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Relevance of ammonium oxidation within biological soil crust communities

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Summary

Thin, vertically structured topsoil communities that become ecologically important in arid regions (biological soil crusts or BSCs) are responsible for much of the nitrogen inputs into pristine arid lands. We studied N_2 fixation and ammonium oxidation (AO) at subcentimetre resolution within BSCs from the Colorado Plateau. Pools of dissolved porewater nitrate/nitrite, ammonium and organic nitrogen in wetted BSCs were high in comparison with those typical of aridosoils. They remained stable during incubations, indicating that input and output processes were of similar magnitude. Areal N_2 fixation rates (6.5–48 μmol C_2H_2 m^{-2} h^{-1}) were high, the vertical distribution of N_2 fixation peaking close to the surface if populations of heterocystous cyanobacteria were present, but in the subsurface if they were absent. Areal AO rates (19–46 μmol N m^{-2} h^{-1}) were commensurate with N_2 fixation inputs. When considering oxygen availability, AO activity invariably peaked 2–3 mm deep and was limited by oxygen (not ammonium) supply. Most probable number (MPN)-enumerated ammonia-oxidizing bacteria ($6.7–7.9 \times 10^3$ cells g^{-1} on average) clearly peaked at 2–3 mm depth. Thus, AO (hence nitrification) is a spatially restricted but important process in the nitrogen cycling of BSC, turning much of the biologically fixed nitrogen into oxidized forms, the fate of which remains to be determined.

Introduction

Because nitrogen is second only to water as a limiting factor in the fertility of arid lands (Schlesinger, 1996; Evans and Lange, 2001), nitrogen inputs into desert ecosystems, either as deposition or as biological fixation, are crucial for their ecology and biogeochemistry. But high rates of nitrogen input resulting from biological fixation are not reflected in an increase in nitrogen content over the long term in arid soils (Peterjohn and Schlesinger, 1990). Clearly, our knowledge of nitrogen cycling and mass transport processes in these ecosystems is insufficient. Because much of the N_2 fixation in arid lands is carried out by microbes, particularly cyanobacteria, associated with topsoil communities known as biological soil crusts (Rychert and Skujins, 1974; Isichei, 1980; Jeffries *et al.*, 1992; Steppe *et al.*, 1996; Belnap, 2002), the study of nitrogen cycling in these communities may hold the key to unravelling this apparent nitrogen paradox.

Biological soil crusts (BSCs) are millimetres to centimetres thick microbial communities that typically cover large portions of the plant interspaces in arid lands (Belnap and Lange, 2001). BSCs have been referred to as microbiotic, microfloral, microphytic, cryptobiotic and cryptogamic crusts (West and Skujins, 1978; Harper and Marble, 1988) or as microbial earths (Retallack, 2001). They are driven by the autochthonous primary productivity of cyanobacteria and/or microalgae (Garcia-Pichel, 2002), either as free-living organisms or as partners in lichen symbioses; mosses may also be found in well-developed crusts (Johansen, 1993; Eldridge and Greene, 1994; Belnap *et al.*, 2001). Highly diverse populations of both phototrophic and non-phototrophic microbes reside in a thin mantle some 1 cm deep, as has been determined by cultivation-independent molecular studies (Garcia-Pichel *et al.*, 2001; 2003; Kuske *et al.*, 2002); there, they attain large population densities compared with bulk desert soil and organize themselves in a vertically stratified manner (Garcia-Pichel *et al.*, 2003), similar to that described for microbial mats (Cohen and Rosenberg, 1989) or biofilms (Doyle, 1999). The microbes remain desiccated, and thus inactive, for most of the time but, upon wetting, become quickly hydrated and active. During pulses of activity, high metabolic rates constrained within small spaces result in the rapid formation of steep chemical gradients and microenvironments, which include oxygen-supersaturated zones close to the surface and anoxic zones some 1–3 mm deep (de Winder, 1990; Garcia-Pichel and Belnap, 1996; 2001). BSCs are important as agents in resisting soil erosion (Campbell, 1979; Schulten, 1985; Belnap, 1993).

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The cycling of nitrogen in BSCs has been studied mostly at the level of N_2 fixation (Englund and Meyerson, 1974; Isichei, 1980; Loftis and Kurtz, 1980; DuBois and Kapustka, 1983; Belnap, 1996); the extent and importance of other nitrogen transformations remains understudied or controversial (Belnap, 2001; Evans and Lange, 2001). A key transformation is ammonium oxidation (the first step in nitrification), because it acts as a 'biogeochemical hinge' that will determine whether local nitrogenous pools remain reduced or are oxidized instead. In its absence, possible nitrogen losses from BSCs will be mainly to the atmosphere through ammonia volatilization (Klubek *et al*., 1978; Peterjohn and Schlesinger, 1991; Evans and Johansen, 1999). If nitrification, on the other hand, and the formation of significant amounts of oxidized nitrogen are relevant processes within the crusts, then possible nitrogen losses will be in the form of dissolved nitrate exiting with percolating water, or possibly as gaseous products of denitrification. Ammonium oxidation (AO) has been studied in detail in many agricultural and other mesic soils because of its impact upon soil fertility (Berg and Rosswall, 1985; Kandeler *et al*., 1999; Bengtsson *et al*., 2003; Templer *et al*., 2003). The study of nitrification in BSCs, in contrast, requires paying close attention to small-scale vertical heterogeneity, which influences not only the three-dimensional distribution of the bacterial populations (Garcia-Pichel *et al*., 2003) but also the availability of oxygen to different microhorizons within the crusts.

In this paper, we attempted to gauge the importance of ammonium oxidation in two types of BSCs. These differ markedly in their appearance and phototroph community composition and are regarded as different stages of colonization: light and dark crusts. Light crusts are dominated by a motile filamentous cyanobacterium, *Microcoleus vaginatus*, whereas dark crusts additionally contain heterocystous filamentous cyanobacteria, such as *Nostoc* spp. and *Scytonema* (Garcia-Pichel and Belnap, 1996; Garcia-Pichel *et al*., 2001). We quantified rates of both AO and N_2 fixation with subcentimetre vertical resolution and interpreted those rates in the light of ancillary chemical analyses of soil, porewater, microsensor determinations of oxygen concentration and the distribution of microbial populations.

