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unclear

Arid ecosystems are characterized by high spatial heterogeneity, being discontinuous vegetation distribution a clear representation of such heterogeneity. Soil biotic and abiotic factors associated with vegetation patches have also been well documented as highly heterogeneous in space. Given the low vegetation cover and little precipitation in arid ecosystems, soil microorganisms are the main drivers of nutrient cycling. Nonetheless, little is known about the spatial distribution of microorganisms and the relationship that their diversity holds with nutrients and other physicochemical gradients in arid soils. In this study, we evaluated the spatial variability of soil microbial diversity and biogeochemical parameters (nutrients and ions content) at local scale (meters) occurring in a gypsum-based desert soil, to gain knowledge on what soil abiotic factors control the distribution of microbes in arid ecosystems. We analyzed 32 soil samples within a 64 m^2 plot and: a) characterized microbial diversity through 16S rDNA-TRFLPs, b) determined soil biogeochemical parameters, and c) identified relationships between microbial diversity and biogeochemical properties. Overall, we show a strong correlation between microbial composition heterogeneity and spatial variation of ions content. Our results could be attributable to spatial differences of soil saline content, favoring the patchy emergence of salt and biological crusts.

Spatial heterogeneity of ions concentration explains microbial diversity distribution in arid soils from Cuatrocienegas Mexico

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ABSTRACT

Arid ecosystems are characterized by high spatial heterogeneity, being discontinuous vegetation distribution a clear representation of such heterogeneity. Soil biotic and abiotic factors associated with vegetation patches have also been well documented as highly heterogeneous in space. Given the low vegetation cover and little precipitation in arid ecosystems, soil microorganisms are the main drivers of nutrient cycling. Nonetheless, little is known about the spatial distribution of microorganisms and the relationship that their diversity holds with nutrients and other physicochemical gradients in arid soils. In this study, we evaluated the spatial variability of soil microbial diversity and biogeochemical parameters (nutrients and ions content) at local scale (meters) occurring in a gypsum-based desert soil, to gain knowledge on what soil abiotic factors control the distribution of microbes in arid ecosystems. We analyzed 32 soil samples within a 64 m^2 plot and: a) characterized microbial diversity through 16S rDNA-TRFLPs, b) determined soil biogeochemical parameters, and c) identified relationships between microbial diversity and biogeochemical properties. Overall, we show a strong correlation between microbial composition heterogeneity and spatial variation of ions content. Our results could be attributable to spatial differences of soil saline content, favoring the patchy emergence of salt and biological crusts.

INTRODUCTION

Spatial heterogeneity is an inherent feature of soils and has significant functional implications, particularly when the activities and distribution of microorganisms are considered. The scale at which environmental variation is considered in association with microbial diversity varies greatly, from tens to thousands of kilometers, to meters and even at the microscale (Vos et al., 2013). Depending on the spatial scale at which microbial diversity is studied, different environmental parameters and ecological processes may be associated to the observed diversity distribution (Martiny et al., 2011). At large spatial scales (tens to thousands of kilometers), soil microbial community structure is correlated to edaphic variables, such as soil pH (Fierer & Jackson, 2006), temperature (Garcia-Pichel et al., 2013), and moisture content (Angel et al., 2010). At smaller scales (tens of meters), plant communities have been shown to have a strong influence on soil microbial diversity through interactions within the rhizosphere (Berg & Smalla, 2009; Hartmann et al., 2009; Ben-David et al., 2011). However, little is known about the effects of small-scale habitat variation on the spatial patterns of microbial diversity and its interactions with the soil abiotic properties (Maestre et al., 2005).

Arid soils have a particularly heterogeneous spatial distribution of abiotic properties (Schlesinger et al., 1996), particularly in nutrients content. Vegetation patches, deemed "fertility or resource islands", are also scarce and sparsely found in arid environments (Cross & Schlesinger, 1999; Hirobe et al., 2001; Schade & Hobbie, 2005). At the same time, there are large areas deprived of vegetation and severely limited in nutrients and water (Evans et al., 2001; Belnap et al., 2005), in which microorganisms are the main drivers of energy input and biogeochemical processes (Titus, Nowak & Smith, 2002; Housman et al., 2007; Bachar, Soares & Gillor, 2012). Among soil microbial communities, biological soil crusts (hereafter termed biocrusts), consisting mainly of cyanobacteria, lichens and mosses, are one of the most important biotic components of arid and semi-arid ecosystems (Belnap, 2003). Biocrusts occur in vegetation interspaces (Belnap, 2003) and contribute actively to natural small-scale soil heterogeneity, not only in terms of biological diversity but also in relation to soil function, including nutrient cycling and physicochemical properties associated with their spatial structure (Maestre et al., 2005).

Is there evidence that the microbes in these hyperarid soils are biologically active? If so, this would add weight to the argument that they are not only drivers of processes here, but also that the processes represent a significant flux in the landscape.

Again, what percentage of landscape process rates occur in these hyperarid soils to make them important?

Given the tight connection between microbial activity and nutrient cycling, it is reasonable to think that microbial distribution in soils might be somehow correlated with nutrients content across space. Despite the idea of resource island formation in arid soils, many studies have shown that spatial distribution of microorganisms and nutrients is not correlated in these ecosystems (Belnap et al., 2005; Housman et al., 2007). Thus, there is a imperative need for understanding both the soil spatial heterogeneity and the complex interactions between these factors, which explain this heterogeneity and its role in arid ecosystem processes (Maestre et al., 2005; Ben-David et al., 2011). Moreover, for desert soils, the biochemical and biological heterogeneity may change depending on the scale analyzed, the type of vegetation and the degree of perturbation (Schlesinger et al., 1996).

There is a large body of work from the polar deserts of Antarctica that may be useful here:

Lee et al. 2012 (DOI 10.1038/ismej.2011.170);
Barrett et al. 2006 (DOI 10.1017/s0954102006000587)
Geyer et al. 2013 (DOI 10.1890/es13-00048.1)

In the present study, we aim to determine the spatial heterogeneity of microbial diversity and soil biogeochemical parameters occurring in an arid soil, in order to gain understanding into which aspects of the soil environment are more strongly associated with differences in microbial community distribution. We hypothesize that the spatial heterogeneity in biogeochemical properties, previously reported for desert soils (Schlesinger et al., 1996), will be reflected in

I would push back at the assertion that this scale is “unexplored.” Again, there are a number of studies from the polar deserts of Antarctica examining geochemical effects on biodiversity (see a few citations above).

microbial diversity distribution at a yet unexplored local scale (order of meters). Thereby, this study aims to: a) characterize microbial community structure, b) determine soil physicochemical and biochemical parameters and, c) identify relationships among microbial community structure and biogeochemical soil properties at a local spatial scale.

The study site, Cuatro Cienegas Basin (CCB), is located in a desert ecosystem in the middle of the Chihuahuan desert in Mexico. This is a gypsum-based system and it is one of the most oligotrophic environments in the world. In contrast, the microbial diversity is very high with respect to other arid soils (López-Lozano et al., 2012). Thus, these soils provide ~~with~~ the opportunity to investigate the spatial relationship between biogeochemical distribution and microbial community structure.

The similarities between this study site and the McMurdo Dry Valleys of Antarctica are again striking, as the polar desert is THE most oligotrophic soil in the world and also has relatively high diversity. See Zeglin et al. 2011 (DOI 10.1007/s00248-010-9782-7) for a comparison of these ecosystems.

