

## NO gas loss from biologically crusted soils in Canyonlands National Park, Utah

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**Abstract.** In this study, we examined N gas loss as nitric oxide (NO) from N-fixing biologically crusted soils in Canyonlands National Park, Utah. We hypothesized that NO gas loss would increase with increasing N fixation potential of the biologically crusted soil. NO fluxes were measured from biologically crusted soils with three levels of N fixation potential (*Scytonema-Nostoc-Collema* spp. (dark) > *Scytonema-Nostoc-Microcoleus* spp. (medium) > *Microcoleus* spp. (light)) from soil cores and field chambers. In both cores and field chambers there was a significant effect of crust type on NO fluxes, but this was highly dependent on season. NO fluxes from field chambers increased with increasing N fixation potential of the biologically crusted soils (dark > medium > light) in the summer months, with no differences in the spring and autumn. Soil chlorophyll *a* content (an index of N fixation potential), percent N, and temperature explained 40% of the variability in NO fluxes from our field sites. Estimates of annual NO loss from dark and light crusts was 0.04–0.16 and 0.02–0.11 kg NO-N/ha/year. Overall, NO gas loss accounts for approximately 3–7% of the N inputs via N fixation in dark and light biologically crusted soils. Land use practices have drastically altered biological soil crusts communities over the past century. Livestock grazing and intensive recreational use of public lands has resulted in a large scale conversion of dark cyanolichen crusts to light cyanobacterial crusts. As a result, changes in biologically crusted soils in arid and semi-arid regions of the western US may subsequently impact regional NO loss.

### Introduction

Biological soil crusts are communities of fungi, lichens, cyanobacteria, and mosses that colonize soil surfaces in arid and semi-arid ecosystems. Associated soil cyanobacteria and bacteria species within biologically crusted soils fix atmospheric N<sub>2</sub> and are an important source of nitrogen (N) in many desert ecosystems (Evans and Ehleringer 1993; Evans and Belnap 1999). Estimates of N fixation in biologically crusted soils on the Colorado Plateau range from 1.3 to 9 kg N/ha/year depending on biological crust composition (Belnap 2002). However, estimates of N accretion in desert ecosystems are less than 1 kg N/ha/year (Peterjohn and Schlesinger 1990), leading to uncertainty regarding the fate of the fixed N by biologically crusted soils.

In a review of N input and loss pathways in southwestern US deserts, Peterjohn and Schlesinger (1990) estimated that up to 77% of annual N inputs may be lost in wind erosion and gaseous pathways. Nitrogen gas loss as NO and N<sub>2</sub>O have been shown to increase dramatically in soils where N-fixing species occur (Virginia et al. 1982; Martin et al. 2003). West and Skujins (1977) suggested that up to 75–80% of the N fixed by biological soil crusts may be lost as N gases. The veracity of these estimates, however, has long been questioned since N gas loss was not measured directly (Evans and Lange 2001). Due to the paucity of soil NO measurements in arid and semi-arid ecosystems, there is a major gap in our understanding of the magnitude and controls on NO loss (Davidson and Kinglerlee 1997). As a result, our objective in this study was to examine the rates and controls on soil NO flux from biologically crusted soils that differed in their N fixation potentials.

Nitric oxide is a gaseous intermediate of both nitrification and denitrification processes. In sites that receive pulsed precipitation events or extended dry periods, wetting of a previously dry soil often results in a large flux of NO (Davidson et al. 1991, 1993; Scholes et al. 1997; Martin et al. 1998; Hartley and Schlesinger 2000). Davidson et al. (1993) showed that the large pulse of NO after wetting of a previously dry soil coincided with an increase in extractable soil NH<sub>4</sub><sup>+</sup> and high rates of gross N mineralization and nitrification. In a Chihuahuan desert grassland and shrubland, short-term NO loss rates (i.e. minutes) soon after water addition were similar to those observed in a tropical savanna and forested ecosystem (e.g. Johansson 1984; Johansson et al. 1988, Sanhueza et al. 1990; Williams and Fehsenfeld 1991; Hartley and Schlesinger 2000). When soil NO emissions are scaled to an annual loss rate in the Chihuahuan desert grassland and shrubland, however, estimates are low and range from 0.15 to 0.38 kg NO-N/ha/year (Hartley and Schlesinger 2000), which is primarily due to pulsed precipitation events resulting in a limited amount of time that soils are moist.

Both chemoautotrophic and heterotrophic bacteria have been shown to produce NO in nitrification, the oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> (Poth and Focht 1985). NO production in nitrification has been closely linked to soil oxygen levels, available NH<sub>4</sub><sup>+</sup> as a reductant, soil pH, moisture and temperature (Firestone and Davidson 1989; Williams et al. 1992; Paul and Clark 1996). Desert soils are generally coarse-textured and well aerated, which favors nitrification. Alkaline desert soils also provide optimal conditions for nitrification, where maximum nitrification occurs at pH levels between 6.6 and 8.0. Optimal temperatures for nitrification range from 30 to 35 °C (Paul and Clark 1996), soil temperatures that commonly occur at the soil surface in the late spring, summer, and early autumn in cool desert sites.

