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Bridging the gap from ice to biofilms: Implications of glacier dust from West Greenland on biofilm production and composition

By:
Nadine Liese
(1174797)

Supervisor:
Miquel Lurling (Aquatic Ecology & Water Quality Management)
Suzanne McGowan (NIOO KNAW)

Chair group:
AQUATIC ECOLOGY & WATER QUALITY MANAGEMENT

NIOO
NETHERLANDS INSTITUTE OF ECOLOGY (NIOO-KNAW)

Abstract

One of the consequences of climate change is the melting of glaciers in Greenland, with dust created by the glaciers' grinding action. This process grinds up rock particles in the glaciers and the bedrock, releasing them suspended in the glacial melt and eventually depositing them on the riverbanks, where they dry out and are released back into the environment by the wind. This study focuses on dust samples collected in the Kangerlussuaq Fjord region, known for its large glacial fluvial river system and numerous lakes where large amounts of dust are released. What effects this glacier dust has on these lake-rich environments in particular is not known exactly. Due to the composition of the dust, it was uncertain whether it served as a fertilizer through the introduction of additional nutrients or whether it functioned as a nutrient trap on biofilm development. In order to address these questions, an exploratory study was conducted over a period of two months to obtain comprehensive answers to the effects of glacier dust. Before starting the dust treatment, biofilms were initially cultivated in a pond at the Netherlands Institute of Ecology and subsequently transferred to the laboratory after a 12-day period of growth and one week incubation. Following that, a pilot study was performed to gather further insights into the growth and variability of the biofilm in the pond. During this phase, the biofilms were cultivated on microscope slides placed in test trays, allowing observation of their growth. Subsequently, investigations were carried out in the laboratory setting to assess the impact of both the microscope slide's position and the tray's position in the pond on biofilm growth. In addition, essential parameters for maintaining the biofilms in the incubator were explored. These included temperatures of 16°C and 20°C and different media types, including nutrient-poor, nutrient-rich, and filtered pond water. In the pilot study, the assessment of chlorophyll-a content and microscopic analysis played an essential role as endpoints. These assessments provided information about the initial conditions. The results of the pilot study showed a notable variability in the growth of the biofilm, which occurred probably due to the randomness of the growth. It was found that using filtered pond water and maintaining a temperature of 16 °C was sufficient to maintain biofilms in the laboratory environment. Finally, the main experiment consisted of exposing the biofilm samples to two different dust concentrations to investigate their response in terms of chlorophyll content, biomass changes and biological community composition. Two different dust concentrations were used in this controlled experiment, determined based on the annual dust load in Greenland. It was found that the group exposed to higher dust concentrations had increased chlorophyll levels and biomass compared to the lower dust concentration group and the control group. However, it was also noted that higher dust concentrations appeared to limit the diversity of the biofilm community composition. Conversely, the group with lower dust treatment showed greater compositional diversity but lower overall chlorophyll levels and biomass development. In response to the dust treatment, a shift away from chlorophytes towards dinoflagellates, diatoms, and streptophytes was observed, contradicting the expected dominance of cyanobacteria, which typically outcompete other organisms under limited nutrient conditions because of the ability to fix nitrogen. These results demonstrate that glacier dust in higher amounts exerts a fertilizing effect on biofilms but limits the diversity of the community.

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1. Introduction

The adverse effects of climate change are shown on global scale. Recognizable consequences such as alteration in sea level, precipitation and temperatures are evident and only one of the many examples that can be correlated to climate change (Harrison et al., 2021). The loss of the Greenland ice sheet is causing sea level rise. Although the Greenland Ice Sheet accounts for 11.2% of Earth's land ice, it contributed significantly 37% to the cryosphere-induced sea level rise in 2012 to 2016 (McCutcheon et al., 2021; Zhang et al., 2023). Glaciers contain not only meltwater but also minerogenic particles that are ground up in situ by the glaciers and bedrock. In addition, surface deposits on the ice may also contain rock debris from cliffs (Earle, 2015; Soreghan et al., 2022). The velocity of meltwater determines the potential to transport released sediments to the base of the glacier. These sediments are deposited as outwash sediments and eventually settle on the riverbanks (Bullard & Austin, 2011). After deposition, the suspended sediments dry out and are released into the environment through deflation (Earle, 2015; Soreghan et al., 2022). Deflation, also known as aeolian transport, refers to the process of wind carrying loose sediment particles (Fairbridge, 2006). The processes of suspended sediment outwash, deposition and deflation are important factors in the transport and distribution of sediments and dust released by glaciers. These processes have the potential to influence the surrounding environment and ecosystems. Therefore, the impact of melting glaciers and the subsequent release of glacial dust should not be underestimated.

In the Kangerlussuaq region of West Greenland, an annual dust load of approximately 50 g/m2 was observed. The primary source of this dust are the large glacial-fluvial river systems and nearby lakes (Soest et al., 2022), where suspended sediments in the meltwater are subsequently deposited in the outwash plains and delta areas (Bullard & Austin, 2011; van Soest et al., 2022). The Kangerlussuaq environment is characterized by numerous lakes where benthic production predominates over pelagic, with an increasing amount of dust deposition (Saros et al., 2019). In Arctic lakes, where nutrients are limited, primary production often takes place on the lake bottom, where nutrients are more accessible. For this reason, it is particularly important to study the effects of glacier dust on freshwater biofilms, as biofilms are an essential component of benthic ecosystems. To this end, a small exploratory study was conducted at the Netherlands Institute of Ecology (NIOO KNAW) to investigate the effects of glacier dust on freshwater biofilms cultured in a pond.

On closer examination, the released glacier dust contains several components that can affect the environment differently. The components include transition metals (e.g., iron, copper, and zinc), lanthanide metals, alkali metals (e.g., sodium and potassium) and alkaline metals (e.g., magnesium and calcium) (Blanckaert et al., 2022). However, because of the composition of glacier dust, it is uncertain whether dust serves as a nutrient supplier or a nutrient trap (Anderson et al., 2017; Rahav et al., 2018; Li et al., 2020; McCutcheon et al., 2021). Dust could serve as a nutrient trap through a process of adsorption, where nutrients adhere to dust particles, and subsequent deposition, where these nutrient-laden particles settle and enrich the environment with important elements such as nitrogen and phosphorus (Hotaling et al., 2021; McCutcheon et al., 2021). With climate change, and rising terrestrial temperatures, more glacial ice will melt and consequently more dust will be released into the environment (Saros et al., 2019; Capron et al., 2021). Hence, accumulated atmospheric dust can be linked to temperature changes due to climate change (Újvári et al., 2022).

It is challenging to simulate an entire ecosystem based on the Kangerlussuaq region in West Greenland, as biological processes and interactions must be accurately understood (Mesple et al.,

1995). Therefore, this study focuses on freshwater biofilms, as they can form ubiquitously in aquatic environments, making them easily accessible (Azeredo et al., 2016). For this reason, the effects of glacier dust are being tested through the development of biofilms. Subsequently, the study investigates what effects the addition of glacier dust could have on the biomass, the chlorophyll content, and the change in composition in the biofilms.

Biofilms consist of microbial life- where different bacterial species colonize densely and develop a community surrounded by a matrix of secreted polymers, including heterotrophic and autotrophic microbes (Steenackers et al., 2012). This implies that bacterial communities in biofilms are of great ecological importance. They not only play a significant role in the production and degradation of organic matter, but also in the recycling of environmental pollutants, the nitrogen and sulfur cycle and many metals (Davey & O'toole, 2000, Ramaraj et al., 2015). The structure of the biofilm varies depending on the abiotic and biotic conditions in the environment. Periphytic or algal biofilms are characterized by their photosynthetic activity and are mainly composed of communities living in symbiosis or communities consisting of, or co-existing with algae, cyanobacteria, and heterotrophic bacteria (Schnurr & Allen, 2015).

Changes in ecosystems can lead to a reduction in ecological resilience, which can adversely impact the biological community and its vitality as certain nutrients, such as nitrogen, phosphate, and others, either diminish or become excessively abundant (Anderson et al., 2009 & 2019; Prater et al. 2022). In summary, processes of change or disturbance, also termed as ecological succession, have the potential to disrupt or alter biofilm communities by affecting species composition, resource availability, microbial interactions, and habitat characteristics. Insights into the interaction between ecological succession and biofilm dynamics are critical to understanding how microbial communities adapt to changes, such as glacial dust, in their environment (Kelly et al., 2019). Saros et al. (2019) demonstrated shifts in the composition of the benthic community in the Kangerlussuaq region that may indicate a link to dust input. Taking this into consideration, it can be hypothesized that glacier dust introduces more nutrients into the biofilm, which can promote or inhibit growth. This may benefit some communities, while others may be displaced (Sabater et al., 2007; Anderson et al., 2017). Specifically, it was assumed that the biofilm samples would be characterized by a prevalence of nitrogen-fixing cyanobacteria as opposed to green algae after the introduction of dust and the associated influx of additional nutrients. With the increasing release of glacial dust, biofilms can serve as indicators of changes in their environment. Sabater et al. (2007) reported that biofilms in rivers and streams are the first to be exposed to nutrients, organic material, or toxins. Under these influences and thus changes in abiotic and biotic nature, biofilms respond particularly through their size and rapid growth rate, species richness and the physiological diversity of which they are composed (Sabater et al., 2007). As a results, biofilms can be the first to exposed to these influences which therefore could function as an early indicator of changes in the ecosystems. In summary, biofilms can provide information on water quality and ecological status (Ramaraj et al., 2015; Balcázar et al., 2015), which makes them of particular interest in this exploratory study.

To this end, the following hypothesis will be tested:

Glacial dust acts as a fertilizer on the productivity on freshwater biofilms, increases biomass production and shifts community composition towards cyanobacteria.

Research aim:

This exploratory study aims to enhance the understanding of how the growth and community composition of periphytic freshwater biofilms are influenced by the addition of glacial dust. The focus of the study is to evaluate the effects of two concentrations of glacier dust on biofilm resistance, nutrient dynamics, chlorophyll a and biological community composition. This study involves the development of biofilms in a selected pond and subsequent laboratory experiments to investigate the dynamics of biofilm growth in the pond. As a result, the cultivation of biofilms is addressed in order to obtain reliable and consistent results for the upcoming experiments. In addition, the optimal conditions (temperature, light, and culture media) for the development of biofilms in experimental channels after transfer of the biofilms to the laboratory will be determined. The results obtained will contribute to the understanding of these interactions and serve as a starting point for further research in this field.

Research objective:

- To gain insights into the methodological development of biofilms.
- To understand biofilm growth dynamics in a pond and determine the optimal conditions (temperature, light, and culture media) for biofilm development in experimental channels in the laboratory.
- To investigate the effects of glacier dust on chlorophyll rate, biomass, biofilm composition and changes in nutrient content in biofilm water samples.

Implementation of the research objectives:

- The study will be conducted on a small scale to investigate the effects of glacier dust on freshwater biofilms.
- The biofilms will be developed in a freshwater pond to simulate a natural habitat.
- A pilot study with several destructive sub-experiments will be conducted to learn more about growth behavior and to identify important parameters such as temperature, light, and culture medium for post-transfer work in the laboratory. In addition, the pilot study will provide more information on which methodologies should be applied to gain optimal biofilm growth.
- In the final experiment, the biofilms are exposed to different concentrations of glacier dust to assess their response in terms of chlorophyll-a content, biomass, community composition and water nutrients.
- The composition of the dust is uncertain, and the study aims to determine whether it acts as a nutrient supplier or a nutrient trap in the biofilms.

For these the following research questions will be answered:

1. Pilot study: How do the position of the trays in the pond and the growth on microscope slides influence biofilm development and what medium should be used for incubation?
2. What is the effect of the addition of different concentrations of glacially derived dust on freshwater biofilm growth?
    a. How will the weight of the biofilm biomass change after the treatment?

b. How does the addition of glacial dust impact the chlorophyll content of the biofilms in the simulated habitat?
c. How do the nutrients in the water samples of the biofilms change after the treatment?
d. How does the addition of different concentrations of glacier dust change the composition of the biofilm community?

2. Methodology

The methodology involved a two-step approach, starting with a pilot study to determine how biofilm growth proceeds in a pond and, after transfer to the laboratory, to determine the optimal conditions (temperature, light, and culture media) for biofilm development in experimental channels. The focus was on creating a low-nutrient environment. The variations in biofilm growth that occurred in the different experiments were investigated to obtain optimizations for the final experimental setup. Comparative analysis between the initial and final chlorophyll measurements was used to identify potential factors influencing biofilm development and then to refine the experimental conditions to obtain reliable results.

Following the pilot study, the final experiment was conducted. In addition, the changes in nutrient content were monitored by examining water samples from the biofilm before and after the addition of glacier.

Furthermore, after the explained methodologies of the pilot study and the final experiment, a chapter follows on the applied response measurements, including phytoplankton PAM (Pulse Amplitude Modulation) and High-performance liquid chromatography (HPLC) to provide information on the implementation of these instruments.

2.1. Pilot project

The pilot study was conducted because there was no experience in working with the development of freshwater biofilms. Therefore, the focus was on creating an approach for biofilm cultivation and ensuring reliable and consistent results for subsequent experiments.

First, the focus was on understanding the effects of variability on microscope slides, temperature, and applied media on biofilm growth. This included exploring the variability of tray and microscope slide positions in the pond and, after transfer to the laboratory, optimizing the growth conditions in the incubator. The objective was to use these preliminary experiments to identify suitable parameters and influencing factors that were incorporated into the planning and implementation of the final experiment.

2.1.1. Variability of tray and microscope slide position in the pond

2.1.1.1. Objective

To investigate the potential spatial variations of biofilm growth in a pond by comparing the results from the left and right sides. To achieve similar initial growth conditions for destructive sampling, samples were taken from sites with comparable biofilm growth. Samples were selected either on different slides to ensure low variability between slides or from the same slides with minimal variability. After comparing the pond's left and right sides, the variability of biofilm growth in the different tray positions (1 to 5) on each side of the pond and the positioning of the slides in the tray were investigated (*Figure 2*). The slides were labelled A to D and placed securely in the tray.

