DISSERTATION

Biogeochemical cycling and N dynamics of biological soil crusts in a semi-arid ecosystem

Submitted by

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We hereby recommend that the dissertation prepared under our supervision by Nichole N. Barger entitled "Biogeochemical cycling and N dynamics of biological soil crusts in a semi-arid ecosystem " be accepted as fulfilling in part requirements for the degree of Doctor of Philosophy.

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ABSTRACT OF DISSERTATION

THE ROLE OF BIOLOGICAL SOIL CRUSTS IN ECOSYSTEM N CYCLING IN A SEMI-ARID ECOSYSTEM

Biological soil crusts are communities of fungi, lichens, cyanobacteria, and mosses that colonize soil surfaces in arid and semi-arid ecosystems. Biological soil crusts fix atmospheric N_2 and are an important source of nitrogen [N] in many aridland ecosystems. Since N accretion in these ecosystems is low, I hypothesized that N inputs via fixation must nearly balance N losses.

I measured NO loss from biological soil crusts with three levels of N fixation potential. The upper limit of annual NO loss from dark, cyanolichen and light, cyanobacterial crusts was 0.13 and 0.07 kg N/ha/yr respectively. Overall, it appears that annual inputs via N fixation greatly exceed estimates of annual N gas losses in dark crusts, whereas N gas loss nearly equals N inputs in light, cyanobacterial crusts.

I also examined the effect of trampling disturbance and biological soil crust composition (dark, cyanolichen vs. light, cyanobacterial crust) on C and N fluxes in surface runoff. Trampling disturbance resulted in higher C and N losses as compared to scraped (crust removed but soil structure intact) and intact biological soil crusts. Biological soil crust composition also impacted C and N losses in runoff, where C and N export was higher in light, cyanobacterial crusts relative to dark, cyanolichen crusts.

I determined acetylene reduction (AR) to N fixation conversion ratios for the soil cyanobacterium *Nostoc commune* collected from three sites (New Mexico, Texas, and Inner Mongolia). Conversion ratios ranged from 4.3-6.2 for the New Mexico sites and

5.7-11.4 for the Texas site. $^{15}N_2$ incorporation was not detectable in samples from the Inner Mongolia site.

In the final chapter, I explored the impacts of sheep grazing on plant composition and soil nutrients in a nine-year grazing study. My results suggest that as grazing intensity increases, litter quantity decreases resulting in C limitation of soil microbial communities. Even though C limitation of microbes results in lower N immobilization and higher plant available N pools, there is also greater potential for N loss from these pools in leaching and gaseous loss pathways.

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Chapter I.

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 biological soil crusts fix atmospheric N_2 and are an important source of nitrogen [N] in many aridland ecosystems (Evans and Belnap 1999, Evans and Ehleringer 1993). On the Colorado Plateau, estimates of N fixation by biological soil crusts range from 1.3-9 kg N/ha/yr depending on biological crust composition (Belnap 2002). However, low rates of N accretion in desert ecosystems are less than 1 kg N/ha/yr (Peterjohn and Schlesinger 1990), leading to uncertainty regarding the fate of fixed N by biological soil crusts and pathways of N loss in aridland ecosystems. Since N accretion in these systems appears to be very low, I hypothesized that increases in N fixation by biological soil crusts would also increase N loss.

In chapter II, I examine NO gas losses from biological soil crusts and explore the potential implications of these losses on ecosystem N budgets. Nitric oxide is a gaseous intermediate produced in both nitrification and denitrification processes. Although there are few measurements of soil NO losses from desert ecosystems, it appears that loss rates may be high. In a Chihuahuan desert grassland and shrubland, NO loss rates (Hartley and Schlesinger 2000) 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).

As NO loss may be a dominant N loss pathway in these systems, I addressed these questions: 1) does NO loss increase with increasing N fixation potential of a biological soil crust? 2) If so, what are the primary controls on NO loss from biological soil crusts? 3) Furthermore, is NO a dominant N loss pathway in these ecosystems? I measured NO loss from biological soil crusts with three levels of N fixation potential in order to better understand N gas losses in relation to inputs via N fixation in biological soil crusts. I hypothesized that NO loss would increase with increasing N fixation potential of the biological soil crust, where higher N fixation and subsequent leakage of carbon (C) and N compounds would provide substrates to microbes in biological N gas loss. I examined controls on NO loss by measuring NO fluxes from biological soil crusts under a wide range of soil moistures, temperatures, and nutrient contents. Furthermore, I discussed the potential implications of NO losses from biological soil crusts on ecosystem N budgets relative to other N loss pathways.

In chapter IIII, I conducted rainfall simulation experiments to examine the role of biological soil crust composition and disturbance on C and N fluxes in surface runoff. Soil surface stability is a critical factor in retention of soil nutrients in arid and semi-arid ecosystems that are characterized by sparse vascular plant cover and exposed soils. Networks of fungal hyphae, cyanobacterial sheaths, and lichen and moss attachment structures (*i.e.* rhizines and rhizoids) in biological soil crusts bind soil particles (Belnap and Gardner 1993). Biological soil crusts often increase soil aggregate stability (Schulten 1985, Eldridge 1993, de Cano *et al.* 1997) surface roughness (Belnap 2001) and porosity (Eldridge 1993), which may subsequently impact surface hydrology via infiltration, soil moisture, and erosional processes (see review by Warren 2001). Numerous studies have

been conducted on the role of biological soil crusts on surface hydrology and erosional dynamics in warm deserts, but there are relatively few studies in cold deserts where frost heaving may be important. In addition, associated element fluxes such as C and N in erosional losses are poorly understood in all ecosystems.

In this study, I conducted rainfall simulation experiments to examine the role of soil surface disturbance and biological soil crust community composition on C and N fluxes in runoff and surface erosion. Disturbances such as livestock grazing and off-road vehicle use, dramatically alter soil surfaces in the arid and semi-arid regions of the western U.S. (Belnap 1996). As a result, I addressed this first question: 1) what effect does disturbance of a dark cyanolichen soil crust have on C and N losses in runoff and soil erosion? Over the longer term, disturbance results in the conversion of a stable, cyanolichen crust to a less stable cyanobacterial crust. To better understand regulation of C and N fluxes by these two biological soil crusts community types, I addressed a second question: 2) does biological soil crust community composition (*i.e* dark, cyanolichen vs. light, cyanobacterial crusts) play a role in regulating C and N fluxes in runoff and erosional processes?

In chapter IV, I determined conversion ratios for acetylene reduction (AR) to N fixation for the soil cyanobacterium *Nostoc commune.* Acetylene reduction (AR) is the most common assay by which to estimate N fixation activity. In AR asssays the enzyme nitrogenase, which catalyzes N_2 reduction, also reduces acetylene to ethylene. Although $^{15}N_2$ incorporation is a true measure of N fixation, AR has been the preferred method to examine N fixation activity over the past several decades since it is less expensive and

easier to implement under field conditions. Conversion ratios may vary dramatically, leading to considerable uncertainty in converting AR rates to actual N fixed.

The theoretical conversion ratio of AR to actual N fixed is 3, based on the biochemistry of the N fixation where 6 electrons are required for N_2 reduction, whereas only 2 electrons are required for reduction of C_2H_2. Field and laboratory studies have shown conversion ratios to vary dramatically and may range from 0.022-22 (Rice and Paul 1971, Nohrstedt 1983, Nohrstedt 1985, Montoya *et al.* 1996, Liengen 1999). Due to the wide range of conversion ratios reported in the literature, it is important to calibrate AR to N fixed for each site and organism. In this study, I examined AR to N fixed conversion ratios for *Nostoc commune*, a ubiquitous N-fixing cyanobacterium in arid and semi-arid soils throughout the world. *N. commune* commonly occurs as free-living colonies or in association with a fungal component in soil lichens. I collected organisms from three arid and semi-arid regions that differed in mean annual precipitation and temperature.

Chapters II, III, and IV, focus primarily on N cycling in biological soil crusts on the Colorado Plateau, USA. In the final chapter, however, I examined the impacts of sheep grazing on plant composition and soil nutrients in a nine-year grazing study in Inner Mongolia Autonoumous Region (IMAR), China. Human population growth and changes in land use practices are rapidly increasing livestock grazing pressure in the steppe regions of the IMAR in northern China, where livestock numbers nearly tripled from 1952 to 1989 (Ellis 1992). Studies conducted in this region during the 1990's indicate that grassland productivity decreased by 30% in some areas, and up to 55% of Inner Mongolian grasslands are now considered to be unusable or "deteriorated" (Ellis

1992). From 1990 to 1998 livestock numbers in the Xilin River Basin, the area of this study, increased by 184%.

Four years after the initiation of a nine-year sheep grazing study in Inner Mongolia, standing aboveground biomass declined with increasing grazing intensity (Wang and Chen 1998). Furthermore, after four years, cover of the dominant grass species, *Agropyron michnoi* and *Leymus chinensis* decreased dramatically with increasing grazing intensity whereas cover of *Potentilla* spp. (*P. acaulis* and *P. tanacetifolia*), indicators of overgrazing, increased at the highest grazing intensities (Wang and Chen 1998, Li *et al.* 1999). I predicted that changes in litter quantity and quality after nine years under varying grazing intensities would alter soil C and N mineralization dynamics given these changes in aboveground biomass and plant community composition,.

I report on changes in plant community composition and soil C and N mineralization dynamics from a nine-year sheep grazing study. I addressed these questions: 1) how does increasing grazing intensity affect plant community composition? 2) how does increasing grazing intensity have affect soil C and N mineralization dynamics? 3) Do changes in soil C and N mineralization dynamics relate to changes in plant community composition via inputs of the quality and quantity of litter?

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Chapter II.

NO gas loss from N-fixing biological soil crusts on the Colorado Plateau

Abstract

Estimates of N fixation by biological soil crusts range from 1.3-9 kg N/ha/yr depending on biological crust composition on the Colorado Plateau. Estimates of N accretion in desert ecosystems is less than 1 kg N/ha/yr, leading to uncertainty regarding the fate of the fixed N by biological soil crusts and pathways of N loss in desert ecosystems. I examined biogenic N gas loss as nitric oxide (NO) from biological soil crusts as one of the potential N gas loss pathways from desert soils and explored the potential implications of these losses for ecosystem N budgets in a Colorado Plateau shrubland. I addressed these questions: 1) does NO loss increase with increasing N fixation potential of a biological soil crust? 2) If so, what are the primary controls on NO flux from biological soil crusts? 3) Furthermore, is NO a dominant N loss pathway from these aridland soils? I measured NO loss from biological soil crusts with three levels of N fixation potential in order to better understand N gas losses in relation to inputs via N fixation in biological soil crusts. Coloration in biological soil crusts is a good indicator of cyanobacterial composition, where light biological soil crusts are dominated by the free-living filamentous cyanobacteria *Microcoleus vaginatus,* medium biological soil crusts contain *M. vaginatus* and darker pigmented cyanobacteria, and dark biological soil crusts contain *M. vaginatus*, darker pigmented cyanobacteria such as *Sytonema myochrous* and *Nostoc commune*, and the N-fixing lichens *Collema tenax and C. coccophorum.* I measured NO fluxes from April 2001-April 2002 in both permanent soil cores incubated

in a common field site to examine the seasonal effect on NO flux and field chambers to examine controls on NO flux. Nitric oxide fluxes from permanent soil cores were highest in dark crusts relative to medium and light crusts. The pattern of NO loss was influenced by season. Nitric oxide fluxes from dark crusts > medium crusts > light crusts during the spring. In the summer months, NO fluxes from dark and medium crusts were greater than light crusts, whereas in the fall I observed no differences in NO flux by crust type.

Results from a regression tree model showed that mean NO flux from field chambers was 3.67 ng NO-N/m^2/s, which was highly variable across sites and over time. The major controlling factor was soil temperature, where NO fluxes averaged 2.75 ng NO-N/m^2/s at temperatures < 31° C. However, when soil temperatures were > 31 ° C, NO fluxes increased by more than 3-fold (9.76 ng NO-N/m^2/s). The majority of measurements (86%) occurred at soil temperatures < 31° C, where soil N was correlated with NO loss. When percent total soil N was < 0.06, NO flux averaged 2.36 ng NO-N/m^2/s, which more than doubled to 5.76 ng NO-N/m^2/s when percent total soil N was >0.06. In the other 17 observations when soil temperatures were > 31 ° C, chlorophyll *a*, reflecting crust type, was the controlling factor in NO loss. Nitric oxide fluxes averaged 5.38 ng NO-N/m^2/s when chlorophyll *a* content was < 2.8 μg/g soil, but more than double to 12.15 ng NO-N/m^2/s when chlorophyll *a* was > 2.8 μg/g soil. The upper limit of annual NO loss from dark and light crusts were 0.13 and 0.07 kg N/ha/yr respectively. Overall, annual inputs via N fixation greatly exceed estimates of annual N gas losses in dark, cyanolichen crusts, whereas N gas loss nearly equals that of N inputs in light, cyanobacterial crusts. Hence in the early successional stages of biological soil crust development N losses may exceed inputs.

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 biological soil crusts fix atmospheric N_2 and are an important source of nitrogen [N] in many desert ecosystems (Evans and Belnap 1999, Evans and Ehleringer 1993). Estimates of N fixation by biological soil crusts range from 1.3-9 kg N/ha/yr depending on biological crust composition on the Colorado Plateau (Belnap 2002). Estimates of N accretion in desert ecosystems is less than 1 kg N/ha/yr (Peterjohn and Schlesinger 1990), leading to uncertainty regarding the fate of the fixed N by biological soil crusts and pathways of N loss in desert ecosystems. 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 to the atmosphere in wind erosion and gaseous pathways.

Nitric oxide (NO) is a gaseous intermediate produced in both nitrification and denitrification processes. Although there are few measurements of soil NO losses from desert ecosystems, rates may be high. In a Chihuahuan desert grassland and shrubland, short-term NO loss rates (*i.e.* minutes) 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, estimates are low and range from 0.15-0.38 kg NO-N/ha/yr (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_4^+ to NO_3^- (Poth and Faucht 1985). NO production in nitrification has been closely linked to soil oxygen levels, available NH_4^+ 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 due to the paucity of precipitation, which would favor nitrification. However when rain events do occur, declining oxygen content may limit nitrification (Garcia-Pichel and Belnap 1996). A negative relationship exists between air porosity and bulk density, where porosity declines to 10% at soil bulk densities in the range of 1.6 g/cm^3, values in the range of what occurs in many desert ecosystems (Paul and Clark 1996). 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 30-35 °C (Paul and Clark 1996), soil temperatures that commonly occur in the late spring, summer, and early fall in cool desert ecosystems.

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), NO may also be lost in denitrification processes. Davidson (1992) observed that chemoautotrophic nitrification was the dominant source of NO and N_2O when water-filled pore space (WFPS) was < 60 percent. However, when WFPS exceeded 60 percent, denitrification was the primary source of NO and N_2O resulting in ratios of N_2O:NO much greater than 1. Denitrification is primarily a biological process, especially in high pH soils, where NO_3^- 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 Sonoran desert site were similar to a temperate hardwood forest (Peterjohn 1991, Peterjohn and Schlesinger 1991). Billings *et al.* (2002) also observed high denitrification enzyme activity in a Mojave desert site, which supports the idea that during certain periods of the year denitrification rates may be high. Deserts are heterogeneous environments likely with hot spots of N loss. The presence of N-fixing organisms may also result in high N gas loss. 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. Rewetting of dry soils results in a large pulse in microbial activity, which draws down soil oxygen levels (Groffman and Tiedje 1988). 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, which may then favor NO losses via denitrification.

In this study I examined NO losses from biological soil crusts and explored the potential implications of these losses on ecosystem N budgets in a Colorado Plateau shrubland. I addressed these questions: 1) does NO loss increase with increasing N fixation potential of a biological soil crust? 2) If so, what are the primary controls on NO flux from biological soil crusts? 3) Furthermore, is NO a dominant N loss pathway in

desert ecosystems? I measured NO loss from biological soil crusts with three levels of N fixation potential in order to better understand N gas losses in relation to inputs via N fixation. I 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. I examined controls on NO loss by measuring NO fluxes from biological soil crusts under a wide range of soil moistures, temperatures, and nutrient contents. Furthermore, I discuss the potential implications of NO losses from biological soil crusts on ecosystem N budgets relative to other N loss pathways.

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 185-226 mm in years 1998-2000 (National Atmospheric Deposition Program, http://nadp.sws.uiuc.edu/). I chose two sites where biological soil crust communities that differ in N fixation potential were present. In the Canyonlands area, the most well-developed biological soil crust communities often occur on soils that were 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 10-50 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.

I chose two sites within this soil type to measure NO gas fluxes from biological soil crusts. The first site was located within an area dominated by *Pinus edulis* and *Juniperus osteosperma* with the presence of *Yucca harrimaniae* (Harriman's yucca) and *Coleogyne ramossisima* (blackbrush), which I refer to as the pinyon-juniper site. Soils at this site were generally less than 10 cm to bedrock. The second site was located in an area dominated by *C. ramossisima* with the presence of *P. edulis* and *Juniperus osteosperma.* Soil depth at this site ranged from 20-40 cm, which I refer to as the blackbrush site.

Biological Soil Crust Type—I measured nitrogen oxide (NO) from three biological soil crust types at each of the fieldsites. Coloration in biological soil crusts is a good indicator of cyanobacterial composition and N fixation potential, where N fixation tends to increase with increasing darkness of the biological soil crust (see appendix I). Light biological soil crusts were dominated by the free-living filamentous cyanobacteria *Microcoleus vaginatus* (Belnap 2002). Dark biological soil crusts contained *M. vaginatus*, darker pigmented cyanobacteria such as *Sytonema myochrous* and *Nostoc commune*, and the N-fixing lichens *Collema tenax and C. coccophorum.* Medium biological soil crusts contained *M. vaginatus* and darker pigmented cyanobacteria, but *Collema* lichens were not present. I 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 permanent soil cores—I measured NO fluxes from April 2001-April 2002 in permanent soil cores to examine seasonal variation in NO loss from the three biological soil crust types. From the same soil type described above, I collected 4, 182 cm^2 soil cores from a depth of 0-5 cm for each soil crust type and placed them in

polyvinyl chloride (PVC) rings with a grated bottom to allow water drainage. Permanent cores were places in a common site dominated by *C. ramossissima* near the USGS laboratory in Moab, UT. NO fluxes in dry soils were always zero. As a result, at each measurement point I added water to simulate a 2.7 mm rain event, which is in the range of an average rain event for this region. Preliminary data from permanent soil cores and field sites showed that peak NO flux generally occurred 1.5-2 hrs after water addition. Therefore NO flux measurements were taken 1.5 hrs after water addition unless otherwise noted.

Field measurements of nitric oxide fluxes—In order to examine controls on NO flux, I placed temporary gas chambers in each of the field sites and measured NO fluxes along with several other soil variables described below. I measured NO fluxes at the pinyon-juniper site in April, June, July, September, and October of 2001 and April of 2002. At the blackbrush site I measured NO fluxes in July, September, and October of 2001 and April of 2002.

I placed 19.7 cm diameter PVC anchors to a depth of 4 cm into each of the three biological soil crusts types. I measured NO flux from 5 blocks of the three biological soil crusts types for a total of 15 measurements at each site within a sample date. In both sites I located areas where the three crust types occurred over a 1-3 m^2 area. At each sample date, the area sampled was approximately 20 x 20 m^2. NO was measured with a portable Scintrex Unisearch LMA-3 chemiluminescent analyzer with an attached LNC-3 converter that oxidizes NO to NO_2. 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. I added water to each ring to simulate a 3.3 mm rain event and measured NO flux after 1.5 hrs. Immediately before a sampling period, I secured a 19.7 cm diameter Teflon-lined PVC chamber top with a height of 18 cm 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, which allowed chamber air to equilibrate with atmospheric air. This resulted in a mixing of atmospheric air and chamber air over the sample period. As a result, I used a point calculation method as described by Martin (1996) to estimate NO flux, which accounted for the mixing of atmospheric air and chamber air. I calculated NO flux 6 minutes after chamber closure since NO accumulation within the chamber was linear over 2-7 minutes from chamber closure.

Ancillary measurements—Immediately after measuring NO flux from each gas ring, I measured soil temperature. I also collected soil cores for analysis of extractable inorganic N, total N, and chlorophyll *a* content. Three 2 cm diameter soil cores were collected from to a depth of 5 cm within each chamber for inorganic N analysis (NO_3^- and NH_4^+). Samples were immediately transported back to the lab and refrigerated. For the July 2001 extractions I added 100 mL of 2 M KCl to 25 g of soil. Since concentrations were near the detection limit of the autoanalyzer, I changed the ratio of salt solution to soil at later dates. For the October and April sample dates I added 50 ml of 2 M KCl to 25 g of soil. Once the salt solution was added to the soil, I placed the samples on a reciprocal shaker for 1 hr. Samples were then filtered with Whatman 4 qualitative grade paper and immediately frozen. Samples were analyzed later on an Alpchem Flow Solution 3 Autoanalyzer.

I also collected soil cores from each gas sample ring 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 I use chlorophyll *a* content as an index of C and N fixation potential, which increases with increasing chlorophyll *a* content (Belnap 2001, Garcia-Pichel and Belnap 1996). I collected 2, 3.1 cm^2 soil cores from the top 0-1 cm of soil. The first core was air-dried in the dark and stored in the freezer 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.

Varying rainfall amount and NO fluxes—In April 2002 I conducted a field experiment to examine the effect of varying rainfall amount on soil NO fluxes. I established 15 sample sites on medium soil crusts at the blackbrush fieldsite. I added 3 levels of water (3.3, 6.6, 13.3 mm) to 5 samples rings each for a total of 15 measurements. I measured NO fluxes 1.5 hrs after water addition. Immediately following NO flux measurements, I collected a soil core from each gas ring to estimate percent water-filled pore space (WFPS). I collected a 7.0 cm^2 soil core 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 I weighed each soil core to obtain a wet weight. Soil samples were then placed in a 100 ° C oven for 24 hrs.

After this time I reweighed each sample to obtain a dry weight. Percent WFPS was calculated as:

% WFPS = (soil water content x bulk density x 100)/(1- (bulk density/2.65)

with bulk density (g/cm^3) = oven dry weight of soil/soil volume. Particle density was set at 2.65 g/cm^3, the density of most mineral particulates (Paul and Clark 1996).

Statistical analysis—I used a factorial analysis of variance 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. I used a Newman-Keuls post-hoc test to examine individual treatment differences. In order to more closely examine factors that regulate NO flux I used a least squares regression tree model (Systat 10.2) with chlorophyll a, percent soil N, soil inorganic N and soil temperature as independent variables in explaining NO fluxes. I report proportional reduction in error (PRE), which is equivalent to a multiple R^2 in a linear regression.

Calculation of annual NO loss budgets—The average NO flux for each season (spring, summer, fall) was multiplied by the minimum and maximum number of hours surface soils were moist in a given season, which was based on soil moisture data generated by time domain reflectometry (TDR) at 5 mm soil depth (Belnap, unpublished results). NO fluxes were near the detection limit of the analyzer in the late fall, therefore I assumed winter NO fluxes would not contribute substantially to annual NO loss. NO loss for each season was then summed to estimate annual NO loss. The number of hours soils were moist, varied by season (minimum/maximum hrs; Spring = 131/251, Summer = 19/154, Fall = 142/408).

Results

Nitric oxide fluxes from permanent soil cores—Nitric oxide fluxes from permanent soil cores were higher in dark crusts relative to medium and light crusts (Fig. 1). The pattern of NO flux, however, from different crust types was influenced by season (crust x season, $F_{4,54} = 52.7$, $P < 0.0001$). Nitric oxide fluxes from dark crusts were higher than medium and light crusts during the spring. In the spring and summer months, NO fluxes from dark > medium > light crusts (Table 1). In the fall I observed no differences in NO flux by crust type.

Field measurements of nitric oxide fluxes—Similar to the permanent soil cores, the interaction of crust and season on NO fluxes from field chambers was significant (crust x season, $F_{4,121} = 2.95$, $P = 0.02$ Fig. 2). NO fluxes were significantly higher in dark crusts relative to medium and light crusts only in the summer months. However there were no differences by crust type in the spring and fall. I observed no differences in NO fluxes in the pinyon-juniper site as compared to the blackbrush site. Although differences were not significant in all seasons, the trend throughout the annual data was higher NO fluxes with increasing darkness of the soil crust.

Ancillary Measurements—Chlorophyll *a* content increased with increasing darkness of the biological soil crust (dark > medium > light), but this again depended on season (crust x season, $F_{4,116} = 2.72$, $P = 0.03$, Table 2). Chlorophyll *a* was similar in dark and medium crust but greater than light crusts in the spring. By the summer and fall months, the pattern changed where chlorophyll *a* content in dark >medium>light crusts. Percent total soil N was significantly higher in dark crusts as compared to medium and light crusts, but this was highly dependent on season (crust x season, $F_{4,117} = 4.30$, $P = 0.002$). In the

spring I observed no differences in percent total soil N, whereas percent total soil N in dark > medium > light crusts in the summer and fall months. Soil crust type had not effect on total soil inorganic N pools ($NO_3^- + NH_4^+$). Although the main effect of crust type on soil NO_3^- was not statistically significant ($P = 0.08$), in the spring months there appeared to be a trend toward higher soil NO_3^- in dark relative to medium and light crusts (Table 2). Season also appeared to be an important factor in soil inorganic N pools. There was an effect of season where soil NO_3^- and NH_4^+ pools were highest in the spring relative to the fall and summer (Table 2). I observed a site by season effect on soil temperature (site x season, $F_{2,121} = 18.5$, $P < 0.0001$) where soil temperatures in the summer were greater than the spring and fall in the blackbrush sites, but in the pinyon-juniper sites soil temperatures were greater in the spring and summer relative to fall (Table 2). There were no differences in soil temperature relative to crust type.