Although most studies show nitrification to be the dominant source of NO production (e.g. Bollman and Conrad 1998; Smart et al. 1999; Godde and Conrad 2000; Jousset et al. 2001; Hartley and Schlesinger 2000; Martin et al. 2003), NO may also be lost in denitrification processes. Denitrification is primarily a biological process, especially in high pH soils, where NO<sub>3</sub><sup>-</sup> is used

by denitrifying bacteria (primarily heterotrophic bacteria) in the absence of  $O_2$  as an electron acceptor. NO is a gaseous intermediate produced along this reduction pathway. Proximal factors regulating denitrification rates are absence of  $O_2$ , available  $NO_3^-$  to serve as an oxidant, and organic C as an energy source for heterotrophic bacteria (Williams et al. 1992). Although desert soils are characterized by low  $NO_3^-$  and C availability as compared to forested sites, denitrification rates in a Chihuahuan desert site were similar to those measured in temperate and tropical forests (Peterjohn and Schlesinger 1991). Denitrification enzyme activity has been observed in several desert soil types (i.e. Chihuahuan, Mojave, Great Basin), which supports the idea that during certain periods of the year denitrification may occur (Peterjohn 1991; Billings et al. 2002).

Denitrification rates under N-fixing desert species have been shown to be quite high. At a Sonoran desert site, Virginia et al. (1982) reported a 58-fold increase in denitrification rates under *Prosopis glandulosa*, an N-fixing shrub, as compared to plant interspaces. Denitrification potentials in desert ecosystems may also be high due to the variable precipitation regimes and extreme wetting and drying cycles at soil surfaces. Groffman and Tiedje (1988) observed a large pulse in microbial activity upon rewetting of dry soils, which resulted in drawing down soil oxygen levels. The combination of low air porosity in high bulk density soils and high rates of microbial activity after a precipitation event may result in anoxic microsites (Garcia-Pichel and Belnap 1996), which may then favor NO losses via denitrification.

In this study, we examined NO losses from biologically crusted soils and addressed these questions: (1) Does NO loss increase with increasing N fixation potential of a biologically crusted soil? (2) If so, what are the primary controls on NO flux from biologically crusted soils? Nitric oxide loss was measured from biologically crusted soils with three levels of N fixation potential in order to better understand N gas losses in relation to inputs via N fixation. We hypothesized that N gas loss would increase with increasing N fixation potential of the biological soil crust, where higher N fixation and subsequent leakage of C and N compounds would provide substrates in N gas loss. Controls on NO loss were examined by measuring NO fluxes from biologically crusted soils under a wide range of soil moistures, temperatures, and nutrient contents.

## Methods

### *Site description*

Sites were located within the Island-in-the-Sky District of Canyonlands National Park, Utah on the Colorado Plateau in southeast Utah, USA. The site was located at 1813 m elevation and annual precipitation ranged between 185 and 226 mm in years 1998–2000 (National Atmospheric Deposition

Program, <http://nadp.sws.uiuc.edu/>). In the Canyonlands area, the most well-developed biological soil crust communities often occur on soils classified as Rizno, dry-Rock outcrop, which is characterized by 45% Rizno gravelly fine sandy loam, 25% rock outcrop and 30% other soils (Lammers 1991). Rizno soils are classified as loamy, mixed calcareous, mesic Lithic Ustic Torriorthents. They tend to be well-drained and shallow, often with depth to underlying sandstone ranging from 10 to 50 cm. Rizno soils cover approximately 30% of the area within Canyonlands National Park. These soils formed in eolian deposits and residuum derived dominantly from sandstone and shale.

Two sites within this soil type were chosen to measure NO gas fluxes from biologically crusted soils. The pinyon-juniper site was located within an area dominated by *Pinus edulis* and *Juniperus osteosperma* with the presence of *Yucca harrimaniae* (Harriman's yucca) and *Coleogyne ramossissima* (blackbrush). Soils at this site were generally less than 10 cm to bedrock. The blackbrush site was located in an area dominated by *C. ramossissima* with the presence of *P. edulis* and *J. osteosperma*. Soil depth at this site ranged from 20 to 40 cm.

#### *Biological soil crust type*

Nitrogen oxide (NO) fluxes were measured from three biological soil crust types at each of the field sites. Visual inspection of the presence of lichen species and coloration in biologically crusted soils is a good indicator of N fixation potential, and cyanobacterial composition and biomass. Nitrogen fixation tends to increase with increasing darkness of the biological soil crust (Figure 1). Dark biologically crusted soils contain *Microcoleus vaginatus*, darker pigmented cyanobacteria such as *Sytonema myochrous* and *Nostoc commune*,

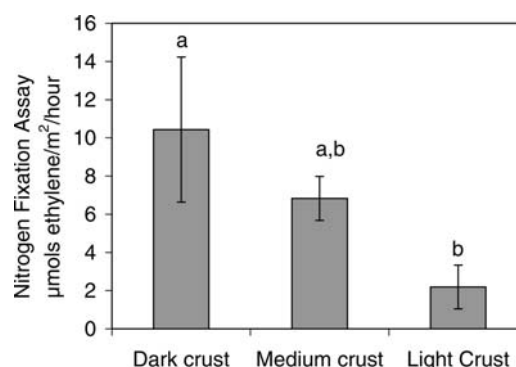


Figure 1. Nitrogen fixation activity in three biologically crusted soils as measured by acetylene reduction. Values are mean ethylene production  $\pm 1$  SE ( $N=6$ ). When a different letter appears above a bar, means are significantly different at  $p < 0.05$  (*post-hoc* Newman-Keuls). Reprinted from Barger (2003).

and the N-fixing lichens *Collema tenax* and *C. coccophorum*. Medium biologically crusted soils contain *M. vaginatus* and darker pigmented cyanobacteria, but the higher N-fixing *Collema* lichens are not present. Light biologically crusted soils are often dominated by the free-living filamentous cyanobacteria *M. vaginatus*. We used these three criteria (dark, medium, and light) to position gas sample rings in the field and collect soil cores for long-term field incubations.

