

Supporting Online Material

Mars-like Soils in the Atacama Desert, Chile and the Dry Limit of Microbial Life

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Science Vol. 302 No. 5647 (7 November 2003).

Supporting Online Text

Labeled release experiment. Microbial respirometry, a technique originally developed for the detection of specific organisms, evolved into the non-specific microbial life detection test known as the labeled release (LR) experiment, that was later used in the Viking landers for the search for life. The LR experiment was designed to detect heterotrophic metabolism in the martian soil by monitoring radioactive gas evolution following the addition of a radioactive ^{14}C -labeled nutrient composed of a blend of selected achiral and chiral molecules with all enantiomers present simultaneously (formate, glycolate, glycine, D- and L-alanine, D- and L-lactate). Because of temperature constraints during the sterilization of the space craft, glucose, that was originally used in the prototype experiments, was eliminated and glycolate substituted. The Viking results showed a rapid evolution of radioactive counts upon addition of the radioactive nutrient to a fresh surface soil sample. At both landing sites the responses were similar, and the magnitude of the evolved counts was consistent with the utilization of only one of the labeled carbon atom positions available in the nutrient, presumably formic acid.

Reactivity of the martian soil. There have been many suggestions regarding the nature of the chemical reactivity of the martian soil but as yet no laboratory experiment

has been able to simulate both the GE and the LR response. Instruments built to further investigate the reactive nature of the martian soil (e.g., MOX for the ill-fated Russian Mars'96 mission (5)) have not yet provided data.

Atacama Desert. The Atacama is a temperate desert that extends across 1000 km with monthly mean air temperatures between 16 to 14°C and is remarkably uniform throughout the year ($\pm 3^\circ\text{C}$). The extreme aridity is due to the combined effects of a high pressure system located on the western Pacific Ocean, the drying effect of the cold north-flowing Humboldt ocean current, the oceanic cloud barrier effect of the Cordillera de la Costa, and the rain shadow effect of the Cordillera de Los Andes intercepting precipitation from the Intertropical Convergence (6-9). The Copiapó river (27°S) marks the southern limit of the extremely arid desert. The area north of Copiapó receives moisture from an occasional fog or a shower event every few decades (6). The region south of Copiapó starts to receive precipitation from the occasional winter incursions of the polar front (6). Proxy temperature records indicate increased precipitation from El Niño events occurred from 10 to 16 kyr ago but yet rains did not penetrate the absolute desert region [J.L. Betancourt, C. Latorre, J.A. Rech, J. Quade, K.A. Rylander, *Science* 289, 1542 (2000)]. In addition, a 106 kyr paleoclimate record from a Salar de Atacama drill core also indicates episodic wet periods [A.L. Bobst, et al., *Paleoecology* 173, 21 (2001)]. Geological and soil mineralogical evidence suggest that the extreme arid conditions have persisted in the southern Atacama for 10-15 Myrs [G.E. Ericksen, *Amer. Scientist* 71, 366 (1983)], making it one of the oldest, if not the oldest desert on Earth. The age and aridity of the Atacama are probably directly responsible for the large nitrate accumulations that are present there. The nitrates are likely to be of atmospheric origin [J.K. Böhlke, G.E. Ericksen, K. Revesz, *Chemical Geology* 136, 1135 (1997)] and are not biologically decomposed or leached away by water flow due to the extreme aridity allowing them to accumulate into significant concentrations over the long age of the desert.

Pyrolysis of biomolecules and bacterial cell mass. Proteins, peptides and free amino acids yield a series of carboxylic acids, saturated nitriles, and saturated, unsaturated and aromatic hydrocarbons; carbohydrates degrade to a series of aliphatic aldehydes, ketones, carboxylic acids, aromatic compounds and furan derivatives; fatty acids pyrolyze to alkanes, alkenes, aromatic compounds, and short chain carboxylic acids; porphyrins degrade to pyrroles; and nucleic acid bases release unsaturated nitriles, and substituted furans. Pyrolysis of Atacama Desert bacteria isolates (*e.g.*, strain AT01-3) releases a mixture of all the above classes of organic compounds.

