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Pastor, A., Freixa, A., Skovsholt, L.J. *et al.* Microbial Organic Matter Utilization in High-Arctic Streams: Key Enzymatic Controls. *Microb Ecol* **78**, 539–554 (2019). <https://doi.org/10.1007/s00248-019-01330-w>

Publication metadata

Title:	Microbial Organic Matter Utilization in High-Arctic Streams: Key Enzymatic Controls
Author(s):	Ada Pastor, Anna Freixa, Louis J. Skovsholt, Naicheng Wu, Anna M. Romaní & Tenna Riis
Journal:	Microbial Ecology
DOI/Link:	https://doi.org/10.1007/s00248-019-01330-w
Document version:	Accepted manuscript (post-print)

This is a post-peer-review, pre-copyedit version of an article published in [Microbial Ecology](#). The final authenticated version is available online at: <https://doi.org/10.1007/s00248-019-01330-w>

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Microbial organic matter utilization in high-Arctic streams: key enzymatic controls

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Keywords

Nutrients, biofilm, extracellular enzymes, geomorphology, Greenland

Abstract

In the Arctic, climate changes contribute to enhanced mobilization of organic matter in streams. Microbial extracellular enzymes are important mediators of stream organic matter processing, but limited information is available on enzyme processes in this remote area. Here, we studied the variability of microbial extracellular enzyme activity in high-Arctic fluvial biofilms. We

22 evaluated twelve stream reaches in North-East Greenland draining areas exhibiting different
23 geomorphological features with contrasting contents of soil organic matter to cover a wide range of
24 environmental conditions. We determined stream nitrogen, phosphorus, and dissolved organic
25 carbon concentrations; quantified algal biomass and bacterial density; and characterized the
26 extracellular enzyme activities involved in catalyzing the cleavage of a range of organic matter
27 compounds (e.g. β -glucosidase, phosphatase, β -xylosidase, cellobiohydrazase, and phenol oxidase).

28 We found significant differences in microbial organic matter utilization among the study
29 streams draining contrasting geomorphological features, indicating a strong coupling between
30 terrestrial and stream ecosystems. Phosphatase and phenol oxidase activities were higher in
31 solifluction areas than in alluvial areas. Besides dissolved organic carbon, nitrogen availability was
32 the main driver controlling enzyme activities in the high-Arctic, which suggests enhanced organic
33 matter mineralization at increased nutrient availability. Overall, our study provides novel
34 information on the controls of organic matter usage by high-Arctic stream biofilms, which is of high
35 relevance due to the predicted increase of nutrient availability in high-Arctic streams in global
36 climate change scenarios.

37

38 Introduction

39 Soil organic carbon (C) pools in the northern circumpolar permafrost region account for
40 approximately 50% of the belowground C global sinks, of which 88% occurs in perennial frozen
41 soils and deposits [1]. Due to global warming, part of this ancient organic C and nutrients are being
42 remobilized [2–9], and the loads reaching Arctic streams are predicted to increase [10, 11]. In
43 temperate streams, fluvial networks are well known to efficiently mineralize terrestrial organic
44 matter [12, 13] and several studies have suggested that ancient organic matter from permafrost is
45 utilized quickly in Arctic streams [14, 15].

46 Microorganisms inhabiting biofilms are responsible for efficient uptake of fluvial organic
47 matter and nutrients, partly due to their ability to synthesize extracellular enzymes. Extracellular
48 enzyme activities (EEA) allow microbes to obtain organic matter and nutrients from a diverse array
49 of compounds, degrading complex organic compounds into assimilable smaller molecules [16–18] .
50 Thus, EEA initially regulate the rate of decomposition and nutrient mineralization and are
51 extremely important under low-resources conditions. Enzyme production represents an investment
52 of resources and energy and is typically induced only under specific circumstances by microbial
53 cells [16].

54 Due to the disproportionate relevance of biofilms for stream biogeochemical fluxes [19],
55 information on biofilm EEA is crucial in order to understand organic matter processing. In polar
56 regions, such information is scarce and limited to seawater [20–22] and river water [23]. Research
57 into biofilm EEA in cold environments is restricted to, for instance, benthic sediments in Alpine
58 streams: [24, 25]. The world's northernmost ecosystems are relevant since they contain the major
59 part of the global freshwater area and are highly sensitive to global change [26], but the controls on
60 stream biofilm EEA remain to be explored.

61 The concentrations of dissolved organic carbon (DOC) and nutrient concentrations
62 (nitrogen [N] and phosphorus [P]) are among the main variables controlling EEA in biofilms [27,
63 28]. Arctic streams are typically oligotrophic. However, differences in geomorphological features
64 and vegetation across landscapes may modulate the input of DOC, N, and P to the Arctic streams
65 [29–31]. Arctic hillslopes are exposed to harsh conditions due to the recurrent freeze-thaw activities
66 of the ground with widespread solifluction features, resulting in gradual mass wasting at the slopes.
67 Thus, uphill areas are typically low vegetated and characterized by low accumulation of organic
68 matter in the soils. In contrast, in the valleys, soils are aggraded, tundra vegetation is established
69 and organic matter is accumulated [32–34]. Furthermore, soil disturbances via thermokrast process
70 such as thaw slumps and rain-induced erosion events are expected to be more intensive in the Arctic
71 due to climate changes [11, 35]. These processes are predicted to enhance chemical weathering as
72 well as to unlock organic matter and nutrients from the permafrost, which will result in enhanced
73 nutrient DOC and nutrients export to the streams [29, 30].

