Polar Biol (2004) 27: 66–74
DOI 10.1007/s00300-003-0580-2

ORIGINAL PAPER

D. R. Mueller · W. H. Pollard

Gradient analysis of cryoconite ecosystems from two polar glaciers

Received: 15 April 2003 / Accepted: 1 November 2003 / Published online: 26 November 2003

Abstract The cylindrical meltholes present in the ablation zones of many glaciers (termed cryoconite holes) contain complex microbial communities. A canonical correspondence analysis (CCA) of community structure and environmental gradients for cryoconite holes on two glaciers was undertaken. The Canada Glacier (77°37′S, 162°55′E) is located in the McMurdo Dry Valleys of Antarctica. The White Glacier (79°27′N, 90°40′W) is located on Axel Heiberg Island, Nunavut Territory, Canada. These glaciers are at similar, yet antipodal latitudes, are roughly the same size and endure approximately the same mean annual temperature. The Canada Glacier cryoconite communities were found to be significantly ($P = 0.001$) associated with six environmental variables, which together explained 55% of the biological variation. The White Glacier cryoconite communities were not significantly associated with environmental variables. The differences in CCA results were attributed to the relative amount of disturbance and isolation between each glacier's cryoconite holes. Canada Glacier cryoconite holes were mostly ice-covered and undisturbed by meltwater flow, whereas high meltwater production and open cryoconite holes on the White Glacier may continually reset the community structure and habitat variability due to inter-hole mixing.

D. R. Mueller (✉) · W. H. Pollard
Department of Geography and Centre for Climate
and Global Change Research,
McGill University, Montreal,
QC, H3A 2K6, Canada
E-mail: derek.mueller@bio.ulaval.ca
Fax: +1-418-6562043

Present address: D. R. Mueller
Département de Biologie and Centre d'études nordiques,
Université Laval, Quebec City,
QC, G1K 7P4, Canada

Introduction

The surface of many glacier ablation zones contain cylindrical meltholes that harbour microbial communities (Wharton et al. 1985; Takeuchi et al. 2000; Mueller et al. 2001). These features, known as cryoconite holes, occur on glaciers worldwide, plus on ice shelves and sea ice in polar regions. Cryoconite holes are created when wind-blown sediment preferentially melts into the ice surface due to its relatively low albedo (Gajda 1958). Over time, the holes become roughly circular and water-filled with brownish-black cryoconite material at the bottom (Nobles 1960). These holes usually range in diameter from 1 cm to 1 m and may reach depths up to 60 cm (Gajda 1958; Brochu 1975), but the coalescence of cryoconite holes may also lead to larger supra-glacial ponds.

Cryoconite holes have been shown to contain heterotrophic bacteria, fungi, cyanobacteria, green algae, diatoms, rotifers, tardigrades and nematodes (Mueller et al. 2001; Christner et al. 2003). These organisms probably originate from local sources, distributed by aeolian processes (Wharton et al. 1985). Regional and long-range dispersal of some of these organisms cannot be ruled out, since several cryoconite taxa live only on ice or snow (Broady and Kibblewhite 1991), thereby necessitating dispersion across large tracts of unsuitable habitat (Wharton et al. 1985; Vincent 2000).

In order to survive, the cryoconite community must be tolerant of extreme conditions. Since this environment is frozen for much of the year, liquid water availability is limited to the short growing season. Further, the cryoconite habitat may be exposed to high levels of radiation (both ultraviolet and photosynthetically active radiation) and subjected to oligotrophic nutrient levels, maximum temperatures near freezing and potential seasonal fluctuations in electrical conductivity from the freeze-fractionation of solutes (Mueller et al. 2001). In addition to the extreme seasonality in the polar regions, the cryoconite habitat may be subjected to varying

degrees of disturbance due to melting (Takeuchi et al. 2000), which might also affect community structure.

Previous studies have identified taxa (Gerdel and Drouet 1960; Christner et al. 2003), described water chemistry (Blanck et al. 1932), measured physical variables (Gajda 1958), examined the micro-structure of the microbial-sediment association (Takeuchi et al. 2001) and demonstrated metabolic activity (Margesin 2002; Säwström et al. 2002) in cryoconite communities. However, quantitative multivariate analysis of cryoconite communities has only just recently been carried out (e.g. Komárek and Komárek 2001). Little is known about the processes of colonization, succession, population dynamics, competition and grazing in these habitats or the effects that various environmental variables may have on cryoconite communities. The aim of this study was to quantify the cryoconite community structure and to determine whether such variations reflect gradients in the cryoconite environment.

According to ecological niche theory, organisms are most abundant when they subsist in optimal conditions. The degree to which the community structure coincides with the environment can be tested by gradient analysis (ter Braak and Verdonschot 1995). Given the random nature of processes that are assumed to introduce organisms to cryoconite microhabitats, a null hypothesis of a random distribution of taxa is proposed. This can be evaluated against the research hypothesis—that assemblages of cryoconite taxa are associated with a combination of environmental variables.

In the present study, a comparison between cryoconite holes on the White Glacier, Axel Heiberg Island, Nunavut and on the Canada Glacier, Taylor Valley, Antarctica was undertaken. Despite the large geographical distance that separates them, these two glaciers share many similarities, including roughly the same mean annual temperature, equivalent latitudes in their respective hemispheres and similar surface areas. Interpretative emphasis was placed on the physico-chemical environment of the two study sites and their relationship to the cryoconite communities.

