Potential environmental controls on nitrogenase activity in biological crusts of the northern Chihuahuan Desert

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We used the acetylene reduction assay to analyse soil nitrogenase activity at the Jornada Long-Term Ecological Research site (northern Chihuahuan Desert, New Mexico, U.S.A.). A three-day irrigation and fertilization experiment showed that water and carbon (glucose) enhanced nitrogenase activity, while NH_4NO_3 reduced activity. Micronutrient (Mo, Fe, Co) and phosphorus fertilization had no significant effect. A survey of Jornada Basin soils did not reveal an inverse relationship between nitrogenase activity and the ratio of available nitrogen (N) to phosphorus (P), as hypothesized; however, the highest rates of nitrogenase activity were detected in tarbush floodplain soils with a low ratio of available N to P. These findings suggest that labile carbon and inorganic N may exert a stronger control on nitrogenase activity than phosphorus or micronutrient levels.

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Introduction

Net primary productivity is often limited by nitrogen availability in terrestrial ecosystems (Vitousek & Howarth, 1991). During desertification of semi-arid grasslands, this limitation may intensify (Dregne, 1983), but relatively little is known about the factors affecting the nitrogen status of desert soils. Atmospheric deposition in deserts of the south-western U.S. is estimated at $3\,kg\,N\,ha^{-1}\,year^{-1}$ (Peterjohn & Schlesinger, 1990), while estimates of N_2 fixation by soil micro-organisms range from <1 to $100\,kg\,N\,ha^{-1}\,year^{-1}$ (West, 1990). Thus, N_2 fixation in surface soils may contribute a significant fraction of the total nitrogen input to arid ecosystems.

Vitousek & Howarth (1991) proposed several mechanisms that regulate biological N_2 fixation and potentially explain which factors influence the contribution of

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N_2-fixing organisms to nitrogen levels in the biosphere. First, N_2 fixation may be limited by energetic constraints. Symbiotic N_2 fixers are limited by metabolites available from their host organism, while asymbiotic N_2 fixers depend on soil carbon levels which are generally low in arid ecosystems (Granhall, 1981; Post *et al.*, 1985). N_2 fixation may be limited by soil nitrogen, phosphorus or micronutrient supplies (Jurgensen, 1973). In arid ecosystems, phosphorus may be unavailable for biotic uptake because it often precipitates as calcium phosphate (Lindsay & Vlek, 1977). Walker & Syers (1976) proposed that phosphorus availability controls the input of nitrogen to terrestrial ecosystems, and the relative availability of nitrogen (N) and phosphorus (P) regulates cyanobacterial activity in some ecosystems. This relationship has been studied intensively in aquatic ecosystems (Redfield, 1958; Schindler, 1977; Cole & Heil, 1981; Howarth *et al.*, 1988), but only rarely in terrestrial ecosystems (Smith, 1992). Eisele *et al.* (1989) demonstrated a strong inverse relationship between the ratio of available N to P and both the growth and nitrogenase activity of cyanobacteria in prairie soils. This study examines the extent to which soil moisture, carbon, nitrogen, phosphorus and micronutrients regulate nitrogenase activity in biological soil crusts of the Jornada del Muerto Basin in southern New Mexico, U.S.A.

Materials and methods

This study was conducted at the Jornada Long-Term Ecological Research (LTER) site, 40 km NNE of Las Cruces in the Jornada del Muerto Basin of southern New Mexico. The basin is located in the northern Chihuahuan Desert (32°37′N, 106°40′W). Study sites were located on the basin floor at approximately 1200 m elevation. Mean annual precipitation is 23 cm with 52% falling in local, convective storms during the summer rainy season (July–September). The mean maximum air temperature of 36°C is typically recorded in June; the mean minimum temperature is 13°C in January.

During the past century, much of the native black grama grassland has been replaced by shrubland communities in the Jornada Basin (Buffington & Herbel, 1965). Putative causes of desertification in this region are short-term drought and overgrazing by domestic livestock (Schlesinger *et al.*, 1990). The dominant plant communities in the Jornada del Muerto Basin are: (1) black grama grasslands (*Bouteloua eriopoda*), shrubland communities dominated by (2) creosote (*Larrea tridentata*), (3) mesquite (*Prosopis glandulosa* or (4) tarbush (*Flourensia cernua*) and (5) mixed grassland communities in topographic depressions (swales dominated by *Pleuraphis mutica* and *Scleropogon brevifolius* and ephemeral lake beds or playas dominated by *Panicum obtusum* and *Pleuraphis mutica*).

