PALAIOS
Emphasizing the impact of life on Earth's history

PALAIOS, 2016, v. 31, 1–9
Research Article
DOI: http://dx.doi.org/10.2110/palo.2015.010

MICROORGANISMS, ORGANIC CARBON, AND THEIR RELATIONSHIP WITH OXIDANT ACTIVITY IN HYPER-ARID MARS-LIKE SOILS: IMPLICATIONS FOR SOIL HABITABILITY

JULIO E. VALDIVIA-SILVA,[1,2] FATHI KAROUIA,[3,4] RAFAEL NAVARRO-GONZALEZ,[5] AND CHRISTOPHER MCKAY[1]
[1]*Space Science and Astrobiology Division, NASA Ames Research Center, Moffett Field, CA 94035, USA*
[2]*Instituto Nacional de Investigación y Capacitación de Telecomunicaciones Universidad Nacional de Ingeniería (INICTEL-UNI), Lima-Perú,*
[3]*Exobiology Branch, NASA Ames Research Center, Moffett Field, CA 94035, USA*
[4]*Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA 94158, USA*
[5]*Laboratorio de Química de Plasmas y Estudios Planetarios, Instituto de Ciencias Nucleares,*
Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria México D.F., 04510, México
e-mail: jvaldivia@inictel-uni.edu.pe

ABSTRACT: **Soil samples from the hyper-arid region in the Atacama Desert in Southern Peru (La Joya Desert) were analyzed for total and labile organic carbon (TOC and LOC), phospholipid fatty acids (PLFA), quantitative real time polymerase chain reaction (qRT-PCR), 4′,6-diamidino-2-phenylindole (DAPI)-fluorescent microscopy, culturable microorganisms, and oxidant activity, to understand the relationship between the presence of organic matter and microorganisms in these types of soils. TOC content levels were similar to the labile pool of carbon suggesting the absence of recalcitrant carbon in these soils. LOC ranged between 2 to 60 μg/g of soil. PLFA analysis indicated a maximum of 2.3×10^5 cell equivalents/g. Culturing of soil extracts yielded 1.1×10^2–3.7×10^3 CFU/g. qRT-PCR showed between 1.0×10^2 and 8×10^3 cells/g; and DAPI fluorescent staining indicated bacteria counts up to 5×10^4 cells/g. Arid and semiarid samples (controls) showed values between 10^7 and 10^{11} cells/g with all of the methods used. Importantly, the concentration of microorganisms in hyper-arid soils did not show any correlation with the organic carbon content; however, there was a significant dependence on the oxidant activity present in these soil samples evaluated as the capacity to decompose sodium formate in 10 hours. We suggest that the analysis of oxidant activity could be a useful indicator of the microbial habitability in hyper-arid soils, obviating the need to measure water activity over time. This approach could be useful in astrobiological studies on other worlds.**

INTRODUCTION

Studies of Mars-like soils on Earth provide an important approach for better understanding the physical, geochemical, and microbiological processes that occur, or could have occurred, on Mars (Navarro-González et al. 2006; Mahaffy 2008; Peters et al. 2008). Appropriate analogues of Mars-like areas on Earth are identified by their similar mineralogy and geochemical context or by current physical and chemical conditions that illustrate preservation mechanisms that might guide the search for fossil and living evidence of microbial life (Preston and Dartnell 2014). Mars today is a cold, dry desert world with surface conditions that are not habitable for even the hardiest known life forms from Earth, yet there is ample evidence of past water activity and the presence of interesting niches for life (e.g., such as subsurface and/or evaporitic minerals). Thus Mars is a prime target for looking for extraterrestrial microorganisms (Davila et al. 2010; McKay 2010).

A Martian soil analogue extensively studied and of great scientific interest is the Atacama Desert, located in northern Chile and southern Peru. This desert lies on the west slopes of the Central Andes between 15°S and 30°S (Houston and Hartley 2003; Hartley et al. 2005) and is considered one of the oldest and driest deserts on Earth. Previous research has identified the Atacama as a key analogue model for life in dry Mars-like conditions (McKay et al. 2003; Navarro-González et al. 2003) based on the very low levels of organic carbon (20–40 ppm), non-biological oxidants, and exotic evaporitic minerals—all characteristics that are expected on Mars (Connon et al. 2007; Fletcher et al. 2012). Detailed multidisciplinary studies comparing those characteristics from the Pampas de La Joya, our sampling site located in the Peruvian hyper-arid region, and Mars have demonstrated that the Pampas de La Joya represents a valuable Martian analogue for studies of oxidative processes that may occur on Mars, and can be used for the testing of instruments designed for future Martian life detection missions (Valdivia-Silva et al. 2009, 2011, 2012a, 2012b). These studies showed different types of abiotic oxidants capable of rapidly destroying organics under several experimental conditions (Quinn et al. 2007; Valdivia-Silva et al. 2012c). Experimental results were extrapolated to Mars to explain the low levels of organic compounds on its surface (Ponnamperuma et al. 1977; Oyama and Berdahl 1979; Quinn and Zent 1999; Quinn et al. 2007; Quinn et al. 2013). Since the return of the Viking data, several hypotheses have been presented to explain oxidative activity on the Martian surface (Quinn et al. 2007, 2013). Hydrogen peroxide, superoxides, UV radiation, peroxide-modified titanium dioxide, peroxinitrites, radiolysis products of perchlorates, etc., are possible candidates (McKay et al. 1998; Zent et al. 2008; Quinn et al. 2011, 2013). Similarly, Pampas de La Joya and other samples from the Atacama have shown the presence of non-chirally specific and as-yet-unidentified oxidants—indicating chemical oxidation rather than biological consumption of amino acids and sugars (Navarro-Gonzalez et al. 2003; Valdivia-Silva et al. 2012c). Although this region is characterized by large amounts of deposited salt (Michalski et al. 2004) and contains highly oxidative species, including iodates (IO^{3-}), chromates (CrO_4^{2-}), perchlorates (ClO_4^-), and probably persulfates ($S_2O_8^{-2}$)

Published Online: January 2016
 0883-1351/16/031-001/$03.00

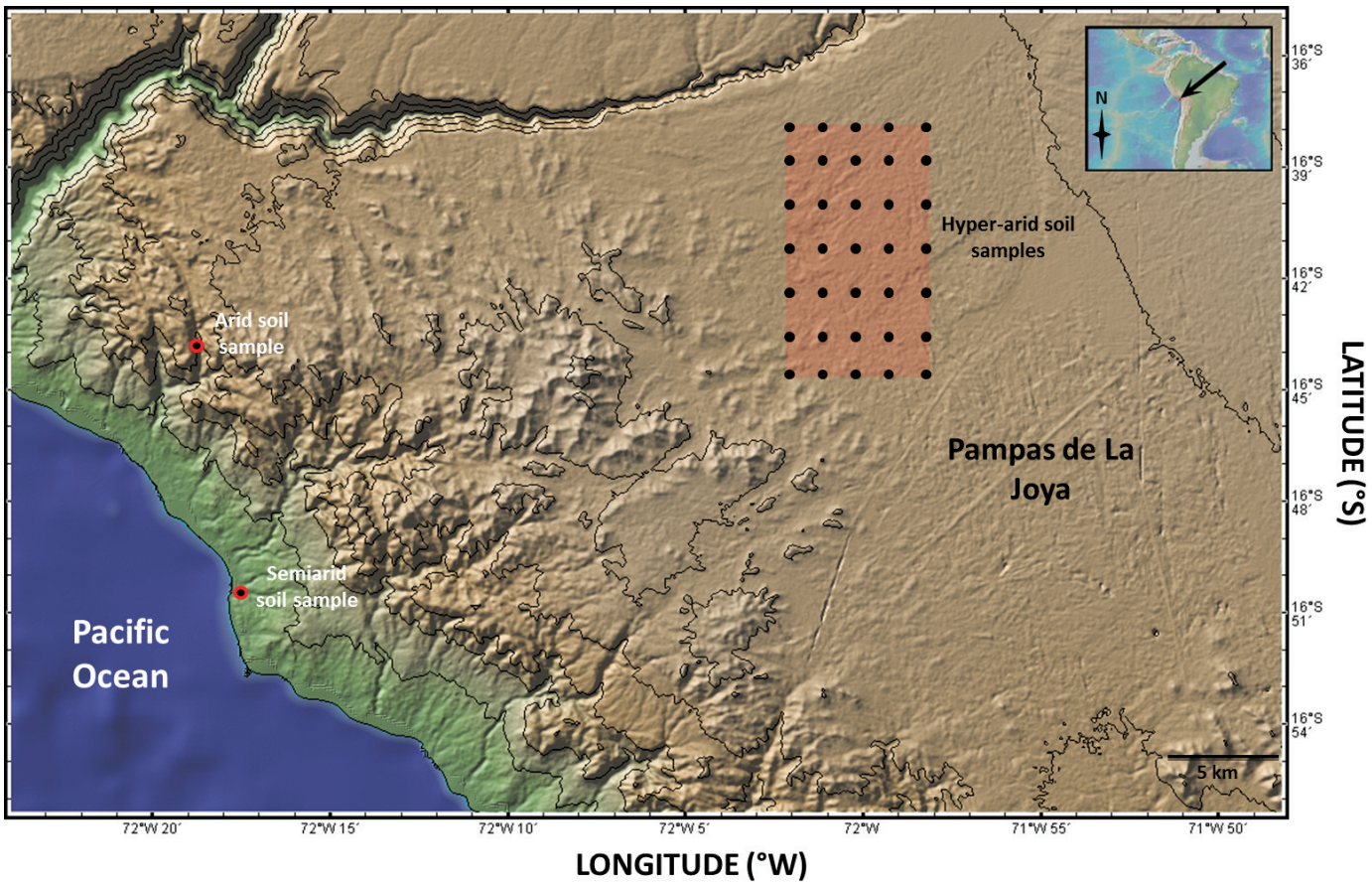

FIG. 1.—Location of the Pampas de La Joya region, SW Peru showing the hyper-arid region analyzed (rectangle), and the arid and semiarid soil sampling locations.

