1 INTRODUCTION

2.1 Isfjord Radio, Svalbard

Svalbard is an archipelago located in the high arctic. Being midway between Norway and the North Pole, it is one of the world's most susceptible regions to climate change (*Figure 1)*. As such, Svalbard is an ideal location for climate change research.[1] The annual temperatures of Svalbard are significantly higher than other areas at the same latitude with Isfjord Radio experiencing an average of -6.3˚C per year.[2] Glaciers cover over sixty percent of the archipelago while the rest of the arctic landscape exhibits mountains, tundra, and fjords.[2]

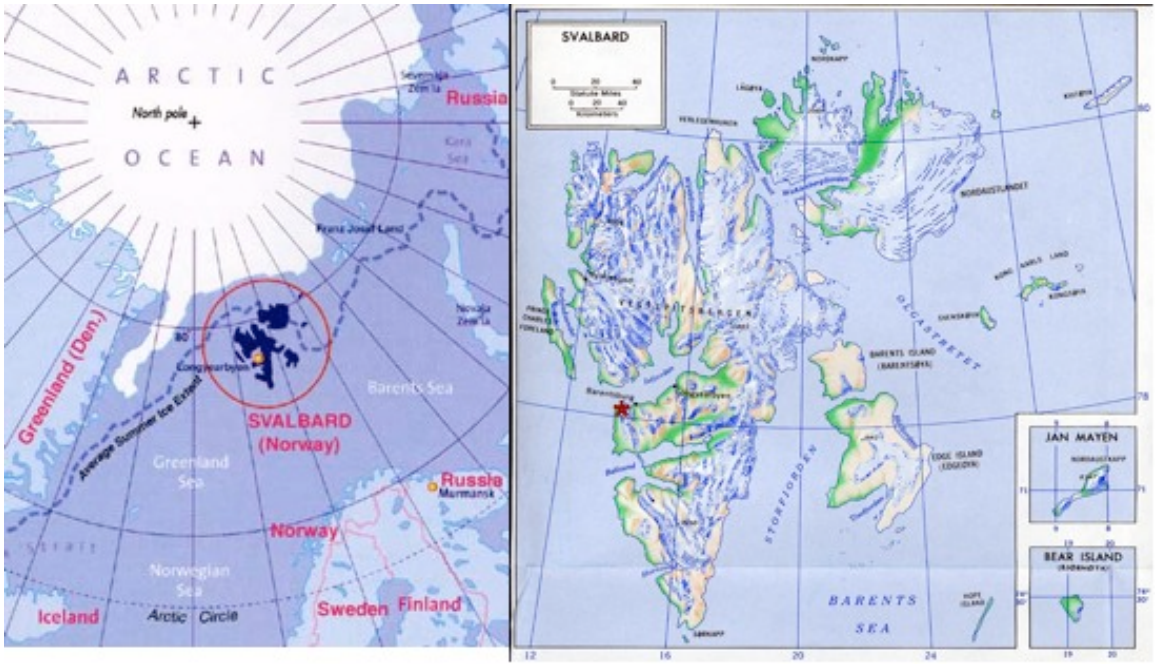

Figure 1. Map of Svalbard
* = Isfjord Radio. Images from svalbardpublilc.org

Isfjord Radio is the site our study and is located on the west coast of Svalbard, surrounded by Svalbard's second longest fjord, Isfjorden.[2] Throughout the year, the area is frequented by a diverse amount of arctic wildlife including birds, reindeer, polar bears, and seals. One of the most stunning features of Isfjord Radio is the Linné Glacier located approximately 15km southeast. This glacier is the main water source for our two lakes of study, Linnévatnet and Kongressvatnet.

2.2 Linnévatnet

Linnévatnet is a glacier fed lake located at 78^{o}3'N and 13^{o}50'E and about four meters above sea level.[3] The lake is surrounded by series of steep and relatively high mountains along both its eastern and western shores and, because of this feature, the region including Linnévatnet and its glacial inlet are commonly referred to as the Linné Valley. *Table 1* provides a solid introduction into morphology of Linnévatnet.[3]

Lake Surface, km^2	4.6
Maximum Depth, m	37
Average Depth, m	18.6
Shoreline, km	10.8
Maximum Length, km	4.7
Maximum Breadth, km	1.3

Table 1. Morphology of Linnévatnet

The waters of Linnévatnet are well mixed due to strong winds and high volumes of water entering into the lake from the glacier and surrounding mountains. The mixing of the lake contributes to a uniform composition of water chemistry and temperature with an average summer water temperature of ~3^oC and pH of 7.5. Additionally, the mean ionic composition of the water column is listed in *Table 2.*

Ca	Mg	Na	K	HCO_3	SO_4	Cl
1.45	0.67	0.15	0.008	0.75	1.32	1.47

Table 2. Ionic Composition of Linnévatnet (milliequivalens/L)

2.3 Kongressvatnet

Kongressvatnet is also fed by the Linné Glaciers and melt water that comes in off the mountains surrounding the lake. It is located at 78^{o}01'N and 13^{o}59'E and a much higher, 94 meters above sea level. Kongressvatnet is significantly smaller than Linnévatnet with a 0.82 km^2 surface area. However, Kongressvatnet can reach depths exceeding 50 meters. Unlike Linnévatnet, Kongressvatnet is a high sulphate lake that exhibits stratification with depth. Notably, Kongressvatnet contains a thermocline near the surface and a chemocline at greater depth. Hydrogen sulfide concentrations in the bottom anoxic water of Kongressvatnet have been recorded as high as 22.60 mL/L.[4]

2.4 Lack of Research on the Nutrient Cycling of Arctic Lakes

Although the geomorphology of the Linné Valley has been well described (*Figure 2*)[5], the microbiology and subsequent nutrient cycling of these waters remains a mystery. Notably, the glacial lakes of the Linné Valley are not an isolated case. Throughout the world, there is an overall lack of research concerning the biogeochemical processes occurring in freshwater systems of polar regions. This is phenomenon is most probably due to the fact that glacial lakes and other arctic freshwater bodies of water are often covered by ice for nine to ten months of the year. However, these microbial processes cannot continue to be ignored. Microbial driven biogeochemical cycling is our environment's first line of defense against climate change and, as global temperatures continue to rise, we need to understand how these microbes are adapting to control our changing environment.[6]

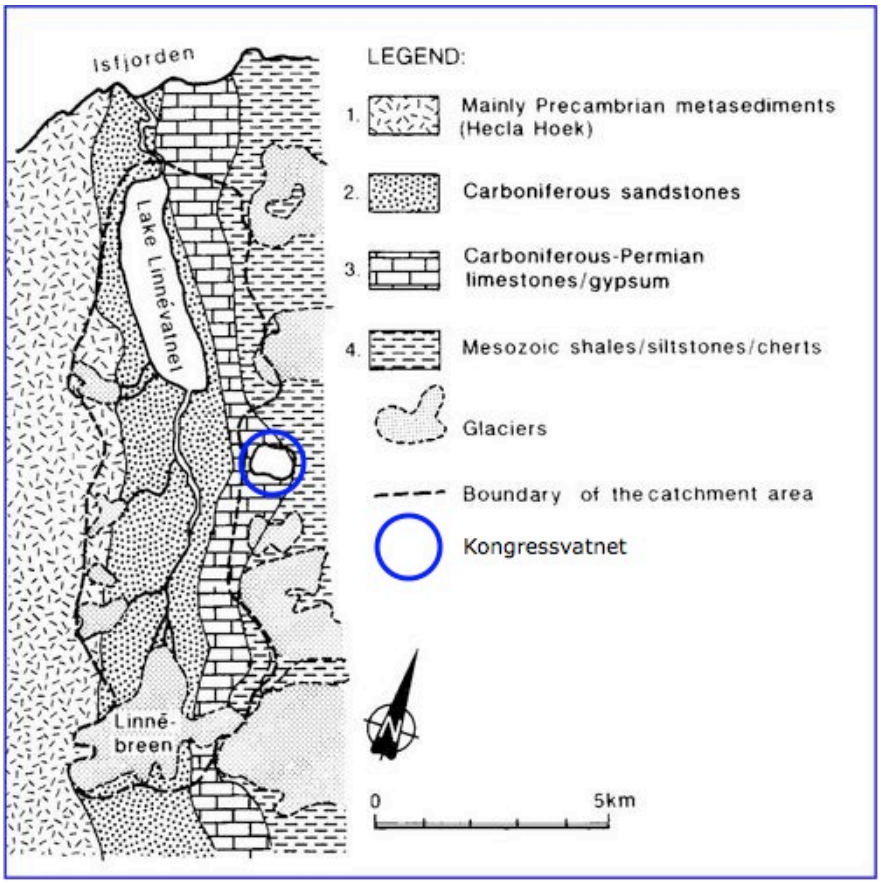

Figure 2. Geomorphology of the Linné Valley

2.5 Anthropogenic Nitrate Deposition in the Linné Valley

Table 4 Spitsbergen precipitation events with associated NO_3^- concentrations

Date	Event type	NO_3^- ($\mu g\ l^{-1}$)	NO_3^- (μM)	Location	Wind direction	Wind speed ($m\ s^{-1}$)	Temperature (°C)
Apr 21, 2001	Fresh snow	124	2.0	Ny Ålesund	N (10°)	2.7	−11.4
Apr 5, 2002	Fresh snow	248	4.0	Ny Ålesund	WSW (260°)	2.9	−2.6
July 26, 2004	Rain	212	3.4	Kapp Linné	SSE to WSW (160–240°)	3.0	10.5
July 29, 2004	Rain	1,271	20.5	Kapp Linné	SSW (180–190°)	6.4	5.2
Aug 4, 2004	Rain	163	2.6	Kapp Linné	SSW to WSW (190–250°)	2.0	6.3
Aug 8, 2004	Rain	1,561	25.2	Kapp Linné	SSW (215°)	2.6	4.4
Feb 4, 2005	Fresh snow	174	2.8	Storfjorden	WSW (240°)	1.7	−9.1
Feb 6, 2005	Fresh snow	61	1.0	Storfjorden	ESE (120°)	2.8	−16.3
July 21–22, 2005	Rain	153	2.5	Kapp Linné	NNE to WSW (20–250°)	3.8	3.7
July 28, 2005	Rain	63	1.0	Kapp Linné	SE to WSW (135–250°)	2.5	4.1

Climate data for the two earliest dates are from Svalbard airport (29 m asl); other data are from the Gruvfjellet station (464 m asl)

Table 3. Spitsbergen Precipitation events with Associated Nitrate Concentrations[7]

Since the late 20th century, anthropogenic nitrogen deposition has been observed in Svalbard. These excess loads of nitrogen are a result of polluted air brought into the arctic from Europe and North America. The majority of the unnatural nitrogen observed is in the form of NO_x and NH_x that are produced as a byproduct of fossil fuel combustion. *Table 3* shows the result of an ice core study performed by Holmgren et al. (2010) to identify the amount of anthropogenic nitrogen entering into the Linné Valley. Notably, Kapp Linné is located in Isfjord Radio and, thus, nitrate measurements from this location provide a solid representation of the amount of anthropogenic nitrogen being deposited in the Linné Valley. Our study is the first of its kind to directly examine how arctic lake ecosystems respond to increased nitrate levels due to anthropogenic nitrogen deposition.

2.6 Primary Production

Primary production is a term used to describe the production of organic compounds from carbon dioxide through the process of photosynthesis and, the less frequent, chemosynthesis. Notably, carbon dioxide has gained considerable attention over the past decade due to recent research that links warming temperatures to, human-induced, increased carbon dioxide levels in our atmosphere.[8] Aquatic cyanobacteria and algae are the earth's largest carbon dioxide sink and primary control mechanism against carbon dioxide entering our atmosphere. These microorganisms account for the removal of over 10 gigatons of carbon dioxide per year via photosynthesis.[9] Due to their dependence on carbon dioxide and sunlight, these primary producers are utilized in climate research as proxies to reconstruct information about global temperatures and nutrient cycling.

2.7 Nitrate to Phosphate Ratios in Lakes

The additional nitrogen being deposited through anthropogenic activities, as mentioned in *Section 1.5,* is an unnatural occurrence. Prior to this study, research has been performed to characterize how additional loads of nitrogen are affecting terrestrial and marine ecosystems.[10 11 12] These non-arctic environments have exhibited shifts in total nitrate to total phosphate supply ratios (TN:TP) and led many freshwater ecosystems to be extremely phosphate limited.[10] These TN:TP shifts have had a direct effect in altering the diversity and ecosystems of various freshwater systems and, in turn, nutrient cycling.[10] Through this study, we explore how the TN:TP supply ratios effect the ecosystems of Linnévatnet and Kongressvatnet and how these results will influence future climate research.

2.8 Purpose of Study

The purpose of this study is to better understand the biogeochemical cycling occurring in arctic freshwater system. In order to do this, two glacial lakes, Linnévatnet and Kongressvatnet, in Svalbard were studied. Through characterization of the biology and nutrient cycling of these two lakes, we have gained valuable insight into how climate change is impacting the ecosystems and biogeochemical processes of this mystique and pristine area.

3 METHODOLOGY

3.1 Pre-Departure Preparation

Funding for this project was obtained through the National Science Foundation's Research Experience for Undergraduates Program (NSF REU). Project planning and preliminary research began in March 2010. In order to prepare for such a trip, an extensive literature search was performed and analyzed. Despite an overall lack of information regarding the biology of the region, the literature search was able to provide a solid description of the geology surrounding Linnévatnet and Kongressvatnet as well as insight on techniques to begin characterizing the biological processes of these two lakes.

Based on the experimental outline created, a packing list was organized. All supplies were purchased and shipped from the University of Southern California to Hampshire College and then flown to Longyearbyen on July 6, 2010. Before fieldwork in Isfjord Radio could commence, my research group and I were sent to safety training at the University Centre in Svalbard (UNIS). At UNIS, we learned arctic survival skills and received rifle training to protect ourselves against Svalbard's natural threats.

3.2 Fieldwork

Fieldwork began on July 14, 2010 when my research team and I traveled from Longyearbyen to Isfjord Radio via UNIS's research boats. Upon arrival to Isfjord Radio, supplies were unpacked and after which we made our first trek into the Linné Valley. Our field season lasted from July 14 – August 11, 2010.

3.2.a. Determination of Sampling Sites

Before any samples were collected from either lake, a thorough survey and analysis of the two water bodies were conducted. Both the surrounding geology of the two lakes and the water flow were taken into consideration when determining where experimental sites would be located (*Figure* 3).

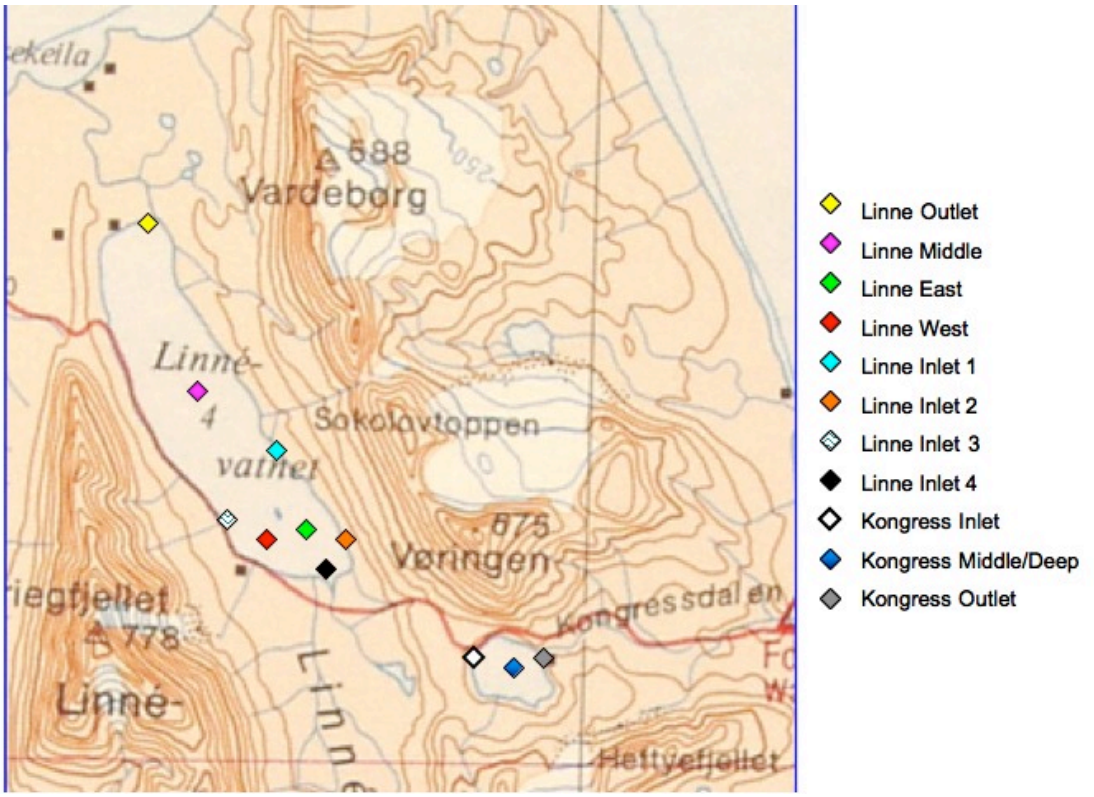

Figure 3. A Schematic Layout of Sampling Sites Throughout Linnévatnet and Kongressvatnet

Due to the variety of inflows coming into Linnévatnet over varying geology's, four inlet locations were selected *Inlet 1* and *Inlet 2* were selected based on their location on Linnévatnet's eastern shore. As mentioned earlier, the eastern shore of Linnévatnet consists mainly of steep limestone and sandstone mountain ridges. *Inlet 1* is the largest inlet on the eastern shore consisting of melt water from the mountains that expels above ground through an alluvial fan into Linnévatnet. *Inlet 2* is a smaller inlet draining melt water down the eastern ridge and travels underground before entering the lake. *Inlet 3* was selected based on its location along the western shore of Linnévatnet. The melt water coming in from *Inlet 3* travels both above and under biologically productive muds surrounding the western shore. Unlike the mountains of the eastern shore, Linnévatnet's western shore is surrounded by Hecla Hoek dominated mountains. Finally, *Inlet 4* is Linnévatnet's largest inlet stream pumping in water directly from the Linné Glacier.