Culturable heterotrophic bacteria along the transect. Figure 2E shows that the colony forming units per gram (CFU/g) in general increase along the precipitation gradient. However there was some degree of patchiness in the samples analyzed between the sites AT01-03/AT02-03 (S 24° 4' 9.6", W 69° 51' 58.8) and AT02-26 (S 24° 54' 15.8", W 69° 54' 28.7"). The samples AT02-24, AT02-27, and AT02-31 show elevated levels of culturable heterotrophic bacteria as compared to sites within the same degree of latitude. One explanation for this patchiness in the number of culturable heterotrophic bacteria recovered is that it could be the result of increased moisture availability at that site due to gaps in the coastal range which allows moisture to get to the more arid region. All of the sites sampled between S24° and S28° had levels of culturable heterotrophic bacteria in the range $<1 \times 10^3$ to 2.2×10^6 CFU/g which is below that found in the Sonoran Desert sample (9.6×10^6). The moisture available from the ocean influences in the coastal region sample AT97-3 a site also devoid of vegetation has an impact on the levels of culturable heterotrophic bacteria surviving there. An upper estimate in the number of culturable heterotrophic bacteria from these samples are shown as solid triangles in Figure 2, panel E. It should be considered that the amount of soil plated, 100 μ l volume of the first dilution of the series is only 0.01g. In order to determine the numbers of heterotrophic bacteria present at such low levels methods that examine larger amounts of sample were explored. Results from using most probable number (MPN) techniques [J.C. de Man, *Eur. J. Appl. Microbiol.* **1**, 67 (1975)] indicate that

culturable heterotrophic bacteria could be detected in larger amounts of soil sample (1g and 10g amounts).

Bacteria in the Yungay area. A number of the colonies recovered from the AT02-03 site after plating of large numbers of replicate samples were identified by 16S rRNA gene sequence analysis. These organisms were found to fall into two distinct phylogenetic lineages, the Actinobacteria and the low G+C Gram positives. The majority of these organisms are members of the *Blastococcus/Geodermatophilus* group, other strains included members of the genera *Bacillus* and *Streptomyces* organisms typically found in arid soils [M.M. Palmisano et al., *Int. J. Syst Evol Microbiol.* **51**, 1671 (2001); J. Wink, R.M. Kroppenstedt, G. Seibert, E. Stackebrandt, *Int. J. Syst Evol Microbiol* **53**, 721 (2003)].

Air sampling. It was carried out at the sample site AT02-03, the area considered to be the core arid region of the desert. Of the ten samples no CFUs were detected on the agar plates. This was very different to the situation in the Mojave Desert, USA where more than 100 CFUs are routinely obtained under the same experimental conditions. These data indicate that there was very little input of microorganisms from the atmosphere onto the surface of the soils in the core arid region.

Toxicity of soil. The soil mixes were dilution plated on 1/10 and 1/100 strength PCA. The CFUs/g for AT02-22 were 8.8×10^6 and 7.8×10^6 , respectively. While no CFUs were detected for the sample AT02-03A. The addition of an equal volume of soil AT02-03A to soil AT02-22 resulted in a reduction of the CFUs/g value by 59% and 50% when the mixture was plated on 1/10 and 1/100 PCA respectively. In the case of the 1:2 mix a reduction of 28% and 33% were observed while the 2:1 mixture reduced the CFUs/g of AT02-22 by 54% and 73% when plated on the 1/10 and 1/100 concentrations of PCA, respectively.

DNA extraction. The lack of recoverable DNA in the soils at Yungay may be surprising but we believe it is correct for several reasons. First we used the same methods that

did recover DNA from other sites. In addition we used an alternative method, the UltraClean Mega Soil DNA Kit (MoBio Laboratories Inc, Carlsbad, Ca) that employs a mechanical disruption bead beating step, which was applied to the same soil samples. The only samples that did not yield DNA using this kit were those from the Yungay site. Soil extracts prepared from these samples were not inhibitory to control PCR reactions adding further evidence to the conclusion that the soils at the most arid zone near Yungay are not toxic to biological activity. Finally the lack of recoverable DNA correlates with sites with no or few culturable organisms and low and oxidized organic content. Thus we believe that the results are valid and there is no recoverable DNA in these Yungay soils.