Results

Bulk soil analysis

Bulk soil analysis indicated that the sand fraction was dominant, with <10% clay or silt, which makes these soils 'loamy sand'. The concentrations of organic carbon and organic matter (≈ 0.17% and ≈ 0.3% respectively) were low, similar to values reported previously on nearby soils (Garcia-Pichel and Belnap, 1996); pH was slightly alkaline (Table 1). Water-holding capacity was 3.25% in the light crust and 3.74% in the dark crusted soil. Overall, water-soluble cation concentration in dark crusts was greater than in the light crust. The dominant water-soluble cation in the light crust was calcium, whereas sodium was dominant in the dark crust. Measured levels of soil phosphorous and total nitrogen in the bulk soil point to significant deviation from the Redfield ratio and potential for nitrogen limitation.

Concentration of nitrogenous compounds in the porewater of intact BSCs

The porewater of wetted light crusts contained ≈ 3.7 mg N l^{-1} as total dissolved nitrogen (N-TN), with ≈ 64% being organic nitrogen (DON), 24% as ammonium and 13% as nitrate and nitrite. Dark BSC porewater showed similar if slightly lower values with the total nitrogen at nearly 3.1 mg N-TN l^{-1}, where roughly 74% was in the organic pool, 11% as ammonium and 15% as nitrate and nitrite (Table 2). The largest nitrogenous pool was thus DON in both types of crusts. Regression of time course data (Fig. 1) could not detect statistically significant trends in net accumulation or depletion in any or the nitrogenous pools measured (R^2 values of regression were under 0.5; linear models are shown, but exponential or quadratic were also low), regardless of the source (light or dark crusts) or incubation conditions (in the light or in the dark), even after 12 h of incubation.

Table 1. Characteristics of bulk soils in the two sites where studied crusts develop (0–10 cm deep).

	Crust type	
Parameter (units)	Light	Dark
Sand (%)	83.28	87.28
Clay (%)	9.00	8.00
Silt (%)	7.72	4.72
Organic carbon (%)	0.15	0.19
Organic matter (%)	0.26	0.33
pH	7.80	7.75
Conductivity (dS m^{-1})	0.40	1.65
Water-holding capacity (%)	3.25	3.74
Ca^{2+} (p.p.m.)[a]	73.85	122.10
Mg^{2+} (p.p.m.)[a]	12.70	22.96
K^+ (p.p.m.)[a]	24.09	79.56
Na^+ (p.p.m.)[a]	9.59	225.90
K^+ (p.p.m.)[b]	64.00	80.00
Sodium absorption ratio	0.27	4.91
NO_3-N (p.p.m.)	2.13	1.95
Total N (p.p.m.)	15.50	6.30
P (p.p.m.)	8.28	6.19

a. Water-soluble fraction.
b. After sodium bicarbonate extraction.
Analyses include both crusted area (0–1 cm) and the undercrust soil (1–10 cm).

Table 2. Concentrations of nitrogenous compounds in the porewater of water-saturated, intact BSC in mg N l^{-1} soil porewater.

	Crust type	
Nitrogenous pool (mg N l^{-1})	Light	Dark
TDN-N	3.73 ± 1.92	3.08 ± 3.51
NH_4 ± N	0.88 ± 0.46	0.35 ± 0.18
(NO_2^- + NO_3^-)-N	0.47 ± 0.69	0.45 ± 0.58
Organic N	2.38 ± 1.72	2.28 ± 3.11

Data are averages of nine independent samples from crusts incubated under water saturation for ≈ 5 min.

Rates of N_2 fixation

Areal rates of N_2 fixation (acetylene reduction) measured in intact cores (n = 4) yielded 6.5 ± 1.9 μmol C_2H_2 m^{-2} h^{-1} in the light crust and at 48 ± 9.3 μmol C_2H_2 m^{-2} h^{-1} in the dark crusts (Table 3), which is in line with previously reported values and with the fact that light crusts do not contain large populations of heterocystous cyanobacteria. Vertical profiles of N_2 fixation through the crusts showed marked variations in activity (Fig. 2). In light crusts, none of the profiles had measurable fixation in the uppermost horizon, and a peak rate of N_2 fixation was always found in the subsurface. In three of the four profiles, measurable activity extended down into the fifth horizon (6–8 mm deep). In dark BSC, in contrast, the peaks of activity were spread over the top 3 mm, with significant activity in the topmost millimetre in three out of four profiles. Generic vertical profiles with averaged rates for both crusts types are presented in Fig. 4. If the profiles of volumetric rates in Fig. 2 are depth integrated to obtain areal rates (Table 3), these are congruent with those measured in dark crusts, but different in light crusts, where direct incubation of whole cores yielded much lower values than integration of core slices.

Oxygen profiles

All vertical profiles of oxygen partial pressure were congruent in overall shape; just below the surface, maximal oxygen concentrations were >100% air saturation, with a sharp decline below this peak, and anoxia was reached by 0.4–3.5 mm. Light crusts achieved anoxia by no more than 1.7 mm depth, with three of the four profiles becoming oxygen depleted by the end of the top millimetre. Dark BSC profiles were similar, but the maximal depth for anoxia was somewhat deeper (3.5 mm), and three of the four profiles reached anoxia by the second millimetre. Figure 3C and D displays averages of four independent replicate samples, and differences between replicates were small.