#### *Nitric oxide fluxes from soil cores*

Nitric oxide fluxes were measured from soil cores to examine seasonal variation in NO loss from the three biological soil crust types. These measurements were taken in April, June, September, and October of 2001 and April and June of 2002. From the same soil type described above, four 182-cm<sup>2</sup> soil cores were collected to a depth of 0–5 cm from each soil crust type and placed in polyvinyl chloride (PVC) rings with a grated bottom to allow water drainage. Soil cores were placed in a common site dominated by *C. ramosissima* approximately 2 km from the USGS laboratory in Moab, UT. NO fluxes in dry soils were always zero. At each sampling period, water was added to simulate an average rain event (3 mm) for this region. In the first field measurement period at the pinyon-juniper field site in April 2001, NO fluxes were measured at 0.5, 1.5, and 4 h after water addition to determine when peak NO fluxes occurred. Once it was determined that fluxes generally peak 1.5 h after water addition, later NO flux measurements were taken only at this time. All NO flux data from soil cores and field chambers were taken 1.5 h after water addition unless otherwise noted.

#### *Field measurements of NO fluxes*

Temporary gas chambers were placed in each of the field sites. At each sampling date, NO fluxes along with several other soil variables were measured. NO fluxes were measured at the pinyon-juniper site in April, June, July, September, and October of 2001 and April of 2002. At the blackbrush site, NO fluxes were measured in July, September, and October of 2001 and April of 2002.

A 19.7-cm diameter PVC anchor was placed to a depth of 4 cm into each of the three types of biologically crusted soils. NO fluxes were measured from five blocks of the three types of biologically crusted soils, for a total of 15 measurements at each site within a sample date. Blocks were located in areas where all three crusts types were present which was typically a 4–5 m<sup>2</sup> area. At each sample date, the total area within which measurements were made was approximately 400 m<sup>2</sup>. NO was measured with a portable Scintrex Unisearch LMA-3 chemiluminescent analyzer with an attached LNC-3 converter that oxidizes NO to NO<sub>2</sub>. The instrument was calibrated each day using a known

concentration of NO from a standard tank mixed with NO-free air. Flow rates from the standard tank were adjusted to obtain different concentrations of NO. A minimum of nine NO concentrations were used in the linear calibration. Water was added to each ring to simulate a 3-mm rain event. Immediately before a sampling period, a 19.7-cm diameter Teflon-lined PVC chamber top with a height of 18 cm was secured to a PVC anchor. To avoid pressurizing the chamber and pulling gas from the soil atmosphere while sampling chamber air, the chamber top was equipped with a small inlet port. This allowed for chamber air to equilibrate with atmospheric air, resulting in a mixture of atmospheric air and chamber air over the sample period. A point calculation method was used to estimate NO flux, which accounted for the mixing of atmospheric air and chamber air (Martin et al. 1998). NO flux was calculated 6 min after chamber closure.

#### *Soil measurements*

Immediately after measuring NO flux from each gas ring, soil temperature was measured and soil cores were collected for analysis of extractable inorganic N, total N, and chlorophyll *a* content. Three 2-cm diameter soil cores were collected down to a depth of 5 cm within each chamber for inorganic N analysis ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ). Samples were immediately transported back to the lab and refrigerated. For the July 2001 extractions 100 ml of 2-M KCl was added to 25 g of soil. Since concentrations were near the detection limit of the autoanalyzer, the ratio of salt solution to soil was changed at later dates. For all later sample dates, 50 ml of 2-M KCl was added to 25 g of soil. Once the salt solution was added to the soil, samples were placed on a reciprocal shaker for 1 h. Samples were then filtered with Whatman 4 qualitative grade paper and immediately frozen. Samples were analyzed later on an Alpchem Flow Solution 3 Autoanalyzer.

Additional soil cores from each gas sample ring were also collected for analysis of chlorophyll *a* and total N. Chlorophyll *a* has traditionally been used as an index of cyanobacterial biomass. However, recent studies have shown a poor relationship between cyanobacterial counts and chlorophyll *a* content (Bowker et al. 2002). As a result, in this study chlorophyll *a* content is used as an index of C and N fixation potential, which increases with increasing chlorophyll *a* content (Garcia-Pichel and Belnap 1996; Belnap 2001). Two 3.1-cm<sup>2</sup> soil cores were collected from the top 0–1 cm of soil. The first core was air-dried in the dark and frozen until chlorophyll *a* analysis could be performed. Samples were ground to a fine powder with a mortar and pestle. Quantitative and qualitative high performance liquid chromatography (HPLC) analysis was performed according to the method of Karsten and Garcia-Pichel (1996). The second soil core collected from each chamber was air-dried and stored in a dark room. These samples were also ground to a fine powder with a mortar and pestle and analyzed for total N on a LECO CHN analyzer.