(Ewing et al. 2006), these compounds do not completely explain the oxidizing activity in the Atacama or on Mars (Navarro-Gonzalez et al. 2003; Quinn et al. 2007; Valdivia-Silva 2009, 2011). The suggestion by Quinn et al. (2013) that the radiolysis of perchlorates may explain the Viking results does not provide a complete explanation for the Atacama oxidants.

On the other hand, an important and contradictory issue to resolve in this type of soil is that the levels of organic material do not correlate with the number of microorganisms or biomass inside the hyper-arid region (Navarro-Gonzalez et al 2003; Valdivia-Silva et al 2011). Although microbiological studies have shown good correlation between organics and microorganisms along precipitation gradients from semiarid, to arid and hyper-arid areas and from the Pacific coast to the core of the desert (Drees et al. 2006; Fletcher et al. 2011), most analyses within the hyper-arid region have shown inconsistent results with regard to number of microorganisms (Orlando et al. 2010; Fletcher et al. 2011).

It is known that in extreme arid deserts, low water activity severely limits microbial growth, abundance, and diversity (Kieft 2002; Warren-Rhodes et al. 2006; Crits-Christoph et al. 2013). However, these environmental conditions do not completely explain the high variability of microorganisms forming patches in the hyper-arid region even a few meters apart. Indeed, although previous reports of near sterile Atacama Desert soils (~ 10 Colony Forming Unit (CFU)/g) have led to the suggestion that the dry limit for microbial life had been crossed (McKay et al. 2003; Navarro-González et al. 2003), other investigations in the region found as many as ~ 10^6 cells/g (Glavin et al. 2004) or even more (Drees et al. 2006; Orlando et al. 2010). Moreover, in a comprehensive survey of the Yungay region, Bagaley (2006) found variations in CFU spanning orders of magnitude over distances of a few hundred meters.

Based on this high heterogeneity of microorganisms, despite almost uniform, albeit low, levels of water in the area, we hypothesize that it is the level of oxidant activity, rather than the amount of water, which determines the survival of microorganisms and maybe organic matter in hyper-arid soils. Indeed, although the presence of water could be beneficial for growth, it could also be detrimental to the survival of microorganisms and organic compounds in these soils since all mineral oxidants require water to trigger chemical reactions.

In this work, we present the results of a variety of microbiological and geochemical analyses of soil samples collected from the hyper-arid soils of Pampa de la Joya in southern Peru (Valdivia-Silva et al. 2011; Preston and Dartnell 2014), and at two points along a latitudinal moisture gradient to the Pacific coast used as controls (from 0.5 mm to 120 mm/y rainfall) (Fig. 1) in order to evaluate: (1) the distribution of biomass and organic carbon in the core of the hyper-arid region, and (2) the relationship between these measurements and the oxidizing processes in the area, which could be limiting the growth of microorganisms.

METHODOLOGY

Soil Samples

Soil samples used in this study were collected from 2008 to 2012 in the hyper-arid area of Pampas de La Joya (alternatively called "La Joya Desert"), located about 70 km from the city of Arequipa, along the South Pacific coast of Peru, (16°S–17°S, 71.5°W–72.5°W), and approximately

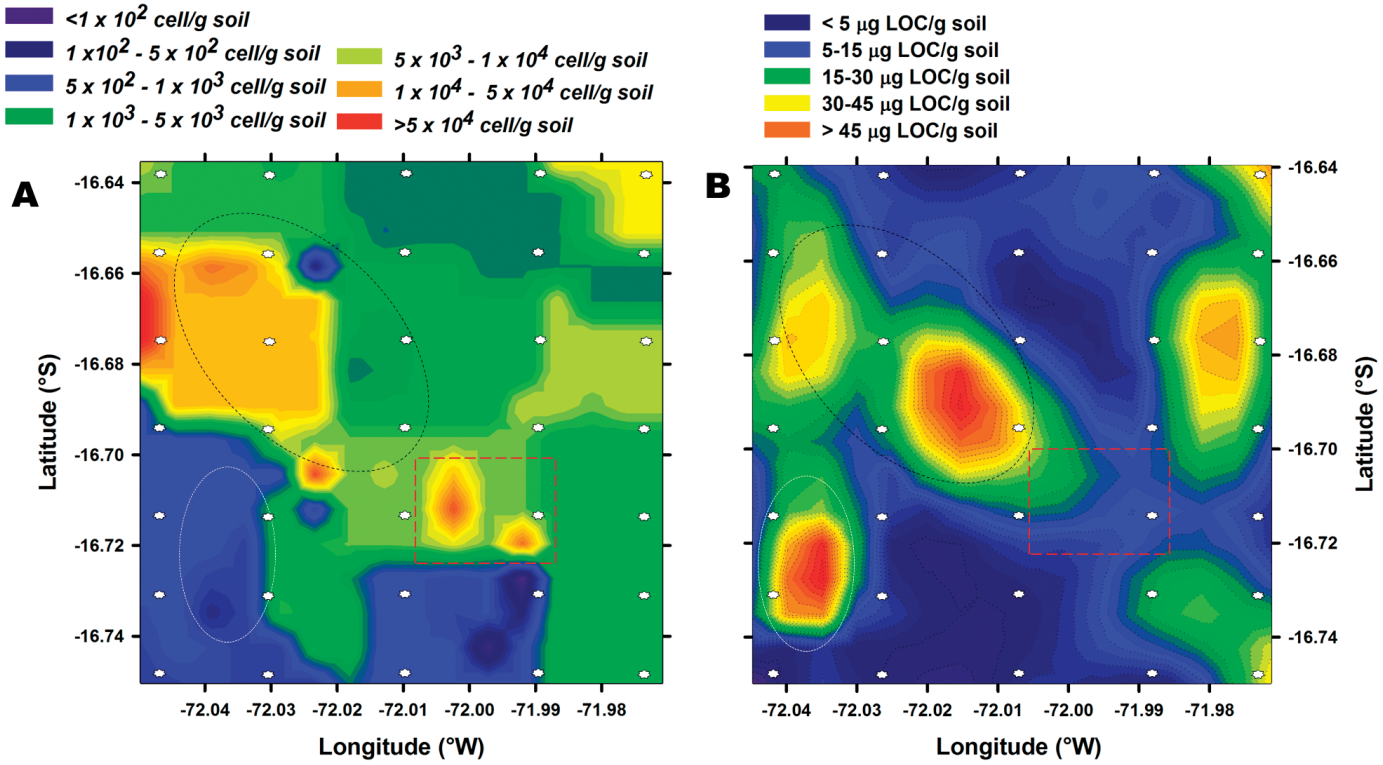

FIG. 2.—Labile organic carbon (A) and microorganism (B) distributions in the study area in Pampa de La Joya. Labile organic carbon was evaluated by permanganate titration in acid media. The population density of microorganisms was analyzed in 35 samples using DAPI staining protocol and fluorescent microscopy. Key: white points=sampling sites; big circles=high values of organic carbon and high levels of microbes; small circles=high levels of organic carbon related to very low levels of microorganisms; rectangles=very low levels of organic carbon and high population density of microorganisms; blue areas=both organic carbon and microorganisms with very low levels (see text for more detail).

between 1000 and 2000 meters above sea level. The entire area is part of the Atacama Desert, inside of the region considered as hyper-arid (Houston and Hartley 2003). This region is considered hyper-arid because the Aridity Index (AI); calculated as the evapotranspiration/precipitation ratio is less than 0.05 (Thornwaite 1948; UNEP 1997). Analyses were carried out on samples from an area of 96 km^2 (12 × 8 km) (16°38.386′S–72°2.679′W and 16°44.986′S–71°58.279′W) (Fig. 1). This quadrangle contains interesting locations of evaporitic minerals, quartz rocks, and soils with high oxidant activity and very low levels of organic matter (Valdivia-Silva et al. 2009, 2011, 2012a).

