Microbial secondary succession in soil microcosms of a desert oasis in the Cuatro Cienegas Basin, Mexico.

Ecological succession is one of the most important concepts in ecology. However for microbial community succession, there is a lack of a solid theoretical framework regarding succession in microorganisms. This is in part due to microbial community complexity and plasticity but also because little is known about temporal patterns of microbial community shifts in different kinds of ecosystems, including arid soils. The Cuatro Cienegas Basin (CCB) in Coahuila, Mexico, is an arid zone with high diversity and endemisms that has recently been threatened by aquifer overexploitation. The gypsum-based soil system of the CCB is one of the most oligotrophic places in the world. We undertook a comparative 16S rRNA 454 pyrosequencing study to evaluate microbial community succession and recovery over a year after disturbance at two sites. Results were related to concurrent measurements of humidity, organic matter and total C and N content. While each site differed in both biogeochemistry and biodiversity, both present similar pattern of change at the beginning of the succession that diverged in later stages. After one year, experimentally disturbed soil was not similar to established and undisturbed adjacent soil communities indicating recovery and succession in disturbed soils is a long process.
Nguyen E. López-Lozano, Karla B. Heidelberg, William C. Nelson, Felipe García-Oliva, Luis E. Eguiarte and Valeria Souza

1 Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, 04510, México

2 Department of Biology, University of Southern California, 3616 Trousdale Parkway, Los Angeles, CA 90089-0371, USA

3 Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, A.P. 27-3 (Santa María de Guido) 58090, Morelia, Mich., México.

*corresponding author: Tel.: 11 52 5556229006; Fax: 11 5255 5622 8995

e-mail: souza@servidor.unam.mx
Introduction

In ecological theory, succession is defined as the predictable manner by which communities change over time during the colonization of a new environment or following a disturbance (primary and secondary succession respectively) (Begon et al. 2006). Bacterial succession has been explored in a variety of environments and over different timescales (Fierer et al. 2010). However, there are many theoretical and methodological questions that remain unanswered. For example the order of occurrence and species turnover in bacterial successional stages can differ and be explained by stochastic or functional factors depending on the use of ecological or functional classifications instead of 16S rRNA taxonomic classifications (Burke et al. 2011a; Burke et al. 2011b). Notwithstanding, there are theoretical models for microbial community succession and assembly, most of them derived from those already well defined for macroorganisms (Prosser et al. 2007). Due to the complexity of the patterns observed in the succession of microbial communities, alternative explanations started to appear based on carbon (C) inputs to the systems, suggesting that species richness and the biomass of specific ecological groups change along successional stages (Fierer et al. 2010).

In recent years, the role of deterministic and stochastic processes in community assembly has been tested. It has been shown that bacterial community assemblages can be regulated by the local environment (species sorting) (Langenheder & Székely 2011), massive immigration that can prevent competitive exclusion of species (mass effect) (Lindström & Langenheder 2012), and neutral process, which assumes that all species are similar in their competitive ability and in dispersal. In the neutral process scenario, changes in community composition result from neutral drift over...
time. (Woodcock et al. 2007; Ofiteru et al. 2010). These mechanisms can co-occur, resulting in microbial communities being structured by more than one process (Langenheder & Székely 2011). However, the development of such theoretical models are based mostly on work done in laboratory environments, and little is known of what happens in natural systems such as soils (Caruso et al. 2011; Navarro et al. 2009; Nemergut et al. 2007; Schmidt et al. 2007). Data on how microbial communities change through time in natural environments are needed in order to refine these theoretical models.

Soils are among the most diverse microbial environments analyzed to date (Youssef & Elshahed 2009; Daniel 2005), which makes the identification of differences in community diversity patterns between stable and disturbed soils challenging. Arid soils are thought to have lower productivity and diversity than other soil habitats, and therefore may provide a better opportunity to validate molecular ecosystems approaches to examine the genetic and functional organization of native microbial consortia. Earth’s arid regions represent today nearly one third of total continental ecosystems (Collins et al. 2008) and are thought to be more vulnerable under most scenarios of global climate change (Intergovernmental Panel on Climate Change, 2007 report).

The Cuatro Cienegas Basin (CCB) in Coahuila, Mexico, is an arid zone that has recently been threatened by aquifer overexploitation. The gypsum-based soil system at CCB is one of the most oligotrophic environments in the world despite the high diversity in comparison with others arid soils (López-Lozano et al. 2012). Nevertheless, due to recent and ongoing overexploitation of the deep aquifer in this oasis, we decided to evaluate the community sensitivity after disturbance. In this study we experimentally
sampled and disturbed soil microcosms with gamma rays and sterilization. Samples were restored to their original site in a mesh bag that allowed migration. Then, community composition as well as nutrient dynamics were followed and compared to neighboring undisturbed soil samples. The objective of this study was to evaluate the recovery of microbial biodiversity and biogeochemical characteristics of the Churince within the CCB during a process of microbial secondary succession over one year period after disturbance. And to find out if microbial succession the shift pattern behaves like any of the previously proposed models. Our approach was designed to be experimentally analogous to sections of a forest cleared by removing the biota of an area, and tracking recolonization by migration.

**Materials and Methods**

**Site description and experimental design.** The sampling sites are located at the Churince oasis system on the west side of the Cuatro Cienegas Basin (CCB) at 740 m above sea level. This valley has < 15.0 cm of annual precipitation falling mainly during the summer. The dominant soil is gypsisol, and the predominant vegetation types are halophile and gypsophile grasses. As described before (López-Lozano et al. 2012), the Churince system consists of a spring, an intermediate lagoon, and a desiccation lagoon (Dry Lagoon), connected by short shallow creeks. Two sites separated by one kilometer were selected. One site borders a small river creek (River) (26°50′43″N/102°8′18″W), and has 60% of plant coverage. The grass *Sporobolus airoides* is the dominant plant. The other site is closer to a dry lagoon (26°50′53″N/102°8′52″W) and has only 10% of plant coverage. At this site the gypsophile grass, *Sesuvium erectum*, is the dominant plant species. The sites have alkaline soils with slight variation in the pH of 8.5-8.8.
In January of 2007, 20 kg of soil were collected from each site. The soil was mixed and used to construct microcosms of 1 kg of soil in permeable bags of nylon mesh (25 x 25 cm). The microcosms were sterilized by autoclaving followed by a dose of 25 kGy of gamma rays (at the Instituto Nacional de Investigaciones Nucleares, La Marquesa, México). One microcosm sample from each site was immediately used for DNA extraction as a control.

