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Biofilm Growth in Two Streams Draining Mountainous Permafrost Catchments in NE Greenland

A. Pastor¹², N. Wu¹, L. J. Skovsholt¹, and T. Riis¹²

¹Department of Biology, Aarhus University, Aarhus, Denmark, ²Arctic Research Centre, Aarhus University, Aarhus, Denmark, ³Aarhus Institute of Advanced Studies, Aarhus University, Aarhus, Denmark

Abstract
The objective of this study was to evaluate how stream water nutrient concentrations influence biofilm accrual in streams draining mountainous permafrost headwaters. We selected six stream locations in the Zackenberg area (NE Greenland, 74°N) subjected to a gradient in the areal contribution of different geomorphological units in the watersheds and channel stability. We used nutrient diffusing substrates to evaluate biofilm growth (autotrophic and total biomass). We found elevated stream nitrate concentrations in samples from upstream reaches draining larger areas of solifluction sheets and bare rock and with higher channel instability. Nitrate had the highest standardized effect on autotrophic biofilm growth as a response to the experimental nutrient additions. The response to nutrient additions via diffusing substrates depended on the altitude gradient. Overall, our results showed stream nitrogen availability to be one of the main drivers of algal biofilm accrual in high-Arctic streams, suggesting that the predicted changes in nutrient exports induced by climate change will have strong impacts on the biogeochemistry and ecological functioning of high-Arctic streams.

Plain Language Summary
Biofilms are complex aggregates of microbes that are responsible of major ecological functions in streams ecosystems, including primary production, nutrient cycling, and food and habitat provision. Despite of the high sensitivity of the Arctic to climate-driven changes, there is still little information on how biofilms would respond to them, hampering our understanding on the ecological effects to freshwaters functioning and the impacts to downstream ecosystems. Here, we evaluate how nutrient concentrations in stream water influence biofilm accrual. We deployed artificial substrates amended with nutrients (nitrogen and phosphorous) in six streams draining mountainous permafrost headwaters in NE Greenland and measured biofilm accrual both the autotrophic fraction and total biomass. Our results showed stream nitrogen concentrations to be one of the main drivers. Thus, we suggest that the predicted changes in nutrient exports induced by climate change will have strong impacts on the biogeochemistry and ecological functioning of high-Arctic streams.

1. Introduction

The Arctic is warming at over 6 times the rate of the global average (Huang et al., 2017). Warming causes permafrost thaw through the gradual deepening of the active layer and increasing soil mass wasting processes (Arctic Monitoring and Assessment Programme, 2017). The active layer will continue to deepen and ice-rich zones will thaw, which will promote slope instability, rapid mass movement (e.g., landslides and active layer failures) and enhanced rates of solifluction activity (Biskaborn et al., 2019; Farquharson et al., 2019; Lewkowicz & Way, 2019). These climate-driven changes will impact the hydrological and related hydrochemical fluxes to aquatic ecosystems (Lafrenière & Lamoureux, 2019). However, while effects of nutrient availability have been extensively documented for upland vegetation in Arctic areas, it is unclear how nutrient input changes may affect high-latitude running waters (Wrona et al., 2016).

Microbial biofilms are complex aggregations of algae, bacteria, and fungi living on stream benthic substrate, and they control stream biogeochemical processing (Battin et al., 2016). Bioassay experiments have been conducted extensively worldwide to study the effects of nutrient amendments on stream biofilm growth. Previous meta-analyses have demonstrated significant growth response to fertilization with the addition of nitrogen (N), phosphorous (P), and the N × P combination, with high effect sizes of N × P treatments for freshwater ecosystems worldwide (Beck et al., 2017; Elser et al., 2007). Moreover, environmental
variables, such as stream nutrients, temperature, and canopy, cover significantly affect stream nutrient limitation. Thus, regional variables, such as land cover and biomes, which are generally linked to local environmental variables, typically present clear trends with nutrient limitation (Beck et al., 2017).

