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Research Article

Functional significance of microbial diversity in arid soils: biological soil crusts and nitrogen fixation as a model system

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Abstract

Microbial communities respond to changes in environmental conditions; however, how compositional shifts affect ecosystem processes is still not well-understood and it is often assumed that different microbial communities will function equally under similar environmental conditions. We evaluated this assumption of functional redundancy using biological soil crusts (BSCs) from two arid ecosystems in Mexico with contrasting climate conditions (hot and cold deserts) following an experimental approach both in the field (reciprocal transplants) and in laboratory conditions (common garden), focusing on the community's composition and potential for nitrogen fixation. Potential of nitrogen fixation was assessed through the acetylene reduction assay. Community composition and diversity was determined with T-RFLPs of *nifH* gene, high throughput sequencing of 16S rRNA gene amplicons and metagenomic libraries. BSCs tended to show higher potential nitrogen fixation rates when experiencing temperatures more similar to their native environment. Moreover, changes in potential nitrogen fixation, taxonomic and functional community composition, and diversity often depended on an interactive effect of origin of the communities and the environment they experienced. We interpret our results as legacy effects that result from ecological specialization of the BSC communities to their native environment. Overall, we present evidence of nonfunctional redundancy of BSCs in terms of nitrogen fixation.

Keywords: arid soils, ecological specialization, functional redundancy, legacy effects, microbial communities, nitrogen cycling, reciprocal transplants

Introduction

Microbial communities are responsible for biogeochemical transformations that occur in all ecosystems and are intricately linked to ecosystem functioning (Falkowski et al. 2008, Cavicchioli et al. 2019). Although it has been widely recognized that microbial community composition changes in response to environmental perturbations, the functional impact of these compositional changes are still a matter of debate (Graham et al. 2014, 2016, Nemergut et al. 2014, Bier et al. 2015). Given their immense diversity, physiological flexibility, and rapid growth, it has been reasonably assumed that different microbial communities may function equally if placed under the same environmental conditions (Allison and Martiny 2008, Bradford and Fierer 2012). While experiments from a limited number of systems demonstrate an influence of microbial community composition on ecosystem functioning (Strickland et al. 2009, Allison et al. 2013, Reed and Martiny 2013, Wagg et al. 2014, Glassman et al. 2018), others support the idea that community composition and ecosystem functioning are uncoupled (Bier et al. 2015, Wood et al. 2015).

There are several potential reasons behind conflicting findings in microbial composition–functioning studies. First, the degree to which functional and compositional changes are related may depend on whether the function of interest is strongly mediated by a limiting abiotic factor such as nutrient availability that overrides any effects of community composition on functioning (Schimel and Schaeffer 2012). Second, the function of interest may be carried out by such a broad range of co-occurring taxa that the community harbours a high degree of functional redundancy, whereas functions performed by fewer taxa are more likely to be affected by compositional changes ('narrow vs. broad hypothesis'; Schimel 1995). Finally, studies that compare communities from contrasting environments are more likely to detect a link between composition and functioning when placed under the same conditions. In particular, if the organisms within a community show signatures of ecological specialization to their environmental setting, then their performance in a new environment might be restricted due to adaptations to the previous environment (Göransson et al. 2013, Hawkes and Keitt 2015, Martiny et al. 2017). Taken together, the

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likelihood of detecting a relationship between composition and functional processes in microbial communities should increase when focusing on functionally 'narrow' processes among communities that are highly specialized on distinct environments when not limited by an abiotic factor.

Arid and semiarid ecosystems offer a convenient system to address hypotheses regarding the composition–functioning relationship in microbial communities. These landscapes cover about 45% of the planet's terrestrial surface and are expected to expand due to climate change (Zeng and Yoon 2009, Schimel 2010). Despite their wide distribution, relatively few studies consider these ecosystems and their soil microbiota, represented mainly by biological soil crusts (BSC). BSCs are spatially stratified communities dominated by cyanobacteria, other prokaryotes, fungi, mosses, and lichens that develop on the surface layer of desert soils and cover up to the 70% of the interplant spaces (Belnap and Lange 2003, Belnap et al. 2016, Ferrenberg et al. 2017, Pombubpa et al. 2020). BSC contributions to arid ecosystem processes are well-documented (Belnap et al. 2003, 2016, Belnap and Weber 2013, Rodríguez-Caballero et al. 2018). In particular, they play a key role in both carbon and nitrogen cycling (Pointing and Belnap 2012), fixing approximately 33 Tg of nitrogen per year or ∼30% of global biologically fixed nitrogen (Elbert et al. 2012).

BSC communities respond compositionally and physiologically to changes in temperature and precipitation, and these responses, in turn, can alter C and N dynamics (fluxes) (Castenholz and García-Pichel 2000, Belnap and Lange 2002, Austin et al. 2004, Zhou et al. 2011, Barger et al. 2013, Fernandes et al. 2018). Most BSC microorganisms are metabolically active only when wet (Noy-Meir 1973, Lange 2001, Belnap et al. 2004); thus, any potential composition–functioning relationships are likely prominent when moisture is not limiting. Hot and cold deserts differ in whether they experience rainy seasons in the summer or winter and, therefore, BSCs experience contrasting environmental temperatures during their rainy, metabolically active, seasons. Indeed, the composition of BSCs differs between hot and cold deserts. Cyanobacteria, one of the dominant and most studied taxa in BSCs, differ in abundance between hot and cold deserts and vary in their psychrophilic or thermotolerant traits (Garcia-Pichel et al. 2013, Bowker et al. 2016, Muñoz-Martín et al. 2019). Further, because cyanobacteria are the primary nitrogen fixers in mature BSCs (Yeager et al. 2004, 2007, 2012, Patova et al. 2016), rainy season temperatures are likely to be an important factor in BSC functioning.

In this study, we reciprocally transplanted BSCs from a hot and cold desert in Mexico to assess the relationship between microbial community composition and functioning. Follow-up experiments in a laboratory setting allowed us to isolate the effect of temperature on the BSCs functioning, assayed by potential nitrogen fixation. Given nitrogen fixation's taxonomic 'narrowness' in BSCs and their potential specialization to the distinct desert environments, we hypothesized that the communities would not be functionally redundant even when exposed to common environments. As such, we predicted that (1) nitrogen fixation would be higher when a BSC community experiences temperatures similar to their native environment; (2) BSC community's taxonomic and functional gene composition would respond to non-native environmental conditions in an origin-dependent way, as a result of legacy effects of its original climate. Finally, (3) taxonomic and functional gene richness would decrease when a BSC community is exposed to non-native environmental conditions.

Materials and methods

Study sites

BSC samples were collected from two geographical regions, a hot desert ecosystem (summer rain) in the Cuatro Ciénegas (CC) locality, and a cold desert ecosystem (winter rain) in the Valle de Guadalupe (VG) locality (Fig. 1). The VG site is located in Baja California state in México (32°023.15N 116° 29 47.77W). We defined the VG site as the 'Cold Desert' location, because the rainy season occurs during the winter (Toro-Guerrero and Kretzschmar 2016) and the mean annual temperature is 17.3°C with a mean maximum temperature of 24.9°C and a mean minimum temperature of 8.3°C (SMN 2017). The CC site is located at the Coahuila state in Mexico (26°5443.15N 102°0052.72W). We defined the CC site as the 'Hot Desert' location, because the rainy season occurs in summer (López-Lozano et al. 2012) and the mean annual temperature is 21.4°C with a mean maximum temperature of 29.1°C and a mean minimum temperature of 14.7°C (SMN 2017).

Experimental design

We carried out a field (reciprocal transplants) and a laboratory experiment (two common garden experiments with reciprocal temperature regimes) to test the relative contribution of community composition versus environmental conditions on the functional response of BSC communities to environmental change (Fig. 2).

Sampling

Between November and December 2012, we collected BSC cores in a 8 m × 8 m transect in each locality of study, the transect was subdivided in 1 m × 1 m quadrants, cores were collected in a checkerboard pattern, being each sample at least 1 m apart. Cyanobacteria-dominated BSCs at both deserts without mosses or lichen cover were collected, aiming to control for differences in developmental stages (Yeager et al. 2004). To preserve the vertical stratification of the BSC, sampling was conducted by using PVC cylinders (5 cm depth and 11 cm diameter). The samples were sealed in black plastic bags for transportation and stored in the dark, at room temperature in the laboratory. Samples collected from the hot desert (CC) in early November 2012 and were stored in the lab for ∼4 weeks while conducting the acetylene reduction assay (ARA; described below). They were then transported to the cold desert (VG) site in early December 2012 for transplantation. Samples collected from the cold desert (VG) in December 2012 underwent the same procedure, but were stored in the lab for ∼7 months before taken to transplantation to hot desert (CC) site in July 2013.

