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3.2 Forms of Nitrogen in Beacon Sandstone Rocks Containing Endolithic Microbial Communities in Southern Victoria Land, Antarctica

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Summary: The forms and amounts of inorganic and organic nitrogen in endolithic sandstone rocks have been determined. Of the total organic nitrogen in these rocks, 70—80%) is contained in amino acid and hexosamines, but of this only 14 and 21% could be apportioned to live biomass amino acid and biomass hexosamine nitrogen respectively, derived from a direct count method. Biomass estimates in the surface (0—15 mm) rock layers did not exceed 10 g m $^{-2}$, considerably lower than earlier estimates. Reasons for these differences are discussed. Only small amounts of mineral nitrogen were produced from incubated rocks and it is suggested that this is due to a large pre-existing pool of abiotically-derived mineral nitrogen (which also reduces biological nitrogen fixation) and the low moisture content of rocks (0.45%).

Zusammenfassung: Form und Mengen anorganisch oder organisch gebundenen Stickstoffs wurden in Sandsteinen mit Endolithen bestimmt. Aminosäuren und Hexoamine bilden 70—80% des gesamten organischen Stickstoffs, hiervon sind 14—21% lebender Biomasse zuzuschreiben (direkte Zählmethode). Unsere Biomassebestimmungen in den obersten 15 mm Gestein ergaben höchstens 10 g m $^{-2}$, also viel geringere Werte als bisher angenommen. Die Gründe hierfür werden diskutiert. Inkubiertes Gestein produzierte nur geringe Mengen an mineralischem Stickstoff. Vermutlich stammt dieser aus größeren schon länger existierenden Vorräten von abiotischen Stickstoff (wodurch die biologische Stickstoffproduktion vermindert wird). Ferner ist dies wohl auch auf den geringen Wassergehalt (0.45%) des Gesteins zurückzuführen.

1. INTRODUCTION

Although visible communities of lower plants occur in sheltered ice-free areas in many lowland regions of the Ross Dependency, in the higher inland regions there occur below the surface of sandstone rocks cryptoendolithic lichens accompanied by non-lichenised algae, fungi and bacteria (FRIEDMANN & FRIEDMANN 1984). The development of these subsurface communities can be such that their presence is manifested by the occasional emergent lichen apothecia (FRIEDMANN 1982) and fungal rhizines (WYNN-WILLIAMS 1986) as well as the shedding of surface rock layers which expose the layered communities.

Some aspects of the biology of these endolithic organisms e.g. gas exchange, microclimate, nitrogen fixation, and biomass, have been investigated, often in situ, in efforts to understand the functioning of these communities (FRIEDMANN et al. 1980, FRIEDMANN & KIBLER 1980, KAPPEN & FRIEDMANN 1983, MCKAY & FRIEDMANN 1985). The aims of the present study were threefold: a) to determine the amounts and forms of nitrogen in rocks using highly sensitive nitrogen analytical techniques; b) to apportion the chemically determined forms of nitrogen into biomass and to compare these latter values with biomass determined by a direct count procedure; c) to estimate the capacity of incubated colonised rocks to produce mineral nitrogen.

2. MATERIALS AND METHODS

The study area (200 m^2) is a stony pavement 2000 m alt., situated between East and West Beacon at 77°50'S and 160°48'E. Varnished dolerite and sandstone rocks cover 90% of the site with the remainder occupied by a gravelly ahumic soil derived from weathered rocks.

A sterilised stainless steel hammer and chisel or dentists drill were used to obtain surface 0—4 mm and 5—15 mm deep horizons from Beacon sandstone.

The 0—4 mm depth rock samples contained narrow black and broader white (showing occasional pink patches) bands of predominantly fungal material whilst the 5—15 mm layers contained an upper broad green band and a lower thin yellow band of algae and/or cyanobacteria. Samples were placed in sterile bags and stored in the dark

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0° C until further analysis. Sterile plastic gloves were used whilst handling rocks and subsamples not only to reduce microbial contamination but to eliminate the transfer of organic nitrogenous substances from the hand to the samples. Collections were made in 1980—1981, 1982—1983, 1984—1985. Two types of sandstones occurred in the study area. The weakly cemented, soft porous type being more heavily colonised than the strongly cemented or hard silicified type. The majority of the rock collected consisted of the soft types (90:10 weight basis).

Rock horizon material apparently devoid of endolithic organisms as determined by eye and fracturing was collected for experimental controls.