Materials and methods

Study sites

The location of Canada Glacier (CG) and White Glacier (WG) are shown in (Figs. 1, 2). Their respective climates, glaciological information and cryoconite holes are compared in Table 1. On the Canada Glacier, ablation occurs mainly through sublimation (70%) rather than the more typical process of melting (Fountain et al. 1998). Although they have opened in previous summers (during the 1980s, D. Andersen, personal communication) and in a subsequent summer (2001/2002, A. Fountain, personal communication), the vast majority of the Canada Glacier cryoconite holes were ice-covered throughout the present study. In contrast, the summer surface of the White Glacier is marked by meltwater, both channelized and non-channelized, and open cryoconite holes.

Sampling

On each glacier, a transect running up the ablation zone of the glacier was established; in addition two samples were taken at the medial edges of the glacier, roughly half way along the transect (Figs. 1b, 2b). Cryoconite holes ($n = 20$ for each glacier) were randomly selected by walking along the transect at a predetermined number of paces and choosing the nearest hole. Cryoconite holes less than 10 cm in diameter were excluded because they did not contain sufficient water for the required sample.

When present, the ice cover of the cryoconite hole was penetrated using a Kovacs hand auger (5 cm in diameter). The sampling device consisted of a PVC tube connected to a 500-ml HDPE sample bottle and then a Nalgene hand pump. The sampling tube was inserted to the bottom of the cryoconite hole and suction from the pump brought a slurry of cryoconite sediment and water from the bottom of the hole into the bottle. The sampling equipment was then rinsed to prevent the cross-contamination of samples.

Hole dimensions (diameter, ice cover and depth) were recorded using a tape measure, and the spatial coordinates of each hole were obtained using a handheld GPS [Trimble Geo (CG), Trimble Flight Mate (WG)]. At the time of this research both GPS systems had a precision of 30–60 m despite averaging 100 positions. The elevation for each hole was estimated using glacier slope values derived from high precision DGPS data for the glacier surface [courtesy of

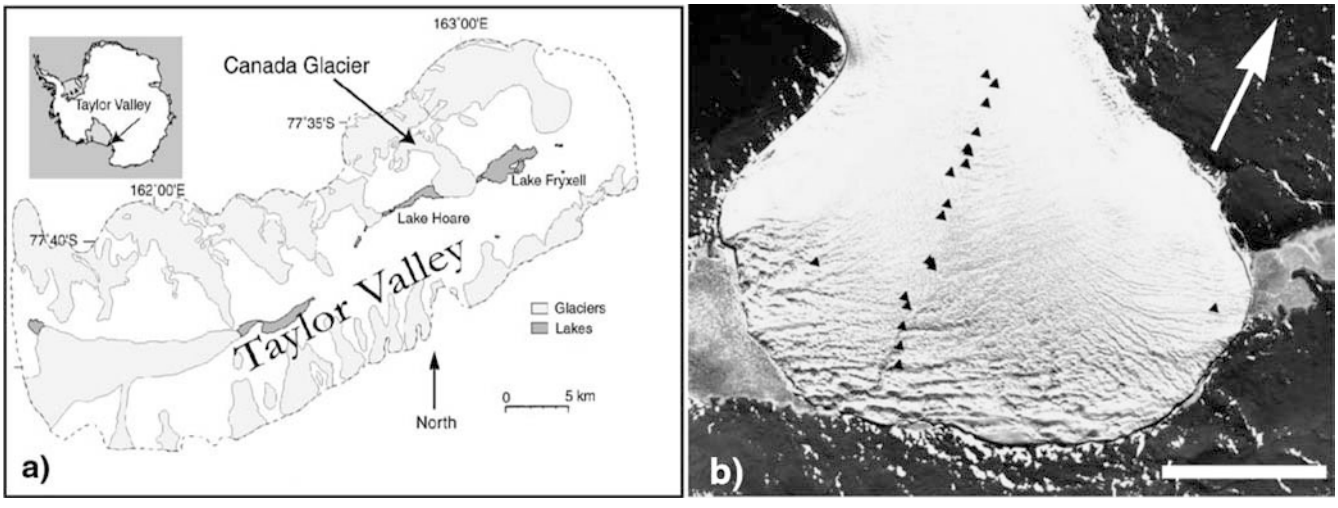

Fig. 1 Southern study site location map (**a**) and airphoto of the Canada Glacier ablation zone (**b**). *Triangles* Cryoconite sample locations, *arrow* indicates north. *Scale bar*: 1 km (modified from McMurdo LTER map and photo 3083-064, 1993)

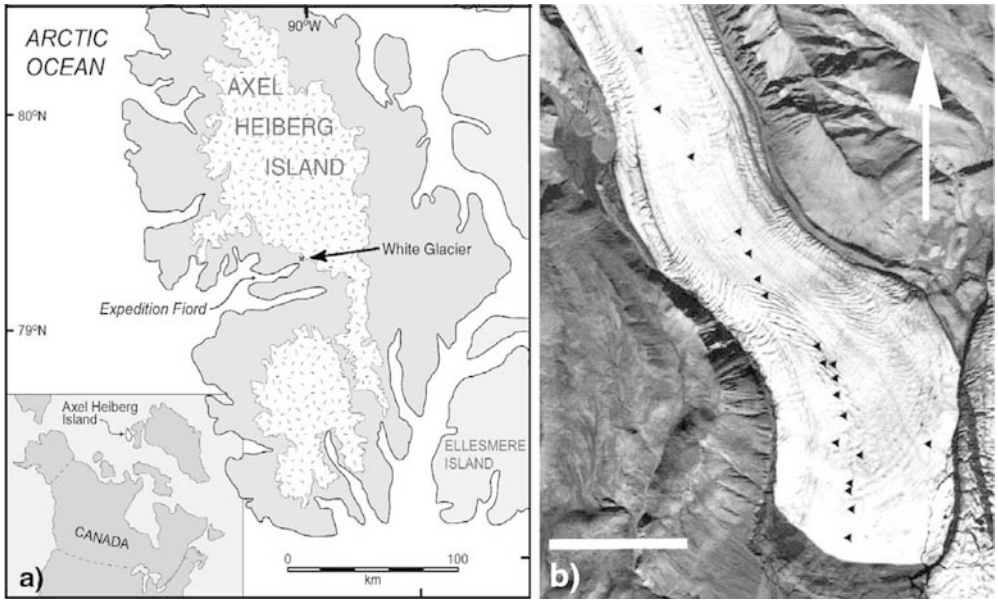

Fig. 2 Northern study site location map (**a**) and airphoto of the White Glacier ablation zone (**b**). *Triangles* Cryoconite sample locations, *arrow* indicates north. *Scale bar*: 1 km. (Map courtesy of Chris Omelon. Photo modified from National Airphoto Library of Canada, photo number A16864-36, 1959)