MATERIALS AND METHODS

Study Area. The study site is locally known as “Churince system”. It is located in the western part of the CCB (26˚ 50’ N, 102˚ 08’ W; **Figure 1**) at 740 m a.s.l. The system consists of a spring, an intermediate lagoon, and a dry desiccation lagoon connected by short shallow creeks. The annual precipitation in the area is less than 250 mm, occurring mainly from May to October. Temperatures fluctuate from 0ºC in January to 45ºC in July, with a mean annual temperature of 21.4ºC (CCB weather station). Halophile and gypsophile grasslands are the main vegetation present in this area (Challenger, 1998), which is also dominated by physical and biological soil crusts. The soil is predominantly basic, rich in calcium and sulfates but very poor in nutrients, and belongs to Gypsisol type (IUSS Working Group WRB, 2007).

How many meters away?

unclear

Sampling design. The sampling plot was closer to the dry desiccation lagoon. It was dominated by the gypsophile grass *Sesuvium erectum* that has only 10% of plant coverage (**Figure 1**). Physical and biological crusts occupied the open areas between plants. The sampling scheme was spatially structured and details of the setting are described elsewhere (Noguez et al., 2005). We used a plot of 8 m x 8 m that consisted of a nested system of four quadrats (*A*-*D* quadrats), which were non-randomly divided in eight 1 m^2 “replicates” (**Figure 1**). Vegetation cover for each sampling “replicate” or site (1 m^2) was registered qualitatively in order to have further ecological context for the results. In August 2007, we collected soil samples (500 g) from the first 10 cm at each site to a total of 32 samples (eight samples for each 4 m^2 quadrat) under the SEMARNAT collection permits 06590/06 and 06855/07. Soil samples were homogenized in the field and divided in two subsamples, which were stored at -20ºC (for molecular analyses) and at 4ºC (for biogeochemical analyses), respectively. Analyses were performed upon arrival to the laboratory.

Physicochemical and biochemical analyses. Soil samples were air dried and sieved through a 2 mm mesh prior to physicochemical and biochemical determinations, which were performed twice for each sample. Total carbon (TC) was determined by dry combustion with a Total Carbon Analyzer (TOC). For total nitrogen (TN) and phosphorus (TP), the samples were acid digested and determined colorimetrically using a Bran-Luebbe Auto-analyzer, according to Bremner (Bremner, 1996) and Murphy & Riley (Murphy & Riley, 1962), respectively. Inorganic N forms (NH_4^+ and NO_3^-) were extracted with 2M KCl, followed by filtration through a Whatman #1 filter, and measured colorimetrically by the phenol–hypochlorite method. Inorganic P (Pi) was extracted with sodium bicarbonate, and determined colorimetrically by the molybdate-ascorbic acid method (Murphy & Riley, 1962). Dissolved organic C (DOC), N (DON) and P (DOP) were extracted with deionized water after shaking for 1 h and then filtered through a Whatman #42 filter. DOC was determined with a TOC module for liquids, while DON and DOP were acid digested and measured colorimetrically.

Electrical conductivity and pH were determined in soil with deionized water (soil solution ratio 1:2). To quantify water-soluble cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) and anions (HCO_3^-, Cl^-, SO_4^{2-}), soil samples were shaken with deionized water for 19 h, centrifuged at 2500 rpm and filtered through a Whatman #42 filter. Ca^{2+} and Mg^{2+} were analyzed by atomic absorption spectrophotometry, while Na^+ and K^+ by flamometry. Anions were determined by liquid chromatography with a mobile phase of borate sodium glucanate. More detail for flamometry is necessary.

Molecular analyses. Microbial community structure was characterized using terminal restriction fragment length polymorphisms (T-RFLPs) of 16S rDNA genes.

Genomic DNA was extracted from the soil samples using the Soil Master DNA Extraction Kit (Epicentre Biotechnology), with an additional previous step based on the fractionation centrifugation technique in order to reduce the high salts concentration (Holben et al., 1988). After extraction, genomic DNA was cleaned with Microcon columns (Fisher Scientific) with the purpose of removing any substance that could inhibit PCR amplification. These protocol modifications gave the best results from various methodologies tested, however we were only ~~just~~ able to amplify the 16S rDNA from 21 out of the 32 soil sampling sites (amplicons obtained in each quadrat: A=3; B=3; C=7; D=8). The low yield in the DNA amplification was due to the high concentration of salts, polysaccharides, and secondary compounds in these soil samples (López-Lozano et al., 2012).

Amplification of the 16S rDNA was carried out in a final volume of 50 µL containing: 0.2 µM of each fluorescently labeled domain-specific primers (VIC-27F and FAM-1492R) (Lane, 1991), 0.2 mM of each dNTP, 1 U of Taq Platinum DNA polymerase (Invitrogen), 2.5 µL DMSO, 2.5 µL BSA, 1 mM $MgCl_2$, 1 mM buffer, and 20 ng of DNA. Five independent PCR reactions were performed for each sample with the following program: 5 min at 94 °C; 30 cycles at 94 °C for 1 min, 52 °C for 2 min, 72 °C for 3min; and 72 °C for 10 min. PCR products were pooled and purified from 2% agarose gel (Gel extraction kit, Qiagen Inc.). The amplicons were restricted with AluI enzyme (Promega) at 37 °C for 3 h and 65 °C for 20 min. Three independent readings of terminal restriction fragments (t-RFs) were performed for each sample, using an ABI 3100-Avant Prism Genetic Analyzer (Applied Biosystems).

Each t-RF was considered to be an operational taxonomic unit (OTU) and only those OTUs with heights ≥50 fluorescent units were used for the analyses (Blackwood et al., 2003). We constructed presence-absence matrices to determine the spatial patterns and diversity of OTUs among the 4 m^2 quadrats (for details of the method see (Noguez et al., 2005)).

No relative abudnance matrices were used?

Statistical analyses. Statistical and diversity analyses were performed in R (R Development Core Team, 2011), mainly with vegan, ggplots and BiodiversityR packages.

Soil properties were analyzed using univariate ANOVA and residuals were explored for normality and variance homogeneity. When residuals did not satisfy these assumptions, a Kruskal-Wallis test was applied. These analyses were followed by multiple pairwise tests, using Tukey's honestly significant difference (HSD), at the 5% level of significance, to identify possible differences in the soil variables between quadrats. The correlations between each pair of variables were calculated using Pearson's correlation coefficient. Soil properties were then standardized and ordered by principal components analysis (PCA), and the sampling points from the four quadrats were visualized with the two first principal components.

Multivariate ANOVA (MANOVA) would be more appropriate here, as there are many (19 in Table 1) repeated tests of significance by quadtrant. Not accounting for these multiple comparisons inflates the chances of making a Type I error.

Alpha diversity indices (Shannon, Simpson, and Berger-Parker) and richness estimates were calculated for each quadrat using the T-RFLPs profiles. Microbial diversity indices were also analyzed using ANOVA type III for unbalanced data and evaluated using Renyi's entropy profiles (Rényi, 1961; Chao et al., 2014). To evaluate sampling effort, rarefaction curves were constructed for each quadrat using EstimateS v.9.1.0 (Colwell, 2005). Microbial community

Renyi's entropy profile is an unusual metric and I would like to see a broader discussion of how it works!

structure was examined through Venn diagrams and ordination analyses. To visualize communities' structure, Bray-Curtis dissimilarity distances were calculated with the T-RFLPs profiles. Similar communities were then clustered using the Ward's hierarchical clustering algorithm, which tries to minimize variances in agglomeration. A heatmap was constructed with dual hierarchical clustering.

