

## 5. PERIDOTITE DISSOLUTION RATES IN MICROBIAL ENRICHMENT CULTURES<sup>1</sup>

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### ABSTRACT

Using peridotite drilled during Ocean Drilling Program Leg 209, a series of enrichment cultures were initiated on board the ship to stimulate microbially enhanced dissolution of olivine. Dissolution was estimated by measured changes in dissolved Li and Si in the media through time (up to 709 days). The results suggest that there was no significant difference between the amounts of dissolved Li and Si in most of the inoculated microbial cultures compared to the control cultures. Alternative explanations for this are that

1. No microbes are living in the culture tubes that can affect the dissolution rates of olivine,
2. The control cultures have microbes effecting the dissolution of olivine as well as the inoculated cultures,
3. Not enough time has passed to build up a large enough microbial population to effect the dissolution of the olivine in the culture tubes,
4. Microbes act to suppress dissolution of olivine instead of enhancing dissolution, and
5. Abiotic dissolution overshadows microbially enhanced dissolution.

Further work is required to test these alternatives.

<sup>1</sup>Josef, J.A., Fisk, M.R., and Giovannoni, S., 2007. Peridotite dissolution rates in microbial enrichment cultures. In Kelemen, P.B., Kikawa, E., and Miller, D.J. (Eds.), *Proc. ODP, Sci. Results, 209*: College Station, TX (Ocean Drilling Program), 1–38. doi:10.2973/odp.proc.sr.209.002.2007

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## INTRODUCTION

Increased interest in microbial interactions with marine basalts began more than 10 yr ago when research revealed that microorganisms can have an effect on the weathering of Fe silicate rocks and minerals such as basalt glass (Thorseth et al., 1992, 1995a, 1995b; Giovannoni et al., 1996; Furnes et al., 1996; Fisk et al., 1998; Bennett et al., 2001). It was suggested that the chemical energy required for chemolithoautotrophs could be provided by both direct interactions of microbes with olivine and microbial utilization of methane and hydrogen, produced when olivine reacts with water (Neal and Stanger, 1983; Stevens et al., 1993; Stevens and McKinley, 1995; Stevens, 1997; Fisk and Giovannoni, 1999; McKinley et al., 2000; Freund et al., 2002). These authors further suggested that the seafloor biosphere on Earth might be an ideal analog to subsurface ecosystems that could exist on other solar bodies.

Over the past 5 yr, the study of olivine dissolution via microbial activity has focused on two different metabolic pathways. Santelli et al. (2001) and Welch and Banfield (2002) used iron-oxidizing bacteria (*Acidithiobacillus ferrooxidans*) in low-pH cultures to examine changes in both the olivine surface morphology and the chemistry in the culture media. Longazo et al. (2001, 2002) placed unidentified bacillus bacteria from the Columbia River aquifer into cultures with olivine but with no added Fe or Mg to show that environmental microbes can create weathering features on olivine. Garcia et al. (2004) used *Escherichia coli* at 37°C in culture with olivine grains to trace changes in the concentration of Fe in the systems.

Research into olivine end-member (fayalite and forsterite) dissolution both with and without microorganisms have created multiple dissolution mechanisms typically based on the pH of the system. Santelli et al. (2001) conducted both biotic and abiotic fayalite (Fe-rich end-member of olivine) dissolution reactions at pH = 2–4 and found that at these pHs Fe<sup>3+</sup> inhibited fayalite dissolution. Welch and Banfield (2002) also conducted biotic and abiotic fayalite dissolution reactions at pH = 2 and also found that the dissolution of fayalite is significantly inhibited by ferric iron. Welch and Banfield hypothesized that the removal of Fe from M1 sites and oxidation of Fe<sup>3+</sup> or adsorption of Fe<sup>2+</sup> into M2 sites create a laihunite-like surface layer that produces the pits and rough texture seen in weathered fayalite and inhibits further dissolution.