Hereafter endolithic rock subsamples are referred to as colonised samples whilst rocks devoid of organisms are referred to as control samples. Subsamples of colonised and control samples were kept intact in sealed containers for determination of density and water loss/gain (a, b, c, d). Other samples were crushed in a sterile mortar and pestle in a laminar flow cabinet and stored in sterile plastic bags at 2° C in the dark. The following methods of analysis were applied to all rock samples.

2.1 *Physical determination*

a) Dry weights — large samples (100—500 g) were heated for 24 hrs at 105° C, cooled in a desiccator and reweighed.

b) Bulk density, particle density and total porosity. Rock subsamples of known dimensions 80—200 g fresh weight for analysis by the procedures given by (BLAKE 1965) and (VOMOCIL 1965) were used. Based on a bulk density of 2.38 g/cm^{-3} it is calculated that one metre square of rock to depths of 4 mm and 15 mm weigh 9.52 and 35.7 kg respectively. Subsequent results are presented on a metre square per depth basis.

c) Water loss and gain. Oven dried (105° C) rocks (10—100 g dry wt) were satured with water by vacuum or boiling, lightly blotted, weighed, and held at 2° C (in a refrigerator) and on a lab bench 20° C for 24 hrs and then reweighed.

d) Loss on ignition was determined by heating rock samples (20—510 g dry wt) for 5 h at 550° C, cooling in a desiccator and reweighing.

2.2 *Biological determinations*

e) Microbial biomass. 2 gm samples of field-moist crushed rock were ultrasonicated by the procedure described by JENKINSON et al. (1976), modified by the use of 10 ml of agar detergent solution and ultrasonication for 60 secs by a probe at full power (300 w). The resultant agar films were stained with phenol aniline blue and mounted as described by (JENKINSON et al. 1976). Stained organisms were counted by bright field microscopy. Four slides were prepared from each of 12 samples (9 colonised and 3 control) and 10 fields of view counted per slide. Only stained microbes with distinct outlines were counted. There was great variation in the size classes of bacteria (0.3 µm diam. — 2 µm x 4 µm). For biomass estimates all bacterial cells were assumed to be spherical with a diameter of 1 µm. Similarly algae were assumed to be spherical with a mean diameter of 8 µm (± 5.0 µm) and fungal hyphal cylindrical with an average hyphal diameter of 1.8 µm (± 0.4 µm). The size approximations used in this study probably result in maximal biovolumes and when used in conjunction with a density of 1.1 g wet weight cm^{-3} (JENKINSON et al. 1976), and dry wt of 20%, they therefore represent maximum biomass values. Black fungal spores, 3 µm diameter, which did not take up the stain were assumed to be spheres and have been included in fungal biomass.

f) Nitrogen fixation. Acetylene reduction and ^{15}N methods were used consecutively on the samples to detect nitrogen fixation in the field. Rock material, 5—20 g fresh weight, was placed in 40 ml serum bottles fitted with rubber seals. Air was replaced with acetylene to give a 10% concentration. Incubations were performed under prevailing field conditions for no longer than 10 hours. Ethylene production was determined using a portable gas chromatograph (DAVEY 1982). Controls to determine endogenous etyhlene production and to monitor trace levels in the acetylene gas bottle were included.

After 10 hours the bottles were opened and flushed with air for 3 hours. Then 0.1 ml sterile water was added to replace losses due to evaporation and the rubber seals replaced. After removing 20 ml of air, 20 ml of ^{15}N gas

(99% enriched) was injected and the bottles incubated in situ for up to 5 days. They were then frozen, returned to New Zealand and digested by the Kjeldahl procedure (see below), modified to include nitrates and fixed ammonium. The resulting distillates were examined for increased ^{15}N abundance levels over and above those natural levels occurring in control rocks which were incubated in ^{15}N free atmosphere.

2.3 *Chemical determinations*
The terminology for the forms of nitrogen investigated follows that given in PAGE (1982).

g) Mineral (exchangeable) nitrogen. 500 g crushed samples were shaken 24 hr with 300 ml 2N KCl at 20° C and filtered through glass fibre filter paper (GF/A). The pH of water extracted samples was determined. Mineral NH_4-N and NO_2-N plus NO_3-N in filtrates were determined on 20 ml aliquots using steam distillation methods employing MgO and Devarda's alloy as described by BREMNER (1965).

h) Fixed (non-exchangeable) NH_4-N. The air-dried residues from (g) were subjected to the procedures described by BREMNER (1965). This involves removal of organic nitrogen with NaOBr followed by HF digestion to release fixed NH_4-N which was determined by steam distillation.

