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Phosphatase Activities of Endolithic Communities in Rocks of the Antarctic Dry Valleys

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ABSTRACT

Phosphorus is scarce in Beacon Sandstone of the McMurdo Dry Valleys, Antarctica, and any input from precipitation is minimal. In endolithic microbial communities recycling of P by the action of phosphatases may therefore be important. The phosphatase activities of three different types of endolithic communities in the McMurdo Dry Valley, Antarctica, were studied in the laboratory. The dominant phototrophs were *Chroococcidiopsis,* mixed *Gloeocapsa* and *Trebouxia,* and *Trebouxia.* Bacteria were also visually conspicuous in the latter two communities, and the *Trebouxia* in both cases formed a lichenized association with fungal hyphae. In each case marked phosphomonoesterase (PMEase) activity was found in assays with 4-methylumbelliferyl phosphate (MUP) or *p*-nitrophenyl phosphate as substrate, and phosphodiesterase activity with bis-*p*-nitrophenyl phosphate as substrate. The pH optimum of PMEase (assayed at 0.5 pH intervals) of the *Chroococcidiopsis, Gloeocapsa–Trebouxia,* and *Trebouxia* communities was 9.5, 5.5, and 8.0, respectively. These values are similar for aqueous extracts of the respective rocks (pH 9.2, 6.2, 7.5). All three communities showed significantly higher PMEase activity at 5° than 1°C, and the first two also showed much higher activity at 5° than 10°C. All three communities also showed slightly lower activity in the light (7 μmol photon m^{-2} s^{-1}) than the dark; this was found with all substrates and substrate concentrations. Prior exposure of a moistened sample to light for 2 h led to a reduction in activity even when the subsequent assay was done in the dark. The rate of PMEase activity (using 100 μM MUP) in the *Gloeocapsa–Trebouxia* and *Trebouxia* communities was approximately linear with time up to 24 h, whereas the *Chroococcidiopsis* community showed a marked decrease after 6 h. At least part of this was due to retention of the 4-methylumbelliferone (MU) hydrolysis product. In spite of the assays being conducted on a whole community, the activity–substrate relationship in each case quite closely resembled a typical Michaelis–Menten relationship. Estimates were made of the apparent half-saturation value and the concentration of MUP required to support half-maximal

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rates. The apparent K_m values were: *Chroococcidiopis,* 230 μM; *Gloeocapsa–Trebouxia* 169 μM; *Trebouxia,* 135 μM. The respective values for apparent V_{max} were 0.053, 0.55, and 0.35 μmol MU g^{-1} h^{-1}. In view of the greater dependence of these communities on the rock for their sole supply of P than for C and probably N, it is suggested that the cycling of P within the communities is a key factor influencing their overall metabolic activity when moisture permits their activation.

Introduction

The McMurdo Dry Valley in Southern Victoria Land, Antarctica, is one of the most extreme microbial habitats on earth, with low biodiversity and exceedingly low productivity [7, 28, 29]. Under the prevailing arid conditions there are extensive areas of rock and soil without snow or ice cover. Apart from infrequent epilithic lichens [19], the microbial communities forming the only biota are confined to a narrow zone beneath the surface of the rock [5, 6]. Such endolithic communities always include one or more phototrophs. The crust of the rock, although permeable to water and gases, is a barrier to penetration of organisms and the cryptoendolithic microenvironment is largely separated from the outside environment. Consequently, the microclimate inside the rock can be distinctly different from the external climate [20, 21].

Since the first detailed description of this community [9], there have been many studies on its environment and the way in which the various microbes respond to it. Melting snow is the only source of water [4]. The upper and lower survival limits of the community are usually sharp. The former is probably determined by the ability to survive damage by UV-B and visible light during the long periods without water and hence without significant metabolic activity; the latter probably reflects the lower limit of light sufficient to permit a net gain from photosynthetic activity [21, 28]. The range of phototrophs in these communities has been reviewed by Friedmann et al. [6] and Broady [1]. Their variety presumably reflects in part the variety of rock types in the desert valleys [11, 27]. The dominant phototrophs are cyanobacteria or eukaryotic algae, but the latter are more frequent and often in lichenized associations [10]. Among the cyanobacteria, *Chroococcidiopsis* was the first to be described and has since been found to be widespread in Antarctic endolithic communities [1].

Some information has been reported on C fixation. Net ecosystem productivity in the lichen-dominated community is only about 3 mg C m^{-2} yr^{-1} [7]. The time required for community lipid carbon turnover has been reported to be ca 20,000 yr [15], although this was revised to 10,000 years by Nienow and Friedmann [19] to account for substantial metabolism below 5°C. The lack of effect of light on incorporation of organic molecules in an endolithic community at one site, Linnaeus Terrace, was assumed to reflect the active metabolism of fungi and nonphotosynthetic bacteria, whereas an increase in ^{14}C-bicarbonate uptake in the light was attributed to the phototrophs [23].

There is a little information on N and P sources for these communities. Phosphate has not been recognized in the Beacon Sandstone of South Victoria Land. The mean value for eight strata of Well-Stratified Sandstone (probably most like the material studied here) was 0.109% P_2O_5 [22], but this P probably consists of grains of apatite, a fairly common heavy mineral in Permian Beacon Sandstone of the central Trans-Antarctic Mountains. Values for inorganic N and inorganic P after extraction with water for a 5-h period have been reported [15] for communities dominated by *Chroococcidiopsis* or lichen. Based on the lack of positive response to nitrate and phosphate additions during short-term assays, the authors concluded that photosynthetic metabolism was nutrient limited. However, their interpretation is open to question, partly because nitrate is only likely to be a natural source of N in these communities if nitrifying bacteria form part of the almost closed ecosystem, but also because a phototroph might use energy preferentially to take up any nutrient which was potentially more limiting than C. Unless a rock has a high content of easily mobilized phosphate, the rate of turnover of P in the community—and hence the availability of P for growing organisms—seems likely to have an important influence on the rate at which the community can metabolize. Long-established communities are presumably almost entirely dependent on P turnover, whereas they may receive further inputs of C from CO_2 and perhaps also N from N_2 fixation, although assays for the latter appear to be lacking for the Antarctic desert communities.