### *NO fluxes in response to rainfall amount and number of events*

In April 2002, a field experiment was conducted to examine the effect of varying rainfall amount on soil NO fluxes. Anchors were placed into medium-colored biologically crusted soils at the blackbrush field site. Three levels of water (3.3, 6.6, 13.3 mm) were added to five samples rings each for a total of 15 measurements. NO fluxes were measured 1.5 h after water addition. Immediately following NO flux measurements, soil cores were collected from each gas ring to estimate percent water-filled pore space (WFPS). A 7.0-cm<sup>2</sup> soil core was collected from the top 5 cm of soil. The soil core was immediately placed in an airtight bag and transported back to the lab. In the lab each soil core was weighed to obtain a wet weight. Soil samples were then placed in a 100 °C oven for 24 h. After this time soils were reweighed to obtain a dry weight. Percent WFPS was calculated as:

$$\%WFPS = (\text{soil water content} \times \text{bulk density} \times 100) / (1 - (\text{bulk density} / 2.65))$$

Particle density was set at 2.65 g/cm<sup>3</sup>, the density of most mineral particulates (Paul and Clark 1996).

In a second experiment, NO fluxes in the soil cores were measured in response to multiple wetting events in September 2001. Air temperatures during the day ranged from 22 to 30 °C during the measurement period. The first event was a 2 mm natural rain event, which occurred during the night. NO fluxes were measured the next morning. After the morning measurement an additional 3 mm was added to the cores. The next morning NO fluxes were measured and immediately following this measurement period a third 3-mm event was simulated. NO flux measurements were taken 1.5 and 3 h after this third event.

### *Statistical analysis*

Factorial analysis of variance was used to examine the effect of crust, season, and site on NO fluxes, chlorophyll *a* content, percent soil N, soil inorganic N content and soil temperature. Individual treatment differences were examined with a Newman–Keuls *post-hoc* test. Treatment effects were considered significantly different at  $p < 0.05$ . We used a multiple regression model to identify important predictors in explaining variability in NO fluxes. Soil chlorophyll *a*, percent N, soil temperature and extractable soil NO<sub>3</sub><sup>−</sup>, soil NH<sub>4</sub><sup>+</sup>, and total inorganic N were used as predictor variables of NO fluxes from our field chambers in a multiple regression model.

### *Calculation of annual NO loss budgets*

Estimates of annual NO loss from light and dark biologically crusted soils were based on seasonal surface soil moisture and temperature data and average flux

rates by crust type and season. Surface soil moisture data was generated by time domain reflectometry (TDR) measurements at 5-mm soil depth that were collected every 0.5 h over a three-year period (Belnap, in prep). Using these data, we calculated both a maximum and minimum number of hours within each season (spring, summer, and autumn) that air temperatures were  $\geq 10^{\circ}\text{C}$  and volumetric water content was  $\geq 1\%$ . Data were collected from 2001 to 2003. Based on knowledge from field studies, NO fluxes were at or near the detection limit of our instrument below these values of soil moisture and air temperature. We assumed winter NO fluxes would not contribute substantially to annual NO loss since NO fluxes were near or below the detection limit of the analyzer in the late autumn.

Mean NO fluxes for each season were determined by the results of a regression tree model. Classification and regression tree (CART) models are ideally suited to identify thresholds at which a response variable, such as NO flux rates change. Both categorical and continuous variables may be used in CART analysis. Data are repeatedly split into mutually exclusive homogeneous groups based on a simple splitting rule (De'ath and Fabricius 2000). The end product is a graphical and easily interpretable tree, which identifies thresholds or categories in the case of continuous and categorical variables, respectively, at which the response variable changes. The initial regression tree that explains the highest variability in the response variable tends to be overly complex and overfit the data. As a result, methods for pruning and subsequent tree selection must be incorporated. For a full description of CART analysis and pruning techniques see Breiman et al. (1998). In our study, we used the data-mining package in Statistica 7.0 to generate a regression tree model. Model selection was based on 10-fold cross-validation (Breiman et al. 1998). In the regression tree model, we entered both categorical (crust type, season) and continuous variables (soil temperature, % soil N, chlorophyll *a* content, soil inorganic N). Nitric oxide flux measurements 1.5 h after water addition from our field chambers were entered as the response variable. Since NO fluxes tended to peak approximately 1.5 h after water addition, these estimates represent an upper limit to NO loss from these crust types. To estimate a lower limit to NO loss, we calculated the percent change in flux rates in measurements taken immediately before (0.5 h) and after (4.0 h) peak NO fluxes. We averaged these values and decreased the maximum NO flux rate by this amount to estimate a lower limit to NO loss.

## Results

### *NO fluxes from soil cores*

Nitric oxide fluxes from soil cores were higher in dark crusts relative to medium and light crusts (Figure 2). The pattern of NO flux, however, from different crust types was influenced by season (crust  $\times$  season,  $F_{4,54} = 52.7$ ,  $p < 0.0001$ ).



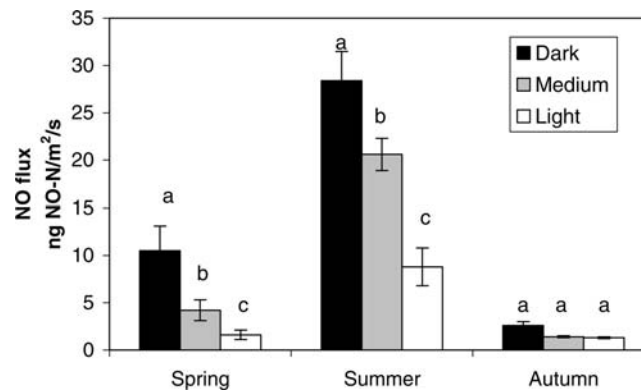


Figure 2. NO fluxes by season from soil cores. Values are mean NO flux  $\pm$  1 SE. Different letters above a bar represent a significant effect of crust within a given season on NO flux evaluated at  $p < 0.05$  (post-hoc Newman-Keuls).