In addition to exploring Linnévatnet's inlets, the outlet of Linnévatnet was selected for analysis to evaluate how the biology and nutrient concentrations of the lake change as the water of the lake drain into the ocean. Notably, the water being pushed into the sea from Linnévatnet is flowing in a counter-clockwise direction. Finally, the eastern and western basins of the lake as well as the deepest point in the lake were selected for analysis based on the large volumes of water that travel through all three areas.

Sampling sites selected in Kongressvatnet were chosen to mimic sites selected in Linnévatnet. Thus, an inlet, mid-lake, and outlet location were identified. Additionally, analysis of Kongressvatnet using a Multi-Parameter TROLL was performed mid-way

through the field season (*See Section 3.2.b*). This analysis identified a distinct thermocline at 12m and a chemocline beginning at approximately 40m. *Kongressvatnet Deep* is a sampling site at this thermocline while analysis of the chemocline is discussed in *Section 4* and *5*.

3.2.b. TROLL Profile

Unlike Linnévatnet, Kongressvatnet is known to exhibit stratification within the lake. Due to this previous knowledge, a TROLL profile was constructed using the Multi-Parameter TROLL 9500. To characterize Kongressvatnet using the TROLL, we used GPS on a small Zodiac® to determine the middle of the lake and then dropped the TROLL to the bottom of the lake and back up three times. Information on how to operate and obtain data using this instrument was found in *WQP-100 Operator's Manual.*[13]

3.2.c. Mesocosms

In order to determine nutrient limitation within Linnévatnet and Kongressvatnet, a series of mesocosms were constructed. Samples were collected as shown in *Table 4* and distributed into four 1L Nalgene® I-Chem Certified Series™ 300 LDPE Cubitainers™ Containers. Each cubitainer was then treated with either 0.5µM of phosphate (PO_4), 7µM of nitrate (NO_3), 0.5µM of phosphate and 7µM of nitrate (cocktail), or nothing (control) and incubated in the shallow waters of Linnévatnet for five days. 200mL water samples were collected from each mesocosm one, three, and five days after collection in addition to the day of collection. These samples were then filtered onto a Whatman® GF/F filter, wrapped in aluminum foil and frozen. Additionally, 50mL of sample were collected at each time point and froze in acid washed Falcon Tubes for nutrient analysis. On the fifth day after collection, 18mL of each mesocosm was preserved in 2mL of 40% formalin and refrigerated.

Location	Method of Collection	Date of Collection
Linnévatnet Outlet	Surface Water Dip	7/16/10
Linnévatnet Middle	Surface Water Dip	7/16/10
Linnévatnet East	200µm Hand Net on Surface	7/30/10
Linnévatnet West	200µm Hand Net on Surface	7/30/10
Linnévatnet Inlet 1	Surface Water Dip	7/24/10
Linnévatnet Inlet 2	Surface Water Dip	7/24/10
Linnévatnet Inlet 3	200µm Hand Net on Surface	7/30/10
Linnévatnet Inlet 4	200µm Hand Net on Surface	7/30/10
Kongressvatnet Inlet	200µm Hand Net on Surface	8/4/10
Kongressvatnet Middle	200µm Hand Net on Surface	8/4/10
Kongressvatnet Deep	Plastic Nansen Bottle w/ TROLL	8/5/10
Kongressvatnet Outlet	200µm Hand Net on Surface	8/5/10

Table 4. Means of Collecting Water for Mesocosms

3.2.d. Nitrogen Fixation Experiment Set-up

Samples were prepared and collected for nitrogen fixation analysis using a 200μm hand net as seen in *Table 5.* Three sets of 20mL of water from each site were placed in individual 27mL serum vials and sealed shut using a rubber stopper and aluminum crimper. Each vial was then injected with 3mL of acetylene made by mixing calcium carbide and water. After acetylene was injected, vials were incubated in the shallow waters of Linnévatnet for a designated time listed in *Table 5.* Once incubation was complete, an airtight syringe was used to extract 2mL of gas from the serum vial to be injected into a 5mL vacuutainer. Vacuutainers were then transported back to Los Angeles for further analysis (*See Section 3.3.c.).*

Location	Date of Collection	Hours Incubated
Linnévatnet Outlet	8/9/10	24
Linnévatnet Middle	8/9/10	24
Linnévatnet East	7/21/10	4, 24
Linnévatnet West	7/21/10	4, 24
Linnévatnet Inlet 1	8/9/10	24
Linnévatnet Inlet 2	8/9/10	24
Linnévatnet Inlet 3	8/9/10	24
Linnévatnet Inlet 4	8/9/10	24
Kongressvatnet Inlet	8/5/10	6
Kongressvatnet Middle	8/5/10	6
Kongressvatnet Deep	8/5/10	6
Kongressvatnet Outlet	8/5/10	6
Kongressvatnet 35.1m	8/10/10	24
Kongressvatnet 37.2m	8/10/10	24
Kongressvatnet 38.6m	8/10/10	24
Kongressvatnet 39.7m	8/10/10	24
Kongressvatnet 40.8m	8/10/10	24
Kongressvatnet 42.4m	8/10/10	24
Kongressvatnet 46.6m	8/10/10	24

Table 5. Information Regarding Nitrogen Fixation Sampling and Experimentation
**For information regarding the chemistry and relationship of acetylene reduction to nitrogen fixation, please refer to *Supp.1*.

3.2.e. Sampling of Chemocline

Due to the mysterious nature of Kongressvatnet's chemocline, an experiment was designed to assess what causes this sharp turbidity and conductivity spike in response to the transition of oxic to anoxic water. Water was collected using a plastic Nansen bottle linked to the TROLL 9500. By linking these two instruments together, we were able to determine the depth of our Nansen bottle in correspondence to the changing conductivity. Water samples for bacteria counts, nutrient analysis, and nitrogen fixation were collected at the depths and dissolved oxygen concentrations seen in *Table 6.*

Depth (m)	35.1	37.2	38.6	39.7	40.8	42.4	46.6
Dissolved Oxygen (μg/L)	10,500	6,678	2,600	2,150	1,042	1,150	2,100

Table 6. Sampling Points of Kongressvatnet's Chemocline

3.2.f. Weather Data

Weather data was collected from an Onset® Weather Station on the south shore of Linnévatnet. The weather station contains a HOBO® weather station logger whose data was downloaded onto a PC using the HOBOware® program.

3.3 Sample Analysis

3.3.a. Chlorophyll a *Analysis*

Chlorophyll *a* samples were analyzed according to Yentsch and Menzel, 1963.[14] This methodology involves soaking each GF/F filter in 4mL of 90% acetone overnight in the freezer. The next day, samples given another 5mL of 90% acetone and placed in the dark at room temperature for two hours. While chlorophyll samples sat in the dark, a standard was prepared using frozen spinach. To prepare such a standard, a pinch of frozen spinach was placed in 10mL of 85% acetone and allowed to soak for one and a half hours. After this predetermined amount of time, the acetone/spinach solution appeared green and a series of dilutions were made as seen in *Supp2*. Standards were then measured in a spectrophotometer at 665nm, 630nm, and 750nm under a 10cm path length and in a fluorometer using a Chlorophyll *a* Acidification/Non-Acidification Module from Turner Designs®. Next, standards were given 2 drops of 1.2M HCl to correct for phaeophytin and measured in the fluorometer and spectrophotometer at 665nm and 750nm. Chlorophyll *a* concentrations (μg/mL) for the standards were then calculated by *Equation 1*.

$$[\text{Chlorophyll } a] \text{ in } \mu g/mL = 11.43(A_{665} - A_{750}) - 0.4(A_{630} - A_{750})$$

where A_{630} = Absorbance at 630nm
A_{665} = Absorbance at 665nm
A_{750} = Absorbance at 750nm

Equation 1. Calculating Chlorophyll *a* Concentrations from Absorbance Values

A standard curve was then prepared by plotting the measured fluorescence versus concentration of chlorophyll *a* on Excel® and a line of best fit was calculated *Supp2*. After the standards were calculated, samples were measured in the fluorometer with and without acidification. Values were then extrapolated from the standard curve.

3.3.b. Bacteria Counts and Microscopy

In order to enumerate bacteria from mesocosms and nitrogen fixation work, bacteria samples were stained with acridine orange according to Hobbie et al., 1977.[15] Stained

samples were filtered onto a Whatman® 0.2μm, 25mm, black PC filter. Notably, depending on the observed densities of samples, 2-5mL of sample were filtered for analysis. Slides were then analyzed at 1000x oil immersion and excited under blue light. All bacteria that fluoresced green were counted on a 10x10 square grid. Each slide was counted ten times. Counted values were then converted to cells/mL using *Equation 2.*

Cells/mL = Average Count x 100 squares x RSF/(# of Squares Counted x Volume Filtered)
where RSF = Area of Sample/Area of the 10x10 Square in Microscope

Equation 2. Calculating Cells/mL from Bacteria Counts

In addition to counting bacteria, microscopy was also used to visualize bacteria and diatoms. Here, both stained and un-stained samples were visualized and photographed under green, blue, and DAPI light. Samples were then taken to diatom expert, Dave Caron, PhD, for diatom identification.

3.3.c. Calculation of Nitrogen Fixation

Nitrogen fixation rates were calculated according to Capone, 1993.[16] In this method, 2mL of gas were collected syringe from the vacuutainers using an airtight and injected in to a gas chromatograph. The acetylene peak height was then recorded for further analysis. Notably, before and after samples were run on the gas chromatograph, a standard was prepared by injecting 2mL of 99.1ppm ethylene into a vacuutainer. 2mL of the standard was then injected into the gas chromatograph. Concentrations of ethylene were then calculated by *Equation 3.*

$$\text{nmol } C_2H_4 = (\text{pk ht, unk})/(\text{pk ht, std}) \times [\text{std}] \times (\text{GPV}) \times (\text{SC})$$

where (pk ht, unk) = ethylene peak height of sample
(pk ht, std) = ethylene peak height of standard
[std] = concentration of ethylene standard (nmol/mL)
GPV = gas phase volume of sample (mL)

$$\text{SC} = 1 + (\alpha \times A/B)$$

where α = Bunsen coefficient for ethylene at the appropriate temperature and salinity
A = volume of aqueous phase in sample
B =volume of gas phase in sample

Equation 3. Calculating nmol/mL of Ethylene

As mentioned in *Supp1,* the reduction of acetylene to ethylene by nitrogenase is a model to demonstrate the breakage one bond in the triple bonded N_2. Despite there being a theoretical 3:1 relationship between the reduction of nitrogen to the reduction of acetylene, in the case of the acetylene reduction method, a 4:1 ratio is more appropriate for application in analysis. This is because a 4:1 ratio considers the hydrogenase reaction of nitrogenase in addition to the N_2 to $2NH_3$ conversion. Thus, *Equation 4* demonstrates how nmol/mL of ethylene were converted to nitrogen fixation rates.

$$\text{nmol Nitrogen Fixed/mL/hour} = [C_2H_4]/(4 \times t)$$
where t = hours acetylene reaction occurred

Equation 4. Conversion of Ethylene Concentration to nmol Nitrogen Fixed/mL/hour

3.3.d. Phosphate Analysis

Phosphate concentrations were analyzed according to Murphy and Riley, 1962.[17] Here, each frozen nutrient sample was thawed and distributed into three 5mL aliquots. A color reagent was then prepared by mixing two parts 32mM ammonium molybdate, five parts 14% sulfuric acid, two parts 0.1M ascorbic acid, and one part 9.0M antimony potassium tartate. 0.75mL of color reagent was added to each 5mL aliquot of sample, briefly vortexed, and left undisturbed for 30-45 minutes. After the designated time, samples were read on a spectrophotometer at 885nm in a 10cm path length cell. Absorbance values were then correlated to a standard curve (*Supp3*) to obtain total dissolved phosphate concentrations. Notably, the phosphate standards were prepared through various dilutions of potassium dihydrogen phosphate (FW 136.09) with milli-Q water.

3.3.e. Nitrate Analysis

In order to calculate nitrate concentrations, samples were thawed and distributed into three 10mL aliquots. One packet of PERMACHEM® REAGENTS NitraVer® 5 Nitrate Reagent was added to each aliquot. These packets contain a mixture of cadmium and sulfanilic acid to reduce nitrate to nitrate and provide a colorimetric way of measuring total nitrate concentrations. Upon addition of the nitrate reagent, the sample was immediately vortexed for one minute to allow the chemical reaction to occur. Samples were then measured on a spectrophotometer at 543nm in a 10cm path length. 543nm was the wavelength selected for analysis due to its ability to resolve a linear relationship between the nitrate standards and absorbance values (*Supp4*). Nitrate concentrations were determined by correlating absorbance values of the samples to the standard curve. Notably, the nitrate standards were prepared through various dilutions of potassium nitrate (FW 101.11) and milli-Q water.

3.3.f. Calculation of Nitrate to Phosphate Ratios

Total nitrate to total phosphate ratios (TN:TP) were calculated using time zero samples and dividing the concentration of total nitrate by the concentration of total phosphate. Relative ratios (RR-N and RR-P) were based on chlorophyll *a* concentrations after the final day of incubation. Nitrate and phosphate chlorophyll *a* concentrations were normalized to the control as described by Elser et al., 2009.[18]

4. RESULTS

The following is a collection of results collected during the 2010 summer field season.

4.1. Daily Temperature and Solar Radiation

In arctic regions, overall temperature and sunlight have shown to be major drivers of primary production in aquatic ecosystems.[19] Their significance necessitates the acquisition of daily temperature and solar radiation data. The data acquired from our weather station are plotted in *Figure 4*. Although there are 24 hours of sunlight during the arctic summer, only temperatures and solar radiation data from the 12:30pm time point were compared. This is because at 12:30pm, the sun reaches its highest point in the sky and consistently contributes to the maximum temperatures and solar radiation readings of the day.