Community structure was also investigated for correlations with biogeochemical parameters following a multivariate analysis. For this, T-RFLPs profiles were ordered by Detrended Correspondence Analysis (DCA) with Hellinger transformation (Blackwood et al., 2003), and correlations between the ordination axes and soil properties were calculated. This eigenvector-based ordination technique uses a chi-square distance measure, and assumes that TRFs have a unimodal distribution along ecological gradients (Legendre & Legendre, 1998), which is a more appropriate assumption than linearity for ecological analysis of T-RFLPs data (Culman et al., 2008). Permutation tests under reduced model were used to identify significant explanatory soil variables. Only the soil variables corresponding to the same sampling sites as the T-RFLPs data were used for this analysis.

RESULTS

Spatial heterogeneity in physicochemical and biochemical parameters

The total plant cover in the experimental plot was only 10%. However, quadrats *C* and *D* were more densely and homogeneously covered than *A* and *B*, since the latter were more distant from the desiccation lagoon (**Figure 1**). Overall there was a high presence of soil crusts and biocrusts, particularly in the *A* quadrat.

Was soil moisture not measured? This seems like an important variable that is mssing!

Soil samples were alkaline (pH between 8.6-8.8) due to the high presence of salts in this arid ecosystem (**Table 1**). The high concentration of Na^+ found in these soils (mean value of 147 cmol kg^{-1}) indicates salinity stress. Ions and C availability (DOC) were the most variable parameters in this small plot. *C* and *D* quadrats had the greatest concentration of cations (except for Na^+), while *A* and *B* quadrats had the highest concentration of anions. Nutrients content was very low in this arid soil, as expected, and it did not show significant differences among quadrats. On the other hand, DOC was significantly higher in *D* quadrat, which means greater substrate availability for microbial metabolism in this quadrat. As expected, the pH was positively correlated with Mg^{2+} and Na^+ (Table S1). The TC was only positively correlated with

Ca^{2+}, while TP was positively correlated with pH and cations, as well as negatively with DOP. The TN was positively correlated with DON and negatively with C:N, NH_4^+, NO_3^- and HCO_3^-. Finally, N inorganic forms were also positively correlated between them and the C:N ratio, which was very low in the four quadrats (from 4.6 to 6.1).

The complex biogeochemical spatial heterogeneity among these four quadrats was explored using a PCA (**Figure 2**). The first component (PC1) explained 54.3%, while the second component (PC2) explained 34.3% of the total variation in the soil parameters among quadrats (**Table S2**). The variables associated with the PC1 were cations and anions, as well as pH, TP, DOC and DOP. The PC2 was mainly related to soil nutrients (TN, C:N, NH_4^+, NO_3^-, DON, DOC:DON). A clear separation between quadrats was observed along the PC1 axis, mainly explained by the spatial heterogeneity distribution of ions.

Spatial heterogeneity of microbial diversity

A total of 184 different OTUs were obtained in the four quadrats. Unfortunately, the number of available samples was unbalanced for the microbial diversity study in this plot (amplicons in each quadrat: *A*=3; *B*=3; *C*=7; *D*=8) due to the high concentrations of salts, which hampered the 16S rDNA amplification from all samples sites. Despite this constraint, rarefaction curves showed a good community sampling for quadrats *A*, *C* and *D*, with evident subsampling for quadrat *B*, which is one of the two quadrats for which only 3 out of 8 samples could be analyzed in terms of microbial diversity (**Figure S1**). Significant variation in alpha diversity indices among quadrats was detected. Although ~~Despite~~ the *A* quadrat was also limited in the number of analyzed samples (3), it was the most diverse and with the highest evenness, followed by *C*, *D*, and *B* (**Table 2**). It was also evident the high variability in microbial diversity among replicates (with the exception of *A*), which reflects the spatial heterogeneity at local scale of this arid soil. A summary of diversity indices was obtained by calculating Renyi´s community profiles, reveling the same pattern of diversity, both in terms of richness and evenness, where the highest diversity was found for quadrat *A* and the lowest for quadrat *B* (**Figure 3**).

Despite the high heterogeneity in microbial diversity in such a small plot, Venn diagram revealed a considerable overlap of OTUs among the four quadrats: 18% of OTUs were shared by all quadrats, and the *C* quadrat had the most “unique” OTUs (12%), followed by quadrat *D*, *B* and *A* in decreasing order (**Figure 4**). Interestingly, *C* and *D* quadrats shared 84 of the 184 recovered OTUs (46%), suggesting that both quadrats had more similar community composition than *A* and *B* quadrats. Moreover, the cluster dendrogram (**Figure 5)** and the heatmap (**Figure S2**) showed the same pattern of grouping as the PCA analysis for soil biogeochemical properties, separating quadrats in two groups: *A*-*B* and *C*-*D*. As in the PCA analysis, there was a sample in the *C* quadrat that clearly deviated from the other samples.

Multivariate analyses of microbial community structure

To explore the association between OTUs abundance and soil parameters, we performed a DCA analysis. The original 19 soil parameters were reduced to 7 non-redundant explanatory variables (TN, DON, Ca^{2+}, K^{+}, HCO_3^{-}, Cl^{-}, and SO_4^{2-}; **Table S3**), which were the factors that contributed significantly to differences in community composition among the four quadrats. The analysis showed a clear separation of the quadrats in two groups, mainly explained by soil salinity: anions (HCO_3^{-}, Cl^{-}, and SO_4^{2-}) significantly correlated with OTUs from the *A* and *B* quadrats, while TN, DON, Ca^{2+} and K^{+} significantly correlated with OTUs from the *C* and *D* quadrats (**Figure 6**). The grouping pattern of these microbial communities showed in the above analyses was also confirmed by the DCA analysis.

DISCUSSION

Biogeochemical heterogeneity at local spatial scale is mainly due to ions concentration variability

The values of TC, TN and TP in the experimental plot were lower than that reported for other deserts (Thompson et al., 2006; Strauss, Day & Garcia-Pichel, 2012), as well as for soils in the CCB (López-Lozano et al., 2012; Tapia-Torres et al., 2015). The very low C:N ratio also suggests relative low return of organic matter, therefore a low nutrient availability to soil microbes and vegetation, limiting the N cycle due to the lack of C availability. The Redfield ratio in this soil was 71:17:1, which indicates that the C is the limiting nutrient in comparison with a general “average” soil C:N:P of 186:13:1 (Cleveland & Liptzin, 2007). On the other hand, this result differs from the Redfield ratio of 104:5:1 reported for the same soil system (López-Lozano et al., 2012). These differences could be attributable to the great heterogeneity of this arid environment and the different time of soil sampling in both studies: February 2007 (dry cold season with low evapotranspiration) in (López-Lozano et al., 2012) and August 2007 (rainy hot season with high evapotranspiration) in this study.

unclear

All soil samples in this study had high alkalinity due to elevated concentrations of ions, which is a general pattern in desert soils (Titus, Nowak & Smith, 2002). The high pH makes even less available the scarce P in these soils, which eventually is bound by Ca^{2+} and Mg^{2+} ions (Cross & Schlesinger, 2001). It is worth to mention that ions varied spatially in identity in this small plot: quadrats *A* and *B* were significantly high in anions, while quadrats *C* and *D* were significantly high in cations. The huge concentration of Na^{+} in the four quadrats is an indicator of the extremely high salinity in these soils, which negatively affects the soil aggregates stability and nutrients and water availability for plants, favoring the development of soil crusts, which are typical in arid and semiarid soils (Belnap, 2003; Zhang et al., 2007). In particular, salt crusts are abundant in this area of CCB. They consist of layers at the soil surface mainly formed by soluble salt crystallizing soil particles at shallow saline groundwater level regions (Zhang et al., 2013).

