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

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The Viking missions showed the martian soil to be lifeless and depleted in organic material and indicated the presence of one or more reactive oxidants. Here we report the presence of Mars-like soils in the extreme arid region of the Atacama Desert. Samples from this region had organic species only at trace levels and extremely low levels of culturable bacteria. Two samples from the extreme arid region were tested for DNA and none was recovered. Incubation experiments, patterned after the Viking labeled-release experiment but with separate biological and nonbiological isomers, show active decomposition of organic species in these soils by nonbiological processes.

The results of the Viking lander analyses of soils on Mars are puzzling in three respects. First is the absence of organic materials at the level of parts-per-billion (ppb), as measured by pyrolysis–gas chromatography–mass spectrometry (pyr-GC-MS) (1, 2). The second unexpected result is the rapid release of molecular oxygen, at levels of 70 to 770 nmol g⁻¹, when soil samples were exposed to water vapor in the gas exchange experiment (3).

The third puzzling result is that organic material in the labeled-release (LR) experiment was consumed as expected if life were present (4) (supporting online text). However, the presence of life is in apparent contradiction with the results from pyr-GC-MS. Currently, the most widely accepted explanation for the reactivity of the martian soil is the presence of one or more inorganic oxidants (such as superoxides, peroxides, or peroxy-nitrates) at the parts-per-million (ppm) level, whereas the lack of organics can be explained by their oxidation because of the presence of such oxidants and/or by direct ultraviolet radiation damage (5) (supporting online text).

We report here results of studies on the soils in the Atacama Desert, an environment that serves as a model for Mars. The Atacama is an extreme, arid, temperate desert that extends from 20°S to 30°S along the Pacific coast of South America (6–9) (supporting online text). Soil samples were collected along the precipitation gradient in a north-to-south transect centered on ~70°W between 24°S and 28°S (Fig. 1). They were analyzed (10) for organic matter by pyr-GC-MS at 750°C under an inert atmosphere and for the presence of viable heterotrophic microorganisms by serial dilution plating on a number of

artificial culture media that contained high and low nutrient levels. The diversity of the bacterial communities in a number of the samples was investigated by isolation of the total DNA, then polymerase chain reaction (PCR) amplification and sequence determination of the 16S ribosomal RNA (rRNA) genes recovered.

Analysis of samples by pyr-GC-MS revealed that the most arid zone of the Atacama, the Yungay area, is depleted of most organic molecules. Only two peaks in the chromatograms corresponding to organic molecules (formic acid and benzene) are detectable (figs. S1 and S2). In contrast, a less arid site, AT01-22, releases a complex mixture of organic compounds on pyrolysis (fig. S2). We carried out a comparative analysis by pyr-GC-MS with major classes of biomolecules (proteins, carbohydrates, fatty acids, porphyrins, and DNA) and bacterial mass (e.g., strain AT01-3 isolated from the Atacama Desert) (supporting online text). We concluded that site AT01-22 contains bacteria and/or all of the major classes of biomolecules at levels within the detection limits of the pyr-GC-MS protocol applied.

The two characteristic compounds released by all pyrolysates are formic acid, a highly oxidized organic compound, and benzene, a thermally stable aromatic compound. These two compounds are typically released by the thermal treatment of monocarboxylic acids, polycarboxylic acids, carbohydrates, polysaccharides, amino acids, and proteins. Formic acid is present at concentrations of ~1 μmol g⁻¹ in the Yungay area, then decreases by an order of magnitude at 26°S and increases again in the less arid zone (Fig. 2C). In contrast, benzene is present at trace levels (~0.01 μmol g⁻¹) at ~24°S, with its concentration increasing to ~1 μmol g⁻¹ in the less arid zone (~28°S). The ratio between formic acid and benzene reaches its highest value (≥12 units) in the Yungay area, and then sharply drops to ≤0.3 from 25°S to 28°S (Fig. 2D). A high formic acid/benzene ratio indicates that the organic matter present in the region is oxidized and possibly composed of refractory organics such as aliphatic and aromatic mono- and polycarboxylic acids.

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The levels of organics released at 750°C from pyr-GC-MS are above the Viking GC-MS reported detection limits for most compounds (1, 2). Therefore additional experiments were performed at 500°C, the highest temperature used by the Viking landers (1, 2). This resulted in a reduction by a factor of 4 in formic acid and no release of benzene from the most arid sample (AT-02-03A) and 20-fold and 100-fold reductions in formic acid and benzene, respectively, in the less arid sample (AT02-22). Consequently, metastable organics (such as salts of aromatic and aliphatic mono- and polycarboxylic acids) may be present in the martian soils but would not have been detected by the Viking landers, because of the lower pyr-GC-MS temperature (500°C) (11, 12). Under our experimental conditions (750°C), organic compounds would have been degraded to formic acid and benzene at detectable levels.

