Title: Importance of nitrogen fixation in soil crusts of southern African arid ecosystems: acetylene reduction and stable isotope studies

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Cyanobacterial soil crusts may be important in arid and semi-arid ecosystems because of their ability to fix atmospheric nitrogen (N_2). These crusts are very sensitive to trampling by animals, and their destruction can decrease ecosystem N inputs, affecting the productivity of the region. The objective of this study was to quantify the nitrogen-fixing activity in soil crusts during the wet season in southern African ecosystems using *in situ* acetylene reduction assays. The average acetylene reduction rates for each site ranged from 88 to 535 nmol m^{-2} h^{-1}, were highly variable, and were lower than previously reported for other arid areas. All soil samples showed acetylene reduction activity; however, soils with crusts supported higher rates than did "non-crusty" soils under litter, moss, or sand. High values of ^{15}N natural abundance ($\delta^{15}N$) indicated that processes other than N fixation were more important in the crusts than N fixation. For example, coupled nitrification/denitrification and ammonia volatilization or atmospheric deposition of ^{15}N-enriched nitrate or ammonium may have caused shifts in $\delta^{15}N$ within the soil crusts. The estimated annual N fixation rates ranged from 8 to 44 g N ha^{-1} $year^{-1}$, orders of magnitude lower than values estimated in other studies. The anomalous wet conditions experienced during the year of the study may have increased the temporal availability of soil mineral N and decreased N fixation rates. However, the presence of N fixation activity in all crusts analysed and their ability to survive at high temperature and after long dry periods may provide ecosystem resilience, facilitating ecosystem recovery after severe droughts.

Keywords: soil crusts; N fixation; Kalahari; acetylene reduction; isotopes; savanna

Introduction

Cyanobacterial soil crusts occur in semi-arid and arid regions throughout the world. Numerous studies have documented the importance of these crusts for stabilizing soils, increasing resistance to wind and water erosion (Campbell *et al.*, 1989; Eldridge, 1993; Belnap & Gillete, 1997, 1998), improving nutrient status of vascular plants adjacent to the crusts (Harper & Pendleton, 1993; Belnap & Harper, 1995), enhancing seedling establishment (St. Clair *et al.*, 1984), and providing a source of ammonium by biological nitrogen (N) fixation (Skarpe & Henriksson, 1986; Zaady *et al.*, 1998). Other N_2-fixing eubacteria and archaebacteria can also be present in soils and affect N_2 fixation rates (Sprent & Sprent, 1990).

Although soil crusts have been widely studied in many deserts of the world, their importance in Southern African drylands has not been well analysed. All of the nitrogenase activity (indicative of N_2 fixation) in the Nyslvley Nature Reserve, South Africa, was reportedly associated with legumes (Grobbelaar & Rosch, 1981; Zietsman *et al.*, 1988; Scholes & Walker, 1993), ignoring the possible role of cyanobacteria in N_2 fixation. Some studies on the Kalahari Desert reported the importance of cyanobacteria to these ecosystems (Shushu, 1996, 2000). Nitrogen fixation activity associated with cyanobacterial crusts and grass roots provided an N input of $1{\cdot}9\,kg\,ha^{-1}\,year^{-1}$ (Skarpe and Henriksson, 1986). Fires and trampling by animals generally decreased crust biomass, cover and diversity (Johansen *et al.*, 1982; Shushu, 2000). For example, the algal ground cover decreased from 13% in undisturbed areas to 8% and 4% in disturbed areas (Shushu, 2000). Recovery rates after disturbances were very slow, estimated on the order of 30–85 years, depending on the availability of inoculating material, intensity of disturbance, and conditions after disturbances (Belnap, 1993).

Nitrogen is an important limiting nutrient for plant production in arid and semi-arid ecosystems (Scholes, 1990; Hooper & Johnson, 1999), as indicated by the biotic and abiotic N pool sizes, low leaf N content and the withdrawal of N from senescent leaves (Ernst, 1975; Skujins, 1981). In addition, southern African drylands are subject to increasing land-use changes with unknown impacts on the biogeochemical cycling and productivity of the ecosystem. A quantification of the inputs of atmospheric N_2 by cyanobacterial N_2 fixation under field conditions is important for the estimation of the potential impacts of disturbances on the N budget.