i) Total Nitrogen. 5—10 g samples of crushed rock were digested for 4 hours within the presence of thiosulphate and a Hg catalyst with a salt: acid ratio of 0.7 after an HF pretreatment to release fixed NH_4-N (BREMNER & MULVANEY 1982).

j) Nitrogen distribution analysis. Large samples (150—200 g) were analysed by the procedure described by BREMNER (1965). Air dried residues from (g) were refluxed for 24 hours at 110° C with 200 mls 6N HCl. The mixtures were cooled and filtered and the insoluble residues washed with water, oven dried and total nitrogen determinations performed, as given in (i). The filtrate and washings were reduced in volume in a rotary evaporator and neutralised to pH 6.5 and diluted to 100 ml. Thereafter forms of nitrogen in the hydrolysate were analysed exactly as described by BREMNER (1965). Hexosamine-N values were corrected for small losses caused by decomposition during hydrolysis.

k) Nitrogen mobilisation. 200—300 g crushed rock were dispensed in triplicate into Roux bottles to give a depth of approximately 1 cm. The samples were moistened to 6—11% (dry wt) by the additions of distilled water, and the flask necks were loosely covered with polythene. The bottles were weighed and incubated at 12 and 20° C for up to 42 days. Negligible water loss occurred over this period. After 42 days flasks were removed for mineral-N determination.

l) Snow nitrogen. Freshly fallen and old snow lying on rock surfaces at the study site near East Beacon was collected using a steel spatula and melted in glass vessels to give 5 litres of water. This water was acidified (pH 5) and evaporated down to 100 ml under a low flame and aliquots analysed for mineral-N by steam distillation in a field laboratory at Cape Bird, Ross Island three days after collection.

All determinations were duplicated and results are expressed on an oven dry basis. Throughout this work double distilled, deionised water was employed and manipulations performed in a sterile laminar flow cabinet.

3. RESULTS

Some physico-chemical properties of colonised and control rocks are given in Table 1. Not only colonised samples containing visible organic matter (LOI data) but also control rocks lost weight on pyrolysis. The weight loss of controls can possibly be ascribed to crystal bound water (GRIM 1968) as they only contained trace quantities of organic nitrogen. Colonised samples probably lost a similar amount of crystal-bound water.

Table 2 shows the amounts of total nitrogen, exchangeable mineral nitrogen and non-exchangeable ammonium nitrogen in colonised and control samples. Although non-exchangeable NH_4-N has been detected in rocks and soils from temperate and tropical regions (STEVENSON 1982), this is believed to be the first report for this form of NH_4-N in rocks from Antarctica. Considerably more total N and organic nitrogen was detected in colonised

		n	Colonised Mean	Range	Control Mean	Range
Moisture	%	(50)	0.41	0.13–1.12	0.11	0.60–0.40
Loss on ignition	%	(50)	0.38	0.29–0.46	0.19	0.11–0.31
Particle Density	g/cm^3	(30)	2.57	2.56–2.59	2.59	2.56–2.62
Bulk Density	g/cm^3	(30)	2.38	2.28–2.44	2.38	2.31–2.42
pH		(24)	4.8	4.6 –5.1	4.4	4.3 –4.7
Total porosity	%	(20)	7	6–7	6	5–7
Total N	%	(90)	0.0093	0.0087–0.0098	0.0013	0.0009–0.0016

Tab. 1: Physico-chemical properties of the 0–15 mm horizon of Beacon sandstone colonised and contol rocks. Figures in parenthesis refer to number of samples.

		Colonised 0–15 mm depth Mean	Range	0–4 mm depth Mean	Range	5–15 mm by difference	Control 0–15 mm Mean	Range
Total N	(30)	3320.1	3205–3511	1561.3	1387–1649	1758.8	464.1	350–517
KCl exchangeable NH_4–N	(20)	63.7	58–66	22.5	18–35	41.2	89.9	68–94
KCl exchangeable NO_3–N	(20)	79.7	71–83	7.9	2–17	71.9	31.8	8–42
Non-exchangeable NH_4–N	(15)	312.4	290–326	61.9	52–68	250.3	342.4	310–373
Organic nitrogen		2864.3		1469		1395.4	trace	

Tab. 2: Total, exchangeable and non-exchangeable nitrogen in colonised and contol Beacon sandstone samples (mg N m^{-2}). Organic nitrogen = Total N minus (exchangeable N + non-exchangeable N).