We determined the total viable counts of culturable heterotrophic bacteria in soil samples from the transect (Fig. 2E) as well as in the coastal desert. We compared these to two samples from the Sonoran Desert. In general, the numbers of culturable heterotrophic bacteria increased along the north-to-south precipitation gradient (supporting online text). This trend is consistent with the increasing levels of benzene released on pyr-GC-MS of soils (Fig. 2C). At the southern end of the gradient sampled, the total heterotrophic bacterial counts do not reach the levels found in the less arid Sonoran Desert sample. The coastal desert sample site AT97-3 has elevated levels [10^3 to 10^4 colony-forming units per gram (CFU/g)] of heterotrophic bacteria compared to sample site AT01-03. The samples from the Yungay area contain levels of heterotrophic bacteria below the detection limits of dilution plating. In many cases, no bacterial colonies were observed at any dilutions on any of the nutrient media used for plating of these samples. Extensive plating with up to 100 replicates of samples AT01-03 and AT02-03 and a sample from a similar site in the same region (AT01-12) provided less than 10 bacterial colonies in total (supporting online text). These data indicate that the Yungay area contains extremely low levels of heterotrophic bacteria. We considered that microorganisms could enter this environment from the atmosphere; however, such microorganisms were not detected in the soil samples analyzed. Air samples were collected at site AT02-03 in order to determine the load of culturable microorganisms that enter this environment from the atmosphere. However, no culturable heterotrophic bacteria were obtained from the air samples collected in the Yungay area, indicating the lack of a local source of airborne bacteria (supporting online text).

The pH of the soils is in the range 5.5 to 8.6, indicating that extreme soil pH values are not the cause of the low microbial numbers found in the most arid zone. Further studies were conducted to determine if the soil from the Yungay area was toxic for the growth of microorganisms. The soil from AT02-03A (in the most arid zone) was mixed with the soil from the less arid site, AT02-22, in the ratios 1:2, 1:1, and 2:1, and then plated on 10% plate count agar to determine the CFU/g number. The CFU/g values for these soil mixtures were not reduced by more than the expected dilution factor (supporting online text), suggesting that the AT02-03A soil is not toxic.

Because only a small percentage of soil microorganisms can be cultured, we assembled and analyzed 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 Mojave sample LRH01-07. Attempts to construct such libraries from samples collected at the Yungay area were unsuccessful. There was no recoverable DNA in the two Yungay soil samples studied (supporting online text).

The degree of bacterial diversity detected at each site was measured 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, and AT01-23), the numbers of distinct taxonomic units ranged from 6 to 26 within the 75 16S rRNA genes examined at each site. The number of distinct taxonomic units observed for the coastal desert sample was 35, showing that increased moisture availability not only influences the amount of culturable bacteria but also 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.

In order to understand the reactivity of the soils, we performed a modified version of the Viking LR experiment (10). In one procedure, desert soil was incubated for several days in an aqueous solution that contained ^{13}C -labeled sodium formate. Any oxidation of formate to ^{13}C could be attributable to either abiotic or biological activity or both. However, we selected formate because it is thought to be the only substrate oxidized in the Viking LR experiment (4) (supporting online text). To distinguish between any abiotic or biological activity, another set of ex-