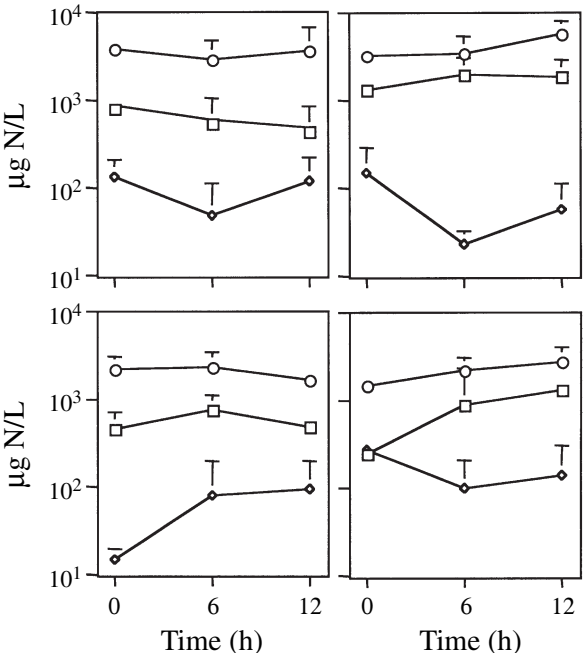

Fig. 1. Dynamics of porewater concentration of nitrogenous compounds during incubation of intact crusts. Circles indicate total dissolved nitrogen (TDN), squares indicate dissolved NH_4^+, and diamonds indicate dissolved NO^{2-} + NO_3^-. Top panels show data obtained from light BSCs, and bottom panels from dark BSCs. Left panels show data obtained from incubation in the light, right panels show data from dark incubation. Each data point is the mean of three independent measurements, for simplicity only 1/2 of the standard deviation is shown through the error bars.

Table 3. Summary of areal rates of nitrogen transformations (in μmol m^{-2} h^{-1}) for dinitrogen fixation and ammonium oxidation.

Process	Measurement conditions	Illumination conditions	Light crust	Dark crust
N_2 fixation (as C_2H_2 reduction)	Intact soil sample	Light	6.53 ± 1.87	48.00 ± 9.31
	Integrated from profile	Light	47.21 ± 27.16	51.86 ± 35.10
AAO (actual)	Intact soil sample	Light	53.38 ± 28.08	41.98 ± 21.08
	Intact soil sample	Dark	28.61 ± 12.99	14.46 ± 10.19
AAO (potential)	Slurried bulk soil	Dark	46.44 ± 13.26	18.74 ± 1.13
	Integrated from profile	Dark	77.91 ± 12.75	59.47 ± 4.64
	Integrated from profile (O_2 corrected)	Dark	20.43 ± 3.85	15.76 ± 2.05

When original data were expressed on a soil weight basis, a density of 1.761 mg mm^{-3} was used to convert into volumetric rates.

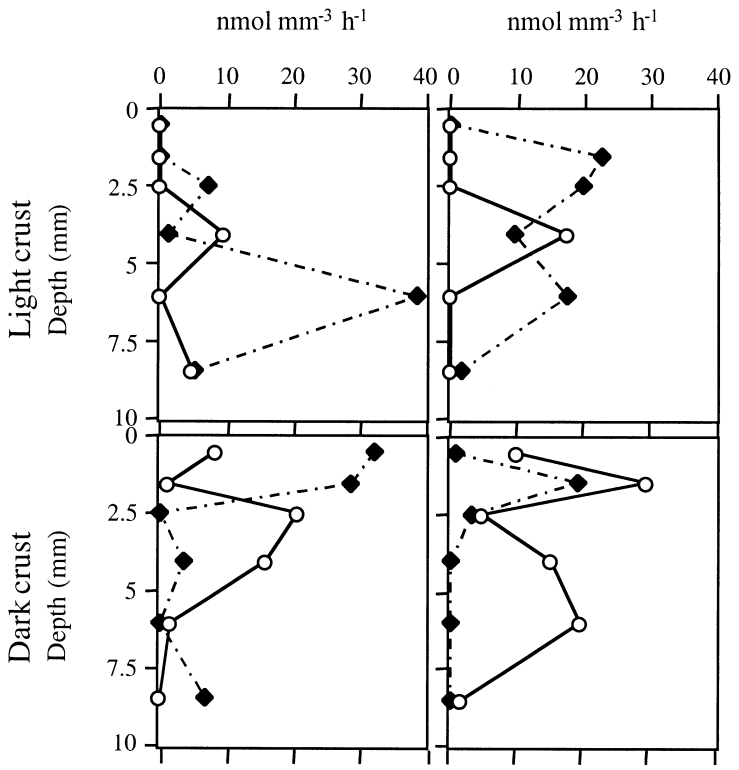

Fig. 2. Vertical profiles of volumetric N_2 fixation (acetylene reduction) rates in the two types of biological soil crust. Top panels show data obtained from light BSCs, and bottom panels from dark BSCs. For each crust type, four replicate profiles are shown (two per panel).

Most probable number (MPN) determination of ammonium-oxidizing bacteria (AOB)

Recoverable (culturable) populations of AOB averaged $6.69 \pm 6.20 \times 10^3$ cells g^{-1} soil ($n = 6$) in light BSCs and $7.93 \pm 5.65 \times 10^3$ cells g^{-1} in the dark BSCs ($n = 6$), even though there was considerable variability among independent profiles. These are relatively large numbers, more typical of organic-rich, mesic soils than of aridosols, and much higher than the 10^2 recoverable AOB g^{-1} found in non-crusted sandy desert soils (S. Johnson, unpublished data). The vertical distribution of AOB (Fig. 4) was clearly not homogeneous: in all but one profile, regardless of crust type, numbers peaked in the second and third horizons (1–3 mm deep) below the surface, with population numbers decreasing steadily with depth below this maximum. Maximal values encountered for light BSCs reached $8.68 \pm 6.70 \times 10^3$ cells g^{-1}, and maximal values were even greater in the dark crust reaching $1.13 \pm 0.65 \times 10^4$ cells g^{-1}.