0—15 mm depth samples than in control rocks. In the latter, non-exchangeable NH_4-N constituted the bulk of the nitrogen and NO_3-N formed a smaller portion of the total exchangeable mineral-N. However, in colonised 0—15 mm samples NO_3-N was present in much larger amounts. The amounts of organic nitrogen in colonised rocks at 0—4 and 5—15 mm depths were similar, although NO_3-N and non-exchangeable NH_4-N were present in greater amounts in the 5—15 mm depth.

Fresh and old snow contained 118 and 437 µg mineral N per litre respectively with NH_4-N predominating (92%). Non-exchangeable NH_4-N becomes fractionated during the hydrolysis procedure to the extent that 70% of this fraction becomes soluble in acid whilst the remainder occurs in the acid insoluble residue (unpubl.). Accordingly, the values for organic forms of nitrogen released by acid hydrolysis in Table 3 have been corrected for this form of N. Negligible quantities of organic nitrogen were detected in the control rock samples. Of the large amounts detected in the colonised samples, appreciable quantities were present in the form of amino acids and hexosamines. It has been shown (GREENFIELD 1972, 1981) that approximately 50% of the nitrogen in the hydrolysable unidentified nitrogen (HUN) fraction is derived from non-α-amino-N or imino acids which do not react with ninhydrin e. g. arginine, proline. The destruction of some amino acids e. g. serine occurs to only a small extent and the NH_4-H values reported are derived mainly from the amides of aspartic and glumatic acid. The insoluble-N fraction consists in part of artifacts containing nitrogen which form between tryptophan and carbohydrates during the hydrolysis procedure. Porphyrins probably contribute only a small amount of nitrogen to this fraction. In the light of this it seems reasonable to place the total amino acid nitrogen values in Table 3 nearer 60—70% of the total organic nitrogen. In addition to the usual protein amino acids being detected by thin layer chromatography, small spots corresponding to the hexosamines glucosamine, galactosamine and muramic acid and the amino acid diamino pimelic acid were also detected.

Acetylene reduction was detected in only two cases out of 40 and at levels approaching the limits of detection. Since no increases in ^{15}N abundance levels over those in control samples were detected, it was concluded that biological nitrogen fixation did not occur in any of the colonised rock samples tested.

	0–15 mm		Colonised 0–4 mm		5–15 mm		Control 0–15 mm
NH_4–N	286	(10)	132	(9)	154	(11)	7
Hexosamine–N	344	(12)	220	(15)	124	(9)	0
α-amino-N	1433	(50)	706	(48)	729	(52)	<5
HUN **	515	(18)	264	(18)	251	(10)	0
Insoluble-N	286	(10)	147	(10)	139	(10)	0

Tab. 3: The distribution of organic nitrogeneous compounds in 0–15 mm depth samples from colonised and control Beacon sandstone rock. Results expressed in mgm m^{-2} with % of total organic nitrogen in parentheses. *By difference i.e. colonised 0–15 minus colonised 0–4. Values are means of 4 samples, each with a S.D. of 4%. **HUN = hydrolysable unidentified nitrogen.

The amounts of mineral nitrogen extracted from crushed rocks is given in Table 4 and have been corrected for mineral-N initially present in the samples before incubation. Levels of non-exchangeable NH_4-N were unchanged during the incubation. In temperate soils, this fraction is regarded as largely unavailable to organisms. In samples which were not pretreated in any way very low levels of mineral-N were observed after incubation and this nitrogen was predominately as NH_4-N. With increasing moisture contents, slightly more mineral-N (NH_4-N) was produced in samples not pretreated in any way although the trend was not consistent. Although considered to be an unrealistic temperature, the maximum amount of mineral-N produced occurred in samples incubated at 20° C for 28 days when 34 mg mineral-N per square metre was formed from organic substrates.

	Moisture (% dry wt)	Mineral-N %
First incubation series (20° C, 28d)		
Colonised rock, untrated (n=12)	0.45	0.3
	6.0	1.2
	11.0	0.9
Colonised rock, treated* (n=12)	0.45	0.2
	6.0	6.4
	11.0	6.1
Second incubation series (12° C, 42d)		
Colonised rock, untreated (n=8)	0.45	0.2
	6.0	0.7
	11.0	0.8
Colonised rock, treated** (n=8)	0.45	0.3
	6.0	6.2
	11.0	9.2
Colonised rock, treated* (n=6)	0.45	0.5
	6.0	9.6
	11.0	10.1
Control rock, untreated (n=4)	0.45	0.0
	6.0	0.0
Control rock, treated* (n=4)	0.45	0.0
	6.0	0.0

Tab. 4: Mineral nitrogen production in incubated 0–15 mm depth samples of colonised and control Beacon sandstone. Mean results expressed as % of total sample organic nitrogen. Moisture on dry wt basis. * = KCl extracted, water washed and air dried before incubation. ** = Water extracted, and air dried before incubation.