Field setting

For the field experiment, the BSC cores were enclosed in cellophane film bags. The chemical properties of cellophane allows water and ions to flow through, but not bacterial cells (Niaounakis 2020). At both sites, 16 cores were placed in each site for 4 months during the corresponding rainy season [hot desert (CC), summer; cold desert (VG), winter] so that the communities experienced the site conditions during the metabolically active season. At each site, we also included four controls, i.e. cores that were collected, enclosed in cellophane bags and placed back into the same site. These controls (samples incubated in the desert of origin) were intended to assess whether BSC composition was affected by the handling and enclosing of transplanted samples and to be used for the functional assays. Due to an unknown disturbance in the hot desert (CC), three out of the four control samples were lost,

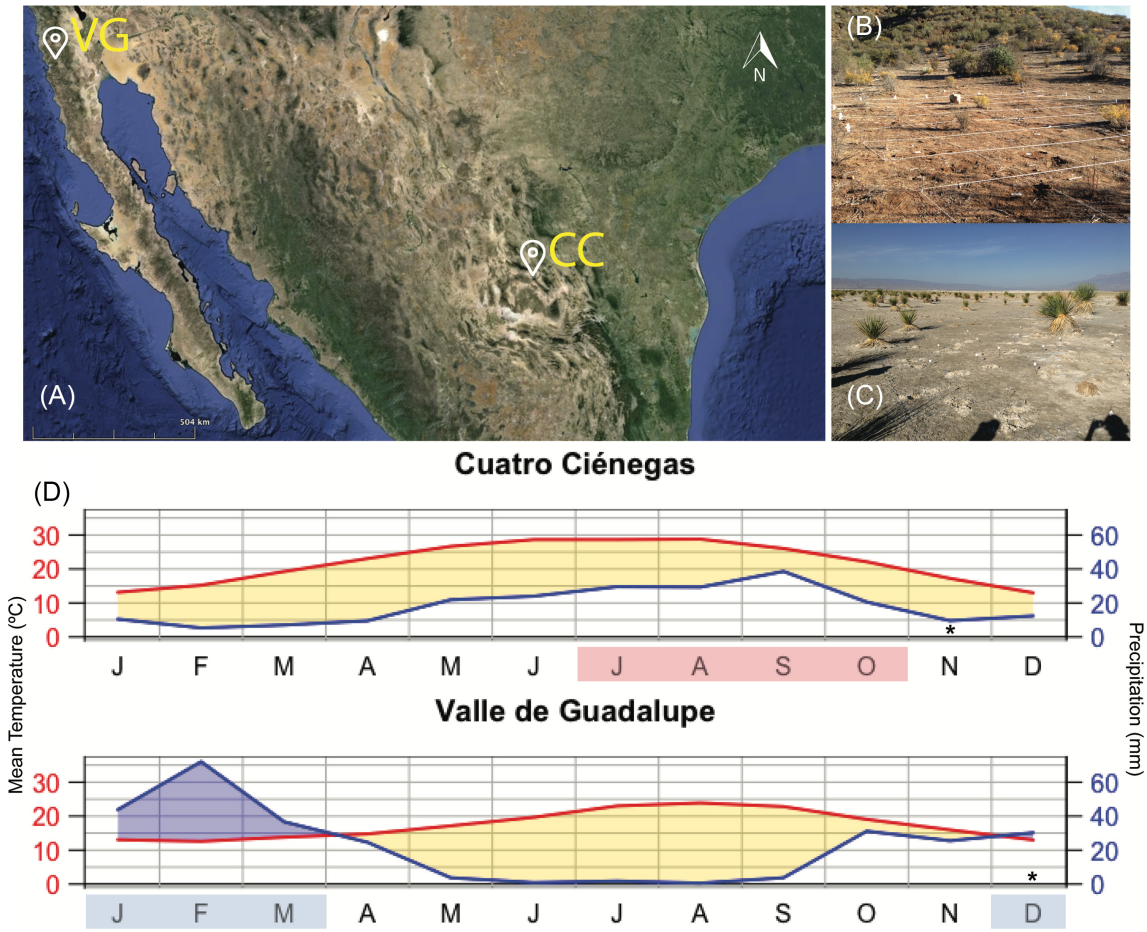

Figure 1. Location, aspect and monthly climate variation of the study sites in Mexico. **(A)** Location of the cold desert site in VG, Baja California and hot desert site in CC, Coahuila. **(B)** VG site at sampling and **(C)** CC site at sampling. **(D)** Climate graph of each site showing monthly mean temperature (red line, left axis) and precipitation (blue line, right axis) (data from Servicio Meteorológico Nacional). The * indicates the sampling month at the two sites. Shaded months on the x-axis indicate the incubation period of the transplants at each site.

affecting the factorial design for the field experiment for the functional assays.

Laboratory setting

To evaluate the influence of temperature as key environmental parameter on N_2-fixation potential, we implemented a common garden experiment with two different temperatures in the lab (15°C and 30°C). The experiments were conducted in environmental incubation chambers (Conviron, G30 model) with lux lamps (500 μmol of photons x m^2s^{-1}) on a 12-h light/dark schedule. Temperature settings inside the chambers were intended to simulate cold desert conditions (15°C, mean temperature in winter for VG locality, Fig. 2) and hot desert conditions (30°C, as mean temperature in summer for CC locality, Fig. 2). In each chamber, we placed 11 BSC cores from both localities and kept them at 100% water saturation throughout the duration of the experiment inside individual PVC cylinders with a clear acrylic cap that was hermetically sealed. The experiment started after an acclimation period of 3 days and lasted 180 days. To evaluate diazotrophic community composition of BCS, we followed a destructive sampling of the cores at four time points throughout the experiment: 0 days (T0), 10 days (T1), 90 days (T2), and 180 days (T3). First, nitrogen fixation activity was evaluated on the whole core with the ARA (see below) at the same four time points described above. After the ARA assay was conducted, we collected a subsample of the BSC core with 15 ml sterile centrifuge tubes (Falcon tubes). Two BSC cores from each treatment were sampled at T0 and three cores at each T1–T3. Samples were frozen at −80°C until further processing for molecular analysis.

Functional analysis of BSC communities

To evaluate the functioning of the BSCs, we measured nitrogen fixation potential via the ARA (Hardy et al. 1973) in laboratory conditions. We considered that in order to evaluate functional responses differences between microbial communities, measurements in laboratory conditions of both types of communities give sufficient information, although not necessarily representative of field conditions. Each of the BSC cores, contained in the PVC cylinders, was air-tight sealed with an acrylic lid with a rubber stopper and plumbing dough. Once sealed, 10% of the headspace volume was extracted with a syringe and replaced with acetylene gas (C_2H_2). After incubation, 1 ml gas sample was extracted and analyzed for C_2H_2 and ethylene (C_2H_4) concentration in a gas chromatographer (Varian 3300). For the laboratory experiment, samples were incubated for 72 h, after the incubation period a single gas sample was analyzed. For the field experiment, after the reciprocal transplant incubation period (4 months), samples were taken to the laboratory and incubated at 27°C under a light regime of 16 h light (2000 lux) and 8 h darkness; samples for ARA measurements were taken at 0, 24, 48, and 120 h. See Supplementary

