Factors influencing bacterial dynamics along a transect from supraglacial runoff to proglacial lakes of a high Arctic glacieri

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Abstract

Bacterial production in glacial runoff and aquatic habitats along a *c.* 500 m transect from the ablation area of a Svalbard glacier (Midre Lovénbreen, 79°N, 12°E) down to a series of proglacial lakes in its forefield were assessed. In addition, a series of *in situ* experiments were conducted to test how different nutrient sources (glacial flour and dissolved organic matter derived from goose faeces) and temperature affect bacterial abundance and production in these ecosystems. Bacterial abundance and production increased significantly along this transect and reached a maximum in the proglacial lakes. Bacterial diversity profiles as assessed by denaturing gradient gel electrophoresis indicated that communities in glacial runoff were different from those in proglacial lakes. Heterotrophic bacterial production was mainly controlled by temperature and phosphorus limitation. Addition of both glacial flour and dissolved organic matter derived from goose faeces stimulated bacterial production in those lakes. The results suggest that glacial runoff sustains an active bacterial community which is further stimulated in proglacial lakes by higher temperatures and nutrient inputs from bird faeces. Thus, as in maritime temperate and Antarctic settings, bacterial communities developing in the recently deglaciated terrain of Svalbard receive important inputs of nutrients via faunal transfers from adjacent ecosystems.

Introduction

During the past decade, glaciers have received increasing recognition not only as an integral part of the cryosphere, but also as ecosystems harbouring microbial life (Skidmore *et al.*, 2000; Christner *et al.*, 2003). Glaciers in alpine and polar regions are highly sensitive to changes in climatic conditions (Oerlemans, 1998). They are characterized by cold temperatures and low nutrient concentrations. In polar glaciers, life is limited also by repeated freeze and thaw cycles that cause extreme hydrochemical conditions and desiccation therein (Tranter *et al.*, 2004). Microbial and metazoan life in glaciers is found in ice cores (Skidmore *et al.*, 2000; Christner *et al.*, 2003), on the glacial surface (Yoshimura *et al.*, 1997), as well as in sub glacial sediments (Foght *et al.*, 2004), drainage systems of glaciers (Řehák *et al.*, 1990), glacier beds (Sharp *et al.*, 1999), and glacial streams (Elster *et al.*, 1997; Battin *et al.*, 2001). Perhaps the best known habitats in glacial environments are the cryoconite holes, which often harbour complex microbial communities (Mueller & Pollard, 2004) within a small, water-filled cylindrical depression (typically less than 1 m in diameter, and not deeper than 0.5 m (Wharton *et al.*, 1985; Takeuchi *et al.*, 2000). These habitats form in the ablation zones of glaciers by preferential melting of dark sediment with low albedo of organic and inorganic origin that rests on the bottom of the hole. These habitats can sustain short food webs mainly dominated by bacteria, viruses, fungi, and autotrophic and heterotrophic protists (Takeuchi *et al.*, 2000; Säwström *et al.*, 2002; Christner *et al.*, 2003). Some metazoans have been reported from cryoconites, for example rotifers (De Smet & Van Romptu, 1994) and tardigrades (Dastych, 2005). Compared with the other glacial habitats, cryoconite holes are thought to contain the highest level of biodiversity (Takeuchi *et al.*, 2000).

Liquid water is available during the summer melting period of glaciers, especially when temperatures rise above zero and large volumes of melt water are produced. This melt forms channels that flush the cryoconite holes (Mueller et al., 2001) and can transport nutrients and microorganisms to newly established proglacial lakes. Thus, glacial melt water runoff has the potential to affect directly the microbial community composition of those lakes. In Taylor Valley, Antarctica, at least 13% of the runoff has its origin in cryoconite holes (Fountain *et al.*, 2004). The main nutrient input to adjacent lakes depends on the drainage flow from the glacier surface and subsurface channels (Priscu *et al.*, 2002).

Previous studies have shown that in cold environments multiple factors may interact to constrain bacterial growth (Wiebe *et al.*, 1992; Gurung & Urabe, 1999; Nedwell, 1999; Pomeroy & Wiebe, 2001). In polar ecosystems, one might anticipate that bacterial metabolism is limited by the availability of inorganic and organic nutrients, and/or by low temperature (Pomeroy & Deibel, 1986; Pomeroy & Wiebe, 2001). Indeed, temperature is an important regulator of bacterial growth in Arctic marine and freshwater environments (Granéli *et al.*, 2004b).

In contrast to polar continental environments, where the main allochthonous input of organic and inorganic matter is from airborne transport and atmospheric deposition (Marshall, 1996), coastal regions benefit from faunal inputs, too (e.g. Hodson, in press a and b). In Svalbard, goose colonies that become established in the summer months produce a remarkable amount of faeces that contribute to the allochthonous carbon and nutrient inputs to local water bodies and soils. An additional source of nutrients for glacial communities is glacial flour, which consists of silt-clay-sized particles of rock generated by glacial erosion and abrasion of solid material by the glacier movement (Hodson *et al.*, 2004, 2005). During summer, melt water with its load of suspended glacial flour is introduced into the adjacent freshwater water bodies such as proglacial lakes or glacial streams. This material is thought to support bacterial growth due to phosphorus content of up to 1 mg P g^{-1} (Hodson *et al.*, 2004). However, as much of the phosphorus is associated with unweathered primary mineral phases (typically apatite), its restricted bioavailability might mean that the high rates of sediment delivery are most important for P supply (Hodson *et al.*, 2004).

