

Carbon fluxes through bacterial communities on glacier surfaces

Alexandre M. ANESIO,¹ Birgit SATTLER,² Christine FOREMAN,³ Jon TELLING,¹
Andy HODSON,⁴ Martyn TRANTER,¹ Roland PSENNER²

¹*Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, University Road, Bristol BS8 1SS, UK
E-mail: a.m.anesio@bristol.ac.uk*

²*Institute of Ecology, University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria*

³*Center for Biofilm Engineering and Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717, USA*

⁴*Department of Geography, University of Sheffield, Winter Street, Sheffield S10 2TN, UK*

ABSTRACT. There is very little information about the activity of microbial communities on the surface of glaciers, though there is an increasing body of evidence to show that they strongly influence the biogeochemistry of these habitats. We measured bacterial abundance and production in cryoconite holes on Arctic, Antarctic and Alpine glaciers in order to estimate the role of heterotrophic bacteria within the carbon budget of glacial ecosystems. Our results demonstrate an active bacterial community on the surface of glaciers with doubling times that vary from a few hours to hundreds of days depending on the glacier and position (water or sediments) within the cryoconite hole. However, bacterial production is only ~2–3% of the published literature values of community respiration from similar habitats, indicating that other types of microbes (e.g. eukaryotic organisms) may also play a role in the C cycle of glaciers. We estimate that only up to 7% of the organic C in cryoconite sediments is utilized by the heterotrophic bacterial community annually, suggesting that the surface of glaciers can accumulate organic carbon, and that this C may be important for biogeochemical activity downstream to adjacent ecosystems.

INTRODUCTION

Recent studies have shown that glaciers and ice caps are vast reservoirs of biological cells and debris, and that melting of the ice surface stimulates microbial activity (Hodson and others, 2008; Anesio and others, 2009). Investigations of cryoconite holes, which are aquatic habitats formed by preferential melting of dark organic and inorganic matter of mostly aeolian origin in the ablation zone, have increased notably in recent years (e.g. Christner and others, 2003; Stibal and others, 2006; Hodson and others, 2008). Studies using both molecular and more traditional microscopic techniques have revealed the existence of a diverse microbial community in cryoconite holes that is able to perform a range of metabolic functions involved in the C, N and P cycles (e.g. Christner and others, 2003; Porazinska and others, 2004; Foreman and others, 2007; Edwards and others, in press). Further, glacial nutrient budgets indicate that microbial communities strongly influence the biogeochemistry of these habitats (Hodson and others, 2005, 2008; Anesio and others, 2009).

Modelling work by Price and Sowers (2004) has demonstrated that microbial metabolism can occur at extremely low temperatures, where liquid water is the most important prerequisite for activity to occur. It has long been known that several species of bacteria can tolerate and maintain growth at temperatures below 10°C and some even have their optimum temperature of growth around 4°C or less (Morita, 1975), which is closer to the water temperatures found at the surface of glaciers (~0.1°C; Anesio and others, 2009). There is still very little information about the activity of the microbial communities on the surface of glaciers, but the few investigations that do exist have shown that the total amount of microbially fixed (via primary production) and oxidized carbon (via respiration) in

cryoconite holes can be substantial and comparable to microbial activities in other warmer and nutrient-rich habitats (Hodson and others, 2007, 2010; Anesio and others, 2009; Telling and others, 2010).

The production of heterotrophic bacteria (hereafter bacterial production) has been used over the last 35 years as an important indicator of the conversion of dissolved organic carbon (DOC) into particulate organic carbon (POC) in the form of bacterial cells (e.g. Fuhrman and Azam, 1980). Part of the carbon within the bacterial biomass will eventually be oxidized via bacterial respiration to CO₂, representing a loss of organic carbon available to higher trophic levels, while the remaining carbon, in the form of bacterial cells, can become available to higher trophic levels via grazing. Whether heterotrophic bacteria in aquatic ecosystems are a link between DOC and higher trophic levels (via production of POC), or a sink (by converting DOC to CO₂), has been under debate since the seminal paper by Pomeroy (1974). Little information is available about levels of bacterial production at the surface of glaciers, with the exceptions of investigations by Foreman and others (2007) providing bacterial production values in the ice-lidded cryoconite holes of the Dry Valleys, Antarctica, and Hodson and others (2007) and Mindl and others (2007) providing similar values in the open cryoconite holes from a Svalbard glacier. These studies showed that bacterial production values are far from trivial, as they are in the same order of magnitude as in other low-temperature soil and lake habitats (Tibbles and Harris, 1996; Stapleton and others, 2005; Laybourn-Parry and others, 2006). However, rates of bacterial production in cryoconite holes are far smaller than rates of gross primary production and community respiration from similar habitats (Anesio and others, 2009; Hodson and others, 2010; Telling and others, 2010), suggesting that C

fixation by primary producers is high enough to sustain heterotrophic bacterial activity in cryoconites.

We measured bacterial abundance and production in cryoconite holes of five glaciers (two Arctic, one Antarctic and two alpine) and one ice field (Patriot Hills, Antarctica), assess and compare for the first time the role of bacteria within the carbon flow of these systems and outline the general importance of heterotrophic bacteria to glacial ecosystems. Cryoconite holes in Antarctica are ice-lidded, imposing different pressures on the bacterial community compared to the open cryoconite systems in Alpine and Arctic glaciers (Hodson and others, 2008). Our study includes closed cryoconite holes of the McMurdo Dry Valleys and Patriot Hills ice field, which are separated from contact with the atmosphere by ice lids up to 30 cm thick that can persist for >10 years (Fountain and others, 2004). Tranter and others (2004) detected conditions in these entombed cryoconites with extremely high pH and extremely low pCO₂ values. Different conditions between open and closed cryoconite holes may thus provide different environmental pressures to bacterial communities in the holes. We compare and discuss our results in the light of previous published data on community respiration and primary production obtained from similar habitats.

METHODS

Study area

1. Arctic

Two valley glaciers in the Kongsfjord region of northwest Spitsbergen (78°53' N, 12°04' E) were investigated: Midtre Lovénbreen and Austre Brøggerbreen. These glaciers have been studied intensively during the last 60 years and are part of a wider glacier mass-balance monitoring network, where melt rates of up to 1–2 m a⁻¹ have been detected for these glaciers (Hagen and others, 2003). Therefore there is copious surface meltwater during the summer months. Their elevation ranges from ~50 m a.s.l. at the terminus to ~600 m at the headwall. Midtre Lovénbreen is a polythermal glacier with a core of temperate ice surrounded by a thick cold surface of ice below the pressure-melting point, while Austre Brøggerbreen is a cold-based glacier (Hagen and Sætrang, 1991). Between 13 and 21 July 2005, samples for bacterial abundance and for bacterial production were collected from the water and sediment from eight cryoconite holes on each of Midtre Lovénbreen and Austre Brøggerbreen. We were also able to use additional data on bacterial production in cryoconite hole sediment collected during different periods of the ablation season in the 2009 summer for these two glaciers.

