Carbon monoxide as a metabolic energy source for extremely halophilic microbes: Implications for microbial activity in Mars regolith

Gary M. King[1]

Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803

Carbon monoxide occurs at relatively high concentrations ($\geq$800 parts per million) in Mars' atmosphere, where it represents a potentially significant energy source that could fuel metabolism by a localized putative surface or near-surface microbiota. However, the plausibility of CO oxidation under conditions relevant for Mars in its past or at present has not been evaluated. Results from diverse terrestrial brines and saline soils provide the first documentation, to our knowledge, of active CO uptake at water potentials (-41 MPa to -117 MPa) that might occur in putative brines at recurrent slope lineae (RSL) on Mars. Results from two extremely halophilic isolates complement the field observations. *Halorubrum* str. BV1, isolated from the Bonneville Salt Flats, Utah (to our knowledge, the first documented extremely halophilic CO-oxidizing member of the Euryarchaeota), consumed CO in a salt-saturated medium with a water potential of -39.6 MPa; activity was reduced by only 28% relative to activity at its optimum water potential of -11 MPa. A proteobacterial isolate from hypersaline Mono Lake, California, *Alkalilimnicola ehrlichii* MLHE-1, also oxidized CO at low water potentials (-19 MPa), at temperatures within ranges reported for RSL, and under oxic, suboxic (0.2% oxygen), and anoxic conditions (oxygen-free with nitrate). MLHE-1 was unaffected by magnesium perchlorate or low atmospheric pressure (10 mbar). These results collectively establish the potential for microbial CO oxidation under conditions that might obtain at local scales (e.g., RSL) on contemporary Mars and at larger spatial scales earlier in Mars' history.

extreme halophiles | Euryarchaeota | carbon monoxide | Mars regolith

The search for past or extant microbial life remains a focal point for astrobiological research on Mars and elsewhere (1–8). Orbiting and rover-based explorations have emphasized historical and contemporary distributions of liquid water as central elements of theories about when, where, and under what conditions life might have existed or continues to exist on Mars. Geological, geochemical, and geomorphological observations have provided definitive evidence for large, ancient fluvial systems that might have been conducive to life (8–13), but the timing, temperature, and composition of surface water have been controversial (14–17). Although unequivocal evidence for contemporary liquid water reservoirs has not yet been obtained, experimental evidence suggests plausible conditions under which brines might form (18), and observations from the Mars Reconnaissance Orbiter suggest that recurring slope lineae (RSL) at latitudes between 32°S and 48°S might develop in association with seasonally moderate conditions during late spring-summer (19–21). McEwen et al. (19) have proposed that these features can be best explained by near-surface liquid water brines that form between temperatures of about −10 °C and 25 °C during late spring-summer.

However, even if liquid water was at one time or is now sufficient to support microbial life, energy sources that could sustain metabolism in near-surface regolith have not been identified. Although the 1976 Viking lander results appeared to exclude surface organic matter (e.g., 22, 23), recent evidence suggests that low organic matter levels might indeed occur in some deposits (e.g., 12). Even so, it is uncertain whether this material exists in a form or concentrations suitable for microbial use.

The Martian atmosphere has largely been ignored as a potential energy source, because it is dominated by CO_2 (24, 25). Ironically, UV photolysis of CO_2 forms carbon monoxide (CO), a potential bacterial substrate that occurs at relatively high concentrations: about 800 ppm on average, with significantly higher levels for some sites and times (26, 27). In addition, molecular oxygen (O_2), which can serve as a biological CO oxidant, occurs at about 1,450 ppm (25). In contrast, Earth's atmosphere contains only about 0.3 ppm CO, a comparatively low concentration that nevertheless supports the activity of numerous soil microbes (28).

One of the few studies that have considered CO as an energy source for putative Mars microbes used estimates of atmospheric CO concentrations and production rates to model uptake by a hypothetical global regolith sink (27). Based on their results, Weiss et al. (27) argued that CO uptake, if it occurs, must take place at very low rates, and that the atmosphere is a marginal energy source. Although this might be true at a global scale, substantial activity could occur at more local scales, such as those of RSL. However, CO utilization has not been previously documented for terrestrial brines or soils experiencing water potential extremes that would characterize putative RSL brines, nor has CO oxidation been demonstrated for extreme halophiles, which have been posited as models for life on Mars (29–32).