2.1.1.2. Method

The biofilms were cultured on microscope slides measuring 76 x 26 mm and placed in trays in a pond provided by the Netherlands Institute of Ecology (NIOO-KNAW). These were attached to a sturdy string so that they remained submerged at a constant depth of 42 cm (*Figure 1*). The microscope slides were obtained from Hirschmann Laborgeräte GmbH & Co KG.

Figure 1: The figure on the left shows the pond used for biofilm development on the NIOO campus. A transect line has been established for the placement of the dishes, with the left and right sides of the pond designated for the placement of the dishes (see red arrow). On the right side are the trays used for the experiment, each containing four slides.

The experimental procedure involved submerging trays with the microscope slides in the pond for a duration of 10-14 days to facilitate biofilm growth. Subsequently, the microscope slides were transferred to the laboratory where the biofilms were removed for chlorophyll a measurement using the phytoplankton PAM (PHYTO PAM) technique. A comparison was made between the obtained chlorophyll contents to identify any potential differences between the biofilms on the left and right sides of the pond.

In addition, differences in biofilm growth were investigated depending on the position of the tray, the position of the slide in the tray. To further analyze these variations, three trays were selected, each with four glass slides, resulting in a total of 12 samples. The same procedure for analyzing the chlorophyll content as before was conducted. *Figure 2* provides a schematic representation of the arrangement of a tray and the different investigated slide position.

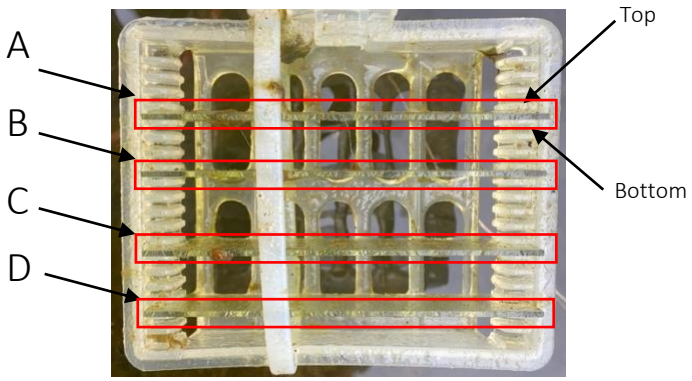

Figure 2: The illustration shows a tray with slides positioned in different places, labelled from A to D (slides highlighted in red), and a top and bottom view.

2.1.2. Variability of the biofilm growth on the slides

2.1.2.1. Objective

This experiment aimed to explore the diversity of biofilm growth on slides and to determine the most effective technique for removing the biofilm for subsequent evaluation. This approach allowed a more accurate comparison between the initial and final stages of the experiment. It ensured that the final sample for incubation was similar to this initial state and variability was minimized.

2.1.2.2. Method

Four different removal methods were tested, involving the removal of the top surface of the biofilm in different orientations. The methods involved vertical, diagonal, and horizontal removal of half of the biofilm, with eight samples used for each method (*Figure 3*). In addition, 36 samples were used, with the entire biofilm from either the top or bottom side for comparison. The higher number of samples in the latter case is due to the use of results from previous trials.

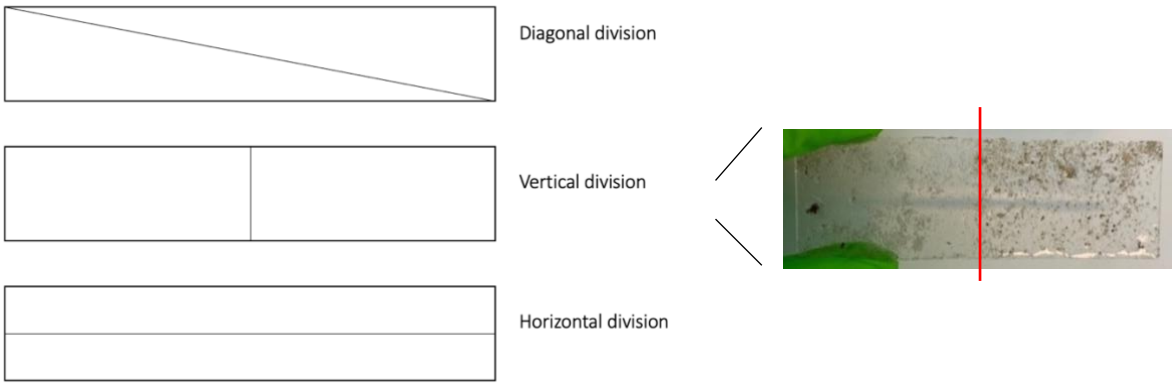

Figure 3: Comparing biofilm growth on microscope slides with different removal methods.
On the right: Biofilm growth after one week, after removing biofilm vertical from the left side.

For chlorophyll determination the PHYTO-PAM instrument was used, one side of the biofilm was removed initially, and after one week of incubation, the remaining side was removed, measured again, and compared to assess any changes in chlorophyll content.

This sub-experiment allowed research into different removal methods and their effects on biofilm growth and chlorophyll content. By analyzing different removal methods, the most optimal approach to capture the initial state of the biofilm was determined and ensured that the final sample for incubation was similar to this state. This allowed a more accurate comparison between the beginning and the end of the experiment.

2.1.3. Optimization of incubator growth conditions

2.1.3.1. Objective

To investigate the facilitation and maintenance of biofilm growth after transfer to the incubator by testing the effects of different temperature, culture media and light intensity conditions.

2.1.3.2. Method

In this sub-experiment, temperatures of 16 °C with a preset light intensity of 52.19 µmol and 20 °C with a preset light intensity of 09.15 µmol were tested. The samples were incubated for one week, with a light period of 16 hours and a dark period of 8 hours. The Economic Lux climate chambers (ECL02) by Snijder Labs were used for incubation. In total, 18 samples were analyzed, with each temperature condition involving nine biofilms. Three different media were used for each temperature condition: filtered pond water, demineralized water, and nutrient rich medium.

To maintain consistent growth conditions, filtered pond water was chosen as a medium for the biofilms, as they originated from a pond environment. To obtain filtered pond water, the collected pond water was filtered with a high-pressure pump from Schleicher & Schuell with two filters to remove larger particles, debris and unwanted organisms that could interfere with the experimental setup or introduce unknown variables into the study. Overall, filtering the pond water helped to create a more controlled environment. A membrane filter with the pore size of 0.2 µm and a GF filter with 135 mm from Whatman was applied for filtration. In addition, a nutrient-rich medium known as Wright and Chu Medium (WC medium) (Guillard et al., 1972) was obtained from NIOO KNAW and prepared following the provided protocols. 1 ml of the WC stock was diluted with 1 liter of demineralized water and then autoclaved for 15 minutes (*Appendix A*). For the demineralized medium no further preparations were needed. Furthermore, an osmotic medium was subsequently produced by removing all trace elements and vitamins (*Appendix B*). In this way, a nutrient-poor environment was created that made it possible to monitor biofilm growth and possible influences closely after the addition of glacier dust. The consideration of a nutrient-poor medium was made because of the nutrient-poor nature of the Kangerlussaq region, so it was important to test the effects under similar conditions. Four samples were subsequently used for this purpose.

The chlorophyll content of the samples was measured using the PHYTO-PAM instrument. The top of the microscope slide was left untouched and placed directly into a beaker with 400 mL medium to obtain the end point. The biofilm on the bottom side was the starting point and was transferred directly to measure the starting chlorophyll content with the PHYTO-PAM.

3. Final experiment

Once the optimal conditions were determined through the pilot study, involving endpoints such as assessed biofilm growth measured by chlorophyll a content and community response, new biofilms were cultivated in the pond for a duration of 12 days. Subsequently, these biofilms underwent a one-week incubation and a period of starvation in the laboratory. Then, the biofilms were exposed to two different concentrations of glacier dust. Before the new samples were introduced into the medium and glacial dust, the biofilms were removed from one side of the glass slides and analyzed to assess either the algal community under the microscopy, the biomass, or the chlorophyll content. The measurements were repeated after the treatment. In addition, in this part of the experiment, the water quality of the biofilm samples before and after the experiment was examined and compared to evaluate the changes in nutrient dynamics.

3.1. What is the effect of the addition of different concentrations of glacially derived dust on biofilm growth?

3.1.1. Objective

Evaluation of the effects of two different concentrations of glacier dust on the development of biofilms and their community composition after a period of one week.

3.1.2. Method

Two different concentrations of glacier dust were applied. To calculate the amount of glacier dust to be added to the biofilm samples, the annual glacier dust load results of van Soest et al. (2022) were used as a calculation approach. The annual glacier dust load is about 50 g/m^2, which corresponds to a daily dose of about 0.06 g/m^2. Assuming a seven-day storm, the total amount is 0.42 g/m^2 (*Table 1*) (Van Soest et al., 2022). These values were determined as an approach to obtain the amount of glacier dust to be added.

Table 1: Annual dust load, as per data from van Soest et al. (2022), was used to calculate the daily and 7-day storm dust loads, as well as the application rate of glacial dust in field.

	Dust load (g/m²)	*Application rate in field (g/cm²)*
*Annual dust load**	50	0.005
Max. daily dose	0.06	0.000006
7-day storm dose	0.42	0.000042

To determine the amount of mass to be applied per beaker (in grams), the diameter of the beakers was multiplied by the surface area (in cm²) and π (3.14). This calculation was used to ensure accurate application rates. Including the beaker diameter in the calculation of the application rate ensured that the intended amount of dust uniformly covered the entire surface of the beaker. This approach guaranteed consistent and accurate application of the dust under the experimental conditions. For the high dust (HD) group, 0.467 g of dust was added to the beakers, while the low dust (LD) group received 0.004 g. The beakers had a diameter of 10.9 cm. Due to supply constraints, smaller beakers with a diameter of 9.3 cm were also used. The application rate for these beakers was calculated using the

same principle to maintain consistent conditions for the final treatment. Specifically, 0.340 g of glacier dust was applied to the HD group with a beaker diameter of 9.3 cm and 0.003 g to the LD group (Table 2).

Table 2: Summary of the calculation of the application rate based on the beaker diameter and surface area. In addition, the applied mass per unit area in the beaker was given in grams, the application rate derived from the annual load data of van Soest et al. (2022) was given in grams per square centimeter, and the estimated amount of dust present on half of the chute after treatment was given in grams. This value was crucial for the biomass assessment and had to be subtracted from the weight.

	Diameter beaker (cm)	*Radius beaker (cm)*	*Beaker's calculated surface (cm^2)*	*Mass of dust applied per beaker (g)*	*Application rate of dust (g/cm^2)*	*Half glass slide (cm)*	*Glacial dust on ½ microscope slide (g)*
High dust	10.9	5.45	93.31	0.467	0.005	3.75	0.019
Low dust	10.9	5.45	93.31	0.004	0.0000420	3.75	0.00016
High dust	9.3	4.65	67.93	0.340	0.005	3.75	0.019
Low dust	9.3	4.65	67.93	0.003	0.0000420	3.75	0.00016

First, the biofilms were placed in the incubator for one week for starvation. Then the chlorophyll a content for each group was measured with the PHYTO-PAM, after that, the treatment with glacier dust started, followed by a subsequent re-incubation of the samples for one week. The chlorophyll content was then measured again and compared with the results before treatment.

Furthermore, in this experiment, the chlorophyll a concentration was additionally analyzed using High-Performance Liquid Chromatography (HPLC) to examine potential differences and variations and the measurement instruments.

3.2. Evaluation of changes in biofilm composition

3.2.1. *Objective*

The aim of this experiment was to determine whether the addition of glacier dust causes changes in the composition of the biofilm that could lead to an altered community structure.

3.2.2. *Method*

This experiment has the same parameters but ends with a different endpoint: the microscopic analysis of the biological composition of the biofilm. To investigate the effects of the addition of glacier dust on the composition of the biofilm community, a qualitative assessment was carried out using microscopic analysis (Leica DMI3000) and microscopic imaging with Cell*D analysis. The study involved the comparison of the samples before and after the treatment. Nine control samples, nine samples from the HD group and nine samples from the LD group were examined. To examine the biofilm community before treatment, one half of the microscope slide was scraped off and preserved in Lugol's solution. After treatment, the remaining side of the microscope slide was used to repeat the taxonomic identification. The NIOO operating protocol entitled "Microscope Leica DMI 3000b" contained the instructions for performing the microscopic analysis. Following the protocol, the biofilms in the

centrifuge tubes were supplemented with 20 ml deionized water and treated with Lugol's solution. The Lugol's solution was used for sample preservation as the samples were stored prior to microscopic analysis. The preserved samples were stored in a well-ventilated, dark environment until microscopic analysis was performed. Before starting the microscopic analysis, the samples were transferred to the objective and allowed to rest for several hours to ensure good visibility of the biofilm structures. Microscopy was performed using magnifications of 10x, 20x, and 40x. After attaching the eyepiece, the total magnifications of 100x, 200x and 400x resulted. In each case, one field of view was picked out and the organisms in the available square were counted. In each sample, five points were chosen and counted. After each count, the magnification was increased to see more or to get a better view in case of identification problems. Afterwards, a file was created in Microsoft Excel to provide better comparisons and to calculate in percentages which taxonomic groups were present before and after the treatment.

3.3. Evaluation of the biofilm biomass change

3.3.1. *Objective*

The aim of this destructive sub-experiment was to assess whether there are changes in dry weight before and after the glacier dust treatment.

3.3.2. *Method*

To determine changes in biomass, all samples were weighed before and after treatment. The biofilm samples were weighed at the beginning and after the incubation period to determine any alterations in biomass. Before weighing, the samples were filtered through the previously weighed WHATMAN GF/C filter (1.2 µm) and then dried at 60 °C for 24 hours. In the next step, the weight of the added glacier dust was subtracted from the glacier dust treated samples to calculate the actual biomass. By subtracting the weight of the glacier dust, the actual biomass could be determined (*Table 2*).

3.4. How do the nutrients in the water samples from the biofilms change after the treatment?

3.4.1. *Objective*

The objective was to investigate how different concentrations of glacier dust affected nutrient concentrations in the water samples.

3.4.2. *Method*

Six samples of filtered water, nine samples of the control group, nine samples of the HD group and nine samples of the LD group were taken during the other experiments and analyzed with the Autoanalyzer AA3. The concentration range of 0.03-5 mg/L (normal range) was investigated for phosphate (PO_4), nitrate (NO_3) and ammonium (NH_4^+) to assess possible changes in nutrient concentrations after the glacier dust treatment. The samples were handed over to research assistant Nico Helmsing to carry out the measurement.