Estimates of N_2 fixation by soil crusts in arid and semi-arid areas vary widely. It is difficult to compare these values because they have been reported with different units (from hourly to annual rates), and at different spatial scales (from cm^2 to ha). The assumptions considered to scale the values measured in the incubations to higher spatial and temporal scales are not always stated. Rychert & Skujins (1974) reported potential N_2 fixation rates in Great Basin deserts (South Curlew Valley, Utah) as high as $84\,g\,N\,ha^{-1}h^{-1}$, which can result in 10–100 kg N fixed $ha^{-1}\,year^{-1}$. Ranges of 10–30 $kg\,ha^{-1}$ per cultivation cycle have been reported in Senegal, $3{\cdot}3$–$9{\cdot}2\,kg\,ha^{-1}\,year^{-1}$ in the Sahel zone of Nigeria (Isichei, 1980), $1{\cdot}3\,g\,N\,ha^{-1}\,h^{-1}$ in semi-arid soils of Texas (Loftis & Kurtz, 1980), and up to $4\,g\ N\,ha^{-1}\,h^{-1}$ in the Sonoran Desert (MacGregor and Johnson, 1971). As much as $2\,kg\,ha^{-1}\,year^{-1}$ of fixed N_2 has been reported for the Negev desert (Zaady *et al.*, 1998), and $1{\cdot}9\,kg\,ha^{-1}\,year^{-1}$ for the Kalahari desert (Skarpe & Henriksson, 1986). Some studies reported acetylene reduction rates, a common technique to measure N_2 fixation, without extrapolating to the whole system. Ethylene (C_2H_4) production rates ranged between 10^5 and $3 \times 10^6\,nmol\,m^{-2}\,h^{-1}$ for Colorado and Negev deserts respectively (Eskew & Ting, 1978; Zaady *et al.*, 1998). Studies in Utah (Arches National Park and Canyonlands National Park) reported considerably lower ethylene production rates, which ranged between 20 and $180\,nmol\,m^{-2}\,h^{-1}$ (Belnap, 1996; Evans & Belnap, 1999).

Stable isotopes of N_2 and C can indicate the importance of N_2 fixation by cyanobacteria. Organic nitrogen derived from atmospheric N_2 has $\delta^{15}N$ values close to 0‰ (Nadelhoffer & Fry, 1994), which makes it possible to estimate the contribution of atmospheric N_2 to the crust biomass. Cyanobacteria possess a CO_2 concentrating mechanism which results in $\delta^{13}C$ signatures similar to those of plants with C-4 photosynthetic metabolism (−12‰) (Palmqvist, 1993; Maguas *et al.*, 1995). Because $\delta^{13}C$ of mosses is generally lower than −23‰, the isotopic signature of soil crusts has been used to identify the contribution of cyanobacteria to the crust carbon (Evans & Belnap, 1999). However, epiphytic algae associated with heterocysts, specialized N-fixing cells of certain cyanobacyteria, showed $\delta^{13}C$ values of −28 to −24‰ on the Orinoco River floodplain (Hamilton & Lewis, 1992).

The objective of this study was to estimate N_2 fixation activities in soil crusts from four relatively undisturbed semi-arid southern African ecosystems, based on *in situ* acetylene reduction assays. The $\delta^{13}C$ and $\delta^{15}N$ of soil crusts were also measured, in order to identify the contribution of atmospheric N_2 to the soil, and the importance of other N cycling processes.

Methods

Study sites

The *in situ* N_2 fixation rates were measured in four sites along a rainfall gradient, as part of a National Aeronautics and Space Administration (NASA)-supported SAFARI 2000 wet-season field campaign, in February–March 2000. The study sites are described in Scholes *et al.* (submitted). The first site, Mongu, was located in Zambia, with vegetation characteristic of Miombo woodlands. The main research site ("undisturbed") had a closed tree canopy cover, and no crusts were observed. Soil crusts were found in an adjacent site that had previously been used for cultivation and abandoned. The vegetation was recovering but the canopy cover was lower than in the "undisturbed" site. Soil crusts were abundant in the open patches of forest, with high light availability. The other three sites Maun, Okwa, and Tshane were located in Botswana and supported a vegetation typical of Mopane woodland, Acacia shrubland, and Acacia savanna respectively. Soil crusts were abundant in these three sites. In addition, soil crusts collected in previous campaigns in the Kalahari (Botswana) and Vastrap sand dunes (South Africa) were analysed for stable isotopes.