In February 2007, plots of 8 x 8 m were established at each site. The plots were divided in 64 quadrants of 1 m², and 40 microcosm bags were placed randomly within the plots as replicates. Every three months, three microcosm bags were collected randomly during a period of one year (3, 6, 9 and 12 months), 12 in total for each site. The remaining microcosms were deposited in the site in order to guarantee enough samples in each sampling period but not analyzed for this experiment. In addition, three samples from 15 cm³ of undisturbed soil next to the microcosms in each plot were randomly collected at the beginning and end (12 months) of the experiment to describe the community that potentially could colonize the microcosms. 50 g of each sample were stored in liquid nitrogen until DNA extraction. The remaining soil was stored in black plastic bags at 4ºC during one month until processing in the laboratory for biogeochemical analysis.

Soil biogeochemical analyses. Separate samples were oven-dried at 75 ºC to constant weight for soil moisture determination by the gravimetric method (Reynolds 1970). Measurements of All carbon (C) forms were determined with a Total Carbon Analyzer (UIC Mod. CM5012), while nitrogen (N) and phosphorus (P) forms were determined
using a Bran-Luebbe Auto Analyzer III (Norderstedt, Germany). Total and inorganic C
were determined by dry combustion and coulometric detection (Huffman 1977). Organic
C was calculated as the difference between total and inorganic C. N and P
concentrations were determined following acid digestion; N was determined using a
modified Kjeldahl method (Bremmer & Mulvaney 1982), and P was determined with the
molybdate colorimetric method following ascorbic acid reduction (Murphy & Riley, 1962).
Microbial C (micC) and microbial N (micN) concentrations were determined from
field-moist samples by a chloroform fumigation extraction method (Vance et al. 1987).

Inorganic N (NH4+ and NO3-) was extracted from fresh sub-samples with 2 M KCl,
followed by filtration through a Whatman No. 1 paper filter (Robertson et al. 1999), and
determined colorimetrically by the phenol-hypochlorite method. Dissolved organic C, N
and P were extracted in two grams of fresh material with deionized water after shaking
for 1 h, and filtering through a Whatman# 42 filter and a 0.45µm nitrocellulose
membrane (Jones y Willett, 2006). Dissolved organic C (DOC) was determined by
combustion and coulometric detection (Huffman 1977). Dissolved organic N (DON) and
dissolved organic P (DOP) were determined after acid digestion. DON was calculated as
the difference between digested soluble N and NH4+ in deionized water extracts. The
DOP was calculated as the difference between digested dissolved P and inorganic P (as
orthophosphate).

Statistical analyses. To evaluate significant differences in biogeochemical soil
parameters between sites and through the time during the microcosms experiment, we
analyzed biogeochemical variables with a repeated measures analysis of variance
(RMANOVA) with one between-subject factor (site: Dry Lagoon and River) and one within factor (sampling date: 3, 6, 9 and 12 months). In order to compare biogeochemical soil parameters between sites, and between disturbed and undisturbed soil in the last sampling date (12 months), we carried out a two-way analysis of variance (ANOVA) (factor 1 levels: sites Dry Lagoon and River; factor 2 levels: undisturbed soil and microcosm).

The relationship between microbial community composition in terms of the most abundant bacterial families, soil characteristics and samples (date and site) was analyzed by canonical correspondence analysis (CCA). In this analysis, the samples are represented by a centroid. Its position is indicative of the relationship between a specific sample and either of the ordination axes. Soil characteristics are represented by vectors. Vectors of greater magnitude and forming smaller angles with an ordination axis are more strongly correlated with that ordination axis. High scores of absolute value for a given family or a given site on a CCA axis indicate that it is highly related to the axis and to the environmental variable exhibiting high correlation to the axis. All soil characteristics were tested for significant contribution to the explanation of the variation in bacterial family community composition with an ANOVA like permutation test to assess the significance of constraints. Only variables that were significant by the permutation test at the $P \leq 0.05$ level were included in the CCA biplot. The CCA was performed using the package Vegan in R (http://www.r-project.org/). In the supplementary material, we added a DCA analysis without the restrictive variables for comparison (figure S2).

**DNA Extraction.** Total DNA was extracted using the Soil Master DNA Extraction Kit
(EPICENTRE Biotechnology) according to the manufacturer’s instructions, with an additional step of bacterial isolation using the fractionation centrifugation technique described in (Holben et al. 1988). This step was performed on frozen 50 g soil samples before DNA extraction as a way of reducing the remaining concentrations of salts, polysaccharides and secondary compounds. DNA was stored at -20 °C.

In order to confirm sterilization in the control microcosms, the DNA region coding for 16S rRNA was PCR amplified using universal primers (Tables S1 and S2). Since no band was recovered in a PCR gel run using the sterilized soil, the sterilization was assumed to be effective.

Pyrosequencing of 16S rRNA tags. 16S rRNA genes were amplified from each sampling date (pooled DNA from the three subsamples was used as template) using the 939F (TTGACGGGGGCCCGCAC) and 1492R (TACCTTGTTACGACTT) paired primers. Sequencing was undertaken using the standard Roche 454 Titanium LIB-A kit with multiplex identifier sequence (MID) tags (Table S3) (Sun et al. 2011) at the Research and Testing Laboratory (Lubbock, TX).

Bioinformatic analysis of barcoded 16S ribosomal RNA gene libraries: Mothur open-source software package (v 1.15.0) (Schloss et al. 2009) was used for processing, clustering, and classification of the quality screened sequence data. Raw sequences were screened for potential chimeric reads using Chimera.slayer (Haas et al. 2011) and the linked SILVA template database (26%). Sequences containing homopolymer runs of 9 or more bases, those with more than one mismatch to the sequencing primer and those longer than 565 bp were eliminated. Group membership was determined prior to
trimming of the bar-code MID and primer sequence. Sequences were aligned against the manually curated bacterial SILVA 16S rRNA gene template using the nearest alignment space termination (NAST) algorithm (Schloss 2010; DeSantis et al. 2006), and manually trimmed for the optimal alignment region (start = 28596, end = 38347), which yielded aligned fragments of 270 bp long, including both the V6 and V7 regions. Pre-clustering, based on the SLP clustering algorithm (Huse et al. 2010), was used to reduce the effect of pyrosequencing errors on subsequent analysis. A pairwise distance matrix was calculate across the non-redundant sequence set, and reads were clustered into operational taxonomic units (OTUs) at 3% distance using the furthest neighbor method (Schloss & Handelsman 2006). This matrix was used to calculate the similarity based on the Bray-Curtis and Jaccard Index between the samples in each site and to create a cluster diagram of sample similarity. The sequences and OTUs were categorized taxonomically using Mothur’s Bayesian classifier and the SILVA bacterial reference set. A single representative sequence from the center of each OTU grouping was classified using a naïve Bayesian approach (Wang et al. 2007). The taxonomic reference database was composed of unique, full-length sequences from the SILVA SSU Ref v.106 database (http://www.mothur.org/wiki/Silva_reference_files) (Pruesse et al. 2007). To account for the effects of different sequencing depths on the alpha-diversity measurements, the sample sets were normalized to equal abundance of the sample with the least sequencing effort (Dry Lagoon, 9 months; 10,441 sequences; Table S3). Alpha-diversity statistics including Chao1 non-parametric species richness estimate (calculates the estimated true species diversity of a sample based in the number of singletons and doubletons), Shannon indices, Shannon-based richness estimate (diversity indices as a quantitative measure that reflects how many different OTUs there are in the dataset, simultaneously takes into account how evenly the OTUs are
distributed), and Good’s coverage estimate (what—percent of the total OTUs—is
represented in the sample), were generated for each sample using the same program.