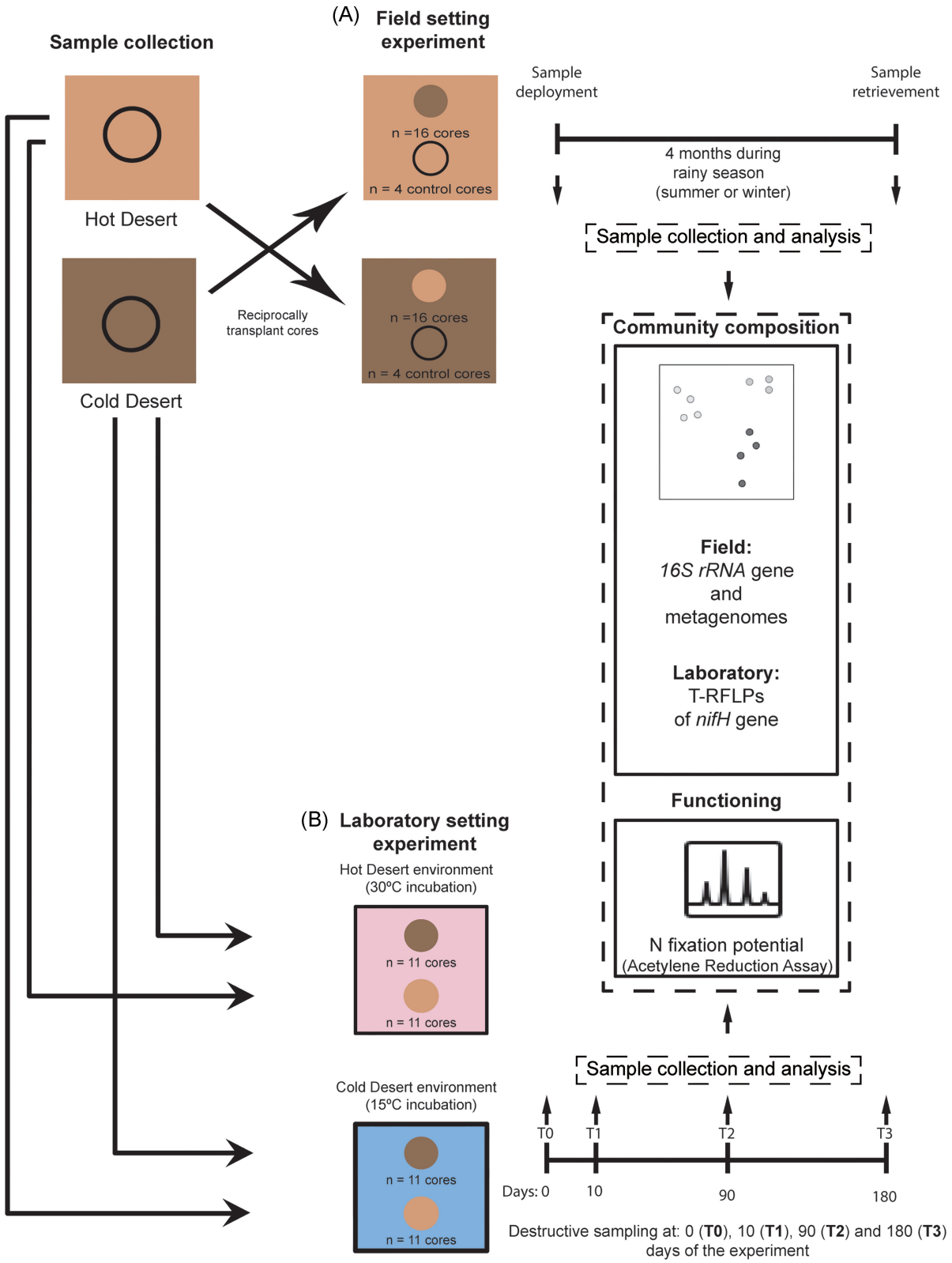

Figure 2. Experimental design depicting a reciprocal transplant experiment in **(A)** field setting and **(B)** laboratory setting. Sampling sites correspond to a cold desert in VG and a hot desert in CC. Two experiments were conducted with the cores collected. (A) A reciprocal transplant experiment was conducted in the field and maintained for 4 months (during rainy season). After the experiment, data on potential for nitrogen fixation (acetylene reduction assay) and microbial composition (*nifH* TRFLP; *16S rRNA* sequencing; metagenomic sequencing) was analyzed. (B) A temperature experiment was conducted in the lab. Two temperatures reflecting local MAT (mean annual temperature) were maintained for 180 days to incubate both sets of BSC samples (15°C and 30°C).

Information for further details about the chromatograph readings and conversion of peak areas to μmoles of each gas. For this comparison, the control samples served as one of the treatments for the statistical tests, i.e. samples that remained in their desert of origin.

Molecular characterization of BSC communities

We conducted three procedures to characterize the composition of BSC communities based on the methods available to us at the time. For the laboratory experiment, we generated terminal restriction fragment length polymorphism (T-RFLP) profiles of the *nifH* gene, which has been widely used to evaluate diversity and abundance of diazotrophic bacteria in different systems as well as BSCs (Zehr et al. 2003, Raymond et al. 2004, Yeager et al. 2007, Gaby and Buckley 2011). For the field experiment, we sequenced 16S rRNA gene amplicon libraries to evaluate diversity and abundance of the entire prokaryotic community of the samples before and after transplants. Finally, to assess nitrogen-cycling gene composition, we sequenced metagenomic libraries from a subset of samples in the field experiment. For all procedures, genomic DNA extraction was performed following the protocol reported by Purdy (2005) with slight modifications (see Supplementary Information). Purified DNA was stored at −20°C.

PCR amplification of the nifH gene

Nested PCR amplification of the *nifH* gene was carried out in triplicate with the primers reported by Yeager et al. (2004). For the first reaction the nifH19F (5′-GCIWTYTAYGGIAARGGIGG-3′), and the nifH3R (5′-ATRTTRTTNGCNGCRTA-3′) were used. The reaction was performed in a 25-μl volume with the following reagent concentrations: 1X buffer, 2.5 mM $MgCl_2$, 2 mM (each) of dNTPs, 2 μM (each) of primers, 2.5 U of Taq DNA polymerase *Platinum* (Thermo Scientific), 10 ng of BSA, and 0.5 μl of DMSO and ∼ 40 ng of DNA. For the second reaction the primers nifH11F-FAM (fluorescently labelled; 5′-GAYCCNAARGCNGACTC-3′) and nifH22R (5′-ADWGCCATCATYTCRCC-3′). Reaction was performed in a 50-μl volume with the same reagent concentration as the previous reaction except for: 2.0 mM $MgCl_2$, 0.1 U of Taq DNA polymerase *Platinum* (Thermo Scientific), 2 μl of the first reaction, and without BSA and DMSO. Thermocycler parameters for the first reaction were as follows: 95°C for 5 min, followed of 20 cycles of 48°C for 1 min, 72°C for 1 min and 95°C for 45 s, with a 72°C final extension step for 10 min. For the second reaction, parameters were as described for the first reaction except that the annealing temperature was 55°C and the reaction cycle was repeated 32 times.

T-RFLP analysis of nifH PCR products

Each of the three individual PCRs per sample were pooled and loaded into a 1.7% agarose gel for fragment separation. A ∼358-bp band was excised from the gel and extracted using the Qiagen QIAquick gel extraction kit. Concentration of the purified amplicons was determined in an 1.7% agarose gel with DNA MassRuler (Thermo Scientific). A total of 80 ng of purified *nifH* amplicons were digested with 2.5 U of *Mae*III (Roche) restriction enzyme in a 25-μl volume reaction at 55°C for 12 h. Purification and precipitation of fragments is described in the Supplementary Information (S1). Precipitated fragments were then suspended in 9.5 μl of formamide (HiDi Applied Biosystems) and 0.25 μl of molecular weight marker GS500 LIZ (Applied Biosystems). The mix was loaded in a sequencing plate and placed in a thermal cycler for 5 min at 95°C and immediately placed on ice for 5 min. Then, the plate was loaded in a capillary electrophoresis sequencer (ABI 3500xL *Genetic analyzer*) with the following electrophoresis run parameters: 60°C, 10 kV, 35 s injection time and running time for 10 000 s. Three runs per sample were performed to identify sequencer artifacts. T-RFLP profiles were generated using the GeneMapper 3.1 software (Applied Biosystems) and for further analysis the GeneMarker software (Softgenetics) was used. T-RFLP profiles data were used to create a table with fragment size (bp), height (fluorescence intensity) and peak area. A fragment was included in the analysis if it had a minimum of 20 bp, a minimum of 50 fluorescence units, and it was present at least in two of the three replicates. Fragments that differed in less than 2 bp were clustered into a single fragment. Peak heights for each sample were converted to a percentage of the total peak heights for that sample and peaks that represented <0.5% of the total peak height were excluded from the analysis. Each of the different fragments was considered an OTU and the percentage of each fragment was considered as the relative abundance.

