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Nitrogen Economy of Endolithic Microbial Communities in Hot and Cold Deserts

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Abstract. The source of combined nitrogen in endolithic microbial communities was studied in samples from desert localities in North and South America, the Middle East, South Africa, and Antarctica. Nitrogen fixation (acetylene reduction) seems to occur only exceptionally. Evidence suggests that, in general, the nitrogen source for endolithic microorganisms in deserts is abiotically fixed nitrogen produced by atmospheric electric discharges (lightning or aurorae), conveyed to the rock by atmospheric precipitation. Nitrogen is apparently not a limiting factor in these low-productivity communities. An incomplete nitrogen cycle seems to be present which includes the following pathways: supply of nitrates and ammonia from the atmosphere; decomposition of organic matter to ammonia; reassimilation of ammonia; ammonia volatilization; loss of organic matter through weathering (only in certain Antarctic rocks); biological nitrogen fixation (exceptional).

Introduction

Endolithic microbial communities have been described from hot deserts by Friedmann, Lipkin, and Ocampo-Paus (20), Friedmann (15, 16), and Friedmann and Galun (19); and from the cold deserts of the Antartctic dry valleys by Friedmann and Ocampo (21) and Friedmann (17, 22). The microorganisms are separated from the outside environment by living under the rock surface. Those existing in the cracks of the rocks are termed chasmoendolithic whereas the organisms occupying the microscopic air spaces between the grains or porous rocks are called cryptoendolithic (Golubic, Friedmann, and Schneider, in preparation). An example (from the Antarctic dry valleys) is shown in Fig. 1. In hot deserts, endolithic organisms are nearly exclusively blue-green algae (cyanobacteria) accompanied by colorless bacteria. In the Antarctic dry valleys, the dominant organisms are endolithic lichens and blue-green algae occur only infrequently. Both lichens and blue-green algae are associated with colorless bacteria.

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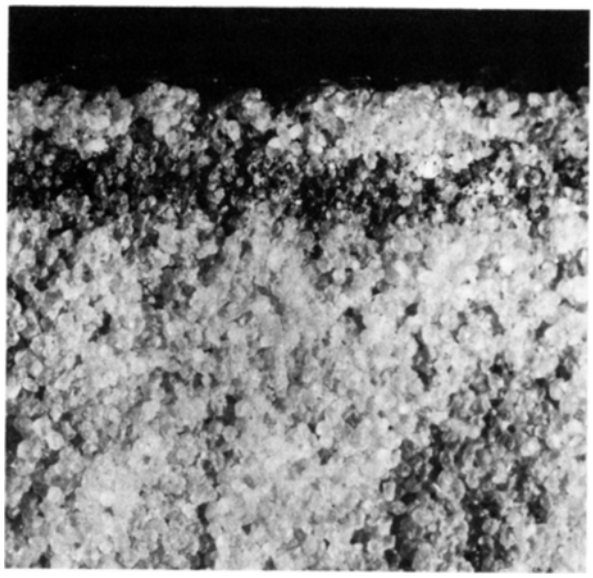

Fig. 1. Vertically fractured rock (Beacon sandstone, orthoquartzite) showing dark zone of endolithic cyanobacteria under the surface. University Valley, southern Victoria Land, Antarctica. ×4

The extreme nature of the endolithic desert environment and the scarcity of plant and animal life raises questions about the possible source of combined nitrogen in these communities. The recent reviews of Rychert and Skujins (39, 40) and Sorenson and Porcella (42) demonstrate convincingly that in hot desert soils, in tundras, as well as in rock outcrops in humid areas, biological nitrogen fixation by microorganisms such as blue-green algae and free-living bacteria is a major source of combined nitrogen. The existence of nitrogen-fixing microorganisms has been reported from Arctic and Antarctic localities (2–5, 7–11, 14, 25, 26, 29). One would expect nitrogen fixation to be the source of combined nitrogen also in endolithic communities. Nitrogen-fixing activity was therefore tested in a variety of rock samples containing cryptoendolithic or chasmoendolithic microorganisms. In addition, rock samples were analyzed chemically for the presence of NO_3^-, NO_2^-, and NH_4^+ and tested for the presence of nitrogen-fixing and nitrifying bacteria.

Materials

Sample Collection

Rock and soil samples were examined from 46 localities, 30 in the polar desert of the dry valleys of southern Victoria Land, Antarctica, and 16 in different hot desert areas of the world. Thirty-eight rock samples contained visible cryptoendolithic or chasmoendolithic microbial growth. Four rock samples without visible microbial growth and four Antarctic soil samples were included for purposes of comparison. The samples were collected by the following: Antarctica—E. I. Friedmann and J. Brunson, 1976–1978; Death Valley—E. I. Friedmann and Dr. D. A. Warnke, 1970; Mexico—E. I. Friedmann and Dr. R. Ocampo-Friedmann, 1971; Chile—Mr. S. Cummings, 1974; South Africa—Dr. M. K. Seely, 1974; Sinai—E. I. Friedmann, 1973–1974; Utah and Arizona—E. I. Friedmann and Dr. R. Ocampo-Friedmann, 1977. Collection sites, substrates, and growth type of microorganisms are listed in Table 1.

Samples used to investigate the presence of Antarctic nitrogen-fixing bacteria were collected by E. I. Friedmann and A. P. Kibler in December 1978. Collection sites, substrates, and growth type of microorganisms are listed in Table 2.