In the spring and summer months, NO fluxes from dark > medium > light crusts. In the autumn, no differences were observed in NO flux by crust type. The duration of NO fluxes after water addition was limited. Immediately after water addition (0.5 h) positive NO fluxes were measured, which peaked at 1.5 h (Figure 3). Four hours after water addition, NO fluxes in medium and light crusts declined to near detection limits, whereas fluxes in dark crusts remained elevated.

#### *Field measurements of NO fluxes*

Similar to the soil cores, the interaction of crust and season on NO fluxes from field chambers was significant (crust  $\times$  season,  $F_{4,121} = 2.95$ ,  $p = 0.02$ ). NO fluxes were significantly higher in dark crusts relative to medium and light crusts only in the summer months (Figure 4). Although NO fluxes were not significant in all seasons by crust type, there was a trend toward higher NO fluxes with increasing darkness of the soil crust throughout the year. NO fluxes were consistently higher from soil cores as compared to field measurements, which were not explained by differences in our measured variables of chlorophyll *a*, percent soil N, and temperature. NO fluxes increased within increasing soil temperature, chlorophyll *a* content, and soil percent N, which explained 40% of the variability in NO fluxes from our field chambers ( $F_{3,126} = 28.9$ ,  $p < 0.0001$ ).

#### *Soil measurements*

Soil chlorophyll *a* content increased with increasing darkness of the biological soil crust, but this again depended on season (crust  $\times$  season,  $F_{4,116} = 2.72$ ,  $p = 0.03$ , Table 1). In the spring, chlorophyll *a* content was similar in dark and

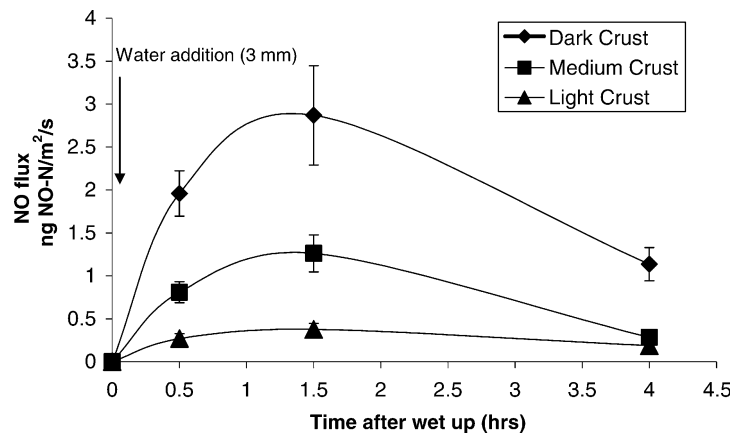


Figure 3. NO fluxes from soil cores in response to a single 3-mm water addition. Values are mean NO flux  $\pm$  1 SE.

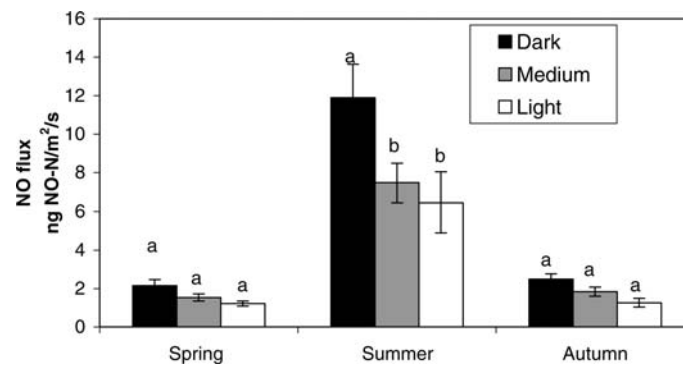


Figure 4. Seasonal NO fluxes from pinyon-juniper and blackbrush field sites. Seasonal means are presented since no differences in NO flux were observed by site. Soil NO fluxes were measured 1.5 h after a 3.3-mm rain event. Values are mean NO flux in ng NO-N/m²/s  $\pm$  1 SE.

medium crusts but greater than light crusts. In the summer and autumn months, the pattern in chlorophyll *a* content was dark > medium > light crusts. Percent total soil N was also increased with crust darkness, but this was also highly dependent on season (crust  $\times$  season,  $F_{4,117}=4.30$ ,  $p=0.002$ ). In the spring no differences were observed in percent soil N, whereas percent soil N in dark > medium = light crusts in the summer and autumn months. Soil crust type had no effect on total soil inorganic N pools ( $\text{NO}_3^- + \text{NH}_4^+$ ). Although the main effect of crust type on soil  $\text{NO}_3^-$  was not statistically significant in the spring months ( $p=0.08$ ), there appeared to be a trend toward higher soil  $\text{NO}_3^-$  in dark relative to medium and light crusts (Table 1). Season was an important factor in soil inorganic N pools. Soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  pools were highest in the spring relative to the autumn and summer (Table 1). There was a site by

Table 1. Soil chlorophyll *a*, percent N, inorganic N (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>), and soil temperature from three biological crust types over three seasons.