2. European Alps

Alpine glacier samples were taken from Rotmoosferner (46°50' N, 11°03' E), Ötztal Alps, Tirol, Austria, in summer 2004. The mean annual temperature in this region is -1.3°C and the mean annual precipitation is 820 mm (Kaufmann, 2001). This glacier has a long history of observation and has retreated ~2.1 km since 1872 and by 600 m since 1969. Its average retreat rate has been 15 m a⁻¹ and is even greater since 2001, at 18 m a⁻¹. The ablation zone, where samples were taken from ten cryoconite holes, ends at 2430 m a.s.l.

Another ten cryoconite samples were collected from Stubacher Sonnblickkees (47°13' N, 12°60' E) in September

2007. This glacier is located in the national park Hohe Tauern, Salzburg, Austria, and has a northwest exposition. The highest elevation of the accumulation zone is 3050 m and the lowest at 2500 m a.s.l. Like Rotmoosferner, this glacier is part of a long-term monitoring programme where mass balance has been studied since 1959 (Slupetzky, 2003). Growth was observed between 1965 and 1981, but there has been a continuous annual loss of ice mass since. Slupetzky and Wiesenegger (2005) predict this glacier's disappearance within the next 50–80 years, since ~25% of the ice mass has been lost since 1981.

3. Antarctica

Patriot Hills (80°18' S, 81°21' W) is a line of rock hills 8 km long, located 4.8 km east of the north end of Independence Hills in Horseshoe Valley, Ellsworth Mountains. Nearby is the only private camp in Antarctica. The hills are surrounded by blue ice areas in which the cryoconite holes were ice-lidded during the sampling period in January 2002. Seven random cryoconite holes were selected across a flat blue ice area.

Taylor Valley (77°00' S, 162°52' E), McMurdo Dry Valleys, runs east to west for 34 km from McMurdo Sound to Taylor Glacier. The McMurdo Dry Valleys form the largest ice-free area on the Antarctic continent and are the coldest and driest desert on Earth. The polar glaciers of the McMurdo Dry Valleys have interior ice temperatures below freezing, and their basal ice is frozen to the ground (Fountain and others, 1998). Precipitation in the valleys is low (<10 cm w.e.), with most of this directly ablating before it is allowed to melt. Mean annual air temperatures for the period 1995–2006 were -17.1°C, with annual ablation rates of 17.7 cm for the same period (Hoffman and others, 2008). During the austral summers of 2005, cryoconites were collected from the ablation zones of Canada, Commonwealth and Taylor Glaciers in Taylor Valley, which are henceforth collectively termed Dry Valley glaciers.

Sampling procedure

Closed cryoconite holes

Frozen holes were cored with a manual ice corer (Kovacs and SIPRE for the Patriot Hills and Dry Valleys, respectively; core diameter 7.25 cm) to a depth where no more cryoconite could be seen. The ice corer was cleaned with ethanol and used to collect cores of frozen cryoconite, encompassing both the overlying ice and the underlying sediment. The extracted ice cores were placed in sterile, high-density polyethylene bags (Whirl-Pak (Lactan, Austria)), stored frozen and transported back to the home institution in a frozen condition or processed on site in Antarctica, for Patriot Hills and Dry Valleys, respectively. They were then cut into upper non-sediment and lower sediment-containing portions using an ethanol-cleaned, deionized-water rinsed saw in a laminar-flow hood. They were placed in sterile plastic containers and allowed to melt in the dark at 4°C. After ~20% of the outer core had melted, the remaining ice from each core was transferred to a second set of sterile containers and allowed to melt completely. This inner portion of the core was used for all analyses reported.

Open cryoconite holes

For bacterial abundance, 50 mL of supernatant in the cryoconite holes was collected using an automatic pipette

and sterile tips, and immediately fixed with 0.02 μm pre-filtered glutaraldehyde (2% final concentration) and 0.2 μm pre-filtered formalin (2% final concentration), for Arctic and Alpine samples, respectively. Sediment samples were collected with a hypodermic syringe, with tubing attached that enabled collection of both sediment and overlying water, and immediately fixed as above. Bacterial production was determined as described below.

Bacterial abundance in water and sediments. Bacteria in the Arctic and Dry Valley samples were counted using an epifluorescence microscope with SYBR Gold stain (Molecular Probes, 10 000 \times original dilution). Samples of 0.1 mL (sediments) or 10 mL (water phase of cryoconite holes and ice cores) were filtered on 0.02 μm Anodisc membrane filters (Whatman), with a 0.45 μm backing membrane filter. Sediment samples were shaken and left to stand for 5 s before 0.1 mL was taken from the surface of the sample and mixed with 2 mL of 0.02 μm filtered distilled water. We did not have the opportunity to sonicate the sediment samples prior to staining, which would have been optimal. However, the very bright fluorescence with SYBR Gold and its specificity gives fairly accurate counts of attached organisms. After filtration, the filter was laid, sample side up, on a drop of SYBR Gold working solution (25 μL of 100 \times diluted SYBR Gold solution and 75 μL of 0.02 μm filtered Milli-Q water) for 15 min in the dark. After drying, the filter was mounted on a glass slide with a drop of Molecular Probes Slow Fade antifade solution. For each filter, >150 bacteria were counted on 20–30 randomly selected fields of view.

For the Alpine and Patriot Hill samples, the bacterial abundance of the liquid fraction was determined from 4', 6-diamidino-2-phenylindole (DAPI, Sigma) stained cells (2 $\mu\text{g mL}^{-1}$ final concentration) (Porter and Feig, 1980). Ten-millilitre aliquots were filtered onto black 0.22 μm pore-sized polycarbonate filters (Nuclepore, USA) with a 0.45 μm backing filter. Staining conditions and time (7 min) were kept constant to obtain even signal intensity for counting. We used a Zeiss Axioplan epifluorescence microscope and counted a minimum of 400 cells on random fields at 1.600 \times magnification.

For sediment samples, the formalin fixed samples were treated with 0.001 M (0.266 g L^{-1}) sodium pyrophosphate. After the incubation, bacteria were dislodged by sonication (sonic probe: 180 s treatment for 5 min) and further treated as described by Epstein and Rossel (1995), before finally an aliquot of 0.1 mL was filtered onto black 0.22 μm polycarbonate filters and counted as above.