Methods

Storage of Samples

Samples were collected in sterile polyethylene bags. Rocks and soils from Antarctica were maintained at 0°C (A767 series) or −20°C (A778 series) in the Antarctic Research Facility at Florida State University. Hot desert rock samples were stored at room temperature.

Nitrogen Fixation

Nitrogenase activity was determined by the acetylene reduction assay technique (23). Rock samples were fractured and aliquots transferred to 35 ml serum bottles under sterile conditions. Samples were moistened with a dilute (1:5) nitrogen-free modification of Kratz and Myers' Medium D [in 1000 ml H_2O: 0.05 g EDTA, 0.15 g $MgSO_4 \cdot 7\ H_2O$, 0.8 g K_2HPO_4, 0.004 g $Fe_2(SO_4)_3 \cdot 6\ H_2O$, 0.005 g $CaCl_2$, 0.016 g Na_2HPO_4, and 1 ml micronutrient solution containing in 1000 ml: 8.82 g $ZnSO_4 \cdot 7\ H_2O$, 0.71 g MoO_3, 1.57 g $CuSO_4 \cdot 5\ H_2O$, and 0.40 g $CoCl_2 \cdot 6\ H_2O$ (personal communication by R. Ocampo-Friedmann]. Cotton-plugged samples were incubated for 3 to 4 weeks at 34°C (hot desert samples) or at 15°C (Antarctic material) on a 16:8 hour light-dark cycle. Bottles were sealed with rubber stoppers and the gas phase then replaced with 0.3% CO_2, 20.7% O_2, and 79% Ar for aerobic incubation, or 0.038% CO_2, 99.96% Ar for microaerophilic nitrogen fixation (strict anaerobic conditions were not realized as illumination during incubation resulted in photosynthesis and oxygen evolution). Replicates of all samples were also tested without prior atmosphere exchange since field studies showed that exchange of atmosphere is unnecessary when the atmospheric concentration is greater than 10% (43). There was no significant difference between results obtained from experiments using a standard aerobic gas phase and those in which no gas exchange was employed. Controls with liquid cultures of the nitrogen-fixing blue-green alga *Fischerella muscicola* were run simultaneously. All tests were carried out in duplicate. Acetylene (commercial grade purified by H_2O and H_2SO_4 bubble traps) was injected (between 10 and 20%) and samples incubated at 15°C and 34°C (cold and hot desert forms, respectively). Ethylene production was determined after 2 hours and subsequently every 24 hours for 4 days using a Hewlett-Packard 5710A gas chromatograph set for dual column operation with two 2 m stainless steel columns packed with Poropak N (Water Assoc. Inc., Framingham, Mass.). The injector port was set at 100°C and the oven temperature was 60°C. The carrier gas was nitrogen with a flow rate of 20 ml/min.

Nitrogen-Fixing and Nitrifying Bacteria

One to three gram aliquots of rock samples with macroscopically visible microbial growth were fractured under sterile conditions and then enriched for nitrogen-fixing bacteria in liquid Modified Burk medium (44) and in Johnstone medium (1). Incubation was at 15°C (Antarctic samples) or 34°C (hot desert samples) for 2 weeks, illuminated by white incandescent lamps on a 16:8 hour light cycle. Visible bacterial growth was transferred into fresh medium and incubated under similar conditions to test the potential of the organism to grow in the absence of combined nitrogen.

The method for testing for the presence of nitrifying bacteria was similar. Samples were incubated at room temperature and illuminated through a north-facing window in Meiklejohn's medium (32). The presence of nitrite in the supernatant was tested by the naphthylamine-sulfanilic acid spot test (13).

Table 1. Rock and soil samples studied at Florida State University

Sample No.	Collection site	Substrate	Growth type[a] and organisms
Antarctica, southern Victoria Land			
A767-5a	Olympus Range, N of Clark Glacier. Alt. 1000 m	Granite	Chasmo blue-green algae
A767-18	Asgard Range, Tyrol Valley, rock outcrop. Alt. 1625 m	Sandstone	Crypto. blue-green algae
A767-35	Victoria Valley, below Sponsor's Peak, small boulder wetted by meltwater. Alt. 1200 m	Sandstone	Crypto. blue-green algae
A767-36	Olympus Range, Plateau N of Mt. Dido, large boulder. Alt. 1750 m	Sandstone	Crypto. lichen and blue-green algae
A767-41	Olympus Range, valley W of Mt. Cerberus. Alt. 1300 m	Granite	Chasmo. blue-green algae
A767-50	Mouth of University Valley,[b] small boulder saturated with meltwater. Alt. 1900 m	Sandstone	Crypto. lichen
A778-5	University Valley, cliff 400 m above valley floor. Alt. 2350 m	Sandstone	Crypto. lichen
A778-6	University Valley, 300 m above valley floor. Alt. 2250 m	Sandstone	Crypto. blue-green algae
A778-7	University Valley, adjacent to A778-6. Alt. 2250 m	Sandstone	Crypto. lichen
A778-13	University Valley, boulder on valley floor. Alt. 1950 m	Sandstone	Crypto. and chasmo. lichen
A778-14	University Valley, small boulder on valley floor. Alt. 1950 m	Sandstone	Crypto. and chasmo. lichen
A778-15	University Valley, boulder on valley floor. Alt. 1950 m	Sandstone	Crypto. and chasmo. lichen
A778-16	University Valley, small boulder. Alt. 1950 m	Sandstone	Crypto. lichen
A778-19	University Valley, small boulder. Alt. 1950 m	Sandstone	Crypto. lichen
A778-20	University Valley, at base of boulder, A778-21. Alt. 1950 m	Soil	No visible algal growth
A778-21	University Valley, small boulder. Alt. 1950 m	Sandstone	Crypto. lichen
A778-22	University Valley, small rock outcrop. Alt. 1950 m	Sandstone	Crypto. lichen
A778-24	University Valley, small boulder. Alt. 2015 m	Sandstone	Crypto. lichen
A778-25	University Valley, at base of cliffs facing E. Alt. 2250 m	Soil	No visible algal growth
A778-26	University Valley, cliffs facing E. Alt. 2250 m	Sandstone	Crypto. blue-green algae
A778-27	University Valley, small boulder at base of cliffs. Alt. 2250 m	Sandstone	Crypto. lichen and blue-green algae
A778-29	University Valley, boulder. Alt. 2216 m	Sandstone	Crypto. lichen and blue-green algae
A778-45	Beacon Valley, mouth of University Valley. Alt. 1900 m	Sandstone	Crypto. lichen