A volume of 1 µl of DNA from each extract was added to a PCR master mix containing: 12.5 μl of QuantaBio's AccuStart II PCR ToughMix (2x concentration, 1x final), 0.2 µM forward primer, and 0.2 µM reverse primer, and H_2O to a final volume of 25 µl. We used the 515 forward primer (GTGYCAGCMGCCGCGGTAA) and 926 reverse primer (CCGYCAATTYMTTTRAGTTT) designed by Caporaso et al. (2012) and modified by Apprill et al. (2015) to target the V4–V5 region of the 16S rRNA gene. Following an initial denaturation step at 94°C for 3 min, PCR was cycled 35 times at 94°C for 45 s, 55°C for 30 s, 72°C for 60 s, with a final extension at 72°C for 10 min.

Amplified samples were pooled based on gel electrophoreses images, with 1.0, 2.0, and 3.0 µl added for strong, moderate, and weak bands respectively, into a low binding tube. After pooling, PCR products were cleaned adding magnetic speed beads (Speed Bead Magnetic Carboxylate, GE Healthcare UK Limited, Buckinghamshire, UK) in a 1:1 ratio and after beads were pulled down with a magnet the bead pellet was washed twice with 80% ethanol. The pellet was dried and resuspended in 50 µl water. The library pool was assessed for quality using a High Sensitivity DNA Assay on an Agilent Bioanalyzer at and sequenced on an Illumina MiSeq sequencer using PE300 v3 chemistry at the Genome High-throughput Facility at the University of California, Irvine using multiplexed paired-end Illumina MiSeq platform. The raw data (paired end files) were deposited in the NCBI Sequence Read Archive with the accession number PRJNA857298.

Preparation of metagenomic libraries

To assess nitrogen cycling gene composition, we created metagenomic libraries from five samples from each site (CC and VG) before and after the transplants for a total of 20 samples. We fragmented the DNA of the samples with the Nextera DNA Library Preparation Kit (Illumina, San Diego, CA. USA) following a modified protocol from Lamble et al. (2013) and Baym et al. (2015). Metagenomic libraries were cleaned using magnetic beads (included in the Nextera DNA Library Preparation Kit) and assessed for quality using a High Sensitivity DNA Assay on an Agilent Bioanalyzer. The libraries were sequenced on the Illumina HiSeq 4000 platform (150-bp paired-end reads) (Illumina) at the Genome Center at the University of California, Davis. The raw data (paired end files) were deposited in the NCBI Sequence Read Archive with the accession number PRJNA857298.

Sequence data processing

16S rRNA gene sequence data

Illumina sequence data was processed using the QIIME2 version 2019.1 (Bolyen et al. 2019) toolkit. After demultiplexing, paired-end sequences were processed with the DADA2 pipeline (Callahan

et al. 2016) for quality filtering and dereplication. Operational taxonomic units (OTUs) were picked at 97% identity using VSEARCH (Rognes et al. 2016). Taxonomy was assigned with SILVA (132 release) (Quast et al. 2013) as the reference database using the q2-feature-classifier (Bokulich et al. 2018) plugin in QIIME2. Representative sequences were aligned with MAFFT (Katoh and Standley 2013), and a ML phylogenetic tree was constructed using FastTree 2 v1.11 (Price et al. 2010). Chloroplast and mitochondrial OTUs (i.e. plant-derived sequences, 0.95% of all sequences) and unidentified OTUs at the kingdom level were excluded. A total of 2 809 997 (1303–98 570) sequences were obtained from 63 samples after removing plant-derived sequences and OTUs unidentified at the kingdom level.

Metagenomic sequence data

Raw sequence reads were quality screened and filtered with Trimmomatic v0.39 (Bolger et al. 2014), and only paired-end matched reads were used for subsequent analysis. The quality-filtered sequences were pooled and assembled with MEGAHIT v1.2.9 using default metagenomic parameters (Li et al. 2016), and contigs greater than 500 bp were retained. The number of contigs in the assembly was 2 234 705 ranging from 500 to 302 388 bp, and the N50 of the assembly was 886 bp. The resulting contigs of the assembly were used to predict ORFs and coding proteins with Prodigal v2.6.3 (Hyatt et al. 2010). Annotation of predicted proteins was conducted using the KEGG database and the GhostKOALA server (Kanehisa et al. 2016). The abundance of each predicted protein was calculated by mapping the high-quality reads of each sample against the predicted ORFs with Bowtie2 v2.3.5 (Langmead and Salzberg 2012). In each metagenomic library, we searched for gene sequences from eight nitrogen cycling pathways previously defined by Nelson et al. (2015). These pathways included nitrification (number of genes targeted: $n = 2$), N fixation ($n = 20$), denitrification ($n = 20$), dissimilatory nitrate to nitrite reduction ($n = 9$), dissimilatory nitrite to ammonia reduction ($n = 4$), assimilatory nitrate to nitrite reduction ($n = 2$), assimilatory nitrite to ammonia reduction ($n = 2$), and ammonia assimilation ($n = 10$).

Statistical analysis

Functional assay

To assess the influence of the origin and environment, we performed ANOVAs on nitrogenase activity (evaluated with the ARA) for both the laboratory and field experiments. For both experiments, origin and environment were considered fixed effects. To correct for non-normality and heteroscedasticity, ARA rates were log transformed for the results of the field experiment; given the loss of one of the treatments [control samples in the hot desert (CC) site] only one-way ANOVAs were performed testing for the effect of origin and environment independently using type III sums of squares. For the laboratory experiment, two-way ANOVAs with sums of squares type III were performed on untransformed data. When there was a significant interaction between the two factors, *post hoc* Tukey's HSD tests were used to interpret the significant interaction terms. Sums of squares type III ANOVAs were performed using the 'car' package (Fox and Weisberg 2019) in R version 4.1.1.

Beta diversity

We used nonmetric multidimensional scaling (NMDS) analyses to visualize differences in community composition between the treatments. For the laboratory experiment, a dissimilarity matrix for each time point was created using Bray–Curtis distances [calculated with the 'vegan' package, (Oksanen et al. 2018) in R version 4.1.1] with the *nifH* T-RFLP data. For the field experiment, comparisons were made between samples from the two origins at the moment of collection and the same samples after the 4-months transplantation in the reciprocal desert. All diversity analyses for the 16S rRNA gene sequences and nitrogen cycling genes were performed on a rarefied table (Weiss et al. 2017) (sequence depth: 16S rRNA gene = 1234 sequences, N cycling genes = 5746 sequences), averaged over 1000 iterations using the 'EcolUtils package' (Salazar 2020). We then analyzed the similarity in bacterial community composition and nitrogen cycling gene composition between samples using weighted UniFrac (Lozupone and Knight 2005) calculated with the 'phyloseq' package (McMurdie and Holmes 2013) in R (version 4.1.1) and Bray–Curtis distances (calculated with the 'vegan' package), respectively, on rarefied tables. For all NMDS plots, a 95% confidence interval around the centroids of each BSC origin was calculated with the 'dataEllipse' function of the 'car' package (Fox and Weisberg 2019). To assess the influence of the two factors (origin and environment), PERMANOVAs were performed to test for significant differences in community composition using PERMANOVA + on PRIMER v6 (Gorley and Clarke 2006, Anderson et al. 2008). All PERMANOVA analyses were conducted using type III partial sum of squares under a reduced model with 999 permutations. To assess the sensitivity of bacterial communities to each experimental factor, we calculated the proportion of variance attributable to each factor of interest. Variance explained by experimental variables was determined by dividing the estimated components of variation from each statistically significant term by the sum of all significant terms and the residuals. Finally, to further visualize compositional differences between treatments we created a heat map with the 400 most abundant OTUs (sorted from most abundant to least abundant) of the whole dataset and a UPGMA clustering was performed on a Bray–Curtis distances matrix calculated with the 'vegan' package in R (version 4.1.1).