Potential and actual rates of aerobic ammonium oxidation (AAO)

Rates of potential AAO (in the dark, with externally supplied ammonium and under agitation) in slurried BSCs ($n = 3$) reached 23.3 ± 6.7 nmol N g^{-1} h^{-1} for light crusts and 9.42 ± 0.57 nmol N g^{-1} h^{-1} for dark crusts, which for our samples corresponds to areal rates of 46.4 ± 13.3 μmol m^{-2} h^{-1} and 18.7 ± 1.1 μmol m^{-2} h^{-1} respectively. Profiles of potential volumetric ammonium oxidation rates (run on sliced cores) obtained from both BSC types displayed a subsurface peak, below which rates decreased somewhat but remained significantly greater than in the top millimetre (Fig. 3G and H). Maximal values in light BSC of 15.5 nmol N g^{-1} h^{-1} were between the second and third horizons and, in dark BSC, maximal rates of 15.0 nmol N g^{-1} h^{-1} were in the third horizon. Vertical integration of these profiles allows for an independent measure of areal rates of potential ammonium oxidation in the dark: 77.91 ± 12.75 and 59.47 ± 1.13 μmol N m^{-2} h^{-1} for light and dark crusts respectively. Comparisons of these values with those obtained from non-sectioned slurries indicate that some limitation may have occurred in the incubations using a single slurry, possibly oxygen supply. In fact, the high rates of potential ammonium oxidation measured below the oxic zone are necessarily artifactual in that each of the horizons had been slurried and shaken separately during incubation, thus relieving certain limitation by lack of oxygen. *Nitrosomonas europaea* (the best studied ammonium oxidizer in pure culture) is not inhibited by microaerophillic conditions, only by anoxia (Laanbroek *et al.*, 1994). Therefore, we can assume that, below the

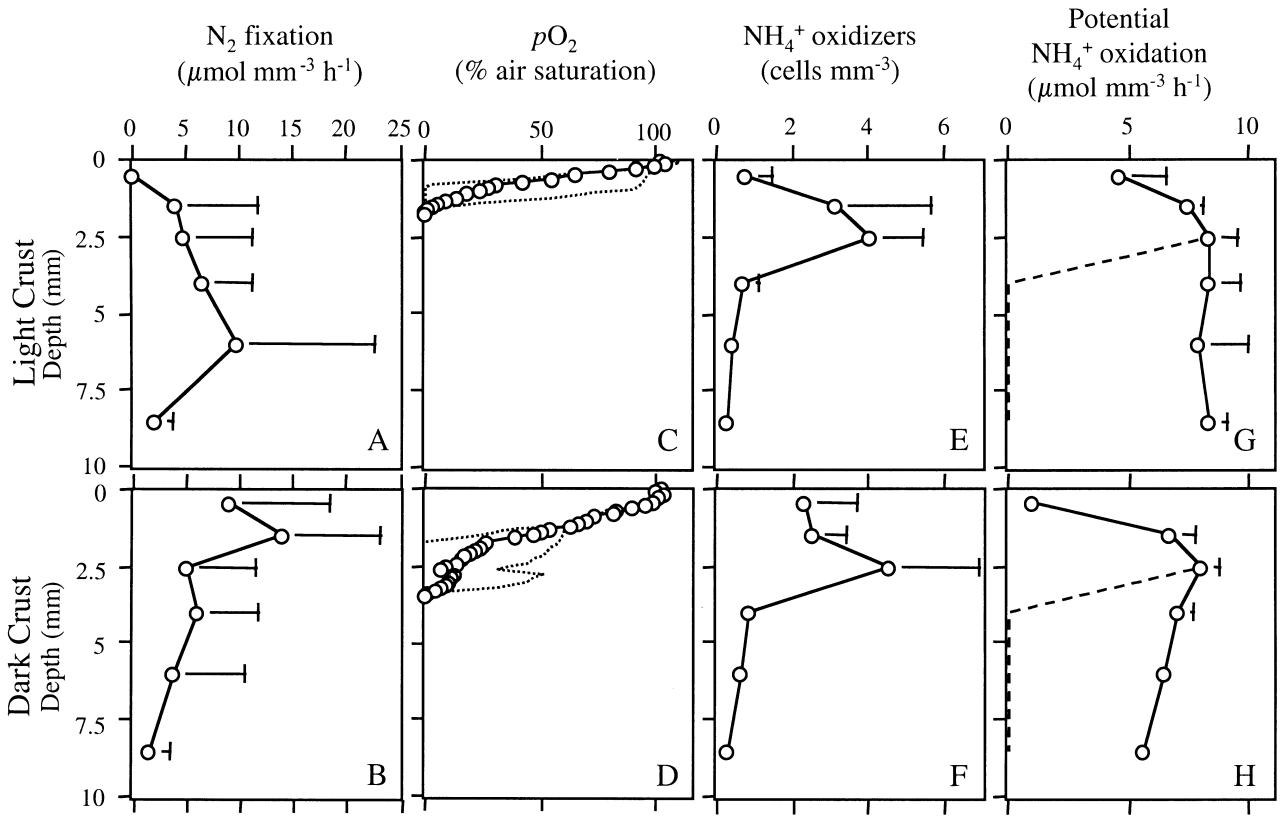

Fig. 3. Vertical profiles of biogeochemical parameters involved in nitrogen cycling in BSCs. Upper row of panels concerns light crusts, bottom row is for dark crusts.
A and B. Average depth distribution volumetric N_2 fixation (data from Fig. 2, $n = 4$).
C and D. Oxygen partial pressure (as percentage of air saturation) in the soil solution as measured with microsensors in intact crusts. Thick line shows an average profile computed from four independent determinations. Dotted profiles delimit maximal range of values.
E and F. Average vertical distribution of ammonium-oxidizing bacteria enumerated by MPNs (data also shown in Fig. 4, $n = 5$).
G and H. Vertical distribution of potential ammonium oxidation rates (solid lines) obtained from sectioned, slurried cores. Dashed line depicts a correction in those potential rates due to oxygen limitation in the lower portion of the profile. For clarity, when error bars are shown, only 1/2 of the standard deviation is depicted ($n = 3$).

second (in the light crust) to third millimetre (in the dark crust), potential ammonium oxidation rates actually become zero (dashed line in Fig. 3G and H), as there is no oxidant available. If profiles are integrated taking into account only those horizons that are oxic or hypoxic, according to our pO_2 profiles, the integrated areal values are much lower: light crusts potentially oxidize 20.4 ± 3.8 μmol N m^{-2} h^{-1} and dark crusts some 15.8 ± 1.1 μmol N m^{-2} h^{-1}.