Unless molecules released during breakdown of one organism are incorporated without degradation into other organisms, P availability for growth depends on there being a range of phosphatases and other enzymes capable of hydro-

Table 1. Features of the sites where the endolithic microbial communities were sampled

Variable	East Beacon	Lower East Beacon	Barwick Valley
Dominant photograph(s)	*Trebouxia*	*Gloeocapsa-Trebouxia*	*Chroococcidiopsis*
Latitude	77° 50' S	77° 50' S	77° 22' S
Longtitude	160° 52' E	160° 51' E	160° 10' E
Altitude (m)	2300	2200	800
Distance to polar ice (km)	15	15	15
Aspect, facing	North	North	North-North-East
Geological formation	Farnell Sandstone	West Beacon Sandstone	Beacon Sandstone
Geological Period	Late Devonian	Late Devonian	Jurassic/Silurian
Grain size: % at feret diam. <250 μm	37	nd	nd
250–500 μm	32	nd	nd
<500 μm	31	nd	nd
Porosity %	6–7[3]	nd	nd
Moisture %	0.13–1.12 (0.41)[3]	nd	nd
Bed rock composition:			
Fe total (μg g^{-1})	1623–1587	nd	nd
Ca total (μg g^{-1})	70–48	nd	nd
K total (μg g^{-1})	507–454	nd	nd
Data obtained in this study			
pH of water with ground rock	9.2	6.2	7.5
FRP in rock extracted by water for 4 h (μg g^{-1})	3.2	nd	nd
TP in rock digested with acid for 45 min (μg g^{-1})	12.1	nd	nd

lyzing organic phosphates [18]. Such enzymes may be released during breakdown of cells, present on the surface of live cells or released extracellularly by live cells. An overview of the features influencing phosphatase activities of phototrophs is given in [13].

The aim of the study reported here is to characterize key features of phosphatase activity in three different types of cryptoendolithic communities in the Antarctic desert. The study is based on samples collected by one of us and was taken as far as availability of material permitted. The experimental methodology involved disruption of the community, so the results should be considered as a guide to the influence of environmental factors rather than an indication of rates of activity likely to occur *in situ*.

Methods

Samples

The Beacon Sandstone samples, all of which were noted in the field to contain obvious endolith communities, were collected by DDW-W in summer (December) 1982. At the time of sampling there was no snow on the valley floor or sides and the weather was calm and above freezing. The samples come from (1) 1-m diameter boulder in Barwick Valley, probably originating from the Beacon Sandstone bed at 1000–1200 m above sea level on the NNE flank of Apocalypse Peaks; (2) sandstone strata from the lower NW flank of East Beacon; (3) sandstone strata from the N flank of the summit of East Beacon. Environmental information is given in Table 1. It was impossible to tell how long ago the Barwick Valley boulder had been dislodged from the Apocalypse cliff and so how long there had been for the endolith community to stabilize to its new environment. However, its aspect and exposure were similar to that of its probable origin. Analysis of a sample of the Barwick rock using differential scanning calorimetry showed that the concentration of water was below the detection limit (M. R. Worland, personal communication).

The surface area of rocks available for study was approximately 25, 6, and 15 cm^2 for *Chroococcidiopsis, Gloeocapsa–Trebouxia,* and *Trebouxia* communities, respectively. The samples included the surface, the endolith community and typically about 5 mm of rock below the community. The upper part of the community was 2–5 mm below the surface and the typical thickness of the communities was 1, 2.5, and 1.5 mm, respectively.

For *Chroococcidiopsis*-containing rock there were two samples and these are considered separately, in case the proportion of cyanobacteria differed between them. Studies using MUP (to detect phosphomonoesterase) on pH, substrate concentration, and time course were done on sample I; those with MUP on temperature and influence of preexposure to light, and those with pNPP (*p*-nitrophenyl phosphate) and bis-*p*-NPP (bis-*p*-nitrophenyl phosphate) were done on sample II. (There was no obvious difference between results from the two samples.) All experiments with the other two communities were conducted on one sample only.

The rocks were stored and transported at approximately -20°C until the time of the experiments.

Preparation for Laboratory Assays

Because of the limited amount of material, it was decided simply to use the mass of rock to standardize the amount material in an assay, rather than some measure of biomass such as the lipid phosphate adopted by some authors based on [24], though a measurement of chlorophyll a content was made for one sample of the *Chroococcidiopsis* community. All the rock material of a particular type was homogenized lightly and the mass of rock plus community used in an assay was utilized to standardize the amount of community present. Rocks from a particular site were fragmented in a shaded part of a cold-room (c 10 μmol photon m^{-2} s^{-1}) with a geological hammer and washed chisel until the material appeared relatively uniform. The process was carried out using a washed high-sided tray to prevent loss and the tray was contained in ice. Gloves were worn to prevent contamination. All the material from a particular site (or sample in the case of *Chroococcidiopsis*) was homogenized lightly and portions of approximately 0.1 g were placed in sample tubes, refrozen and stored at -20°C until use; the exact mass for each assay was weighed at the end of an experiment.

Chlorophyll a Analysis

Chlorophyll *a* was extracted from 0.2 g rock with the *Chroococcidiopsis* community (sample II). The methanol extraction procedure of Marker [17] was used.

Phosphatase Assay

PMEase (phosphomonoesterase) activity was assayed routinely fluorometrically using the fluorogenic substrate MUP. Some assays for PMEase activity were also conducted using the less sensitive methodology with the substrate pNPP and one set of assays for PDEase (phosphodiesterase) activity was made using bis-pNPP. Many of the practical details are given in [26]. Assays were carried out in a P-free version of the medium described in [16], but buffered to give a range of pH values using 100 μM final concentrations of DMG (3,3-dimethylglutaric acid) (pH 4.5–5.5), HEPES (*N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid)) (pH 6.0–7.5) or glycine (pH 8.0–10.0). For each replicate, a universal bottle was filled with 5.9 mL cold sterile medium and the homogenized rock was added to each bottle (excluding one of set of controls).

Assays were conducted in a refrigerated water-bath with gentle shaking. A temperature of 5°C was used unless stated otherwise. Temperature was measured at the end of assays to ensure that there was no difference between samples in the light or dark. The light flux was about 6–8 μmol photon m^{-1} s^{-1} in routine experiments, but other conditions were tested, including dark and higher values. Dark conditions were obtained by wrapping the universal bottles, including caps, twice in aluminum foil. Particular care was taken in one experiment to ensure that the rock was kept in the dark all the time it was exposed to medium; otherwise "dark" samples were exposed to low light for about a minute prior to the addition of substrate. The influence of high light flux was tested using a Phillips H P I, 400-watt light source, to provide a flux of 800 μmol photon m^{-2} s^{-1} for the sample.