4. Response measurements

4.1. Phytoplankton Pulse Amplitude Modulation (PHYTO-PAM)

The measurement of chlorophyll a before and after the test runs was performed to provide an indication of phytoplankton biomass and composition of algal community (Antoine et al., 1996). Different excitation wavelengths were possible to set to detect the presence of phytoplankton and photosynthetic performance based on a fluorescent signal (Heinz Walz GmbH, 2019).

The measurement followed the NIOO operating protocol for the PHYTO-PAM phytoplankton analyzer and Phyto-Win software and involved the following steps. First, each biofilm was detached from the slide and transferred to a 50 mL centrifuge tube into which 20 mL of demineralized water was added. This procedure served to detach the biofilm from the slide and suspend it for subsequent chlorophyll analysis. Subsequently, 2 mL of the sample was used for the determination of the chlorophyll content with the PHYTO-PAM. Due to the inhomogeneity of the samples, they were shaken before analysis, and each sample was measured three times to calculate the average chlorophyll a content. Following these measurements, the remaining samples were filtered through a 25-diameter glass microfiber filter (GF/F) using a vacuum pump at low pressure (<60 hPa). These filtered samples were then stored in aluminum foil at -18°C in a freezer until analysis by high performance liquid chromatography (HPLC).

4.2. High-performance liquid chromatography (HPLC)

Pigment analysis with High-performance liquid chromatography (HPLC) can reveal information about the taxonomic composition of the phytoplankton community, as the presence or absence of marker pigments can be used to determine composition (Roy et al., 2011).

In this study, the HPLC Ultimate 3000 system was used because it has a low detection limit and does not require a hydrochloric acid (HCl) step, which simplified the analytical procedure. While most samples were treated with filtered pond water, implying a higher nutrient content, those exposed to a nutrient-poor medium benefited from the measurement that allowed even minor changes to be detected after the introduction of glacial dust. Furthermore, this additional analysis of chlorophyll a served as a complementary method to the PHYTO-PAM application. The NIOO operating protocol entitled "Measuring Chlorophyll-a Concentration with HPLC Ultimate 3000" was used to prepare samples for analysis. First, a water bath was heated to 80°C and the filters were taken out of the freezer. After about 30 minutes of thawing, the filters were transferred to tubes filled with 1.5 ml of 80% ethanol and incubated in the water bath for 10 minutes. The samples were then centrifuged and placed on ice. In the final preparation step, 1 ml of the ethanol extract was taken with a syringe and filtered through a syringe filter into brown HPLC vials. The prepared samples were then handed over to technical assistant Erik Reichmann to set up and initiate the measurement protocol. This involved diluting the samples five times to create a calibration curve, configuring two blank samples, and starting the system.

A direct comparison to the PHYTO-PAM instrument is not provided, as there are differences in the units and measurement methodology. PHYTO-PAM measures chlorophyll fluorescence and provides an estimate of chlorophyll content in living cells, while HPLC measures the chlorophyll a concentration in samples directly. The values from both methods describe different aspects of chlorophyll concentration and distribution.

5. Results

5.1. Pilot study

5.1.1. How does microscope slide variability, temperature, and media affect biofilm growth?

5.1.1.1. Variability of biofilms among trays and microscope slide position in the pond

The concentrations were similar on the left and right side of the pond. The statistical analysis showed that the p-value associated with the t-statistic (-0.82) was 0.41. Consequently, no statistically significant difference was found between the left and right side of the pond. The results show that biofilm growth is comparable on both sides of the pond, which is confirmed by the non-significant p-value (*Figure 4*).

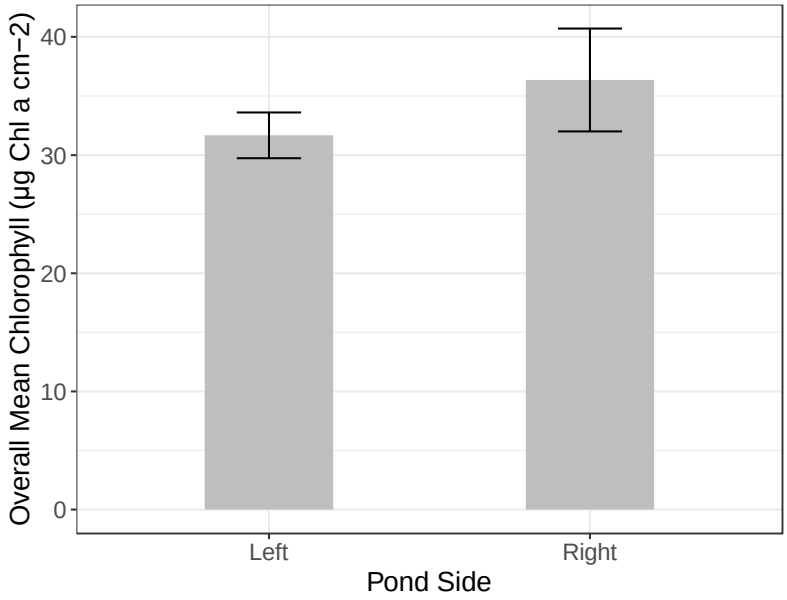

Figure 4: Chlorophyll mean compared to the right and left pond side after 12 days of biofilm growth in the trays. Samples obtained from the microscope slide. Results show no significant difference between both side (p= 0.41).

Figure 5 represents the tray on the left pond side and consisted of five trays. The ANOVA results showed a p-value less than 0.05 (F (4, 15) = 52.54, p = < .01) and revealed that the position of the trays lead to significantly different biofilm growth. The Tukey HSD test for multiple comparisons showed that the position of tray 1 was significantly different from the other positions with a p-value of less than 0.01 (M = 161.73, SD = 31.04). There was no statistically significant difference in the results between tray 2 and tray 3 (p = 0.89), tray 2 and tray 4 (p = 0.19), tray 2 and tray 5 (p = 0.31), tray 3 and tray 4 (p = 0.05), tray 3 and 5 (p = 0.76) or between tray 4 and tray 5 (p = 0.13).

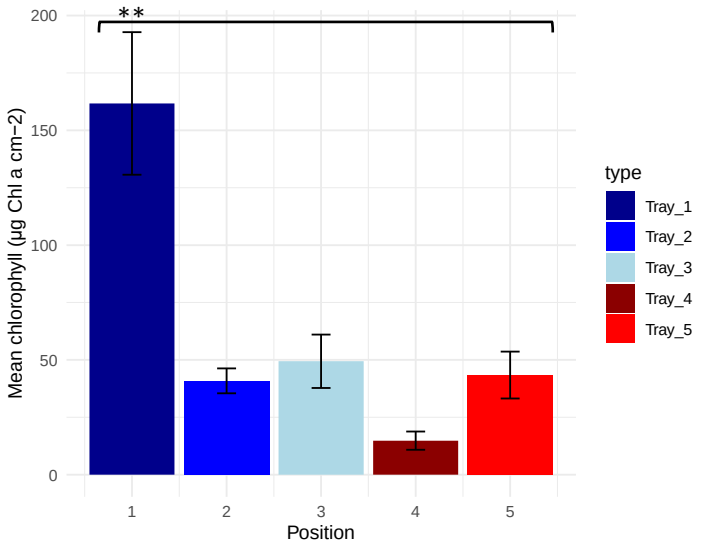

Figure 5: The average chlorophyll content (µg Chl a cm-2) after 12 days of growth on the left side of the pond is displayed for various tray positions (1 to 5). Tray position 1 is indicated with two asterisks (**), signifying a p-value of less than 0.01.

Next, the influence of the position of the microscope slides (A-D) within the tray was inspected. The results of the position of the microscope slides (A to D) on the left side were not significantly different from each other and had no influence on biofilm growth ($F(3,20) = 0.15$, $p = 0.93$) (*Figure 6*).

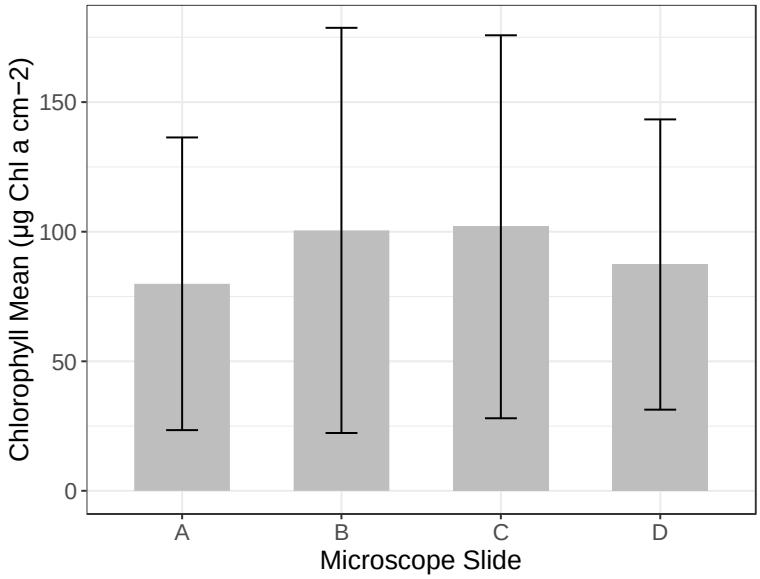

Figure 6: Average chlorophyll measurements taken from microscope slides (A to D) on the left pond side with no significant differences within the slide positions.

The right side of the pond consisted of three trays. The results indicated a difference in biofilm growth depending on the position of the trays. The p-value (.012) corresponding to the F-statistic (7.52) of the ANOVA was less than 0.05 and revealed that a preference in the tray position can be assumed (*Figure 6*). The Tukey HSD confirmed the difference in growth between tray position 1 and 3 with p-values of 0.023 and 0.018.

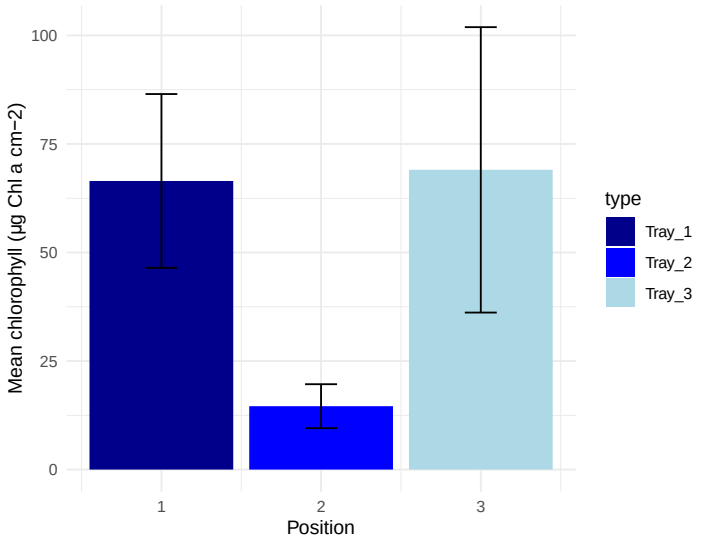

Figure 7: Chlorophyll average (µg Chl a cm^{-2}) after 12 days in different tray positions (1 to 3) on the right side of the pond.

After testing the position of the trays on the right side of the pond, the glass slides (A to D) inside were examined (*Figure 8*). The results of the ANOVA for the glass slides position A to D showed a p-value of more than 0.05, indicating non-significance (F (3,20) = 1.39, p = 0.27).

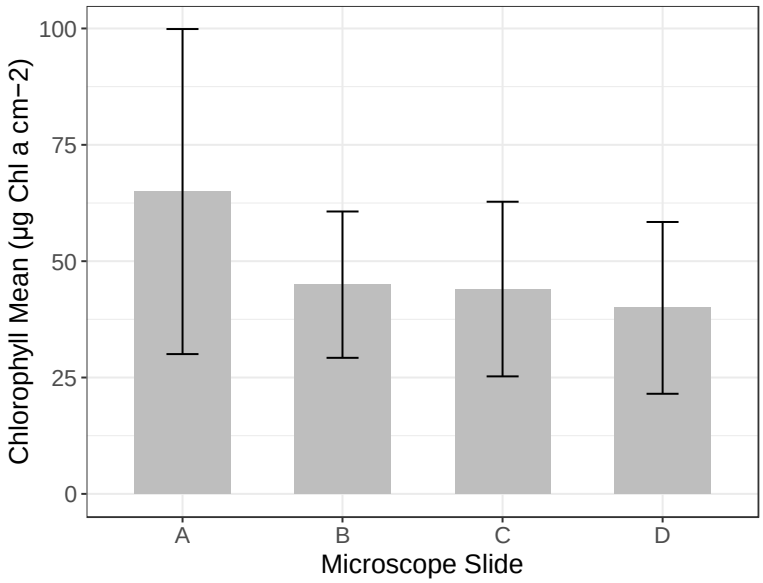

Figure 8: Average chlorophyll measurements taken from microscope slides (A to D) on the right pond side with no significant differences within the slide positions (p=0.27).

5.1.1.2. Variability of the biofilm growth on the microscope slides

First, the chlorophyll content from the top and the bottom side were compared. The results indicated a random variability in growth and no significant differences between the top (M = 92.15, SD = 59.45) and bottom surfaces (M = 98.16, SD = 65. 18) (t (35) = 0.63, p = 0.53) (*Figure 9*). Based on the results, it was subsequently decided to focus and test the biofilm growth on the top slide.

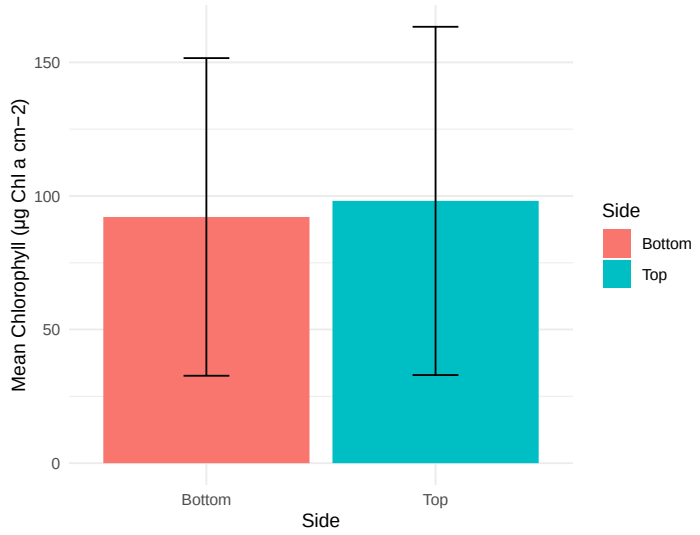

Figure 9: Chlorophyll average measured for the bottom and the top side after one week incubation with no significant difference.

