Denitrification from nitrogen-fixing biologically crusted soils in a cool desert environment, southeast Utah, USA

Nichole N Barger[1*], Sarah C Castle[2] and Gavin N Dean[1]

Abstract

Introduction: Nitrogen fixation by microorganisms within biological soil crust ("biocrust") communities provides an important pathway for N inputs in cool desert environments where soil nutrients are low and symbiotic N-fixing plants may be rare. Estimates of N fixation in biocrusts often greatly exceed that of N accretion rates leading to uncertainty regarding N loss pathways.

Methods: In this study we examined nitrogen fixation and denitrification rates in biocrust communities that differed in N fixation potential (low N fixation = light cyanobacterial biocrust, high N fixation = dark cyanolichen crust) at four temperature levels (10, 20, 30, 40°C) and four simulated rainfall levels (0.05, 0.2, 0.6, 1 cm rain events) under controlled laboratory conditions.

Results: Acetylene reduction rates (AR, an index of N fixation activity) were over six-fold higher in dark crusts relative to light crusts. Dark biocrusts also exhibited eight-fold higher denitrification rates. There was no consistent effect of temperature on denitrification rates, but there was an interactive effect of water addition and crust type. In light crusts, denitrification rates increased with increasing water addition, whereas the highest denitrification rates in dark crusts were observed at the lowest level of water addition.

Conclusions: These results suggest that there are no clear and consistent environmental controls on short-term denitrification rates in these biologically crusted soils. Taken together, estimates of denitrification from light and dark biocrusts constituted 3 and 4% of N fixation rates, respectively suggesting that losses as denitrification are not significant relative to N inputs via fixation. This estimate is based on a previously published conversion ratio of ethylene produced to N fixed that is low (0.295), resulting in high estimates of N fixation. If future N fixation studies in biologically crusted soils show that these ratios are closer to the theoretical 3:1 ratio, denitrification may constitute a more significant loss pathway relative to N fixed.

Keywords: Biological soil crust, Colorado Plateau, Cool desert, Denitrification, Nitrogen fixation, Soil nitrogen cycling

Introduction

Biological soil crusts ("biocrusts") are diverse communities of cyanobacteria, algae, lichens, mosses, fungi, and other bacteria, which exist in open soil areas not favorable for the growth of higher autotrophs. They comprise up to 70% of the living cover at many sites in arid and semi-arid regions of the western United States (Belnap et al. 2001), contributing to a broad range of ecological functions. One such function of primary importance in dryland ecosystems is the ability of microorganisms within biocrusts to fix atmospheric nitrogen (N) (Mayland and McIntosh 1966; Zaady et al. 1998; Evans and Belnap 1999; Hartley and Schlesinger 2002; Billings et al. 2003; Johnson et al. 2007). Nitrogen fixation by microorganisms within biocrust communities provides an important pathway for N inputs in cool desert environments that are characteristically low in nutrient availability and have a paucity of symbiotic N-fixing plants.

What has been less clear is the fate of the N fixed by biocrust organisms and whether N fixed is retained

* Correspondence: nichole.barger@colorado.edu
[1]Department of Ecology and Evolutionary Biology, University of Colorado, Campus Box 334, Boulder, CO 80309-0334, USA
Full list of author information is available at the end of the article

within the ecosystem. Estimates of N fixation in bio-crusts often greatly exceed that of N accretion rates (Peterjohn and Schlesinger 1990), leading to uncertainty regarding the fate of the fixed N by biologically crusted soils and the potentially important N loss pathways. Soils in dryland ecosystems are characterized by pulse precipitation events resulting in wet and drying cycles. These pulsed dynamics in soils may result in N "leakage" by biological soil crust organisms to the surrounding soil environment in such forms as NH_4^+ and other soluble organic nitrogen compounds (Mayland and McIntosh 1966; Johnson et al. 2007). Nitrogen leakage from biocrusts may not only enhance soil nutrient availability to support plant growth (Mayland and McIntosh 1966; Belnap and Harper 1995 but may also be lost from the system via gaseous N loss in transformations related to nitrification and denitrification processes (Zaady 2005; Barger et al. 2005; Johnson et al. 2007; Strauss et al. 2012; Brankatschk et al. 2013).

Denitrification is the biological process that occurs under reducing conditions where NO_3^- is used by de-nitrifying bacteria (primarily heterotrophic bacteria) in the absence of O_2 as an electron acceptor. NO_3^- is converted to NO, N_2O and N_2 along a reduction pathway. Factors regulating denitrification rates are low O_2 partial pressure, available NO_3^- to serve as an oxidant, and organic C as an energy source for heterotrophic bacteria (Williams et al. 1992). Previously, it was thought that denitrification in dryland ecosystems should be low because anaerobic conditions should rarely occur in arid environments. However, the presence of anaerobic microsites within dryland soils is not as rare as previously believed with oxygen levels reaching near zero in the surface soils (Garcia-Pichel and Belnap 1996; Johnson et al. 2007). A large portion of the microbial community resides in the top few millimeters of dryland soils. Thus a pulse in microbial activity after a rain event may quickly reduce soil oxygen levels (Garcia-Pichel and Belnap 1996; Johnson et al. 2007).

Estimates of denitrification are highly variable in desert ecosystems ranging from 0.4 to 9 kg N ha^{-1} year^{-1} in hot North American deserts (e.g., Chihuahuan and Sonoran) (Virginia et al. 1982; Peterjohn and Schlesinger 1991; Guilbault and Matthias 1998; Schade et al. 2002). In soils from a cold desert site on the Colorado Plateau, denitrification was estimated as high as 19 kg N ha^{-1} year^{-1} (West and Skujins 1977). These highly variable estimates are likely driven by high spatial and temporal variability (e.g., hot spots and hot moments) in denitrification rates. Both biotic and abiotic processes drive patterns in resource distribution in dryland ecosystems resulting in accumulation of nutrients beneath shrub and tree canopies relative to interspace soils in what has been previously described as "islands of fertility" (Noy-Meir 1985; Schlesinger and

Pilmanis 1998). 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. These patterns in denitrification suggest that high N availability associated with N fixation is an important driver of this process.