Bacterial carbon production in Arctic, Alpine and Patriot Hill samples. Bacterial production in the sediment and water were estimated by the incorporation of ^3H -leucine by the microcentrifuge method as modified from Kirchman (2001) and by the filtration method of Bell (1993), respectively. Incubation for both water and sediment samples took place under in situ temperature conditions (0.1–1 $^{\circ}\text{C}$). ^3H -leucine was added to a final concentration of 40 nM and 100 nM for water and sediment samples, respectively. For water samples, triplicate 20 mL samples and two formalin-killed control samples were incubated for 3 hours and the incubation was terminated by the addition of trichloroacetic acid (TCA; 5% final concentration). Samples were filtered through 0.22 μm cellulose acetate filters and washed with three volumes (5 mL) of 5% ice-cold TCA. Thereafter, the filter was dissolved with 1 mL

ethylacetate, 10 mL of scintillation fluid (Beckman Ready Safe) and counts conducted with a Beckman LS6500 scintillation counter.

For sediment samples, 1.5 mL samples collected with a hypodermic syringe, with a tube attached to its end in order to collect sediment material and water, were added into 2 mL microcentrifuge tubes. Triplicate samples and duplicated killed controls were incubated for 3 hours. After incubation, 90 μL of 100% TCA was added to the samples. The tubes were then centrifuged at 16 000g for 10 min, following washing, centrifugation and aspiration of the supernatant with 5% TCA and 80% ethanol. The final supernatant was aspirated and the remaining sediment weighted for the calculation of bacterial production. Finally, 1 mL of scintillation cocktail (Ecoscint and Beckman, Ready Safe for Arctic and Alpine/Patriot Hill samples, respectively) was added and the samples counted by liquid scintillation (Beckman LSC 6000 IC). Although the method for measuring bacterial production in the sediments produced substantially higher blanks (i.e. higher amounts of residual non-incorporated radioisotope in the sample) than in water samples, the radioisotope activity of the control samples was typically $\sim 20\%$ of the measured activity in non-control samples. There was a positive correlation between the amount of debris added to the tubes and background DPM, and thus all counts in DPM were first individually normalized by the amount of sediments used in each sample, and the DPM values in the killed controls were discounted accordingly before results were calculated in $\text{ng C g}^{-1} \text{h}^{-1}$. Leucine incorporation rates were transformed into bacterial carbon production following the methodology of Smith and Azam (1992).

Bacterial carbon production in the Dry Valleys. Bacterial productivity was measured via the incorporation of ^3H -thymidine (20 nM final concentration) into DNA from the ice overlying the sediment layer and the sediment layer from the Dry Valley cryoconites, following the methodology of Takacs and Priscu (1998). Triplicate ^3H -thymidine assays and duplicate formalin-killed controls (5% final concentration, 30 min prior to ^3H -thymidine addition) were analysed for each sample. Samples were incubated in the dark for 20 hours at 4 $^{\circ}\text{C}$, before the incubation was terminated by the addition of 10 mL of ice-cold 10% TCA. The solution was filtered onto 0.2 μm , 47 mm polycarbonate filters, rinsed three times with ice-cold 5% TCA and the filter placed in a 20 mL scintillation vial with Cytoscint ES scintillation cocktail. The vials were counted on a Beckman LS6500. Thymidine incorporation was converted to carbon incorporation using conversion factors of 2.0×10^{18} cells (mol thymidine) $^{-1}$ and 11 fg C cell^{-1} (Takacs and Priscu, 1998; Takacs and others, 2001).

Doubling times. Doubling times (i.e. the time it takes for the bacterial community to double its biomass) were calculated following the methodology of Crump and others (2007), where doubling time = $\ln 2 / \{\ln[(\text{BB} + \text{BP})/\text{BB}]\}$, where BP is bacterial carbon production and BB is the bacterial biomass. Bacterial biomass was obtained by converting bacterial abundance in cells mL^{-1} to biomass in $\mu\text{g L}^{-1}$ using a conversion factor of 11 fg C cell^{-1} . We recognize that only a fraction of aquatic bacteria are actively growing in most habitats, so these calculations allow exploration of whether glaciers are habitats for nutrient transformations by the bacterial community or whether they only act as a repository of cells.

Table 1. Average \pm std dev. (range) of bacterial abundance and production in the water and sediments of cryoconite holes in six glaciers (ABR: Austre Brøggerbreen; MLO: Midtre Lovénbreen; ROT: Rotmoosferner; STU: Stubacher Sonnblickkees; PAT: Patriot Hills; and DRY: Dry Valleys). ANOVA tests for each variable show significant statistical differences between the glaciers (ANOVA, $P < 0.05$)

Glacier	Water		Sediment	
	Cell abundance 10^3 mL^{-1}	Production $\text{ng CL}^{-1} \text{ h}^{-1}$	Cell abundance 10^9 g^{-1}	Production $\text{ng C g}^{-1} \text{ h}^{-1}$
ABR	54.0 ± 16.2 (21.5–69.9)	3.26 ± 2.62 (0.50–7.44)	0.48 ± 0.25 (0.29–0.99)	8.62 ± 6.41 (2.06–21.6)
MLO	34.4 ± 11.7 (22.3–56.2)	5.27 ± 1.75 (2.88–7.90)	1.40 ± 0.79 (0.39–2.93)	39.7 ± 17.9 (16.9–70.3)
ROT	41.3 ± 38.0 (7.66–100.6)	16.7 ± 15.8 (1.20–43.0)	0.54 ± 0.85 (0.03–2.95)	24.6 ± 21.4 (6.00–67.0)
STU	37.1 ± 13.6 (18.3–58.9)	0.05 ± 0.02 (0.03–0.07)	0.42 ± 0.36 (0.002–0.95)	0.13 ± 0.14 (0.02–0.38)
PAT	12.9 ± 8.18 (4.50–28.8)	0.22 ± 0.31 (0.02–0.78)	0.05 ± 0.02 (0.02–0.09)	11.2 ± 4.11 (8.62–19.4)
DRY	44.0 ± 24.4 (12.7–79.4)	0.04 ± 0.02 (0.01–0.06)	0.22 ± 0.05 (0.16–0.29)	23.4 ± 11.8 (9.20–37.1)

RESULTS

Bacterial abundance and production

We detected significant differences in the bacterial abundance and production in water and sediment between glaciers. Average bacterial abundance in water ranged from 12.9 to 54.0×10^3 cells mL^{-1} , with an average of $37.2 \pm 13.8 \times 10^3$ cells mL^{-1} (Table 1). Average bacterial abundance in the sediments of cryoconite holes was more variable, ranging from 0.051×10^9 cells g^{-1} in Patriot Hills to 1.40×10^9 cells g^{-1} in Midtre Lovénbreen (Table 1). Midtre Lovénbreen had the highest bacterial abundance in the sediments of cryoconite holes ($P < 0.05$, one-way ANOVA, followed by Duncan analyses).