The results of the ANOVA revealed a variance between the four tested removal methods (F (3.32) = 9.67, p = <.001) (*Figure 10*). The Tukey HSD test demonstrated a significant difference in the chlorophyll results (t (3) = 15.95, p = 0.01) in the treatment groups where the biofilm was removed vertically (M = 17.45, SD = 3.64) compared to the horizontal removal (M = 45.89, SD = 10.83). In addition, a significant difference was found between the horizontal and diagonal (M = 12.66, SD = 4.63) removal methods (t (3) = 18.63, p = .001).

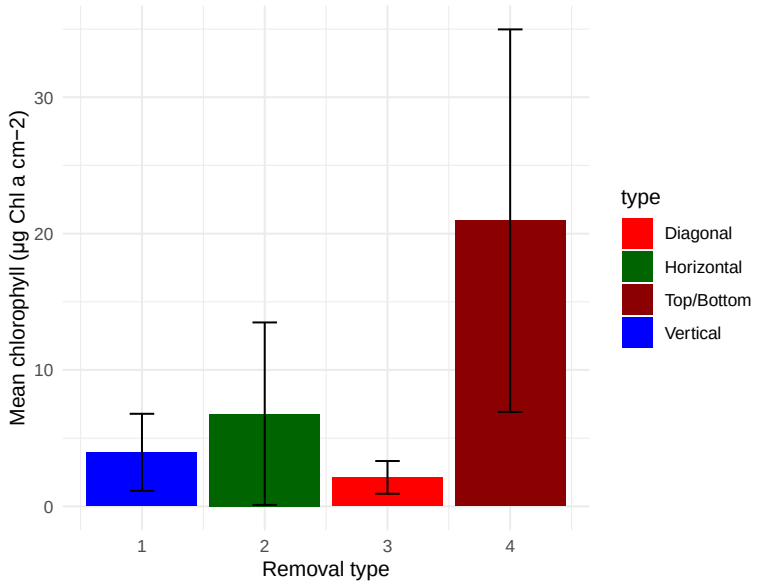

Figure 10: Difference measurement shows variability of biofilm growth (Chlorophyll measured in (µg Chl a cm^{-2}) on microscope slides, tested and compared with different removal methods.

No significant differences were found between the vertical and diagonal sampling methods. In addition, chlorophyll measurements after the incubation period were similar to the initial measurements, which was an important endpoint. Considering these results, the vertical sampling method was selected for its practicality and low variability instead of the diagonal removal.

5.1.1.3. Biofilm development and optimization of incubator growth condition

Based on daily observations of the biofilms in the pond, it was determined that a growth period of 12 days provided satisfactory biofilm development. Therefore, this duration was chosen as the optimal period before transferring the biofilms to the laboratory for further analysis.

After the first removal of six biofilms from the top of the microscope slide without any treatment, the measurement with the PHYTO PAM showed an average chlorophyll content of 91.94 (SD = 106.04). This average was used for comparison to the different treated media to be able to identify influences.

The next step was to determine the influence of temperature and medium on the biofilms. Using the results of the PHYTO-PAM, a two-sided paired-sample t-test was performed to discover the effect of three different media and 16 °C and 20 °C.

The following end results of the demineralized water (DIW) at two different temperatures after one week of incubation are illustrated in *Figure 11*. The results at 16 °C (M = 14.4, SD = 2.63) compared to 20 °C (M = 14.11, SD = 2.31) showed no significant effect on biofilm development (t (2) = 0.22, p = 0.85). The filtered pond water (FPW) produced higher mean chlorophyll levels at 16 °C (M = 880.18, SD = 698.48) than at 20 °C (M = 289.19, SD = 263.84). The results for using FPW indicated no significant preference between the two temperatures (t (2) = 1.51, p = 0.27). The nutrient-rich medium indicated more chlorophyll rates at 16 °C (M = 275.99, SD = 220.02) compared to 20 °C (M = 58.61, SD = 15.96). However, the stats found no significant preference in temperature preference for the nutrient-rich medium (t (2) = 1.82, p = 0.21).

A two-factor analysis of variance (ANOVA) with replication was performed to investigate the effects of temperature and medium on the biofilm samples. The p-value for the interaction between temperature and medium showed no significant interaction (F (2) = 1.32, p = 0.30), indicating that the combined effects of temperature and medium had no significant influence on the biofilm development. When analyzing the temperature factor separately, no statistical significance was found (F (1) = 3.23, p = 0.097), indicating that the temperature differences did not have a significant effect on the biofilm samples. However, significant differences were observed between the different media treatments (F (2) = 5.17, p = 0.02), which indicated that the choice of media had a significant effect on biofilm growth and therefore more chlorophyll outcome.

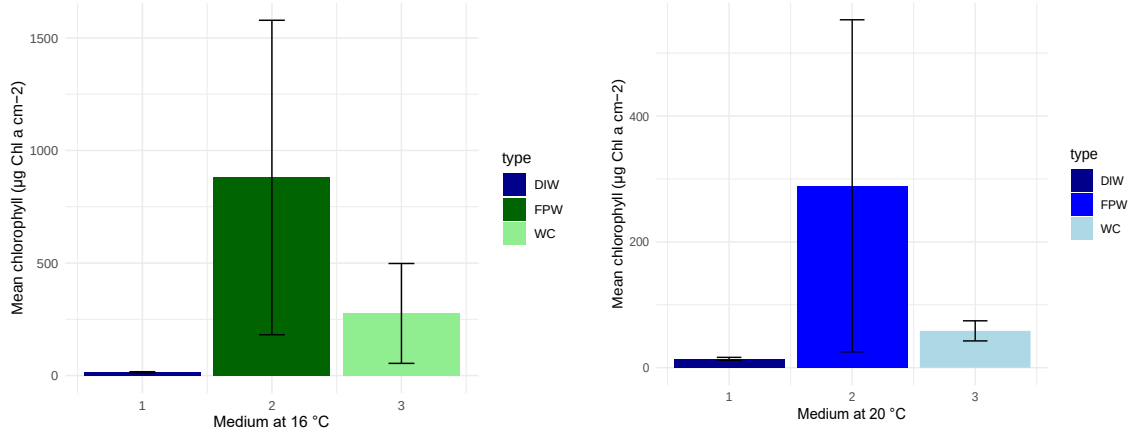

Figure 11: The chlorophyll levels (µg Chl a cm-2) were measured following one week of incubation, considering three distinct applied media and temperatures of both 16 °C and 20 °C.

Due to an inaccurate composition of the nutrient-rich medium, additional samples were subjected to incubation at 16 °C (mean initial value: 16.32, SD = 3.16). As a result, there was a significant

change in biofilm growth, so that the use of the nutrient-rich medium had a favorable effect on biofilm growth (final mean = 131.88, SD = 26.25) (t (3) = -8.9, p = .003).

Another biofilm was transferred to the osmotic medium and measured a chlorophyll average of 49.43 before the treatment (SD = 11.63). After one week incubation, the osmotic medium had a negative growth rate (M = 17.66, SD = 7.57) (*Figure 12*). The biofilm growth rate was calculated using the natural logarithm (ln) of the doubling time (d-1), the measure of the exponential increase in biofilm biomass within the one-week treatment. Furthermore, the t test confirmed a significant influence of the osmotic medium on biofilm growth (t (3) =12.82, p = .001). As a reference, filtered water was added to the nutrition-depleted medium. Before the biofilm was placed in the filtered medium, the chlorophyll average was 40.89 (SD = 5.43). A significant change and an increasing growth rate after one week in the filtered pond water was discovered (M = 115.29, SD = 14.38) (t (3) = -8.73, p = .003).

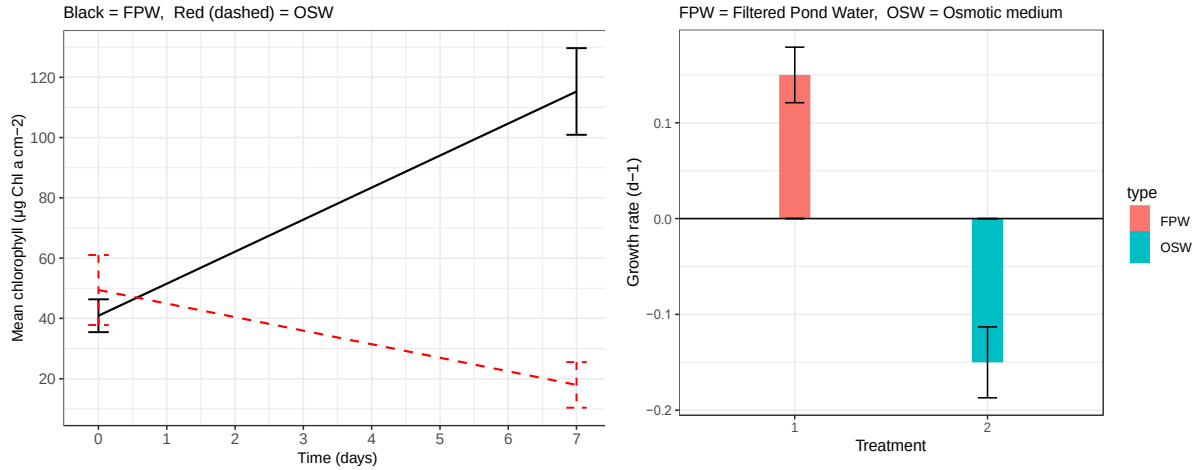

Figure 12: On the left side, the variations in the average chlorophyll content (µg Chl a cm-2) over a period of 7 days in both filtered pond water (FPW) and osmotic medium (OSW). On the right side, the growth rates (d-1) originating from the filtered pond water and osmotic medium are presented.

In summary, based on the results of this experiment, it was found that a temperature of 16 °C combined with the use of filtered pond water yielded the most reliable and favorable outcomes. Consequently, these conditions were selected for further investigations.

5.2. What is the effect of the addition of different concentrations of glacially derived dust on biofilm growth?

5.2.1. Results of the PHYTO-PAM measurement

The t test confirmed a significant change in chlorophyll after adding the glacier dust in the high dust (HD) group with (0.467 g) (t (3) = - 5.40, p = 0.016). The low dust group (LD) (0.004 g) showed no impact after the addition of the glacier dust (t (3) = - 1.67, p = 0.23). In summary, the biofilms in the HD group had higher chlorophyll levels after the treatment phase compared to the biofilms in the LD group (HD group: from M = 15.79, SD = 2.11 to M = 41.34, SD = 6.45) than those that received a smaller amount of glacier dust (LD group: from M = 13.04, SD = 1.03 to M = 17.58, SD = 5.66) (*Figure 13*).

These results were tested statistically using the TUKEY HSD test, with the HD group significantly different (p = < .001) and LD group non-significant (p = 0.61) after the treatment. The ANOVA indicated the effects of glacier dust on the chlorophyll levels as significant (F (3,5) = 4.066, p = <.001). The post-

hoc analysis revealed that the addition of glacier dust in higher concentration had a significant effect on chlorophyll growth in the HD group compared to the LD group (p = 0.61).

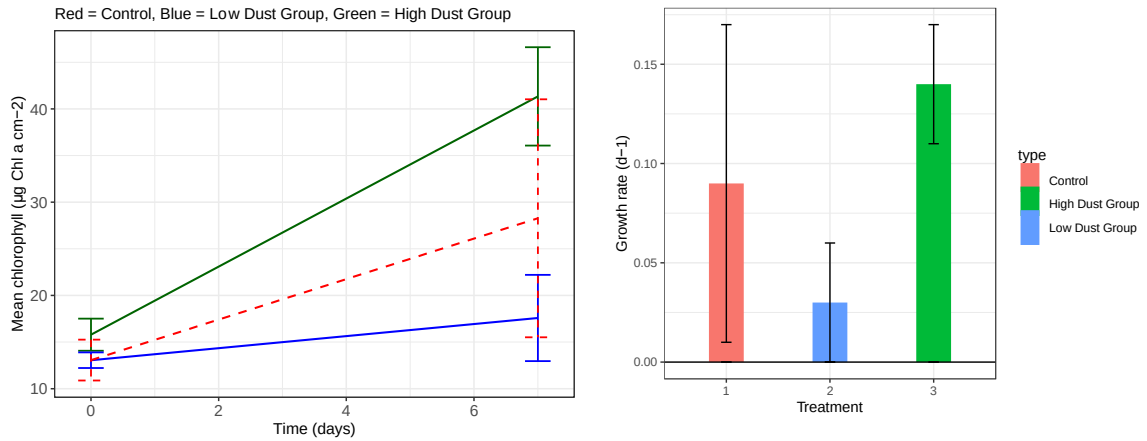

Figure 13: Left: The average chlorophyll content (µg Chl a cm-2) before and after the glacier dust treatment is depicted for three groups: the HD group, the LD group, and the control group. On the right side, the growth rates (d-1) within each of these groups, both before and after the treatment, are presented.

In summary, the results indicate that higher concentrations of glacier dust led to increased chlorophyll levels and therefore a higher growth rate in the biofilm compared to lower dust concentrations.

5.2.2. Results of the High-Performance Liquid Chromatography (HPLC) measurement

The HPLC results showed a response of the biofilm to the addition of glacier dust, as well as an increasing growth rate. After the addition of glacier dust in the HD group, a significant influence was observed in the chlorophyll levels (M = 5.99E-05, SD = 1.04599E-05) compared to before (M = 1.68E-05, SD = 8.3976E-06) (t (2) = -17.09, p = 0.003) (*Figure 14*). In the LD group, as in the PHYTO-PAM results, no effect of the glacier dust treatment on the biofilms were detected (t (1) = -1.08, p = 0.46). The average chlorophyll values before (M = 1.29E-05, SD = 1.01672E-05) and after treatment (M = 1.31E-05, SD = 5.8553E-06) revealed only slight differences.