Acetylene reduction activity

Nitrogen fixation rates were estimated using the acetylene reduction assay (ARA), which is based on the ability of the nitrogenase enzyme to catalyse the reduction of N_2 to ammonium (nitrogen fixation), as well as the reduction of acetylene to ethylene (Bergersen, 1980). Acetylene gas was obtained from the reaction between calcium carbide and water, and was trapped in gas-sampling bags. A surface of 4·95 cm^2 of soil crust with a thickness of about 0·5 cm was taken with a syringe core, placed in 13 ml hungate tubes, wetted to field capacity and then covered with gas-tight septa. Ten percent of the tube air was replaced with acetylene using disposable syringes with stopcocks, and the septa were covered with silicone sealant. The soil crusts were incubated in the field for 48 h, after which 7–12 ml of air sample were removed to a second hungate tube, pressurizing it. The hungate tube septa were covered with silicone sealant and the tubes were stored upside down in water to reduce contamination with air. An additional set of tubes was incubated for only few minutes, and these air samples were used as "time 0" references (t_0). The ethylene

concentration of the air samples was measured in a Hewlett-Packard 5890 gas chromatograph, fitted with flame ionization detector, and Poropak R column. The oven and detector temperatures were 80°C and 220°C respectively. The samples were injected into 250 µl sample loops and calibrated with ethylene (Scott Specialty Gases, Plumsteadville, PA, USA). The ratio of ethylene produced to N fixed varies widely for different soils, microbial communities and environmental conditions. Conversion factors ranged from 1 to 15·7 for different soils and water contents in Sweden (Nohrstedt, 1983), and from 0·022 to 4 for different microbial communities in the high Arctic (Liengen, 1999). However, existing literature commonly reports N_2 fixation applying the theoretical 3:1 ratio (Rychert & Skujins, 1974; Ischei, 1980; Skarpe & Henriksson, 1986). For the purpose of comparison, this ratio was also used in this study to calculate the amount of N fixed ($g\,N\,m^{-2}$). The number of replicates was eight for Mongu, Maun, and Okwa, and 15 for Tshane. However, owing to the heterogeneity of the soil, the replicates were separated into different blocks, according to the visual characteristics and spatial distributions of the crusts, including location under grasses, bushes or trees, or according to their color. Data were logarithmically transformed in order to normalize them for statistic tests. Statistical differences between shaded and non-shaded crusts and between crusts and other surfaces (sand, soil under litter, and moss) were analysed with *t*-tests at $p = 0{\cdot}01$ and $0{\cdot}05$.

The relative amount of crust cover was estimated in Mongu, Maun, and Okwa, by visually determining the presence or absence of crusts along random transects. Although no transects were run in Tshane, the crust cover was comparatively high, so a crust cover of 40% was assumed for this site. However, the crust covers varied within the period of study following rain events. The amount of N fixed per hectare was estimated using the percent crust cover obtained from the transects. The number of rainy days at each place obtained from Tyson & Crimp (2000) was assumed to represent half the number of days during which the crusts were at field capacity, because soil crusts can rapidly take up water, swell to several times their original volume and retard their dehydration rate (Campbell *et al.*, 1989).

Isotopic analysis

The crust samples used for the ARA were analysed for isotopic analysis of C and N ($\delta^{13}C$ and $\delta^{15}N$). In addition, the cyanobacteria of eight composite crust samples were separated from the sand matrix by sonication and subsequent centrifugation, to analyse the algal component for $\delta^{13}C$ and $\delta^{15}N$. These analyses were performed with a Micromass Optima isotope ratio mass spectrometer (IRMS) coupled with an elemental analyser (EA), with an overall precision better than 0·5‰. The data are reported relative to a standard, and expressed in δ notation as

$$\delta_{\text{sample}}\ (‰) = (R_{\text{sample}}/R_{\text{standard}} - 1)1000$$

where δ_{sample} represents either $\delta^{13}C$ or $\delta^{15}N$, and R is the molar ratio of the heavier to the lighter isotope for the standard or sample. The standards are atmospheric N_2 for N and PeeDee Belemnite for C, and are defined to be equal to 0·0‰ (Hoefs, 1997).