Results presented here establish the plausibility of atmospheric CO as an energy source for hypothetical RSL brine microbes. We
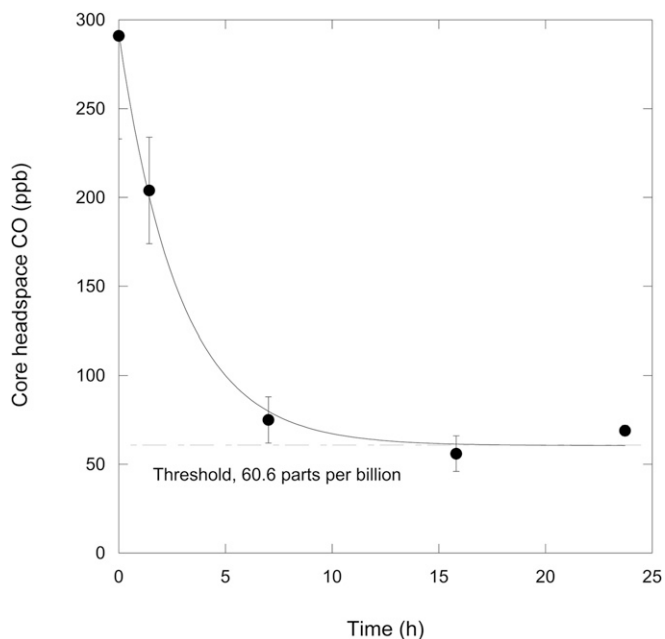
Fig. 1. Headspace CO concentrations (ppb; means ± 1 SE, $n = 3$) as a function of time for intact cores from Wendover, Utah, saline soil (July 2014); soil surface water potentials were approximately −41 MPa. Initial CO concentrations represented ambient atmospheric CO; the dashed line indicates the apparent CO uptake threshold.

observed that intact saline soil cores with surface water potentials of about −41 MPa consumed atmospheric CO, and that surface soil (0–2 cm) from this site oxidized exogenous CO, as did saline soils, sediments, and crusts from three other systems with water potentials consistent with brines that might exist on Mars (33, 34). These systems included salinized volcanic ash and sand from Hawai'i Island (water potentials from about −29 to −117 MPa), mixed gypsum and halite deposits from solar salterns in the Atacama Desert, Chile (water potentials of about −8 to −12 MPa), and salt-encrusted sediments from the Bonneville Salt Flats, Utah (BSF; water potentials of about −42 MPa).

We also observed CO uptake using a medium with 5.3 M NaCl and a water potential of −39 MPa with *Halorubrum* str. BV1 (from BSF), the first extremely halophilic euryarchaeote isolated, to our knowledge, as a CO oxidizer. In addition, we documented CO uptake by two other extremely halophilic euryarchaeotes that possess form I CO dehydrogenase genes: *Natronorubrum bangense* and *N. sulfidifaciens*. Finally, we demonstrated that a gammaproteobacterial halophile, *Alkalilimnicola ehrlichii* MLHE-1, oxidized CO at −19 MPa, at atmospheric pressures (10 mbar) similar to those on Mars, and under suboxic and anoxic conditions with nitrate present. *A. ehrlichii* MLHE-1 also tolerated perchlorate concentrations comparable to those that occur in Mars' regolith.

Results and Discussion

Atmospheric CO (about 290 parts per billion; ppb) was consumed at rates of 0.40 ± 0.16 mg CO·m^{-2}·d^{-1} by intact saline soil cores from a Wendover, Utah (WEND), site with surface water potentials (0- to 2-cm-depth interval) of −41 MPa (Fig. 1). The threshold for uptake, 61 ppb, was well below ambient CO concentrations, and more than four orders of magnitude lower than levels in Mars' atmosphere. WEND CO uptake rates were somewhat less than values reported for Maine forest soils and Hawaiian volcanic deposits (35, 36), but activity by the latter was substantially inhibited at water potentials below −0.1 to −1 MPa (37). Thus, results from WEND provide the first evidence, to our

knowledge, for active atmospheric CO uptake under conditions of extreme water limitation.

Active CO uptake at −41 MPa was also confirmed using WEND soil from the 0- to 2-cm-depth interval incubated with exogenous CO (100 ppm). Apparent maximum CO uptake rates by these soils (0.30 ± 0.04 nmol g^{-1}·h^{-1}) were similar to rates for soil from the 8- to 10-cm interval (0.24 ± 0.03 nmol g^{-1}·h^{-1}) with water potentials of −6.3 MPa. This indicated that CO-oxidizing bacterial communities in the saline surface soils were adapted to high salt and low water potential. The fact that these soils have subsequently yielded a CO-oxidizing extremely halophilic isolate provided further support for the presence of bacterial communities adapted to salt stress.