A 0.1-mL aliquot of substrate was added to the bottles to give a final concentration of 100 μM for routine assays and the universal bottles then capped tightly. Details of time course studies or length of assays are given in Results. Two controls with assay medium were sampled at each time interval: substrate, but no rock; rock, but no substrate. There was no detectable change in absorbance or fluorescence in either case. After an assay, the supernatant was passed through a GF/C filter and activity ended using 10% (v/v) of the correct base/acid terminator. The filters were put in the appropriate universal bottles with the remainder of the endolithic biomass. Using excitation at 356 nm, fluorescence emission was measured at 444 nm. The absorbance of pNP (*p*-nitrophenol) and bis-pNP (moieties remaining after hydrolysis) was measured at 405 nm. The bottles containing the filters were dried overnight at 105°C. The mass of the filter and biomass were recorded while the amount of rock and associated biomass was determined after correction for the mass of the filter. The results are expressed as product (MU, pNP, bis-pNP) formed g^{-1} d · wt h^{-1} (MU = 4-methylumbelliferone; pNP = *p*-nitrophenol; bis-pNP = bis-*p*-nitrophenol).

Microscopy and Staining for Localization of Phosphatase

Observations on the microbial communities were made using visible light and fluorescence microscopy. The dimensions of *Chroococcidiopsis* cells were determined using a Leitz Ploemopak epifluorescence system with a green excitation filter (N2, 530 nm excitation) for phycocyanin fluorescence and an image analyzer (Sonata Seescan, Cambridge). The attachment to the substratum of untreated but carbon-coated *Chroococcidiopsis* cells was imaged using a scanning electron microscope (Leica Cambridge Stereoscan 360).

Potential localization of enzyme activity in field samples was studied by visible light microscopy using BCIP (bromo-4-chloro-3-indolyl phosphate) as an organic phosphate substrate. A rock sample was washed three times, resuspended in 1 mM BCIP in assay medium at pH 7.0, left for 30 min at 32°C, and again washed three times. BCIP is first hydrolyzed, releasing one molecule of soluble, colourless indole and one molecule of phosphate. This is followed by an oxidation step, leading to the production of the highly insoluble blue pigment 5-bromo-4-chloro indigo. Staining therefore depends on both enzyme activity and the presence of O_2 [2].

Phosphate Analysis

FRP (filtrable reactive phosphate-P) was measured using the method in [3]. A measurement was made on water (5 mL) in which Barwick rock (0.1 g total) had been shaken for 4 h (5°C, 6–8 μmol photon m^{-2} s^{-1}). Total P was also measured after extraction from 0.1 Barwick rock for 45 min using the digestion mixture in [3].

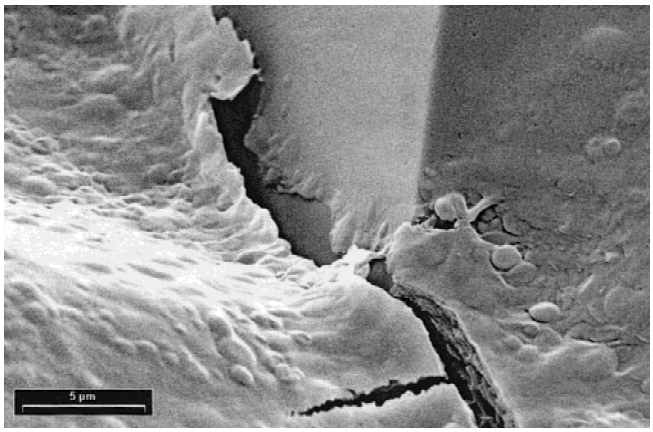

Fig. 1. Scanning electron micrograph of Beacon Sandstone, showing cells of *Chrococcidiopsis* embedded in an exopolysaccharide matrix on a quartzite crystal. Cell dimensions: mean = 1.8 µm, cv = 15%. (The cells are dehydrated, which probably explains why their dimensions are slightly less than those shown by light microscopy.)

pH of rocks

To determine the approximate pH of the environment for the endolith, rock fragments (0.5 g) were suspended in deionized water (5 mL) and shaken for 4 h at 5°C and 6–8 µmol photon m^{-2} s^{-1}. The pH of the supernatant was then read.

Statistical Analysis

Mean values and standard deviations are given in the figures. To determine the significance of differences between treatments, a *t*-test with a significance level of 0.05 was performed. Owing to insufficient rock material, some experiments were conducted on single representative samples and therefore do not have error bars.

Results

The dominant phototrophs in each rock type are listed in Table 1. Epifluorescence microscopy with green excitation of the *Chroococcidiopsis*-dominated community in the Barwick boulder (both replicates) showed that >90% of the cyanobacterial cells appeared viable. They are partially embedded in an exopolysaccharide matrix adhering to the quartzite substratum (Fig. 1). Rod-shaped and coccoid bacteria were evident, but constituting far less biomass than the cyanobacteria. The cyanobacterial cells shown by epifluorescence image analysis immediately after mounting crushed rock in water consisted entirely of *Chroococcidiopsis,* frequently associated in pairs, with the following dimensions (mean ± c.v., n = 100): length 2.05 µm ± 12% and width 1.77 µm ± 11%.

In the *Gloeocapsa–Trebouxia* community of Lower East Beacon, the prevalence of the brown sheaths of *Gloeocapsa* suggested that the cyanobacterium was the more abundant component of the community. However, fluorescence microscopy indicated that only a minority of the sheaths included viable cells. Bacteria and fungal hyphae were abundant, the latter presumably being the mycobiont of lichenized *Trebouxia.* The *Trebouxia*–fungal hyphal association in the East Beacon rock was more closely integrated than the similar association in the Lower East Beacon rock. The bacterial population in the East Beacon rock was more abundant than in the Barwick rock, but less than in the Lower East Beacon rock.

1 cm^2 of sample II of the Barwick rock weighed approx. 2.3 g. The single measurement of chlorophyll a gave a value of 3.4 µg chl g^{-1} rock.

Lower pH, as determined after rehydration of the rock samples, favored eukaryote-dominated communities more than the *Chroococcidiopsis*-dominated community (Table 1). For clarity in the remainder of the Results, the communities are named according to their dominant phototroph.

The effect of pH on the PMEase activity of the three endolith communities is shown in Fig. 2. The *Chroococcidiopsis* community showed a pH optimum of 9.5; below pH 7.0, the rates were either near the detection limit or the cells had lysed. The *Trebouxia* community peaked at pH 8.0, whereas the *Gloeocapsa–Trebouxia* community had two pH optima, 5.5 and 8.5–9.5. Subsequent assays were carried out at the optimum pH for each community (pH 9.5 for *Chroococcidiopsis,* 5.5 for *Gloeocapsa–Trebouxia,* and 8.0 for *Trebouxia*). These values are quite similar to the pH values determined for rock shaken in water (Table 1: pH 9.2, 6.2, 7.5, respectively).