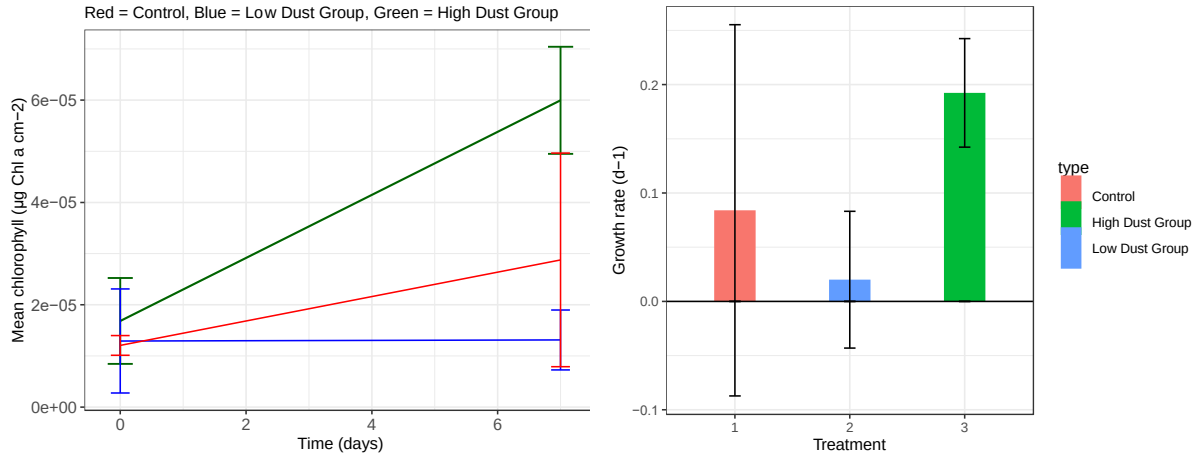

Figure 14: HPLC results. Left: The average chlorophyll content (µg Chl a cm-2) before and after the glacier dust treatment is depicted for three groups: the HD group, the LD group, and the control group. On the right side, the growth rates (d-1) within each of these groups, both before and after the treatment, are presented.

The results of both test methods showed a similar trend in the HD group, where the addition of glacier dust led to higher chlorophyll results. Both test methods showed a high variance of growth in the groups. In the LD group, more growth was observed with the HPLC method compared to the control group, which was not evident in the PHYTO-PAM analysis. However, a growth rate was detected in all groups, regardless of whether glacier dust was present or not.

5.2.3. Results in biofilm composition change

Figure 15 shows the percentage changes of the different taxa with glacier dust treatment compared to the control group. The percentage change was calculated using the following formula: Percent change = (value - control value) / control value * 100. In this case, the percentage change is calculated in relation to the control value itself and not the change between the initial and final value, as is the case with the chlorophyll a metric. These percentage changes represented the deviation from the control group, with negative values representing a decrease and positive values representing an increase in the various taxa. In these calculations, it was assumed that the control group represents the baseline with a value of 100.

In the control group, the taxa Chlorophyta and filamentous Cyanobacteria were mainly found, followed by Streptophyta and Dinoflagellata and then Bacillariophyceae (Diatoms), which accounted for the smallest proportion. After one week, the samples showed empty cells, partly no spots with organisms and generally less diversity than before. In addition, a new, unidentifiable species was discovered that resembled Spirogyra, but was narrower, more elongated and had alternating black and white compartments (*Figure 16*).

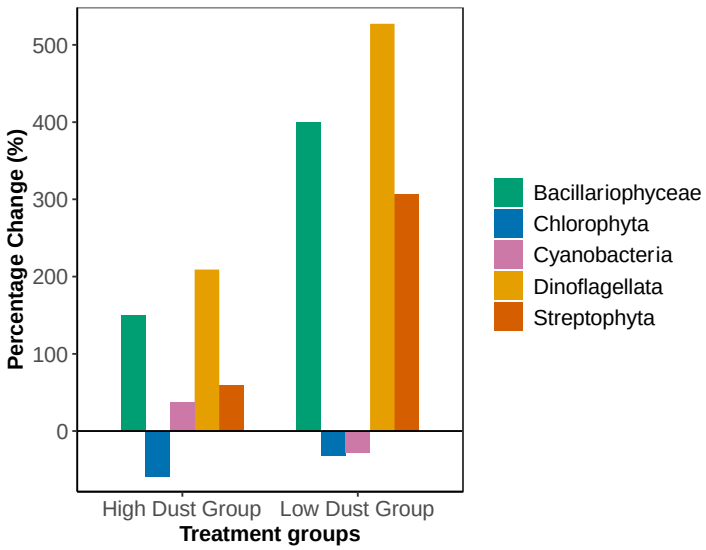

Figure 15: Main taxa composition after the glacier dust treatment compared to the control group (100%) in percentage change.

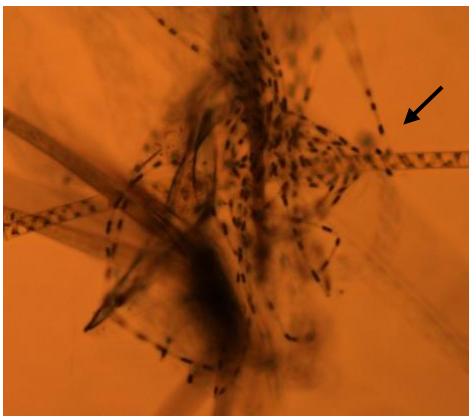

Figure 16: Unidentified species, similar to Spirogyra, but narrower, more elongated with alternating black and white compartments (Magnification 20 x).

In comparison to the control group, the HD group showed a shift towards the taxa Dinoflagellata and Bacillariophyceae (Diatoms) and a decrease in chlorophytes. The Cyanobacteria consisted mainly of the genus *Gloeotrichia* (Order Nostocales), which had not been found in the samples before. In addition, they accumulated at one point and formed accumulation sites with

Chlorophyta, mostly with the genus of *Gloeocystis* and/ or *Dictyosphaerium* (*Figure 16*). *Figure 17* presents the percentage changes in species diversity for the HD group compared to the control group and the LD group. It was noticeable that the overall species diversity in the HD group had decreased, as the values of several species had decreased compared to the control group and the LD group. This suggests a possible impact on species diversity within the HD group after treatment, as indicated by the observed declining trends.

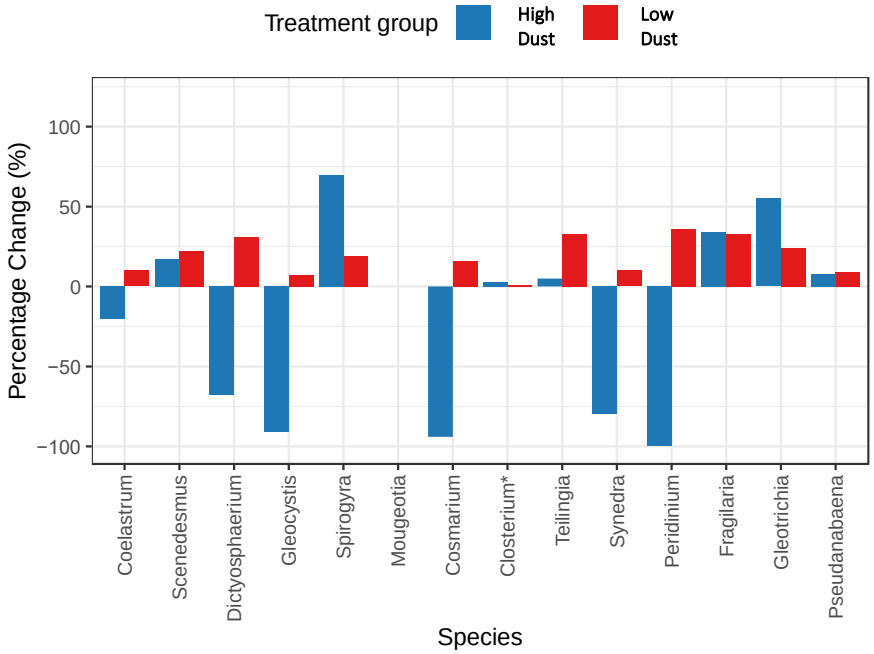

Figure 17: Detailed biofilm composition after the glacial dust treatment compared to the control group (100%) in percentage.

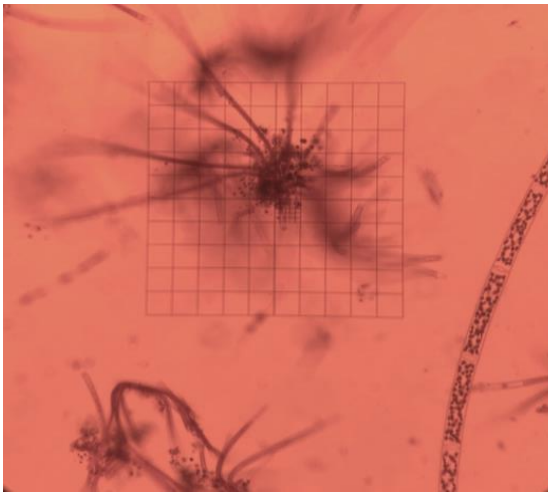

Figure 18: Gloeotrichia accumulation sites with Chlorophyta, mostly with the genus of Gloeocystis and/ or Dictyosphaerium.

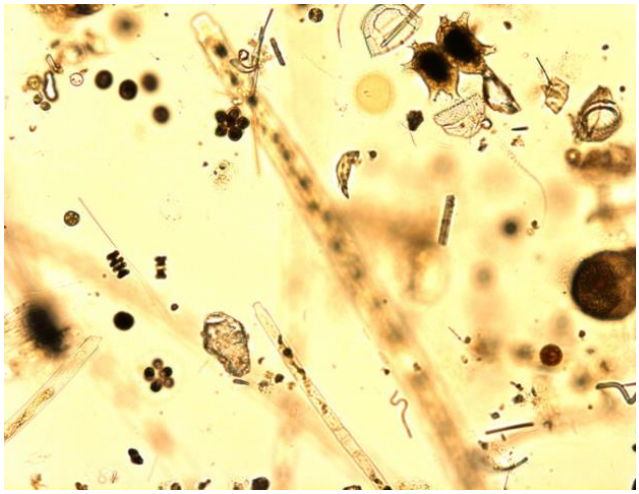

*Figure 19: LD group showed high diversity compared to HD group. Image by Microscope Imaging with Cell*D.*

The LD group showed an increase in all previously mentioned groups after the treatment, resulting in highest biodiversity compared. The general composition tended towards Dinoflagellata and Bacillariophyceae (Diatoms), followed by Streptophyta (green algae), and finally a decrease in Chlorophyta and Cyanobacteria. Within the taxon of Bacillariophyceae, many *Synedra* and *Fragilaria* were identified, which did not appear in the control group or represented only a small proportion. The

Cyanobacteria accounted for the smallest proportion and decreased compared to the other groups. Similarly, the Cyanobacteria showed characteristic clustering at specific locations with *Gloeotrichia*, but to a reduced extent. As in the HD group, the unidentifiable long organisms similar to *Spirogyra* were found, as well as apparently empty short sticks and empty cells.

The results indicate that the introduction of a small amount of glacial dust had a stronger effect on the community composition than larger amounts. This effect led to a clear transition from chlorophytes to a new dominant composition consisting mainly of dinoflagellates and diatoms.

5.2.4. Results of the biomass change

All groups studied indicated an increase in dry weight after one week of incubation. The weight of glacier dust was calculated in *Table 2* and subtracted from the weight of biomass to obtain accurate measurements. For the HD group, a weight of 0.019 g was subtracted, while for the LD group a weight of 0.00016 g was subtracted. This correction ensured that the biomass measurements reflected the actual biological components without the contribution of the added glacial dust particles. Compared to the control group, the filters of the HD group appeared to be more grey, with many particles, whereas the LD group appeared to be more green and had fewer particles. Considering the HD group before (M = 37.53, SD = 0.49), a significant increase in dry weight after the one-week treatment (M = 47.93, SD = 1.6) was observed (t (2) = -10.47, p = .009). When calculating the growth rate in the HD group, the results showed an average gain of 0.034 g per day (*Figure 20*).

The dry weight of the samples from the LD group showed an average of 36.95 g (SD = 0.37) and increased slightly after one week to 37.44 g (SD = 0.45). No significance was found in the execution of the t test (t (2) = -3.63, p = 0.07). The t test was performed to assess whether the observed differences in the dry weight of samples from the LD group, before and after one week, were statistically significant. The growth rate per day of the LD group proved to be slightly higher (0.002 g) compared to the control group (0.001 g).

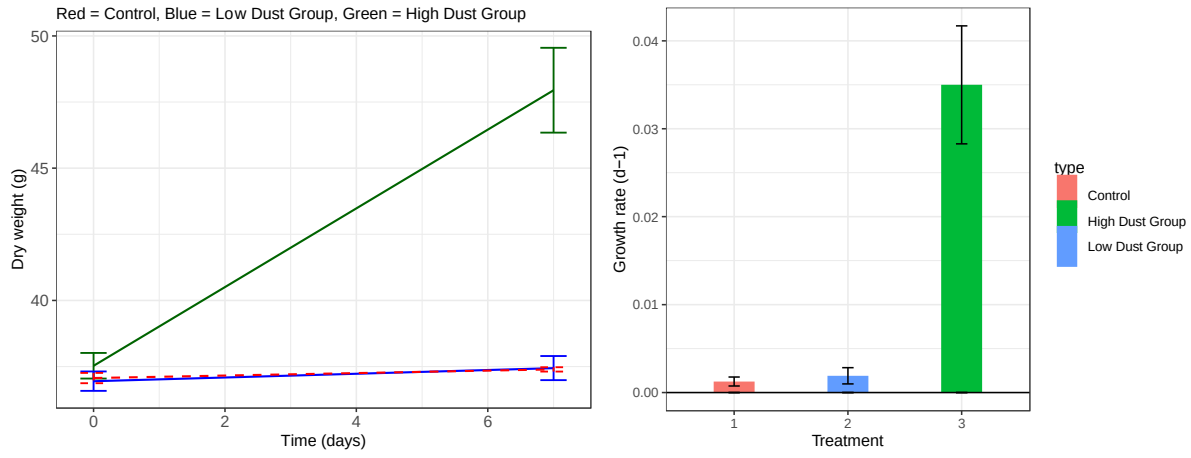

Figure 20: On the left side are the changes in dry weight after one week (before and after glacier dust treatment). The right side shows the biomass growth rate (d-1) both before and after treatment.