Past studies of denitrification in biologically crusted soils have yielded a wide range of denitrification rates. Denitrification rates within surface soils (i.e., top 1 cm) containing biocrusts were negligible relative to other N loss pathways (Johnson et al. 2007; Strauss et al. 2012) or increased with biocrust development (Brankatschk et al. 2013). These studies, however, evaluated denitrification from biologically crusted soils at a single temperature and soil moisture level, environmental conditions that may strongly influence not only N fixation but also denitrification rates.

Following this, our objective was to examine the influence of biocrust community on denitrification rates across a range of environmental conditions in the lab to better understand the potential for denitrification from biocrust communities in dryland ecosystems. We hypothesized that denitrification rates would increase with increasing N fixation potential of the biocrust community and that environmental conditions such as temperature and soil moisture would further influence these rates. In this study, we examined nitrogen fixation and denitrification rates in two biocrust communities that differed in their N fixation potential (light cyano-bacterial biocrust and dark cyanolichen crust) at four temperature levels (10, 20, 30, 40°C) and four simulated rainfall levels (0.05, 0.2, 0.6, 1 cm rain events) under controlled laboratory conditions.

Methods

Site description

In the spring of 2009, intact biocrust cores were collected to a 5 cm depth near the Island-in-the-Sky District of Canyonlands National Park, UT (38°33'19.7" N, 109°44'38.8" 1,883 m.a.s.l.). Mean annual precipitation in this area was 231 mm ranging from a low of 179 mm to a high of 327 mm in the 10 years prior to our study (NADP site UT09). Throughout Canyonlands, well-developed biocrust communities often occur on Rizno, dry-Rock outcrop soils, which are classified as loamy, mixed calcareous, mesic Lithic Ustic Torriorthents (Web Soil Survey, http://websoilsurvey.nrcs.usda.gov/app/Home Page.htm). High sand content and low organic C and N characterize these soils.

Sample collection

Visual inspection of the presence of lichen species and coloration in biocrusts is a strong indicator of biocrust community structure and function across the cool desert

regions of the western U.S. (Barger et al. 2005; Belnap et al. 2008). In our study we used visual inspection of biocrust color in the field to evaluate the N fixation potential of the biocrust community. "Light" biologically crusted soils are often dominated by the free-living filamentous cyanobacteria *Microcoleus vaginatus* and *M. steenstruppi* (Gundlapally and Garcia-Pichel 2006) with little to no presence of dark pigmented cyanobacteria (Yeager et al. 2004). In contrast, "dark" biologically crusted soils contain not only *Microcoleus* spp. but also the darker pigmented cyanobacteria such as *Scytonema myochrous* and *Nostoc commune* (Yeager et al. 2004; Gundlapally and Garcia-Pichel 2006). In addition to these cyanobacterial organisms, the N-fixing lichens *Collema tenax* and *C. coccophorum* may also be present on dark biologically crusted soils. Coloration is not only an indicator of microorganism composition but also biocrust function. Chlorophyll a (chl*a*), exopolysaccharide, and soil aggregate stability are strongly and positively correlated with biocrust darkness (Belnap et al. 2008).

In the field, biocrusts were gently covered with a towel and wet by a watering can with distilled water in order to minimize breaking of the crust surface during sampling. We used a 5.3 cm diameter polyvinyl chloride (PVC) cylinder to core the soil and then gently retrieved the core with a spatula and fitted the bottom with a plastic cap. Sampling was done within the soil type described above within a 30 × 30 m area. We collected 64 cores from both light and dark biocrusts for a total of 128 cores. Following collection, soils within the PVC cylinders were allowed to dry immediately in overcast/sunny conditions and transported to the laboratory.

Although we based our field collections on visual assessment of coloration, we further quantified biocrust cover in the lab. Biocrust cover was measured for each 22 cm^2 core using the point-intercept method. A small wire grid with evenly spaced cells was placed on the surface of each core. Cover at each point on the grid was classified using the six different cover classes: light cyanobacteria, dark cyanobacteria, *Collema* lichens, all other lichens, moss, and rock.

To examine the influence of water addition and temperature on nitrogen fixation and denitrification rates, we conducted a full factorial design of biocrust color (dark, light), simulated rain event size (0.05, 0.2, 0.6, 1 cm), and temperature (10, 20, 30, 40°C) in the laboratory. Within the light and dark biocrusts, each core received water addition to simulate rain events of a specific size. The four rain event sizes were chosen to represent the amount of water these biocrust organisms may experience under field conditions. Each of these cores was then incubated at one of the four experimental temperatures. There were four replicates within each level of rain event and incubation temperature.

Acetylene reduction assay

Incubation chambers were constructed of mason jars that were pre-drilled through the glass bottom and plugged with a stopper that had a small section of glass tubing fitted with half-hole septa. When incubation chambers were assembled, crust cores were placed on the jar lid and the jar was turned upside down, allowing cores to be fully exposed to the incubation lights. Biocrusts were wetted with deionized water at the levels described above and placed in incubators in open mason jars for a period of 4 h at the desired temperature. Following the pre-incubation period, jars were plugged with rubber stoppers to form an airtight chamber and a 10% acetylene atmosphere was created in each chamber by first removing a volume of headspace and replacing that same volume with acetylene gas. Acetylene gas was made in the lab by reacting calcium carbide with water and trapping the gas generated from the reaction in an airtight gas sampling bag. After the 4 h pre-incubation, biocrust cores were immediately incubated in the presence of the acetylene atmosphere for an additional 2 h. Following the 2 h incubation, a gas sample was drawn from the chambers using an airtight syringe fitted with a needle and injected into pre-evacuated Exetainers (Labco Limited, UK). Ethylene and N_2O gas were simultaneously analyzed by gas chromatography (Shimadzu, Columbia, MD) equipped with both an FID and an ECD, such that there was a front flush mechanism in order to prevent acetylene exposure to the ECD, and fitted with a 1 cm sample loop.