74 To gain insight into the use of microbial organic matter in Arctic streams, we studied key
75 microbial enzymatic activities on epilithic stream biofilms linked to the utilization of organic matter
76 of distinct origin and composition (polysaccharides, plant derived compounds, lignin, organic
77 phosphorus compounds). Specifically, we pursued two objectives. First, we aimed to test the
78 variability of EEA in contrasting geomorphological landforms (i.e. solifluction versus alluvial
79 sedimentation areas) and, second, to identify the factors controlling EEA variability with focus on
80 stream nutrient environment and biofilm characteristics. We sampled twelve stream reaches in
81 Northeast Greenland (Zackenbergs area, 74°N) during late summer when Arctic streams have the
82 lowest discharge and are most dependent on soil water and less dependent on melt water from snow
83 packs [7, 36]. Thus, the most intensive interaction of water solutes and stream benthos takes place
84 in late summer, expectedly enhancing the EEA machinery in benthic biofilms. We hypothesized

85 that geomorphological features determine the amount of organic matter and nutrients reaching the
86 streams and thus strongly affect the microbial processing of organic matter. In particular, we
87 expected that microbial biofilms in stream reaches highly influenced by solifluction landforms with
88 low vegetation density would have access to limited allochthonous organic matter sources derived
89 from the watershed, whereas stream biofilms in low-land alluvial areas depend on organic matter
90 derived from tundra vegetation (e.g. cellulose and hemicellulose compounds derived from plant
91 material). Furthermore, we aimed to identify linkages between organic matter availability and
92 nutrients and EEA, which would help predict organic matter processing in Arctic streams in climate
93 change scenarios.

94 Material and methods

95 Study site

96 The study was carried out within the Northeast Greenland National Park located near
97 Zackenberg Research Station (<http://zackenberg.dk/>; 74°28' N, 20°34' W, Fig. 1) in the high-Arctic
98 zone, which has a mean annual air temperature of -9.2°C and an annual precipitation of 203 mm,
99 August being the warmest month (mean 5.1°C; Hasholt and Hagedorn 2000). The study region is
100 underlain by continuous permafrost with a modelled depth of 200-300 m and an active layer
101 thickness varying between 0.3 and 0.65 m [38]. The lithology is composed mostly of Cretaceous
102 and Tertiary sediments (mainly sandstones, conglomerates, and black shales) covered by superficial
103 Quaternary deposits in the lower parts of the valleys. In the western part of the study area, west of
104 Lindeman river, crystalline rocks are predominant [37]. The areas east and west of Zackenberg river
105 exhibit geological differences and thus a different geomorphology. The eastern area consists of
106 glacial landforms with barren areas at the hillslopes, delivering fine-grained sediment to the lower
107 hillslopes. In contrast, solifluction is more prevalent on the western hills characterized by with

108 frequent shallow slumps, which contributes to alluvial sedimentation downhill [33]. In the whole
109 region, fellfields and barrens predominate at the slopes, but on the plains vegetation is well-
110 established with presence of grasslands, *Cassiope* heath, *Dryas* heath, *Salix* snow-beds, and fens
111 [39].

112 We selected 12 sites with three independent fluvial networks (Grænseelv, Kærelv, and
113 Zackenberg tributaries; Fig 1), which were sampled in late summer 2017. Grænseelv and Kærelv
114 are snow-fed streams with relatively high stability, flowing parallel in the eastern Zackenberg area
115 [31]. Four sampling reaches were selected in Kærelv (0, A, B, C; altitude range: 245 m) and three
116 in Grænseelv (A, B, C; altitude range: 105 m, Fig. 1; Table 1). Furthermore, we sampled
117 Zackenberg River as well as Lindeman, Palnatokeelv, Aucellaelv, and unnamed tributaries
118 demonstrating different geomorphological landforms (Fig. 1; Table 1). Lindeman is the largest
119 tributary feeding the Zackenberg mainstream followed by Store Sødal, which has the highest glacial
120 contributions and drains a large watershed connected to two lakes . In this way we covered the main
121 landforms in the area [33], including upstream reaches influenced by solifluction and nivation
122 processes (hereafter referred to as “solifluction” sites), alluvial areas (“alluvial” sites) and mixed
123 areas (“mixed” sites). Solifluction sites (≥ 120 m a.s.l.) are situated on the hillslopes draining areas
124 with solifluction sheets and sorted stripe formations and are characterized by low vegetation cover.
125 Mixed sites (20-120 m a.s.l.) are situated on the lower slopes with intermediate vegetation cover,
126 and alluvial sites (<20 m a.s.l.) are completely vegetated areas situated at lower elevations. In the
127 Zackenberg area, the differences in vegetation and geomorphological landforms are related to
128 differences in soil organic C in the area, with a lower content of soil organic C in solifluction sheets
129 (0 to 30 cm soil: 0.1 ± 0.4 kg C m⁻²) than in deltaic deposits (0 to 30 cm soil: 7.3 ± 3.6 kg C m⁻² [40]).

130 **Stream physicochemical parameters**

131 We used a portable meter to measure conductivity (CyberScan CON 11, Eutech
132 Instruments, Singapore). Water samples were collected and filtered in the field through membrane
133 filters (0.22 μm ; Supor 200 PES, Pall Corporation, Port Washington, New York, USA) for nutrient
134 analyses and through 0.7 μm pre-combusted glass-fiber filters (GF/F, Whatman, UK) for dissolved
135 organic C (DOC) determination. Ammonium (NH_4^+), nitrate (NO_3^-), and soluble reactive
136 phosphorus (SRP) were analyzed using a Lachat QC-8500 Flow Injection Autoanalyzer (Lachat
137 Instruments, Loveland, Colorado, USA). The coefficients of variation among replicate water
138 samples ($n=3$) were ≤ 0.1 for DOC, ≤ 0.4 for NH_4^+ , ≤ 0.2 for NO_3^- , and ≤ 0.7 for SRP. Dissolved
139 inorganic N (DIN) was calculated as the sum of NH_4^+ and NO_3^- . DOC concentration was
140 determined using a Shimadzu TOC Analyzer TOC-VCSH. We characterized DOC composition
141 using the slope ratio (SR), shown to be negatively correlated with molecular weight [41], and UV
142 absorbance at 254nm (SUVA_{254}) as an indicator of DOC aromaticity [42]. UV–Vis absorbance
143 spectrum was measured using a Shimadzu UV-1800 spectrophotometer with a 1-cm quartz
144 cuvette. SR was calculated as the log-transformed absorption slope between 275-295 nm and 300-
145 350 nm. To calculate SUVA_{254} ($\text{L mg}^{-1} \text{ m}^{-1}$), spectra average sample absorbance between 700 and
146 800 nm was subtracted from the spectrum in order to correct for offsets due to instrument baseline
147 drift [43]. Then, sample absorbance at 254 nm was divided by DOC concentration and cell length.
148 The high ratio of iron cations to DOC concentration in the Aucellaelv stream ($1.82 \text{ mg Fe mgC}^{-1}$)
149 prevented us from calculating optical DOC descriptors at this site [44].