Between 50 and 100 g of soil, representing a composite of five individual samples within a ~ 1.5 m radius, were collected from the surface to a depth of 5–10 cm using sterile scoops and stored in sterile polyethylene (Whirlpak TM) bags for transport until analysis. The samples were homogenized to compensate for any small scale spatial heterogeneity (Girvan et al. 2003). Immediately after collection, the samples were stored in a cool, dry, and dark location before being shipped to NASA ARC. Transport required four days, and after arrival, the samples were stored in a −20°C refrigerator until the analyses of TOC, LOC, PLFA, qRT-PCR, DAPI, and oxidation activity. Culture-dependent assays were initiated 14 days after sample collection and separate bags of soil were used for microbiology analysis and chemical studies. Although storage conditions could change the structure and function of communities, several studies have shown that microorganisms in hyper-arid soils are in a dormant state due to very low water activity, and storage in similar conditions (mainly low humidity) did not show significant change in the microbial community (Navarro-Gonzalez et al. 2003; Glavin et al. 2004; Drees et al. 2006; Connon et al. 2007; Lester et al. 2007; Orlando et al. 2010; Fletcher et al. 2011; Crits-Christoph et al. 2013). Furthermore, in this study we track changes in total microbial biomass and not microbial community structure.

Because of the high spatial heterogeneity reported in these soils (Peeters et al. 2009; Valdivia-Silva et al. 2012b), sampling was conducted on a systematic "grid" type (Webster and Oliver 1990) (Fig. 1). For labile organic carbon (LOC) and fluorescence microscopy (DAPI-FM), 35 soil samples spaced 2 km apart were analyzed; for total organic carbon (TOC), quantitative polymerase chain reaction (qRT-PCR), and cultures, 12 samples spaced 4 km apart were evaluated; and finally for phospholipid fatty acid analysis (PLFA), five samples from the vertices and at the midpoint of the quadrangle were analyzed. Additionally, two more samples were collected (positive controls) following a short precipitation gradient from an arid area (P/PET > 0.05; coordinates 16°43.945′S–72°18.645′W) and a semiarid area (P/PET > 0.2; coordinates 16°50.31′S–72°17.16′W) (Fig. 1). LOC and DAPI analyses were first used to first evaluate the presence of a correlation between both variables (Fig. 2) and this was followed by the application of the other methods used in this study to confirm and validate the number of bacteria obtained in the DAPI results. LOC and DAPI are faster than the other methods and can be used to build a preliminary map of variations and correlations on which to base further specific analysis (Fletcher et al. 2011, 2014).

Organic Carbon Analysis

The labile organic carbon (LOC) content was evaluated by permanganate titration in acid media (Valdivia-Silva et al. 2011; Fletcher et al. 2012). This technique has proven to be simple, accurate, sensitive, and reproducible for the quantification of LOC in hyper-arid soils (Fletcher et al. 2012, 2014). These soils have shown negligible levels, almost absence, of recalcitrant carbon. The labile form is the most abundant in these soils and includes

molecules with biological importance such as amino acids, nucleotides, lipids, sugars, and aliphatic and aromatic hydrocarbons (Skelley et al. 2007; Ewing et al. 2008). In order to demonstrate the low levels of other forms of organic carbon we evaluated the total organic carbon (TOC) using an Elemental Analyzer model EA1108 (Fisions, Loughborough, U.K.) at 1200°C. The inorganic carbon from carbonates and bicarbonates was removed by acid treatment (Navarro-Gonzalez et al. 2006).

Negative controls were made by heating some soil samples to 500°C for eight hours.

Microbiological Analyses

The quantification of microorganisms in hyper-arid soils has proven be a real challenge since established methods each have specific limitations. Because of this, in this work we analyze the number of microorganisms using different culture-independent methods and a variety of culture-based techniques. A more comprehensive comparison between molecular enumeration techniques used in this type of dry soil was published by Fletcher et al. (2011, table 3).

DAPI Staining by Fluorescence Microscopy.—Soil samples from La Joya were analyzed under the fluorescence microscope by means of 4′,6-diamidino-2-phenylindole (DAPI) stain protocol, which is a fluorescent stain that binds strongly to double-stranded DNA. For fluorescence microscopy, DAPI is excited with ultraviolet light (absorption at 358 nm) emitting blue light at 461 nm. Despite being less specific for DNA than previously thought, DAPI has been used as the bacterial stain of choice for a wide range of sample types and is particularly useful for quantifying the total number of nonviable and viable bacteria in natural samples (Kepner and Pratt 1994). The protocol of this technique has been previously reported for hyper-arid soils demonstrating good sensibility and reproducibility (Glavin et al. 2004; Fletcher et al. 2011). Analysis of images was done using NIH Image-J, which enabled the calculation of bacterial biomass in terms of pixels (Kemp 1993; Posch et al. 2001).

Phospholipid Fatty Acid Analysis (PLFA).—PLFA analyses on 50 g soil samples were performed by Microbial Insights (Rockford, TN). The limit of detection for PLFA analysis is ~ 50 picomoles of total PLFA and the limit of quantification is ~ 150 picomoles of total PLFA (10^4 cell/g). Microbial biomass was determined from the total concentration of ester-linked phospholipid fatty acids in the samples (White and Ringelberg 1997). PLFA analysis could detect phospholipids from dead microorganisms which can remain for a long time in the dry soils due to very low, if any, bacterial consumption or chemical activity. Preliminary results of the structural community profiles were generated for each sample by community-level PLFA analyses based on six major PLFA structural groups, each of which corresponds to a broad phylogenetic group of microorganisms (White 1979; Lehman et al. 1995; White et al. 1996). The complete protocol of this technique is published on the Microbial Insights website (http://www.microbe.com/).

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR).—The DNA extraction and purification of soil samples was done using the Ultra-Clean Mega Soil DNA extraction kit (MoBio Laboratories Inc.) following the recommended instructions.

Amplifications were performed using a Cepheid, Inc., Smart Cycler automated real-time PCR system with 25 μL reaction tubes. The necessary enzymes were added by using OmniMix PCR Beads (Cepheid, Inc.), a premade enzyme mix containing reagents for the PCR reaction that are stable in a field environment. The 16S rRNA genes were amplified using the universal primers 8F (5′-AGA GTT TGATCM TGG CTC AG-3′), and 1492R (5′-GGY TAC CTT GTT ACG ACT T-3′). A calibration curve was generated by running a serial dilution series using purified *Bacillus globigii* DNA at known concentrations, from a starting concentration of 10^6 copies/μL. Klappenbach et al. (2001) evaluated a variety of soil samples and determined that a reasonable estimate for copy number in common soil organisms was 5.5 copies of 16S rRNA per bacterium, so this value was used as a conservative number of copies of operons per bacteria for the purposes of this study in the estimation of the number of bacteria per gram of soil from DNA extraction and amplification. Details of the PCR protocol was reported by Fletcher et al. (2011).

Culturable Microbial Populations.—Five grams of sieved-homogenized soil were mixed with 10 mL of a sterile 5 mM sodium pyrophosphate buffer (pH 8.0) + 1% Tween 80 solution and vortexed for five minutes. The suspension was allowed to settle until a clear layer appeared on top, approximately two hours. Four mL of the clear layer were removed to create the extract; 100 mL of the extract were plated in triplicate on R2A and TSA medium plates (Hardy Diagnostics, Santa Maria, CA) and incubated at 26°C for three weeks. Sterile water aliquots plated under identical conditions yielded no growth.

Oxidant Activity in Soils

In order to evaluate the decomposition rates of organic molecules in soil samples, we monitored the release of $^{13}CO_2$ produced by the oxidation of 0.25 cc (0.25 mM) of ^{13}C labeled sodium formate (IsoTec Inc.) added to the samples. The aqueous organic solution was introduced into a sample vial that contained 1.2 g of soil and incubated for 24 hours. The headspace $^{13}CO_2$ (ion 45 m/z) released was syringe-extracted each hour using an autosampler coupled to a gas chromatograph-ion trap mass spectrometer (Varian® serie CP-3800, MS 4000). Measured levels of $^{12}CO_2$ (44 m/z) were used to correct for the natural abundance of $^{13}CO_2$ (~ 1.1%) present in the sample cells. The detection limit for the method was 2 nmol of carbon. Control experiments in which sodium formate was not added or where soil was pre-heated to 500°C for eight hours were run in triplicate.