Alpha diversity

For the laboratory experiment, the Shannon diversity index was calculated with the relative abundances of each OTU from the *nifH* T-RFLP data, and a three-way ANOVA was used to evaluate the effects of origin, incubation temperature, and sampling time as fixed factors using a type III sum of squares with the 'car' package in R. For the field experiment, comparisons were made between samples from the two origins at the moment of collection and the same samples after the 4-months transplantation in the reciprocal desert. Alpha diversity analyses were conducted using rarefied tables (described above). A two-way ANOVA with type III sums of squares was used to evaluate the effect of origin (i.e. community composition) and incubation site (i.e. the environment) on the Shannon and Pielou's evenness index for both the 16S rRNA gene and nitrogen cycling gene data. All analyses were performed with R version 4.1.1 (R Core Team 2017) using the 'vegan' (Oksanen et al. 2018), 'phyloseq' (McMurdie and Holmes 2013), and 'car' (Fox and Weisberg 2019) packages.

Results

We evaluated, in laboratory and field settings, the functional and compositional response of BSC communities from climatically contrasting sites to new environmental conditions. Three lines of evidence supported our hypotheses showing that hot and cold desert BSC communities are not functionally equivalent, at least in terms of their nitrogen fixation potential, and that this difference is, at least in part, a consequence of changes in community

composition of the communities when exposed to a non-native environment.

BSC functional responses

First, the BSC communities from different desert origins were not functionally equivalent in terms of nitrogen fixation potential, showing that performance is generally higher under the native environmental conditions in both experimental settings. Nitrogen fixation rates in the BSCs were influenced by both microbial community composition and the environment. In the field experiment, potential nitrogen fixation depended on both the origin (community) and the site (environment) ($P < .01$ for both main effects; Table 1). As expected, the cold desert BSC had higher nitrogen fixation in its original, cold desert environment (Fig. 3A). Because the hot desert cores incubated in the hot desert site were disturbed during the experiment, we could not test for an interactive effect of origin and site.

Similar functional trends were confirmed in the laboratory experiment. Both temperature and community origin influenced nitrogen fixation potential at the beginning of the experiment (T0). Generally, the cold desert community fixed N at a higher rate than the hot desert community, and both communities fixed N at a higher rate at 30°C than 15°C. However, after some time to acclimate, the BSC communities displayed higher nitrogen fixation potential under their native temperature than in the contrasting temperature (Fig. 3B), resulting in a significant temperature-by-origin interaction for the next three time points (Table 1). Thus, the BSCs were not functionally equivalent when exposed to a common environment and showed higher functional potential when placed under temperatures similar to their place of origin.

BSC compositional responses

Second, the taxonomic and functional gene composition of the BSCs was highly constrained in its response to a new environment—i.e. the compositional response of a BSC depended on its original community. As expected, the composition of the BSC communities (as assayed by 16S rRNA gene sequences) differed by their origin (Fig. 4A; Table 2). In the field experiment, origin still accounted for 43% of the compositional variation observed after 4 months of transplantation (CC samples December 2012–March 2013; VG samples July 2013–November 2013). The hot and cold desert environments also altered BSC composition by the end of the experiment, accounting for a further 10% of variation. Moreover, the communities responded to the two environments in a community-dependent manner (an origin × sample type, explaining 24% of variation). Thus, the response of the BSC communities to a new environment was constrained by the original community. This result is clearly illustrated by the most abundant OTUs, where each community harbours a unique set of OTUs and very few are shared between the hot and cold desert communities (Fig. 4B).

These observations are further supported by data on the composition and abundance of nitrogen cycling genes (assayed by metagenomic sequencing) in the field experiment. The composition of nitrogen cycling genes differed between BSCs from different origins (Figure S1, Supporting Information); origin accounted for most of the variation in composition (48%). Further, both the site environment and an origin-by-sample type interaction accounted for 4% and 10% of variation in nitrogen cycling genes, respectively (Table 2). Thus, although community composition changed after communities were exposed to the reciprocal site conditions, after 4 months composition remained more similar among communities from the same origin.

In the laboratory experiment, the BSC communities showed similar trends in response to changes in temperature, although few of these trends were statistically significant (Fig. 5; Table 2). BSC composition (assayed by *nifH* gene T-RFLPs) only differed significantly between cold desert (VG) and hot desert (CC) after 120 days, accounting for 29.49% of variation (Table 2). Similarly, an origin by temperature interaction was only significant at 90 days (T2), accounting for 22.73% of variation in community composition at this time-point.

BSC richness responses

Finally, richness (overall number of OTUs and number of nitrogen cycling genes) of the BSC communities tended to be highest in the original environment and declined when exposed to a new environment. Transplanting the BSC communities to a new environment reduced overall taxonomic diversity. While initial alpha-diversity did not differ between sites, alpha-diversity was significantly reduced in the field experiment after 4 months of transplantation to the contrasting environment as measured by taxonomic (16S rRNA gene) gene diversity (Fig. 6A; Table 3). Likewise, Pielou's evenness index results show a reduction in evenness after the 4 months transplant period (Figure S3 and Table S3, Supporting Information). Similarly, in the two cases when there was a significant or marginally significant origin-by-sample type effect, transplanting the communities to a new environment tended to decrease alpha-diversity of nitrogen cycling genes for ammonia assimilation, denitrification, and dissimilatory nitrite to ammonia (Fig. 6B, Table 3).

Discussion

Using BSCs from two climatically contrasting deserts, we tested the functional significance of microbial community composition in the face of environmental change. Our three predictions were confirmed, supporting our hypothesis that hot and cold desert BSCs are not functionally redundant as a result of the taxonomic narrowness of the nitrogen fixation process and the specialization of the communities to their native environment. In particular, BSCs tended to show higher potential nitrogen fixation rates when experiencing temperatures more similar to their native environment. Moreover, changes in potential nitrogen fixation, taxonomic and functional community composition, and diversity often depended on an interactive effect of origin of the communities and the environment they experienced. Such an effect is indicative of ecological specialization of the BSC communities to their native environment.

Functional consequences of compositional differences

The BSC community composition from the hot desert (CC) is highly dissimilar from the cold desert (VG) and also shows different functional responses in terms of N fixation potential (Fig. 3, Table 1), although BSC communities from the cold desert seem to be more affected when exposed to non-native conditions. By controlling for environmental conditions in both the laboratory and the field, the reciprocal transplants allowed us to test the hypothesis of nonredundancy in nitrogen fixation potential. Previous studies have shown that cyanobacteria are the main microbial taxa involved in nitrogen fixation in BSCs (Yeager et al. 2004, 2007, 2012, Pepe-Ranney et al. 2016). Given the phylogenetic narrowness of