Around pH = 9, the mechanism of forsterite (Mg-rich end-member of olivine) dissolution changes from a system controlled by the production of a Si-rich surface layer through Mg-H exchange (at pH < 9) to one with a Mg-rich surface layer because of the preferential release of Si (at pH > 9) (Pokrovsky and Schott, 2000a, 2000b). At pH < 9, the dissolution rate of forsterite decreased with increasing pH, but at pH > 9, the dissolution rate remained constant with increasing pH. In alkaline conditions, the dissolution processes may lead to the formation of a brucitelike or Mg-bearing sheet silicate layer. However, once both systems reached equilibrium, the molar ratio of Mg:Si released into solution was the same (~2); at pH = 3, equilibrium was reached within 100 min, and at pH = 11, equilibrium was attained after 150 hr (Pokrovsky and Schott, 2000b). Oelkers (2001) found that at pH = 2 forsterite dissolution rates are independent of aqueous Mg and Si concentrations and indicate that dissolution occurs in a two-step process. First, the surface of the forsterite is protonated, forming rate-controlling precursor com-

plexes. Second, the Mg–O octahedral chain linking bonds break, which liberates both Mg and Si from the forsterite structure. Pokrovsky and Schott (2000b) showed that at pH > 9 the dissolution rates of forsterite decrease as a function of increasing dissolved Si or CO<sub>2</sub> concentrations. However, Mg concentrations had no effect on the dissolution rate. For olivine, the initial step in dissolution is the formation of a leached layer (either M1 sites in fayalite, or the removal of Mg or Si in forsterite), which can either inhibit or enable further dissolution.

The focus of this research is to quantify the rates of dissolution that can be achieved by a consortium of environmental bacteria. This work builds on the culturing work started by M.M. Moeseneder et al. (unpubl. data) in 2002 with basalt glass and olivine cultures, which resulted in enhanced dissolution of both basalt glass and olivine in the presence of a basalt glass inoculum. M.M. Moeseneder et al. (unpubl. data) collected samples of pillow lavas from seamounts in the Cobb-Eickelburg chain to inoculate cultures with either sterile basalt glass or sterile olivine. The cultures and controls were incubated at 4° and 15°C for 3 yr. Periodically, the media within the cultures were analyzed for changes in dissolved Si and Li. The inoculated cultures showed enhanced concentration of dissolved Li and Si through time over the uninoculated controls (M.M. Moeseneder et al., unpubl. data). The idea behind this research was to estimate microbially driven dissolution rates of peridotite in the seafloor using a subsurface peridotite as our source of inoculating microbes. We wanted to have an environmental source of microbes for our cultures so that our estimates of microbially driven dissolution would reflect in situ rates. Our experimental design did not test for the presence of microorganisms in the enrichments. Instead, we assayed the enrichment cultures and controls for soluble products of olivine dissolution.

## **MATERIALS AND METHODS**

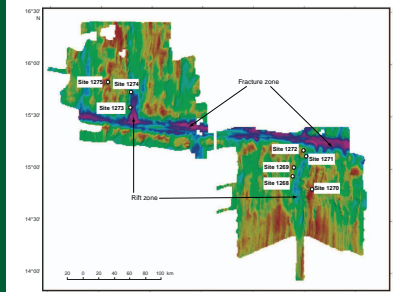
### **Sample Collection**

Samples of subseafloor peridotite were collected during Ocean Drilling Program (ODP) Leg 209. Transects were cored along two segments of the Mid-Atlantic Ridge bounding the 15°20′ Fracture Zone (Fig. F1). Whole-round cores of drilled peridotite from 14 to 146 meters below seafloor (mbsf) from six drill holes were collected to set up cultures on board the ship (Table T1).

Ten 10- to 15-cm-long whole-round core samples (Table T1) were taken for microbiological studies directly from the cutting room immediately after the cores were brought on deck; eight of these samples were hard rock and two were composed of clay-sized grains. Once on deck, each sample was rinsed with nanopure water and the water was collected and examined for the presence or absence of fluorescent microspheres, which were used as a tracer of drilling contamination. The selected core was then flame sterilized. Each of the samples was transported in a sterile nitrogen-filled container and immediately placed in the nitrogen-filled anaerobic chamber in the microbiology laboratory. Subsamples of the whole-round samples were preserved for analysis by electron microscope, electron microprobe, and organic carbon and deoxyribonucleic acid (DNA) extraction as well as culture experiments.

The cultures from samples from Sections 209-1274A-8R-16 and 15R-7 were selected for initial detailed analysis because they are from the

**F1.** Map of Mid-Atlantic Ridge, p. 15.



**T1.** Leg 209 samples used for peridotite culture experiments, p. 24.

some location, which allows them to have a similar volcanic and hydrothermal history while having two different concentrations of olivine within them (20% and 0%). The differing amounts of fresh olivine may have provided two different microbial populations for the cultures, which in turn could lead to different results.