The influence of temperature on the PMEase activity of these communities is shown in Fig. 3. In all three cases activity was significantly higher (p = <0.01) at 5°C than at 1°C, and in two cases significantly lower (p = <0.01) at 10°C than at 5°C.

In view of the mixed composition of a community, assays on the influence of substrate concentration (Fig. 4) gave a surprisingly close approximation of what might be expected with a pure enzyme (see Discussion). Estimates were therefore made of the apparent half-saturation value and the concentration of MUP required to support half maximal rates. The apparent K_m values for the communities were *Chroococcidiopis,* 230 µM; *Gloeocapsa–Trebouxia,* 169 µM;

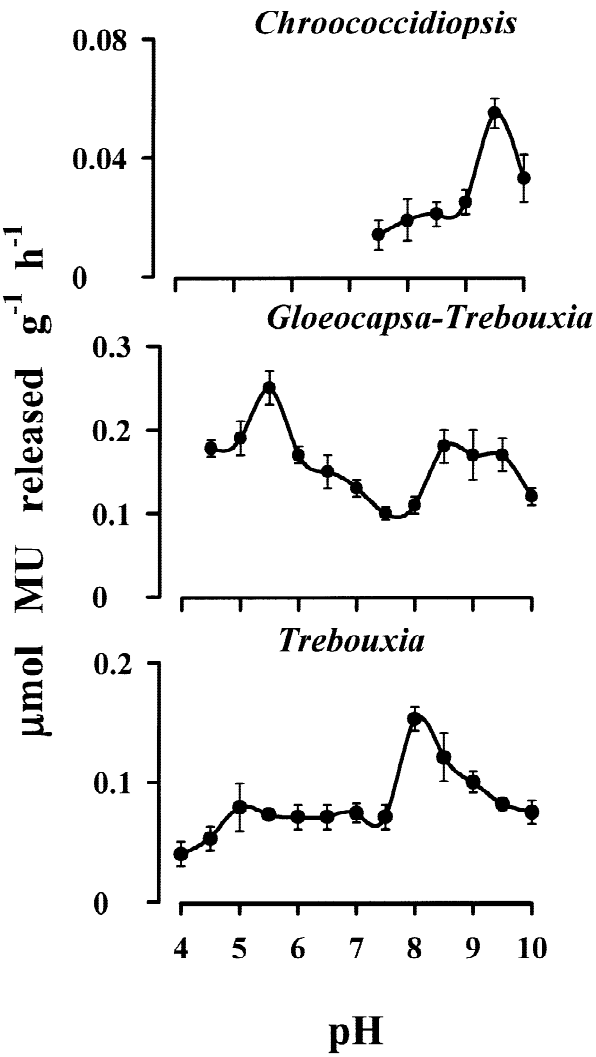

Fig. 2. Effect of pH on PMEase activity (100 μM MUP, 5°C, 7 μmol photon m^{-2} s^{-1}) of *Chroococcidiopsis, Gloeocapsa–Trebouxia,* and *Trebouxia* communities; assays conducted for totals of 6, 4, and 4 h, respectively, but rates expressed per hour. Mean ± S.D., $n = 3$.

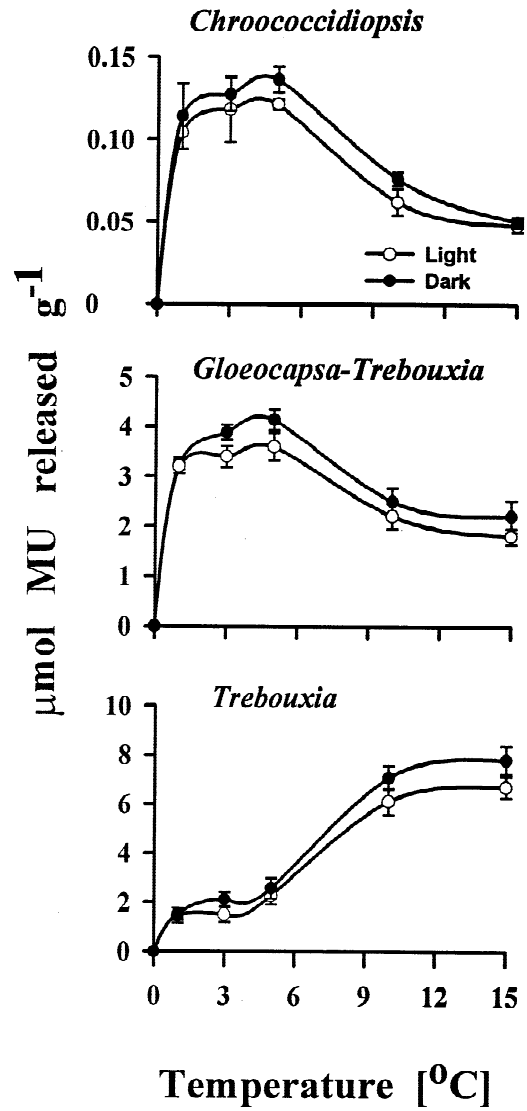

Fig. 3. Effect of temperature on PMEase activity (100 μM MUP,) of *Chroococcidiopsis, Gloeocapsa–Trebouxia,* and *Trebouxia* communities in light (7 μmol photon m^{-2} s^{-1}) and dark. Results are presented for the full 10-h period (not per hour). Mean ± S.D., n = 3.

Trebouxia, 135 μM. The respective apparent V_{max} values were 0.053, 0.55, and 0.35 μmol MU g^{-1} h^{-1}.

As light is essential for the long-term maintenance of these endolithic communities, its influence was examined. All three communities showed lower PMEase activity in the light (6–8 μmol photon m^{-2} s^{-1}) than the dark (Fig. 5). Although the difference in activity between light and dark at individual times was not always significant, the fact that all 10 measurements for a community were lower in the light is highly significant ($p < 0.01$) for each community. The response with time of *Chroococcidiopsis* community differed from that of the other two communities (Fig. 5); its PMease activity showed a marked decrease in rate in both light and dark after about 6 h. Much of the difference between total activity in light and dark seemed to develop in the first 2 h with *Chroococcidiopsis,* but became more evident with time with the other communities. Nevertheless, the difference at 12 h was similar for all three: 9%, 11%, and 20%, respectively.