KCl-extractable soil NH_4^+ and NO_3^-

At the study sites of the 2000 field campaign, composite samples (eight samplings) were taken from the top 5 cm of soil and sieved through a 2-mm sieve. Three 20-g samples were removed from the composite sample and extracted with 2-M KCl in the field. The soil was weighed into a whirl-pack (Nasco) bag and 60 ml of KCl (2 M) added. The sample was shaken and the soil allowed to settle over a 6-h period. The supernatant was filter-sterilized (0·45 µm, Gelman supor filter) and stored refrigerated

in sterile whirl-pack bags until returned to the United States for analysis. Ammonium was determined colorimetrically by an automated indophenol method, and NO_3^- by copperized cadmium reduction in combination with diazotization. All were analysed using an Alpkem "Flow Solution" autoanalyser, equipped with a model 510 spectrophotometer.

Results

Acetylene reduction rates (indicative of nitrogenase activity and N_2 fixation) were highly variable in the samples, but all the incubations yielded higher rates than the "time 0" references. The crust samples supported significantly higher rates ($p = 0{\cdot}01$) than "non-crusty" soils under the litter, moss, and sand surfaces (Fig. 1 and Table 1).

The average acetylene reduction rates for crusts shaded by trees, shrubs or grasses was 546 nmol m^{-2} h^{-1}, whereas it was 89 nmoles m^{-2} h^{-1} for crusts located within the canopies, but with no shading. However, the difference was not significant ($p = 0{\cdot}05$). In Mongu, acetylene reduction activity was higher in a disturbed site with higher light availability and lower plant cover than in the relatively undisturbed site. The high variability in acetylene reduction rates between the crust samples was not related to the $\delta^{15}N$ values of the crusts (Figure 2).

The % crust cover was between 30% and 40% in Maun and Okwa, and a value of 40% was assumed for Tshane, based on visual estimates. The undisturbed site at Mongu was covered by moss or litter, with less than 5% of bare soil, while the disturbed site had a bare soil and crust cover of 40% (Table 2). However, the crust cover increased greatly after a rain event. The estimated annual N fixation rates, calculated from average values of the ARA for each site, percent crust cover and number of rain days, ranged from 8 to 44 g N ha^{-1} $year^{-1}$ (Table 1).

The $\delta^{15}N$ values of the crusts were higher than what is expected if all N was derived from atmospheric N_2 fixation. The $\delta^{13}C$ signatures were between typical values of plants with C-3 and C-4 photosynthetic metabolism (Table 2).

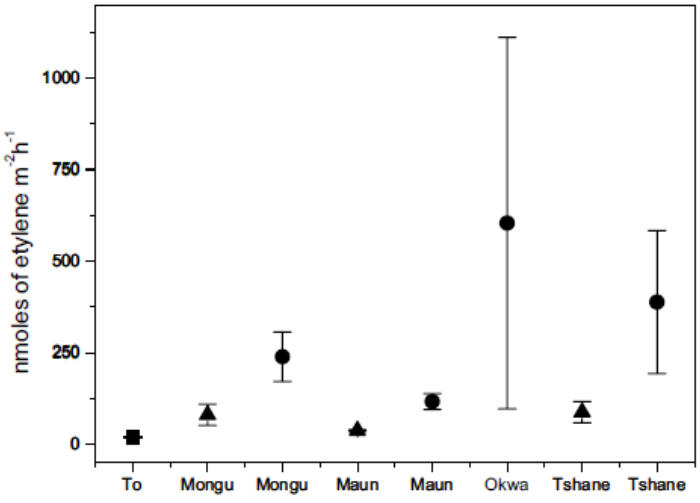

Figure 1. Nitrogense activity of soil crusts and "non-crusty" surfaces in four sites of Southern Africa: (■) "time 0" reference; (▲) non-crusty surfaces; (●) soil crusts. The symbols are average values and the bars are standard errors.

Table 1. *Average nitrogenase activity (nmol ethylene fixed $m^{-2} h^{-1}$) and annual N_2 fixation rates (g N fixed ha^{-1} $year^{-1}$) for different sites and soil surfaces*

Site and crust characteristics	nmol ethylene fixed $m^{-2} h^{-1}$
Mongu crusts	239 (117)
Mongu under muss	52 (8)
Mongu open sand	138
Maun crusts	117 (44)
Maun under litter	38 (3)
Okwa crusts	535 (1149)
Tshane crusts	388 (647)
Tshane non-crusty sand	88 (65)
Time 0 reference	20 (3)
Site	**g N fixed ha^{-1} $year^{-1}$**
Mongu	8
Mongu disturbed	30
Maun	14
Okwa	44
Tshane	37

Nitrogen fixation rates were calculated considering the % crust cover and number of rain days at each site. Numbers in parentheses denote standard errors for $n \geq 3$.