M. Ecclestone, Trent University (WG) and K. Lewis, University of Colorado (CG)].

Each sample was kept chilled (1–5°C) until it could be processed. Sub-samples were preserved with buffered gluteraldehyde/formaldehyde (WG) or gluteraldehyde (CG) fixative (1% final concentration, v/v) for microscopy. Filtered [Whatman glass fibre GF/F (CG) and GF/C (WG)] cryoconite water was frozen to preserve it for nutrient analysis. The pH was measured with an Orion pH probe at each hole on the White Glacier. The pH of Canada Glacier holes was measured in the laboratory, after sub-sampling, using a Beckman pH probe. Electrical conductivity was measured after sub-sampling for both glaciers.

Ammonium, nitrate, nitrite and dissolved reactive phosphorus (DRP) concentrations were measured colourmetrically using an autoanalyser [Antarctic Support Associates, Crary Lab, McMurdo, Antarctica (CG) and National Laboratory for Environmental Testing, Burlington, Ontario, Canada (WG)]. Detection limits were 2 μg-N l^{-1} for Canada Glacier ammonium, 10 μg-N l^{-1} for nitrate, 5 μg-N l^{-1} for nitrite and 2 μg l^{-1} for dissolved reactive phosphorus (DRP). For the White Glacier, detection limits were 5 μg-N l^{-1} for ammonium, 10 μg-N l^{-1} for nitrate, 1 μg-N l^{-1} for nitrite and 0.2 μg l^{-1} for DRP. For nutrient concentrations, which were frequently below detection limits (CG nitrite, WG ammonium and WG nitrate), the variable precision was downgraded to binary (detected = 1, not detected = 0). This strategy allowed these variables to be included in the multivariate analysis while simultaneously exercising caution concerning measurement limitations. For variables where only some values were below the detection limits, 'not detected' was substituted by a value representing half the detection limit. This was thought to be a conservative approach since variance is reduced through this procedure, and no justification can be given for substituting the value of the detection limit or zero (P. Legendre, personal communication).

Microscopy/enumeration

The present study emphasizes cyanobacteria, algae and protists. Despite their presence in the samples, data were not collected on heterotrophic bacteria, fungi, rotifers and tardigrades. In addition, only living individuals were recorded, which excluded the empty diatom frustules that occurred with some regularity. Individuals were confirmed to have been living (at the time of preservation) by the presence of a visible cytoplasm, nucleus or chloroplast. Instead of identifying to the species level, we divided taxa into morphotypes (Broady and Kibblewhite 1991). Each morphotype may represent a species (sensu stricto), a variant/ecophene of a certain species or may include several species that were morphologically undifferentiated. When identification was possible, the cyanobacterial nomenclature of Anagnostidis and Komárek (1985) and the diatom nomenclature of Krammer and Lange-Bertalot (1986) were used.

A known volume from each sample was scanned using a bright-field phase haemacytometer with an improved Neubauer ruling (Hauser Scientific). Preparations were counted at 320× magnification (N.A. 1.32) using bright-field illumination on a Leitz Diaplan microscope. Since these samples contained colonial forms, individual cells were not always enumerated; rather only natural units (colonies or cells, depending on habit) were considered (Smayda 1978). Counting proceeded until 400 natural units were enumerated, and each natural unit was measured to obtain a biovolume using standard geometrical formulae (sphere, cylinder and hemispherical rod). Biovolume data were analysed instead of percentage abundance data since many of the observed organisms were colonial. This reconciled some of the disparity between natural unit size and abundance and gave recognition to the physical space occupied in the community by each taxon (much like percentage cover in vegetation ecology). However, it should be realized that this method de-emphasizes small yet populous organisms in favour of large yet rare taxa.

It should be noted that this methodology was prone to several different sources of error. Every effort was made to treat each sample in the same manner, however errors are associated with sub-sampling (Venrick 1978b), counting (Student 1907; Venrick 1978a), identification, measurement and extrapolation. Fixative induced cell shrinkage and/or swelling has been shown to occur with a variety of phytoplankton species (Meden-Deuer et al. 2001), and this may have affected biovolume measurements. In addition, it is acknowledged that morphology does not necessarily reflect functionality or distinguish between species (e.g. Nadeau et al. 2001), nor do these methods fully investigate the species diversity or enumerate the microbial consortia fully (cf. Curtis et al. 2002).