The high concentration of ions can be attributed to the gypsum-rich nature of the CCB soils, where groundwater rises to the surface by soil capillarity action, and water evaporation promotes salt accumulation. This results in rivers with a steep salinity gradient (Cerritos et al., 2011) and pools surrounded by saline soils rich in sulfates and extremely poor in nutrients (López-Lozano et al., 2012). Therefore, it is not surprising to find that the soil properties variation in this small plot was mainly explained by ions concentration, grouping the four quadrats in two broad clusters: *A*-*B* and *C*-*D*. These clusters had a qualitative pattern associated with the vegetation cover, being quadrats *C* and *D* more densely and homogeneously covered than *A* and *B*. Although the present research refers to soil communities, a previous study of microbial communities of the water system associated with the studied plot showed a clear correlation of microbial composition and water conductivity gradients (Cerritos et al., 2011). Thus, the spatial variation in these physicochemical properties among the four quadrats may be a consequence of differences in moisture content due to the proximity to a subterranean water flow, indirectly evidenced by the marked patchy distribution of the vegetation cover and the "open" areas occupied by soil crusts (López-Lozano et al., 2012).

Heterogeneity in microbial diversity at local spatial scale is explained by physicochemical factors, not by vegetation cover neither by nutrients content

Despite important advances in our knowledge of the structure, composition and physiology of biotic components in arid soils (Belnap et al., 2005; Caruso et al., 2011; Maestre et al., 2015; Makhalanyane et al., 2015), little is known about the spatial variability of microbial diversity at local scales and its interactions with biogeochemical heterogeneity in these ecosystems (Housman et al., 2007; Castillo-Monroy et al., 2011; Andrew et al., 2012). Thus, T-RFLPs fingerprinting was used in this study to assess the relationship between microbial structure and the small-spatial heterogeneity of soil biogeochemical properties. We are aware that this technique cannot recognize taxonomic groups and accounts mainly for relatively abundant microbial groups, while rare groups, comprising the majority of the population, are unaccounted for. Nevertheless, it has been shown that T-RFLPs fingerprinting facilitates replication, allowing comparisons among communities' diversity and pattern identification by the analysis of large number of samples (Fierer & Jackson, 2006). In addition, it is a robust method capable of revealing reproducible spatial patterns of soil microbial communities (Angel et al., 2013). In line with this, the number of total OTUs identified with this technique is within the expected range, given a previous report from the same study area using 16S rDNA clone libraries (López-Lozano et al., 2012). However, the heterogeneity in OTUs diversity among these quadrats is evident, being the *A* quadrat the most different with respect to the other quadrats.

Despite the fact that the *A* quadrat had scarce plant cover and similar nutrients and ions concentrations to the *B* quadrat, it showed the greatest microbial diversity, which could be related to the high presence of biocrusts. Soil crust communities occur in vegetation interspaces (Belnap, 2003) and contribute importantly to soil fertility and stability, favoring microbial activity and diversity and enhancing the overall spatial patterns of ecosystem processes (Maestre et al., 2005; Castillo-Monroy et al., 2011). For instance, autotrophic groups, such as Cyanobacteria and Chloroflexi, usually dominate biocrusts in arid soils (Nagy, Pérez & Garcia-Pichel, 2005; Gundlapally & Garcia-Pichel, 2006; Rajeev et al., 2013) and facilitate available nutrients to opportunistic heterotrophs that growth during the rainy season (from May to October in CCB). This is also the case for this arid system, where López-Lozano et al., (2013) reported a high abundance of Chloroflexi and Cyanobacteria groups using a 16S rDNA 454 pyrosequencing strategy to evaluate microbial community succession over a year after disturbance.

On the other hand, the *B* quadrat had the lowest microbial diversity, which could be related to the lowest values of DOC found in this quadrat. Labile organic matter fractions, such as DOC, are the primary energy source for soil microorganisms and are characterized by rapid turnover (Bolan et al., 2011). It has been reported that even in disturbed sites, DOC is the main source of C influencing the composition of the microbial community (Churchland, Grayston &

Bengtson, 2013). Then, changes of soil microbial community could be regulated by C availability through labile soil organic matter pools.

See Geyer et al. 2013 (DOI 10.1890/es13-00048.1) for further evidence of OC as a driver of bacterial richness in arid soils!

Regarding similarity in microbial composition among the four quadrats, cluster dendogram and multivariate analyses showed two clear groups, which were *A*-*B* and *C*-*D*, corresponding to the same clustering of quadrats based on soil biogeochemical parameters. A common explanation for the soil microbial composition patterns is related to the presence of plants controlling levels of microbial diversity and driving community assembly (Singh et al., 2007; Berg & Smalla, 2009; Ben-David et al., 2011). However, in our study the observed spatial pattern of microbial diversity distribution at such local scale does not seem to be associated with vegetation cover. For example, the *A* quadrat is the most diverse in microbial community and the less vegetated, suggesting that microbial diversity in this arid soil could be more related to the presence of "open" areas occupied by biocrusts. On the other hand, abiotic factors, such as ionic content, are statistically explanatory variables in the spatial ordering of the microbial communities analyzed. Abiotic drivers of microbial diversity in arid soils has been also reported for the Sonoran desert (Andrew et al., 2012), where location, pH, cation exchange capacity and soil organic C were highly correlated with microbial composition. Therefore, we showed that microbial community diversity and distribution responds to and/or influences local soil physicochemical characteristics at a small spatial scale in this arid ecosystem.

CONCLUSIONS

To our knowledge, this is the first study reporting the high spatial heterogeneity and the strong relationship of soil physicochemical and microbiological diversity at local scale (plot of 64 m^2) in an arid ecosystem. In desert areas, such as CCB, soil moisture is one of main limiting factors affecting vegetation growth and distribution, as well as soil microbiology. The gypsum-based water system controls the soil physicochemical factors and ultimately the microbial community distribution in this arid ecosystem. Thus, the high heterogeneity in the soil properties and microbial community among these small four quadrats seems to be a consequence of differences in the soil saline content. In addition, the high concentration of Na^+ favors the emergence of both salt and biological crusts and the irregular plant cover distribution in this system. Local spatial variability of physicochemical properties and microbial diversity observed in this arid ecosystem is likely to exist in most soils ecosystems, and needs to be considered when making ecological inferences and when developing strategies to sample the soil environment. A better understanding of the role of spatial heterogeneity in biotic and abiotic factors will help to determine the relevance of small-scale studies for large-scale patterns and processes.

ACKNOWLEDGEMENTS

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FIGURES AND TABLES

Figure 1. Sampling scheme. An 8x8 m plot was selected in the Churince System within the Cuatro Cienegas Basin, México. A checkerboard sampling scheme was followed (Noguez et al., 2005) to a total of 32 samples, eight for each of the four quadrats (*A*, *B*, *C*, and *D*). Soil parameters were determined for the 32 samples. Numbers with asterisks indicate samples that were also analyzed for microbial diversity. Green colored areas indicate presence of vegetation.