The aim of this study was to assess the temporal and spatial shifts in bacterial abundance and production, and diversity along a longitudinal transect in the ablation zone down to proglacial lakes. In addition, the effect of limiting bacterial growth factors such as nutrients and temperature along an elevational transect were evaluated. Of particular interest was assessing the effect of inorganic (glacial flour) and organic (goose faeces) nutrients upon the bacterial abundance and productivity of supraglacial melt water streams and pelagic communities of the adjacent lakes along this transect.

Materials and methods

Study site

The glacier Midre Lovénbreen was an alpine type valley-glacier located in the Kongsfjord region of northwest Spitsbergen (78.53°N, 12.04°E). The glacier was *c.* 6 km long, with an area of *c.* 5.5 km^2, and a maximum thickness of *c.* 180 m (Björnsson *et al.*, 1996). It ranged from *c.* 50 m at the terminus to *c.* 600 m above sea level at the head wall. Midre Lovénbreen was a polythermal glacier with a warm-based core, cold-based ice around its snout and margins, and cold ice at its surface (Hagen & Saetrang, 1991; Björnsson *et al.*, 1996).

Sampling

Samples were collected on three occasions on the 19th, 22nd and 27th of July 2004 (hereafter addressed as day 1–3) from supraglacial melt water runoff and cryoconites with throughflow along a longitudinal transect consisting of three stations (*c.* 150 m apart) on the glacial ablation zone (Tran 2–Tran 4) and two proglacial lakes. On the third sampling occasion, an additional sampling station high on the glacier was included (Tran 1, Fig. 1). Samples from the proglacial lakes were collected at 0.5 m depth and *c.* 1.5 m from the edge. For sample collection, 10 L acid-washed polyethylene bottles previously rinsed with distilled and sample water were used. All samples were immediately transported to the laboratory at the Natural Environment Research Council (NERC) station in Ny Ålesund for further processing.

Experimental design

Nutrient limitation

A laboratory experiment was conducted to test the effects of different sources of carbon and phosphorus, i.e. glucose, KH_2PO_4, goose faeces, and glacial flour on the dynamics of bacteria. The water used for the experiments was collected from the supraglacial melt water runoff and cryoconites with throughflow to have samples unaffected by the different nutrient sources. Approximately 30 L melt water were collected from station Tran 2 (Fig. 1) and transported to the laboratory, where incubations were started within 2 h after sampling. Samples for phosphorus analyses were taken directly from the 10-L polyethylene bottles. For dissolved organic carbon (DOC) analyses, 20 mL were filtered through precombusted (450 °C, 2 h) glass fibre GF/F filters (Whatman, 25 mm). For the experiments, the water was gently filtered through 0.8 μm polycarbonate filters to remove particulate matter and bacterial grazers. Subsamples were taken for determinations of the initial bacterial abundance,

