



# How does nutrients and light affect algal growth in Swedish headwater streams?

A study using nutrient diffusing substrate and natural gradients of light

Jannika Andersson

**Jannika Andersson**

Degree Thesis in biology 15 ECTS

Bachelor's Level

Report passed:

Supervisor: Ryan Sponseller



# How does nutrients and light affect algal growth in Swedish headwater streams?

A study using nutrient diffusing substrate and natural gradients of light

Jannika Andersson

## Abstract

Gaining knowledge about what factors determine benthic algal biomass and productivity is vital for understanding food webs in aquatic systems, especially in woodland streams with naturally low rates of primary productivity. The aim of this study was to investigate what factors determine algal growth in Swedish headwater streams. Nutrients, in terms of nitrogen (N) and phosphorus (P), and light are factors known to affect algal growth. By using nutrient diffusing substrate (NDS) and different gradients of light, it was possible to test the importance of these factors. To determine the effect of the experiment, the study was carried out in a forested reference stream, which is largely shaded with extreme low nutrient levels, and in a stream running through a clear-cutting plantation with high nutrient levels and incident light. In the forested reference stream it became clear that algal growth increased by experimentally adding N ( $P < 0.005$ ), although light did not affect the productivity. In the stream running through the clear-cut, algal productivity increased significantly with higher levels of light ( $P < 0.005$ ), regardless of nutrient addition. The results from this study suggest that light only becomes the depending factor when sufficient amounts of nutrients are available. However, it is still unclear at what nutrient levels this shift occur, and therefore future research is recommended.

Key Words: boreal streams, algal productivity, N limitation, nutrient diffusing substrate, incident light



# Table of Contents

1 Introduction.....	1
1.1 Background.....	1
1.2 Factors that determine algal growth in streams .....	1
1.3 Anthropogenic effects of nutrient leakage .....	2
1.4 General aim .....	2
2 Methods .....	2
2.1 Study area.....	2
2.2 Sampling method .....	4
2.2.1 Nutrient diffusing substrate .....	4
2.2.2 Water chemistry and stream velocity .....	4
2.3 Statistical analyses.....	5
3 Results .....	5
3.1 Temperature, depth and light .....	5
3.2 Comparison amongst streams.....	6
3.2.1 Treatment response .....	6
3.2.2 One-way ANCOVA .....	7
3.3 Velocity.....	8
4 Discussion .....	9
4.1 “Benthic algae are more likely to be inhibited by lack of nitrogen than phosphorus” .....	10
4.2 “With higher levels of light the growth rate of algae will increase” .....	10
4.3 Velocity.....	11
4.4 Grazing.....	11
4.5 Wider implications .....	11
4.6 Conclusion .....	12
5 Acknowledgements .....	12
6 References .....	13

# 1 Introduction

## 1.1 Background

Periphyton is defined as a community consisting of microorganisms, such as algae, bacteria and fungi that grow on submerged surfaces of streams and rivers and can create extremely complex assemblages (Austin et al., 1981). Research on the controls over periphyton growth in streams and rivers has commonly addressed concerns about unwanted algal blooms and eutrophication (Dodds and Welch, 2000; Biggs 2000), which has become a problem due to a great increase in phosphorus and nitrogen inputs from anthropogenic sources into water bodies, mostly through the runoff of fertilizers and wastewater (Conley et al., 2009). However, gaining knowledge about what factors determine benthic algal biomass and productivity is also vital for understanding food webs in aquatic ecosystems. This understanding is likely to be particularly important in woodland streams with naturally low rates of primary productivity, because benthic algae serve as a high-quality resource base for microbial and metazoan heterotrophs, as well as fish. Thus, even small amounts of algal production have disproportionately large and widespread ecological effects (Mayer and Likens, 1987; McCutchan and Lewis, 2002). Variation in periphyton biomass has been shown to affect not only the aquatic food web, but also community structure, and biogeochemical cycling (Bernhardt and Likens, 2004; Hill et al., 2009; Sanderson et al., 2009)

## 1.2 Factors that determine algal growth in streams

There are several physical, chemical, and biological factors that are thought to determine the biomass and the growth rate of algae in streams. First, in order for algae to photosynthesize and grow, sunlight needs to be available (Bernhardt and Likens, 2004; Hill et al., 2009; Sanderson et al., 2009). In addition, algal productivity in streams may also be constrained by the amount of nutrients available to support growth, in terms of nitrogen (N) and phosphorus (P). Many studies in lakes have been able to relate variation in phytoplankton and periphyton biomass to light and nutrient availability (Jansson et al., 1996; Vadeboncoeur et al., 2003). However, it is often more difficult to make these connections in running waters, where light and nutrient availability can be dynamic in space and time, depending on the degree of shading and nutrient export through unidirectional water flow from the surrounding forest (Tank and Dodds., 2003). Although high levels of incident light can be present, algal productivity does not necessarily increase which indicates that in order for algae to reach its' optimum growth rate, both light and nutrient need to be available (Häder et al., 1998; Sanderson et al., 2009). Although it is well known that stream algae can be co-limited by light and nutrients, few studies have been made to investigate the small-scale effects of shading, or how variation in light and nutrient availability interact to govern variation algal growth.

Stream hydrological regimes are an additional potential source of variation in periphyton growth. First, scouring by seasonal flood disturbance is well known to influence the standing biomass of stream algae, for example changes in growth rates or patchy losses (Biggs 2000). As a result, algal biomass can indeed differ between individual substrata over time (Downes et al., 2000). An additional factor, which has been hypothesized for decades, but not tested as frequently, is stream velocity. Townsend et al. (2012) linked current velocity with the amount of algal biomass. According to this study, benthic algal biomass increases with increasing water velocity. With accumulating stream rapids, the thickness of a diffuse boundary layer around the algal cells reduces. When the layer becomes thinner, nutrient uptake becomes more accessible for the cells.

Since periphyton serve as a high-quality resource base for invertebrates in lotic systems, it can be the primary choice of food for certain taxa, even when the amount of primary producers is small. Macroinvertebrates, such as scrapers, can have a strong top-down effect by heavily reducing their food resource, which can lead to a decrease in biomass and growth of benthic algae (McNeely et al., 2007; Wallace and Webster., 1996). However, grazing invertebrates are

probably not a primary controlling factor for algae growth in many streams, given that high abundance of macroinvertebrates are often found in streams with high chlorophyll concentrations (Lewis and Mccutchan, 2010).

### **1.3 Anthropogenic effects of nutrient leakage**

Man has altered the landscape for centuries, by for example urban and agricultural land use and logging. In Sweden, forestry actions increased by the 20<sup>th</sup> century and went from small-scale to today's current large-scale plantations and clear-cuttings (Nylund 2009). Forestry is known to affect the hydrology of the ground water and streams (Laudon et al. 2009; Löfgren et al. 2009; Magnusson 2009). When vegetation is removed, the water balance changes, which leads to increased run-off and soil erosion (Sørensen et al. 2009; Löfgren et al. 2009). Enhanced run-off and erosion may increase nutrients leakage, such as carbon (C), nitrogen (N) and phosphorus (P), into surrounding aquatic habitats (Steedman and France, 2000) and because of these disturbances the primary production as well as decomposition processes will be affected. Also, removing of vegetation can lead to an increase in water temperature, a decrease in shading over water bodies, and increase in periphyton growth (Holapainen and Huttunen., 1992). By taking advantage of the effects of clear cutting on nutrient leakage and incident light, it is possible to make algae experiments in a high nutrient environment.