In the Arctic, the information on the effects of increasing nutrient availability on stream microbial communities is scarce. Most of the available data on nutrient limitation are for low-Arctic ecosystems, although high-Arctic region (located above 70°N on the east coast of Greenland) is distinct in various environmental, geomorphological, and biological characteristics (Olson et al., 2001). The combination of N and P additions has been shown to have the highest effects on biofilm productivity in worldwide freshwaters (Beck et al., 2017; Elser et al., 2007) and across the Arctic (Docherty, Riis, Hannah, et al., 2018; Myrstener et al., 2018; Peterson et al., 1983; Tank & Dodds, 2003). These studies have also usually found a higher effect of N than P when added alone (but see Peterson et al., 1983; Tank & Dodds, 2003). Moreover, most previous studies exclusively focus on the algal fraction of the biofilm (measured as chlorophyll, chl $a$), but autotrophic and heterotrophic communities of biofilms might respond differently to nutrient addition (Kendrick & Huryn, 2015; Peterson et al., 1985).

Here, we aimed to evaluate how nutrient availability influences biofilm accrual in streams draining montaneous permafrost landscapes with contrasting geomorphological features in the High Arctic. We hypothesize that N stream concentration is one of the main drivers controlling biofilm accrual due to the low N concentrations usually found in high-Arctic streams and following what has previously been found for the low-Arctic region (Myrstener et al., 2018).

We selected six stream locations subjected to a gradient in the areal contribution of different geomorphological units in the watersheds, thus potentially covering a wide range of stream hydrochemical characteristics and stream nutrient concentrations (Docherty, Riis, Milner, et al., 2018). The study was carried out in Zackenberg valley, which represents one of the most well-studied areas on the periglacial coast of NE Greenland with a recent field-validated geomorphological map available (Cable et al., 2018).

We used nutrient diffusing substrates (NDS), a widely applied method in more southerly situated freshwaters (Beck et al., 2017; Tank et al., 2017). We incubated the NDS in the streams to evaluate the effects of nutrient availability on biofilm autotrophic and total biomass accrual (as chl $a$ and carbon content, respectively). The experiments were conducted during late summer when the maximal seasonal thaw depth and a only limited low snow cover in the watershed, and the highest hydrological connectivity between the geomorphological landforms drained in the watershed and downstream ecosystems occurs.

We had two specific aims: (1) to evaluate how biofilm accrual responds to a gradient of nutrient availability by measuring biomass accrual (incubated on control substrates and related the responses to the stream environment) and (2) to determine nutrient biofilm limitation along the in-stream nutrient gradient by comparing biofilm biomass on nutrient-amended and control substrates (i.e., response ratios).

2. Materials and Methods

2.1. Study Sites

The study was carried out in the Zackenberg valley (74°28′N, 20°34′W; Figure 1) in the high-Arctic zone. The climate here is high Arctic with a mean annual air temperature of −9.2 °C and an annual precipitation of 203 mm, the warmest month being August (mean 5.1 °C; Hasholt & Hagedorn, 2000). The area is characterized by Cretaceous and Tertiary sediments (mainly sandstones, conglomerates, and black shales) covered by superficial Quaternary deposits in the lower parts of the valleys (Pedersen et al., 2013). Fellfields and barrens predominate at the slopes, but on the plains vegetation is well developed with presence of grasslands, Cassiope and Dryas heaths, Salix snow beds, and fens (Elberling et al., 2008).

We sampled the Grønseelv and Kærelv streams which are closely located to each other (Figure 1) and have similar characteristics, being mainly sourced from small and seasonal snow patches on an annual basis (<0.01 km$^2$; Docherty, Riis, Milner, et al., 2018). Within each stream, we selected three sampling reaches (~80 m long) from upstream to downstream A, B, and C (Figure 1 and Table 1). These six stream reaches within the two streams drained small watersheds (0.7–3.7 km$^2$, Table 1) covering contrasting landforms in the area. Upstream watersheds on the hillslopes have a higher coverage of bedrock, solifluction sheets, and nivation hollows (i.e., shallow pits that are formed due to the accumulation of snow, associated to
headword weathering and erosion on the backwall of the snow patch) and alluvial sedimentation areas occurs mostly in the valley (Figure 1 and Table 1; Cable et al., 2018; Christiansen, 1998; Christiansen et al., 2002). The vegetation cover increases downstream the sampled watersheds (Elberling et al., 2008). The channel stability of the sampling reaches increases downstream as well (i.e., low values of the Pfankuch stability index Docherty, Riis, Milner, et al., 2018; Table 1). The Pfankuch index (Pfankuch, 1975) is a subjective, visual, and easily measured index of channel stability that is appropriated to characterizing disturbance regimes of mountain streams (Peckarsky et al., 2014).