Overall, bacterial production in water and sediments was more variable across the glaciers than bacterial abundance, with values of bacterial production differing by >300 times. Average bacterial production in the waters of cryoconite holes ranged from $0.05 \text{ ng CL}^{-1} \text{ h}^{-1}$ in Stubacher Sonnblickkees to $16.7 \text{ ng CL}^{-1} \text{ h}^{-1}$ in Rotmoosferner (Table 1), while average bacterial production in the sediments ranged from $0.13 \text{ ng C g}^{-1} \text{ h}^{-1}$ in Stubacher Sonnblickkees to $39.7 \text{ ng C g}^{-1} \text{ h}^{-1}$ in Midtre Lovénbreen (Table 1).

With a few exceptions, we did not find correlations between bacterial abundance and bacterial production, either in the water or in the sediments of the cryoconite holes (Table 2). Table 3 shows the average bacterial production in the sediments measured in the Arctic glaciers

in two different years and during different periods of the ablation season. Overall, bacterial production did not show any marked seasonality.

Doubling times

Average doubling times across the glaciers ranged from 1.8 days in the water of Rotmoosferner and the sediment of Patriot Hills to 2057 days in the sediment of Stubacher Sonnblickkees (Fig. 1). The fastest doubling times occurred in the water of the Arctic glaciers and the Alpine Rotmoosferner, and in the sediments of the Antarctic cryoconites. Doubling times of Stubacher Sonnblickkees were extremely high in both water and sediments. This glacier also showed the lowest bacterial carbon production associated with sediments and in water despite the fact that bacterial abundance was not significantly lower than in other glaciers, suggesting that most bacterial cells in Stubacher Sonnblickkees are not active and/or do not contribute to the biogeochemical transformations occurring at this glacier.

Considering the short summers in polar regions during which liquid water is available for microbial growth, we consider that doubling times in bacterial communities higher than 60 days are not fast enough for the bacterial community to contribute significantly to local biogeochemical cycles. This implies that the bacterial community cannot duplicate within the ablation period, suggesting that only a small portion of all cells are active. This was particularly the case for the water phase of the Antarctic cryoconites and the

Table 2. Pearson correlation between bacterial abundance and production in water and sediments for each glacier. N is number of cryoconite holes with the full dataset. ABR: Austre Brøggerbreen; MLO: Midtre Lovénbreen; ROT: Rotmoosferner; STU: Stubacher Sonnblickkees; PAT: Patriot Hills; and DRY: Dry Valleys

Glacier	Water		Sediment	
	r^2	N	r^2	N
ABR	0.593*	7	0.664*	7
MLO	0.139	8	0.026	8
ROT	0.002	10	0.055	10
STU	0.0003	5	0.018	9
PAT	0.003	8	0.106	7
DRY	0.289	5	0.864*	5

*Statistically significant correlation ($P < 0.05$).

Table 3. Comparison of bacterial production data available in sediments of cryoconite holes ($\text{ng C g}^{-1} \text{ h}^{-1}$) from different times of the season in two different years. ABR: Austre Brøggerbreen; MLO: Midtre Lovénbreen. NA: not analysed

	MLO	ABR
Summer 2005*		
13–21 July	39.7 ± 17.9	8.62 ± 6.41
Summer 2009†		
8 July	17.9 ± 3.64	NA
17 July	NA	6.40 ± 3.77
19 August	19.3 ± 10.3	NA
20 August	36.2 ± 8.45	NA

*Average of eight different cryoconites. †One cryoconite (average of triplicate measurements).

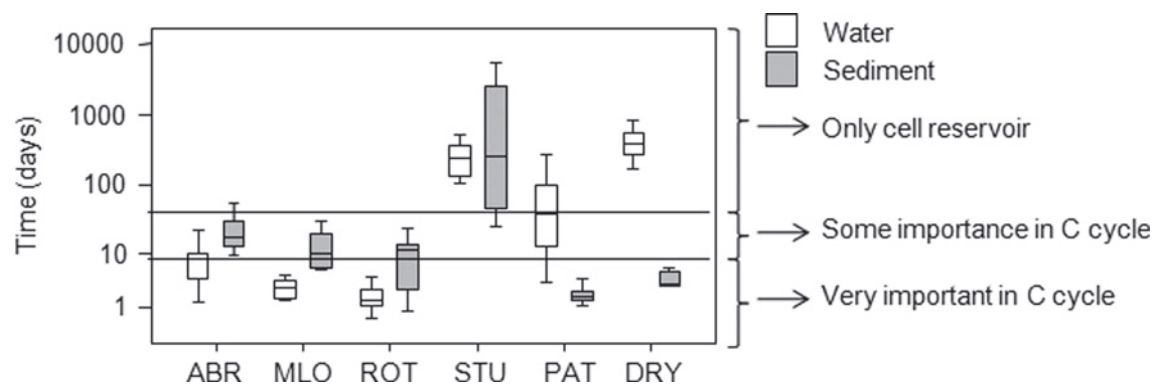


Fig. 1. Box plot of doubling times in the water and sediments of cryoconite holes in six glaciers (ABR: Austre Brøggerbreen; MLO: Midtre Lovénbreen; ROT: Rotmoosferner; STU: Stubacher Sonnblickkees; PAT: Patriot Hills; DRY: Dry Valleys). Bacterial doubling times less than 10 days suggest that bacterial activity plays an important role in the C cycle of glaciers. Bacterial doubling times of 10–60 days suggest that bacteria have a less prominent role in the C cycle of glaciers, while doubling times higher than 60 days are not sufficient for the bacterial community to double its biomass during the course of one ablation season.

water and sediments of Stubacher Sonnblickkees. However, in most cases, doubling times in bacterial communities were <60 days and in some cases even <5 days, suggesting that the majority of microbes in glaciers can divide and thus do have an impact on biogeochemical cycles of glacial habitats in the course of an ablation season.