Soil processing

Following incubation, biocrust cores were cut into cross-sections of the 0–2 and 2–5 cm sections. Total soil mass of each sub-sample was recorded. Each horizon was homogenized and then split for different analyses. A sub-sample of each horizon was extracted immediately with 40 mL of 2M KCl for 1 h on a reciprocal shaker. Soil extracts were filtered (Whatman #1) and were immediately frozen. NH_4^+ concentrations were analyzed using the phenol-hypochlorite reaction described by Weatherburn (1967), and NO_3^- was determined using a method adapted from Doane and Horwáth (2003). Both NH_4^+ and NO_3^- were determined colorimetrically using a microplate reader (Biotek, Winooski, VT). An additional subsample of each horizon was dried at 105°C for gravimetric water content determination. Samples from each horizon were split and oven dried (60°C) for organic carbon [C] and N analysis. Total organic soil C and N measurements were made on a CHN 4010 Elemental Combustion System (Costech Analytical Technologies, Valencia, CA). All C and N analyses were calibrated with an atropine standard. Soil inorganic carbon was determined using the modified pressure transducer method

(Sherrod et al. 2002). A subsample of the 0–2 cm sample was removed for chl*a* analysis and air-dried, sieved, and ground. Chl*a* was extracted with ethanol (Castle et al. 2011) and assessed spectrophotometrically (Beckman DU-64, Beckman Instruments Inc., Fullerton, CA). Soil texture was determined using a modified rapid method procedure described by Kettler et al. (2001), which involved removing carbonates and organic matter using sodium acetate and sodium hypochlorite, respectively.

Statistical analyses

Acetylene reduction (AR) assay data and denitrification rates were log transformed to meet the assumptions of parametric statistical tests. Denitrification rates were highly variable across samples. Thus denitrification rates that were greater than three standard deviations from the mean were removed before analysis. When this cut-off was applied, only one dark biocrust core incubated at 30°C with a 0.5 cm water addition was removed from the analysis. We conducted a three-way analysis of variance (ANOVA) of AR activity, denitrification rates, and inorganic N pools evaluating the two crust types (light, dark), four levels of temperature, and four levels of moisture (IBM SPSS Statistics 20.0).

Results and discussion

Soil characterization

The "light" and "dark" visual characterization of biocrust types when collecting the soil samples in the field delineated clear differences in cyanobacterial composition and N-fixing lichen cover. The light biocrust cores were composed of 95% light cyanobacterial crust cover and only 3% dark crust cover. In contrast the percent dark crust cover in the dark crust cores was 87% with only 5% cover characterized as light crust (Table 1). In addition to darker pigmented cyanobacteria, the N-fixing *Collema* lichens comprised approximately 6% cover in the dark crust cores. Chl*a* content, which is an index of cyanobacterial biomass and N fixation potential of the biocrusts, was nearly two-fold higher in the dark crusts relative to the light crusts (Table 1). Moss cover across both light and dark was low and ranged from 2 to 3% (Table 1).

Nitrogen fixation

Acetylene reduction rates were over six-fold higher in dark biocrusts relative to light biocrusts [mean (± 1 SE) μmol m^2 h^{-1} light = 0.13 (0.05), dark = 0.86 (0.26)]. There was an interactive effect of biocrust type and water additions, in that water additions increased AR rates only in dark biocrusts but had no effect on light biocrusts (Figure 1A, B; crust × water; F = 4.6, P = 0.005). Temperature also influenced AR rates, but again, temperature effects were only observed in dark biocrusts (crust × temperature; F = 4.6, P = 0.005). Within dark

Table 1 Biocrust biological, physical, and chemical characteristics

Soil measurement	Unit	Number	Light	Dark
Chlorophyll a	μg g soil^{-1}	64	3.8 (0.24)	6.5 (0.28)
Crust cover				
Light crust	% cover	64	94.8 (1.0)	4.5 (1.7)
Dark crust	% cover	64	3.3 (0.9)	87.0 (2.6)
Collema	% cover	64	0.1 (0.1)	6.0 (1.6)
Other lichen	% cover	64	0.0 (0.0)	1.1 (0.4)
Rock	% cover	64	0.1 (0.1)	0.1 (0.1)
Moss	% cover	64	1.8 (0.4)	2.5 (0.6)
Soil texture (0–5 cm)				
Sand	% by mass	16	82.3 (0.6)	82.6 (0.6)
Silt	% by mass	16	13.3 (0.8)	13.5 (0.7)
Clay	% by mass	16	4.4 (0.4)	3.9 (0.4)
Chemical characteristics				
Soil organic C 0–2 cm	% by mass	64	0.32 (0.02)	0.41 (0.01)
Soil organic C 2–5 cm	% by mass	64	0.31 (0.01)	0.27 (0.01)
Soil total N 0–2 cm	% by mass	64	0.032 (0.001)	0.041 (0.001)
Soil total N 2–5 cm	% by mass	64	0.028 (0.001)	0.026 (0.001)

Values are mean ± 1 SE.

crusts, optimal temperatures for N fixation were in the range of 10–30°C with a peak at 20°C and declined to near zero at 40°C (Figure 1B).