RESULTS

Distribution of Organic Carbon

Baseline levels of organic carbon in samples from the study area, as well as from the arid and semiarid comparison locations, were assessed using acid hydrolysis extraction followed by permanganate oxidation, whereas that the total organic carbon (TOC) was evaluated with elemental analysis (Table 1). The range of labile organic carbon in the hyper-arid quadrangle raged from 2 to 60 μg C/g of soil (mean value 31.6 ± 25 μg C/g of soil) (Table 1, Fig. 2B), whereas the arid and semiarid sites showed mean values of 85.8 ± 33 μg C/g and 167.2 ± 66 μg C/g of soil, respectively. Notably, all sites showed areas with high heterogeneity and variability of organic carbon content (Fig. 2B). Values lower than 15 μg C/g of soil represented approximately 50% of the study area and values close to 60 μg C/g of soil were ~ 10% of the total study area. By comparison, the range of TOC was from 10 to 75 μg C/g of soil (mean value 34.9 ± 30 μg C/g of soil). Interestingly, the TOC values did not show a statistically significant difference from the LOC values (U-MW test, $p=0.8$) implying that non-labile forms of organic carbon, mainly recalcitrant, are not present or are at negligible levels in the sample sites.

On the contrary, the arid and semiarid areas showed high values of TOC compared to the LOC levels (Table 1), demonstrating the presence of recalcitrant organic carbon not susceptible to oxidation, such as complex molecules and debris from plants or insects.

Distribution of Microorganisms

Because previous attempts to detect microorganisms from hyper-arid soil samples indicated high variability and heterogeneity even a few meters

TABLE 1.—*Levels of organic carbon and microorganisms in dry soils from Pampas de La Joya Desert.*

Sampling regions	TOC (μg C/g)	LOC (μg C/g)	DAPI-FM (cell/g)	RTq-PCR (cell/g)	PLFA (cell/g)	%Total PLFA						Culturable counts (CFU/g)	
						Tb	*Mb*	*Ns*	*Mo*	*Br*	*Po*	TSA	R2A
Hyperarid	34.9 ± 30	31.6 ± 25	1.2×10^4	2.2×10^3	1.4×10^5	6.5	3.4	19	70	1.1	Nd	1×10^3	2×10^3
Range	10–75	2–60	10^2–10^4	10^2–10^3	10^4–10^5							Nd–10^3	Nd–10^3
Arid	207 ± 88	85.8 ± 33	4.3×10^7	9.7×10^7	6.6×10^7	15	7	20	56	1.5	0.5	2×10^7	2×10^7
Semiarid	620.9 ± 126	167.2 ± 66	8.7×10^{11}	2.2×10^{11}	9×10^{11}	18	4	25	46	2	5	2×10^{10}	3×10^{11}

Abbreviations: TOC=total organic carbon; LOC=labile organic carbon; DAPI-FM=DAPI staining by fluorescent microscopy; RTq-PCR=real time quantitative polymerase chain reaction; PLFA=phospholipid fatty acid analysis; Tb=terminal-branched satured; Mb=mid-branched saturated; Ns=normal saturated; Mo=monoenoic; Br=branched monoenoic; and Po=polyenoic; Nd=not detected. The data represent the mean values (+/− standard deviation for TOC and LOC). Errors for culturable counts are all less than 50% of reported values based on quadruplicate plating results.

apart (Navarro-Gonzalez et al. 2003; Bagaley 2006), here we report the number of microorganisms and their distribution evaluated by a variety of culture-dependent and culture-independent techniques (Table 1).

Direct counting methods using DNA-specific fluorochromes, such as DAPI, have traditionally been used to count total microbial cells for a wide range of sample types (Kepner and Pratt 1994) and are particularly useful for quantifying the total number of bacteria in natural samples in large areas for initial mapping (Glavin et al. 2004). Using the DAPI staining method, we measured bacterial counts from hyper-arid, arid, and semiarid soil samples at 1.2×10^4 cells/g, 4.3×10^7 cells/g, and 8.7×10^{11} cells/g, respectively. As expected, these values compared reasonably well to the amount of organic carbon present along the precipitation gradient. However, a more detailed analysis within the study area showed high variability with values between 10^2 and 10^4 cells/g of soil (Fig. 2A). Moreover, four of the 35 samples in the sampling area showed values higher than 5×10^4 cells/g of soil, similar to the number of bacteria found in Yungay region with the same method (Glavin et al. 2004). Importantly, the mean of biomass calculated for the study area was 34.9 ± 12.1 ng C/g of soil. Knowing that ~ 10^6 bacteria provide 1 μg C/g of soil (1 ppm) of organic carbon (Smayda 1978), the contribution of carbon by microorganisms in this type of soil is almost negligible (Valdivia-Silva et al. 2011), as indicated by the lack of correlation between both variables (r~0; Pearson correlation). The four types of relationships corroborate this statement: (1) one where the concentration of organic carbon (> 30 μg C/g of soil) was related to relatively high levels of microorganisms (> 10^4 cells/g of soil) (i.e., big circles in Fig. 2); (2) another where a high concentration of organic carbon (> 45 μg C/g of soil) was related to extremely low levels of microorganisms (< 5×10^2 cells/g of soil) (i.e., small circles in Fig. 2); (3) regions where very low levels of organic carbon (< 15 μg C/g of soil) were related to high values of microorganisms (> 5×10^3 cells/g of soil) (i.e., rectangles in Fig. 2); and (4) areas where very low levels of organic carbon (< 15 μg C/g of soil) were related to low levels of microorganisms (< 10^3 cells/g of soil) (i.e., blue areas in Fig. 2A, 2B). Clearly, our results demonstrate a lack of any correlation between these variables in hyper-arid soils; however, the carbon contribution from microorganism is more effective in areas where there is arguably more moisture, as is the case of the arid and semiarid areas used in this work as positive controls.

qRT-PCR analyses in these soils showed values between 1×10^2 and 8×10^3 bacteria per gram of soil (mean of 6.2×10^3 cells/g of soil). The low values obtained by this method could be mainly due to problems with the efficiency of DNA extraction in these types of soils (Fletcher et al. 2011). Importantly, culturing of soil extracts yielded similar levels of bacteria between 1.1×10^2–3.7×10^3 CFU/g excluding two soil samples which showed no bacterial growth after 60 days of incubation.

PLFA analyses, in terms of cell equivalents, in the five surface samples averaged 2.3×10^5 cell/g—higher than the corresponding fluorescent values. However, it is important to note that the interpretation of PLFA biomass as viable biomass when investigating hyper-arid soils could be in error, because this interpretation rests on the assumption that microbial activity rapidly degrades PLFA after cell death (Connon et al. 2007). Since water activity rarely rises above the metabolic activity threshold in these soils, the extracted PLFA might represent both current viable communities and previous communities whose cellular remains are preserved due to the lack of microbial activity in these soils. The PLFA profile from these soils showed that the detectable microbial community is primarily composed of *Proteobacteria, Actinobacteria*, and *Firmicutes* (Table 1). For hyper-arid soil samples, the PLFA composition was dominated by monoenoic PLFA (Mo), ranging from 64 to 72%, indicating the presence of Gram-negative *Proteobacteria*. The monoenoic PLFA content in arid and semiarid samples were ~ 15 and 25% lower than hyper-arid samples, with a corresponding increase in terminally branched saturated PLFA (Tb) up to 15 and 18% respectively, indicating the presence of a *Firmicutes* population. In the same way, mid-branched saturated PLFAs (Mb) increased from ~ 3.5% in hyper-arid to 10 and 14% in arid and semiarid samples respectively, indicating the presence of *Actinobacteria*. Branched-monoenoic (Br) of micro-aerophiles and anaerobes, and Polyenoic PLFAs (Po) found in the cell membranes of eukaryotes, showed very low levels and were completely absence in hyper-arid soils, unlike wetter regions where these PLFAs reach up to ~ 5%. Normal saturated PLFAs, found in all organisms, showed relatively high values indicating less diverse populations in these types of soils. Importantly, the very low ratios of cyclopropyl-PLFA to cis-PLFA and cis-PLFA to trans-PLFA indicate that metabolic activity was arrested before cellular responses to stress could be initiated.

Soil Oxidant Activity

To determine soil oxidant activity we measured the decomposition of sodium formate to $^{13}CO_2$. We analyzed two samples taken in the driest part of the study area known as "Mar de Cuarzo", and in two wetter areas corresponding to arid and semiarid regions. Similar to our previous results, there were significant differences in the kinetic behavior between soil samples under similar conditions depending on the moisture and organic content (and microorganisms) (Valdivia-Silva et al. 2012c). The samples belonging to the most arid environment showed a very high release of carbon dioxide ~ 40 nmoles in a few hours. The arid soil sample showed a slight peak up to ~ 20 nmoles of $^{13}CO_2$ in a few hours, and after 24 hours the kinetics of release were dominated by an evident biological decomposition of the nutrients. In contrast, the semiarid soil sample showed kinetics of decomposition consistent with biological activity, because it did not show any abrupt peak of carbon dioxide in the first few hours, and the kinetic curve increased at a steady rate of ~ 0.2 nmol/h in the first 24–30 h. Then, it rose rapidly in a manner consistent

TABLE 2.—*Oxidant activity and microorganisms in dry soils from Pampas de La Joya Desert.*

Oxidant activity	LOC (µg C/g)	Microorganisms (cell/g)*	$^{13}CO_2$ (nmol/h)
Low	< 15 and >45	$> 5 \times 10^4$	0.8–1
Moderate	15–45	10^3–10^4	1.5–2
High	Any concentration	$< 5 \times 10_2$	4–6

Abbreviations: LOC=labile organic carbon; *=range of the number of microorganisms obtained by different methods (DAPI staining by fluorescent microscopy, real time quantitative polymerase chain reaction, phospholipid fatty acid analysis and culture). The absence of correlation with organic matter is explained in the text.

with the growth and metabolism of microorganisms (Valdivia-Silva et al. 2012c).