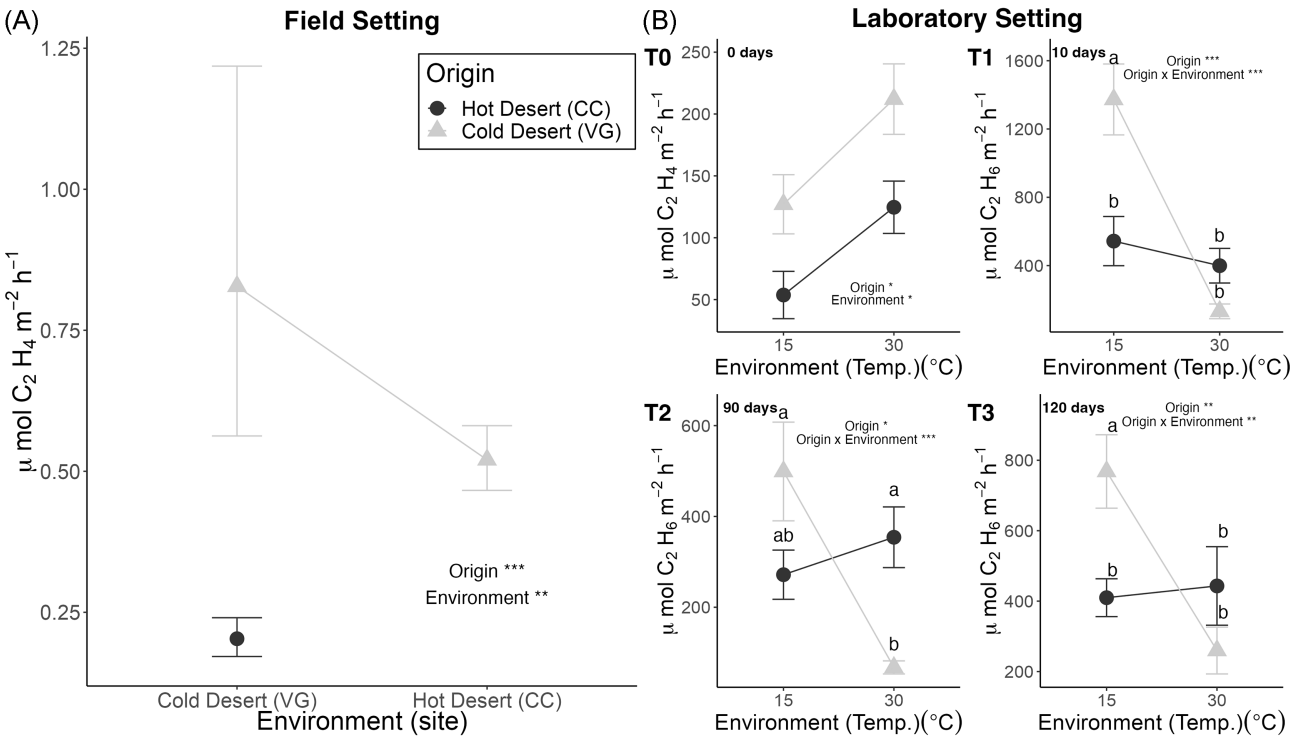

Figure 3. Effects of contrasting environmental conditions on functioning of BSCs **(A)** Field setting *interaction plot* of ARA for all reciprocally transplanted samples and controls [hot desert (CC) and cold desert (VG)]. Data is presented as log back-transformed values for the mean ± SE of acetylene production (mmol$C_2H_4m^{-2}h^{-1}$). Significant terms are indicated with ** ($P < .01$), *** ($P < .001$). **(B)** Laboratory setting *interaction plots* of ARA data at each sampling point in time for hot desert (CC) and cold desert (VG) samples and both temperature regimes (15°C and 30°C). Data is presented as mean ± SE of acetylene production (mmol$C_2H_4m^{-2}h^{-1}$). Significant terms are indicated with * ($P < .05$), ** ($P < .01$), and *** ($P < .001$). Letters show significant differences between treatments as detected by *post hoc* Tukey's HSD test, in the cases that the there was a significant interaction between the two factors.

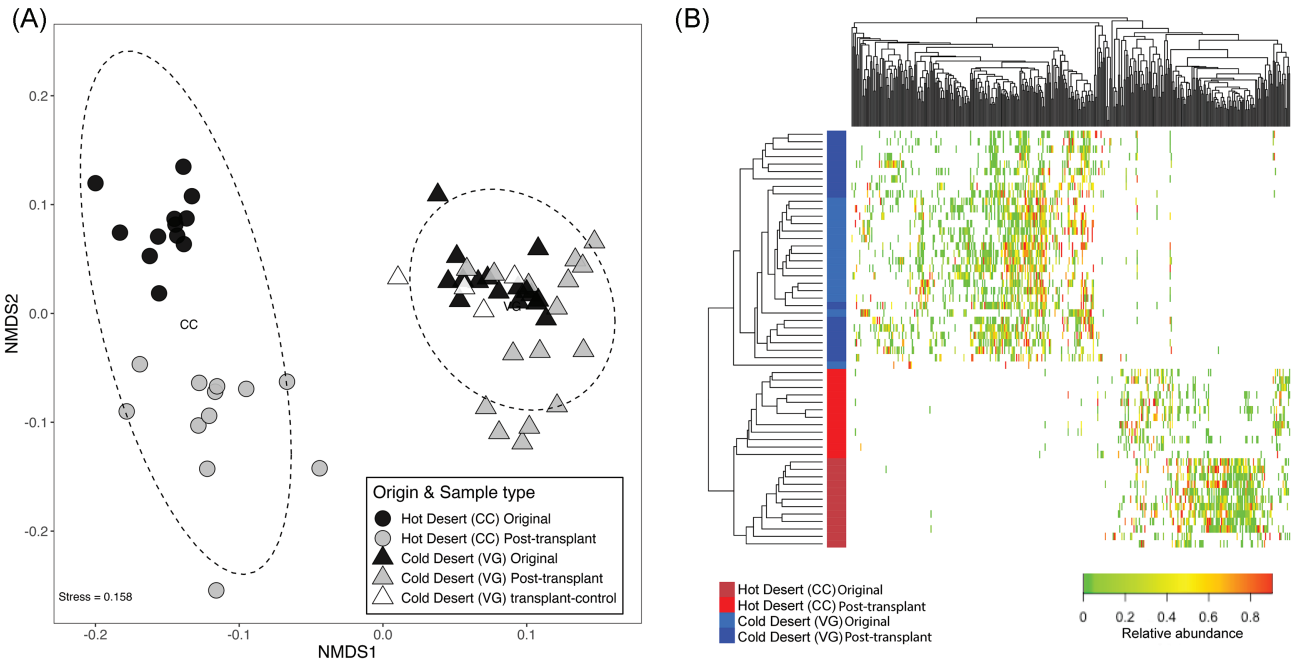

Figure 4. Compositional response of the BSCs to the field transplants. **(A)** NMDS plot based on UniFrac distances of 16S rRNA amplicon composition. Ellipses show 95% confidence intervals for the distribution of samples for the factor 'Origin'. **(B)** Heatmap showing the abundance and distribution of 16S rRNA OTUs (x-axis) across samples (y-axis) from both sites. Clustering (UPGMA) analysis of the 400 most abundant OTUs from the field samples were performed using Bray–Curtis distances.

Table 1. ANOVA tests evaluating the effect of composition (origin) and environment (temperature and site) in nitrogenase activity in both experimental settings.

Field setup			
Factor	Origin	26.62	**< .001**
	Site	11.52	**.001**
Laboratory setup			
Time	**Factor**	**F_1**	***P***
T0 (0 days)	Origin	4.90	**.033**
	Temperature	4.58	**.039**
	Temp × Origin	0.09	.766
T1 (10 days)	Origin	18.12	**< .001**
	Temperature	0.55	.462
	Temp × Origin	15.84	**< .001**
T2 (90 days)	Origin	5.32	**.024**
	Temperature	0.70	.405
	Temp × Origin	13.60	**< .001**
T3 (120 days)	Origin	8.39	**.005**
	Temperature	0.07	.788
	Temp × Origin	9.59	**.003**

Significant *P*-values (< .05) are highlighted in bold.

Table 2. PERMANOVA tests evaluating the effect of origin and environment (temperature and site) on community composition in both experimental setups.

Field setting				
Marker gene	**Factors**	**Pseudo-F**	**% of var. explained**	***P***
16S rRNA	Origin	53.79	43.25	**<.001**
	Sample type	13.31	10.08	**<.001**
	Origin × Sample type	15.77	24.20	**<.001**
N cycling genes	Origin	16.23	48.03	**<.001**
	Sample type	2.45	4.58	**<.001**
	Origin × Sample type	2.61	10.17	**<.001**
Laboratory setting				
Time	Factors	Pseudo-F	% of var. explained	*P*
T0 (0 days)	Origin	1.17	-	.383
	Temperature	0.21	-	.923
	Origin × Temp.	0.21	-	.888
T1 (10 days)	Origin	0.61	-	.641
	Temperature	0.45	-	.770
	Origin × Temp.	0.69	-	.582
T2 (90 days)	Origin	1.61	-	.156
	Temperature	0.44	-	.822
	Origin × Temp.	2.96	39.47	**.025**
T3 (120 days)	Origin	3.37	30.73	**.021**
	Temperature	0.70	-	.646
	Origin × Temp.	0.61	-	.777

P-values are based on 999 permutations. Significant *P*-values are highlighted in bold.

nitrogen fixation, ecological specialization of cyanobacteria may account for differences observed in potential nitrogen fixation rates between BSCs of different deserts (Schimel 1995). Indeed, cyanobacteria isolated from hot and cold deserts BSCs grow better in laboratory conditions at their corresponding temperatures, suggesting specialization of specific bacterial groups and functional consequences associated to specific temperatures or environmental conditions (Garcia-Pichel et al. 2013, Giraldo-Silva et al. 2020). Additionally, it has been reported that BSCs samples originating from hot and cold deserts incubated under the same conditions displayed different rates of potential nitrogen fixation, showing an influence of community composition on this process (Zhou et al. 2016). Still, the nonredundancy result should be interpreted considering N fixation as a narrow function, since other studies addressing other N cycling transformations such as ammonification (a broader process) have observed that rates are not affected by changes in abundance and diversity of the communities along an environmental gradient, whereas nitrification rates (a narrower process) are (Isobe et al. 2020). Beyond N cycling, previous studies on broader processes, including litter decomposition as part of carbon cycling, have also presented evidence of nonfunctional redundancy (Ayres et al. 2009, Strickland et al. 2009, Schimel and Schaeffer 2012, Keiser et al. 2014, Chávez-Vergara et al. 2018). Altogether, our results on N fixation potential strongly suggest that ecological specialization may account for nonfunctional redundancy observations in response to environmental changes.