Soil physical and chemical characteristics are summarized in Table 1. Black grama grassland and creosote shrubland soils are coarse-textured, derived from quartz monzonite alluvium from the Doña Ana Mountains (Wierenga *et al.*, 1987; Lajtha & Schlesinger, 1988). Mesquite shrubland soils are eolian deposits with > 90% sand (Virginia *et al.*, 1992). Playa grassland and tarbush shrubland soils are derived from ancient Rio Grande floodplain deposits and have the highest clay content. Soils at two of the mesquite sites lack diagnostic horizons, and are classified as Entisols. The remaining soils are Aridisols, which typically have an argillic horizon near the surface subtended by a calcic, petrocalcic or cambic horizon (Gile *et al.*, 1981).

Biological soil crusts host N_2-fixing micro-organisms, including free-living bacteria and cyanobacteria, and symbiotic cyanobacteria in lichens. Free-living aerobic N_2-fixing bacteria such as *Azotobacter* are widely distributed in desert soils of the southwestern U.S. (Fuller & Hanks, 1982), and they have been cultured from Jornada soils (R.P. Herman, pers. comm.). Free-living cyanobacteria (e.g. *Nostoc commune*) are

Table 1. *Physical and chemical properties of Jornada soils*

Ecosystem	% Sand*	% Clay*	pH in H$_2$O*	Depth (cm)*	% Organic C[†]	% CaCO$_3$[†]
Black grama grassland	68·5	10·0	6·8	0–15	0·3	0·03
Creosote shrubland	71·0	7·5	7·6	0–18	0·4	0·02
Mesquite shrubland	90·0	7·0	7·8	0–81	0·3	0·13
Tarbush shrubland	66·0	18·0	7·9	0–15	0·5	0·63
Playa grassland	18·0	51·0	7·9	0–8	1·3	0·28

*Upper horizon data from Virginia & Jarrell, unpub (sampling depth variable).
[†]Carbon data published in Gallardo & Schlesinger (1992); soils sampled to 10 cm.

sparsely distributed in the grassland and playas, but are frequent on floodplain soils that support tarbush. Dark pedicelled lichen crusts are also a prominent feature of tarbush soils, hosting cyanobacterial lichens of the genera *Collema, Psora, Peltula* and *Heppia* (P. Claire, pers. comm.).

Nitrogenase activity in soil crusts was measured by ethylene production from acetylene during lab incubation (Hardy *et al.*, 1968). The following protocol was used to analyse nitrogenase activity. Before soils were sampled, 1 l of deionized water was sprinkled into 22·5-cm diameter PVC rings to simulate a 2-cm rain. Twenty-four hours after watering, a soil core was collected randomly within each ring (6·4-cm diameter, 3-cm deep), transferred to a 237-cm^3 mason jar, then transported to New Mexico State University. The same day, soils were weighed in the laboratory and remoistened with approximately 6·3 ml H$_2$O per sample. Jars were sealed and 10% acetylene injected into the headspace. Headspace samples were collected in 2-ml Vacutainer® vials and stored at room temperature until analysis. Ethylene (C$_2$H$_4$) concentrations were measured on a Varian Gas Chromatograph (Model 3700) using a flame ionization detector and a 180-cm × 0·3-mm Poropak N column with He as a carrier gas. After gas analysis, soils were passed through a 2-mm screen. Inorganic P was extracted from a 2-g subsample in 0·5 N NaHCO$_3$, adjusted to pH ≈8·5 (Olsen *et al.*, 1954). Inorganic N (NH$_4^+$ and NO$_3^-$) was extracted from a 5-g subsample in 2 N KCl. Soil extracts were analyzed colorimetrically on a TRAACS autoanalyser (Bran & Luebbe, 1986). We calculated a ratio of available N to P using KCl-extractable inorganic N (NH$_4^+$ + NO$_3^-$) divided by bicarbonate-extractable P.