Table 1.—Continued

Sample No.	Collection site	Substrate	Growth type[a] and organisms
A778-57	Mountain between University Valley and Farnell Valley. Alt. 2400 m	Sandstone	Crypto. lichen
A778-61	Asgard Range, Linnaeus Terrace, rock table. Alt. 2350 m	Sandstone	Crypto. lichen
A778-62	Near Don Juan Pond, 1 m above water level. Alt. 120 m	Soil	No visible algal growth
A778-66	Gneiss Point, small boulder. Alt. 50 m	Marble	Crypto. blue-green algae
A778-69	Gneiss Point, small outcrop. Alt. 50 m	Marble	Crypto. blue-green algae
A778-71	Gneiss Point, green-colored soil. Alt. 50 m	Soil	No visible algal growth
Middle East			
Si734-5a	Sinai Desert, Mt. Sinai. Alt. 2200 m	Granite	No visible algal growth
Si734-12	Sinai Desert, 134 km S of Elat on the road between Dahab and Nuweiba. Alt. 300 m	Sandstone	Crypto. blue-green algae
Si734-13	Sinai Desert, coastal road 35 km S of Elat. Alt. 50 m	Granite	Chasmo. blue-green algae
Si734-14	Sinai Desert, coastal road 35 km S of Elat. Alt. 50 m	Granite	No visible algal growth
North America			
71-1	Sonoran Desert, Mexico, near Puerto Peñasco. Rock outcrop with desert varnish. Alt. 30 m	Granite	No visible algal growth
71-20	Northern Baja California, Mexico, on Highway 5, 50 km from Mexicali. Alt. 150 m	Granite	Chasmo. blue-green algae
70-6	Death Valley, California, at Scotty's Castle. Alt. 970 m	Breccia	Crypto. blue-green algae
70-6a	Death Valley, California, 1 km from Ubehebe Crater. Alt. 760 m	Breccia	No visible algal growth
U77-02	Utah, Route 128, 10 km S of Cisco. Alt. 1340 m	Sandstone	Crypto. blue-green algae
U77-07	Utah, Route 313, near Seven Mile Canyon. Alt. 1400 m	Sandstone	Crypto. blue-green algae
U77-09	Utah, Route 313, near Seven Mile Canyon. Alt. 1400 m	Sandstone	Crypto. blue-green algae
U77-17	Utah, NE foot of Henry Mts. Alt. 1500 m	Sandstone	Crypto. blue-green algae
U77-19	Utah, NE foot of Henry Mts. Ceiling of shallow cave. Alt. 1500 m	Sandstone	Crypto. blue-green algae
U77-28	Arizona, Virgin River Gorge, N of Santa Clara, vertical cliff. Alt. 800 m	Limestone	Chasmo. blue-green algae
South America			
74-1	Chile, Guanaqueros Peninsula at Punta La Zanuca, 100 m from sea. Alt. 10 m	Granite	Chasmo. blue-green algae

Table 1.—Continued

Sample No.	Collection site	Substrate	Growth type[a] and organisms
Africa			
Swa74-5a	Namibia (SW Africa), near Namib Desert Research Station, Gobabeb	Granite	Crypto. blue-green algae

[a]Chasmo., chasmoendolithic—organisms living in the cracks of rocks. Crypto., cryptoendolithic—organisms living in the microscopic air spaces between the grains of porous rocks.
[b]University Valley—the third lateral valley of Beacon Valley to the SE, 77° 52′ S, 160° 39′ E.
[c]Linnaeus Terrace NE of Oliver Peak, facing Wright Valley, 77° 36′ S, 161° 05′ E.

Qualitative Spot Test Analysis for NO_3-, NO_2-, *and* NH_4+

Samples were homogenized in a rock crusher. The diphenylbenzidine test (sensitivity 0.07 μg/g) was used for the detection of nitrates (13), the naphthylamine-sulfanilic acid test (sensitivity 0.010 μg/g), for nitrites and the phenol-hypochlorite test (sensitivity 0.10 μg/g) for ammonia (24). Semiquantitative estimates of a possible nitrate gradient were based on diphenylbenzidine spot test by placing single drops of the reagent on the fractured rock surface at intervals and by visually estimating the intensity of the color reaction.