In the case of *Chroococcidiopsis,* PMEase activity decreased slightly toward the end of the period in assays using 100 μM MUP substrate (Fig. 5), and markedly so in those using 300 μM MUP (data not shown). Because of this decrease in activity after 16 h, an experiment was conducted to see whether all the nonphosphate moiety (MU) from hydro-

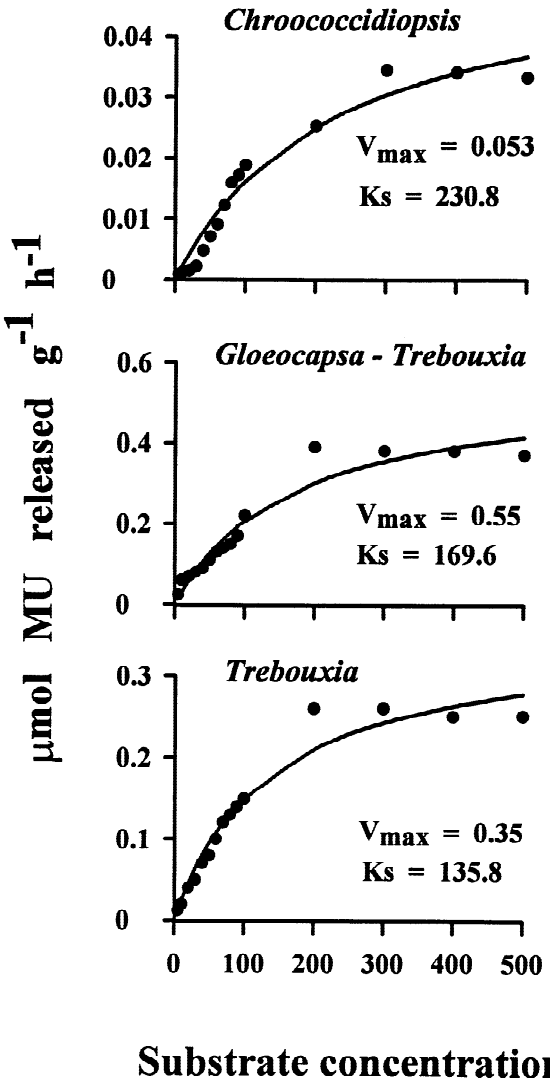

Fig. 4. Relationship between PMEase activity (5 °C, 7 µmol photon m^{-2} s^{-1}) and substrate (MUP) concentration for the *Chroococcidiopsis, Gloeocapsa–Trebouxia,* and *Trebouxia* communities. Activities measured over 6-, 4-, and 4-hour periods, respectively.

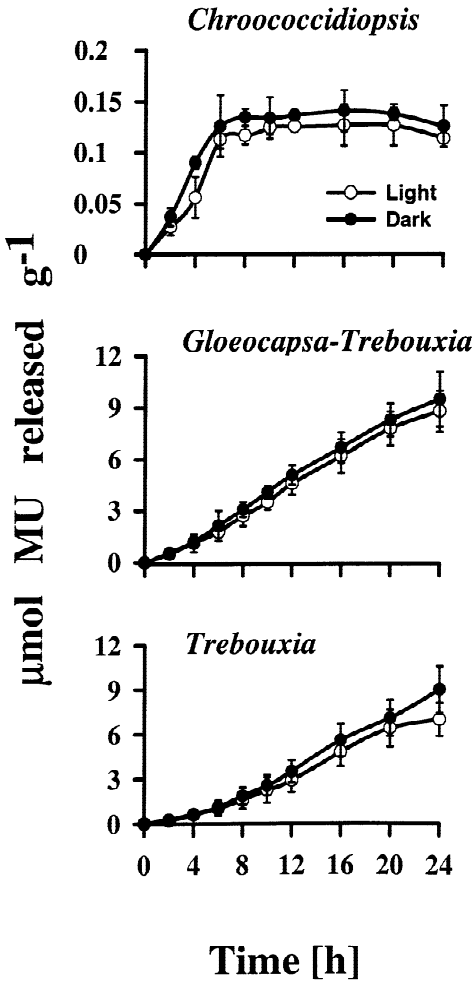

Fig. 5. Effect of time on total PMEase activity of the *Chroococcidiopsis, Gloeocapsa–Trebouxia,* and *Trebouxia* communities. Assays conducted with 100 µM MUP (5°C, 7 µmol photon m^{-2} s^{-1}) and assessed as concentration of MU in the medium. Mean ± S.D., $n = 3$. (The lack of linearity with time using *Chroococcidiopsis* is in part due to retention of MU by particulate material: see text.)

lyzed MUP phosphomonoester added was still present in the medium when fluorescence was measured. A homogenized rock sample at the end of an assay was washed thoroughly and resuspended in fresh buffered medium without substrate. There was a slow release of MU up to at least 1 h (Table 2).

The rates for various assays are compared in Table 3. The various experiments gave quite similar values for the communities with *Gloeocapsa–Trebouxia* and *Trebouxia,* but less so with *Chroococcidiopsis.*

Two other substrates (100 µM pNPP and bis-pNPP) were also used to assay changes in PMEase and PDEase activities with time in light and dark (Fig. 6). The absolute values of PMEase were slightly higher using pNPP than using MUP (compare with Fig. 5). PDEase activity was about half that of PMEase activity with *Chroococcidiopsis* and *Gloeocapsa–Trebouxia* communities, but less with the *Trebouxia* com-

Table 2. MU (non-phosphate moiety) released by *Chroococcidiopsis* community after completion of assays with MUP for 16 h in the light (7 µmol photon m^{-2} s^{-1}) at three substrate concentrations

Substrate concentration (µM)	µmol MU g^{-1} rock
5	0.01
100	0.022
300	0.031

Table 3. Comparison of rates of PMEase and PDEase activities using 100 μM substrate in different experiments with the three communities[a]

Substrate purpose of expt.	*Chroococcidiopsis*		*Gloeocapsa–Trebouxia*		*Trebouxia*	
MUP	Time (h)	μmol MU $g^{-1}h^{-1}$	Time (h)	μmol MU $g^{-1}h^{-1}$	Time (h)	μmol MU $g^{-1}h^{-1}$
pH	6	0.05	4	0.25	4	0.15
Temperature	10	0.012	10	0.35	10	0.22
Temperature, dark	10	0.0135	10	0.41	10	0.25
Substrate concentration	6	0.018	6	0.23	6	0.15
Time course	6	0.018	6	0.30	6	0.17
Time course, dark	6	0.02	6	0.35	6	0.18
pNPP		μmol pNP g^{-1} h^{-1}		μmol pNP g^{-1} h^{-1}		μmol pNP g^{-1} h^{-1}
Time course	6	0.046	6	0.46	6	0.36
Time course, dark	6	0.06	6	0.48	6	0.40
bis-pNPP		μmol bis-pNP g^{-1} h^{-1}		μmol bis-pNP g^{-1} h^{-1}		μmol bis-pNP g^{-1} h^{-1}
Time course	6	0.033	6	0.13	6	0.08
Time course, dark	6	0.035	6	0.16	6	0.10

[a] Rates are expressed as product released per hour, though assays have been run for different lengths of time (as shown in table). The estimate for *Chroococcidiopsis* based on the temperature study is an underestimate of the extrapolated value, as the assay was continued beyond the period of linear increase in total activity with time. Experiments were carried out at 5 °C and at 7 μmol photon m^{-2} s^{-1}, apart from the four in the dark.

munity. The rates were higher in dark than light with both substrates and all three communities.