### Rock Descriptions

Section 209-1274A-8R-2 (Piece 16) was part of the harzburgite lithologic unit. The microbiology sample was part of the core that was moderately altered spinel-rich dunite that had little orthopyroxene. The core's primary mineralogy consisted of olivine (83%–98%), orthopyroxene (0%–15%), clinopyroxene (<0.5%), and spinel (1%–1.5%) (Kelemen, Kikawa, Miller, et al., 2004). The secondary mineralogy showed that the core was composed of highly serpentine altered harzburgite and dunite. The olivine was altered to serpentine with mesh textures with some fresh olivine remaining in the cores, as much as 10% in the orthopyroxene-rich areas (Kelemen, Kikawa, Miller, et al., 2004). Figure F2 shows the mineralogy of the core as seen in a photomicrograph.

Section 209-1274A-15R-2 (Piece 7) was also part of the harzburgite lithologic unit. The microbiology sample was taken from a section of highly altered harzburgite with varied amounts of interstitial orthopyroxene (12%–15%) and small amounts of spinel. The primary mineralogy of this core was olivine (84%–89%), orthopyroxene (10%–15%), clinopyroxene (<1%), and spinel (<1%) (Kelemen, Kikawa, Miller, et al., 2004). The secondary mineralogy reveals that the core was composed completely of serpentine altered harzburgite and dunite. Additionally, there was subsequent overprinting of reddish brown iron oxyhydroxides and carbonates. Fresh olivine is very rare in this lithologic unit. Figure F3 shows a photomicrograph from this lithologic unit.

### Culture Experiment Setup

These experiments were designed to test the microbial utilization of seven different electron donors (Table T2) in conjunction with sterile olivine as a source of either oxidants or reductants. The seven different electron donors were chosen based on their theoretical activation energy ( $E_0'$ ) values. Culture experiments were set up with all eight hard rock samples. Once each sample was in the anaerobic chamber, it was divided into three subsamples. One subsample was frozen at  $-80^{\circ}\text{C}$  for DNA extraction directly from the drilled sample. The second subsample was preserved for total organic carbon analysis, and the third subsample was crushed and placed into the culture tubes. The exterior of the core was mechanically removed from each whole-round to eliminate the region of highest potential contamination. Once the exterior was removed, the samples were crushed using a sterile mortar and pestle. Once crushed, 1 g of sample was added into each culture tube. Throughout the process the peridotite samples were kept cool with ice (from below) and  $4^{\circ}\text{C}$  sterile artificial seawater (in the mortar and pestle).

The enriched media solution consisted of artificial seawater and one of seven electron donors (Table T2). The artificial seawater was produced based on ZoBell's recipe (ZoBell, 1946) (Table AT1; see "Appendix A," p. 13). Once 20 L of artificial seawater was made, it was sparged with  $\text{CO}_2$  for 12 hr. At this point, the entire volume was divided into twenty 1-L containers. One of the seven electron donors or acceptors

F2. Sample 209-1274A-8R-2, 101–103 cm, p. 16.



F3. Sample 1274A-15R-1, 102–105 cm, p. 17.



T2. Electron donors used in the culture experiments, p. 25.

was added to two 1-L containers of artificial seawater (Tables T2, AT2; see “Appendix A,” p. 13). Each 1-L container was sparged with N<sub>2</sub> gas for 5 hr to drive off any oxygen that accumulated during transport. Next, the enriched media were placed in the anaerobic chamber (N<sub>2</sub>). Once sealed in the chamber, the pH was adjusted to 7.5 or 9.5.

The olivine that was used as a mineral source of reduced Fe was obtained from Nickel Mountain, an uplifted peridotite in Roseburg Oregon (Table AT3; see “Appendix A,” p. 13). The Nickel Mountain peridotite was crushed by hand and then in a jaw crusher. The crushed samples were sieved and the 0.250- to 0.150-mm grains were collected. The olivine grains were rinsed three times with milliQ water and allowed to air dry. Approximately 4 g of olivine was added to each culture tube and sealed with aluminum foil. The tubes were packed together into packages of 56. Each package was heated at 210°C for 12 hr to kill any organisms living on or in the olivine. Once on board the ship, the packages of culture tubes were placed within the anaerobic chamber. One package of 56 preprepared tubes was used for each sample.