The higher N fixation rates in dark crust were likely due not only to the presence of the N-fixing *Collema* spp. but also the higher level of N-fixing cyanobacteria as supported by the nearly two-fold greater soil chl*a* in dark biocrust soils relative to light biocrusts (Table 1). Overall, these results are consistent with patterns and controls on N fixation in biologically crusted soils in cool desert environments, confirming that dark biologically crusted soil has higher N fixation potential than light crusts with optimal temperatures for fixation occurring around 20°C (Belnap 2002; Johnson et al. 2007).

Denitrification

Denitrification rates were on average eight-fold higher in dark biocrusts as compared to light biocrusts [mean denitrification (μg N$_2$O-N m^2 day^{-1}): light = 48, dark = 418; Figure 2), which supports our hypothesis that denitrification increases with increasing N fixation potential of the biocrust community. Overall denitrification rates were in the mid range of previously reported estimates for similar light and dark biocrust communities in this region (Johnson et al. 2007; Strauss et al. 2012) but dramatically lower than values reported for early and later successional biocrusts located on a central European inland dune
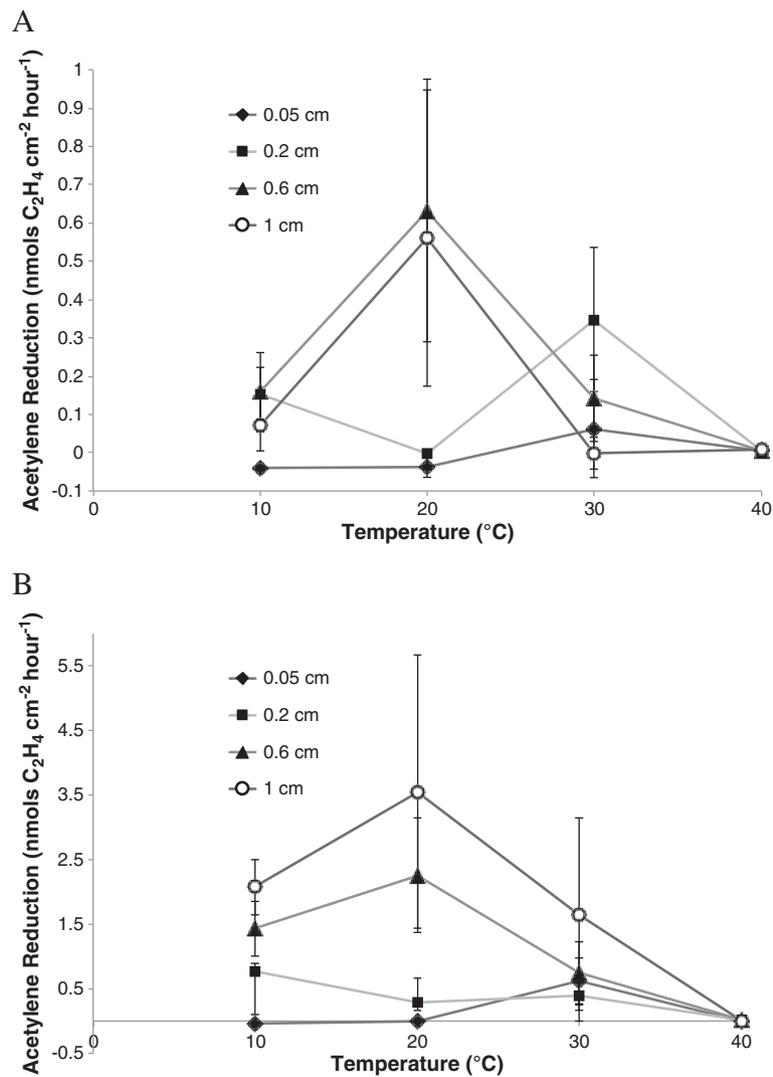
Figure 1 Acetylene reduction assays of biological nitrogen fixation activity in biologically crusted soils incubated under a range of temperature and moisture regimes. Values and means ± 1 SE. (**A**) Light crust and (**B**) dark crust. Legend values indicate water additions to simulate 0.05, 0.2, 0.6, and 1 cm rainfall events.

(Brankatschk et al. 2013; Table 2). The high denitrification rates observed on the central European dune systems may be due to high N deposition rates in the region, which ranged from 12 to 15 kg N ha^2 year^{-1} Elevated soil NO$_3^-$ due to high N deposition rates in these European dune systems may promote high rates of denitrification that would not be observed in biocrusts near Canyonlands National Park, UT, where N deposition rates are much lower (<2 kg N ha^2 year^{-1}; Sullivan et al. 2011).

Although denitrification rates were higher in dark biocrusts that exhibited higher N fixation rates relative to light biocrusts, denitrification rates were much lower than those observed beneath the N-fixing shrub, *P. glandulosa,* in the Sonoran desert. Denitrification rates were two orders of magnitude lower in our

study of N-fixing biocrusts relative to the N-fixing shrub *P. glandulosa* (27,840 μg N m^{-2} day^{-1}; Virginia et al. 1982). Following this, we would predict that denitrification rates will be enhanced by the presence of N-fixing cyanobacteria and lichens in biocrusts, but rates are not on par with those observed in soils beneath symbiotic N-fixing plants.