Differences between closed and open cryoconite holes

We have removed Stubacher Sonnblickkees, for reasons outlined below, from the comparison between open and closed cryoconite holes, since this glacier behaved as an outlier with regard to microbial activity. We have grouped the other Alpine glacier (Rotmoosferner) and the two Arctic glaciers together as open cryoconite holes, while the Antarctic cryoconites were grouped together as entombed cryoconite holes. Significant differences in the abundance of bacteria (water and sediment), production (water) and doubling times (water and sediment) were observed between open and entombed cryoconite holes (Table 4; *t* tests). Generally, bacterial abundance and production were higher in open holes than in closed holes. However, doubling times were generally faster in the water of open holes compared to entombed holes, while doubling times in the sediments were faster in entombed holes than in open holes.

DISCUSSION

Our results demonstrate that the surface of glaciers is colonized by an active bacterial community and that

carbon flow and transformation through heterotrophic bacteria may have a strong impact on the biogeochemistry of these habitats. The exception was Stubacher Sonnblickkees, which had very low bacterial production despite bacterial abundance values similar to those of the other investigated glaciers. The typical cryoconite structure (well described by Takeuchi and others, 2001, 2010; Hodson and others, 2010) has been found at all investigated glaciers in this study, except in the cryoconites from Stubacher Sonnblickkees. Contrary to the relatively loose structures caused by the gentle rolling and incorporation of inorganic and organic matter as water circulates through the holes, the cryoconites of Stubacher Sonnblickkees were found to be of a dense, black and smeary consistency. Furthermore, heavy metals such as Co (65.6 ppm), Cr (11.8 ppm) and Pb (49.8 ppm) were prominent in the solid phase of Stubacher (H. Lettner, unpublished data). Another reason for this difference might be the unusually high concentration of ^{137}Cs at Stubacher Sonnblickkees (up to $140\,000\text{ Bq kg}^{-1}$) originating from the Chernobyl accident in 1984. The cryoconite material of alpine glaciers in Austria has been found to have the highest known dosage of artificial radionuclides compared to all other environmental compounds, some of them deriving from the bomb tests in the 1950s and 1960s (Tieber and others, 2009), which supports the hypothesis that cryoconite holes, even on temperate glaciers, are much older than previously assumed. This leads to the assumption that bacterial cells in cryoconites of Stubacher Sonnblickkees are hampered in their activity by a number of different factors.

Table 4. Bacterial abundance, production and doubling times in water and sediments for pooled open and closed cryoconite holes. *P* indicates the *t*-test significance for the comparison between open and closed cryoconite holes. NS: differences not significant

	Open cryoconites*	Closed cryoconites	<i>P</i>
Cell abundance in water (10^3 mL^{-1})	7.70–106 (43.7 ± 26.6)	4.50–79.4 (24.5 ± 23.0)	0.037
Production in water ($\text{ng C L}^{-1}\text{ h}^{-1}$)	1.03–43.0 (9.07 ± 11.2)	0.005–0.78 (0.16 ± 0.26)	0.001
Cell abundance in sediment (10^9 g^{-1})	0.03–2.95 (0.83 ± 0.81)	0.02–0.29 (0.12 ± 0.09)	<0.001
Production in sediment ($\text{ng C g}^{-1}\text{ h}^{-1}$)	2.06–70.3 (25.1 ± 20.5)	8.62–37.1 (16.3 ± 9.98)	NS
Doubling time in water (days)	0.33–21.9 (4.04 ± 4.69)	3.39–807 (221 ± 251)	0.009
Doubling time in sediment (days)	0.65–56 (15.4 ± 12.4)	1.04–5.68 (2.67 ± 1.51)	<0.001

*Stubacher was excluded.

Table 5. Carbon utilization (bacterial production (P) + bacterial respiration (R)) in water and sediments, and annual bacterial C utilization as a fraction of the organic carbon available in the sediments of open and entombed cryoconites. Each column represents a different bacterial growth efficiency (BGE) scenario for the estimation of bacterial importance to glacial C budgets. See text for full discussion of conversion factors used to calculate annual bacterial C utilization as a fraction of the organic carbon (OC) available in the sediments

BGE scenario	Open cryoconites				Closed cryoconites			
	5%	10%	20%	30%	5%	10%	20%	30%
C utilization								
In water ($P+R$) (ng CL ⁻¹ h ⁻¹)	181	91	45	30	3.2	1.6	0.8	0.5
In sediments ($P+R$) (ng C g ⁻¹ h ⁻¹)	502	251	125	84	326	163	82	54
As proportion of OC available in sediments (%)	2.4	1.2	0.6	0.4	7.0	3.5	1.8	1.2

Bacterial community doubling times across all cryoconite holes on the glaciers had a wide range from 0.5 day to >5 years, depending on the glacier of origin or whether they were found in water or associated with debris. Except for the Dry Valley samples, where cores were melted before bacterial production incubations were performed in laboratory conditions at 4°C, all bacterial production measurements (Arctic and Alpine) were performed under in situ temperature conditions (i.e. 0.1°C). All glaciers except Stubacher contained enough active bacteria to double their biomass more than twice within the period of an ablation season, whether they were in water, associated with sediments or both. Clear differences were observed between open and entombed cryoconite holes, with bacterial doubling times in open cryoconite holes faster in water than in sediments, while the opposite was the case in entombed cryoconite holes. The fact that entombed cryoconites need to be melted before bacterial production is measured may add experimental artefacts to our doubling times and carbon flux calculations (see discussion below). It is possible that thin layers of liquid water are still available in entombed sediments, which are enriched in nutrients, thus stimulating microbial activity. On the other hand, poor nutrient conditions in the frozen lids offer limiting conditions for active microbial metabolism associated with the water phase. Concerning the open cryoconite holes, allochthonous inputs of cells and nutrients are far more likely to contribute to the 'aquatic' supraglacial habitats, stimulating microbial activity associated with water, while flushing of the holes may dilute the nutrient concentration in the sediments. There is clearly more scope to investigate the differences in microbial activity between entombed and open cryoconite holes under better-constrained laboratory conditions.