150 **Biofilm sampling and characterization**

151 Three replicates of biofilm extract were obtained from the combination of four to eight
152 stones randomly collected at close proximity to obtain a minimum sampled biofilm area of 250 cm^2
153 for each replicate. Biofilm from the light-exposed side of cobbles (stone minimum size: 6 cm b-

axis) was sampled by brushing the selected stones with a soft brush and rinsing it with unfiltered stream water to collect the biofilm extract (250-900 mL). Total surface areas brushed were estimated with a ruler. Biofilm extracts were kept at 4°C and transported to the laboratory for the analysis of ash-free dry mass (AFDM), chlorophyll *a* (chl *a*), C and N content, bacterial density, and extracellular enzyme activity.

For each replicate sample we measured AFDM using an aliquot (10-60 mL) that was filtered onto ignited, pre-weighed GF/F filter (Whatman, UK) and chl *a* using an aliquot (10-60 mL) filtered onto GF/C filters (Whatman, UK). The filters for AFDM analysis were oven-dried at 60°C, while those for chl *a* were wrapped in aluminum foil and frozen at -20 °C until analysis. To determine the concentration of total bacterial cells, 2-mL samples were fixed with formaldehyde (3% final concentration) and kept refrigerated at 4°C. For total organic C (TOC) and total N (TN) analyses, a 30-mL aliquot was kept frozen. Extracellular enzyme activities were measured on the fresh samples within 24 h of collection (see below).

To determine AFDM, the difference in mass between dry and combusted filters after four hours at 450 °C was calculated and reported relative to cobble surface (units in mg cm⁻²). For the determination of chl *a* content (units in µg cm⁻²), samples were extracted in ethanol for 24 h and analyzed by spectrophotometry following methods previously published [31]. For bacterial density, biofilm extracts were sonicated for 10 s (Selecta, 40 W and 40 kHz) and were then stock-still for 5 min, after which the supernatant was collected and diluted 1:4 with MilliQ water. Aliquots of diluted samples (400 µL) were stained with 4 µL of Syto13 (Molecular Probes, Invitrogen) for 15 min in the dark. Next, 10 µL of an internal standard of beads (concentration: 10⁶ beads/mL, Fisher 1.0 µm) were added to each sample and vortexed. Samples were measured in a FACSCalibur cytometer (Becton-Dickinson) running at low flow rate until 10,000 events were achieved. The number of bacterial cells was determined by the number of events in a log plot of the side scatter vs.

the green fluorescence, taking into account the bead events. The concentration of beads was tested by counting them under a fluorescence microscope (Nikon Eclipse, E600, Tokyo, Japan). To obtain an estimate of the relative contributions of the heterotrophic and autotrophic community on the biofilm, we calculated the Heterotrophic Index (HI; bacterial cells:μg chl *a*) as the number of bacterial cells (bacteria cm⁻²) divided by chl *a* (μg chl *a* cm⁻²). The reported HI is an adaptation of the commonly used autotrophic index (AFDM:chl *a*) that takes into account heterotrophic organisms and nonliving organic material [45]. Finally, biofilm extracts were analyzed for TOC and TN using a Shimadzu TOC Analyzer TOC-VCSH.

Extracellular enzyme activities

The extracellular enzymatic activities involved in catalyzing the cleavage of a range of organic matter compounds were investigated for all stream biofilms sampled (Table 2; phenol oxidase and cellobiohydrolase measured only at Grænseelv and Kærelv streams). In the laboratory, assays for hydrolytic enzymes were conducted on fresh scrapped biofilm samples (2 mL biofilm extract) using methylumbelliferone (MUF) linked substrates and determined fluorometrically. Samples were incubated in the dark for 1.5 h at room temperature at 0.3 mM final substrate concentration for β-glucosidase (GLU), phosphatase (PHOS), and β-xylosidase (XYL) and with 0.8 mM for cellobiohydrolase (CEL). At the end of the incubation, glycine buffer (pH 10.4, 1:1 vol:vol) was added to stop the reaction and maximize MUF fluorescence. MUF standards and blanks of stream water were run using the same conditions. Samples for phenol oxidase (PHEN) were incubated in the dark using L-DOPA at a final concentration of 5 mM with acetate buffer, together with blanks for biofilm and acetate buffer, for 2 h at room temperature [18].

Each hydrolytic enzyme sample was placed in triplicate (350μL) into 96-well black microplates (Cell grade, Brand plates) for fluorescence measurements using a fluorimeter microplate reader (Victor Perkon Elver). Fluorescence was measured at 355 nm (40 nm range)/460

(8 nm range) excitation/emission wavelengths. Enzymatic activities were expressed as the rate of MUF released per hour in relation to biofilm area ($\text{nmol MUF cm}^{-2} \text{ h}^{-1}$), bacteria-specific activity ($\text{nmol MUF bacterial cell}^{-1} \text{ h}^{-1}$), and, for PHOS activity only, chlorophyll-specific activity ($\text{nmol MUF } \mu\text{g chl } a^{-1} \text{ h}^{-1}$). For PHEN activity, absorbance was measured at 460 nm using a Shimadzu UV-1800 spectrophotometer. PHEN values were expressed as $\mu\text{mol DIQC (2,3-dihydroindole-5,6-quinone-2-carboxylate) cm}^{-2} \text{ h}^{-1}$ and $\mu\text{mol DIQC bacterial cell}^{-1} \text{ h}^{-1}$. We calculated extracellular enzyme ratios, which are indicative of the origin or the type of organic matter and P deficiency [41–43; Table 2].