Nitrogenase activity was measured in light- *vs.* dark-incubated tarbush soils. Starting 1–2 h after soil sampling, ten soil cores each were incubated in the dark (25°C) and in the light (12·5 h day-length, 150 PPFD, 25°C day, 20°C night) in a growth chamber. Headspace samples were collected at 15 and 30 h.

On June 15 1991, we surveyed soils throughout the Jornada del Muerto Basin. Five soil cores were removed at five sites in each of the five major plant communities in the Jornada Basin. Soils were incubated in acetylene within hours after the cores were removed from the field. In this experiment, soils were incubated in the dark at 25°C only and headspace samples were collected at 15 and 30 h.

On May 16, 1992, we conducted a factorial experiment in the tarbush shrubland in which 150 soils in 22·5-cm diameter rings were assigned randomly to one of five treatment groups that received different solutions: (1) water only (1 l deionized H$_2$O), (2) carbon+water (9 g glucose l^{-1}), (3) nitrogen + water (1·7 g NH$_4$Cl l^{-1} and 0·1 g KNO$_3$ l^{-1}), (4) phosphorus + water (4·4 g KH$_2$PO$_4$ l^{-1}) and (5) micronutrients + water (0·6 g FeDTPA and 0·45 mg MoO$_3$ + 0·38 CoCl$_2$ l^{-1}). C$_2$H$_4$ production in watered soil served as the control for carbon (C), N, P and micronutrient treatments, which were applied in solution. The carbon amendment was approximately 12 times higher than background available C levels (Peterjohn & Schlesinger, 1991). Inorganic
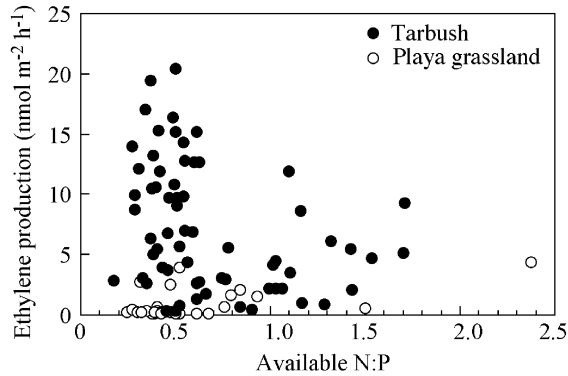
Figure 1. The relationship between C_2H_4 production and available N-to-P ratio in tarbush shrubland and playa grassland soils of the Jornada Basin.

N and P treatments were ten times higher than background concentrations of NH_4^+, NO_3^- and P in Jornada soils Fig. 1. Micronutrient concentrations were applied in the proportions used in Hoagland's solution. All solutions were adjusted to pH $\approx 8\cdot 0$ using NaOH. Soil samples within each 30-sample treatment group were treated 1, 2 or 3 times at 24-hour intervals before soil collection. Two additional sets of ten untreated soil samples served as controls for C_2H_4 production in treated soils. Soil cores were sampled and analysed for C_2H_4 production as described above. Two procedural changes were made in 1992: (1) soils were not remoistened before incubation in the laboratory, and (2) headspace samples were extracted at 6 and 12 h, instead of 15 and 30 h.

The results of the soil survey were analysed using a linear model with C_2H_4 production as the dependent variable and various soil factors as independent variables. A one-way ANOVA was used to test the response to irrigation with frequency (# of daily watering treatments = 0–4) as the main effect. The effect of water, carbon and nutrient additions was analysed using two-way ANOVA with treatment and frequency (# treatments = 1–3) as main effects. C_2H_4 production values were log-transformed in all but the first analysis to meet the assumptions for normal distribution and homogeneity of variance. Differences between treatment means were analysed using a Scheffe post hoc test.

Results

Mean C_2H_4 production was not significantly different in light- *vs.* dark-incubated soils after 15 or 30 h. Dark-incubated soils produced 80% less C_2H_4 between 15 and 30 h ($10\cdot 1$ *vs.* $2\cdot 1$ nmol C_2H_4 m^{-2} h^{-1}; $p \leq 0\cdot 05$) which prompted a change in protocol for the irrigation/fertilization experiment to 6 and 12 h sampling.