Actual ammonium oxidation was also measured in intact crusts without the addition of external ammonium. Here, we carried out incubations in the light and in the dark. Intact light crusts ($n = 3$) incubated in the light oxidized 53.4 ± 28.1 μmol N m^{-2} h^{-1} and 28.6 ± 13.0 μmol N m^{-2} h^{-1} when incubated in the dark. Intact dark crusts oxidized 42.0 ± 21.1 μmol N m^{-2} h^{-1} when in the light and 14.8 ± 10.2 μmol N m^{-2} h^{-1} when in the dark (Table 3).

Discussion

Dynamics of porewater nitrogen

The concentrations of dissolved nitrogenous compounds found in the porewaters of BSCs were very high in comparison with the range of typical values in soil solution for a variety of soils (Fitzhugh *et al.*, 2001; Critchley *et al.*, 2002; Schade *et al.*, 2002; Bragazza *et al.*, 2003; Yeakley *et al.*, 2003) and also with the concentration of total N in bulk soil at the same sites (Table 1). This is perhaps not too surprising if one takes into account the unavoidable loss of low-molecular-weight cellular matter imparted by the wetting/drying regime that is characteristic of these communities (Klubek *et al.*, 1978; Millbank, 1978; Potts, 1984; Dodds *et al.*, 1995) and the high concentrations of microbes present (Garcia-Pichel *et al.*, 2003). The cellular source of the nitrogenous compounds in the soil solution is evidenced by the importance of the organic N

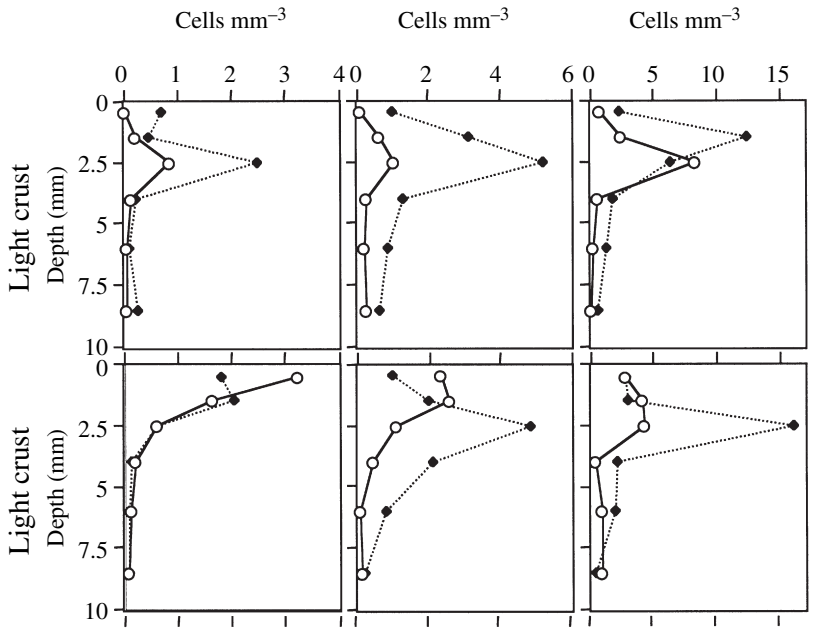

Fig. 4. Vertical distribution of MPN for ammonium-oxidizing bacteria through the top centimetre of two types of BSC. Top panels show data obtained from light BSCs, and bottom panels from dark BSCs. Replicate profiles are shown from left to right. Data shown are representative of six independent profiles for each soil crust type.

fraction within the TDN. The fact that no measurable temporal trends of accumulation or depletion in TDN, NH_4^+ or $[NO_2^- + NO_3^-]$ were detected in the time course experiment (Fig. 1) is interesting. Obviously, steady-state concentrations are the result of both input and output rates of the soil solution system. Biological inputs of N are ultimately all from N_2 fixation, as litter input on these crusts is insignificant making ammonification of allochthonous organic matter negligible. Because inputs by N_2 fixation are expected to be high, these results point to the presence of commensurate output processes within the bounds of the BSC itself. Much of this may result from reuptake of leaked matter by microbes themselves, which are known to have diverse and specialized uptake systems for both inorganic and organic nitrogenous compounds.

N_2 fixation rates and distribution

A wealth of studies have reported widely varying rates for BSC collected from the same general area (Mayland *et al*., 1966; MacGregor and Johnson, 1971; Rychert and Skujins, 1974; West and Skujins, 1977; Rychert *et al*., 1978; Isichei, 1980; West, 1990; Jeffries *et al*., 1992; Evans and Belnap, 1999; Evans and Johansen, 1999). This is probably due to a combination of patchiness in the distribution of activity, perhaps some seasonal variability and methodological differences as well. Particularly, preconditioning (wetting the crusts for a specified period of time before measurement) seems to play a large role, and the various studies clearly differ in this respect. In any event, this made it important for our study to assess N_2 fixation potential in the same samples in which ammonium oxidation was studied. Our rates measured on intact cores yielded values within the range of previously published results and showed that rates in dark crust were significantly higher than those in light crust ($P < 10^{-10}$). This is in agreement with previous studies in which a distinction between the two crust types was made (Jeffries *et al*., 1992; Belnap, 2001; 2002). It is also in agreement with the fact that dark crusts have all been shown to contain the sunscreen-bearing, heterocystous cyanobacteria (*Nostoc* spp. and *Scytonema*) and light crusts do not (Garcia-Pichel and Belnap, 1996). However, when areal rates were calculated from vertical profiles, the difference in rates between light and dark crusts was no longer significant ($P = 0.51$), largely because of a highly statistically significant ($P = 0.0003$) increase in the estimate of N_2 fixation in light crusts. It is possible that the difference in estimates resulted from a methodological problem in using whole cores for the N_2 fixation assay.

