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Nitrogen cycling in desert biological soil crusts across biogeographic regions in the Southwestern United States

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Abstract Biological soil crusts (BSCs) are thought to be important in the fertility of arid lands as gateways for carbon (C) and nitrogen (N). Studies on the Colorado Plateau have shown that an incomplete internal N cycle operates in BSCs that results in significant exports of dissolved organic N, ammonia and nitrate into the bulk soil through percolating water, thus mechanistically explaining their role as a N gateway. It is not known if this pattern is found in other arid regions. To examine this, we measured rates of major biogeochemical N-transformations in a variety of BSCs collected from the Colorado Plateau and the Mojave, Sonoran and Chihuahuan Deserts. Dinitrogen fixation and aerobic ammonia oxidation were prominent transformations at all sites. We found anaerobic ammonia oxidation (anammox) rates to be below the detection limit in all cases, and at least 50-fold smaller than rates of N_2-fixation, making it an irrelevant process for these BSCs. Heterotrophic denitrification was also of little consequence for the flow of N, with rates at least an order of magnitude smaller than those of N_2-fixation. Thus we could confirm that despite the demonstrable differences in microbial community composition and soil material, BSCs from major biogeographic regions in arid North America displayed a remarkably consistent pattern of internal N cycling. The implications for arid land fertility drawn from previous studies in the Colorado Plateau appear applicable to BSCs across other arid regions of the Southwestern United States.

Keywords Biological soil crust · Desert · Nitrogen · Bacteria · Denitrification · Ammonia-oxidation · Dinitrogen-fixation

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Introduction

Next to water, nitrogen (N) is often regarded as the factor most limiting to productivity in arid terrestrial ecosystems of the Southwestern USA (Peterjohn and Schlesinger 1990; Hooper and Johnson 1999). Nitrogen enters these desert ecosystems mostly via biological dinitrogen (N_2) fixation (Belnap 2002) because atmospheric deposition is typically low (Peterjohn and Schlesinger 1990). Organosedimentary assemblages of microorganisms that develop in the uppermost centimeters of soil and that are driven by primary production of edaphic microbial phototrophs are hotspots for N_2-fixation in arid lands (Evans and Johansen 1999). These assemblages, known as biological soil crusts (BSCs), are not only important in

sustaining soil fertility (Harper and Belnap 2001), but can also stabilize soils, reducing erosion (Belnap and Gillette 1998), improve moisture retention (Aguilar et al. 2009), and lixiviate a variety of elements (Beraldi-Campesi et al. 2009). Microbial activity in these communities is restricted to typically brief periods when precipitation or dew hydrates microbial cells allowing metabolic activity of the otherwise dormant biota (Garcia-Pichel and Belnap 1996).

Instantaneous rates of carbon (C; Garcia-Pichel and Belnap 1996; Evans and Lange 2001; Jeffries et al. 1993) and nitrogen (Johnson et al. 2005; Abed et al. 2010) fixation in BSCs during periods of microbial activity are high enough to expect noticeable long-term increases in BSC soil C and N content. However, biomass does not seem to accumulate in a commensurate manner in BSCs during succession to maturity, and loss processes are deemed to be important (Garcia-Pichel and Belnap 1996). The sustained and widespread presence of N_2-fixing primary producers in BSCs, such as heterocystous cyanobacteria (Yeager et al. 2007), indicates a state of secular N-limitation (Johnson et al. 2007). Potential processes that would result in loss of N include: (i) denitrification to N_2, which would require prior oxidation of reduced N to provide the necessary nitrate (NO_3^-), (ii) loss of nitric oxide (NO) or nitrous oxide (N_2O) during nitrification or denitrification, (iii) volatilization of ammonia (NH_3) and, (iv) leaching of dissolved N-containing species with runoff or percolation. Processes (i)–(iii) represent losses to the atmosphere that, if relevant, would partly or completely nullify the role of BSCs as gateways of N to the ecosystem beyond their own small-scale boundaries. In BSCs of the Colorado Plateau, which have been the subject of most investigations in this respect, rates of aerobic ammonia-oxidation (AAO), the first step in nitrification, were found to be similar to those of N_2-fixation (Johnson et al. 2005), effectively providing a pool of oxidized N that could potentially enable denitrification within BSCs. Surprisingly, however, BSC denitrification rates were negligible in spite of the availability of NO_3^-, organic C, and internal anaerobic conditions in the soil solution (Johnson et al. 2007). The paradoxical lack of denitrification was confirmed by the virtual absence of culturable (most probable number) denitrifying bacteria (Johnson et al. 2007). Other potential gaseous losses in BSCs from the Colorado Plateau were also proportionally small, including fluxes of NO and NH_3 (Barger et al. 2005). Thus, the internal N cycle of BSCs, based on these Colorado Plateau experiments, seems to be active but incomplete with BSCs effectively exporting excess dissolved N into the bulk soil with percolating water (Johnson et al. 2007).