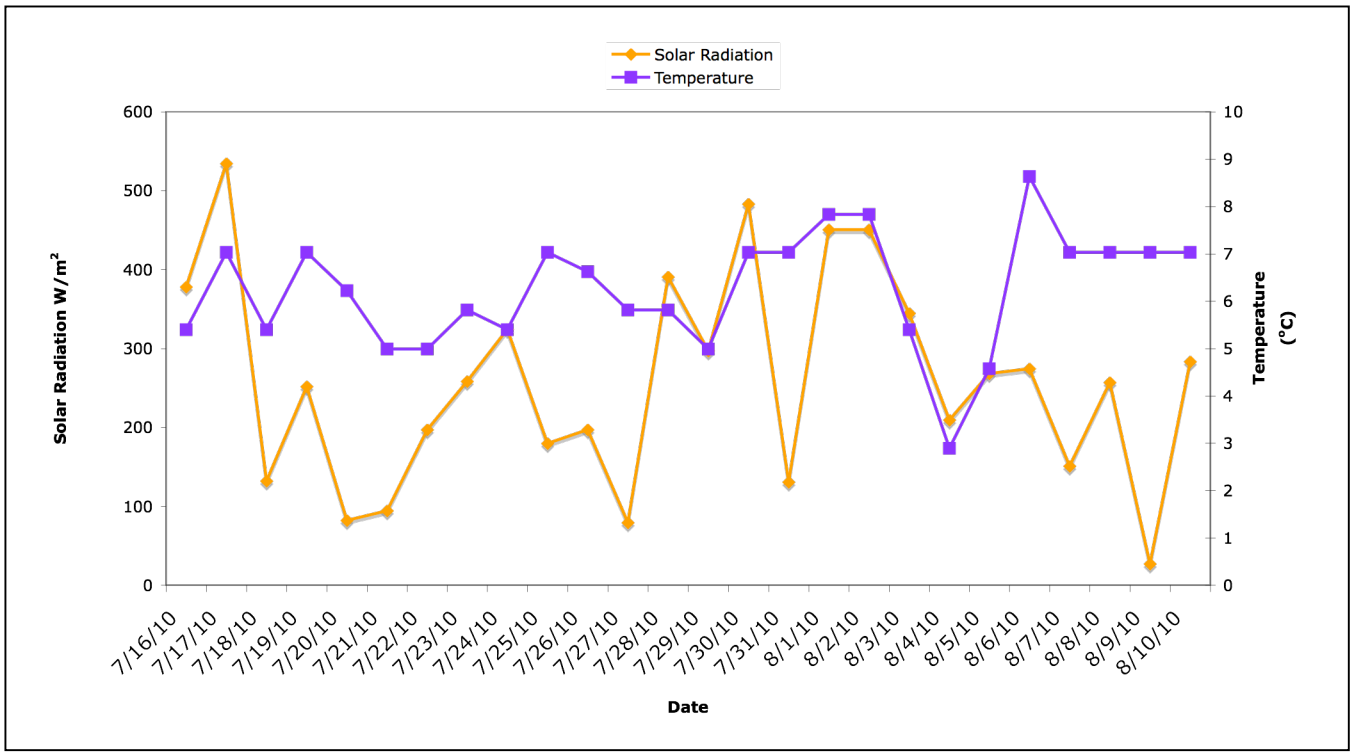

Figure 4. Daily Temperature and Solar Radiation

4.2 Total Dissolved Nitrate and Phosphate Concentrations

Nitrate and phosphate concentrations were measured at each sampling point's time zero (*Table 7*). Notably, the inlets of both Linnévatnet and Kongressvatnet displayed the highest concentrations of nitrate. On the other hand, phosphates were most abundant in Linnévatnet's west basin and Kongressvatnet's outlet. Total nitrate (TN) to total phosphate (TP) ratios were calculated and listed in *Table 7*. Notably, Kongressvatnet Inlet had the highest TN:TP while Kongressvatnet Deep had the lowest TN:TP ratio.

Relative ratios (RR) of chlorophyll *a* production are also listed in *Table 7.* Here, total chlorophyll *a* concentrations on the final day of treatment (either nitrate (N) or phosphate (P)) were normalized to the control's final chlorophyll *a* concentration. RR-N/RR-P grants insight into what kind of nutrient limitation is occurring within the various sample sites. Notably, the greater the RR-N/RR-P is for a given location, there is a greater chance that the location will exhibit nitrate limitation.

Location	t=0 μM Nitrate	t=0 μM Phosphate	TN:TP	RR-N	RR-P	RR-N/RR-P
Linnévatnet Inlet 1	11.67	0.087	134.6	0.28	0.24	1.14
Linnévatnet Inlet 2	49.22	0.308	159.8	0.50	2.69	0.18
Linnévatnet Inlet 3	21.3	0.158	135.0	0.46	4.69	0.10
Linnévatnet Inlet 4	45.33	0.211	214.8	5.82	17.13	0.34
Linnévatnet Outlet	13.44	0.104	128.8	1.07	2.95	0.36
Linnévatnet Middle				1.15	1.12	1.03
Linnévatnet East	19.00	0.096	197.3	1.41	0.67	2.10
Linnévatnet West	18.11	1.155	15.7	1.38	4.87	0.28
Kongressvatnet Inlet	51.67	0.043	1215.7	0.25	3.56	0.07
Kongressvatnet Middle	23.78	0.093	8.6	0.52	0.74	0.71
Kongressvatnet Deep	0.75	0.157	4.8	1.32	1.11	1.18
Kongressvatnet Outlet	0.80	0.228	104.3	0.95	1.75	0.54

Table 7. Nutrient Concentrations and TN:TP Throughout Linnévatnet and Kongressvatnet

4.3 Linnévatnet Inlet 1

The results of Linnévatnet Inlet 1's mesocosm experiments are shown in *Figure 5.* As previously indicated, the water coming in to Linnévatnet from Inlet 1 is a result of melt water flowing in from a limestone and sandstone mountain ridge surrounding the lake. *Figure 5a,* shows the water source that samples were taken from. A graphical representation of bacteria growth throughout the various nutrient treatments is shown in *Figure 5b.* Notably, bacteria growth was enhanced in the phosphate and cocktail treatments and bacteria growth was inhibited when treated with nitrate. These results indicate that, at the time of experimentation, the bacteria of Inlet 1 were phosphate limited.

Figure 5c illustrates the concentrations of chlorophyll *a* recorded over a period of time that is indicative of primary production. Here, an increase in chlorophyll *a* is directly correlated to the growth of photosynthesizing organisms (primary producers). Notably, from time zero to day three, there was no significant growth; however, when compared to the weather data of *Table 8,* this period of slow growth paralleled the low amounts of solar radiation. These results indicate that the primary producers of Inlet 1 were light limited. Finally, in comparing *Figure 5c* and *Figure 5d,* the cocktail treatment shows a large drop in phosphate concentration from day 1 to 3 showing that the organisms in this mesocosm took advantage of the extra nutrients in their mesocosm.

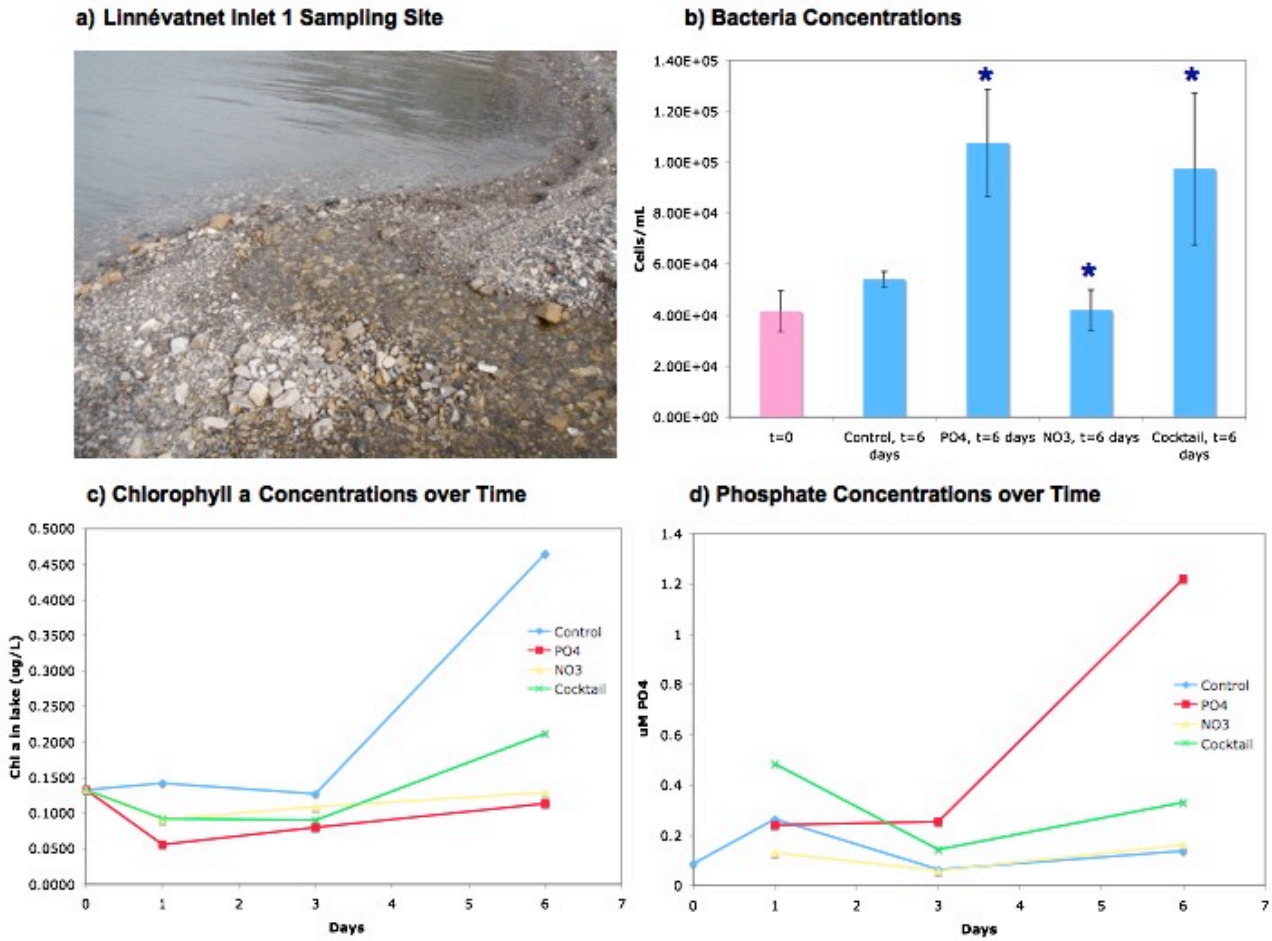

Figure 5. Linnévatnet Inlet 1 Mesocosm Experiment Results
* = significant difference between nutrient addition and t=6 days control ($p<0.05$)

Day	0	1	2	3	4	5	6
Temperature (^{o}C)	5.40	7.03	6.62	5.81	5.81	4.99	7.03
Solar Radiation (W/m^2)	324.4	179.4	196.9	79.4	390.6	269.6	483.1

Table 8. Daily Temperature and Solar Radiation for Linnévatnet Inlet 1 and 2 Mesocosm Experiments

4.4 Linnévatnet Inlet 2

Figure 6 shows the results of Linnévatnet Inlet 2's mesocosm experiments. *Figure 6a* is a photograph of the geology surrounding Inlet 2. Notably, the water flowing down this limestone ridge seeps underground (about mid-point on is image) and enters Linnévatnet through the lake sediments. *Figure 6b* shows that bacteria growth was enhanced when treated with phosphate and inhibited when nitrate was added. Unlike Inlet 1, *Figure 6c* shows that chlorophyll *a* concentrations increased in the phosphate mesocosm over time. Additionally, primary production was independent of the corresponding temperature and solar radiation measurements of *Table 8*. These results indicate that the microorganisms of Inlet 2 were phosphate limited. These results are confirmed by the steep drop in phosphate concentrations for the phosphate treatment in *Figure 6d.*

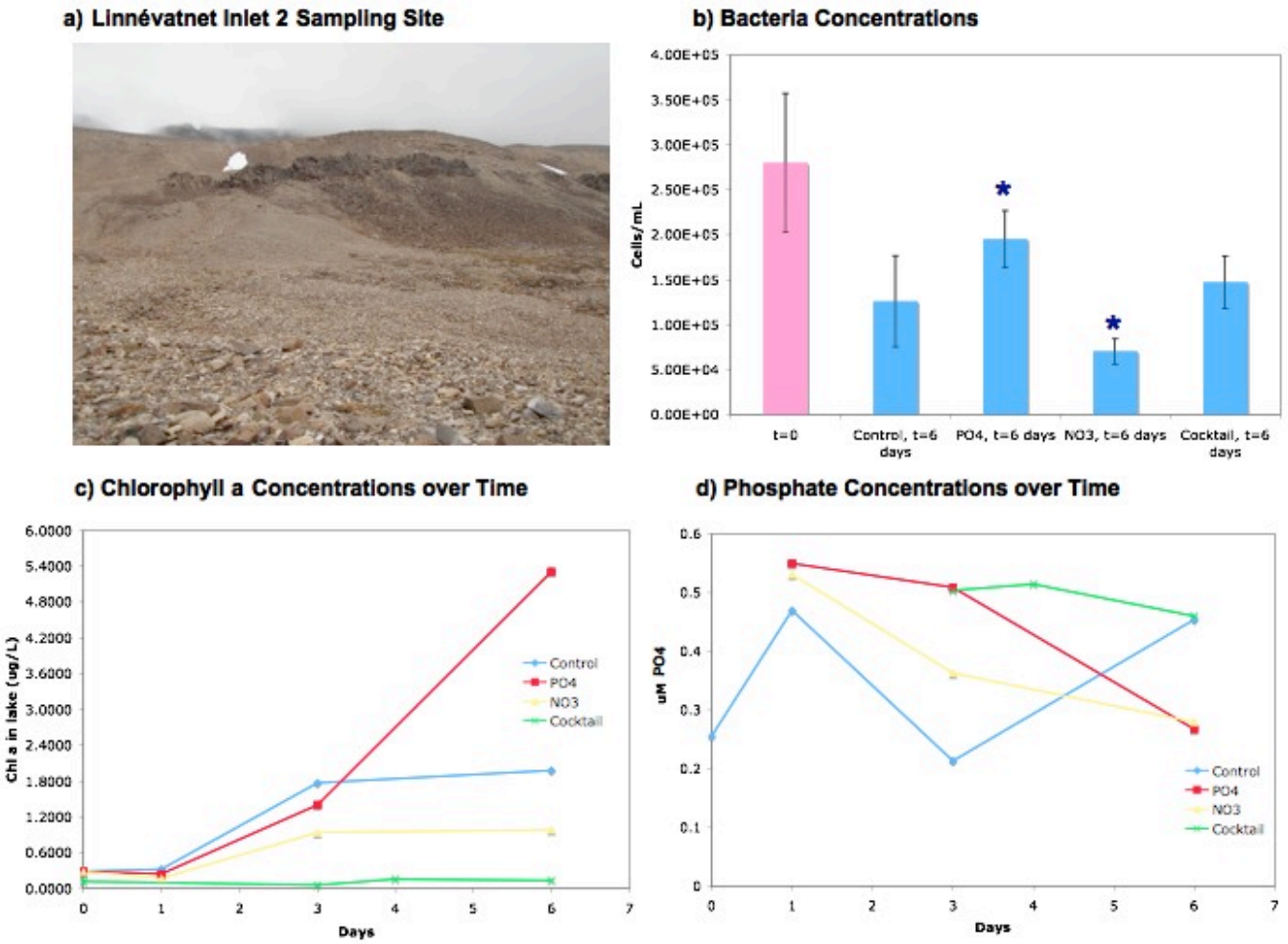

Figure 6. Linnévatnet Inlet 2 Mesocosm Experiment Results
* = significant difference between nutrient addition and t=6 days control ($p<0.05$)

4.5 Linnévatnet Inlet 3

Figure 7 shows the results of Linnévatnet Inlet 3's mesocosm experiments. Here, the phosphate and cocktail treatments exhibited a steep drop in phosphate concentrations over time (*Figure 7c*) in coordination with a large increase in chlorophyll *a* concentrations over time (*Figure 7c*). These results indicate that the primary producers of Inlet 3 were phosphate limited during the time of experimentation. On the other hand, the cocktail treatment inhibited the growth of bacteria while phosphate and nitrate additions showed no effect. Finally, temperature and solar radiation seemed to have no effect on the growth of the primary producers (*Table 9*).