Quantitative NO_3- *and* NH_4+ *Analysis of Rocks*

Rock samples were homogenized and duplicate aliquots of 5 to 15 g eluted in 20 ml of H_2O for 30 min. Nitrate was determined in the elute by the Szechrome method. This is based on two organic reagents, *Szechrome NAS*

Table 2. Samples tested for the presence of nitrogen-fixing bacteria at Eklund Biological Laboratory, McMurdo Station, Antarctica

Sample No.	Collection site	Substrate	Growth type and organisms
Antarctica, southern Victoria Land			
A789-2	Beacon Valley, at mouth of University Valley, boulder. Alt. 1900 m	Sandstone	Crypto. lichen and blue-green algae
A789-4	Beacon Valley, at mouth of University Valley, flat rock table. Alt. 1900 m	Sandstone	Crypto. lichen
A789-12	Beacon Valley, at mouth of University Valley. Alt. 1900 m	Sandstone	Crypto. blue-green algae
A789-27a	Olympus Range, Bull Pass, at base of granite boulder. Alt. 700 m	Soil	No visible algal growth
A789-30	Olympus Range, Bull Pass. Alt. 700 m	Soil	No visible algal growth
A789-45L	Olympus Range, top of Mt. Dido. Lower surface of rock table. Alt. 2070 m	Sandstone	Crypto. lichen
A789-45 v	Olympus Range, top of Mt. Dido. Upper surface of rock table. Alt. 2070 m	Sandstone	Crypto. lichen
A789-48	Marble Point, rock outcrop. Alt. 50 m	Marble	Crypto. blue-green algae
A789-49	Marble Point, rock outcrop. Alt. 50 m	Marble	Crypto. blue-green algae and green algae

and *Szechrome NB*.[1] These reagents produce blue or violet colors with nitrates. Szechrome NAS is 4-diphenylbenzidine-4-sulfonic acid and is used for samples in the range of 1–20 mg NO_3–/liter. Szechrome NB, diphenylbenzidine, is used in the range of 0.05–1.0 mg/liter. The NO_3– content was quantified colorimetrically with a ratio-recording spectrophotometer by comparing the color reaction of the sample to KNO_3 concentration standard curves. Ammonium was determined by the Nesslerization technique (sensitivity 2μg/g) according to the standard methods of the American Public Health Association (6).

NO_3– *Gradient in Rocks*

Samples from various depths in the rock were removed using a carbide drill. The nitrates were determined by the Szechrome method as described above.

Determination of NO_3– *and* NH_4+ *in Antarctic Snow*

Snow samples were collected in Beacon Valley, southern Victoria Land, Antarctica, on December 20, 1978, on sheets of aluminum foil. The samples were melted, membrane filtered, and analyzed for nitrate (6) using the cadmium-reduction technique and for ammonium by the phenol-hypochloride method (31). Colorimetric quantification was carried out by spectrophotometry in the Eklund Biological Laboratory at McMurdo Station, Antarctica.

Results

Acetylene Reduction in Rocks

Samples listed in Table 1 (with the exception of A767-5a, A767-18, A778-13, A778-25, A778-71, Si734-5a, and 70-6) were tested for nitrogen fixation. Among the 39 samples tested, after both aerobic and microaerophilic incubation, only one sample (A778-26) showed acetylene reduction. This was a sandstone from the Antarctic dry valleys containing a unicellular reddish blue-green alga and colorless bacteria. Of the other 38 samples, none showed acetylene reduction.

As nitrogenase synthesis (and thus nitrogenase activity) is inhibited by the presence of inorganic combined nitrogen compounds (NO_3–, NO_2–, and NH_4+), the failure to fix nitrogen may have been due to the presence of these ions in the substrate. In order to test this possibility, the acetylene reduction tests were repeated in all samples after removal of soluble salts. Aliquots of crushed rocks and soils were leached in triple glass distilled water four times for four consecutive 24 hour periods. Following leaching, the samples were incubated under both aerobic and microaerophilic conditions. In addition to A778-26, another sample, A778-18, showed acetylene reduction following leaching under both microaerophilic and aerobic incubation, but not before leaching. The rest of the samples failed to show acetylene reduction after leaching.

The cold-stored Antarctic samples were tested 5 months (A778 series) and 18 months (A767 series) after collection. Hot desert samples were tested after 1 to 8 years of storage. As far as could be determined, all photosynthetic endolithic microorganisms in the rocks were in viable condition. Experience in our laboratory shows that hot desert endolithic cyanobacteria remain fully viable even after storage of over 15 years, and at least some of the nonphotosynthetic bacteria are viable. Therefore it was felt that the inability to reduce acetylene was not due to the death of microorganisms during

[1] Yedatek Ltd., Rimon 10, Omer, Israel.

transportation and storage. In order to test further this possibility, additional tests were carried out with samples immediately after collection (see Enrichment Cultures for Nitrogen-Fixing Bacteria).