Figure 2. Biplot generated from a Principal Component Analysis (PCA) of the standardized soil variables for the four quadrats. Symbols represent the different quadrats. Each vector points to the direction of increase for a given variable and its length indicates the strength of the correlation between the variable and the ordination scores. The first component of the PCA analysis accounted for 54.3% of the total variation, and the second component accounted for 34.3% of the variation.

Figure 3. Renyi's entropy profiles for the studied quadrats (*A*: 3 samples; *B*: 3 samples; *C*: 7 samples; *D*: 8 samples). Profiles were calculated with the OTUs abundance matrix. The alpha scale shows the different ways of measuring diversity in a community. Alpha=0 is richness, alpha=1 shows Shannon diversity, alpha=2 is Simpson index (only abundant species are weighted), and alpha= Infinite only dominant species are considered (Berger-Parker index). The height of *H*-alpha values show diversity.

Figure 4. Venn diagrams displaying the degree of overlap of OTUs composition among the four studied quadrats (*A*: 3 samples; *B*: 3 samples; *C*: 7 samples; *D*: 8 samples).

Figure 5. Cluster dendrogram of similar microbial communities from the TRFLPs profiles of the four studied quadrats (*A*: 3 samples; *B*: 3 samples; *C*: 7 samples; *D*: 8 samples) using Bray-Curtis dissimilarity distances and the Ward's hierarchical clustering algorithm.

Figure 6. Detrended Correspondence Analysis (DCA) of the TRFLPs profiles with respect to the soil properties. Sample sites for the four quadrats are represented by symbols, and OTUs are represented by grey crosses. Vectors stand for significant soil variables ($p < 0.1$). Each vector points to the direction of increase for a given variable and its length indicates the strength of the correlation with the axes.

Table 1. Soil physicochemical parameters (mean ± standard deviation) of the four studied quadrats within Churince System in the Cuatro Cienegas Basin (Mexico).

Variable	Quadrat	Overall mean

	A	*B*	*C*	*D*	
Total C (mg g^{-1})	2.4 ± 0.8	2.4 ± 0.4	2.6 ± 0.4	2.8 ± 0.6	2.6 ± 0.6
Total N (mg g^{-1})	0.57 ± 0.13	0.48 ± 0.18	0.59 ± 0.1	0.60 ± 0.18	0.56 ± 0.15
Soil C:N	4.6 ± 2.3	6.1 ± 4	4.6 ± 0.7	5.0 ± 1.3	5.1 ± 2.4
Total P (mg g^{-1})	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01
NH_4^+ (µg g^{-1})	4.0 ± 0.6	4.2 ± 0.8	4.0 ± 0.6	3.6 ± 1	4.0 ± 0.7
NO_3^- (µg g^{-1})	1.8 ± 1.9	1.5 ± 1.5	1.6 ± 1.7	2.3 ± 1.5	1.7 ± 1.6
Dissolved organic C (µg g^{-1}) *	97.3 ± 27.8^{ab}	75.8 ± 30^{a}	83.0 ± 33^{ab}	124 ± 21.3^{b}	95.1 ± 33.2
Dissolved organic N (µg g^{-1})	14.6 ± 3.4	18.1 ± 12.3	17.9 ± 8.7	19.6 ±9.2	17.6 ± 8.7
Dissolved organic C:N	7 ± 2.9	6.4 ± 4.8	5.2 ± 2.4	7.9 ± 3.9	6.4 ± 3.5
Dissolved organic P (µg g^{-1})	4.3 ± 3.6	5.6 ± 2.1	2.6 ± 3.5	3.3 ± 3.7	3.9 ± 3.3
pH *	8.6 ± 0.1^{a}	8.7 ± 0.1^{a}	8.7 ± 0.1^{ab}	8.8 ± 0.1^{b}	8.7 ± 0.1
Electrical conductivity (dSm^{-1})	1.4 ± 0.1	1.4 ± 0.3	1.6 ± 0.2	1.4 ± 0.4	1.4 ± 0.3
Mg^{2+} (cmol kg^{-1}) *	27.1 ± 5.2^{a}	28 ± 6.3^{a}	35.2 ± 4.3^{ab}	36.5 ± 4.5^{b}	31.7 ± 6.5
Ca^{2+} (cmol kg^{-1}) *	0.56 ± 0.03^{a}	0.55 ± 0.02^{a}	0.64 ± 0.07^{ab}	0.65 ± 0.07^{b}	0.59 ± 0.07
Na^+ (cmol kg^{-1})	140 ± 15.9	127 ± 16.1	166 ± 38.1	157 ± 28.4	147 ± 29.1
K^+ (cmol kg^{-1}) *	0.95 ± 0.14^{a}	0.82 ± 0.19^{a}	1.27 ± 0.14^{b}	1.33 ± 0.25^{b}	1.09 ± 0.28
HCO_3^- (cmol kg^{-1}) *	2.8 ± 0.2^{b}	2.3 ± 0.6^{b}	1.2 ± 0.2^{a}	1.3 ± 0.3^{a}	1.9 ± 0.8
Cl^- (cmol kg^{-1}) *	2.8 ±0.2^{b}	2.5 ± 0.3^{b}	1.1 ± 0.4^{a}	1.3 ± 0.3^{a}	1.9 ± 0.8
SO_4^{2-} (cmol kg^{-1}) *	15.1 ± 2.2^{b}	16.4 ± 2.5^{b}	7.6 ± 0.8^{a}	7.2 ± 0.9^{a}	11.6 ± 4.6

Variable acronyms: C, carbon; N, nitrogen; P, phosphorous.

* Significant difference among quadrants ($p < 0.05$).

Different letters indicate that means are significantly different among quadrats.

Table 2. Alpha diversity estimates. OTUs diversity indices (mean ± standard deviation) from the TRFLPs data of the four quadrats (*A*: 3 samples; *B*: 3 samples; *C*: 7 samples; *D*: 8 samples).

Quadrat	Richness (S)	Shannon (H)*	Simpson (1/D)*	Berger-Parker*
A	48 ± 9	3.31 ± 0.08^{a}	0.944 ± 0.004^{a}	0.153 ± 0.004^{b}
B	36 ± 15	1.98 ± 0.62^{b}	0.704 ± 0.145^{b}	0.497 ± 0.144^{a}
C	45 ± 13	2.56 ± 0.44^{ab}	0.8 ± 0.105^{ab}	0.393 ± 0.138^{a}
D	47 ± 19	2.3 ± 0.77^{ab}	0.738 ± 0.189^{b}	0.426 ± 0.203^{a}

* Significant difference among quadrats ($p < 0.05$).

Different letters indicate that means are significantly different among quadrats.

Figure 1(on next page)

Sampling scheme

An 8x8 m plot was selected in the Churince System within the Cuatro Cienegas Basin, México. A checkerboard sampling scheme was followed (Noguez et al., 2005) to a total of 32 samples, eight for each of the four quadrats (A, B, C, and D). Soil parameters were determined for the 32 samples. Numbers with asterisks indicate samples that were also analyzed for microbial diversity. Green colored areas indicate presence of vegetation.