Statistical analysis

Hypotheses were tested using the multivariate direct ordination technique of canonical correspondence analysis (ter Braak and Smilauer 1998b). This method was chosen because its asymmetrical

Table 1 Comparison between Canada and White Glacier

	Canada Glacier	White Glacier
Site		
Location	Taylor Valley, Antarctica	Axel Heiberg Island, Nunavut, Canada
Coordinates	77º37'S, 162º55'E	79º27'N, 90º40'W
Site climate		
Mean annual temperature[a] (ºC)	−20	−15.2
Annual precipitation[a] (mm)	< 100	58–117
Glacier		
Surface area (km^2)	27	38
Approximate elevation of the equilibrium line (m.a.s.l.)	450	800
Glacier maximum elevation (m.a.s.l.)	1,750	1,650
Glacier minimum elevation (m.a.s.l.)	15	65
Slope of accumulation zone	Fairly constant, approximately 6%	Variable, 5–30%
Climate during study		
Study period	December and January 1998–1999	June and July 1999
Average downwelling shortwave radiation[b] ($W\ m^{-2}$)	324	217
Maximum temperature[b] (ºC)	3	14.1
Minimum temperature[b] (ºC)	−13	−2.6
Cryoconite hole characteristics		
Cryoconite sampling dates	December 23–24, 1998	July 9–11, 1999
Average depth[c] (cm)	32.4	18.7
Average diameter[c] (cm)	38.7	27.1
Average ice cover thickness[c] (cm)	14	0
Nitrate[c] (μg-N l^{-1})	< 10–255	< 10–33
Nitrite[c] (μg-N l^{-1})	< 5–18	1–6
Ammonium[c] (μg-N l^{-1})	5–140	< 5–9
Dissolved reactive phosphorus[c] (μg l^{-1})	< 2–19	0.6–13.5
Conductivity[c] (μS cm^{-1})	3–61	7–17
pH[c]	5.9–9.6	3.4–4.5

[a]Antarctic Study Site: Fountain et al. (1998); Arctic Study Site: Cogley et al. (1995); Doran et al. (1996)
[b]McMurdo Long-Term Ecological Research Project data, McGill Arctic Research Station data (N.B. measurements approximately 2 km from glacier)
[c]Mueller et al. (2001). Small holes (< 10 cm diameter) were not included in this analysis. Diameter is an average of length and width

approach considers biological data as response variables and relates them directly to the independent (environmental) variables (Legendre and Legendre 1998). Significant results were projected in an ordination diagram to assist in interpreting the community's relationship to various environmental factors. None of the biovolume data were transformed prior to the analysis; however, non-normally distributed environmental variables were transformed with the natural logarithm to remove skewness.

Canonical correspondence analysis (CCA) was carried out using CANOCO 4.0 (ter Braak and Smilauer 1998a). Biological data were initially assessed for suitability for this analysis using detrended correspondence analysis (DCA). The lengths of gradients, or dispersion, given for each axis are a measure of unimodal response, an assumption required for CCA (ter Braak and Smilauer 1998b). Dispersion values higher than 4 are strongly unimodal and are suitable for CCA; dispersion values lower than 2 are linear, indicating that a linear technique should be employed, while between these thresholds, either approach may be suitable (ter Braak and Smilauer 1998b). The significance of each solution (or each variable during forward selection) was determined by 999 unrestricted Monte Carlo permutations. The first step was to eliminate rare taxa from the analysis through a backward selection process. An all-environmental variable solution was calculated using all of the available biovolume data. The morphotype variables were sorted according to their respective frequencies, and those with the lowest frequencies were removed from the solution in a stepwise fashion. After each removal, the solution was recalculated until a noticeable decrease in inertia and canonical eigenvalues was noticed. Those variables whose removal caused the general community structure to change were reinserted and kept for the remainder of the analysis. All the environmental variables were subjected to an automatic forward selection procedure to determine which variables were the most important ones and to increase the overall significance of the model (Legendre and Legendre 1998).

Results

A list of the taxa found, their average abundance as percentage total biovolume and a brief description are given in Table 2. The most numerous taxon on both glaciers was the ubiquitous filamentous cyanobacterium Osc45. Due to the use of biovolume, the less numerous, albeit large desmids in the White Glacier cryoconite holes became dominant, and to some extent, this is also the case with Canada Glacier *Chroococcus* spp.

Canada Glacier

The environmental variables, specifically elevation, cryoconite hole depth, electrical conductivity and pH, were normally distributed and did not require transformation. Cryoconite hole surface area, ammonium and DRP concentrations were successfully normalized using a natural logarithm transform; however nitrate concentration failed to become normalized with this procedure (Shapiro-Wilks, $\alpha = 0.05$). None of the morphotype