Varying rainfall amount and NO fluxes—Rainfall amount was not an important factor in regulating NO fluxes from biological soil crusts. I observed no relationship between NO flux and percent WFPS when rainfall amount was varied (Fig. 3). Multiple water additions, however, did alter NO flux dynamics. Immediately after water addition positive fluxes were measured after 0.5 hrs, which peaked at 1.5 hrs (Fig. 4a). Four hrs after water addition, NO fluxes in medium and light crusts had declined to near detection limits. However, NO fluxes from dark crusts were nearly three-fold higher than medium and light crusts during this time. NO fluxes after several water additions extending over two days had very different dynamics. The morning after a 2 mm natural rain event, positive NO fluxes were measured in all cores (Fig. 4b), but I observed no differences in NO flux by crust type. Immediately after this first measurement period I added an

additional 2.7 mm of water and returned the next morning. NO fluxes in dark crusts more than tripled, whereas fluxes from medium and light crusts remained unchanged. A third 2.7 mm water addition had no effect on NO fluxes, which remained unchanged throughout that day.

Factors controlling NO field fluxes—Results from a regression tree model showed that mean NO flux for all plots was 3.67 ng NO-N/m^2/s, which was highly variable across sites and over time (Fig. 5). The model explained 42% of the variability in NO fluxes. The major controlling factor was soil temperature, where NO fluxes averaged 2.75 ng NO-N/m^2/s at temperatures < 31.3° C. However, when soil temperature was > 31.3 ° C, NO fluxes increased by more than 3-fold (9.76 ng NO-N/m^2/s). The majority of measurements (86%) occurred at soil temperatures < 31° C, where soil N was correlated with NO loss. When percent soil N was < 0.06 NO flux averaged 2.36 ng NO-N/m^2/s, which more than doubled to 5.76 ng NO-N/m^2/s when percent soil N was > 0.06 (Fig. 5). There was an additional temperature threshold when percent soil N was > 0.06, where NO fluxes averaged 3.32 ng NO-N/m^2/s at temperatures < 27 ° C, but increased to 9.68 ng NO-N/m^2/s at temperatures > 27 ° C. In the other 17 observations when soil temperatures were > 31 ° C, chlorophyll *a,* reflecting crust type, was the controlling factor in NO loss. Nitric oxide fluxes averaged 5.38 ng NO-N/m^2/s when chlorophyll *a* content was < 2.8 μg/g soil, but more than double to 12.15 ng NO-N/m^2/s when chlorophyll *a* was > 2.8 μg/g soil.

Discussion

Factors controlling NO loss from biological soil crusts—Temperature was the primary control on NO loss from biological soil crusts at the two sites. However chlorophyll *a* and soil N, factors that generally increase with increasing N fixation potential of the

biological soil crust, were important secondary correlates. NO fluxes increased with increasing chlorophyll *a* content at high soil temperatures (> 31° C), conditions that persist over a limited period of time during the summer months on the Colorado Plateau. Jones and Stewart (1969) showed a strong temperature effect on N release, where up to 75% of the total N in the N-fixing cyanobacteria *Calothrix scopulorum* was in the extracellular N pool at 40 °C. Mayland (1966) showed that N leakage from biological soil crusts was primarily in the form of NH_4^+ and simple organic N compounds. Hence N leakage to underlying soil at high soil temperatures may provide substrates for nitrifiers resulting in increased NO loss. Nitrogen release only occurs when adequate moisture is available to organisms, resulting in a strong pattern in pulsed N release in desert soils. Nitrogen leakage may also increase with increasing N fixation of the biological soil crust. In a laboratory experiment, Barr (1999) showed that NH_4^+ flux in leachate beneath a soil crust dominated by dark, *Nostoc* spp. was 20-fold higher than a light, *M. vaginatus* soil crust.

During most of the year soil temperatures at these sites are well below 31 ° C, where soil N appears to be an important control on NO loss. Total soil N in relation to gas loss may reflect differences in a variety of factors such as standing pools of inorganic N, N mineralization rates, and soil organic matter (SOM) content. In this study, there was no relationship between total soil N and standing pools of inorganic N. This may be partially due to scale differences at which total N (0-1 cm depth) and inorganic N (0-5 cm depth) were measured. Since a majority of the microbial biomass resides in the top few mm (Garcia-Pichel and Belnap 1996), integrating inorganic N pools over the first 5 cm may not adequately reflect standing pools of N in the top few mm. I did not measure

N mineralization in this study, but it is often assumed there is a relationship between total soil N and mineralizable N, but results are often mixed. In a review of controls on N mineralization rates, total N was a poor predictor of short-term (< 15 weeks) N mineralization dynamics (Vigil *et al.* 2002). In contrast, the relationship between total soil N and soil organic matter is more clear, where total N increases with increasing SOM content (Vigil *et al.* 2002). Thus higher NO loss with increasing soil N may also reflect increased availability of C substrates for dentrifiers.

Although NO losses may be produced in both nitrification and denitrification processes, losses from aridland soils are more often associated with nitrification (Hartley and Schlesinger 2000, Smart *et al.* 1999). Standing soil NH_4^+ pools, the initial substrate in nitrification, were a poor predictor of NO fluxes in this study. However, seasonal NO_3^-: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 site.

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) showed that dark respiratory activity quickly drew down soil oxygen levels in the surface 4 mm of a biological soil crust. 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 pronounced anoxic conditions in dark crusts may also partially explain the higher NO losses. Organic C availability,

which may serves as an energy source in heterotrophic denitrification, may also be an important factor regulating NO loss. In a study where biological soil crusts were labeled with $^{14}CO_2$, results showed that newly fixed labile C was quickly released to underlying soils (Beymer and Klopatek 1991). Fluxes of labile C compounds to underlying soils may be higher in dark crusts than light crusts, due to the higher net photosynthesis and C gain (Garcia-Pichel and Belnap 1996).

Increasing water filled pore space at 0-5 cm depth had no impact on NO fluxes. In biological soil crusts 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.*, submitted). Microprobe studies showed that soil NO_3^- pools are also highest in the soil surface between 1-2 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 mm 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.

Biological soil crust N budget—NO gas losses are decoupled from N inputs via fixation. Nitrogen fixation peaked in the spring and fall, when air temperatures were less than 20 °C (Belnap 2001). However, NO fluxes peak in the summer months especially at temperatures > 31 °C. Combining seasonal fluxes and soil moisture, total NO loss during the summer months equals that of the spring and fall combined. In an unusually wet summer with a strong summer monsoon effect, NO losses may be unusually high.

The upper limit of annual NO loss from dark and light crusts was 0.13 and 0.07 kg N/ha/yr respectively for the two sites studied. There was evidence from NO flux data

that fluxes in dark crusts may occur for a longer period relative to light crusts (Fig. 4a). As a result, annual NO loss from dark crusts may be slightly underestimated. Including estimates of denitrification losses as N_2O and N_2 (appendix II) and NH_3 volatilization (appendix III) from biological soil crusts, the upper limit of total N gas loss was 1.6 kg N/ha/yr from all biological soil crusts. Estimates of N fixation, however, ranged from an upper limit of 9 kg N/ha/yr down in dark crusts down to 1.4 kg N/ha/yr in light crusts (Belnap 2002). I expect N fixation in dark crusts from this study to be lower due to the low cover of N-fixing lichens than those used in the study by Belnap (2002). I measured N fixation activity by acetylene reduction (AR) in soil crusts at these sites, where AR was 4.6 fold higher in dark crusts relative to light crusts (appendix I). Since light crusts in this study were similar in composition to those used in the study by Belnap (2002), I estimated N inputs by dark crusts by multiplying the N inputs by light crusts by 4.6, resulting in an N input for dark crusts at these sites of 6.4 kg N/ha/yr. Overall, annual inputs via N fixation greatly exceed estimates of annual N gas losses in dark crusts, whereas N gas loss nearly equals N inputs for light crusts. Hence in the early successional stages of biological soil crust development N losses may exceed inputs. Higher losses relative to inputs in light crusts effectively mine soil N pools and may result in depletion of soil N in early successional stages. As a biological soil crust develops over time, N inputs via fixation should increase with little change in N gas loss, resulting in accumulation of N as soil crusts develop.

Standing pools of total soil N in the top 20 cm of soil in light and dark crusts in these sites were approximately 650 kg N/ha and 710 kg N/ha respectively, an increase of 60 kg N/ha during successional development. A light, cyanobacterial crust develops into

a dark, cyanobacterial crust with < 5% lichen in approximately 50 years (J. Belnap, pers. comm.), which would result in an N accretion rate of 1.2 kg N/ha/yr. Taking into account dissolved and sediment bound N loss in surface runoff (Chapter III, dark = 0.6 kg N/ha/yr, light = 6.3 kg N/ha/yr), inputs exceed losses by 5.8 kg N/ha/yr in a dark biological soil crust. In contrast, N losses in light biological soil crusts exceed inputs by 4.9 kg N/ha/yr.

The current N budget does not take into account all N loss pathways such as losses in wind erosion and leaching of dissolved N through the soil profile. Cyanobacterial crusts are more susceptible to wind erosion as compared to cyanolichen crusts. Belnap and Gillette (1998) showed a more than 5-fold increase in threshold friction velocity (a measure of wind velocity) was needed to move soils in a lichenized crust as compared to a cyanobacterial crust. Therefore, if N losses via wind erosion were taken into account, losses should be much greater on light cyanobacterial crusts than dark cyanolichen crusts. Nitrogen leaching losses in these sites are not well understood. Vertical transport of water through the soil profile, in a sandy soil similar to those within this study, reached 80 cm in a season (J. Neff, unpublished results). In sites used in this study soil depth ranges from 20-40 cm. In a lab experiment examining leaching of inorganic N from biological soil crusts, Barr (1999) estimated annual losses in the range of 0.55-0.75 kg N/ha/yr. In chapter III, I show that organic N is the dominant form of N in surface runoff. Therefore leaching and transfer of N loss through the soil profile may also constitute a substantial N loss pathway.

Nitrogen uptake by the surrounding plant community may also be an important pathway for N loss from biological soil crusts. Biological soil crusts have been shown to

leak significant amounts of N to extracellular N pools, which may then be taken up by the surrounding plant community (Belnap *et al.* 2001a). A study of ^{15}N natural abundance showed that N fixation by biological soil crusts was a dominant N source in a pinyon-juniper woodland (Evans and Ehleringer 1993).

Overall, plant interspaces colonized by biological soil crusts appear to lose N in early successional stages, but shift to retaining N in later stages. Retention of plant-limiting nutrients such as N, especially in ecosystems where soil nutrient content is low, is critical in maintaining plant productivity. Human impacts such as livestock grazing and recreational use of public lands have greatly affected biological soil crust communities on the Colorado Plateau where well-developed cyanolichen crusts are rapidly being converted to light cyanobacterial crusts (J. Belnap, pers. comm.). Although NO fluxes in this study were higher from well-developed biological soil crusts; I observed no strong difference in N gas loss by crust type when considering all N gas losses pathways. Biological soil crusts make up to 70% of the living cover in many sites on the Colorado Plateau (Belnap *et al.* 2001b). If these patterns in N input and loss pathways are similar for other vegetation and soil types, conversion of a well-developed resource-conserving biological soil crust to a resource-losing cyanobacterial crust may have a dramatic impact on ecosystem N cycling in this region.

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Table 1. NO fluxes by season from permanent soil cores. Values are mean NO flux ± 1 SE. Different letters within a row represent a significant effect of crust on NO flux evaluated at $P < 0.05$ (post-hoc Newman-Keuls).

	Dark	Medium	Light
Spring	10.5 (2.6) a	4.2 (1.1) b	1.6 (0.5) c
Summer	28.4 (3.1) a	20.6 (1.7) b	8.8 (2.0) c
Fall	2.6 (0.4) a	1.4 (0.1) a	1.3 (0.1) a

Table 2. Soil chlorophyll *a*, percent N, inorganic N (NO_3^- + NH_4^+), soil temperature, and NO loss from the three biological crust types over several seasons. Values are seasonal means within a crust type. We examined the main effects of season (spring, summer, and fall) and crust type (dark, medium, and light). Significant main effects, listed at the top of the table, were considered significant at $P < 0.05$. Values are significantly different at $P < 0.05$ (post-hoc Newman-Keuls test) when a different letters follows a mean within a season. In the absence of a two-way interaction, individual treatment effects are listed directly below significant main effects where Sp = Spring, Su = Summer, and Fa = Fall. I observed no crust x season x site interaction in any of the soil measurements.

	Chl *a* µg/g soil	**% Soil N**	**NO_3^- µg/ g soil**	**NH_4^+ µg/ g soil**	**Soil Temperature °C**	**NO flux ng NO-N/m^2/s**
Significant effects	Crust x Season	Crust x Season	Season Sp>Su=F	Season Sp>Su=F	Site x Season	Crust x 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	2.16 (0.31) a
Medium	6.28 (0.79) a	0.038 (0.005) a	1.94 (0.28) b	0.88 (0.25) a	26.9 (1.0) a	1.53 (0.19) a
Light	3.93 (0.69) b	0.034 (0.003) a	1.57 (0.22) b	0.53 (0.10) a	27.4 (1.0) a	1.21 (0.14) 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	11.88 (1.75) 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	7.47 (1.01) b
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	6.46 (1.57) b
Fall						
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	2.49 (0.27) 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	1.84 (0.24) 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	1.26 (0.23) a

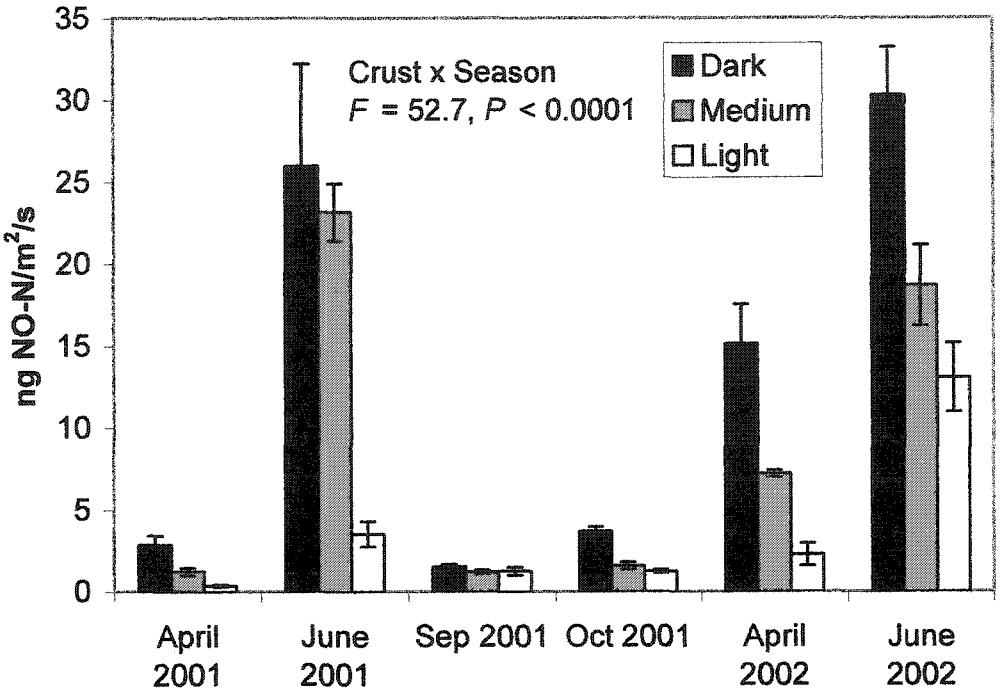

Figure 1. Nitric oxides fluxes from permanent soil cores. Soil NO fluxes were measured 1.5 hrs after a simulated 2.6 mm rain event. Values are mean NO flux in ng NO-N/m^2/s ± 1 SE. I observed a significant crust x season interaction on NO loss throughout the year.

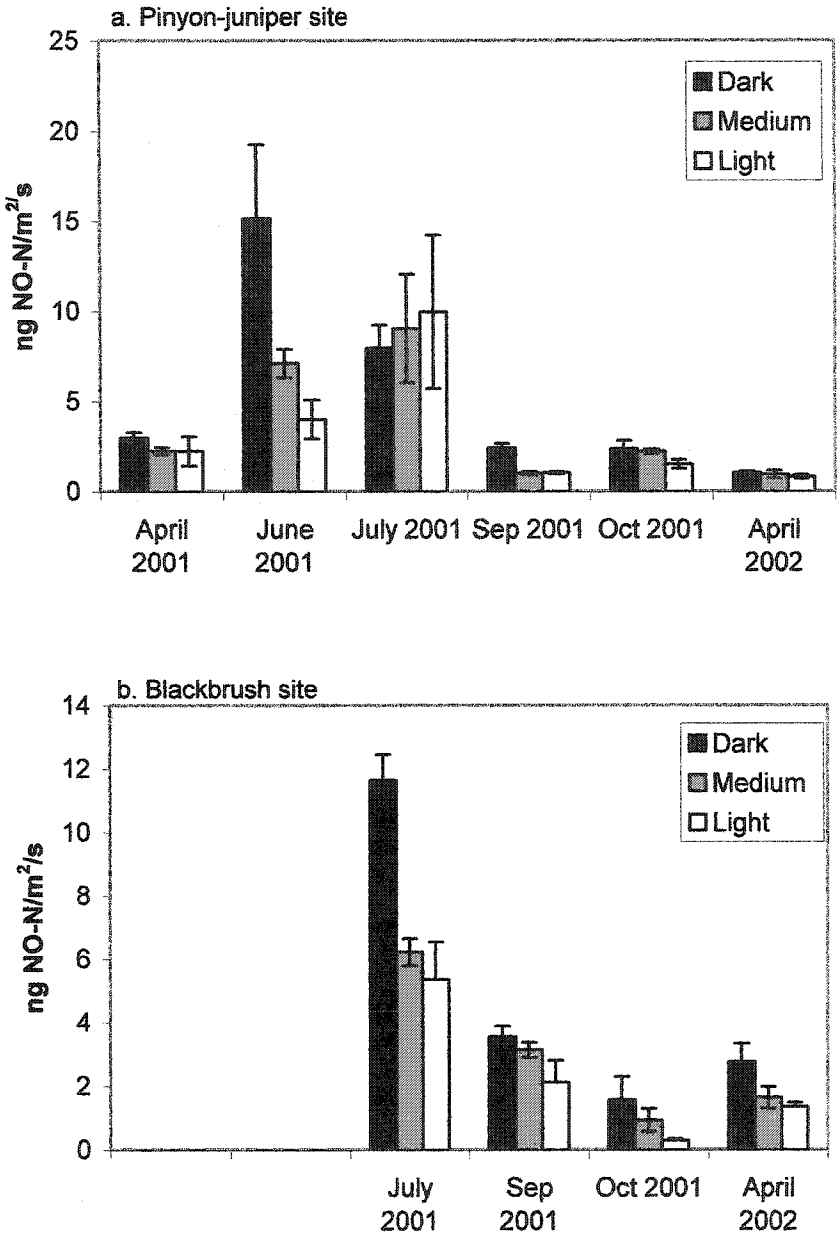

Figure 2. Nitric oxide fluxes from field sites. Soil NO fluxes were measured 1.5 hrs after a 3.3 mm rain event. Values are mean NO flux in ng NO-N/m^2/s ± 1 SE. I observed a significant crust x season interaction on NO loss throughout the year. However there was no site effect. Note different scales on y-axes.

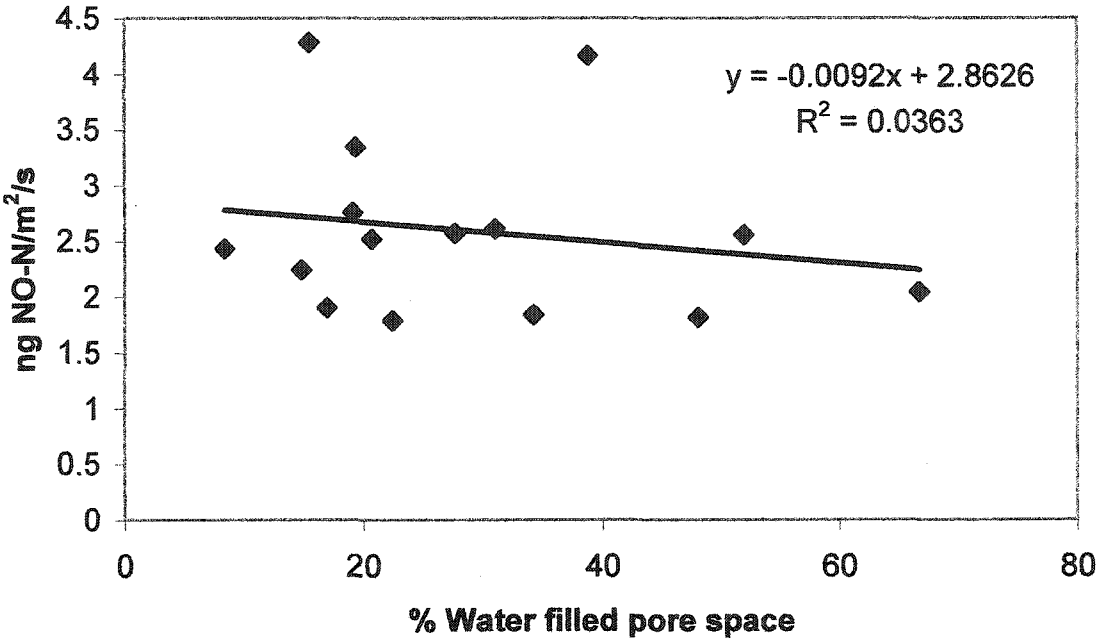

Figure 3. Soil NO flux as a function of percent water-filled pore space (WFPS). To examine the effect of increasing percent WFPS on NO loss, I simulated a 3.3, 6.6, and 13.3 mm rainfall.

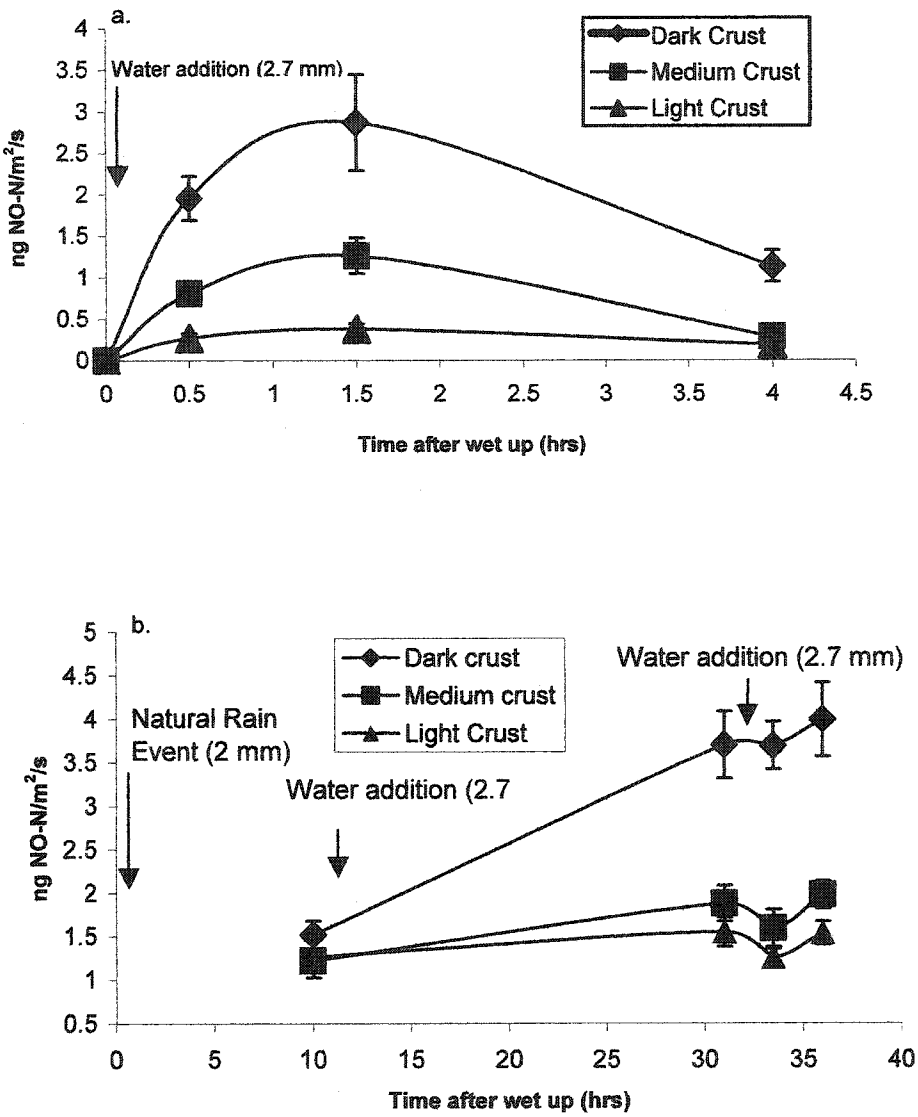

Figure 4. Diurnal NO fluxes in response to a) a single water addition and b) multiple water additions. I conducted this experiment on the permanent soil cores. Values are mean NO flux in ng NO-N/m^2/s ± 1 SE.