Day	0	1	2	3	4	5
Temperature (^oC)	7.03	7.03	7.83	7.83	5.40	2.89
Solar Radiation (W/m^2)	483.1	130.6	450.6	450.6	344.4	209.4

Table 9. Daily Temperature and Solar Radiation for Linnévatnet Inlet 3, Inlet 4, East and West Mesocosm Experiment

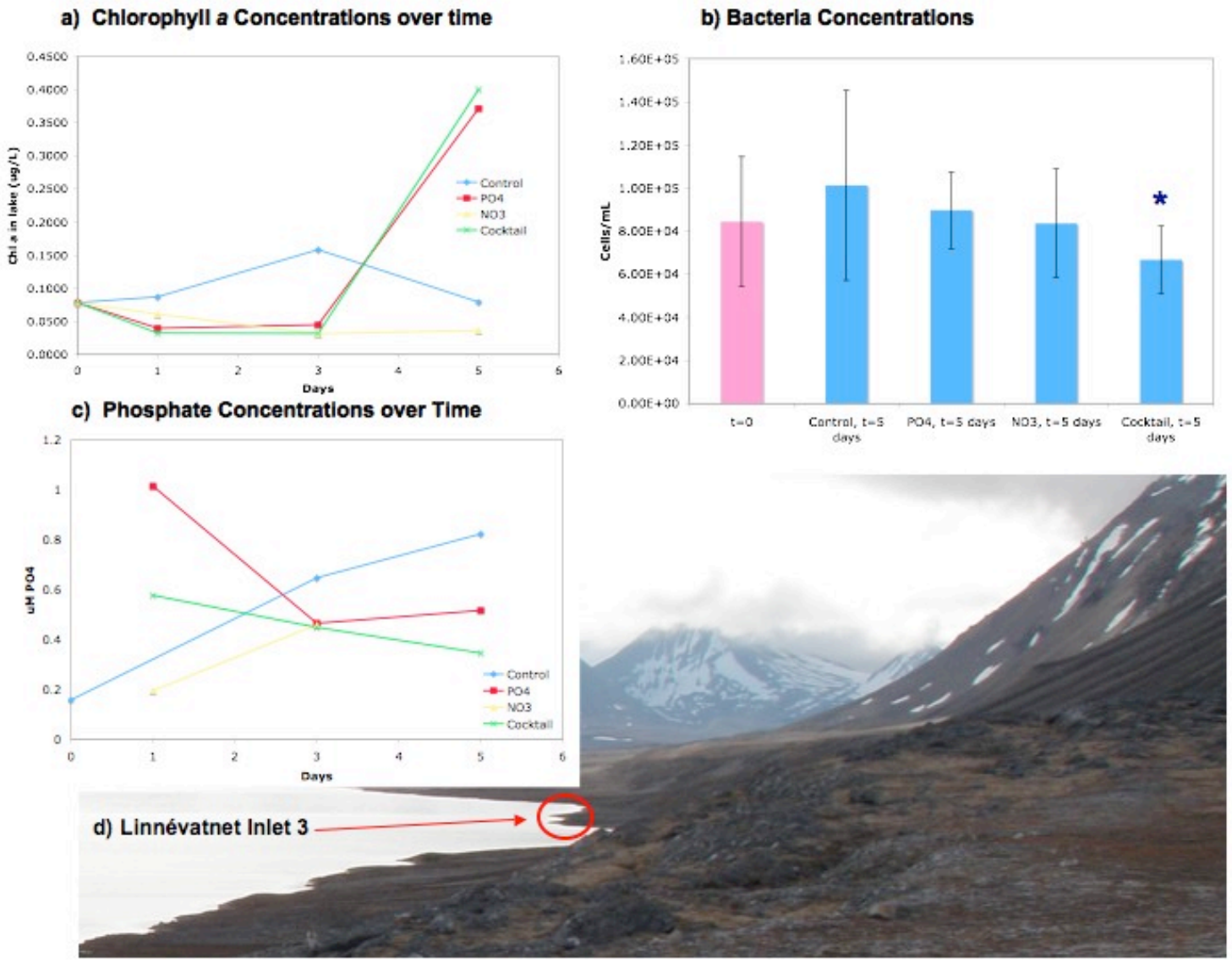

Figure 7. Linnévatnet Inlet 3 Mesocosm Experiment Results
* = significant difference between nutrient addition and t=5 days control ($p<0.05$)

4.6 Linnévatnet Inlet 4

Inlet 4 is the largest inlet into Linnévatnet. This inlet brings in water all the way from the Linné Glacier approximately 7km away (*Figure 8a*). *Figure 8b* shows that when samples from Inlet 4 were given a phosphate or cocktail treatment chlorophyll *a* concentrations skyrocketed while the control and nitrate treatment chlorophyll *a* concentrations remained relatively constant. These results are indicative of phosphate limitation. Additionally, in comparing the results of *Figure 8b* and *Table 9,* day 1 exhibited the lowest amounts of solar radiation and chlorophyll *a* concentrations indicating that light limited the primary production in this area as well. Notably, reliable bacteria counts were unobtainable due to the high amount of sediment in this area.

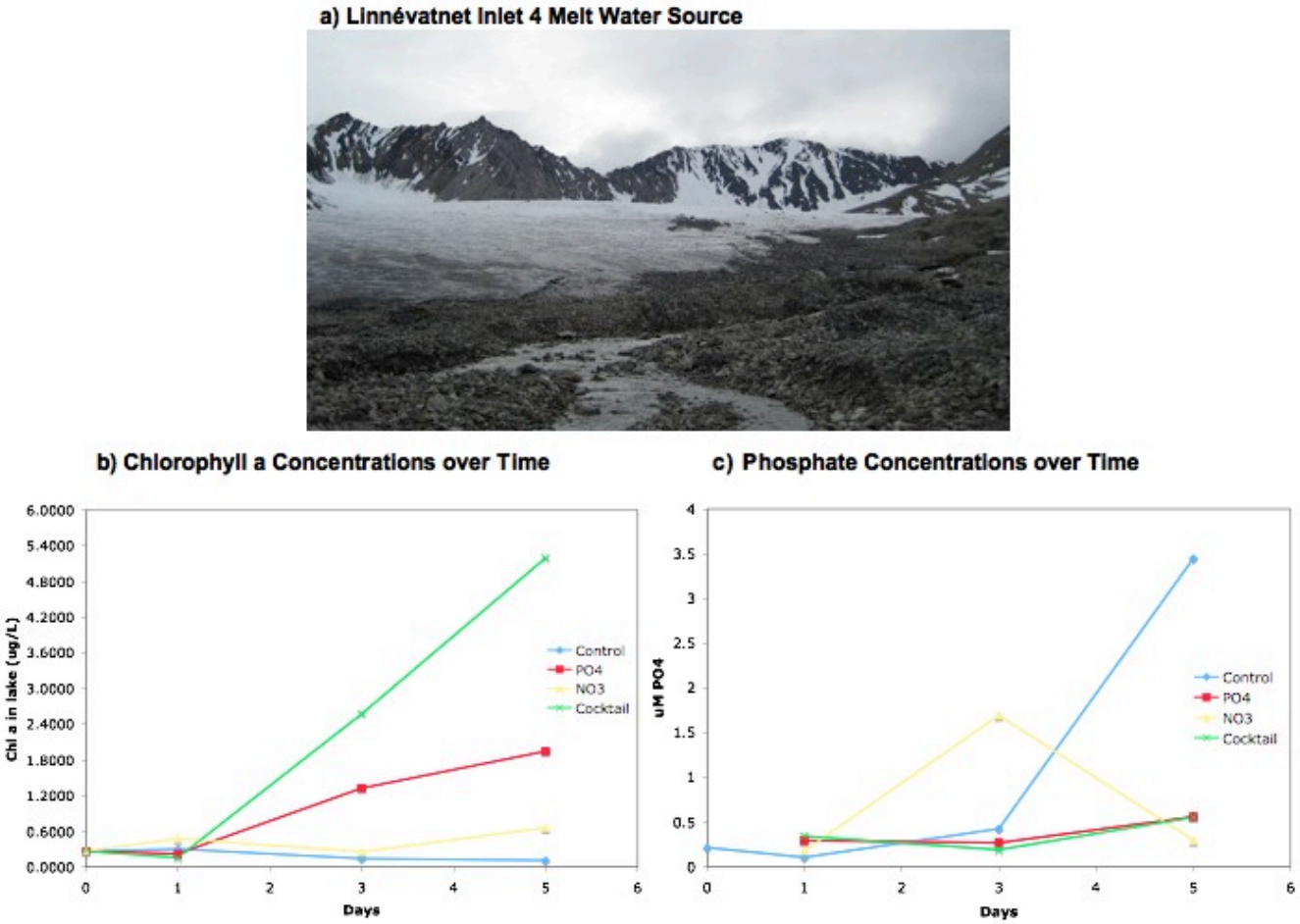

Figure 8. Linnévatnet Inlet 4 Mesocosm Experiment Results

4.7 Linnévatnet Outlet

Figure 9 shows the results of Linnévatnet Outlet's mesocosm experiments. The microorganisms in Linnévatnet Outlet exhibited a strong phosphate limitation that was observed through the rapid depletion of available phosphate (*Figure 9d*). Here, all treatments, excluding the cocktail, consumed nearly all of the dissolved phosphate by day 2. Phosphate limitation is further illustrated by the significant increase in bacteria concentrations upon phosphate additions (*Figure 9b*) and the greater concentrations of chlorophyll *a* in the phosphate-treated mesocosm (*Figure 9c*). These chlorophyll *a* concentrations remain high until all available phosphate is depleted (*Figure 9d*). Finally, the anomaly as to why the cocktail treatment, in spite of its high phosphate content (*Figure 9d*), exhibited minimal bacteria and chlorophyll growth until the day 5 sampling time, is most likely due to the microbiota's difficulty in dealing with the 7µM nitrate addition. This is because Linnévatnet Outlet typically exhibits a mere 13.44µM nitrate environment (*Table 7*). On the other hand, when we treated these organisms with nitrate, nitrate concentrations nearly doubled. Thus, a culture effect occurred in the mesocosm in which the microorganisms needed to adjust their metabolism to deal with these newly raised nutrient levels.

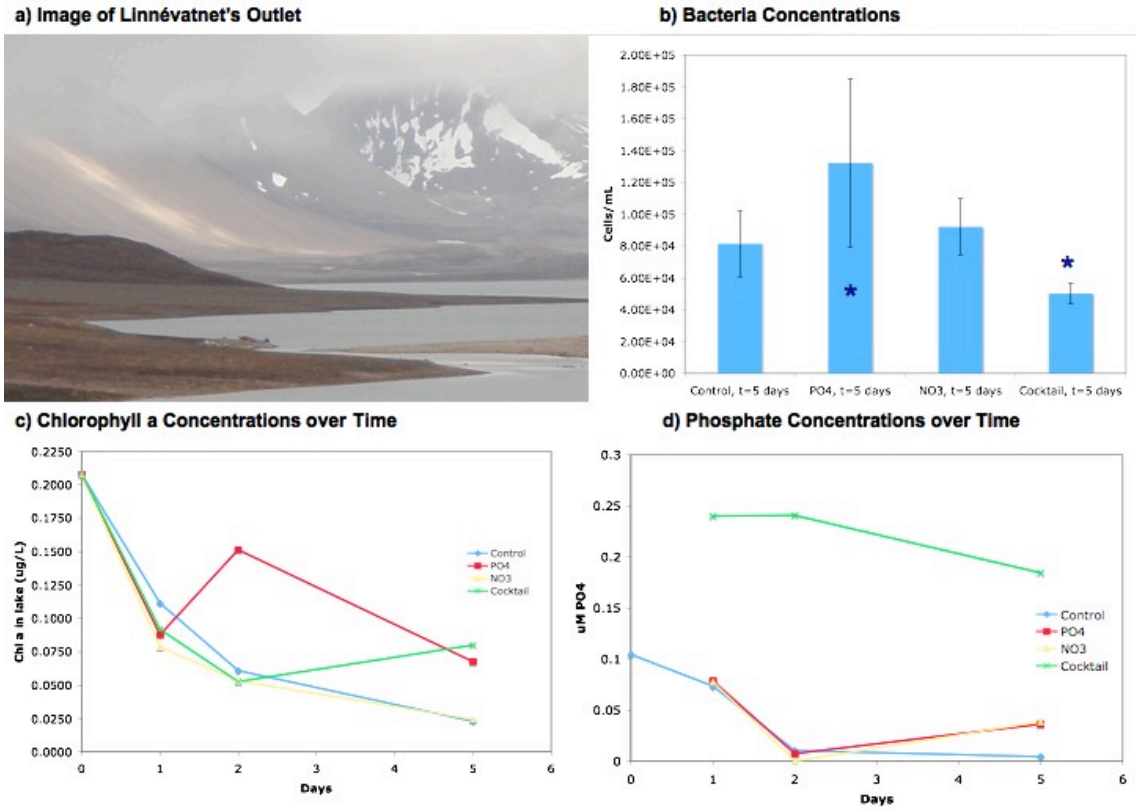

Figure 9. Linnévatnet Outlet Mesocosm Experiment Results
* = significant difference between nutrient addition and t=5 days control ($p<0.05$)

Day	0	1	2	3	4	5
Temperature (^oC)	5.4	7.03	5.4	7.03	6.22	4.99
Solar Radiation (W/m^2)	378.1	534.4	131.9	251.9	81.9	94.4

Table 10. Daily Temperature and Solar Radiation for Linnévatnet Outlet and Middle Mesocosm Experiments

4.8 Linnévatnet Middle

The results of Linnévatnet Middle's mesocosm experiments are shown in *Figure 10*. *Figure 10b* shows that bacteria growth was stimulated by the addition of nitrate; however, *Figures 10c* and *10d* indicate that the primary producers in all of the mesocosms struggled. Notably, the jumps in phosphate concentration (*Figure 10d*) in both the nitrate and control treatments are indicative of high biological death frequencies. This is because the phosphate jumps that appear in *Figure 10d* were a result of cell death and lysis that released DNA and, subsequently, increased phosphate concentrations due to the phosphate in the DNA's phosphodiester backbone. Notably, the increased growth in cocktail and nitrate treated bacteria (*Figure 10b*) indicate that the bacteria in Linnévatnet Middle were more efficient at consuming introduced phosphate than the primary producers. Ultimately, since neither phosphate nor nitrate stimulated primary production (*Table 10*), Linnévatnet Middle's primary production was limited by some other nutrient (i.e. Iron or Molybdate) or outside factor.

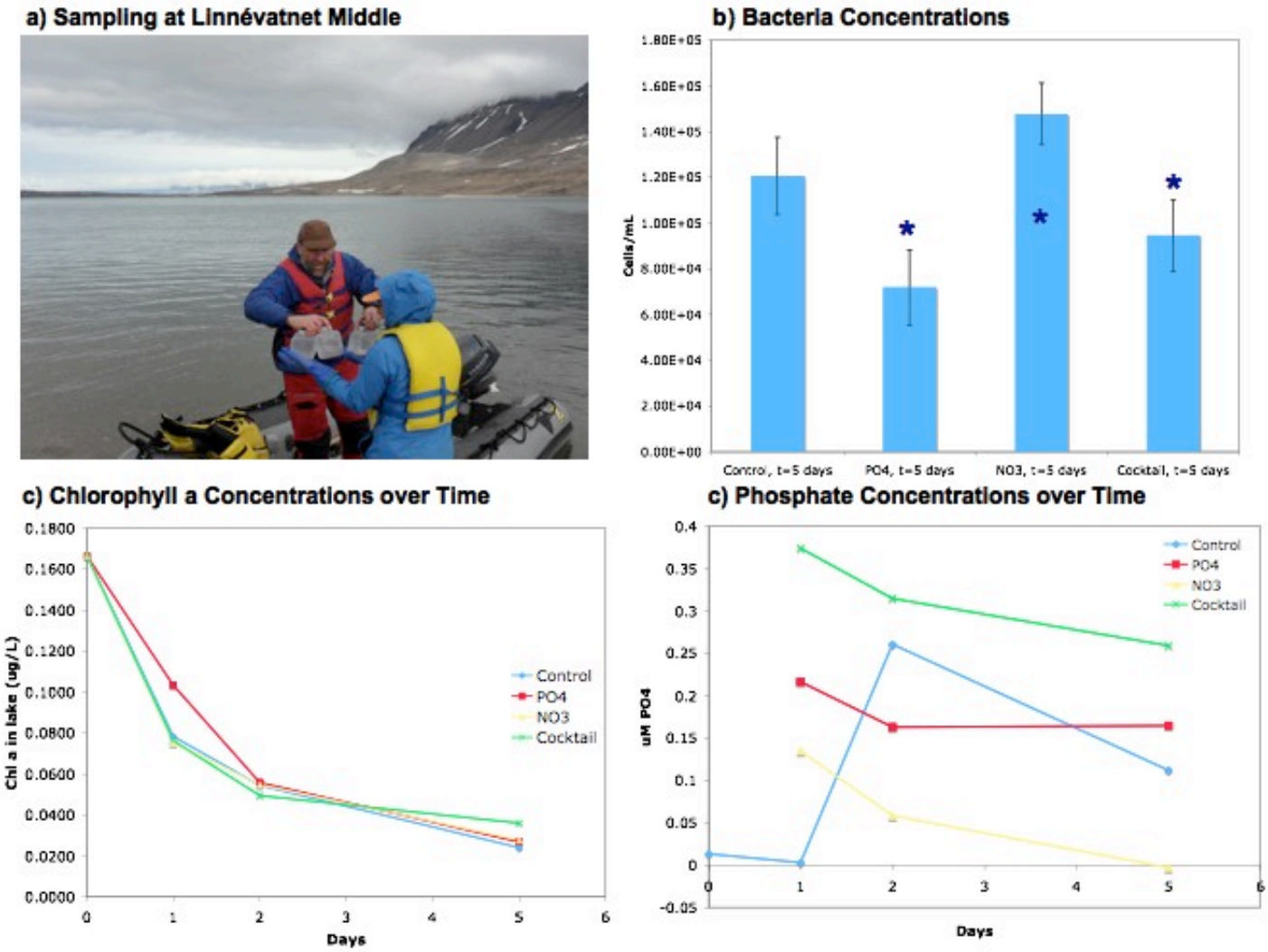

Figure 10. Linnévatnet Middle Mesocosm Experiment Results
* = significant difference between nutrient addition and t=5 days control ($p<0.05$)

4.9 Linnévatnet East

Figure 11 shows the result of Linnévatnet East's mesocosm experiments. *Figure 11c* shows that both the nitrate and cocktail mesocosms had increased chlorophyll *a* concentrations over time while *Figure 11b* indicates that there was increased bacteria growth in the nitrate mesocosm. These results, in addition to the relatively low nitrate concentration (19.00μM, *Table 7*) of the region, demonstrate that bacteria and primary production are nitrate limited in Linnévatnet East. Finally, in comparing the weather data of *Table 9* and chlorophyll *a* concentrations of *Figure 11c*, temperature and solar radiation did not play a limiting role in primary production.