Each culture tube (Balch glass tube) consisted of 4 g of sterile olivine, 1 g of the drilled peridotite (inoculate), and 20 mL of an enriched media solution. Each tube was assembled within the anaerobic chamber and sealed with N<sub>2</sub> gas in the headspace (referred to as inoculated cultures throughout the paper). Duplicates of each tube were designed to culture the microbes at both 4°C and 15°C. One set of controls was set up with each sample. The control tubes were identical to the culture tubes described above, but once sealed, 0.25 mL of HgCl (saturated solution) was added to each control tube to sterilize the sample (referred to as the control cultures throughout the paper). This control allowed us to examine the abiotic chemical changes resulting from the peridotite inoculate and the sterile olivine reacting with the enriched media.

### BET Analysis

Specific surface area (SSA) of the sterile olivine grains from two random cultures was measured with BET analysis at Oregon State University by Mark Nielsen (Gregg and Sing, 1982). The sieved olivine within the enrichment cultures began with an average of 0.75 m<sup>2</sup>/g surface area.

### Contamination Testing

Testing was performed on board the ship to examine the extent of microbial contamination into the cores as a result of the drilling process. The techniques used here are based on the work done by Smith et al. (2000).

### Analysis of Media

The tubes were gently mixed to bring the crushed olivine into suspension, and a subsample (400 µL) was taken with a sterile syringe. The 400-µL samples were centrifuged at 8000 revolutions per minute for 10 min, and the supernatant was transferred to a new tube and fixed with nitric acid (final concentration = 1%) for inductively coupled plasma-mass spectrometer (ICP-MS) measurements and the pellet frozen at -80°C for DNA extraction.

A 50- $\mu$ L aliquot of the fixed ICP-MS samples was diluted 102 $\times$  with 1% nitric acid, and 1 part per billion (ppb) beryllium was added as an internal standard. The samples were run on the Oregon State University Keck Colaboratory's Axiom SC (VG Elemental, Thermo Elemental Corporation) equipped with an autosampler (Gilson). Olivine dissolution was measured by following the amounts of dissolved lithium (Li) and silicon (Si) in the particle-free solution over  $\sim$ 2 yr. Subsamples of liquid media were taken on days 12, 36, 237, and 709 for Section 209-1274A-8R-2 (42 mbsf) and on days 10, 40, and 204 for Section 209-1274A-15R-2 (76 mbsf). The instrument was calibrated with lithium and silicon standards before each run, with a detection limit of 0.1 parts per trillion (ppt) Li and 1 ppt Si.

### Calculation of Total Amount

Each amount of dissolved Li or Si in Tables AT4 and AT5 (see "Appendix A," p. 13) are the average of two instrument measurements. The total counts were converted into concentrations using the Axiom SC-generated standard curve created on each day of analysis. To calculate the total amount of dissolved Li and Si in each culture, the ICP-MS measured concentration was multiplied by 102 to find the concentration of dissolved Li and Si in the 50  $\mu$ L of media analyzed (diluted with 5 mL of nitric acid and 1 ppb Be internal standard). The concentration was multiplied by 20 to calculate the total amount of dissolved Li and Si within the tube (20 mL). Then parts per billion and parts per million were converted into nanograms and micrograms, respectively.

## RESULTS

### Contamination Testing

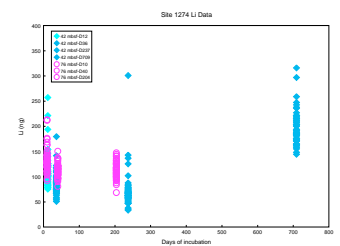
Table T3 lays out the samples tested for contamination and the results. For statistical purposes no microspheres reached the core exterior of Section 209-1274A-27R-1; however, when the entire filter was examined, one microsphere was found, indicating that the microspheres did reach the core but at a very low concentration.