Statistical analyses

Variables were ln-transformed to meet requirements for analyses. We used Tukey's test analysis after significant factor of the variance (one-way ANOVA) to determine differences between variables among landforms (solifluction, mixed, and alluvial). The significance level for indicating differences and relationships was set to $p < 0.05$. We explored the contribution of the stream nutrient environment (DOC, SUVA_{254} , SR, SRP, NH_4^+ , NO_3^- , the molar ratios of DOC to DIN, DOC to SRP, and DIN to SRP) and the biofilm characteristics (AFDM, chl *a*, bacterial abundance, HI, C content, N content, and the molar ratio of C to N) to the EEA variability. For each group of variables, we built all possible linear models and controlled for model complexity by limiting models up to three variables without interaction terms. Candidate models were identified using the Akaike Information Criterion corrected for small sample size (AIC_c) as those differing by less than two units from the best model [49]. We automated this process using the R package for automated model selection 'glmulti' [50]. For the best single model, we estimated the relative importance of each variable by using the R package "relaimpo" [51]. Finally, we explored the role of autotrophic and heterotrophic biofilm compartments in PHEN by evaluating the relationship between chlorophyll-specific activity and bacteria-specific activity and SRP concentration. We first

226 conducted Spearman's rank order correlations and further estimated breakpoints (i.e. visually
227 detected) using the R package 'segmented' [52]. All data analyses were conducted using R version
228 3.4.1 [53].

229 Results

230 Stream characteristics

231 The sampled streams were characterized by low nutrient concentrations (Table 1).
232 Conductivity varied between sites (from 71 to 566 $\mu\text{S cm}^{-1}$), the lowest occurring in the rivers with
233 highest glacial contribution (i.e. Palnatokeelv and Zackenberg; Table 1). DOC and SRP were also
234 very low, especially for Zackenberg river and its tributaries. The stream characteristics differed
235 statistically among landforms for conductivity, the concentration of NO_3^- , and the stoichiometric
236 ratios. In particular, conductivity was statistically higher at solifluction-influenced sites than at
237 alluvial sites (Tukey's HSD; $p=0.018$, Table 1) and NO_3^- was lowest at the alluvial sites (Tukey's
238 HSD; $p<0.012$). The stoichiometric ratios were highest for DIN:SRP and lowest for DOC:DIN at
239 the solifluction sites (Tukey's HSD; for both $p<0.001$). There were no significant differences for
240 DOC, SUVA_{254} , SR, SRP, and NH_4^+ between the landforms (ANOVA: $p<0.05$). DOC:SRP was
241 higher for the alluvial than for the mixed sites (Tukey's HSD; $p=0.031$). Along the streams, the
242 concentrations of NO_3^- , NH_4^+ , and SRP decreased downstream Kærelv and for NO_3^- also
243 downstream Grænseelv (Table 1).

244 Biofilm characterization

245 The concentrations of chl *a* (0.01 to 0.30 $\mu\text{g cm}^{-2}$) and AFDM (0.14 to 0.36 mg cm^{-2}) were
246 low at all sampling sites, the highest averaged values emerging for Aucellaelv and Unnamed
247 reaches (Table 3). Bacterial density varied two orders of magnitude, with the lowest values being
248 recorded at Zackenberg and Lindeman and the highest at Grænseelv A (Table 3). The Kærelv and

Grænseelv streams had a higher relative contribution of the heterotrophic community than the streams on the Zackenberg fluvial network (Table 3). Chl *a*, AFDM, bacteria, and biofilm contents of C and N were higher at the solifluction sites than at the alluvial sites (Tukey's HSD: $p = 0.024$, $p < 0.001$, $p = 0.012$, $p < 0.001$ and $p < 0.001$, respectively). There were no differences in C:N or HI among the landform categories (ANOVA: $p < 0.05$). Along the streams, the concentrations of chl *a* and AFDM tended to decrease downstream in Kærelv, and HI increased. The same pattern applied to Grænseelv, except for chl *a* where the highest values occurred at site B (Table 3).

Variability and controls for EEA in Arctic streams

Of the total measured EEA, PHOS showed the highest contributions (ranging from 5.8 to 68.3 nmol MUFcm⁻² h⁻¹), followed by GLU (from 0.2 to 8.9 nmol MUFcm⁻² h⁻¹). The ratio between GLU and PHOS was lower than 0.8 in all the samples measured (Fig. 2). For the predominant landforms, PHOS and PHEN were higher at the solifluction than at the alluvial sites (Tukey's HSD: $p < 0.05$; Fig. 2), while no significant differences between landforms emerged for the other measured enzymes. The bacteria-specific activities showed that GLU was higher and XYL lower at the solifluction sites than at the alluvial and mixed sites (Tukey's HSD: $p < 0.05$; see Table S1). The enzyme activity ratios revealed that the proportion of organic matter used was greater when derived from cellulose/cellobiose (β -D-glucose bonds) than from hemicellulose/xylobiose (β -D-xylose bonds; XYL:GLU < 0.2 ; Fig. 2). In addition, a higher capacity for decomposition of complex polysaccharides – as shown by the lowest (GLU + XYL):CEL – was found at the alluvial sites than at the solifluction sites (Tukey's HSD: $p < 0.05$; Fig. 2).

The stream nutrient environment explained the majority of the variability in EEA (adjusted r^2 from 0.35 to 0.69, Fig. 3, Table S2), whereas the explanatory capacity for biofilm variables was lower (adjusted $r^2 < 0.44$, Fig. S1, Table S3). DOC-related variables (DOC, SUVA₂₅₄, and SR) were the most predictive variables and were included in all EEA linear models. The direction of the

relationship between these variables and enzymatic activities differed among EEA. PHOS, GLU, and CEL were positively related, whereas XYL and SUVA₂₅₄ and SR as well as PHEN and SR were negatively related (Fig. 3; Table S2). Interestingly, also the concentration of NO₃⁻ was included in all the best-performing linear models for EEA, with the exception of XYL, which had a positive relationship for all EEA (Fig. 3, Table S2).