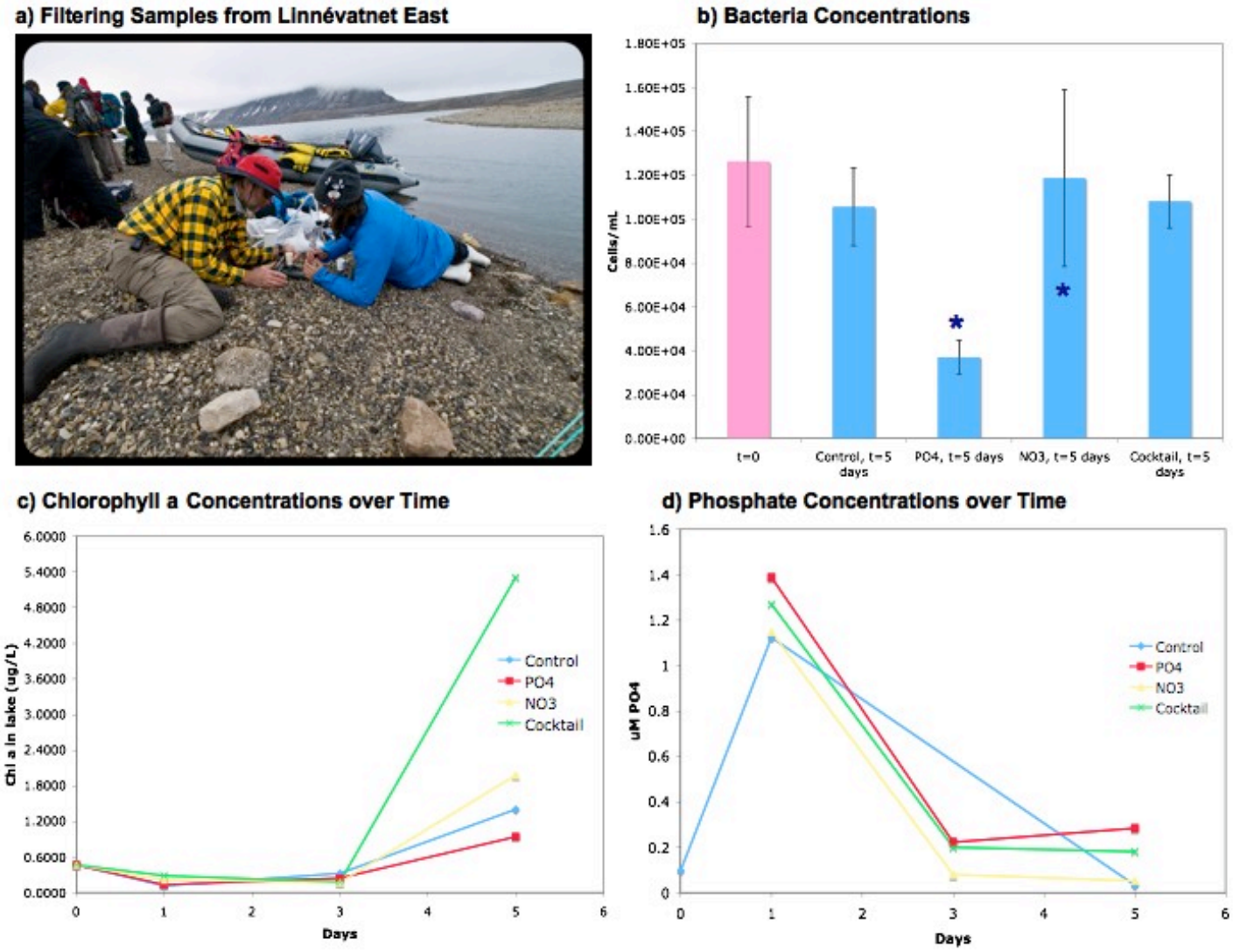

Figure 11. Linnévatnet East Mesocosm Experiment Results
* = significant difference between nutrient addition and t=5 days control ($p<0.05$)

4.10 Linnévatnet West

The results of Linnévatnet West's mesocosm experiments are shown in *Figure 12.* Notably, none of the nutrient additions increased bacteria or chlorophyll *a* production (*Figures 12b* and *12c*). Additionally, there was no correlation between the weather data of *Table 9* and chlorophyll *a* concentrations (*Figure 12c*). These results indicate that the microorganisms in Linnévatnet West are limited by a nutrient other than phosphate and nitrate or some other unknown factor.

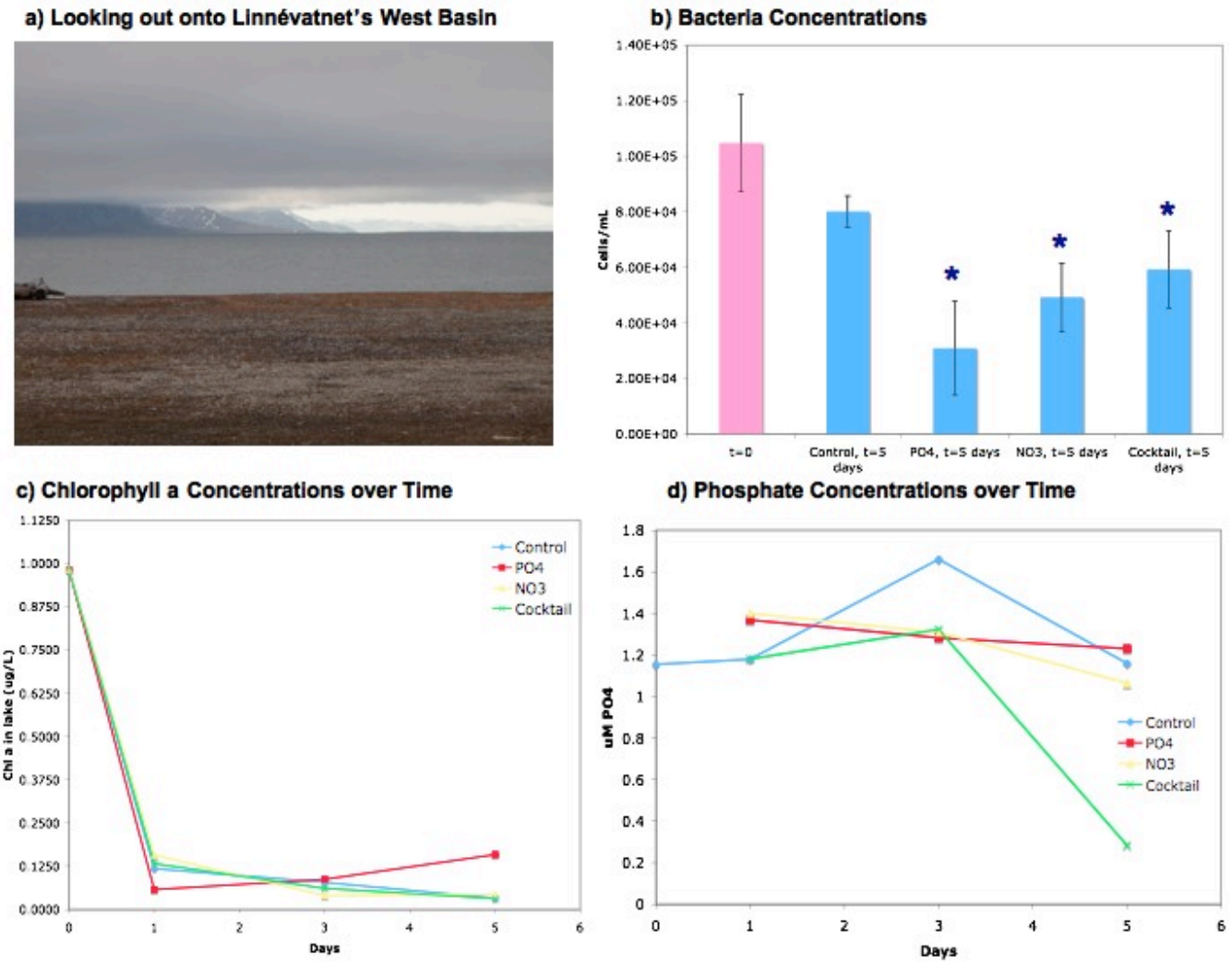

Figure 12. Linnévatnet West Mesocosm Experiment Results
* = significant difference between nutrient addition and t=5 days control ($p<0.05$)

4.11 Kongressvatnet Inlet

Figure 13 shows the results of Kongressvatnet Inlet's mesocosm experiments. Notably, *Figures 13c* and *13d* do not show a significant response to nutrient additions and phosphate consumption until after day 3. This delay in microbial growth occurs until temperatures in the region surpass 7°C (*Table11*) indicating that the greatest limiting factor of microbial growth at this location was temperature. However, once temperatures surpassed 7°C, growth in the mesocosms treated with phosphate (*Figures 13b* and *13c*) surpassed all other nutrient treatments.

Day	0	1	2	3	4	5
Temperature (°C)	5.4	2.89	4.57	8.23	7.03	7.03
Solar Radiation (W/m^2)	344.4	209.4	268.1	274.4	150.6	256.9

Table 11. Daily Temperature and Solar Radiation for Kongressvatnet Inlet and Middle Mesocosm Experiments

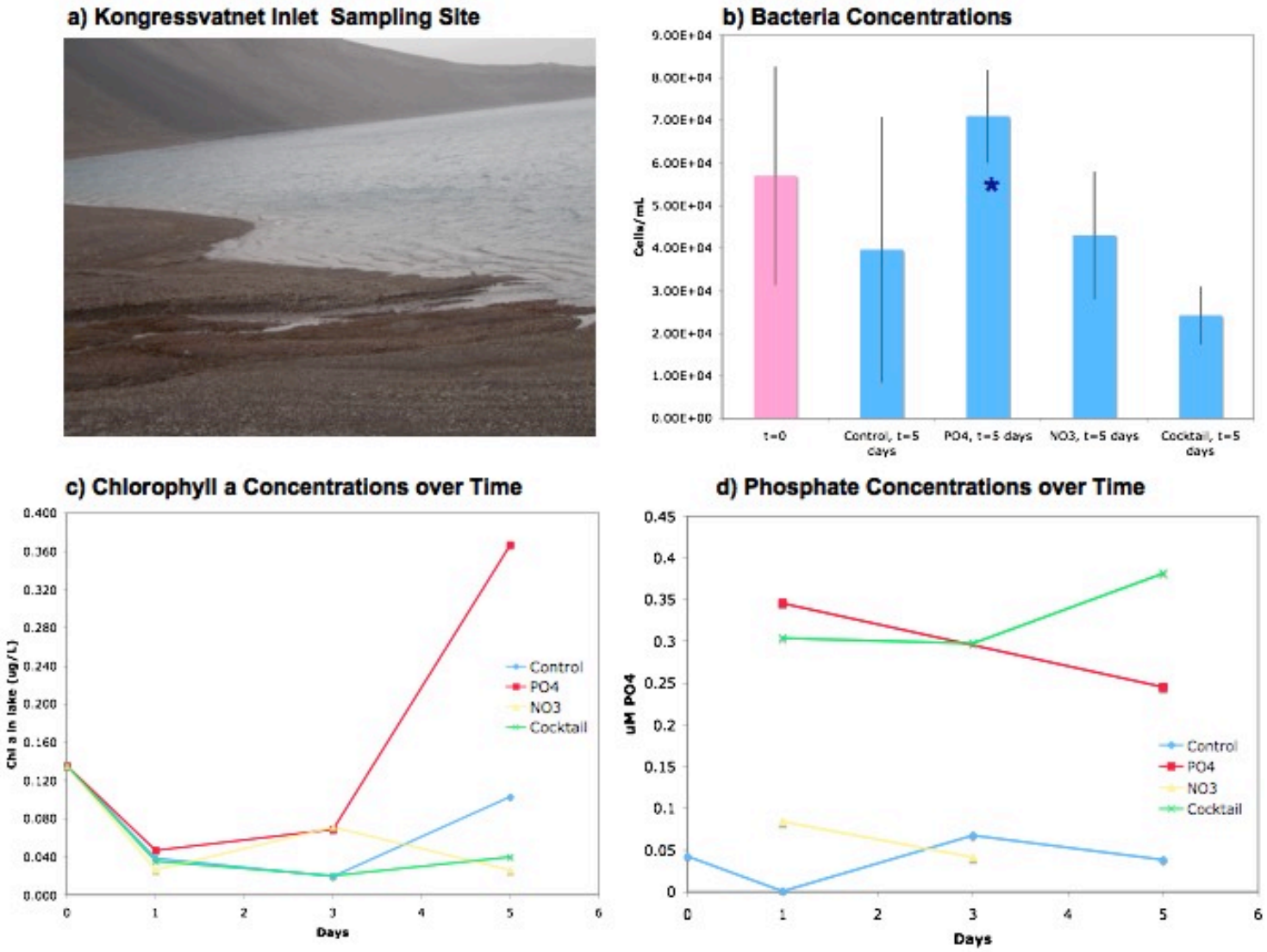

Figure 13. Kongressvatnet Inlet Mesocosm Experiment Results
* = significant difference between nutrient addition and t=5 days control ($p<0.05$)

4.12 Kongressvatnet Middle

The results of Kongressvatnet Middle's mesocosm experiments are shown in *Figure 14.* Unlike the inlet of Kongressvatnet, primary production in the middle of the lake did not show any correlation to external temperature or solar radiation (*Figure 14c* and *Table 11*). *Figure 14d* displays a sharp decrease in phosphate concentration between days 1 and 3 for the phosphate treated mesocosm indicating that there is a phosphate limitation. Notably, it was the bacteria rather than the primary producers who were consuming the phosphate (*Figure 14b*) and, thus, out-competing the primary producers for available nutrients.

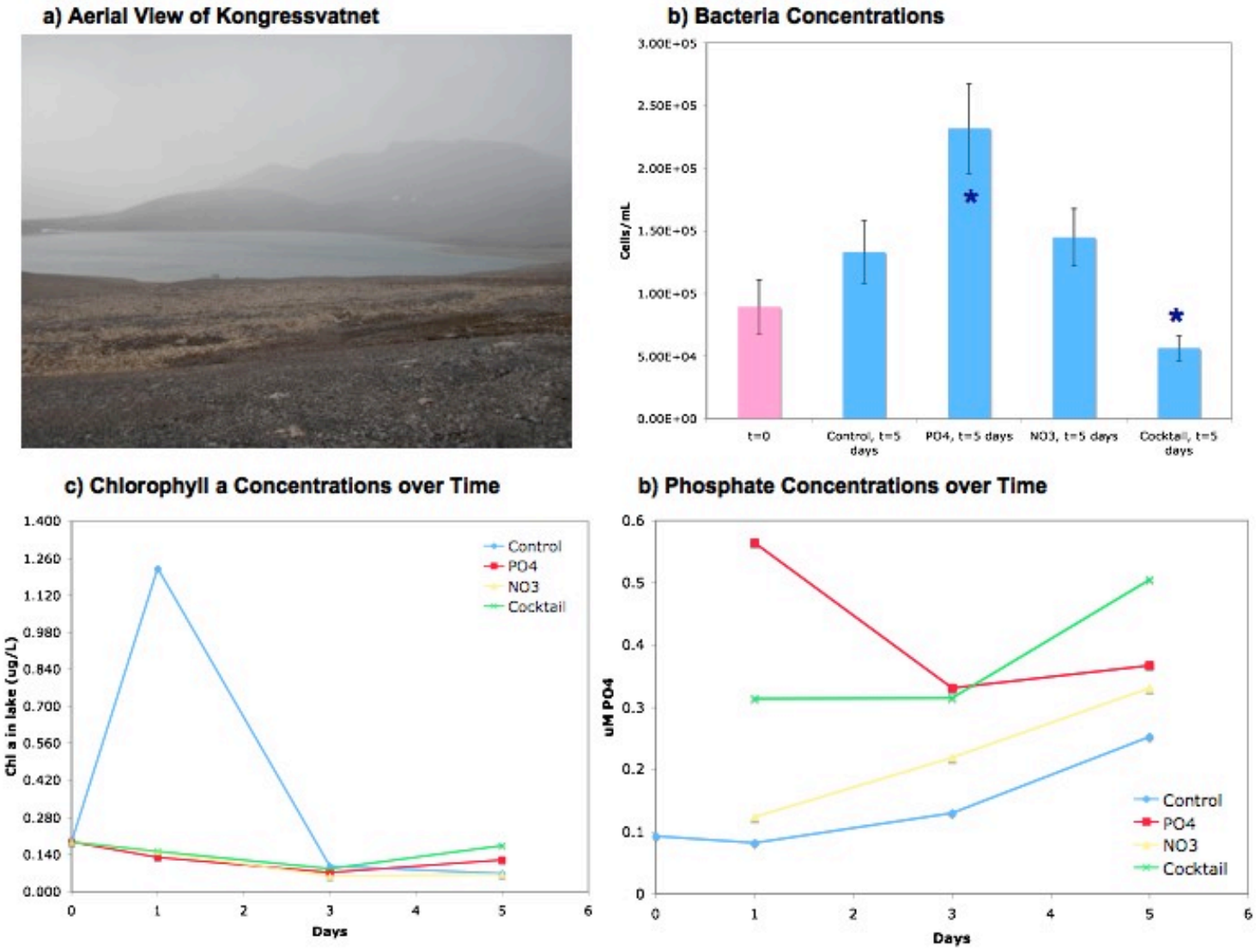

Figure 14. Kongressvatnet Middle Mesocosm Experiment Results

- = significant difference between nutrient addition and t=5 days control ($p<0.05$)

4.13 Kongressvatnet Deep

Figure 15 shows the results of Kongressvatnet Deep's mesocosm experiments. As mentioned earlier, Kongressvatnet Deep contained samples drawn from the thermocline of Kongressvatnet and, thus, were less susceptible to varying air temperatures and solar radiation. After a five day incubation period, all mesocosms exhibited increased bacteria growth from the time zero concentrations (*Figure 15b*). Notably, the nitrate mesocosm exhibited the most significant growth. On the other hand, the greatest growth of primary producers was seen in the cocktail mesocosm (*Figure 15c*). Due to the results of *Figures 15b* and 15*c* as well as the minimal phosphate consumption in *Figure 15d*, Kongressvatnet Deep exhibited nitrate limitation during the time of experimentation.