In a first approximation, one can characterize one-dimensional diffusive transport by noting that the root-mean-square displacement of molecules will be proportional to the square root of time (Berg, 1983), $<x^2> = (2Dt)^{1/2}$, where D is the apparent diffusion coefficient for the gas in question, which for small molecules is $\approx 10^{-5}$ cm^2 s^{-1}. In our case, and using a value of $D = 2 \times 10^{-5}$ cm^2 s^{-1} to account for the tortuosity of diffusive path imposed by the mineral particles, it would take a time, $t \approx x^2/2D$, of 16 min for acetylene to diffuse to depth of 2 mm and about the same time for any ethylene to diffuse back out and be detected. This is much smaller than the times used for incubations (4 h). However, it will take an average of ≈1.75 h for acetylene to diffuse to a depth of 5 mm and thus 3.5 h for ethylene produced at

that depth to make a significant contribution to the levels detected in the overlying gas phase. Similarly, contributions of acetylene reduction originating from a depth of 7 mm will only be detectable after incubations of 7 h. These calculations clearly point out that a vertical distribution of nitrogen fixation activity skewed towards the deep end can lead to a significant underestimation of the rates measured using whole cores, and that this will be relieved to a large extent by using slices 1–3 mm in thickness. A more detailed analysis of this possible bias is obviously needed in future studies. As shown in Fig. 4, this is the case for the vertical distribution of nitrogenase activity, where peaks of activity in light crusts are much deeper than those in dark crusts. This finding underscores the need to take into account microbial small-scale architecture in natural communities where active turbulent transport is restricted. Thus, we believe that the estimates obtained by integration of vertical profiles are more accurate than those obtained from whole core measurements. One can also surmise from the vertical profiles of diazotrophic activity that N_2 fixation activity in light crusts may have an important heterotrophic, non-cyanobacterial component, as peaks of cyanobacterial populations and N_2 fixation do not coincide in space as determined by vertical profiles of chlorophyll *a* (Garcia-Pichel *et al*., 2003), even though alternative explanations are also possible. This would be in agreement with recent molecular work demonstrating the large diversity of eubacterial *nif* genes found in BSCs (Steppe *et al*., 1996).

In spite of the uncertainties associated with relating acetylene reduction to actual N_2 reduction by nitrogenase (Belnap, 2001), it seems clear that, when wet, our samples displayed high areal rates of N_2 fixation. These were comparable to, if not greater than, those measured in other very productive ecosystems such as tropical forests and salt marshes (Jordan *et al*., 1983; Schlesinger, 1996).

The importance of aerobic ammonium oxidation in BSCs

AAO, the first step in nitrification, uses oxygen as an electron acceptor for the oxidation of ammonium to nitrite and is a significant transformation in the cycling of nitrogen in many nutrient-rich soils. We demonstrated here that the process is also important within BSCs at the small scale. Recoverable populations of AOB within the crusts themselves were comparable to those found in estuarine sediments, farm soils and grasslands soils (Bruns *et al*., 1999; Phillips *et al*., 2000a; Hesselsøe *et al*., 2001). They peaked 2–3 mm below the surface, where sufficient oxygen is still present but penetration of solar radiation and speed of desiccation are lessened (Garcia-Pichel and Pringault, 2001). We are aware that the culturable portion of the AOB community may only be a small fraction of the actual population. A previous work (Hermansson and Lindgren, 2001) using real-time polymerase chain reaction (PCR) found actual populations of AOB to be 10- to 100-fold greater than those culturable. Rates of AAO, as exemplified by *Nitrosococcus oceanus*, are typically 2.16×10^{-8} μmol per cell h^{-1} (Ward, 1987). To account for the rates measured here in view of such cell-specific rates, the true population size in our crusts must also have been underestimated by some two orders of magnitude. Not only were the rates of potential ammonium oxidation high in wet crusts regardless of crust type, but the actual rates in the absence of added ammonium remained high for hours after wetting (Table 3) as well. Potential rates can clearly be an overestimation, but they are interesting because they allow the comparison of activity in BSCs with published values in other environments. In cultivated agricultural soils, where ammonium oxidation is regarded as a major nitrogen transformation, potential rates typically vary in the range of 10–30 nmol N h^{-1} g^{-1} soil (Højberg *et al*., 1996; Badía, 2000; Phillips *et al*., 2000a,b), a range that includes our determinations. Rates in arid, gypsiferous soils are reported at around 6 nmol N h^{-1} g^{-1} soil (Badía, 2000) and below 1 nmol N h^{-1} g^{-1} soil in flooded alkaline soils (Chen *et al*., 1998). If our rates are converted to volumetric units, residence times for ammonium in the porewater can be constrained. These residence times are in the order of 15–30 h, based on outputs due to AO alone, indicating the strength of nitrogen cycling within the crusts. More importantly, perhaps, is that the areal rates for actual ammonium oxidation measured in intact crusts are very close to the input rates of N_2 fixation measured in the same set of samples (Table 3). In a first reading, this implies that most of the net biological inputs of reduced nitrogen in the system must be transformed into oxidized nitrogen within the bounds of the desert crust themselves in a spatially coincident manner. It is well known, however, that the relationship between estimates of N_2 fixation rates obtained by the acetylene reduction assay and direct estimates using stable isotope tracers can be notoriously divergent and system dependent. This implies that a significant uncertainty remains in the absolute ratio of inputs and outputs in the reduced nitrogen pools in the system. In any event, it can be said with confidence that AAO represents a significant cycling process in the crusts, and studies using ^{15}N will be necessary to obtain a finer picture of this balance. Recent work on ammonia volatilization (another possible output process for reduced nitrogen) in BSCs from the same area yielded rates ranging from 0.18 to 0.95 μmol NH_3 m^{-2} h^{-1}, with no statistically significant differences between crust types (Barger, 2003). This range is about two orders of magnitude smaller than our rates for ammonia oxidation (Table 3), indicating that nitrification, not volatilization, is the major avenue for reduced N loss from the system. There was a good match (in both