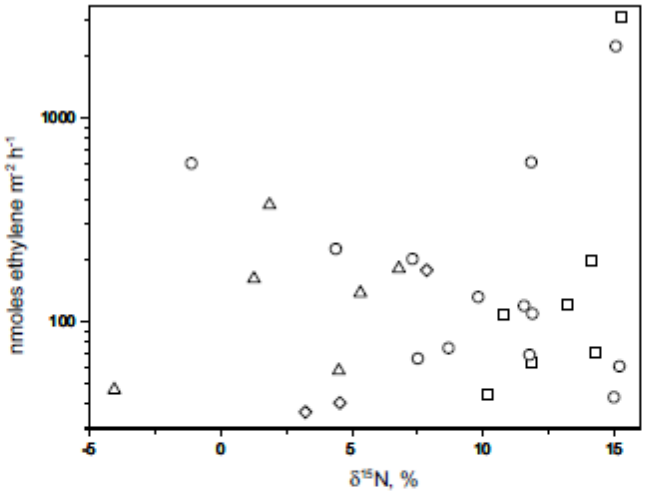

Figure 2. Relation between $\delta^{15}N$ and nitrogenase activity of samples from Mongu (△), Maun (◇), Okwa (□), and Tshane (○).

The mineral N concentrations (ammonium and nitrate) were higher below than between plant canopies, and the highest values were present at Tshane, the most arid site. These ammonium and nitrate concentrations are similar to those reported for dry savannas with sandy soils (Cardenas *et al.*, 1993; Parsons *et al.*, 1996).

Table 2. *Site characteristics, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of soil crusts, and soil mineral N from Zambia (Mongu), Botswana (Maun, Okwa, Tshane, and Kalahari), and South Africa (Vastrap)*

Site	Lat. and long.	% crust cover	Year collected	$\delta^{13}C$ of crusts (‰)	$\delta^{15}N$ of crusts (‰)	NH_4^+ ($\mu g N g soil^{-1}$)	NO_3^- ($\mu g N g soil^{-1}$)
Mongu	15·43S 23·25E	Undisturbed site: < 5%; abandoned field: 30–40% of sand and crust cover	2000	−23·5 (0·6)	3·8 (1·8)	Undisturbed: 1·2 Abandonded field: 0·7	Undisturbed: 0·4 Abandonded field: 0·7
Maun	19·92S 23·59E	White crusts: 22% Brown crusts: 20%	2000		7·8	U. canopy: 1·6 U. crusts: 0·4 B. canopy: 0·4	U. canopy: 4 U. crusts: 0·7 B. canopy:0·7
Okwa	22·40S 21·71E	Brown crusts: 29%	2000	−24·5 (1·2)	12·8 (0·7)	U. canopy: 1·1 B. canopy: 0·4	U. canopy: 1·9 B. canopy: 0·4
Tshane	24·16S 21·89E	No data available Assumed value of 40% based on observations	2000	−24·1 (1·9)	9·9 (1·3)	U. canopy: 2 B. canopy: 0·8	U. canopy: 7·2 B. canopy: 0·7
Tshane isolated algal filaments	24·16S 21·89E		2000	−21·0 (0·5)	7·5 (0·6)		
Kalahari	24·22S 25·22E		1998	−19·7	14·5		
Kalahari	24·22S 25·22E		1998	−20·2	15·1		
Kalahari	24·28S 25·17E		1998	−19·7	19·8		
Kalahari	22·05E 25·17S		1998	−19·7	14·2		
Vastrap	27·75S 21·42E		1995	−17·4	3·7		

Numbers in parentheses indicate standard errors for $n \geq 3$. U = under; B = between.