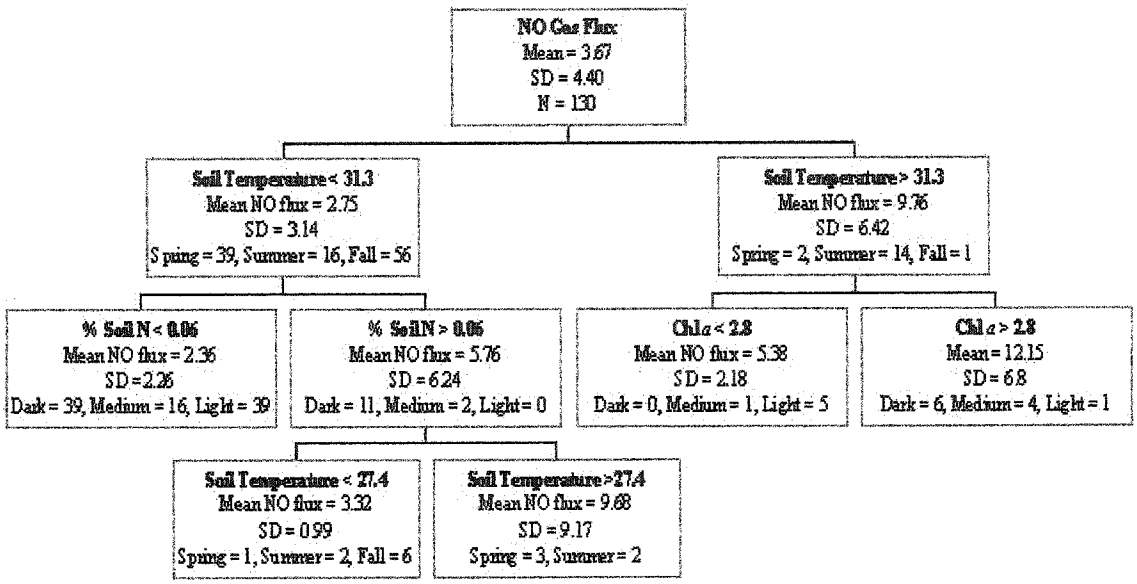

Figure 5. Nitric oxide fluxes from field cores as a function of soil temperature, percent N, inorganic N, and chlorophyll *a* in a regression tree model. The proportional reduction in error (similar to an R^2 in an linear regression) for the model was 0.42. At each split in the tree, or mobile, I list the mean NO flux and standard deviation (ng NO-N/m^2/s). I list the sample number by season when there was a temperature effect on NO flux. When there was a soil N or chlorophyll *a* effect I list sample number by crust type.

Chapter III.

The role of biological soil crust composition and disturbance on C and N fluxes in runoff: a rainfall simulation experiment

Abstract

Biological soil crusts are assemblages of lichens, fungi, cyanobacteria, and mosses that modify soil surface structure, and thus are expected to impact surface hydrology and element flux. In this study, I addressed two questions: 1) what effect does trampling disturbance have on C and N losses in runoff and soil erosion? 2) Does biological soil crust community composition (dark, cyanolichen vs. light, cyanobacterial crust) play a role in regulating C and N fluxes in runoff and erosional processes? I compared runoff and sediment C and N fluxes during a 30-minute rainfall simulation from intact dark, well-developed biological soil crusts (controls) to both trampled soil crusts (trampled) and those where the top 1 cm of the soil surface was removed but the subsurface physical structure was left intact (scraped). In a later experiment, I conducted rainfall simulations on light biological crusts and compared C and N flux in runoff and sediments to intact dark cyanolichen crusts.

Total runoff was significantly higher from trampled plots as compared to scraped and control plots. Both higher runoff and concentrations of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) led to higher organic C and N fluxes from trampled plots relative to scraped and control plots. Ammonium losses from trampled plots were significantly higher than from scraped and control plots, but only in the first 10 minutes of the simulation with no differences thereafter. Dissolved organic nitrogen was the dominant form of dissolved N loss in all the simulations. Average DON loss

ranged from 80-88% of the total dissolved N flux within all treatments. Trampling significantly increased sediment export relative to controls, with scraped plots intermediate between the two. Sediment N loss was significantly higher from trampled plots relative to scraped and control plots throughout the duration of the simulation.

Runoff from light crusts was greater than from dark crusts throughout the simulation. DOC flux from light crusts was higher than from dark crusts during the first 10 minutes of the rainfall simulation. Total DON flux from dark crusts was higher relative to light crusts during the 30-minute simulation. Nitrate export appears to be regulated by cyanobacterial community (as measured by chlorophyll *a*), where volume-weighted mean concentration of NO_3^- in runoff decreases with increasing chlorophyll *a* content. Biological soil crust biomass and composition also regulates runoff dynamics, where chlorophyll *a* content explained 79% of the variability in surface runoff. Sediment loss in light crusts was greater relative to dark crusts, resulting in 5-fold higher loss of sediment-bound C and N. Sediments from all treatments were highly enriched in N relative to the surface mineral soil early in the rainfall simulation. Sediment N concentration in the first minutes after runoff was more than 5 times higher than the percent N of the top 1 cm of soil, suggesting that even short-term runoff events may have a high potential for N loss due to the movement of sediments highly enriched in N.

Introduction

Soil surface stability is a critical factor in retention of soil nutrients in arid and semi-arid ecosystems that are characterized by sparse vascular plant cover and exposed soils. Biological soil crusts (*i.e.* cryptogamic, cryptobiotic, microphytic, microbiotic) are assemblages of lichens, fungi, cyanobacteria, and mosses that colonize soil surfaces in arid ecosystems. Networks of fungal hyphae, cyanobacterial sheaths, and lichen and moss attachment structures in biological soil crusts bind soil particles (Belnap and Gardner 1993). Biological soil crusts often increase soil aggregate stability (Schulten 1985, Eldridge 1993, de Cano *et al.* 1997) surface roughness (Belnap 2001) and porosity (Eldridge 1993), which may subsequently impact surface hydrology via infiltration, soil moisture, and erosional processes (see review by Warren 2001). Numerous studies have been conducted on the role of biological soil crusts on surface hydrology and erosional dynamics in warm deserts, but there are relatively few studies in cold deserts where frost heave plays an important role. Associated element fluxes such as carbon (C) and nitrogen (N) from erosional losses, are poorly understood in all ecosystems.

The functioning of biological soil crusts in plant interspaces, which may comprise as much as 70% of the living ground cover in arid shrubland and woodland communities (Belnap *et al.* 2001), is critical to understanding nutrient retention and transfer in arid ecosystems. Surface runoff and associated sediments from plant interspaces is a dominant pathway of resource transfer in arid and semi-arid ecosystems (Ludwig *et al.* 1997). Therefore runoff from biological soil crust communities may be critical in distributing water and associated nutrients to vascular plants downslope (Eldridge *et al.* 2000, Maestre *et al.* 2002). Alternatively, higher runoff from biological soil crusts may

also result in loss of water and nutrients at the watershed level, both of which are highly limiting to plant productivity. In a Chihuahuan desert shrubland, Schlesinger *et al.* (1999) showed that runoff was higher from intershrub plots than vegetated plots. Interestingly this did not translate to significantly higher dissolved (inorganic + organic) N loss from intershrub plots due to lower volume-weighted N concentrations. In contrast to these results, Fierer and Gabet (2002) showed that C and N export in surface runoff was highest in bare soils and decreased as vegetation cover increased in a coastal California grassland.

Biological soil crusts do not have a single, uniform impact on surface runoff and infiltration rates. Studies from the Great Basin and Chihuahuan deserts showed that removal of biological soil crusts increased infiltration (Osborn 1952, Fletcher 1960, Harper and St. Clair 1985, Harper and Marble 1988). Removal of biological soil crusts in the absence of disturbance had no effect or decreased infiltration rates on the Colorado Plateau (Williams *et al.* 1995, Williams *et al.* 1999). In an extensive review of the role of biological soil crusts on infiltration rates, Warren (2001) argued that when sand content exceeds 80% in soils with no microtopography (*e.g.* hot deserts or recently disturbed soils in any desert), biological soil crusts generally have a negative effect on infiltration. However, infiltration rates were still much higher on sandy soils relative to fine-textured soils. Organisms associated with biological soil crusts expand when wet, which may result in "clogging" water flow through matrix pores resulting in reduced hydraulic conductivity at the soil surface, which, in some cases, may even become hydrophobic (Bond and Harris1964, Roberts and Carbon 1972, Kidron *et al.* 1999). On more fine-textured soils where there are fewer textural macropores, fungal hyphae and

cyanobacterial filaments help to maintain continuous structural pores throughout the soil, which may result in increased infiltration (Warren 2001). Soil surface roughness may also be affected by biological soil crusts, which may impact infiltration rates. Undisturbed biological soil crusts in cool and cold deserts are mostly pinnacled (15 cm microtopography), whereas those in hot deserts are flat or rugose (1-3 cm microtopography) (Belnap 2001). The microrelief in cold desert biological soil crusts is believed to be the effect of frost-heaving and subsequent downward erosion of uplifted surfaces. Runoff from pinnacled biological soil crusts follows a more tortuous pathway compared to a flat soil surface, resulting in slower surface flow and longer residence times, which may increase infiltration rates.

Disturbance affects both the physical and biological structure of the soil, which makes it difficult to separate the effect of biological crust damage from physical changes such as increased compaction and bulk density. Surface disturbances such as grazing and trampling have mixed effects on infiltration rates. In an Australian grassland, Graetz and Tongway (1986) showed that infiltration rates were three times higher at heavily grazed sites without biological soil crusts than ungrazed areas on similar soils with intact biological soil crusts. On the Colorado Plateau, livestock grazing had no impact on infiltration rates (Loope and Gifford 1972), whereas another study within the same region showed that disturbance of lichen and algal crusts increased infiltration (Brotherson and Rushforth 1983). However none of the above-mentioned studies used plots large enough to detect the influence of surface roughness on runoff.

In contrast to the mixed role of biological soil crusts on infiltration, biological soil crusts reduce soil erosion by water (McCalla 1946, Booth 1941, Fletcher and Martin

1948, Osborn 1952, Faust 1970, Tchoupopnou 1989, Eldridge and Greene 1994, Eldridge and Kinnell 1997, Eldridge 1998). Soil erosion by water occurs when raindrop impact detaches soil particles from the surface, which may then be moved downslope via sheet flow. Functional group composition of the biological soil crust impacts surface erosion. Lichens are more effective than cyanobacteria in reducing splash erosion and sediment production (Tchoupopnou 1989). In lichenized soil crusts, thalli cover the soil surfaces which may dissipate energy of incoming raindrops. In cyanobacterial-dominated crusts, cyanobacteria reside within the mineral soil matrix and soil particles may be more easily detached from the surface. Once biological soils crusts are disturbed sediment loss increases dramatically (*e.g.* Loope and Gifford 1972, Warren *et al.* 1986, Eldridge 1993, Eldridge 1998).

In this study, I conducted rainfall simulation experiments to examine the role of soil surface disturbance and biological soil crust community composition on C and N fluxes in runoff and surface erosion. Disturbances such as livestock grazing and off-road vehicles dramatically alter soil surfaces in arid and semi-arid regions of the western U.S. (Belnap 1996). I addressed this first question: what effect does disturbance of a cyanolichen soil crust have on C and N losses in runoff and soil erosion? Over the longer term, disturbance results in the conversion of stable, cyanolichen soil crust to a less stable cyanobacterial crust. In order to better understand regulation of C and N fluxes by these two biological soil crusts community types, I addressed a second question: does biological soil crust community composition (*i.e* cyanolichen vs. cyanobacterial crusts) play a role in regulating C and N fluxes in runoff and erosional processes?

Methods

Site Description—This study was conducted near the Island-in-the-Sky District of Canyonlands National Park, Utah on the Colorado Plateau. The site was located at 1813 m elevation and mean annual precipitation ranged between 185-226 mm in years 1998-2000. Soils are classified as Rizno, dry-Rock outcrop, which is characterized by 45% Rizno gravelly fine sandy loam, 25% rock outcrop and 30% other soils. Rizno soils tend to be shallow often with depth to underlying sandstone ranging from 10-50 cm. Dominant plant species at the site were *Coleogyne ramosissima* (blackbrush), *Pinus edulis* (pinyon pine), *Juniperus osteosperma* (Utah juniper) and *Yucca harrimaniae* (Harriman's yucca). I selected 2 x 3 m plots where biological soil crusts occurred but also vascular plants were absent with an average slope of 3.6 % ± 1.7 S.D. In the first experiment I examined the impact of disturbance on runoff. Plots within this experiment were located on dark biological soil crusts containing both lichens and free-living cyanobacteria. "Dark" crusts are often dominated by the cyanobacteria *Microcoleus vaginatus*, but also contain large amounts of the darker pigmented cyanobacteria *Scytonema myochrous* and *Nostoc commune* and the soil lichens, *Collema tenax* and *C. coccophorum*. I conducted the disturbance experiment in October 2001. I selected seven plots that were then divided into three subplots, all of which received the rainfall treatment at the same time. The following treatments were randomly assigned to the subplots: 1) trampled, 2) scraped, and 3) controls. In the trampled treatment, I made 100 passes over the plot by foot (jogging in hard-soled hiking boots), moving from the downslope to the upslope side of the plot on each pass. Since trampling affects both the biological and physical structure of the soil I also implemented a scraped treatment. In

this treatment, I identified the lowest point in the plot and removed the top 1 cm of soil in addition to all crust pinnacles above the soil surface with a straight edged flat trowel, while leaving the subsurface soil structure intact. Soils were dry at the time the treatments were applied. Simulations were completed within 6 hours of treatment application to prevent interactions with eolian processes. In a later experiment in November 2001, I conducted rainfall simulations on seven light crust plots. Light crusts were dominated by the cyanobacteria *Microcoleus vaginatus* with no presence of lichens, mosses or darker-pigmented cyanobacteria.

Presimulation soil measurements—Soil sampling was completed following treatment application and prior to simulation. Bulk density and antecedent moisture content were measured on one 0-5 cm deep sample per treatment plot, composited from four 5 cm-diameter cores. Soil texture was determined using the hydrometer method (Gee and Bauder 1979) on one 0-2 cm deep sample per plot. Each of these samples was also a composite of four subsamples for each treatment. I generated an index of soil aggregate stability for the top 5 mm for eight samples per plot using a field soil stability test. Each 6-8 mm diameter crust fragment was immersed in deionized water on a 1.5mm sieve for 5 minutes, then pulled completely out of the 2.5 cm deep water 5 times at a rate of one cycle every two seconds and rated on a scale from one to six (Herrick *et al.* 2001). I measured surface roughness with a 696 mm flexible chain. The chain was placed on the soil surface at 4 different locations perpendicular to the downward slope within each plot. Once the chain was place on the surface, I measured the length between to the ends. Chain length at the surface was divided by total chain length to get an index of surface roughness, where 1 was equal to a completely flat surface and values near 0 displayed a

large amount of microtopography. I measured soil chlorophyll *a* content, which I use as an indicator of cyanobacterial biomass, on one sample per plot composited from six, five mm deep soil cores. In the lab, soil samples were ground to a fine powder with a mortar and pestle. Quantitative and qualitative HPLC analysis was performed according to the method of Karsten and Garcia-Pichel (1996).

Rainfall Simulation—I applied water simultaneously to all three 71 x 71 cm treatment plots for 30 minutes at an average rate of 227 mm/hr with a VeeJet 80/100 nozzle located 2.0 m above the soil surface. The high simulation rate was applied to ensure runoff on these relatively porous soils. The first 10 minutes effectively simulate an extreme for this region, and reflect the relatively large amount of water that may be generated by less extreme events on these sites due to runon from upslope exposed bedrock.

Water pressure was controlled at 31.0 kPa and the nozzle was moved once across the plots every 4 seconds using a hand-pulley system, generating an extremely uniform spatial distribution: the coefficient of variation of the 15 precipitation gauges located in the plots was generally less than 5%. The N composition of natural rainfall that occurs within Island-In-The-Sky District of Canyonlands National Park (National Atmospheric Deposition Program, http://nadp.sws.uiuc.edu/) was simulated using water purified by reverse osmosis supplemented with NH_4OH and HNO_3. Ammonium and NO_3^- concentrations in rainfall simulation water ranged from 0.05-0.079 and 0.09-0.13 mg/L, respectively. Runoff was collected continuously and measured once every minute. Runoff samples were retained for sediment samples every minute for the first five minutes and every five minutes thereafter.

Analysis of C and N in sediment and water samples—I collected runoff samples for chemical analysis every minute for the first five minutes of runoff and every five minutes thereafter. Once all the samples were collected in the field, I immediately transported them to the laboratory where they were kept frozen. In the laboratory I thawed small subsets of the runoff waters and separated sediments from the water. I centrifuged each sample and then filtered the water through a 0.75 μ Millipore glass fiber filter. The filtered waters were refrozen and the remaining sediments were dried in a 70 °C oven. Once all the waters were filtered I analyzed them for dissolved organic carbon (DOC), total dissolved nitrogen, NO_3^-, and NH_4^+. NO_3^- and NH_4^+ were measured colorimetrically on an Alpchem autoanalyzer. Total dissolved nitrogen and TOC was measured on a Shimadzu TOC analyzer coupled with a total nitrogen analyzer. Disssolved organic nitrogen was calculated as total dissolved nitrogen - inorganic nitrogen. Total C and N in sediments were measured on a LECO CHN analyzer. Total organic C in sediments was calculated as total C – inorganic C. Inorganic C was measured by modified pressure-calcimeter as described by Sherrod *et al.* (2002).

Statistical analysis—I used a linear extrapolation of changes in concentrations between consecutive samples, since water and sediment samples were not collected for chemical analysis every minute during the 30-minute simulation. Once an estimate of concentration was calculated for each minute, I multiplied the concentration by the total runoff for that minute.

I analyzed runoff and sediment data from the disturbance experiment with a one-way analysis of variance. I used a Newman-Keuls post-hoc test to evaluate differences

among treatments. I analyzed runoff and sediment data from light and dark crusts with a Student's t-test and treatments were considered significantly different at $P < 0.05$.

Results

Disturbance Experiment—Dissolved organic carbon flux from trampled plots was significantly higher relative to scraped and control plots throughout the duration of the simulation (Fig 1a). The higher DOC fluxes were driven primarily by a trend toward higher runoff in the first 10 ($F_{2,18} = 2.45$, $P = 0.11$) and higher runoff in the first 20 minutes and not by concentration differences (Table 1). By 30 minutes both volume-weighted mean concentration (total constituent loss / total runoff) of DOC and runoff were significantly higher in trampled plots relative to scraped and control plots (Table 1).

Dissolved organic nitrogen fluxes were similar to patterns observed in DOC, where losses were significantly higher from trampled plots relative to scraped and control plots during the first 10 minutes and a trend toward higher DON loss after 30 minutes (Fig 1b). During the first 20 minutes DON flux was significantly higher in trampled plots relative to scraped plots, with control plots intermediate between then two. Over the course of 30 minutes, however, both runoff and volume-weighted mean concentrations of DON were higher in trampled plots relative to control and scraped plots (Table 1).

Ammonium export from trampled plots was significantly higher than scraped and control plots, but only in the first 10 minutes. Even though there were no significant differences by treatment in NH_4^+ export after the first 10 minutes, this general pattern was maintained throughout the simulation (Fig. 1c). The difference in NH_4^+ export in the first 10 minutes was due to both higher runoff and NH_4^+ concentrations in trampled plots as compared to scraped and control plots (Table 1). Nitrate export displayed a very

different pattern from that of NH_4^+ and DON losses. There were no differences in NO_3^- export with disturbance in the first 10 minutes of the simulation (Fig. 1d). By 30 minutes, NO_3^- losses increased significantly in trampled and scraped plots relative to controls. In control plots, NO_3^- inputs approximately equaled outputs resulting in no net flux of NO_3^- in the presence of intact biological soil crusts. The higher NO_3^- fluxes from scraped plots over the 30-minute period were due to significantly higher volume weighted mean concentrations of NO_3^- (Table 1). In contrast to scraped plots, the higher NO_3^- export from trampled plots was driven by both higher runoff and NO_3^- concentrations. DON was the dominant form of dissolved N loss within all treatments. Average DON loss ranged from 80-88% of the total dissolved N flux in all treatments. The higher runoff in trampled plots may be explained by the decrease in infiltration rates (Fig. 2) due to higher bulk density in the top 5 cm of soil after disturbance (Table 2). Although surface roughness decreased in scraped as compared to control plots (Table 2), there was no difference in runoff between these two treatments. Sediment export within the disturbance experiment was nearly linear within all treatments throughout the course of the rainfall simulation. Trampling led to a significant increase in sediment export relative to controls, with scraped plots intermediate between the two (Fig. 3a). Sediment-bound C was significantly higher in trampled plots relative to scraped and control plots during the first 10 minutes of the simulation (Fig. 3b). During the first 20 minutes, sediment C export was significantly higher from both trampled and scraped plots relative to controls. This pattern held over the 30-minute simulation, although differences were not significant. Sediment N loss was significantly higher from trampled plots relative to scraped and control plots throughout the duration of the simulation. Trampling resulted

in a nearly 4-fold increase in sediment N export relative to controls during the entire 30 minutes (Fig. 3c). Higher sediment C and N export in trampled plots was due to higher sediment export and not differences in sediment C concentration (data not shown). There were no differences in sediment loss per unit of runoff with scraping or trampling relative to controls, which suggests that increased sediment loss was driven by runoff amount.

Impact of biological soil crust community—There was a trend toward higher DOC flux from light crusts relative to dark crusts during the first 10 minutes ($t = -1.79$, $P = 0.09$), but no differences were observed thereafter (Fig. 4a). Runoff from light crusts was significantly greater than dark crusts throughout the simulation (Table 1). However cumulative volume-weighted mean concentrations of DOC were significantly higher in dark crusts during the first 10 and 20 minutes relative to light crusts. Cumulative DON flux from dark crusts was significantly higher relative to light crusts when compared over 30 minutes, with no significant differences at earlier timepoints (Fig. 4b). Although runoff from light crusts was higher than dark crusts, the 3-fold increase in volume weighted mean DON concentration from dark crusts (Table 1) led to significantly higher overall DON loss.

Ammonium losses did not differ by crust community (Fig. 4c). There was a trend toward higher NO_3^- losses from light crusts compared to dark crusts after 20 ($t = -1.65$, $P = 0.12$) and 30 minutes ($t = -1.9$, $P = 0.08$). Although runoff and was higher in light crusts during the first 10 minutes, this did not result in significantly higher NO_3^- export. Overall, NO_3^- export appears to be regulated by cyanobacteria biomass, where volume-weighted mean concentration of NO_3^- in runoff decreases with increasing chlorophyll *a*

content (Fig. 5). Biological soil crust community also regulates runoff dynamics, where chlorophyll *a* content explained 79% of the variability in surface runoff (Fig. 6).

Sediment loss in light crusts was greater than dark crusts (Fig. 7a), resulting in an increase in sediment-bound C and N loss (Fig. 7b,c). After 30 minutes, sediment-bound C transport was 26 and 8 g/m^2 in light and dark crusts respectively (Fig. 7b), whereas sediment-bound N transport was on the order of 2.5 g/m^2 in light crusts compared to 0.5 g/m^2 in dark crusts (Fig. 7c). Even though mass-weighted mean concentrations of C (total C in sediment / total sediment loss) were significantly higher in dark crusts relative to light crusts by 30 minutes (mean mg C/g sediment, dark crust = 14, light crust = 9, t = 1.89, P = 0.05), total C export was higher in light crusts due to greater sediment export. Mass-weighted mean concentrations of N were also significantly higher in dark crusts relative to light crusts during the first 10 (mean mg N/g soil, dark crust = 4.5, light crust = 1.4, t = 3.46, P = 0.004), 20 (mean mg N/g soil, dark crust = 3.4, light crust = 1.1, t = 2.45, P = 0.03), and 30 (mean mg N/g soil, dark crust = 2.8, light crust = 0.77, t = 2.32, P = 0.03) minutes. Sediment loss per unit of runoff was three-fold higher in light crusts relative to dark crusts (sediment g/L; dark crust = 8.5, light crust = 29.7, t = -2.49, P = 0.02). These data suggest that biological soil crusts are highly effective in stabilizing soil surfaces, which is supported by data on aggregate stability. Surface aggregate stability of dark crusts was significantly greater than that of light crusts (Table 2).

Sediments were highly enriched in N relative to the surface mineral soil early in the rainfall simulation. Sediment percent N in the first minutes after runoff was more than 5 times higher than the percent N of the top 1 cm of soil (Fig. 8), which suggests that

even short-term runoff events have a high potential for N loss due to the movement of sediments highly enriched in N.

Discussion

Runoff dynamics and associated C and N fluxes—Both higher runoff and volume-weighted mean concentrations contributed to higher DOC and a trend in higher DON and NH_4^+ fluxes from trampled plots relative to control and scraped plots. Surface disturbance by trampling breaks down the biological matrix of fungal hyphae, cyanobacterial sheaths, and lichen and moss rooting structures, which may expose previously protected sites to leaching of organic and inorganic constituents. Trampling may also damage cell structure in soil organisms resulting in further exudation of organic compounds. Biological soil crusts have been shown to release large amounts of N compounds, mostly upon initial wetting. In a biological soil crust that was exposed to an atmosphere elevated in ^{15}N, nearly 19% of the newly fixed N leaked to the surrounding environment in the form of NH_4^+ (Klubek *et al.* 1978). Mayland (1966) showed that 1-2% of the total N in biological soil crusts was found in the NH_4^+ pool.