In order to evaluate possible heterogeneity of oxidant activity in the hyper-arid soils within the study area that could explain the non-homogeneous distribution of organics and microorganisms, a more comprehensive analysis was done in this region. We classified the samples soils into three groups—high, moderate and low—based on the oxidant activity present, as evidenced by the release of $^{13}CO_2$ in the first 10 hours of the experiment (Table 2; Fig. 3). Our results showed a strong inverse correlation between the levels of decomposition of sodium formate with the presence of microorganisms. Soil samples with oxidation rates greater than 4 to 6 nmol/h were always associated with the lowest levels of microorganisms (< 5×10^2 cells/g of soil as indicated by DAPI). In contrast, the soil organic carbon content showed a wide range of values between very low (< 15 µg C/g of soil) and high levels of LOC (> 45 µg C/g of soil). After 10 hours of incubation, there was a sharp fall in $^{13}CO_2$ between 20 and 50% over the next 20 hours. The abrupt fall of headspace $^{13}CO_2$ levels after the initial increase is apparently explained by an uptake of $^{13}CO_2$ into solution due a shift in soil/solution pH in the absence of biological activity (Quinn et al. 2007). These results corresponded with the lowest levels of microorganisms detected by qRT-PCR and cultures, and in two cases, the soils with a release rate greater than 6 nmol/h of $^{13}CO_2$ did not show any type of bacterial growth after 60 days of culture. Soils with moderate activity between rates of 1.5 to 2 nmol/h were associated with organic carbon content between 15 and 45 µg C/g of soil and between 1×10^3 and 1×10^4 cells/g of soil. After 11 hours of releasing carbon dioxide, the levels remained steady with small fluctuations for the next 20 hours. This type of soil had a similar oxidant activity behavior to the arid soil samples used as controls. Finally, soils

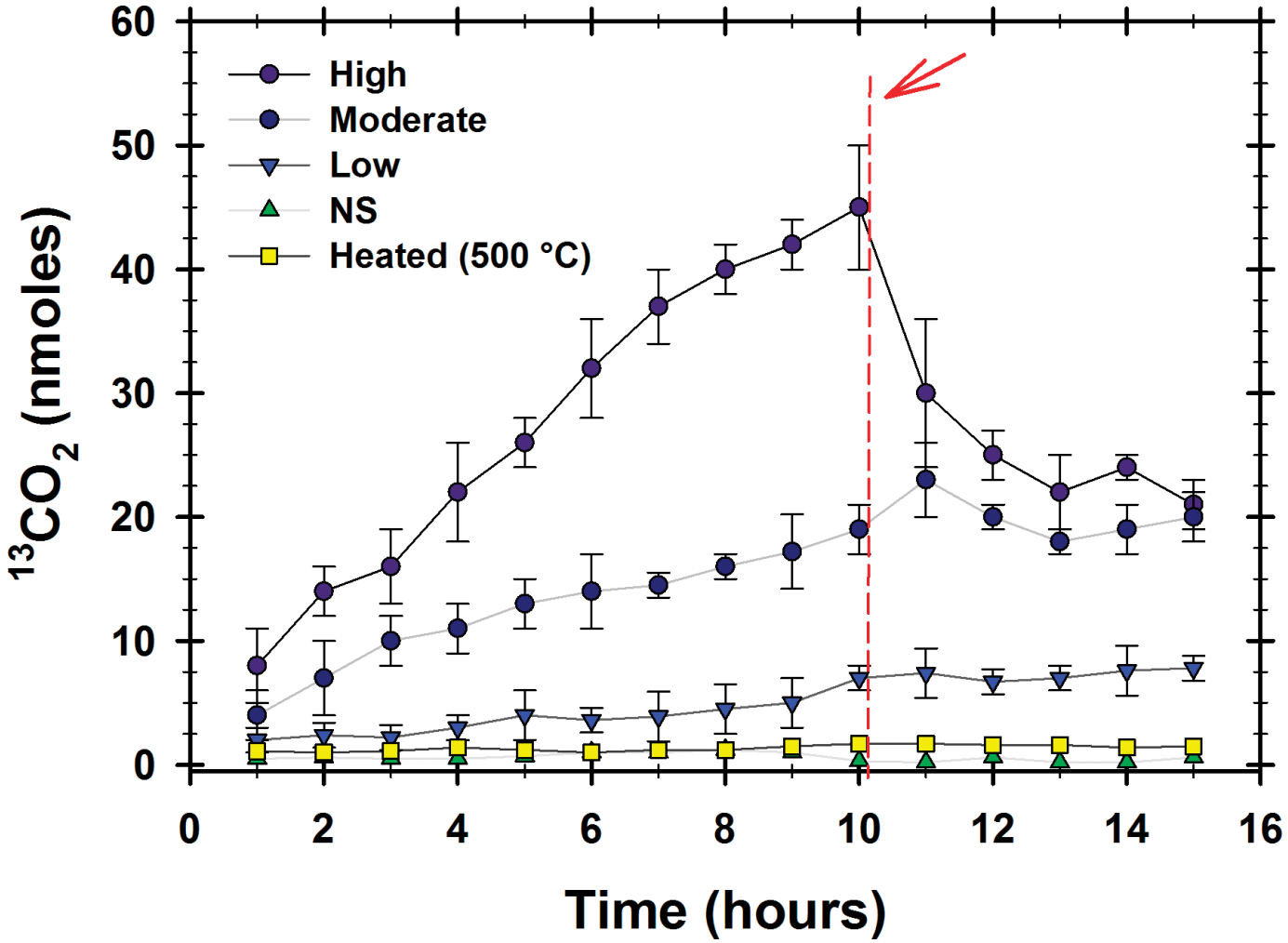

FIG. 3.—Oxidant activity trends in hyper-arid soils from Pampas de La Joya. Oxidant activity was evaluated using the rates of decomposition of ^{13}C-labeled sodium formate over 24 hours (graphic shows 16 hours of experiment). This experiment shows three replicates per location. The dose of sodium formate added was 0.25 mM at 0.25 cc of solution. Control experiments were run in triplicate where sodium formate was not added, or where soil was pre-heated to 500°C for 8 hours.

with low oxidant activity (0.8–1 nmol/h) were strongly associated with high content of microorganisms ($> 5 \times 10^4$ cells/g of soil as indicated by DAPI), independent of the values of the labile organic carbon in these soils. The variable values of organic concentration confirm the presence of different types of oxidants as described above. Control and pre-heated soil samples did not show any activity for the 24-hour experiment (Fig. 3).

DISCUSSION

For the past 10 years, different studies have demonstrated that 1000 km north from the most studied Mars-soil analog area known as Yungay, there is another hyper-arid region which contains interesting geochemical and biological characteristics similar to the Yungay area and constitutes an important point of comparison (Valdivia-Silva et al. 2005; Valdivia-Silva et al. 2011). This area, denominated as Pampas de La Joya in the Atacama Desert of southern Peru (Fig. 1), has been described showing levels of precipitation lower than 2 mm/y and the virtual absence of available liquid water in the soil for long periods throughout the year (Valdivia-Silva et al. 2012b). The extremely low water input in Pampas de La Joya soils provides an example of how life survives at the lower limit of water availability. Comparing the distribution of habitats and microorganisms between different hyper-arid environments provides lessons for the survival of life in extreme dryness and a quantitative means of extrapolating to the surface of Mars. In this context, preliminary data in this area (Valdivia-Silva et al. 2011) has motivated further microbiological studies.