### **1.4 General aim**

The overall aim of this project is to understand the factors which control algal growth boreal headwater streams of northern Sweden. A previous survey of algal biomass accrual on tiles in this region suggested that inorganic nitrogen, light, and stream depth explain most of the variation in periphyton growth among 45 locations (Sponseller, unpublished). Based on these results, two streams were selected with large differences in nutrient-input and light but with approximately the same depth to test these following hypotheses, which are thought to determine biomass and growth of benthic algae in boreal headwater streams.

1. Benthic algae are more likely to be inhibited by lack of nitrogen than phosphorus
2. With higher levels of light the growth rate of algae will increase

The first hypothesis can be tested by experimentally adding N and P to the streams. If N is the primary limiting nutrient, the growth rate of algae should only increase by N addition. The second hypothesis can be tested by deploying biofilm samplers along different gradients of light. If light is the most important factor, the growth rate should increase with higher incident light, regardless of nutrient addition.

## **2 Methods**

### **2.1 Study area**

The experiments were carried out in two streams within the Balsjö paired catchment study (277 Balsjö, N 64° 1' 37" E 18° 55' 43") , in the central part of Swedish boreal forest (figure 1) during July 2014. Balsjö is located in Västerbotten County, which is a major forest landscape, with a great majority of lakes, water courses, mires and wetlands (Länsstyrelsen, 2008). The vegetation is characteristic of boreal regions in Scandinavia, including Norway spruce (*Picea abies*) in the lower and middle locations and Scots pine (*Pinus sylvestris*) at higher elevations. Around the wetlands and streams within these catchments, are Birch (*Betula* ssp.) and various bryophytes abundant (Schelker et al., 2014). The surrounding catchment of one of the streams (CC-4) was clear cut in 2006, leaving no riparian buffer zone and is thus a high light and high nutrient environment, with concentrations of inorganic nitrogen (N) approaching 1000 µg/L in the years after clear felling (Löfgren et al., 2009). Although now the nutrient-levels appear to be decreasing year by year since the clear cutting occurred (table 1). The other stream (RF-3) is a forest reference site that is largely shaded and extremely nutrient-poor (Table 1). Before the clear cutting took place, the average concentration for each chemical-physical compound

studied were relatively similar between the two streams, both exhibited low concentrations of inorganic N although the reference stream had a higher P concentration than the clear-cutting stream. Field surveys from these sites in the summer of 2013 showed that average riparian canopy cover was 88 and 14 % at RF-3 and CC-4, respectively. Not surprisingly, estimates of incident light on benthic surfaces during this same summer period were 1951 ( $\pm 288$  SE,  $n=3$ ) and 6830 ( $\pm 3009$  SE) lux at RF-3 and CC-4, respectively (Ryan Sponseller, 2014, pers. comm, July).

Table 1. Nutrient concentrations ( $\mu\text{g/l}$ ) and pH from summer 2013 in the studied streams. Source: Ryan Sponseller, 2013

Site name	Soluble reactive phosphorus (SRP)	Ammonium ( $\text{NH}_4\text{-N}$ )	Nitrate ( $\text{NO}_3\text{-N}$ )	Dissolved inorganic nitrogen (DIN)	pH
Forest reference (RF-3)	4.165	3.41	6.99	10.4	5.2
Clear cut (CC-4)	10.715	10.325	95.86	106.185	5.74

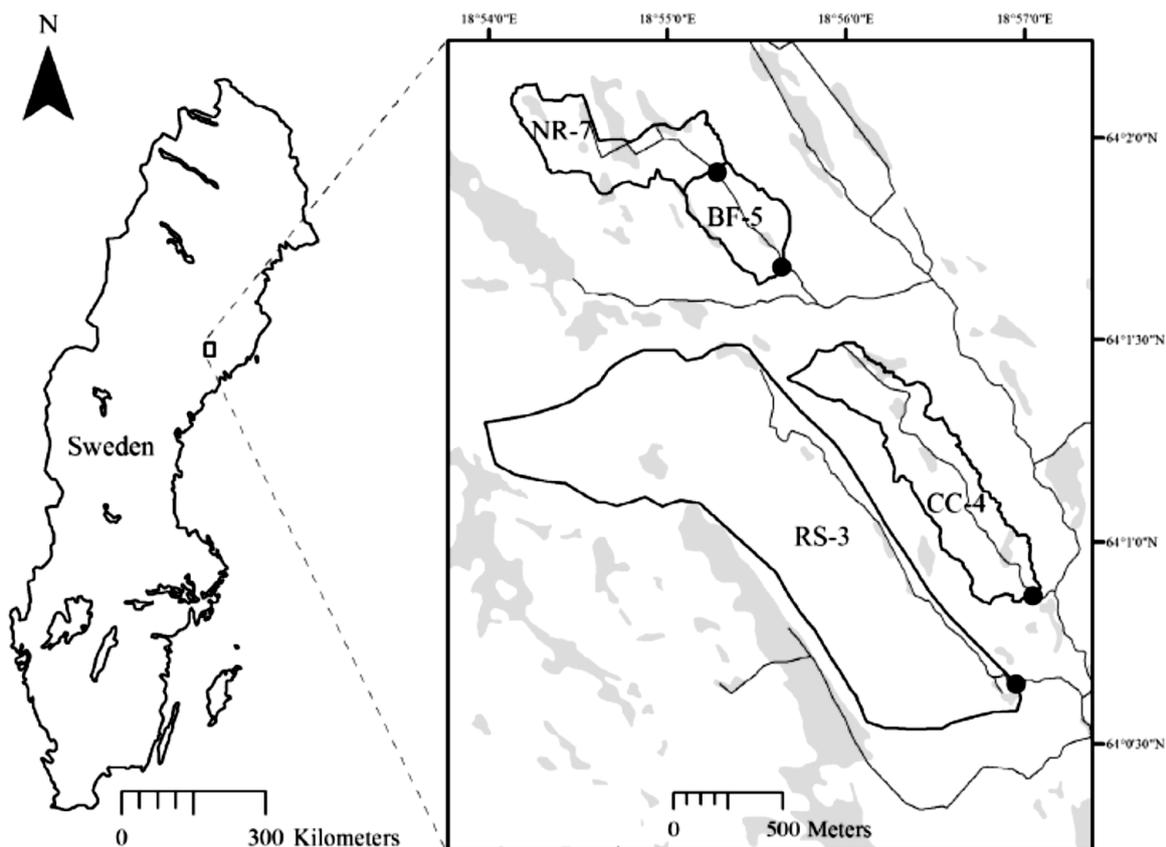


Figure 1. Map of the catchment area where the experiments were conducted. CC-4, which was clear cut all the way down to the stream, is the treated catchment while RF-3, displayed as RS-3 on the map, is used as a forested reference site. The thin black lines mark the streams, and the thick black lines mark the catchment boundaries. Source: Schelker et al., 2014.

## 2.2 Sampling method

### 2.2.1 Nutrient diffusing substrate

By using nutrient diffusing substrata (NDS), a mean measure can be provided to predict if heterotrophic algae are nutrient-limited, or not (Tank et al., 2007). The NDS treatments were constructed using 30 mL plastic cups filled with agar solution of 0.5 M NaNO<sub>3</sub> (N treatment), 0.5 M KH<sub>2</sub>PO<sub>4</sub> (P treatment), N and P together (NP treatment), or an unmodified control agar solution (C), making it a total of 240 cups (60 for each nutrient treatment). The cups were fitted on the top with a porous ceramic cover, enabling the nutrients to diffuse through the surface during incubation in the streams.