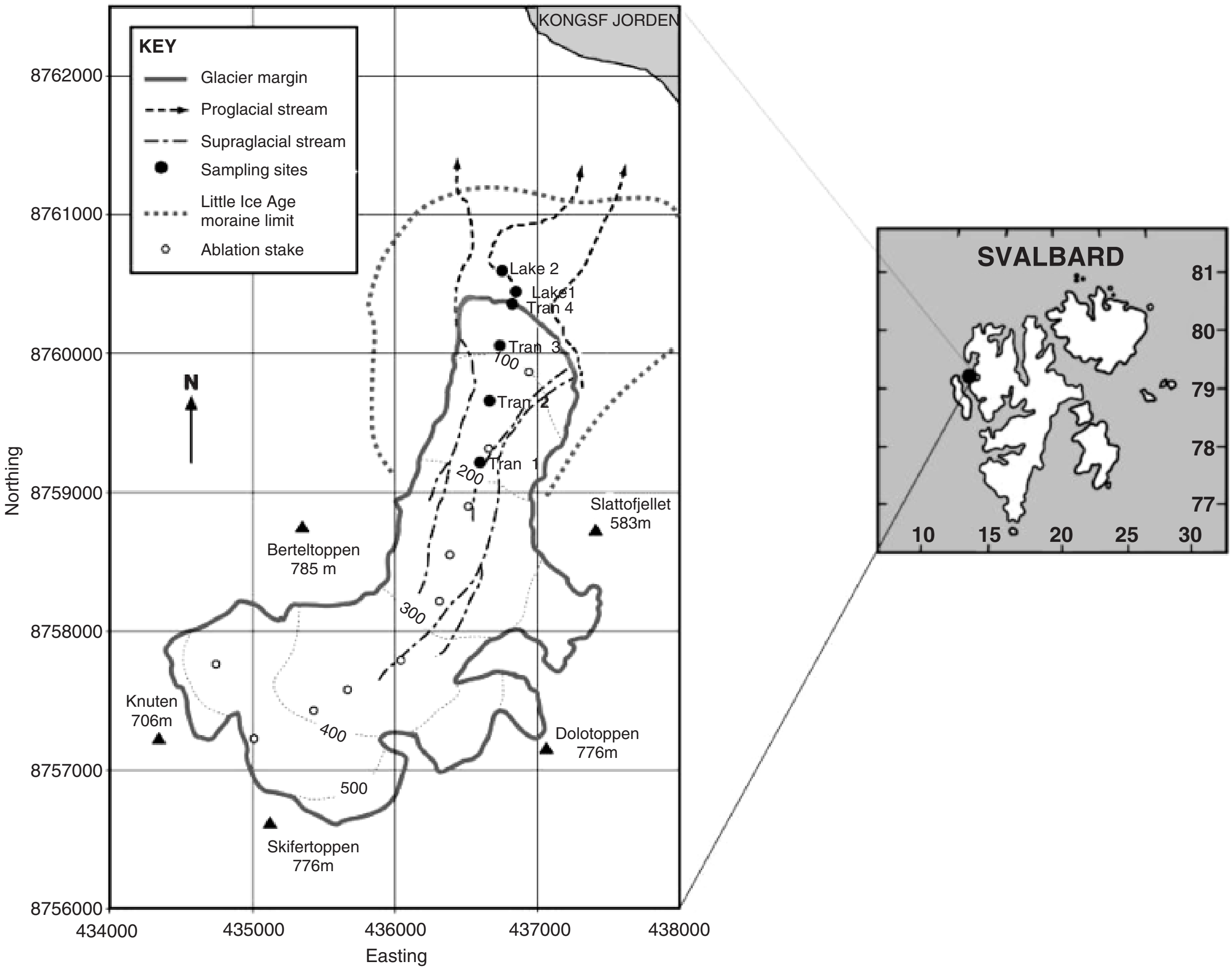

Fig. 1. Location of Midre Lovénbreen at Kongsfjord and sampling sites (modified after Hodson and Irving-Fynn).

bacterial production, DOC, and phosphorus concentration. All treatments were performed in triplicate.

Experiment I

Experiment I was conducted to examine whether P or C (or both) were limiting bacterial growth. The bacterial community collected from Tran 2 at day 1 was amended by the independent addition of single sources of carbon and inorganic phosphorus, and a combination of both. Subsamples of 900 mL from the 0.8 μm filtered melt water were allocated to three treatments consisting of the addition of glucose (+C) at a final concentration of 100 μM, phosphorus (+P) as K_2HPO_4 at a final concentration of 2 μM, and both (+CP). Batch cultures were run for 1 week. The concentration of nutrients chosen was the same as used in previous studies investigating limiting factors for bacteria (Ellis-Evans *et al*., 2001).

Experiment II

Experiment II was conducted to assess whether bacteria from glacial melted water were stimulated by the independent addition of a sterile extract of goose faeces and glacial flour. Goose faeces were collected on the lake shore and dissolved in 0.2 μm filtered melt water. The solution was homogenized and shaken for 3 h to give a stock solution concentration of 1.54 mg fresh weight mL^{-1}. An aliquot was twice filtered through 0.2 μm sterile filters (Sartorius) and 2 mL of this extract was added to 900 mL of the 0.8 μm filtered glacial melted water. The targeted amount of goose faeces added to the amended treatments was calculated to reach a final concentration of phosphorous of *c*. 10 times higher than those in the melt water. We have considered concentrations of nutrients reported for lakes where geese are present (Ellis-Evans *et al.*, 2001) and used unpublished data on chemical analyses of the faeces (personal communication, Geir Wing-Gabrielsen, 2005). The final DOC, total phosphorus (P_{tot}), and total dissolved phosphorous (P_{diss}) concentration was 2.08 mg L^{-1}, 13.1 μg L^{-1}, and 8.8 μg L^{-1}, respectively.

The glacial flour from melted water was collected onto a polycarbonate filter (0.8 μm) autoclaved for 20 min and dried at 60 °C overnight. Then, the flour was added to 900 mL of 0.8-μm filtered melted glacial water to a final concentration of 280 mg L^{-1}. DOC, P_{tot}, and P_{diss} concentrations were 1.64 mg L^{-1}, 2.9 μg L^{-1}, and 2.2 μg L^{-1}, respectively. The concentration of P_{tot} and P_{diss} in the control (no addition) was 0.6 and 0.5 μg L^{-1}, respectively. The DOC concentration for the control was 1.00 mg L^{-1}.

Temperature limitation

Both experiments (I and II) were run at 0.1 °C and 12 °C to test the effect of temperature on bacterial growth. The selected temperature range corresponded to that observed in the melt water runoff and lake surface. In experiment I, only bacterial abundance was assessed, whereas in experiment II, bacterial production was also measured.

Physico-chemical parameters

Air temperature

Air temperature data were obtained from the meteorological station in Ny Ålesund and kindly provided by the Alfred--Wegener-Institute, Bremerhaven, Germany. Water temperature was measured with a pin-tipped digital thermometer (Dailymate).

DOC and phosphorus

DOC was measured with a Shimadzu TOC-5000 following recommendations of Sharp & Peltzer (1993). Total phosphorous (unfiltered sample) and total dissolved phosphorous (GF/F filtered sample) were measured spectrophotometrically following the molybdate-blue method (Schmid & Ambuehl, 1965).