In an attempt to address the potential geographical or climatic variability of this unusual mode of C and N cycling, we present a set of biogeochemical rate determinations, including net photosynthesis, dark respiration, N_2-fixation, aerobic ammonium-oxidation, and denitrification on a variety of BSCs collected in the Colorado Plateau region, and the Mojave, Sonoran and Chihuahuan Deserts. Additionally, we assessed rates of anaerobic ammonia oxidation (anammox; van de Graaf et al. 1995) in BSCs. Anammox is a potential mechanism of N release to the atmosphere that has not been previously measured in BSCs.

Methods

Soil sampling and preparation

Biological soil crusts were collected from two sites within the Sonoran Desert, two sites within the Chihuahuan Desert, four sites within the Mojave Desert and one site in the Colorado Plateau (Table 1). Sampling, soil preparation, and preservation followed the methods described by Soule et al. (2009). Prior to collection, soils were lightly wetted with sterilized ultra-pure laboratory grade (Milli-Q) water using a spray bottle. A thin metal frame (19.5 × 16 × 5 cm) was inserted into the BSC and underlying soil and gently removed to allow the sample to be extracted intact. Samples were then air-dried and sealed in plastic bags and transported to Arizona State University. Drying times were between 1 and 8 h, and were unlikely to artificially alter the composition of the microbial communities (Soule et al. 2009). BSCs were collected from the Colorado Plateau in September of 2007, Sonoran and Chihuahuan Deserts in February of 2008, and from the Mojave Desert in March of 2008 and all were stored in the dark at 20°C until measurements were made. For all measurements, sub-samples were collected intact using the bottom of a 15 mm Petri plate to excise the top one cm of soil.

Table 1 Site denomination, locations and characteristics of the BSCs and soil under the BSCs including total N (*TN*), total C (*TC*), total organic C (*TOC*), and total inorganic C (*TIC*) concentrations and pH

Location	Site	Location	Sample type	TN (%)	TC (%)	TOC (%)	TIC (%)	pH
Mojave desert	Cactus Plain	34°07′46.6″N	BSC	0.02	0.34	0.15	0.19	7.7
		114°14′28.5″W	Under BSC	0.01	0.26	0.07	0.19	
	Hayden	35°02′01.3″N	BSC	0.07	0.73	0.66	0.07	7.7
		115°34′30.2″W	Under BSC	0.02	0.21	0.19	0.02	
	Soda Lake	35°15′11.4″N	BSC	0.01	1.53	0.12	1.40	8.1
		115°58′38″W	Under BSC	0.01	0.38	0.09	0.29	
	Searles Lake	35°38′21.3″N	BSC	0.04	1.20	0.60	0.60	8.1
		117°22′30.4″W	Under BSC	0.01	0.90	0.24	0.66	
Chihuahuan desert	Willcox Playa	32°07′07.5″N	BSC	0.13	1.56	1.14	0.42	7.8
		109°54′04.6″W	Under BSC	0.08	1.02	0.71	0.31	
	Jornada LTER	32°31′58.9″N	BSC	0.07	1.41	0.64	0.77	7.7
		106°43′41.5″W	Under BSc	0.05	1.26	0.44	0.82	
Sonoran desert	Chandler	33°15′21.8″N	BSC	0.09	0.76	0.64	0.11	7.7
		111°56′29.5″W	Under BSC	0.03	0.26	0.22	0.04	
	Yuma	32°45′6.4″N	BSC	0.06	1.73	0.82	0.91	8.6
		113°39′4.1″W	Under BSC	0.03	1.22	0.50	0.72	
Colorado plateau	Green Butte	38°43′6.4″N	BSC	0.01	0.68	0.29	0.40	8.0
		109°41′15.7″W	Under BSC	0.01	0.67	0.16	0.51	

For N_2-fixation, ammonia-oxidation, denitrification, anammox, net photosynthetic and dark respiration rates, three independent samples from each site were measured. A single sample per site was used for the measurements of soil characteristics, including microbial diversity and richness.

Soil geochemical parameters

Soil geochemical parameters (total organic C, total inorganic C, and total N) for each sample were determined commercially at the Environmental Research Laboratory, University of Arizona, Tucson. For total inorganic C and N measurements, air-dried soil samples were ground in a ball mill and analyzed on a nitrogen/carbon/sulfur analyzer (NA 1500 Carlo Erba Instruments). For total organic C measurements, ground air-dried soil samples were treated with phosphoric acid before being analyzed using the same N/C/S analyzer. For these analyses, we distinguished BSC proper from the soil upon which they developed. The latter gives a better assessment of the geological/pedological character of the bulk soil, whereas the former may integrate characteristics of both the soil and the microbial community.

Soil solution pH was measured in double-dionized water with a 1:2 volume ratio of BSC: double-dionized water. The slurry was mixed and allowed to equilibrate for 24 h. The pH was measured using a benchtop pH meter (VWR Symphony Model SP70P) after a pH 7 standard was measured to verify calibration.