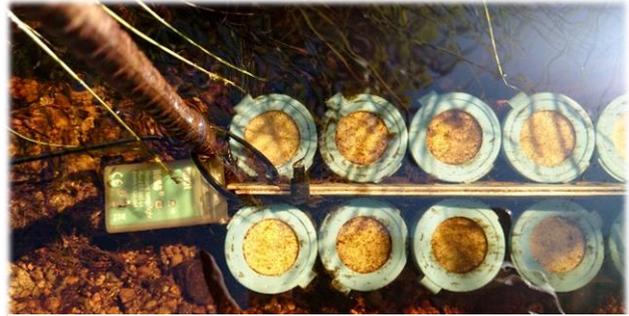


Figure 2. Picture of one rack deployed in the CC-4 stream

The cups were randomly placed onto L-shaped metal racks, containing 5 replicates of each of the 4 treatments, and secured with cable ties. The metal racks were then covered in plastic wrap and stored in a refrigerator until the next day, when 6 racks were put out in each stream parallel to the flow, and placed in different levels of light by using natural shading, at an average depth of 116 mm. In order to determine the relative amount of light present to the NDS-cups, two light and temperature loggers (HOBO, UA-002-64), were attached to each rack (figure 2)

During the incubation time, it became necessary to repeatedly clean the racks located in CC-4 due to an accumulation of unwanted material which could possibly inhibit growth and create shading. By sweeping away the water above the cups, and not touching the ceramic plates, unattached material could be removed. In addition, potential grazing insects were observed, counted, and removed from the cups.

Algal growth was measured in RS-3 and CC-4 after 21 and 22 days of incubation, respectively. The amount of chlorophyll *a* was established directly at the sites by using a field fluorometer (bbe BenthosTorch). The BenthosTorch is a fairly new measurement instrument, which is easy to use and effectively quantifies algal biomass (chlorophyll *a*) in-situ. Kahlert and McKie (2013) tested how trustworthy the results from the BenthosTorch were, in terms of the total amount of chlorophyll *a* and assessments of taxonomic composition in comparison to conventional microscope-based methods. When it comes to quantifying total chlorophyll *a*, there were no measurement differences between the BenthosTorch and the conventional method. However, this instrument did not always generate accurate characterizations of the different algal groups. Therefore, only the total amount of chlorophyll was analyzed in this study. Kahlert and McKie (2013) also discuss the importance of measurements, concluding that at least 15 measurements are necessary in order to get a good representative value, since algal biomass is seldom evenly distributed on the surfaces. In this study, 3 measurements were performed on different parts of each cup, making it a total of 15 of measurements per treatment and rack.

### 2.2.2 Water chemistry and stream velocity

Water samples to determine pH, dissolved organic carbon (DOC), nitrate (NO<sub>3</sub>), ammonium (NH<sub>4</sub>), and phosphate (PO<sub>4</sub>) were collected at the bottom, middle, and top of both stream reaches on three different occasions with approximately seven days interval. Samples were filtered in the field (0.45 µm; Sarstedt, Nümbrecht, Germany), and water for nutrients was frozen prior to analysis. DOC was analysed on a Shimadzu TOC<sub>VCPH</sub>; NO<sub>3</sub>, NH<sub>4</sub>, and PO<sub>4</sub> were determined using standard colorimetric techniques on a Seal Autoanalyzer (AA3; Seal Analytical). Stream velocity (m/s) was estimated from 5-10 locations around each NDS-rack during the same occasions using a velocity meter (Valeport Model 801 Open Channel Flow Meter. Valeport Ltd, St. Peter's Quay, Totnes, Devon, TQ9 5EW United Kingdom).

### 2.3 Statistical analyses

The total average chlorophyll a from both streams were not normally distributed, and was square root transformed in Excel 2013 prior to statistical analyses. The differences in algal biomass for each stream were then tested by using a one-way ANCOVA with average median light as co-variate. Median light was chosen in order to exclude extreme values that probably did not affect algae growth under this short period of time. Where the ANCOVA suggested significant treatment effects, a Tukey Contrast test was used to identify which treatments were significantly different from each other when combining effects of treatment with light.

For testing if current-speed had an effect on biomass response ratio (BRR) within the streams, a linear regression analysis was performed. The BRR was calculated by dividing the average chlorophyll of the nutrient-treated cups by the average of the control (C) cups and tested against the average velocity for each rack.

## 3 Results

Unfortunately, the water chemistry samples for pH, DOC, NO<sub>3</sub>, NH<sub>4</sub> and PO<sub>4</sub> were not analyzed in time, and therefore no results from this year can be provided in this report. The grazers, identified as Trichoptera larvae, that were noted and removed from the cups at CC-4 were evenly distributed amongst the different treatments and racks, with the C cups having the most (16) and the NP treatments having the fewest (10), and no further analyses were done. No grazing insects were observed on cups at RF-3.

### 3.1 Temperature, depth and light

The average mean temperature in RF-3 and CC-4 was 12.87° C ( $\pm 0,039$  SE) and 10.36 ° C ( $\pm 0,021$  SE), with mean range of 5.05 ° C and 3.06 ° C, respectively, meaning that the temperature was higher in the stream located in the forest, but also with higher fluctuations. The water depth was similar between the two streams. When deploying the racks the depth was 116 mm, compared to 97 mm when retracting them. Both the highest and lowest levels of light available to the racks were found in CC-4, with average median light ranging from 492,45-3186,15 lux ( $\pm 380$  SE), while the average median light in RF-3 ranged from 936,45-2303,46 ( $\pm 295$  SE) lux (figure 3).

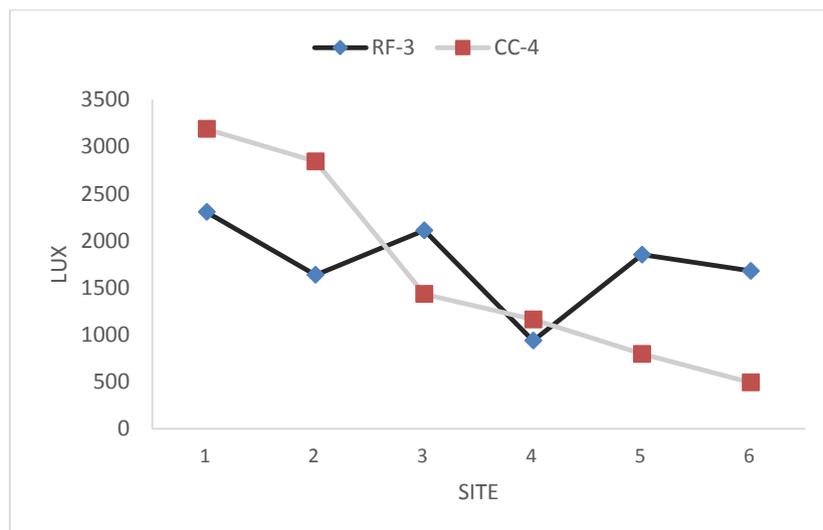


Figure 3. Average median light amongst the six rack in both streams.