Main effects	Chl <i>a</i> µg/g soil		% Soil N		NO <sub>3</sub> <sup>-</sup> µg/g soil		NH <sub>4</sub> <sup>+</sup> µg/g soil		Soil temperature °C	
	Crust × Season		Crust × Season		Season Sp > Su = Au		Season Sp > Su = Au		Site × Season	
Spring										
Dark	6.85	(0.84) a	0.036	(0.004) a	2.89	(0.86) a	0.88	(0.20) a	26.3	(1.1) a
Medium	6.28	(0.79) a	0.038	(0.005) a	1.94	(0.28) a	0.88	(0.25) a	26.9	(1.0) a
Light	3.93	(0.69) b	0.034	(0.003) a	1.57	(0.22) a	0.53	(0.10) a	27.4	(1.0) a
Summer										
Dark	8.81	(1.20) a	0.067	(0.007) a	1.02	(0.21) a	0.41	(0.11) a	29.7	(1.4) a
Medium	5.42	(0.81) b	0.045	(0.003) b	1.02	(0.13) a	0.50	(0.10) a	30.8	(1.9) a
Light	2.26	(0.35) c	0.031	(0.005) b	1.33	(0.33) a	0.51	(0.08) a	30.2	(1.7) a
Autumn										
Dark	10.10	(0.88) a	0.054	(0.005) a	1.84	(0.42) a	0.48	(0.11) a	22.7	(1.2) a
Medium	5.25	(0.88) b	0.031	(0.002) b	1.51	(0.22) a	0.49	(0.12) a	23.7	(1.3) a
Light	2.53	(0.35) c	0.022	(0.002) b	1.04	(0.24) a	0.51	(0.15) a	23.3	(1.5) a

Values are seasonal means ± 1 SE within a crust type. We examined the main effects of season, crust type, and site. Significant main effects are listed at the top of the table. In the absence of a two-way interaction, individual treatment effects are listed directly below significant main effects (Sp = Spring, Su = Summer, and Au = Autumn). When a different letters follows a mean within a season there was a significant effect of crust type evaluated at *p* < 0.05 (*post-hoc* Newman-Keuls).

season effect on soil temperature (site  $\times$  season,  $F_{2,121} = 18.5$ ,  $p < 0.0001$ ). Soil temperatures in the summer were greater than the spring and autumn in the blackbrush site, whereas soil temperatures in the pinyon-juniper site were greater in the spring and summer relative to autumn (Table 1). There were no differences in soil temperature relative to crust type.

#### *NO fluxes in response to rainfall amount and number of events*

Percent WFPS was not an important factor in regulating NO fluxes from biologically crusted soils at our field sites, where NO fluxes remained consistent even though rainfall amount varied (Figure 5). Multiple water additions, however, did alter NO flux dynamics from the cores. The morning after a 2-mm natural rain event, positive NO fluxes were measured in all cores (Figure 6), but no differences were observed in NO flux by crust type. Immediately after this first measurement period an additional 3 mm of water was added and by the next morning NO fluxes in dark crusts more than doubled, whereas fluxes from medium and light crusts remained unchanged. A third 3-mm water addition had no effect on NO fluxes, which remained unchanged throughout that day.

#### *Regression tree analysis of NO fluxes*

Categorical predictors (season, crust type) were more important than continuous predictors (soil temperature, chlorophyll *a*, soil percent N, inorganic N) in explaining the variability in NO fluxes. Season and crust type explained 60% of the variability in NO fluxes from our field chambers (Figure 7). The first

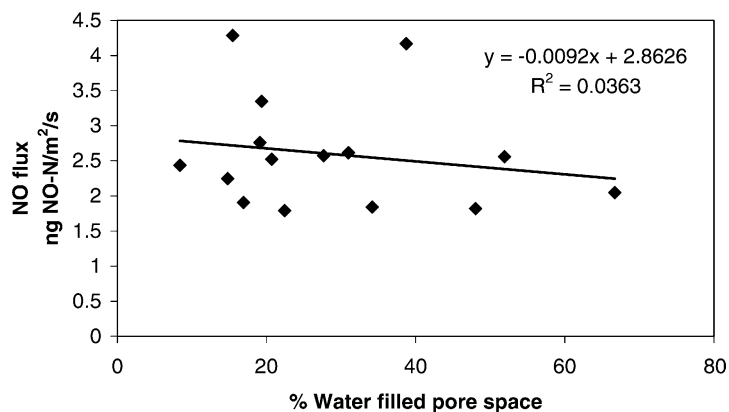


Figure 5. Soil NO flux as a function of percent water-filled pore space (WFPS). To obtain a range of WFPS values, 3 levels of water were added (3.3, 6.6, and 13.3 mm).

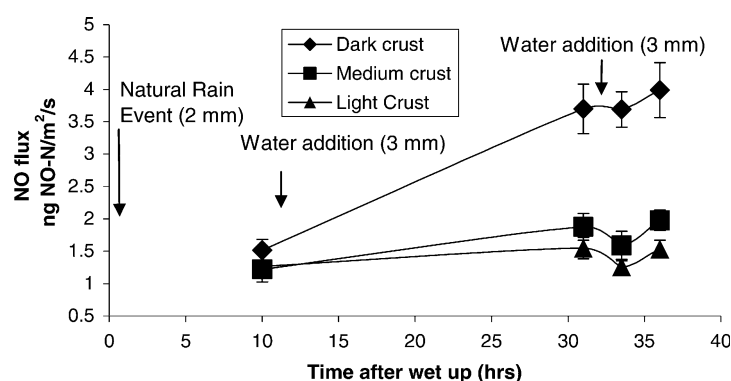


Figure 6. Diurnal NO fluxes in response to multiple water additions from soil cores. Values are mean NO flux in  $\text{ng NO-N/m}^2/\text{s} \pm 1 \text{ SE}$ .