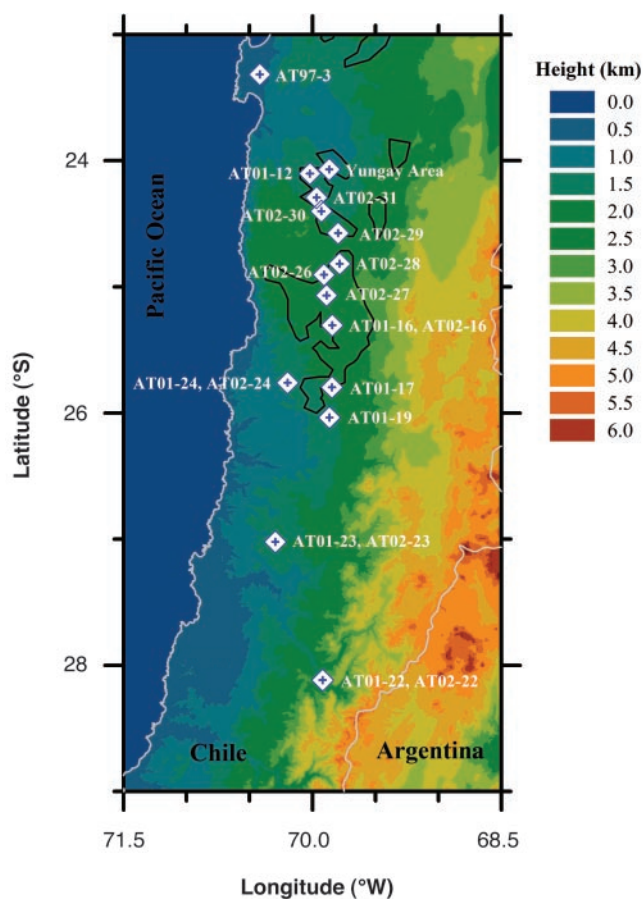


Fig. 1. Digital elevation map of the Atacama Desert in northern Chile, showing the historic nitrate mining sites (black counter lines) and the soil sampling sites. The Yungay area was sampled at the following sites: AT01-03, AT02-03A to AT02-03E, and AT03-33 to AT03-39 (10).

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periments was performed in which desert soil was incubated for several days in an aqueous mixture of two ^{13}C -labeled chiral substrates,

sodium alanine and glucose. Different combinations of these enantiomers were used, so that any microorganisms present in the soil

could (L-alanine and D-glucose) or could not (D-alanine and L-glucose) carry out metabolism. A set of soil samples across the north-to-south transect was monitored for their LR responses (Fig. 3). A fraction (3 to 12 μmol) of the formic acid added ($\sim 50 \mu\text{mol}$) was decomposed in the Yungay area (samples AT02-03A to AT02-03E), even where no culturable heterotrophic organisms were detected (Fig. 2). In the less arid zones, the degree of conversion of ^{13}C -formate to $^{13}\text{CO}_2$ rose as expected on the basis of the increased biological activity, but this does not rule out biological activity in the Yungay area. The LR response of the enantiomeric mixtures of alanine and glucose (Fig. 3) showed equal quantities of $^{13}\text{CO}_2$ ($\sim 0.4 \mu\text{mol}$) released from both the D-alanine and L-glucose and L-alanine and D-glucose mixtures in the Yungay area, and it was three orders of magnitude higher than those measured in the blank experiments. Therefore, any biological explanation for the reactivity of the soil in this part of the desert is highly unlikely. We observed this abiotic response with similar strength in all soils of the north-to-south precipitation gradient, using D-alanine and L-glucose. In contrast, the amount of $^{13}\text{CO}_2$ released from the L-alanine and D-glucose mixture rises as one moves toward less arid zones, indicating that biological activity dominates versus the abiotic reactivity of soils. The ratio between the biotic plus abiotic responses and the abiotic response alone increases from 1 in the Yungay area, where the soils were chemically reactive, to nearly 5 at $\sim 25.8^\circ\text{S}$, where the soils were biologically active. Degradation of the ^{13}C -labeled molecules was observed in the presence of hydrogen peroxide and sodium peroxide in control experiments, whereas no reactivity was observed in the presence of nitrates.

The redox potential (E_h) of several Atacama Desert samples was oxidizing with values ranging from 365 to 635 mV. We performed chemical assays for superoxides and hydrogen peroxide, because these are the most plausible oxidants and are those suggested as explanations for the reactivity observed by the Viking landers. Our results rule out these oxidants as the cause of the reactivity seen at the Yungay area, because the concentrations are too low (0.05 to 0.14 ppm) to explain our results and because the variation with latitude does not follow the variation seen in the organic or microbial concentrations. Nitrates are present in the soil in high levels (10 to 140 ppm), but they alone are not oxidizing enough to account for the reactivity seen in our samples. Nitrates may lead to the formation of peroxonitrite (NOO_2^-), and this has been suggested as a possible martian oxidant (13). However, the nitrate concentrations needed are in the percent level (10), much higher than in the Atacama. Thus, although our results show the presence of a strong oxidant in the soils in the