Results of similar assays for several additional sites indicated that CO oxidation at low water potentials was widespread among diverse saline systems (Fig. 2 and Table S1). CO uptake by BSF saline crusts and sediments and Hawaiian volcanic ash and cinders (Holei Sea Arch, HSA; MacKenzie State Park, MSP) occurred over a range of water potentials from about −3.6 to −117 MPa. Rates varied across this range, but with no obvious relationship to water potential (Fig. 2 and Table S1). CO was oxidized at −28.5 MPa at a saline MSP site [0.40 nmol gfw (g fresh weight)$^{-1}$·h^{-1}], whereas no activity was detected for two adjacent samples with water potentials of −39.1 to −39.7 MPa. In contrast, CO uptake was readily observed for four of five HSA sites with water potentials ranging from −33.3 to −117.4 MPa; notably, two of three HSA samples with water potentials >−10 MPa showed no uptake, whereas activity at the HSA site with the highest water potential (0.32 nmol g^{-1}·h^{-1}, −3.6 MPa) was comparable to that for the site with the lowest water potential (0.26 nmol g^{-1}·h^{-1}, −117.4 MPa). Active CO uptake was also observed for crystalline sediment from two Atacama Desert solar salterns (ASL), Salar de Llamara and Laguna Cejar, at −7.5 to −11.8 MPa (Fig. 2 and Table S1). ASL rates (0.06–0.17 nmol g^{-1}·h^{-1}) were somewhat lower than those for HSA and MSP; however, ASL CO uptake potential was stable for 9 mo without exogenous CO (Fig. 2 and Table S1), suggesting that ASL CO oxidizers tolerated both low water potential and starvation substrate regimes. Activity for
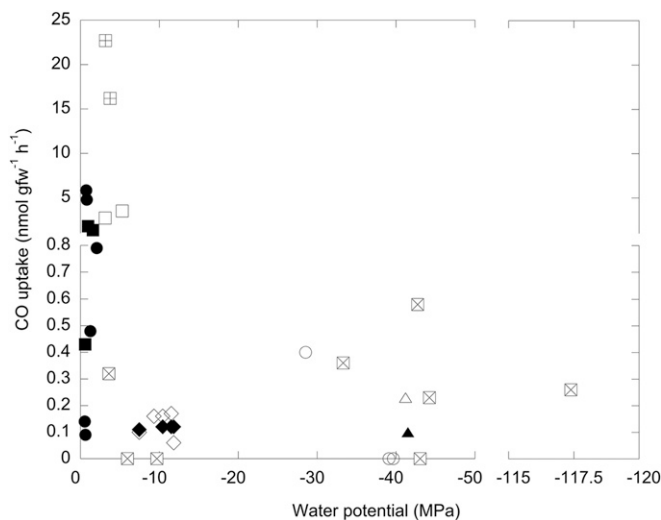
Fig. 2. CO uptake rates (nmol gfw^{-1}·h^{-1}) for individual samples versus sample water potential (MPa). ⊞, ○, and □ represent MacKenzie State Park samples from sites 1, 2, and 3 in April 2014, respectively; ● and ■ represent MacKenzie State Park samples from sites 2 and 3 in July 2014, respectively; ⊠ represents HSA samples from July 2014; ◇ and ◆ represent Atacama samples from June 2013 and April 2014, respectively; △ and ▲ represent Wendover, Utah, soil (0–2 cm) and Bonneville Salt Flats sediment, respectively.

Fig. 3. CO uptake rates [nmol (mg cell dry weight)$^{-1}$·h^{-1}; means ± 1 SE, n = 3] for *Halorubrum* sp. BV1 incubated at varied NaCl concentrations (molar, ●) and water potentials (MPa, ■).