Enrichment Cultures for Nitrogen-Fixing Bacteria

To test for heterotrophic nitrogen fixation, enrichment cultures were set up with all rock and soil samples listed in Table 1. All samples were tested in both Modified Burk medium (44) with sucrose and Johnstone medium (1) with glucose as carbon source. None of these enrichment cultures showed visible bacterial growth, including samples A778-19 and A778-26 that were able to reduce acetylene. Sample A778-26 contains cyanobacteria, potential nitrogen-fixing organisms. Sample A778-19 contains an endolithic lichen with a green alga (*Trebouxia*) as phycobiont. Noncyanophilous lichens are not known to fix atmospheric nitrogen and no potential nitrogen-fixing organisms have been detected in this sample.

In order to test experimentally the possibility that failure to demonstrate nitrogen fixation in Antarctic samples was due to the death or damage of the organisms during storage, a series of enrichment cultures was set up for aerobic nitrogen-fixers at the Eklund Biological Laboratory at McMurdo Station, Antarctica, in December 1978–January 1979. Seven rock and two soil samples listed in Table 2 were tested immediately following collection in both full- and half-strength Johnstone medium with mannitol as carbon source. None of these enrichment cultures showed visible bacterial growth.

Enrichment Cultures for Nitrifying Bacteria

The presence of nitrifying bacteria was tested in illuminated enrichment cultures. The medium used contained NH_4+ as nitrogen source but no organic carbon source. All samples listed in Table 1 were tested in duplicate. No nitrites could be detected by spot tests in 2-week-old enrichment cultures and no visible bacterial growth appeared.

Nitrate, Nitrite, and Ammonium Determinations

As the general absence of nitrogen fixation indicated that other sources of combined nitrogen may be available in the endolithic microbial environment, substrate samples were tested for the presence of NO_3-, NO_2-, and NH_4+. Nitrates were present in all but two samples. Both of these samples were different from the others in some respect. Sample 71-1 is granite, without cracks formed by weathering, covered by desert varnish on the surface and apparently without microbial colonization. The lack of microscopic cracks and the impermeability of the hard varnish crust may account both for the absence of microorganisms and for the absence of nitrates. The second sample, U77-28, from the Virgin River Gorge in Arizona did not originate from an arid locality. This sample was collected from a vertical rock wall (with occasional chasmoendolithic algal growth in the rock) that, in contrast to nearly all other samples, is not directly exposed to rain.

Nitrites were generally absent except in two Antarctic samples. These samples were different from the others by being wetted by snowmelt. Sample A767-35 was saturated

Table 3. Nitrate and ammonium contents in homogenized rock samples containing endolithic microorganisms (μg/g rock)

Sample No.	NO_3-	NH_4+
A778-7	25.9	4.0
A778-13	0.6	< 2.0
A778-19	1.3	< 2.0
A778-26	0.4	< 2.0
A778-29	0.1	< 2.0
A778-69	5.6	< 2.0
U77-07	3.1	2.0
U77-19	0.8	13.0

by a snowmelt stream at the time of collecting and the site of A778-62, collected near Don Juan Pond, is near a freshwater stream originating from Wright Upper Glacier.

Ammonium ion was found in desert samples from the Utah Desert and in many Antarctic samples (exceptions: A767-5a, A767-36, A767-41, A767-50, A778-6, A778-19, A778-20, A778-21, A778-22, A778-25, A778-26, A778-61, A778-69). Only one sample from Mexico (70-6a) and none from the Sinai desert contained NH_{4+}. Sample 74-1 (from Chile) contained ammonium ion while SWa 74-5a (S. Africa) did not.

Quantitative analyses were carried out on eight homogenized rock samples that represented characteristic localities. Nitrate content showed a wide range of variation from 0.1 to 25.9 μg/g rock (Table 3). Ammonium content was generally 2 μg/g rock or less but A778-7 and U77-19 contained 4 and 13 ppm NH_{4+}, respectively (Table 3).

As solutes in porous desert rocks are likely to be concentrated at the surface due to evaporation, the possible formation of a nitrate concentration gradient was tested in a number of substrates. The relative concentration of nitrates was estimated near the surface layer and below from the intensity of the spot test color reaction. Of 29 samples tested, 22, or 76% showed significantly higher conentration on the surface than below.

In 10 representative substrates in which the presence of a gradient was indicated by spot tests, nitrate was determined quantitatively at different depths (Table 4). The depths of the samples in the substrates vary to some extent due to differences in size and morphology of the rock material. The steepest gradient was found in a rock from the Utah desert (U77-02) where nitrate concentration decreased to nearly 1/40 between the surface and 2.5 cm depth.

As snow was considered as a possible source of inorganic nitrogen compounds in Antarctic dry valley rocks, nitrate and ammonium were determined in freshly fallen snow. The analysis showed 1 μg/g nitrate, 0.17 μg/g ammonium, and no detectable nitrite.