There was no consistent effect of temperature on denitrification rates, but there was an interactive effect of water addition and biocrust type. In light crusts, denitrification rates increased with increasing water addition, whereas the highest denitrification rates (but also the most variable) in dark crusts were observed at the lowest level of water addition (Figure 2A, B, crust × water, F = 3.93 *P* = 0.001). Previous studies of denitrification
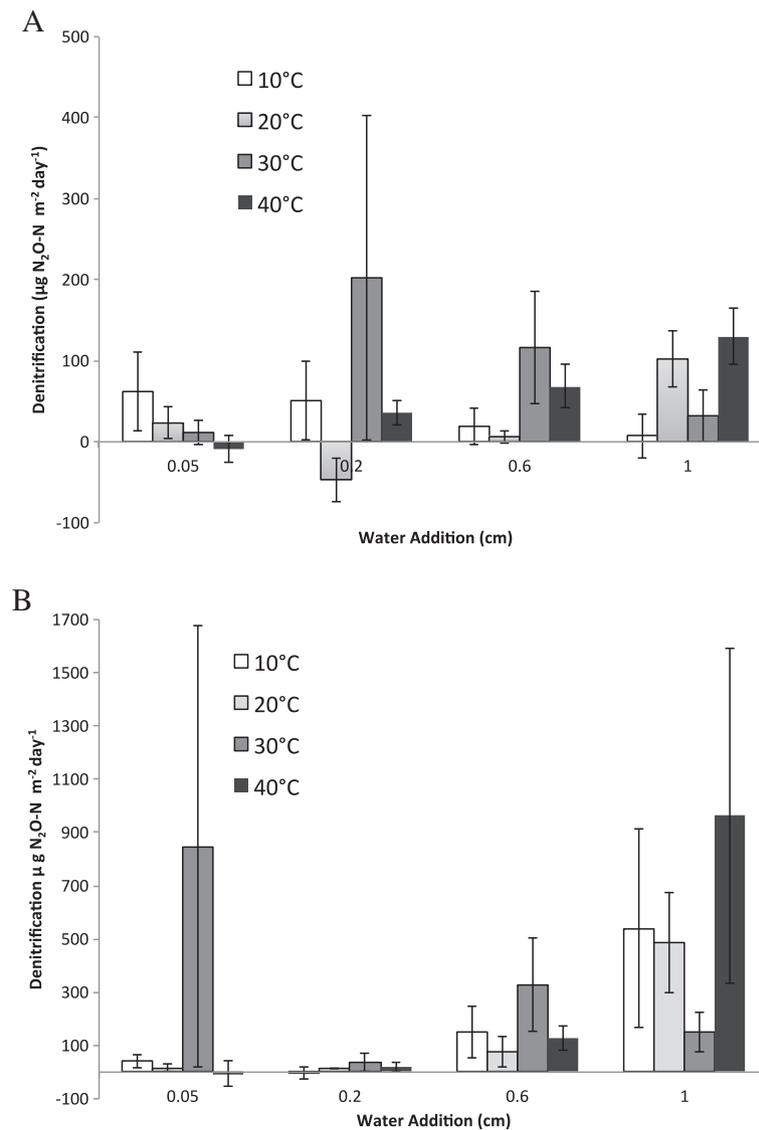
Figure 2 Denitrification rates in biologically crusted soils incubated under a range of temperature and moisture regimes. Values and means ± 1 SE. (**A**) Light crust and (**B**) dark crust. Legend values indicate temperatures at which soils were incubated.

enzyme activity (DEA) in desert soils showed optimal activities at 40°C (Peterjohn 1991). In contrast to these results, some the highest denitrification rates in dark biocrusts in our study occurred at 10 and 20°C after a 1 cm simulated rain event (Figure 2B). Although the amount of water added did influence denitrification rates, it did not do so in a predictable manner. Percent soil moisture ranged from less than 1% in the 0.05 cm rainfall simulation up to 11% in the 1 cm rainfall in the top 0–2 cm of soil (mean % soil moisture; 0.05 cm = 0.05%; 0.2 cm = 3%; 0.6 cm = 7%; 1.0 cm = 12%). These results are in contrast to previous studies that have shown strong differences in denitrification rates with varying soil moisture levels (Groffman and Tiedje

1988). Overall the lack of a consistent response to our temperature and water manipulations suggests that more traditional modeling approaches of denitrification dynamics, which are often based on environmental variables, would fail to adequately describe denitrification rates in biocrust soil systems.

The higher denitrification rates in dark biocrusts may be partially explained by the 22% higher soil organic C (Table 1) in addition to higher inorganic N pools relative to light biocrusts. Extractable soil NO_3^- in dark crusts was nearly two-fold higher than light crusts [Figure 3A, B; mean NO_3^- (mg NO_3^- g soil^{-1}): light = 0.68, dark = 1.24]. Similar to the pattern in denitrification rates, there was an interactive effect of crust type and water addition

Table 2 Estimates of N gas loss from biocrusts

Nitrogen fixation and gas loss from biocrusts	Light crust*	Dark crust*
	Early successional†	Later successional†
	µg N m^{-2} day^{-1}	µg N m^{-2} day^{-1}
Denitrification		
This study*	48	418
(Colorado Plateau)		
Johnson et al. 2007*	5	7
(Colorado Plateau)		
Strauss et al. 2012*	672	Not reported
(Colorado Plateau)		
Brankatschk et al. 2013†	3,360	6,720
(Germany inland dune)		
Nitric oxide		
Barger et al. 2005*	229	335
(Colorado Plateau)		
Ammonia volatilization		
Evans and Johansen 1999	Not reported	15
Barger 2003*	302	173
(Colorado Plateau)		

Values were converted to mean N loss in µg N m^{-2} day^{-1}. A * indicates that the light and dark biocrust characterization was used to characterize biocrust type, whereas a † indicates an early and late successional biocrust.

on soil NO_3^- pools (crust × water; F = 2.70, P = 0.05). Soils were extracted for standing inorganic N pools at the completion of the experiment, thus our reported values may not only reflect the NO_3^- pool remaining at the end of the experiment. In light crusts, patterns in NO_3^- pools were what we would expect, in that treatments that exhibited the highest denitrification rates had the lowest NO_3^- pools at the end of the experiment. Results from soil NO_3^- pools in the dark biocrusts were more mixed, but the 0.2 cm water addition that had exceedingly low denitrification did exhibit the highest extractable NO_3^-.