Further experiments on the influence of light were conducted on the *Chroococcidiopsis* community. Part of a homogenized rock sample was exposed at 5°C to 2 h continuous light (7 mol photon^{-2} s^{-1}) before the start of an assay; a comparison was then made of the PMEase activity (100 μM MUP) of this sample and of one for which care had been taken to avoid any preexposure to light. Preexposure to light led to a marked reduction in PMEase activity, whether the assays were run in light or dark (Fig. 7; $p < 0.01$). Under all treatments the rate of activity slowed markedly between 30 and 60 min. Another experiment with preexposure for 2 h at 800 μmol photon^{-2} s^{-1} led to cessation of activity (data not shown).

FRP was measured in filtrates after 16 h of assay (Table 4). The values ranged from 5.7 μg P g^{-1} rock (*Chroococcidiopsis*) to 24.8 μg P g^{-1} rock (*Trebouxia*). Staining the rock samples with BCIP showed purplish-blue color around the sheath of the *Chroococcidiopsis* community. For *Gloeocapsa-Trebouxia* and *Trebouxia* communities this color was not only found on the outer surface of the organisms, but also elsewhere on rock fragments.

Discussion

Despite their existence in xeric habitats near the limits of life, phosphatase activity associated with endolithic cyanobacteria and algae was demonstrated on rehydration. However, before discussing the results in detail, it is necessary to consider how the samples had been treated before the experiments were carried out. The rocks were collected during the summer season of continuous light, though it is very doubtful if they were metabolically active at the time of sampling, because of the absence of snow and consequent desiccation and the known absence of water in the Barwick community. The *Chroococcidiopsis*-dominated community was in a fallen boulder originating from an upper stratum of Beacon Sandstone. The boulder was well-bedded and partly entrapped by subsequent rockfalls, so it is unclear how long ago this occurred. However, its aspect (including shading) and orientation were similar to those of endolithic communities of the original rock outcrop, and the relatively small difference in altitude (c. 200 m) is unlikely to have a significant influence on such slow-growing communities. The rocks were kept below 0°C immediately after sampling and stored at approximately -20°C in the dark for some 16 yr (c.f. dark winter temperatures of -19° to -45°C) [8]. These conditions are not abnormal for communities with a 10,000-year C-turnover time [19]. The procedure for homogenizing the rocks for experimental purposes was carried out in the cold-room at low light intensity, and the temperature of the material probably rose little above 0°C. This may be compared with the surface of N-facing exposed sandstone on a calm sunny day, when the temperature can reach as high as +15°C [8]. Following homogenization, the material was then re-

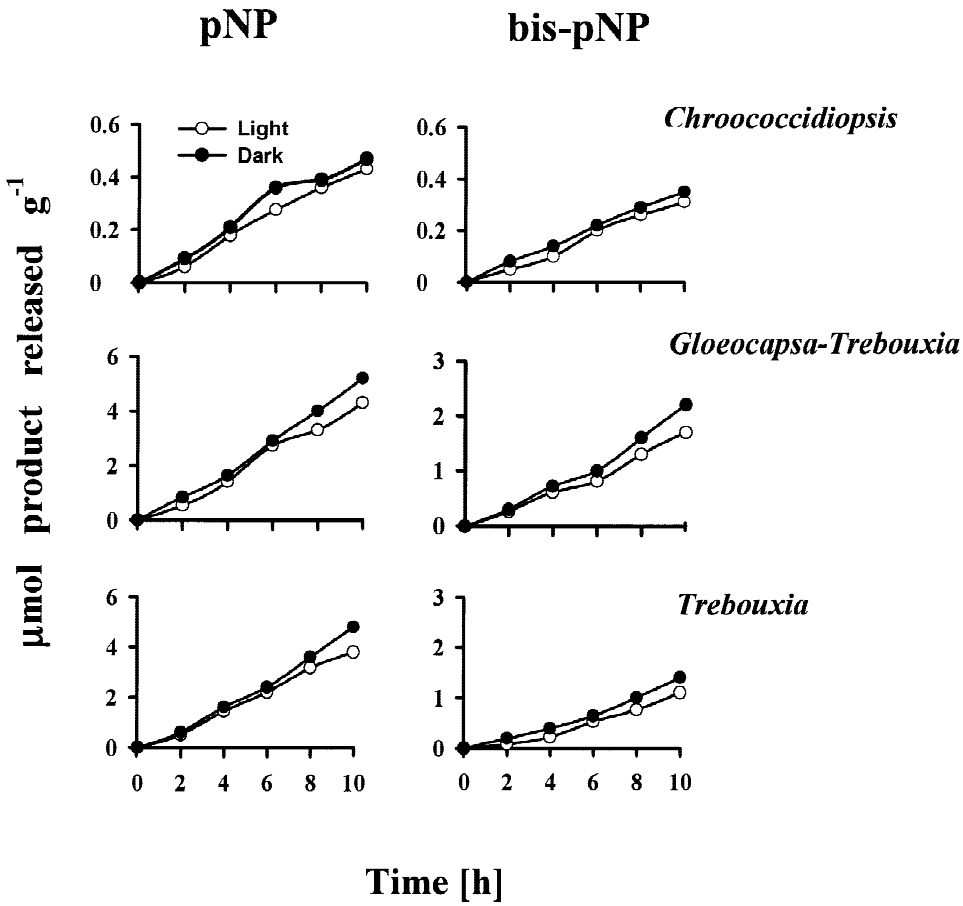

Fig. 6. Effect of time on total PMEase activity (assayed with 100 µM pNPP) and PDEase activity (assayed with 100 µM bis-pNPP) of *Chroococcidiopsis*, *Gloeocapsa–Trebouxia*, and *Trebouxia* communities at 5°C in the light and dark.

turned to -20°C until required. Apart from one experiment when special care was taken to ensure total darkness, samples were exposed to low light for about a minute before starting an assay.