Acetylene reduction assay for potential N_2 fixation rates

Potential N_2-fixation rates were determined according to the methods described by Belnap (2002) and modified by Johnson et al. (2005) to avoid diffusion limitations. BSC sub-samples were placed in clear plastic tubes closed with rubber stoppers at both ends and preconditioned by leaving the samples wet and in approximately 350 μmol m^{-2} s^{-1} PAR at 20°C for 2 h before incubation. The samples were then incubated with acetylene in the light for 8 h at 20°C. Headspace gas samples were taken at 4 and 8 h for ethylene analyses. Ethylene was measured using a gas chromatograph (Hewlett-Packard 5890 Series II) equipped with a thermal ionization detector and a 6′ × 1/8″ SS Porapak N 80/100 column (Ohio Valley

Specialty Chemical). The nitrogenase enzyme may not catalyze the reduction of acetylene to ethylene as efficiently as it does N_2; therefore conversion factors are used to determine N_2-fixation rates when using the acetylene reduction method (Hardy et al. 1973). These factors range from 1 to 4.9 in cultures (Hardy et al. 1973) and between 1 and 3 in soils (Nohrstedt 1983), and it is unclear which is the most accurate or applicable in each case (Belnap 2002). We used a conversion factor of 1 to obtain the most conservative rate of N_2-fixation. Areal rates of N_2-fixation were obtained by dividing total production by the original surface area of the sample.

Ammonium oxidation rates

Potential (ammonium-amended) and actual (unamended) aerobic ammonium oxidation (AAO) rates were determined according to the methods described by Johnson et al. (2005). For the potential AAO measurements, BSC sub-samples were slurried with 75 mM sodium chlorate ($NaClO_3$; an inhibitor of nitrite reduction) and 1 mM ammonium sulfate ($(NH_4)SO_4$). These samples were incubated for 10 h in the dark on a shaker at 20°C. Aliquots of 2.5 ml were removed every 2 h, mixed with 500 ml KCl and frozen until analysis. Once thawed, samples were centrifuged at 13,000 × *g* for 10 min to pellet debris. The supernatant was incubated at 20°C for 15 min with 60 mM ammonium chloride (NH_4Cl; pH 8.5) and a color reagent for nitrite (NO_2^-) determination. The absorption at 520 nm was compared to a set of standards. Areal rates were obtained by dividing total production by the original surface area of the sample. Actual AAO rates were determined preserving the community architecture. Intact BSC sub-samples were placed in double chamber Petri dishes previously designed and described by Johnson et al. (2005) and were incubated under 350 μmol m^2 s^{-1} PAR with 20 ml of 75 mM $NaClO_3$ for 24 h at 20°C. Aliquots of the pore water were collected at 0 and 24 h and treated and analyzed in the same manner as those collected for potential AAO. Areal rates were obtained by dividing total production by the original surface area of the sample.

Anaerobic ammonium oxidation (anammox) and nitrate denitrification rates were measured using a series of tracer incubations to determine the extent of N_2 resulting from each process (Thamdrup and Dalsgaard 2002). Anammox organisms couple NO_3^- reduction to NH_4^+ oxidation, whereby N_2 is formed with one N from NO_3^- and one from NH_4^+ (van de Graaf et al. 1995). This combination allows the use of ^{15}N tracer incubations to distinguish the N_2 produced by anammox from that produced by denitrification. Three different combinations were used: (i) $Na^{15}NO_3^- + {}^{14}NH_4Cl$, which would result in $^{29}N_2$ through anammox or $^{30}N_2$ through denitrification, (ii) $Na^{14}NO_3^- + {}^{15}NH_4Cl$, which would result in $^{29}N_2$ through anammox and $^{28}N_2$ through denitrification, and (iii) $Na^{15}NO_3^- + {}^{15}NH_4Cl$, which would result in doubly labeled N_2 for both anammox and denitrification.

BSC sub-samples from each location were homogenized and divided into three 12-ml Labco Exetainer (Labco Limited) vials, each with a total volume of 1.46 cm^3. Vials were purged with helium (He) for 10 min. Solutions in combinations of labeled and unlabeled 100 mM $NaNO_3^-$ and NH_4Cl as listed above were flushed with He before being injected into the vials (2 μl of each solution; natural abundance or $^{15}NH_4Cl$ (98 atm%), $Na^{15}NO^3$ (98 atm%; Isotec). Headspace $^{29}N_2$ and $^{30}N_2$ concentrations were determined every 12 h using a mass spectrometer (Thermo Finnigan Delta V Advantage) equipped with a gas bench and an autosampler (CTC Combi PAL). Using a $^{29}N_2$ and $^{30}N_2$ calibration curve, the amount of $^{29}N_2$ and $^{30}N_2$ produced in excess of natural abundance was determined using the method of Thamdrup and Dalsgaard (2002). Areal rates were obtained by dividing total production by the original surface area of the sample. A sample collected from the anoxic portion of a Winogradsky column served as a positive control and had an unamended denitrification rate of 0.20 μmol m^{-2} h^{-1} and an anammox rate of 0.10 μmol m^{-2} h^{-1}.