Extractable soil NH_4^+ was nearly 40% higher in dark crusts relative to light crusts [Figure 3C, D; mean NO_3^- (mg g soil^{-1}): light = 0.66, dark = 1.00]. There was also an interactive effect of temperature and water addition on soil NH_4^+, in which levels were two- to three-fold higher at the 40°C and highest levels of water additions (Figure 3C, D). High extractable NH_4^+ levels at 40°C may be due to increased mineralization at this temperature but also declines in NH_4^+ uptake by biocrusts. Although microorganisms within biocrusts fix N, biocrusts may also exhibit high uptake rates of NH_4^+ when active. Biocrust photosynthetic activity, however, declines dramatically at temperatures at or above 40°C (Lange et al. 1998). This pattern of NH_4^+ release has
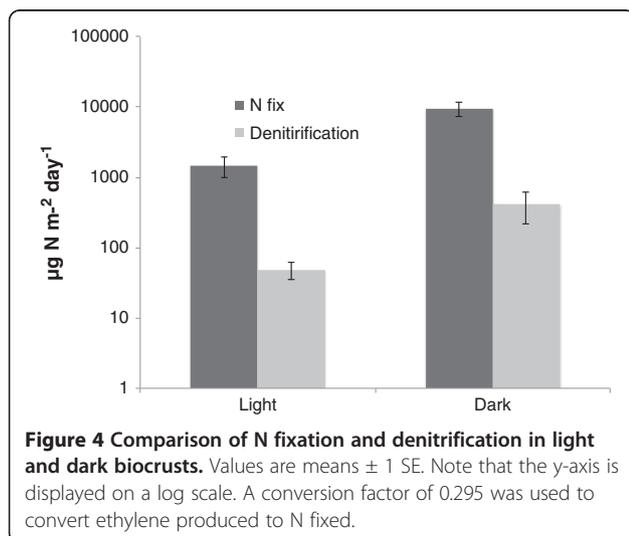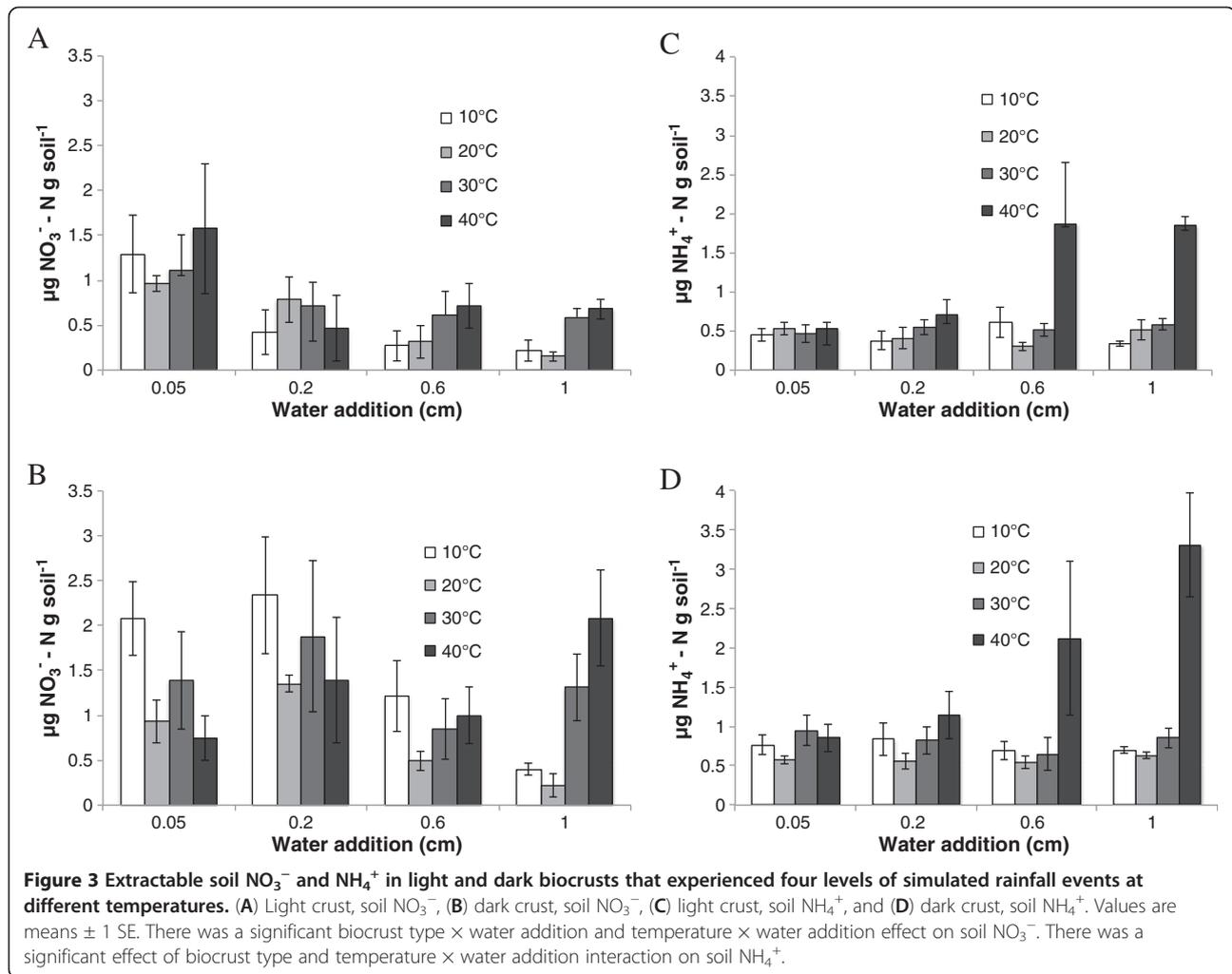
been observed in previous studies, in which NH_4^+ production in soils peaked at approximately 50–60°C (Chantigny et al. 2010). Overall the higher soil organic C in addition to NO_3^- levels in dark biocrusts relative to light biocrusts may support the observed higher denitrification rates in our study.