Table 2 Taxa (morphotypes) found in Canada and White Glacier cryoconite holes

Glacier	Taxa	Taxa code	Mean biovolume (%)	Mean abundance (%)	Frequency (out of $n = 20$)
Canada	Unidentified cyanobacterial unicell	Cyano	0.2	0.9	15
	Aphanothece cf. *nidulans* Richter	Anidu	1.9	5.7	13
	cf. *Synechococcus aeruginosa* Nägeli	Saerug	10.2	2.2	9
	cf. *Gloeocapsa* spp. Kützing (irregular arrangement in small colonies)	Gloeo1	11.1	0.6	11
	cf. *Gloeocapsa* spp. Kützing (regular arrangement in tetrads)	Gloeo2	0.2	0.1	9
	cf. *Microcystis* spp. Kützing	Microc	4.5	0.5	5
	Chroococcus spp. Nägeli	Chrooc	40.1	19.7	18
	Oscillatiotoriales (may include *Oscillatoria* spp., *Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (cell walls discernable at 320 × magnification)	Osc123	0.9	11.7	20
	Oscillatiotoriales (may include *Oscillatoria* spp., *Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (cell walls not discernable at 320 × magnification)	Osc45	5.1	50.0	20
	Oscillatiotoriales (may include *Oscillatoria* spp., *Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (brown trichome with distinct cross walls; width: 3–6 μm)	Osc6	2.2	1.3	10
	Oscillatiotoriales (may include *Oscillatoria* spp., *Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (green trichome with distinct cross walls)	Osc7	10.5	2.2	6
	Oscillatiotoriales (may include *Oscillatoria* spp., *Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (trichome pale-brown to purple-brown, distinct cell walls, conspicuous sheath)	Osc8	1.1	0.2	4
	cf. *Spirulina* spp. (spiral trichome with polar aerotopes, pale colour)	Spir1	1.7	1.1	12
	cf. *Spirulina* spp. (tightly coiled filament, pale-brown colour)	Spir2	0.1	0.0	2
	Crinalium glaciale Broady	Cglac1	5.6	1.3	12
	Crinalium glaciale var. *helicoides* Broady	Cglac2	2.0	0.3	7
	cf. *Anabaena* spp. Bory (trichome width: 7 μm)	Anab1	0.0	0.0	2
	cf. *Anabaena* spp. Bory (trichome width: less than 5 μm)	Anab2	0.1	0.2	3
	Nostoc cf. *commune* Vaucher	Ncom	0.0	0.0	1
	Nostoc cf. *punctiforme* (Kützing) Hariot	Npunct	0.1	0.0	2
	Unidentified chlorophyte	Chloro	0.3	0.5	10
	Chlamydomonas spp. Wille	Chlamy	0.3	0.3	12
	Luticola cf. *gaussii* (Heiden) D.G. Mann	Lgaus	0.0	0.0	1
	Luticola cf. *murrayi* West et West	Lmurray	0.0	0.0	1
	Luticola cf. *mutica* Kützing	Lmutica	0.0	0.0	1
	Muellaria spp. (West et West) Spaulding et Stoermer	Muell	0.9	0.4	7
	cf. *Dinobryon* Ehrenberg	Dinob	0.0	0.0	1
	Heterotrophic flagellates	Flag	0.2	0.2	7
	Ciliates	Ciliate	0.6	0.2	6
	Unidentified	UnId	0.0	0.0	2
White	Unidentified cyanobacterial unicell	Cyano	0.2	1.6	15
	cf. *Microcystis* spp. Kützing	Microc	0.4	0.1	1
	Chroococcus spp. Nägeli	Chrooc	0.4	2.1	15
	Oscillatiotoriales (may include *Oscillatoria* spp., *Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (cell walls discernable at 380× magnification)	Osc123	0.8	6.8	20
	Oscillatiotoriales (may include *Oscillatoria* spp., *Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (cell walls not discernable at 380× magnification)	Osc45	12.4	70.1	20
	Oscillatiotoriales (may include *Oscillatoria* spp., *Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (trichome with a pale colour, apical cell rounded, sometimes 'stepped' or swollen)	OscA	0.2	0.2	10
	Oscillatiotoriales (may include *Oscillatoria* spp.,*Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (trichome with a green colour, apical cells blunt, conspicuous aerotopes seen at cross walls)	OscB	0.1	0.1	4
	Oscillatiotoriales (may include *Oscillatoria* spp., *Lynbya* spp., *Phormidium* spp., *Leptolyngbya* spp. and *Microcoleus* spp.) (golden-green trichome with striations along its edges, granular cells)	OscC	0.2	0.1	5
	cf. *Scytonema* sp. Agardh	Scyto	1.7	0.8	6
	Unidentified chlorophyte	Chloro	0.2	0.9	10
	Autotrophic flagellates	Aflag	0.0	0.1	4

Table 2 (Contd.)

Glacier	Taxa	Taxa code	Mean biovolume (%)	Mean abundance (%)	Frequency (out of $n=20$)
	Cylindrocystis brebissonii (Ralfs) de Bary (syn. *C. cylindrospora*)	Cylindro	60.0	4.5	20
	Mesotaenium berggrenii (Wittrock) Lagerheim (includes *Ancyclonema nordenskioldii*)	Mbergg	20.9	12.2	20
	Ciliates	Ciliate	2.5	0.4	13
	Heterotrophic flagellates	Hflag	0.0	0.0	2
	Unidentified	UnId	0.1	0.0	1

biovolume variables followed a normal distribution. The preliminary DCA of biovolume data gave a dispersion of 3.0 for the first axis and 3.6 for the second axis, indicating that while morphotype distributions were not strongly unimodal, they were sufficiently so to undertake canonical correspondence analysis.

From a total of 30 morphotypes, the backward selection of taxa proceeded until ten of the least frequent morphotypes were eliminated. The removal of these taxa from the analysis did not unduly influence the overall community response to the explanatory variables. The forward selection of the all-environmental variable solution (69% of biovolume variance explained, $P=0.002$) revealed that the binary variable nitrite concentration along with nitrate concentration, DRP concentration, electrical conductivity, elevation and depth had conditional probabilities of 0.11 or less. Together, these six variables explained 54.6% of the biovolume variance (all axes: $P=0.001$; first axis: $P=0.004$).

The six environmental variable correlation biplot of the Canada Glacier cryoconite morphotypes is presented in Fig. 3. The abscissa represents the first canonical axis, which accounts for 21% of the total biological variance in this example. The ordinate represents the second canonical axis, which accounts for a further 12% of the biovolume variance. The most important environmental variable was found to be nitrite concentration, which generated much of the variance explained in the first canonical axis. Nitrate concentration, electrical conductivity and DRP concentration also contributed to this axis. The variation in the second canonical axis was dominated by depth, elevation and electrical conductivity.