Discussion

It is clear that soil crusts supported higher acetylene reduction activity than all other non-crusty surfaces such as moss, litter, or sand. The difference between crusts and non-crusty surfaces was significant ($p = 0{\cdot}01$). Acetylene reduction rates in the crusts were not related to $\delta^{15}N$, visual characteristics (dark or white crusts), or location of the crusts (under the shade of a canopy or within canopies). Although the difference between shaded and non-shaded crusts was not significant ($p = 0{\cdot}05$), acetylene reduction was generally higher in shaded crusts. The three highest values of acetylene reduction activity observed (3137, 2238, and 605 nmol $m^{-2} h^{-1}$) were found in samples shaded by shrubs or grasses. The incubation tubes may have acted as a "greenhouse", increasing the temperatures and inhibiting N_2 fixation, particularly in non-shaded samples. The optimum temperatures of acetylene reduction activity reported for different ecosystems ranged from 19°C to 23°C for crusts from the Great Basin Desert (MacGregor & Johnson, 1971), and from 30°C to 40°C for Nigerian crusts (Ischiei, 1980). Below and above those temperatures, acetylene reduction activity decreased by more than half and was inhibited above a threshold of 40°C. Cyanobacteria of the South African highveld can survive even at 75°C, although it is not known whether any N_2 fixation takes place at such a high temperature (Buzer *et al.*, 1985). Moisture and relative humidities also affect N_2 fixation rates. The Nigerian crusts started to fix N_2 within 24 h after rewetting, and the activity increased exponentially until at least 72 h (Ischiei, 1980). Relative humidities of 75% caused the maximum activity, while at less than 40%, no reduction took place (Ischiei, 1980). Although the optimum conditions have not been determined for the Kalahari soil crusts, it is possible that the incubation tubes altered them and the measured values underestimate the real N_2 fixation rates.

The average acetylene reduction activity of the crusts at each site ranged from 117 to 535 nmol $m^{-2} h^{-1}$ (Table 1). These values are much lower than previously reported for other arid areas (Table 3), representing the total activity over the two incubation days. The actual rates at any time during the day may be higher. Our results are similar within an order of magnitude to those from the Arches and Canyonlands National Parks, Utah (Belnap, 1996; & Evans & Belnap, 1999), which reported acetylene reduction rates of 20–180 nmol $m^{-2} h^{-1}$, measured during 4 h of incubation at 26°C and under light.

The high $\delta^{15}N$ values observed in the crusts suggest that other processes in addition to atmospheric N fixation affected their ^{15}N composition (Table 2). If most of the organic N was derived from N fixation, the $\delta^{15}N$ signature would be approximately 0‰ (Nadelhoffer & Fry, 1994). Soil crusts from Utah had $\delta^{15}N$ values of 0–4‰, even under disturbed conditions, and the surface soil $\delta^{15}N$ ranged from 4‰ to 6‰ (Evans & Ehleringer, 1993; Evans & Belnap, 1999). The $\delta^{15}N$ values of our samples indicate that the crust N is affected by other processes of the N cycle, including coupled nitrification/denitrification, ammonia volatilization, or atmospheric deposition of enriched nitrate or ammonium. Soil $\delta^{15}N$ of other sites in the Kalahari ranged from 1‰ to 15‰ (Aranibar *et al.*, accepted). Aerosols from the region had $\delta^{15}N$ values centered at 14·2‰ and as high as 21‰ in Etosha National Park (Namibia), and $8{\cdot}2 \pm 3{\cdot}9$‰ in Victoria Falls (Zimbabwe) (Swap, 1996; Swap *et al.*, 1996). Part of the fixed N_2 may be lost as gaseous N (NO and N_2O) by denitrification or nitrification, enriching the remaining crust N. Denitrification needs anaerobic conditions to occur, and can enrich the remaining nitrate by 15–20‰ (Mariotti *et al.*, 1981). Preliminary laboratory analysis of soils from South African savannas showed that 20% of the mineralized ammonium is nitrified and subsequently lost by denitrification when soils were at field capacity (Aranibar, unpublished data). Biogenic emissions of NO in Kruger National Park (South Africa), which can also enrich the remaining soil N, peaked after light rains or wetting events (Harris *et al.*, 1996; Levine *et al.*, 1996). The

Table 3 *Reported ethylene production rates (nmol $C_2H_4 m^{-2} h^{-1}$) and estimated nitrogen fixation rates for different arid and semi-arid areas*