Materials and Methods

SAMPLING SITES

Soil samples used in this study were collected in the Atacama Desert (AT) along the precipitation gradient in a north-to-south transect centered on ~70° W between 24°S and 28°S in October 1997, October 2001, 2002 and April 2003. The sampling sites are identified by the following nomenclature: ATxx-yy; where xx and yy refer to the year and site sampled, respectively. They are listed in a north-to-south sequence: **AT97-3** (S 23° 18' 56.4", W 70° 25' 4.4"), **AT03-39** (S 24° 3' 33.0", W 69° 52' 11.3"), **AT03-38** (S 24° 3' 38.8", W 69° 52' 5.3"), **AT03-44** (S 24° 3' 41.8", W 69° 54' 29.8"), **AT03-37** (S 24° 3' 44.0", W 69° 51' 53.3"), **AT03-36** (S 24° 3' 50.2", W 69° 51' 51.2"), **AT03-35** (S 24° 4' 0.4", W 69° 51' 49.7"), **AT03-48** (S 24° 4' 0.9", W 69° 52' 11.6"), **AT03-34** (S 24° 4' 6.2", W 69° 51' 48.4"), **AT03-33** (S 24° 4' 6.8", W 69° 51' 58.1"), **AT02-03D** (S 24° 4' 7.1", W 69° 51' 57.7"), **AT02-03E** (S 24° 4' 8.3", W 69° 52' 50.1"), **AT02-03C** (S 24° 4' 8.9", W 69° 51' 54.5"), **AT01-03**, **AT02-03A** (S 24° 4' 9.6", W 69° 51' 58.8"), **AT02-03B** (S 24° 4' 11.1", W 69° 51' 58.1"), **AT03-50** (S 24° 4' 27.2", W 69° 52' 55.4"), **AT03-49** (S 24° 4' 32.6", W 69° 52' 38.3"), **AT01-12** (S 24° 6' 10.2", W 70° 1' 9.7"), **AT02-31** (S 24° 17' 29.8", W 69° 58' 0.3"), **AT02-30** (S 24° 24' 1.9", W 69° 55' 45.2"), **AT02-29** (S 24° 34' 26.2", W 69° 47' 44.8"), **AT02-28** (S 24° 49' 1.2", W 69°

47' 2"), **AT02-26** (S 24° 54' 15.8", W 69° 54' 28.7"), **AT02-27** (S 25° 4' 6", W 69° 53' 17.1"), **AT01-16, AT02-16** (S 25° 18' 17.4", W 69° 50' 32.2"), **AT01-24, AT02-24** (S 25° 45' 36.9", W 70° 11' 46.5"), **AT01-17** (S 25° 47' 46.7", W 69° 50' 39.2"), **AT01-19** (S 26° 2' 9.2", W 69° 51' 52.2"), **AT01-23, AT02-23** (S 27° 1' 17.4", W 70° 17' 40.7"), and **AT01-22, AT02-22** (S 28° 7' 4.5", W 69° 55' 8"). With the exception of samples AT01-19, AT01-23, AT02-23, AT01-22, AT02-22 all sites were devoid of vegetation. Representative views of these sites are shown in Fig S3 to S5.

Soil samples were also collected from the Mojave (LRH01-07: N 35° 23' 11.5", W 116° 15' 50.8") and Sonoran (S97-3: N 32° 49' 14.5", W 111° 12' 14.1") deserts. The Mohave and Sonoran sites had vegetation similar to that observed at the Atacama site, AT01-22 and AT02-22.