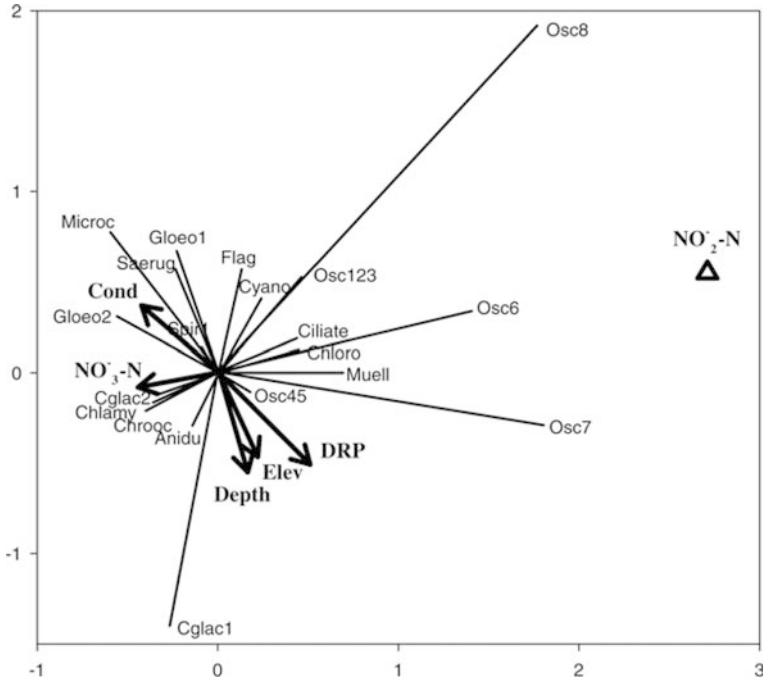

Fig. 3 Canada Glacier CCA biplot. Environmental variables are depicted by *arrows* (or a *triangle* for the binary variable). Their importance to the overall solution is indicated by their *length* (the longer, the more important), and the degree to which they contribute to each axis is indicated by the *cosine of the angle* between the *arrow* and the *axis*. Morphotypes (*simple lines*) are ranked according to their association with environmental variables by projecting a *perpendicular line* from the *environmental arrow* to the *tip of the morphotype line*. Morphotype codes are explained in Table 2. *Cond* Electrical conductivity (μS cm^{-1}), *Elev* elevation (m.a.s.l.), *NO_2-N* nitrite concentration (μg-N l^{-1}), *NO_3-N* nitrate concentration (μg-N l^{-1}), *DRP* dissolved reactive phosphorus concentration (μg l^{-1}), *Depth* cryoconite hole depth (cm)

White Glacier

The non-normally distributed variables (Shapiro-Wilks test, $\alpha=0.05$) DRP and cryoconite hole surface area

were transformed to a normal distribution using the natural logarithm. Ammonium, nitrate and nitrite concentrations could not be transformed to a normal distribution. In general, biovolume variables did not follow the normal distribution, except for the morphotypes Osc123 and Osc45. The assumption of unimodal distribution was marginally met, as the DCA dispersion measurement was 2.1 for the first axis and 2.0 for the second. For internal and statistical consistency we decided to use the same analysis technique for both data sets. All 16 morphotypes were included in the multivariate analysis since backward selection of morphotypes determined that the removal of even the rarest taxa unduly affected the analysis.

An all-environmental variable solution was not found to be significant ($P = 0.632$), despite explaining 44% of the biovolume variance. A forward selection procedure found that cryoconite hole surface area and nitrate concentration were the most promising variables, having conditional probabilities of 0.213 and 0.164, respectively. Although these variables were not individually significant, they were retained in the model while the other variables ($P > 0.250$) were rejected. This improved the overall probability of the environment solution somewhat, but it remained insignificant ($P = 0.138$), explaining only 15% of the biovolume variation. These results show that the White Glacier cryoconite community structure was not influenced by environmental variables, which led to the rejection of the research hypothesis.

Discussion

Canada Glacier

The Canada Glacier biplot (Fig. 3) depicts the optima for each taxa along the synthetic gradients of the first two canonical axes (ter Braak and Verdonschot 1995). Taxa with the least total biovolume exert relatively little influence on the overall solution because of their small numerical contribution (Legendre and Legendre 1998). These taxa will occasionally appear on the edge of the scatterplot because they are associated with variables at a particular site or their presence is due to chance (e.g. Osc8). Conversely, taxa near the origin of the biplot either have their optimum at this location, or they are not associated with the canonical axes in question (e.g. Osc45, Spir1). The taxa that are neither in the centre nor at the edges of the biplot exhibit less ambiguous relationships with the canonical axes (ter Braak 1995).

The six environmental variable CCA solution given above was based heavily on nitrite concentration, whose presence was significantly associated with variation in community composition. Nitrate and DRP concentrations were also found to be significant variables, possibly indicating that some cryoconite taxa were nutrient-limited. Nutrient sources include the deposition of sea salts and aeolian transport of material into the cryoconite holes. The Canada Glacier cryoconite holes had generally low, but variable electrical conductivity values. Elevation and depth were the only significant physical variables. Elevation may indicate a change in temperature or snow-cover as the higher cryoconite holes are closer to the equilibrium line altitude. Depth may also be indicative of cryoconite temperatures or, alternatively, available light.

White Glacier

The low explanatory power of the environmental variables suggests that our set of environmental measurements did not include the primary forcing variables (Legendre and Legendre 1998). For example, glacier slope may be an important missing variable. Slope has been linked to cryoconite hole stability and size (Adams 1966; Takeuchi et al. 2000). Perhaps small variations in the slope of the White Glacier cause enough change in the energy balance and hydrologic regime that cryoconite hole communities are affected in some way.

It was found that the Canada Glacier community structure is significantly linked to measured environmental variables while the White Glacier community structure is not. The reason why this is the case may be based on hole morphology. The presence or absence of a cryoconite hole ice cover may have a large impact on the biogeochemistry of the hole. If inputs and outputs to the cryoconite community are restricted, biogeochemical cycles and population dynamics within each hole will persist in isolation. With inputs and outputs relatively unhindered, cryoconite holes will be less secluded, thereby increasing the chance for homogenization. The Canada Glacier cryoconite holes at the time of the study were typically ice-covered, whereas White Glacier cryoconite holes were all found to be without an ice cover.