Coupling N fixation to denitrification

The difficulty in estimating N loss via denitrification relative to N fixation in our experimental approach lies in uncertainties in estimating actual N fixed from AR assays. The theoretical conversion ratio of AR to actual N fixed is 3, based on the biochemistry of the N fixation where six electrons are required for N_2 reduction, whereas only two electrons are required for reduction of C_2H_2. Field and laboratory studies, however, have shown conversion ratios to vary dramatically, ranging from 0.022 to 22 (Rice and Paul 1971; Nohrstedt 1983, 1985; Montoya et al. 1996; Liengen 1999). In order to compare N losses in denitrification in this study to N fixation we used a conversion ratio of 0.295, which was reported specifically for *Nostoc commune* cyanobacterial crusts in high arctic environments (Liengen 1999). These cyanobacterial communities are the most similar to the desert biocrusts evaluated in our study. Based on this, estimates of denitrification constitute 3 and 4% of N fixation rates of light and dark biocrusts, respectively (Figure 4). This proportion of N loss in denitrification is similar to Strauss et al. (2012) in that denitrification was ca. 3% of N fixation rates. In contrast denitrification rates from biocrusts at an inland dune in Germany were five- to ten-fold higher than N fixation rates (Brankatschk et al. 2013). It is important to note, however, that the Brankatschk et al. (2013) study was conducted in an area of high N deposition. In contrast, biocrusts from sites in our study and Strauss et al. (2012) were collected on the Colorado Plateau from areas of low N deposition. In areas of high atmospheric N deposition, we would predict that wet deposition of NO_3^- would elevate soil NO_3^- levels and may increase denitrification rates. Comparisons of denitrification in biocrusts relative to other gaseous N loss pathways in ecosystems with low N deposition rates suggest that denitrification rates are highly variable across studies but are generally within the range of nitric oxide and ammonia volatilization losses reported from previous studies (Table 2).

Conclusions

Our hypothesis that denitrification rates would increase with increasing N fixation potential of biologically crusted soils was supported in this study. Denitrification rates from dark cyanolichen biocrusts were eight-fold higher than light cyanobacterial crusts. In contrast to numerous studies across a broad range of ecosystems,

Figure 3 Extractable soil NO_3^- and NH_4^+ in light and dark biocrusts that experienced four levels of simulated rainfall events at different temperatures. (**A**) Light crust, soil NO_3^-, (**B**) dark crust, soil NO_3^-, (**C**) light crust, soil NH_4^+, and (**D**) dark crust, soil NH_4^+. Values are means ± 1 SE. There was a significant biocrust type × water addition and temperature × water addition effect on soil NO_3^-. There was a significant effect of biocrust type and temperature × water addition interaction on soil NH_4^+.

Figure 4 Comparison of N fixation and denitrification in light and dark biocrusts. Values are means ± 1 SE. Note that the y-axis is displayed on a log scale. A conversion factor of 0.295 was used to convert ethylene produced to N fixed.

soil moisture and temperature had inconsistent and mixed effects on denitrification rates. Taken together, estimates of denitrification from light and dark biocrusts in this lab experiment constituted 3 and 4% of N fixation rates, respectively, indicating that denitrification rates are unlikely to be significant relevant to N inputs via fixation.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
N.B. provided financial support for the experiment, designed the experiment, collected the field samples, analyzed the data, and wrote the Introduction, Results and Discussion section. S.C. collaborated with NB in experimental design and field sample collection, conducted all the laboratory analyses, assisted in organizing and analyzing the data, wrote the initial draft of the Methods section, and edited the final drafts. G.D. researched a range of appropriate statistical analyses for the data, assisted in writing the Introduction, compiled all the references, and edited the manuscript. All authors read and approved the final manuscript.

Author details
[1]Department of Ecology and Evolutionary Biology, University of Colorado, Campus Box 334, Boulder, CO 80309-0334, USA. [2]Department of Ecosystem and Conservation Sciences, College of Forestry and Conservation, University of Montana, Missoula, MT 59812, USA.

References
Barger NN (2003) Biogeochemical cycling and N dynamics of biological soil crusts in a semi-arid ecosystem. PhD Dissertation, Colorado State University, Ft. Collins

Barger NN, Belnap J, Ojima DS, Mosier A (2005) NO gas loss from biologically crusted soils in Canyonlands National Park, Utah. Biogeochemistry 75:373–391

Belnap J (2002) Nitrogen fixation in biological soil crusts from southeast Utah, USA. Biol Fertil Soils 35:128–135

Belnap J, Harper K (1995) Influence of cryptobiotic soil crusts on elemental content of tissue of two desert seed plants. Arid Soil Res Rehabil 9:107–115

Belnap J, Rosentreter R, Leonard S, Kaltenecker JH, Williams J, Eldridge D (2001) Biological soil crusts: ecology and management. US Department of the Interior Technical Reference 1730–2, US Dept of the Interior, Denver

Belnap J, Phillips SL, Witwicki DL, Miller ME (2008) Visually assessing the level of development and soil surface stability of cyanobacterially dominated biological soil crusts. J Arid Environ 72:1257–1264

Billings SA, Schaeffer SM, Evans RD (2003) Nitrogen fixation by biological soil crusts and heterotrophic bacteria in an intact Mojave Desert ecosystem with elevated CO_2 and added soil carbon. Soil Biol Biochem 35:643–649

Brankatschk R, Fischer T, Veste M, Zeyer J (2013) Succession of N cycling processes in biological soil crusts on a Central European inland dune. FEMS Microbiol Ecol 83:149–160

Castle SC, Morrison CD, Barger NN (2011) Extraction of chlorophyll a from biological soil crusts: a comparison of solvents for spectrophotometric determination. Soil Biol Biochem 43:853–856