In the survey of Jornada soils, tarbush soils produced the highest levels of C_2H_4, and playa soils ranked second (Table 2). C_2H_4 production was negligible in the black grama grassland and in creosote and mesquite shrublands. Extractable NH_4-N and P were highest in playa soils and lowest in mesquite soils (Table 2). Two playa sites (College and Small) had significantly higher NH_4-N and P contents than all other sites. Soil NH_4-N and P concentrations were strongly linearly related in playa soils ($r^2 = 0\cdot 86$, $p \leq 0\cdot 0001$). Available N:P of Jornada soils ranged from $0\cdot 3$ in Fox Playa to $1\cdot 5$ in Gypsum Playa. Extractable NH_4-N and NO_3-N, P and available N:P were not significantly linearly related to C_2H_4 production (Fig. 1).

Table 2. *C_2H_4 production and soil chemistry in a survey of Jornada soils (mean $\pm$ 1S.E.; n = 5)*

Ecosystem	Site	Mean C_2H_4 production (nmol m^{-2} h^{-1})	P	NH$_4$-N	NO$_3$-N	N:P
			Extractable (ug g^{-1} soil)			
Black grama grassland	Basin	0·05 (0·03)	16·8	9·0	0·1	0·6
	Transect	0·06 (0·00)	13·8	9·1	0·1	0·6
	College	0·11 (0·01)	10·3	4·3	0·2	0·4
	Summerford	0·18 (0·02)	22·2	13·9	0·3	0·6
	IBP	0·33 (0·02)	7·5	4·5	0·1	0·6
Creosote	Transect	0·04 (0·01)	8·8	3·9	0·1	0·5
	Caliche	0·08 (0·02)	28·0	9·2	0·1	0·4
	Gravel	0·09 (0·01)	10·2	4·2	0·1	0·4
	Sand	0·10 (0·01)	9·7	8·7	0·1	0·9
	Isaack	0·20 (0·01)	17·9	10·1	0·2	0·6
Mesquite	College Ranch	0·19 (0·01)	7·2	3·0	0·1	0·4
	Rise	0·23 (0·03)	5·1	2·4	0·1	0·5
	West Well	0·28 (0·03)	7·3	2·7	0·1	0·4
	North	0·29 (0·03)	7·0	2·6	0	0·4
	Rabbitbrush	0·33 (0·02)	7·4	3·5	0·2	0·5
Tarbush	West	3·47 (0·39)	13·2	12·3	0·4	1·0
	High	3·58 (0·72)	14·4	6·1	0·2	0·4
	Taylor	3·80 (0·98)	14·2	11·3	0·3	0·8
	East	4·16 (1·51)	10·5	11·0	0·1	1·1
	Cross	12·69 (3·16)	9·0	5·4	0·1	0·6
Playa	College	0·12 (0·01)	86·0	44·8	0·7	0·5
	Small	0·20 (0·01)	84·4	30·5	0·4	0·4
	Fox	0·31 (0·04)	17·8	5·7	0·2	0·3
	Gypsum	1·63 (0·67)	10·1	7·0	0·9	1·5
	Tobosa	2·45 (0·52)	16·7	13·6	0·3	1·1

In the experimental manipulation, C_2H_4 production rates were based on a 12 h incubation time, but C_2H_4 production was not significantly different between soils incubated for 6 *vs.* 12 h. In the tarbush shrubland, watered soil produced 14 times more C_2H_4 than dry soils after a single treatment (Fig. 2: $p \leq 0·1$); however, C_2H_4 production did not increase significantly when soils were watered for 2 or 3 consecutive days. Glucose stimulated C_2H_4 production significantly ($p \leq 0·01$), while inorganic N ($p \leq 0·001$) inhibited production (Fig. 3). C_2H_4 production in micronutrient- and P-treated soil was not significantly different from watered soil.

C_2H_4 production rates for desert ecosystems are displayed in Table 3. These rates were not adjusted for community composition or percent cover.