Acetylene inhibition assay for potential denitrification rates

Potential denitrification rates were determined according to the methods described by Groffmann and Tiedje (1989). BSC sub-samples of approximately 50 g were placed in 125 ml glass bottles with 50 ml of sterile denitrification media (per liter: 100 mg of KNO_3^- and 100 mg of dextrose). Each bottle was purged with N_2 gas to attain anoxia, after which 10 ml of acetylene was injected through the

septum in each lid. Gas samples were drawn at 10 min and 4 h following the addition of acetylene using 2 ml purged and capped scintillation vials and double-ended needles. Nitrous oxide (N_2O) concentration was quantified using a gas chromatograph (Shimadzu GC-14A) equipped with an electron capture detector and a $6' \times 1/8''$ SS Poropak Q 50/80 (Ohio Valley Specialty Chemical). The positive control was a sediment sample from a retention basin from the Arizona State University campus with a measured denitrification rate of 7.21 μmol N m^{-2} h^{-1}. Areal rates were obtained by dividing total production by the original surface area of the sample.

Net Photosynthesis and dark respiration measurements

Rates of net photosynthesis and respiration of wetted, intact BSC samples were determined using an open infra-red gas analyzer (IRGA) system (Li-COR Li-6400) equipped with a gas chamber made of a transparent acrylic tube (inner diameter 10.2 cm, height 12 cm) lined inside with Teflon tape. Samples were preconditioned by watering the samples until moist and incubating them under 350 μmol m^2 s^{-1} PAR for 1 h before measurement, equivalent to the light intensity during a rain event. Samples were placed on an acrylic base lined with Teflon tape and covered with the chamber. The base and chamber were sealed airtight with putty. Net photosynthetic rates were measured for 15 min after the gas chamber was sealed and dark respiration rate measurements followed for 5 min after covering the chamber with a black cloth. All measurements had an incoming air CO_2 concentration of 370 μl l^{-1} supplied by a CO_2 injector system (Li-COR 6400-01), incoming air-flow maintained at 200 μmol s^{-1} and relative humidity maintained at 80%. Net photosynthetic rates were measured as the net uptake of CO_2 by the crust (presented as positive values), while dark respiration rates were measured as a net release of CO_2 from the crust (presented as negative values). All measurements were made at 20°C.

Isolation of DNA

DNA was isolated from approximately 1 g of air-dried BSC from each site with the commercial UltraClean Soil DNA isolation kit (MoBio Laboratories). An additional three freeze–thaw cycles using liquid N (1 min each) and a 65°C water bath (15 min each) were performed before isolating the DNA according to the manufacturer's protocol. The isolated DNA was checked for quality by standard agarose gel electrophoresis followed by ethidium bromide staining. The concentration of DNA in the extracts was quantified from the agarose gels using a Fluor-S Multi-Imager system (BioRad Laboratories) with an EZ Load Precision Molecular Mass Standard (BioRad Laboratories) as a standard.

PCR Amplification of 16S rRNA gene

To obtain a bacterial community fingerprint, universal eubacteria primers GM5f(GC) and 907r (Muyzer et al. 1995) were used for amplification of ca. 590-bp-long 16S rRNA gene fragments from the community DNA. Each 50 μl PCR reaction mix contained 5 μl Takara ExTaq PCR buffer, 4 μl Takara dNTP mixture (2.5 mM each), 50 pmol of each primer (synthesized by Operon Biotechnologies), 5–10 ng template DNA, and 1 unit Takara ExTaq DNA polymerase (Takara Bio) and sterile Milli-Q water. The PCR reaction performed was modified from the procedure described by Garcia-Pichel et al. (2003). The first 20 cycles involved a touchdown method with a decrease in annealing temperature from 65 to 55°C with 1°C per cycle. Each of these 20 cycles included 1 min at 94°C (denaturation), 1 min at 65°C (annealing) and 2 min at 72°C (extension). These 20 cycles were followed by an additional 6 cycles of 1 min at 94°C (denaturation), 1 min at 55°C (annealing) and 2 min at 72°C (extension) and a final extension at 72°C of 6 min. All reactions were done in a thermocycler (BioRad iCycler).

To obtain a cyanobacterial community fingerprint, universal cyanobacteria primers CYA106f(GC) and CYA781r (Nübel et al. 1999) were used for amplification of ca. 600-bp-long 16S rRNA gene fragments from the community DNA. Each 50 μl PCR reaction mix contained the same mixture as used for the universal eubacteria primers (see above). The PCR reaction was performed according to the program described by Garcia-Pichel et al. (2002). An initial denaturation at 94°C for 5 min was followed by 30 cycles of 1 min each at 94°C (denaturation), 60°C

(annealing), and 72°C (extension), with a final extension at 72°C for 9 min.