Species composition of biological soil crusts impacts soil surface hydrology and associated C and N fluxes in runoff, where runoff is negatively correlated with chlorophyll *a* content on soils with 79% sand content. These results are in contrast to the those reviewed by Warren (2001), which suggested that infiltration rates tend to decrease in biological soil crusts on coarse-textured soils (*i.e.* sand content > 80%). Studies cited by Warren (2001) were conducted primarily in hot deserts with little microtopography and surface roughness. In addition, several studies were conducted at such small spatial scales that surface characteristics may not have had much impact on infiltration rates.

There are several factors that may contribute to decreased runoff with increasing chlorophyll *a* content. Soil surfaces in dark soil crusts are pinnacled, whereas light crusts display some surface roughness but are nearly flat. Depressions between pinnacles in the dark crusts may allow water to pond and increase infiltration relative to light crusts. In addition to differences in soil microrelief, surface aggregate stability is a good indicator of the ability of raindrops to detach soil particles from the surface, where soil detachment decreases as aggregate stability increases (Blackburn *et al.* 1992). The 4-fold lower surface aggregate stability in light crusts may have resulted in increased particle detachment and subsequent clogging of soil pores, leading to decreased infiltration rates. Differences in antecedent soil moisture may also affect runoff, where higher soil moisture often leads to lower infiltration at the beginning of a rainfall event and greater total runoff. Soil moisture in light crusts ranged from 1-3%, while dark crusts were lower and ranged from 0.3-1%. Although soil moisture was significantly higher in light crusts, it is unlikely that the small differences in antecedent soil moisture fully explain the nearly 2-fold increase in runoff from light crusts relative to dark crusts.

Runoff from light crusts was higher relative to dark crusts, but volume-weighted mean concentrations of DOC, DON, and NH_4^+ were generally higher in dark crusts relative to light crusts. In a study where biological soil crusts were labeled with $^{14}CO_2$, newly fixed labile C was quickly released to underlying soils (Beymer and Klopatek 1991). Fluxes of labile C compounds to underlying soils should be higher in dark crusts than light crusts, due to the higher net photosynthesis and C gain (Garcia-Pichel and Belnap 1996, Lange *et al.* 1998), which may partially explain the increase in DOC concentrations in surface runoff. Leakage of newly fixed N may also result in elevated

dissolved N loss from soil crusts, where cyanobacteria have been shown to release significant amounts of NH_4^+ and organic N (Mayland *et al.*1966, Glibert and Bronk 1994). In a study of the marine cyanobacteria, *Trichodesmium* spp., Glibert and Bronk (1994) showed that dissolved organic N release was 50% of the N fixation rates. Since N fixation is higher in dark crusts relative to light crusts, I expect N leakage from dark biological soils crusts, which may partially explain the higher NH_4^+ and DON loss in runoff.

Nitrate export decreased with increasing chlorophyll *a* content of the biological soil crust. It is well known that cyanobacteria are able to take up NO_3^- (*e.g.* Jones and Stewart 1969, Singh *et al.* 1996), which may result in lower soil NO_3^- concentrations beneath biological soil crusts and decreased NO_3^- concentrations in surface runoff. During the summer on the Colorado Plateau, soil NO_3^- concentrations were shown to be significantly higher in light crusts relative to dark crusts (see appendix II), which may partially explain the increased NO_3^- concentrations in runoff from light crusts.

Erosional losses—It is widely recognized that biological soil crust cover in sparsely vegetated arid and semi-arid ecosystems may greatly decrease soil erosion by water (Williams *et al.* 1995, Eldridge and Green 1994, Eldridge and Kinnell 1997). Following this, any surface disturbance such as livestock grazing or trampling that reduces biological soil crust cover, often results in increased water erosion (Loope and Gifford 1972, Eldridge 1993, Eldridge 1998). In this study, aggregate stability decreased significantly with trampling and scraping relative to controls, but only trampled plots had higher sediment loss. Sediment loss per unit volume of runoff was also similar among treatments, which further supports the idea that changes in surface aggregate stability

does not increase sediment production. Therefore, it appears that sediment loss at this small plot scale is more strongly influenced by energy available for removal and transport than by surface stability. In other words, infiltration capacity controls sediment loss.

Even small runoff events may be an important N loss pathway from biological soil crusts. Nitrogen concentrations in sediments were highly enriched relative to the surface mineral soil early in the rainfall simulation. Finer soil fractions, which are generally elevated in N content, may be mobilized early in a runoff event. There are several other mechanisms by which surface sediments are elevated in N content. Biological soil crusts trap atmospheric dust, which may partially explain the elevated N content of sediments (Danin and Ganor 1991). Blank *et al.* (1999) showed that aqueous soluble NH_4^+ and NO_3^- in dust were much greater than the top 5 cm of mineral soil, which suggests that dust inputs may elevate N concentrations at the soil surface. Nitrogen-fixing cyanobacteria and cyanolichens within sediment samples may have also been important in elevating the N content of sediments. Runoff may disperse soil microorganisms, especially lichens and cyanobacteria living at the soil surface. In a study of microphyte dispersal in surface runoff in Australian woodland, *Collema coccophorum,* a common soil lichen on the Colorado Plateau, was the most abundant lichen collected in the early stages of runoff (Eldridge 1996). In my study, there was strong evidence that soil cyanobacteria were detached from the soil surface and transported in runoff waters, where *Microcoleus vaginatus* was the most commonly observed cyanobacteria in sediment samples.

Implications for ecosystem C and N budgets—Sediment-bound C and N transport appears to be a dominant loss pathway. In this study, >98% of the total C and N flux was from

sediment sources, whereas dissolved C and N made up very little of the total export budget. Light crusts or *Microcoleus*-dominated soil crusts are representative of a post-disturbance soil surface in the western US, where surface disturbance often results in mortality of the lichen and moss community. Interestingly, C and N export was higher in light crusts relative to trampled crusts. Trampling of a biological soil crust results in changes in soil physical structure and the breakdown of the biological matrix at the soil surface. Over the long-term, however, mortality of lichens and cyanobacteria after disturbance may further destabilize soil surfaces.

In the following discussion comparing fluxes of C and N in runoff to other loss pathways, I use total C and N flux values from the first ten minutes of the rainfall simulation. An average of 38 mm of rainfall was applied to plots over the first ten minutes. This is comparable to the upper end of 32 mm/hr of rainfall recorded in Canyonlands National Park, which occurred once over a four year period when individual rain events were recorded (http://climchange.cr.usgs.gov/info/sw/clim-met/) assuming that, as is typical for convective storms, most of the rainfall fell during a relatively short period of time. Carbon flux (sediment + dissolved organic C) was on the order of 7.9 and 3.4 g C/m^2 for light and trampled crusts and 0.76 g/m^2 for dark crusts. Although this constitutes <1% of the organic C in the top 5 cm of surface soil at these sites, C flux of this magnitude is comparable to annual C inputs via photosynthesis by cyanobacteria. Using data collected by Jeffries *et al.* (1993a,b), Evans and Lange (2001) estimated C inputs via photosynthesis by light, *Microcoleus* and dark, *Scytonema* dominated crusts at 0.64 and 2.3 g $C/m^2/yr$. Based on these data, C export in a large runoff event from light or disturbed crusts may be 5-12 times the annual C fixation. However, if this magnitude

of C flux occurs only one out of every four years, C losses in runoff are closer to annual C inputs. In contrast, it appears that estimates of annual C fixation from dark crusts are 3-fold greater than total C export. Model estimates of sediment C flux by Fierer and Gabet (2002) based on rainfall simulation data are 0.2 g $C/m^2/yr$ 3-fold lower than C export in this study. However, these model estimates are based on a rainfall intensity of 60 mm/hr.

Due to the paucity of symbiotic N fixers in these sites, N inputs via fixation by cyanobacteria in biological soil crusts is an important pathway for N to enter these ecosystems. Estimates of N fixation in dark and light crusts were 9 and 1.4 kg N/ha/yr (Belnap 2002), whereas N export from dark crusts (0.6 kg N/ha) was an order of magnitude lower than light crusts (6.3 kg N/ha/yr). Following this, N inputs via fixation in dark crusts may be 15-fold higher than N losses in runoff. This pattern would be further exacerbated if N losses of this magnitude in runoff occur only one out of every four years. In light crusts, however, N export in runoff is nearly 5 times greater than N fixation. Estimates of N transport in suspended sediments were 0.05 and 0.19 g N/m^2 in pinyon-juniper and grassland sites in New Mexico and Arizona, values that are comparable to this study (Bolton *et al.* 1991).

Although C and N fluxes were relatively high from runoff plots, watershed level C and N loss dynamics may function very differently. In a Sonoran desert watershed Fisher and Grimm (1985) estimated net N accumulation after a summer storm. Wilcox *et al.* (in press) examined runoff and erosional dynamics at several spatial scales in a semi-arid woodland and showed that runoff decreased by 96% from the plot to the hillslope scale. Patterns in erosional losses in that same study were similar to runoff dynamics where

sediment losses from the plot level ranged from 1000-4000 kg/ha, but decreased to < 100 kg/ha at the level of the hillslope. Nutrient redistribution via physical processes such as wind and water erosion is recognized as an important feature in nutrient cycling in arid ecosystems (Parsons *et al.* 1992, Schlesinger *et al.* 1996, Schlesinger and Pilanis 1998, Abrahams *et al.* 1994, Ludwig *et al.* 1997). Ludwig *et al.* (1997) proposed that transfer of resources between source areas (plant interspaces) to sink areas (vegetated patches) is a critical process in maintaining plant productivity. Disturbance of these source/sink relationships may alter nutrient delivery downslope and negatively impact plant communities. In this study, runoff and sediment export increased with disturbance, which suggests that downslope plant communities receive a higher flux of nutrients in water and sediments in a post-disturbance environment. Alternatively, higher runoff and sediment transport from plant interspaces may also result in higher nutrient losses from the watershed. In a post-disturbance environment with light biological soil crusts, soil nutrients should be more heterogenously distributed, with plant interspaces being highly depleted in nutrients relative to tree and shrub canopies due to higher fluxes of water and sediments in runoff. In a community dominated by late successional dark cyanolichen crusts, nutrient distribution may become more homogeneous relative to disturbed and early successional biological soil crust communities; as runoff and sediment production decreases nutrients will be retained in the plant interspaces.

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Table 1. Cumulative runoff and volume-weighted mean concentrations of DOC, DON, NH_4^+, and NO_3^- at 10, 20, 30 minutes during the rainfall simulation. Values are means within each treatment ± 1 SE. I compared differences by crust community using a Student's t-test (light crust vs. dark crust control) and the effects of disturbance (control, scraped, trampled) with a one-way ANOVA. Within each row the capital letters signify treatment differences by crust community (A and B) and the second letter represents differences within the disturbance experiment (a, b, and c). If a different letter appears by a treatment the means are significantly different at $P < 0.05$. Negative N concentrations indicate that runoff concentrations were less than initial N content of the water added as simulated rainfall.

	Light Crust	**Dark Crust Control**	**Dark Crust Scraped**	**Dark Crust Trampled**
10 minutes				
Runoff L/m^2	24.30 (0.77) A	5.12 (1.48) Ba	4.02 (1.40) a	8.69 (1.77) a
DOC mg/L	1.29 (0.49) A	2.76 (0.57) Ba	3.28 (0.80) a	2.47 (0.31) a
DON µg/L	67 (19) A	245 (56) Ba	206 (44) a	260 (43) a
NH_4^+ µg/L	7 (8) A	34 (16) A a	25 (12) a	95 (32) b
NO_3 µg/L	25 (15) A	9 (7) A a	48 (7) b	2 (8) a
20 minutes				
Runoff L/m^2	52.86 (1.19) A	21.25 (3.00) Ba	19.18 (2.62) a	30.03 (2.95) b
DOC mg/L	0.04 (0.01) A	1.49 (0.26) Ba	1.20 (0.13) a	1.44 (0.16) a
DON µg/L	4 (11) A	192 (67) Ba	112 (24) a	173 (27) a
NH_4^+ µg/L	8 (5) A	34 (15) A a	24 (12) a	57 (21) b
NO_3 µg/L	71 (25) A	-0.98 (7) B a	29 (7) b	6 (6) a
30 minutes				
Runoff L/m^2	80.6 (2.4) A	41.4 (4.0) Ba	39.5 (3.5) a	53.8 (3.8) b
DOC mg/L	0.56 (0.20) A	0.77 (0.08) Aa	0.71 (0.11) a	1.12 (0.13) b
DON µg/L	26 (8) A	82 (23) Ba	63 (16) a	139 (18) b
NH_4^+ µg/L	-4 (10) A	21 (10) Ba	11 (9) a	40 (14) a
NO_3 µg/L	10 (4) A	-0.6 (5) Aa	27 (7) b	12 (3) c

Table 2. Pre-rainfall simulation soil measurements. Values are means ± 1 SE (N=7). I compared differences by crust community using a Student's t-test (light crust vs. dark crust Control) and the effects of disturbance (control, scraped, trampled) with a one-way ANOVA. Within each row the first letter signifies treatment differences by crust community (a and b) and the second letter represents differences within the disturbance experiment (c, d, and e). If a different letter appears by a treatment the means are significantly different at $P < 0.05$ unless otherwise noted.

	Light Crust	**Dark Crust Control**	**Dark Crust Scraped**	**Dark Crust Trampled**
Texture				
% Sand	79.94 (2.09) A	79.02 (0.67) Ac	81.21 (0.86) c	79.54 (1.49) c
%Silt	13.39 (2.35) A	13.76 (1.70) Ac	10.91 (0.62) d	13.22 (1.34) c
%Clay	6.67 (0.57) A	7.22 (1.03) Ac	7.82 (0.53) c	7.24 (0.45) c
Bulk Density	1.70 (0.03) A	1.71 (0.03) Ac	1.71 (0.03) c	1.96 (0.03) d
Soil Stability Class	3.38 (0.34) A	5.09 (0.24) Bc	1.16 (0.11) d	1.00 (0.00) d
Chlorophyll *a*	1.48 (0.16) A	11.40 (1.82) Bc	0.72 (0.14) d	2.67 (0.16) e
% Soil Moisture	2.13 (0.32) A	0.61 (0.09) Bc	0.72 (0.12) c	0.65 (0.09) c
Roughness class	Not measured	0.853 (0.012) a	0.984 (0.002) b	0.973 (0.001) b

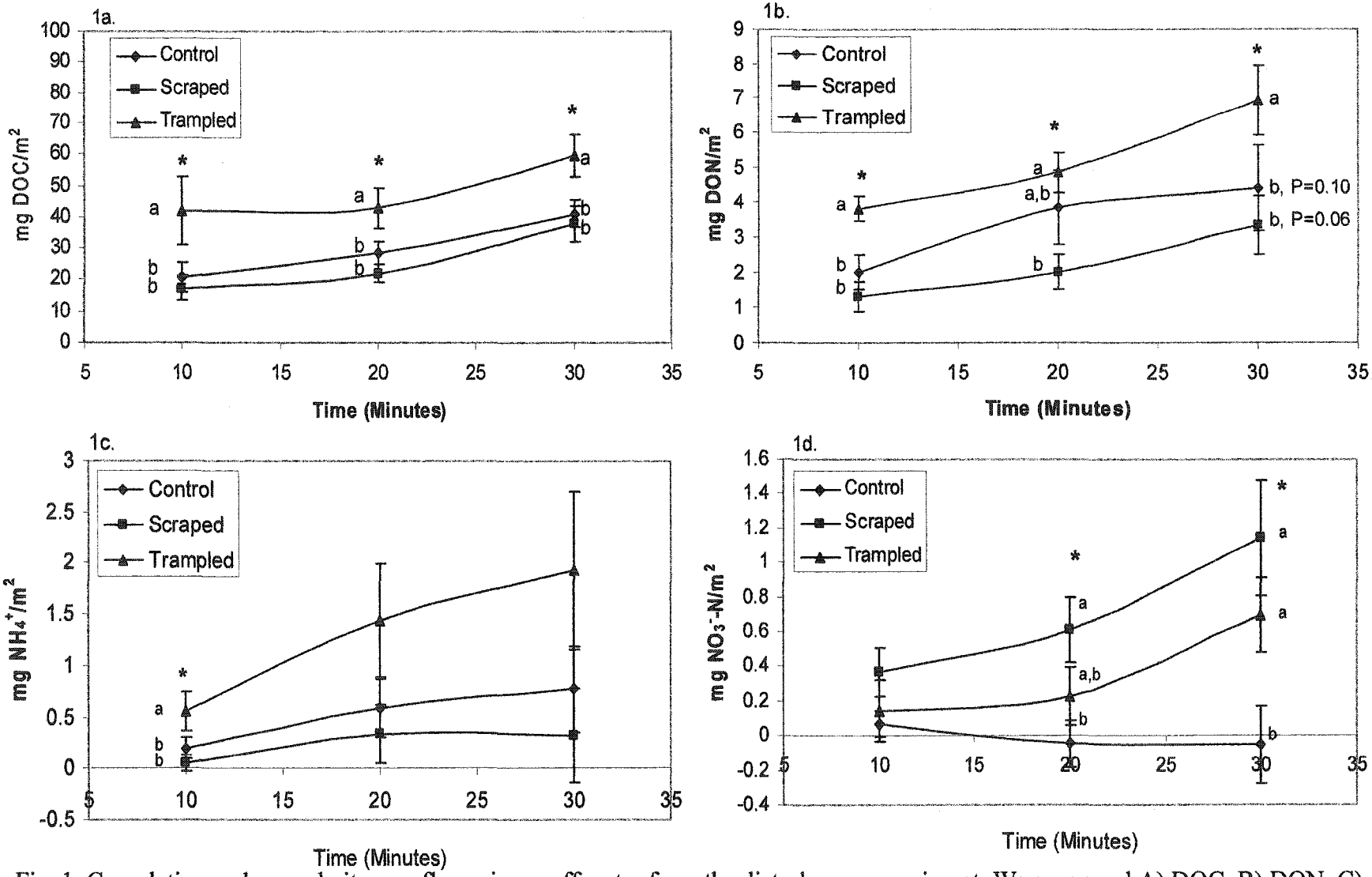

Fig. 1. Cumulative carbon and nitrogen fluxes in runoff water from the disturbance experiment. We measured A) DOC, B) DON, C) NH_4^+, and D) NO_3^- concentrations in runoff and calculated a total flux of each constituent over the first 10, 20, and 30 minutes of the rainfall simulation. Values are means ± 1 SE (N=7). An asterisk denotes a significant effect of treatment using a one-way ANOVA. When a different letter appears next to a treatment symbol, means are significantly different at $P < 0.10$ (Post-hoc Newman-Keuls).

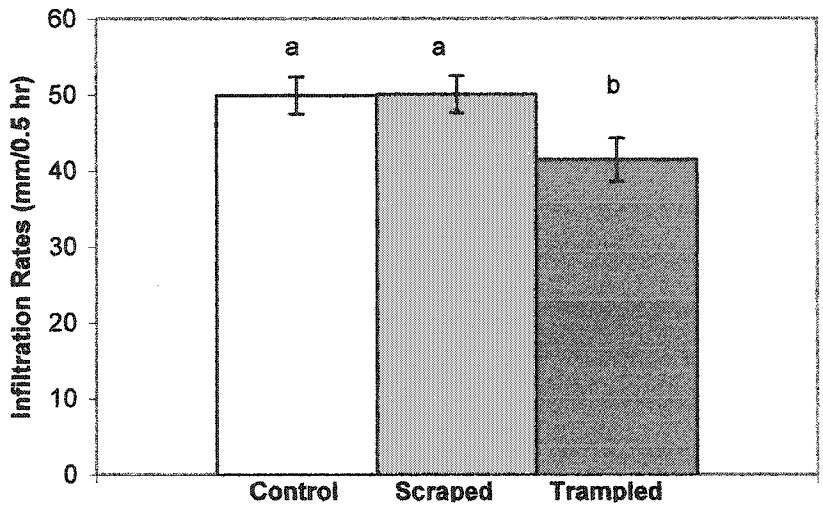

Fig. 2. Disturbance impacts on infiltration rates in a 30-minute rainfall simulation. Infiltration rates were calculated as (total rainfall input (mm)- runoff (mm)) / 0.5 (hrs). Values are means ± 1 SE (N=7). When a different letter appears above a bar, values are significantly different at $P < 0.05$ (Post-hoc Newman-Keuls).

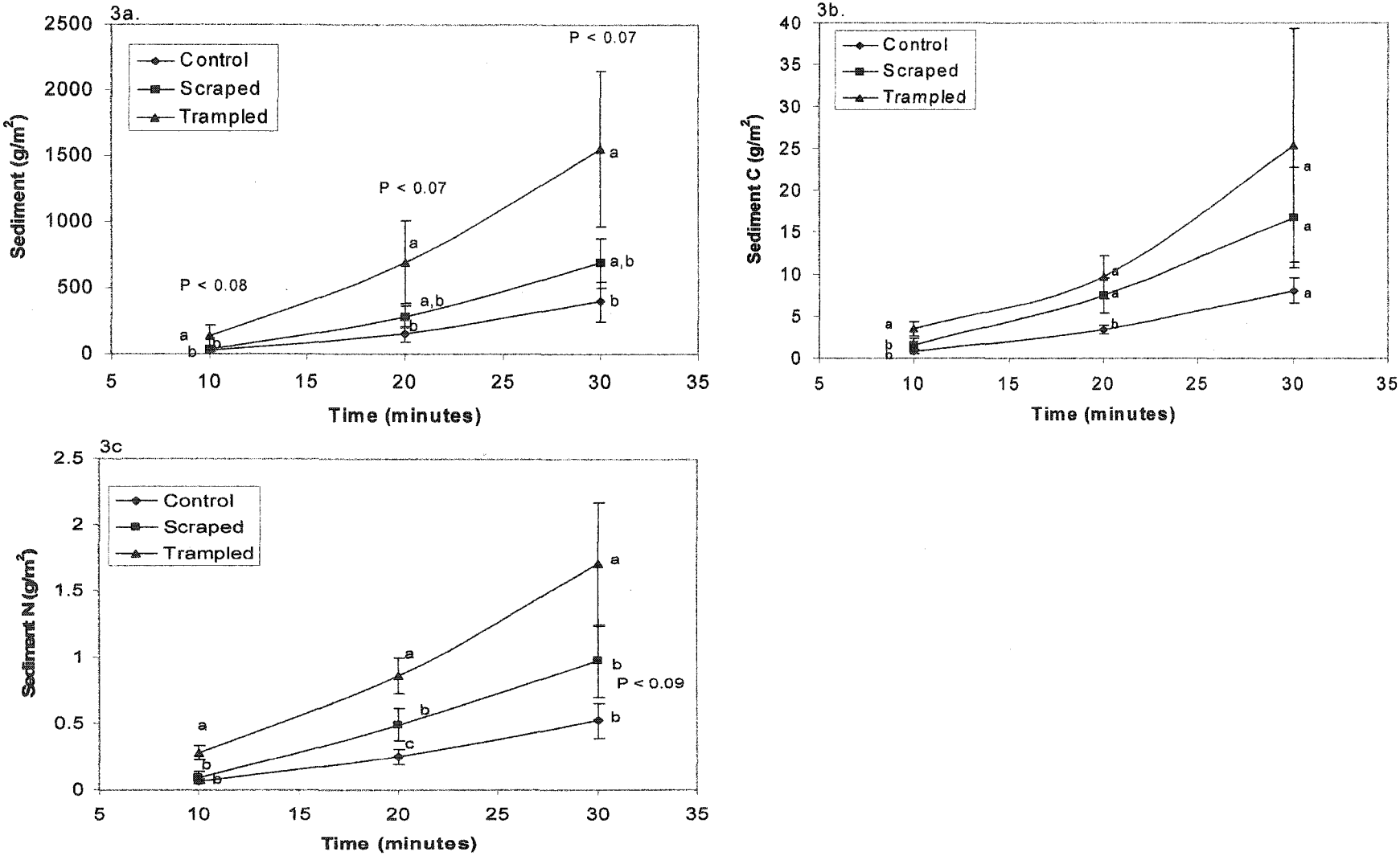

Fig. 3. Total sediment loss and sediment-bound C and N loss with surface disturbance and scraping. We measured total organic C and N in sediments and estimated a sediment C and N flux at 10, 20, and 30 minutes during the rainfall simulation. Values are means ± 1 SE (N=7). When a different letter appears next to a treatment symbol, values are significantly different at $P < 0.05$ (Post-hoc Newman-Keuls).