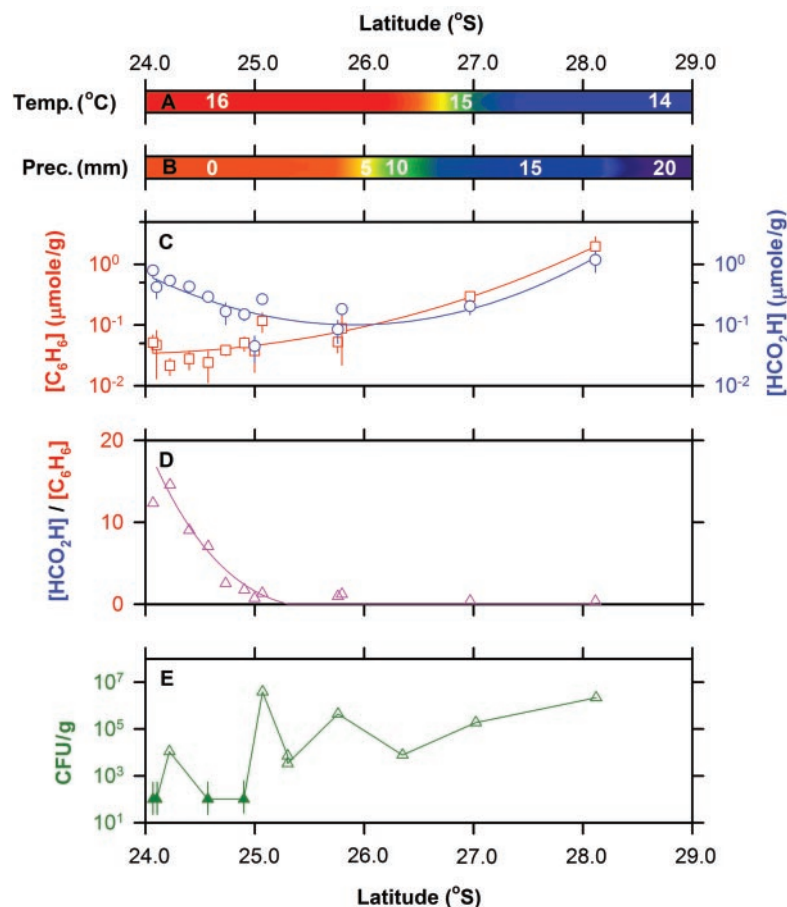
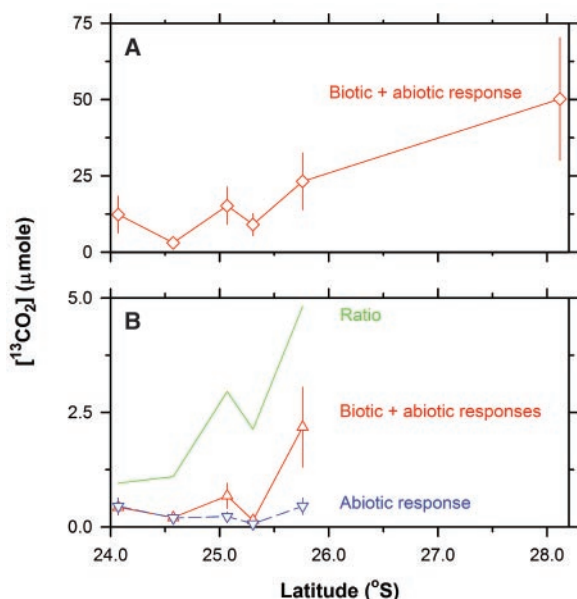


Fig. 2. Characteristic meteorological, chemical, and microbiological profiles of soils along the Atacama Desert: (A) mean annual air temperature, (B) mean annual precipitation, (C) organics released by pyr-GC-MS (benzene, squares; formic acid, circles), (D) the ratio of formic acid to benzene in pyrolyzed soils, and (E) culturable heterotrophic bacteria. Solid triangles represent an upper estimate of CFU/g in samples where no culturable heterotrophic bacteria were detected with classical microbiological culture approaches.

Fig. 3. LR responses of Atacama Desert soil samples with (A) achiral and (B) chiral nutrients: \diamond sodium formate; Δ L-alanine and D-glucose; and ∇ D-alanine and L-glucose. Sodium formate and alanine were labeled with ^{13}C in all carbon atoms, whereas glucose was labeled at the carbon-1 position with ^{13}C .



Yungay area, the nature of the oxidant remains unexplained. Photochemical reactions initiated by sunlight continually produce oxidants in the lower atmosphere and surface. However, in most soils, biological production of reduced organic material completely dominates the net redox state of soils. When biological production is less than the photochemical production of oxidation, then the soil will become oxidizing. The transition from biologically dominated soils to photochemically dominated soils appears to be abrupt. Whichever process dominates will shift the redox state in one direction or another. In the Atacama, there is a gradual decline in biological activity as conditions became drier, yet near the extreme arid region there is an abrupt transition to very low bacterial levels and low organic content.