Discussion

Nitrogen Fixation in Desert Microbial Communities

The salient information emerging from the present study is that in deserts, biological nitrogen fixation has no significant role in the nitrogen economy of endolithic microbial

Table 4. Nitrate content at various depths in rocks colonized by endolithic microorganisms (μg/g rock)

Sample No.	A767-35	A778-6	A778-19	A778-26	A778-61	Si734-12	Si734-13	70-6a	U77-02	U77-17
Approximate depth, cm										
Surface	125.3	13.9	11.9	0.5	1.3	18.4	17.4	0.9	3.9	2.1
1.0		4.4		0.2		1.3				
2.0						0.7	1.0			0.9
2.5									0.1	
3.0		2.0		0.2		0.8				
3.5	45.4				1.0					
4.0						0.6				
5.0						0.4		0.4		
5.5			0.5							

communities. This contrasts with the high level of nitrogen-fixing acitivity of algal soil crusts in deserts (see Introduction). This disparity becomes understandable when rates of productivity are compared. The yearly amount of biologically fixed nitrogen in soil crusts in the Great Basin desert of Utah was calculated by Rychert and Skujins (40) as ranging from 1 to 10 g m^{-2}. The yearly productivity rate of endolithic communities can be estimated only tentatively. The total organic nitrogen in rocks colonized by endoliths ranges in North American and Middle Eastern deserts from 1.5 to 8.0 g m^{-2} (Friedmann and Ocampo-Friedmann, unpublished), and in the dry valleys of Antarctica from 1.29 to 7.07 g m^{-2} (22). Due to the very limited turnover, yearly productivity can not amount to more than a small fraction of standing biomass, so that the rate, even by conservative estimate, is significantly lower than in soil crusts. Nitrogen, therefore, is not a limiting factor in the slow growing endolithic communities. In this respect, these communities seem to resemble lichens in general where, due to their slow growth rate, nutrient limitation seems to be rare (41).

Endolithic Microorganisms and Nitrogen Fixation

In cyanobacteria the ability to fix nitrogen under aerobic conditions is mostly coupled with the presence of heterocysts. Although desert soil crusts are dominated by heterocystous genera, none of the known endolithic forms have heterocysts (16, 21; Friedmann and Ocampo-Friedmann, unpublished). Although some nonheterocystous unicellular forms are capable of nitrogenase production in aerobic environments (37, 47), this phenomenon seems to be rare. In hot desert rocks the most common genus of endolithic cyanobacteria is *Chroococcidiopsis* (Friedmann and Ocampo-Friedmann, unpublished). This coccoid form is known to fix nitrogen in laboratory experiments under strictly anaerobic conditions when oxygen evolution by photosynthesis is blocked by the Photosystem II inhibitor DCMU (38). Yet it is unlikely that such conditions can ever exist inside the rocks in nature.

The occurrence of nitrogen fixation in two Antarctic samples is apparently incidental. Sample A778-19, in which nitrogen fixation started only after the nitrates have been removed from the substrate, indicates that organisms with nitrogen-fixing ability may be present without this potential being manifest. The identity of the nitrogen-fixing organisms in these samples has not been determined to date.

Approximately one-half of the rock samples tested contained ammonium salts. No ammonium-oxidizing activity could be demonstrated, although the data are not sufficient to draw general conclusions.

Origin of Combined Nitrogen Compounds in Desert Rocks

Reports of the presence of nitrates in deserts have been known for many years (30, 33, 35). In our analyses one of the two samples that did not contain nitrate originated from a nonarid locality and the other had a compact physical structure that precluded the penetration of water, the potential carrier of NO_3-.

Available evidence indicates that combined nitrogen compounds in desert rocks originate from the atmosphere. Rain and snow over hot deserts contain up to 1.5 ppm NO_3- and up to 0.2 ppm NH_4+ (28). Antarctic snow with 0.1 ppm NH_4+ has been reported by Parker and Wodehouse (36), and Antarctic glacial ice (which probably was derived from snow) contained 0.3–0.4 ppm NO_3- (Dr. B. C. Parker, personal communication). Our analyses of Antarctic snow in the dry valleys revealed an NH_4+ content of approximately 0.2 ppm and a NO_3- content of 1.0 ppm. These values are significantly higher than those obtained for snow over the Antarctic ice cap. The difference could be due to the conspicuous presence of windblown sand grains in the snow from the dry valleys. The sand grains obviously originated from soils or crumbling rocks that also contain soluble nitrogen compounds, and these may have increased the values found in the analysis of the snow.

Noxon (34) reported approximately 0.1 ppb NO_2- in the troposphere over the United States. These low values concur with our data as we were unable to detect any NO_2- in North American samples. Apparently atmospheric NO_2- is photooxidized in rainwater to NO_3-.

Deposition on the rock surface occurs in various ways. In hot deserts nitrogen compounds dissolved in rain or in snow may penetrate rocks that have a porous structure or have microscopic cracks due to weathering. In the Antarctic cold desert, melting snow is the source of moisture for endolithic microorganisms (18). Snow that sublimes in the extreme arid conditions of the dry valleys deposits its salt contents on the surface of rocks. According to the estimate of Junge (27), up to 70% of the atmospheric contribution of nitrogen compounds in hot arid regions comes from dry fallout. The nitrate gradient present in many rock samples is an indication that nitrogen compounds reach the rock from the surface (another factor that may produce this gradient is the upward capillary movement of water). The most significant source of nitrogen compounds in the atmosphere is probably abiotic nitrogen fixation by electric discharges, which in hot deserts take place as lightning (28), whereas in Antarctica they occur as aurorae (46). In polar regions, galactic cosmic ray ionization is also likely to produce nitrogen compounds in the atmosphere (7a). Other potential sources, partly abiotically produced and partly of organic origin, are listed in the review of West (45).