brine-saturated BSF sediment and salt crusts was comparable to that of the ASL samples, but at water potentials of approximately −41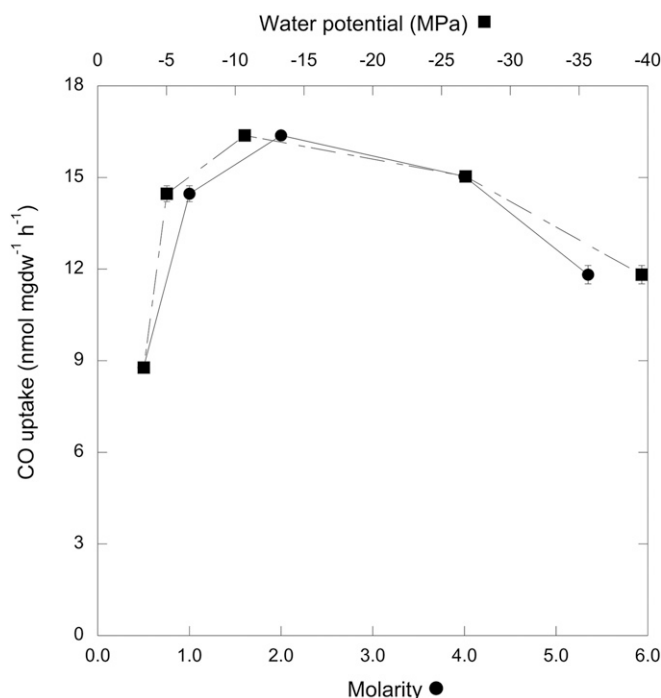 MPa (Fig. 2 and Table S1). Collectively, these results documented a previously unsuspected capacity of extreme halophiles to oxidize CO in geographically dispersed hypersaline systems with ecologically distinct characteristics (e.g., saline soils versus saltern sediments).

CO uptake by BSF samples at −41 MPa was consistent with results from the first documented extremely halophilic CO-oxidizing euryarchaeote, *Halorubrum* str. BV1, which was isolated from BSF sediment (Fig. S1). *Halorubrum* str. BV1 oxidized CO maximally at about −10.7 MPa, but activity was reduced by only 28% at −39.6 MPa (5.3 M NaCl; Fig. 3), which suggested that CO uptake was well-adapted for water stress and the high salt regimes that typify BSF. Additional assays have also revealed CO oxidation by the extremely halophilic euryarchaeotes *N. bangense* and *N. sulfidifaciens*, both isolated from Chinese salt lakes (38, 39). Collectively, these results suggested that novel extremely halophilic CO oxidizers derived from diverse terrestrial systems might prove suitable as models for metabolism and survival on Mars or elsewhere.

To assess the responses of halophilic CO oxidation to conditions relevant for Mars, washed cells of the gammaproteobacterial halophile *A. ehrlichii* MLHE-1 (40) were incubated on basalt cinders. *A. ehrlichii* MLHE-1 oxidized CO at 6 °C (Table S2), which was within the range of RSL temperatures reported by McEwen et al. (19). However, significant CO uptake likely occurs at even lower temperatures in other halophiles, because *Octadecabacter antarcticus* 307, a marine psychrophile with form I genes for CO oxidation, grows at 4 °C or lower in brines with at least twice the salinity of full-strength seawater (41). Extreme halophiles have also been isolated from and are active in subzero Antarctic and Arctic brines (42–48). In addition, atmospheric CO uptake has been documented for Maine forest soils incubated at 0 °C (35), which indicates that cold-tolerant CO-oxidizing populations might be widely distributed, and provides important insights

about the behavior of extraterrestrial systems, such as the RSL, that experience large seasonal shifts in temperature.

Although high salt concentrations and low water potential partially inhibited *A. ehrlichii* MLHE-1, no adverse effect on CO uptake was observed for 50 mM magnesium perchlorate (Table 1), a concentration that can be anticipated for regolith with volumetric water contents of 10% and total perchlorate levels by weight of 0.1%. Perchlorate has been previously reported at somewhat higher levels for two locations on Mars' surface (49–51), but higher concentrations might not prove to be a serious constraint, because Oren et al. (32) have reported tolerance of up to 0.4 M sodium perchlorate by several extreme halophiles. Indeed, in addition to tolerating perchlorate, some CO oxidizers might be able to use it as a respiratory electron acceptor. Perchlorate reduction using organic substrates has been reported for several extremely halophilic euryarchaeotes (32, 52), but *A. ehrlichii* MLHE-1 did not oxidize CO with perchlorate (Fig. S2), nor has perchlorate-linked CO oxidation been documented yet for other CO oxidizers. Nonetheless, perchlorate-coupled CO oxidation is thermodynamically favorable (ΔG° −1,042 kJ·mol^{-1}), and an analogous process, perchlorate-dependent hydrogen oxidation, has been used for perchlorate bioremediation (53). These observations suggest that Mars' regolith could support communities of novel CO-oxidizing perchlorate reducers when or if conditions are favorable for the development of near-surface brines.