split in the data was by season, which explained 51% of the variability in NO fluxes. Mean NO fluxes in the summer were  $8.7 \text{ ng NO-N/m}^2/\text{s}$ , nearly 5-fold higher than the spring and autumn with a mean of  $1.8 \text{ ng NO-N/m}^2/\text{s}$ . Within the summer months, crust type explained an additional 9% of the variability in NO fluxes. Mean NO fluxes from dark crusts were 70% higher relative to medium and light crusts during the summer months. Using the results from the regression tree analysis and TDR data we estimated that approximately 0.11 and 0.16 kg NO-N/ha/year is lost each year from light and dark crusts (Table 2). We were also interested in a lower limit of NO loss from light and dark crusts. Following the diurnal pattern in NO fluxes (Figure 3), measurements preceding (0.5 h after water addition) and following (4.0 h after water

Table 2. Estimates of annual NO loss by season and crust type.

	Light crust	Dark crust
Spring		
NO flux ( $\mu\text{g/m}^2/\text{h}$ ) (min/max)	3.3/6.5	3.3/6.5
Hours available (min/max)	48/70	48/70
Spring NO loss	0.002–0.005	0.002–0.005
Summer		
NO flux ( $\mu\text{g/m}^2/\text{h}$ ) (min/max)	12.6/25.2	21.4/42.8
Hours available for NO loss (min/max)	151/330	151/330
Summer NO loss	0.02–0.09	0.03–0.14
Fall		
NO flux ( $\mu\text{g/m}^2/\text{h}$ ) (min/max)	3.3/6.5	3.3/6.5
Hours available for NO flux (min/max)	40/218	40/218
Fall NO loss (kg N/ha)	0.001–0.014	0.001–0.014
Annual NO loss (kg N/ha)	0.02–0.11	0.04–0.16

Maximum NO loss rates are based on results from a regression tree model (Figure 7). Minimum NO fluxes are 50% of the maximum rate. Hours available for NO loss is based on air temperature and surface soil moisture data over a three-year period (2001–2003).

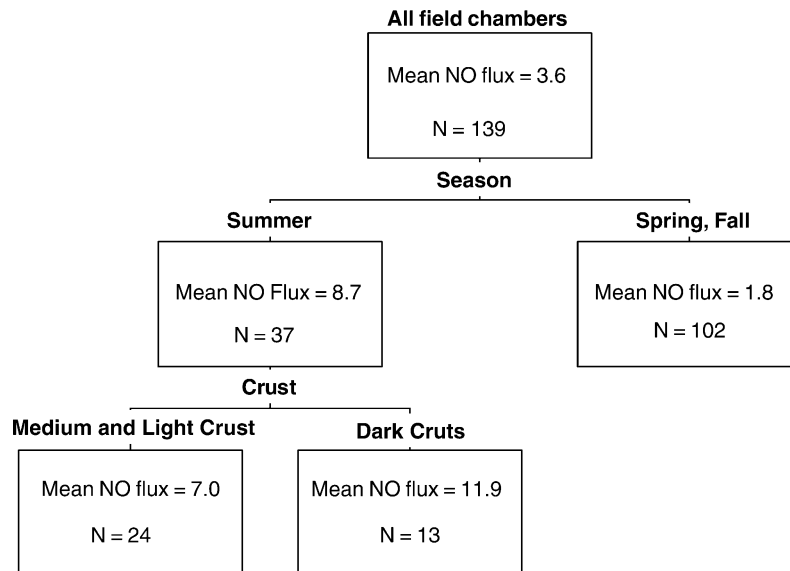


Figure 7. Regression tree model of NO fluxes from field chambers. At each split in the data, mean NO flux and sample number is listed.

addition) peak NO flux was 50% lower than the peak NO flux at 1.5 h. Following this, we estimated a lower limit to NO loss using mean NO flux values within each season and crust type that were 50% lower than the peak values (Table 2). The lower limit for NO loss was 0.02 and 0.04 kg N/ha/year for light and dark crusts, respectively.

## Discussion

Nitric oxide loss increased with increasing N fixation potential (as inferred from crust color) of biologically crusted soils in both soil cores and at our field sites, but this effect was highly dependent on season. NO fluxes were 5-fold higher in the summer months when soils temperatures frequently exceeded 30 °C, as compared to the cooler spring and fall months. Jones and Stewart (1969) showed a strong temperature effect on N release from a N-fixing cyanobacteria. After a 12-day incubation at 40 °C, 85% of the total N in *Calothrix scopulorum* was in the extracellular N pool, whereas only 34% of the total N was in the extracellular N pool at 25 °C. Nitrogen release from N-fixing cyanobacteria to the extracellular N pool is primarily in the form of  $\text{NH}_4^+$  and simple amino acids (Mayland et al. 1966; Jones and Stewart 1969). Furthermore, N fixation and N leakage rates in biologically crusted soils appear to be closely related. In a laboratory experiment, Barr (1999) showed that  $\text{NH}_4^+$  concentrations in leachate beneath a soil crust dominated by dark *Nostoc* spp.

was 20-fold higher than a light *M. vaginatus* soil crust. Thus, N leakage to underlying soil may provide available  $\text{NH}_4^+$  for nitrification and the potential for NO loss, which we expect to increase with increasing N fixation potential of the biologically crusted soil.

