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

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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 & LOC), phospholipid fatty acids analysis (PLFA), quantitative real time polymerase chain reaction (qRT-PCR), 4',6-diamidino-2-phenylindole (DAPI)-fluorescent microscopy, culturable microorganisms, and oxidant activity, in order 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. The range of LOC was from 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). The value of Mars-like areas relies on the similarity of the analogue to its target, either in terms of their mineralogical or geochemical context, or current physical or chemical environmental conditions. They illustrate preservation mechanisms that could guide the search for fossil and biological remnants of microbial life, which could be extrapolated to the ancient or current Mars (Preston and Dartnell, 2014). Although, Mars today is a cold dry desert world with surface conditions that are not habitable even for the hardiest “known” life forms from Earth, there is ample evidence of past water activity and the presence of interesting niches for life, such as subsurface or/and evaporitic minerals, that make the Red Planet in a prime target for looking for extraterrestrial microorganisms (Davila et al., 2010; McKay, 2010).

In this regard, 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 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 desert 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). Hyper-arid soils from the Chilean and Peruvian region have represented an ideal test bed for constraining the limits of life or its preserved remnants (McKay et al., 2003; Drees et al., 2006). Previous studies have shown the presence of very low levels of organic carbon (20-40 ppm), non-biological oxidants and exotic evaporitic minerals, which all together are common characteristics expected on Mars (Connon et al., 2007; Fletcher et al., 2012). Importantly, 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 this area 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 with strong chemical activity on the accelerated destruction of organics under several experimental conditions (Quinn et al., 2007; Valdivia-Silva et al., 2012c), and were extrapolated to Mars trying to explain the low levels of organics 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 Martian surface (Quinn et al., 2007). 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 a chemical oxidation not 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 ($S_2O_8^{-2}$) (Ewing et al., 2006); these candidates do not completely explain our results, nor experiments similar to those made by the Viking Lander on Mars (Navarro-Gonzalez et al., 2003; Quinn et al., 2007; Valdivia-Silva 2009; 2011). The Quinn et al. (2013) proposal based on radiolysis of perchlorates may explain the Viking results but would not provide a complete explanation for the Atacama oxidants.

On the other hand, an important and contradictory issue to resolve in this type of hyper-arid soils is that the levels of organic content have no shown a direct correlation with the number of microorganisms or biomass inside the dry core region (Navarro-Gonzalez et al 2003; Valdivia-

Silva et al 2011). Although the microbiological studies have shown good correlation curves between organics and microorganisms in precipitation gradients throughout semiarid, arid, and hyper-arid areas and/or from the Pacific coast to the core of the desert (Dress et al 2006; Fletcher et al., 2011); most analyses in the hyper arid core have shown conflicting results with regard to number of microorganisms as discussed later (Orlando et al., 2010; Fletcher et al., 2011).

It is known that in extreme arid deserts, the 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 core even 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 produced results ranging values near to ~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 regions, Bagaley (2006) found variations in CFU by orders of magnitude over distances of few hundred meters.

In this regard, this high heterogeneity in the presence of microorganisms despite having 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 habitability, on the other hand 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 multi-component investigation involving different microbiological and geochemical analyses of soil samples collected in the hyper-arid soils from Pampa de la Joya in southern Peru (Valdivia-Silva et al., 2011; Preston and Dartnell, 2014), and in 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 biomass and organic carbon distribution 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, or alternatively called “La Joya Desert”, located about 70 km from the city of Arequipa, Peru, along the South Pacific coast, between latitudes of 16°S–17°S, longitudes of 71.5°W–72.5°W, and approximately between 1000 and 2000 m.a.s.l. 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 ratio P/PET (evapotranspiration / precipitation) is less than 0.05 (Thornwaite, 1948; UNEP, 1997). Our specific area of analyses encompasses an area of 96 km^2 (12 x 8 km) referred in this paper as the “quadrangle of interest” (diagonal coordinates: 16°38.386’S – 72°2.679’W and 16°44.986’S – 71°58.279’W) (Fig. 1). Importantly, 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 g and 100 g, representing a composite of 5 individual nearby sites (~1.5 m in radius) of each sampling site, 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 obtain a representative sample for each time 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. The transport required 4 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. Importantly, separate bags of soil were used for microbiology analysis than those used for chemical studies. Although, storage conditions could change 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 the storage in similar conditions (mainly low humidity) did not show significantly change 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) the sampling was 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) 5 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 (P/PET > 0.05; coord.16°43.945'S – 72°18.645'W) and a semiarid (P/PET > 0.2; coord.16°50.31'S – 72°17.16'W) area (Fig. 1). Importantly, LOC and DAPI analyses were 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 we use and can to complete many samples over large areas in order 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 as our group has reported before (Valdivia-Silva et al., 2011; Fletcher et al., 2012). This technique has proven to be simple, accurate, sensitive, and reproducible for the quantification of labile organic carbon in hyper-arid soils (Fletcher et al., 2012; 2014). Since, these soils have shown negligible levels, or almost absence, of recalcitrant carbon (or passive pool), the labile form (or active pool) is the most abundant in these soils and includes molecules with biological importance such as amino acids, nucleotides, lipids, sugars, aliphatic and aromatic hydrocarbons, etc (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 as has been reported before (Navarro-Gonzalez et al., 2006).