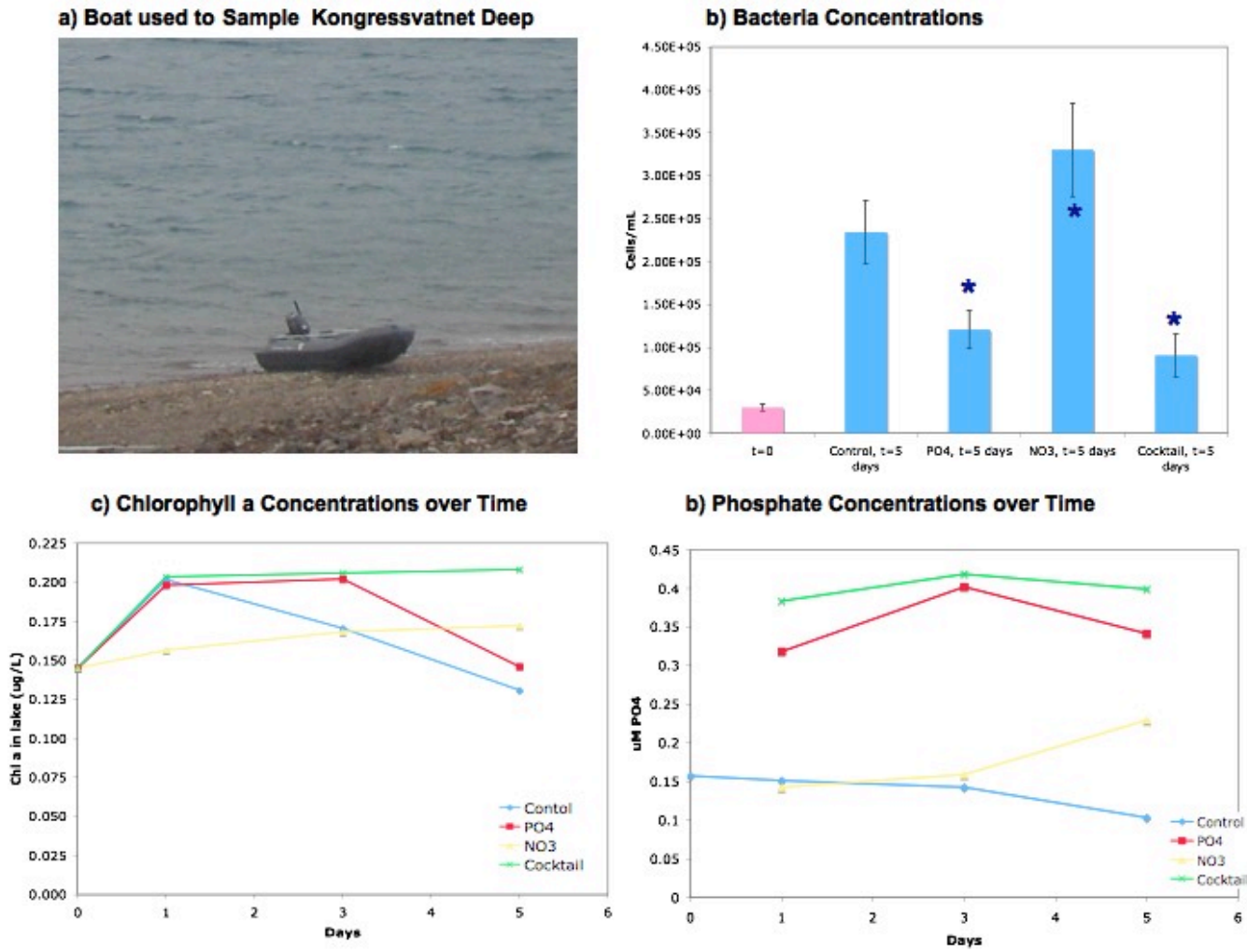

Figure 15. Kongressvatnet Deep Mesocosm Experiment Results
* = significant difference between nutrient addition and t=5 days control ($p<0.05$)

4.14 Kongressvatnet Outlet

The results of Kongressvatnet Outlet's mesocosm experiments are shown in *Figure 16.* Here, addition of phosphate and/or nitrate had an inhibitory effect on the growth of bacteria (*Figure 16b*). Additionally, nutrient addition had no effect on chlorophyll *a* concentrations over time (*Figure 16c*). Finally, there was no correlation between temperature, solar radiation, and chlorophyll a concentrations (*Table 12* and *Figure 16c*) and, thus, the growth of microorganisms in Kongressvatnet Outlet were limited by a nutrient other than phosphate and nitrate or some other external factor.

Day	0	1	2	3	4	5
Temperature (^{o}C)	4.57	8.23	7.03	7.03	7.03	7.03
Solar Radiation (W/m^2)	268.1	274.4	150.6	256.9	26.9	283.1

Table 12. Daily Temperature and Solar Radiation for Kongressvatnet Outlet Mesocosm Experiment

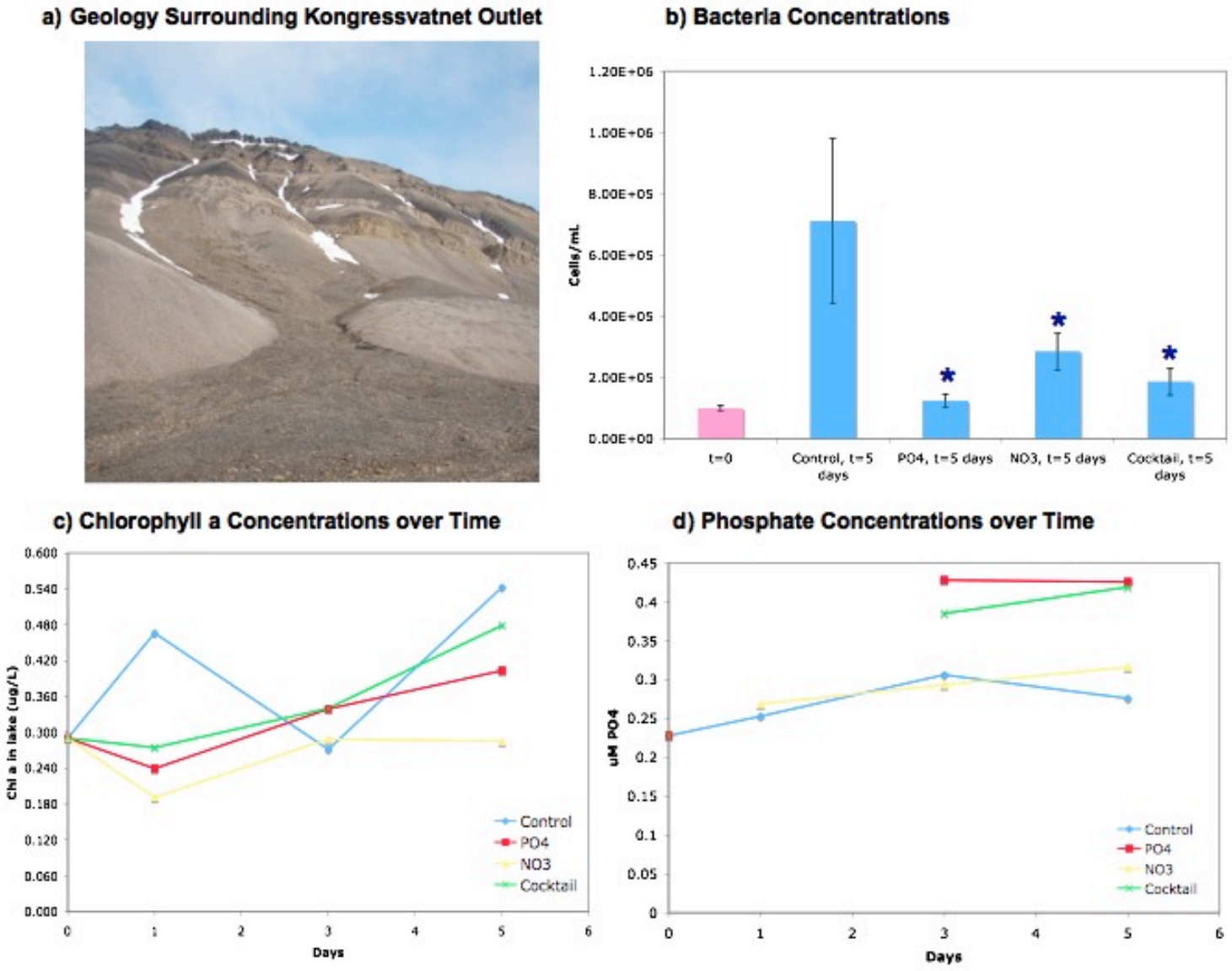

Figure 16. Kongressvatnet Outlet Mesocosm Experiment Results
* = significant difference between nutrient addition and t=5 days control ($p<0.05$

4.15 Nitrogen Fixation Rates

The results of the nitrogen fixation experiment are shown in *Figure 17*. This chart shows the activity and efficiency of the nitrogenase enzyme. Kongressvatnet Outlet had the highest rates of nitrogen fixation (0.22 nmol Nitrogen Fixed/mL/hr) followed by Linnévatnet East. Notably, Linnévatnet Inlet 4, Kongressvatnet Inlet, Kongressvatnet Deep did not exhibit any nitrogenase activity. Finally, when Linnévatnet East and West were incubated for four and twenty four hour periods, the shorter incubation time exhibited greater nitrogen fixation rates.

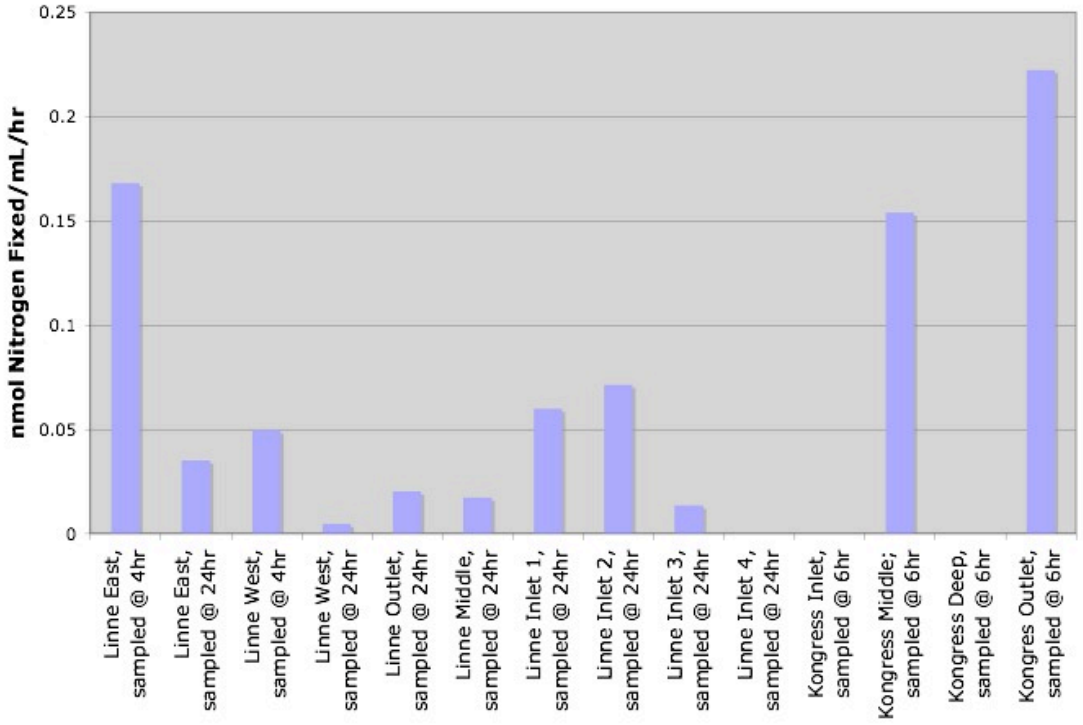

Figure 17. Nitrogenase Activity

4. 16 Kongressvatnet's Chemocline

TROLL profiling revealed that Kongressvatnet contains a very distinct chemocline that occurred at approximately 40m (*Figure 19*). Through the chemocline, water switched from oxic to anoxic (*Figure 19a*) in coordination with a turbidity spike (*Figure 19c*). When preserved bacteria samples were counted, bacteria concentrations mimicked the turbidity curve (*Figure 19b*). In addition to observing a wide variety of bacteria (*Figure 18a*) through microscopy, several nodular iron-sulfate precipitates were also found (*Figure 18b*). Lastly, *Figure 19d* represents a conductivity spike while traveling through the chemocline and *Figure 19c* demonstrates that pH and temperature remained relatively neutral during this transition.

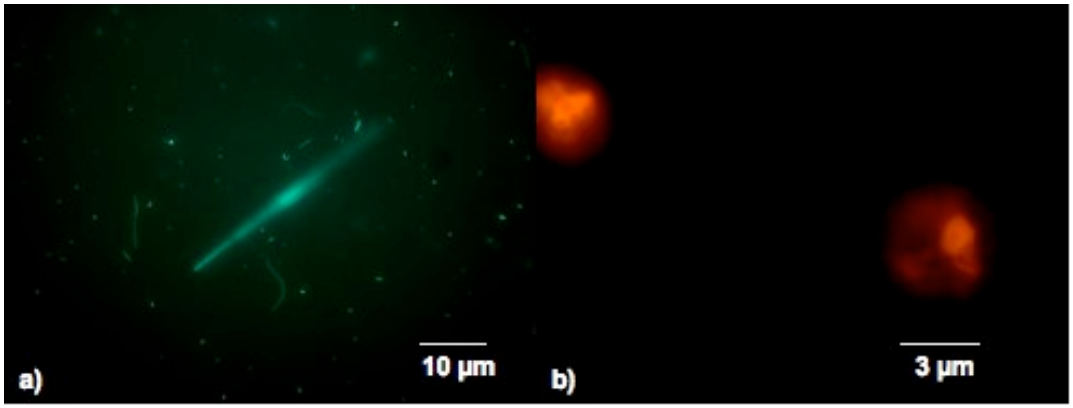

Figure 18. Microscope Images of Chemocline
Acridine orange stained samples at 1000x under blue light. DNA fluoresces green and other "gunk" is red.