## 3.2 Comparison amongst streams

### 3.2.1 Treatment response

The distribution of algae growth at RF-3 varied amongst the different treatments, with the highest average values of chlorophyll a on the N and NP-treated cups, and the lowest values on the C and P-treated cups (figure 4a). The value of chlorophyll a on the N and NP treated cups ranged from 0,42-2,20 and 0,14-2,38  $\mu\text{g cm}^{-2}$ , respectively, whereas the value of chlorophyll a on the C and P treated cups ranged from 0,17-0,75 and 0,09-0,70  $\mu\text{g cm}^{-2}$ , respectively.

In contrast to RF3, there was no major differences amongst the average chlorophyll between the different treatments at CC-4. The highest values were found on the N and C treated cups 2,15 and 1,95  $\mu\text{g cm}^{-2}$ , respectively. However the NP and P average were not far behind with 1,63 and 1,24  $\mu\text{g cm}^{-2}$ , respectively (figure 4b). Overall, the amount of chlorophyll a was higher in CC-4, with the highest value of 5,8  $\mu\text{g cm}^{-2}$  found on a C cup.

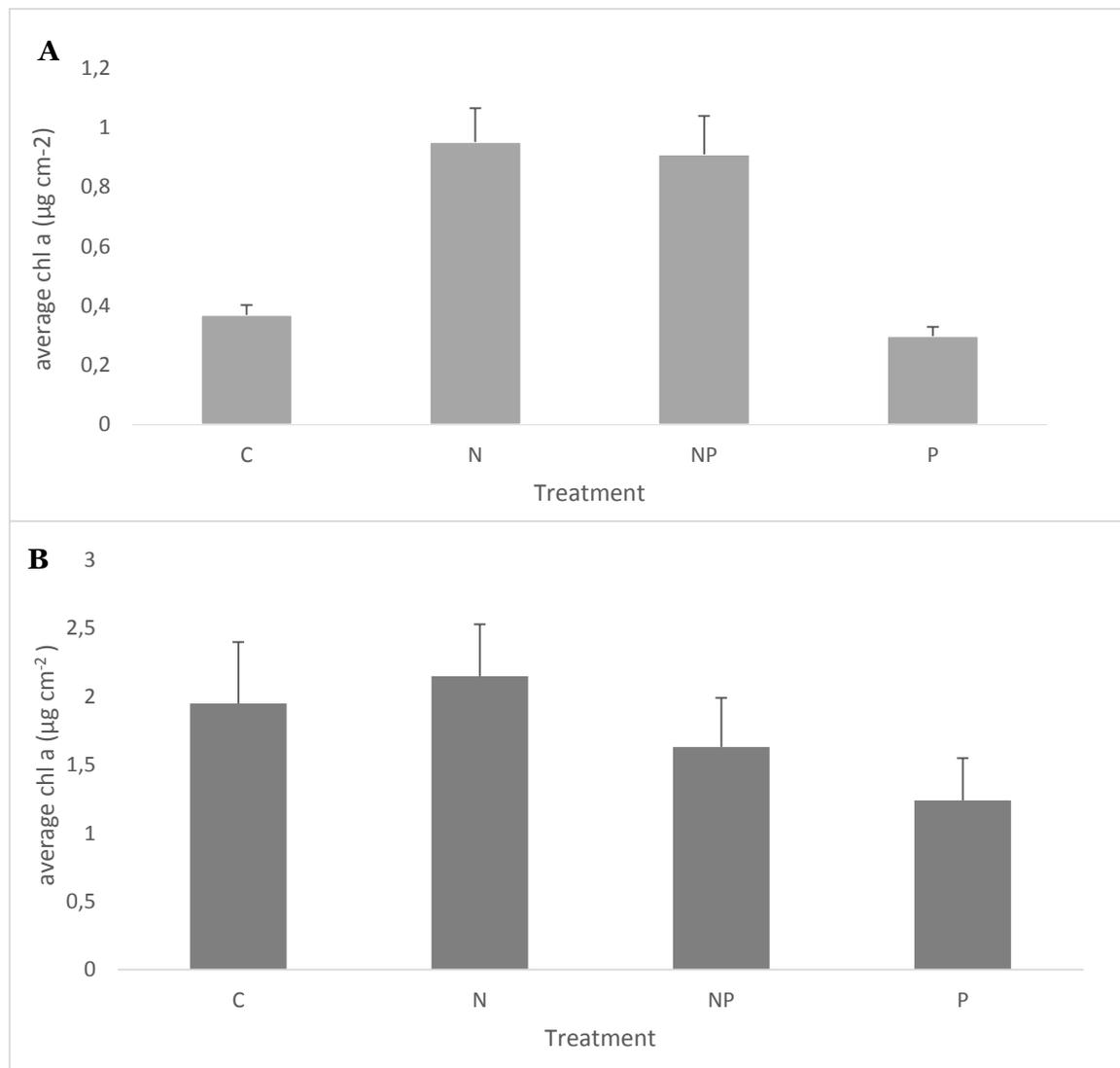


Figure 4. Average chlorophyll a ( $\pm$ SE) amongst the different nutrient treatments. **(A)** RF-3, with significant treatment response from N and NP addition **(B)** CC-4, with a minor significant N-response.

When comparing the distribution of algae found on the racks placed in the lowest level of light to the rack placed in the highest levels of light in RF-3, the concentration of chlorophyll a differed on the N and NP treated cups, from 0,63 and 0,55  $\mu\text{g cm}^{-2}$ , respectively to 1,13 and 1,15  $\mu\text{g cm}^{-2}$ , respectively (figure 5a), meaning that the biomass response to N addition was greater in the highest level of light. When looking at the distribution of chlorophyll a in the lowest level

of light compared to the highest level of light in CC-4, the algal biomass is greater on the rack placed with high incident light. However, there is no real difference amongst the different treatments (figure 5b)