The GenBank accession numbers for 16S rRNA pyrosequences in this work are in the
BioProject: PRJNA167137. The samples identifications for microcosms in the Dry
Lagoon are: before sterilization SRS346170, at 3 months SRS346171, at 6 months
SRS346172, at 9 months SRS346173 and at 12 months SRS346174. The samples ID
for microcosms in the River are: before sterilization SRS346176, at 3 months
SRS346177, at 6 months SRS346178, at 9 months SRS346179 and at 12 months
SRS346180. The samples ID for the undisturbed adjacent soil without disturb at 12
months are SRS346175 for the Dry Lagoon, and SRS346181 for the River.

Results

Nutrient fluctuations during the secondary succession. In order to understand the
relationships between the changes in community composition and nutrient
transformations that occurred during the secondary succession process in the Churince
soils, the soil samples were biogeochemically characterized and compared. We found
that the CCB soil at the Dry Lagoon site was more oligotrophic (C:N:P ratio of 125:5:1)
than the River site (C:N:P ratio 300:16:1) and in comparison with a general “average"
soil C:N:P of 186:13:1 (Cleveland & Liptzin 2007). RMANOVA analysis revealed
consistent differences in the soil nutrients between sites across time (Table 1). The Total
Organic C was higher at the site adjacent to the River throughout the sampling, even
though it showed a fluctuating pattern during the secondary succession experiment,
probably due to both seasonal effects and succession stages (Table 1). The same
pattern was observed for Total N and Total P. On the other hand, microbial C (micC) as
an indicator of microbial biomass showed similar fluctuations in both sites (Table 1). Values increased during the first three and six months after sterilization, then micC decreased at 9 months and increased again in the last sampling date. Ammonium showed contrasting patterns between sites. At the Dry Lagoon, ammonium increased showing higher values at 6 months, and then decreased gradually. At the River, ammonium increased gradually to drop in the last sampling. The microbial N (micN) increased in samples near the River, but presented fluctuations in the Dry Lagoon. Only the nitrate was higher in the Dry Lagoon microcosms but there were not significant differences by time. ANOVA analysis of soil nutrients in microcosms samples and undisturbed adjacent soil at 12 months showed significant differences between treatments in Total Organic C, dissolved organic N (DON), dissolved organic P (DOP) and ammonium (Table 2). However, undisturbed soil samples were similar to the perturbed samples in their total N and P content (TN, TP and DIP) (Table 2). These data suggests that, in general, the sampling sites are different and as such, respond differently to perturbation.

Evaluation of community structure by 16S rRNA amplicons. The number of high quality sequences per library ranged from 10,441 to 41,074 per sample (Suppl. Table S3). Once the dataset was normalized to the sample with the lowest sequencing effort, 79,303 OTUs at 97% similarity were identified. The majority of sequences (> 80%) belonged to one of the nine major phyla (arranged in order of abundance): Acidobacteria, Proteobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Deinococcus-Thermus, Gemmatimonadetes, Actinobacteria, or Candidatus division TM6 (Table 3, figure S1). A higher number of unclassified sequences were observed at the Dry Lagoon site than in the River site. Interestingly, none of the sequences belonged to
any eukaryotic specie which mitochondria and chloroplast would be amplified by these techniques. Suggesting that while wind and water could help the colonization processes; nematodes and other invertebrates did not play an important role in the migration of bacteria between disturbed and undisturbed sites.

Despite the differences observed in nutrient content between sites, the 16S rRNA sequence analysis showed similar variations in the diversity and community composition of the sites, even though the overall diversity was higher in the River during the entire sampling period (Table 4, Figure 1). At both sites, during the first six months (3 and 6 months samples) there was an increase in diversity, then the diversity decreased by nine months and increased again after one year (12 months) following a similar fluctuating pattern to the biomass. Contrasting the diversity in the last sampling date with the diversity of the adjacent undisturbed soil, we observed that the undisturbed soil had higher diversity than microcosms samples (Table 4).

Prior to disturbance, both Dry Lagoon and River communities were dominated by Acidobacteria (44% and 60%, respectively) (Table 3). The Dry Lagoon had a large proportion of bacteria that were unclassified at the 97% identity threshold (around 12 to 33% of the sequences). Proteobacteria were also abundant, comprising 9% of the Dry Lagoon sequences and 35% of the River sequences. Overall there was a gradual increase in the abundance of the Bacteroidetes during the succession process, which were not abundant in the first sampling (less than 0.24%) but increased with time (around 7-9%). Autotrophic phyla such as Chlorobi, Chloroflexi and Cyanobacteria, increased in abundances the first nine months and then decrease their abundance in the last sampling (12 months) (Table 3).
A more detailed analysis at the family level showed that the families present before the sterilization in low abundance were not found after 3 months, but many of them were recovered by 6 months and onward (Table S4). The initial colonizers at 3 months were different between sites, but in both cases, the majority of the identified families were related to known opportunistic heterotrophs. In the Dry Lagoon the most abundant families were Moracellacea, Streptococcaceae, Alcaligenaceae, Pseudomonadaceae, Micrococcaceae and Bacillaceae. At the River site at 3 months, Holophagaceae, Acidobacteriaceae, GIF3 (Chloroflexi), Enterobacteriaceae, Streptococcaceae, Listeriaceae and Bacillaceae were found. By 6 months the abundance of unclassified taxa increased, but also the abundance of families that include previously described members with other metabolic capabilities besides heterotrophy (Table S4). The relative abundances of some bacterial groups at different taxonomic levels correlated significantly with Total Organic C, Total N, humidity and NO$_3^-$ (Table 5), and these trends were corroborated with the CCA (Figure 2).

Similarity between sites and samples through the time, measured with the Bray-Curtis distance and Jaccard coefficient using OTUs at 97% of identity, achieved a peak three months after sterilization, while it was lower before sterilization and six, and nine months after sterilization. After a year the microcosms communities were more similar to the communities from undisturbed soil at each site (Figure 3).