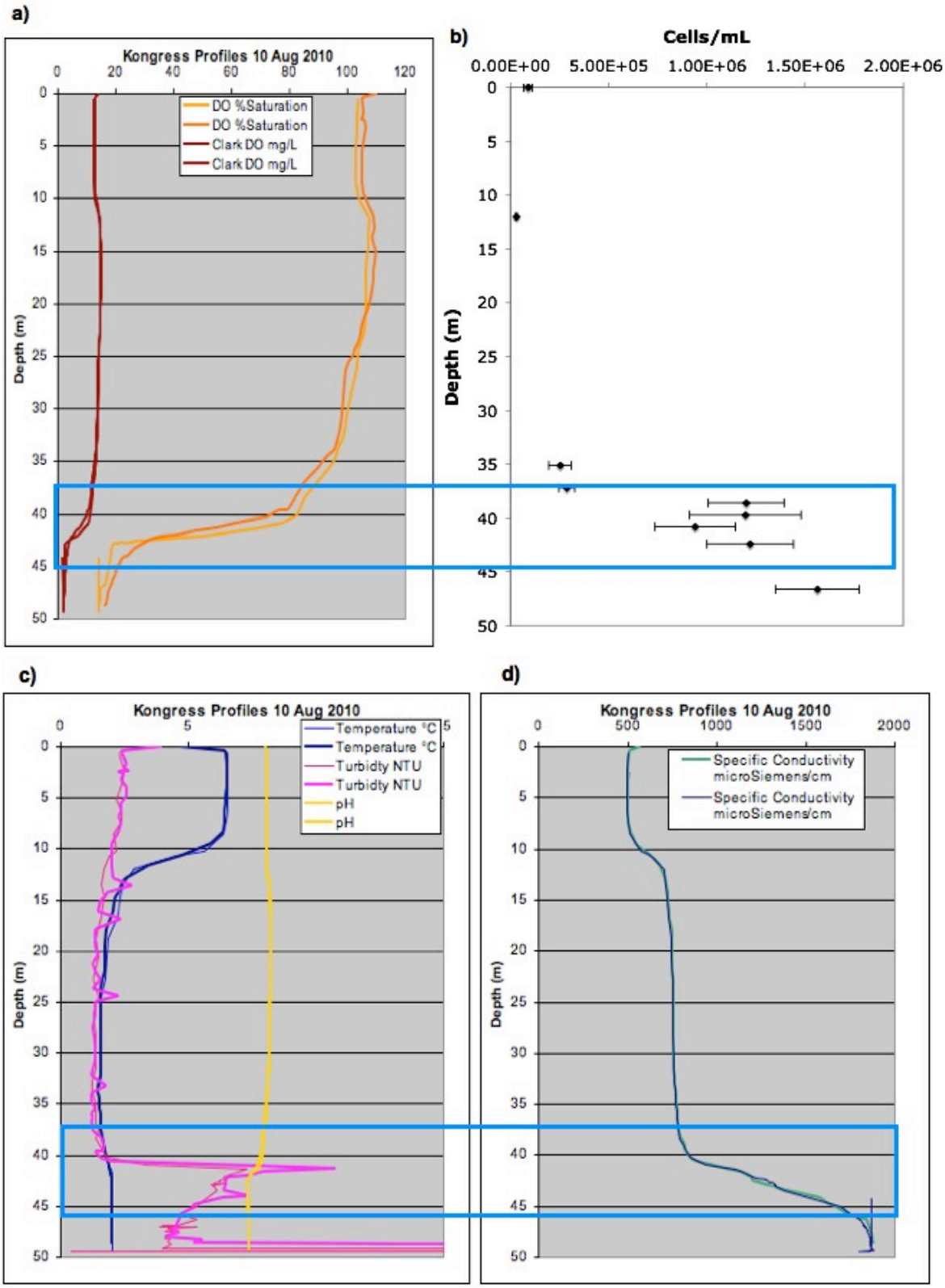

Figure 19. The Biology and Chemistry of Kongressvatnet's Chemocline
The chemocline is circled in blue on all four images. a) Transition from oxic to anoxic water (oxicline). b) Bacteria concentrations throughout the chemocline. c) Temperature, turbidity and pH throughout the chemocline. d) Conductivity throughout the chemocline.

4.17 Microorganism Identification

Diatoms were identified with the help of Dr. Dave Caron. *Figure 20a* is an image of a *Skeletonema*, *Figure 20b* is a *Licmophora*, *Figure c* was unable to be identified, and *Figure 20d* is an *Oscillatoria* (which is actually a nitrogen-fixing filamentous cyanobacteria). Additionally, *Table 13* diagrams all diatoms observed and their relative abundance. Unfortunately, photographs of the predominant *Nitzschia* population were unavailable.

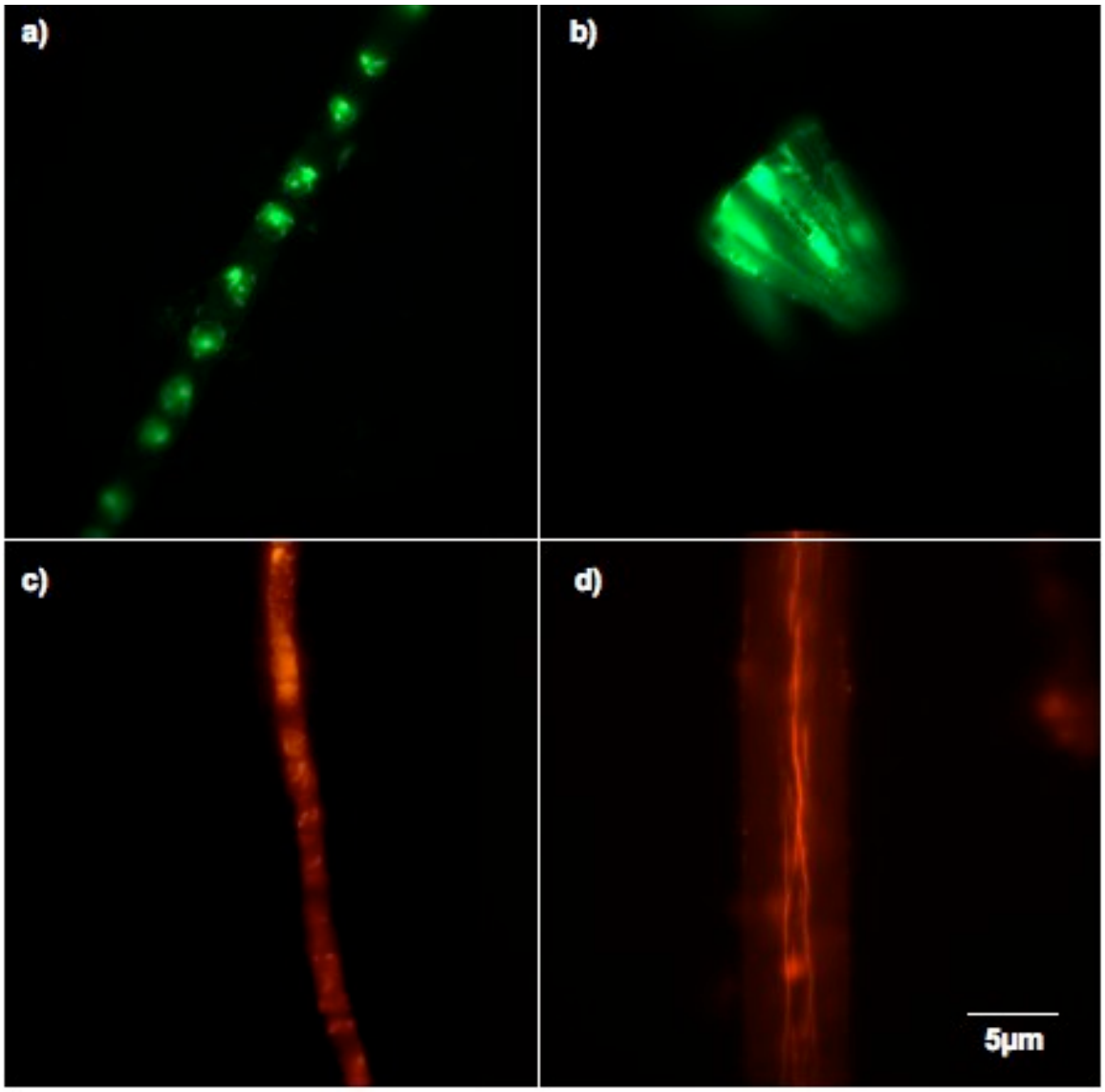

Figure 20. Diatoms Present in Linnévatnet
Figures (a) and (b) are samples stained with acridine orange and observed at 1000x under blue light. Figures (c) and (d) are also stained with acridine orange but are observed at 1000x under green light. All images are scaled to the 5µm bar in the bottom right corner of image.

Lake	Most Abundant	Other Protists
Linnévatnet	**Genus:** *Nitzschia* (many species) **Location:** Linnévatnet Inlet 3	*Pseudonitzschia* *Thalassiosira* *Licmophora* *Dinobryon* (East Only)
Kongressvatnet	**Genus:** *Nitzschia* (many species) **Location:** Kongressvatnet Deep	N/A

Table 13. Diatoms and Protists Found in Lake Linnévatnet and Kongressvatnet

Figure 21. Looking Down into the Linné Valley from the Northwest Shore

5 DISCUSSION

5.1 Linnévatnet and Kongressvatnet N:P Stoichiometry

The results of the mesocosm experiments describe how various mini-ecosystems throughout Linnévatnet and Kongressvatnet respond to nitrate and phosphate additions. Regions that experienced enhanced growth upon the addition of nutrients were described as either phosphate or nitrate limited. Other locations, like Linnévatnet Inlet 1 and Linnévatnet Inlet 2, were light limited while Kongressvatnet Inlet was temperature and phosphate limited. The overall nutrient and light limitations throughout Linnévatnet is illustrated in *Figure 22*.

Figure 22 shows that Linnévatnet's main source of melt water comes in through Inlet 4 off the Linnévatnet Glacier. As water from Inlet 4 flows into Linnévatnet a wealth of nitrate (*Table 7*) and sediment are brought into the lake. This thick sediment plume limits the sun's ability to penetrate the water and, thus, the primary producers exhibit a sunlight limitation. Additionally, the extra nitrate (an N:P ratio of 214:1 rather than the Redfield 16:1[20]) pushes the region into phosphate limitation.

Notably, Inlet 4 is not an isolated case. All inlets of both Linnévatnet and Kongressvatnet are carrying water with elevated TN:TP ratios (*Table 7*) that force the microbiology of the region to become phosphate limited. The best explanation for this phenomenon is that the high amounts of anthropogenic nitrogen coming into Kapp Linné from Western Europe are precipitating out of the atmosphere and onto the mountains surrounding the Linné Valley (*Figure 21*). For most of the year, the precipitation into the valley is snow and, thus, nitrates accumulate within the mountain snow pack until the summer melt season occurs. Then, as temperatures rise, these nitrate rich waters are flushed into Linnévatnet and Kongressvatnet. Kongressvatnet shows even greater nitrate enrichment through glacial and snow pack melt (*Table 7*) due to its smaller size.

Although there are elevated levels of nitrate coming into Linnévatnet and Kongressvatnet through the inlets, results indicate that as water circulates counter-clockwise through Linnévatnet and across Kongressvatnet, nitrate concentrations are lowered through diffusion and biological consumption. When water reaches the east basin of Linnévatnet and the thermocline of Kongressvatnet enough nitrate has been depleted that these regions actually become nitrate limited. On the other hand, in the west basin and middle of Linnévatnet, the N:P of Linnévatnet West balances out to nearly resemble the Redford 16:1 while Linnévatnet Middle's RR-N/RR-P value close to 1 shows that phosphate and nitrate additions had a neutral effect. Notably, these regions are far enough removed from the nitrate loaded inlets that they do not feel a shift in their N:P stoichiometry.

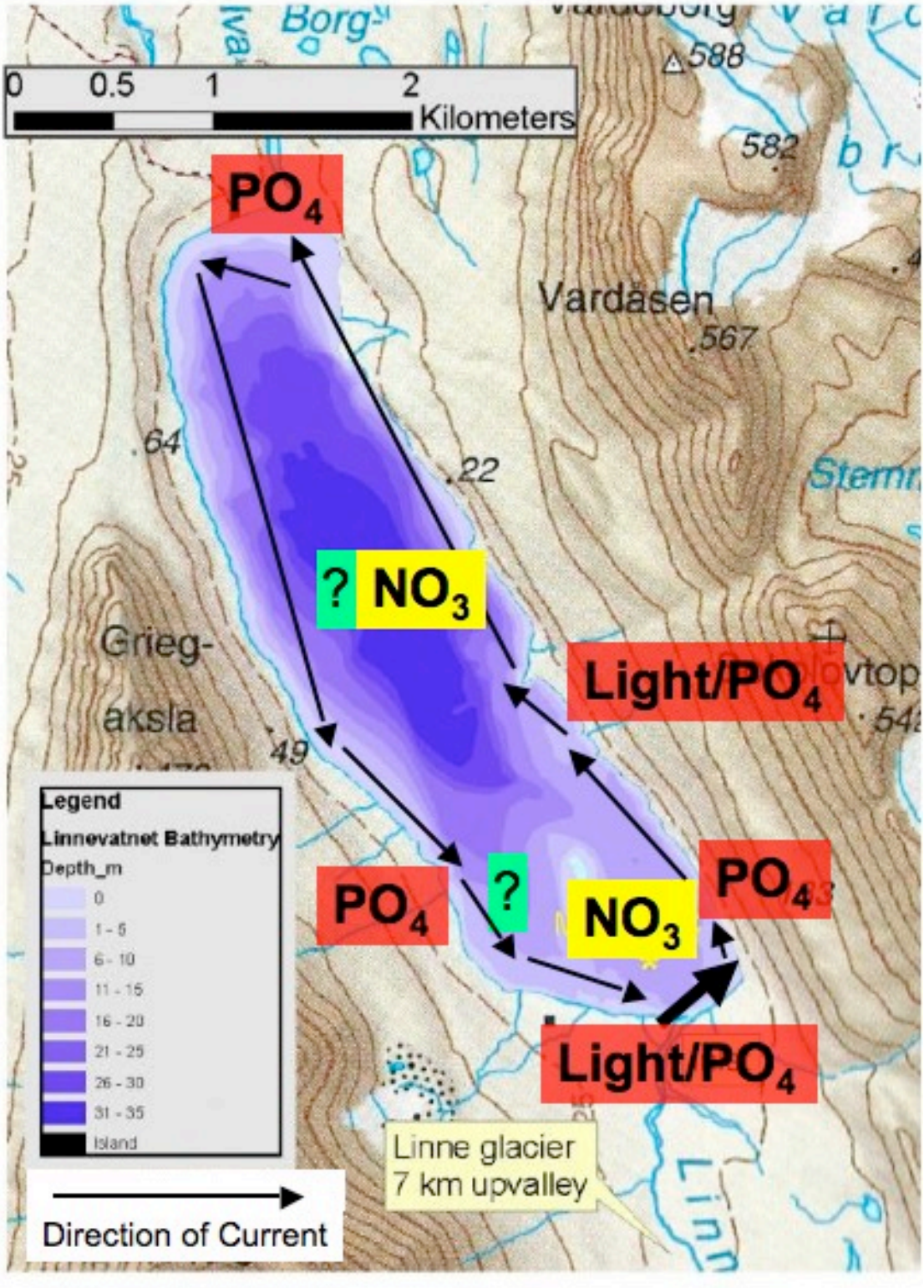

Figure 22. Schematic of the Nutrient Limitation in Lake Linne

5.2 N:P Supply Ratio's Influence on the Biological Assemblages of the Lakes

The overwhelming dominance of the diatom genus *Nitzschia* in both Linnévatnet and Kongressvatnet is not a coincidence. We believe that the dominance of this organism is a result of the elevated N:P ratios of the lakes. According to Elser et al, 2009, elevated N:P supply ratios often limit photosynthetic diversity due to the intense competition for phosphate. In this scenario, organisms that are incapable or bad at utilizing the scarce amounts of available phosphate in the water column will be out-competed by organisms better suited for phosphate limited environments. Notably, *Nitzschia* has been found to be particularly good at utilizing low concentrations of phosphate[21] which would explain their overall dominance in the two lakes.

Prior to this study, diatoms in Kongressvatnet had only been identified within sediment cores. The most recent analysis was performed on a core sampled in 1999 by Guilizzoni et al (*Supp*).[22] This study identified the genus *Cyclotella* as the most dominant diatom entering into the 21st century. On the other hand, the most dominant diatom genus, *Nitzschia,* was merely a minority within their sediment core. In comparing the diatom populations of Guilizoni et al and our study, it is apparent that there has been an ecological shift in diatom assemblages. In fact, during our analysis, not a single *Cyclotella* was observed. Previous studies have shown that members of the *Cyclotella* genus are very phosphate limited[2324] and, thus, their disappearance is most likely due to their inability to compete for the low levels of phosphate associated with elevated N:P supply ratios.