In the present work, using multiple techniques, we evaluated the distribution of organic carbon and microorganisms in these types of dry soils in order to understand the relationship between both variables. Our results revealed and confirmed a high degree of heterogeneity with the greatest variability in organic carbon and number of microorganisms between geographical locations (Fig. 2), which is a common characteristic in hyper-arid regions (Ewing et al. 2006; Lester et al. 2007; Crits-Christoph et al. 2013). The very low levels of organic carbon and microorganisms are also associated with low diversity in the La Joya Desert soil microbiome as determined by preliminary PLFA analyses. Heterotrophic soil bacteria encountered in the driest environments, such as Yungay in the Atacama Desert or the upper elevations of the Dry Valleys in Antarctica, were common in our samples and mainly included *Proteobacteria* and *Actinobacteria* communities (Lester et al. 2007; Pointing et al. 2009; Crits-Christoph et al. 2013), although more studies of the microbiome are necessary to validate our assertion. Interestingly, a survey of multiple desert environments appears to suggest that the community composition is mainly composed of heterotrophic α-*Proteobacteria, Actinobacteria, Flexibacteria, Firmicutes*, *Gemmatimonadetes, Planctomycetes,* and *Thermus*/*Deinococcus* (Navarro-Gonzalez et al. 2003; Drees et al. 2006).

Water availability is the primary controlling factor for microbial activity, biomass, and diversity in desert soils (Warren-Rhodes et al. 2006; Connor et al. 2007). Previous studies in hyper-arid soils showed direct correlations between microbial presence and organic carbon with water availability, and an inverse relationship with soil conductivity that is associated with high salt content (Ewing et al. 2006; Crits-Christoph et al. 2013). However, these studies did not evaluate the physicochemical properties of different potentially habitable substrates in the hyper-arid areas; rather, they focused on precipitation gradients without considering in detail the high heterogeneity in the hyper-arid core. In our work, changes in soil organic carbon and microbiology along the precipitation transect were closely correlated with water availability, described as mean air relative humidity (Valdivia-Silva et al. 2012b) and mean annual rainfall (Fig. 1). However, within the hyper-arid soils this correlation broke down—we found all possible types of relationships between organic carbon or number of microorganisms and the reported water availability in the study area. Importantly, the analyzed study area is located in flat terrain with similar wind direction and intensity at all sampled sites. This removes the possibility that heterogeneity is due to differences in eolian deposition.

The most likely alternative explanation for the lack of correlation is the distribution of exotic minerals with oxidizing capacity and evaporitic salts with high spatial variability at the scale of several meters (Ewing et al. 2006; Peeters et al. 2009; Valdivia-Silva et al. 2011). Oxidant activity in soils frequently requires water to activate geochemical reactions, so that water availability could be harmful rather than protective to microorganisms and/or organic carbon, depending on the minerals present (Valdivia-Silva et al. 2012c).

Previous experiments using hyper-arid soil samples from different deserts on Earth, including Pampas de La Joya, showed high rates of oxidation as different organic substrates added into the soil decomposed to CO_2 (Quinn et al. 2007; Peeters et al. 2009). Using similar methods, we evaluated this capacity in the hyper-arid study area. Interestingly, we found that all of the samples with very low levels of microorganisms were from soils with high oxidant activity of more than 4–6 nmol/h in the first 10 hours of the experiment. In addition, soils with high oxidant activity were soils in which we could not detect microbial growth. Moderate and low oxidant activities were related to an increasing number of microorganisms, and the kinetic of release of CO_2 was rapidly dominated by biological processes (Fig. 3). Importantly, our study suggest that the levels of CO_2 released after 10 hours is the clearest indicator of the type of soil and can predict the concentration of microorganisms in these soils, but not the concentration of organic carbon, which appears to be dependent on the type of oxidant present. Indeed, previous work has demonstrated that $^{13}CO_2$ release from ^{13}C-labeled formate was completely different from ^{13}C-labeled D- or L- alanine, indicating the presence of more nonbiological chemical decomposition mechanisms in this region similar to those found in Yungay soils and indicated by the Viking Labeled Release experiment on Mars (Levin and Straat 1976; Levin and Straat 1977; Navarro-González et al. 2003). Those results seemed to suggest a different "susceptibility" to oxidation of the organic molecules and the presence of non-chirally specific and as-yet-unidentified oxidants. An additional factor contributing to the non-homogenous distribution may be the preservation of organic materials for geologically long periods in sulfate minerals, which are abundant in these soils (Aubrey et al. 2008).

Recently, the Sample Analysis at Mars (SAM) instrument on board the Mars Science Laboratory Curiosity Rover, confirmed the presence of organic molecules on the Martian's surface (Freissinet et al. 2015). The identification of chlorinated hydrocarbons, such as chlorobenzene (150–300 parts per billion by weight [ppbw]) and C_2 to C_4 dichloroalkanes (up to 70 ppbw) in samples from the Cumberland drill hole in the Sheepbed mudstone at Yellowknife Bay, were explained as products from reactions during pyrolysis between Martian perchlorates and organic aromatic and aliphatic compounds derived from different Martian sources (Freissinet et al. 2015). The presence of these compounds near the surface means some organic molecules have survived despite the abundance of oxidants and significant high-energy radiation exposure. Five years ago, Navarro-Gonzalez et al. (2010) found the production of similar chlorinated organic compounds when Mars-like soils from the Atacama Desert containing 32 ± 6 ppm of organic carbon were mixed with 1 wt% magnesium perchlorate and heated. In this manner, these fascinating results on Mars corroborated previous experiments in Mars analogs to provide new explanations for high variability of organic carbon in hyper-arid soils in the presence of different type of oxidants.

Although we did not analyze hypolithic and endolithic niches for life in these harsh environments (i.e., within evaporitic minerals, beneath quartz, and other translucent rocks), we suggest that the heterogeneity that we

observed in these soils might also be limiting factor of cyanobacterial colonization (Wierzchos et al. 2011; Wierzchos et al. 2013). So, the presence of different oxidants in the soil adjacent to these niches and in cracks and crevices could trigger oxidative reactions during wet events.

Finally, although this study is far from exhaustive in identifying the diversity of bacteria present in these driest regions, it is evident that microbes are capable of enduring extremes of aridity, and importantly, we found an inverse trend that could be used to evaluate the distribution of potential habitability in different dry regions, including the surface of Mars. Other studies about the nature of these oxidants with some specificity for some organic molecules and a more comprehensive distribution of the microbiome in this particular hyper-arid region are under investigation by our group.

CONCLUSIONS AND REMARKS

Together, our results show that: (1) There is no correlation between the soil labile organic carbon and the number of microorganisms within hyper-arid soils, and both of them have a distribution that is highly heterogeneous; (2) The number of microorganisms in the hyper-arid soils of La Joya is inversely correlated with the rate of oxidant activity in the soil, although this trend is not observed with organic matter. Importantly, the study area has a uniform input of eolian deposit of particles and humidity (fog or rain). This suggests that, the variation observed is due to intrinsic characteristics of the soil; and (3) The analyses of oxidant activity in hyper-arid soils, using the decomposition rates of labeled sodium formate over 10 hours, is a useful way to evaluate the microbial concentration in these types of soils, independently of water availability and organic carbon content. Indeed, we suggest that a release rate of 4–6 nmol/h of $^{13}CO_2$ is related to very low microbial content.

The search for life on Mars is one of the more important objectives for astrobiology, and Martian analogs give to us a better chance to understand where and how life could survive on that planet. Our results strongly suggest where the microorganisms in extreme dry environments, like the Atacama Desert, and maybe Mars, can or could have survived despite the presence of different oxidants in the soil. Importantly, minimum levels of water and humidity could be beneficial for life in low-oxidant activity environments, but harmful in settings with high chemical activity. Determining where to look and how to search for evidence of microorganisms on Mars is therefore a key task for astrobiology in the near future.

ACKNOWLEDGMENTS

This research was supported by grants from the National Aeronautics and Space Administration, National Autonomous University of Mexico (DGAPA IN106013), the National Council of Science and Technology of Mexico (CONACyT 98466), and the Sociedad Cientifica de Astrobiologia del Peru (SCAP). We thank to Mr. Saul Perez and the two anonymous reviewers for their comments and suggestions to improve this paper.

REFERENCES

Aubrey, A.D., Chalmers, J.H., Bada, J.L., Grunthaner, F.J., Amashukeli, X., Willis, P., Skelley, A.M., Mathies, R.A., Quinn, R.C., Zent, A.P., Ehrenfreund, P., Amundson, R., Glavin, D.P., Botta, O., Barron, L., Blaney, D.L., Clark, B.C., Coleman, M., Hofmann, B.A., Josset, J.L., Rettberg, P., Ride, S., Robert, F., Sephton, M.A., and Yen, A., 2008, The Urey instrument: an advanced in situ organic and oxidant detector for Mars exploration: Astrobiology, v. 8, p. 583–595, doi: 10.1089/ast.2007.0169.

Bagaley, D.R., 2006, Uncovering bacterial diversity on and below the surface of a hyper-arid environment, the Atacama Desert, Chile: M.Sc. thesis, Louisiana State University, 158 p.

Connon, S., Lester, E., Shafaat, H., Obenhuber, D., and Ponce, A., 2007, Bacterial diversity in hyperarid Atacama Desert soils: Journal of Geophysical Research, v. 112, p. G04S17.