Negative controls were made by heating some soil samples to 500°C for 8 hours.

Microbiological analyses

The quantification of microorganisms in hyper-arid soils has shown to be a real challenge for microbiologists since each method has specific limitations. Because of this, in this work we analyze the number of microorganisms using different independent and dependent culture-based techniques in order to improve our approach to the number of microorganisms present in the soil. A more comprehensive comparison between molecular enumeration techniques used in this type of dry soils was published for our group (see Table 3 in Fletcher et al., 2011).

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). The analysis of the images was done by the program 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). Importantly, the PLFA analysis could detect phospholipids from dead microorganisms which can remain for long time due to very low, if any, bacterial metabolism and the lack of chemical activity in the dry soils. 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 the OmniMix PCR Beads (Cepheid, Inc.) as they are 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. Because of this evaluation, the resulting number of copies of DNA per gram of soil was divided by 5.5 operons/bacteria to determine the estimated number of bacteria per gram of soil. More details of the PCR protocol used in this study analyzing similar soils was previously discussed and reported by our group (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 5 minutes. The suspension was allowed to settle until a clear layer appeared on top, approximately 2 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 3 weeks. Control negative culturing experiments consisting of 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 during 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 were run in triplicate where sodium formate was not added, or where soil was pre-heated to 500° C for 8 hours.

RESULTS

Distribution of Organic Carbon

Baseline levels of organic carbon in samples from the quadrangle of interest, 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 label organic carbon in the hyper-arid quadrangle was from 2 to 60 µg C/g of soil (mean value 31.6 ± 25 µg C/g of soil) (Table 1, Fig. 2B), while that the arid and semiarid sites showed mean values of 85.8 ± 33 µg C and 167.2 ± 66 µg C/g of soil, respectively. Interestingly 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 area of the quadrangle and values close to 60 µg C/g of soil were ~10% of the total sampling area. By comparison, the range of TOC found in the quadrangle 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 other forms of organic carbon, mainly recalcitrant, are not present or are at negligible levels in the quadrangle 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 few meters apart (Navarro-Gonzalez et al., 2003; Bagaley, 2006), here we report the number of microorganisms and their distribution evaluated by different 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 in the quadrangle of interest showed high variability with values between 10^2 and 10^4 cell/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, which are 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 this quadrangle was 34.9 ± 12.1 ng C/g of soil. Knowing that $\sim10^6$ bacteria provide 1 μg C/g of soil (1 ppm) of organic carbon (Smayda, 1978), the carbon contribution of microorganisms to the carbon source in these type of soils is almost negligible

(Valdivia-Silva et al., 2011). Indeed, this assertion was demonstrated when we compared the patterns of distribution of labile organic carbon and the number of microorganisms (Fig. 2). There was no correlation between both variables (r~0; Pearson correlation) and we could find four interesting types of relationships which are discussed below: a) 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 Figure 2); b) another where a "high" concentration of organic carbon (>45 μg C/g of soil) was related to extremely low levels of microorganisms (<5 x 10^2 cells/g of soil) (i.e. small circles in Fig. 2); c) regions where very low levels of organic carbon (<15 μg C/g of soil) were related to high values of microorganisms (>5 x 10^3 cells/g of soil) (i.e. rectangles in Fig. 2); and d) 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 Figs. 2A and B). 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 x 10^2 and 8 x 10^3 bacteria per gram of soil (mean 6.2 x 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, which have been extensively reviewed formerly in another publication (Fletcher et al., 2011). Importantly, culturing of soil extracts yielded similar levels of bacteria between 1.1 x 10^2–3.7 x 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 5 surface samples averaged 2.3 x 10^5 cell/g -- higher values 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 the 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*. Interestingly, 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 *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) and Polyenoic PLFAs (Po) found in the cell membranes of micro-aerophiles and anaerobes, and eukaryotes respectively, showed very low levels and they were completely absence in hyper-arid soils unlike wetter regions where these PLFAs reached 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 indicated that metabolic activity was arrested before cellular responses to stress could be initiated.