In contrast to N availability, the concentration of SRP was only selected in the best-performing linear model for PHOS but contributed less than 7% to the overall relative explained variability (Fig. 3). However, when assessing the specific role of the autotrophic and heterotrophic community, chl *a*-specific PHOS was negatively related to the SRP concentration (Spearman correlation, $\rho = -0.65$, $p < 0.001$; Fig. 4), while bacteria-specific PHOS was positively related to the SRP concentration ($\rho = 0.45$, $p = 0.006$; Fig. 4). This relationship was rather clumpy for bacteria-specific PHOS but visually presented a change in the response, separated by a breakpoint for chl *a*-specific PHOS. We obtained the optimum breakpoint of SRP at $5.7 \pm 1.1 \mu\text{P L}^{-1}$ by using breakpoint regression analysis that demonstrated a limited impact on chl *a*-specific PHOS by the effects of the SRP increase (Fig. 4).

Discussion

Extracellular enzymes are important mediators in nutrient and organic matter microbial processing, and they provide relevant information on what controls the persistence or decomposition of organic compounds along the fluvial networks. In this work, we present the findings of the first EEA study of microbial biofilms in high-Arctic streams. We found significant differences in organic matter usage of the aquatic microbiota depending on landscape features, including uphill areas characterized by low soil organic matter and nutrients due to high solifluction and nivation processes and alluvial valley bottoms where organic peat soils are aggraded and tundra

296 vegetation is established [32, 33, 54]. We showed that there is a spatial shift in microbial organic
297 matter usage between these two contrasting Arctic landforms; thus, the largest use of PHOS and
298 PHEN is found in solifluction areas, while a higher capacity for the use of complex polysaccharides
299 occurs in the alluvial areas (Fig. 5). Seen in a broader context, EEA in Arctic streams were lower
300 and showed a lower capability of decomposing plant originated materials than streams at lower
301 latitudes. Moreover, our results emphasize the relevance of DOC and nutrient availability in
302 controlling EEA in Arctic streams, which is a finding worth considering due to the predicted
303 increase in nutrient availability in a global change scenario.

304 **Organic matter utilization in high-Arctic stream biofilms**

305 The distinct extracellular enzyme activities and ratios measured in our study across
306 landform formations suggest that the interaction between terrestrial sources and environmental
307 settings determines the microbial processing of the available sources of organic matter and
308 ultimately also the in-stream organic matter cycling. As expected, the EEA suggest a greater impact
309 of the input of phenolic compounds in solifluction than in alluvial areas as shown by the fact that
310 the highest phenol oxidase activity was measured in solifluction-characterized reaches. Despite the
311 importance of phenolic compounds in peatlands and wetlands [55], which might be similar in
312 alluvial areas, terrestrial soil sources in solifluction deposits may provide an even greater
313 availability of these compounds for microbial degradation. In Arctic Siberia, an observed increase
314 in stream phenolic compounds concentrations has been attributed to weaker retention of the soil
315 because of shallow active layers in continuous permafrost areas [56]. Despite lower soil organic
316 storage in uphill areas [40, 54], low organic matter processing through soil passage (i.e. high slope,
317 shallow active layer, and low soil organic matter) might result in larger proportions of phenolic
318 compounds entering the streams. Alternatively, the higher PHEN activity in the solifluction areas
319 could be due to a priming effect where the available easy-degradable algal exudates may enhance

320 PHEN [57]. This is supported by the higher chl *a* content in solifluction biofilms, indicating greater
321 algal biomass and availability of algal exudates for microbial heterotrophs [49]. Moreover, the
322 reduced microbial degradation of phenolic compounds in alluvial areas suggests that stream
323 processing may reduce phenolic concentrations at the downstream reaches due to microbial and
324 ultraviolet photolytic decomposition. Alluvial reaches are located in the bottom valley and have
325 higher stream orders than the reaches on the hillslopes [33]. Thus, the higher residence water time
326 might promote higher DOC processing in the alluvial areas [26]. In particular, phenolic reduction
327 via light could be important since previous research has shown that photochemical processes
328 potentially account for 70-95% of DOC processing in Arctic unshaded streams [58].

329 In contrast, in alluvial areas, microbial degradation of allochthonous plant originated
330 material is more important than in solifluction areas. This is indicated by the lower
331 (GLU+XYL):CEL ratio recorded in the alluvial areas, indicating greater degradation of cellulose-
332 derived polysaccharides, which suggests that a high amount of plant-derived organic matter reaches
333 the streams. Moreover, the efficiency of the bacterial community in decomposing hemicellulose is
334 higher in alluvial areas (as shown by the specific bacterial XYL activity). Peat vegetation
335 communities such as grasslands, *Cassiope* heath, *Dryas* heath, *Salix* snow-bed, and fens dominated
336 in the alluvial areas [4] and organic matter accumulated in the soil [54, 59], while – in contrast –
337 only scarce vegetation developed in the solifluction areas.

338 Placing the biofilm extracellular enzyme activities in Arctic stream in a broader context
339 and comparing them with those measured at lower latitudes, EEA were generally lower in high-
340 Arctic Greenland streams than in the warmer Atlantic and Mediterranean regions (Fig. S2).
341 Latitudinal gradients, i.e. those related to temperature and moisture, determine the soil organic
342 matter stock in the watershed [60, 61] and, thus, the availability for transport to aquatic ecosystems,
343 and eventually the utilization of the organic matter of the fluvial biofilms. Across latitudinal

344 gradients, temperature has been shown to affect enzymatic responses in marine systems [62], but its
345 effect in soil ecosystems warrants further elucidation [63, 64]. In streams, the differences occurring
346 between Mediterranean and Atlantic systems for both GLU and XYL have been related positively
347 to stream temperature [65]. Along the temperature gradient in our study, we found a strong
348 correlation between temperature and enzyme activity (Fig. S2), and this inclusion of the high-Arctic
349 area supports the reliability of the suggested climate effect on EEA.