Fieldwork was conducted at the end of summer (from 17 August to 3 September 2017), during maximum seasonal thaw of the active layer. Data on temperature, precipitation, snow depth, and photosynthetic active radiation 5 days before the deployment and throughout the deployment are given in the supporting information (Figure S1). Daily photon flux density during this period ranged from 9 to 41 mol·m$^{-2}$·day$^{-1}$, which indicates no light limitation for biofilm production (<7 mol·m$^{-2}$·day$^{-1}$; Hill et al., 1995). The active layer depth during summer 2017 is also provided in the supporting information (Figure S2). For each of the study site, the catchment drainage area was delimitated by performing a flow model analysis on a digital terrain model (obtained from the Greenland Ecosystem Monitoring Programme) using the hydrology tools of the ArcMap software (version 10.5.1 © ESRI). Catchment composition of geomorphology was then extracted by overlaying areas of the catchment with the geomorphological map (Cable et al., 2017, 2018), and areal cover was converted to percent.

2.2. Nutrient-Diffusing Substrata

Nutrient-diffusing substrata (NDS) were made using 30-ml Polycon cups following standard methodology (Tank et al., 2017). The cups were filled with agar solution (2% by weight) and enriched with 0.5-M NH$_4$Cl (N treatment), 0.5-M KH$_2$PO$_4$ (P treatment), and N and P (NP treatment) supplemented with none-nriched agar controls (C treatment). The cups were capped with 30-mm diameter fritted glass disks on top of the agar. We chose to use fritted glass disks to best emulate the natural stream substrates, which consisted
mainly of cobbles and limited organic substrates. The choice of NDS rather than an organic substrate likely promoted autotrophic over heterotrophic colonization (Johnson et al., 2009). For each site and each treatment, six cups were randomly attached to plastic bars, deployed on the streambed parallel to stream flow and secured by rocks. At deployment time, the water depth above the substrates was between 2.5 and 18 cm. For each treatment and site, three disks were used to measure chlorophyll $a$ ($\text{chl}_a$), as a proxy for algae biomass growth, and three disks for carbon determination (total carbon; hereafter TC), as a proxy for both algae and heterotrophic biomass growth on the disk. The NDS were deployed for 15 days, which is within the time period for maintaining constant rates of nutrient diffusion (Tank & Dodds, 2003). Upon removal, the disks were frozen until further analyses in the laboratory (analyzed within 3 months of collection). For $\text{chl}_a$, unfrozen disks were directly extracted with 6‐ml 96% ethanol and measured for absorbance after 12 hr on ultraviolet (UV) 1700 spectrophotometer (Shimadzu, Japan). $\text{chl}_a$ was calculated as (Docherty, Riis, Hannah, et al., 2018): 

$$\text{Chl}_a = \frac{(\text{Abs}_{665} - \text{Abs}_{750}) \times E}{83.4 \times A}$$

where $E$ is volume of ethanol (ml), 83.4 is the absorption of $\text{chl}_a$ in ethanol, and $A$ is the sample area (cm$^2$).

For TC, unfrozen disks were sonicated for 30 min in MilliQ water, brushed with a soft brush, and centrifuged. The pellet was dried and encapsulated to quantify C using an Elementar vario EL cube. To estimate the proportion of algae on the biofilm, we calculated the autotrophic index (AI, %) as the percentage of $\text{chl}_a$ ($\mu$g C of $\text{chl}_a$/cm$^2$) in TC ($\mu$g C/cm$^2$). Note that for the calculation of AI, we expressed $\text{chl}_a$ in terms of C weight of the molecule (i.e., C$_{55}$H$_{72}$MgN$_4$O$_5$).

### 2.3. Environmental Variables

We measured water temperature and collected water samples at each site during the incubation. Water temperature was recorded every 30 min using Tinytag Underwater data loggers (Gemini Data Loggers Ltd., Chichester, UK) during the incubation and an average of all data points was calculated for each stream reach. Discharge was estimated at deployment time site by conducting a cross-sectional profile (every 20 cm) of water depth and measuring water velocity (HFA; Höntzsch Instruments, Waiblingen, Germany). Water velocity above the NDS racks was also measured at the beginning and the end of the incubations.