crusts) between areal rates obtained from vertical profile integration of potential rates (which were incubated in the dark), as corrected for anoxia, and the actual rates in intact crust incubated in the dark. This is evidence that, once the availability of oxygen is taken into account, no other limiting factor is significant for AAO. By extension, one can infer that the supply of ammonium in wet BSCs is sufficient, as was surmised from the dynamics of porewater concentration, and that it is oxygen that limits the rates of AAO in BSCs. Interestingly, actual AO rates in intact crusts incubated in the light were higher than those obtained for incubations in the dark (Table 3). The difference was not significant for either light BSCs ($P = 0.26$) or dark BSCs ($P = 0.09$). Light is generally considered to be an inhibitor of AO in aquatic habitats (Hooper and Terry, 1973; Hyman and Arp, 1992; Kaplan *et al*., 2000) but, in benthic environments and biofilms, it has been shown to have an enhancing effect (Eriksson, 2001). Mechanistically, it is possible that a direct coupling is created between photosynthesis and AO, either through an enhancement of N_2 fixation or, as has been shown previously (Garcia-Pichel and Belnap, 1996), by the increased oxygenation levels within the crusts caused by photosynthetic activity.

Conclusions and outlook

Our results of small-scale analyses in BSCs indicate that AAO is an important process in the biogeochemistry of BSCs, with populational and activity parameters more typical of mesic soils than those of bulk arid soils. It is restricted in space to a thin subsurface layer some 1–3 mm thick; it is limited by the availability of oxygen, not ammonium, in these communities that create internal oxic microenvironments. Rates of AAO are similar in magnitude to N_2 fixation inputs, but more refined studies are still needed to obtain robust estimates. AO should be considered as a potential link to explain the continued nitrogen limitation of BSCs in particular and arid environments at large, but the immediate and ultimate fate of the oxidized products has yet to be determined. Possibilities to be considered include export to the bulk soils with percolating water, uptake by plant roots outside the crusts bounds and *in situ* use in denitrification.

Experimental procedures

Soil sample preparation

We studied two distinct crust types representing end-members in the compositional variation of cyanobacterial populations. These were collected from two separate sites outside the town of Moab, UT, USA. All samples of *Microcoleus vaginatus*-dominated BSCs (hereafter called light crust) were collected from the 'Slick Rock' site (GPS: N38°34′-W109°31′), and samples of *Nostoc* spp./*M. vaginatus*-dominated BSCs (hereafter called dark crust) were collected from a site known as 'Sunday Churt' (GPS: N38°38′-W109°39′). All samples were taken from soil wetted with distilled water and stored dry until experimentation (Garcia-Pichel *et al*., 2003).

Cores of crusted soil (5–7 cm deep) were taken with a 2.5 cm (internal diameter) PVC corer. For all experiments designed to obtain vertical profiles at subcentimetre resolution, the cores were sliced into millimetre-thick horizons with a microtome. For this, cores were embedded with ≈ 20 ml of a 1.5% solution of Bacto agar (Sigma) in distilled water. After setting, a subcore was taken from it with a 1.5 cm internal diameter plastic corer. The subcore was then extruded using a piston on to a table microtome, and the top centimetre was sliced into six horizons (from top to bottom: three horizons of 1 mm in thickness, two horizons of 2 mm thickness and one horizon of 3 mm thickness). Each single soil horizon was placed into a tube containing 3.2 ml of 25% Ringer's solution (Lorch *et al*., 1995) and vortexed briefly. Each tube was then treated with 16 units of β-agarase (Sigma), and the dissolution of the agar was carried out for 20 min in a 37°C water bath. If the horizon sample was to be used for culturing, 6 mg of sodium pyrophosphate was added as a disbursing agent (Lorch *et al*., 1995); the sample was then vortexed and allowed to settle for another 20 min at room temperature.

Two other sample types were collected: those for testing of porewater chemistry and those for microsensor studies. Samples taken for bulk chemical analyses were collected using small (15 × 60 mm) Petri dishes. Samples to be used for microsensor analyses were collected using custom-made open-bottom Plexiglas boxes (6.0 cm wide × 9.5 cm long × 5.75 cm tall). The box was pushed at least 5 cm into the soil, providing needed depth, and a metal sheet base was inserted from the side in order to lift the intact contents of the box.

Standard analysis of bulk soil

Bulk soil characteristics were done commercially (Soil and Plant Analysis Laboratory at Brigham Young University) on 200 g samples of mixed topsoil taken from the top 0–10 cm of soil. The results of these analyses are shown in Table 1.

Determination of nitrogenous compounds in the porewater of intact BSCs

Intact pieces of both crust types were weighed and placed into incubation dishes constructed from 15 × 60 mm Petri dishes. Each incubation dish was designed to allow the porewater to be pulled through into a collection chamber upon application of vacuum. This was achieved by melting several small holes in the bottom of one dish and fixing it over an inverted lid, sealing the junction with silicone, so that each set-up contained an upper container and a lower collecting chamber. To allow for removal of the porewater from the collecting chamber, a single hole was melted into the bottom of the chamber and sealed watertight with silicone. Sampling from the collecting chamber was with a syringe, the needle of which was inserted through the silicone seal. In order to

ensure particulate-free samples, two washed Whatman GF/F filters were included in the set-up, the first on the bottom of the sample container, and the second placed in line with the syringe.