Crits-Christoph, A., Robinson, C., Barnum, T., Fricke, W., Davila, A., Jedynak, B., McKay, C., and DiRuggiero, J., 2013, Colonization patterns of soil microbial communities in the Atacama Desert: Microbiome, v. 1, p. 28.

Davila, A., Duport, L., Melchiorri, R., Janchen, J., Alea, S., De Los Rios, A., Fairen, A., Mohlmann, D., McKay, C., Ascaso, C., and Wierzchos, J., 2010, Hygroscopic minerals and the potential for life on Mars: Astrobiology, v. 10, p. 617–628.

Drees, K.P., Neilson, J.W., Betancourt, J.L., Quade, J., Henderson, D.A., Pryor, B.M., and Maier, R.M., 2006, Bacterial Community Structure in the Hyperarid Core of the Atacama Desert, Chile: Applied and Environmental Microbiology, v. 72, p. 7902–7908.

Ewing, S.A., Macalady, J.L., Warren-Rhodes, K., McKay, C.P., and Amundson, R., 2008, Changes in the soil C cycle at the arid-hyperarid transition in the Atacama Desert: Journal of Geophysical Research-Biogeosciences, v. 113, doi: 10.1029/2007jg000495.

Ewing, S.A., Sutter, B., Owen, J., Nishiizumi, K., Sharp, W., Cliff, S.S., Perry, K., Dietrich, W., McKay, C.P., and Amundson, R., 2006, A threshold in soil formation at Earth's arid-hyperarid transition: Geochimica Et Cosmochimica Acta, v. 70, p. 5293–5322, doi: 10.1016/j.gca.2006.08.020.

Fletcher, L.E., Conley, C.A., Valdivia-Silva, J.E., Perez-Montaño, H.S., Condori-Apaza, R., Kovacs, G.T.A., Glavin, D., and McKay, C.P., 2011, Determination of low bacterial concentrations in hyper-arid Atacama soils: of biochemical and microscopy methods with real-time Quantitative-PCR: Canadian Journal of Microbiology, v. 57, p. 953–963, doi: 10.1139/w11-091.

Fletcher, L.E., Valdivia-Silva, J.E., Perez-Montaño, H.S., Condori-Apaza, R., Conley, C.A., and McKay, C.P., 2012, Variability of organic material in surface horizons of the hyper-arid Mars-like soils of the Atacama Desert: Advances in Space Research, v. 49, p. 271–279, doi: 10.1016/j.asr.2011.10.001.

Fletcher, L.E., Valdivia-Silva, J.E., Perez-Montaño, S., Condori-Apaza, R.M., Conley, C.A., Navarro-Gonzalez, R., and McKay, C.P., 2014, Field method for rapid quantification of labile organic carbon in hyper-arid desert soils validated by two thermal methods: International Journal of Astrobiology, v. 13, p. 182–189, doi:10.1017/S1473550414000019.

Freissinet, C., Glavin, D.P., Mahaffy, P.R., Miller, K.E., Eigenbrode, J.L., Summons, R.E., Brunner, A.E., Buch, A., Szopa, C., Archer Jr, P.D., Franz, H.B., Atreya, S.K., Brinckerhoff, W.B., Cabane, M., Coll, P., Conrad, P., Des Marais, D.J., Dworkin, J.P., Fairén, A.G., François, P., Grotzinger, J.P., Kashyap, S., ten Kate, I.L., Leshin, L.A., Malespin C.A., Martin, M.G., Martin-Torres, F.J., McAdam, A.C., Ming, D.W., Navarro-González, R., Pavlov, A.A., Prats, B.D., Squyres, S.W., Steele, A., Stern, J.C., Sumner, D.Y., Sutter, B., Zorzano, M.-P., and the MSL Science Team, 2015, Organic molecules in the Sheepbed Mudstone, Gale Crater, Mars: Journal of Geophysical Research-Planets, v. 120, p. 495–514, doi:10.1002/2014JE004737.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., and Ball, A.S., 2003, Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils: Applied and Environmental Microbiology, v. 69, p. 1800–1809, doi: 10.1128/aem.69.3.1800-1809.2003.

Glavin, D., Cleaves, J., Schubert, M., Aubrey, A., and Bada, J.L., 2004, New method for estimating bacterial cell abundances in natural samples by use of sublimation: Applied Environmental Microbiology, v. 70, p. 5923–5928.

Hartley, A., Chong, G., Houston, J., and Mather, A., 2005, 150 million years of climatic stability: evidence from the Atacama Desert, northern Chile: Journal of the Geological Society, v. 162, p. 421–424.

Houston, J. and Hartley, A.J., 2003, The Central Andean West-slope rainshadow and its potential contribution to the origen of hyperaridity in the Atacama Desert: International Journal of Climatology, v. 23, p. 1453–1464.

Kemp, P.F., 1993, Handbook of methods in aquatic microbial ecology: Lewis Publisher, Boca Raton, Florida.

Kepner, R.L.J. and Pratt, J.R., 1994, Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present: Microbiological Reviews, v. 58, p. 603–615.

Kieft, T.L., 2002, Hot Desert Soil Communities, Encyclopedia of Environmental Microbiology: Wiley, New York, p. 1576–1586.

Klappenbach, J.A., Saxman, P.R., Cole, J.R., and Schmidt, T.M., 2001, rrndb: the ribosomal RNA operon copy number database: Nucleic Acids Research, v. 29, p. 181–184, doi: 10.1093/nar/29.1.181.

Lehman, R.M., Colwell, F.S., Ringelberg, D.B., and White, D.C., 1995, Combined microbial community-level analyses for quality assurance of terrestrial subsurface cores: Journal of Microbiological Methods, v. 22, p. 263–281.

Lester, E., Satomi, M., and Ponce, A., 2007, Microflora of extreme arid Atacama Desert soils: Soil Biology Biochemistry, v. 39, p. 704–708.

Levin, G.V. and Straat, P.A., 1976, Labeled release—experiment in radiorespirometry: Origins of Life and Evolution of the Biosphere, v. 7, p. 293–311.

Levin, G.V. and Straat, P.A., 1977, Life on Mars—Viking Labeled Release Experiment: Biosystems, v. 9, p. 165–174.

Mahaffy, P., 2008, Exploration of the habitability of Mars: development of analytical protocols for measurement of organic carbon on the 2009 Mars science laboratory: Space Science Reviews, v. 135, p. 255–268, doi: 10.1007/s11214-007-9223-1.

McKay, C.P., 2010, An origin of life on Mars: Cold Spring Harbor Perspectives in Biology, v. 2, doi: 10.1101/cshperspect.a003509.

McKay, C.P., Friedmann, E.I., Gomez-Silva, B., Caceres-Villanueva, L., Andersen, D.T., and Landheim, R., 2003, Temperature and moisture conditions for life in the extreme arid region of the Atacama Desert: four years of observations including the El Nino of 1997–1998: Astrobiology, v. 3, p. 393–406.

McKay, C.P., Grunthaner, F.J., Lane, A.L., Herring, M., Bartman, R.K., Ksendzov, A., Manning, C.M., Lamb, J.L., Williams, R.M., Ricco, A.J., Butler, M.A., Murray, B.C., Quinn, R.C., Zent, A.P., Klein, H.P., and Levin, G.V., 1998, The Mars Oxidant experiment (MOx) for Mars '96: Planetary and Space Science, v. 46, p. 769–777.

Michalski, G., Bohlke, J.K., and Thiemens, M., 2004, Long term atmospheric deposition as the source of nitrate and other salts in the Atacama desert, Chile: new evidence from mass-independent oxygen isotopic compositions: Geochemica et Cosmochemica Acta, v. 68, p. 4023– 4038.

Navarro-Gonzalez, R., Rainey, F., Molina, P., Bagaley, D., Hollen, B., de la Rosa, J., Small, A., Quinn, R., Grunthaner, F., Caceres, L., Gomez-Silva, B., and McKay, C., 2003, Mars-like soils in the Atacama Desert, Chile, and the dry limit of microbial life: Science, v. 302, p. 1018–1021.

Navarro-Gonzalez, R., Rainey, F., Molina, P., Bagaley, D., Hollen, B., de la Rosa, J., Small, A., Quinn, R., Grunthaner, F., Caceres, L., Gomez-Silva, B., and McKay, C., 2003, Mars-like soils in the Atacama Desert, Chile, and the dry limit of microbial life: Science, v. 302, p. 1018–1021.

Navarro-González, Vargas, E., de la Rosa, J., Raga, A.C., and McKay, C.P., 2010, Reanalysis of the Viking results suggests perchlorate and organics at midlatitudes on Mars: Journal of Geophysical Research-Planets, v. 115, p. E12, doi: 10.1029/2010JE003599.

Orlando, J., Alfaro, M., Bravo, L., Guevara, R., and Carú, M., 2010, Bacterial diversity and occurrence of ammonia-oxidizing bacteria in the Atacama Desert soil during a "desert bloom" event: Soil Biology and Biochemistry, v. 42, p. 1183–1188, doi:10.1016/j.soilbio.2010.03.025.