Water samples were collected for UV spectroscopy analysis and to measure ambient concentrations of dissolved organic carbon (DOC), nitrate, ($\text{NO}_3^-$), ammonium (NH$_4^+$), soluble reactive phosphorous (SRP), and cations. In the field, samples for DOC, spectroscopy, and cations analyses were filtered using

| Geographical Characteristics and Channel Stability (Measured as Pfankuch Index; Data Obtained From Docherty, Riis, Milner, et al., 2018) for the Sampling Reaches |
|---------------------------------|---------|---------|---------|---------|---------|---------|
|                                  | Granseelv | Kærelv  |
|                                  | A       | B       | C       | A       | B       | C       |
| Sampling reaches                 |         |         |         |         |         |         |
| UTM (Zone 27X) north             | m 8,266,481 | 8,265,718 | 8,264,813 | 8,267,213 | 8,266,073 | 8,266,648 |
| UTM (Zone 27X) east              | m 515,400 | 515,035 | 514,728 | 514,951 | 513,888 | 514,347 |
| Elevation at site                | m a.s.l. | 125 | 99 | 20 | 172 | 44 | 18 |
| Pfankuch index                   | % 104 | 83 | 78 | 93 | 70 | 74 |
| Watershed area                   | km$^2$ | 1.4 | 1.6 | 2.9 | 0.7 | 2.6 | 3.7 |
| Watershed geomorphology          |         |         |         |         |         |         |
| Bedrock                          | % 15.8 | 13.0 | 7.5 | 16.5 | 5.6 | 3.9 |
| Nivation hollow                  | % 2.6 | 2.9 | 2.1 | 10.5 | 4.4 | 3.4 |
| Solifluxation                    | % 71.2 | 69.0 | 48.4 | 59.4 | 54.8 | 38.2 |
| Glacial (lateral moraine and lodgment till) | % 1.3 | 1.1 | 6.8 | 0.0 | 6.2 | 12.1 |
| Alluvial (alluvial fans and peat bogs) | % 5.4 | 9.5 | 28.2 | 8.0 | 19.5 | 24.6 |
| Deltaic                          | % 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 5.5 |
| Others                           | % 3.7 | 4.5 | 7.0 | 5.7 | 9.6 | 12.4 |

Note: Geomorphological analyses of the watersheds drained at each sampling site. The category “Others” includes fluvial and unclassified areas from the original classification (data derived from Cable et al., 2018).
pressed GF/F filters (Whatman, UK) and samples for NO$_3^-$, NH$_4^+$, and SRP analyses onto Supor filters (0.22 μm; Supor 200 PES, Pall Corporation, Port Washington, New York, USA). For preservation, samples for DOC were acidified using 10% HCl to pH = 2–3, whereas samples for NO$_3^-$, NH$_4^+$, SRP, and cations were frozen until analysis.

The water analyses were conducted using standard methods (APHA, 2005). Concentrations of DOC were analyzed by combustion catalytic oxidation on a Shimadzu TOC Analyzer TOC-VCSH. Inorganic nutrient concentrations were measured using a Lachat QC-8500 Flow Injection Autoanalyzer (Lachat Instruments, Loveland, Colorado, USA). Cations were determined by inductively coupled plasma optical emission spectrometry (Optima 2000 DV; Perkin Instruments). The sum of the four major cations (Mg$^{2+}$, Na$^+$, K$^+$, and Ca$^{2+}$, reported in meq/L) was used as a proxy for mineral soil weathering (Barnes et al., 2018). The absorbance spectrum was measured using a Shimadzu UV-1800 spectrophotometer with a 1-cm quartz cuvette. The average sample absorbance between 700 and 800 nm was subtracted from the spectrum in order to correct for offsets due to instrument baseline drift (Green & Blough, 1994). We calculated SUVA$_{254}$ (L·mg$^{-1}$·m$^{-1}$) by dividing sample absorbance at 254 nm by DOC concentration and cell length, as a measure of the aromatic C content (Weishaar et al., 2003).