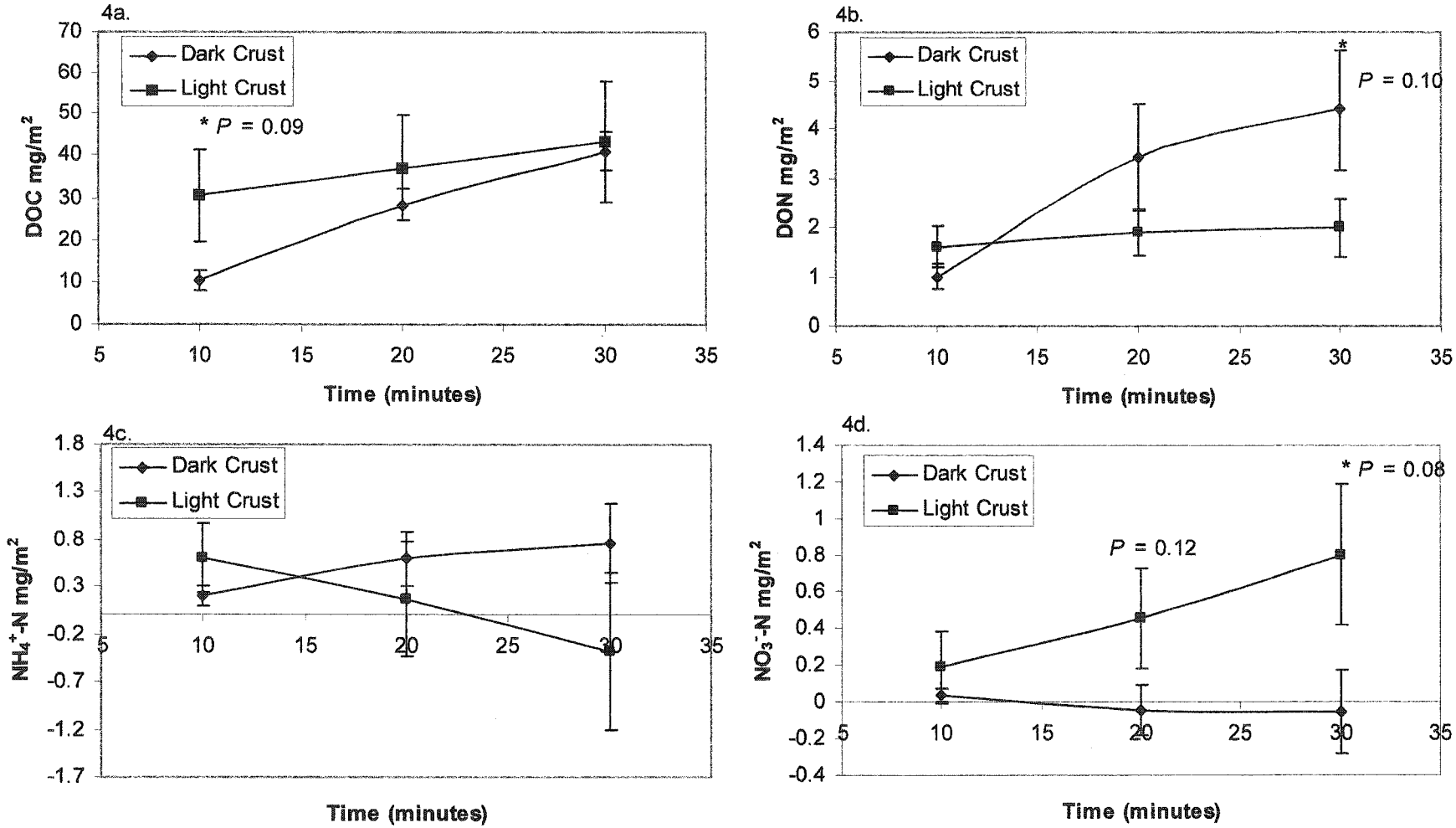

Fig. 4. Cumulative carbon and nitrogen fluxes in runoff water from lichen/cyanobacteria (dark) and cyanobacterial (light) soil crusts. We measured A) DOC, B) DON, C) NH_4^+, D) NO_3^- concentrations in runoff and calculated a total flux of each constituent over the first 10, 20, and 30 minutes of the rainfall simulation. Values are means ± 1 SE (N=7). An asterisk above the means indicates a significant effect of crust type on element flux evaluated at $P < 0.10$ using a Student's t-test.

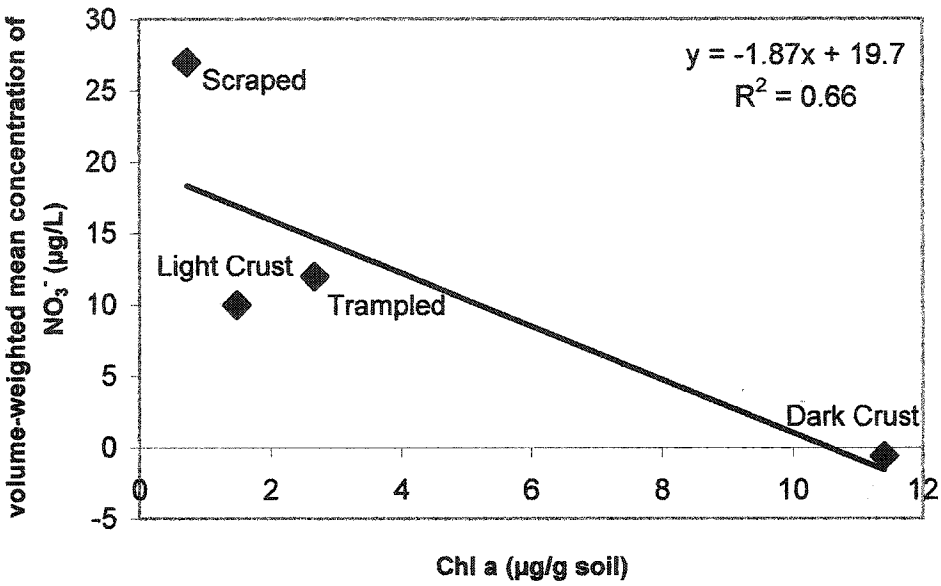

Fig. 5. Volume-weighted mean concentration of NO_3^- as a function of chlorophyll *a* content in the surface soil (top 1 cm). Chlorophyll *a* content, an index of cyanobacterial biomass, explained 66% of the variability in volume-weighted mean concentration of NO_3^- in all treatments.

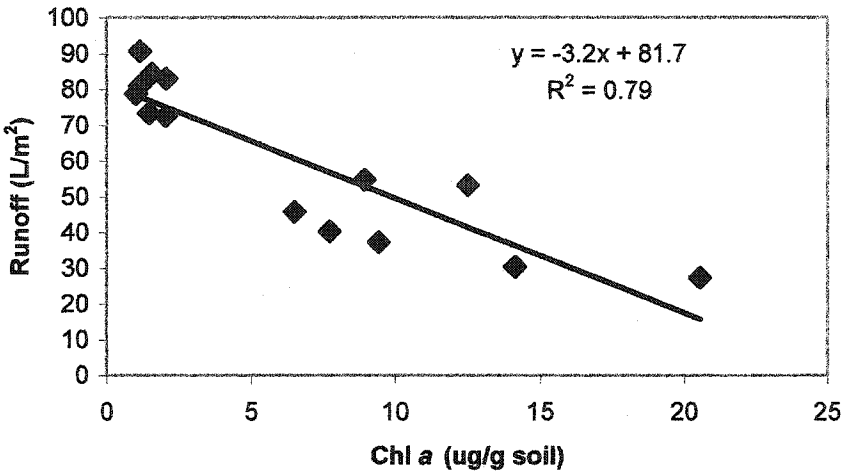

Fig. 6. Total runoff after the 30 minute simulation as a function of chlorophyll *a* content in the surface soil (top 1 cm). Chlorophyll *a* content, and index of cyanobacterial biomass, explained 79% of the variability in surface runoff, where runoff decreases with increasing chlorophyll *a* content.

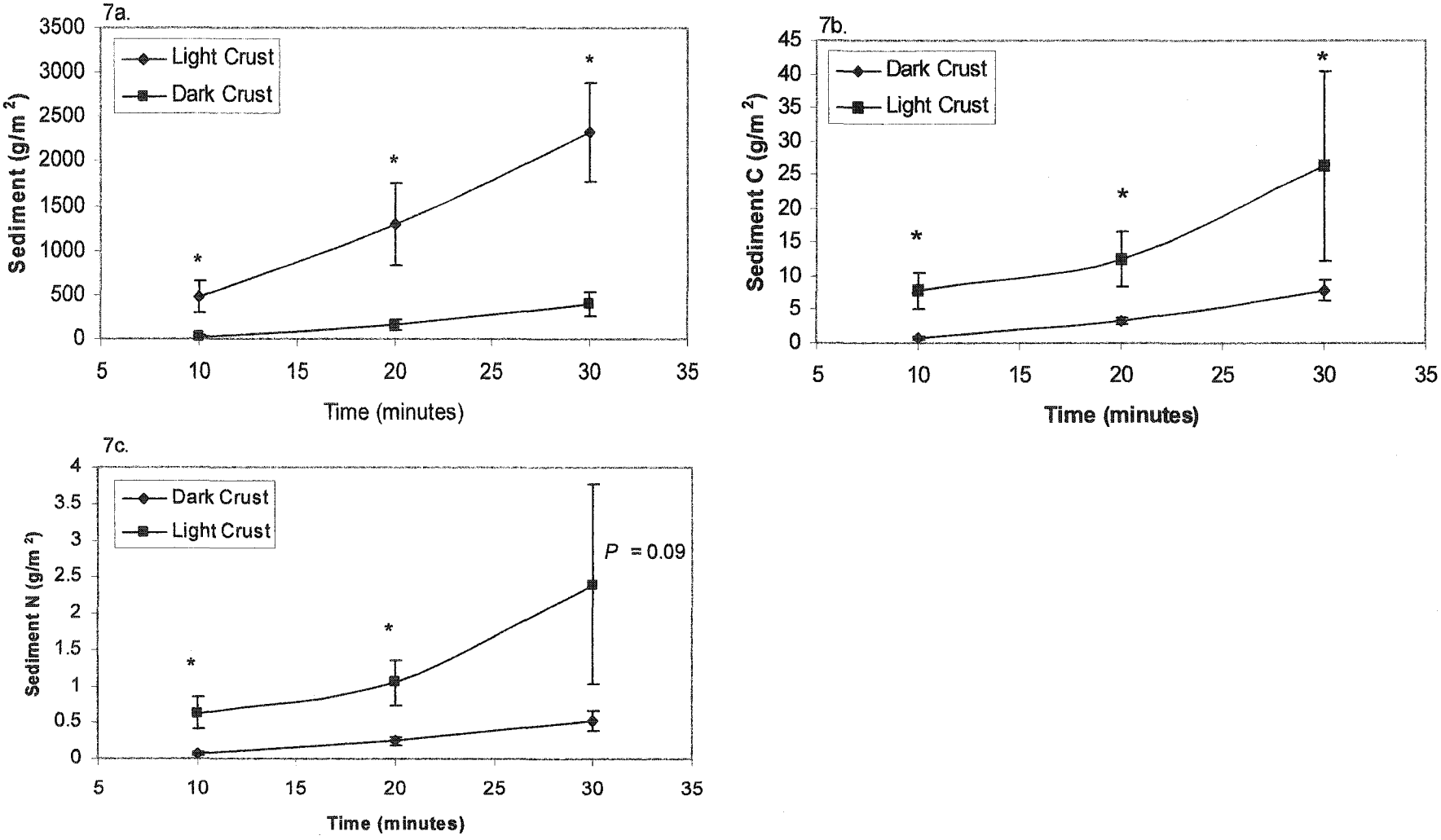

Fig 7. Total sediment loss and sediment-bound C and N loss in dark cyanobacteria/lichen crusts as compared to light cyanobacterial crusts. Values are means ± 1 SE (N=7). When a * appears above two symbols, means are significantly different at $P < 0.05$ (Student's T-test).

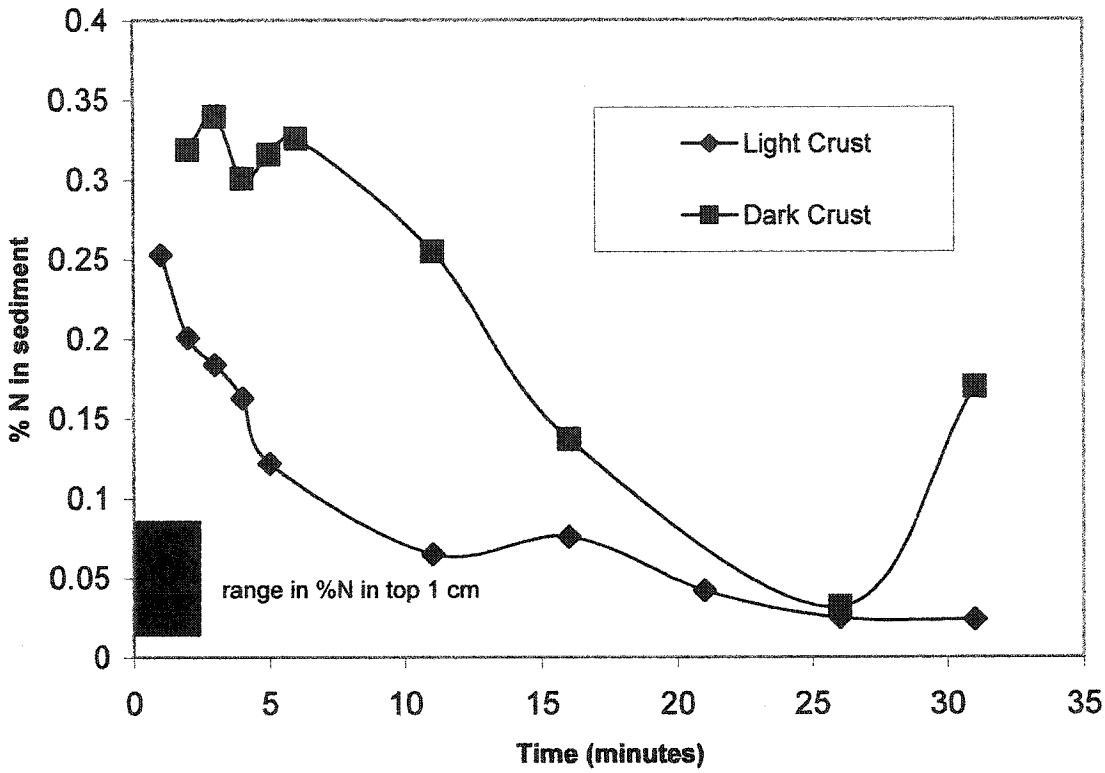

Fig. 8. Nitrogen content in sediment throughout the duration of the rainfall simulation. Time course N concentrations in sediments for a dark and light crust plots. The solid black bar represents the range of soil % N in the top 1 cm.

Chapter IV.

Acetylene reduction to N fixation conversion ratios for the soil cyanobacterium *Nostoc commune*

Abstract

The theoretical conversion ratio of acetylene reduction (AR) to nitrogen (N) fixed is 3 based on the biochemistry of the N fixation. However, conversion ratios of AR to N fixed from field and laboratory studies have been shown to vary dramatically. In this study, I examined AR to N fixed conversion ratios for *Nostoc commune*, a N-fixing cyanobacterium that is ubiquitous in arid and semi-arid soils throughout the world. I collected organisms from three regions (southern New Mexico, southeastern Texas, and central Inner Mongolia) that differed in mean annual precipitation and temperature. I measured AR and N fixation by $^{15}N_2$ incorporation on the same replicates over two incubation periods (method 1) and on different replicates over a single incubation period (method 2). In method 1, I examined variability in N fixation rates over time, whereas in method 2 I examined variability within different *N. commune* colonies. Nitrogen fixation by $^{15}N_2$ incorporation was not detectable in *N. commune* from the Inner Mongolia sites due to unusually low N fixation rates. Consequently, I present results only for New Mexico and Texas sites. Conversion ratios were greater than the theoretical 3-4 for the New Mexico and Texas site using methods 1 and 2. However ratios were highly variable depending on the method of calculation. Using method 1, ratios for the two sites were similar (New Mexico = 6.2, Texas = 5.7). However calculating AR to N fixation ratios using method 2, ratios of AR to N fixed from Texas were significantly higher relative to

New Mexico (New Mexico = 4.3, Texas = 11.4). Within site differences in conversion ratios were less clear. Ratios nearly doubled from Texas, using method 1 and 2 respectively, whereas conversion ratios for the New Mexico site were more similar across methods. Due to the variability in conversion ratios these data suggest that N fixation experiments must include a calibration component if AR is to be used as an index of N fixation.

Introduction

Acetylene reduction (AR) is the most common assay by which to estimate N fixation activity. In AR assays the enzyme nitrogenase, which is responsible for catalyzing N_2 reduction, also reduces acetylene to ethylene. Although $^{15}N_2$ incorporation is a true measure of N fixation, AR is less costly than $^{15}N_2$ methods and has a higher sensitivity. The reliance on AR was primarily due to the costliness of conducting $^{15}N_2$ experiments and the higher sensitivity of AR as compared to $^{15}N_2$ incorporation. In the past decade, declines in costs and increased sensitivity in isotope ratio mass spectrometry has made calibration of AR to N fixation for a variety of organisms and sites more practical.

The theoretical conversion ratio of AR to actual N fixed is 3, based on the biochemistry of the N fixation where 6 electrons are required for N_2 reduction, whereas only 2 electrons are required for reduction of C_2H_2. Hardy *et al.* (1968) showed that conversion ratios ranged between 3-4 for bacterial cultures and legumes under laboratory and field conditions. Since this time, results from field and laboratory studies have shown that conversion ratios vary dramatically and may range from 0.022-22 (Rice and Paul 1971, Nohrstedt 1983, Nohrstedt 1985, Montoya *et al.* 1996, Liengen 1999). Conversion ratios higher than 3 may be a result from H_2 evolution in N_2 reduction, which consumes electrons. In contrast, reduction of protons to hydrogen is completely inhibited at high concentrations of C_2H_2 (Jensen and Cox 1983). Jensen and Cox (1983) suggested that theoretical ratios were closer to 4, owing to H_2 evolution and the higher electron requirement in N fixation. In addition, the higher solubility of C_2H_2 in water as compared to the solubility of N_2 may also lead to ratios greater than the theoretical values

(Rice and Paul 1971, Nohrstedt 1983), where higher solubility of C_2H_2 may result in higher C_2H_2 at sites of N fixation. Enzyme system preference within an N-fixing organism may result in differences in conversion ratios, where vanadium nitrogenase shows lower ratios as compare to molybdenum nitrogenase (Smith *et al.* 1987). Other factors such as C_2H_2 toxicity to microbes and adsorption of acetylene on to soil colloids may also result in a decrease in conversion ratios (Rennie *et al.* 1978).

Due to the wide range of conversion ratios reported in the literature, it may be important to calibrate AR to N fixed for each site and organism. Nitrogen fixation by soil cyanobacteria is an important pathway by which N enters desert ecosystems (Evans and Ehleringer 1993). Furthermore, a better understanding of AR rates and the relationship to ecosystem N inputs is an important link in balancing ecosystem N budgets. In this study I examined AR to N fixed conversion ratios for *Nostoc commune*, a N-fixing cyanobacterium that is ubiquitous in arid and semi-arid soils throughout the world. *N. commune* commonly occurs as free-living colonies or in association with a fungal component in soil lichens. I collected organisms from three arid and semi-arid regions that differed in mean annual precipitation and temperature.

Methods

Site description—Free-living *N. commune* colonies were collected from a Chihuahuan desert site in southern New Mexico, an oak savanna in Texas, and a semi-arid grassland in Inner Mongolia, China. The New Mexico colonies were collected near the Jornada Experimental Range in southern New Mexico, the Texas colonies were collected from the College Station Rangeland Experimental Range Area, and the Inner Mongolia colonies were collected near the Inner Mongolia Grassland Ecosystem Research Station

(IMGERS), located in the Inner Mongolia Autonomous Region, China. Mean annual temperature for New Mexico, Texas and Inner Mongolia is 24, 21, and O ° C, whereas mean annual precipitation for these same sites is 240, 743, and 350 mm/yr (New Mexico, http://www.lternet.edu/sites/jrn/; Texas, B. Wilcox, pers. comm.; Inner Mongolia, http://www.ncdc.noaa.gov/oa/ncdc.html).

AR and N fixation experiment—Immediately after collection, *N. commune* samples were air-dried and stored in a dark, dry area. Inner Mongolia samples were collected in July 1998, Texas samples were collected in February 2002, and New Mexico samples were collected in December 2001. I conducted this laboratory study in April 2002. I sorted and weighed individual *N. commune* colonies into replicates from the three sites. Weights in each replicate ranged from 0.015-0.020 g for the New Mexico colonies to 0.12-0.19 g for Texas and Inner Mongolia colonies. I had a total of 16 replicates from the New Mexico site and 20 replicates from the Texas and Inner Mongolia sites. I filled 31 cm^3 round plastic containers with sand and placed the pre-weighed *N. commune* colonies on the surface. I added water to each of the replicates to saturation and placed them in a growth chamber at 25 °C and light constant light of 600 umol/m^2/s. I misted DI water on the samples over the next 24 hrs to keep them near saturation. After 24 hrs I measured AR on all samples (Fig. 1). I placed each replicate in a 60 ml glass jar equipped with a gas sample port. After sealing all the jars, I removed 6 ml of air from the jar with a gas tight syringe and replaced it with 6 ml of acetylene. Samples were left to incubate for 4 hrs. After this time, I collected a 3 ml gas sample, which was stored in an air-tight evacuated glass vial. Once the gas sample was collected, I opened all the jars and let them equilibrate with atmospheric air for 0.5 hrs. After this time I sealed the jars and randomly

assigned each replicate to one of three treatments: 1) AR, 2) $^{15}N_2$ incorporation, or 3) control (Fig. 1). In the AR treatment, I measured AR on 6 New Mexico replicates and 10 each from Texas and Inner Mongolia. I assigned 6 replicates from each site for measurement of N fixation by $^{15}N_2$ incorporation. I removed 5 ml of air from each jar and replaced it with 5 ml of 99.9% $^{15}N_2$. Chamber volume was 29 ml once I took into account volume displacement by the plastic container filled with sand. As a result, the atom % enrichment of $^{15}N_2$ within the chamber was 22.1 %. The remaining 4 replicates assigned to the control treatment were sealed and left untreated. All samples were left to incubate for 4 hrs.

After this time, I opened the jars and all *N. commune* samples were air-dried in a dark area. Samples from the control and $^{15}N_2$ incubation were ground with a mortar and pestle and run for atom % ^{15}N and total N on a Europe 20/20 isotope ratio mass spectrometer equipped with a flash combustion nitrogen and carbon analyzer. Gas samples from the AR experiment were analyzed gas chromatographically on a Shimadzu GC 14-A equipped with a Flame Ionization Detector.

Calculations of conversion ratios for AR to N fixation—Nitrogen fixation rates as $^{15}N_2$ incorporation were calculated using the following equation:

N fixed (ng N/g *N. commune*/hr) = (Total N (g) x (% Ndfa / 100) x 10^9) / incubation time (hrs)

where %Ndfa (percent N derived from atmosphere) was calculated as:

%Ndfa = (^{15}N atom % excess in sample / ^{15}N atom% excess in atmosphere) x 100

I calculated ^{15}N atom % excess for each replicate as:

^{15}N atom % excess in sample = ^{15}N atom % of samples incubated in $^{15}N_2$ – mean ^{15}N atom % of control

$^{15}N_2$ atom % excess in atmosphere was calculated as:

^{15}N atom % excess in atmosphere = atom% $^{15}N_2$ – atom% $^{15}N_2$ in N_2 of ambient air.

AR and N fixation rates vary over time and also between individual colonies, leading to a high level of variability in conversion ratios. As a result, I explored different methods by which to calculate conversion ratios. In method 1, I conducted AR and N fixation on the same samples to examine variability over N fixation rates over time. I calculated the ratio of AR during incubation 1 to N fixation during incubation 2 (Fig. 1). Since AR and N fixation rates may change from one sampling period to the next, I also measured AR during both incubations in order to examine how rates changed over time and whether the pattern was predictable (Fig. 1). In method 2, I examined the variability between samples and calculated AR to N fixed for samples that were incubated during the same time period but on separate samples (Table 1).

Statistical analysis—I compared within (method 1 vs. 2) and between site differences in conversion ratios of AR to N fixed using a Student's t-test. Treatments were considered significantly different at $P < 0.05$. Since AR and N fixation were conducted on independent samples in method 2, I was unable to generate a standard error of the mean using the traditional calculation. As a result, standard error of the mean in method 2 was calculated as:

$$Ratio = \lambda = \overline{R_1} / \overline{R_2}$$

$$\text{Standard Error of } \lambda = s(\lambda) = \frac{1}{\overline{R}_1}\sqrt{\frac{\text{var}(R_2)}{n_2} + \frac{\lambda^2\,\text{var}(R_1)}{n_1}}$$

where :

$$\overline{R}_1 = {}^{1}\!/_{n_1} \sum_{i=1}^{n_1} R_{1i}$$

$$\text{var}(R_k) = \frac{1}{n_k - 1}\sum_{i=1}^{n}(R_{ki} - \overline{R}_k)^2$$

n_k = Number in group k (k = 1,2)

R_1 = mean ethylene production rate (nmol C_2H_4/g/hr)

R_2 = mean N fixation rate (nmol N/g/hr)

n_1 = AR sample size

n_2 = N fixation sample size

λ = mean ethylene production (ng C_2H_4/g/hr) / mean N fixation (ng N/g/hr).

Results

Nitrogen fixation in *N. commune* from the Texas and New Mexico was high enough to observe a significant enrichment in ^{15}N (Table 1). Nitrogen fixation was near zero based on AR, and $^{15}N_2$ incorporation was not detectable in *N. commune* from the Inner Mongolia site. Atom % ^{15}N in $^{15}N_2$-enriched samples from these sites was not different from controls. As a result, I was unable to calculate a ratio of AR to N fixation ratio for the Inner Mongolia site. Conversion ratios were greater than the theoretical 3-4 for the New Mexico and Texas site (Table 1). Conversion ratios were highly variable depending on the method of calculation. Using method 2 where conversion ratios were calculated on separate replicates, ratios for the two sites were similar (New Mexico = 6.2, Texas = 5.7). However calculating AR to N fixation ratios using method 1, the ratio of AR to N fixed from Texas was significantly higher relative to New Mexico (Table 1, t = -

2.31, $P = 0.04$). Within site differences in conversion ratios was less clear. Ratios more than doubled from 5.7 to 11.4 for *N. commune* from Texas, but due to the high variability this was not statistically significant. Conversion ratios for the New Mexico site using methods 1 and 2, however, were more similar at 4.3 and 6.2.