CO oxidation need not depend on perchlorate, however. Molecular oxygen occurs in the Martian atmosphere at concentrations sufficient to support aerobic activity [about 1,450 ppm (25)]. When incubated with 2,000-ppm headspace oxygen concentrations in the absence of nitrate, *A. ehrlichii* MLHE-1 consumed CO nearly as rapidly (92.4%) and completely as it did in the presence of 21% oxygen (Table 1 and Fig. S3); the small difference between fully oxic and suboxic was not significantly different (ANOVA, P = 0.59, Bonferroni post hoc test).

Nitrate might also serve as an electron acceptor for CO oxidation. Although the availability of nitrate has not yet been established, evidence from Martian meteorites suggests that it could be distributed as widely as perchlorate (54). Previous studies have shown that both denitrification and dissimilatory nitrate reduction can be coupled to CO oxidation (55) and, in this study, *A. ehrlichii* MLHE-1, a nitrate respirer, oxidized CO anaerobically in the presence of 5 mM nitrate, albeit at rates significantly slower (14.3–15.4%) than under oxic or suboxic conditions (Table 1 and Fig. S3).

Table 1. CO uptake observed for *A. ehrlichii* MLHE-1 incubated with varied electron acceptors (suboxic, 0.2% O$_2$; anoxic, +5 mM nitrate) or magnesium perchlorate (50 mM) or magnesium sulfate (500 mM)

Uptake units	Treatments		
	Oxic	Suboxic	Anoxic
Rate, nmol^{-1}·mgdw^{-1}·h^{-1}	1.61 ± 0.39	1.49 ± 0.26	0.23 ± 0.03
	Control	Mg(ClO$_4$)$_2$	MgSO$_4$
Rate, nmol^{-1}·mgdw^{-1}·h^{-1}	0.49 ± 0.07	0.52 ± 0.05	0.63 ± 0.09
	Control	Vacuum control	10 mbar
Rate constant, h^{-1}	0.91 ± 0.04	1.25 ± 0.31	0.67 ± 0.16

Uptake rate constants (h^{-1} ± 1 SE, n = 3) were observed for ambient and low-pressure incubations. Rates for varied electron acceptor and magnesium salt availability [nmol (mg cell dry weight)$^{-1}$·h^{-1} ± 1 SE, n = 3] were determined for cells incubated with initial headspace CO concentrations of 8 ppm and were based on first-order uptake rate constants. Rate constants for varied pressure incubations were obtained for treatments initiated at 6 ppm CO and 1 atm pressure (controls), for treatments with 6 ppm CO and 1 atm pressure reestablished after reducing pressure to 10 mbar (vacuum controls), and for treatments with 600 ppm CO at 10 mbar pressure. mgdw, mg cell dry weight.

Although temperature and water availability might change spatially and temporally at Mars' surface, atmospheric pressure is consistently low and potentially constrains metabolic activity. Hypobaria inhibits growth of several *Bacillus* species, *Deinococcus radiodurans* R1, and *Escherichia coli* K12 (56, 57). Nonetheless, hypobaria did not affect *A. ehrlichii* MLHE-1 CO uptake over a range from 10 to 1,020 mbar, because first-order CO uptake rate constants were equivalent regardless of incubation pressure (Table 1). Low-pressure tolerances have also been observed for growth of *Carnobacterium* isolates, *Psychrobacter cryohalolentis* K5, *Serratia liquefaciens* ATCC 27592, and a *Vibrio* isolate (58). These results show collectively that bacterial responses to hypobaria vary, but they also suggest that hypobaria need not constrain atmosphere–regolith CO exchanges.