METEOROLOGICAL DATA FOR THE YUNGAY AREA

In each data file for each year there are 16 columns (name: YXX.out where XX is 94,95,96,97 or 98):

1. Time in days from the beginning of the present year (1-365, or 366);
2. Air temperature, °C;
3. Air relative humidity, %;
4. Rain, mm;
5. Dew scaled from 1 to 10;
6. Wind speed, m/s;
7. Wind direction, degrees;
8. Photosynthetically Active Radiation (400-700 nm), $\mu\text{mole m}^{-2} \text{ s}^{-1}$;
9. Rock Temperature, °C ;
10. Soil Relative humidity 10 cm below soil surface, %;
11. Relative humidity beneath rock 1, %;
12. Relative humidity in soil between rocks, %;
13. Conductivity beneath rock 1, in micro-Seimens ($\mu\Omega$); and
14. Conductivity beneath rock 2, in micro-Seimens ($\mu\Omega$);
15. Month (1-12);

16. Year (94-98).

In the single file of daily averages (Name:yungay_daily.out), there are the following 16:

1. Time in days from the beginning of the year 1994 (268-1749);
2. Daily average Air temperature, °C;
3. Daily average Air relative humidity, %;
4. Daily total Rain, mm;
5. Daily average dew scaled from 1 to 10;
6. Daily average Wind speed, m/s;
7. Daily average Wind direction, degrees;
8. Daily average Photosynthetically Active Radiation (400-700 nm), $\mu\text{mole m}^{-2} \text{ s}^{-1}$;
9. Daily average Rock Temperature, °C ;
10. Daily average Soil Relative humidity 10 cm below soil surface, %;
11. Daily average Relative humidity beneath rock 1, %;
12. Daily average Relative humidity in soil between rocks, %;
13. Daily average Conductivity beneath rock 1, in micro-Seimens ($\mu\Omega$); and
14. Daily average Conductivity beneath rock 2, in micro-Seimens ($\mu\Omega$).

For monthly summaries of this data and analysis see McKay, C.P., E.I. Friedmann, B. Gomez-Silva, L. Caceres-Villanueva, D.T. Andersen, and R. Landheim, Temperature and moisture conditions for life in the extreme arid region of the Atacama Desert: Four years of observations including the El Nino of 1997-98, *Astrobiology*, 3, 393 (2003).

SAMPLES, CONTROLS AND BLANKS

Approximately 500 g representing a composite of 6 individual nearby sites (~2 m in radius) of the upper 10 cm soil layer were collected using sterile polyethylene scoops and stored in sterile polyethylene (Whirlpak™) bags. The samples were kept at ambient temperature until analysis.

The transect from north-to-south provided a control in that we compared the exact same methods described below. The “wet” sites provided positive controls for the dry sites and show that the relative lack of detection in the dry areas was not a failure of the methods. Blank tests were also run simultaneously with all methods in which no soil was added to each assay.

NITRATE MEASUREMENTS

Ten grams of soil were sonicated for several minutes in 50 ml of deionized water and then the suspension was centrifuged. The nitrate dissolved in solution was reduced to nitrite using powdered cadmium. The nitrite is then determined by diazotizing sulfanilamide and coupling with N-(1 naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically [R. Navarro-González and S. Castillo-Rojas, *Educ. Chem.* 32, 161 (1995)]

SUPEROXIDES AND HYDROGEN PEROXIDE MEASUREMENTS

They were determined by measuring the absorbance of crystal violet at 592 nm at pH 4, formed by the oxidation of leuco crystal violet by H₂O₂ in the presence of the enzyme horseradish peroxidase [L.S. Zhang, G.T.F. Wong, *Talanta* 41, 2137 (1994).]

E_h AND pH MEASUREMENTS OF SOILS

The E_h and pH and of 1:2 (by weight) soil/ultra high purity water slurries were measured for one hour using a Thermo Orion 635 pH/ORP/Temperature meter and probe. The following samples were examined: AT02-3, ATO2-16, AT02-22, AT02-24, AT02-27 and AT02-29.