### Analysis of Media

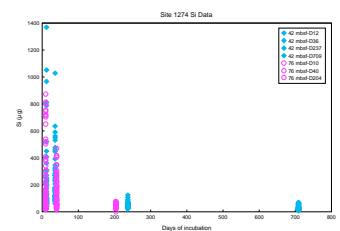
The amounts of both dissolved Li and Si in the culture media show similar trends for both sample sets for the first three time points (Figs. F4, F5). Both sets of cultures from Hole 1274A have similar dissolved Li and Si amounts and show a similar range in values at each time point. The amount of dissolved Li or Si does not break into separate groups for the inoculated vs. control cultures, nor is there an apparent difference in dissolved Li or Si based on the media pH or incubation temperature. Over the first two time points in both culture sets the amounts of dissolved Li maintain similar averages and ranges (Fig. F4), then the average dissolved Li drops by the third time point ( $\sim$ 200 days of incubation) and the range increases (Fig. F4). At 709 days in the cultures inoculated with the 42 mbsf peridotite, the average amount of dissolved Li increased above the initial amount. The general trend for the dissolved Si in both sets of cultures is one of decreasing amounts through time (Fig. F5; Table T4). Both sets have similar averages and ranges of dissolved Si

T3. Microsphere tracer results, p. 26.

F4. Site 1274 Li data, p. 18.



F5. Site 1274 Si data, p. 19.



T4. Hole 1274A average dissolved Si, p. 27.

at all three time points; at 709 days, the 42-mbsf cultures continue the trend of decreasing dissolved Si (Fig. F5).

It is noteworthy that the average amount of dissolved Li in the inoculated 42-mbsf cultures at the initial time point is always lower than the average of the control cultures (Table T5). Additionally, the average amounts of dissolved Li measured at day 709 were lower in the inoculated cultures than the control culture, except for the dimethyl sulfoxide (DMSO) cultures (Table T5; Fig. F6C). This contrasts with the 76-mbsf cultures, where the DMSO, fumarate, and MnO<sub>2</sub> enrichment media had higher average amounts of dissolved Li in the inoculated cultures relative to the controls, which remained higher at day 204 (Table T5; Fig. F6C, F6E, F6M).

### Enrichment Media Results

Four generalities about the dissolved Li in the cultures can be made:

1. The average dissolved Li decreased over the first ~250 days then increased by day 709.
2. The final average amount of Li at 709 days was 200 ng with two exceptions.
3. The lowest amount of dissolved Li in Hole 1274A 42-mbsf cultures (inoculated and control) was 50 ng on average; yet, the lowest amount of dissolved Li in Hole 1274A 76-mbsf cultures (inoculated and control) was 100 ng on average.
4. None of the cultures decreased to 0 ng dissolved Li.

The dissolved Si results also show three trends across all the cultures:

1. The average dissolved Si decreased through the entire length of incubation for both sample sets.
2. Most of the culture set started with an average amount of dissolved Si of 200 µg or more.
3. While the final amount of dissolved Si averaged 50 µg, a number of culture sets decreased to 0 µg.

Finally, the largest decrease in dissolved Si through time was 550 µg over 204 days (Hole 1274A 76 mbsf, MnO<sub>2</sub> inoculated).

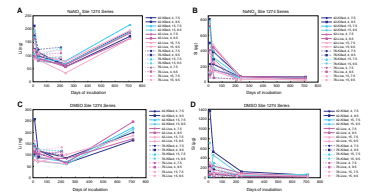
## DISCUSSION

The lack of significant differences in the averages of dissolved Li and Si in the inoculated and control cultures suggests that the influence of the inocula on the rates of olivine dissolution was insignificant during the course of the experiment or perhaps that the cultures did not grow at all. At 709 days only two experiments, 1274-18, MnO<sub>2</sub> pH 7.5, 4°C (control), and 1274-15, fumarate pH 7.5 15°C (inoculated culture), had a noticeable increase in Li over the other enrichment cultures at this time point.

Although previous work showed that biotic cultures can actually inhibit dissolution (Santelli et al., 2001; Welch and Banfield, 2002), the authors' hypothesis was that increased dissolution would occur within the inoculated cultures. This hypothesis is based on previous work showing a significant increase in biologically mediated mineral dissolution (M.M. Moeseneder et al., unpubl. data) for experiments with basalt

T5. Hole 1274A average dissolved Li, p. 28.