The lack of variation in the White Glacier community and the insignificant association with the environmental variables may reflect micro-habitat patchiness within the White Glacier cryoconite holes that could allow for contemporaneous disequilibrium, as suggested by Richerson et al. (1970). Alternatively, a higher level of disturbance and a greater degree of connectivity between cryoconite holes on the White Glacier relative to the Canada Glacier cryoconite holes is probably responsible for the lack of correspondence. Recent models show that Cl^- freeze concentration is indicative of cryoconite hole age (Fountain et al. 2004). Half the cryoconite holes in this study on the Canada Glacier were shown to be hydrologically isolated, including one hole that persisted in that state for over 10 years. When present, connections between cryoconite holes were thought to be via interstitial pores and therefore likely limited to relatively small spatial and temporal scales. Elsewhere, precipitation and meltwater production has been shown to promote the degradation of cryoconite holes (Adams 1966; Takeuchi et al. 2000). White Glacier cryoconite holes are therefore short-lived compared to Canada Glacier holes.

Furthermore, cascading meltwater is able to physically mix cryoconite organisms between holes without ice covers. This opportunity does not exist to the same extent on the Canada Glacier due to a paucity of meltwater and the prevalence of ice-covered holes. In contrast to the White Glacier, Canada Glacier cryoconite holes are more isolated and less disturbed. This would lead to the development of a community whose structure reflects the environment within individual cryoconite holes—and not the environment of the glacier surface as a whole. Our data do not allow us to quantify the time and exact conditions required to structure these communities or the amount of meltwater disturbance needed to remove this structure. We suggest community structure may become discernable within weeks to years, becoming more pronounced with increasing isolation time. However, the differences between cryoconite hole communities may be erased suddenly in a single event.

This study highlights the ecological implications of connectivity between individual cryo-ecosystems. The high degree of sublimation and concomitant reduction in meltwater observed in the McMurdo Dry Valley glaciers are rarely observed in arctic and alpine ablation environments (Fountain et al. 1998). Our contrasting results from the two glaciers in this study suggest that the large differences in cryoconite community structure may be bipolar in scope, however we do not have enough evidence to conclude this. These glaciers demonstrate how considerable selection pressures could exist in cryoconite refugia during colder periods in the Earth's history. However, organisms must be able to survive environmental disturbance during relatively warmer periods, as evidenced on the White Glacier. It is possible that repeated cycling between warmer and colder periods could have evolutionary significance, especially during past eras when cryo-environments were more important (e.g. Vincent and Howard-Williams 2000).

Conclusions

The cryoconite communities studied were diverse and variable. Cryoconite communities found on the White Glacier were often dominated by desmids, whereas those on the Canada Glacier were mostly dominated by cyanobacteria. A canonical correspondence analysis of morphotype volumetric abundance versus environmental and spatial variables showed that 55% of the Canada Glacier cryoconite community structure could be accounted for by six environmental variables; however a significant relationship was not found in White Glacier cryoconite holes.

These contrasting results likely stem from the differences in the surfaces of both glaciers (i.e. energy balance, precipitation, meltwater production, slope and aspect). These differences contribute to a morphological divergence between their respective cryoconite holes. This in turn affects the stability and age of the cryoconite holes. Disturbance from meltwater flushing and hole destruction on the White Glacier prevented the development of unique and varied cryoconite hole conditions and communities. On the Canada Glacier, ice covers on cryoconite holes promoted the isolation of these ecosystems. The lack of meltwater and relative longevity of the cryoconite holes may have caused these cryoconite communities to become structured along environmental gradients. This structure likely disappeared during one of the warmest summers (2001/2002) on record in the Dry Valley when most of the cryoconite holes were without ice covers and meltwater flowed freely on the surface of the Canada Glacier (A. Fountain, personal communication). This development serves to highlight the dynamic nature of these ecosystems, despite their apparent stability at the time of this study.

Acknowledgements The Antarctic portion of this research was carried out with the support of Dr. Peter Doran of the McMurdo LTER program (NSF grant OPP-92-11773), Dr. Christian Fritsen (NSF grant OPP-98-14972) and the Polar Continental Shelf Project—Arctic-Antarctic Exchange Program (grant given to WHP). Field assistance was provided by Karen Lewis, Paul Langevin and Kathy Welch, while logistical support was made available by Antarctic Support Associates. The Arctic portion of this research was conducted at the McGill Arctic Research Station with logistical support from the Polar Continental Shelf Project, Natural Resources Canada (this is PCSP/ÉPCP manuscript no. 021–03). Field assistance was provided by Tom Holloway. Financial assistance was provided by an NSERC graduate fellowship to (DRM) and an operating grant (WHP). The authors would like to thank Miles Ecclestone, Trent University, Karen Lewis, University of Colorado and Peter Doran, University of Illinois at Chicago, for sharing unpublished data. This manuscript has benefited from comments from Prof. Gail Chmura, McGill University, Prof. Pierre Legendre, Université de Montréal, Prof. Warwick Vincent, Université Laval, Prof. John Smol, Queen's University and anonymous reviewers.