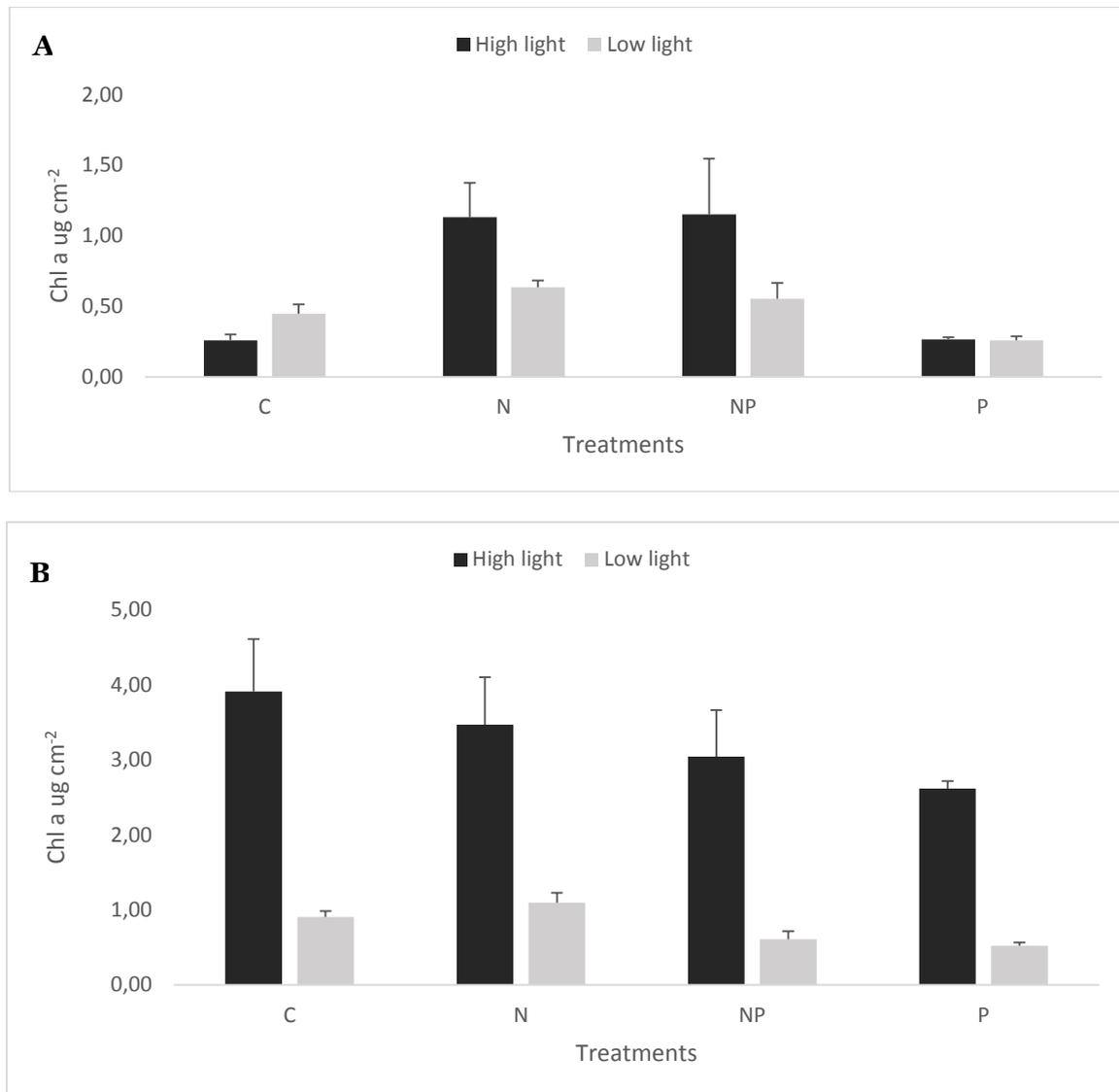


Figure 5. Average chlorophyll in the lowest and highest levels of light. (A) RF-3. (B) CC-4)

### 3.2.2 One-way ANCOVA

Figure 6a illustrates how the algae responded to the different treatments as a function of incident light in RF-3. The algae accrual rate responded best on the N and NP treated cups, whereas there was no light response on the P and C cups. The results from one-way ANCOVA ( $p < 0,05$ ) revealed a significant positive treatment effect, and also a significant light effect ( $p = 0.033$ ). When identifying which treatments were significantly different from each other, by using the Tukey Contrast test, it showed that both N and NP were significantly different to C and P ( $p < 0.001$ ). There was no significant difference between C and P, nor between N and NP. In contrast to the results from the RF-stream, the Tukey Contrast test did not show any significant difference between N and C, nor between NP and C at CC-4. The Tukey contrast did, however, show a significant difference between N and P treated cups ( $p < 0.04$ ), which potentially suggests P-inhibition. However, the one-way ANCOVA revealed a greater

significant effect in terms of light compared to the RF-3 stream ( $p < 0.001$ ) (6b).

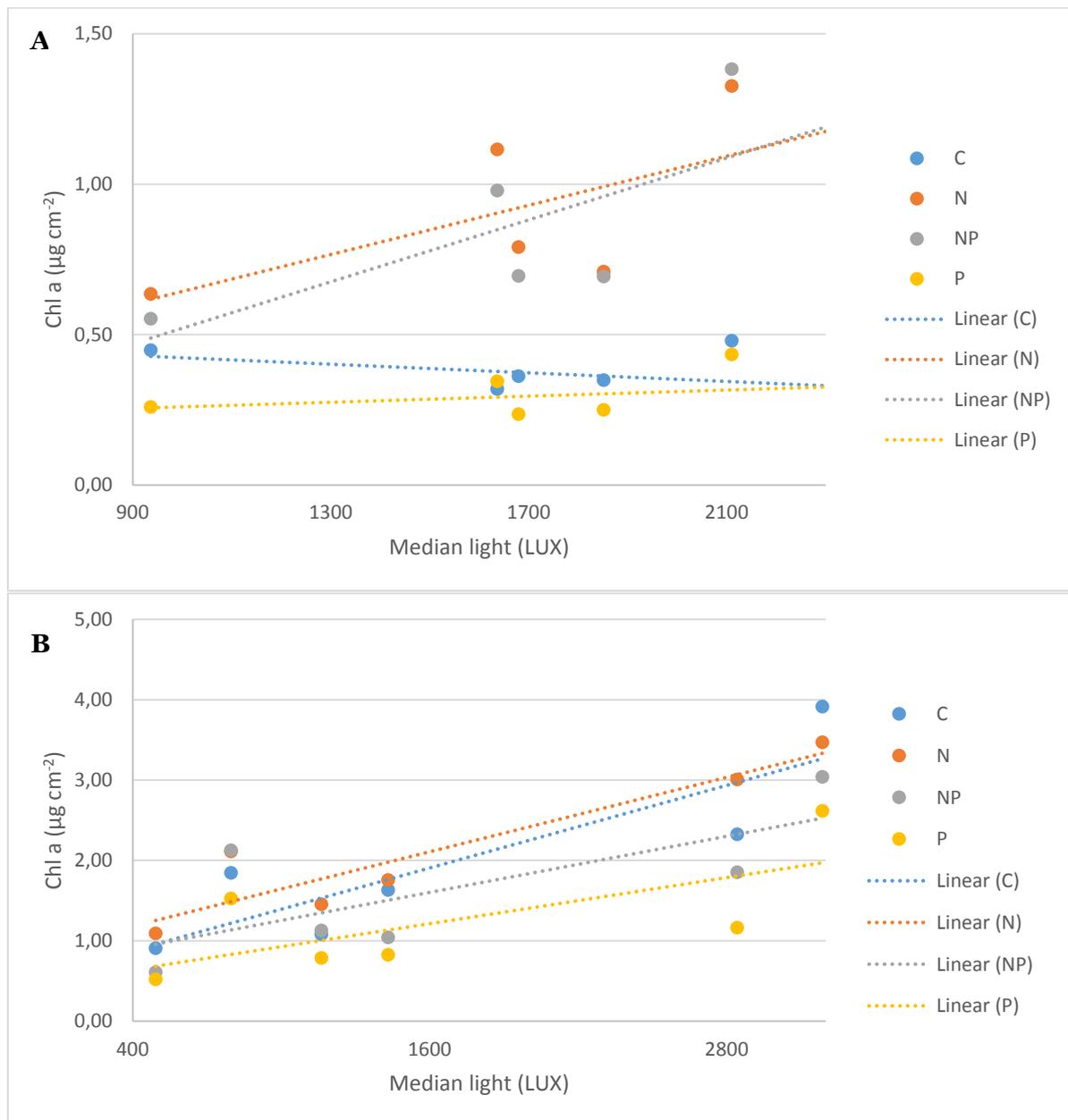


Figure 6. Average chlorophyll a amongst the different treatments compared to light input. **(A)** RF-3 **(B)** CC-4

### 3.3 Velocity

The velocity was higher in the RF-3 stream ranging from 16,28-32,19 cm/s compared to the CC-4 stream where the velocity ranged from 4,57-10,56 cm/s. When testing the relationship between biomass response ratio (BRR) and velocity by using a linear regression analysis, no significant response was found in the RF-3 stream (figure 7a)  $p = 0,709$ , nor in the CC-4 stream (figure 7b),  $p = 0,47$ .