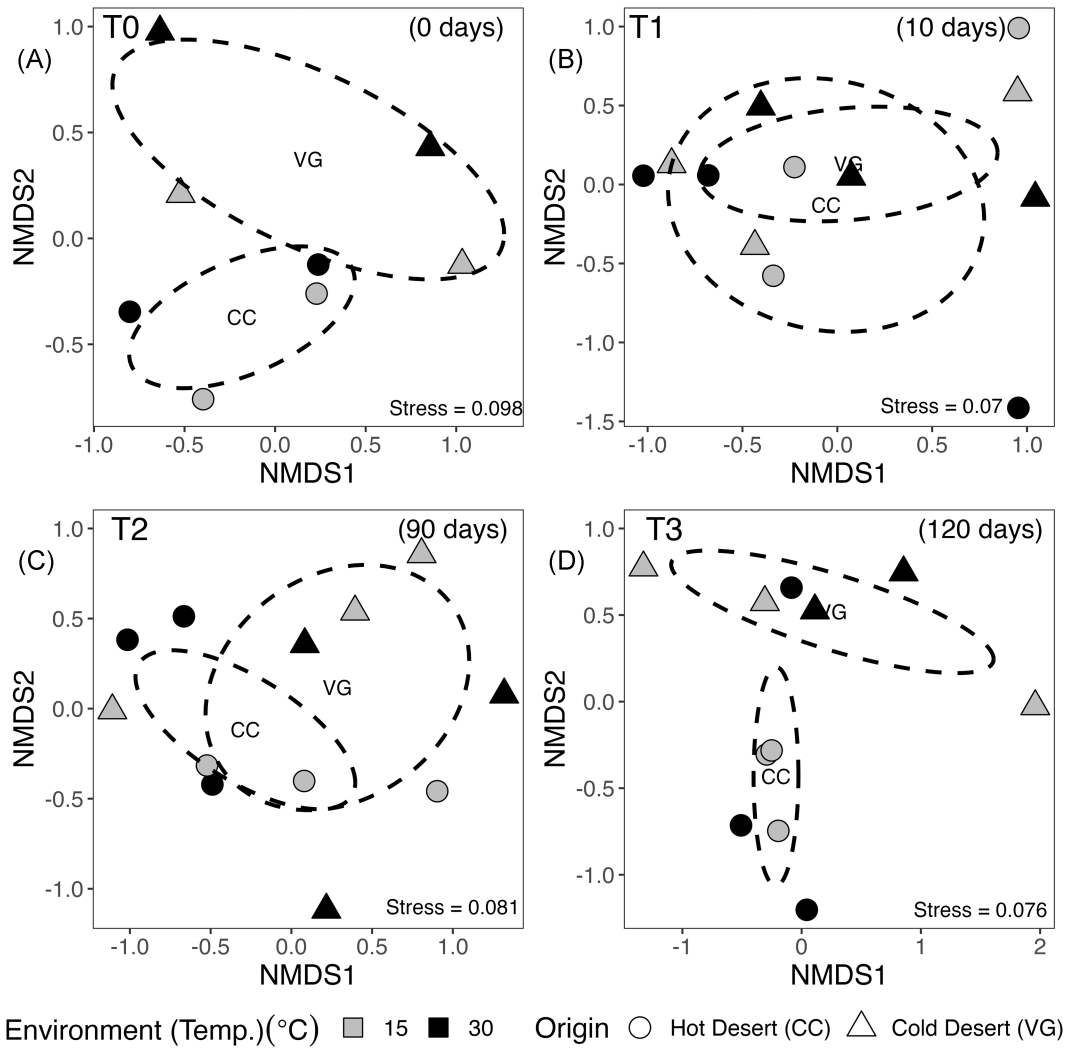

Figure 5. Effects of temperature on BSC community composition. Laboratory setting NMDS with Bray–Curtis distance of *nifH* TRF composition at sampling times **(A)** T0, **(B)** T1, **(C)** T2, and **(D)** T3 for hot (CC) and cold (VG) desert samples and both temperature regimes (15°C and 30°C) Ellipses show 95% confidence intervals for the distribution of samples for the factor 'Origin'.

Legacy effects of community composition

The compositional responses of the BSCs were origin-dependent and persisted throughout the duration of both laboratory and field experiments (Figs 4 and 5, Table 2). Historical contingencies may account for the initial compositional differences we observe between hot and cold desert BSCs communities (Bahl et al. 2011, Hagemann et al. 2015, Bowker et al. 2016, Becerra-Absalón et al. 2019, Muñoz-Martín et al. 2019). Further, when comparing the taxonomic composition across samples (beta diversity) and timepoints, the communities showed different trajectories when responding to a new environment. We interpret this as an indication of a constraint, or legacy effect, of the original community. The legacy effect interpretation of our results is supported by observations on community structure when responding to novel conditions. For instance, conditions of the native environments regarding land use history, vegetation type, drought history, thermal disturbances, and precipitation patterns have proven to have long lasting effects in soil microbial community composition, and not only contemporary soil or environmental conditions (Waldrop and Firestone 2006, Elgersma et al. 2011, Jangid et al. 2011, Banerjee et al. 2016, Bond-Lamberty et al. 2016, Jurburg et al. 2017, Waring and Hawkes 2018, Phillips et al. 2022). Given that dispersal was restricted in our experiments, community compositional changes and nonconvergence in composition between samples from the different deserts is evidence of the legacy effects, which results from ecological specialization by the BSC communities to their native environment (Hawkes and Keitt 2015).

Further evidence of legacy effects is provided by taxonomic alpha diversity analyses. Initial alpha diversity of BSCs is similar between samples from different origins and, as predicted, both communities lose diversity after experiencing the contrasting environmental conditions of the reciprocal desert (Fig. 6A, Table 3). When analyzing the evenness of the communities we see a similar trend where communities decrease in evenness after experiencing the conditions of the reciprocal desert (Figure S3 and Table S3, Supporting Information). These results suggest that some hot and cold desert taxa do not persist at low abundance in the reciprocal desert, or at least are not able to respond to the new conditions within the time frame of this study and by the implemented methods. Indeed, previous studies report that the dominant members of BSCs are susceptible to environmental changes (Garcia-Pichel et al. 2013, Fernandes et al. 2018, Muñoz-Martín et al. 2019, Giraldo-Silva et al. 2020). However, no such differences were detected in the laboratory experiment