All primers were obtained commercially (Operon Biotechnologies, Huntsville, AL, USA). All PCR products were checked for quality on 1% agarose gels. Quantification of all PCR products was performed as described above for DNA quantification.

Denaturing gradient gel electrophoresis

Each heterogeneous mixture of PCR-amplified 16S rRNA gene alleles was separated by denaturing gradient gel electrophoresis (DGGE) using a 30–60% chemical gradient of denaturants (urea and formamide) on a 6% polyacrylamide gel following the methods described by Gundlapally and Garcia-Pichel (2006). Approximately 200 ng of PCR product from each site was loaded in a lane of the DGGE gel and electrophoresed for 6 h at 150 V in a D-code Universal Mutation Detection System (BioRad Laboratories). Standard lanes, containing known mixtures of three 16S rRNA alleles from cultivated isolates, were run to ensure correct gel properties. The gels were stained for 30 min with 3 µl of SYBR Gold Nucleic Acid Gel Stain (Invitrogen) in 15 ml of TAE buffer and de-stained in TAE buffer for 5 min. The gels were visualized under a Fluor-S Multi-Imager (BioRad Laboratories). The most visible bands were excised with sterile scalpels and allowed to elute for 3 days at 4°C in 10 mM Tris buffer.

Sequencing and phylogenetic analysis

The eluted DGGE bands were amplified using the unclamped versions of the original primers. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced commercially using both forward and reverse primers. Similarity searches were performed using the BLASTN (Altschul et al. 1998) function available from GenBank (http://www.ncbi.nlm.nih.gov).

Quantification and analysis of DGGE fingerprint

The DGGE fingerprint was quantified according to the methods described by Gundlapally and Garcia-Pichel (2006) using the image analysis software Quantity One (BioRad Laboratories). Phylotype richness was calculated based on the number of bands per lane and the intensity of each band was used to calculate the relative abundance of each band. This information was used to calculate the Shannon–Weaver diversity indices (H′; Nübel et al. 1999). Similarity between sites based on their community fingerprints was analyzed by automated UPGMA run on the digitized DGGE gels using the "Phylogenetic Analysis" option of the Quantity One software, according to the methods described by Rothrock and Garcia-Pichel (2005).

Statistics

To determine if the BSCs were enriched in total organic C, total inorganic C, and total N compared to the soil under the BSCs, we calculated the ratio of each parameter (BSC:under crust; ratios not shown). We used the Wilcoxon signed rank test following the method described by Beraldi-Campesi et al. (2009) to test if the mean of the distribution of ratios for each parameter was significantly larger than unity, which would confirm an enrichment trend. Two-sample *t*-tests were used to determine statistical differences between N_2-fixation and ammonium-oxidation, N_2-fixation and denitrification, and ammonium-oxidation and denitrification rates. Paired *t*-tests were used to determine statistical differences between net photosynthetic and the absolute value of dark respiration rates at each site. For all statistical tests, differences were considered signification at the $P < 0.05$ level. All data sets analyzed parametrically gave Ryan-Joiner test normality values of >0.97. Analyses were done using the Minitab statistical package (Minitab 2006).

Results

Soil geochemistry

Total organic C, total inorganic C, and total N varied among sites by as much as an order of magnitude (Table 1), but were within the typical ranges found in aridisols (Peterjohn and Schlesinger 1990; Walvoord et al. 2003; Lajtha and Schlesinger 1986; Beraldi-Campesi et al. 2009). BSCs tended to show clear enrichments over their corresponding bulk soil in all geochemical parameters measured ($P < 0.05$). BSC pH was typically slightly alkali, ranging from 7.7 to 8.6 (Table 1).

Microbial community

BSC microbial DNA concentrations were between 6.03 and 9.05 ng g^{-1} (Table 2), which are within the expected range based on concentrations found in BSCs of the Colorado Plateau (Garcia-Pichel et al. 2003). Bacterial phylotype richness varied among sites by a factor of 2, with a mean of 17.55 distinguishable alleles per sample (Table 2). In contrast, bacterial phylotype diversity, calculated with the Shannon–Weaver index, did not vary as much among sites, with a mean of 2.46 (Table 2). At all sites, cyanobacteria accounted for at least 36% of the bacterial richness (Table 2). A cursory sampling of individual DGGE bands with subsequent sequencing showed that most BSC inhabitants matched closely with cyanobacterial phylotypes common to other desert BSCs, including *Scytonema* sp., *Microcoleus steenstrupii* and *Microcoleus vaginatus*, and *Pseudanabaena* sp. (Garcia-Pichel et al. 2001).

Due to the dominance of cyanobacteria in these BSC communities, we focused our analysis of composition differences on changes in the cyanobacterial community. There was a clear difference in cyanobacterial community composition among sites, as shown in the DGGE fingerprints, which revealed distinct banding patterns in different samples (Fig. 1). An unweighted average pair-group (UWPGA) similarity analysis of these patterns, as determined from the cyanobacterial fingerprint (Fig. 1), showed that samples tended to group by geographic origins, with geographically close samples similar in composition.