PYROLYSIS-GC-MS

Soil samples were freeze-dried and then were finely grounded with an agate mortar mill. A portion of this powder (~40 mg) was loaded in a capillary quartz tube and held in place using small plugs of quartz wool. Blanks were prepared with no soil added. Each tube was then mounted in the center of a platinum coil filament-type pyrolyzer (Pyroprobe 2000 from CDS Analytical, Inc). Atmospheric air was removed from the pyrolysis cavity by flushing a stream of helium (99.9999%) at 60 PSI for 3 min. The material was subjected to a thermal treatment of 200°C for 10 s, then to 750°C with a heating rate of 10 °C/ms, and finally holding this temperature for 60 s. The resulting pyrolyzed gases and volatiles were injected 60 s after pyrolysis into the injection port of the Hewlett Packard (HP) gas chromatograph 5890 series by an automatic six-port gas-

sampling valve. The pyrolyzer probe is mounted on the top of the injection port of the gas chromatograph. The pyrolyzer interface and the gas chromatograph injection port were maintained at 250°C. The gas chromatographic column used was a PoraPlot Q fused-silica 25 m long × 0.32 mm I.D. with a 2.5 m particle trap. The column program temperature was isothermal at 60°C for 2 min, and then a rate of 10°C min⁻¹ up to 240°C, and finally isothermal for 35 min. The carrier gas used was helium with a flow of 1.2 ml min⁻¹. The gas chromatograph is interfaced in parallel with a HP FTIR-detector (model 5965) and a HP quadrupole mass spectrometer (5989B) operating in electron impact mode at 70 eV. The temperatures at the interfaces were at 260°C. The mass analyzer was scanned from m/z 45 to 200 at a scan rate of 4.4 scan/s. The electron impact chamber and the quadrupole were maintained at 250°C and 100°C, respectively. Each compound was identified by its characteristic mass fragmentation pattern (see SFig 1).

HETEROTROPHIC BACTERIA COUNTS

The total number of culturable heterotrophic bacteria present in each soil sample collected was determined using a serial dilution plate-count technique on a number of artificial culture media with both low (1/10 and 1/100) strength Plate Count Agar (PCA) and high nutrient single strength PCA media. Plate Count Agar was obtained from Difco (USA) and prepared according to the suppliers instructions. The samples plated were a composite of the sample collected at the individual sites. One gram of soil was used for the initial dilution and all dilutions were plated in triplicate. Temperatures either side of and including the environmental mean were used for incubation. The majority of incubations were carried out at 26°C.

DIVERSITY OF THE BACTERIAL COMMUNITIES

16S rRNA gene sequence clone libraries each comprising ~75 16S rRNA gene PCR products for the environmental samples AT01-16, AT01-17, AT01-19, AT01-22, AT01-23, AT97-3 and LRH01-07 were assembled and analyzed by partial and full sequence determination. Attempts to construct such libraries from samples collected at the AT01-03 and AT02-03 sites were unsuccessful. This would seem to be due to the

extremely low numbers of microorganisms present in these samples and the resulting lack of DNA as template for PCR amplification. The DNA extraction method used included three freeze-thaw steps to maximize cell and spore lysis. In order to verify the lack of recoverable DNA was independent of the DNA extraction method used, the UltraClean Mega Soil DNA Kit (MoBio Laboratories Inc, Carlsbad, Ca) that employs a mechanical disruption bead beating step was applied to the same soil samples. The only samples that did not yield DNA using this kit were AT01-03 and AT02-03. Soil extracts prepared from these samples were not inhibitory to control PCR reactions adding further evidence to the conclusion that the soils at the most arid zone (AT01-03 and AT02-03) are not toxic to biological activity. The degree of diversity detected at each site was observed as the number of novel taxa detected within the group of 16S rRNA clones examined. The level of diversity was found to increase from north-to-south along the precipitation gradient. For the five samples within the central desert area of the Atacama Desert (AT01-16, AT01-17, AT01-19, AT01-22, AT01-23) the numbers of distinct taxonomic units ranged from 6 to 26 within the 75 16S rRNA genes examined at each site. The numbers of distinct taxonomic units observed for the Atacama coastal desert sample was 35 showing that increased moisture availability not only influences the numbers of culturable bacteria but the diversity of the total bacterial community. By comparison the sample studied from the Mojave Desert contained 48 distinct taxonomic units within the 75 16S rRNA gene sequences recovered and studied.