Pretreatment of colonised rock samples with KCl or water markedly increased mineral-N production at 6 and 11% moisture contents over the incubation periods regardless of temperature. This observation suggests, but direct support is lacking, that many indigenous organisms were not markedly affected by pretreatments. Conversely, pretreated samples held at 0.45% moisture levels produced very little mineral-N. In KCl extracted samples incubated at 12° C and 11% moisture, maximal amounts of mineral-N were produced corresponding to 289 mg mineral-N m^{-2}. With the exception of samples extracted with water before incubation and held at 12° C, in all other samples NH_4-N was the predominant form of mineral-N appearing. In the samples pretreated with water and incubated at 12° C, NO_3-N constituted 26, 15 and 10% of the total mineral-N found at the end of the incubation period. Subject to further testing (cf. FRIEDMANN & KIBLER 1980) this observation suggests that some of the endolithic microbes possess nitrifying capacity but that this ability is reduced in untreated samples because of large abiotically derived mineral-N levels. After 42 days incubation at 12° C no significant increases in mineral-N could be detected in control rock samples.

Estimates of microbial biomass in endolithic communities are given in Table 5. Fungi contributed most to the total biomass followed by algae with bacteria contributing only a very small fraction. No animals were detected in crushed rocks during microscopic examination. Microbes were detected in control rocks but the amounts were negligible e. g. <10 m fungal hyphae, <1000 algal and <10.000 bacterial cells g^{-1}. This observation is in agreement with only trace amounts of organic nitrogen compounds being detected in these rocks.

Colonised and control rocks did not exhibit any differences in their ability to absorb and retain water. Saturated rocks absorbed 5% (± 1%, n = 40) of their dry weights as water. Rocks in which interstitial air was not removed by boiling or vacuum when immersed in water for 1 and 24 hrs absorbed 1.7% (± 1%, n = 67) and 2.2% (± 1%, n = 52) respectively of their dry weights as water. Rocks lost their absorbed water within 24 hrs when kept at 20°

Rock horizon	Numbers/length g^{-1} rock		Biomass g m^{-2} (mean±SD)	
0–15 mm	fungal hyphae	452 m	9.03	(± 0.06)
	algal cells	244,315	0.51	(± 0.01)
	bacterial cells	3,442, 700	0.014	(± 0.002)
0–4 mm	fungal hyphae	1494 m	7.95	(± 0.04)
	algal cells	39,530	0.02	(±0.003)
	bacterial cells	2,110, 500	0.002	(±0.001)
5–15 mm	fungal hyphae		1.08	
(by difference)	algal cells		0.49	
	bacterial cells		0.012	

Tab. 5: Estimates of microbial biomass of endolithic communities (dry wt basis) determined by direct counting procedures on colonised rocks to a depth of 15 mm. 0–15 mm rock weighs 35.7 kg m^{-2} and 0–4 mm rock weighs 9.5 kg m^{-2}.

C on a laboratory bench or in a refrigerator at 2° C. Field moist rocks kept at 20° C or 2° C lost virtually all their water within 12 hours.

4. DISCUSSION

The endolithic environment has been extensively reviewed by FRIEDMANN & FRIEDMANN (1984) and it has been suggested that the standing biomass, estimated by indirect methods, is 32—177 g m^{-2}. The direct biomass estimates in this study are considerably lower than those suggested by FRIEDMANN et al. (1980) and KAPPEN & FRIEDMANN (1983) who used a variety of indirect methods. On close inspection their unmodified micro Kjeldahl methods were adjudged insufficiently sensitive to determine such low levels of nitrogen, including non-exchangeable ammonium in rocks. Furthermore these workers did not estimate nitrogen and "organic matter" in control rocks and could not take account of crystal lattice water in their samples which would tend to overestimate biomass. The biomass conversion factor, N x 25, used by FRIEDMANN et al. (1980) is not explained. In the present study, the direct counting procedure with all its attendant defects, produced biomass estimates of 10 g m^{-2} to a depth of 15 mm. VESTAL (1988) has recently produced biomass estimates for colonised rocks based on carbon metabolism which suggest that biomass may be of the order 0.5 g m^{-2}. Other workers (JENKINSON & LADD 1981, SCHNURER et al. 1985) who compared techniques for determining biomass have commented that vital staining procedures estimate as true biomass only a very small proportion of those cells counted using direct procedures. Shrinkage of cells during the preparation of films for microscopic analysis, which may cause underestimates in biovolume, was not examined in this study but losses of 20% have been reported by JENKINSON et al. (1976). Microscopic examination of sedimented sand grains produced during the dispersion procedure revealed the presence of microbial cells still attached to sand grains and cell debris. Despite probable overestimation of cell dimensions it is likely therefore that the method used in this study underestimates true biomass, although it was selected as a compromise to give maximum dispersion with minimum destruction of cells. Uncertainty exists as to which of the literature values for cell density and dry weight used in conversion of biovolume to biomass are most applicable.