Net photosynthesis and dark respiration

All BSCs displayed net photosynthetic CO_2 drawdown in the light at 350 μmol m^{-2} s^{-1}. The rates varied among samples by more than 10-fold with a mean of 317.4 μmol m^{-2} h^{-1} (Fig. 2). Dark respiration was much more homogenous, varying among samples maximally by 3-fold with a mean of −274.4 μmol m^{-2} h^{-1} (Fig. 2). All rates, however, were within typical ranges measured in other BSCs (Lange 2001). Net photosynthetic rates were not significantly different from the absolute value of dark respiration rates at the Chandler ($P = 0.72$), Willcox ($P = 0.97$), and Jornada ($P = 0.39$) sites (Fig. 2). However, net photosynthetic rates were greater than the absolute value of dark respiration rates at the Yuma, Cactus, Hayden, Soda, Searles, and Green Butte sites.

Tracer experiments: absence of anammox and denitrification in BSCs

Rates of anammox at all sites were very low and close to the detection limit of 0.005 μmol m^{-2} h^{-1} (Fig. 3). Heterotrophic denitrification rates were also low regardless of the technique used. The acetylene inhibition method (data in Fig. 3) tended to give higher estimates, likely because it included a C

Table 2 Diversity indices of cyanobacterial and bacterial communities in BSCs as determined from DGGE cyanobacterial and bacterial community fingerprints

Location	Site	Total microbial DNA (ng g^{-1})	Cyanobacteria		Bacteria	
			Richness	Shannon–Weaver index of diversity	Richness	Shannon–Weaver index of diversity
Mojave desert	Cactus Plain	6.04	10	1.72	13	2.41
	Hayden	6.79	8	1.67	22	2.70
	Soda Lake	5.16	n/d	n/d	19	2.47
	Searles Lake	8.28	10	1.90	15	2.20
Chihuahuan desert	Willcox Playa	8.22	10	1.78	16	2.41
	Jornada LTER	8.17	13	2.09	15	2.34
Sonoran desert	Chandler	9.05	10	1.89	23	2.67
	Yuma	8.15	9	1.72	21	2.70
Colorado plateau	Green Butte	8.41	6	1.05	14	2.25

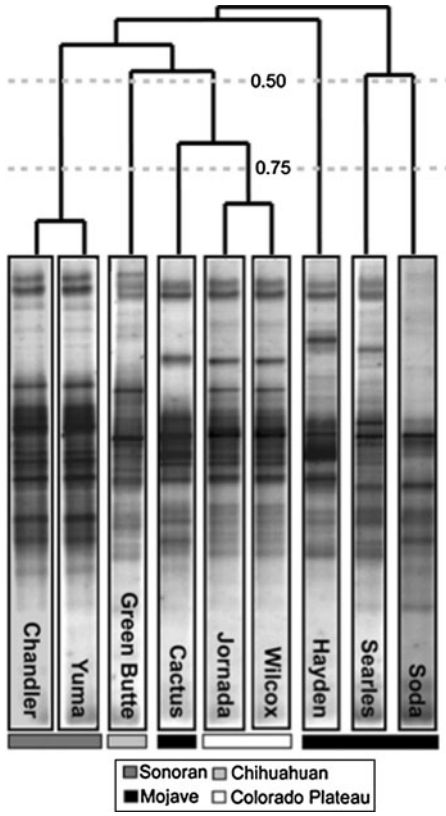

Fig. 1 16S rRNA gene based fingerprint of bacterial communities in BSCs used in this study. The similarity of the banding patterns was analyzed using an unweighted average pair-group method (UWPGA), with percentage of similarity indicated by the *dashed lines*

amendment absent from the tracer technique. Regardless of this difference, all denitrification rates were significantly ($P < 0.05$) and appreciably (>10-fold) lower than N_2-fixation rates measured by acetylene reduction (Fig. 3).

N_2-fixation and aerobic ammonia-oxidation

All BSCs exhibited N_2-fixation (Fig. 3). While the rates varied among samples by more than 5-fold with a mean of 70 μmol m^{-2} h^{-1}, all were within the range measured in other BSCs (Belnap 2002; Johnson et al. 2005). Aerobic ammonia-oxidation was also detectable in all samples (Fig. 3), with rates that varied 10-fold and had a mean of 110 μmol m^{-2} h^{-1}. At 7 of the nine sites, N_2-fixation and AAO rates were not significantly different from each other on a per N basis ($P > 0.42$; Fig. 3). However, in two of the Mojave sites, Cactus ($P = 0.012$) and Soda ($P < 0.01$), N_2-fixation rates were significantly lower than AAO rates (on a per N basis) by at least 10-fold.