Bacterial abundance

Duplicate 50-mL samples for bacterial enumeration were immediately fixed with prefiltered (0.02 μm pore size filter) glutaraldehyde (2% final concentration). Bacteria were counted by epifluorescence microscopy (Axioplan, Zeiss) using SYBR Gold stain (10 000 × original dilution; Molecular Probes) according to the method of Chen *et al.* (1992). Samples of 10 mL were filtered on 0.02 μm pore size Anodisc membrane filters (Whatman), with a 0.45-μm pore size backing membrane filter. The filter was then laid, sample side up, on a drop of SYBR Gold working solution [25 μL of 100 μL diluted SYBR Gold solution and 75 μL of filtered (0.02 μm pore size filter, Milli-Q water)], for 15 min in the dark. After drying, the filter was mounted on a glass slide with a drop of Slow Fade® antifade solution (Molecular Probes). For each filter, at least 150 bacteria were counted on 10–15 fields of view randomly selected. The samples from the Experiment II with glacial flour were treated with 0.1 M tetrasodium pyrophosphate for 1 h and subsequently sonicated for 90 s, 40-W output) and finally centrifuged at 1000 ***g*** for 10 min to remove the bacteria attached to the particulate material before staining (Griebler *et al.*, 2001).

Bacterial production

Samples were incubated at *in situ* temperature, except for those tested at 12 °C. Bacterial production was determined using the leucine incorporation method (Kirchman *et al.*, 1993). ^{14}C-Leucine (specific activity 86 Ci $mmol^{-1}$; GE Healthcare, UK; Little Chalfont, Bucks, UK) was added to a final saturating concentration of 40 nM. On each sampling occasion, triplicate 20-mL samples and one formaldehyde-killed control incubation were run. After 3 h, the incubation was terminated by the addition of 1 mL formaldehyde and 2.5 mL trichloric acetic acid (TCA) 50%. Samples were filtered through 0.22-μm cellulose acetate filters and washed with three volumes (5 mL) of 5% ice-cold TCA. The filter was dissolved with 1 mL ethyl acetate, 10 mL of scintillation fluid (Beckman Ready Safe) was added and counted in a scintillation counter (Beckman, LS6500) with internal quench curve. Bacterial carbon production was calculated as described by Simon & Azam (1989).

Bacterial diversity profiles by DGGE

Samples (2 L) collected at each station along the transect and at the proglacial lakes were gently filtered onto 0.2-μm pore size polycarbonate filters which were frozen immediately after return to the station. DNA was extracted directly from the filters following the user instructions for the Nucleo-spin tissue DNA-extraction kit (Machery & Nagel™). For the enzymatic amplification of 16S rRNA gene, each PCR mixture (50 μL) contained 10 ng of extracted DNA as template, 20 pmol of each primer, and 46 μL of PCR Master Mix (Promega). Primers used were 63f (CAGGCCTAACA-CATGCAAGTC, 5′–3′) and 1378r (CGGTGTGTA-CAAGGCCCGGGAACG, 5′–3′). Amplification reactions were performed with a PHC-3 thermocycler (Techne, Minneapolis, MN) using the following protocol: an initial denaturation step consisting of 94 °C for 3 min, 30 cycles consisting of 94 °C for 1 min, 62 °C for 1 min, 72 °C for 2 min, and a final elongation step consisting of 72 °C for 10 min with subsequent cooling. Controls containing no DNA were used to ensure that contaminants were not being amplified. According to Muyzer *et al.* (1993), a nested PCR reaction is recommended to achieve stronger PCR products. The second PCR reaction was performed with the amplificates and a GC-clamp and following primers: GC338 (CTTCTTGATTCTGCATCACC, 5′–3′) and 907r (CCGTCA ATTCCTTTRAGTTT, 5′–3′), with a slightly modified protocol for amplification as follows: initial denaturation for 5 min at 94 °C, 35 cycles with 94 °C for 1 min, 48 °C for 1 min, 72 °C for 1 min, final elongation for 5 min at 72 °C, and subsequent cooling. To assure amplification had occurred, the PCR products were loaded and visualized on a 1% agarose gel where electrophoresis was conducted at 100 V for 15 min.

The PCR products (25 μL) were separated by DGGE as described by Pearce (2000) using a formamide gradient of 60–40%. The gel was loaded with 2 μL of template and 2 μL loading dye and was run for 16 h at 60 °C with 70 V, before staining with silver nitrate with an automated gel stainer (Hoefer, Amersham Pharmacia Biotech). The gel was visualized with a UV transilluminator. Bands were not excised for further sequencing. Clustering of bands was done with the software GELCOMPAR (version 4.0; Applied Maths, Sint Martens-Latem, Belgium). Similarities in the bacterial diversity profile among sampling stations were evaluated by Dice correlation coefficient (Nei & Li, 1979) and expressed in a Distance matrix. Cluster analysis was performed and dendrograms were calculated using the Unweighted Pair group Method using Arithmetic averages.

Statistical analyses

To test for the effect of nutrients and temperature in the experiments, we used a 2-way ANOVA with posthoc comparisons using Bonferroni-corrected *t*-tests. For the field survey, Spearman correlations were examined between bacterial and chemical parameters. All statistical analyses were performed using SIGMASTAT (Version 2.03, SPSS). To control the overall experimental Type I error, we corrected the significance level according to the Bonferroni method as $\{\alpha/[n(n-1)/2]\}$, where α is the probability level and n is the number of observations.