Discussion

The absence of nitrogenase activity in dry soils suggests that moisture is the primary factor limiting N_2 fixation in desert soils. The increase in nitrogenase activity with watering may reflect the activity of N_2-fixing micro-organisms after extended drought.
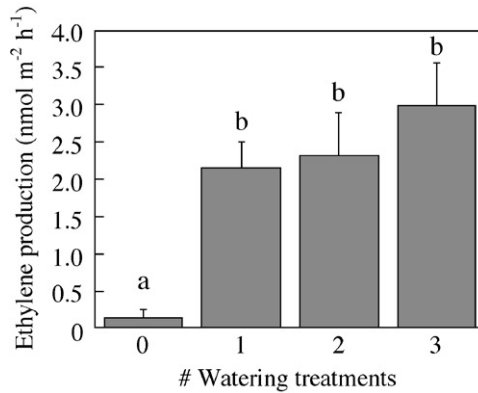
Figure 2. C_2H_4 production in tarbush soils (at Tarbush-West; mean ± 1 S.E.; $n = 10$; 12-h dark incubation results) treated daily *in situ* with up to three applications of a 2-cm simulated rain. Treatment bars followed by the same letters are not significantly different at 0·05 based on a Scheffe post-hoc test.
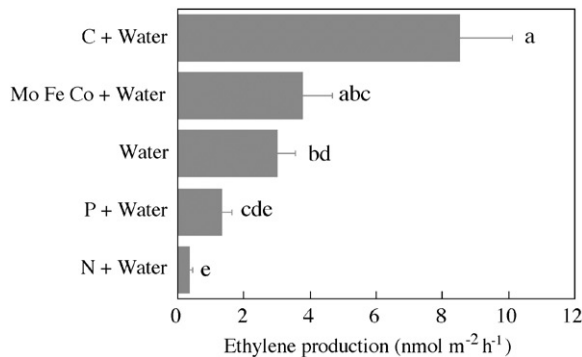

Figure 3. C_2H_4 production (mean ± 1 S.E.; $n = 10$; 12-h dark incubation results) in tarbush soils treated with three applications of water, carbon (glucose), inorganic N (NH_4^+ and NO_3^-), phosphorus or micronutrient (Mo, Fe and Co) solution. Treatment bars followed by the same letter are not significantly different at 0·05 based on a Scheffe post-hoc test.

Both the duration and severity of drought affect recovery rates of cyanobacterial lichens (Henriksson & Simu, 1971; Kershaw & Dzikowski, 1977), but recovery can be rapid. After 6 months of desiccation, nitrogenase activity in Great Basin desert cyanobacterial lichen crusts increased to 80–98% of maximum levels within 3 days of watering (Jeffries *et al.*, 1992). Cyanobacterial soil crusts from the Sahel exhibited nitrogenase activity as early as 2 h after rewetting of a sample that had been desiccated for 3 years (Malam Issa *et al.*, 2001).

  Soil moisture enhances the metabolic activity of N_2 fixers directly and promotes nitrogenase activity by increasing C and energy supplies. Nitrogenase activity in tarbush soils is strongly C-limited, based on the three-fold increase in activity observed in glucose-amended soils. Klubek & Skujins (1980) reported a similar increase in C_2H_4 production (15–60%) in Great Basin desert soil crusts treated with 2% glucose. They attributed the nitrogenase activity in glucose-amended, dark-incubated soils to heterotrophic bacteria; however, glucose stimulated cyanobacterial nitrogenase activity in both the light and dark (Fay, 1976; Huang & Chow, 1988). As with moisture, we expected light incubation to promote photosynthetic activity in

Table 3. *Ethylene production rates in biological soil crusts of desert ecosystems*