Discussion

The nine different BSCs locations we sampled were diverse in terms of their climate, microbial communities, and geochemistry. In the Colorado Plateau, snow, frost and overall low winter temperatures yield to moderately warm summers. In the Sonoran Desert, by contrast, freezing and snow are rare, and summers are very hot (Bailey 1994; MacMahon 1987). Precipitation patterns also differ among these regions: some receive three annual periods of precipitation (Colorado Plateau) and some only one (Mojave and Chihuahuan Deserts; Bailey 1994; MacMahon 1987). Our analysis of community structure revealed that significant variations in the microbial community composition were also present among the samples (Fig. 1). While geographically close samples tended to be more similar to each other, the set as a whole encompassed a minimal similarity of 32%. Sequences from our samples, and that of other researchers (Nagy et al. 2005; Gundlapally and Garcia-Pichel 2006; Yeager et al. 2007), indicate that the dominant cyanobacterial species in BSCs do show a significant geographic overlap. However, even if the composition of primary producers is comparable across regions, the net primary production may differ. Southern sites (Chandler, Willcox, and Jornada) had similar net photosynthetic and absolute dark respiration rates (Fig. 2) compared to the more northern sites, suggesting different physiological responses at the moderate PAR we used for our measurements. As noted in Table 1, variation among samples can also be extended to several other parameters, including major biogenic elemental concentrations of various pools (C and N). Non-biogenic elements within BSCs from at least two of these sites also vary (Beraldi-Campesi et al. 2009).

In spite of this variability, the overall character of the N cycle of the BSCs we examined, as revealed by our rate measurements, was remarkably consistent. Dinitrogen fixation and AAO played a much larger role than denitrification in all samples. All sites examined had high areal rates of N_2-fixation and AAO but extremely low rates of heterotrophic

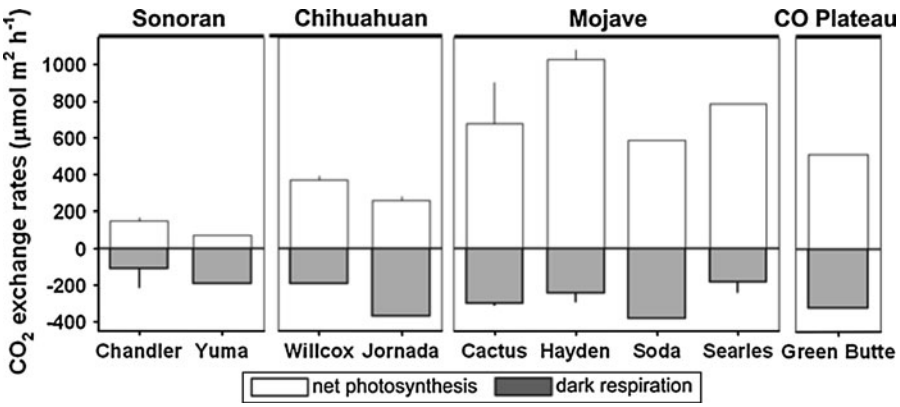

Fig. 2 Net photosynthetic and dark respiration rates at all sites. Net photosynthetic rates were measured as a net uptake of CO_2 by the soil (and presented as positive values), while dark respiration rates were measured as a net release of CO_2 from the soil (and presented as positive values). All measurements were made at 350 µmol m^2 s^{-1} PAR and 20°C. Values are mean ± SE (n = 3)