It is unlikely that the oxidizing conditions are due to high ultraviolet flux, because the site is only 1 km above sea level. Instead, the dry conditions in the Atacama must inhibit biological production of reductants and possibly enhance the survival of photochemically produced oxidants. Our results suggest that in the extreme arid core of the Atacama, we have crossed the dry limit of microbial survival in extreme environments. The net result is that photochemical processes dominate. Thus, in the Atacama Desert, we find almost no microorganisms and low levels of organic material, and the organic material present appears to have been oxidized. The LR experiments confirm the presence of as-yet-identified oxidants in the Atacama soil. In many respects, these soils are similar to the Mars soils investigated by the Viking Biology Experiment and may provide a valuable testing ground for instruments and experiments designed for future Mars missions.

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- The temperature of pyrolysis was set to any of three temperatures: 200°, 350°, or 500°C. The detection limit for benzene was less than 0.5 to 5 ppb; for smaller molecules such as HCO₂H, the detection limit was poor, at best in the ppm range.
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- Materials and methods are available as supporting material on Science Online.
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Supporting Online Material
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 Figs. S1 and S2
 Data Tables S1 to S6

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A Reservoir of Nitrate Beneath Desert Soils

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A large reservoir of bioavailable nitrogen (up to ~10⁴ kilograms of nitrogen per hectare, as nitrate) has been previously overlooked in studies of global nitrogen distribution. The reservoir has been accumulating in subsoil zones of arid regions throughout the Holocene. Consideration of the subsoil reservoir raises estimates of vadose-zone nitrogen inventories by 14 to 71% for warm deserts and arid shrublands worldwide and by 3 to 16% globally. Subsoil nitrate accumulation indicates long-term leaching from desert soils, impelling further evaluation of nutrient dynamics in xeric ecosystems. Evidence that subsoil accumulations are readily mobilized raises concern about groundwater contamination after land-use or climate change.

Increased deposition of bioavailable nitrogen (N) at the land surface has adversely affected water quality, biodiversity, and ecosystem functioning around the world (1–6). Understanding such impacts requires quantification of N sources, reservoirs, and cycling rates (1, 5, 7, 8). Desert soils, which cover approximately one-fourth of the conterminous United States and one-third of the land surface worldwide, are reportedly low in total N (9, 10). Studies of N cycling in terrestrial ecosystems have traditionally examined only the biologically active soil zone, defined operationally as extending to ~1 m in depth (9, 11). Within this zone, N turnover is rapid (6), and N concentrations decrease with depth (7, 10, 11). Natural sources of N in desert ecosystems include nitrate (NO₃⁻) and ammonium (NH₄⁺) in precipitation, eolian deposition of nitrate salts, and biological assimilation of atmospheric N₂ by N-fixing organisms (5, 7, 8, 10). Mechanisms of N removal include

plant uptake, volatilization to ammonium and other gases, wind erosion, and denitrification (6, 7, 12). Nitrogen loss from the soil zone by leaching is generally assumed to be negligible in desert ecosystems (5, 10, 12). Our findings challenge this assumption, demonstrating that substantial quantities of N, as NO₃⁻, have leached and accumulated beneath the soil zone over millennial time frames.

Soil-water N generally follows a nutrient-type profile, with concentrations that decrease sharply with depth because of biological uptake and cycling (11). In contrast, soil-water chloride (Cl⁻) follows a conservative solute-type profile, with concentrations that increase with depth because of progressive evaporation and water extraction by plant roots. In desert settings, Cl⁻ typically exhibits an exaggerated conservative solute-type profile resulting from the accumulation of thousands of years of atmospheric Cl⁻ deposition (13). A recently developed model (14) (supporting online material) quantitatively explains these Cl⁻ profiles by considering geothermally driven water vapor transport toward the atmosphere, together with the hydraulic sink created in the soil by the roots of desert plants. Physical and biological processes selectively remove water, concentrating Cl⁻ (Fig. 1A).

Surprisingly, soil-water concentration profiles of NO₃⁻ N in five arid-to-semiarid sites in the western United States (Fig. 2) (15) follow the conservative solute-accumulation profiles of Cl⁻ (Fig. 3) rather than the expected progressive nutrient depletion profiles. Maximum NO₃⁻ N

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