In this study, we show that bacterial production in cryoconite holes is of the same order of magnitude as previous estimates from similar glaciers (Foreman and others, 2007; Hodson and others, 2007; Mindl and others, 2007) and other polar soils and fresh waters (Tibbles and Harris, 1996; Stapleton and others, 2005; Laybourn-Parry and others, 2006). In order to calculate the total carbon flux through the bacterial community, it is important to have data on the growth efficiency or bacterial respiration, since bacterial production only represents the transformation of DOC into bacterial biomass, and does not account for the amount of carbon respired by the bacterial community (Jahnke and Craven, 1995). Bacterial production can be measured routinely as we did in this study. However, respiration and growth efficiency are difficult to measure

and values are still relatively rare in the literature. The necessary physical separation between bacterial respiration and community respiration (i.e. which includes all other members of the microbial community such as autotrophs, heterotrophic protozoa, fungi, etc.) in benthic debris-associated communities is particularly difficult, if not impossible, with the methodology currently available. Bacterial respiration can be estimated from a theoretical bacterial growth efficiency (BGE), considering that $BGE = BP/(BP + R)$, where BP and R are bacterial production and respiration, respectively. The range of empirical measurements of BGE available in the literature is large, varying from almost 0% to 100% depending on a number of environmental factors and the substrate used (Jahnke and Craven, 1995). Cole and Pace (1995) calculate that the average BGE varies between 30% and 60%, but it is closer to 30% in oligotrophic habitats and 60% in eutrophic habitats. There are very few measurements of BGE in cold environments, although some recent studies suggest that it could actually be higher than in warmer environments because, although both bacterial production and respiration increase with temperature, the increase in bacterial respiration is proportionally greater than the increase in bacterial production (Apple and others, 2006; Hall and Cotner, 2007). If we assume a range of different scenarios for BGE in cryoconites between 5% and 30%, then total C utilization (i.e. bacterial production plus respiration) at the surface of glaciers could potentially be 3.3–20 times higher than bacterial production values alone, where higher BGEs will result in more efficient systems with less C utilization for generation of bacterial biomass (Table 5).

Based on the above observations and assumptions, we can calculate the annual bacterial carbon utilization as a fraction of the total organic carbon available at the surface of glaciers. Cryoconite debris of glaciers in Svalbard has an average organic carbon composition of 3% (Kastovska and others, 2005), while Antarctic cryoconite debris has an organic carbon composition of 0.5% (Barrett and others, 2007). Further, we assume that microorganisms are active ~ 2 months a^{-1} in Svalbard and ~ 1.5 months a^{-1} in Antarctica. Based on these assumptions, bacterial communities associated with the debris at the surface of Arctic and Antarctic glaciers can utilize annually (for both biomass production and respiration) 0.4–2.4% and 1.2–7%, respectively, of the C stored in the sediments, depending on which BGE is assumed for the calculation of bacterial respiration. These calculations suggest that the organic carbon stored in the debris of cryoconite holes is enough to fuel heterotrophic bacterial activity at the surface of glaciers.

The fact that no significant correlation was found between bacterial abundance and production in most glaciers (the only exceptions are in the sediments of the Dry Valley glacier and water and sediment of Austre Brøggerbreen) suggests that either only a variable fraction of all bacteria were active or top-down controls (i.e. grazing and viral infection) may play a strong role in controlling bacterial abundance in cryoconite holes. This may be true especially in cases where high bacterial production is not reflected by a higher bacterial abundance and where doubling times in the bacterial community are fast enough for the bacterial biomass to double several times during one annual cycle. In these cases, production of new bacterial biomass may either be directed to higher components of the food web (i.e. through grazing) or recycled back to DOC by viral infection and lysis. The first process is still little understood in cryoconite holes, but the presence of flagellates and ciliates in cryoconites, which are important grazers of bacteria (Sävström and others, 2002; Christner and others, 2003), suggests that grazing should not be discounted. Viral infection in cryoconites has been demonstrated to be high, compared to other marine and freshwater habitats (Sävström and other, 2007b), and a strong correlation between viruses and bacteria in Arctic cryoconites suggests that viruses play an important role in controlling the bacterial community in these ecosystems (Anesio and others, 2007). However, we do not imply that bottom-up controls (e.g. nutrient limitation) are not important in controlling bacterial production in glaciers. Several recent studies show that phosphorus is an important limiting factor for heterotrophic bacterial growth in cryoconite holes, both in the Arctic and Antarctic (Foreman and others, 2007; Mindl and others, 2007; Sävström and others, 2007a; Stibal and others, 2008).

Comparison between bacterial production and literature values of primary production and community respiration

The comparison of bacterial production data obtained in this study with the published literature values of primary production (Sävström and others, 2002; Anesio and others, 2009; Telling and others, 2010) indicates that carbon fixation by algae is more than enough to fuel bacterial production. Compared to an average gross primary production of $720 \text{ ng g}^{-1} \text{ h}^{-1}$ in Telling and others (2010), bacterial production is only $\sim 3.5\%$ of the gross primary production. There may be a number of reasons for this. First, there may be a decoupling between primary and heterotrophic bacterial production during the ablation season, where heterotrophic activity is likely to be higher at the beginning and/or end of the ablation season. We note that the majority of measurements in this study were conducted during the peak of the summer. However, the few data points that we have from Midtre Lovénbreen and Austre Brøggerbreen that were obtained during the thaw and the end of the season showed no significant differences from the data obtained during the middle of the season (Table 3). Considering the small differences in bacterial production measured in the same glaciers between different years and different periods of the ablation season, we suggest that there is no major seasonality within the ablation season in the heterotrophic bacterial activity of cryoconite holes. Therefore we also suggest that a decoupling between heterotrophic and autotrophic activity is not the main reason why bacterial

production is low relative to gross primary production and respiration. Secondly, the low rates of bacterial production relative to primary production indicate that only a small fraction of the bacterial community may be active. Previous investigations in Svalbard have shown that temperature, in addition to the factors mentioned above, is an important limiting factor for heterotrophic bacterial community growth (Mindl and others, 2007; Sävström and others, 2007a). Nevertheless, bacterial cells released during ablation could potentially become active colonizers of adjacent habitats. For instance, a previous study demonstrated how bacterial production can substantially increase from the surface of glaciers towards proglacial streams and lakes (Mindl and others, 2007). Finally, the reason for the low bacterial production in relation to low gross primary production in cryoconite habitats could be due to the fact that bacterial growth efficiency (BGE) is very low in these cold systems. If we assume that the average community respiration obtained by Anesio and others (2009) of $1250 \text{ ng C g}^{-1} \text{ h}^{-1}$ was all due to bacterial respiration, BGE in cryoconite debris would have to be only $\sim 3\%$, which is considerably lower than empirically measured BGE from other cold habitats and experiments conducted in low-temperature conditions (Carlson and others, 1999; Apple and others, 2006; Hall and Cotner, 2007). Considering the large range of BGE available in the literature and the possible scenarios shown in Table 5, there is an urgent need to empirically measure bacterial respiration and BGE in cryoconite holes. Most importantly, the fact that bacterial production is just a small fraction of the total community respiration, particularly when high BGE is assumed, suggests that there are potentially many other members of the microbial community in cryoconite holes (e.g. prokaryotic and eukaryotic communities) that are an active part of the C cycle at the surface of glaciers.