5.2.5. Results of the nutrient analysis with the Autoanalyzer 500

ANOVA was used to determine differences between the means of the HD groups, the LD group, and the control group. The analysis of the alteration in nitrate levels before and after the dust treatment revealed no statistically significant differences (F (2,15) = 3.01, p = 0.08) (*Figure 21*).

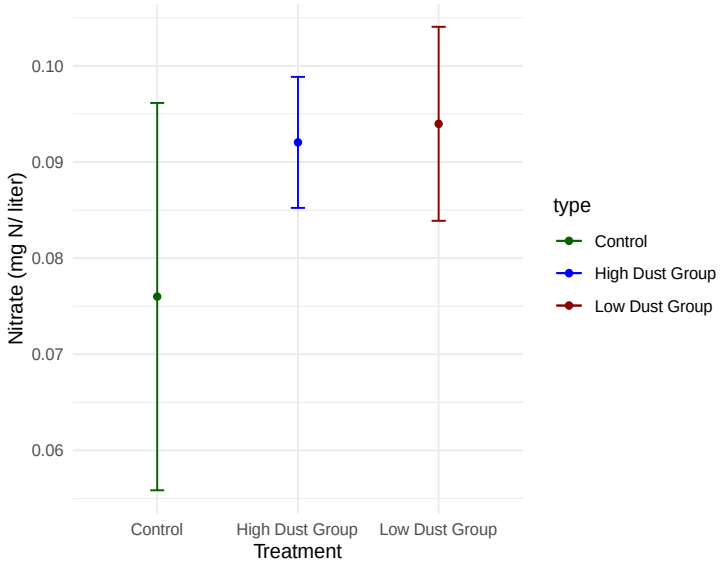

Figure 21: Nitrate results. Shows the results of the water quality tests (10 mL) obtained from the biofilm samples, highlighting in particular the presence of nitrate in different treatments.

Similarly, no significant change could be detected in the phosphate (f (2,15) = 0.11, p = 0.9) and ammonium values (f (2,15) = 0.13, p = 0.88) (*Figure 22).*

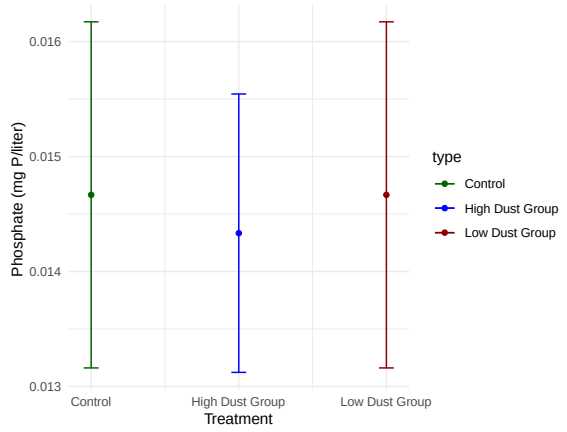

Figure 21: Ammonia results. Shows the results of the water quality tests (10 mL) obtained from the biofilm samples, highlighting in particular the presence of ammonia in different treatments.

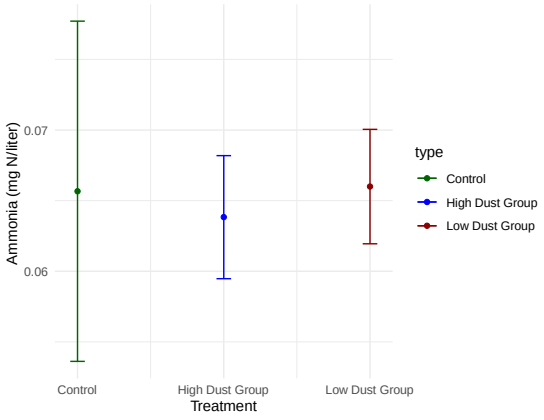

Figure 22: Phosphate results. Shows the results of the water quality tests (10 mL) obtained from the biofilm samples, highlighting in particular the presence of phosphate in different treatments.

The HD group displayed a reduction in the average nitrate content (M = 0.076 mg N/liter, SD = 0.02) after treatment compared to the control group (M = 0.094 mg N/liter, SD = 0.01). No change in phosphate and ammonia contents was observed (M_p = 0.015 mg P/liter, SD = .002, M_A = 0.066 mg N/liter, SD = 0.012). The LD Group recorded only a slight reduction in nitrate content compared to HD group and control group (M = 0.092 mg N/liter, SD = 0.06). The phosphate and ammonium concentrations changed only very marginally (M = 0.014 mg P/liter, SD = .001) (M = 0.064 mg N/liter, SD = 0.04).

6. Discussion

The inevitable melting of glaciers and the release of sediment particles will lead to increased dispersion of dust particles in the environment. The consequences of this on the surrounding ecosystems are not yet fully understood. By studying the effects of glacier dust in simulated habitats with biofilms, new insights into biofilm handling and the dynamics of growth, biomass and chlorophyll rates could be analyzed, providing a better understanding of future ecosystem dynamics.

6.1. Pilot study

6.1.1. The impact of tray position in the pond

In summary, the results indicate that no statistically significant differences in biofilm growth were observed between the two sides of the pond. Nevertheless, it is important to note that the study took place in April and that possible variations in environmental conditions related to the time of year could have influenced biofilm growth. For example, variations in light incidence due to the position of the sun in April could have contributed to the differences between the pond sides. Previous studies have demonstrated that biofilm responses can vary significantly depending on the season, highlighting the importance of considering seasonal factors (Schmidt et al., 2016). This study did not include comparisons with other seasons, meaning it can only suggest a tendency in the outcomes without making direct comparisons to other seasonal data. Further investigations in different seasons would provide a more comprehensive understanding of the seasonal variability in biofilm growth. The development time of the biofilm is a crucial factor that should be considered. While a period of 12 days was chosen in this study, a longer development period of one month could be more reliable. With different development times, other biotic communities can possibly be detected in the biofilm.

Future studies should consider the influence of the sample locations, especially given the significant differences observed, for example, in tray position 1 (close to the wall) and position 3 (open orientation to the pond). Factors and conditions contributing to these different orientations should be studied to ensure a more comprehensive understanding of biofilm dynamics in similar aquatic environments. On both sides of the pond, tray 1 was closer to the wall, where more algae were present and therefore possibly showed higher growth, suggesting that the proximity to the algae source may have promoted biofilm development. Position 3, facing the open pond side, was characterized by less algal growth, but possibly allowing the trays to receive more sunlight, which favored biofilm growth (Laderriere et al. 2022). However, the position of the microscope slides (A-D) within the trays on both pond sides did not show significant effects on biofilm growth and it can be assumed that the biofilm grows randomly after attaching to a substrate. Overall, these findings highlight the importance of the strong influence of ecosystem characteristics, including physical, chemical, and biological factors, on biofilm responses (Pesce et al., 2009; Laderriere et al. 2022). Future biofilm research should consider the complex dynamics of the cultivation system. In the present study, these dynamics were not taken into account, which is a limitation that restricts the formulation of precise conclusions. It is therefore advisable to include seasonal variations, light intensity and angle of incidence, potential competition between different biofilm growth areas within the pond, nutrient availability, and temperature fluctuations. This approach can contribute to a more comprehensive understanding of biofilm growth patterns and behaviors.

6.1.2. Comparative analysis of biofilm removal methods

The results for the different biofilm removal techniques showed the presence of random variability in growth when comparing chlorophyll content between the top and bottom of the slides. This variability made it difficult to assess significant differences ($t(35) = 0.63$, $p = 0.53$), and when variability is high, the use of destructive sampling techniques based on inter-sample comparisons becomes difficult. These results highlight the fact that biofilm growth can show considerable variability even within a single slide. It is advisable to include multiple samples and replicates in future studies in order to effectively account for variability and avoid confounding results.

Removal methods should be considered when treating biofilms, as shown by the results of this study ($F(3,32) = 9.67$, $p < 0.001$). The Tukey HSD test showed significant differences between the vertical and horizontal removal methods ($t(3) = 15.95$, $p = 0.01$) and between the horizontal and diagonal removal methods ($t(3) = 18.63$, $p = 0.001$). No significant difference was observed between the vertical and diagonal removal methods, suggesting that both methods could have been used to remove the biofilm. At the end, the vertical removal method was selected as it proved to be more practical and adaptable. Furthermore, the vertical removal method allowed a more uncomplicated and more precise technique, as it offered better grip and minimized the risk of dragging the entire biofilm with the removal process. In contrast, the horizontal removal method often resulted in incomplete separation of the slimy biofilm, making it challenging to perform and potentially leading to contamination from the other side.

Furthermore, the different treatment methods underline the random character of biofilm formation, as biofilms usually develop when they encounter suitable surfaces for attachment (Steenackers et al., 2012; Schnurr & Allen, 2015). This observation suggests that biofilm growth is characterized by randomness rather than a specific directional preference. In view of this, few replicates were carried out in this study, therefore it would be of interest to perform more in the future and repeat the removal methods to obtain more meaningful and reliable results.

6.1.3. Optimization of incubation parameters for biofilm growth

In this sub-experiment, it was focused on investigating various parameters that contribute to optimal biofilm growth in the incubator. Temperature did not significantly affect biofilm development in demineralized water and filtered pond water. Although the ANOVA results gave insignificant results, it remains to be mentioned that the nutrient-rich medium showed increased chlorophyll activity at 16 °C. Considering the lack of experience in dealing with biofilms, especially with the transfer process from the pond to the laboratory, it was initially considered to use the nutrient-rich medium to ensure the survival and growth of the biofilms. However, after the successful transfer of the biofilm with filtered pond water alone, the medium was no longer applied. This was also because the focus was on a nutrient-poor habitat. To achieve the goal of simulating the ecosystem of the Kangerlussuaq region in West Greenland, the original plan was to develop a low-nutrient medium for biofilm development. This would have allowed a more detailed analysis of how glacier dust provides important nutrients and promotes biofilm growth. Unfortunately, it was not possible to implement the application of the low-nutrient medium originally intended for the simulation, as an unforeseen reduction in the growth rate was detected, which ultimately led to a deviation from the original simulation approach. The filtered pond water maintained the natural growth and simulated their habitat condition to the pond.

Based on the results of the study by van der Grinten et al. (2004), a temperature of 16 °C and overall lower light intensities were chosen for biofilm growth. According to van der Grinten et al. (2004),

diatoms prefer colder seasons, while cyanobacteria dominate in summer or autumn. From these findings, it was decided to create conditions that would mainly favor diatom growth. Diatoms showed less density increase at higher light intensities (200 µmol m^2 s^{-1}) than at lower intensities. Although diatoms can grow in a wide range of light intensities, high light intensities promoted the dominance of cyanobacteria (O'Neil et al., 2012, Zhao et al., 2018). Therefore, the preset light intensity of 52.13 µmol m^2 s^{-1} was chosen to create a habitat that favored diatom growth and prevented cyanobacteria dominance (van der Grinten et al., 2004). In contrast, the results of the study by Zhao et al. (2018), showed the highest values for biofilm biomass and chlorophyll a at moderate temperature (25 °C). Both high and low temperatures appeared to inhibit biofilm proliferation. From this it can be concluded that other factors also have an influence on the development of biofilms, as sufficient results were found at 16 °C in this study. Nevertheless, it is recommended to carry out investigations using several temperatures and media that provide new information on the optimal formation and preferences of biofilms. It should be noted that the biofilm sample was cultured in a freshwater pond and not in a controlled laboratory environment. This difference could potentially affect the composition of the biofilm from the beginning, as environmental factors and the natural variability of the pond introduced additional complexity. In addition, the quality and characteristics of the biofilms could not be determined during growth.

The pilot study served as a learning phase to explore and improve the methodology with biofilms and to establish necessary methods for the successful growth and maintenance of biofilms in the laboratory environment. In summary, the results provided valuable insights into spatial variations and position preferences in biofilm growth. It was shown that biofilms had the ability to randomly establish and colonize the microscope slides (Azeredo et al., 2016). The findings demonstrated the significance of considering multiple ecosystem characteristics and parameters when assessing biofilm responses to environmental conditions and glacial gust. Future studies should consider growing biofilms in different seasons and increasing the number of replicates to obtain more robust and meaningful results regarding biofilm variability.

6.2. Final study: Exploring the effects of glacier dust treatment on biofilms.

The final study aimed to investigate the hypothesis regarding the fertilizing effect of glacier dust on biofilm growth and the possible shift in composition from chlorophytes to cyanobacteria. To investigate this, the biofilms were exposed to two different dust concentrations: The HD group with a higher dust concentration and LD group with a lower dust concentration. In the following sections, the results of the microscopic examination, the effects on biomass and nutrient changes after treatment with glacier dust were discussed to test the hypothesis regarding the influence of glacier dust on the biofilm habitat.

When assessing algal biomass with two different measurement methods, the results of the PHYTO-PAM and HPLC, showed a similar trend in the HD group, indicating that the addition of glacier dust resulted in increased biofilm response. However, a high variability of growth between the different groups was observed with both measurement methods. As discussed in the pilot study, the growth of biofilms proved to be random and could vary even within one slide side. Interestingly, more growth was detected in LD group using the HPLC method compared to the control group, which was not the case with the PHYTO-PAM analysis. This difference could be attributed to the higher sensitivity of the HPLC method in detecting low chlorophyll concentrations in the samples (Thermo Fisher Scientific - IE, n.d.).