2.4. Statistical Analyses

We first tested the relationship between biofilm (chl $a$, TC, and AI) on control disks with the explanatory variable altitude and Pfankuch index (i.e., stability index). We used altitude as an integrative variables of the physiographic and geomorphological changes in the watershed (Table 1). To control for the nested design of this study, and thus the nonindependence among data points, we used linear mixed models including stream:site as random intercept. We tested the models against the null model including only stream:site as random effect to check significance (i.e., p value).

Second, we explored the contribution of the nutrient environment to both autotrophic (chl $a$) and TC on the control disks. This was done by building all possible linear mixed models including discharge, velocity, average stream temperature, and stream nutrient concentration, that is, NH$_4^+$, NO$_3^-$, dissolved inorganic nitrogen (DIN; as the sum of NH$_4^+$, NO$_3^-$), SRP, DIN:SRP, DOC, DOC:DIN, DOC:SRP, and SUVA$_{254}$. We also included stream:site as random intercept to account for the nested design of this study. We used the R package “lme4” to build the global linear mixed model (Bates et al., 2015) and used the function “dredge” in the “MuMIn” package to generate a set of models from the global model (Barton, 2016). We controlled for model complexity by limiting the models to one predictor and selected the best-performing models using the Akaike information criterion corrected for small sample size (AICc < 2; Burnham & Anderson, 2002). Models were visually checked using “mcheck” function in R (Crawley, 2012). We reported standardized effect sizes based on partial standard deviations, as a measure of variable importance, and the two complementary $r^2$ values that comprised the variance explained by only the fixed effects, that is, marginal $r^2$, and the variance explained by the whole model including fixed and random effects, that is, conditional $r^2$ (Cade, 2015; Harrison et al., 2018).

Finally, to assess differences between treatments, we conducted a mixed model analysis of variance for chl $a$ and TC using treatments as fixed factors (four levels: control, N amended, P amended, and N × P amended) and stream:site as random intercept. For each model, post hoc Tukey contrasts were used to test, which treatments differed from each other. Finally, we calculated the response ratios to N (RR$_N$), P (RR$_P$), and NP (RR$_{NP}$) by dividing chl $a$ or TC on amended NDS disks by the average control disk. Thus, ratios of 1 indicate a treatment response identical to the control value (i.e., no response) and ratios $>1$ or ratios $<1$ denote a positive or negative response to the nutrient added in relation to the control, respectively.

To meet the requirements of the analyses (Crawley, 2012), all data were transformed using natural logarithms. All analyses were conducted using R, Version 3.4.3 (R Core Team, 2017).

3. Results

3.1. Stream Characteristics

The sampling sites encompassed a high variability of environmental characteristics, following a altitudinal gradient downstream the two streams (Table 2). Upstream reaches (Kærelv A and Greneelv A) were more
unstable (measured with the Pfankuch scores) with higher NO₃ and lower DOC concentrations than downstream sites (Kærelv C and Grenseelv C). SRP concentrations were generally low (<4.2 μg P/L), being relatively high for Grenseelv B and Kærelv A (respectively, 10.2 and 6.9 μg P/L; Table 2). Stream water DIN:SRP ratios >16 suggest P limitation, which was observed at all sites except Grenseelv B (11), which may be N limited. The average temperature (every half an hour) during the NDS incubations was similar for the six study sites (3.5–4.0 °C; Table 2).

3.2. Biofilm Accrual on Control NDS

The variability of biomass on the control disks among sampling sites was high for both chl a (from 0.06 to 4.11 μg/cm²) and TC (from 0 to 245.36 μg/cm²; Figures 2 and S4). Both streams had the highest chl a on control disks at the upstream sites A with a sharp decrease of one order of magnitude at the B and C sites. Accordingly, we found a strong correlation between altitude and chl a (marginal r² = 0.83; p < 0.001; Figure 2a). Also TC was highest at the Upstream Sites A, but the decrease downstream was less steep than for chl a, with a reduction of 50% of the biomass in Kærelv B and 20% in Grenseelv B (Figure 2c). Thus, the proportion of autotrophic biofilm was also higher at the upstream sites (AI = 0.60 and 1.17 for Grenseelv A and Kærelv A, respectively) compared to the downstream sites (AI increased from 0.04 to 0.17; Figure 2f). Similar to altitude, the Pfankuch index was also related to chl a (marginal r² = 0.59; p = 0.003; Figure 2b) and, to a lower extent, to TC and AIC (Figures 2d and 2f), indicating a link between higher channel instability and biofilm accrual.