Although hypobaria might adversely affect some bacteria, it could also reduce potentially inhibitory effects of high CO_2 (Mars atmosphere contains 96% CO_2). Elevated CO_2 concentrations have long been known to inhibit bacterial food spoilage and inhibit growth of several isolates (59–64). Consistent with these observations, CO uptake by *A. ehrlichii* MLHE-1 was partially inhibited during incubation in an atmosphere with 95% CO_2/4.8% N_2/0.2% O_2 at 1,020 mbar relative to activity in an atmosphere with 99.8% N_2/0.2% O_2 (Fig. 4). However, no inhibition was observed when cells were incubated with 9.5% CO_2/89.5% N_2/1% O_2 (Fig. 4). Because previous analyses have shown that oxygen concentrations from 0.2% to 21% did not affect *A. ehrlichii* MLHE-1 CO uptake (Table 1), the lack of inhibition by 9.5% CO_2 can be attributed to the lower CO_2 concentration. This is significant, because CO_2 concentrations in a near-surface Mars RSL brine in equilibrium with a CO_2 partial pressure (pCO_2) of 0.96 at 7 mbar would be more than 10-fold lower than in similar brines equilibrated with a pCO_2 of 0.095 at 1,020 mbar. Thus, despite its high CO_2 partial pressure, the low total pressure for Mars might preclude significant CO_2 inhibition of bacterial activity. A similar conclusion has been drawn from growth analyses of two facultative anaerobes, *E. coli* ATCC 35218 and *S. liquefaciens* ATCC 27592, and an obligately anaerobic *Carnobacterium* spp. (65, 66).

Collectively, these results establish the plausibility of CO as a substrate that can contribute to the energy needs of microbes in extraterrestrial environments with conditions that permit formation of stable brines. Such conditions might exist on Mars at RSL, and on the moons Enceladus and Europa (67, 68). If extremely halophilic CO oxidizers exist on contemporary Mars, they could use multiple electron acceptors, including molecular oxygen. Any such microbes might represent relics from earlier periods in Mars' history when atmospheric CO concentrations could have been much higher than at present (69, 70). The plausibility of microbial CO oxidation on Mars also suggests that extremely halophilic CO oxidizers as well as other CO-oxidizing microbes might be exploited in efforts to manipulate Mars' atmosphere and to establish multifunctional microbial communities in the regolith or in engineered environments.

Of course, it must be noted that the persistence of extant microbial populations, if any exist, or populations transplanted in the future, depends on factors other than energy substrates. For example, the availability for biosynthesis of major and trace nutrients in the regolith (e.g., phosphorus, sulfur, molybdenum, etc.) could prove an important constraint. Results from Meslin et al. (71) indicate that Mars soil elemental compositions are comparable to terrestrial soils, including volcanic deposits known to support assemblages of CO oxidizers (36), but additional effort is certainly needed to establish the limits of regolith as a growth medium.

Materials and Methods

Sites and Sampling. Samples were collected from two sites on the southeastern coast of Hawai'i Island during April and July 2014. MacKenzie State Park sites were located in the vicinity of 19.439389 x 155.862472 and 19.355878 x 155.86222; Holei Sea Arch sites were located in the vicinity of
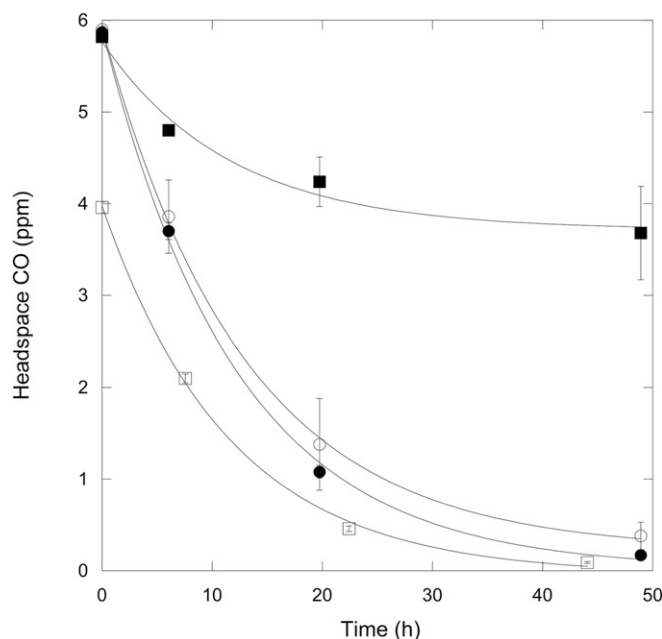

Fig. 4. Headspace CO concentrations (ppm; means ± 1 SE, $n = 3$) as a function of time for *A. ehrlichii* MLHE-1 incubated on basalt cinders with the following treatments: ○ and ● represent ambient atmosphere and 99.8% N_2/0.2% O_2, respectively; ■ represents 95% CO_2/4.8% N_2/0.2% O_2; and □ represents 9.5% CO_2/89.5% N_2/1% O_2.