There was a significant positive linear relationship between ethylene produced and N fixation by *N. commune* from the New Mexico site ($F = 25.2$, $P = 0.007$, Fig. 2a.). Furthermore, the slope in this relationship was close to the theoretical 3 (2.96) with an intercept close to zero. There was no significant relationship between ethylene produced and N fixed for Texas *N. commune* ($F = 3.24$, $P = 0.14$) (Fig. 2b). The lack of a relationship between AR and N fixation may be due to changes in AR between incubation 1 and 2 at the Texas site, where there was no generalizable trend in ethylene production by *N. commune* (Fig. 3b). Points fell both above and below the 1:1 line showing that AR rates both increased and decreased over the two incubation periods. The relationship between AR at incubation 1 and 2 for *N. commune* from the New Mexico site was more clear. Except for one outlier where AR rates increased, points generally fell on the 1:1 line (Fig. 3a).

Discussion

Conversion ratios in this study using methods 1 and 2, were greater than the theoretical 3 for all sites and ranged from 4.3-11.4. These results are in contrast to a previous study where the conversion ratio was 0.366 for *N. commune* collected from the same sites in New Mexico (Belnap, unpublished results). Conversion ratios for *N. commune* from a high arctic ecosystem were also low and ranged from 0.11-0.48 (Liengen 1999). Liengen (1999) suggested that lower conversion ratios may be due to

the thick mucilaginous gel surrounding *N. commune* from arctic environments, resulting in diffusional differences between acetylene or ethylene and N_2. In this study, higher conversion ratios may be partially explained by faster diffusion by acetylene relative to N_2 through the polysaccharide sheath. Since acetylene is 60-65 times more soluble in water than N_2 (Bergerson 1970, Rice and Paul 1971), faster diffusion of C_2H_2 through a thick mucilaginous sheath may result in C_2H_2 being more available to enzyme sites. Polysaccharide content, an index of mucilaginous gel development, in *N. commune* from the New Mexico sites appears to be high relative to other regions. In a recent study of *N. commune* collected from the same sites in New Mexico and Inner Mongolia, polysaccharide content from the New Mexico sites was 15-fold higher relative *N. commune* from Inner Mongolia (Belnap, unpublished results). Leakage of newly fixed N may also result in conversion ratios higher than the theoretical 3. Cyanobacteria have been shown to release significant amounts of NH_4^+ and organic N (Mayland 1966, Glibert and Bronk 1994). In a study of the marine cyanobacteria, *Trichodesmium* spp., Glibert and Bronk (1994) showed that dissolved organic N release was 50% of the N fixation rates. In another study up to 19% of the newly fixed N was released to the surrounding environment (Klubek *et al.* 1978).

Conversion ratios of AR to N fixed varied depending on experimental design. For the New Mexico site, there was a strong relationship between AR rates from incubation 1 and N fixation (R^2=0.86). Using this same method Vitousek and Hobbie (2000) also showed a strong relationship between AR and N fixation conducted on the same samples over different incubation periods (R^2 = 0.94). Within the New Mexico sites the coefficient of variation was also lower using method 1 as compared to method 2.

Overall, the conversion ratio of 4.3 using method 1 for the New Mexico sites appears to the best method of calculation.

In contrast method 2 appears to be the best method of calculation for the Texas sites. AR rates were highly variable over the period of the two incubations with no generalizable relationship, which resulted in a high conversions ratio using method 1 (11.4). However, using method 2 the conversion ratio was closer to the theoretical values (5.7) and the coefficient of variation was low as compared to method 1.

Based on the wide range of variability in published conversion ratios and the highly variable conversion ratios in this study that are highly dependent on the method of calculation, there is no evidence that supports the use of the theoretical ratio of 3 (Rice and Paul 1971, Montoya *et al.* 1996, Ley and D'Antonio 1998). The between site variability in conversion ratios strongly suggests that all experiments must include a calibration component, if AR is to be used as an index of N fixation. If investigators are only interested in the presence of N fixation activity, calibration would not be as important. Once an organism shows a positive response to AR then more detailed studies using calibration of AR and N fixation may be conducted.

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Table 1. Nitrogen fixation by *Nostoc commune* as related to AR. I report two conversion ratios for ethylene produced to N fixed. In method 1, I measured AR and N fixation on the same samples during two different incubation periods. In method 2, I measured AR and N fixation on different samples during the same incubation period. When a different letter follows a mean conversion ratio within a column (a's and b's) or row (c's and d's), these ratios are significantly different evaluated at $P < 0.05$. Values are means (± 1 SE). N.D. = not determined.

Site	Atom % ^{15}N	Percent N	N fixed nmol/g/hr	C_2H_4 produced nmol/g/hr	Method 1 C_2H_4:N_{fix}	Method 2 C_2H_4:N_{fix}
New Mexico	0.3719 (0.0022)	3.32 (0.06)	137 (43)	659.4 (174)	4.3 (0.9) a,c	6.2 (1.63) a,c
Texas	0.3804 (0.0043)	2.45 (0.03)	181 (55)	800.8 (124)	11.4 (3.3) b,c	5.7 (0.88) a,c
Mongolia	0.3653 (0.0002)	1.94 (0.18)	N.D.	0.70 (0.26)	N.D.	N.D.

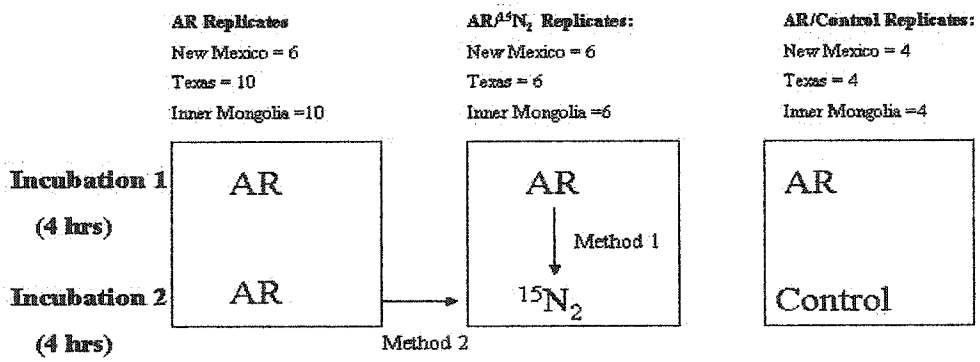

Fig. 1. Experimental design of AR and N fixation experiment on *Nostoc commune* collected from three sites. I measured AR on all samples 24 hrs after water addition to *N. commune* colonies. Immediately following this, I conducted the N fixation experiment by comparing samples incubated in an elevated $^{15}N_2$ atmosphere and compared to controls that were incubated under ambient $^{15}N_2$. Values below each treatment are samples size for each site.

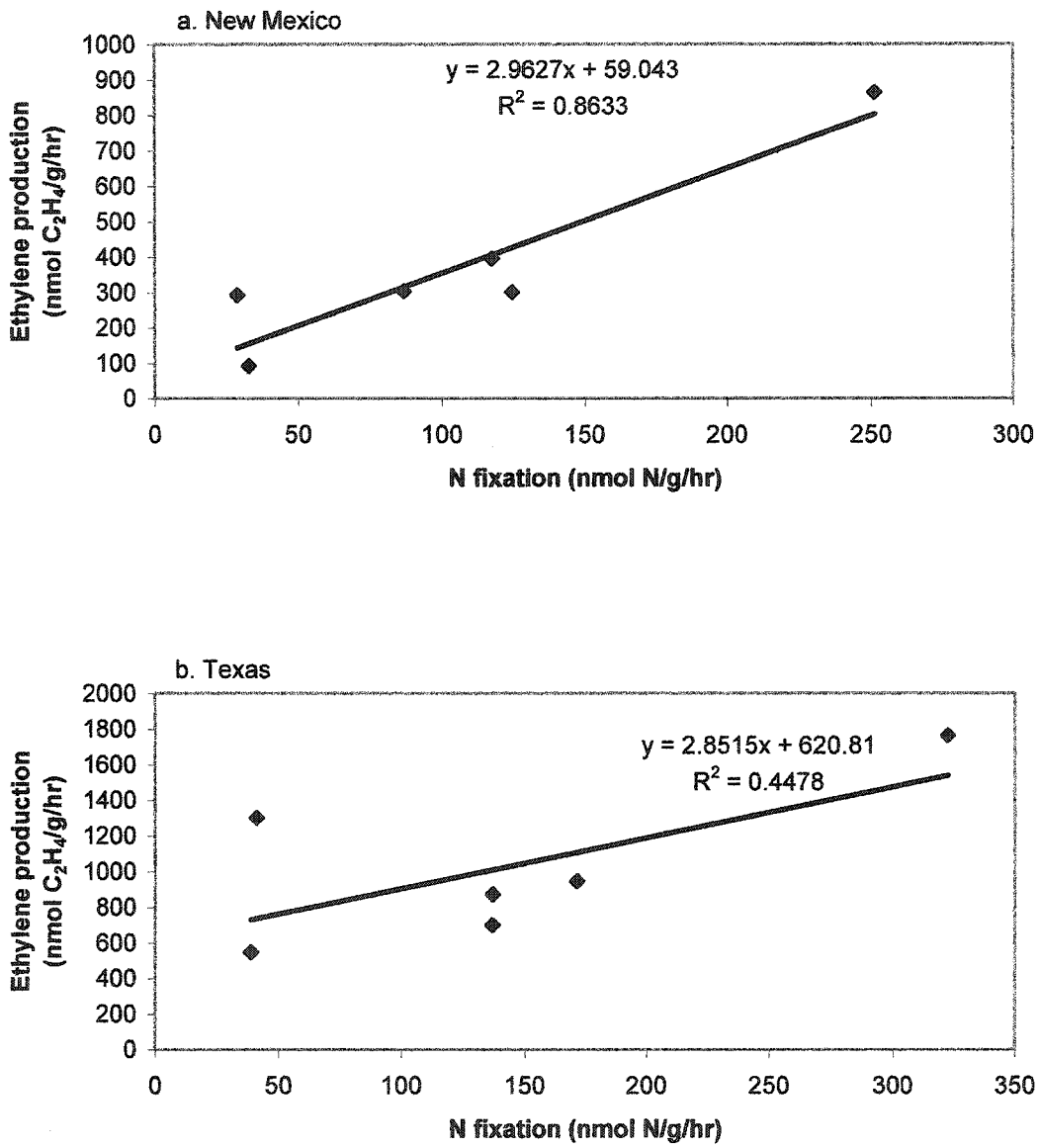

Fig. 2. Ethylene production as a function of ^{15}N fixation. AR and ^{15}N fixation were measured on the same replicates over two, four hr incubation periods for *Nostoc commune* from New Mexico and Texas.

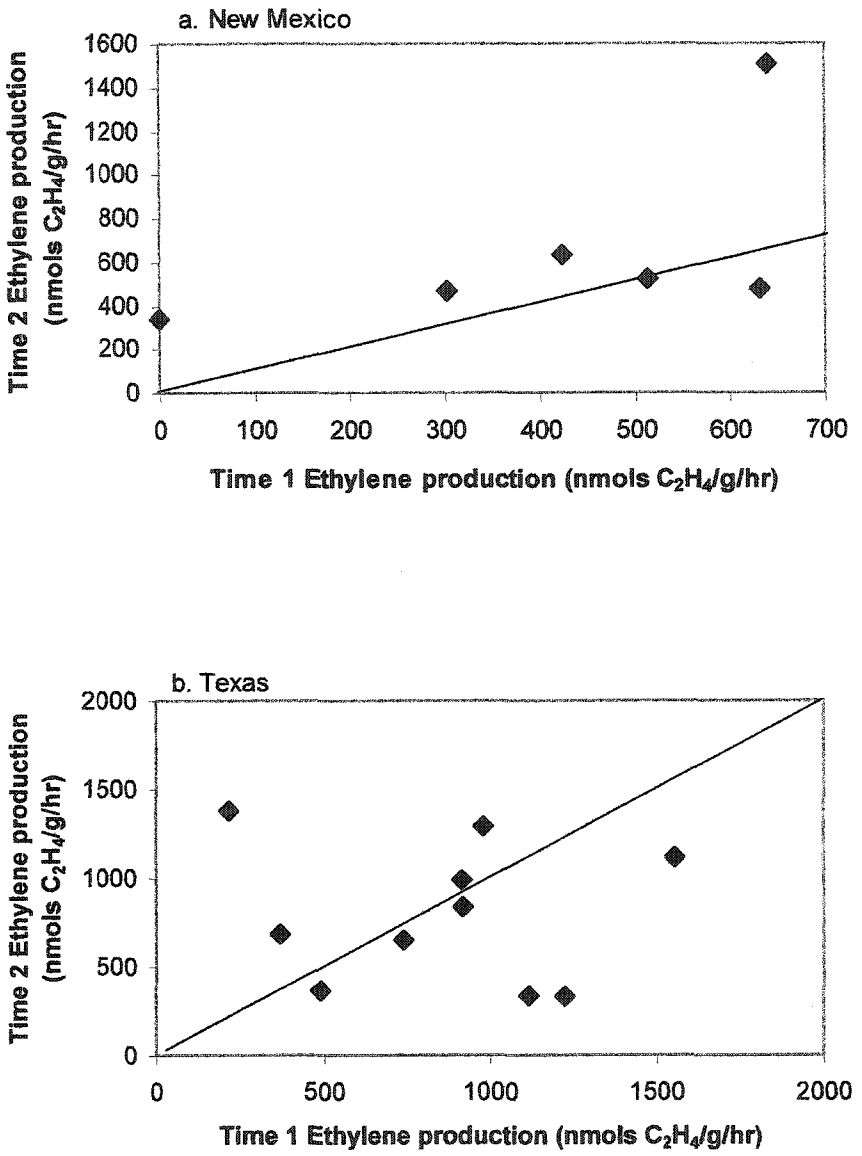

Fig. 3. AR rates at incubation 1 as compared to incubation 2. AR was conducted on the same replicates over two, four hr incubations for the New Mexico and Texas sites. Triangles within each graph are actual data points.

Chapter V.

Impacts of Sheep Grazing on Plant Composition and Soil Nutrients in an Inner Mongolian Grassland

Abstract

Human population growth and changes in land use practices are rapidly increasing livestock grazing pressure in the steppe region of the Inner Mongolia Autonomous Region in northern China. These changes have led to declines in grassland productivity, with 55% of Inner Mongolian grasslands now unusable for livestock grazing. These declines in plant productivity and functional changes in plant community composition may have large effects on soil carbon (C) and nitrogen (N) cycling. In this study I report on changes in plant community composition and soil C and N mineralization dynamics from a nine-year sheep grazing study in Inner Mongolia. I addressed these questions: 1) how does increasing grazing intensity affect plant community composition? 2) how does increasing grazing intensity alter soil C and N mineralization dynamics? 3) do changes in soil C and N mineralization dynamics relate to changes in plant community composition via inputs of the quality and/or quantity of litter? Grazing plots were set up near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) with five grazing intensities: 1.33, 2.67, 4.00, 5.33, and 6.67 sheep/ha/yr. After nine years total plant cover decreased with increasing grazing intensity, primarily due to a dramatic decline in grasses, *Carex duriuscula*, and *Artemesia frigida*. *Potentilla* spp. (*P. acaulis* and *P. tanacetifolia*), an indicator of overgrazing, increased at the higher grazing intensities. Litter mass was significantly lower at the higher grazing intensities of 4.00 and 5.33

sheep/ha/yr compared to 1.33 and 2.67 sheep/ha/yr. Changes in litter mass and percent organic C in the litter caused a significant decrease in total C in the litter layer at 4.00 and 5.33 sheep/ha/yr compared to 2.67 sheep/ha/yr. A similar pattern was observed in total litter N, which was significantly lower at 5.33 sheep/ha/yr compared to 2.67 sheep/ha/yr. Litter C:N ratios, an index of litter quality, were significantly lower at 4.00 sheep/ha/yr relative to 1.33 and 5.33 sheep/ha/yr. The lower C:N ratios at 4.00 sheep/ha/yr were mainly driven by higher percent C in the litter layer, since there were no significant differences in percent N under different grazing intensities. Cumulative C mineralized after 16 days decreased with increasing grazing intensity. In contrast, net N mineralization ($NH_4^+ + NO_3^-$) after a 12-day incubation from soils collected at the same depth increased within increasing grazing intensity. Changes in C and N mineralization resulted in a narrowing of CO_2-C:net N_{min} ratios with increasing grazing intensity. Grazing explained 31% of the variability in the ratio of CO_2-C:net N_{min}. High CO_2-C:net N_{min} ratios reflect growth in the microbial population by immobilization of N, whereas lower ratios under high intensity grazing indicate low demand and microbial growth. The ratio of CO_2-C:net N_{min} was positively correlated with litter mass, which explained 94% of the variability in the ratio of CO_2-C:net N_{min}. Furthermore, there was a positive correlation between litter mass and *A. frigida* cover, where *A. frigida* cover explained 94% of the variability in litter mass. These results suggest that C quality for microbial decomposition was enhanced under low intensity grazing as compared to high intensity grazing. Therefore as grazing intensity increases microbes may become more C limited resulting in lower microbial growth and demand for N. In addition, long-term monitoring of *A. frigida* may be used as an indicator of potential changes in C quality for microbial

decomposition and soil C and N mineralization dynamics in typical steppe regions of Inner Mongolia.

Introduction

Human population growth and changes in land use practices are rapidly increasing livestock grazing pressure in the steppe region of Inner Mongolia in northern China, where livestock numbers nearly tripled from 1952 to 1989 (Ellis 1992). Studies conducted in this region during the 1990's indicate that grassland productivity decreased by 30% in some areas, and up to 55% of Inner Mongolian grasslands are now considered to be unusable or "deteriorated" (Ellis 1992). From 1990 to 1998 livestock numbers in the Xilin River Basin, the area of this study, increased by 184%.

Declines in plant productivity and functional changes in plant community composition may have large effects on soil C and N cycling. In central Inner Mongolia 12 % of the soil organic carbon (SOC) in the top 20 cm was estimated to be lost over a 40-year period as a result of overgrazing (Li *et al.* 1998). Another study in the same region indicated that 17 years of grazing had no effect on soil organic C in the top 35 cm (Wang and Chen 1998). These data suggest that grazing effects on soil C and N dynamics may be observed only after several decades, with no discernible differences occurring in the shorter term. Livestock grazing affects soil organic C and N storage via changes in litter quality and quantity (Willms *et al.* 2002, Olofsson and Okansen 2002, Mapfumo *et al.* 2002, Fuhlendorf *et al.* 2002, Reeder and Schuman 2002), belowground biomass (Biondini *et al.* 1998, Wang and Wang 1999, Johnson and Matchett 2001, Frank *et al.* 2002), soil microclimate (Rietkerk *et al.* 2000, Wan *et al.* 2002), and wind and water erosion (Bach 1991, Bird *et al.*1992).

Increased offtake by animals often results in decreased litter inputs (Huang *et al.* 1997), whereas selective foraging may cause changes in litter quality as a result of

changes in plant community composition (*e.g.* Pastor *et al.* 1993, Oloffsson and Oksanen 2002). Four years after the initiation of a nine-year sheep grazing study in Inner Mongolia, standing aboveground biomass declined with increasing grazing intensity (Wang and Chen 1998). Furthermore, after four years, cover of the dominant grass species, *Agropyron michnoi* and *Leymus chinensis* decreased dramatically with increasing grazing intensity whereas cover of *Potentilla* spp. (*P. acaulis* and *P. tanacetifolia*), an indicator of overgrazing, increased at the highest grazing intensities (Wang and Chen 1998, Li *et al.* 1999). Given these changes in aboveground biomass and plant community composition, I hypothesized that changes in litter quantity and quality after nine years under varying grazing intensities would alter soil C and N mineralization dynamics.

In this study I report on changes in plant community composition and soil C and N mineralization dynamics from a nine-year grazing study in Inner Mongolia. I addressed these questions: 1) how does increasing grazing intensity affect plant community composition? 2) How does increasing grazing intensity affect soil C and N mineralization dynamics? 3) Do changes in soil C and N mineralization dynamics relate to changes in plant community composition via inputs of the quality and quantity of litter?

Methods

Site description—Study sites were located in the Xilin River Basin near the Inner Mongolian Grassland Ecosystem Research Station (IMGERS), Inner Mongolia Autonomous Region, China (43°38′ N, 116°42′ E, Fig. 1). Mean annual temperature in this region is 0° C with warm summers and cold winters dominated by northern arctic

fronts. Annual precipitation ranges between 250-450 mm/yr with up to 80% of annual precipitation occurring from May through September. Vegetation is characterized as typical steppe, which is dominated by *Artemesia frigida, Carex duriuscula* and the grasses *Stipa grandis*, *Leymus chinensis*, and *Agropyron michnoi* (Wu and Loucks 1992, Li *et al.* 1999). Experiments were conducted in one ha sheep grazing plots that were established nine years prior to this experiment (1989-1998). Grazing plots were set up as a restricted complete block design with 5 grazing intensities of 1.33, 2.67, 4.00, 5.33, and 6.67 sheep/ha/yr within 3 blocks (Fig. 2), except during the first year of the experiment (1989) when grazing intensities within the plots were 1.33, 2.00, 2.67, 3.33, and 4.00 sheep/ha/yr. The grazing schedule was 15 days on, 30 days off rotation from May 20 to October 5 each year.

Plant community composition—I surveyed plant species and functional groups within the grazing plots in July of 1998. I delineated four transects within each plot. At 10 equally spaced points along each transect I estimated percent cover of four groups: 1) *A. frigida* 2) *Potentilla* spp. (*P. acaulis* and *P. tanacetifolia*) 3) *Carex duriuscula* and 4) grass. I estimated cover using one 25 x 25 cm gridded quadrat with a total of sixteen point hits within a quadrat. Cover was estimated at 10 locations every 10 m along 4 random lines for a total of 40 quadrats in each 1 ha grazing plot.

Soil carbon and nitrogen mineralization—I conducted a laboratory incubation to determine the impact of grazing on soil C and N mineralization. I collected three soil cores from each depth of 0-5, 5-10, and 10-20 cm within each plot. Since I was primarily interested in laboratory C and N mineralization potentials and not field rates of C and N mineralization, soils were immediately air-dried and stored at room temperature for

several months. To measure net N mineralization, I used a double chamber sterile filtration unit to leach the soils for inorganic N (NH_4^+ and NO_3^-). The upper chamber was lined with a Whatman Glass Fiber Filter. I placed 50 g of dry soil into the chamber. I leached soil samples with 100 ml of 0.01 M $CaCl_2$ as described by Wedin and Pastor (1993) on days 12, 29, 45, 109, and 151 after the initial wet up. I allowed the salt solution to equilibrate with the soil for one hour. After this time I applied a vacuum to the lower chamber to remove excess solution. The leachate was then stored in polypropylene bottles and frozen. Ammonium and NO_3^- concentrations were analyzed colorimetrically using an Alpchem Autoanalyzer (Perstorp Analytical).

I measured soil respiration 4, 16, and 29-43 days after wet up on the same soil samples. Filtration units were placed in 1.89 L glass jars with metal screw top lids. Each lid was equipped with a Wheaton septum in order to withdraw gas samples from the jar. Before each sample period I purged each chamber with compressed air of a known concentration of CO_2. On each sample date I randomly chose six jars and measured initial CO_2 concentrations immediately after the jars were capped. I calculated a mean CO_2 concentration from these jars, which I used as the initial CO_2 concentration for all samples. On sample days 4 and 16, soils were incubated for 24 hrs. Soil CO_2 fluxes were low by day 29, therefore CO_2 concentrations were allowed to build up in the chamber over 12 days. CO_2 gas samples were drawn from the jars using polypropylene disposal syringes and analyzed on an Infrared Gas Analyzer (Licor-6252).

Litter quantity and quality—To determine the effect of increasing grazing intensity on litter quality and quantity, I established two transects within each 1 ha plot in July 1999 and sampled litter on the soil surface at 10 equally spaced points along each transect at

grazing intensities of 1.33, 2.67, 4.00, and 5.33 sheep/ha/yr. I was unable to collect litter samples from the highest grazing intensity of 6.67 sheep/ha/yr due to the paucity of surface litter. I collected litter from a 10 x 10 cm area at each point along each transect. The litter samples were immediately air dried in the field and then dried in a 65°C oven once they were transported back to the laboratory. I first removed the free-living soil cyanobacteria *Nostoc commune* and the soil lichen *Xanthoparmelia camtschadalis* (Ach.) Hale from the litter samples. The remaining organic material was considered surface litter material. After weighing the litter samples, I chose four samples (two from each transect) from within each plot to measure total C and N. I ground the litter samples with a mortar and pestle and analyzed the samples for total C and N on a Leco CHN-1000 Elemental Analyzer.

Statistical Analysis—I analyzed soil C and N mineralization data using an analysis of covariance (ANCOVA) with grazing as the main effect and blocking as a covariate. I analyzed litter and vegetation cover data using a 2-way analysis of variance (ANOVA) with the 2 main effects of grazing and block and their interaction, because there were significant grazing x block interactions using the ANCOVA. Overall F-tests were considered significant at $P < 0.05$. I used a Newman-Keuls post-hoc test to evaluate differences among individual grazing treatments in the 2-way ANOVA.