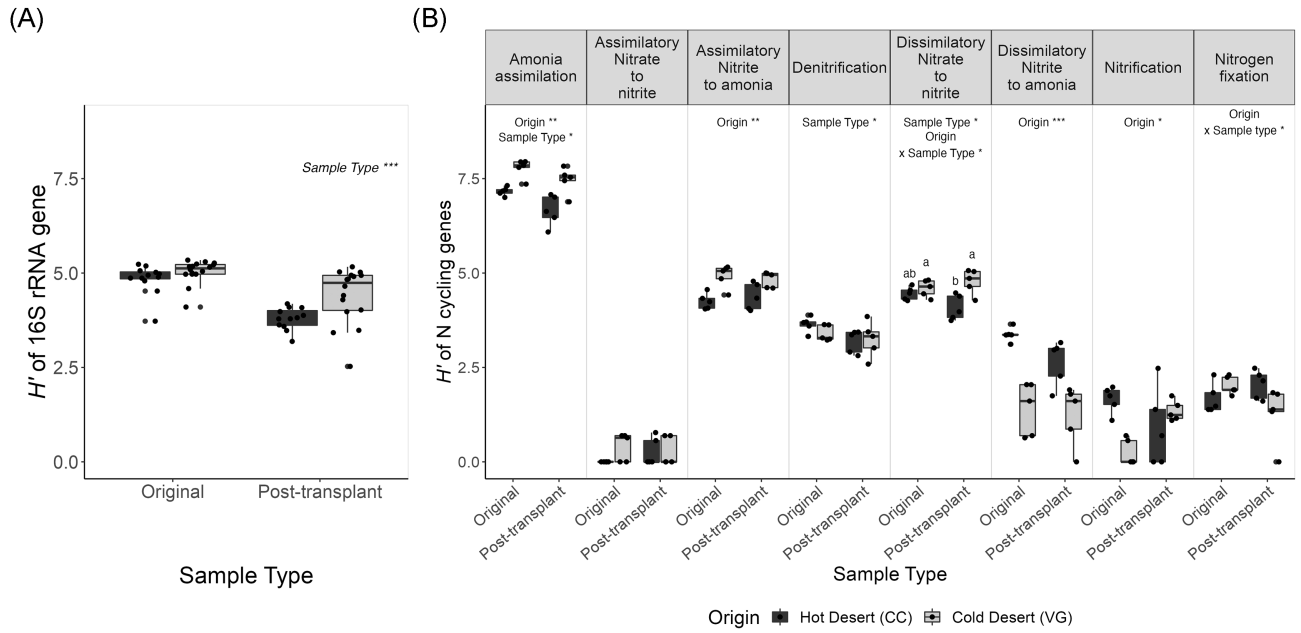

Figure 6. Effects of contrasting environmental conditions on taxonomic and functional gene diversity of BSCs. **(A)** Alpha diversity in the field setting experiment including hot desert (CC) and cold desert (VG) samples, diversity estimated based on 16S rRNA gene amplicon sequences. **(B)** Nitrogen cycling pathways gene diversity, estimates based on metagenomic sequences obtained from the field experiment. Letters show significant differences between treatments as detected by *post hoc* Tukey's HSD test, in the cases that the there was a significant interaction between the two factors. Significant terms are indicated with * ($P < .05$), ** ($P < .01$), and *** ($P < .001$). For nitrogen fixation no significant differences were detected by *post hoc* Tukey's HSD test.

Table 3. ANOVA tests evaluating the effect of the experimental factors on diversity (*H'*) of bacteria and nitrogen cycling genes.

Marker	**Factor**	***F***	***P***
Taxonomic (16S rRNA)	Origin	1.12	.294
	Sample type	27.78	**< .001**
	Origin × Sample type	2.48	.121
N cycling genes	**Factor**	***F***	***P***
Amonia assimilation	Origin	10.51	**.005**
	Sample type	7.13	**.017**
	Origin × Sample type	0.48	.497
Assmiliatory nitrate to nitirite*	Origin	3.89	.066
	Sample type	1.70	.211
	Origin × Sample type	1.85	.192
Assmiliatory nitirite to amonia	Origin	14.78	**.001**
	Sample type	0.52	.481
	Origin × Sample type	0.76	.396
Denitrification	Origin	1.39	.255
	Sample type	5.05	**.039**
	Origin × Sample type	1.05	.320
Dissimilatory nitrate to nitrite	Origin	0.67	.424
	Sample type	4.77	**.044**
	Origin × Sample type	5.20	**.037**
Dissimilatory nitrite to amonia	Origin	25.36	**< .001**
	Sample type	3.61	.075
	Origin × Sample type	1.07	.316
Nitrification	Origin	6.77	**.025**
	Sample type	0.14	.714
	Origin × Sample type	2.66	.131
Nitrogen fixation*	Origin	3.18	.095
	Sample type	3.24	.092
	Origin × Sample type	7.47	**.015**

Significant *P*-values (< .05) are highlighted in bold. Nitrogen cycling genes results come from the field setup. * denotes data sets that were log-transformed to fit a normal distribution.

(Table S2, Supporting Information). This may be explained by the different techniques employed to assess the BSC composition in this experiment. Nonetheless, we were able to detect significant changes in community composition.

In addition to that observed for taxonomic composition, metagenomic sequencing also revealed evidence of legacy effects in functional gene composition. Specifically, N cycling pathways in the BSCs did not converge to similar composition under the same environmental conditions leading to an interactive effect between origin and environment (Table 2, Figure S1, Supporting Information). Previous studies in BSCs have found that changes in taxonomic composition are reflected in changes in functional gene composition (Steven et al. 2014, 2015). Such correspondence between taxonomic and functional gene composition has been interpreted as evidence against functional redundancy (Galand et al. 2018). Thus, changes in climate, and in particular temperature, appear to select for traits that are correlated with N cycling genes, leading to both compositional and functional changes. Similarly, aridity selects for stress tolerance genes that are correlated with the overall functional gene composition of the communities (Song et al. 2019).

Finally, the alpha-diversity of some nitrogen cycling pathways was reduced when the BSCs were transplanted to a new environment (resulting in an origin by environment interaction) (Fig. 6B, Table 3). This trend suggests that while some taxa may have benefited from the new conditions, overall, more responded negatively. However, this trend was only significant for ammonia assimilation and denitrification genes. Ammonia assimilation genes are in fact distributed throughout the prokaryotic phylogenetic tree, whereas the rest of the nitrogen pathways are more narrowly distributed (Nelson et al. 2016), contradicting the idea that phylogenetically narrow functions are more likely to be affected by changes in community composition. At the same time, these N pathways were also quite abundant (Figure S2, Supporting Information); thus, the nonsignificant results in the other pathways may be explained by the depth of sequencing (and therefore, accurate assessment of the pathway richness) than any biological difference. Nonetheless, interpreting these results should be done cautiously, as it is still uncertain on how changes in the abundance and composition of functional genes affect process rates (Prosser 2015, Rocca et al. 2015).

Legacy effects on functioning

The responses in the taxonomic and functional composition of the BSCs communities paralleled their functional response. Potential nitrogen fixation rates of BSCs in the field experiment after 4 months of transplantation did not converge between samples. A similar pattern was observed for BSCs in the laboratory as well; after 180 days of incubation, potential nitrogen fixation rates did not converge between communities placed in the same temperature (Fig. 3, Table 1). In our experiments, the maximum levels of potential nitrogen fixation from all samples was achieved in their native (or closest to native) environment, suggesting that given an environmental perturbation or change, functionality was not resilient at least in the time frame of our study. This has been also observed in nitrogen cycling processes (Banerjee et al. 2016, Chen and Peng 2020) and in other processes such as microbial respiration (a broad function) (Göransson et al. 2013, Strickland et al. 2015, Martiny et al. 2017, Dacal et al. 2019, Min et al. 2019, Hawkes et al. 2020) with signatures of local specialization to soil moisture levels (Hawkes et al. 2017) where lack of resilience has been interpreted as evidence of legacy effects in function. Nonetheless, these interpretations should be taken cautiously, as resilience times for different conditions and functions may vary greatly, therefore, longer time frames may be required for solid conclusions in this regard (Shade et al. 2012).

Finally, we note that there are limitations to the reciprocal transplant design. In particular, an origin effect on potential nitrogen fixation could reflect differences in uncontrolled variables such as the physicochemical properties of the soil. Indeed, both pH and total organic carbon (TOC) differed between samples from the hot (CC) and cold (VG) desert, (Table S1, Supporting Information), whereas phosphorous availability did not. However, these differences persisted after the experiment, suggesting that the differences in soil properties did not cause of functional and compositional changes in the BSCs communities.

Conclusion

A prevalent paradigm in the ecosystem processes modelling has considered that such processes are mainly determined by environmental conditions (Bradford and Fierer 2012, Treseder et al. 2012). Both the compositional and functional legacies of the BSCs suggest that these communities are specialized to their native environment, and combined with limited dispersal, preclude convergence (at least in the time frame here studied) of the community to the native BSC community. If BSCs are not functionally redundant, then future BSC functioning under climate change scenarios cannot be predicted from their current distributions. Given the vast extension of land surface covered by arid ecosystems, changes in the biogeochemical cycling in these ecosystems are expected to have global repercussions (Poulter et al. 2014). Therefore, understanding when and where microbial diversity will influence ecosystem level processes may help to highlight areas for improvement of ecosystem and Earth system models predictions.

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Supplementary data

Supplementary data are available at *FEMSEC* online.

Conflict of interest statement. None declared.

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