19.389361 x 155.249028. All sites were near the edge of a lava escarpment at the sea margin. Sea spray from waves breaking at the escarpment base created small pools of seawater on top of the escarpment; water potentials in the pools varied from less than seawater due to rainfall input up to those of salt-saturated brines due to evaporation. Sea spray also moistened weathered basalt, cinders, and ash on top of the escarpment; water potentials for this material also varied due to rainfall and evaporation. Sediment from the bottom of small shallow pools was collected using a sterile spatula and transferred to sterile Whirl-Pak sample bags for subsequent ex situ analyses of water potential and CO uptake rates at a nearby field facility. Weathered basalt, cinder, and ash deposits were collected and analyzed similarly; these samples included depths of 2 cm or less. Samples from Laguna Cejar and Salar de Llamara, Chile were collected by B. Bebout (NASA Ames Research Center, Moffett Field, CA) and C. Kelley (University of Missouri, Columbia, MO) at approximately −23.062444 x 68.215278 and −21.266667 x 69.616667, respectively. These sites have been described previously (72). Samples of saline sediments were collected aseptically during May 2013, and then transferred to 50-mL conical disposable centrifuge tubes that were capped and shipped to Louisiana State University for water potential and initial CO uptake analysis in June 2013. Samples from the Bonneville Salt Flats were collected in July 2013 and again in July 2014. The July 2013 samples were collected aseptically from a small salt-crusted pool (40.737722 x 113.858694; BSF-1) and transferred to Whirl-Pak bags for transport to Louisiana State University for analysis. July 2014 samples were collected similarly, but were assayed within a few hours of collection at a field facility. Sites included the original July 2013 site plus an additional site (40.774583 x 113.858917; BSF-2). A third set of samples was collected from a saline soil (WEND) supporting sparse vegetation south of the Bonneville Salt Flats (40.515472 x 114.044917). Saline soil was collected from the 0- to 2-cm interval and transferred to Whirl-Pak and Ziplock food storage bags. A set of intact triplicate cores was also collected at WEND using 7.5-cm (inner diameter) × 30-cm-long aluminum tubes; the tubes were sealed and transported with minimal disturbance to a nearby field facility for analysis of atmospheric CO uptake.

CO Uptake Assays. Subsamples (5–10 gfw) from the upper two 2 cm of BSF-1, BSF-2, HSA, and MSP and from the 0- to 2-cm and 8- to 10-cm intervals of WEND were transferred to either 135-cm^3 serum bottles or 110-cm^3 glass jars (HSA and MSP) that were sealed with neoprene rubber stoppers; samples from ASL were processed similarly, except that 75-cm^3 serum bottles were used. CO was added to all bottles at a final concentration of about 100 ppm, and headspaces were sampled at intervals for assays using a Peak Laboratories

reduced gas detector operated according to the manufacturer's instructions. CO was separated using a 1-m stainless steel column (3.2-mm outer diameter × 1.8-mm inner diameter) packed with Molecular Sieve 5A (Sigma-Aldrich) and operated at 122 °C with a carrier gas of air at a flow of about 35 $cm^3 \cdot min^{-1}$. All samples were incubated at ambient temperature (about 20–23 °C). For atmospheric uptake assays, the upper ends of WEND core tubes were sealed with plastic caps containing a septum, thus entraining ambient air (36). Atmospheric CO uptake was monitored by sampling at intervals, and assaying CO was as described above.

Water Potential. Water potential was measured using a Decagon Instruments WP4T dew point hygrometer standardized with −2.2 MPa (0.5 M KCl) and −190 MPa (0.25 a_w LiCl; aw = water activity) standards obtained from the manufacturer. Subsamples from all sites were transferred to disposable sample cups and analyzed within several hours of collection following the manufacturer's protocols as described by Weber and King (37).

Isolate Assays. The responses of CO uptake by *Halorubrum* strain BSF-1 to varied salt concentrations and water potentials were determined by growing the isolate in CM1 medium to an absorbance of 0.8 at 600 nm. Five equal volumes of medium were centrifuged at 10,000 × g for 10 min at 4 °C to harvest cells. Cell pellets were resuspended in one of five modified versions of the CM1 medium containing 0.5, 1.0, 2.0, 4.0, or 5.3 M NaCl and 2.5 mM sodium pyruvate as a maintenance substrate. Cells were centrifuged a second time (10,000 × g, 10 min), and then resuspended in the appropriate modified CM1 medium. Triplicate aliquots for each sodium chloride treatment were incubated in 160-cm^3 serum bottles at 40 °C with shaking at 100 rpm and initial headspace CO concentrations of 100 ppm. CO uptake was assayed as described above for other samples.