Results

Physico-chemical parameters

During the sampling period, the air temperature at midday varied from 7.5 °C on 27 July to 9.2 °C on 19 July 2004. The temperature of supraglacial melt water runoff was between − 0.2 °C and 0.2 °C, whereas the temperature range of Lake 1 was from 0.6 to 7.4 °C (Fig. 2). Water temperatures in Lake 2 were higher and changed during the study period from 4.2 °C to 14.4 °C (Fig. 2). Contrary to the relatively stable temperature found in the glacial runoff, lake surface water temperatures varied significantly depending with weather

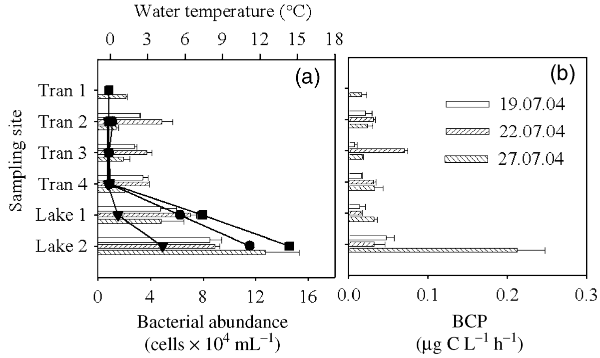

Fig. 2. Bacterial abundance (a), bacterial carbon production (BCP) (b) and temperature along the transect data points (Tran 1–4) on the Midre Lovénbreen glacier and two proglacial lakes on three different sampling days (mean ± SD, temperature lines: ● day 1, ▼ day 2, ■ day 3).

conditions. For example, a heavy rainfall between sampling days 1 and 2 caused a temperature decrease of 5–7 °C in both lakes, due to increased input of glacial melt water runoff.

The concentration of phosphorus in the glacial melt water runoff was low, with P_{tot} concentrations ranging from 1.2 to 13.5 $\mu g\,L^{-1}$, and P_{diss} from 0.9 to 1.0 $\mu g\,P\,L^{-1}$. In Lake 1, the average P_{tot} and P_{diss} concentrations were 18.8 and 0.8 $\mu g\,L^{-1}$, respectively, whereas in Lake 2, it was 4.1 and 0.5 $\mu g\,L^{-1}$, respectively. The DOC concentration showed minimum values with 1.02 $mg\,L^{-1}$ on day 3 for all sampling points. In melt water samples, DOC ranged from 1.10 $mg\,L^{-1}$ in Tran 3 (day 3) to 1.65 $mg\,L^{-1}$ in Tran 2 on the first sampling day. DOC content in lakes showed no substantial variation in concentration. The lowest concentration was 1.02 $mg\,L^{-1}$ (day 3, Lake 1) and the highest was 1.43 $mg\,L^{-1}$ (day 1, Lake 1) (Fig. 2).

Bacterial abundance

An increase in bacterial abundance was observed along the transect towards the lakes, although bacterial numbers showed less variation among sampling points on the glacier (Tran 1–4, Fig. 2). Bacterial numbers ranged from $1.38 \times 10^4\,mL^{-1}$ (Tran 3, day 3) to $4.84 \times 10^4\,mL^{-1}$ (Tran 2, day 2). The lakes showed significantly higher (ANOVA, $n = 6$, $P < 0.001$) bacterial abundances than in the glacial runoff with a minimum of 4.74×10^4 cells mL^{-1} (Lake 1) (day 1) and a maximum of 12.7×10^4 cells mL^{-1} (Lake 1) (day 3). During the sampling period, bacterial abundance was significantly correlated with water temperature (Spearman correlation: $r = 0.527$, $P < 0.05$, $n = 16$).

Bacterial production

There was little variation in bacterial production among sampling sites from Tran 1–4 (Fig. 2). Values ranged from 0.008 $\mu g\,C\,L^{-1}\,h^{-1}$ (Tran 3, day 1) to a maximum value of

$0.071\,\mu g\ C\,L^{-1}\,h^{-1}$ (Tran 3, day 2). In the lakes, bacterial production was generally higher than in the glacial melt water samples and ranged from $0.014\,\mu g\,C\,L^{-1}\,h^{-1}$ (Lake 1) on day 1 to $0.213\,\mu g\,C\,L^{-1}\,h^{-1}$ (Lake 2) on day 3. Bacterial production in Lake 2 was significantly higher than at the other sampling points along the elevational transect (ANOVA, $n=6$, $P<0.001$) (Fig. 2).

Bacterial diversity profiles

In general, the banding patterns from the glacier runoff samples showed some degree of similarity among them, whereas samples from the proglacial lakes displayed no significant similarities either with each other or the samples from the glacial surface (Fig. 3). The banding patterns of the samples from the last sampling day (day 3) Tran 1–3 showed a similarity of 71% (cluster A in Fig. 3), whereas the samples Tran 1–3, from the first day (day 1) were 100% similar (cluster B in Fig. 3), as well as cluster C, which includes the samples Tran 1–3 from the second sampling day (day 2). No bands were detected in samples from Lake 1 from day 1 and 3 (Fig. 3).

Effect of nutrients and temperature

After incubation for 1 week at 0.1 °C, bacterial abundance showed no increase in any of the amendment treatments (i.e. +C, +P, and +CP). In contrast, at 12 °C bacterial abundance approximately doubled in the +P and in +CP treatments compared to the control (Fig. 4). The addition of organic carbon (+C) alone did not affect bacterial abundances relative to the control.