AIR SAMPLING OF MICROORGANISMS

Samples of 500 liters were collected on the surface of 1/10 and 1/100 strength PCA plates using a Hi-VAC bacterial air sampler from Cherwell Laboratories (United Kingdom) air sampler. The plates were then incubated for up to 20 days at 26°C.

LABELED RELEASE EXPERIMENT

Achiral substrate. One cm³ of desert soil was transferred to a sterile 5 cm³ glass serum bottle using a sterile scoop and 1 cm³ of 50 mM aqueous ¹³C-labeled sodium formate solution was added using a sterile syringe fitted with a 0.22 µm filter. The bottle was immediately gas-tight sealed and stored at ambient temperature at the University of

Antafogasta Desert Research Station at Yungay where the experiment was conducted until it was brought to the GC-MS facility at the Universidad Nacional Autónoma de México for immediate analysis.

Chiral substrate. One cm³ of an equimolar (5 mM) aqueous mixture of sodium alanine and glucose, were added to 1 cm³ soil with the different combinations of enantiomers so that any microorganisms present in the soil could (L-alanine + D-glucose) or could not (D-alanine + L-glucose) carry out metabolism. Sodium alanine was labeled with ¹³C in all three carbon atoms while glucose was labeled at the carbon-1 position with ¹³C.

Blank and Control experiments. Experiments were run in triplicate with achiral and chiral substrates in which no soil was added (blank experiments), or 1 cm³ of hydrogen peroxide (3% aqueous solution), sodium peroxide or sodium nitrate were added instead of the soil (control experiments).

Analyses. The experiments were analyzed 3 to 5 days after initiation of incubation. One cm³ of head space gas of each vial was injected into a 5890-HP gas chromatograph equipped with a chromatographic column type PoraPlot Q fused-silica (25 m long × 0.32 mm I.D) with a 2.5 m particle trap. The column program temperature was isothermal at 60°C for 2 min, and then a rate of 10°C min⁻¹ up to 240°C, and finally isothermal for 35 min. The carrier gas used was helium with a flow of 1.2 ml min⁻¹. The gas chromatograph was interfaced to a 5989B-HP quadrupole mass spectrometer operating in electron impact mode at 70 eV. The temperatures at the interfaces were at 260°C. A special single-ion monitoring technique was employed to detect only the ions 44 and 45 corresponding to ¹²CO₂ and ¹³CO₂, respectively. ¹²CO₂ was monitored due to the conversion of carbonates present in the soils to carbon dioxide after the acid hydrolysis (0.5 cm³ of 3.7% HCl solution) treatment just prior to GC-MS analysis. The ion 45 signal was corrected for the expected natural abundance of ¹³C (1.11%) determined by the counts of ion 44 detected in each experiment.

