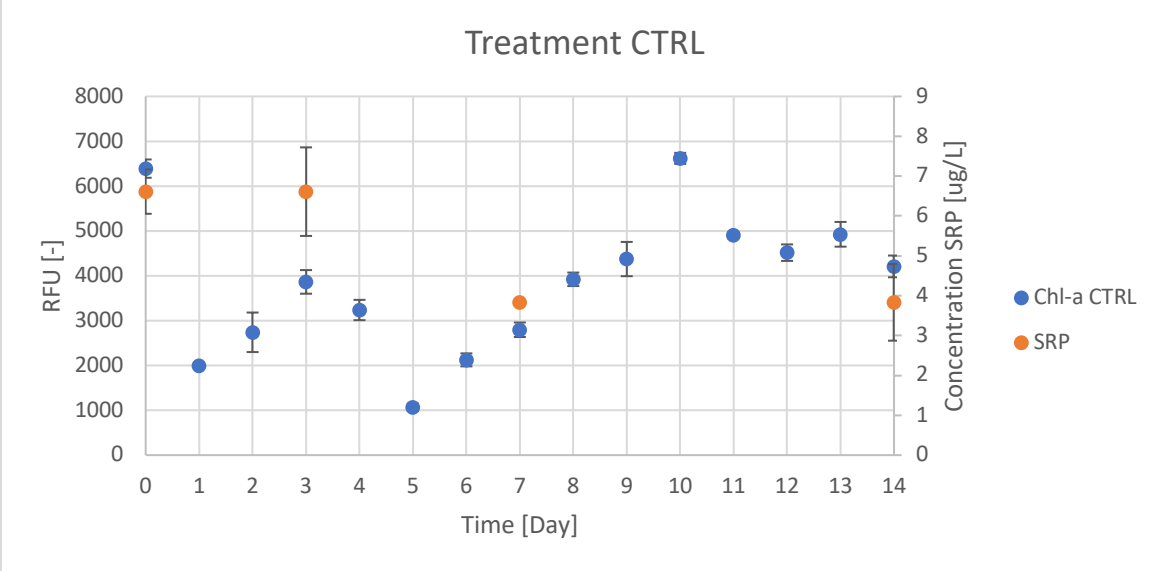

Figure X. The RFU of chl-a for average of CTRL treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

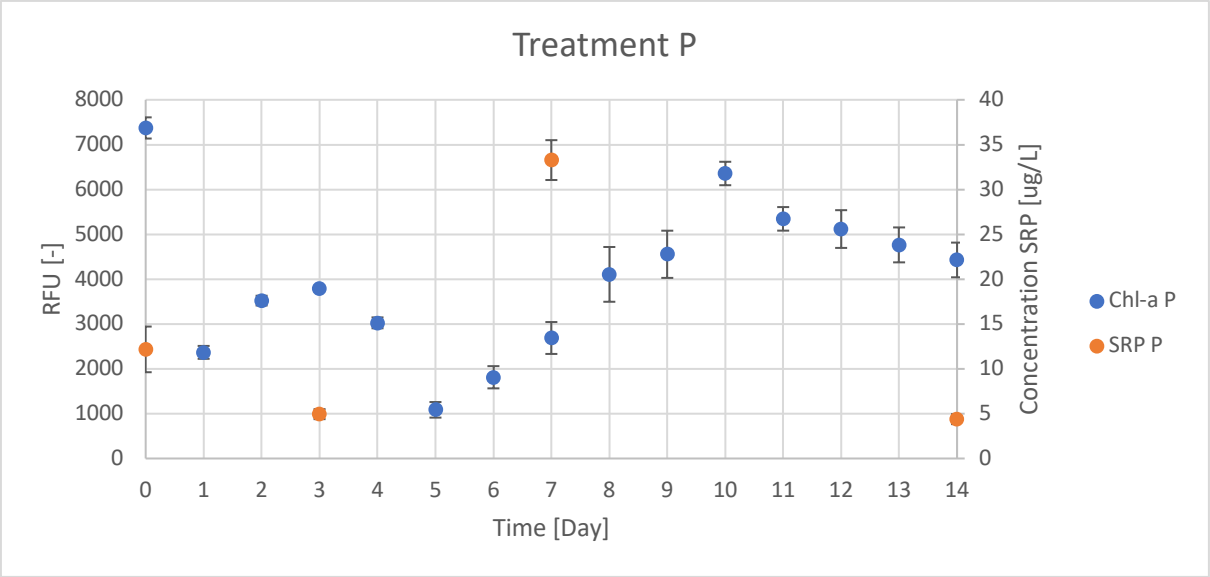

Figure X. The RFU of chl-a for average of P treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

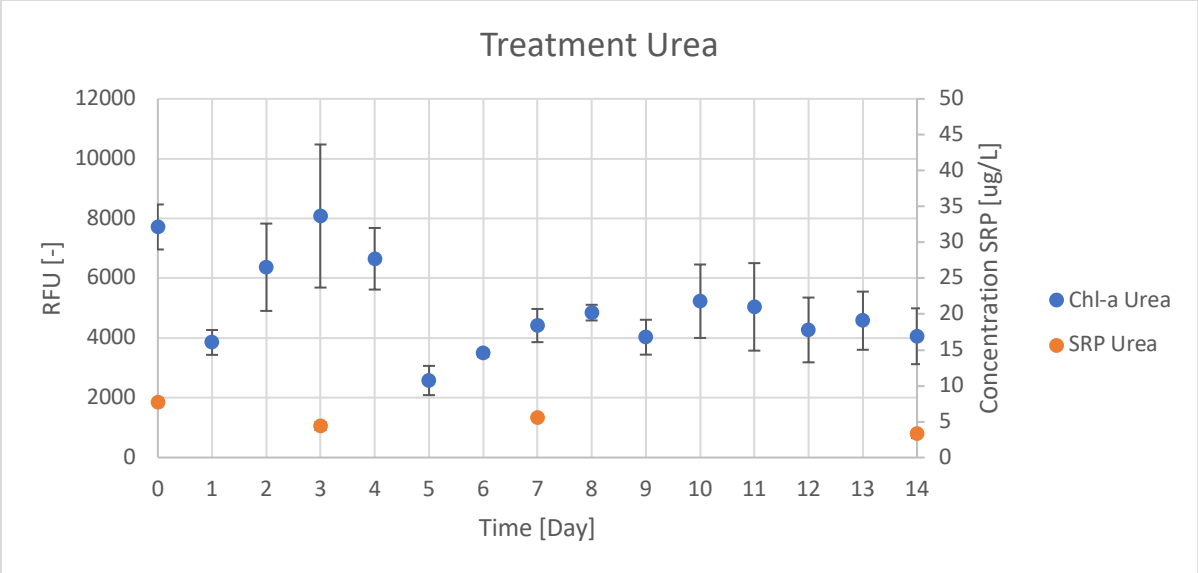

Figure X. The RFU of chl-a for average of Urea treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

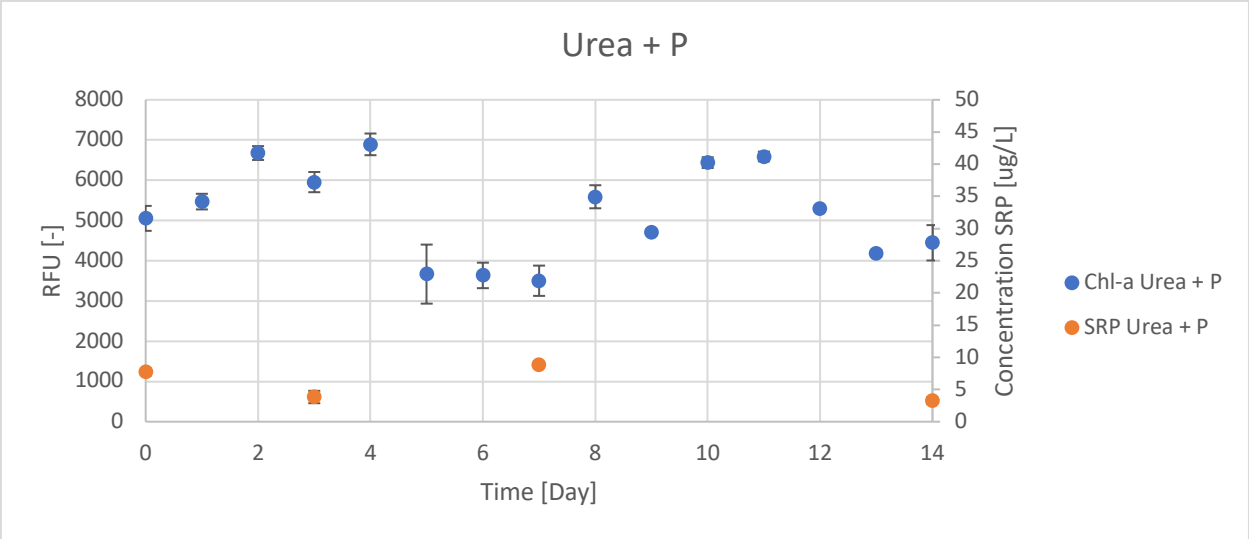

Figure X. The RFU of chl-a for average of Urea + P treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

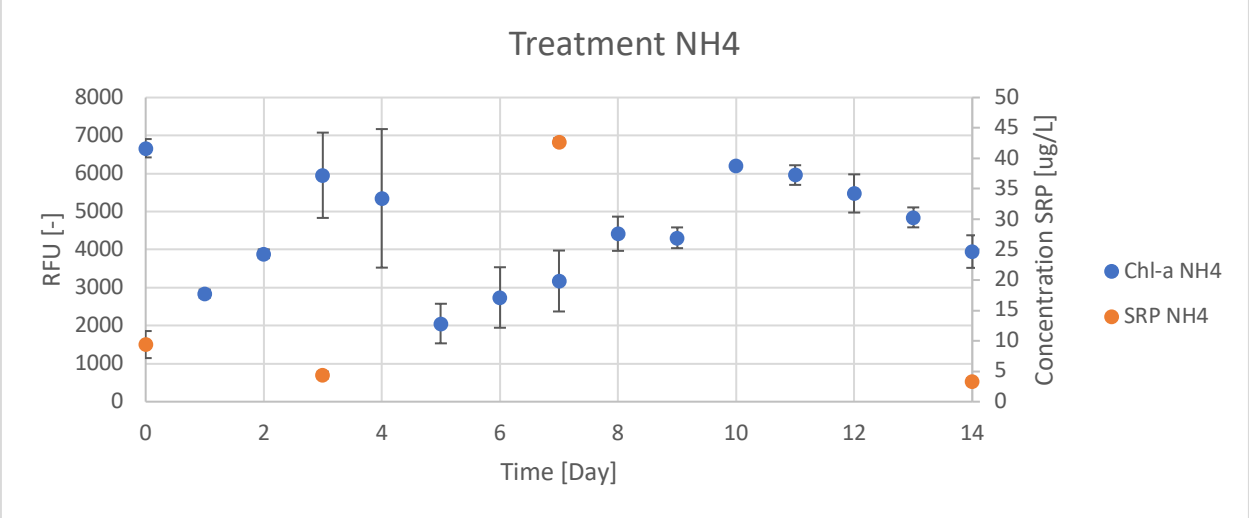

Figure X. The RFU of chl-a for average of NH4 treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

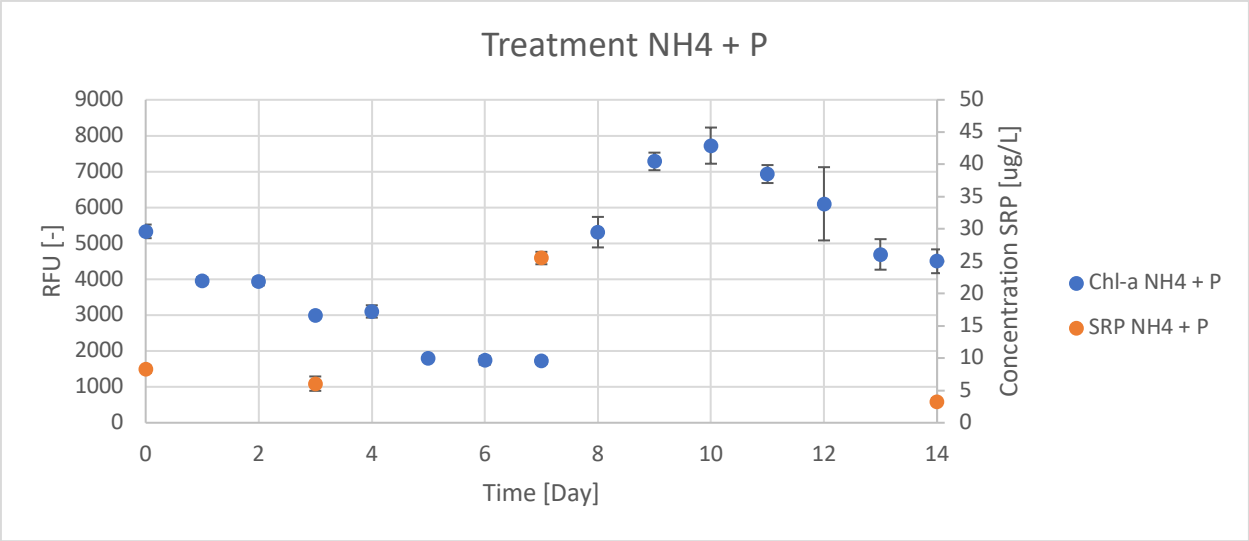

Figure X. The RFU of chl-a for average of NH4 + P treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

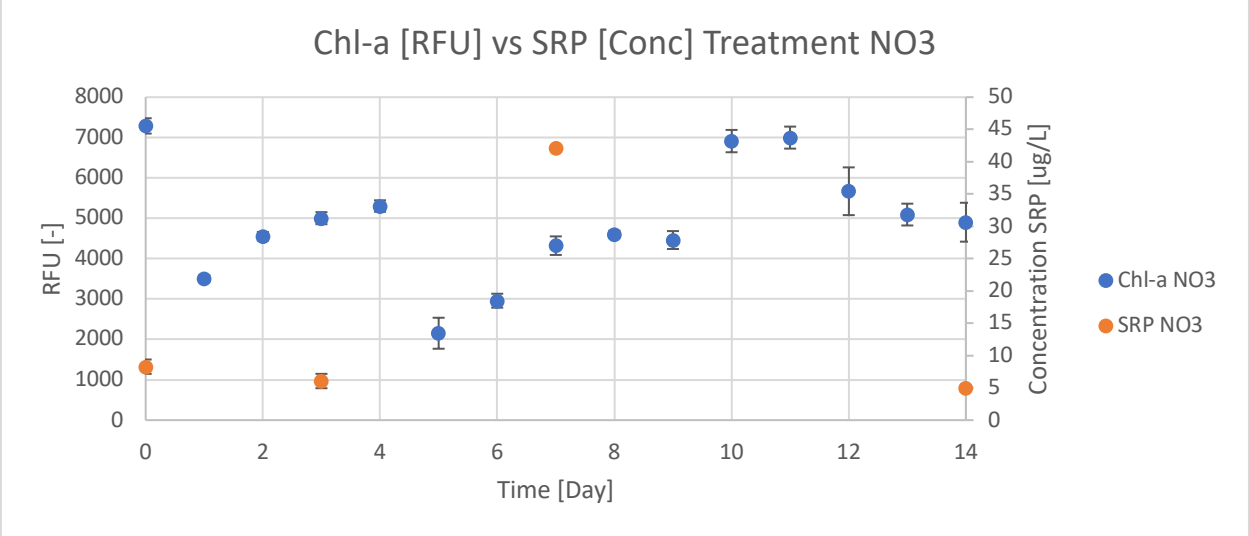

Figure X. The RFU of chl-a for average of NO3 treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