Several members of the genus *Natronorubrum* were obtained from either the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) or the Japan Collection of Microorganisms (JCM): *N. aibiense* (JCM-13488), *N. bangense* (JCM-10635), *N. sulfidifaciens* (JCM-14089), *N. texcoconense* (JCM-17497), and *N. tibetense* (DSM-13204). Isolates were grown in DSMZ Medium 371. Cells were harvested by centrifugation as above, and then resuspended in 5 mL Medium 371 with 0.05% yeast extract and no added carbon source; cells were incubated with 100 ppm CO in sealed 60-cm^3 serum bottles with uptake assays conducted as described previously. Genome sequences for *N. bangense*, *N. sulfidifaciens*, and *N. tibetense* (available from Integrated Microbial Genomes) contained the canonical form I genes for CO dehydrogenase (27); however, CO uptake by these isolates has not been previously reported.

Assays with the extremely halophilic proteobacterium *A. ehrlichii* MLHE-1 were conducting using a strain obtained from the DSMZ (DSM-17681). *A. ehrlichii* MLHE-1 was grown in Artificial Mono Lake medium [AML; (73)] supplemented with 25 mM pyruvate as a carbon and energy source and 0.05% yeast extract (YE). NaCl concentrations were varied from 0.5 to 3.1 M. For all assays, cells in AML were grown to an absorbance of about 1.4 at 600 nm, and then harvested by centrifugation (10 min at 4 °C and 10,000 × g) and

resuspended in AML with 0.05% YE. One-milliliter volumes of resuspended cells were pipetted onto 10-g dry weight masses of washed, organic-free (fired at 550 °C, 3-h minimum) basalt cinders in 110-cm^3 glass jars, the contents of which were gently but thoroughly mixed to homogenize the cells and cinders. After sealing with neoprene rubber stoppers, CO was added to jar headspaces for uptake assays as described above.

The effect of oxygen availability on CO uptake was assessed using triplicate sets of neoprene-stoppered 110-cm^3 jars with headspaces at ambient pressure and temperature and headspace compositions of ambient air, 99.8% N_2 plus 0.2% O_2, or 100% oxygen-free nitrogen. Anoxic treatments included incubations with or without 5 mM sodium nitrate. Nitrate supports dissimilatory nitrate reduction by *A. ehrlichii* MLHE-1 (40). The effect of perchlorate was assessed at ambient temperature using ambient air headspaces and treatments (in triplicate) with no added perchlorate or 50 or 500 mM magnesium perchlorate; *A. ehrlichii* MLHE-1 was also incubated with perchlorate anaerobically, and in anoxic treatments with no added electron acceptors.

The effect of subambient pressure on CO uptake was determined by incubating at ambient temperature four triplicate sets of neoprene-stoppered 110-cm^3 jars with ambient air headspace pressures of 10 mbar and initial CO concentrations of about 600 ppm. Sealed jars were incubated in an aluminum vacuum chamber maintained at 10 mbar. At intervals, the chamber was returned to ambient pressure, and one set of triplicates was removed for CO assays as described above after raising the jar headspaces to ambient pressure using CO-free air. The vacuum chamber was resealed and the remaining jars were incubated at 10 mbar for up to 34 h. Results were compared with two treatments. One set of triplicate jars was incubated with ambient pressure and air with 6 ppm CO; the headspace pressures of a second set of triplicate jars were reduced to 10 mbar and then returned to ambient, at which point a final concentration of 6 ppm CO was added.

The effects of CO_2 on CO uptake were determined with two separate assays. One used triplicate 110-cm^3 jars with *A. ehrlichii* MLHE-1 incubated at ambient temperature on basalt cinders as described above for each of three headspace treatments: ambient air, 95% CO_2/4.8% N_2/0.2% O_2, or 99.8% N_2/0.2% O_2. The second assay was conducted similarly, but with the exception that CO uptake from an ambient air atmosphere was compared with uptake from an atmosphere of 9.5% CO_2/89.5% N_2/1% O_2. CO was added at final concentrations of 4–6 ppm for both assays, and initial CO uptake rates were estimated using a nonlinear curve-fitting algorithm (35).

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MICROBIOLOGY

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