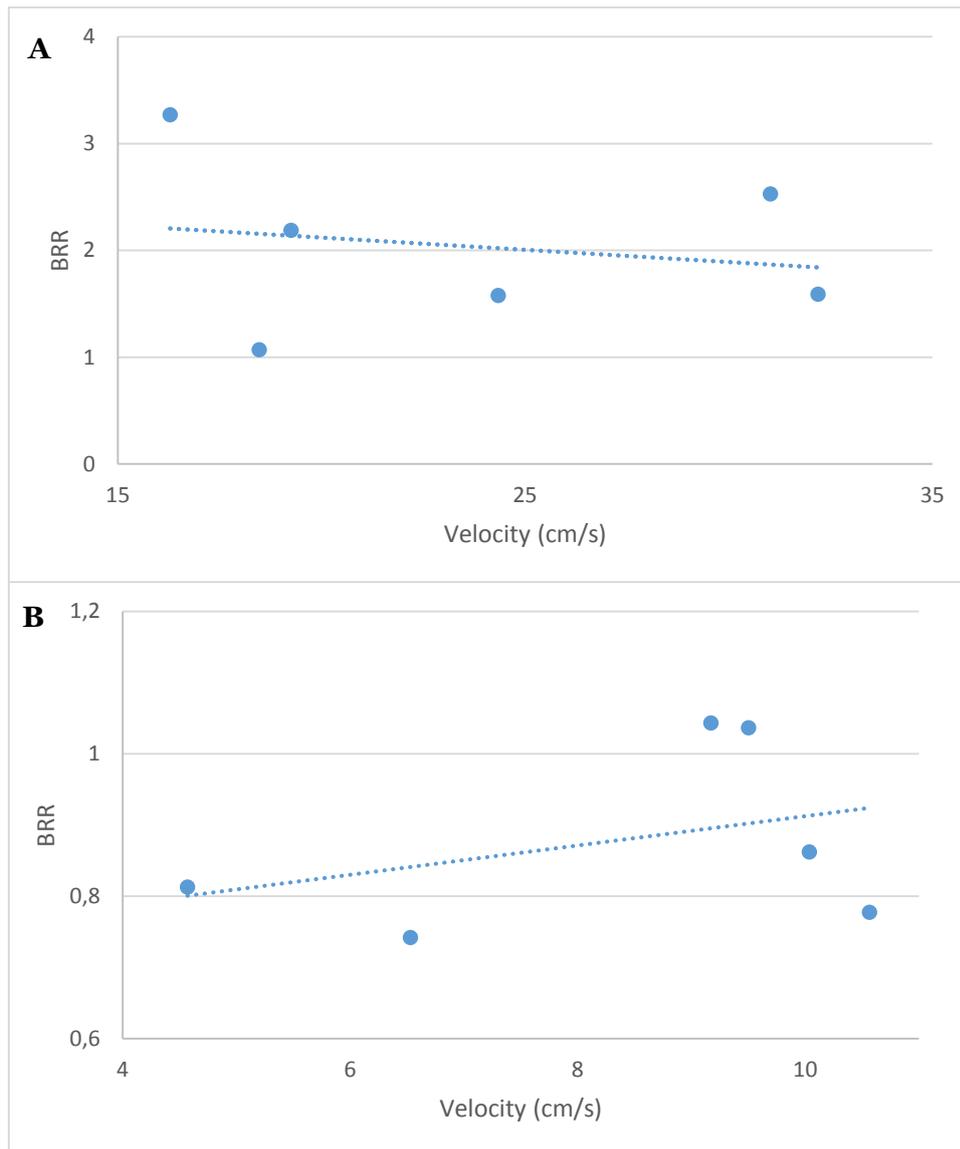


Figure 7 shows the biomass response ratio (BRR) of algae compared to the velocity. **(A)** is the BRR compared to the velocity in the RF-stream,  $BRR = 2,581 (\pm 1,4 SE) * Velocity (cm/s)$ . **(B)** is the BRR compared to the velocity in the CC-4 stream,  $BRR = 0,707 (\pm 0,26 SE) * Velocity (cm/s)$ . Neither relationships is statistically significant.

## 4 Discussion

This experiment was set out in two streams, one largely shaded and extremely nutrient-poor and one in a high light and high nutrient environment. By selecting streams with large differences in nutrient-input and light but approximately with the same depth it was possible to test hypotheses about how light and nutrients interact to influence algal growth in boreal streams. Overall, experimental results highlight the role of both incident light and inorganic N availability, with the relative importance of these abiotic factors differing between the study sites. However, since the study was carried out in only two streams the results cannot necessarily be interpreted as general for all boreal head water streams. That said, these results do provide experimental evidence to support the broader descriptive survey of algal accumulation across many streams in which inorganic N and incident light were identified as important predictors.

The hypothesis were:

- H<sub>1</sub>, “Benthic algae are more likely to be inhibited by lack of nitrogen than phosphorus”.
- H<sub>2</sub>, “With higher levels of light the growth rate of algae will increase”.

In the section below, I will discuss these hypotheses and evaluate these results in the broader context of boreal streams of northern Sweden.

#### **4.1 “Benthic algae are more likely to be inhibited by lack of nitrogen than phosphorus”**

In the RF-3 stream, which is extremely nutrient poor with concentrations of SRP and NO<sub>3</sub>-N as low as 4,165 µg/l and 6,99 µg/l, respectively, there was a significantly greater algal growth in response to the addition of N and NP. In contrast, there was no treatment effect in response to P alone, which had the lowest values of chlorophyll a. Overall, this indicates that the algae in this stream suffer solely from strong N-limitation. This result supports the hypothesis, and the results from the previous descriptive study of algal growth in the region.

In the CC-4 stream, which is a high light and high nutrient environment due to the clear-cutting in 2006, with concentrations of SRP and NO<sub>3</sub>-N of 10,715 µg/l and 95,86 µg/l, there was no significant positive treatment effect in terms of N and NP additions. However, there was a minor significant treatment effect when comparing P with N, which seems to have resulted from a slight P-inhibition. Observations by (Schanz and Juon 1983; Lohman et al., 1991) suggest that N limitation is assumed when the molar ratio of N to P (N:P) is <10, whereas P limitation is assumed when N:P is >20. Those observations strengthen this study's result, because the streams have chemical conditions that are comparable to those observations. Bergström et al (2005) discuss that the use of P for buildup of algal biomass will increase with increasing N deposition. It is possible that a similar effect - but from forestry rather than deposition - is reflected in this study, since the slight P-inhibition is detectable in a high N environment. Bergström et al (2008) also suggest that N deposition shift phytoplankton from being N limited to becoming P limited. The results from this study suggest that forestry may also reduce N-limitation, however in contrast to atmospheric deposition where P deposition is usually very low, forestry can also elevate the levels of P entering the water bodies.