Despite the observed differences, the responses in LD group can be explained with following observation. Prater et al. (2022) described Katabatic winds, which blow away from the ice sheets contribute to frequent dust storms. However, the strength of these winds weakens as they move away from the ice sheet, typically about 10 km away (Bullard & Mockford, 2018). As a result, there is less dust input in areas further away from the ice sheet, which may explain lower total phosphorus (TP) levels and limited microbial growth due to phosphorus (P) limitation (Brutemark et al., 2006; McCutcheon et al., 2021). The assumption that biofilm growth in the LD group was limited by nutrient availability was confirmed by the fact that low doses of glacier dust did not cause a significant response in this study ($p = 0.23$). The expectation was that the limited nutrient content in LD group due to the lower dust concentration limited biofilm growth and resulted in a lower effect compared to HD group.

The results of this study supported the hypothesis that glacier dust stimulates biofilm growth, as evidenced by the observed growth rate in the HD group. However, the stimulating effect seemed to be dependent on the addition of glacier dust. Enhanced biofilm growth promoted by glacial dust may have implications for processes such as increased melting and the possible spread of algal blooms on ice sheets. This is because mineral dust can promote the development of glacial algal blooms by providing P to supraglacial algal communities (Uetake et al., 2010; McCutcheon et al., 2021). In addition, it can be assumed that glacier dust particles could serve as a physical substrate for the attachment and colonization of new biofilms (Lutz et al., 2014).

6.2.1. Impact of glacial dust treatment on biofilm composition

The microscopic evaluation did not confirm the initial hypothesis of a shift towards cyanobacteria in the composition of the biofilm. Instead, the analysis revealed a shift towards dinoflagellates, diatoms and streptophytes compared to the control group. It was expected that cyanobacteria would dominate the biofilm after the addition of dust due to their ability to fix nitrogen (McCutcheon et al., 2021; Prater et al, 2022). Cyanobacteria can transform atmospheric nitrogen gas into biologically useful ammonia through specialized enzymes called nitrogenases and can thereby contribute nutrients to ecosystems (El-Seedi et al., 2023). However, their growth decreased in contrast to the control group. This unexpected finding could be attributed to various factors, including the presence of cell residues, and dissolved organic matter from algae, which may have influenced the growth dynamics of the biofilm community (Northington et al., 2019; Prater et al., 2022). Additionally, factors such as nutrient availability, sediment composition, and light penetration could have influenced the observed composition changes (Mills et al., 2004; Guieu et al., 2014; Zhang et al., 2019; Northington et al., 2019).

Finally, the dominance of dinoflagellates was observed in both groups, which can be attributed to a sufficient supply of nutrients in the samples (Malik & Saros, 2016; Northington et al., 2019). The presence of dinoflagellates can be explained by their ubiquitous occurrence, whether in freshwater or marine environments. They can adapt pelagic and benthic habitats, living freely, parasitically or in an endosymbiosis. In addition, dinoflagellates have the ability to lead a heterotrophic lifestyle, which could potentially be advantageous in a biofilm environment. In such an environment, which is rich in bacteria, the dinoflagellates could use their ability to consume these bacteria to feed (Hackett et al. 2004). In general, freshwater biofilms are frequently inhabited by typical genera such as diatoms and *Scenedesmaceae*/ *Desmidiaceae* (green algae, Chlorophyceae) (Amaral-Zettler et al., 2020; Di Pippo et al.,2022). These findings align with the identification in the analyzed samples, further confirming their presence within biofilms. The coexistence of dinoflagellates, diatoms, and streptophytes within the

biofilm samples can be also explained by their preference for similar environmental conditions and their ability to occupy overlapping ecological niches. This phenomenon can be observed among phototrophic algal families, as they adapt and thrive in similar habitats (Di Pippo et al., 2022).

After one week of treatment, cell residues and dissolved organic matter from algae were observed, particularly in the HD group. This condition probably provided a favorable environment for heterotrophic organisms such as dinoflagellates, which could use these organic substances as a food source. This finding, along with the lower diversity in the biofilm composition, could provide an explanation for the increase in nitrogen-fixing cyanobacteria and mixotrophic algae (dinoflagellates) in the samples (Northington et al., 2019; Prater et al., 2022). Unexpectedly, the HD group had lower diatom production compared to the LD group. Previous studies by Prater et al. (2022) suggest that diatom biomass increases in lakes closer to the ice sheet and sandurs due to phosphorus-rich sediments. In this case, HD group, which received a greater amount of glacial dust and may be closer to the sandurs in the real case, had lower diatom production than LD group. The observed discrepancy in diatom production between the two groups may suggest that factors other than glacial dust played a role in shaping the algal community. Relating it back to a the Kangerlussuaq region nutrient composition and availability in lakes may possess other influential factors that determine algal community composition (Northington et al., 2019; Prater et al., 2022). As nutrients are mainly stored in the benthic areas, these play an important role in diatom production (Vadeboncoeur et al. 2002; Whalen et al. 2008).

Furthermore, light availability may have been another factor that influenced algal composition. It is important to note that the presence of dust on the filters could have resulted in reduced light availability, which could have affected the growth of photosynthetic organisms such as chlorophytes in these samples. The reduced light penetration caused by dust coverage could have hindered their photosynthetic activity and subsequently influenced their growth dynamics in the biofilm community (Northington et al., 2019; Prater et al., 2022). However, it can be assumed that the larger filamentous species had a competitive advantage as they could grow far above the dust layer, giving them access to more light resources and thriving despite the lower light availability below.

Previous studies conducted in marine ecosystems have demonstrated that dust input in nutrient-rich and chlorophyll-poor areas can trigger phytoplankton blooms, particularly benefiting diatoms (Boyd et al., 2007). Given that the current study examined the impact of glacial dust obtained from filtered pond water, which can be presumed to be nutrient-rich, it is possible that future occurrences may result in increased algal blooms, primarily driven by diatoms and dinoflagellates. Rapid climate change and glacial melt have the potential to accelerate this process, resulting in the rapid release of nutrients into the environment through the dispersion of dust particles. These nutrients support algal growth, which can have significant impacts on existing ecosystems. As a result, these ecosystems may be subject to increased stress and experience changes in their composition and dynamics (Boyd et al., 2007; Northington et al., 2019). In marine regions characterized by low nutrient and chlorophyll levels, dust deposition can have a direct impact by providing essential nutrients, stimulating nitrogen fixation, and influencing phytoplankton growth (Mills et al., 2004; Guieu et al., 2014; Zhang et al., 2019). Additionally, dust particles contain trace metals like manganese (Mn) and cobalt (Co), which can further contribute to the promotion of phytoplankton growth (Sunda, 2012). In the context of the Kangerlussuaq case study, the increased deposition of dust can be expected to support phytoplankton growth and potentially lead to the formation of phytoplankton blooms.

In general, the presence of glacier dust has been observed to impact the composition of biofilms. Higher exposure to dust has been associated with a decrease in biodiversity, potentially

attributable to increased nutrient competition among organisms and light availability, leading to the displacement of certain species through possibly interference competition (van der Grinten et al., 2014). Conversely, lower concentrations of dust have been found to exert positive effects on biofilm biodiversity, facilitating the growth of multiple organisms. Consequently, the concentration of dust input plays a crucial role in determining the influence on biofilm composition, with higher concentrations potentially limiting biodiversity and lower concentrations promoting it.

In this study, the algal composition of the biofilms was investigated qualitatively. However, to gain more detailed insights into the effects on biovolume and cell density, it is recommended to conduct a quantitative study. A quantitative analysis would provide a more detailed understanding of the changes in algal abundance after glacier dust treatment in biofilms.

6.2.2. Influence of glacier dust on biofilm biomass

The objective of this sub-experiment was to examine the influence of glacial dust on biomass development and assess its potential impact. The one-week treatment, indicated in both groups an increase in dry weight. Visual observations of the filters revealed that the HD group had a greyer appearance with numerous particles, while the LD group appeared greener and had fewer particles. This can be concluded with the different amount of dust input on the biofilms. Also, the results suggest a greater appearance in green algae (Streptophyta) in the LD group, such as desmids, which explain the green coloration.

Regarding the dry weight, a significant increase was observed in the HD group (before: M = 37.53, SD = 0.49; after: M = 47.93, SD = 1.6) ($t(2) = -10.47$, $p = .009$). The growth rate in the HD group was calculated to be an average gain of 0.034 g per day. The contribution of Dinoflagellata taxa, known for their relatively large size, probably played an important role in the total biomass, as their size can range from 14 to 20 µm (Franzè & Menden-Deuer, 2020; Fulfer et al., 2021). In addition, the presence of empty or dead cells in the samples probably also contributed to the total weight.

In the LD group, the dry weight of the samples had an average of 36.95 g (SD = 0.37) and slightly increased to 37.44 g (SD = 0.45) after one week. However, there was no significant difference found in the t-test analysis. The growth rate per day in the LD group was slightly higher (0.002 g) compared to the control group (0.001 g). As expected, the LD group had a lower weight than the HD group. This was to be assumed as the chlorophyll rate measured with PHYTO-PAM and HPLC was lower in the LD group than in the HD Group. However, despite the lower chlorophyll rate, the samples in the LD group had a higher weight than the control group, which can be attributed to the increase in total biomass through the glacial dust input.

The biomass of biofilms is considered to be a reliable indicator of long-term contaminant effects. However, it may not be sensitive enough to detect subtle variations in biofilm responses. (Sabater et al., 2007; Laderriere et al., 2022). Nevertheless, in the present study, a significant change in biofilm biomass was observed in the HD group, suggesting that the biofilms responded to the addition of glacier dust. This finding is consistent with other results of the study, further supporting the effects of glacier dust on biofilm dynamics. Sabater et al. (2007) indicated that biofilms react under changes in abiotic and biotic nature through their size and rapid growth rate, which can be observed here in the HD group. As a results, biofilms could function as early indicators of changes in the ecosystems and provide information on water quality and ecological status (Ramaraj et al., 2015; Balcázar et al., 2015).

Overall, the investigation of the biofilm biomass confirms the nutrient-supplying influence of glacier dust, leading to increased biomass accumulation. The observed increase in biomass is consistent

with the hypothesis that higher amounts of glacier dust serve as a nutrient source for biofilms, stimulating their growth and development.

6.2.3. Evaluating the influence of glacier dust on nutrient composition in water samples from biofilms

In general, there were no detectable changes in nutrient content after the glacier treatment when comparing the different groups in the water samples collected from the biofilms. However, it is worth noting that the nitrate content in the HD group decreased after treatment compared to the control group, while the LD group showed a slight decrease. However, the changes in phosphate and ammonium concentrations were minimal in all groups. Although previous studies suggest that phosphorus (P) and nitrogen (N) are important elements for diatom production, especially in areas closer to the ice sheet with higher dust input (Prater et al., 2022), the results showed no statistically significant differences in the amount of those between the groups. This suggests that the nutrients provided through the glacier dust treatment may have either been unavailable for uptake or quickly utilized by the biofilms (Lutz et al., 2014). Based on this, it can be assumed that biofilms can take on a dual role, as a sink for nutrients and a source for pollutants (Flemming et al.,2014).

In nutrient-poor regions, such as the numerous lakes in Kangerlussuaq, the presence of dust particles can bring important nutrients such as iron, nitrogen, and phosphorus, which are needed for the growth and development of chlorophyll-producing algae. These nutrients are often limiting factors and the deposition of glacial dust can serve as an additional source of nutrients (Mills et al., 2004). However, this could not be demonstrated in this study. This may be because the study period of one week was too short, and more replicates should have been used.

In the context of climate change, it can be assumed that the deposition of glacial dust in the Kangerlussuaq region will increase. This will have implications for the availability of nutrients in lakes, which in turn may influence algal community composition as they occur in a nutrient-dependent manner (Northington et al., 2019; Prater et al., 2022). In addition, the influence of temperature should be taken into account, as the samples in this study were collected in spring, when temperatures are generally higher. With the results, it could also be assumed that the combination of increased nutrient availability due to dust input and favorable temperatures in spring promotes biofilm growth (Laderriere et al., 2022). Despite the influence of the temperature, climate change effects on the carbon cycle and the connections between glacial, proglacial, aquatic, and terrestrial systems. Higher temperatures in spring and a longer melt season expand the melt zone and create favorable conditions for microbial colonization and growth. This leads to increased microbial production and fluxes of dissolved organic carbon (DOC) and particulate organic carbon (POC) into subglacial environments and glacial runoff streams (Prater et al., 2022). The composition of dust includes carbon as well, which would double the carbon input into the environment and could enhance benthic production even more (Prater et al., 2022).

As also shown in this study, the input of dust can have a fertilizing effect and influence the dynamics of biofilm growth. However, this effect could not be explained or confirmed by nutrient changes in the water. In the future, a comprehensive investigation of the nutrient dynamics within these biofilms should be conducted. Nevertheless, it can be assumed that dust deposition can lead to changes in sediment dynamics, which in turn can influence the availability of nutrients in lakes as a result of glacier advance and retreat (Anderson et al., 2017; Anderson et al., 2018).

7. Conclusion

In summary, this study aimed to investigate the hypothesis that glacier dust acts as a fertilizer on freshwater biofilms, leading to increased productivity, biomass production and a shift in community composition towards cyanobacteria. The results of this study should fill the knowledge gap regarding the effects of glacier dust on biofilm growth and dynamics. While the hypothesis that glacier dust causes a shift towards cyanobacteria was not confirmed by the microscopic evaluation, the study showed that higher amounts of glacier dust act as a nutrient supplier, leading to increased chlorophyll content and biomass production. This aspect of the hypothesis can therefore be confirmed. The results highlighted the complex interactions between glacier dust and biofilms.

The variability of trays and microscope slides position influenced biofilm growth, and removal methods played a crucial role in obtaining reliable chlorophyll values. Nevertheless, the initial discovery implies that growth is relatively random, with the biofilm only needing a substrate to attach and begin its growth. Through a detailed analysis of removal methods, the study settled on the most practical and reliable vertical removal approach to obtain similar results of initial and final chlorophyll content to minimize variance. In addition, the study identified optimal temperature and filtered pond water as suitable conditions for biofilm development. However, it is recommended to further research the effects of biofilm development applied in osmotic medium, as this would be more reliable for comparing the region in Kangerlussuag.

Lower concentrations of glacier dust showed no significant effects on chlorophyll levels or changes in biomass. However, they did affect the community structure of the biofilms, resulting in a greater diversity of organisms. Neither group showed a significant effect on nutrient dynamics, suggesting that the nutrients provided by the glacier dust were either rapidly consumed by the biofilms or unavailable for uptake. Overall, the study confirms that glacier dust serves as a source of nutrients in higher amounts and stimulates biofilm growth and productivity.