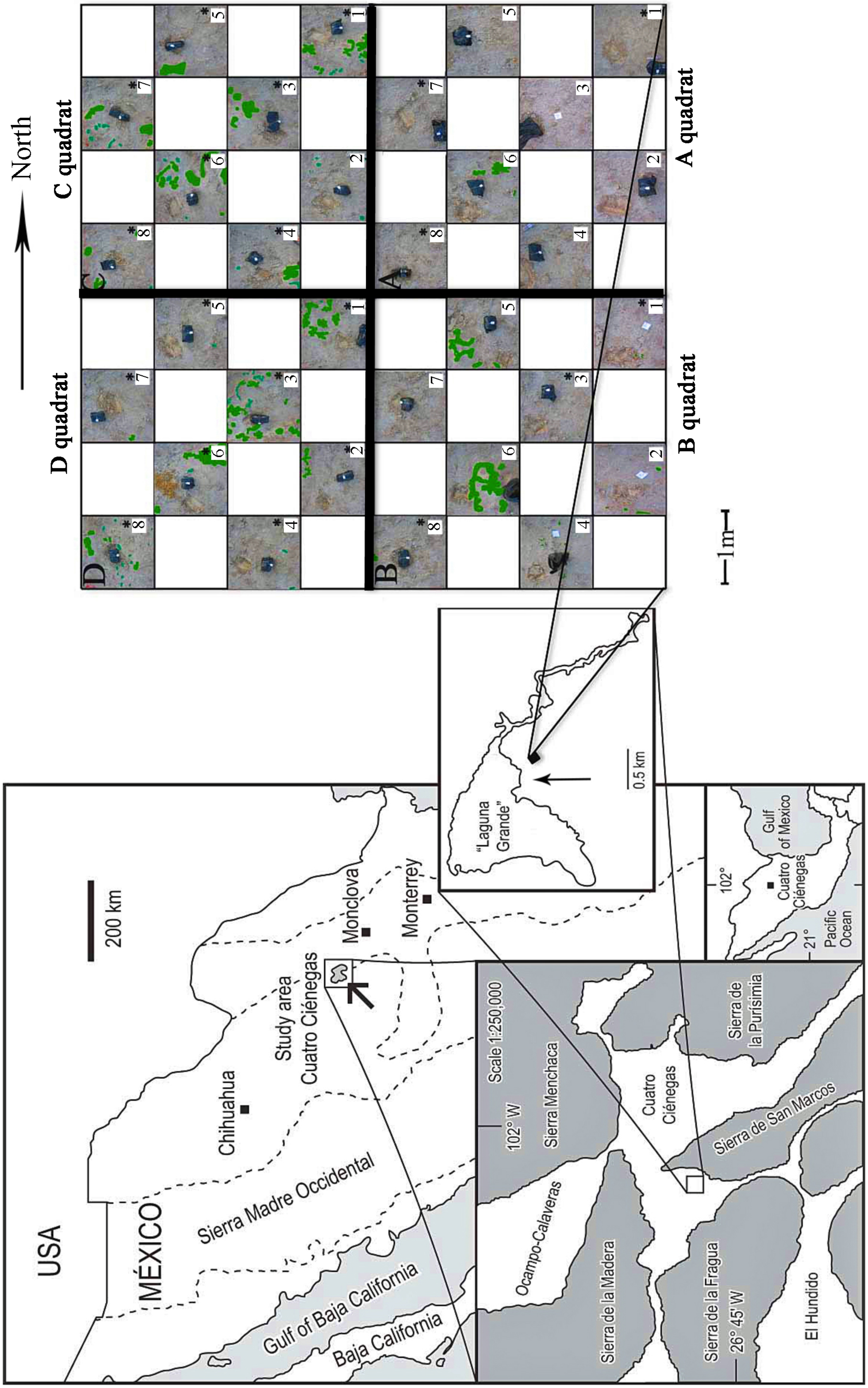
USA
MÉXICO
Sierra Madre Occidental
Chihuahua
Study area
Cuatro Ciénegas
Monclova
Monterrey
200 km
Gulf of Baja California
Baja California
Gulf of Mexico
102°
21°
Cuatro Ciénegas
Pacific Ocean
Scale 1:250,000
102° W
Sierra Menchaca
Cuatro Ciénegas
Sierra de la Purísima
Sierra de San Marcos
Ocampo-Calaveras
Sierra de la Madera
Sierra de la Fragua
26° 45' W
El Hundido
"Laguna Grande"
0.5 km
North
C quadrat
D quadrat
A quadrat
B quadrat
A
B
C
D
1m
*1
2
*3
*4
*5
*6
*7
*8

Figure 2(on next page)

Biplot generated from a Principal Component Analysis (PCA) of the standardized soil variables for the four quadrats

Symbols represent the different quadrats. Each vector points to the direction of increase for a given variable and its length indicates the strength of the correlation between the variable and the ordination scores. The first component of the PCA analysis accounted for 54.3% of the total variation, and the second component accounted for 34.3% of the variation.

Ellipses of standard deviation around each of the quandrants would highlight the separation between A/B and C/D. This could replace the dendrogram (Fig. 5) which I feel is unnecessary.

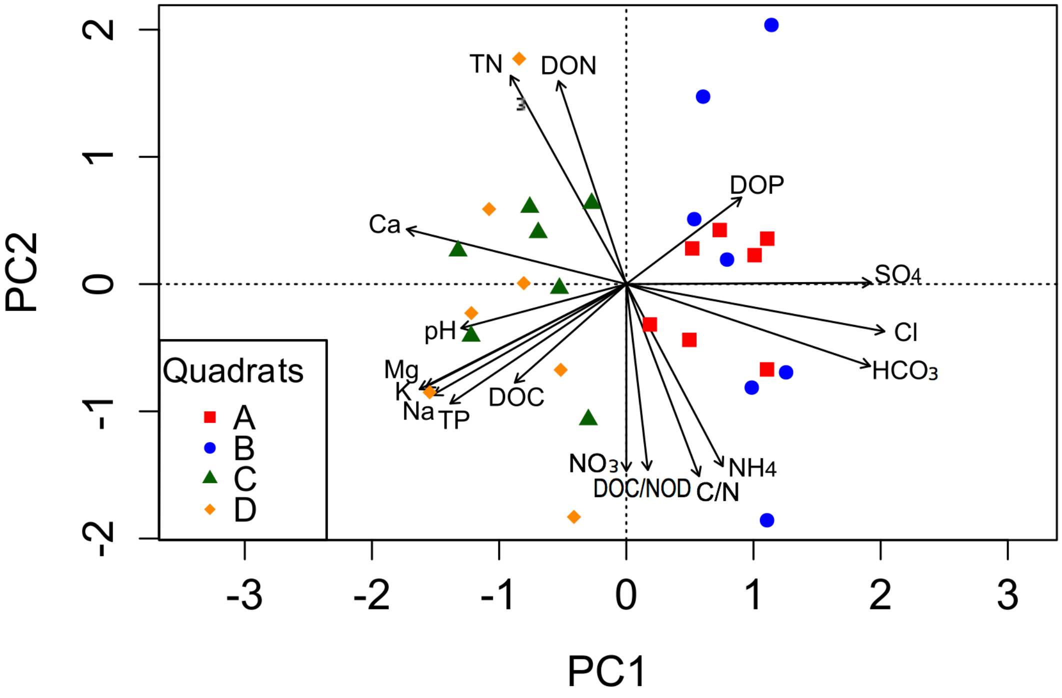
PC2
PC1
2
1
0
-1
-2
-3
-2
-1
0
1
2
3
TN
DON
DOP
Ca
SO4
pH
Cl
HCO3
Mg
K
Na
TP
DOC
NO3
DOC/NOD
C/N
NH4
Quadrats
A
B
C
D

Figure 3(on next page)

This figure is unclear to me. A broader discussion of how Renyi's entropy profile works in the Results section would be useful. Are the richness, Shannon diversity, and Simpson index calculated independently and then plotted on a single figure, or are there other embedded calculations that make these estimates unique?