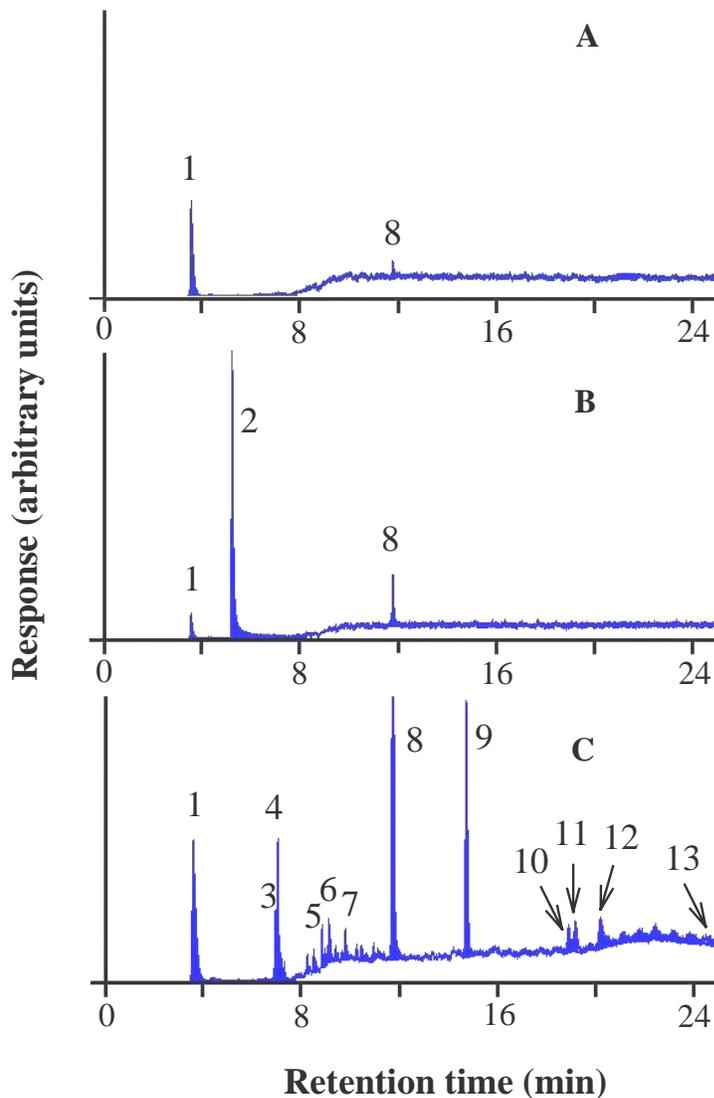


Fig. S1. Reconstructed ion gas chromatograms of the volatile fraction released during flash pyrolysis at 750°C of Atacama Desert soils along the north-to-south precipitation gradient: (A) AT01-03, (B) AT01-16, and (C) AT01-22. Peak identification: 1. Formic acid; 2. Sulfur dioxide; 3. 2-Butene; 4. 1,2-Butadiene; 5. Propenenitrile; 6. 1,3-Pentadiene; 7. 2-Methylfuran; 8. Benzene; 9. Methylbenzene; 10. Ethylbenzene; 11. 1, 2-dimethylbenzene; 12. Styrene; 13. Benzenenitrile. For simplicity only the major peaks are labeled in the chromatogram but the following chemical groups were formed by the thermal degradation treatment: alkanes, alkenes, dienes, alcohols, aldehydes, ketones, ethers, carboxylic acids, saturated and unsaturated nitriles, amines, alkyl-hydrazines, aromatic, N- and O-heterocycles.

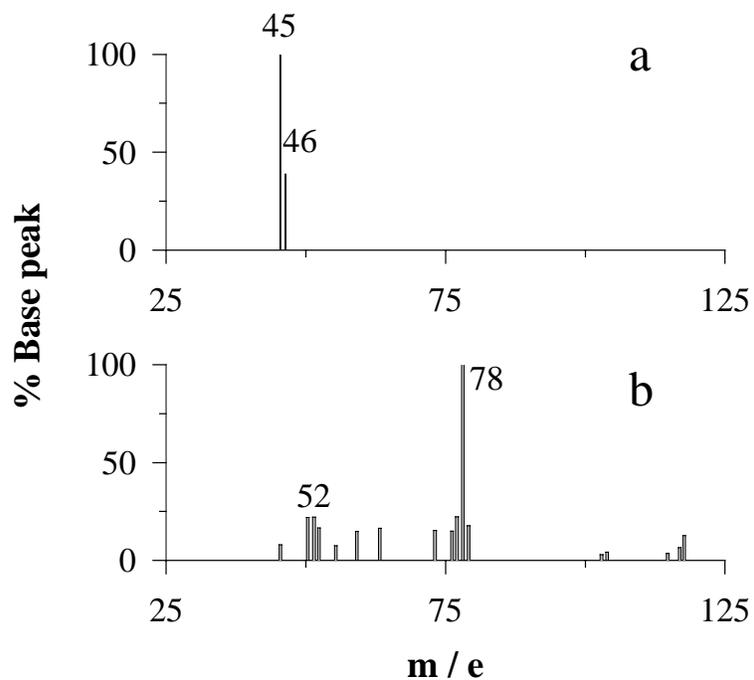


Fig S2. Mass spectra pattern of (a) formic acid and benzene released pyrolytically in soil samples from AT01-03.