Place	Characteristics	Ethylene production rates (nmol C_2H_4 m^{-2} h^{-1})	Nitrogen fixation rates, (kg N ha^{-1} $year^{-1}$)	Reference
Negev Desert	Macrophytic patches	$1{\cdot}8 \times 10^6$	$1{\cdot}8 \times 10^2$	Zaady *et al.* (1998)
	Microphytic patches	1×10^6	10^2	
Kalahari Desert	Dark crusts	$6{\cdot}8 \times 10^6$	$6{\cdot}85 \times 10^2$	Skarpe & Henriksson (1986)
	Light crusts	6×10^5	$6{\cdot}05 \times 10^1$	
Tucson, Desert	Algal crusts	$7{\cdot}8 \times 10^5$	$7{\cdot}9 \times 10^1$	MacGregor & Johnson (1971)
Utah, Great Basin Desert	Lab incubations	$3{\cdot}7 \times 10^4$	$3{\cdot}7$	Rychert & Skujins (1974)
	Field incubations	$6{\cdot}5 \times 10^3$	7×10^{-1}	
Colorado desert	Cyanobacteria-lichen crusts	2×10^4–$5{\cdot}8 \times 10^5$	2–$5{\cdot}8 \times 10^1$	Eskew & Ting (1978)
Semiarid soils of West Texas	Crusts at 56°C	1×10^4	1	Loftis & Kurtz (1980)
	Crusts at 22°C	$1{\cdot}7 \times 10^5$	17	
Camp Floyd and Rush Valley, Deserts, Utah	Grazed, burned and undisturbed sites	$1{\cdot}2 \times 10^3$–$7{\cdot}4 \times 10^4$	$1{\cdot}2 \times 10^{-1}$–$7{\cdot}5$	Terry & Burns (1987)
Kalahari Sands	Maximum value measured	3×10^3	$3{\cdot}2 \times 10^{-1}$	This study
Kalahari Sands	Okwa site	5×10^2	$5{\cdot}3 \times 10^{-2}$	This study
Kalahari Sands	Tshane site	$3{\cdot}9 \times 10^2$	$3{\cdot}9 \times 10^{-2}$	This study
Kalahari Sands	Mongu site	$2{\cdot}4$	$2{\cdot}4 \times 10^{-2}$	This study
Kalahari Sands	Maun site	1×10^2	$1{\cdot}2 \times 10^{-2}$	This study
Canyonlands National Park, Grasslands, Utah	Undisturbed site	5×10^1	5×10^{-3}	Evans & Belnap (1999)
	Disturbed site	2×10^1	2×10^{-3}	
Arches National Park, Cold Desert, Utah	Cyanobacterial crusts	4×10^1	4×10^{-3}	Belnap (1996)

The N_2 fixation rates were calculated assuming 30% crust cover, 3:1 ratio of C_2H_4 to N_2 fixed, and 150 days of active N_2 fixation per year.

anomalous wet conditions experienced during the year of study were likely to increase mineral N_2 availability since the beginning of the rainy season and thereby inhibit N_2 fixation, and increase anaerobic conditions that favor nitrification/denitrification (Bothe, 1982; Haynes, 1986). Although the mineral N concentrations measured in our study (Table 2) are typical of dry savannas with sandy soils (Cardenas *et al.*, 1993; Parsons *et al.*, 1996), they probably do not represent the conditions of the whole rainy season because mineral N concentrations are highly variable. The higher temporal availability due to more rainy days may be more important than the absolute concentrations during a single day. In addition, ammonium concentrations obtained with a different methodology yielded considerably higher values (Feral *et al.*, this issue). Crusts collected in a more typical year (1998) in the Kalahari showed similarly high $\delta^{15}N$ values (Table 2). Only the crusts of Mongu and Vastrap suggest an unambiguous and unaltered contribution of N_2 to the crust N based on $\delta^{15}N$ (Table 2).

Crusts collected in Vastrap, South Africa, in 1995 had the lowest $\delta^{13}C$ (−17·4‰), suggesting a CO_2 concentrating mechanism. Crusts from Mongu, Okwa, and Tshane have $\delta^{13}C$ similar to filamentous cyanobacteria from the Orinoco river floodplain, that ranged between −28 ‰ and −24‰ (Hamilton & Lewis, 1992). Crusts from the other sampling sites had $\delta^{13}C$ signatures similar to those reported for deserts in Utah (−21 to −19‰) (Table 2). The crusts analysed in our study had a wide range of $\delta^{13}C$, which implies differences in their CO_2 metabolism and probably the contribution of other micro-organisms besides cyanobacteria (Table 2).