CONCLUSIONS

Considering that our estimates reveal that only up to 7% of the organic C stored in the debris is utilized by the heterotrophic community annually, we infer that the surface of glaciers can accumulate organic carbon of autochthonous origin, albeit until physical processes such as melt-water sloughing remove it from ice, and thus this C may be important for biogeochemical activity in ecosystems downstream. There is indeed an increasing amount of evidence showing that DOC from glacier outflow has a strong microbial component (Lafrenière and Sharp, 2004; Hood and others, 2009). Nevertheless, allochthonous organic carbon (potentially also of microbial origin) may be present in substantial amounts in cryoconite holes (Stibal and others, 2008). This may be the case especially in large ice-sheet ecosystems with large slush zones where heterotrophic processes could prevail, potentially using wind-blown organic matter (Stibal and others, 2010). The proportion of allochthonous and autochthonous carbon that is fuelled through the bacterial community still needs to be investigated.

ACKNOWLEDGEMENTS

We thank the two anonymous referees and J. Laybourn-Parry for constructive comments on the manuscript. This study was funded by grants from the UK National Environment

Research Council (NERC; NE/D007321/1 and NE/G00496X/1) to A.M.A., from the Austrian Academy of Science (ÖAW) and Planetary Studies Foundation (PSF) to B.S., and from the US National Science Foundation's Office of Polar Programs (NSF OPP 0338342 and OPP 0838970) to C.F. We thank everyone who provided support in the field and the laboratory during this study, especially N. Cox at the NERC Arctic Research Station, and G. Fitz and I. Antlinger at the University of Innsbruck.

REFERENCES

- Anesio, A.M., B. Mindl, J. Laybourn-Parry, A.J. Hodson and B. Sattler. 2007. Viral dynamics in cryoconite holes on a high Arctic glacier (Svalbard). *J. Geophys. Res.*, **112**(G4), G04S31. (10.1029/2006JG000350.)
- Anesio, A.M., A.J. Hodson, A. Fritz, R. Psenner and B. Sattler. 2009. High microbial activity on glaciers: importance to the global carbon cycle. *Global Change Biol.*, **15**(4), 955–960.
- Apple, J.K., P.A. del Giorgio and W.M. Kemp. 2006. Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. *Aquat. Microbial Ecol.*, **43**(3), 243–254.
- Barrett, J.E. and 8 others. 2007. Biogeochemical stoichiometry of Antarctic Dry Valley ecosystems. *J. Geophys. Res.*, **112**(G1), G01010. (10.1029/2005JG000141.)
- Bell, R.T. 1993. Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine. In Kemp, P.F., B.F. Sherr, E.B. Sherr and J.J. Cole, eds. *Handbook of methods in aquatic microbial ecology*. Boca Raton, FL, Lewis.
- Carlson, C.A., N.R. Bates, H.W. Ducklow and D.A. Hansell. 1999. Estimation of bacterial respiration and growth efficiency in the Ross Sea, Antarctica. *Aquat. Microbial Ecol.*, **19**(3), 229–244.
- Christner, B.C., B.H. Kvitto and J.N. Reeve. 2003. Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. *Extremophiles*, **7**(3), 177–183.
- Cole, J.J. and M.L. Pace. 1995. Why measure bacterial production? A reply to the comment by Jahnke and Crave. *Limnol. Oceanogr.*, **40**(2), 441–415.
- Crump, B.C., C. Peranteau, B. Beckingham and J.C. Cornwell. 2007. Respiratory succession and community succession of bacterioplankton in seasonally anoxic estuarine waters. *Appl. Environ. Microbiol.*, **73**(21), 6802–6810.
- Edwards, A. and 7 others. In press. Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard. *ISME J.* (10.1038/ismej.2010.100.)
- Epstein, S.S. and J. Rossel. 1995. Enumeration of sandy sediment bacteria: search for optimal protocol. *Mar. Ecol. Progr. Ser.*, **117**(1–3), 289–298.
- Foreman, C.M., B. Sattler, D.L. Mikucki, D.L. Porazinska and J.C. Prisco. 2007. Metabolic activity and diversity of cryoconites in the Taylor Valley, Antarctica. *J. Geophys. Res.*, **112**(G4), G04S32. (10.1029/2006JG000358.)
- Fountain, A.G., G.L. Dana, K.J. Lewis, B.H. Vaughn and D.M. McKnight. 1998. Glaciers of the McMurdo Dry Valleys, southern Victoria Land, Antarctica. In Prisco, J.C., ed. *Ecosystem dynamics in a polar desert: the McMurdo Dry Valleys, Antarctica*. Washington, DC, American Geophysical Union, 65–75. (Antarctic Research Series 72.)
- Fountain, A.G., M. Tranter, T.H. Nylén, K.J. Lewis and D.R. Mueller. 2004. Evolution of cryoconite holes and their contribution to meltwater runoff from glaciers in the McMurdo Dry Valleys, Antarctica. *J. Glaciol.*, **50**(168), 35–45.
- Fuhrman, J.A. and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British-Columbia, Antarctica, and California. *Appl. Environ. Microbiol.*, **39**(6), 1085–1095.
- Hagen, J.O. and A. Sætrang. 1991. Radio-echo soundings of sub-polar glaciers with low-frequency radar. *Polar Res.*, **9**(1), 99–107.
- Hagen, J.O., J. Kohler, K. Melvold and J.-G. Winther. 2003. Glaciers in Svalbard: mass balance, runoff and freshwater flux. *Polar Res.*, **22**(2), 145–159.
- Hall, E.K. and J.B. Cotner. 2007. Interactive effect of temperature and resources on carbon cycling by freshwater bacterioplankton communities. *Aquat. Microbial Ecol.*, **49**(1), 35–45.
- Hodson, A.J., P.N. Mumford, J. Kohler and P.M. Wynn. 2005. The High Arctic glacial ecosystem: new insights from nutrient budgets. *Biogeochemistry*, **72**(2), 233–256.
- Hodson, A.J. and 10 others. 2007. A glacier respire: quantifying the distribution and respiration CO₂ flux of cryoconite across an entire Arctic supraglacial ecosystem. *J. Geophys. Res.*, **112**(G4), G04S36. (10.1029/2007JG000452.)
- Hodson, A. and 7 others. 2008. Glacial ecosystems. *Ecol. Monogr.*, **78**(1), 41–67.
- Hodson, A. and 6 others. 2010. The structure, biological activity and biogeochemistry of cryoconite aggregates upon an Arctic valley glacier: Longyearbreen, Svalbard. *J. Glaciol.*, **56**(196), 349–362.
- Hoffman, M.J., A.G. Fountain and G.E. Liston. 2008. Surface energy balance and melt thresholds over 11 years at Taylor Glacier, Antarctica. *J. Geophys. Res.*, **113**(F4), F04014. (10.1029/2008JF001029.)
- Hood, E. and 6 others. 2009. Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature*, **462**(7276), 1044–1047.
- Jahnke, R.A. and D.B. Craven. 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: a need for respiration rate measurements. *Limnol. Oceanogr.*, **40**(2), 436–441.
- Kaštovská, K., J. Elster, M. Stibal and H. Šantrěčková. 2005. Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (High Arctic). *Microbial Ecol.*, **50**(3), 396–407.
- Kaufmann, R. 2001. Invertebrate succession on an Alpine glacier foreland. *Ecology*, **82**(8), 2261–2278.
- Kirchman, D. 2001. Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environment. *Meth. Microbiol.*, **30**, 227–237.
- Lafrenière, M.J. and M.J. Sharp. 2004. The concentration and fluorescence of dissolved organic carbon (DOC) in glacial and nonglacial catchments: interpreting hydrological flow routing and DOC sources. *Arct. Antarct. Alp. Res.*, **36**(2), 156–165.
- Laybourn-Parry, J., N.J. Madan, W.A. Marshall, H. Marchant and S.W. Wright. 2006. Carbon dynamics in an ultra-oligotrophic epishelf lake (Beaver Lake, Antarctica) in summer. *Freshwater Biol.*, **51**(6), 1116–1130.
- Mindl, B., A.M. Anesio, K. Meirer, A.J. Hodson, J. Laybourn-Parry and R. Sommaruga. 2007. Factors influencing bacterial dynamics along a transect from supraglacial runoff to proglacial lakes of a high Arctic glacier. *FEMS Microbiol. Ecol.*, **59**(2), 307–317.
- Morita, R.Y. 1975. Psychrophilic bacteria. *Bacteriol. Rev.*, **39**(2), 144–167.
- Pomeroy, L.R. 1974. The ocean's food web, a changing paradigm. *BioScience*, **24**, 499–504.
- Porazinska, D.L., A.G. Fountain, T.H. Nylén, M. Tranter, R.A. Virginia and D.H. Wall. 2004. The biodiversity and biogeochemistry of cryoconite holes from McMurdo Dry Valley glaciers, Antarctica. *Arct. Antarct. Alp. Res.*, **36**(1), 84–91.
- Porter, K.G. and Y.S. Feig. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**(5), 943–948.
- Price, P.B. and T. Sowers. 2004. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proc. Natl. Acad. Sci. USA (PNAS)*, **101**(13), 4631–4636.
- Sävström, C., P. Mumford, W. Marshall, A. Hodson and J. Laybourn-Parry. 2002. The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard 79°N). *Polar Biol.*, **25**(8), 591–596.