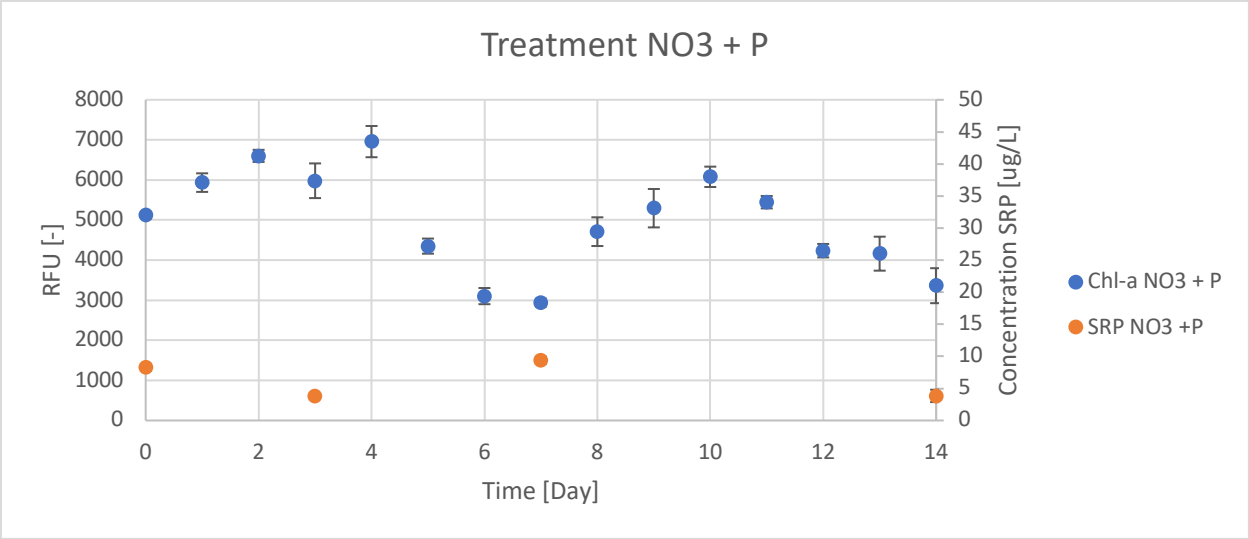

Figure X. The RFU of chl-a for average of NO3 + P treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

CHL-A RFU TDN

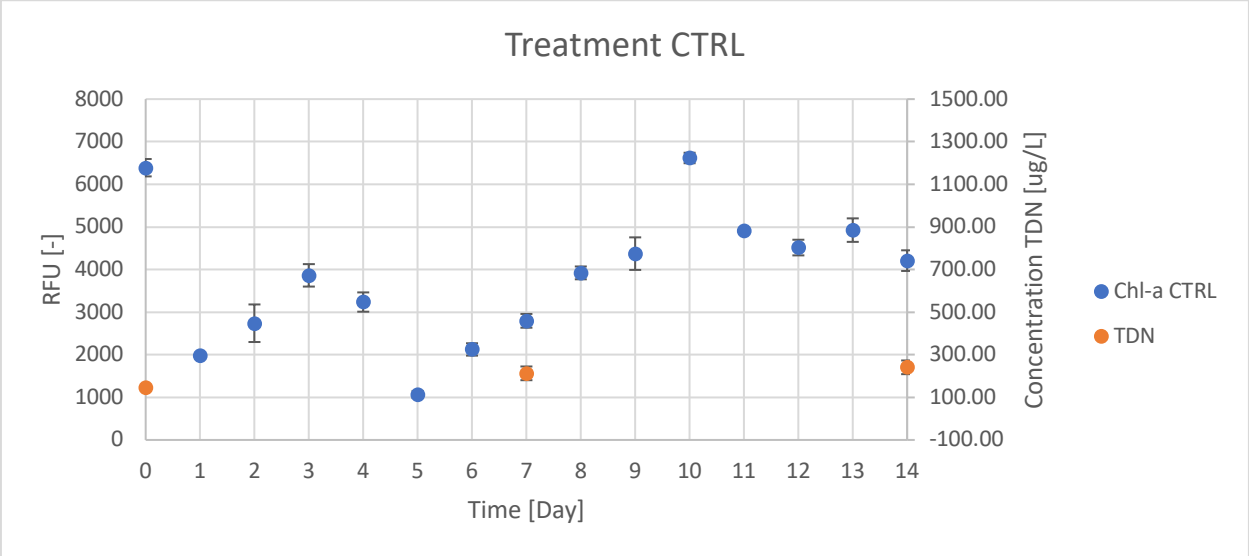

Figure X. The RFU of chl-a for average of CTRL treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

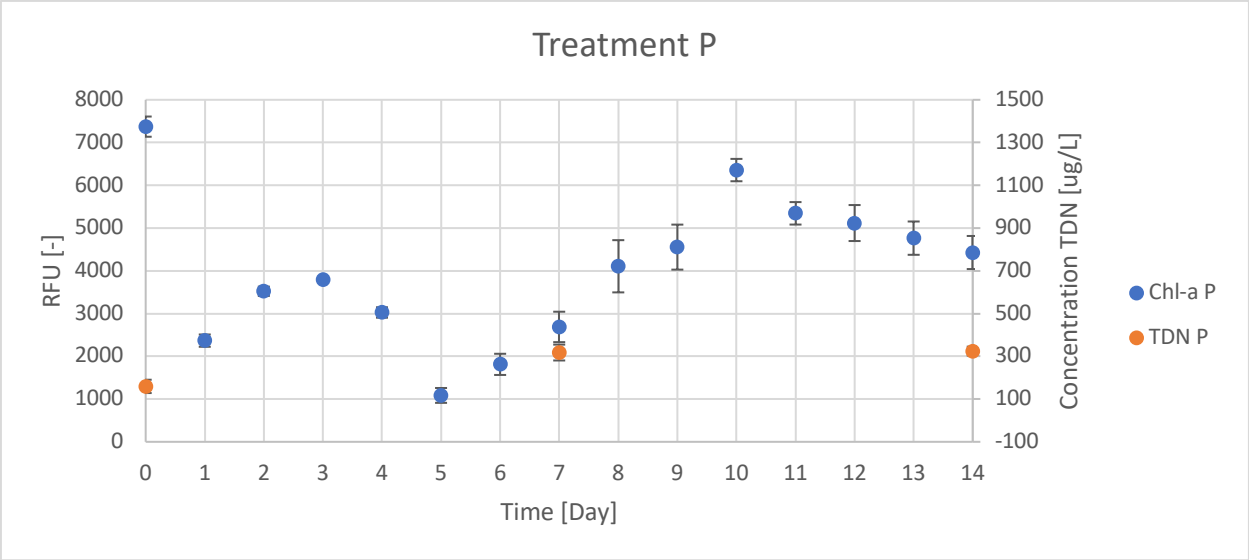

Figure X. The RFU of chl-a for average of P treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

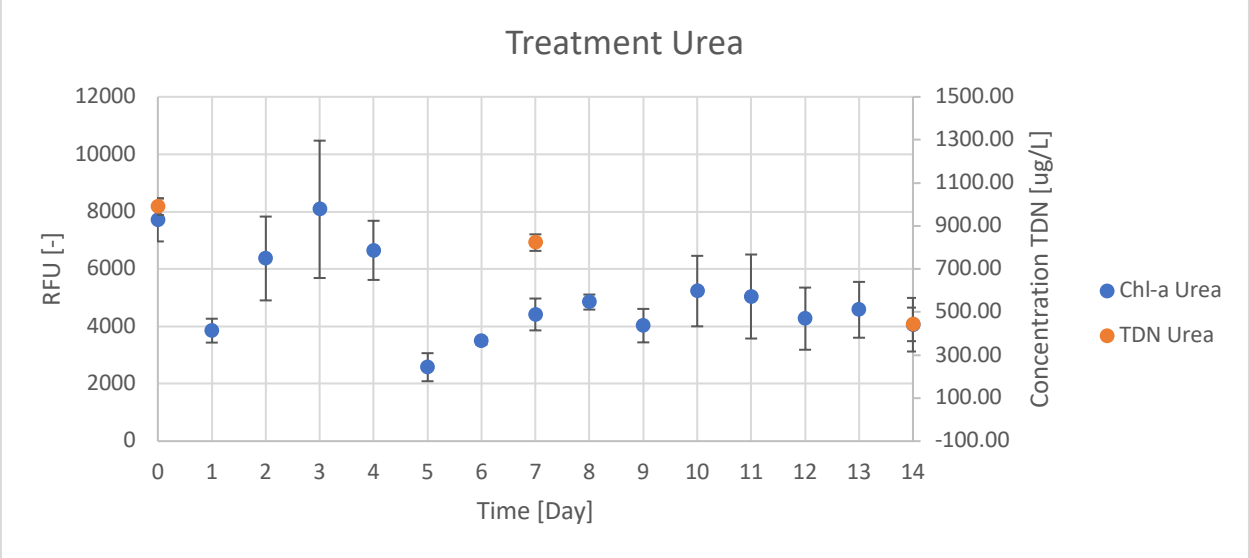

Figure X. The RFU of chl-a for average of Urea treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

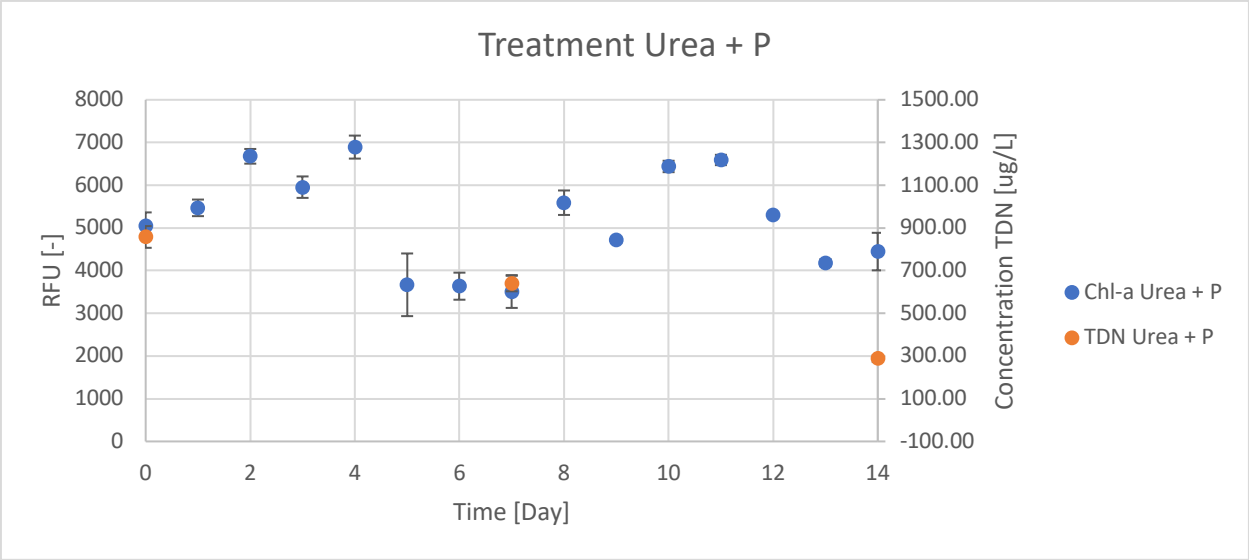

Figure X. The RFU of chl-a for average of Urea + P treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

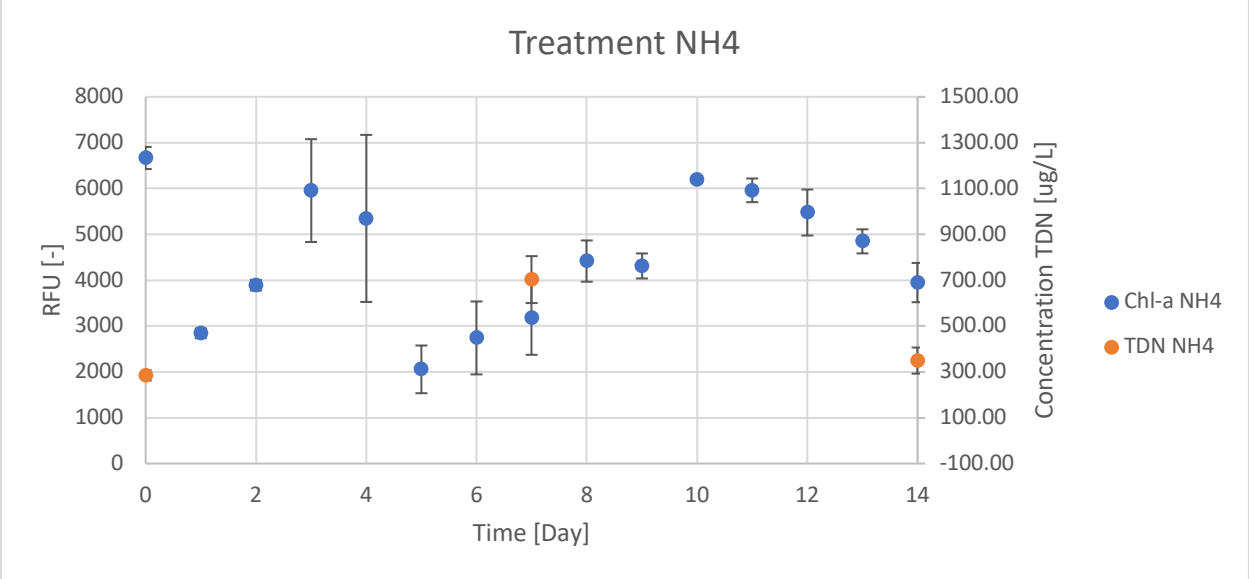

Figure X. The RFU of chl-a for average of NH4 treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

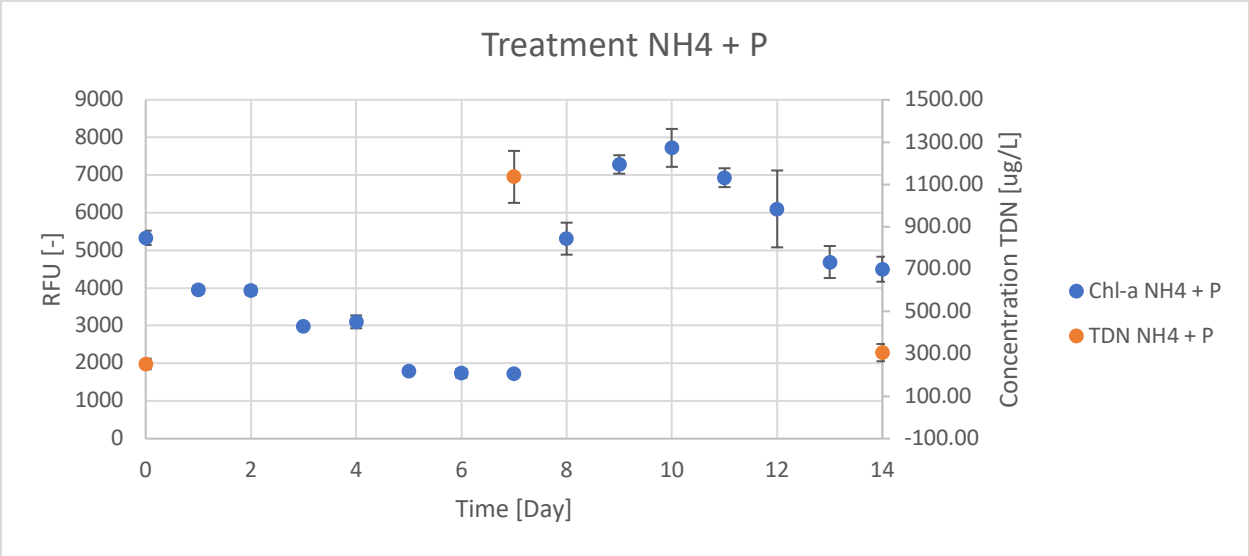

Figure X. The RFU of chl-a for average of NH4 + P treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