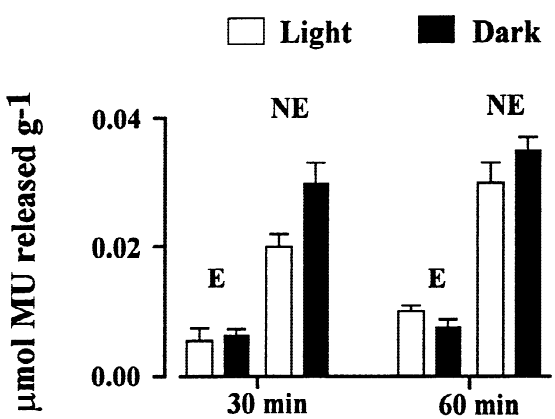

Fig. 7. Influence of preexposure to 2 h light (7µmol photon m^{-2} s^{-1}) on the *Chroococcidiopsis* community prior to assays of total PMEase activity (100 µM MUP) in the light and dark (5°C). Mean ± S.D., n = 3. E = exposed: NE = not exposed.

All the samples showed phosphatase activities, with maxima close to the ambient pH. This and the staining of cyanobacterial sheaths suggest that "surface" phosphatases of the organisms make an important contribution to the activity detected. Apart from any inorganic phosphate released from the rock, almost all the phosphate available for growing organisms must be that released from decaying organisms and thus initially organic phosphates, together with an extremely limited occasional input from snow meltwater and aeolian dust. It is likely that the phosphate released in the present experiments was enhanced considerably from that in nature due to the disturbance of the community, the optimal hydration state, experimental mixing, and high concentrations of substrate. The inorganic phosphate extracted from an endolithic lichen-dominated community by Vestal and Friedmann [23] may well have arisen from organic phosphate hydrolyzed by phosphatases.

Two of the communities assayed showed their highest activity at 5°C (compared with 1° or 10°C). Short-term assays of surface phosphatases of organisms isolated from temperate and tropical climates often show optima at consider-

Table 4. Comparison of the concentration of phosphate-P measured in the assay medium with the concentration which would be expected based on measurements of MU in the medium following incubation with 100 μM MUP for 16h (5 °C, 7 μmol photon m^{-2} s^{-1})[a]

Communities	P present (μg g^{-1} rock)	MU released (μmol g^{-1} rock)	Estimated P released (μg^{-1} rock)	P measured as % estimated P released
Chroococcidiopsis	5.8 ± 0.78	0.127+	3.9+	67%
Gloeocapsa–Trebouxia	13.1 ± 0.37	6.2	192	7%
Trebouxia	24.8 ± 0.5	4.8	148	17%

[a] Results are expressed as μg P g^{-1} rock in order to provide a comparison with the other studies.

ably higher temperatures than those of their natural habitat [13]. This emphasizes the significance of the adaptation of enzymes to low temperature in these Antarctic communities. Although temperatures above 10°C are uncommon in endolithic habitats, PMEase activity continued to increase above 5°C in the *Trebouxia*-dominated community, which may reflect the importance of flexibility to allow for extremes that could denature sensitive enzymes.

Some of the features of the phosphatase activities were similar to those of many other organisms or communities, such as the higher PMEase activity found when using pNPP rather than MUP as substrate. This is probably due to the hydrolysis of pNPP by a range of enzymes and not just PMEases [18]. Comparison of PMEase and PDEase activities using pNPP and bis-pNPP showed approximately half the activity with the latter in two of the communities. A similar result has been reported from a number of laboratory cultures [14], suggesting that the same enzyme may possibly be involved in both processes. However, the ratio between the two activities is by no means universal in assays with other organisms taken from nature. Although the phosphatase activities assayed are presumably due to a range of enzymes, nevertheless the data in Fig. 4 suggest that the activity–substrate relationships resemble quite closely a typical Michaelis–Menten relationship.

The lack of linearity of PMEase activity (assayed with MUP) of the *Chroococcidiopsis* community with time (Fig. 5) is at least in part due to retention of MU by particulate material (Table 2). There are several possible explanations for the slow release of some of the nonphosphate moiety of an organic phosphate molecule by particulate material. If hydrolysis takes place inside the cell (in addition to hydrolysis at or external to the cell wall), the product released in the cell might move slowly to the exterior. Another explanation could be that MU released by PMEase activity in the thick sheath of *Chroococcidiopsis*(Fig. 1) tends to be retained there.

There was some uncertainty concerning the fate of phosphate hyrolyzed during the assays. The phosphate present in medium at the end of a 16-h assay with the *Chroococcidiopsis* community was approximately the same as that released from MUP, suggesting that uptake by the cyanobacteria and any other bacteria present is slow. However, only a low proportion of the phosphate released by the other two communities was detectable in the medium (Table 4), suggesting that there is either a very high rate of uptake by organisms or immobilization on the rock.

The inhibitory effect of light on PMEase activity with either substrate and also on PDEase activity was unexpected. Much of the effect appeared to occur during the early stage of assays with *Chroococcidiopsis,* so the percentage decrease dropped during long-term assays. Nevertheless, this effect was consistent and repeatable, and there was no obvious source of an artifact, such as might occur if light influenced the temperature in the assay bottles or hydrolysis of the substrate. Most studies on surface phosphatases show no effect of light during short-term studies, though a few studies on seaweeds have shown enhancement in the light [13], where the effect is thought to reflect the demand for energy to transport the phosphate released by hydrolysis into the cell. The red alga *Corallina elongata* is apparently the only organism reported to show higher activity in the dark than under low light flux [12]. It was suggested that phosphatase activity may help fulfill P requirements in this organism when direct uptake of inorganic P is reduced under conditions of limited reducing power. This might apply to the endolithic community, though this would not fit with the interpretation in [23] that photosynthetic metabolism in the endolithic communities studied showed no sign of nutrient limitation (including P limita-

tion). Possibly the surface phosphatase(s) in the endolithic communities undergoes some form of change on transfer from dark to light. The minimal input of P into the endolithic ecosystem from meltwater and aeolian dust makes the recycling of organic P very important for the transient metabolism of endolithic microbial communities. Any factor that affects the metabolism of this element, which is crucial for biomolecules associated with energy exchange and genetic processes at the limits of survival, is of great importance.

These studies have been made under conditions (shaking, dilution with medium) likely to enhance greatly the rates of activity occurring in nature. In addition it is unclear how well the hydrolysis of MUP simulates that of the substrates present *in situ*. We also do not know whether organic phosphates are more likely to be available at very low concentrations for much of the time or whether growing organisms depend on higher concentrations supplied in pulses, though fungal hyphae may aid the lichenized *Trebouxia* to mobilize nutrients from scattered microzones of microbial breakdown.