Nitrogen Economy of Endolithic Microbial Communities: General Considerations

Endolithic microbial communities in desert rocks are closed systems in the sense that they are practically isolated from organisms of the outside environment. From the point

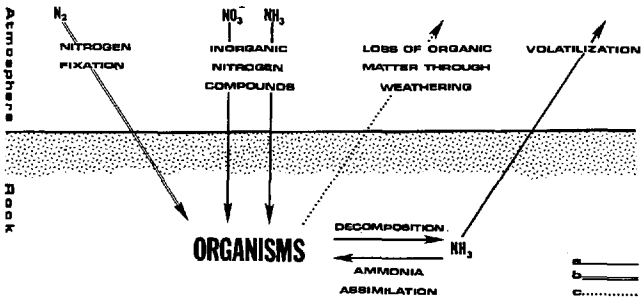

Fig. 2. Nitrogen pathways in endolithic microbial communities in deserts. **a** Pathway generally occurring; **b** pathway not generally occurring; **c** pathway occurring with exfoliating weathering in Antarctica.

of view of nitrogen economy, the most conspicuous feature of this system, other than the apparent rarity of biological nitrogen fixation, is the absence of a complete nitrogen cycle. Although the data reported in this paper are still incomplete, it is possible to summarize the nitrogen pathways that may be present in desert endolithic environments (Fig. 2).

The endolithic microbial community generally depends on abiotically formed nitrogen compounds from the atmosphere. This is sufficient to satisfy the needs of the community, and the general presence of nitrates in the rocks is an indication that nitrogen is not a limiting factor. Biological nitrogen fixation that occurs exceptionally seems to play only a minor role. Decomposition resulting in ammonia formation is likely to occur, although there is no evidence to date that it actually takes place. It is probable that the greater part of the ammonia thus produced is utilized again by the microorganisms. At least a partial loss of ammonia through volatilization is also likely. In the Antarctic desert some organic matter is lost to the outside environment when rocks with exfoliating weathering crumble and some of the endolithic organisms are released (25). As nitrification and denitrification may be lacking, a complete nitrogen cycle in the usual sense probably does not exist.

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References

1. Aaronson, S.: Experimental Microbial Ecology, Academic Press. New York (1970)
2. Alexander, V.: Nitrogen fixation by blue-green algae in polar and sub-polar regions. In W. D. P. Stewart (ed.): Nitrogen Fixation by Free-living Microorganisms, pp. 175-187. International Biological Programme 6 (1975)

3. Alexander, V., M. Billington, and D. Schell: The influence of abiotic factors on nitrogen fixation rates in the Barrow, Alaska, artic tundra. Rep. Kevo. Subartic Res. Stat. **11**, 3-11 (1974)
4. Alexander, V., and S. Kallio: Nitrogenase activity in *Peltigera aphtosa* and *Steracaulon paschale* in early spring. Rep. Kevo. Subartic Res. Stat. **13**, 12-15 (1976)
5. Alexander, V., and D. M. Schell: Seasonal and spatial variation of nitrogen fixation in the Barrow, Alaska, tundra. Arct. Alp. Res. **5**, 77-88 (1973)
6. American Public Health Assoc.: Standard Methods for the Examination of Water and Wastewater, 13th Ed. Washington, D.C. (1971)
7. Benoit, R. E., and C. L. Hall: The microbiology of some dry valley soils of Victoria Land, Antarctica. In M. W. Holdgate (ed.): Antarctic Ecology, Vol. 2, pp. 697-701. Academic Press, London (1970)
7a. Bauer, E.: Non-biogenic fixed nitrogen in Antarctic surface waters (letter to the editor), Nature **276**, 96 (1978)
8. Boyd, W. L., and J. W. Boyd: Soil microorganisms of the McMurdo Sound area, Antarctica. Appl. Microbiol. **11**, 116-121 (1962)
9. Boyd, W. L., and J. W. Boyd: Presence of *Azotobacter* species in polar regions. J. Bacteriol. **83**, 429-430 (1966)
10. Boyd, W. L., and J. W. Boyd: Studies of soil microorganisms, Inuvik, Northwest Territories. Arctic **24**, 162-176 (1971)
11. Cameron, R. E.: Ecology of blue-green algae in Antarctic soils. In T. V. Desikachary, (ed.): Taxonomy and Biology of Blue-Green Algae, pp. 353-386. University of Madras, The Bangalore Press, Bangalore (1972)
12. Claridge, G. G. C., and I. B. Campbell: Origin of nitrate deposits. Nature **217**, 428-430 (1968)
13. Feigel, F., V. Anger, and R. Oesper: Spot Tests in Inorganic Analysis. Elsevier, Amsterdam (1972)
14. Fogg, G. E., and W. D. P. Stewart: In situ determination of biological nitrogen fixation in Antarctica. Br. Antarc. Surv. Bull. **15**, 39-46 (1968)
15. Friedmann, E. I.: Light and scanning electron microscopy of the endolithic desert algal habitat. Phycologia **10**, 411-428 (1971)
16. Friedmann, E. I.: Ecology of lithophytic algal habitats in Middle Eastern and North American deserts. In L. E. Rodin (ed.): Ecophysiological Foundation of Ecosystems Productivity in Arid Zones, Nauka, pp. 182-185. U.S.S.R. Academy of Sciences, Leningrad (1972)
17. Friedmann, E. I.: Microorganisms in Antarctic desert rocks from dry valleys and Dufek Massif. Ant. J. U. S. **12**, 26-29 (1977)
18. Friedmann, E. I.: Melting snow in the dry valleys is a source of water for endolithic microorganisms. Ant. J. U. S. **13**, 162-163 (1978)
19. Friedmann, E. I., and M. Galun: Desert algae, lichens, and fungi. In G. W. Brown (ed.): Desert Biology, Vol. II, pp. 165-212. Academic Press, New York (1974)
20. Friedmann, E. I., Y. Lipkin, and R. Ocampo-Paus: Desert algae of the Negev (Israel). Phycologia **6**, 185-200 (1967)
21. Friedmann, E. I., and R. Ocampo: Endolithic blue-green algae in the dry valleys: primary producers in the Antarctic desert ecosystem. Science **193**, 1247-1249 (1976)
22. Friedmann, E. I.: Endolithic microbial life in hot and cold deserts. Origins of Life **10** (1980, in press)
23. Hardy, R. W. F., R. C. Burns, and R. Holsten: Applications of the acetylene-ethylene test for measurement of nitrogen-fixation. Soil Biol. Biochem. **5**, 47-81 (1973)
24. Harwood, J. E., and A. L. Kuhn: A colorimetric method for ammonia in natural waters. Water Res. **4**, 805-811 (1970)
25. Holm-Hansen, O.: Algae: nitrogen-fixation by Antarctic species. Science **139**, 1059-1060 (1963)
26. Horne, A. J.: The ecology of nitrogen fixation on Signy Island, South Orkney Islands. Br. Antarc. Surv. Bull. **27**, 893-902 (1972)
27. Junge, C. E.: The distribution of ammonia and nitrate and rainwater over the United States. Trans. Am. Geophys. Union **39**, 241-248 (1958)
28. Junge, C. E.: Air Chemistry and Radioactivity. Academic Press, New York (1963)
29. Kallio, P., and S. Kallio: Nitrogen fixation by free-living microorganisms in the Kevo district. Rep. Kevo Subartic Res. Stat. **12**, 28-33 (1975)