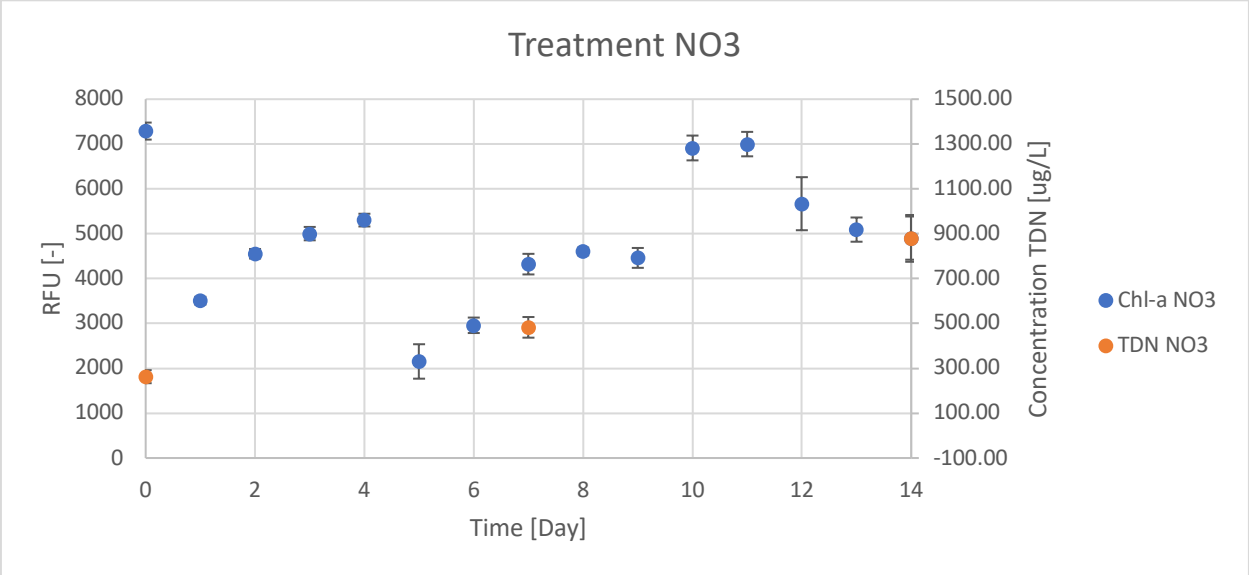

Figure X. The RFU of chl-a for average of NO3 treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

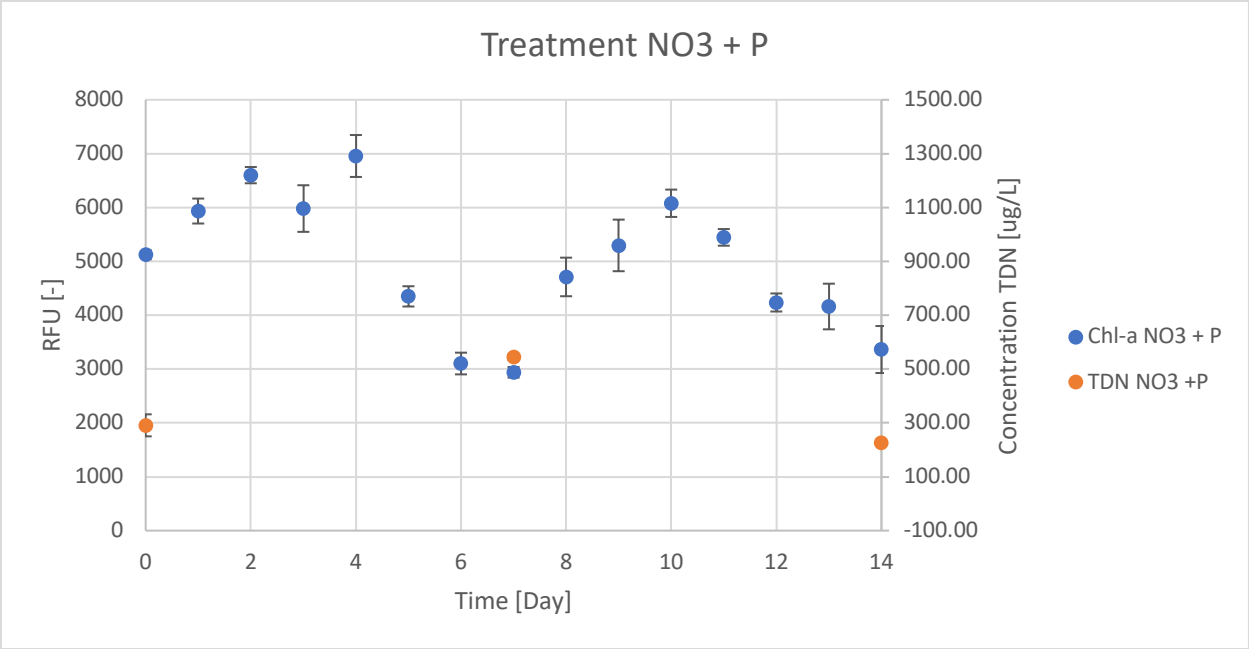

Figure X. The RFU of chl-a for average of NO3 + P treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

1.0 Chl-a (concentration) vs. Nutrient (concentration)

2.0 Chl-a (ug/L) vs. SRP (ug/L)

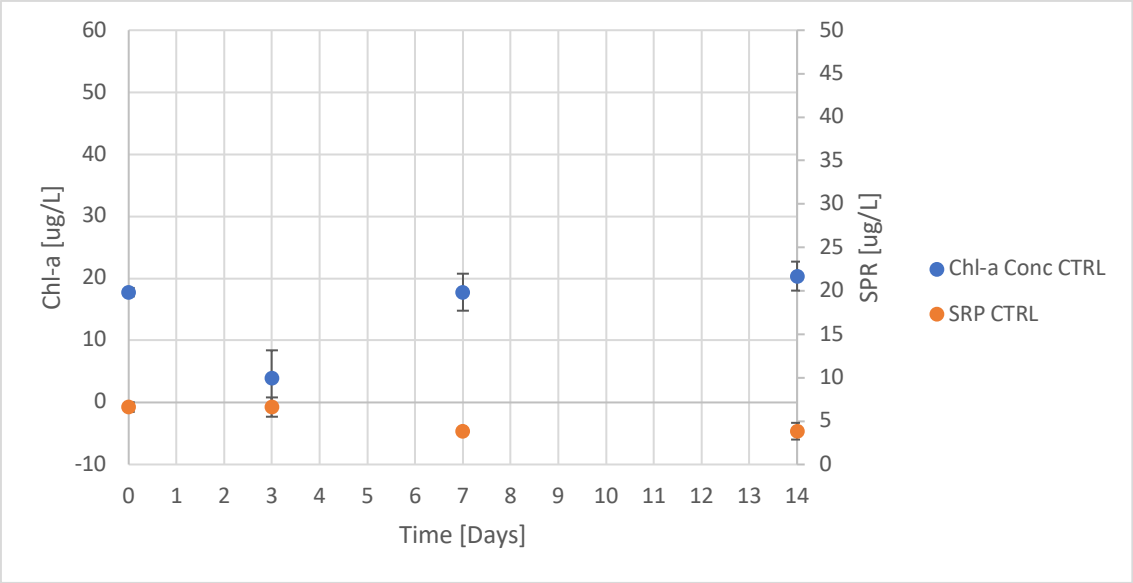

Figure X. The concentration of chl-a for average of CTRL treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

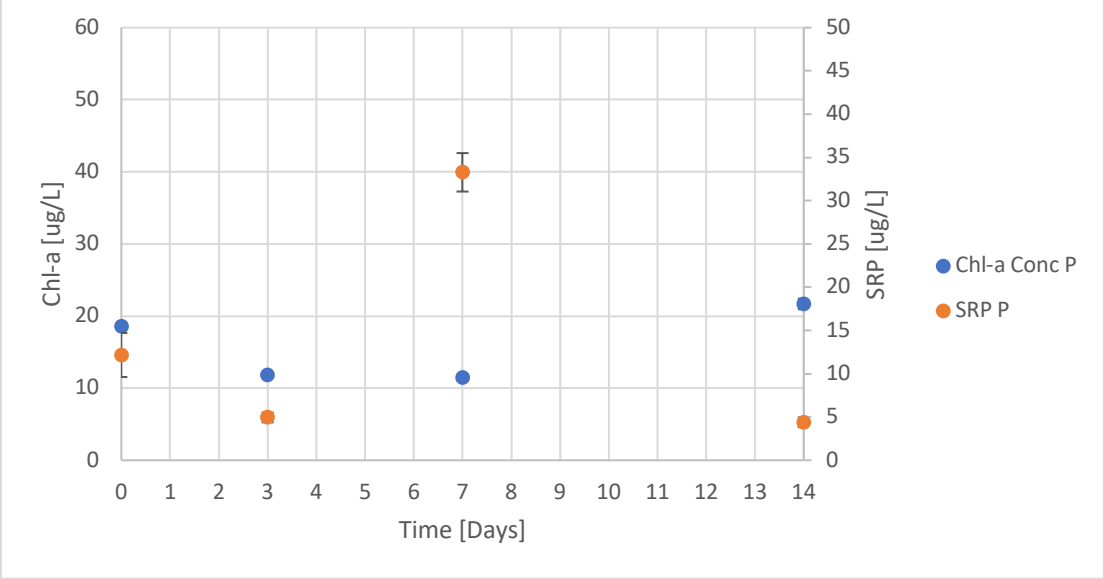

Figure X. The concentration of chl-a for average of P treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

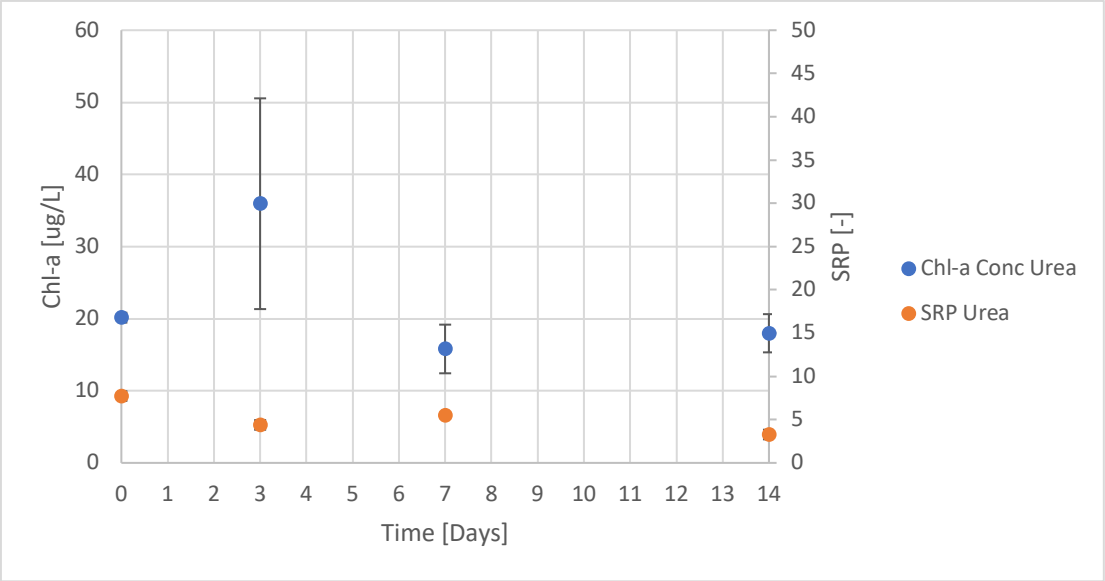

Figure X. The concentration of chl-a for average of Urea treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

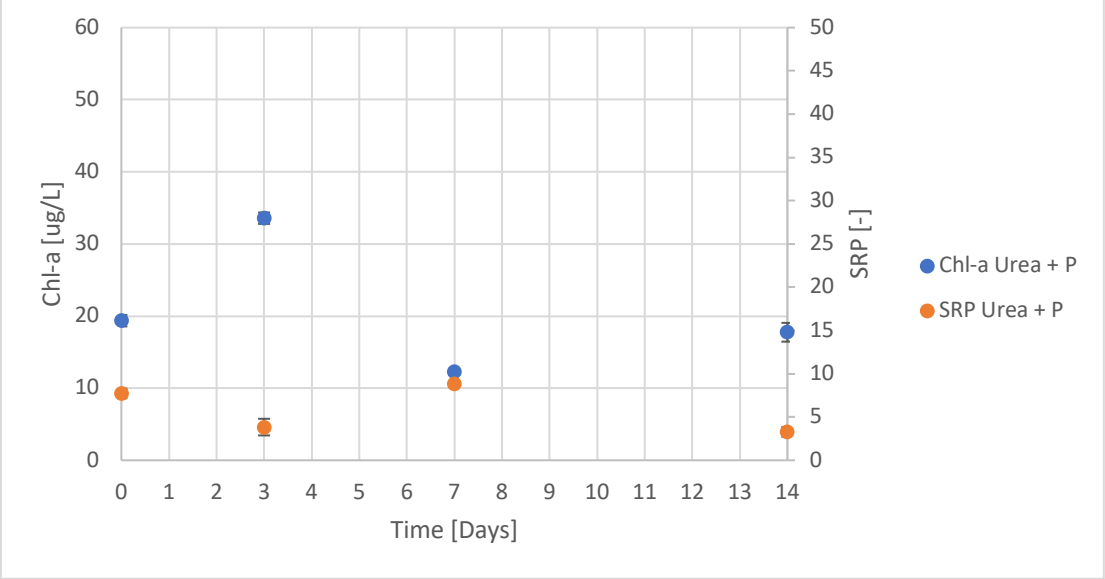

Figure X. The concentration of chl-a for average of Urea + P treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

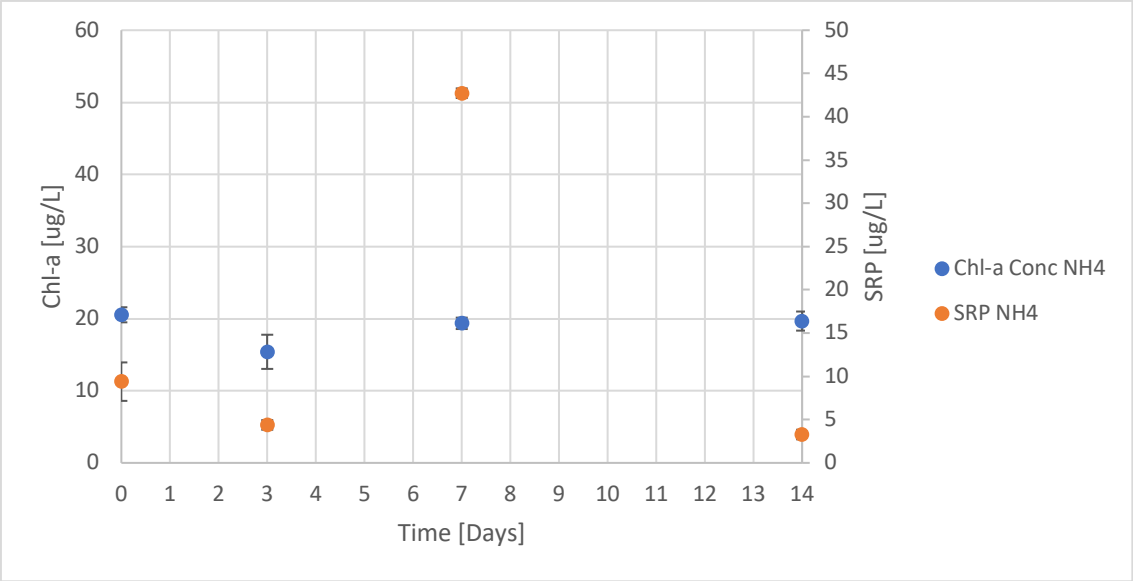

Figure X. The concentration of chl-a for average of NH4 treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