This study of algal nutrient limitation is the first one ever made in the center of Swedish boreal forest, which are generally considered to be N-limited with a closed loop leading to available N being efficiently taken up by roots and soil organisms. The leaching of N to nearby lakes and streams is thus considered to be minimal, and Bergström et al (2008) established that phytoplankton in lakes situated in the north are N-limited. The results from this study further indicates that inorganic N is a critical resource in aquatic ecosystems of northern Sweden.

#### **4.2 “With higher levels of light the growth rate of algae will increase”**

Consequently, the second hypothesis was not supported by the results from the RF-3 stream. Because, higher light did not affect the algal growth without the experimentally added nitrogen. In RF-3, light had a significant effect on the magnitude of algal response to N and NP addition ( $p=0,033$ ), with an increase of 78 % and 108% of chlorophyll a, respectively, from the lowest input of light to the highest input. When comparing algal growth on the P treated cups and C cups from lowest to highest light input, there was only a 2 % increase and a 43 % decrease of chlorophyll a, respectively, meaning light had no significant effect of algal growth for the control and P cups. The results suggest that there was a significant effect of N-addition in the nutrient poor stream, however the algal accrual rate increased significantly in high light input when nutrients were also experimentally supplied. Considering this, algae growing in natural substrates should therefore not be affected by variation of light, since there is not enough nutrient to support algal growth.

In contrast to RF-3, the second hypothesis was supported by patterns at CC-4. The major factor in the CC-4 stream was indeed the variation in light with a strong significant response in algal growth among NDS racks, regardless of experimental nutrient addition ( $p<0.001$ ). The highest values of average chlorophyll a were found on the C cups (1,09 µg cm<sup>-2</sup>) with the highest light input, but when comparing all the cups with the lowest light input to the cups with the highest

light input the amount of chlorophyll *a* increased dramatically, C (332%), N (216%), NP (401%), P (403%). The added nitrogen did not increase algal growth, which indicates that there is no N-inhibition in the CC-4 stream and the biggest differences in algal accrual rate are dependent on light. These results suggest that, variation in light becomes the primary determining factor of boreal stream algal growth when sufficient amount of nutrients are available. Future research is recommended to investigate when the controlling productivity factor shifts from nutrient to light.

### **4.3 Velocity**

According to Townsend et al. (2012), benthic algal biomass will increase with increasing water velocity and they manage to make a connection between biomass response ratio (BRR) and stream velocity at speed of 27-98 cm/s. The explanation of why algal biomass will increase with greater water velocity is that the thickness of a boundary layer around the algal cells reduces, and nutrient uptake becomes more accessible for the cells. Although, no connection between BRR and velocity was established in RF-3 or CC-4. The highest velocity was in RF-3, ranging from 16-32 cm/s and it is possible that the velocity was too low for the boundary layer to reduce. It is also possible that the high light response conceals the effect of velocity, considering that the highest velocity did not correspond to the location with the highest light.

### **4.4 Grazing**

In this study, caddisfly larvae (Order:Trichoptera) were only found on the cups located in the CC-4 stream, which is not surprising since high abundance of macro invertebrates are often found in streams with high chlorophyll concentrations (Lewis and McCutchan., 2010). It is hard to say what role they have played in this study, but it is possible that the grazing invertebrates have affected the results of algal biomass accrual. The caddisflies that were noted did not seem to favor any particular nutrient treatment, since the abundance was equally divided among the different treatments. However since the caddis flies were only noted and removed, it is uncertain if the larvae grazed upon the cups, and if so on which cups. It is also possible that the larvae did not affect the results at all considering that when abiotic constraints, such as sunlight, become available to the algae, the rate of productivity can outpace the pressure of consumption (Hill et al., 2009).

### **4.5 Wider implications**

Gaining knowledge about what factors determine benthic algal biomass and productivity is likely to be particularly important in woodland streams with naturally low rates of primary productivity. As primary producers, alga productivity can have a bottom-up effect in the aquatic food webs, the abundance of grazers may increase with productivity and there can be a positive response in the food chain, meaning that any changes in algal productivity can have wide spread ecological effects (Mayer and Likens, 1987). Understanding what controls algal growth in running waters has proven to be much harder than in lakes or ponds, because changes in resources can be much more dynamic in space and time. It can also be difficult to detect where the main water input to streams comes from, and how the different inputs affect, for example, the water chemistry, turbidity and temperature. For example, during this three-week experiment, the temperature was unusually high for northern Sweden, with air temperatures around 30 ° C. Even though the temperature was high and the unshaded stream running through the clear-cut area was only 100-150 mm deep, the water temperature did not increase during the experiment period, which indicates that the stream gets water from a ground water source.

In this study the hypotheses were largely confirmed through small-scale experiments conducted at only two streams. The reason why this method and design was chosen had to do with the short time frame of the study and the need to ensure that the water chemistry within each stream would be the same, and thus the effects of light could be tested while controlling for background nutrient availability. However, since the study was carried out at just two streams, the results cannot be interpreted as general for all boreal streams. This study was

within the Västerbotten County, a vast forest landscape with countless water sources, making it difficult to determine how changes in the landscape effect the forest streams. However, probably there are many streams that have the similar light and nutrient inputs as the RF-3 stream. Thus, the importance of N availability and limitation is likely to be common in the region. The fact that a previous survey in nearby streams indicated a correlation between inorganic N concentration and algal biomass also supports this hypothesis and the generality of the experimental results described here.

In addition, clear-cut plantations are also common in Västerbotten County, which can affect the stream conditions. However, according to Swedish forestry acts a riparian buffer should always be left, to avoid any damages (Skogsstyrelsen, 2014) and therefore it is possible that the high levels of both nutrient and light input are not typical for streams going through clear-cutting areas. Nevertheless, the results from this study suggest that anthropogenic land use can affect the food web composition in nearby streams. Streams in the northern parts of Sweden are often limited by N, which the results from RF-3 strengthens. Sørensen et al (2009) showed that the average runoff was 35 % higher in the CC-4 stream compared with a reference stream, which may influence macro invertebrates. Even though a riparian zone is remaining, nutrient run-off will effect streams, however with reducing effects (Holopainen and Huttunen., 1992). Also with increasing incident light, which are often common when clear-cutting, both periphyton and grazers can increase (Hill et al., 2005), however, the composition of periphyton changes. Holopainen and Huttunen (2005) saw an increase of the most common periphytic species, however the more unusual species were eliminated with increasing nutrient, which can open up for new species to enter the current stream reach. In conclusion, clear-cutting activities not only increase the algal productivity, but it can also effect species composition among periphyton and create a whole new assemblage of species.

#### **4.6 Conclusion**

The hypotheses related to the controls over algal growth in boreal streams were largely confirmed. In the nutrient-poor stream algae responded to the experimental N-addition, and in CC-4 stream the productivity increased with higher light inputs. However, since the study is small, one cannot draw any general conclusions from it. This study is the first one ever to test for nutrient limitation of stream algae in northern Sweden and for further knowledge it is necessary to expand these efforts to include more than two streams and also for a longer time frame. Water chemistry measurements from previous years reveal that the nutrient levels in the CC-4 stream are declining since the clear-cutting in 2006, which indicates that the absence of nitrogen limitation will eventually disappear as the forest regrows. In addition to this, N and P inputs to streams can change seasonally (Bergström et al. 2008), and these temporal patterns may also influence the strength of algal nutrient limitation. Therefore, in order to make any further conclusions on periphyton productivity and species composition in Swedish boreal streams, more studies are recommended.