Discussion

Microbial diversity and secondary succession in a gypsum based soil. In this study we
analyzed the bacterial secondary succession of experimentally disturbed soil microcosms at two sites over one year. While each site differed in both biogeochemistry and biodiversity, both present similar community composition at the beginning of the experiment. However, successional patterns differed in later stages. This suggests an species sorting effect at the beginning of the experiment, followed by a more neutral process in later stages. At the end of the experiment, comparing the adjacent soil samples with the experimentally disturbed soil, the microbial communities at either site do not resemble adjacent undisturbed microbial communities. Both disturbed soils were most closely related to each other (Figure 3).

Four factors contribute strongly to differences in our sites: humidity, Total N, Total Organic C and Total P. The first three factors were identified as the most correlated variables with the relative abundance of the bacterial groups. These factors have been considered as part of the best group of soil variables for predicting microbial community composition in arid lands (Collins et al. 2008; Wall & Virginia 1999). We also found that Total P in the soil explained some variability. This was expected, as P is the most limiting nutrient in CCB (Elser et al. 2005).

In a previous 16S rRNA Sanger-based clone library analysis (López-Lozano et al., 2012), 15 phyla and 40 classes were identified from 293 sequences in the harsher Dry Lagoon. The River site had 16 phyla and 36 classes identified from 223 sequences. Even with the higher sequencing effort afforded by 454 pyrosequencing, we still only captured 52% to 81% of the microbial communities in our samples (Figure 1 and Table 4). This was surprising, since in more fertile and wet soils analyzed with similar sequencing effort, the maximum number of OTUs at 97% of identity seldom exceeds
~5,600 OTUs (Roesch et al. 2007), while in our more diverse sample, we estimated ~9,000 OTUs at 97% identity. However, the sites analyzed in this study differed in diversity, with soil adjacent to the River being more diverse than the Dry Lagoon if we compare the OTUs at 97% (Table 4). In the previous clone library study the same pattern was observed (López-Lozano et al. 2012). We also evaluated the community composition in terms of “ecological groups”, considering groups in which abundance correlated with high or low C mineralization rates (copiotrophs or oligotrophs, respectively) in general soil surveys (Fierer et al. 2007, 2010), and widely known autotrophs (phototrophs such as Cyanobacteria and Chloroflexi). Also we determined some metabolic capabilities by comparison of the sequences with the closer cultivated organisms. Despite the broad ecological classification based only in 16S rRNA gene sequences, our results showed interesting trends in groups considered as oligotrophs, copiotrophs (organism that need more nutrients) as well as heterotrophs and autotrophs.

The 16S rRNA libraries of both sites are dominated by Acidobacteria in all samples. This is one of the most common phyla found in soil libraries worldwide (Janssen 2006). The Acidobacteria are in general oligotrophic (Eichorst et al. 2007; Fierer et al. 2007) and have been shown to comprise 50% of clone libraries in arid soils (Dunbar et al. 1999; Kuske et al. 1997), but they are less abundant in more nutrient-rich agricultural soils (Nagy et al. 2005; Roesch et al. 2007). Hence, it is not surprising that they represent ~30-60% of bacteria in all our libraries in CCB sampling sites. In contrast, Bacteroidetes have been classified as copiotrophs in general (Fierer et al. 2007). In our libraries Bacteroidetes become much more abundant at the 6 months sampling, and it is possible that these phyla appear when the accumulation of organic material is enough to sustain the mineralization rate of this group. In fact, between 6 and 9 months there was a drop
in the Dissolved Organic C while the microbial C increase in the soil microcosms. Autotrophic groups, such as Cyanobacteria are abundant at both sites. This phylum is common in most environments including soil and biological soil crust of arid zones (Gundlapally & Garcia-Pichel 2006; Nagy et al. 2005). The abundance of yet another autotrophic group, the Chloroflexi, increased during the initial stages of succession (3-9 months) but decreased in the last sampling date (12 months). With the exception of high Chloroflexi in the River sample during the first samplings, the majority of the colonizers are opportunistic heterotrophs (a greater part members of Firmicuttes, Betaproteobacteria and Gammaproteobacteria). It is possible that especially in the Dry Lagoon site, the initial colonizers were benefited by the Dissolved Organic C, released during the sterilization process. The nutrient pulse could have facilitated colonization by heterotrophs that benefited from available resources in the disrupted microcosm. The finding of preferential growth of opportunistic heterotrophs during early succession agrees with the findings from another study using outdoor sterile microcosms seeded by rainwater bacteria (Langenheder & Székely 2011). These authors found that neutral and species sorting processes interacted during the assembly of bacterial communities, and the importance of each depended on how many generalists and specialists were present in the community. We suggest that in our microcosms, the initial faster growing community (generalists) depleted the nutrients, and then were out competed by groups that have alternative energy sources (specialists). At 6 months the abundance of groups with more specialized metabolic capabilities increased, and the taxa with low abundances before the sterilization, “the rare biota” that were not present after three months returned to the community from this sampling date. The observed nitrate accumulation in the Dry Lagoon site could be due to an increase in nitrification, an alternative mechanism of obtaining energy that could persist under those conditions.
(Montaño et al. 2007). Also, the higher DOP in the Dry Lagoon site suggests that P mineralization is not occurring as efficiently as in the River site, perhaps because of limited energy for exo-enzyme production necessary for acquisition (F. Garcia-Oliva, personal communication). Hence, the Dry Lagoon site might be supporting a more oligotrophic microbiota than the River site. Conversely, changes in DOC at the River site correlated with changes in microbial C. Additionally, ammonium correlated with microbial N, suggesting that the initial surge of nutrients could have facilitated a faster migration of the neighboring heterotrophic biomass in this site than in the Dry Lagoon site. Photosynthetic autotrophs (such as Chloroflexi) seem to be sufficient for nurturing the community in the River site providing C sources, since nitrates and DOP are scarce, indicating low nitrification rates.

Regarding the diversity shifts, both sites showed similar patterns. There is an initial increase in the Shannon index, followed by a decrease at 9 months and an increase at 12 months. We do not discard the possibility that these convergent patterns only in the diversity index shifts can be related to climatic factors. At nine months a decrease in diversity was observed, which coincided with an unusually heavy precipitation event (Table S5).

The Bray-Curtis similarity index and Jaccard similarity coefficient analyses, suggest that even though the initial communities are similar in composition, they differ in later stages. In addition, the analysis by phylogenetic and ecological groups, suggest that the communities respond in a similar trajectory of initial colonization first by heterotrophic generalists and later specialists. However the River site was characterized by higher nutrients and diversity, together with the continuous presence of Cloroflexi in high
abundance suggest an early food web based on primary production. This pattern is similar to the early stages of the autotrophic succession suggested by Fierer et al. (2010). Our experimental design cannot be used to test this autotrophic model, but our data show that the colonizers were a mix of heterotrophs and autotrophs.