In the present study the amounts and forms of nitrogen in sandstone rocks were estimated with more sensitive and extensive methods than was possible in earlier studies (FRIEDMANN et al. 1980, KAPPEN & FRIEDMANN 1983). Although the nitrogen chemistry of isolated endolithic microbes is not yet known it would be unusual to expect that their N contents were significantly different from the values reported in the literature for similar groups of microbes. The average nitrogen contents of fungi, algae and bateria are taken to be 4, 10 and 10% of the dry weight mass (see Tab. 7). Similarly, the hexosamine-N content has been found to be of the order 20, 5 and 10% of the total nitrogen content for a wide range of fungi, algae and bacteria respectively (GREENFIELD 1981 and unpubl.). These values can be used to calculate the likely amounts of total nitrogen and hexosamine-N in the biomass determined by the direct count method. Furthermore the derived biomass hexosamine-N value can be compared with the chemically determined hexosamine-N value.

Table 6 shows a large discrepancy between derived and experimental values with only 14% of the biomass derived organic nitrogen (0.411) and 21% (0.0721) of the biomass derived hexosamine-nitrogen being accounted for in direct chemical estimates. Several reasons can be advanced for this: a) biomass estimate obtained by direct counts are far too low; b) endolithic microbes have abnormally high hexosamine nitrogen and total nitrogen contents; c) large amounts of necromass and/or organic matter occur in rocks. If the direct biomass estimates in this study

Organism	Biomass*	Biomass-N**	Biomass hexosamine-N**
Fungi	9.03	0.36	0.07
Algae	0.51	0.05	0.002
Bacteria	0.01	0.001	0.0001
calculated total	9.55	0.411	0.0721
Analysed total	NA	2.864	0.344

Tab.6: Biomass and derived biomass nitrogen (Biomass-N) compared with direct chemical estimates (analysed total) of organic and hexosamine-N in endolithic organisms. Values in g m^{-2} per 15 mm. * = direct counts (see Tab. 5). ** = derived data assumes fungi, algae and bacteria have N contents of 4, 10 and 10% dry wt basis respectively with 20, 5 and 10% of N in the form of hexosamine-N. Analysed total = obtained by direct chemical analysis (see Tab. 3) NA = not applicable.

	Algae	Fungi	Lichens	Bacteria
NH_4-N	10	9	9	10
Hexosamine-N	2	21	10	3
α-amino-N	54	42	45	52
HUN	29	21	27	32
Insoluble	5	7	9	3

Tab. 7: Mean values for the nitrogen composition of microbial groups isolated from Antarctic soils and rocks. Values expressed as % of total microbial nitrogen. HUN = Hydrolysable unidentified nitrogen. Algae = *Chlamydomonas, Chlorella, Anabaena, Nostoc* (2 spp.) from Antarctic soils). Fungi = *Penicillium, Aspergillus, Humicola, Mortierella, Malbranchea* (from Antarctic soiles). Lichens = *Usnea, Xantoria, Buellia, Lecanora, Umbilicaria* (on Antarctic rocks). Bacteria = *Bacillus* spp. (2). *Rhodopseudomonas, Aerobacter, Azotobacter* (from Antarctic soils).