Fig S3. Landscape (A) and close up (B) views of the Yungay Area: Sites AT01-03 and AT02-03A.

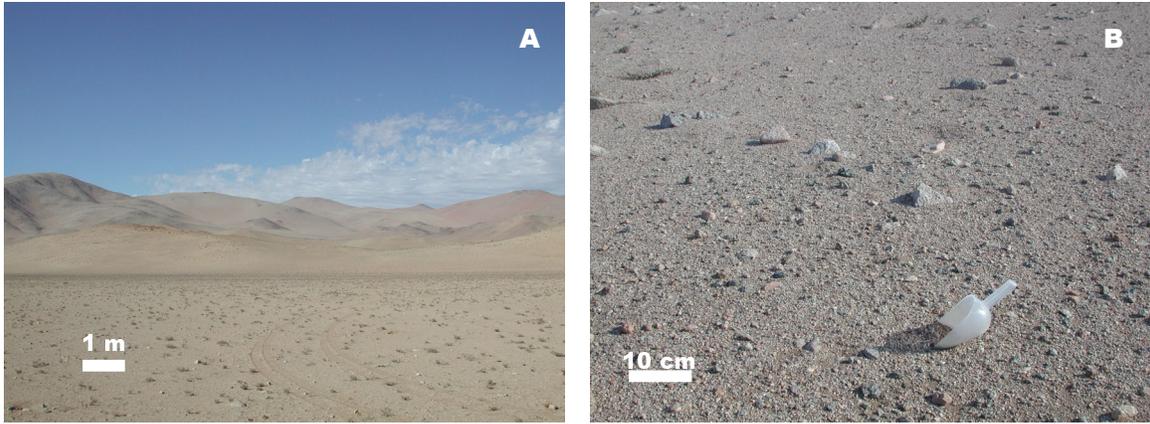


Fig S4. Landscape (A) and close up (B) views of Sites AT01-23 and AT02-23.

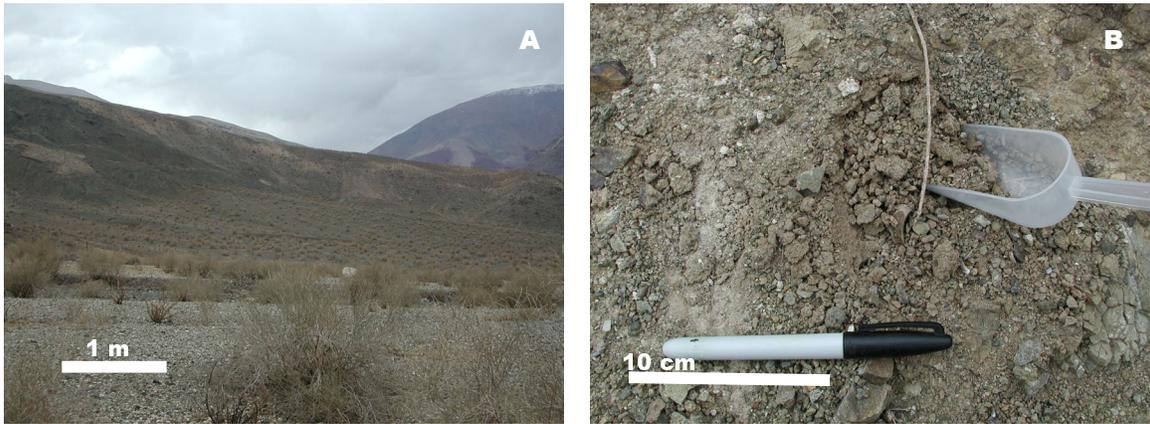


Fig S5. Landscape (A) and close up (B) views of sites AT01-22 and AT02-22.