350 The capacity of benthic microbial communities to decompose simple C molecules (i.e.
351 cellobiose) was between 5 to 100 times higher than their capacity to decompose xylobiosic
352 molecules (XYL:GLU ranged from 0.01 to 0.19; average: 0.1). These ratios are strikingly low
353 compared with the values around 0.5 reported for Atlantic and Mediterranean streams [65] but
354 similar to those reported for a stream draining a peat-land dominated watershed in Pennsylvania
355 (USA, 41°N;[57]). This indicates that in high-Arctic streams, there is a low input of allochthonous
356 plant-derived material. Furthermore, PHEN was relatively high compared with GLU relative to the
357 values found in southern ecosystems (a Mediterranean stream [66] and a temperate stream [67]),
358 suggesting that DOC with a high content of aromatic rings and resistant to microbial degradation is
359 transported to high-Arctic streams.

360 The results from the PHOS activity analyses suggest a high sensitivity of biofilm microbes
361 to inorganic P availability in high-Arctic streams. High PHOS has been shown to be a sensitive
362 indicator of P limitation in stream biofilm communities [e.g. 78] and the PHOS:GLU ratio is used
363 to describe differences in stoichiometric relations to organic nutrient acquisition among
364 environments [46]. Our results showed low GLU:PHOS ratios (below <0.8) that indicate low
365 nutrient availability and negatively correlated along the latitudinal gradient. These results are
366 consistent with the ratios derived from river water in Arctic Siberia (median: 0.36; interquartile
367 range from 0.18 to 0.70; [23]). The unbalanced ratios suggest that microorganisms need to scavenge

368 on organic matter to obtain nutrients for metabolism and growth [46]. Thus, oligotrophy under
369 high-Arctic conditions might induce microbial investment in phosphorus-acquiring strategies.

370 **Main controlling factors on organic matter utilization in high-Arctic streams and potential** 371 **effects of global change**

372 Our results revealed DOC quantity and composition to be the main drivers regulating the
373 microbial enzymatic machinery, despite the low variability of the variables detected in this study.
374 The concentrations of stream DOC in the Zackenberg area were relatively low (<1.5 mgC/L)
375 compared with the large Arctic rivers [69], which might be explained by the low soil organic C
376 contents in the barren ground drained in this area [54]. Previous freshwater studies have shown
377 lower values of SUVA₂₅₄ in mineral than organic soils in ponds across the circumpolar North [70].
378 In contrast, in this study, DOM optical analyses did not reveal significant differences between the
379 streams draining the different geomorphological landforms. Contrasting organic matter sources,
380 both autochthonous from stream production and allochthonous from the watershed, may mask the
381 differences between study sites. Our results suggest that DOC generally has a positive effect on
382 biofilm EEA (for GLU and CEL), as shown in previous studies [27]. However, this response might
383 be the result of the complex nature of the available organic molecules and their reactivity. We found
384 contrasting EEA responses to DOC composition measured with optical proxies; yet, our analysis
385 does not allow inferring the accurate source and reactivity of those molecules. In Mediterranean
386 streams, for example, large organic molecules originating from riparian leaf litter have been
387 associated with higher reactivity than small molecules [71]. In small streams in the high-Arctic, the
388 link between stream DOC origin, composition, and reactivity needs further study, which is a
389 challenging task due to the low DOC concentrations. Notwithstanding, despite the small local
390 differences in DOC concentrations and composition, we revealed a strong effect on EEA,
391 supporting the results of previous studies [27, 28].

Besides DOC-related variables, our data identified NO_3^- availability as one of the main factors explaining the spatial variability of EEA. In oligotrophic systems, microbial activity may be limited by nutrient availability, which might constrain organic matter degradation. Specifically, high N availability has been reported to alter the EEA involved in the breakdown of stream leaf litter, a likely consequence of reduced N limitation on microbial activity [72]. Indeed, N has been shown to strongly limit biofilm accrual in Arctic Sweden [73], and the same phenomenon was observed in our sampling area during early summer [31]. Furthermore, in the case of PHOS, not only inorganic P (as discussed below) but also the N and P balance may regulate its activity [27]. Our results agree with those of a recent soil meta-analysis showing that total N was the most important factor positively affecting the observed spatial gradients in PHOS in terrestrial environments [63]. Noteworthy, the highest NO_3^- availability was recorded in solifluction areas. In the Zackenberg area, uphill areas are those most exposed to bedrock weathering [33] and they may thus act as a significant N source [74]. Consequently, N availability might be responsible for the fact that the highest overall microbial biomass was recorded here (both algal and bacterial), which may have contributed to higher enzyme activities for PHOS and PHEN. This is supported by our results where algal biomass positively explained an important part of the variability in these two activities (Fig. S1), reinforcing the view of algae being an important regulator of heterotrophic enzymatic activity [65, 75].

P availability is known to control PHOS activity [28], but our results did not identify a positive relationship between SRP availability and areal PHOS activity. However, the strong relationship between SRP availability and chl *a*-specific biomass across sites, as well as the fact that chl *a* explained more than 75% of the variability of PHOS, suggest that autotrophic biofilm compartments could strongly influence PHOS. The direct origin of the EEA measured in this study is unknown, but PHOS likely originated not only from microbial heterotrophs but – even more

416 importantly – also from autotrophs [76, 77]. Our data suggest that the autotrophic biofilm
417 compartment was more responsive to limited SRP concentrations, as demonstrated by the increased
418 efficiency of PHOS production in the low concentration range ($<5.7 \pm 1.1 \mu\text{P L}^{-1}$). This is in
419 agreement with previous research and suggests high internal cycling in the biofilm at similar low
420 SRP concentration ranges [78]. In contrast, bacteria-specific PHOS did not show a negative
421 relationship with SRP. Lower production of PHOS when SRP exceeds P requirements has
422 previously been reported for bacterioplankton despite a higher P affinity of bacteria than of algae
423 [79]. As for the non-downregulation of P availability, it has previously been hypothesized that
424 bacteria may use organic moiety as an organic C source (if limited by assimilable organic C) or,
425 even more intriguingly, that bacteria could P-fertilize algae, making rich-C algal leachates available
426 for their own heterotrophic metabolism [79].