References

Adams WP (1966) Studies of ablation and run-off on an Arctic glacier. PhD thesis, McGill University, Montreal

Anagnostidis K, Komárek J (1985) Modern approach to the classification system of cyanophytes. 1. Introduction. Archiv Hydrobiol/Algologic Stud 38/39:291–302

Blanck E, Poser H, Oldershausen E (1932) Über Kryokonitvorkommnisse im ostgrönländischen Packeis und ihre chemische Zusammensetzung. Chem Erde 7:434–440

Broady PA, Kibblewhite AL (1991) Morphological characterization of Oscillatoriales (Cyanobacteria) from Ross Island and southern Victoria Land. Antarct Sci 3:35–45

Brochu M (1975) Les trous à cryoconite du Glacier Gilman (Nord de l'Ile d'Ellesmère). Polarforschung 45:32–44

Christner BC, Kvitko BH, Reeve JN (2003) Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. Extremophiles 7:177–183

Cogley JG, Adams WP, Ecclestone MA, Jung-Rothenhäusler F, Ommanney CSL (1995) Mass balance of Axel Heiberg glaciers 1960–1991. NHRI Report No. 6, National Hydrology Research Institute, Environment Canada, Saskatoon

Curtis TP, Sloan WT, Scannell JW (2002) Estimating prokaryotic diversity and its limits. Proc Natl Acad Sci USA 99:10494–10499

Doran PT, McKay CP, Adams WP, English MC, Wharton RA, Meyer MA (1996) Climate forcing and thermal feedback of

residual lake-ice covers in the high Arctic. Limnol Oceanogr 41:839–848
Fountain AG, Dana GL, Lewis KJ, Vaughn BH, McKnight DM (1998) Glaciers of the McMurdo Dry Valleys, Southern Victoria Land, Antarctica. In: Priscu JC (ed) Ecosystem dynamics in a polar desert: the McMurdo Dry Valleys, Antarctica. Antarctic Research Series. American Geophysical Union, Washington D.C., pp 65–76
Fountain AG, Tranter M, Nylen TH, Lewis KJ, Mueller DR (2004) Evolution of cryoconite holes and their contribution to melt-water runoff from glaciers in the McMurdo Dry Valleys, Antarctica. J Glaciol (in press)
Gajda RT (1958) Cryoconite phenomena on the Greenland Ice Cap in the Thule area. Can Geogr 12:35–44
Gerdel RW, Drouet F (1960) The cryoconite of the Thule area, Greenland. Trans Am Microsc Soc 79:256–272
Komárek O, Komárek J (2001) Contribution to the taxonomy and ecology of green cryosestic algae in the summer season 1995–96 at King George Island, S. Shetland Islands. Nova Hedwigia Beiheft 123:121–140
Krammer K, Lange-Bertalot H (1986) Bacillariophyceae. Suesswasserflora von Mitteleuropa, Teil 1. Naviculaceae. Fischer, Stuttgart
Legendre P, Legendre L (1998) Numerical ecology. Developments in environmental modelling, vol 20. Elsevier, Amsterdam
Margesin R (2002) Characterization of heterotrophic microorganisms in alpine glacier cryoconite. Arctic Antarct Alpine Res 34:88–93
Meden-Deuer S, Lessard EJ, Satterberg J (2001) Effect of preservation on dinoflagellate and diatom cell volume and consequences for carbon biomass predictions. Mar Ecol Progr Ser 222:41–50
Mueller DR, Vincent WF, Pollard WH, Fritsen CH (2001) Glacial cryoconite ecosystems: a bipolar comparison of algal communities and habitats. Nova Hedwigia Beiheft 123:173–197
Nadeau T-L, Milbrandt EC, Castenholz RW (2001) Evolutionary relationships of cultivated Antarctic oscillatorians (cyanobacteria). J Phycol 37:650–654
Nobles LH (1960) Glaciological Investigations, Nunatarssuaq Ice Ramp, Northwest Greenland. Technical Report 66, U.S. Army Snow Ice and Permafrost Research Establishment, Corps of Engineers, Hanover
Richerson P, Armstrong R, Goldman CR (1970) Contemporaneous disequilibrium, a new hypothesis to explain the ''Paradox of the Plankton''. Proc Natl Acad Sci USA 67:1710–1714
Säwström C, Mumford P, Marshall W, Hodson A, Laybourn-Parry J (2002) The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard 79°N). Polar Biol 25:591–596
Smayda TJ (1978) Estimating cell numbers: What to count. In: Sournia A (ed) Phytoplankton manual. UNESCO, Paris, pp 165-166
Student (1907) On the error of counting with a haemacytometer. Biometrika 5:351–360
Takeuchi N, Kohshima S, Yoshimura Y, Seko K, Fujita K (2000) Characteristics of cryoconite holes on a Himalayan glacier, Yala Glacier Central Nepal. Bull Glaciol Res 17: 51–59
Takeuchi N, Kohshima S, Seko K (2001) Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: a granular algal mat growing on the glacier. Arctic Antarct Alpine Res 33:115–122
Ter Braak CJF (1995) Ordination. In: Jongman RHG, Ter Braak CJF, Van Tongeren OFR (eds) Data analysis in community and landscape ecology. Cambridge University Press, Cambridge, pp 91–173
Ter Braak CJF, Smilauer P (1998a) CANOCO for WINDOWS: a program for canonical community ordination by [partial] [detrended] [canonical] correspondence, principal component analysis and redundancy analysis (version 4.0). Microcomputer Power, Ithaca, N.Y.
Ter Braak CJF, Smilauer P (1998b) CANOCO release 4 reference manual and user's guide to CANOCO for Windows - Software for canonical community ordination. Microcomputer Power, Ithaca, N.Y., pp 352
Ter Braak CJF, Verdonschot PFM (1995) Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat Sci 57:255–289
Venrick EL (1978a) Estimating cell numbers: How many cells to count? In: Sournia A (ed) Phytoplankton manual. UNESCO, Paris, pp 167–180
Venrick EL (1978b) The implications of subsampling. In: Sournia A (ed) Phytoplankton manual. UNESCO, Paris, pp 75–87
Vincent WF (2000) Evolutionary origins of Antarctic microbiota: invasion, detection and endemism. Antarct Sci 12:374–386
Vincent WF, Howard-Williams C (2000) Life on snowball earth. Science 287:2421
Wharton RA, McKay CP, Simmons GM, Parker BC (1985) Cryoconite holes on glaciers. BioScience 35:499–503