F6. Site 1274 changes in Li and Si concentrations, p. 20.



glasses and olivine. The rationale for the somewhat contrary hypothesis is also based on the fact that, with the exception of the study by M.M. Moeseneder et al. (unpubl. data), previous studies were conducted over periods ranging from 24 hr (Longazo et al., 2001) to 25 days (Santelli et al., 2001). We expected that over a longer time period the microbes would eventually enhance dissolution. By 709 days the Hole 1274A 42-mbsf cultures' dissolved Li suggests that something has started to release Li into solution; however, since there is not a corresponding increase in Si, stoichiometric olivine dissolution may not be occurring.

At least five hypotheses can be formulated to explain experiments performed here:

1. The first, and most straightforward, hypothesis is that there are no microbes that interact with olivine growing within the cultures because there is no significant difference between the amounts of dissolved Li and Si in the inoculated and control cultures sets. One scenario is that there were no olivine-active microbes within the inoculating peridotites to begin with; therefore, the necessary forms of microbial life were not introduced at the initiation of the experiment. Alternatively, olivine-active cells were introduced into the cultures but the physical-chemical conditions were not sufficient to support microbial growth.
2. A second hypothesis is that the mercuric chloride did not kill the microbes; therefore, both the inoculated and control cultures have live microbes in them.
3. The third possible, and perhaps most likely, hypothesis is dependent on the doubling time of these microbes. If these microbes are growing very slowly because of low nutrients or low temperatures, our cultures may not have a long doubling phase. D'Hondt et al. (2002) suggested that most subsurface organisms are in a dormant state. Without an active doubling, 709 days may not be enough time for the microbes to build up a metabolically active population that can significantly enhance the dissolution of peridotite.
4. A fourth hypothesis is that, while it has been shown that some microbes enhance dissolution of basalt glass over the rate of abiotic dissolution (M.M. Moeseneder et al., unpubl. data), microbes cannot use olivine for similar metabolism (Welch and Banfield 2002; Santelli et al., 2001; Garcia et al., 2004).
5. A final hypothesis is that the microbes may be causing the peridotite to dissolve, but the Si and Li released into solution was taken up into secondary minerals within the cultures. There is a small amount of flocculated material within the cultures, which may have adsorbed any dissolving Si or Li. However, since both the inoculated and control cultures seem to reach such similar amounts of dissolved Li and Si through time, it is difficult to find evidence that one set of cultures has significantly more Li and Si being released into the solution.

To assist in the evaluation of these five hypothesis, we estimated how much Li should be dissolved in the media at the same time points we analyzed based on abiotic olivine dissolution rates calculated by Pokrovsky and Schott (2000b), the composition of the Nickel Mountain olivine in the enrichment cultures (Pecora and Hobbs, 1942), and the BET-measured SSA in the enrichment cultures. The estimates found in Tables T6

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T6. Hole 1274A at 42 mbsf concentration of dissolved Li and Si, p. 29.



and T7 are maximum values that were calculated with the forsterite dissolution rates measured by Pokrovsky and Schott (2000b). Their dissolution reactions were established with a reaction temperature of 25°C; therefore, the dissolved Li amounts calculated are maximum estimates. Dissolution rates of forsterite decrease with temperature at a constant pH (Oelkers, 2001). Details of these calculations can be found in "Appendix B," p. 14. The rate used for both Li and Si dissolution from olivine are  $1.75 \times 10^{-14}$  mol/cm<sup>2</sup>/s for pH 7.5 and  $3.5 \times 10^{-15}$  mol/cm<sup>2</sup>/s for pH 9.5 (Pokrovsky and Schott, 2000b).

There is a clear difference between the measured concentrations of Li and Si and the estimated amount of dissolved Li and Si. While the estimated concentration of dissolved Li at the first time point is similar to the actual concentration of dissolved Li by days 36 and 40, the measured concentrations of dissolved Li are much lower than the estimated values. However, the measured concentration of dissolved Si and the estimated concentration of dissolved Si are very different from the initial time point and continue to diverge at all later time points. The data support the hypothesis that the Li and Si are being taken up into secondary minerals within the enrichment cultures instead of remaining in solution. The idea of secondary mineral formation is supported by research that shows aqueous Si is commonly formed in marine sediment experiments at the low temperatures of the enrichment cultures (Von Damm, 1995). The discrepancy between the measured and the estimated Li and Si concentrations is likely because of the formation of aqueous Si and uptake of Li into it or other secondary minerals within the culture tubes.