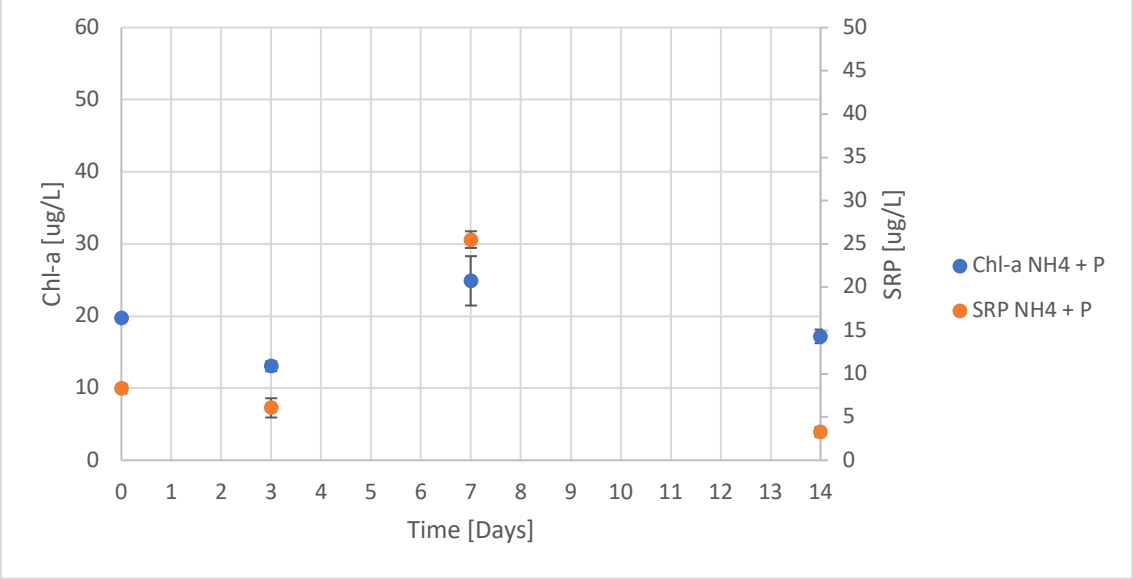

Figure X. The concentration of chl-a for average of NH4 + P treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

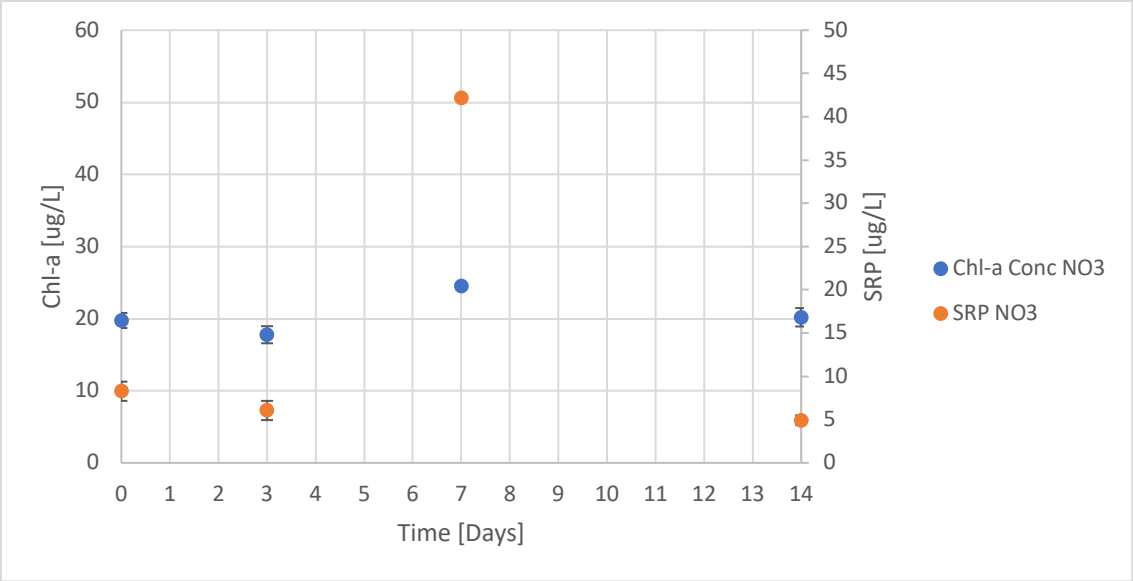

Figure X. The concentration of chl-a for average of NO3 treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

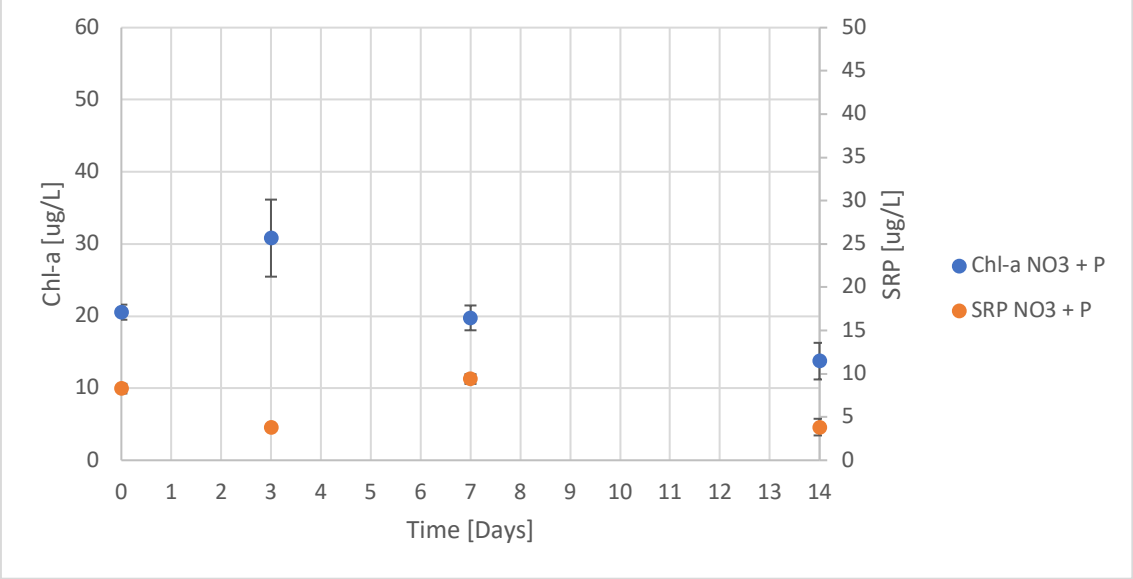

Figure X. The concentration of chl-a for average of NO3 + P treatment verse the concentration of SRP over Day 0 to 14 of trial 3.

3.0 Chl-a (ug/L) vs. TDP (ug/L)

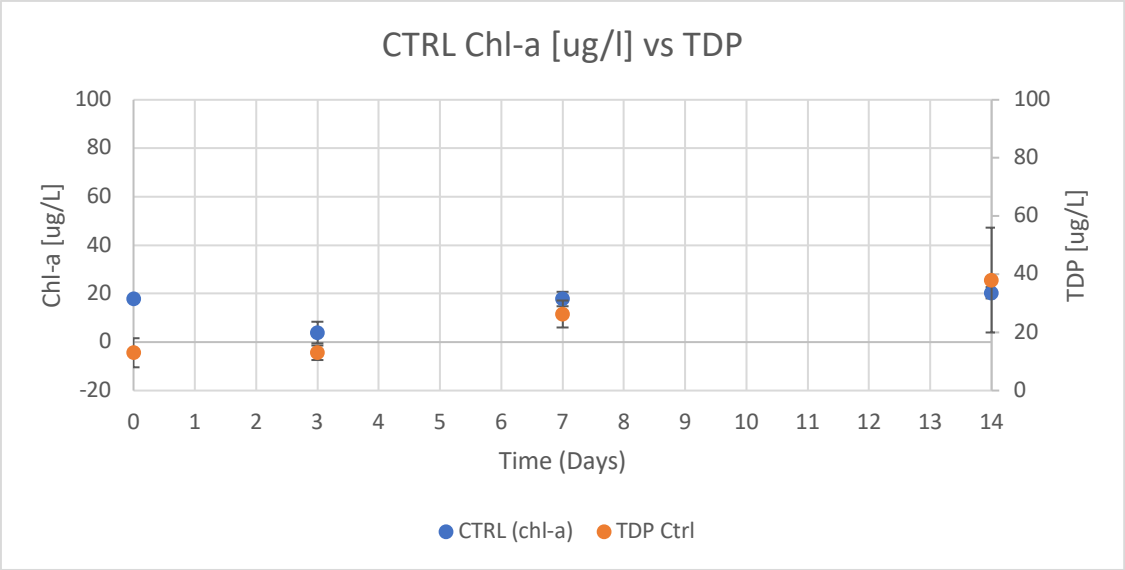

Figure X. The concentration of chl-a for average of CTRL treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

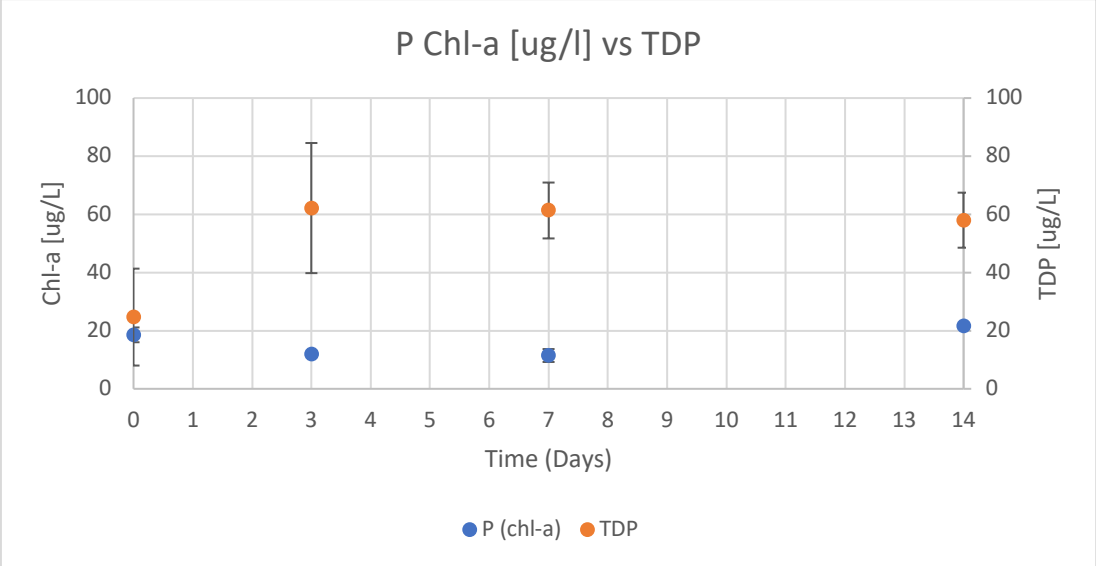

Figure X. The concentration of chl-a for average of P treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

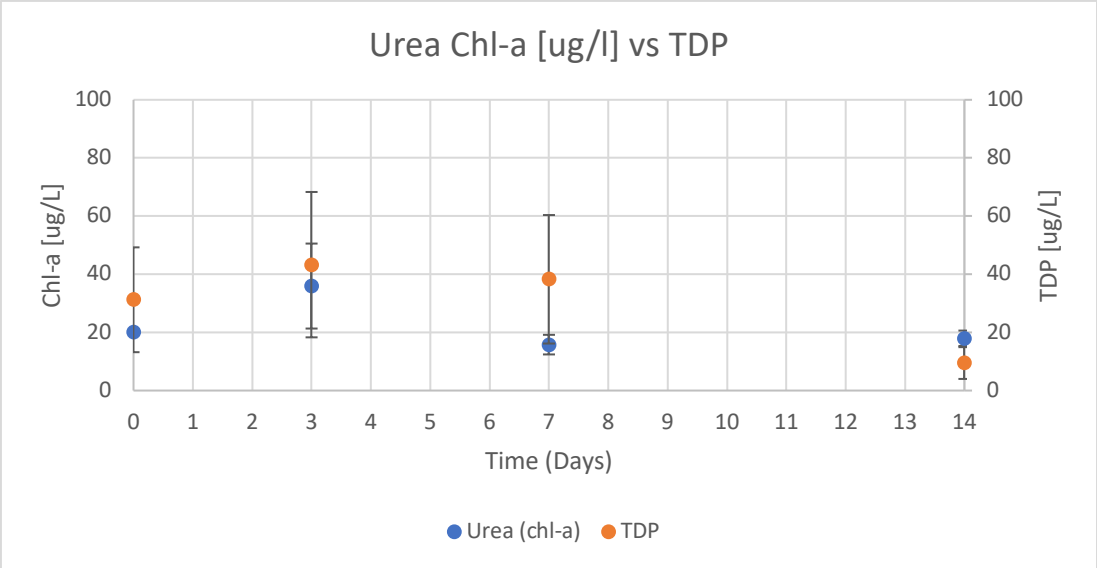

Figure X. The concentration of chl-a for average of Urea treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

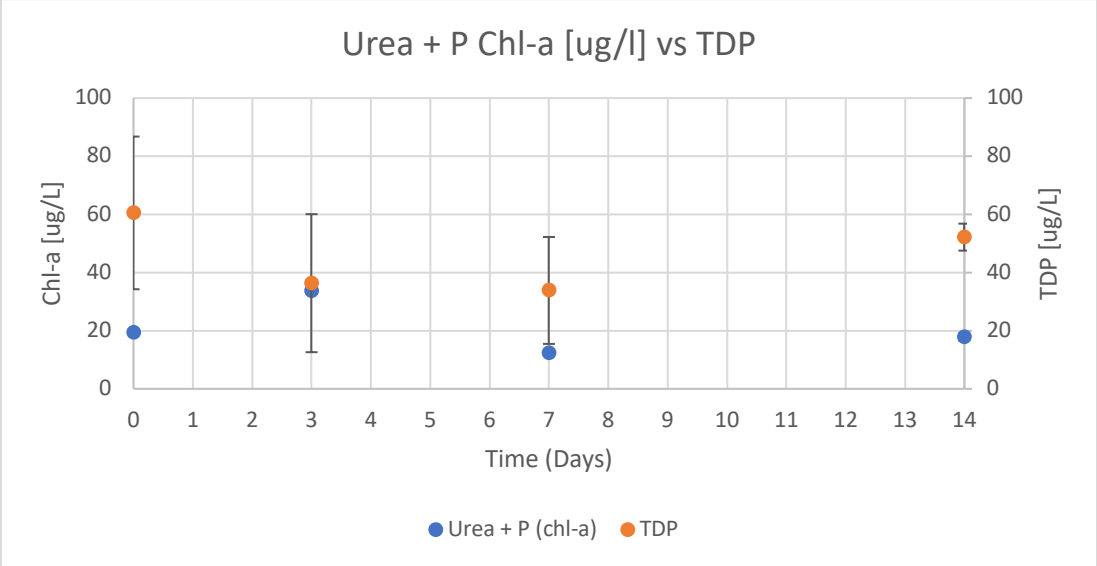

Figure X. The concentration of chl-a for average of Urea + P treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