30. Killian, C., and D. Feher: Recherches sur la microbiologie des sols désertiques. Encyclopédie Biologique **21.** Paul Lechevalier, Paris (1939)
31. Martin, D. F.: Marine Chemistry. Marcel Dekker, New York. 451p. (1972)
32. Meiklejohn, J.: Iron and nitrifying bacteria. J. Gen. Microbiol. **8**, 58-65 (1953)
33. Noble, L. F., G. R. Mansfield, et al.: Nitrate deposits in the Amargosa region, southeastern California. U.S. Geol. Surv. Bull. **724**, 1-96 (1922)
34. Noxon, J. F.: Tropospheric NO_2−. J. Geophys. Res. **83**, 3051-3057 (1978)
35. Odintsova, S. V.: Nitre formation in deserts. Doklady A. N. SSSR. **32**, 578-580 (1941)
36. Parker, B. C., and E. B. Wodehouse: Atmospheric ammonia-nitrogen: a potential source of nitrogen eutrophication of fresh-water Antarctic ecosystems. Antarct. Res. Ser. **30** (in press)
37. Rippka, R., A. Neilson, R. Kunisawa, and G. Cohen-Bazire: Nitrogen-fixation by unicellular blue-green algae. Arch. Mikrobiol. **76**, 341-348 (1971)
38. Rippka, R., and J. B. Waterbury: The synthesis of nitrogenase by non-heterocystous cyanobacteria. FEMS Microbiol. Lett. **2**, 83-86 (1977)
39. Rychert, R. C., and J. Skujins: Microbial activity in arid soils. Utah Sci. **34**, 96-98 (1973)
40. Rychert, R. C., and J. Skujins: Nitrogen fixation by blue-green algae-lichen crusts in the Great Basin Desert. Soil Sci. Soc. Am. Proc. **38**, 768-771 (1974)
41. Smith, D. C.: Symbiosis and the biology of lichenized fungi. Symp. Soc. Exp. Biol. **29**, 373-405 (1975)
42. Sorenson, D. L., and D. B. Porcella: Nitrogen erosion and fixation in cool desert soil-algal crusts in northern Utah. US/IBP Desert Biome Res. Memo. 74-37. Utah State University, Logan (1974)
43. Stewart, W. D. P., T. Mague, G. P. Fitzgerald, and R. H. Burris: Nitrogenase activity in Wisconsin lakes of differing degrees of eutrophication. New Phytol. **70**, 497-509 (1971)
44. Strandberg, G. W., and P. W. Wilson: Formation of the nitrogen-fixing enzyme system in *Azotobacter vinelandii*. Can. J. Microbiol. **14**, 25-31 (1968)
45. West, N. E.: Physical inputs of nitrogen to desert ecosystems. In N. E. West and J. Skujins (eds.): Nitrogen in Desert Ecosystems, pp. 165-170. Dowden, Hutchinson and Ross, Stroudsburg, Pa. (1978)
46. Wilson, A. T., and D. A. House: Fixation of nitrogen by aurora and its contribution to the nitrogen balance of the earth. Nature **205**, 793-794 (1965)
47. Wyatt, J. T., and J. K. G. Silvey: Nitrogen fixation by *Gloeocapsa*. Science **165**, 908-909 (1969)