Based on the mixed model selection with the lowest AICc, nitrate availability was one of the most important variables driving biomass accrual for chl a on the control disks, which explained most of the variability (marginal r² = 0.84; Figure 3 and Table S1) and, to a lower extent, to AI (marginal r² = 0.42; Figure 3 and Table S1). In contrast to ammonium availability, DOC and the DOC to DIN ratio in the water were negatively related to biofilm accrual on the disks (Figure 3). The effect of temperature was relatively small, with a standardized effect size close to 0 (Figure 3).

3.3. Biofilm Biomass Response to Nutrient Amendments

The mixed model analyses of variance in chl a and TC on the amended disks showed that treatment had statistically significant effects (compared with the null model p < 0.001). However, the differences among treatments explained only a small fraction of the relative variability of both chl a (marginal r² = 0.08) and TC (marginal r² = 0.24) compared with the total variance explained by both fixed and random effects (conditional r² being 0.70 and 0.69, respectively). Results from the post hoc comparisons of chl a and TC between the treatments showed a negative response for single addition of N and P (Table S2), indicating no single nutrient limitation but inhibition of biofilm growth on the amended disks. The levels of chl a and TC in the biofilm on the N × P amended disks were similar to those of the control disks but higher than for single P and single N for TC (Table S2).
The generally negative effects of nutrient supply revealed in this study are depicted on the RR graphs (Figure 4); the majority of the values are <1, indicating that the control disks had higher biofilm biomass than the nutrient-amended substrates. However, for the downstream sites (sites C), the values tended to...
be higher than 1 for chl a on disks amended with N, but not for TC (Figures 4a and 4b). The relationship between the RR and N amendment with altitude was negative for biofilm chl a (Figure 5a; marginal $r^2 = 0.49; p < 0.001$) and positive for biofilm TC (Figure 5b; marginal $r^2 = 0.56; p = 0.005$).

4. Discussion

Microbial biofilms serve many critical ecological functions in streams ecosystems, including primary production, nutrient cycling, and food and habitat provision. Given the ongoing rapid changes in the Arctic, understanding how microbial biofilms will respond to these changing environmental conditions is highly relevant. Here, we present the findings of a NDS experiment in six streams reaches differing in their geomorphological watershed composition to study the effects of altered resource supply (i.e., N and P) in biofilm growth in high-Arctic NE Greenland. We discovered a large variation in biofilm biomass despite the small geographical distribution of the studied sampling reaches ($< 6$ km$^2$). Thus, high biomass values were recorded at the upstream sites, especially for the autotrophic fraction with the highest chl a values occurring at the Kærelv A site (1.6–4.1 μg chl a/cm$^2$ on control disks). The values are conspicuously high compared with those recorded for unamended inorganic substrates in other NDS studies in the Arctic region (Table S3), including the same downstream reaches (C sites) sampled during early summer (0.33 and 0.79 μg chl a/cm$^2$; Docherty, Riis, Hannah, et al., 2018). Concurrently with the large variability in biofilm biomass, we found large variation in water N concentrations among the six sampled stream reaches. In particular NO$_3^–$ concentrations at the upstream sites ($< 160$ μg NO$_3^–$/N/L) were more than 10 times higher than at the downstream sites for both study streams, and they are also among the highest DIN concentrations measured in other resource limitation experiments Arctic streams (7–59 μg N/L; Table S4). The variability in both chl a and TC on the control disks responded positively to this nutrient gradient, as evidenced by the model selection. This is in agreement with other high-latitude studies that found N as a key constraint for biofilm metabolism found in Swedish streams by Myrstener et al. (2018) and the significance of algal growth in Icelandic streams recorded by Friberg et al. (2009). Our study expands these previous results to the high-Arctic region.