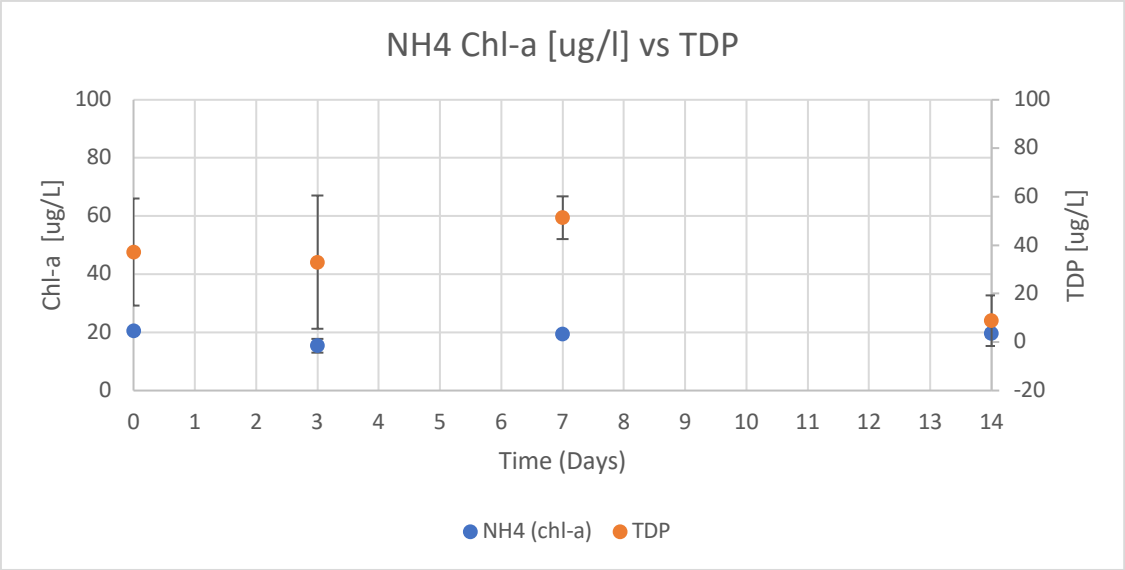

Figure X. The concentration of chl-a for average of NH4 treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

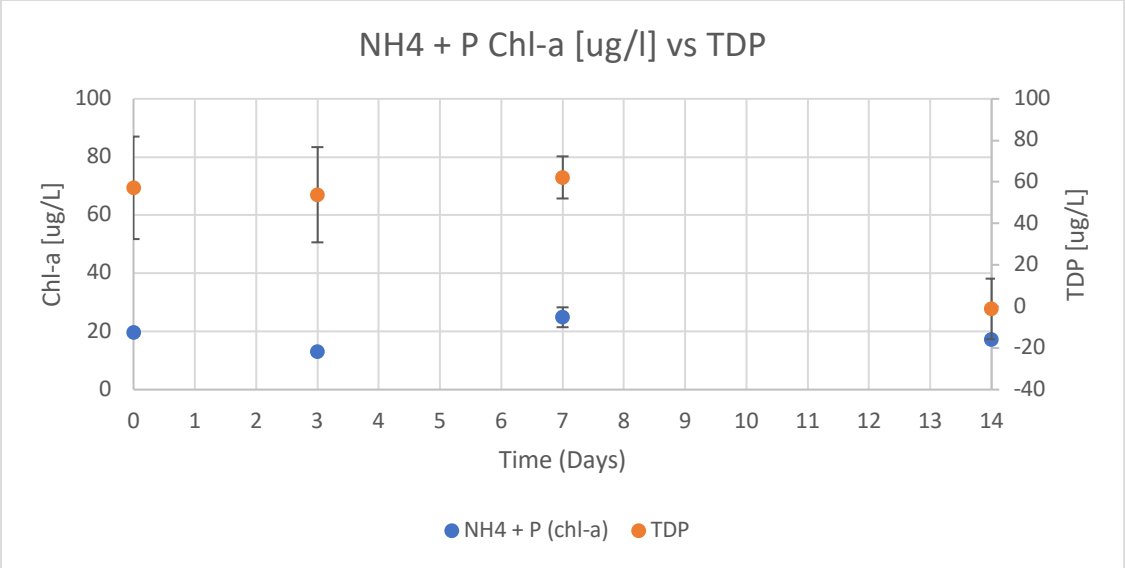

Figure X. The concentration of chl-a for average of NH4 + P treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

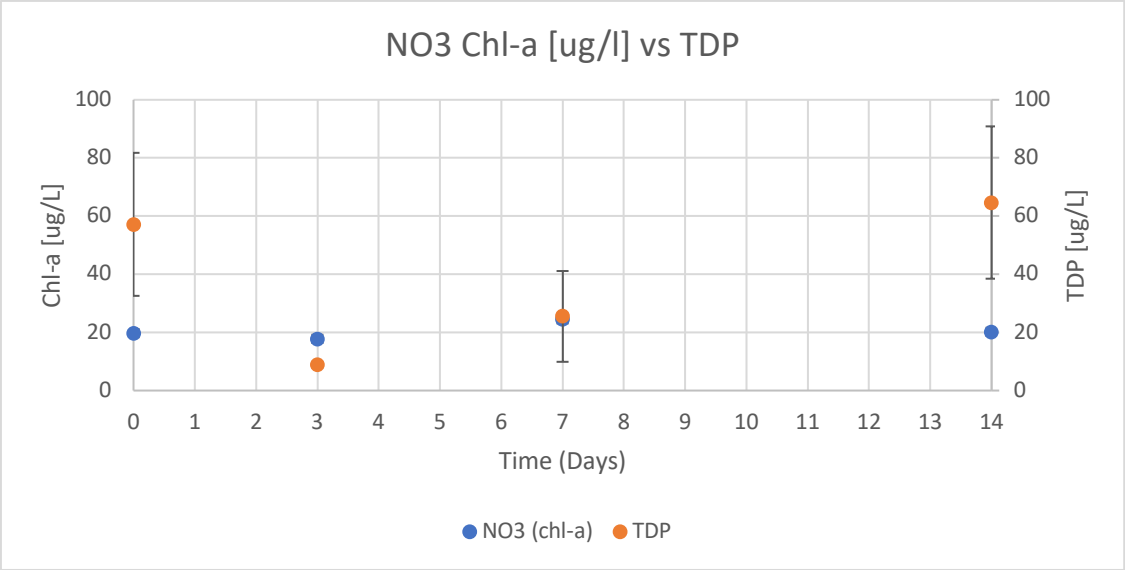

Figure X. The concentration of chl-a for average of NO3 treatment verse the concentration of TDP over Day 0 to 14 of trial 3.

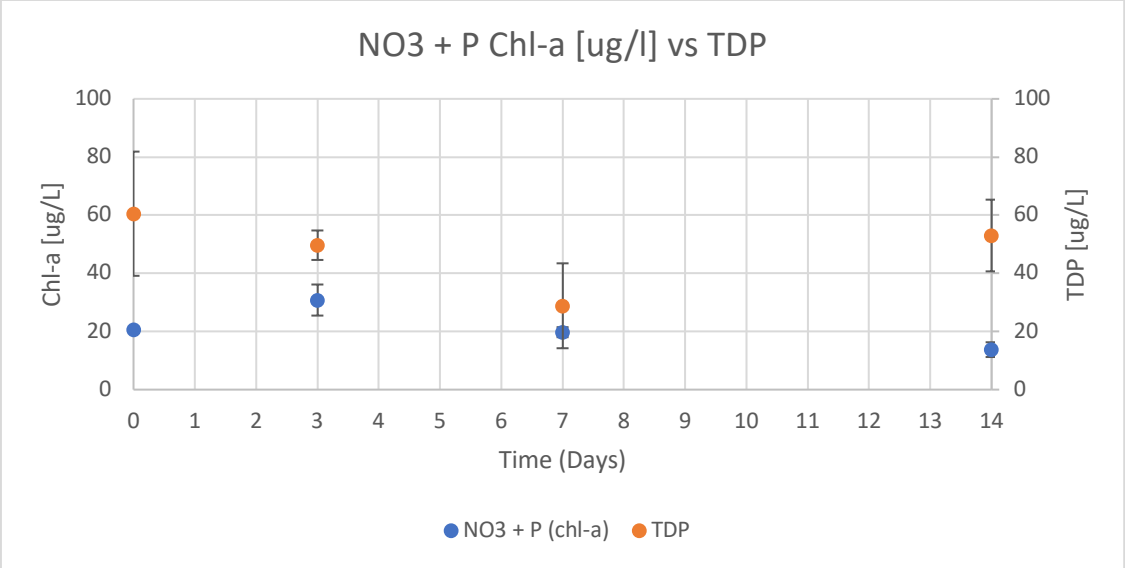

Figure X. The concentration of chl-a for average of NO3 + Ptreatment verse the concentration of TDP over Day 0 to 14 of trial 3.

4.0 Chl-a (ug/L) vs. TDN (ug/L)

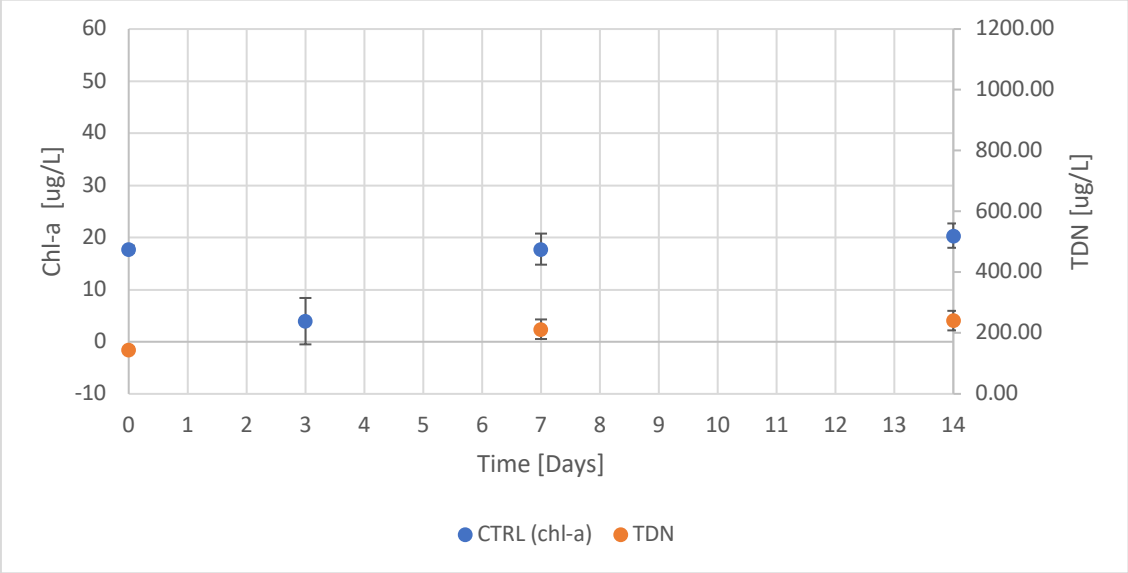

Figure Y. The concentration of chl-a for average of CTRL treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

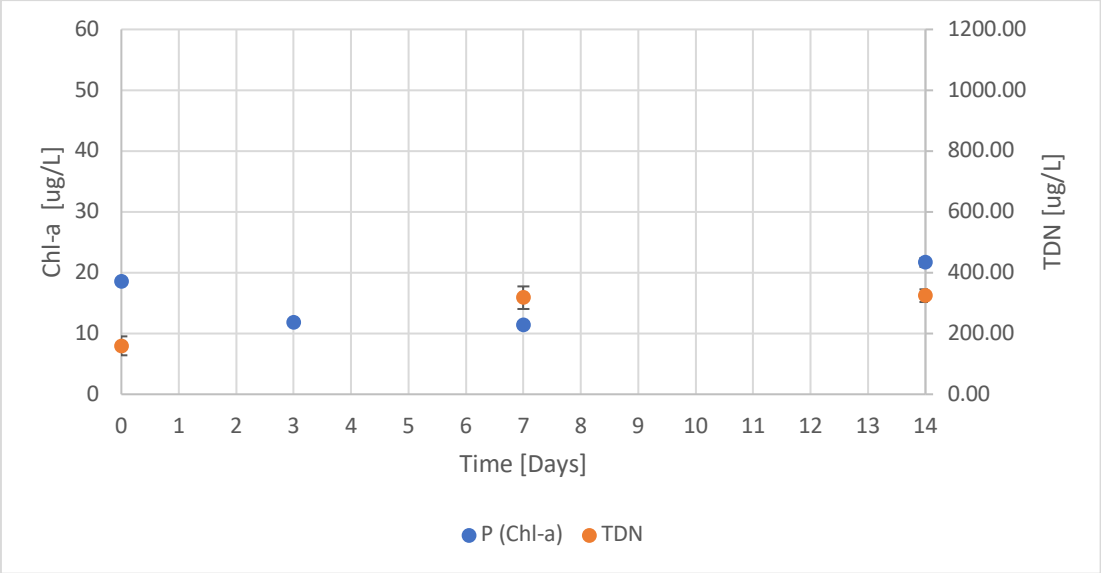

Figure Y. The concentration of chl-a for average of P treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

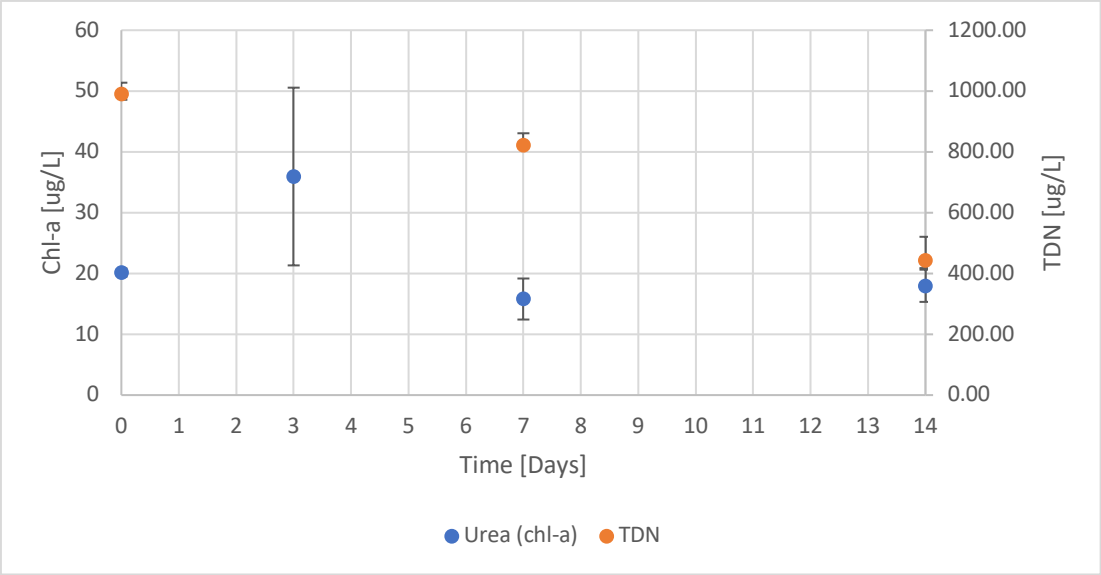

Figure Y. The concentration of chl-a for average of Urea treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