Renyi's entropy profiles for the studied quadrats (A-D).

Profiles were calculated with the OTUs abundance matrix. The alpha scale shows the different ways of measuring diversity in a community. Alpha=0 is richness, alpha=1 shows Shannon diversity, alpha=2 is Simpson index (only abundant species are weighted), and alpha= Infinite only dominant species are considered (Berger-Parker index). The height of *H*-alpha values show diversity. N for studied quadrats is A*: 3 samples; B*: 3 samples; C*: 7 samples; D*: 8 samples).

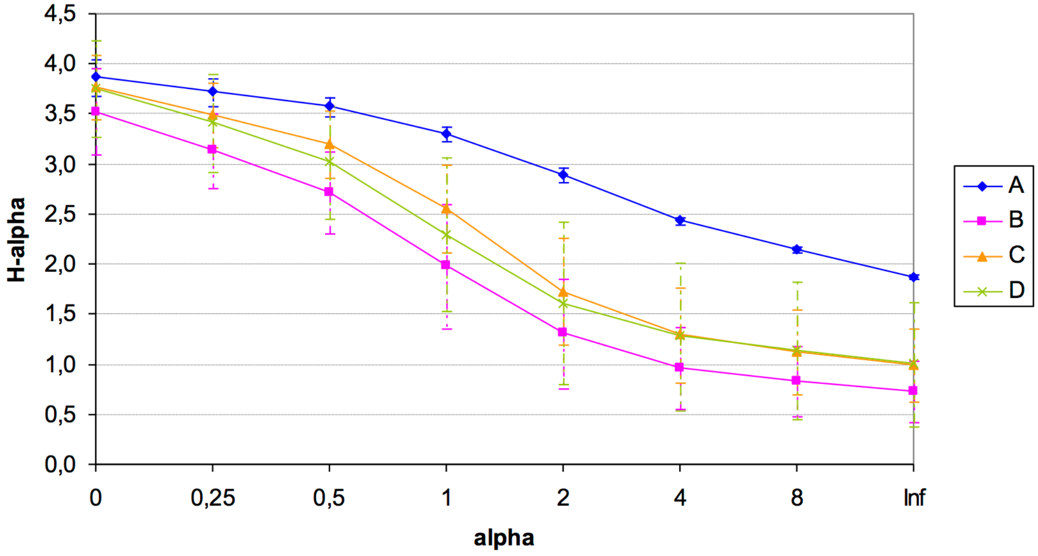
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
H-alpha
0
0,25
0,5
1
2
4
8
Inf
alpha
A
B
C
D

Figure 4(on next page)

Venn diagrams

Displaying the degree of overlap of OTUs composition among the four studied quadrats (A*: 3 samples; B*: 3 samples; C*: 7 samples; D*: 8 samples).

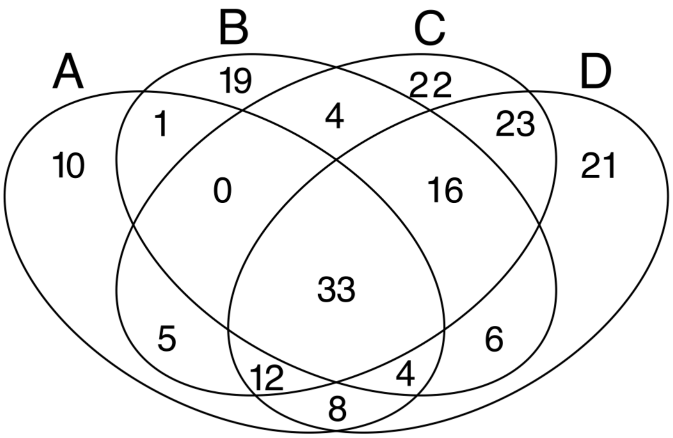
A
B
C
D
19
22
1
4
23
10
21
0
16
33
5
6
12
4
8

Figure 5(on next page)

Cluster dendrogram

I don't see this figure as necessary.

Cluster of microbial communities based on the TRFLPs profiles of the four studied quadrats (A*: 3 samples; B*: 3 samples; C*: 7 samples; D*: 8 samples) using Bray-Curtis dissimilarity distances and the Ward's hierarchical clustering algorithm.

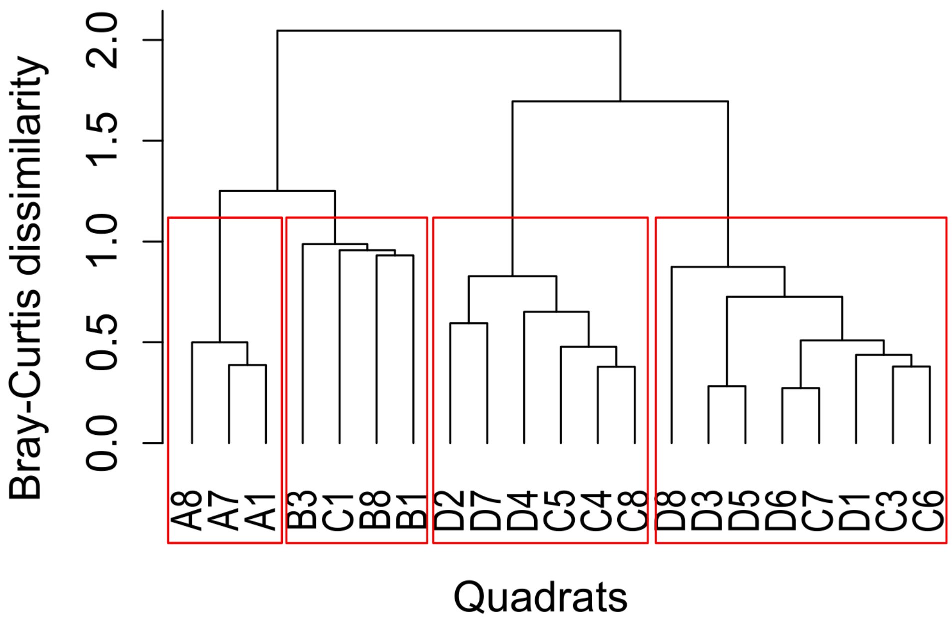
Bray-Curtis dissimilarity
0.0
0.5
1.0
1.5
2.0
A8
A7
A1
B3
C1
B8
B1
D2
D7
D4
C5
C4
C8
D8
D3
D5
D6
C7
D1
C3
C6
Quadrats

Figure 6(on next page)

Detrended Correspondence Analysis (DCA) of the TRFLPs profiles with respect to the soil properties

Sample sites for the four quadrats are represented by symbols, and OTUs are represented by grey crosses. Vectors stand for significant soil variables ($p < 0.1$). Each vector points to the direction of increase for a given variable and its length indicates the strength of the correlation with the axes