In this study we found community composition of the soil microcosms after perturbation did not recover to similar communities found in the undisturbed soil community after one year. We are not assuming that there is only one “climax” community in terms of composition. What we suggest is that a community would be “recovered” when it has similar performing conditions to the neighboring undisturbed community. The performance of the community can be inferred by the soil nutrient content and characteristics. Due to the significant differences in physicochemical parameters between microcosms and control sites at 12 months, we can conclude that despite the diversity found at the Churince soils, the small patches (1 kg mesh bags) did not recover to resemble undisturbed soil in either site, after a year of migration and succession. Nevertheless, it seems they followed a parallel paths for such recovery at the beginning of the succession and diverged in later stages.

Conclusions

This is the first study on bacterial soil dynamics conducted at the CCB. Our results provide important insight into microbial community dynamics in response to a disturbance (secondary succession). In this study we found changes in community composition across time that were indicative of the successional process. Our data showed evidence of similar initial colonizers in both sites, but the divergence in later successional stages reflects stochastic factors suggesting a species sorting and neutral
effects. While community descriptions based only on 16S rRNA gene analysis do not reflect the full metabolic plasticity of the communities, this study provides important insight into microbial succession patterns. For further research metagenomics and measures of the rates of the physiological traits are necessary to corroborate our results. Notwithstanding, it is interesting that succession of small, perturbed spots is very slow; this reveals the temporal scale is important for this community in terms of resilience, but general long-term monitoring is necessary to better understand the temporal patterns and natural variability of this area. Changes in microbial communities due to disturbance may directly affect ecosystem processes, which are vital in a protected natural area, threatened by over-exploitation of aquifers such as is occurring in the CCB.

Acknowledgements: We are grateful to Rodrigo González Chauvet, German Bonilla Rosso and other members within the laboratory of Evolución Molecular y Experimental for assistance with the sample collection; to Celeste Martínez-Piedragil, Rodrigo Velázquez-Durán and Maribel Nava-Mendoza for assistance with soil chemical analysis; to Laura Espinosa Asuar and Erika Aguirre Planter for help with molecular, sequencing work and logistic support; to Ana Gutiérrez-Preciado and Jaime Gasca-Pineda for bioinformatics assistance. We specially thank the APFF of Cuatro Cienegas for their support and logistics.

References

Begon M, Townsend CR & Harper JL (2006) Ecology: from individuals to ecosystems. 4th ed. Wiley-Blackwell.

Bremmer JM & Mulvaney CS (1982) Total Nitrogen. Methods of Soil Analysis Agronomy
Burke C, Steinberg P, Rusch D, Kjelleberg S & Torsten T (2011) Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences* 108: 14288–14293.

Burke C, Thomas T, Lewis M, Steinberg P & Kjelleberg S (2011) Composition, uniqueness and variability of the epiphytic bacterial community of the green alga Ulva australis. *ISME Journal* 5: 590–600.

Caruso T, Chan Y, Lacap DC, Lau MCY, McKay CP & Pointing SB (2011) Stochastic and deterministic processes interact in the assembly of desert microbial communities on a global scale. *ISME Journal* 5: 1406–1413.

Cleveland CC & Liptzin D (2007) C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85: 235–252.

Collins SL, Sinsabaugh RL, Crenshaw C, Green L, Porras-Alfaro A, Stursova M & Zeglin LH (2008) Pulse dynamics and microbial processes in aridland ecosystems. *Journal of Ecology* 96: 413–420.

Daniel R (2005) The metagenomics of soil. *Nature Reviews Microbiology* 3: 470–478.

DeSantis T Z et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* 72: 5069–5072.

Dunbar J, Takala S, Barns S M, Davis J a & Kuske C R (1999) Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. *Applied and Environmental Microbiology* 65: 1662–1669.
Eichorst S a, Breznak J a & Schmidt TM (2007) Isolation and characterization of soil bacteria that define Terriglobus gen. nov., in the phylum Acidobacteria. *Applied and Environmental Microbiology* 73: 2708–2717.

Elser JJ, Schampel JH, Garcia-Pichel F, Wade BD, Souza V, Eguiarte L, Escalante A & Farmer JD (2005) Effects of phosphorus enrichment and grazing snails on modern stromatolitic microbial communities. *Freshwater Biology* 50: 1808–1825.

Fierer N, Bradford M a & Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* 88: 1354–1364.

Fierer N, Nemergut Diana, Knight R & Craine JM (2010) Changes through time: integrating microorganisms into the study of succession. *Research in Microbiology* 161: 635–642.

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. *Microbial Ecology* 52: 345–357.

Haas BJ et al. (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR ampicons. *Genome research* 21: 494–504.

Holben WE, Jansson JK, Chelm BK & Tiedje JM (1988) DNA Probe Method for the Detection of Specific Microorganisms in the Soil Bacterial Community. *Applied and Environmental Microbiology* 54: 703–711.

Huffman EWD (1977) Performance of a new automatic carbon dioxide coulometer. *Microchemical Journal* 22: 567–573.

Huse SM, Welch DM, Morrison HG & Sogin ML (2010) Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environmental Microbiology* 12:
1889–1898.

Janssen PH (2006) Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Applied and Environmental Microbiology* 72: 1719–1728.

Kuske C.R., Barns S.M. & Busch JD (1997) Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. *Applied and Environmental Microbiology* 63: 3614.

Langenheder S & Székely AJ (2011) Species sorting and neutral processes are both important during the initial assembly of bacterial communities. *ISME Journal* 5: 1086–1094.

Lindhström ES & Langenheder S (2012) Local and regional factors influencing bacterial community assembly. *Environmental Microbiology Reports* 4: 1–9.

López-Lozano NE, Eguiarte LE, Bonilla-Rosso G, García-Oliva F, Martínez-Piedragil C, Rooks C & Souza V (2012) Bacterial communities and the nitrogen cycle in the gypsum soils of cuatro ciénegas basin, coahuila: a Mars analogue. *Astrobiology* 12: 699–709.

Montaño NM, García-Oliva F & Jaramillo VJ (2007) Dissolved organic carbon affects soil microbial activity and nitrogen dynamics in a Mexican tropical deciduous forest. *Plant and Soil* 295: 265–277.

Murphy J & Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27: 31–36.

Nagy ML, Pérez A & Garcia-Pichel F (2005) The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). *FEMS Microbiology Ecology* 54: 233–245.
Navarro JB, Moser DP, Flores A, Ross C, Rosen MR, Dong H, Zhang G & Hedlund BP (2009) Bacterial succession within an ephemeral hypereutrophic Mojave Desert playa Lake. *Microbial Ecology* 57: 307–320.