The results of this research have important implications for understanding the ecological consequences of glacial dust deposition in freshwater ecosystems, particularly in the context of glacier melt and climate change. They provide valuable insights into the potential impacts of glacier dust on aquatic habitats and contribute to the understanding of how these ecosystems might respond to future changes in glacier dynamics. However, further research is needed to fully understand the impacts on biofilm community composition and especially nutrient dynamics.

8. References

Amaral-Zettler, L. A., Zettler, E. R., & Mincer, T. J. (2020). Ecology of the plastisphere. *Nature Reviews Microbiology*, *18*(3), 139-151.

Anderson, N. J., Curtis, C. J., Whiteford, E. J., Jones, V.J., McGowan, S., Simpson, G.L., Kaiser, J., (2018). Regional variability in the atmospheric nitrogen deposition signal and its transfer to the sediment record in Greenland lakes. Limnol Oceanogr 63:2250–65.

Anderson, N. J., Saros, J. E., Bullard, J. E., Cahoon, S. M., McGowan, S., Bagshaw, E. A., ... & Yde, J. C. (2017). The Arctic in the twenty-first century: Changing biogeochemical linkages across a paraglacial landscape of Greenland. *BioScience*, *67*(2), 118-133.

Antoine, D., André, J. M., & Morel, A. (1996). Oceanic primary production: 2. Estimation at global scale from satellite (coastal zone color scanner) chlorophyll. *Global biogeochemical cycles*, *10*(1), 57-69.

Azeredo, J., Azevedo, N. F., Briandet, R., Cerca, N., Coenye, T., Costa, A. R., ... & Sternberg, C. (2017). Critical review on biofilm methods. *Critical reviews in microbiology*, *43*(3), 313-351.

Balcázar, J. L., Subirats, J., & Borrego, C. M. (2015). The role of biofilms as environmental reservoirs of antibiotic resistance. *Frontiers in microbiology*, *6*, 1216.

Blanckaert, A. C., Omanović, D., Fine, M., Grover, R., & Ferrier-Pagès, C. (2022). Desert dust deposition supplies essential bioelements to Red Sea corals. *Global Change Biology*, *28*(7), 2341-2359.

Boyd, P. W., Jickells, T., Law, C. S., Blain, S., Boyle, E. A., Buesseler, K. O., ... & Watson, A. J. (2007). Mesoscale iron enrichment experiments 1993-2005: synthesis and future directions. *science*, *315*(5812), 612-617.

Brutemark, A., Rengefors, K., & Anderson, N. J. (2006). An experimental investigation of phytoplankton nutrient limitation in two contrasting low arctic lakes. *Polar Biology*, *29*, 487-494.

Bullard, J. E., & Austin, M. J. (2011). Dust generation on a proglacial floodplain, West Greenland. *Aeolian Research*, *3*(1), 43-54.

Bullard, J. E., & Mockford, T. (2018). Seasonal and decadal variability of dust observations in the Kangerlussuaq area, west Greenland. *Arctic, Antarctic, and Alpine Research*, *50*(1), S100011.

Capron, E., Rasmussen, S. O., Popp, T. J., Erhardt, T., Fischer, H., Landais, A., ... & White, J. W. C. (2021). The anatomy of past abrupt warmings recorded in Greenland ice. *Nature Communications*, *12*(1), 2106.

Davey, M. E., & O'toole, G. A. (2000). Microbial biofilms: from ecology to molecular genetics. Microbiology and molecular biology reviews, 64(4), 847-867.

Di Pippo, F., Crognale, S., Levantesi, C., Vitanza, L., Sighicelli, M., Pietrelli, L., ... & Rossetti, S. (2022). Plastisphere in lake waters: Microbial diversity, biofilm structure, and potential implications for freshwater ecosystems. Environmental Pollution, 310, 119876.

Earle, S., (2015). *16.4 Glacial Deposition*. Pressbooks. https://opentextbc.ca/geology/chapter/16-4-glacial-deposition/.

El-Seedi, H. R., El-Mallah, M. F., Yosri, N., Alajlani, M., Zhao, C., Mehmood, M. A., ... & Shou, Q. (2023). Review of Marine Cyanobacteria and the Aspects Related to Their Roles: Chemical, Biological Properties, Nitrogen Fixation and Climate Change. *Marine Drugs*, *21*(8), 439.

Fairbridge, R., W., (2006). Deflation. In Kluwer Academic Publishers eBooks (pp. 246–247). https://doi.org/10.1007/3-540-31060-6_84

Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology, 14(9), 563-575.

Franzè, G., & Menden-Deuer, S. (2020). Common temperature-growth dependency and acclimation response in three herbivorous protists. *Marine Ecology Progress Series*, *634*, 1-13.

Fulfer, V. M., & Menden-Deuer, S. (2021). Heterotrophic dinoflagellate growth and grazing rates reduced by microplastic ingestion. *Frontiers in Marine Science*, *8*, 716349

Guieu, C., Dulac, F., Ridame, C., & Pondaven, P. (2014). Introduction to project DUNE, a DUst experiment in a low Nutrient, low chlorophyll Ecosystem. *Biogeosciences*, *11*(2), 425-442.

Guillard, R. R., & Lorenzen, C. J. (1972). Yellow-green algae with chlorophyllide C 1, 2. *Journal of Phycology*, *8*(1), 10-14.

Hackett, J. D., Anderson, D. M., Erdner, D. L., & Bhattacharya, D. (2004). Dinoflagellates: a remarkable evolutionary experiment. *American journal of botany*, *91*(10), 1523-1534.

Harrison, S. P., Kohfeld, K. E., Roelandt, C., & Claquin, T. (2001). The role of dust in climate changes today, at the last glacial maximum and in the future. *Earth-Science Reviews*, *54*(1-3), 43-80.

Hotaling, S., Lutz, S., Dial, R. J., Anesio, A. M., Benning, L. G., Fountain, A. G., ... & Hamilton, T. L. (2021). Biological albedo reduction on ice sheets, glaciers, and snowfields. *Earth-science reviews*, *220*, 103728.

Kelly, M. G., & King, L. L. (2019). As trees walking: the pros and cons of partial sight in the analysis of stream biofilms. *Plant Ecology and Evolution*, *152*(2), 120-130.

Laderriere, V., Morin, S., Eon, M., & Fortin, C. (2022). Vulnerability and tolerance to nickel of periphytic biofilm harvested in summer and winter. *Environmental Pollution*, *315*, 120223.

Lutz, S., Anesio, A. M., Jorge Villar, S. E., & Benning, L. G. (2014). Variations of algal communities cause darkening of a Greenland glacier. *FEMS Microbiology Ecology*, *89*(2), 402-414.

Malik, H. I., & Saros, J. E. (2016). Effects of temperature, light, and nutrients on five Cyclotella sensu lato taxa assessed with in situ experiments in arctic lakes. *Journal of Plankton Research*, *38*(3), 431-442.

McCutcheon, J., Lutz, S., Williamson, C., Cook, J. M., Tedstone, A. J., Vanderstraeten, A., ... & Benning, L. G. (2021). Mineral phosphorus drives glacier algal blooms on the Greenland Ice Sheet. *Nature communications*, *12*(1), 570

Mesple, F., Casellas, C., Troussellier, M., & Bontoux, J. (1995). Some difficulties in modelling chlorophyll an evolution in a high-rate algal pond ecosystem. *Ecological modelling*, *78*(1-2), 25-36.

Mills, M. M., Ridame, C., Davey, M., La Roche, J., & Geider, R. J. (2004). Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature*, *429*(6989), 292-294.

Northington, R. M., Saros, J. E., Burpee, B. T., & McCue, J. (2019). Changes in mixing depth reduce phytoplankton biomass in an Arctic Lake: Results from a whole-lake experiment. *Arctic, Antarctic, and Alpine Research*, *51*(1), 533-548.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful Cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334.

Pesce, S., Lambert, A. S., Morin, S., Foulquier, A., Coquery, M., & Dabrin, A. (2018). Experimental warming differentially influences the vulnerability of phototrophic and heterotrophic periphytic communities to copper toxicity. *Frontiers in Microbiology*, *9*, 1424.

PHYTO-PAM-II Phytoplankton & Photosynthesis Analyzer | WALZ. (n.d.). https://www.walz.com/products/chl_p700/PHYTO-PAM/introduction.html

Prater, C., Bullard, J. E., Osburn, C. L., Martin, S. L., Watts, M. J., & Anderson, N. J. (2022). Landscape Controls on nutrient stoichiometry regulate lake primary production at the margin of the Greenland Ice Sheet. Ecosystems, 25(4), 931-947.

Ramaraj, R., Tsai, D. D. W., & Chen, P. H. (2015). Biomass of algae growth on natural water medium. *Journal of Photochemistry and Photobiology B: Biology*, *142*, 124-128.

Roy, S., Llewellyn, C. A., Egeland, E. S., & Johnsen, G. (Eds.). (2011). *Phytoplankton pigments: characterization, chemotaxonomy and applications in oceanography*. Cambridge University Press.

Sabater, S., Guasch, H., Ricart, M., Romaní, A., Vidal, G., Klünder, C., & Schmitt-Jansen, M. (2007). Monitoring the effect of chemicals on biological communities. The biofilm as an interface. *Analytical and bioanalytical chemistry*, *387*, 1425-1434.

Schmidt, H., Thom, M., King, L., Wieprecht, S., & Gerbersdorf, S. U. (2016). The effect of seasonality upon the development of lotic biofilms and microbial biostabilisation. *Freshwater Biology*, *61*(6), 963-978.

Schnurr, P. J., & Allen, D. G. (2015). Factors affecting algae biofilm growth and lipid production: A review. *Renewable and Sustainable Energy Reviews*, *52*, 418-429.

Schnurr, P., Espie, G., Allen, G., (2013). Algae biofilm growth and the potential to stimulate lipid accumulation through nutrient starvation. Bioresour Technol 136:337–344

Schüpbach, S., Fischer, H., Bigler, M., Erhardt, T., Gfeller, G., Leuenberger, D., ... & Wolff, E. W. (2018). Greenland records of aerosol source and atmospheric lifetime changes from the Eemian to the Holocene. *Nature communications*, *9*(1), 1476.

Soreghan, G. S., Pfeifer, L. S., Sweet, D. E., & Heavens, N. G. (2022). Detecting upland glaciation in Earth's pre-Pleistocene record. *Frontiers in Earth Science*, *10*, 904787.

Steenackers, H. P., Parijs, I., Foster, K. R., & Vanderleyden, J. (2016). Experimental evolution in biofilm populations. *FEMS microbiology reviews*, *40*(3), 373-397.

Sunda, W. G. (2012). Feedback interactions between trace metal nutrients and phytoplankton in the ocean. *Frontiers in microbiology*, *3*, 204.

Thermo Fisher Scientific - IE. (n.d.). *UltiMate 3000 UHPLC and HPLC Manuals and Quick Installations.*https://www.thermofisher.com/co/en/home/industrial/chromatography/chromatography-learning-center/liquid-chromatography-information/liquid-chromatography-resources/hplc-manuals-quick-installation-guides/ultimate-3000-uhplc-manuals-quick-installation-guides.html.

Újvári, G., Klöti, U., Stevens, T., Svensson, A., Ludwig, P., Vennemann, T., ... & Formenti, P. (2022). Greenland ice core record of last glacial dust sources and atmospheric circulation. Journal of Geophysical Research: Atmospheres, 127(15), e2022JD036597.

van der Grinten, E., Janssen, A. P., de Mutsert, K., Barranguet, C., & Admiraal, W. (2005). Temperature-and light-dependent performance of the cyanobacterium Leptolyngbya foveolarum and the diatom Nitzschia perminuta in mixed biofilms. Hydrobiologia, 548, 267-278.

van Soest, M. A., Bullard, J. E., Prater, C., Baddock, M. C., & Anderson, N. J. (2022). Annual and seasonal variability in high latitude dust deposition, West Greenland. Earth Surface Processes and Landforms.

Zhang, C., Yao, X., Chen, Y., Chu, Q., Yu, Y., Shi, J., & Gao, H. (2019). Variations in the phytoplankton community due to dust additions in eutrophication, LNLC and HNLC oceanic zones. *Science of the Total Environment*, *669*, 282-293.

Zhang, L., Delgado-Baquerizo, M., Hotaling, S., Li, Y., Sun, X., Xu, Y., & Chu, H. (2023). Bacterial diversity and co-occurrence patterns differ across a worldwide spatial distribution of habitats in glacier ecosystems. *Functional Ecology*.

Zhao, Y., Xiong, X., Wu, C., Xia, Y., Li, J., & Wu, Y. (2018). Influence of light and temperature on the development and denitrification potential of periphytic biofilms. *Science of the Total Environment*, *613*, 1430-1437.

9. Appendix

Appendix A: WC Medium

Table 3: WC-Stock prepared from NIOO KNAW (1 g in 1 L) (Source: NIOO Operating Protocol: Recipe WC Medium)

Major nutrients	*mg/ L*	*Trace elements*	*mg/ L*	*Vitamins*	*mg/ L*
NaNO3	85.01	Na2EDTA*2H2O	4.36	Biotin B1	0.00005
*MgSO4*7H2O*	36.97				
CaCL2.2H2O	36.76	FeCl3*6H2O	1.00	B12	0.00005
*NaSiO2*9H2O*	28.42	MnCl2*4H2O	0.18	Thiamine HCl	0.0001
H3BO3	24.00	CuSO4*5H2O	0.001		
NaHCO3	12.60	ZnSO4*7H2O	0.022		
K2HPO4	8.71	NaMoO4*2H2O	0.022		
		CoCl2*6H2O	0.012		
		Na3Vo4	0.0018		
		H2SeO3	0.0016		

Appendix B: Osmotic medium

Table 4: Ingredients of the osmotic medium for 1L (Source: NIOO Operating Protocol: Recipe WC Medium)

Major nutrients	*mg/ L*
*MgSO4*7H2O*	36.97
CaCL2.2H2O	36.76
*NaSiO2*9H2O*	28.42
H3BO3	24.00
NaHCO3	12.60
KCl	7.40