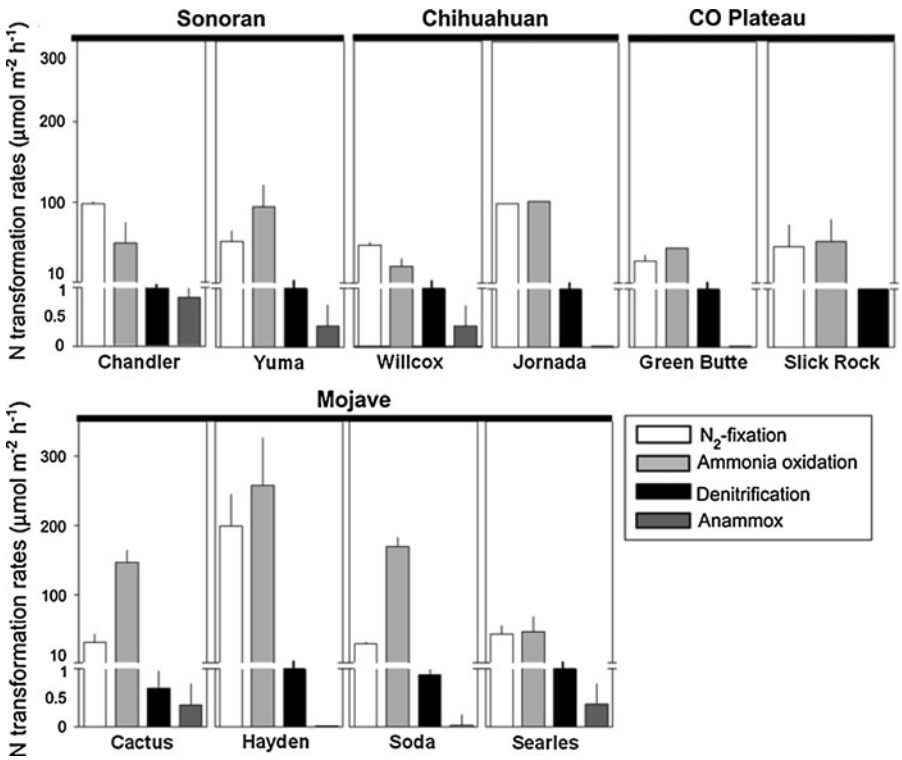

Fig. 3 N_2-fixation (acetylene reduction), aerobic ammonia-oxidation (AAO), potential (C-amended) heterotrophic denitrification and anaerobic ammonia oxidation (anammox) rates at all sites. Values are mean ± SE (n = 3). Data for the Colorado Plateau site named Slick Rock is from Johnson et al. (2005, 2007)

denitrification and anammox by comparison (Fig. 3). Thus, the pattern of N cycling previously found in the Colorado Plateau (Johnson et al. 2005, 2007) was also found in BSCs from other Southwest deserts subject to distinct climatic regimes.

The magnitude of the N cycling processes in these BSCs may vary as seasonal changes in soil temperature and moisture levels affect microbial metabolic processes (Belnap et al. 2004; Cable and Huxman 2004), perhaps even differentially. However, even if the balance of processes may be altered under varying conditions, this is unlikely to overcome the order-of-magnitude mismatches demonstrated here. Our measurements provide an estimate of these processes, matching standard conditions. All of our measurements occurred at 20°C, which is within the

range of average annual temperatures of the Southwestern US deserts (10–24°C; Bailey 1994; MacMahon 1987) and within the range of optimal temperatures for N_2-fixation in BSCs (20–30°C; Belnap 2002). While optimal denitrification temperatures were measured in soils at 40°C (Peterjohn 1991), rain events (even in summer) are associated with cooler temperatures. As most biological activity occurs during or immediately after rain events (Garcia-Pichel and Belnap 1996), all of our measurements were made in saturated BSCs. Partially unsaturated BSCs would be expected to only decrease the relevance of denitrification and anammox.

The reason for the widespread lack of heterotrophic denitrification remains paradoxical, given that the presence of soil organic C and NO_3^- (Table 1; Johnson et al. 2005, 2007), and the typical formation of internal, temporary anoxic microzones within wet BSCs (Garcia-Pichel and Belnap 1996). Instead, some other common factor (or combination of factors) appears to prevent substantial denitrification in most BSCs. The microscale hydrology of desert BSCs could possibly contribute to removing NO_3^- from micro-niches that contain the organic C, but in reality, any explanation we offer is speculative.

We also definitively show that anammox (Fig. 3) is not a mechanism of any relevance in the potential N release to the atmosphere from the BSCs we examined. This is again somewhat surprising given the internal anoxic conditions that typically develop inside the BSC (Garcia-Pichel and Belnap 1996), the large N_2-fixation rates constituting a source of necessary NH_4^+, and the equally active AAO providing necessary NO_3^-. However, anammox organisms seem to require high concentrations of NH_4^+ and NO_3^- at the same location (Jetten et al. 1998), and it is possible that the interstitial concentrations of NH_4^+ and NO_3^- in BSCs are insufficient for anammox organisms. Whatever the reason, this seems to add to the N cycle paradox of BSCs.

Despite the consistent lack of denitrification and anammox, one characteristic of BSC N cycling showed significant variation: in two sites, both in the Mojave, AAO rates were significantly greater than N_2-fixation. To sustain such AAO rates, an input of reduced N other than N_2-fixation would be required. While this could be in the form of localized atmospheric NH_3 deposition, no data is available to support or reject this conjecture. If one looks at total wet inorganic N deposition, which is available for locales close to our sampling sites (National Atmospheric Deposition Program; http://nadp.sws.uiuc.edu/), high deposition rates are not apparent at the sites with high AAO rates. An alternative explanation could be a variation in the efficiency of N_2-fixing organisms. As explained in the Methods, our conversions of acetylene-reduction to N_2-fixation rates are very conservative, and one cannot exclude that this may account for the apparent mismatch.

Aside from differences in N_2-fixation and AAO rates between sites, and irrespective of our inability to provide satisfactory explanations for the lack of anaerobic processes, the common trait of an incomplete BSC N cycle has important implications for the role of BSCs to desert N fertility. The N fixed and partly oxidized by the BSC biota may be exported and enter the surrounding soil or groundwater through leaching or runoff (Johnson et al. 2007). It is also possible that this N may leave the BSC system to drive abiotic volatilization, as found in deeper Mojave Desert soils (McCalley and Sparks 2008; 2009) or to reach denitrification hotspots elsewhere (McClain et al. 2003). Regardless of the fate of the fixed and partly oxidized N, our biogeochemical survey makes this implication applicable to BSCs beyond the Colorado Plateau across several biogeographic provinces, to BSCs with distinct microbial community compositions and developing on different soil types.

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