Results

Increasing grazing after nine years resulted in a decline in total plant cover (Fig. 3a; $F_{2,493} = 8.8$, $P = 0.0001$). Under the lowest grazing intensity, percent cover was 53% and significantly declined to 37% under the highest grazing intensity. The trend in decreasing plant cover with increasing grazing intensity was not linear. Total plant cover

was significantly higher at 2.67 and 5.33 sheep/ha/yr relative to 4.00 and 6.67 sheep/ha/yr. This effect was primarily due to the increase in *Potentilla* spp. cover with increasing grazing intensity. *Potentilla* spp. cover increased at grazing levels of 2.67 sheep/ha/yr, with a further increase to 25% cover at the two highest grazing intensities (*i.e.* 5.33 and 6.67)(Fig. 3b). In contrast to *Potentilla* spp., cover of all other groups decreased with increasing grazing intensity. Grass cover decreased by 50% when grazing increased from 1.33 to 2.67 sheep/ha/yr (Fig. 3c), but did not decline further until the highest grazing intensity was reached. *A. frigida*, which had the highest cover at the lowest grazing intensity (17%), declined nearly linearly with increasing grazing intensity (Fig. 3d), whereas, *Carex* spp. cover decreased at 2.67 sheep/ha/yr and again at 5.33 sheep/ha/yr (Fig. 3e).

Changes in plant community composition after nine years of sheep grazing appeared to affect both quantity (*i.e.* mass) and quality (*i.e.* C or N content) of surface litter. Litter mass was significantly lower at the higher grazing intensities of 4.00 and 5.33 sheep/ha/yr compared to the 1.33 and 2.67 sheep/ha/yr intensities (Fig. 4). Changes in litter mass in combination with percent C in the litter led to a significant decrease in total C in the litter layer at 4.00 and 5.33 sheep/ha/yr compared to 2.67 sheep/ha/yr (Table 1). A similar pattern was observed in total litter N, which was significantly lower at 5.33 sheep/ha/yr compared to 2.67 sheep/ha/yr. Litter C:N ratios were significantly lower at 4.00 sheep/ha/yr relative to 1.33 and 5.33 sheep/ha/yr. The lower C:N ratios at 4.00 sheep/ha/yr were mainly driven by higher percent C in the litter layer, since no significant differences were observed in percent N under different grazing intensities.

Cumulative C mineralized after 16 days decreased with increasing grazing intensity (Fig. 5; ANCOVA, grazing: $F_{1,44} = 4.08$, $P = 0.05$). In contrast, net N mineralization ($NH_4^+ + NO_3^-$) after a 12-day incubation from soils collected at the same depth increased within increasing grazing intensity (Fig. 6; ANCOVA, grazing: $F_{1,44} = 6.79$, $P = 0.01$). Changes in C and N mineralization resulted in a narrowing of CO_2-C:net N_{min} ratios with increasing grazing intensity (Fig. 7; ANCOVA, grazing: $F_{1,39} = 9.89$, $P = 0.003$). Grazing explained 31% of the variability in the ratio of CO_2-C:net N_{min} when adjusted for the blocking effect. The ratio of CO_2-C:net N_{min} was positively correlated with litter mass, which explained 94% of the variability in the ratio (Fig. 8). After the first two weeks of the incubation, there was no effect of grazing on C and N mineralization in surface 0-5 cm soils. At no time did I observe any effect of grazing on C and N mineralization in subsurface soils (5-10 cm and 10-15 cm). The decrease in standing litter pools was also positively correlated to *A. frigida* cover, where percent cover of *A. frigida* explained 94% of the variability in litter mass (Fig. 9).

Discussion

High CO_2-C:net N_{min} ratios reflect growth in the microbial population by immobilization of N, whereas lower ratios under high intensity grazing indicate low demand and microbial growth (Schimel 1986, Burke *et al.*1989, Holland and Detling 1990). Results from this study suggest that C quality for microbial decomposition was enhanced under low intensity grazing as compared to high intensity grazing. Similar to results from this study, Fahnestock and Detling (2002) showed that field net N mineralization was 4 times higher in areas grazed by prairie dogs as compared to areas off prairie dog colonies. In an earlier study, Holland and Detling (1990) observed an

increase in CO_2-C:net N_{min} ratios with increasing grazing intensities and suggested the decrease in root biomass along a grazing sequence was a result of decreased C allocated belowground resulting in C limitation of the microbial community. Belowground biomass also decreased after seven years of grazing at the Inner Mongolia sites (Wang and Wang 1999), which may partially explain the higher CO_2-C:net N_{min} ratios with increasing grazing intensity in this study.

The decline in C quality for microbial decomposition was driven more by litter quantity (*i.e.* mass) as compared to litter C or N content. Litter quantity at the soil surface, which generally decreases with increasing grazing intensity, was the best predictor of C quality as reflected in CO_2-C:net N_{min} ratios. As grazing intensity increases, microbes may become more C limited resulting in lower microbial growth and demand for N. Indeed, four years after the initiation of the grazing experiment, microbial biomass and numbers decreased with increasing grazing intensity in these sites (Zhao 1999). Field data on available N also support the results from the laboratory incubation, which showed increasing N availability with increasing grazing intensity (Zhao 1999).

Although lower microbial demand for inorganic N results in higher plant available N in soils, during times of low plant N demand, N may also be more easily lost via gaseous and leaching pathways. After four years of experimental grazing at the Inner Mongolia sites, percent soil organic N decreased in the top 10 cm of soil at the highest grazing intensity relative to light and moderately grazed plots (Zhao 1999). Whether this was due to decreased inputs as a result of declines in N-fixing organisms (Belnap *et al.*, in prep) or increased outputs via changes in leaching or gaseous N loss is not clear. In this study, however, there were no differences in soil organic N in the top 5 cm of soil.

Carbon and N mineralization dynamics were not correlated with litter C:N ratios, an index of litter quality, whereas litter mass explained a large amount of the variability in CO_2-C:net N_{min} ratios. C:N ratios of the surface litter pool are integrated over several years. A narrower C:N ratio may reflect not only a difference in litter quality but microbial processing of litter. Microbial decomposition of litter results in a narrowing of C:N ratios where C may be lost as CO_2 but N is retained. As a result, C:N ratios of fresh litter may be a better indicator of litter quality rather than surface litter pools, used in this study.

Potentilla spp. are often used as an indicator of overgrazing in grassland ecosystems (Li *et al.* 1999). In addition to the presence of *Potentilla* spp., *A. frigida* cover may also be used to predict changes in soil C and N mineralization dynamics via changes in litter quantity. *A. frigida* cover explains a large amount of the variability in the surface litter pools, which is strongly related to C and N mineralization dynamics. Based on these relationships, long-term monitoring of *A. frigida* may be an important indicator of changes in C quality and subsequent effects on soil C and N mineralization dynamics in typical steppe regions of Inner Mongolia.

Conclusions

Overall, future increases in livestock grazing on the Inner Mongolian steppe, and the resulting transition from a grass, *C. duriuscula,* and *A. frigida* community to one that is dominated by *Potentilla* spp. may impact soil C and N mineralization dynamics. As grazing intensity increases, litter quantity should decrease, resulting in C limitation of soil microbial communities. Since there wass a strong relationship between litter quantity and *A. frigida* cover, long-term monitoring of *A. frigida* may be an important indicator of

litter inputs and subsequent changes in soil C and N mineralization dynamics. Even though C limitation of microbes results in lower N immobilization and higher plant available N pools, there is also greater potential for N loss from these pools in leaching and gaseous loss pathways. Even though I present evidence that litter quantity plays a role in soil C and N mineralization dynamics, other factors such as changes in allocation to belowground biomass may be important in contributing to these changes.

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Table 1. Carbon and nitrogen in the surface litter layer. Surface litter samples were collected from a 10 x 10 cm^2 area and analyzed for percent C and N. Total litter C and N was calculated as (g litter/m^2 * %C or %N)/100. Values are means per grazing treatment ± 1 SE. When a different letter occurs within a column there was a significant effect of grazing at $P < 0.05$ (Post-hoc Newman-Keuls).

Animal units (sheep/ha/yr)	Litter % C	Litter %N	Litter C (g/m^2)	Litter N (g/m^2)	Litter C:N
1.33	31.6 (1.2) a	1.36 (0.06) a	24.3 (3.7) a,b	1.1 (0.18) a,b	23.3 (0.82) a
2.67	28.2 (1.1) a,b	1.37 (0.06) a	26.5 (4.5) a	1.3 (0.20) a	20.7 (0.59) a,b
4.00	26.1 (1.2) b	1.44 (0.07) a	14.9 (2.5) b	0.84(0.15) a,b	18.7 (1.06) b
5.33	29.5 (1.8) a,b	1.26 (0.06) a	13.0 (3.7) b	0.56(0.15) b	23.9 (1.81) a

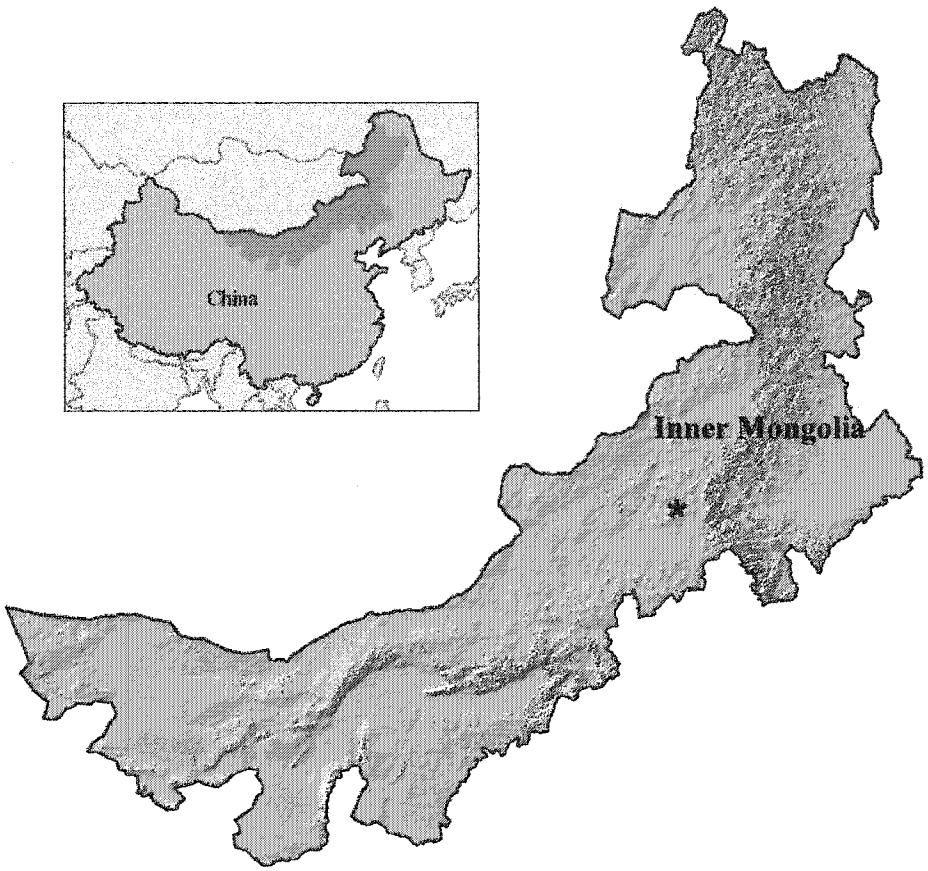

Figure 1. Site location. The darker shaded area within the inset shows the Inner Mongolia Autonomous Region (IMAR). The field site location is designated by a ★.

Block I	Block II	Block III
4.00	2.67	1.33
6.67	5.33	4.00
2.67	1.33	6.67
5.33	4.00	2.67
1.33	6.67	5.33

Figure 2. Grazing plot experimental design. Grazing plots were set up as a restricted randomized complete block design with 5 grazing intensities of 1.33, 2.67, 4.00, 5.33, and 6.67 sheep/ha/yr within 3 blocks. In the first year of the experiment (1989) grazing treatments were 1.33, 2.00, 2.67, 3.33, and 4.00 sheep/ha/yr. Soil samples were collected in the summer of 1998 and litter samples were collected in the summer of 1999.

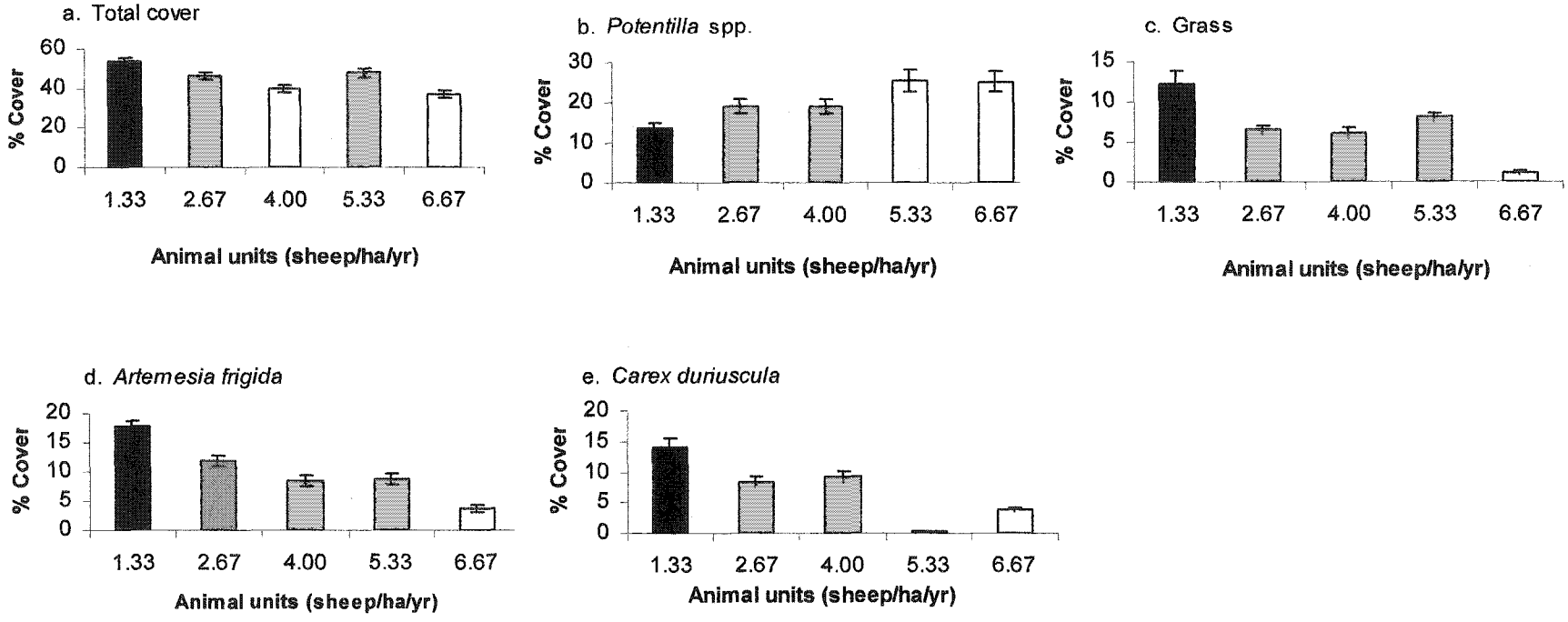

Figure 3. Changes in plant community composition with livestock grazing after nine years. Values are mean percent absolute cover (N = 40) for a) total plant b) *Potentilla* spp. c) grass d) *Artemesia frigida* and e) *Carex duriuscula* ± 1 SE. A different colored bar within each graph represents a significant effect of grazing evaluated at $P < 0.05$ (Post-hoc Newman-Keuls).

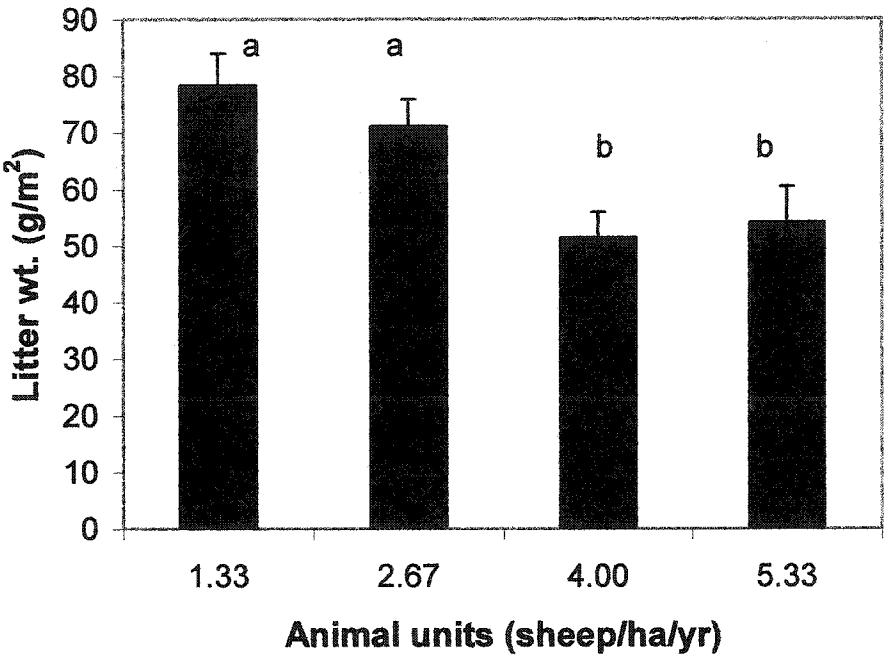

Figure 4. Soil surface litter mass. Surface litter was collected from 10 x 10 cm quadrats within each grazing plot. Values are mean litter mass within each grazing treatment ± 1 SE (N = 20). There was a significant effect of grazing at $P < 0.05$ where a different letter appears above two treatment means (Post-hoc Newman-Kreuls).

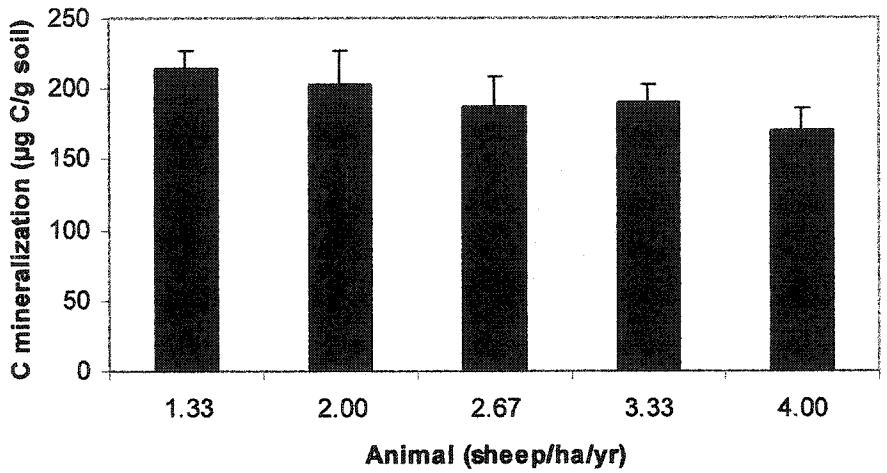

Figure 5. Cumulative C mineralization after 16 days. Values are mean C mineralized (µg C/g soil) ± 1 SE. Grazing explained 21% of the variability in cumulative C mineralized when adjusted for the blocking effect using an ANCOVA ($F = 4.08$ $P = 0.05$).

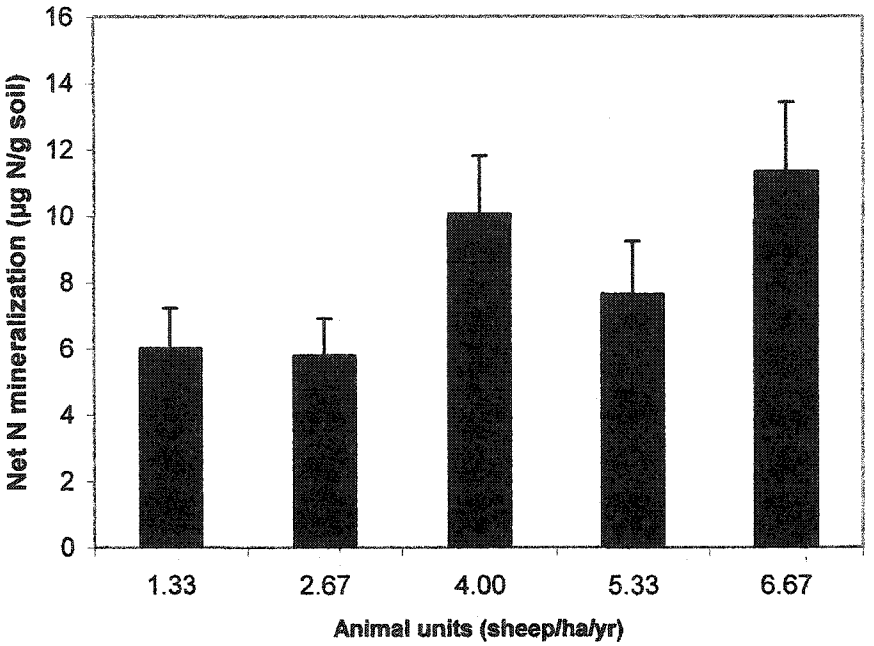

Figure 6. Net N mineralization after 12 days in the lab. Soils were leached with a salt solution on day 1 of the experiment and then incubated for 12 days. Values are means of total mineral N (NO_3 + NH_4^+) from soils collected at 0-5 cm depth ± 1 SE. Grazing explained 28% of the variability in cumulative N mineralized when adjusted for the blocking effect using an ANCOVA ($F = 6.79$, $P = 0.01$).

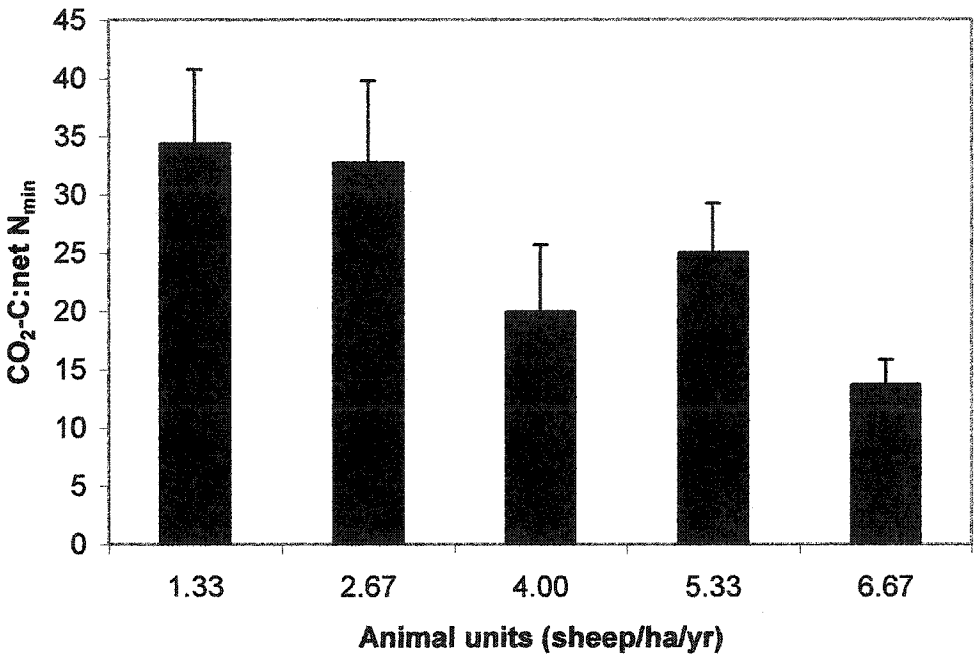

Figure 7. Ratio of C mineralized as CO_2 after 16 days to net N mineralized (NO_3^- + NH_4^+) after 12 days. CO_2-C:net N_{min} from soils. Values are means within each grazing treatment ± 1 SE. Grazing explained 31% of the variability in cumulative CO_2-C:net N_{min} when adjusted for the blocking effect using an ANCOVA ($F = 9.89$ $P = 0.003$). ($F = 4.08$ $P = 0.05$).