427 High-latitude freshwaters are among the most sensitive areas affected by climate change in
428 the world. Even when considering only changes in runoff, organic C decay rates in freshwaters are
429 predicted to increase by up to 10%, which are among the highest predicted global variations in
430 organic decomposition rates [26]. Moreover, plant communities will likely respond strongly to
431 environmental changes due to the effects of warming [80] and water availability [81]. The predicted
432 vegetation changes will have important implications for the aquatic microbial processing of organic
433 matter since, as demonstrated by our results, there is a high coupling between terrestrial sources and
434 the stream biofilm processing of organic matter. In particular, the predicted increases in woody
435 species in tundra regions [80] could promote the synthesis of enzymes associated with the
436 decomposition of plant material in streams, such as XYL and CEL (Fig. 5). Biofilm enzymatic
437 capabilities to degrade recalcitrant compounds have been shown to be highly responsive to
438 increasing temperatures [66, 82, 83], and our results suggest efficient utilization by microbes of
439 organic matter and woody terrestrial inputs in Arctic streams.

Moreover, nutrient exports reaching high-Arctic streams are expected to rise in consequence of the global climate change [10]. First, an increase of atmospheric reactive N deposition, emitted and transported from industrialized areas, has been reported in remote Arctic areas [84, 85]. The accumulated N in snowpacks can be flushed into the streams during snowmelt but also released after biological recycling at a later stage [86, 87]. Second, the thawing permafrost is likely to enhance dissolved inorganic N exports in Arctic streams [8, 10, 30, 88, 89]. Our results suggest that high N availability enforces the EEA machinery in high-Arctic streams, thus promoting fast organic matter mineralization along the fluvial networks. In our sampling area, small-erosional landforms such as slide scars and thaw slumps are predominant at the slopes in the solifluction and nivation zones [33, 90], which is associated with the observed high NO_3^- concentrations in the stream water. Moreover, solifluction areas are highly sensitive zones in that they are low vegetated and generally have low soil N -uptake and denitrification, inducing rapid flow of N to the water outlets [91].

Overall, our results provide increasing evidence on the potential for climate change to affect Arctic ecosystems. The strong terrestrial-aquatic coupling and the clear response of N availability to the microbial use of organic matter suggest that organic matter mineralization along the fluvial networks could rise in a climate change setting. Thus, the potentially enhanced organic matter mineralization in the future could have system-wide consequences via changes in resource availability at higher trophic levels and positive C-climate feedback.

Acknowledgements

The authors thank Biobasis, Geobasis, and Zackenberg logistics for assistance at the Zackenberg Research Station. We are grateful to the GeoBasis Programme of the Zackenberg research station and Stefanie Cable for providing DEM and geomorphological data, respectively. Special thanks to

463 Lone J. Ottosen, Malin Camilla Håckansson and Birgitte Kretzschmar Tagesen for their support in
 464 laboratory analysis. This work was financially supported by the Carlsberg Foundation (grant
 465 number CF16-0325). AP has received additional support from the Ramón Areces Foundation
 466 postgraduate studies program.

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689 Tables

690 **Table 1** Stream characteristics of the sampling sites and the predominant landform.

Site	Altitude (m)	Major landform	Conductivity ($\mu\text{S cm}^{-1}$)	DOC (mg C L^{-1})	SUVA ₂₅₄ ($\text{L mg}^{-1}\text{ m}^{-1}$)	SR	NO_3^- ($\mu\text{g N L}^{-1}$)	NH_4^+ ($\mu\text{g N L}^{-1}$)	SRP ($\mu\text{g P L}^{-1}$)	DOC:DIN (molar ratio)	DOC:SRP (molar ratio)	DIN:SRP (molar ratio)
Grønselv A	125	Sol.	387	1.3	1.3	2.0	175.7	22.2	3.1	7.6	1074.5	145.8
Grønselv B	59	Mix.	237	1.2	2.1	1.3	13.0	36.5	10.2	28.8	308.5	10.8
Grønselv C	20	Allu.	252	1.6	1.9	1.3	6.3	46.3	4.2	36.5	1004.6	27.2
Kærelv 0	263	Sol.	302	1.3	1.6	1.6	134.0	105.0	5.2	8.0	814.7	101.4
Kærelv A	172	Sol.	362	1.0	1.4	1.8	161.0	22.2	6.9	6.4	377.1	59.0
Kærelv B	44	Mix.	299	1.2	1.6	1.5	10.4	26.3	2.3	37.0	1323.0	35.8
Kærelv C	18	Allu.	293	1.5	2.0	1.0	4.6	22.6	2.1	63.7	1847.0	29.0
Aucellaelv	135	Sol.	472	0.5	n.a.	n.a.	231.0	38.4	4.3	2.2	309.2	139.1
Lindeman	84	Mix.	566	0.6	1.4	1.6	23.5	28.2	3.5	12.5	404.9	32.4
Palnatokeelv	80	Mix.	161	0.5	1.3	13.0	7.7	29.2	9.5	16.5	142.3	8.6
Unnamed	107	Mix.	381	1.1	1.5	2.0	33.1	42.1	5.8	17.1	489.4	28.7
Zackenberget	12	Allu.	71	0.6	1.2	1.9	18.4	36.3	8.4	11.8	170.8	18.8

691 Sol. = solifluction; Mix. = mixed; Allu. = alluvial; n.a. = not available

692

693 **Table 2** Enzyme assays measured, their function and the artificial substrates used. Enzyme ratios and their ecological meaning as well as
694 bibliographic references are also included. EC: enzyme commission number.