Chihuahuan Desert (New Mexico, U.S.A.)	
$0\cdot001$ nmol $C_2H_4\,cm^{-2}\,h^{-1}$	This study (tarbush rates)
20 nmol $C_2H_4\,cm^{-2}\,h^{-1}$	Pavlicek, unpubl. data
Sonoran Desert (California and Arizona, U.S.A.)	
$6\cdot4$–13 nmol $C_2H_4\,cm^{-2}\,h^{-1}$	Eskew & Ting (1978)
78 nmol $C_2H_4\,cm^{-2}\,h^{-1}$	MacGregor & Johnson (1971)
Great Basin Desert (Utah, U.S.A.)	
$0\cdot004$ nmol $C_2H_4\,cm^{-2}\,h^{-1}$	Belnap (1996)
$0\cdot15$–$2\cdot7$ nmol $C_2H_4\,cm^{-2}\,h^{-1}$	Terry & Burns (1986)
$6\cdot8$–12 nmol $C_2H_4\,cm^{-2}\,h^{-1}$	Jeffries *et al.* (1992)
11–90 nmol $C_2H_4\,cm^{-2}\,h^{-1}$	Rychert & Skujins (1974)
Negev Desert (Israel)	
34 nmol $C_2H_4\,cm^{-2}\,h^{-1}$	Zaady *et al.* (1998)
Kalahari Desert (Botswana)	
60–680 nmol $C_2H_4\,cm^{-2}\,h^{-1}$	Skarpe & Henriksson (1987)
Sahel Desert (Niger)	
$0\cdot001$–$4\cdot2$ nmol $C_2H_4\,cm^{-2}\,h^{-1}$	Malam Issa *et al.* (2001)

cyanobacteria, providing additional C and energy for N_2 fixation. Though not significant, four times higher C_2H_4 production in light- *vs.* dark-incubated tarbush soils after 30 h suggests the importance of this effect.

Exogenous nitrogen often inhibits nitrogenase activity. In tarbush soils, the addition of the inorganic N solution reduced nitrogenase activity. Great Basin desert soils responded similarly when treated with $(NH_4)_2SO_4$ (Klubek & Skujins, 1980). The addition of NH_4NO_3 to a temperate grassland soil reduced nitrogenase activity and decreased the proportion of N_2 fixers in the population of heterotrophic bacteria (Kolb & Martin, 1988). In a previous study in the Jornada Basin, NH_4NO_3 and water increased the number of N_2-fixing bacteria in the rhizosphere of potted grasses from the Jornada Basin (Herman *et al.*, 1993). Nitrogenase activity was not explicitly analysed, but the authors hypothesized that N additions spared bacteria the metabolic cost of N_2 fixation, allowing for accelerated reproduction.

In general, low phosphorus availability inhibits nitrogenase activity by lowering the supply of ATP (Stewart, 1977; Layzell, 1990). In past experiments, the addition of KH_2PO_4 or P in ash stimulated cyanobacterial growth and nitrogenase activity in tallgrass prairie soils (Eisele *et al.*, 1989). P fertilization also stimulated cyanobacterial nitrogenase activity in arctic tundra (Chapin *et al.*, 1991) and agricultural soils (Stewart, 1977; Wilson & Alexander, 1979; Bisoyi & Singh, 1988). In contrast to previous work, P fertilization did not promote nitrogenase activity in tarbush soils. In the survey of Jornada Basin soils, nitrogenase activity was highest in soils with a low available N-to-P ratio, but an inverse relationship was not obtained. The survey results suggest that phosphorus is a necessary, but not sufficient, soil factor to predict nitrogenase activity in this arid ecosystem.

Iron (Fe) and molybdenum (Mo) are essential components of nitrogenase (Kim & Rees, 1992), and cobalt (Co) is involved in coenzyme activity and nucleotide reductase activity (Evans & Russell, 1971). Various soil properties, including redox potential, pH, the presence of clay minerals and calcium carbonate, determine micronutrient availability. Oxidizing and alkaline conditions cause Fe to precipitate as oxides and hydroxides (Kabata-Pendias & Pendias, 1992). Thus, Fe may be

unavailable for biotic uptake despite an abundance of Fe minerals (magnetite or Fe_3O_4) in Jornada soils. Similarly, Co adsorbs onto iron and aluminum oxides and clay minerals (McLaren et al., 1986; Bibak, 1994), which may limit its availability in fine-textured alluvial soils. Typically, arid soils are not deficient in Mo, but as with P, the solubility of Mo may decrease due to adsorption to $CaCO_3$ (Kabata-Pendias & Pendias, 1992; but see Goldberg & Foster, 1998). Nitrogenase activity in calcareous Egyptian soils was not enhanced by single or combined applications of Mo, Fe, V or Mn (Abd-El-Malek, 1971). The absence of a significant stimulatory effect of Mo, Fe and Co on nitrogenase activity in Jornada Basin soils provides additional evidence that micronutrients do not limit N_2 fixation in arid soils.