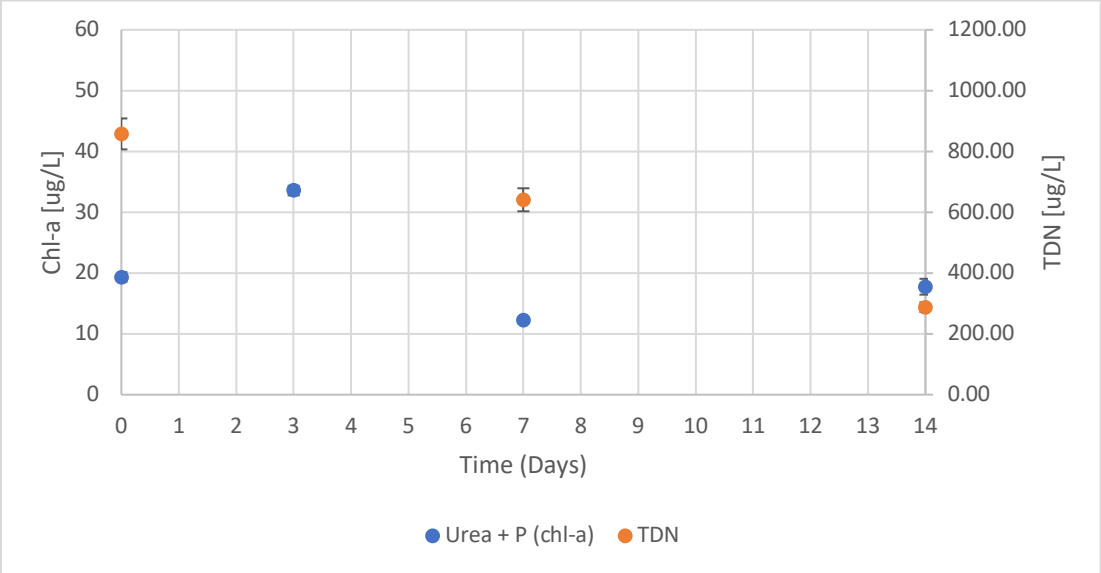

Figure Y. The concentration of chl-a for average of Urea + P treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

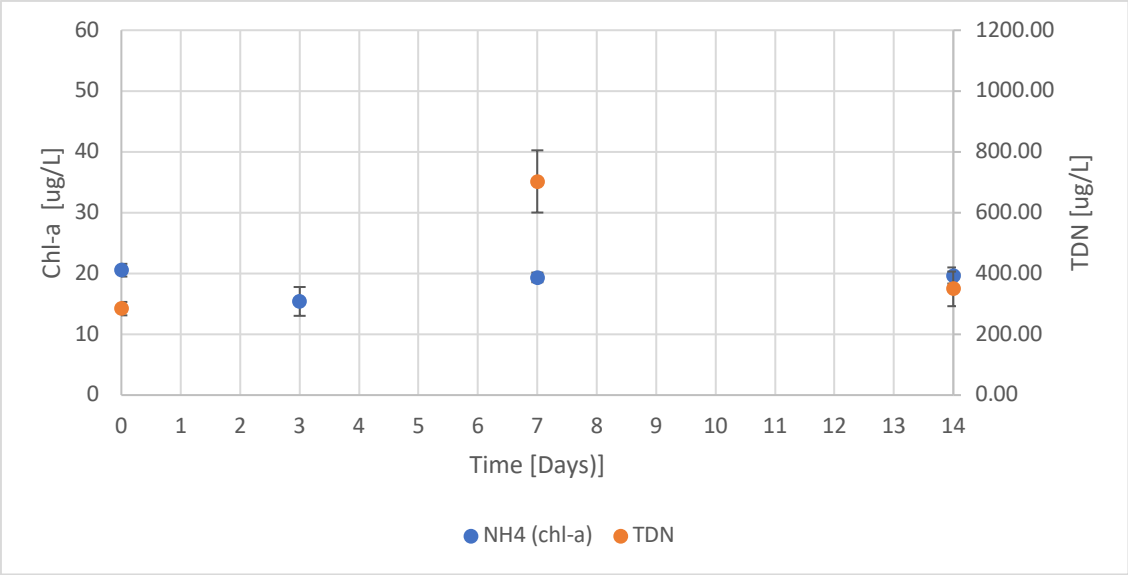

Figure Y. The concentration of chl-a for average of NH4 treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

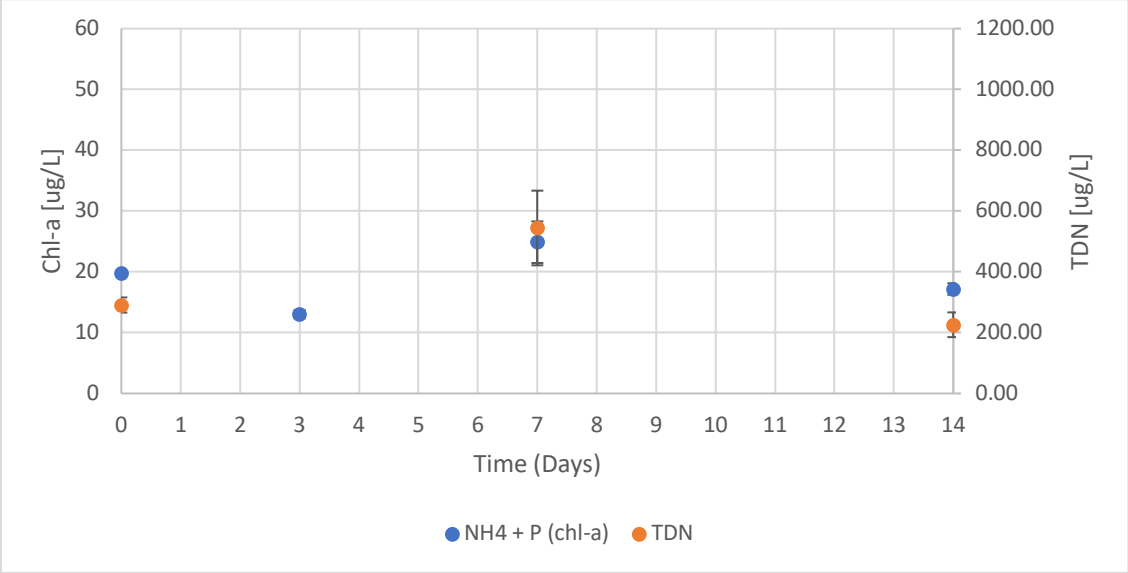

Figure Y. The concentration of chl-a for average of NH4 + P treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

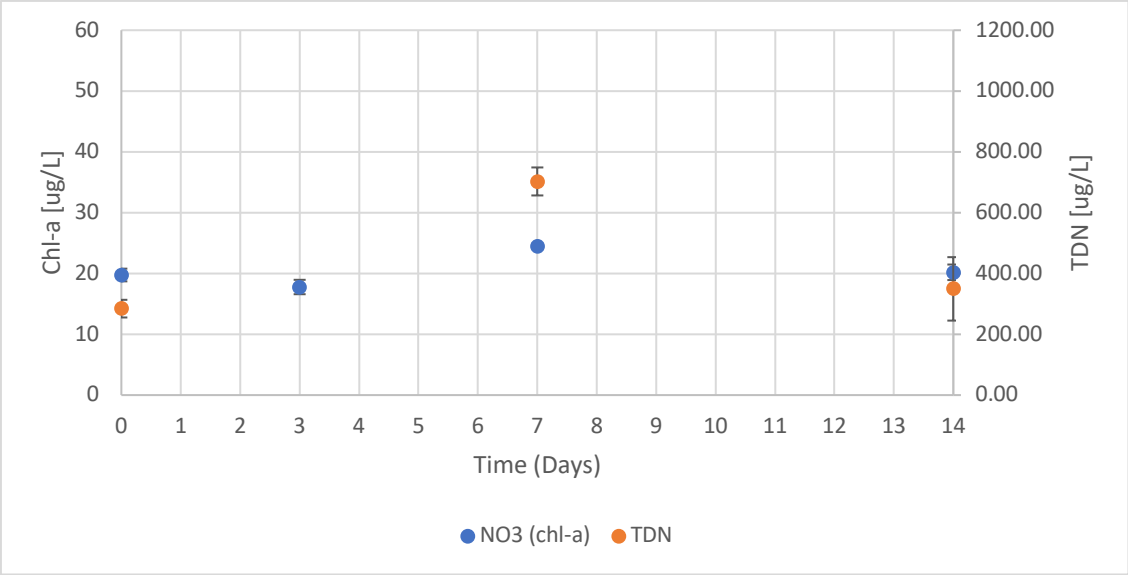

Figure Y. The concentration of chl-a for average of NO3 treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

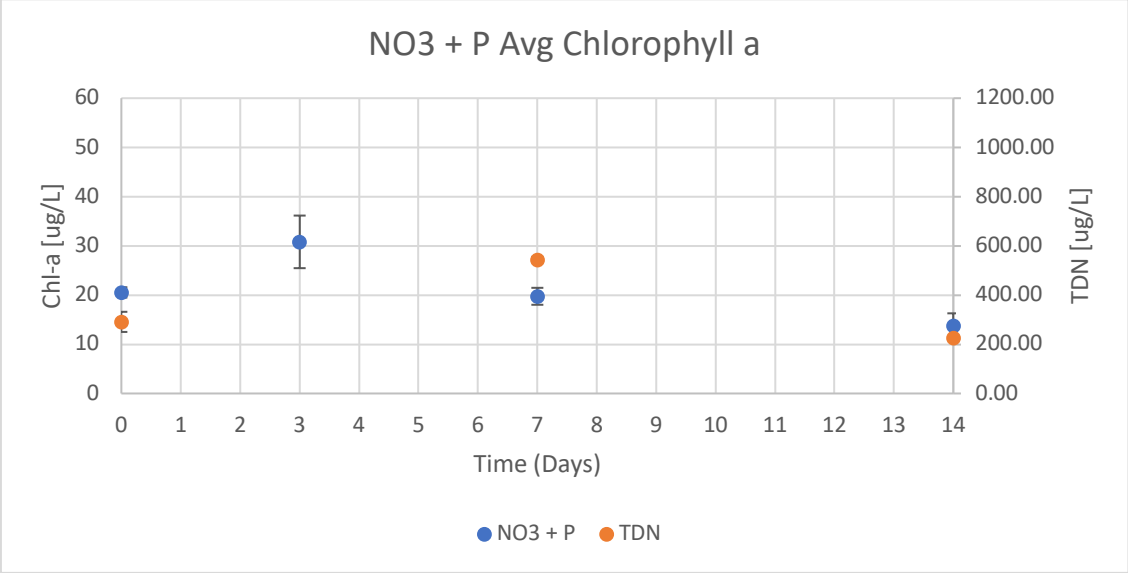

Figure Y. The concentration of chl-a for average of NO3 + P treatment verse the concentration of TDN over Day 0 to 14 of trial 3.

5.0 Chl-a (ug/L) vs. NH4 (ug/L)

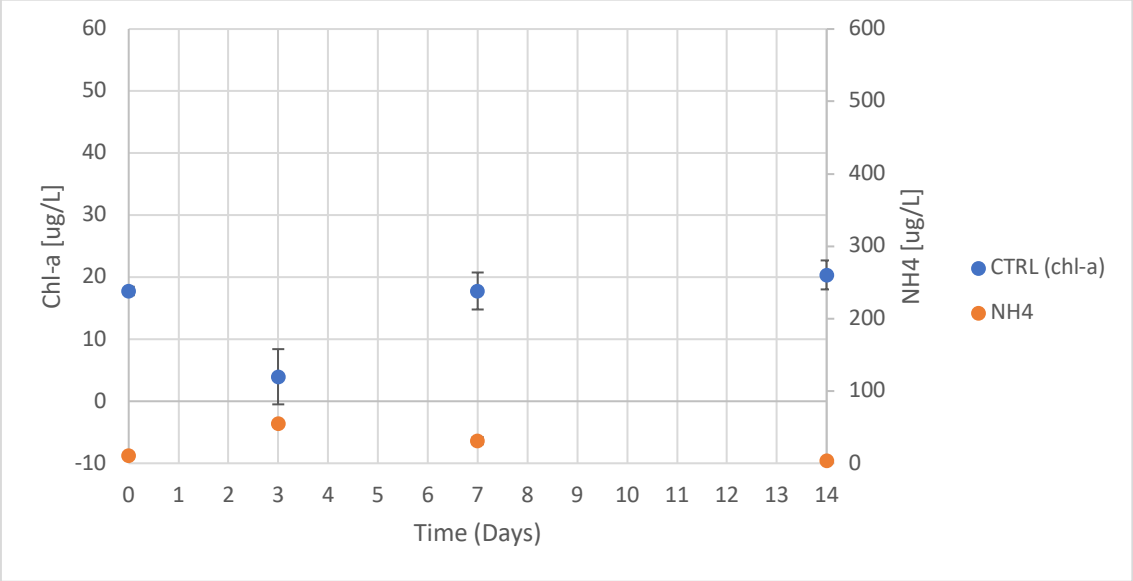

Figure K. The concentration of chl-a for average of CTRL treatment verse the concentration of NH4 over Day 0 to 14 of trial 3.

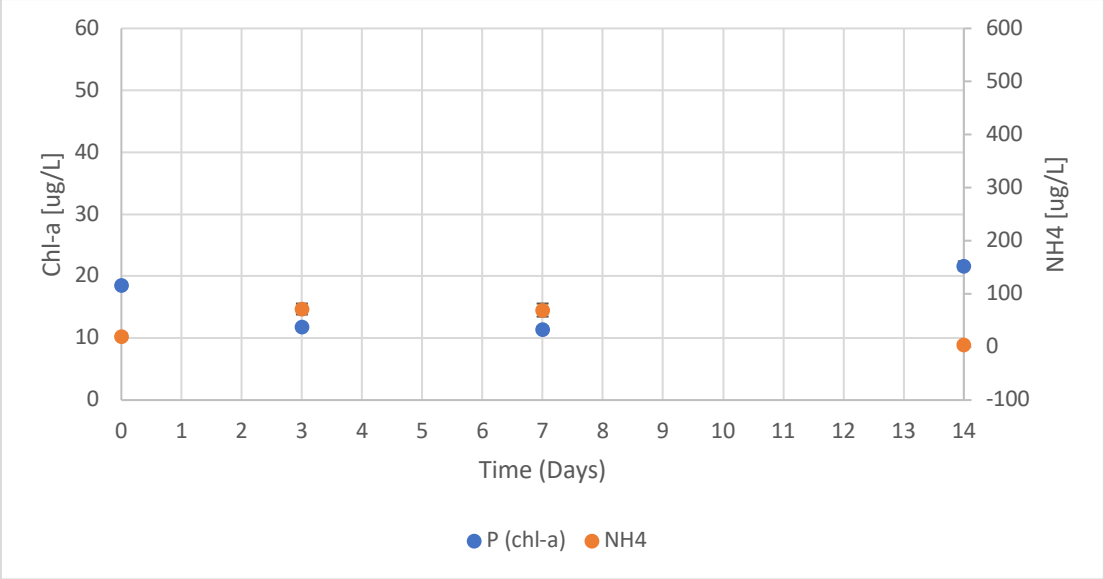

Figure K. The concentration of chl-a for average of P treatment verse the concentration of NH4 over Day 0 to 14 of trial 3.