Although there are five hypotheses presented above, only two are likely possibilities. It is likely that the enrichment culture conditions were insufficient to promote growth. Then the most plausible hypothesis is that not enough time has passed for the microbes within the enrichment cultures to double into a large enough population to influence the dissolution of olivine. This hypothesis can encompass the hypothesis that the Li and Si are taken up by secondary minerals. If the microbial population is not large enough to influence the dissolution of olivine, then naturally abiotic dissolution reactions and secondary mineral formation dominate the cultures. It is reasonable to propose that the microbes in culture may be barophiles, in which case the culture conditions are not adequate for growth because the enrichment cultures are at atmospheric pressure. The other likely hypothesis is that there were no olivine-active microbes in the inocula. If this is the case then abiotic reactions will dominate the cultures as well. At this point the data do not favor one hypothesis over another. In time, additional sampling of the cultures may reveal biotic influences upon the olivine dissolution reactions to support the hypothesis that more time is needed.

## CONCLUSIONS

The goal of this research was to quantify long-term microbially mediated dissolution of olivine in culture in comparison to abiotic dissolution. With the use of dissolved Li and Si, the extent of microbially driven dissolution in the seafloor may have been estimated. Despite other success with using an environmental source of microbes to examine biotic dissolution of olivine (Santelli et al., 2001; Welch and Banfield, 2002; Longazo et al., 2001, 2002; M.M. Moeseneder et al., unpubl.

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T7. Hole 1274A at 76 mbsf concentration of dissolved Li and Si, p. 30.

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data), our cultures with drilled seafloor peridotite show little difference in the amounts of dissolved Li and Si between our inoculated and control cultures. There are a number of possible explanations for this observation. Currently there is no evidence to support or reject the presence of microbes within our cultures. In time it may be possible to answer this question with DNA extraction. The cultures are being maintained at their incubation temperatures and are available for further study.

### **ACKNOWLEDGMENTS**

This research used samples and/or data provided by the Ocean Drilling Program (ODP). ODP is sponsored by the U.S. National Science Foundation (NSF) and participating countries under management of Joint Oceanographic Institutions (JOI), Inc. Funding for this research was provided by NSF ODP and NSF IGERT (Integrated Graduate Education Research and Traineeship). Thank you to my committee at Oregon State University, to the ODP staff for finding my supplies and getting them on board the ship, Mark Nielsen for the BET analyses, and Andy Unger for his assistance with the ICP-MS.

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## APPENDIX A

The artificial seawater recipe for 1 L of sterile H<sub>2</sub>O is in Table AT1 (Zobell, 1946). The electron donors and acceptors added to the 1-L artificial seawater solutions to make culture medias are in Table AT2. The composition of Riddle Nickel Mountain peridotite used in the enrichment cultures and the SSA measurements are in Table AT3.

The amount of dissolved Li in media through time for the two culture sets measured and used to calculate the averages in Table T5 are in Table AT4 (plotted in Figs. F4, F6A, F6C, F6E, F6G, F6I, F6K, F6M).

The amount of dissolved Si in media through time for the two culture sets measured and used to calculate the averages in Table T4 are in Table AT5 (plotted in Figs. F5, F6B, F6D, F6F, F6H, F6J, F6L, F6N).

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AT1. Artificial seawater recipe, p. 32.

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AT2. Electron donors and acceptors, p. 33.

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AT3. Composition of peridotite and SSA measurements, p. 34.

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AT4. Dissolved Li for two culture sets, p. 35.

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AT5. Dissolved Si for two culture sets, p. 36.

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## APPENDIX B

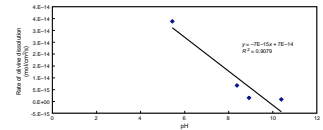
### Estimating the Abiotic Dissolution of Olivine

Based on the equation trend line from Pokrovsky and Schott's (2000b) data of olivine dissolution rates at 25°C and changing pH (Fig. AF1), an approximate dissolution rate of olivine was extrapolated at 25°C and pH 7.5 and 9.5 (Table AT6).

The moles of olivine that should have dissolved at each of the time points when ICP-MS analysis was used (Tables T6, T7) was calculated with the rates from Table AT6, the BET analysis SSA (Table AT3; see "Appendix A," p. 13.), and the time converted to seconds. A step-by-step layout of how the values shown in Table T6 were calculated is in Table AT7. Table T7 was calculated the exact same way but over slightly different lengths of time.