(9.5 g) are hugely inaccurate then from the chemically determined hexosamine-N values it can be calculated that impossibly huge biomvolumes (more than the total rock porosity) and therefore huge biomasses would be required to account for all the chemically determined hexosamine-N. The accuracy of the hexosamine determinations is supported by the quantitative recovery of the nitrogen in compounds such as chitin and glucosamine which were added to rocks undergoing the hydrolysis and fractionation procedure. It is not impossible that a considerable quantity of necromass (particularly as cell wall material) or organic matter occurs in rocks. This has been discussed for temperate soils (GREENFIELD 1981) and supported in the present study where 'ghost' hyphae were detected using phase contrast microscopy. Inspection of the loss on ignition value for oven dry colonised rocks in Table 1 indicates that there is 135 g of "organic material" in a square meter of these rocks but it is possible that a substantial part of this value represents crystal lattice water which is not removed at 105° C but is driven off at much higher temperatures (GARDNER 1965, GRIM 1968). There are 68 g of this lattice bound water in control rocks and therefore 67 g (135 minutes 68) would seem to be a more reasonable value for organic matter content in endolithic rocks. Since there is 10 g biomass included in the value of 67 g organic matter then at this stage it does not appear unreasonable to suggest that much nitrogen particularly in the form of hexosamines which occurs in microbial cells walls resides in a large necromass of organic matter pool. It has been shown (GREENFIELD 1981) that dead microbial cells are essentially devoid of N-rich proteinaceous cytoplasmic contents and thereofore contain a larger proportion of their total nitrogen in the form of cell wall hexosamines, more than with live cells. Although it constitutes a potentially rich source of nutrients including N, cell wall hexosamine may be difficult to decompose. Only very small amounts of mineral-N were produced from this source under optimum moisture and temperature conditions in the laboratory. These latter are unlike those in situ. It may be that the endolithic system is saturated with substantial amounts of mineral nitrogen (not including non-exchangeable NH_4-N) derived abiotically from snow fall (FRIEDMANN & KIBLER 1980), and either free dust or dust accumulation in old snow as found in this study, but also to a lesser extent from active enzymatic mineralisation. This suggestion would help to explain the absence of biological nitrogen fixation in the samples tested as the large amounts of existing mineral nitrogen would reduce the dependence of procaryotes on fixation processes. The low temperature drying experiment in this study using colonized and control rocks supports physiological and microclimatic observations (KAPPEN & FRIEDMANN 1983, MCKAY & FRIEDMANN 1985) which indicate that factors such as freeze/thaw events, limited light and short growing season all combine to preclude the generation of adequate energy for nitrogen fixation over and above that required for basic maintenance and some slight seasonal growth by the members of the endolithic community. It would also follow that if the biomass is low in these rocks and growth is sporadic and very much dependent on water and temperature, then only small amounts of mineral nitrogen are required to sustain this biomass. The rapid loss of absorbed water from rocks held at low temperature and humidity in this study together with the production of very small amounts of mineral nitrogen in pretreated or untreated rocks at low (0.45%) moisture levels is in keeping with the suggestion (MCKAY & FRIEDMANN 1985) that opportunities for sustained growth by endolithic microbes are infrequent in the field.

Although FRIEDMANN & KIBLER (1980) could not detect nitrifying organisms in endolithic rocks, the presence in this study (Tab. 2) of large amounts of nitrate in the 5—15 mm rock layers with the abundance of algae and bacteria in this zone suggests that nitrifiers might exist. The weakly acidic nature of endolithic rocks does not preclude nitrifiers for they have been detected in quite acid soils (WALKER & WICKRAMASINGHE 1979).

The % nitrogen distribution analyses in Table 3 for colonised rock layers bear similarities to those derived from pure cultures of Antarctic microbes (Tab. 7). This suggests that the microbial constituents of endolithic communities are similar in their overall biochemistry to their free living counterparts elsewhere.

The results reported here are in keeping with the suggestion that the endolithic system is largely a closed one (FRIEDMANN & FRIEDMANN 1984). The habitation space and the rapidly fluctuating environmental conditions together with the presence of a pool of mineral nitrogen, which may be largely derived from snowfall, exert fundamental effects on metabolism. That the endolithic organisms are successful is testified by their widespread occurrence in surface Beacon sandstones. Although growth conditions may be limiting, the biological rate of weathering of these rocks (exfoliation) far exceeds the slow physico-chemical processes. Shed rock layers may be considered as inoculants and will contain a small proportion of their organic matter in the form of biomass but the large necromass content will provide an important source of preformed organic substrates (or starter energy rich substrates) which may be advantageous for developing heterotrophic micro-organisms.

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References

Blake, G. R. (1965): Particle and Bulk Density. — In: C. A. Black (ed.) Methods of Soil Analysis, Amer. Soc. Agronomy 9: 371—390. Madison, Wisconsin.