Chantigny MH, Curtin D, Beare MH, Greenfield LG (2010) Influence of temperature on water-extractable organic matter and ammonium production in mineral soils. Soil Sci Soc Am J 74:517–524

Doane TA, Horwáth WR (2003) Spectrophotometric determination of nitrate with a single reagent. Anal Lett 36:2713–2722

Evans RD, Belnap J (1999) Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. Ecology 80:150–160

Evans RD, Johansen JR (1999) Microbiotic crusts and ecosystem processes. Crit Rev Plant Sci 18:183–225

Garcia-Pichel F, Belnap J (1996) Microenvironments and microscale productivity of cyanobacterial desert crusts. J Phycol 32:774–782

Groffman PM, Tiedje JM (1988) Denitrification hysteresis during wetting and drying cycles in soil. Soil Sci Soc Am J 52:1626–1629

Guilbault MR, Matthias AD (1998) Emissions of N_2O from Sonoran Desert and effluent-irrigated grass ecosytems. J Arid Environ 38:87–98

Gundlapally SR, Garcia-Pichel F (2006) The community and phylogenetic diversity of biological soil crusts in the Colorado Plateau studied by molecular fingerprinting and intensive cultivation. Microb Ecol 52:345–357

Hartley A, Schlesinger W (2002) Potential environmental controls on nitrogenase activity in biological crusts of the northern Chihuahuan Desert. J Arid Environ 52:293–304

Johnson SL, Neuer S, Garcia-Pichel F (2007) Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. Environ Microbiol 9:680–689

Kettler TA, Doran JW, Gilbert TL (2001) Simplified method for soil particle-size determination to accompany soil-quality analyses. Soil Sci Soc Am J 65:849–852

Lange OL, Belnap J, Reichenberger H (1998) Photosynthesis of the cyanobacterial soil-crust lichen *Collema tenax* from arid lands in southern Utah, USA: role of water content on light and temperature responses of CO_2 exchange. Funct Ecol 12:195–202

Liengen T (1999) Conversion factor between acetylene reduction and nitrogen fixation in free-living cyanobacteria from high Arctic habitats. Can J Microbiol 45:223–229

Mayland HF, McIntosh TH (1966) Availability of biologically fixed atmospheric nitrogen-15 to higher plants. Nature 209:421–422

Montoya JP, Voss M, Kahler P, Capone DG (1996) A simple, high-precision, high-sensitivity tracer assay for N_2 fixation. Appl Environ Microbiol 62:986–993

Nohrstedt H (1983) Conversion factor between acetylene reduction and nitrogen fixation in soil: effect of water content and nitrogenase activity. Soil Biol Biochem 15:275–279

Nohrstedt H (1985) Nonsymbiotic nitrogen fixation in the topsoil of some forest stands in central Sweden. Can J For Res 15:715–722

Noy-Meir I (1985) Desert ecosystem structure and function. In: Evenari M (ed) Hot deserts and arid shrublands. Elsevier Science, Amsterdam

Peterjohn W (1991) Denitrification: enzyme content and activity in desert soils. Soil Biol Biochem 23:845–855

Peterjohn WT, Schlesinger WH (1990) Nitrogen loss from deserts in the southwestern United States. Biogeochemistry 10:67–79

Peterjohn WT, Schlesinger WH (1991) Factors controlling denitrification in a Chihuahuan desert ecosystem. Soil Sci Soc Am J 55:1694–1701

Rice WA, Paul EA (1971) The acetylene reduction assay for measuring nitrogen fixation in water-logged soil. Can J Microbiol 17:1049–1056

Schade JD, Marti E, Welter JR, Fisher SG, Grimm NB (2002) Sources of nitrogen to the riparian zone of a desert stream: implications for riparian vegetation and nitrogen retention. Ecosystems 5:68–79

Schlesinger WH, Pilmanis AM (1998) Plant-soil interactions in deserts. Biogeochemistry 42:169–187

Sherrod LA, Dunn G, Peterson GA, Kolberg RL (2002) Inorganic carbon analysis by modified pressure-calcimeter method. Soil Sci Soc Am J 66:299–305

Strauss SL, Day TA, Garcia-Pichel F (2012) Nitrogen cycling in desert biological soil crusts across biogeographic regions in the southwestern United States. Biogeochemistry 108:171–182

Sullivan TJ, McDonnell TC, McPherson GT, Mackey SD, Moore D (2011) Evaluation of the sensitivity of inventory and monitoring of National Parks to nutrient enrichment effects from atmospheric nitrogen deposition. Nat Resour Rep NPS/NRPC/ARD/NRR 2011/321, US Dept of the Interior, Denver

Virginia RA, Jarrell WM, Franco-Vizcaino E (1982) Direct measurement of denitrification in a *Prosopis* (Mesquite) dominated Sonoran Desert ecosystem. Oecologia 53:120–122

Weatherburn MW (1967) Phenol-hypochlorite reaction for determination of ammonia. Analytical Chem 39:971–974

West NE, Skujins JJ (1977) The nitrogen cycle in North American cold-winter semi-desert ecosystems. Oecol Plant 12:45–53

Williams EJ, Hutchinson GL, Fehsenfeld FC (1992) NO_x and N_2O emissions from soil. Glob Biogeochem Cycles 6:351–388

Yeager CM, Kornosky JL, Housman DC, Grote EE, Belnap J, Kuske CR (2004) Diazotrophic community structure and function in two successional stages of biological soil crusts from the Colorado Plateau and Chihuahuan Desert. Appl Environ Microbiol 70:973–983

Zaady E (2005) Seasonal change and nitrogen cycling in a patchy Negev Desert: a review. Arid Land Res Manage 19:111–124

Zaady E, Groffman PM, Shachak M (1998) Nitrogen fixation in macro- and microphytic patches in the Negev desert. Soil Biol Biochem 30:449–454