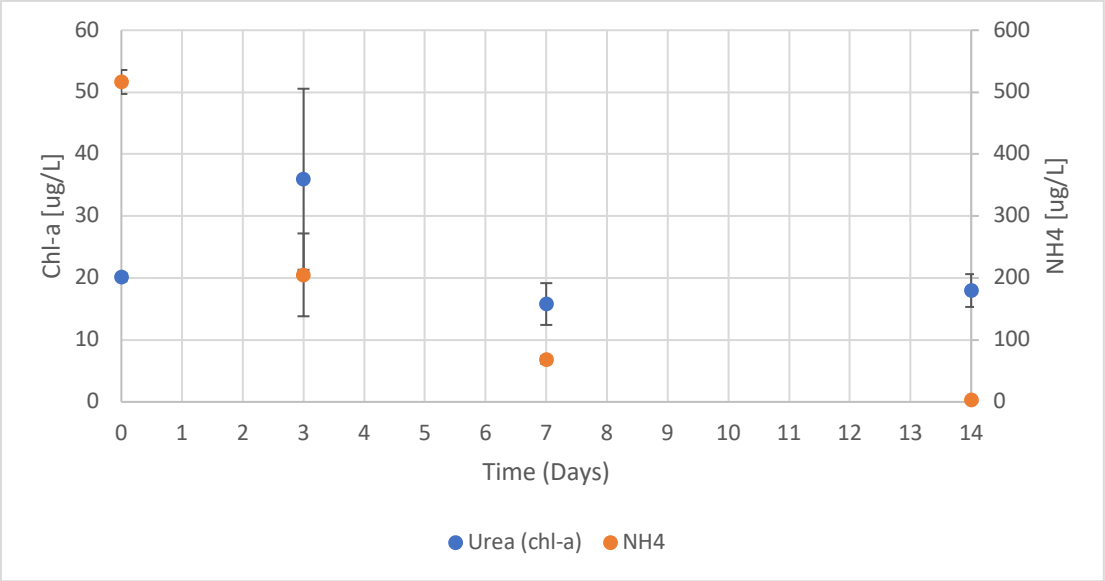

Figure K. The concentration of chl-a for average of Urea treatment verse the concentration of NH4 over Day 0 to 14 of trial 3.

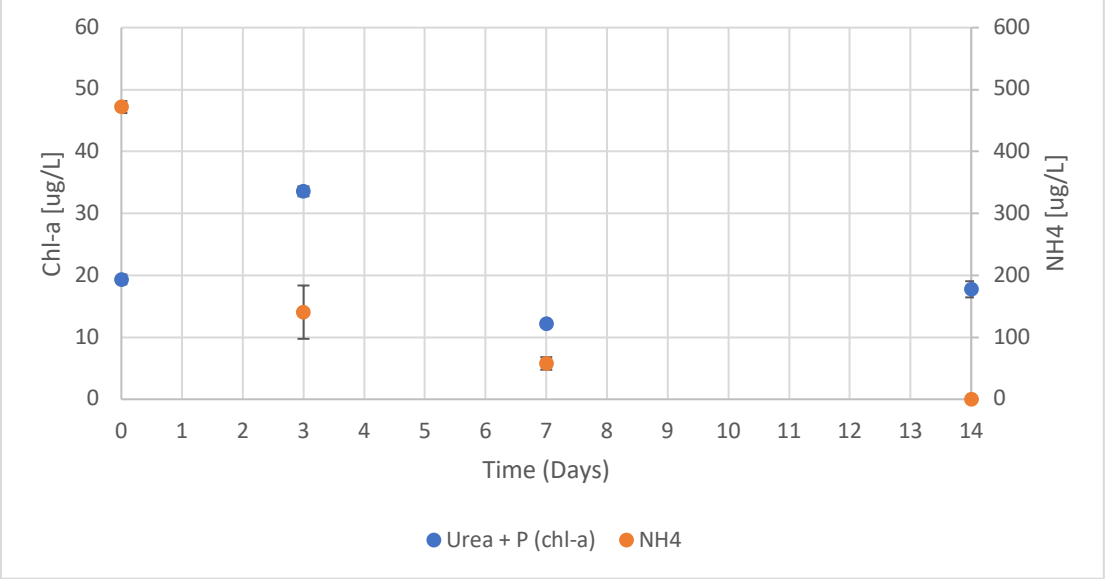

Figure K. The concentration of chl-a for average of Urea + P treatment verse the concentration of NH4 over Day 0 to 14 of trial 3.

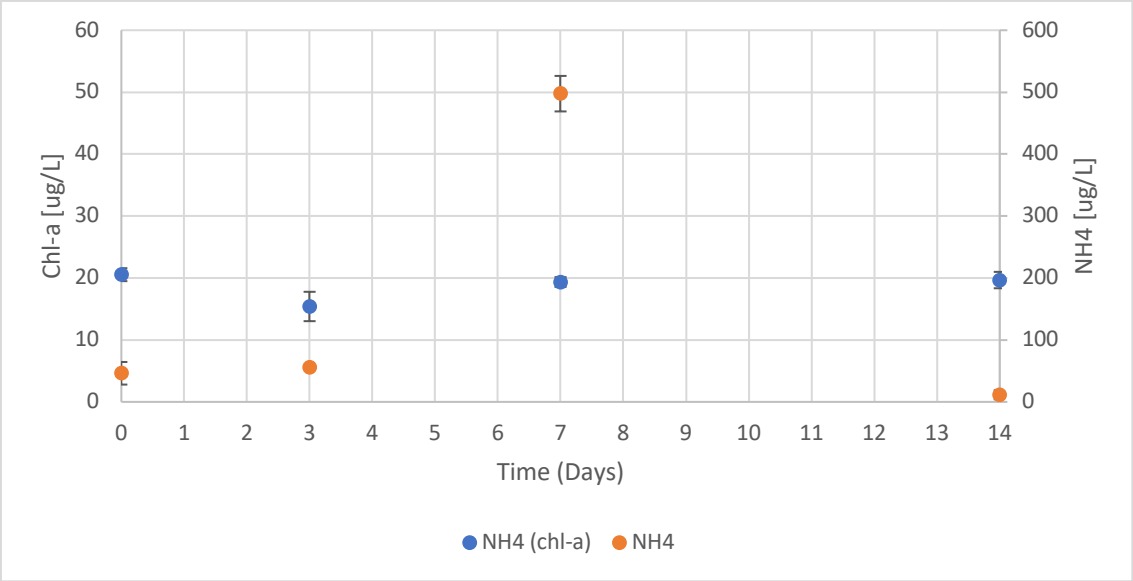

Figure K. The concentration of chl-a for average of NH4 treatment verse the concentration of NH4 over Day 0 to 14 of trial 3.

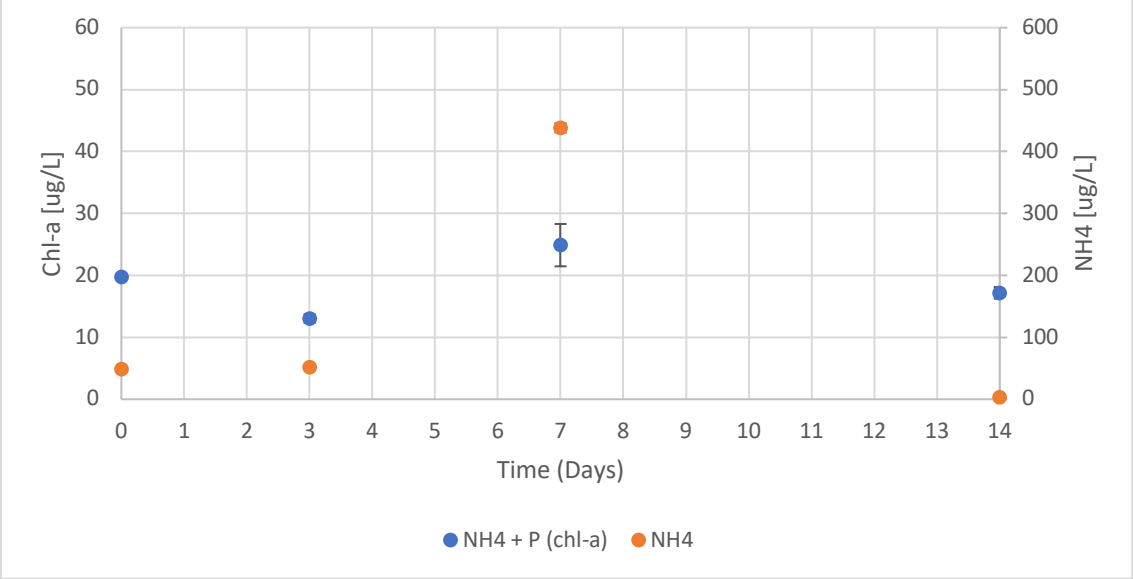

Figure K. The concentration of chl-a for average of NH4 + P treatment verse the concentration of NH4 over Day 0 to 14 of trial 3.

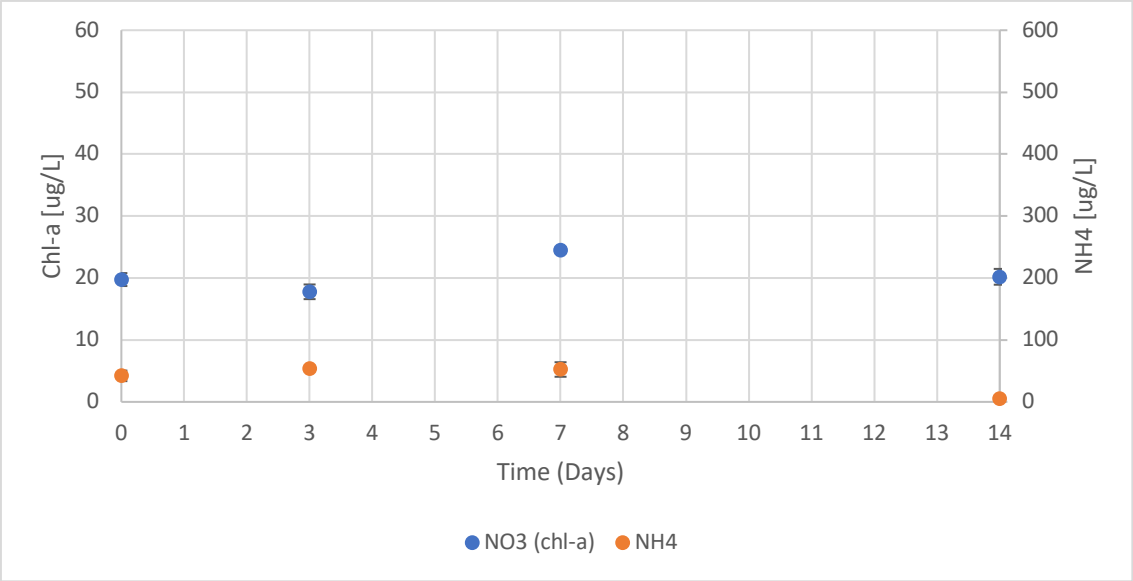

Figure K. The concentration of chl-a for average of NO3 treatment verse the concentration of NH4 over Day 0 to 14 of trial 3.

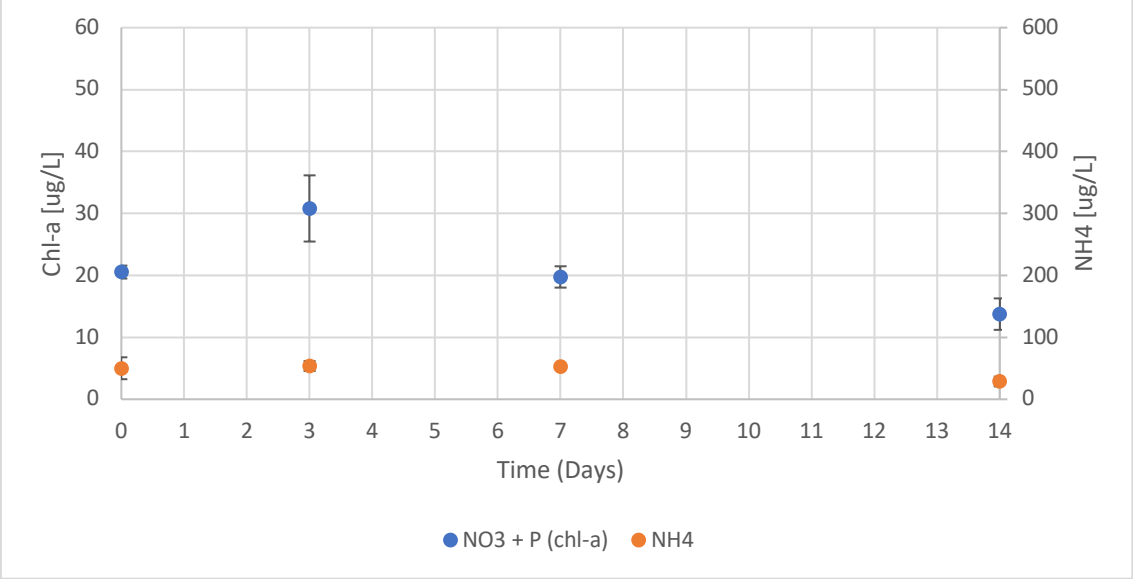

Figure K. The concentration of chl-a for average of NO3 + P treatment verse the concentration of NH4 over Day 0 to 14 of trial 3.

6.0 Chl-a (ug/L) vs. NO3 (ug/L)

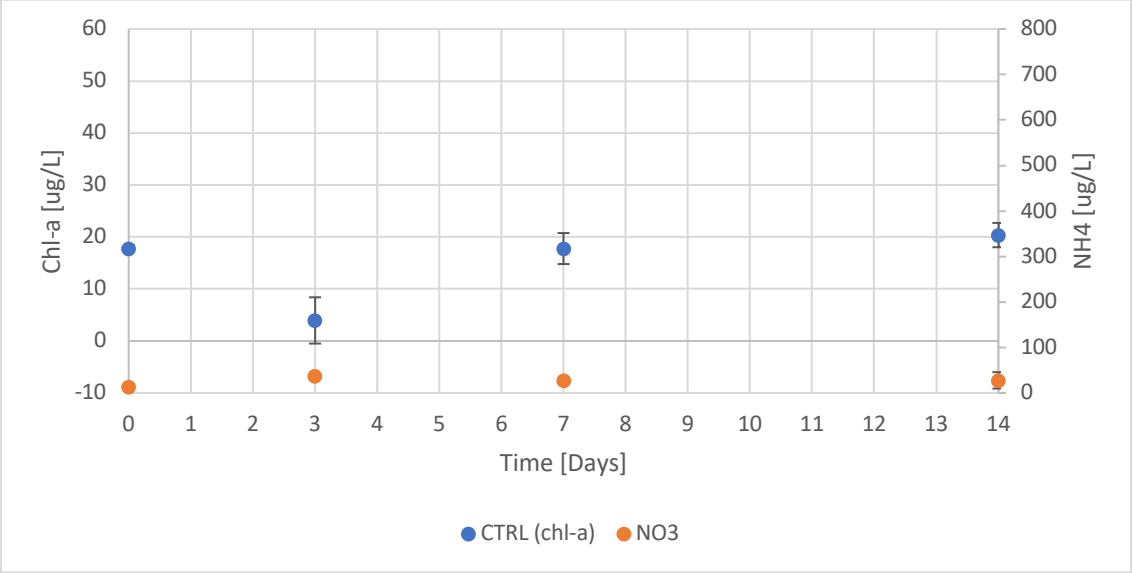

Figure C. The concentration of chl-a for average of CTRL treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