Bremner, J. M. (1965): Total Nitrogen, Inorganic Form of Nitrogen and Organic Forms of Nitrogen. — In: C. A. Black (ed.) Methods of Soil Analysis, Amer. Soc. Agronomy 9: 1149—1254 Madison, Wisconsin.

Bremner, J. M. & Mulvaney, C. S. (1982): Nitrogen Total. — In: A. L. Page (ed.) Methods of Soil Analysis Part 2, Second edition, Amer. Soc. Agronomy 9: 595—624 Madison, Wisconsin.

Davey, A. J. (1982): In situ determination of nitrogen fixation in Antarctica using a high sensitivity portable gas chromatograph. — Aust. J. Ecology 7: 395—402.

Friedmann, E. I. & Kibler, A. P. (1980): Nitrogen Economy of Endolithic Microbial Communities in Hot and Cold Deserts. — Microbial Ecology 6: 95—108.

Friedmann, E. I., LaRock, P. A. & Brunson, J. O. (1980): ATP, chlorophyll and organic nitrogen in endolithic microbial communities and adjacent soils in the dry valleys of Southern Victoria Land. — Ant. J. US. 15: 164—166.

Friedmann, E. I. (1982): Endolithic microorganisms in the Antarctic cold desert. — Science 215: 1045—1053

Friedmann, E. I. & Friedmann, R. O. (1984): Endolithic microorganisms in Extreme Dry Environments. — In: M. J. Klug & C. A. Reddy (eds.) Current Perspectives in Microbial Ecology, Analysis of a Lithobiontic Microbial Habitat: 175—185.

Gardner, N. H. (1965): Water Content. — In: C. A. Black (ed.) Methods of Soil Analysis, Amer. Soc. Agronomy 9: 82—127 Madison, Wisconsin.

Greenfield, L. G. (1972): The nature of the organic nitrogen of soils. — Plant and Soil 36: 191—198.

Greenfield, L. G. (1981): A re-examination of some Arid Indian soils for hexosamine-N content and the origin of hexosamines in Mull and Mor soils. — Mauri Ora 9: 83—95.

Grim, R. E. (1968): Clay Mineralogy. — McGraw-Hill Inc.

Jenkinson, D. S., Powlson, D. S. & Wedderburn, R. M. (1976): The Effects of Biocidal Treatments on Metabolism in Soil III. The Relationship between soil biovolume, Measured by Optical Microscopy, and the Flush of decomposition caused by Fumigation. — Soil Biology and Biochemistry 8: 189—202.

Jenkinson, D. S. & Ladd, J. N. (1981): Microbial Biomass in Soil: Measurement and Turnover. — In: E. A. Paul & J. N. Ladd (eds.) Soil Biochemistry, Vol. 5: 415—471, Marcel Dekker Inc.

Kappen, L. & Friedmann, E. I. (1983): Ecophysiology of Lichens in the Dry Valleys of Southern Victoria Land, Antarctica II CO_2 Gas Exchange in Cryptoendolithic Lichens. — Polar Biol. 1: 227—232.

McKay, C. P. & Friedmann, E. I. (1985): The Cryptoendolithic Microbial Environment in the Antarctic. Cold Desert: Temperature Variations in Nature. — Polar Biol. 4: 19—25.

Page, A. L. (1982): Methods of Soil Analysis Part 2, Second edition. Amer. Soc. Agronomy 9, Madison Wisconsin.

Schnürer, J., Clarholm, M. & Rosswall, T. (1985): Microbial Biomass and activity in an agricultural soil with different organic matter contents. — Soil Biol. Biochem. 17: 611—618.

Stevenson, F. J. (1982): Nitrogen in Agricultural Soils. — Amer. Soc. Agronomy 22, Madison, Wisconsin.

Vestal, J. R. (1988): Carbon metabolism in the cryptoendolithic microbiota. — Polarforschung 58.

Vomocil, J. A. (1965): Porosity. — In: C. A. Black (ed.) Methods of Soil Analysis, Amer. Soc. Agronomy 9: 299—314 Madison, Wisconsin.

Walker, N. & Wickramasinghe, K. N. (1979): Nitrification and autotrophic nitrifying bacteria in acid tea soils. — Soil Biol. Biochem. 11: 231—236

Wynn-Williams, D. D. (1986): Microbial Colonization of Antartic Fellfield Soils, 191—200. In: F. Megusar (ed.), Proceedings of the Fourth International Symposium Microbial Ecology, Ljublana.