### **5 Acknowledgements**

I would like to give the biggest thank you to my supervisor, Ryan Sponseller, for all the support and guidance. Thank you for the dedication, I have learned a lot when working with you. I would also like to thank Ryan Burrows, post-doc at SLU, for the help with preparing the NDS-racks, and for answering all my silly questions. Finally, I would like to thank Johan Lidman for helping me in the field.

## 6 References

- Austin, A., Lang, S. and Pomeroy, M. 1981. Simple methods for sampling periphyton with observations on sampler design criteria. *Hydrobiologia*, 85:33-47
- Bergström, A-K., Blomqvist, P. and Jansson, M. 2005. Effects of atmospheric nitrogen deposition on nutrient limitation and phytoplankton biomass in unproductive Swedish lakes. *Limnology and Oceanography*, 50: 987-994
- Bergström, A-K., Jonsson, A. and Jansson, M. 2008. Phytoplankton responses to nitrogen and phosphorus enrichment in unproductive Swedish lakes along a gradient of atmospheric nitrogen deposition. *Aquatic biology*, 4: 55-64
- Bernhardt, E.S. and Likens, G.E. 2004. Controls on periphyton biomass in heterotrophic streams. *Freshwater Biology*, 49: 14-27
- Biggs, Barry J.F. 2000. Eutrophication of streams and rivers: dissolved nutrient- chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society*, 19:17-31
- Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Habens, K.E., Lancelot, C. and Likens, G.E. 2009. Controlling Eutrophication: Nitrogen and Phosphorus. *Science*, 323:1014-1015
- Dodds, W.K. K. and Welch, E.B. 2000. Establishing nutrient criteria in streams. *Journal of the North American Benthological Society*, 19:186-196
- Downes, B.J., Lake, P.S., Schreiber, E.S.G. and Glaister, A. 2000. Habitat structure, resources and diversity: the separate effects of surface roughness and macroalgae on stream invertebrates. *Oecologia*, 123: 569-581
- Hill, W.R., Fanta, S.E. and Roberts, B.J. 2009. Quantifying phosphorus and light effects in stream algae. *Limnology and Oceanography*, 54: 368-380
- Holopainen, A-I. and Huttunen, P. 1992. Effects of forest clear-cutting and soil disturbance on the biology of small forest brooks. *Hydrobiologia* 243/244: 457-464
- Häder, D.-P., Kumar, H.D., Smith, R.C. and Worrest, R.C. 1998. Effects on aquatic habitats. *Journal of Photochemistry and Photobiology B*, 46(1-3): 53-68
- Jansson, M., Blomqvist, P., Jonsson, A. and Bergström, A-K. 1996. Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanoflagellates in Lake Örträsket. *Limnology and Oceanography*, 41: 1552-1559
- Kahlert, M. and McKie, B. 2013. Comparing new and conventional methods to estimate benthic algal biomass and composition. *Environmental science: Processes & Impacts*, 1-3: 3-12
- Laudon, H., Hedtjärn, J., Schelker, J., Bishop, K., Sørensen, R. and Ågren, A. 2009. Response of dissolved organic carbon following forest harvesting in a boreal forest. *Ambio*, 38: 381-386
- Lewis, W.M. and McCutchan, J.H. 2010. Ecological responses to nutrients in streams and rivers of the Colorado Mountains and foothills. *Freshwater biology*, 55: 1973-1983
- Lohman, K., Jones, JR. and Baysinger, D.C. 1991. Experimental-evidence for nitrogen limitation in a northern ozoark stream. *Journal of the North American Benthological Society*, 10: 14-23
- Länsstyrelsen. 2008. Strategi för skydd och restaurering av våtmarker i Västerbottens län. <http://www.lansstyrelsen.se/vasterbotten/SiteCollectionDocuments/Sv/Publikationer/2008/Strategi%20v%C3%A5tmarker.pdf>. Collected 2014-09-01
- Löfgren, S., Ring, E., von Brömssen, C., Sørensen, R. and Högbom, L. 2009. Short-term effects of clear-cutting on the water chemistry of two boreal streams in Northern Sweden: A paired catchment study. *Ambio*, 38:347-356
- Magnusson, T. 2009. Skogsbruk, mark och vatten. *Skogsskötselserien no 13*. Skogsstyrelsen.
- Mayer, M.S. and Likens, G.E. 1987. The Importance of Algae in a Shaded Headwater Stream as Food for an Abundant Caddisfly (Trichoptera). *Journal of the North American Benthological Society*, 6: 262-269
- McCutchan, J.H. and Lewis, W.M. 2002. Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. *Limnology and Oceanography*, 47:742-752

- McNeely, C., Finlay, J.C. and Power, M.E. 2007. Grazer traits, competition, and carbon sources to a headwater-stream food web. *Ecology*, 88: 391-401
- Nylund, J.E. 2009. Forestry legislation in Sweden. Report no 14. Department of forest products. SLU, Uppsala
- Sanderson, B.L., Coe, H.J., Tran, C.D., Macneale, K.H., Hardstadt, D.L. and Goodwin, A.B. 2009. Nutrient limitation of periphyton in Idaho streams: results from nutrient diffusing substrate experiments. *Journal of the North American Benthological Society*, 28:832–845
- Schanz, F. and Juon, H. 1983. Two different methods of evaluating nutrient limitations of periphyton bioassays, using water from the River Rhine and eight of its tributaries. *Hydrobiologia*, 102: 187-195
- Schelker, J., Öhman, K., Löfgren, S. and Laudon, H. Scaling of increased dissolved organic carbon inputs by forest clear-cutting – What arrives downstream?. *Journal of hydrology*, 508: 299-306
- Skogsstyrelsen. 2014. Skydds zoner. <https://www.skogsstyrelsen.se/Aga-och-bruka/Mina-sidor1/Systemsidor/Hjalpsidor/Mina-sidor/Natur--och-kulturmiljohansyn-/Skydds zoner/>. Collected 2014-09-01
- Steedman, R.J. and France, R.L. 2000. Origin and Transport of Aeolian Sediment from New Clearcuts into Boreal Lakes, Northwestern Ontario, Canada. *Water, air and soil pollution*, 122: 139-152
- Sørensen, R., Ring, E., Meili, M., Högbom, L., Grabs, T., Laudon, H. and Bishop, K. 2009. Forest harvest increases runoff most during low flows in two boreal streams. *Ambio*, 38:357-363
- Tank, J.L. and Dodds, W.K. 2003. Nutrient limitation of epilithic and epixylic biofilms in 10 North American streams. *Fresh water biology*, 48:1031-1049
- Tank, J.L., Bernot, M.J. and Rosi-Marshall, E.J. 2007. Nitrogen limitation and uptake. In *Methods in stream ecology*. Hauer, R.F. and Lamberti, G.A. (editors), second edition.
- Townsend, S.A., Garcia, E.A. and Douglas, M.M. 2012. The response of benthic algal biomass to nutrient addition over a range of current speeds in oligotrophic rivers. *Freshwater Science*, 31:1233–1243
- Vadeboncoeur, Y., Jeppesen, E., Vander Zanden, J.M., Schierup, H-E., Christoffersen, K. and Lodge, D.M. 2003. From green-land to green lakes: cultural eutrophication and the loss of the benthic pathways in lakes. *Limnology and Oceanography*, 48: 1408-1418
- Wallace, J.B. and Webster, J.R. 1996. The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology*, 41: 115-139