Nemergut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A & Schmidt SK (2007) Microbial community succession in an unvegetated, recently deglaciated soil. *Microbial Ecology* 53: 110–122.

Ofiteru ID, Lunn M, Curtis Thomas P, Wells GF, Criddle CS, Francis C a & Sloan WT (2010) Combined niche and neutral effects in a microbial wastewater treatment community. *Proceedings of the National Academy of Sciences of the United States of America* 107: 15345–15350.

Prosser JI et al. (2007) The role of ecological theory in microbial ecology. *Nature reviews. Microbiology* 5: 384–392.

Pruesse, E., C. Quast, K. Knittel, B. Fuchs, W. Ludwig JPFOG (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* 35: 7188–7196.

Reynolds S (1970) The gravimetric method of soil moisture determination Part IA study of equipment, and methodological problems. *Journal of Hydrology* 11: 258–273.

Roesch LFW et al. (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME Journal* 1: 283–290.

Schloss PD et al. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75: 7537–7541.

Schloss PD (2010) The effects of alignment quality, distance calculation method,
sequence filtering, and region on the analysis of 16S rRNA gene-based studies. 

*PLoS Computational Biology* 6: e1000844.

Schloss, PD & Handelsman J (2006) Introducing SONS, a tool for OTU-based comparisons of membership and structure between microbial communities. *Applied and Environmental Microbiology* 72: 6773–6779.

Schmidt S, Costello E & Nemergut DR (2007) Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* 88: 1379–1385.

Sun Y, Wolcott RD & Dowd SE (2011) Tag-Encoded FLX Amplicon Pyrosequencing for the Elucidation of Microbial and Functional Gene Diversity in Any Environment. *Methods in Molecular Biology* 733: 129–141.

Vance E, Brookes P & Jenkinson D (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19: 703–707.

Wall DH & Virginia RA (1999) Controls on soil biodiversity: insights from extreme environments. *Applied Soil Ecology* 13: 137–150.

Wang Q, Garrity GM, Tiedje JM & Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73: 5261–5267.

Woodcock S, van der Gast CJ, Bell T, Lunn M, Curtis Thomas P, Head IM & Sloan WT (2007) Neutral assembly of bacterial communities. *FEMS Microbiology Ecology* 62: 171–180.

Youssef NH & Elshahed MS (2009) Diversity rankings among bacterial lineages in soil. *ISME Journal* 3: 305–313.
Figure 1

sampling effect

Rarefaction curves of the A) Dry Lagoon and B) River in all sampling dates (3, 6, 9 and 12 months). OTUs were determined at 97% sequence identity.
**Figure 2**

relationship between diversity and environment

CCA ordination biplot of the most abundant family composition during the secondary sucessional process. Vectors represent the soil characteristics, while centroids indicate different 454 pyrosequence 16S rRNA gene amplicon libraries across the time. Temporal replicates that are close together on the ordination diagram are more similar (in terms of their microbial community structure) than replicates that are farther apart.
Figure 3

relationship between samples

Figure 3. Clustering of the 16S rRNA community composition at 97% similarity based on the Bray-Curtis algorithm of ecotype abundance. The cluster diagram of sample similarity generated using the Jaccard Index showed the same pattern (data not shown).
Table 1 (on next page)

Soil and nutrients

Soil biogeochemical parameters in each sampling date for both sites. Statistical analysis was done as a 2 way ANOVA. Numbers represent the mean ± SD with F values and significance levels: * = P < 0.05, ** = P < 0.01, *** = P < 0.0001, ns = not significant.
Table 1. Soil biogeochemical parameters in each sampling date for both sites Dry Lagoon and River. Analysis was done as a 2 way ANOVA, the mean ± SD with F values and significance levels: * = P < 0.05, ** = P < 0.01, *** = P < 0.0001, ns = not significant.

| Effect | Dry Lagoon | River | Site | Time | site*time | F values |
|--------|------------|-------|------|------|----------|----------|
| Sterilized soil | 0.3±0.13 | 0.15±0.01 | 0.02±0.00 | 1 | 0.4±0.1 | 508±53 | 19.76** | 3.91* |
| TOC (mg g⁻¹) | 1.8±0.60 | 0.16±0.06 | 0.01±0.00 | 1 | 0.08±0.08 | 817.4±14 | 41.44*** | 10.68*** | 7.67*** |
| TN (mg g⁻¹) | 2.5±0.03 | 0.23±0.01 | 0.05±0.00 | 1 | 0.1±0.01 | 218.4±26 | 10.98** | 3.37* | 0.36ns |
| TP (mg g⁻¹) | 3.9±0.20 | 2 | 2 | 1 | 0.007±0.00 | 6 | 10.98** | 3.37* | 0.36ns |
| DOC (μg g⁻¹) | 1.01±0.03 | 1.02±0.06 | 0.005±0.00 | 1 | 0.007±0.00 | 170±206 | 10.98** | 3.37* | 0.36ns |
| DON (μg g⁻¹) | 6.5±0.60 | 0.6±0.08 | 0.08±0.04 | 1 | 0.01±0.00 | 239±33 | 10.98** | 3.37* | 0.36ns |
| DOP (μg g⁻¹) | 9.8±0.86 | 0.6±0.03 | 0.03±0.00 | 1 | 0.015±0.00 | 712±132 | 10.98** | 3.37* | 0.36ns |
| Ammonium (μg g⁻¹) | 2.9±0.20 | 0.1±0.2 | 0.02±0.00 | 1 | 0.007±0.00 | 830±375 | 10.98** | 3.37* | 0.36ns |
| Nitrates (μg g⁻¹) | 6.2±0.20 | 0.0±0 | 0.0±0 | 1 | 0.0±0 | 94.3±47 | 10.98** | 3.37* | 0.36ns |
| micC (μg g⁻¹) | 0±0 | 0.0±0 | 0.0±0 | 1 | 0.0±0 | 3.75ns | 10.98** | 3.37* | 0.36ns |
| micN (μg g⁻¹) | 0±0 | 0.0±0 | 0.0±0 | 1 | 0.0±0 | 9.37** | 10.98** | 3.37* | 0.36ns |
Changes in biogeochemical parameters

Mean ± SD of biogeochemical parameters of microcosms and undisturbed soil samples of each site for the last sampling date, 12 months (n=3). A two way ANOVA was used for statistical comparison and results are presented as F values with significance levels: ∗ = P < 0.05, ∗∗ = P < 0.01, ∗∗∗ = P < 0.0001, ns = not significant.
Table 2. Biogeochemical parameters of microcosms and undisturbed soil samples of each site for the last sampling 12 months (n=3). Mean ± SD with F values and significance levels: * = P < 0.05, ** = P < 0.01, *** = P < 0.0001, ns = not significant a.