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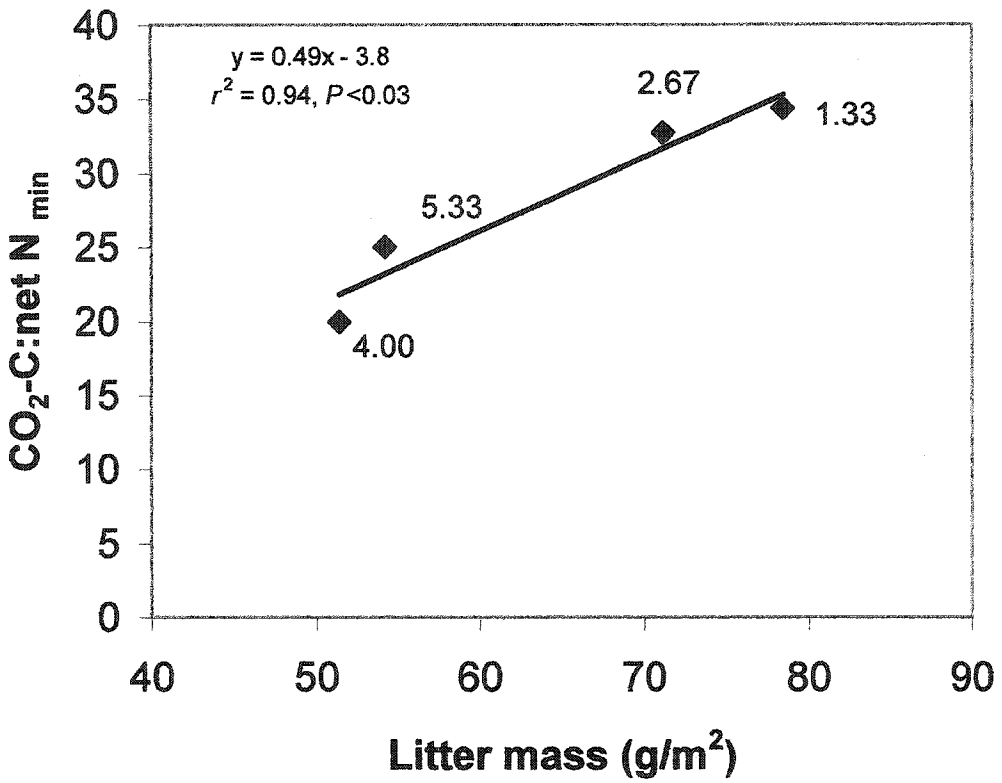

Figure 8. Ratio of CO_2-C:net N_{min} as a function of litter mass. Litter mass at the soil surface explains 90% of the variability in CO_2-C:net N_{min}. These data show that as litter mass increases the ratio of CO_2-C:net N_{min}, and indicator microbial immobilization, (See Fig. 5) also increases. Values are treatment means, with the numbers next to the triangle representing the grazing treatment.

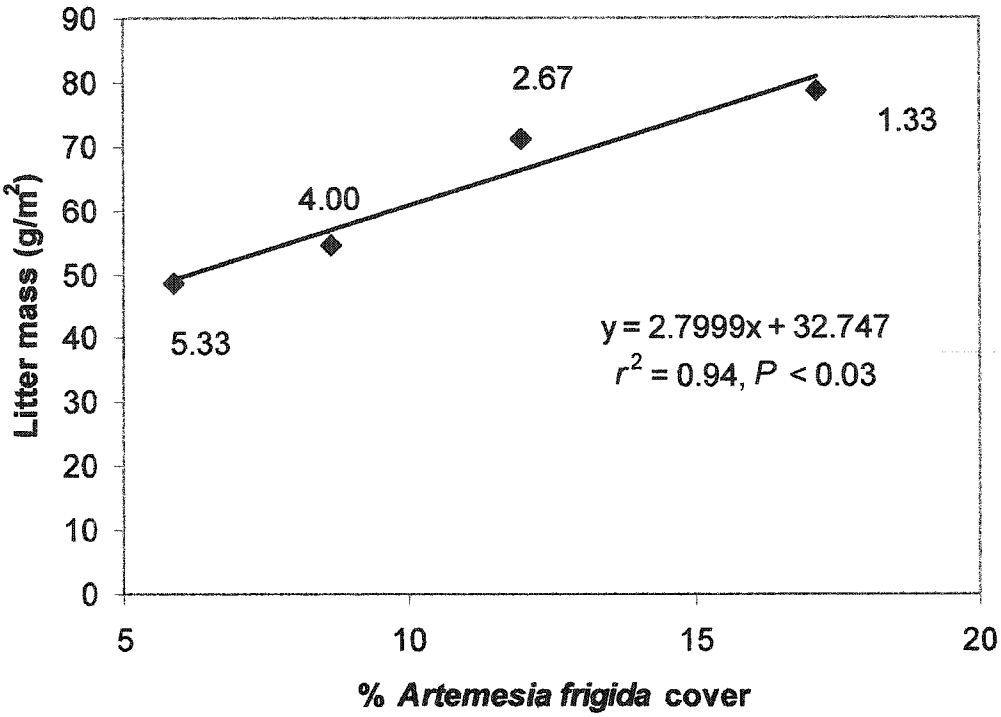

Figure 9. Litter mass as a function of *Artemesia frigida* cover. Percent cover of *A. frigida* at the soil surface explains 91% of the variability in litter mass. As *A. frigida* cover increases with decreasing grazing intensity, litter mass also increases.

Chapter VI.

Conclusions

N loss from biological soil crusts and impacts on ecosystem N budgets (chapters II, III, and IV)—In chapter II, the hypothesis that NO gas loss increases with increasing N fixation potential of biological soil crusts was supported. The upper limit of annual NO loss from dark and light biological soil crust was 0.13 and 0.07 kg N/ha/yr respectively. Once estimates of denitrification losses as N_2O and N_2 (appendix II) and NH_3 volatilization (appendix III) from biological soil crusts were considered, there appears to be no strong differences in N gas loss by crust type. Estimates of the upper limit of total N gas loss were 1.6 kg N/ha/yr from all biological soil crusts.

The major factor controlling NO flux was soil temperature where NO fluxes averaged 2.75 ng NO-N/m^2/s at temperatures < 31° C. However, when soil temperature was > 31 ° C, NO fluxes increased by more than 3-fold (9.76 ng NO-N/m^2/s). The majority of measurements (86%) occurred at soil temperatures < 31° C where soil N, which increased with increasing N fixation of biological soil crusts, was a controlling factor in NO loss. At soil temperatures > 31 ° C, chlorophyll *a*, reflecting crust type, was the controlling factor in NO loss.

In chapter III I examined the impact of disturbance and biological soil crusts composition on C and N fluxes in surface runoff. Total C and N export (sediment + dissolved) increased significantly with trampling disturbance as compared to intact dark cyanolichen crusts. More than 98% of the total C and N flux in surface runoff was from sediment sources, whereas dissolved C and N made up very little of the total export

budget. Total C flux was on the order of 7.9 and 3.4 g C/m^2 for light and trampled crusts and 0.76 g/m^2 for dark crusts. Although this constitutes <1% of the organic C in the top 5 cm of surface soil at these sites, C flux of this magnitude is comparable to annual C inputs via photosynthesis by cyanobacteria. Biological soil crust composition also impacted C and N flux. Total N export from dark crusts (0.6 kg N/ha) was an order of magnitude lower than light crusts (6.3 kg N/ha/yr).

Overall, runoff and sediment export increased with disturbance, which suggests that downslope plant communities receive a higher flux of nutrients in water and sediments in a post-disturbance environment. Alternatively higher runoff and sediment transport from plant interspaces may also result in higher nutrient losses from the watershed. In a post-disturbance environment represented by light cyanobacterial crusts, soil nutrients may be more heterogenously distributed, with plant interspaces being highly depleted in N relative to tree and shrub canopies due to higher fluxes of water and sediments in runoff. In a community dominated by late successional dark cyanolichen crusts, nutrient distribution may become more homogeneous relative to disturbed and early successional biological soil crust communities; as runoff and sediment production decreases in late successional stages, nutrients will be retained in the plant interspaces.

These results still present a discrepancy in N budgets for biological soil crusts, where N inputs greatly exceed losses in well-developed biological soil crusts, but N losses greatly exceed inputs in light cyanobacterial crusts. In order to close ecosystem N budgets in these ecosystems alternative N loss pathways such as vertical leaching of dissolved N and losses due to wind erosion must be considered. Furthermore the

discrepancy in N inputs via fixation relative to losses may also be due to overestimates of N fixation.

Result from the study of conversion ratios of acetylene reduction (AR) to N fixation for *Nostoc commune*, a free-liviing soil cyanobacterium, showed that ratios were greater than the theoretical 3 used in most studies. Conversion ratios were highly variable by site and method of calculation and ranged from 4.3 to 11.4. If these results hold for *N. commune* on the Colorado Plateau, N inputs via fixation may be currently overestimated.

Chapter V.—Increasing livestock grazing after nine-years resulted in a transition from a grass, *Carex duriuscula,* and *Artemesia frigida* community to one that is dominated by *Potentilla* spp. These changes in plant composition resulted in a decrease in litter quantity, which was strongly related to soil C and N mineralization dynamics. The ratio of soil CO_2-C:net N_{min}, an index of microbial growth and demand for N, decreased with decreasing litter quantity. These results suggest that soil microbial communities become more C limited with increasing grazing intensity. Although C limitation of microbes results in lower N immobilization and higher plant available N pools, there is also greater potential for N loss from these pools in leaching and gaseous loss pathways. Even though I present evidence that litter quantity plays a role in soil C and N mineralization dynamics, other factors such as changes in allocation to belowground biomass may be important in contributing to these changes.

Appendix I.

Nitrogen fixation as measured by acetylene reduction in biological soil crusts

Methods

I measured N fixation activity using the acetylene reduction (AR) method in three biological soil crusts types. In July 2001, I collected 44 cm^2 soil cores to a depth of 5 cm in the deep soil fieldsite (described in chapter II). I collected 6 cores from each of the three biological crust types (*i.e.* dark, medium, and light, see chapter II for criteria of the three types) for a total of 18 cores. I added water to the soil cores to bring them up to field moisture. Percent soil moisture for all cores averaged 17% ± 0.9 SE. I placed the cores in 1.79 L glass jars with metal screw top lids. During the experiment, jars were left to incubate at the fieldsite. Three hours after water addition, I sealed the jars and injected acetylene (C_2H_2) to a 10% (v/v) atmosphere. I let the cores incubate for one hour and collected a gas sample from the headspace of each jar. Gas samples were stored in Labco Exetainers and transported to the laboratory. Gas samples were analyzed for ethylene gas chromatographically on a Shimadzu GC 14-A equipped with a Flame Ionization Detector.

Results

Nitrogen fixation activity in dark crusts was higher than light crusts (Fig. 1, $F_{2,15} = 2.89$ $P = 0.08$), with medium crust showing intermediate N fixation activity. Nitrogen fixation activity in dark crusts was more than 5 fold higher than light crusts.

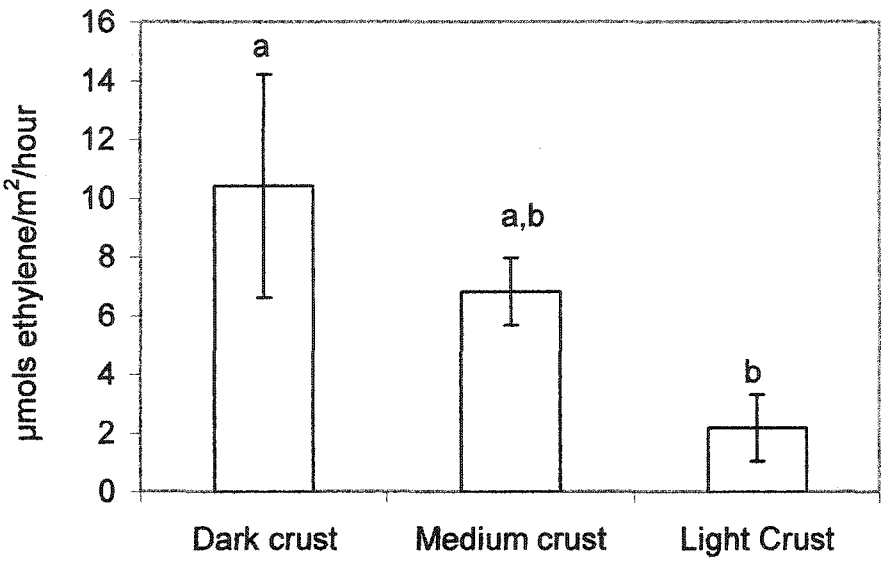

Fig. 1. Nitrogen fixation activity in three biological soil crusts as measured by acetylenereduction. Values are mean ethylene production ± 1 SE (n = 6). When a different letter appears above a bar, means are significantly different at $P < 0.10$.

Appendix II.

Denitrification from biological soil crusts

Introduction

Estimates of denitrification are highly variable in desert ecosystems ranging from 0.4-7 kg N/ha/yr in hot North American deserts (*i.e.* Chihuahuan and Sonoran) (Virginia *et al.* 1992, Peterjohn and Schlesinger 1991, Guilbault and Mathias 1998). In soils from a cold desert site on the Colorado Plateau, denitrification was estimated as high as 19 kg N/ha/yr (West and Skujins 1977). Deserts are heterogeneous environments with hot spots of N loss, where N-fixing organisms may also impact denitrification rates. 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. In this study, I examined denitrification losses ($N_2O + N_2$) from biological soil crusts that varied in N fixation potential.

Methods

In July 2001 I collected 44 cm^2 soil cores to a depth of 5 cm in the deep soil fieldsite (described in chapter II) to measure total denitrification. I collected 6 cores from each of the three biological crust types (*i.e.* dark, medium, and light, see chapter II for criteria of the three types) for a total of 18 cores in each experiment. I added water to the soil cores to bring them up to field moisture. Percent soil moisture for all cores averaged 17% ± 0.9 SE. In the denitrification experiment I placed the cores in 1.79 L glass jars with metal screw top lids. During this experiment, jars were left to incubate at the fieldsite. After 1.5 hrs the jars were sealed and gas samples were collected at 0, 15, and 30 minutes.

After this measurement period, the jars were opened the chamber was left to equlibrate with atmospheric air for 0.5 hrs. I then sealed the jars and injected acetylene (C_2H_2) to a 10% (v/v) atmosphere, which blocks the reduction of N_2O to N_2. I let the cores incubate for one hour in order for the acetylene to diffuse into the soil cores. Gas samples were collected at 60, 75, and 90 minutes after chamber closure. Gas samples were stored in Labco Exetainers and transported to the laboratory. Gas samples were analyzed for N_2O and CO_2 chromatographically using a Shimadzu 14A GC as described by Mosier and Mack (1980).

Immediately after the soil incubation, I collected three 0-5 cm cores within each chamber for inorganic N analysis (NO_3^- and NH_4^+). Samples were immediately transported back to the lab and refrigerated. 25 g of soil were weighed out and extracted with 100 ml of 2 M KCl. Once the salt solution was added to the soil, I placed the samples on a reciprocal shaker for 1 hr. Samples were then filtered with Whatman 4 qualitative grade paper and immediately frozen. Samples were analyzed later on an Alpchem Flow Solution 3 Autoanalyzer.

I also collected soils from within each gas ring for analysis of chlorophyll *a,* total N, and extractable inorganic N immediately after each incubation period. 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. As a result, in this study I use chlorophyll content as an index of C and N fixation potential. I collected 2, 3.14 cm^2 soil cores from the top 0-1 cm of soil. The first core was air-dried in the dark and stored in the freezer until chlorophyll *a* analysis could be performed. After removing samples from the freezer they were ground to a fine

powder with a mortar and pestle. Quantitative and qualitative 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.

Results

N_2O fluxes from in situ field cores were low and with mean fluxes ranging from 0.38-1.67 ng N_2O-N/m^2/s (Fig. 1). There were no significant differences in N_2O fluxes by crust type. Total denitrification (N_2O+N_2) was considerably higher than N_2O alone, with a 15 and 35 fold increase in total denitrification in dark and light crusts respectively (Fig. 1). These data suggest that N_2 losses dominate denitrification loss pathways. Denitrification in light crusts was significantly higher relative to medium and dark crusts ($F_{2,15} = 2.8$, $P = 0.09$). The higher denitrification in light crusts may be partially explained by differences in soil inorganic N pools. Although there were no differences in percent soil N (Table 1), there was an increasing trend in NO_3^- pools in light crusts rtelative to medium and dark crusts (Table 1, $F_{2,15} = 2.45$, $P = 0.12$), which is primarily due to higher NO_3^- concentrations.

Discussion

Denitrification rates were significantly higher in light crusts relative to dark crusts. The higher denitrification rates from light crusts may be partially explained by higher inorganic N pools. I conducted the denitrification experiment during July when temperatures were too high for N fixation (Belnap 2001), whereas maximum net photosynthesis in the lichen *Collema tenax,* occurs during the summer months (Lange *et*

al. 1998). During these times when C fixation peaks in the absence of N fixation, cyanobacteria must take up an external source of N. As a result, during the hotter months biological soil crusts may rely more on soil inorganic N rather than N fixation. Inorganic N uptake should be higher in dark biological crusts, which supports higher cyanobacterial biomass, as opposed to light crusts resulting in a higher soil inorganic N pool from which losses may occur.

Ratios of N_2:N_2O loss in denitrification ranged from 13-35 in the field incubation. It has been suggested that an excess of the oxidant (NO_3^- or NO_2^-) relative to a reductant (primarily organic C) in denitrification results in higher production of intermediates such as N_2O (see review by Firestone and Davidson 1989). Nitrate pools were extremely low and ranged from 0.2-0.5 μg NO_3^- /g soil, which may have resulted in higher ratios of N_2:N_2O loss in denitrification. Parton *et al.* (1996) observed N_2:N_2O ratios in the range of 17-24 at low soil NO_3^- levels, which decreased with increasing NO_3^- concentrations. Firestone and Tiedje (1979) also showed that N_2 was the dominant product in denitrification the first 3 hrs after the onset of anaerobic conditions in the soil. Hence in biological soil crusts that dry down quickly after a rain event, N_2 loss may dominate denitrification losses in these sites.

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Table 1. Total inorganic N from the soil cores in the denitrification experiment. Values are mean inorganic N (NO_3^- and NH_4^+) expressed in ng N/g soil ± 1 SE. I used a one-way ANOVA to evaluate the overall effect of crust type on soil inorganic N. When a different letter appears aboves a bar, treatment differences were significantly different at $P < 0.05$ (Post-hoc Newman-Keuls).

	Chlorophyll *a* µg/g soil	Percent soil N	Extractable NO_3^- ng NO_3^--N/g soil	Extractable NH_4^+ ng NH_4^+-N/g soil
Dark	5.58 (0.70) a	0.026 (0.004) a	208 (80) a	459 (106) a
Medium	3.17 (0.12) b	0.023 (0.004) a	260 (43) a,b	481 (80) a
Light	2.83 (0.38) b	0.020 (0.003) a	548 (180) a	669 (102) a

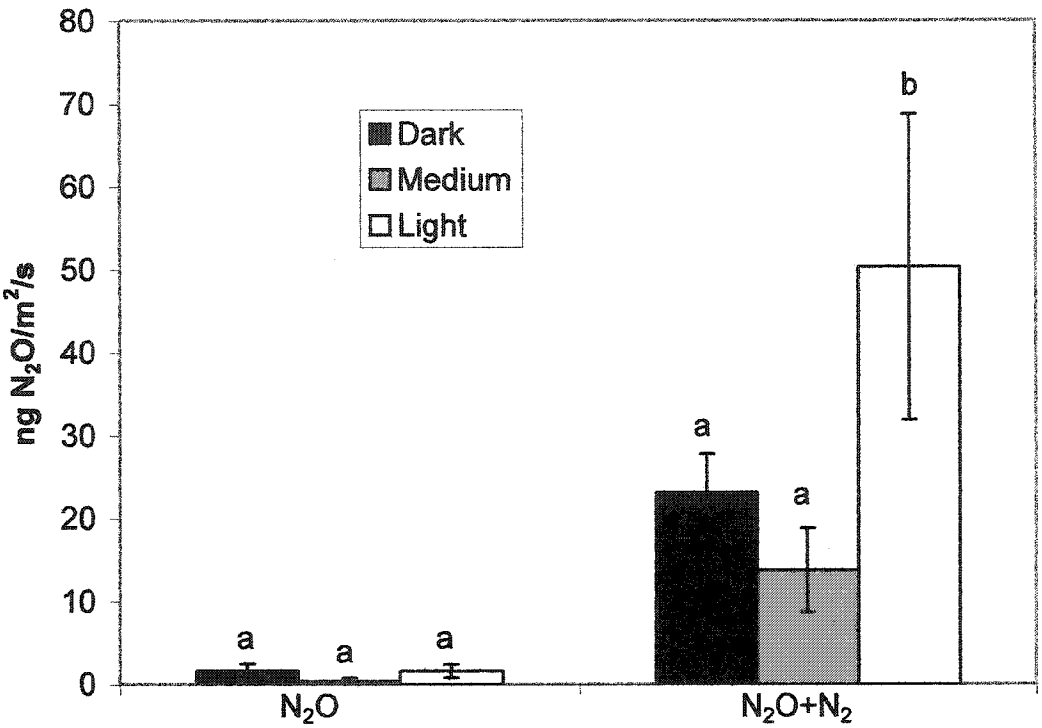

Fig. 1. Denitrification from biological soil crusts with varying N fixation potentials. I incubated soil cores in the field containing each crust type in July 2001. I first measured N_2O only, and then injected acetylene into the chambers to measure total denitrification as N_2O+N_2. Values are mean denitrification fluxes ± 1 SE. I used a one-way ANOVA to evaluate the effect of crust type on denitrification rates. When a different letter appears above a bar, individual treatment differences were significantly different at $P < 0.05$ (Post-hoc Newman-Keuls).

Appendix III.

Ammonia loss from biological soil crusts

Introduction

Ammonia volatilization may also be a major loss pathway for N in desert ecosystems. Desert soils provide optimal conditions for ammonia volatilization, since rates are often positively correlated with soil pH, $CaCO_3$, and total salt content, but negatively correlated with organic matter content, CEC, and clay content (Duan and Hongland 2000). Schlesinger and Peterjohn (1991) reported low rates of NH_3 volatilization in a Chihuahuan desert site with rates ranging from 15-95 $\mu g\ N/m^2/day$, 150-fold lower than denitrification at these same sites. In a Mojave desert site, Billings *et al.* (2002) reported higher NH_3 volatilization rates in the range of 120 $\mu g\ NH_3\text{-}N/m^2/day$. There is evidence that biological soil crusts have higher NH_3 volatilization rates relative to bare soils. In Colorado Plateau soils, Evans and Lange (2001) reported NH_3 losses of 5 $\mu g\ NH_3\text{-}N/m^2/day$ in bare soils, which more than tripled when biological soil crusts were present. In this study I examined NH_3 losses from biological soil crusts that vary in N fixation potential.

Methods

In July 2001 I collected 44 cm^2 soil cores to a depth of 5 cm in the deep soil fieldsite (described in chapter II) to measure ammonia volatilization. I collected 6, field moist 0-5 cm soil cores from dark, medium and light soil crusts (chapter II for criteria of the three types) for a total of 18 cores. I placed the cores in the same glass jars that were used in the denitrification experiment (see appendix II) and transported them back to the

laboratory. A 10ml vial of 5% H_2SO_4 was placed in each jar to trap NH_3 that was volatilized during the incubation period. The jars were immediately sealed and left to stand in the dark for 24 hrs. The samples were transported to the lab and the H_2SO_4 solution was analyzed for NH_4^+ on an Alpchem Autoanalyzer (Perstorp Analytical).

Results

Ammonia volatilization from different crust types was highly variable and ranged from 0.7-3.7 ng NH_3-N/m^2/s (Fig. 8). I observed no differences in NH_3 loss by crust type.

Discussion

The low NH_3 flux rates relative to N_2O and NO may due to the high soil moisture content under which they were incubated. Ammonia dissolves easily in water where soils at high water content may take up NH_3 resulting in enhanced loss rates as soils dry down (Fleisher *et al.* 1987, Rochester *et al.* 1991). In contrast to patterns observed by Evans and Lange (2001), NH_3 volatilization did not increase with increasing N fixation potential of the biological soil crust. Ammonia volatilization rates in this study ranged from 86-345 μg/m^2/day, an order of magnitude higher than those reported for a similar biological soil crust type (Evans and Lange 2001). These rates, however, are in the range of those observed by Billings *et al.* (2002) at a Mojave desert site.

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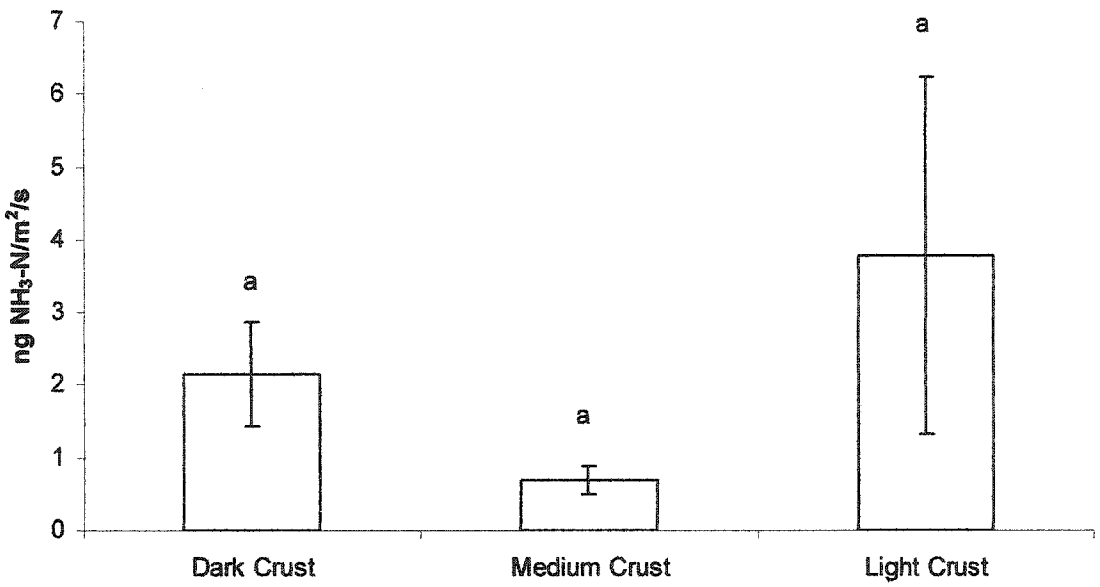

Fig. 1. Field incubation for NH_3 loss from biological soil crust with varying N fixation potentials. Values are mean NH_3 flux in ng $N/m^2/s \pm 1$ SE. There were no significant differences in NH_3 flux by crust type.