5.3 Complexity of Linnévatnet and Kongressvatnet Ecosystems

Figure 22 illustrates the complexity of nutrient limitation within Linnévatnet (and Kongressvatnet) that must be taken into account when describing the lake as a whole. Due to the varying geology and rapid cycling of water in and out of these two glacial lakes, it is important to acknowledge that we are observing is not just one, but several smaller ecosystems interacting together to form Linnévatnet and Kongressvatnet. Thus, in contrast to what Elser and Ptacnik[25] suggest, it is impossible to assess to the nutrient limitation of either glacial lake as one complete entity. Rather, one must consider all major inlet channels, outlets, and current circulation patterns to understand the N:P stoichiometry in its entirety.

Further fueling this argument is the fact that recent research on the two lakes reveals that Kongressvatnet may occasionally drain significant volumes of water into Linnévatnet.[26] If events like this do, in fact, occur, Linnévatnet's nutrient and water chemistry would be susceptible to rapid and drastic change. In particular, Kongressvatnet's iron and sulphate rich waters would introduce ample amounts of trace nutrients to Linnévatnet's diverse ecosystems.

5.4 Biology and Chemistry of Kongressvatnet's Chemocline

Due to the fact that Kongressvatnet exhibits a very distinct chemocline and oxicline, we know that there is a tremendous amount of chemistry occurring at depth within the lake. From ~37m to ~ 43m, Kongressvatnet is fueling one giant redox reaction that releases an abundance of nutrients at depth. This surge in newly available nutrients encourages the growth of a diverse array of specialized microorganisms as seen in *Figure 18a and Figure 19b.* Notably, the abundance of bacteria observed in *Figure 18a* in addition to the iron-sulfate precipitates of *Figure 18b* are the main contributing factors to the turbidity spike seen in *Figure 19c.*

One of the most interesting facts about Kongressvatnet's chemocline is the sulfur chemistry that occurs. Kongressvatnet is a high sulphate lake and, thus, its chemocline an ideal environment for sulfur oxidizing bacteria that utilize hydrogen sulfide abundant at the lake bottom *(Section 2.3)*. Notably, the gradual mixing of oxic to anoxic water provide creates electron gradients that the sulfur bacteria are eager to take advantage of. Typically, the oxidation of hydrogen sulfide will occur in a highly acidic environment; however, due to the nearly neutral pH levels across the chemocline, the sulfur oxidizing bacilli present in *Figure 18a* are most likely neutral sulfur oxidizers of the genus *Thiobacillus*[27] (*Figure 23*[28]). Furthermore, the iron-sulphate precipitates that occur within the chemocline are a result of ferrous ions being oxidized to ferric hydroxysulfates. This reaction is most probably due to *Thiobacillus ferrooxidans,* or a similar species, within the chemocline.[29]

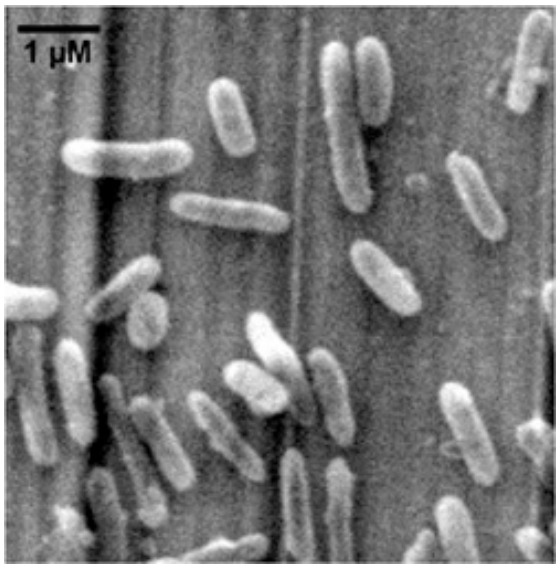

Figure 23. *Thiobacillus denitrificans* under SEM courtesy of Beller et al (2006)

5.5 Limiting Factors in Nitrogen Fixation Rates

Nitrogen fixation is a major driver of primary production by making nitrogen gas biologically available; however, nitrogen fixation can be limited by a variety of factors. In this study, Linnévatnet Inlet 4, Kongressvatnet Inlet, and Kongressvatnet Deep all

failed to exhibit nitrogenase activity. We believe the lack of nitrogen fixation in these regions is associated with various external limiting factors that prevented nitrogen fixation from occurring. In the case Linnévatnet Inlet 4, the heavy sediment plume entering into the lake most probably blocked a majority of the sunlight from reaching nitrogen-fixing cyanobacteria. Thus, as described by Karl (2001), nitrogen fixation was light limited.[30] Additionally, we believe light limitation prevented nitrogen fixation from occurring at depth, within the thermocline. On the other hand, nitrogen fixation in Kongressvatnet's inlet was most probably temperature limited much like the microbial growth in the mesocosm experiment. This hypothesis is based on the fact that if the nitrogen fixers are not expending energy to replicate, they will not expend the energy required to unnecessarily break the triple bond of N_2 to create available nitrogen.

5.6 Comments on Nitrogen Fixation Incubation Times

During nitrogen fixation experimentation, a wide variety of incubation times were used. Through analysis of Linnévatnet East sampled at 4 hours, Linnévatnet East sampled at 24 hours, Linnévatnet West sampled at 4 hours and Linnévatnet West sampled at 24 hours, Linnévatnet East and West sampled at 4 hours yielded much higher nitrogen fixation rates. Notably, longer incubation times were employed under the suspicion that nitrogenase activity would be slowed due to the cold temperatures of Svalbard. Ironically, these extended incubation times proved to be detrimental to our data set. Rather than providing the most accurate description of nitrogenase activity, the extended presence of acetylene in our samples actually inhibited the reoxidation of H_2 by hydrogenase.[31] Without the reoxidation of H_2, nitrogenase is no longer provided the reducing power to transform the acetylene to ethylene (*Supp*) and, thus, nitrogenase activity is halted. In order to prevent hydrogenase inhibition in the future, all acetylene incubation times in Linnévatnet and Kongressvatnet will be performed for less than 6 hours.

6 CONCLUSIONS

This study indicates that there has been an increase in anthropogenic nitrogen deposition into the Linné Valley over the past decade. The additional nitrogen load is precipitating into the mountains surrounding Linnévatnet and Kongressvatnet and, subsequently, flushing melt water enriched with nitrate into these lakes. This additional nitrate coming into Linnévatnet and Kongressvatnet through these inlet streams, is distorting the natural N:P stoichiometry of the region into a highly phosphate limited environment. As a result, the decrease in available phosphate has had a detrimental effect on diatom diversity, especially in Kongressvatnet. Notably, the waters of these two glacial lakes have shifted from *Cyclotella* dominated ecosystems to a heavily *Nitzschia* dominated one. The resulting study is the first to look at the nutrient dynamics and biogeochemical processes of arctic freshwater systems and will provide an insightful background for future climate research

7 FUTURE WORK

This study provides the first steps towards understanding the biology and nutrient cycling of the Linné Valley. As mentioned in the *Conclusion*, Linnévatnet and Kongressvatnet are displaying strong responses to the additional nitrate precipitation as a consequence of industrialization. However, the most recent analysis on anthropogenic nitrogen deposition into the Linné Valley July 25, 2005 and, subsequently, a more expansive survey on the seasonal deposition of anthropogenic nitrogen should be performed. In coordination with this study, measuring nitrate levels within Linnévatnet and Kongressvatnet before and after summer rainstorms would be beneficial in furthering our understanding of how N:P supply ratios are distorted by the inflow of nitrate rich melt waters and run-off during precipitation events.

Additionally, our observation of the sensitivity of these two lakes to anthropogenic nitrogen deposition warrants further research on other glacial and non-glacial lakes throughout the high arctic. Notably, a recent study by Holmgren et al., (2010), characterized diatom populations throughout the sediment cores of four non-glacial lakes. Therefore, it would be of value to investigate whether or not increased anthropogenic nitrogen deposition is creating an ecological shift within these waters as well.

Ultimately, this study opens the door for future biological and biogeochemical research in Linnévatnet and Kongressvatnet. In particular, nitrogen fixation rates in the high arctic still remain a bit of a mystery. Therefore, future nitrogen fixation experiments within Linnévatnet and Kongressvatnet should be performed with incubation times less than or equal to 6 hours as well as on lakes in the surrounding areas to provide a greater understanding of the nitrogen cycling in the region. Finally, Kongressvatnet provides an extraordinary ecosystem to understand sulfur cycling in high arctic regions and, thus, should be further explored.

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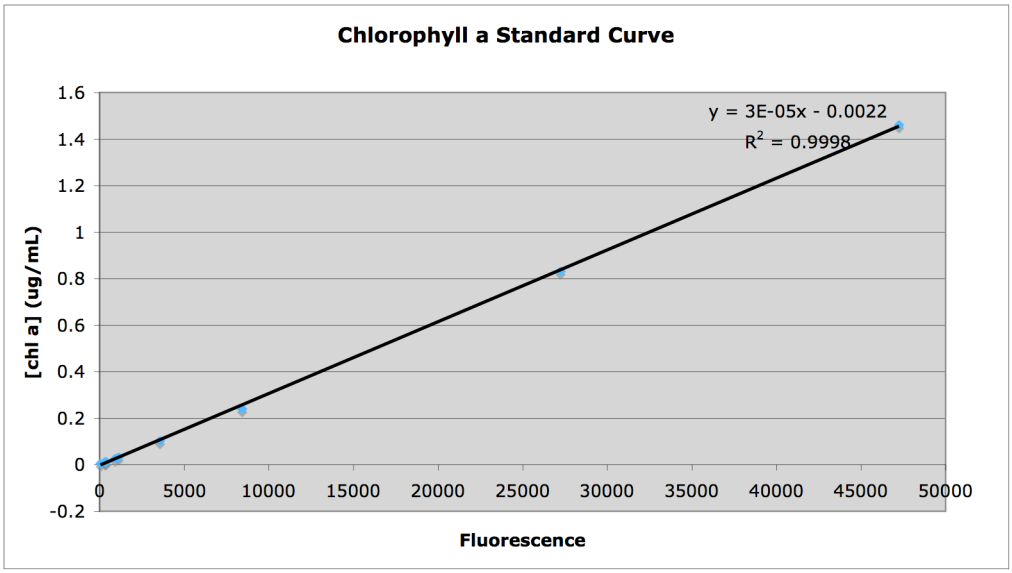
SUPPLEMENTARY FIGURES

1. Relationship of Nitrogen Fixation and Acetylene Reduction by Nitrogenase

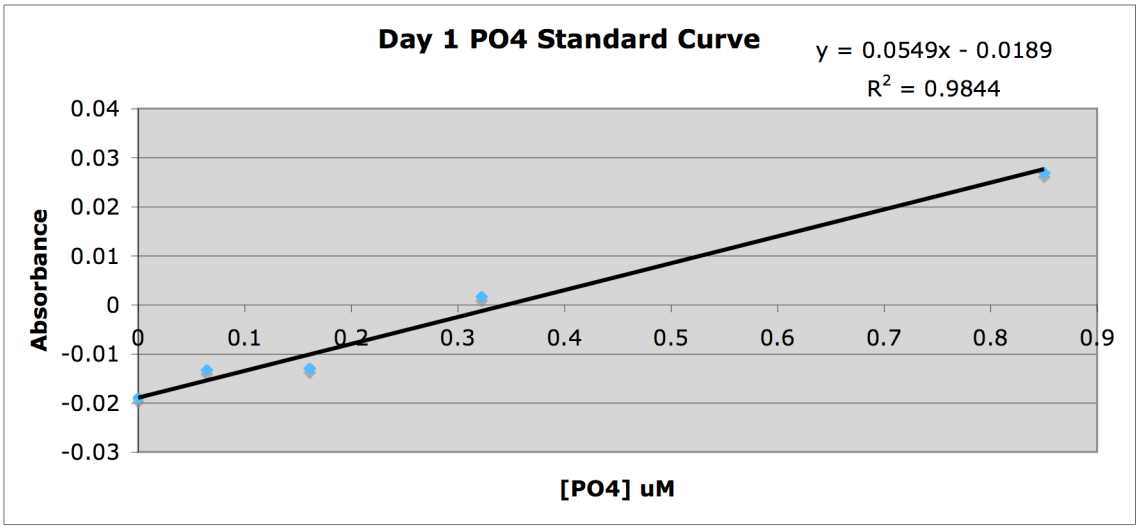
Nitrogen Fixation:
$N_2 + 8\ H^+ + 8e^- \rightarrow 2\ NH_3 + H_2$

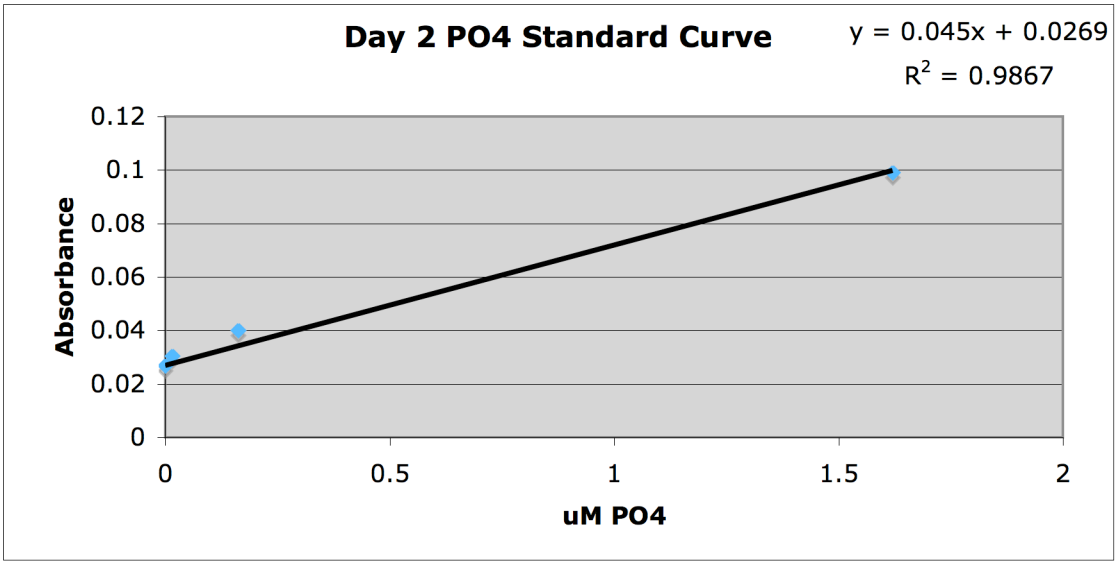
Acetylene Reduction:
$C_2H_2 + 2\ H^+ + 2e^- \rightarrow C_2H_4$

2. Chlorophyll *a* Standard Curve

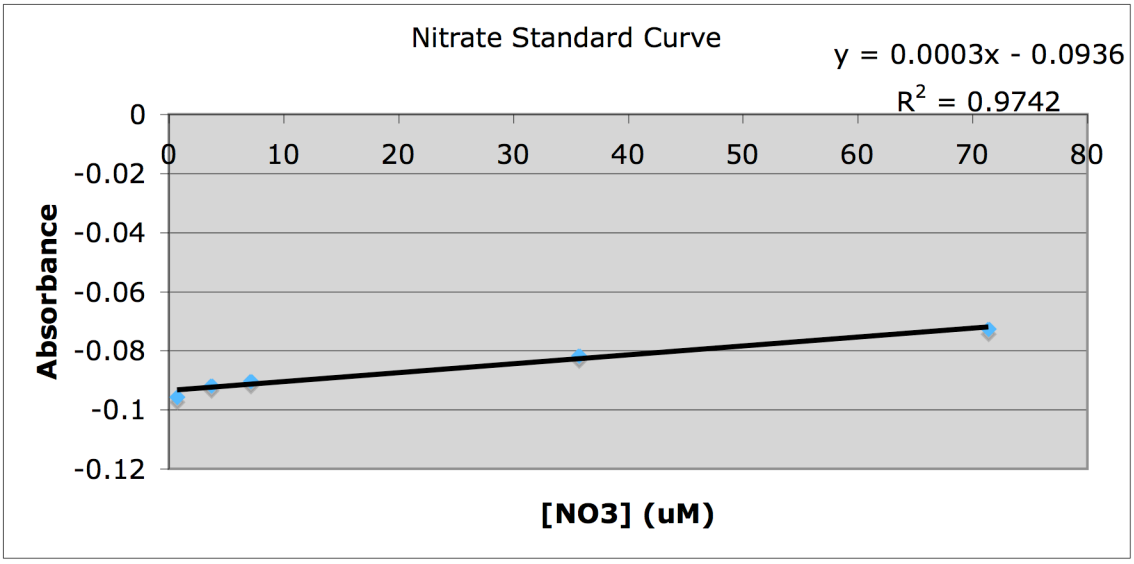


3. Phosphate Standard Curve





4. **Nitrate Standard Curve**



5. Major Fossil Diatom Taxa in Kongressvatnet over Time (Guilizzoni 2006)