| Effect | Treatment       | Site          | F values |
|--------|-----------------|---------------|----------|
|        | Dry Lagoon undisturbed | Dry Lagoon microcosms | River undisturbed | River microcosms |        |
| TOC (mg g⁻¹) | 2.9±0.3 | 2.9±0.2 | 23.2±3.9 | 4±1.7 | 19.7** | 24.8** |
| TN (mg g⁻¹)  | 0.2±0.005 | 0.24±0.015 | 1.1±0.2 | 0.7±0.06 | 5.02ns | 54.7*** |
| TP (mg g⁻¹)   | 0.023±0.001 | 0.024±0.001 | 0.06±0.008 | 0.04±0.006 | 2.3ns | 26.9*** |
| DOC (μg g⁻¹) | 13.5±9.4 | 94.3±35.4 | 20.4±9.4 | 110±12.3 | 0.2ns | 38.8*** |
| DON (μg g⁻¹)  | 1.4±0.5 | 8.2±4.5 | 0.5±0.12 | 9.5±0.6 | 24.2*** | 0.15ns |
| DIP (μg g⁻¹)  | 0.0±0 | 0.005±0.003 | 0.0±0 | 0.004±0.004 | 3.7ns | 0.36ns |
| DOP (μg g⁻¹)  | 1.5±0.04 | 1.8±0.06 | 1.1±0.05 | 1.43±0.04 | 45.5*** | 77.82*** |
| Ammonium (μg g⁻¹) | 0.95±0.2 | 1.3±0.1 | 0.6±0.1 | 1.45±0.1 | 13.4** | 0.26ns |
| Nitrates (μg g⁻¹) | 0.8±0.4 | 0.34±0.2 | 0.0±0 | 0.0±0 | 0.86ns | 5.4* |
| MicC (μg g⁻¹)  | 43.5±20 | 92.1±25.7 | 206±17 | 219±38 | 1.4ns | 32*** |
| MicN (μg g⁻¹)   | 4.9±0.7 | 2.1±0.3 | 11.2±2.6 | 5.5±0.5 | 9.1* | 12.3** |
Table 3 (on next page)

Microbial diversity

Relative abundance of bacterial phyla for each sample. Values represent the percentage of each group in the respective library.
Table 3. Distribution of bacterial phyla. The relative abundance of bacterial phyla in each of the sampling dates and site. Values represent the percentage of each group in the respective library.

**Dry lagoon**

| Phylum               | Before sterilization | 3 months | 6 months | 9 months | 12 months | Undisturbed soil | Before sterilization | 3 months | 6 months |
|----------------------|----------------------|----------|----------|----------|-----------|------------------|----------------------|----------|----------|
| Acidobacteria        | 44.41                | 30.56    | 42.02    | 24.26    | 42.68     | 33.36            | 59.60                | 49.32    | 47.45    |
| Bacteroidetes        | 4.68                 | 0.24     | 2.40     | 7.40     | 7.34      | 3.16             | 9.03                 | 0.00     | 5.35     |
| Chloroflexi          | 0.98                 | 0.01     | 1.25     | 4.61     | 0.97      | 9.36             | 2.05                 | 8.37     | 2.85     |
| Gemmatimonadetes     | 0.70                 | 0.02     | 1.71     | 0.82     | 1.48      | 0.48             | 1.92                 | 0.02     | 3.25     |
| Deferrribacteres     | 0.00                 | 0.00     | 0.00     | 0.00     | 0.00      | 0.02             | 0.90                 | 1.08     | 1.62     |
| TM6                  | 0.46                 | 0.00     | 0.13     | 4.53     | 0.51      | 0.80             | 0.89                 | 0.02     | 0.94     |
| Firmicutes           | 0.31                 | 10.73    | 0.59     | 2.04     | 0.37      | 2.44             | 0.82                 | 21.69    | 0.86     |
| Actinobacteria       | 0.63                 | 4.69     | 1.00     | 0.74     | 0.43      | 1.74             | 0.60                 | 0.19     | 1.24     |
| Deinococcus-Thermus  | 2.80                 | 0.33     | 6.09     | 2.26     | 0.69      | 1.21             | 0.45                 | 0.00     | 6.27     |
| Cyanobacteria        | 0.20                 | 0.31     | 0.20     | 0.06     | 0.21      | 0.46             | 0.39                 | 0.16     | 0.55     |
| Verrucomicrobia      | 0.08                 | 0.00     | 0.22     | 2.38     | 1.24      | 0.72             | 0.31                 | 0.00     | 0.56     |
| Chlamydiae           | 1.01                 | 0.01     | 0.38     | 0.29     | 0.85      | 1.62             | 0.54                 | 0.00     | 0.34     |
| Planctomycetes       | 0.51                 | 0.00     | 0.20     | 0.08     | 0.01      | 0.03             | 0.12                 | 4.62     | 1.23     |
| TM7                  | 0.23                 | 0.00     | 0.05     | 3.65     | 0.42      | 0.22             | 0.39                 | 0.00     | 0.39     |
| TA06                 | 0.00                 | 0.00     | 0.00     | 0.00     | 0.00      | 0.00             | 0.00                 | 2.97     | 0.08     |
| OP11                 | 0.00                 | 0.00     | 0.00     | 0.00     | 0.00      | 0.00             | 0.02                 | 0.00     | 0.47     |
| unclassified          | 33.59               | 12.01    | 33.56    | 26.63    | 18.21     | 30.55            | 0.00                 | 0.00     | 0.00     |

**Proteobacteria**

| Phylum               | Before sterilization | 3 months | 6 months | 9 months | 12 months | Undisturbed soil | Before sterilization | 3 months | 6 months |
|----------------------|----------------------|----------|----------|----------|-----------|------------------|----------------------|----------|----------|
| Alphaproteobacteria  | 1.88                 | 0.16     | 1.14     | 3.52     | 4.32      | 2.47             | 7.23                 | 0.15     | 4.37     |
| Betaproteobacteria   | 0.04                 | 6.56     | 0.03     | 0.41     | 0.40      | 0.03             | 1.35                 | 0.01     | 0.19     |
| Deltaproteobacteria  | 0.54                 | 8.95     | 0.30     | 1.04     | 0.59      | 3.64             | 4.31                 | 0.85     | 4.96     |
| Gammaproteobacteria  | 5.84                 | 24.95    | 7.32     | 12.27    | 17.47     | 3.86             | 21.40                | 10.52    | 15.25    |
| Other groups (< 1%)  | 1.10                 | 0.47     | 1.41     | 3.02     | 1.80      | 3.86             | 1.70                 | 0.04     | 1.92     |
Table 4 (on next page)