Experiments to test the effects of goose faeces and glacial flour on the dynamics of bacteria, showed no effect on bacterial abundances or production at 0.1 °C, except on day 2 when bacterial abundance in the glacial flour treatment was significantly higher (ANOVA, $P<0.05$) than in the control (Fig. 5). On the other hand, bacterial abundance at 12 °C in the + goose faeces treatment was significantly higher than in the control on the last sampling day (Fig. 5). Bacterial abundance in the + glacial flour did not significantly differ from that in the control. Bacterial production increased significantly in both + goose faeces and + glacial flour at 12 °C.

Discussion

The evolution of life strategies in naturally cold environments such as Arctic, Antarctic, or alpine regions has attracted the attention for many years due to growing awareness of the sensitivity of these areas to climate change. With ongoing glacial retreat (as is the case in Svalbard), the interaction between melt waters and moraines in the glacial forefield is becoming more significant. Here solute and nutrient acquisition rates are influenced by the development of long residence time flowpaths through terrestrial moraines prior to drainage into proglacial lakes (e.g. Hodson *et al.*, 2002). Thus, glacial retreat can affect solute and nutrient fluxes at the catchment level and the lake plankton communities that develop (Anderson *et al.*, 2000; Engstrom *et al.*, 2000). Melt waters from glacial ablation can also dramatically increase stream discharge, expand channel networks, and thus enhance hydrological connectivity in proglacial plains (Battin *et al.*, 2004), resulting in the potential for increased transport of organic and inorganic

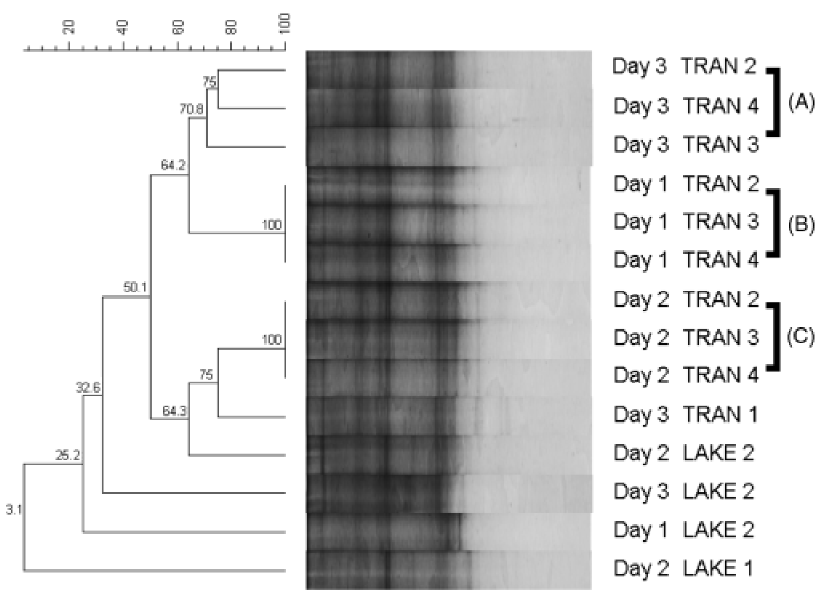

Fig. 3. Results of the cluster analysis based on DGGE band position for the stations located along the transect data points (Tran 1–4) on the Midre Lovénbreen glacier and the two proglacial lakes. Banding patterns were subjected to GELCOMPAR cluster analysis (UPGMA; Dice correlation coefficient). The similarity value is indicated at the branch of each cluster.

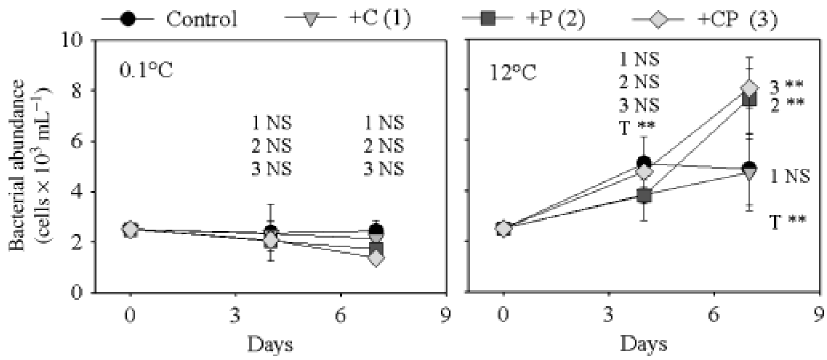

Fig. 4. Response of bacterial abundance to nutrient addition (C-Glucose, P-K_2HPO_4) at two different temperatures (T) (mean ± SD, $n=3$, NS, not significant; $^{**}P < 0.01$). Sampling days: July 19, 22 and 27, 2004.