In an attempt to compare this study with others from African and American deserts, annual N_2 fixation rates for the whole ecosystem were estimated (Table 1). Our values were much lower than those reported in other previous studies, with differences of several orders of magnitude (Rychert & Skujins, 1974; Isichei, 1980; Loftis & Kurtz, 1980; Skarpe & Henriksson, 1986; Zaady *et al.*, 1998). Even if the lowest conversion factor reported by Nohrstedt (1983) is used (ratio of ethylene to nitrogen of 1:1), the results would differ by orders of magnitude with other reported values. In our calculations (Table 1) we assumed the crusts to be active only during twice the number of rainy days, which is probably an underestimate because these crusts can rapidly take up water, swell to several times their original volume and retard the rate of dehydration (Campbell *et al.*, 1989). The crust cover also varies depending on the moisture conditions of the soil and the time since the last rain. After each rain, the crust cover and the darkness of the crusts clearly increased at our study sites. The estimated crust cover of 30–40% is higher than previously reported for the Kalahari (Skarpe & Henriksson, 1986; Shushu, 2000), probably because of the exceptionally wet conditions of the year of study. Nitrogenase synthesis and activity are inhibited by availability of a mineral N source (Bothe, 1982), which in this year was probably increased by cycles of drying and rewetting associated with the more frequent rains (Haynes, 1986). Thus, the N_2 fixation rates may have been lower than in other years. Annual N_2 fixation rates were calculated for different ecosystems, based on previously reported acetylene reduction activities (Table 3). Although different assumptions were used to report annual N_2 fixation rates in the original publications, we considered the same assumptions for all the sites (3:1 ratio of ethylene to N_2 fixed, 30% crust cover, and 150 days during which the crusts were active), in order to show the variability of the data reported. The acetylene reduction rates varied by 5 orders of magnitude in these studies. This is reflecting not only ecosystem characteristics, but also different incubation conditions (temperature, light, moisture, crust volume, and incubation time). In order to get accurate annual ecosystem N_2 fixation rates, temporal variability of temperature and moisture in the ecosystem as well as the dynamics of nitrogenase activity after rewetting must be considered.

Nitrogen fixation by soil crusts was lower in our study than in other deserts (Table 3) (Rychert & Skujins, 1974; Eskew & Ting, 1978; Isichei, 1980; Loftis & Kurtz, 1980;

Skarpe & Henriksson, 1986; Zaady *et al.*, 1998), with the exception of deserts and grasslands in Utah (Belnap, 1996; Evans & Belnap, 1999). Other processes likely altered the isotopic signatures of the crusts, including ammonia volatilization, coupled nitrification/denitrification, atmospheric deposition or uptake of soil mineral N. These soil crusts are so resistant and widely distributed that they may play a role in providing resilience of the ecosystem to environmental events, especially where N-fixing plants are not abundant. Crust recovery after disturbances such as trampling and frequent fires is slow, on the order of decades (Belnap, 1993), and can have negative effects on ecosystem resilience. The nitrogenase activity present in the crusts from Vastrap (Aranibar, unpublished data), even after storage for 4 years, demonstrated the capacity of these crusts to recover from water stress. Crusts in the Miombo woodlands are more abundant in patches of low vascular plant cover, which result from low-frequency fires (Belnap *et al.*, 1997). In the Miombo woodland site (Mongu, Zambia) we found soil crusts only in disturbed areas (cultivated fields that had been abandoned), suggesting that crusts may contribute to the recovery of disturbed soil ecosystems. Nitrogen fixation rates by soil crusts following disturbances, even if they are low, can provide the necessary N-inputs to allow plant establishment, especially in the Kalahari sands, where N_2-fixing plants are limited (Frost, 1996; Aranibar *et al.*, accepted). In the sites located in Botswana, N_2-fixing vascular plants were restricted to some forbs of low biomass and cover, and none of the *Acacia* species presented evidence of N_2-fixation (Aranibar *et al.*, accepted). This study shows the presence of nitrogenase activity in all the soil crusts analysed in several sites of Botswana, Zambia, and South Africa, but N supplied by soil crust N_2-fixation was not shown to be quantitatively important. The period of study was extremely wet, especially in the more arid areas, so it may represent the lowest threshold of soil crust N_2 fixation in decades. However, the impacts of cyanobacteria in providing N inputs following periods of environmental stress may be crucial to maintaining ecosystem stability in semi-arid and arid southern Africa. Studies of N_2 fixation in dry years and along land-use gradients, including temporal variability, would help us to assess the importance of soil crusts in ecosystem recovery and stability.

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