Soil oxidant activity

First, in order to verify the influence of the precipitation gradient (moisture) and the levels of organic matter content and microorganisms over the decomposition of sodium formate to $^{13}CO_2$, we analyzed two samples taken in the driest place of the quadrangle 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). Briefly, 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 was 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 so as to coincide to the growth and metabolism of microorganisms (Valdivia-Silva et al., 2012). Second, in order to evaluate a possible heterogeneity of oxidant activity into the hyper-arid soils from the quadrangle which could explain the non-homogeneous distribution of organics and microorganisms, a more comprehensive analysis was done in this region. Importantly, we classified the samples soils in 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 experiment (Table 2; Fig. 3). Interestingly, our results showed a strong inverse correlation between the levels of decomposition of sodium formate with the presence of microorganisms. Soil samples with “high” oxidation at rates greater than 4 to 6 nmol/h were always associated with soils with the lowest levels of microorganisms (< 5 x 10^2 cells/g of soil as indicated by DAPI), but not so with the soil organic carbon content, which 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 subsequent sharp fall in $^{13}CO_2$ of between 20 and 50% in the next 20 h. The abrupt fall of headspace $^{13}CO_2$ levels after the initial increase is apparently explained an uptake of $^{13}CO_2$ into solution due a shift in soil/solution pH in absence of biological activity (Quinn et al., 2007). Importantly, these results were corroborated 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 with rates of 1.5 to 2 nmol/h, were associated with soil samples with organic carbon content between 15 and 45 µg C/g of soil, and number of microorganisms between 1 x 10^3 and 1 x 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. Interestingly, this type of soils had a similar oxidant activity behavior to the arid soil samples used as controls. Finally, soils with “low” oxidant activity (0.8 – 1 nmol/h) were strongly associated with soil samples with high content of microorganisms (> 5 x 10^4 cells /g of soil as indicated by DAPI), independently 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. Importantly, control and pre-heated soil samples did not show any activity for the 24-hour experiment (Figure 3).

DISCUSSION

For the past 10 years, our group has been demonstrating that 1000 km north from the most studied Mars-soil analog area knew as Yungay, there is another hyper-arid region which contains interesting geochemical and biological characteristics similar to that area and constitutes an important point of comparison (Valdivia-Silva et al., 2005; Valdivia-Silva et al., 2011). This area, known as Pampas de La Joya in the Atacama Desert of southern Peru, have 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 into Pampas de La Joya soils, similar to the Yungay region in Chile, allows us to probe how life can survive without water, and compare the distribution, the type of habitats, and the type of microorganisms between different hyper-arid environments providing lessons for life survival including on the surface of Mars. In this context, preliminary results in La Joya Desert showed very low levels of microorganisms similar to Yungay (Valdivia-Silva et al., 2011), however a more detailed microbiological study had not been done.

In the present work, using multiple techniques, we first evaluated the distribution of organic carbon and microorganisms in these types of dry soils in order to understand the relationship between both variables. Importantly, our results revealed and confirmed the high degree of heterogeneity with the greatest variability in organic carbon and number of microorganisms between geographical locations (Fig. 2), which is a shared characteristic in different 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 a low microbial diversity of the La Joya Desert soil microbiome found by preliminary PLFA analyses. Soil heterotrophic bacteria encountered in the driest environments, such as Yungay in Atacama Desert or the upper elevations of the Dry Valleys in Antarctica, were common with 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 indicate that the heterotrophic community composition is mainly composed of α-*Proteobacteria, Actinobacteria, Flexibacteria, Firmicutes*, *Gemmatimonadetes, Planctomycetes,* and *Thermus/Deinococcus* (de la Torre et al., 2003; Navarro-Gonzalez et al., 2003; Nagy et al., 2005; Chanal et al.,2006; Drees et al., 2006). A more exhaustive study of the structure and composition of the communities inhabiting this region is being conducted at this time in order to demonstrate that La Joya Desert also shares similar interactions between those communities.