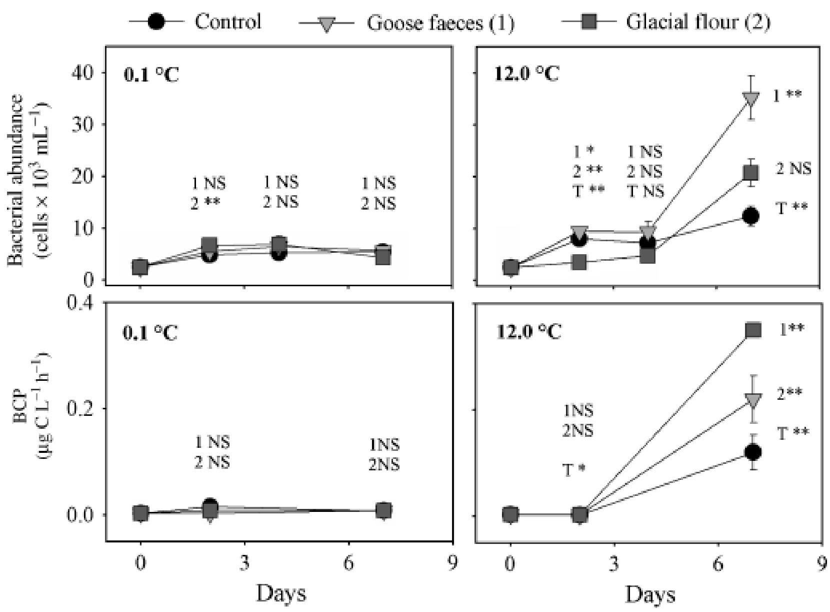

Fig. 5. Response of bacterial abundance and bacterial carbon production (BCP) to nutrient addition (DOM-goose faeces and glacial flour) at two different temperatures (T) (mean ± SD, $n=3$, NS, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$).

matter through the system. For these reasons, the following discussion gives emphasis to the effects of both temperature and nutrient concentration upon bacterial communities occupying such a dynamic water course.

Effect of nutrients and temperature

The data clearly showed a combined positive effect of temperature and P on bacterial abundance in the glacial melt water, whereas no significant carbon limitation was observed (Fig. 4). No significant increase in bacterial abundance could be detected at *in situ* temperatures but in the treatment at 12 °C where a significant rise in bacterial numbers was observed. Despite the finding that sufficient nutrient supply could compensate for low temperatures (Felip *et al.*, 1995; Pomeroy & Wiebe, 2001), a distinct synergistic effect of both temperature and nutrients was observed in our experiment. The results are therefore in agreement with those from Skidmore *et al.* (2000), who assessed nutrient limitation at various temperatures in a Canadian high Arctic glacier. In their study, bacterial growth and production remained unchanged in spite of nutrient amendment at *in situ* temperatures. Numerous other studies have shown a strong relationship between growth rate and temperature (White *et al.*, 1991; Hoch & Kirchman, 1993; Pomeroy & Wiebe, 2001). However, the interaction between nutrient and temperature limitation of bacterial growth is potentially complex: low temperature, for instance, limits bacterial growth when there is an excess of C and P (Felip *et al.*, 1996). The same results were also found in different aquatic environments where bacteria are P-limited (Toolan *et al.*, 1991; Elser *et al.*, 1995; Granéli *et al.*, 2004a). Thus, it seems that in cold aquatic systems, heterotrophic bacteria might require very high substrate concentrations to be active probably because of reduced substrate affinity at low temperature (Wiebe *et al.*, 1992). Furthermore, it has been demonstrated that specific growth rates of bacteria in the subarctic sea are enhanced not only by the addition of DOC but also by the increase of temperature, and that the temperature response is faster than the response to nutrient additions (Kirchman *et al.*, 1993).

Effect of different nutrient sources

Both goose faeces and glacial flour had a positive effect upon bacterial growth and production (Fig. 5). However, this effect was only significant at elevated temperatures. Therefore, glacial runoff transporting these two nutrient sources is expected to have its largest effect on proglacial lakes where water temperature is higher. The drainage area of the proglacial lakes has a poor vegetation cover, but it is highly influenced by goose colonies (Barnacle Goose, *Branta leucopsis*), which often rest in the moraines after feeding. Importantly, their nutrient-rich faeces fertilize the lakes within the moraines, but not the aquatic environments upon the glacier. Here, however, bird faeces were also observed because kittiwakes frequently flew over the glacier whilst commuting to and from a local colony to the east (personal communication, Geir Wing-Gabrielsen, 2005). However, the occurrence of kittiwake droppings upon the glacier is best described as occasional, and in no way as commonplace as goose droppings in the forefield.

Though goose droppings are abundant in the Ny Ålesund region, about 36% are removed by reindeer foraging (van der Wal & Loonen, 1998). Despite this, up to 18 droppings per m^2 could be counted in this area, suggesting that it is almost certain that the geese are important input factors for phosphorus and other nutrients inside the moraine complex of the glacier (van der Wal & Loonen, 1998). Moreover, with the ongoing glacial retreat, vegetation cover in the ablated moraines would increase and eventually enlarge the habitat for barnacle geese. Thus, rather like the Pacific salmon of Glacier Bay forefields in Alaska (Milner & Bailey, 1989) and the penguin colonies of Signy Island in the maritime Antarctic (Hodson, in press a and b), fauna most probably play a significant role in the fertilization of microbial foodwebs trying to become established in the newly deglaciated terrain of this Svalbard glacier basin.

Hodson (in press, a and b) show that a significant phosphorus yield ($530\,kg\,P\,h^{-1}\,a^{-1}$) is transported by glacial streams draining the study site, of which a vast majority is transported by subglacial streams (Hodson *et al.*, 2004). However, less than 1% is base extractable and thus operationally defined as bioavailable (Hodson *et al.*, 2004). Thus, as the vast majority of the phosphorus is associated with unweathered apatite and other primary mineral phases, the bioavailability of this critical productivity-limiting nutrient is compromised and limited by low dissolution rather than rapid desorption. Such a mechanism, like bacterial metabolism, is almost certainly likely to be enhanced at 12 °C.