Relationship between soil and diversity

Spearman’s rank correlations between the relative abundances of the most abundant bacterial phyla, proteobacterial classes and bacterial families, and the soil properties in CCB. Bold numbers: $P < 0.05$; Bold and underlined numbers $P < 0.001$. 
Table 4: Bacterial 16S rRNA amplicon diversity analysis of two arid soil sites in Cuatro Cienegas, Coahuila, Mexico.

|                      | OTUs a | singlets | doublets | Chao1     | CI b      | Shannon   | CI b      | Simpson | CI b      |
|----------------------|--------|----------|----------|------------|-----------|-----------|-----------|---------|------------|
| **Dry lagoon**       |        |          |          |            |           |           |           |         |            |
| Before sterilization | 6586   | 4917     | 882      | 43226      | (39437-47450) | 7.31      | (7.27-7.34) | 0.007   | (0.006-0.007) |
| 3 months             | 4106   | 2760     | 587      | 20166      | (18253-22337) | 6.07      | (6.03-6.11) | 0.020   | (0.019-0.021) |
| 6 months             | 7518   | 5830     | 1093     | 42815      | (39583-46372) | 7.59      | (7.56-7.63) | 0.004   | (0.004-0.004) |
| 9 months             | 3552   | 2433     | 451      | 10097      | (9334-10961) | 7.17      | (7.15-7.21) | 0.002   | (0.002-0.003) |
| 12 months            | 6882   | 5114     | 808      | 60661      | (54582-67515) | 7.70      | (7.66-7.73) | 0.004   | (0.004-0.005) |
| Undisturbed soil     | 8258   | 7157     | 1135     | 53773      | (49545-58434) | 8.16      | (8.13-8.19) | 0.001   | (0.001-0.001) |
| **River**            |        |          |          |            |           |           |           |         |            |
| Before sterilization | 8268   | 6797     | 1085     | 53676      | (49534-58233) | 7.80      | (7.76-7.83) | 0.005   | (0.004-0.005) |
| 3 months             | 3785   | 2878     | 657      | 17395      | (15706-19323) | 6.04      | (6.00-6.08) | 0.013   | (0.013-0.014) |
| 6 months             | 9001   | 7289     | 1148     | 61455      | (56784-66583) | 8.24      | (8.21-8.26) | 0.001   | (0.001-0.002) |
| 9 months             | 6530   | 6607     | 1202     | 33236      | (30685-36056) | 7.35      | (7.32-7.38) | 0.003   | (0.003-0.003) |
| 12 months            | 6356   | 4728     | 734      | 27928      | (25926-30135) | 7.80      | (7.75-7.82) | 0.002   | (0.002-0.002) |
| Undisturbed soil     | 8461   | 7019     | 1171     | 52934      | (48931-57332) | 8.23      | (8.20-8.25) | 0.001   | (0.001-0.001) |

All samples were normalized to 10, 441 sequences (table S3) for diversity comparison, values in parentheses represent the lower and upper 95% confidence interval associated with the Chao1 nonparametric estimator.

a. values calculated based on 97% threshold
b. Lower and upper 95% confidence intervals associated with the diversity parameter.
c. Good’s coverage estimator
Table 5 (on next page)

16S diversity

Bacterial 16S rRNA amplicon diversity analysis of two arid soil sites in Cuatro Cienegas, Coahuila, Mexico.
Table 5. Spearman’s rank correlations between the relative abundances of the most abundant bacterial phyla, proteobacterial classes and bacterial families, and the soil properties in CCB. Bold numbers: $P < 0.05$; Bold and underlined numbers $P < 0.001$.

| Taxonomic Group | Humidity | Total Organic Carbon | Total Nitrogen | Nitrate |
|-----------------|----------|-----------------------|----------------|--------|
| **Phyla**       |          |                       |                |        |
| Acidobacteria   | 0.54     | **0.59**               | **0.57**       | **-0.66** |
| Bacteroidetes   | 0.46     | 0.46                  | 0.51           | -0.16  |
| Chloroflexi     | **0.71** | **0.65**               | 0.51           | -0.24  |
| Firmicutes      | 0.24     | 0.27                  | 0.26           | -0.01  |
| Deinococcus-Thermus | 0.02    | -0.13                 | -0.23          | 0.05   |
| Gemmatimonadetes| 0.50     | 0.43                  | 0.51           | -0.27  |
| Actinobacteria  | -0.07    | -0.30                 | -0.16          | 0.42   |
| TM6             | **0.58** | **0.59**               | 0.49           | 0.03   |
| Cyanobacteria   | 0.50     | 0.32                  | 0.50           | -0.12  |
| **Class**       |          |                       |                |        |
| Alphaproteobacteria | 0.49   | 0.47                  | **0.64**       | -0.10  |
| Betaproteobacteria | -0.06 | -0.02                 | 0.24           | 0.22   |
| Deltaproteobacteria | 0.44   | 0.37                  | 0.53           | -0.01  |
| Gammaproteobacteria | -0.12 | -0.02                 | 0.22           | 0.07   |
| **Family**      |          |                       |                |        |
| Acidobacteriaceae | **0.69** | 0.62                  | 0.63           | **-0.60** |
| Holophagaceae   | **0.75** | **0.84**               | **0.78**       | **-0.57** |
| RB25 (Holophagae) | **0.71** | **0.68**               | **0.71**       | **-0.65** |
| SJA-36 (Holophagae) | **0.67** | 0.74                  | **0.71**       | -0.70  |
| Unclassified Acidobacteria | **-0.93** | **-0.89**              | **-0.82**      | **0.60** |
| Moraxellaceae   | -0.12    | -0.22                 | -0.29          | -0.05  |
| Chitinophagaceae| 0.42     | 0.41                  | 0.53           | -0.04  |
| Truepera        | -0.11    | -0.24                 | -0.30          | 0.07   |
| Anaerolineaceae | 0.63     | 0.47                  | 0.44           | 0.09   |
| Pseudomonadaceae| -0.45    | -0.24                 | -0.10          | 0.18   |
| Streptococcaceae| -0.50    | -0.43                 | -0.53          | 0.24   |
| Enterobacteriaceae | **0.56** | 0.51                  | 0.39           | **-0.84** |
| GIF3 (Chloroflexi) | **0.76** | **0.79**              | **0.76**       | **-0.73** |
| Family          | 0.15 | 0.20 | 0.18 | -0.46 |
|-----------------|------|------|------|-------|
| Alcaligenaceae  | -0.37| -0.24| -0.03| 0.50  |
| unclassified    | -0.80| -0.85| -0.82| 0.66  |
| Deltaproteobacteria |      |      |      |       |
| Listeriaceae    | 0.15 | 0.20 | 0.18 | -0.46 |