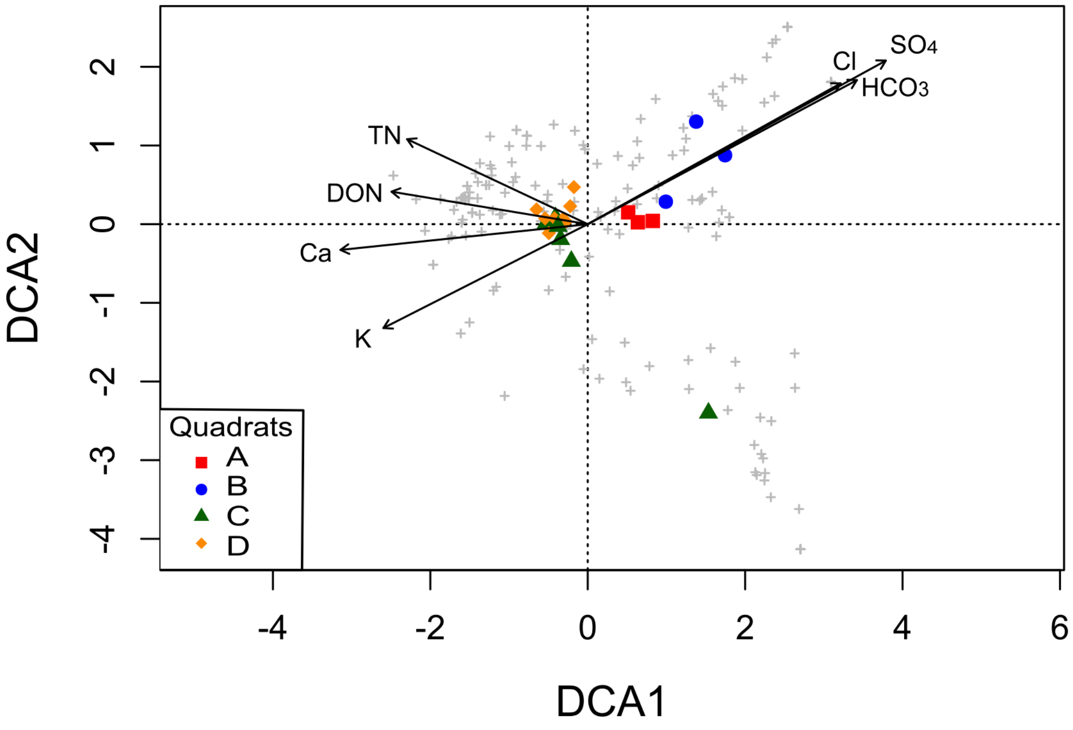
DCA2
DCA1
2
1
0
-1
-2
-3
-4
-4
-2
0
2
4
6
TN
DON
Ca
K
SO4
Cl
HCO3
Quadrats
■ A
● B
▲ C
◆ D

Table 1(on next page)

Soil physicochemical parameters (mean ± standard deviation) of the four studied quadrats within Churince System in the Cuatro Cienegas Basin (Mexico).

Table 1. Soil physicochemical parameters (mean ± standard deviation) of the four studied quadrats within Churince System in the Cuatro Cienegas Basin (Mexico).

Variable	Quadrat				Overall mean
	A	*B*	*C*	*D*	
Total C (mg g^{-1})	2.4 ± 0.8	2.4 ± 0.4	2.6 ± 0.4	2.8 ± 0.6	2.6 ± 0.6
Total N (mg g^{-1})	0.57 ± 0.13	0.48 ± 0.18	0.59 ± 0.1	0.60 ± 0.18	0.56 ± 0.15
Soil C:N	4.6 ± 2.3	6.1 ± 4	4.6 ± 0.7	5.0 ± 1.3	5.1 ± 2.4
Total P (mg g^{-1})	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01
NH_4^+ (µg g^{-1})	4.0 ± 0.6	4.2 ± 0.8	4.0 ± 0.6	3.6 ± 1	4.0 ± 0.7
NO_3^- (µg g^{-1})	1.8 ± 1.9	1.5 ± 1.5	1.6 ± 1.7	2.3 ± 1.5	1.7 ± 1.6
Dissolved organic C (µg g^{-1}) *	97.3 ± 27.8^{ab}	75.8 ± 30^{a}	83.0 ± 33^{ab}	124 ± 21.3^{b}	95.1 ± 33.2
Dissolved organic N (µg g^{-1})	14.6 ± 3.4	18.1 ± 12.3	17.9 ± 8.7	19.6 ±9.2	17.6 ± 8.7
Dissolved organic C:N	7 ± 2.9	6.4 ± 4.8	5.2 ± 2.4	7.9 ± 3.9	6.4 ± 3.5
Dissolved organic P (µg g^{-1})	4.3 ± 3.6	5.6 ± 2.1	2.6 ± 3.5	3.3 ± 3.7	3.9 ± 3.3
pH *	8.6 ± 0.1^{a}	8.7 ± 0.1^{a}	8.7 ± 0.1^{ab}	8.8 ± 0.1^{b}	8.7 ± 0.1
Electrical conductivity (dSm^{-1})	1.4 ± 0.1	1.4 ± 0.3	1.6 ± 0.2	1.4 ± 0.4	1.4 ± 0.3
Mg^{2+} (cmol kg^{-1}) *	27.1 ± 5.2^{a}	28 ± 6.3^{a}	35.2 ± 4.3^{ab}	36.5 ± 4.5^{b}	31.7 ± 6.5
Ca^{2+} (cmol kg^{-1}) *	0.56 ± 0.03^{a}	0.55 ± 0.02^{a}	0.64 ± 0.07^{ab}	0.65 ± 0.07^{b}	0.59 ± 0.07
Na^+ (cmol kg^{-1})	140 ± 15.9	127 ± 16.1	166 ± 38.1	157 ± 28.4	147 ± 29.1
K^+ (cmol kg^{-1}) *	0.95 ± 0.14^{a}	0.82 ± 0.19^{a}	1.27 ± 0.14^{b}	1.33 ± 0.25^{b}	1.09 ± 0.28
HCO_3^- (cmol kg^{-1}) *	2.8 ± 0.2^{b}	2.3 ± 0.6^{b}	1.2 ± 0.2^{a}	1.3 ± 0.3^{a}	1.9 ± 0.8
Cl^- (cmol kg^{-1}) *	2.8 ±0.2^{b}	2.5 ± 0.3^{b}	1.1 ± 0.4^{a}	1.3 ± 0.3^{a}	1.9 ± 0.8
SO_4^{2-} (cmol kg^{-1}) *	15.1 ± 2.2^{b}	16.4 ± 2.5^{b}	7.6 ± 0.8^{a}	7.2 ± 0.9^{a}	11.6 ± 4.6

Variable acronyms: C, carbon; N, nitrogen; P, phosphorous.

* Significant difference among quadrants ($p < 0.05$).

Different letters indicate that means are significantly different among quadrats.

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Table 2(on next page)

Alpha diversity estimates

OTUs diversity indices (mean ± standard deviation) from the TRFLPs data of the four quadrats (A*: 3 samples; B*: 3 samples; C*: 7 samples; D*: 8 samples).

Table 2. Alpha diversity estimates. OTUs diversity indices (mean ± standard deviation) from the TRFLPs data of the four quadrats (*A*: 3 samples; *B*: 3 samples; *C*: 7 samples; *D*: 8 samples).

Quadrat	Richness (S)	Shannon (H)*	Simpson (1/D)*	Berger-Parker*
A	48 ± 9	3.31 ± 0.08[a]	0.944 ± 0.004[a]	0.153 ± 0.004[b]
B	36 ± 15	1.98 ± 0.62[b]	0.704 ± 0.145[b]	0.497 ± 0.144[a]
C	45 ± 13	2.56 ± 0.44[ab]	0.8 ± 0.105[ab]	0.393 ± 0.138[a]
D	47 ± 19	2.3 ± 0.77[ab]	0.738 ± 0.189[b]	0.426 ± 0.203[a]

* Significant difference among quadrats ($p < 0.05$).

Different letters indicate that means are significantly different among quadrats.