In a desert basin, the accumulation of water in playa and tarbush soils, which receive runoff from higher elevations, and the high water-holding capacities of these fine-textured soils would be expected to enhance nitrogenase activity. Higher inputs of water can also affect N_2 fixers indirectly by stimulating net primary production, thereby increasing soil organic matter inputs, and by transporting dissolved organic carbon and nutrients downslope (Wierenga et al., 1987). Clay colloids protect organic matter and act as anaerobic microsites for enhanced microbial activity (Tiedje et al., 1984). Despite higher availability of C, P and moisture, the soils in playas show limited nitrogenase activity, which may be inhibited by high inorganic N concentrations.

C_2H_4 production rates in this study are lower than most of the published rates for arid ecosystems (Table 3). A theoretical ratio of 3 or 4 mol of C_2H_4 produced to 1 mol of N_2 fixed has been used as a conversion factor in past studies; however, the actual ratio can differ by several orders of magnitude (Hardy et al., 1968). Without $^{15}N_2$ calibration of the acetylene reduction assay in each study, C_2H_4 production rates cannot be assumed to be equivalent to N_2 fixation potential.

Nitrogenase activity may be underestimated in this study. The experiments were conducted at the end of the dry season when soil nitrogenase activity is potentially limited by moisture, C and energy. In addition, soil inorganic N concentrations are highest at the end of the dry season (Hartley & Schlesinger, 2000). N_2 fixers may become more active as the rainy season progresses and soil inorganic N pools are depleted by biotic uptake, volatilization and leaching (Schlesinger & Peterjohn, 1991; Gallardo & Schlesinger, 1995; Schlesinger et al., 1999; Hartley & Schlesinger, 2000). There is also the potential for long-term inhibition of nitrogenase activity due to disturbance. A large portion of the Jornada Basin has been grazed by cattle. Nitrogenase activity in cyanobacterial lichen soil crusts of the Great Basin desert required at least 50 years to recover from surface disturbance (Belnap, 1995, 1996).

For a coarse estimate of N_2 fixation potential in tarbush soils, we used a ratio of 0·3 mol of C_2H_4 produced to 1 mol of N_2 fixed obtained for N. commune on arid soil surfaces (Belnap, unpublished data). This ratio falls within the range reported for N. commune in a high arctic ecosystem (0·1–0·5; Liengen, 1999). Assuming that the ratio of 0·3:1 is closer to the actual ratio for tarbush soils than the theoretical ratio, we estimate that these soils have an N_2 fixation potential of $0·01\,g\,N_2\,ha^{-1}\,h^{-1}$. This rate is at the low end of the range of N_2 fixation potentials published for desert soils ($0·002\,g\,ha^{-1}\,h^{-1}$, assuming 10-h activity per day, Mayland et al., 1966; 12–$78\,g\,ha^{-1}\,h^{-1}$, Rychert & Skujins, 1974).

Vascular plant N_2 fixers contribute to the N budget of the Jornada Basin, but their inputs are localized. When ^{15}N abundance was used to estimate in situ N_2 fixation by phreatophytic mesquite shrubs in the Sonoran Desert (Rundel et al., 1982; Shearer et al., 1983), the proportion of fixed nitrogen in leaf tissue was 45–61%. In the Jornada Basin, mesquite also acquires approximately half of its nitrogen through symbiotic fixation (Lajtha & Schlesinger, 1986). Assuming that mesquite shrubs in the Jornada Basin fix N at the rate of 30–$40\,kg\,ha^{-1}\,year^{-1}$ (Rundel et al., 1982), N inputs from this source may well exceed those of surface soil micro-organisms in this shrubland

ecosystem. The expansion of mesquite shrubland in the Jornada Basin during the last century may have secured its status as the dominant N_2-fixing plant (Buffington & Herbel, 1965; West & Klemmedson, 1978). There are few perennial streams in the Jornada Basin; however, in Sycamore Creek in the Sonoran Desert, annual rates of cyanobacterial nitrogenase activity were 2–3 times higher than estimated for mesquite (Grimm & Petrone, 1997). Further study is needed to determine whether biological soil crusts are equally important contributors of nitrogen to Jornada Basin soils.

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