The reason for the overall high and spatially variable NO$_3^–$ concentrations during the sampling period remains unclear. The geomorphological and vegetation differences, together with channel stability, existing along the altitudinal gradient at the Grænseelv and Kærelv streams, suggest that those might be related to the sources of N which ultimately control biomass accrual. Indeed, the geographical variable “altitude” explained 83% of the variability in chl a on the control disks and 59% of the variability in TC, as well as the response to N amendment (49% for chl a and 56% for TC responses). Thus, the importance of altitude might indicate the highest importance of geomorphological features driving biofilm biomass, rather than local variables such as discharge. However, determining the origin of the N reaching the stream depending on the landscape features goes beyond the scope of this study and requires additional investigations with the application of tracers such as isotopes. Moreover, the sample sizes were small, and thus, statistical tests must be interpreted cautiously.

The hillslopes in the Zackenberg area have scarce vegetation cover (Elberling et al., 2008), low stream channel stability (Docherty, Riis, Milner, et al., 2018), and widespread solifluction areas (Cable et al., 2018). Thus, the higher mineral weathering, high erosion, and low N demand of plants at the hillslopes can lead to higher runoff of solutes and nutrients to headwater streams. For example, plant N uptake in the Arctic riparian areas has been hypothesized to increase with stream order, whereas net nitrification might decline downstream (Pinay et al., 2018). Rock weathering has been demonstrated to be a major source of N in tundra biomes (Houlton et al., 2018). We found high concentrations of mineral weathering products found at the sampling sites (the sum of major cations ranged from 2.2 to 4.2 mEq/L; Table 2), compared to the ones found during early summer (from 0.17 to 0.27 mEq/L; Docherty, Riis, Milner, et al., 2018). Thus, suggesting the occurrence of deep flow paths and long water residence time in the soils during late summer, leading to enhanced N weathering rates. In addition, N accumulated in snow patches due to deposition in winter may reach the streams during melting; this may, however, be more relevant during the snowmelt flush in early summer and less important in late summer when the snow patches have shrunk (Tye & Heaton, 2007). In our study, only small snow patches fed into Kærelv and Grænseelv during the late summer sampling period (Figure S3), implying that only low proportions of stream water directly dripped from snow.
The sharp decrease of NO$_3^-$ concentrations down through the streams compared with the generally stable concentrations of the sum of cations (i.e., conservative tracers, Blaen et al., 2014) supports the existence of a strong in-stream biological control of NO$_3^-$.

Alternatively, diluted NO$_3^-$ soil inputs from the watershed and the downstream use of N by assimilation may explain the decrease in NO$_3^-$ along the streams. Finally, the effect of hydrological changes and soil thermal perturbation due to climate change is likely to increase N export to streams but still a large uncertainty exists (e.g., Lafreniére & Lamoureux, 2019; Wrona et al., 2016).

In general, in our study a negative response to single nutrient amendments was found for both autotrophic and total biofilm biomass, which contrasts the results of early summer investigations conducted in the same streams where increased chl a with N addition was recorded (Docherty, Riis, Hannah, et al., 2018). Nutrient inhibition of growth has previously been observed in NDS research worldwide (Beck et al., 2017; Francoeur, 2001) and for Arctic studies (Figure S5), but the mechanisms behind remain equivocal. Methodological aspects such as amendments with too high nutrient concentrations, long incubation times,

Figure 4. Response ratios to nitrogen amendment (RR$_N$), phosphorus (RR$_P$), and nitrogen × phosphorus (RR$_{NP}$) for chlorophyll a (a–c) and total carbon (d–f). The errors bars are standard deviation of the mean. Dashed vertical lines at 1 separates negative respond (left-hand side) and positive response (right-hand side) to nutrients additions.

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Figure 5. Relationships between the altitude with (a) the response ratio to N amendment (RR$_N$) for chlorophyll a (marginal $r^2 = 0.49; p < 0.001$ and (b) for total carbon (marginal $r^2 = 0.56; p = 0.005$). The two sampled streams are denoted with unfilled circles for Grænseelv and filled circles for Kærlev.
selection of salt type, or preparation of the agar (i.e., boiling vs. autoclaving; stirring) have been suggested as possible explanations of nutrient inhibition (Beck et al., 2017). Certainly, aspects such as these may affect biofilms growing on NDS, but the overall lack of consistent inhibition of biofilms in NDS studies shows that the effect is not ubiquitous (Reisinger et al., 2016). We advocate future studies to test the mechanisms behind nutrient inhibition in NDS settings, which will provide recommendations for a better use of this methodology in low-nutrient environments, such as the use of lower nutrient enrichment than the ones that are generally used (Tank et al., 2017). Another possible explanation could be related to selective grazing on the high-nutrient biofilm growing on nutrient-amended substrates by invertebrates (Bernhardt & Likens, 2004). Yet as our field observations generally showed rare grazer occurrence on NDS, we find it unlikely that the differences in algal growth among nutrient amendments can be attributed to invertebrates. The effect of nutrient additions to consumers remains elusive for high-Arctic streams and future studies should focus on these trophic relationships.