Each crust sample was wetted with 20 ml of distilled water and incubated for various periods of time (0, 6 or 12 h). In order to account for some of the intrinsic heterogeneity of the soils, three separate samples were incubated and extracted for each of the crusts and for each time point. The porewater was then removed by applying gentle vacuum with the syringe. Between 75% and 95% of the added water could be recovered with this procedure. The porewater sample was then split three ways for determination of nitrogenous compounds. Nitrate and nitrite were determined colorimetrically as a combined value by the hydrazine sulfate reduction method (Rand *et al*., 1975) while ammonium testing was carried out using the phenohypochlorite method, as described previously (Solórzano, 1969). Total dissolved nitrogen (TDN) was analysed using a Lachat Quick Chem 8000 flow injection analyser with in-line digestion. The concentrations of organic nitrogen were calculated by subtracting the inorganic nitrogen (NH_4^+ and $NO_2^- + NO_3^-$) from the TDN, after all values had been converted to equivalent units based on N atoms.

Rate of N_2 fixation

Acetylene reduction assays were carried out as described previously (Jeffries *et al*., 1992), by incubating the samples in clear plastic tubes in the light (around 350 μmol photons m^{-2} s^{-1}) under acetylene and measuring the evolution of ethylene in the gas phase using a gas chromatograph. Samples taken for acetylene reduction assays were measured ≈ 72 h after collection; during the intervening time, they were kept dry and in the dark. Both intact soil cores and sectioned cores were preconditioned by leaving the samples wet and in the light for 2 h before testing. Rates for intact soil cores were determined as originally described. For sectioned cores, each individual horizon was incubated separately. To mimic *in situ* illumination conditions, the intensity of the illumination was reduced according to their original position in the typical light gradient, which has been published previously (Garcia-Pichel and Belnap, 1996): by 90% for the second horizon or in the dark for the remaining subsurface horizons. Four replicate vertical profiles and four intact core determinations were done for each type of BSC.

Quantification of ammonium-oxidizing bacteria (AOB) populations by MPN

Microplate MPN analysis was carried out as described previously (Rowe *et al*., 1977) for the quantification of AOB populations, with the following minor modifications. The medium used was 'ATCC *Nitrosolobus* medium', formulation #929 (http://www.atcc.org), containing 10 mM ammonium (in the form of NH_4SO_4) as substrate. Additionally, the volume of liquid in each well was increased from 50 μl to 100 μl to help against desiccation during incubation. While the soil slicing procedure does add a small amount of sugar to the solution, the amount is negligible (0.03%) and so should not affect the counts. Six independent replicate profiles were carried out for each of the BSC types.

Quantification of ammonium oxidation rates

Potential nitrification rates in BSC samples were measured using the colorimetric determination of nitrite after incubation in the presence of sodium chlorate ($NaClO_3$; an inhibitor of nitrite reduction) on slurried samples with added NH_4SO_4 so that neither substrate, O_2 nor NH_4^+, was limiting. The method followed was a modified version of that described by Berg and Rosswall (1985). Incubations were carried out in 75 mM $NaClO_3$ and 1 mM NH_4SO_4. Aliquots from the incubations were taken, mixed with 500 mM KCl and frozen until analysis. Once thawed, samples were centrifuged at 13 000 *g* for 10 min to pellet debris. For determination of nitrite, the supernatant was incubated at room temperature for 15 min with 60 mM NH_4Cl (pH 8.5) and a colour reagent (Berg and Rosswall, 1985) for NO_2^- determination. Their absorption at 520 nm was compared with a standard nitrite curve. Both whole crusts and crusts sectioned into horizons were analysed for potential ammonium oxidation rates. Whole soil samples (2 cm deep) were individually slurried, as were individual vertical horizons, then incubated with the $NaClO_3$/NH_4SO_4 solution. Both incubations were carried out under the same conditions: 10 h, on a shaker, in the dark, at room temperature. Data from each of the analyses represent the mean of three individual samples for each soil type, where samples were taken at five time points during each incubation, giving four rates.

Actual AO rates with preservation of the community architecture, internal microgradients and based on endogenously generated ammonium were also determined by a modified chlorate inhibition assay. Here, intact BSC samples were placed into the double chamber dishes described above and incubated in $NaClO_3$ without added NH_4SO_4. This assay was carried out under illumination (300 μmol photons m^{-2} s^{-1}), for 24 h, at 30°C, with aliquots taken at 0 and 24 h (Berg and Rosswall, 1985). In separate experiments (not shown), 75 mM $NaClO_3$ did not affect crust photosynthetic activity, as judged by profiles of photosynthetically produced levels of oxygen supersaturation within the crusts (see below).

Oxygen microsensor profiling

Before each experiment, a soil sample containing an intact piece of BSC and at least 4 cm of soil below was wetted with distilled water so that a thin film of water remained on the surface. This water was replenished as necessary to avoid desiccation of the sample and the sensor. Only enough water was added to the samples to achieve field water-holding capacity and leave a minimally thin film on the soil surface, as we believed the soils to be rarely flooded under natural circumstances. Samples were exposed to ≈ 400 μmol photons m^{-2} s^{-1} light during experimentation. All sensors (Unisense) were inserted through the samples in 100–500 μm increments, depending on apparent rates of change, using a motorized micromanipulator (Microx; Precision Sensing). Four separate oxygen microsensor profiles were performed on different spots within each BSC type, using

commercial microoptodes and an electronic unit obtained from Precision Sensing. These sensors are fibre optic based, and their tips contain a fluorescent dye that can be quenched by oxygen (Klimant *et al*., 1995). The signal was calibrated against air-bubbled, fully oxygenated water (100% saturation) and N_2-flushed water (0% saturation).

Statistical analyses

Two-sample *t*-tests were used for direct comparison of data where appropriate and are represented by *P*-values.

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