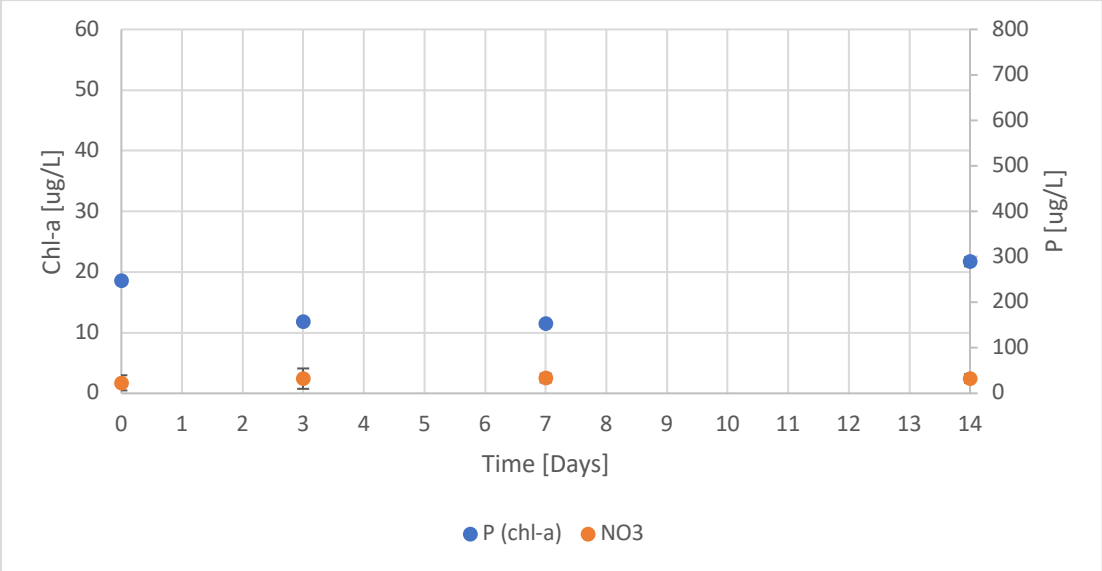

Figure C. The concentration of chl-a for average of P treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

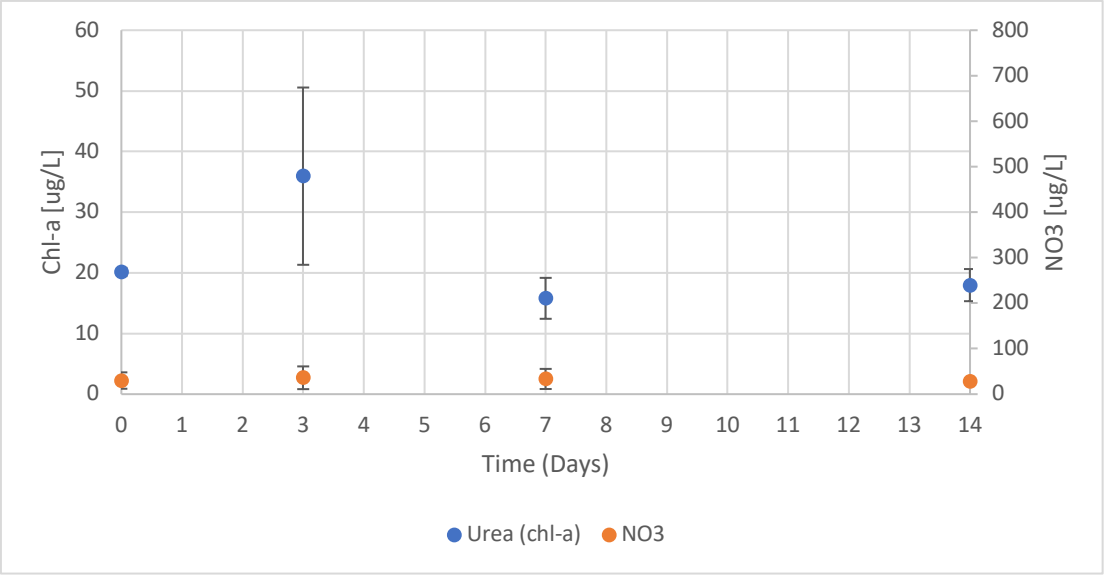

Figure C. The concentration of chl-a for average of Urea treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

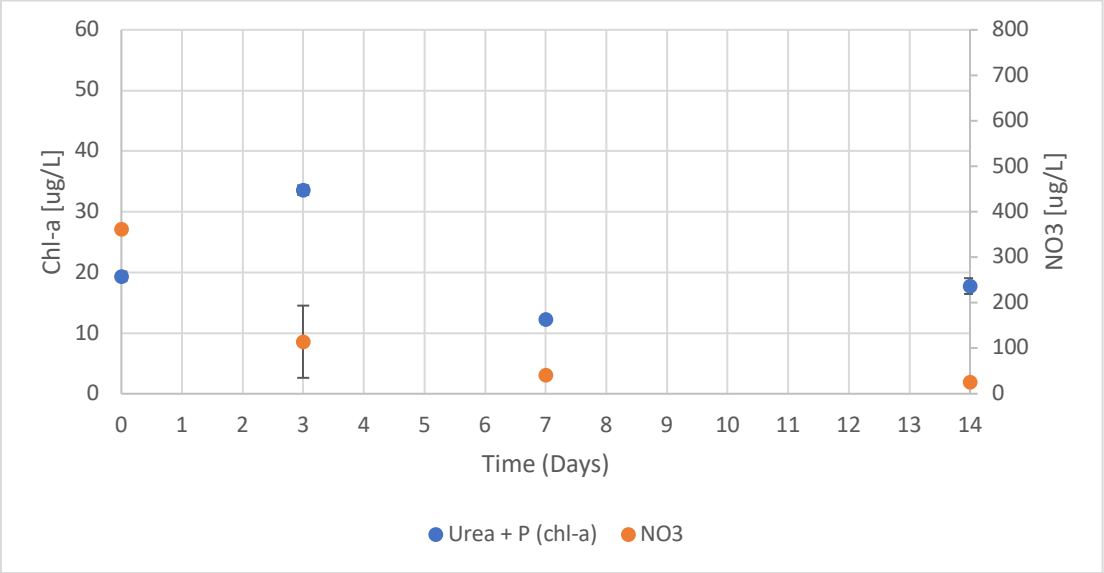

Figure C. The concentration of chl-a for average of Urea + P treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

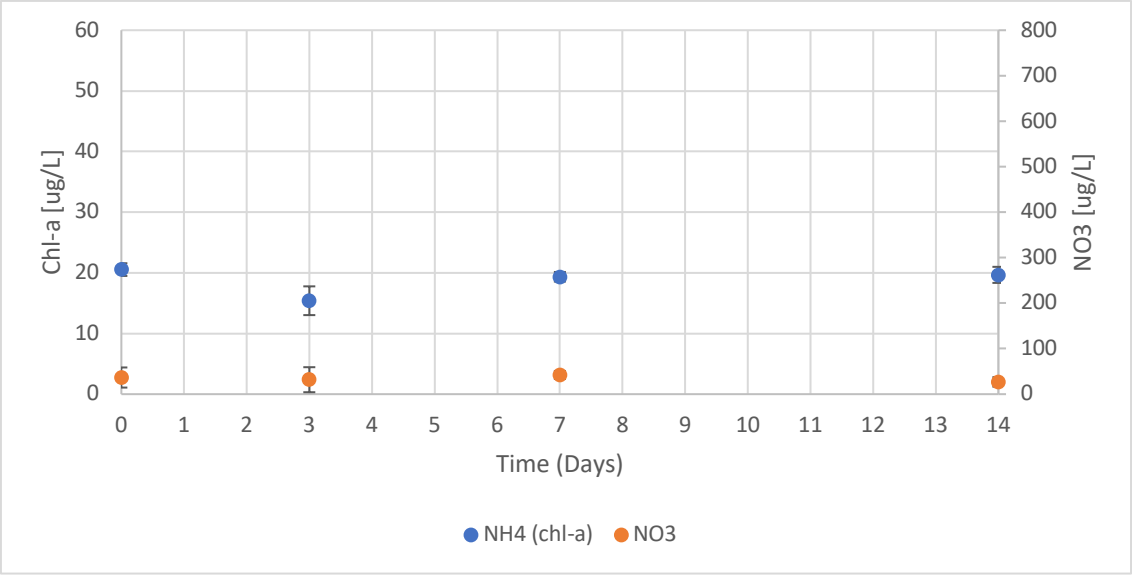

Figure C. The concentration of chl-a for average of NH4 treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

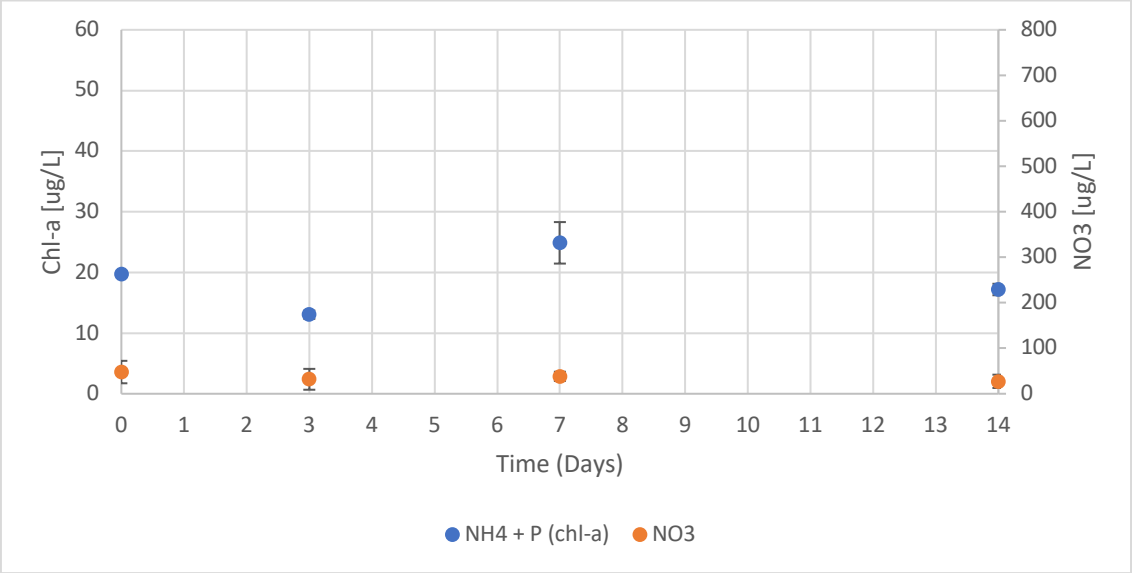

Figure C. The concentration of chl-a for average of NH4 + P treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

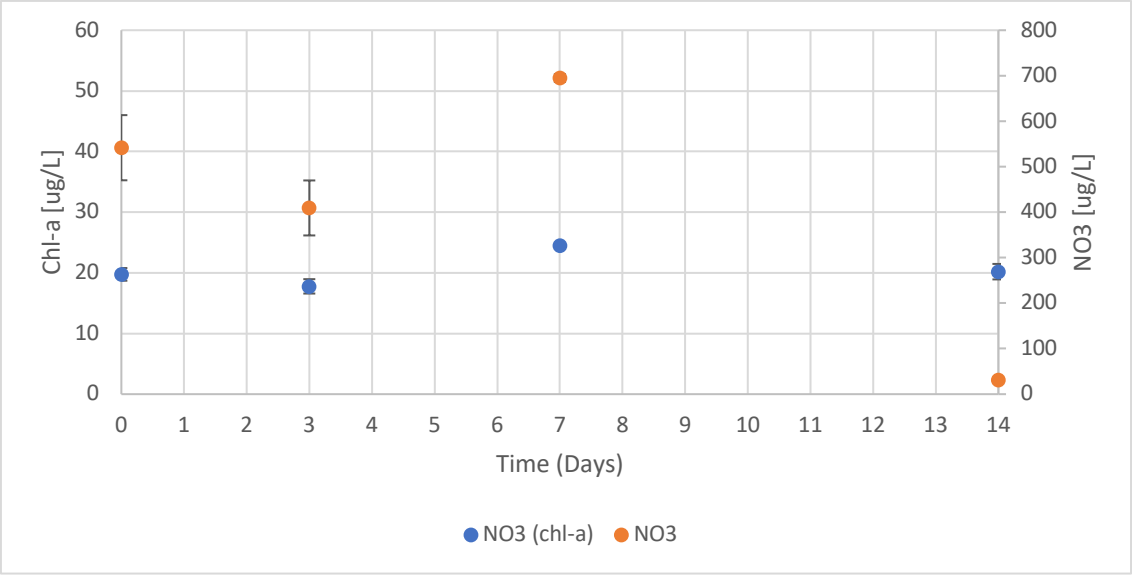

Figure C. The concentration of chl-a for average of NO3 treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

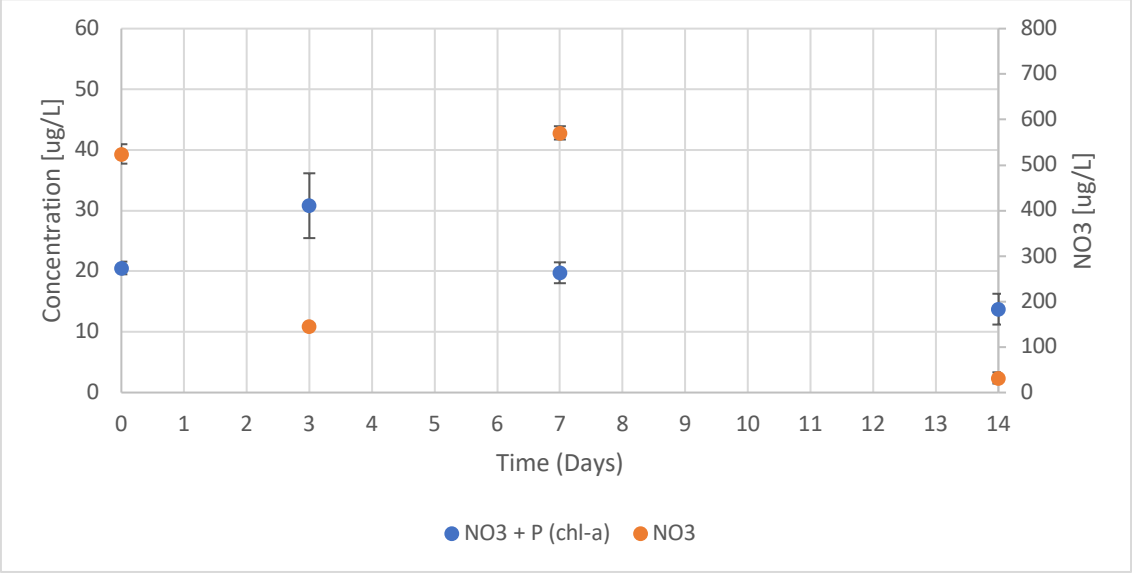

Figure C. The concentration of chl-a for average of NO3 + P treatment verse the concentration of NO3 over Day 0 to 14 of trial 3.

Discussion and Challenges

There were several challenges faced during the field work section of the placement. Firstly, trial 1 and 2 were unusable due to punctures in the plastic mesocosm bags throughout their field deployment. Secondly, we faced multiple equipment failures out in the field which lead to changes in sampling and measurement techniques. This meant our sampling methods were not standard throughout individual trials or between trials. Thirdly, the experiment was subjected to environmental conditions and sources of error could marginally be mitigated for. A main source of error could have occurred through the addition of nutrients from external sources. The mesocosm bags were exposed to bird droppings, small insects, human interference and plant material. Often when arriving to NaFIRRI Farm the invasive water hyacinth would surround our mesocosm bags and birds would fish off of the metal cage. This could be the reason for the high RFU units recorded in Urea 1.