It is well known that one of the most important characteristics in hyper-arid environments on Earth is the scarcity of water. 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 interesting direct correlations between microbial presence and organic carbon with water availability, and an inverse relation with soil conductivity that is associated with high salt contents (Ewing et al., 2006; Crits-Christoph et al., 2013). However, these studies have not evaluated the physicochemical properties of different substrates potentially habitable into the hyper-arid areas, and they have been focused on precipitation gradients without considering the high heterogeneity into 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, a more detailed analysis of these variables into the hyper-arid soils did not corroborate this assumption since we found all the different types of relationships between organic carbon or number of microorganisms and the reported water availability in the quadrangle area. Importantly, the analyzed quadrangle is located in flat terrain which has similar influence of inlet and outlet winds. This removes the possibility that heterogeneity is due to differences in the deposit of aeolian transported cells. Because of this, in the second part of this work, we looked for a more detailed explanation that will help us to a better understanding of the high variability of LOC and microorganisms in the quadrangle of interest. First, since that the very low contribution of carbon from the bacterial mass to the labile organic carbon pool in the soil ranged between 0.01 and 0.2%, the absence of a direct correlation is explained somewhat, but not completely (Fig. 2). So, the most logical option that came out from these results was that the soil mineralogy present in this region played some role in explaining the variation. Indeed, several studies in hyper-arid soils, including Pampas de La Joya, have also shown the presence of exotic minerals with oxidizing capacity, and evaporitic salts with high spatial variability even several meters apart (Ewing t al., 2006; Peeters et al., 2009; Valdivia-Silva et al., 2011). Importantly, the oxidant activity in soils frequently needs the presence of water to activate geochemical reactions, so that water availability could be harmful instead of being protective to microorganisms and/or organic carbon depending on adjacent minerals (Valdivia-Silva et al., 2012c).

Previous experiments using hyper-arid soil samples of different deserts on Earth, including Pampas de La Joya, showed high rates of oxidation evaluated as the decomposition to CO_2 of different labeled organic substrates added into the soil (Quinn et al., 2007; Peeters et al., 2009). Using similar methods, in this work we evaluated this capacity in the hyper-arid quadrangule. Interestingly, we found that all of the samples with very low levels of microorganisms were from soils with *high* activity of more than 4 - 6 nmol/h in the first 10 hours of the experiment. In addition, soils with high oxidant activity were from soil in whic we could not detect microbial growth. *Moderate* and *low* oxidant activities were related with an increasing number of

microorganisms, and the kinetic of releasing of CO_2 was rapidly dominated by biological processes (Figure 3). Importantly, our study suggest that the levels of CO_2 released after 10 hours of experiment exhibit the most significant difference between the types of soils and can predict the concentration of micoorganisms in the sample, but not the concentration of organic carbon - which appears to be dependent on the type of oxidant present. Indeed, previously we demonstrated that $^{13}CO_2$ release from ^{13}C-labeled formate was completely different to the releasing 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). Importantly, 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. In addition, organic materials may also be preserved for geologically long periods in sulfate minerals, which are abundant in these soils. It could be an additional factor contributing to the non-homogenous distribution (Aubrey et al., 2008).
Although, in this work we have not analyzed hypolithic and endolithic niches for life in these harsh environments such as within evaporitic minerals, beneath quartz, and other translucent rocks, we could suggest that the process observed in these soils might be extrapolated as a limiting factor of cyanobacterial colonization in these niches since the abundance of spatial distribution in the hyper-arid core is not also homogeneous (Wierzchos et al., 2011; Wierzchos et al., 2013). So, the presence of different oxidants in the adjacent soil of these niches and their deposition in cracks and crevices could trigger oxidative reactions during wet events in the same way as in the soil. This hypothesis warrants further investigation.

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 important 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 shows is inversely correlated with the rate of oxidant activity in the soil, although this trend is not observed with organic matter. Importantly, since this region has the same input of aeolian deposit of particles and humidity (fog or rain), the variation effect observed is due to intrinsic characteristics of the soil. 3) The analyses of oxidant activity in hyper-arid soils, using the decomposition rates of labeled sodium formate in the first 10 hours of experiment, is an interesting 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 - 6nmol/h of $^{13}CO_2$ is related with 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 the life could survive in that

Planet. In this work, our results strongly suggest where the microorganisms in extreme dry environments like this desert, and maybe Mars can or could have survived despite the presence of several different oxidants in the soil. Importantly, minimum levels of water and humidity could be beneficial for life in low-oxidant activity environments, while harmful in other ones 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.

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Figure Captions

Figure 1-Location of the Pampas de La Joya region, S-W Peru showing the hyper-arid region analyzed (quadrangle), and the arid and semiarid soil samples.

Figure 2-Labile organic carbon (A) and microorganisms (B) distributions in the study area in Pampa de La Joya. Labile organic carbon was evaluated by permanganate titration in acid media. The number of microorganisms in the graphic was analyzed in 35 samples using DAPI staining protocol and fluorescent microscopy. The geometric figures represent some examples of the type of relationships between both variables (see text).

Figure 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 during 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.