The following assessment of the relevance of phosphatase activities for growth of the *Chroococcidiopsis*-dominated community *in situ* is presented as a guideline for future researchers. Suppose that the chlorophyll content of the cyanobacterium is 1.8% dry weight and that any contribution to PMEase activity from heterotrophic organisms is ignored. Then, based on the chlorophyll *a* content (3.4 μg g^{-1} rock) and the values for PMEase activity in Table 3, 1 mg cyanobacterium releases approx. 3 μg P h^{-1} from 100 μM substrate. Assuming P is and remains at 1% dry weight (based on other studies of cyanobacteria) [25], it would take about 30 h of metabolic activity at 5°C for the cyanobacterium to obtain enough P to double in mass. As there is an approximately linear relationship between activity and substrate concentration over the lower part of the concentration range, a 10-fold decrease in substrate concentration would lead to a 10-fold increase in the time required. In addition, phosphodiesters, which were hydrolyzed at about half the rate of phosphomonoesters (Fig. 6), constitute an important component of cell P, thus also increasing the time required. In view of the spasmodic activity of these communities during very transitory periods of rehydration, this activity may be sufficient to support the phosphorus metabolism necessary to accompany the carbon and nitrogen metabolism of these remarkable slow-growing communities.

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References

1. Broady PA (1996) Diversity distribution of Antarctic terrestrial algae. Biodiversity and Conservation 5:1307–1335
2. Coston S, Holt SJ (1958) Kinetics of aerial oxidation of indolyl and some of its halogen derivatives. Proc Royal Soc B 148:506–510
3. Eisenreich SJ, Bannerman RT, Armstrong DE (1975) A simplified phosphorus analysis technique. Environ Lett 9:43–53
4. Friedmann EI (1978) Melting snow in the dry valleys is a source of water for endolithic microorganisms. Antarctic J US 13:162–163
5. Friedmann EI (1982) Endolithic microorganisms in the Antarctic cold deserts. Science 215:1045–1053
6. Friedmann EI, Hua M, Ocampo-Friedmann R (1988) Cryptoendolithic lichen and cyanobacterial communities of the Ross Desert Antarctica. Polarforschung 58:251–259
7. Friedmann EI, Kappen L, Meyer MA, Nienow JA (1993) Long term productivity in the cryptoendolithic microbial community of the Ross Desert Antarctica. Microb Ecol 25:51–69
8. Friedmann EI, McKay CP, Nienow JA (1987) The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Satellite-transmitted continuous nanoclimate data, 1984 to 1986. Polar Biol 7:273–287
9. Friedmann EI, Ocampo-Friedmann R (1976) Endolithic blue-green algae in the dry valley: Primary producers in the Antarctic desert ecosystem. Science 193:1247–1249
10. Friedmann EI, Ocampo-Friedmann R (1984) Endolithic microorganisms in extreme dry environments; analysis of a lithobiontic habitat. In: Klug MJ, Reddy CA (eds) Current Perspectives in Microbiology. Amer Soc Microbiol, Washington DC, pp 177–185
11. Friedmann EI, Weed R (1987) Microbial trace fossil formation, biogenous and abiotic weathering in Antarctic cold desert. Science 236:703–705
12. Hernández I, Niell FX, Fernández JA (1996) Alkaline phosphatase activity of the red alga *Corallina elongata* Ellis et Solander. Sci. Mar. 60:297–306

13. Hernández I, Niell FX, Whitton BA (2000) Phosphatase activity of benthic marine algae: An overview. In: Whitton BA, Hernández I, (eds) Phosphatases in the Environment. Kluwer, Dordrecht, in press
14. Islam MR, Whitton BA (1992) Phosphorus content and phosphatase activity of the deepwater rice-field cyanobacterium (blue-green alga) *Calothrix* D764. Microbios 69:7–16
15. Johnston CG, Vestal JR (1991) Photosynthetic carbon incorporation and turnover in Antarctic cryptoendolithic microbial communities: Are they the slowest growing communities on earth? Appl Environ Microbiology 57:2308–2311
16. Mahasneh IA, Grainger SLJ, Whitton BA (1990) Influence of salinity on hair formation and phosphatase activity of the blue green alga (cyanobacterium) *Calothrix viguieri* D253. Brit Phycol J 25:25–32
17. Marker AFH (1995) Chlorophyll Analysis: Standard Methods. National Rivers Authority, Bristol, UK
18. McComb RB, Bower GN, Posen S (1979) Alkaline Phosphatase. Plenum Press, New York
19. Nienow JA, Friedmann EI (1993) Terrestrial lithophytic (rock) communities. In: Friedmann EI (ed) Antarctic Microbiology. Wiley-Liss, New York, pp 353–412
20. Nienow JA, McKay CP, Friedmann EI (1988a) The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Mathematical models of the thermal regime. Microb Ecol 16:253–270
21. Nienow JA, McKay CP, Friedmann EI (1988b) The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Light in the photosynthetically active region. Microb Ecol 16:271–289
22. Roser BP, Pyne AR (1989) Whole rock geochemistry. In: Barrett PJ (ed) Antarctic Cenozoic History from the CIROS-1 Drillhole, McMurdo Sound. DSIR Bulletin 245:175–184 (DSIR, Wellington, New Zealand)
23. Vestal JR, Friedmann EI (1982) In situ carbon metabolism by the cryptoendolithic microbial community in the antarctic cold desert. Antarct J US 17:190–191
24. White DC, Davis CWM, Nickels JS, King JD, Bobbie RJ (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40:51–62
25. Whitton BA (1992) Diversity, ecology and taxonomy of the cyanobacteria. In: Carr NG, Mann NH (eds) Photosynthetic Prokaryotes. Plenum Press, pp 1–51
26. Whitton BA, Grainger SLJ, Hawley GRW, Simon JW (1991) Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb Ecol 21:85–98
27. Wynn-Williams DD (1996) Antarctic microbial diversity: The basis of polar ecosystem processes. Biodiversity and Conservation 5:1271–1293
28. Wynn-Williams DD (2000) Cyanobacteria in deserts—Life at the limits. In: Whitton BA, Potts M (eds) Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer, Dordrecht. pp 341–366
29. Wynn-Williams DD, Russell NC, Edward HGM (1997) Moisture and habitat structure as regulators for microalgal colonists in diverse Antarctic terrestrial habitats. In: Lyon WB, Howard-Williams C, Hawes I (eds) Ecosystem Processes in Antarctic Ice-free Landscapes. Balkema, Rotterdam, pp 77–88