Although NO losses may be produced in both nitrification and denitrification processes, losses from aridland soils are more often associated with nitrification (Smart et al. 1999; Hartley and Schlesinger 2000; Martin et al. 2003). Standing soil  $\text{NH}_4^+$  pools, the initial substrate in nitrification, were a poor predictor of NO fluxes in this study. However, seasonal  $\text{NO}_3^-:\text{NH}_4^+$  ratios were always  $>1$ , which suggests that nitrification does occur at these sites under a variety of soil conditions. Other important factors that enhance nitrification, such as high soil temperatures, sand content (ca. 80% at sites in this study) that enhances soil aeration, and soil pH values in the range of 6–8 were optimal for nitrification at these sites.

Although NO loss is often associated with nitrification, development of anaerobic microsites within well-aerated soils, which may support denitrification, is not uncommon. Garcia-Pichel and Belnap (1996) reported oxygen levels near zero in the surface (4 mm) of a biological soil crust, while measuring dark respiration. Furthermore, the zones of anoxia were much more extensive in dark crusts relative to light crusts. A large pulse in microbial activity after a rain event, resulting in anoxic conditions in dark crusts, may also partially explain the higher NO losses.

Increasing water filled pore space at 0–5 cm depth had no impact on NO fluxes. In biologically crusted soils, a large proportion of the microbial community resides in the top 4 mm of the soil surface (Garcia-Pichel and Belnap 1996; Garcia-Pichel et al. 2003a). Microprobe studies showed that soil  $\text{NO}_3^-$  pools are also highest in the soil surface between 1 and 2 mm, but declined to nearly undetectable levels by 4 mm, suggesting that soil nitrifiers reside primarily near the soil surface (Garcia-Pichel and Belnap 2001). As a result, small rain events that wet up the first few millimeter of the soil surface may be adequate to activate soil microbes associated with N gas loss, and further wetting of the soil profile below this region may have no additional effect.

#### *Estimates of annual NO loss*

Nitric oxide emissions from biologically crusted soils appear to be temporally decoupled from N inputs via fixation. Nitrogen fixation peaks in the spring and autumn, when air temperatures are less than 20 °C (Belnap 2001), whereas NO fluxes in our study peaked in the summer months. Over 80% of annual NO losses were estimated to occur during the summer months in both light and dark biologically crusted soils. High NO emissions during the summer months were due not only to high flux rates, but also a greater number of hours that soils are moist. In years when the summer monsoon brings above-normal precipitation we would expect NO losses to be unusually high.

The magnitude of NO fluxes was consistently higher from cores as compared to field chambers, which cannot be explained by differences in any of our measured variables (i.e. chlorophyll *a* content, percent soil N, soil temperature). Increased drought conditions in cores as compared to field chambers may partially explain differences in NO flux rates. Cores were positioned above the soil surface which may have exposed the soils to more rapid drying as compared to field chambers. In addition, water was allowed to drain through the cores into a collection container, whereas at our field sites drainage was inhibited by bedrock at 10–20 cm depth and water often pooled after rain events. Drought conditions in the cores over the one-year measurement period may have resulted in mortality of cyanobacteria and lichens, leading to release of substrates for use by nitrifying and denitrifying bacteria.

Estimates of annual NO loss from dark and light crusts were in the range of 0.04–0.16 and 0.02–0.14 kg NO-N/ha/year, respectively, for the two sites studied. These estimates are on the lower end, but in the range of NO loss rates of 0.15–0.38 kg NO-N/ha/year reported from Chihuahuan desert sites (Hartley and Schlesinger 2000). NO loss from light crusts may be overestimated, since soil moisture data were collected only from dark crusts. Nitric oxide fluxes occurred over a shorter period after a wetting event in a light relative to a dark crust (Figure 6), which was most likely due to differences in moisture retention by crust type. Observations from the field indicated that dark crusts tend to retain moisture for a longer period than light crusts.

Previous estimates of N fixation ranged from an upper limit of 9 kg N/ha/year down in dark crusts to 1.4 kg N/ha/year in light crusts (Belnap 2002). We expect N fixation in dark crusts from this study to be lower due to the lower cover of N-fixing lichens than those used in the study by Belnap (2002). Based on acetylene reduction assays on crusts at these sites, we expect N fixation rates for dark crusts to be in the range of 5 kg N/ha/year. Given these N fixation rates, NO gas loss accounts for approximately 3–10% of the N fixed by dark and light crusts.

Land use change such as livestock grazing and increased recreational use of public lands in the western US is currently impacting biological soil crust communities. Of the 100 million hectares of grazing lands in this region, approximately 40% of this area is covered by biological soil crusts (Garcia-Pichel and Belnap 2003b). Before the introduction of domestic livestock and changes in recreational use of public lands, much of the area covered by biological soil crusts was thought to be dominated by dark cyanolichen crusts. Recent estimates of light cyanobacterial crusts are upwards of 70%, with only 30% remaining in dark cyanolichen crusts. Following this, conversion of dark cyanolichen to light cyanobacterial crust may lead to regional declines in NO emissions with further land use change. As better information becomes available on land use impacts on cover and composition of biologically crusted soils, our simple model of NO emissions based on season and crust type may be used to better estimate regional NO emissions and identify sites of high NO loss.



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