Enzyme	Reference EC	Abbreviation	Enzymatic function	Substrate
Phosphatase	3.1.3.1-2	PHOS	Decomposition of phosphomonoesters	4-MUF-phosphate
β-D-1,4-glucosidase	3.2.1.21	GLU	Last step of cellulose decomposition, decomposition of cellobiose or small oligomers containing β-D-glucose linkages	MUF- β -D-glucoside
β-xylosidase	3.2.1.37	XYL	Last step of hemicellulose decomposition, decomposition of xylobiose or xylooligosaccharides	MUF- β -D-xyloside
Cellobiohydrase	3.2.1.	CEL	Degradation of cellulose	4-MUF- β-D-cellobioside
Phenol oxidase	1.10.3.2	PHEN	Transformation of a broad spectrum of phenolic molecules like lignin	L-3,4-dihydroxyphenylalanine (L-DOPA)
Enzyme ratios		Abbreviation	Ecological meaning	References
β-glucosidase:phosphatase		GLU:PHOS	1:1 ratios indicate an equilibrium between the elemental composition of available organic matter, microbial biomass, and nutrient assimilation; it can be used to detect P limitation.	[46]
β-xylosidase:β-glucosidase		XYL:GLU	Indicates the origin of polysaccharides; high values indicate that the organic matter used is derived from hemicellulose rather than cellobiose, which may indicate greater use of allochthonous plant material.	[48, 65]
(β-glucosidase+β-xylosidase):cellobiohydrolase		(GLU+XYL):CEL	Shows the complexity of the available polysaccharides; higher values indicate higher capacity for decomposition of simple polysaccharides (i.e. cellobiose and xylobiose) rather than the use of complex polysaccharides (i.e. cellulose).	[48]
β-glucosidase:phenol oxidase		GLU:PHEN	Referred to as the “recalcitrant index”; lower values indicate a potentially greater use of recalcitrant organic matter compounds	[47]
Cellobiohydrolase:phenol oxidase		CEL:PHEN	Points to the origin of the complex material; high values imply that the origin of the organic matter origin is rather cellulolytic (cellulose) than ligninolytic (phenolic compounds).	[48]

695

696 **Table 3** Biological characteristics of the biofilm. Values are mean and standard deviation for each sampling site (n=3).

Site	AFDM (mg cm ⁻²)	Chl <i>a</i> (μg cm ⁻²)	Bacteria (cell×10 ⁶ cm ⁻²)	HI (bacterial cell ×10 ⁶ μg chl <i>a</i> ⁻¹)	C content (μg C cm ⁻²)	N content (μg N cm ⁻²)	C:N (molar ratio)
Grønselv A	0.25 ± 0.10	0.02 ± 0.01	124 ± 28	6175±2677	62.94±36.20	7.33±1.91	9.6±3.3
Grønselv B	0.24 ± 0.01	0.04 ± 0.04	8 ± 5	575±716	38.93±4.43	7.81±1.85	6.0±0.9
Grønselv C	0.14 ± 0.03	0.01 ± 0.00	22 ± 13	2547±2321	17.76±2.82	3.46±0.78	6.1±0.5
Kærelv 0	0.23 ± 0.07	0.07 ± 0.02	49 ± 23	745±120	19.83±5.44	2.93±0.33	7.8±1.8
Kærelv A	0.27 ± 0.08	0.09 ± 0.06	40 ± 29	825±1072	23.02±7.65	4.98±2.07	5.5±0.9
Kærelv B	0.21 ± 0.10	0.02 ± 0.02	39 ± 31	2127±1174	15.64±6.28	3.18±1.16	5.7±0.3
Kærelv C	0.17 ± 0.04	0.01 ± 0.01	24 ± 8	2589±546	19.03±8.08	2.90±1.65	8.2±1.3
Aucellaelv	0.32 ± 0.04	0.30 ± 0.15	16 ± 2	64±34	50.98±9.61	8.32±0.86	7.1±0.7
Lindeman	0.18 ± 0.06	0.04 ± 0.02	7 ± 2	240±187	23.81±11.48	3.67±1.60	7.4±0.6
Palnatokeelv	0.22 ± 0.06	0.14 ± 0.03	14 ± 11	93±63	33.41±11.42	5.14±1.85	7.7±0.6
Unnamed	0.36 ± 0.22	0.30 ± 0.16	39 ± 26	129±28	25.68±14.68	3.63±1.01	7.9±2.2
Zackenberg	0.08 ± 0.01	0.08 ± 0.08	4 ± 3	57±12	62.94±36.20	7.33±1.91	7.1±0.7

697

698 Figure captions

699 **Fig. 1** Location of the Zackenberg study area in Greenland and the sampled reaches in the area (red
700 dots), including a solifluction uphill area (a) and a depositing alluvial area (b) adjacent to the
701 streams. Geomorphological map obtained from: <https://doi.pangaea.de/10.1594/PANGAEA.883484>
702 [33]

703 **Fig. 2** Dot plots for extracellular enzyme activities (EEA; nM MUF cm⁻² h⁻¹) grouped by
704 predominant landforms for each enzyme type: β -glucosidase (n = 36), phosphatase (n = 36), β -
705 xylosidase (n = 33), cellobiohydase (CEL, n = 21), and phenol oxidase (n = 21). Enzyme ratios are
706 also included. Significantly different landform groups (Tukey's test after significant one-way
707 ANOVA at $p < 0.05$) are annotated by different letters. Colors correspond to the three watersheds
708 sampled. Mean $\pm$ SD values are included in a black point range. Note the different scales for each
709 enzyme.

710 **Fig. 3** The relative contribution of stream variables to variance of extracellular enzyme activities
711 (EEA) based on the results of the best-predicting multiple regression model for each activity. The
712 symbol “-” indicates that the variable shows a negative coefficient in the selected model, no symbol
713 implies positive coefficients. Further information on the multiple regression models can be found in
714 the supporting information (Table S2).

715 **Fig. 4** Relationships between chl *a*-specific phosphatase and bacteria-specific phosphatase with
716 stream SRP concentration (Spearman correlation: $\rho = -0.65$, $p < 0.001$, $\rho = 0.45$, $p = 0.006$,
717 respectively). The red line is the fitted regression model with segmented relationship with an
718 estimated breakpoint at $5.7 \pm 1.1 \mu\text{P L}^{-1}$.

719

720 **Fig. 5** Conceptual diagram of the microbial organic carbon utilization of Arctic streams related to
721 the key organic terrestrial sources for the main geomorphological landforms. Created with Inkscape
722 (0.92.2) with images courtesy of the Integration and Application Network, University of Maryland
723 Center for Environmental Science (ian.umces.edu/symbols/).

724

729 Fig 3

730

731 Fig 4

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735 Fig 6

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