Oyama, V.I. and Berdahl, B.J., 1979, Model of Martian surface-chemistry: Journal of Molecular Evolution, v. 14, p. 199–210.

Peeters, Z., Quinn, R., Martins, Z., Sephton, M.A., Becker, L., van Loosdrecht, M.C.M., Brucato, J., Grunthaner, F., and Ehrenfreund, P., 2009, Habitability on planetary surfaces: interdisciplinary preparation phase for future Mars missions: International Journal of Astrobiology, v. 8, p. 301–315, doi:10.1017/S1473550409990140.

Peters, G.H., Abbey, W., Bearman, G.H., Mungas, G.S., Smith, J.A., Anderson, R.C., Douglas, S., and Beegle, L.W., 2008, Mojave Mars simulant-characterization of a new geologic Mars analog: Icarus, v. 197, p. 470–479, doi: 10.1016/j.icarus.2008.05.004.

Pointing, S.B., Chan, Y., Lacap, D.C., Lau, M.C.Y., Jurgens, J.A., and Farrell, R.L., 2009, Highly specialized microbial diversity in hyper-arid polar desert: Proceedings of the National Academy of Sciences, v. 106, p. 19964–19969, doi: 10.1073/pnas.0908274106.

Ponnamperuma, C., Shimoyama, A., Yamada, M., Hobo, T., and Pal, R., 1977, Possible surface-reactions on Mars—implications for Viking Biology results: Science, v. 197, p. 455–457.

Posch, T., Loferer-Krößbacher, M., Gao, G., Alfreider, A., Pernthaler, J., and Psenner, R., 2001, Precision of bacterioplankton biomass determination: a comparison of two fluorescent dyes, and of allometric and linear volume-tocarbon conversion factors: Aquatic Microbial Ecology, v. 25, p. 55–63.

Preston, L.J. and Dartnell, L.R., 2014, Planetary habitability: lessons learned from terrestrial analogues: International Journal of Astrobiology, v. 13, p. 81–98, doi:10.1017/S1473550413000396.

Quinn, R.C., Ehrenfreund, P., Grunthaner, F.J., Taylor, C.L., and Zent, A.P., 2007, Decomposition of aqueous organic compounds in the Atacama Desert and in Martian soils: Journal of Geophysical Research-Biogeosciences, v. 112, p. G04S18, doi: 10.1029/2006jg000312.

Quinn, R.C., Grunthaner, P.J., Taylor, C.L., Bryson, C.E., and Grunthaner, F.J., 2011, The radiolytic decomposition of soil perchlorates on Mars, 42nd Lunar and Planetary Science Conference, LPI Contribution No. 1608, p. 2003

Quinn, R.C., Martucci, H.F.H., Miller, S.R., Bryson, C.E., Grunthaner, F.J., and Grunthaner, P.J., 2013, Perchlorate radiolysis on Mars and the origin of Martian soil reactivity:Astrobiology, v. 13, p. 515–520.

Quinn, R.C. and Zent, A.P., 1999, Peroxide-modified titanium dioxide: a chemical analog of putative Martian soil oxidants: Origins of Life and Evolution of the Biosphere, v. 29, p. 59–72.

Skelley, A.M., Aubrey, A.D., Willis, P.A., Amashukeli, X., Ehrenfreund, P., Bada, J.L., Grunthaner, F.J., and Mathies, R.A., 2007, Organic amine biomarker detection in the Yungay region of the Atacama Desert with the Urey instrument: Journal of Geophysical Research-Biogeosciences, v. 112, doi: G04s1110.1029/2006jg000329.

Smayda, T., 1978, From phytoplankters to biomass, *in* Sournia, A. (ed.), Phytoplankton Manual: UNESCO, UK, p. 273–280.

Thornwaite, C.W., 1948, An approach toward a rational classification of climate: Geographical Review, v. 38, p. 55–94.

UNEP, 1997, World atlas of desertification (second edition), *in* Programme, U.N.E.(ed.), Nairobi, p. 182.

Valdivia-Silva, J.E., Fletcher, L.E., Navarro-Gonzalez, R., McKay, C.P., Perez-Montaño, S., Condori, R., and Conley, C.A., 2005, Organic matter analysis of the hyper-arid Peruvian Desert in comparison to other hyper-arid environments: EOS Transactions, American Geophysical Union, Fall Meeting., v. 86, p. Abstract #P51D–0949.

Valdivia-Silva, J.E., Navarro-González, R., Fletcher, L., Perez-Montaño, S., Condori-Apaza, R., and Mckay, C.P., 2012a, Soil carbon distribution and site characteristics in hyper-arid soils of the Atacama Desert: a site with Mars-like soils: Advances in Space Research, v. 50, p. 108–122.

Valdivia-Silva, J.E., Navarro-González, R., Fletcher, L.E., Pérez-Montaño, S., Condori, R., Ortega-Gutierrez, F., and McKay, C.P., 2012b, Climatological characteristics in the extreme hyper-arid region of Pampas de La Joya, Peru. Astrobiological approach in four years of observation: 2004–2008: International Journal of Astrobiology, v. 11, p. 25–35.

Valdivia-Silva, J.E., Navarro-Gonzalez, R., and McKay, C., 2009, Thermally evolved gas analysis (TEGA) of hyperarid soils doped with microorganisms from the Atacama Desert in southern Peru: implications for the Phoenix mission: Advances in Space Research, v. 44, p. 254–266, doi: 10.1016/j.asr.2009.02.008.

Valdivia-Silva, J.E., Navarro-González, R., Ortega-Gutierrez, F., Fletcher, L.E., Perez-Montaño, H.S., Condori-Apaza, R., and McKay, C.P., 2011, Multidisciplinary approach of the hyperarid desert of Pampas de La Joya in southern Peru as a new Mars-like soil analogue: Geochimica Et Cosmochimica Acta, v. 75, p. 1975–1991, doi: 10.1016/j.gca.2011.01.017.

Valdivia-Silva, J.E., Navarro-González, R., Rosa, J.D.L., and McKay, C.P., 2012c, Decomposition of sodium formate and L- and D-alanine in the Pampas de La Joya soils: implications as a new geochemical analogue to Martian regolith: Advances in Space Research, v. 49, p. 821–833.

Warren-Rhodes, K.A., Rhodes, K.L., Pointing, S.B., Ewing, S.A., Lacap, D.C., Gomez-Silva, B., Amundson, R., Friedmann, E.I., and McKay, C.P., 2006, Hypolithic cyanobacteria, dry limit of photosynthesis, and microbial ecology in the hyperarid Atacama Desert: Microbial Ecology, v. 52, p. 389–398, doi: 10.1007/s00248-006-9055-7.

Webster, R. and Oliver, M.A., 1990, Statistical methods in soil and land resource survey: Oxford University Press, Oxford, Inglaterra.

White, D.C., 1979, Lipid Analysis of Sediments for Microbial Biomass and Community Structure, *in* Litchfield, C.D. and Seyfreid, P.L. (eds.), Methodology for Biomass Determinations and Microbial Activities in Sediments: American Society for Testing and Materials, Philadelphia, PA., p. 87–103.

White, D.C. and Ringelberg, D.B., 1997, Utility of the signature lipid biomarker analysis in determining the in situ viable biomass, community structure, and nutritional/physiologic status of deep subsurface microbiota, *in* Amy, P.S. and Haldeman, D.L. (eds.), The Microbiology of the Terrestrial Deep Subsurface: CRC Press, New York, NY, p. 119–136.

White, D.C., Stair, J.O., and Ringelberg, D.B., 1996, Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis: Journal of Industrial Microbiology, v. 17, p. 185–196.

Wierzchos, J., Camara, B., de Los Rios, A., Davila, A., Sanchez Almazo, I., Artieda, O., Wierzchos, K., Gomez-Silva, B., McKay, C., and Ascaso, C., 2011, Microbial colonization of Ca-sulfate crusts in the hyperarid core of the Atacama Desert: implications for the search for life on Mars: Geobiology, v. 9, p. 44–60.

Wierzchos, J., Davila, A., Artieda, O., Camara-Gallego, B., De Los Rios, A., Nealson, K., Valea, S., Garcia-Gonzalez, M., and Ascaso, C., 2013, Ignimbrite as a substrate for endolithic life in the hyper-arid Atacama Desert: implications for the search for life on Mars: Icarus, v. 224, p. 334–346.

Zent, A.P., Ichimura, A.S., Quinn, R.C., and Harding, H.K., 2008, The formation and stability of the superoxide radical (O-2(-)) on rock-forming minerals: band gaps, hydroxylation state, and implications for Mars oxidant chemistry: Journal of Geophysical Research-Planets, v. 113, doi: 10.1029/2007je003001.

Received 22 February 2015; accepted 15 November 2015.