- Sävström, C., J. Laybourn-Parry, W. Granéli and A.M. Anesio. 2007a. Heterotrophic bacterial and viral dynamics in Arctic freshwaters: results from a field study and nutrient-temperature manipulation experiments. *Polar Biol.*, **30**(11), 1407–1415.
- Sävström, C., W. Granéli, J. Laybourn-Parry and A.M. Anesio. 2007b. High viral infection rates in Antarctic and Arctic bacterioplankton. *Environ. Microbiol.*, **9**(1), 250–255.
- Slupetzky, H. 2003. Do we need long term terrestrial glacier mass balance monitoring for the future? *Geophys. Res. Abstr.*, **5**, EAE03-A-11390.
- Slupetzky, H. and H. Wiesenegger. 2005. Glacial changes, water cycle observations and mass balance developments on Stübacher Sonnblickkees, Salzburg, in recent years. *Geophys. Res. Abstr.*, **7**, EGU05-A-07870.
- Smith, D.C. and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ³H-leucine. *Mar. Microb. Food Webs*, **6**(2), 107–114.
- Stapleton, L.M. and 6 others. 2005. Microbial carbon dynamics in nitrogen amended Arctic tundra soil: measurement and model testing. *Soil Biol. Biochem.*, **37**(11), 2088–2098.
- Stibal, M., M. Šabacká and K. Kaštovská. 2006. Microbial communities on glacier surfaces in Svalbard: impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. *Microbial Ecol.*, **52**(4), 644–654.
- Stibal, M., M. Tranter, L.G. Benning and J. Reháč. 2008. Microbial primary production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input. *Environ. Microbiol.*, **10**(8), 2172–2178.
- Stibal, M., A.M. Anesio, C.J.D. Blues and M. Tranter. 2009. Phosphatase activity and organic phosphorus turnover on a high Arctic glacier. *Biogeosciences*, **6**(5), 913–922.
- Stibal, M., G.P. Lis, E. Lawson, K.M. Mak, J.L. Wadham and A.M. Anesio. 2010. Organic-matter content and quality in supraglacial debris across the ablation zone of the Greenland ice sheet. *Ann. Glaciol.*, **51**(56) (see paper in this issue).
- Takacs, C.D. and J.C. Priscu. 1998. Bacterioplankton dynamics in the McMurdo Dry Valley Lakes, Antarctica: production and biomass loss over four seasons. *Microbial Ecol.*, **36**(3), 239–250.
- Takacs, C.D., J.C. Priscu and D.M. McKnight. 2001. Bacterial dissolved organic carbon demand in McMurdo Dry Valley lakes, Antarctica. *Limnol. Oceanogr.*, **46**(5), 1189–1194.
- Takeuchi, N., S. Kohshima and K. Seko. 2001. Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: a granular algal mat growing on the glacier. *Arct. Antarct. Alp. Res.*, **33**(2), 115–122.
- Takeuchi, N., H. Nishiyama and Z. Li. 2010. Structure and formation process of cryoconite granules on Ürümqi glacier No. 1, Tien Shan, China. *Ann. Glaciol.*, **51**(56) (see paper in this issue).
- Telling, J. and 7 others. 2010. Measuring gross and net rates of photosynthesis in cryoconite holes: a comparison of field methods. *Ann. Glaciol.*, **51**(56) (see paper in this issue).
- Tibbles, B.J. and J.M. Harris. 1996. Use of radiolabelled thymidine and leucine to estimate bacterial production in soils from continental Antarctica. *Appl. Environ. Microbiol.*, **62**(2), 694–701.
- Tieber, A., H. Lettner, P. Bossew, A. Hubmer, B. Sattler and W. Hofmann. 2009. Accumulation of anthropogenic radionuclides in cryoconites on Alpine glaciers. *J. Environ. Radioact.*, **110**(7), 590–598.
- Tranter, M. and 6 others. 2004. Extreme hydrochemical conditions in natural microcosms entombed within Antarctic ice. *Hydrol. Process.*, **18**(2), 379–387.