High concentrations of glacial flour, rather than high concentrations of labile phosphorus adsorbed to it, are therefore most likely to make it a valuable source of phosphorus for bacteria and other organisms. The intensity of glacial erosion certainly ensures an abundance of fresh mineral surfaces and high surface areas to volume ratios for sediments in most glacial habitats. In addition, attachment to particles is well known to favour bacterial growth and has been demonstrated in Antarctic lakes (Laybourn-Parry *et al.*, 2004). Moreover, in another polythermal high Arctic glacier in Canada, Skidmore *et al.* (2000) observed enhanced bacterial activities with increasing sediment concentration in subglacial waters. Similar findings have also been obtained for alpine subglacial environments (Sharp *et al.*, 1999).

The transect

The course of a supraglacial stream is extremely dynamic and variable depending on cracks on the glacier surface which can open and close within short time periods leading to variable conditions. Therefore, conditions can vary since the runoff might flush cryoconite holes during the sampling period or even end up subglacially in Moulins, which are tubular chutes through which water enters a glacier from the surface and carries melt water. The sampling transect in this study was placed along the largest melt water channel upon Midre Lovénbreen, which was characterized by a relatively clean ice surface at Tran 1–4 and greatly increased debris cover towards the boundary between glacier and moraines. Battin *et al.* (2004) showed that glacial melt waters were a shaping factor for the hydrology and biogeochemistry of glacial streams resulting from the terminus, as well as for the structure and function of microbial communities therein.

In general, bacterial numbers along the transect showed clearly that the abundance in the lakes was much higher than in the melt water runoff. A steady and small decrease was observed from the higher sampling points down to the terminus of the glacier (Tran 1–4). The sudden drop in lake water temperature did not have a significant effect on bacterial cell numbers and so bacterial abundance in fact increased in the lakes, probably due to the input of bacteria flushed from cryoconite holes which experience a through flow during melting period. Given that up to 6% of the ablation area of the studied glacier consists of cryoconite material (Hodson *et al.*, 2005), the holes themselves might represent an important nutrient and microbial source for the entire system since they are connected with the streams and lakes.

Bacterial abundances were within the range of a runoff-transect on an alpine polythermal glacier (Rotmoosferner in Ötztal Alps; B. Sattler *et al*., unpublished data) but clearly below those from melt water streams on an Antarctic glacier in the McMurdo Dry Valley sampled during the austral summer (Mikucki *et al*., 2004). The bacterial abundance from glacial melt water runoff (Fig. 2) was very similar to that reported for cryoconite holes of the same glacier (Säwström *et al*., 2002). Along the investigated transect, bacterial abundance and production significantly increased in the proglacial lakes and both parameters were one order of magnitude higher than in the supraglacial melt water (Fig. 2). Bacterial numbers in the proglacial lakes were in the range of seasonally ice-covered high-mountain lakes in the Alps (Psenner *et al*., 1999). Glacial runoff is crucial for the nutrient supply to proglacial lakes of glaciers worldwide. However, compared with Antarctic glacier systems, arctic and alpine proglacial water bodies have more allochthonous input sources because the ice cover is in most cases seasonal as opposed to perennial, as is the case in key sites such as the McMurdo Dry Valleys (McKay *et al*., 1985; Priscu *et al*., 2002) where the stream flow enters the lakes via the ice-free moats during the austral summer. In addition, microbial communities of cryoconite holes in Antarctic systems are sealed by an ice lid for most of the year (Tranter *et al*., 2004) and cannot contribute to proglacial lakes as directly as we have observed in Svalbard. Instead, an indirect effect is likely because the nutrient status of supraglacial melt changes markedly as runoff percolates slowly through the nutrient impoverished cryoconite holes of the McMurdo Dry Valleys and other continental Antarctic environments.

At Midre Lovénbreen, a strong degree of hydrological interconnectivity between cryoconite holes and other supraglacial habitats can be observed on account of the high melt rates (*c*. 2 m year^{-1} at Trans 1: Hodson, in press a and b). Thus, it is not surprising that no severe differences in bacterial composition were encountered along the transect. This is also reflected in the results from the cluster analysis of bacterial diversity profiles (Fig. 3), which shows clearly separated clusters consisting of melt water runoff samples on one hand and lake water samples on the other hand. The latter could possibly receive more input from subterrestrial sources if a connection with subsurface melt water were assumed. However, hydrological studies are needed to confirm this assumption. Despite the increase in bacterial numbers increased in the lakes and the fact that they seem to be influenced by the outwash of cryoconite holes at first sight, a subterrestrial inflow contributing additional bacterial cells could be also responsible for this observation. This process has been proven on other glaciers (Skidmore *et al*., 2000) but not for Midre Lovénbreen so far. This hypothesis could then explain the different clusters of melt water runoff and lake samples obtained from DGGE profiles.

Conclusions

This study has shown that increased temperature and additional phosphorus sources in the form of both faeces and glacial flour enhance bacterial growth and production in glacial and proglacial bacterial habitats in the European High Arctic. Along the transect investigated, it was found that bacterial abundance and production significantly increased in the proglacial lakes and it was shown that the synergistic effects of temperature and substrate supply on the heterotrophic community in this area are most likely strong. This synergistic effect is not only crucial for glacial environments but also for the ice-marginal habitats, which will receive more bacterial biomass following warming and further glacier retreat. Thus, there exists a potential for glacial and proglacial microbial habitats to provide an additional carbon and nutrient resource for higher trophic levels during increased glacier ablation.

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