Interestingly, nutrient addition has been hypothesized to stimulate the growth of heterotrophic organisms, thereby reducing algal growth by competition (Bernhardt & Likens, 2004). However, the hypothesis is poorly tested since most NDS studies exclusively report the chl a response (Beck et al., 2017). Our results showed clear differences in AI on control substrates across the N concentration gradient, with the highest algae proportion occurring at the highest N availability. The experimental N addition had contrasting effects on the relative growth of algae across sites with different stream water N concentrations. At the highest NO$_3^-$ concentration (A sites), the inhibition effects were most pronounced for the autotrophic fraction, while the highest proportional algal growth appeared at low nutrient concentrations (C sites). Although our results cannot be used for appraising the competition for resources between heterotrophic and autotrophic organisms in a biofilm, as previously suggested by Bernhardt and Likens (2004), we showed a decoupled response of algal and bacterial growth to N availability.

The low temperatures in our sampled streams (average ≤ 4.0 °C) compared to other resource limitation work in Arctic streams (Table S3) may have contributed to the weak response of the biofilms to NDS fertilization. In a recent extensive compilation of NDS studies, the response ratio to nutrient additions was much lower during winter than in the other seasons (Beck et al., 2017). While temperature does not significantly affect the diffusion rates from NDS (Rugenski et al., 2008), it was positively related to chl a accrual on NDS in a New Zealand study, being least responsive to nutrient amendments in winter (Francoeur et al., 1999). Also, nutrient inhibition of biofilm respiration was prevalent during the winter months in a study of Swedish boreal streams (Burrows et al., 2016).

Also interestingly, our study showed that the response of biofilm growth to nutrient amendments depended on stream ambient nutrient concentrations. We found the strongest inhibitory effects on nutrient amendments at the highest stream DIN availability and the most pronounced biofilm growth after nutrient amendments at low nutrient availability along the altitudinal gradient. The response capacity of biofilm communities to short-term increases in N is primarily driven by the ambient DIN concentrations at which they develop (Ribot et al., 2013). Moreover, algal strategies of nutrient acquisition are likely to vary along nutrient availability gradients. Thus, organisms inhabiting low-nutrient environments, such as many high-Arctic streams, are likely to be dominated by those that are adapted to resource scarcity and whose efficiency of using short-term nutrient enrichment is low (Litchman et al., 2007). Together, based on the responses of stream biofilm growth to nutrient amendments found in earlier and the present study, we conclude that the capacity of biofilms to retain inorganic nutrients at low temperatures and low nutrient supply is strongly limited. This conclusion calls for further experimental investigations in the High Arctic to further elucidate this topic.

5. Conclusions

Our study suggests that high stream NO$_3^-$ concentrations, which likely related to periglacial activity on the hillslopes, have a strong effect on biofilm accrual in high-Arctic streams, in particular on the autotrophic fraction. Our results support those of previous studies that have shown increased N concentration in streams draining hillslopes with mass-wasting processes (i.e., solifluction and stream channel instability), and they also demonstrate that unlocked N has strong biological effects on the abundance and composition of biofilms. Our results revealed no nutrient limitation at the end of summer; however, the response to nutrient
amendments depended on stream N availability. In sum, our study provides support of the role of N on stream biofilm production in the High Arctic. The results suggest that climate change effects on the nutrient supply, for example, via periglacial activities (e.g., solifluction), will likely affect biomass and growth of both autotrophic and heterotrophic components of biofilms, thus affecting stream energetics and the nutrient export to downstream ecosystems. Future studies should determine N sources reaching Arctic streams depending on the landscape features within the climate change context and the effects of N additions to higher trophic levels.

References


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