Based on Oelkers (2001) data of changing temperature with a constant pH of 2.0, a trend line was created to extrapolate dissolution rates for 4°C and 15°C. However, the data yield negative dissolution rates for both temperatures. Because of this, the lower temperature dissolution rates were not used to calculate an estimate of abiotic olivine dissolution within the enrichment cultures and controls.

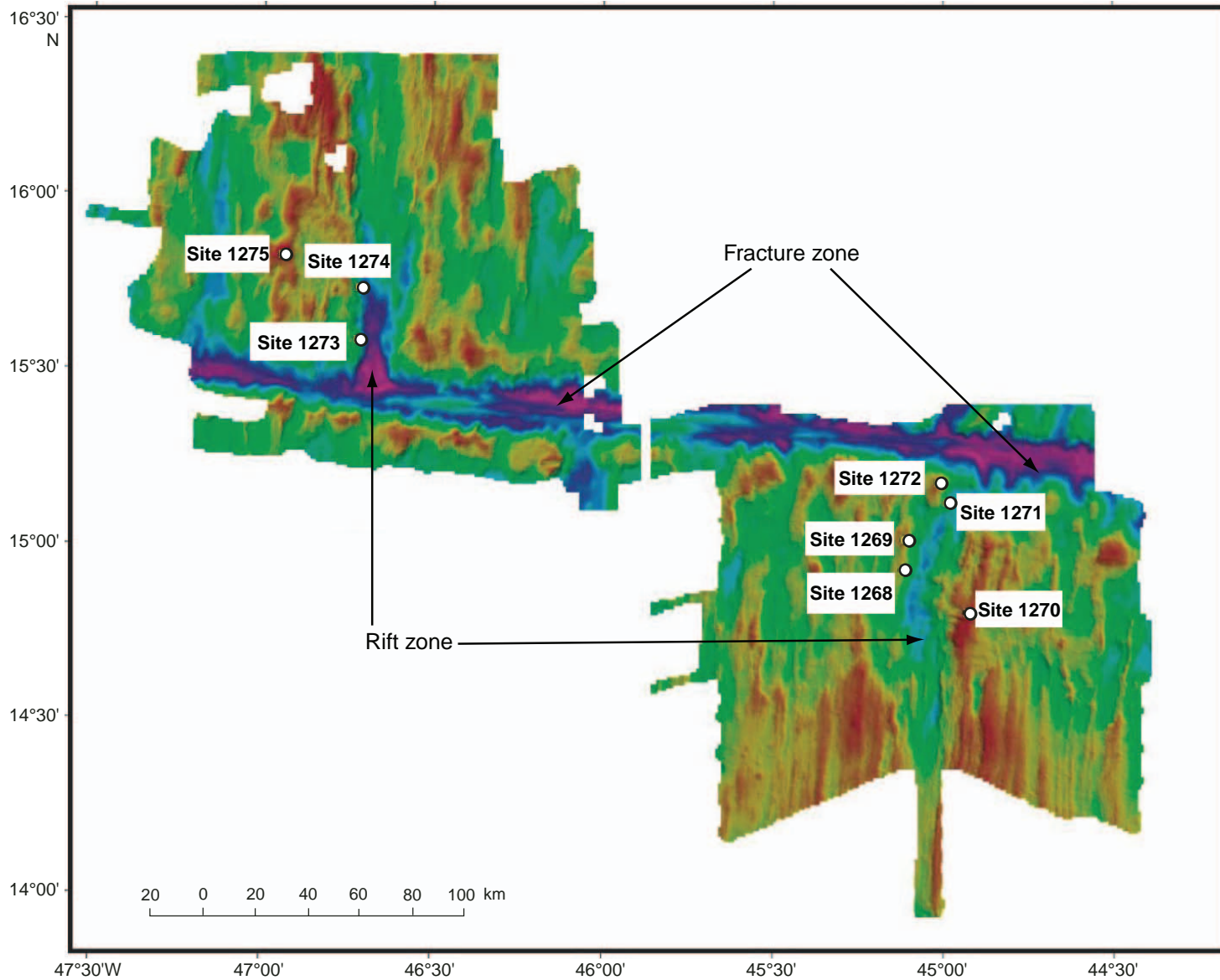
AF1. Pokrovsky and Schott (2000b) rates, p. 31.



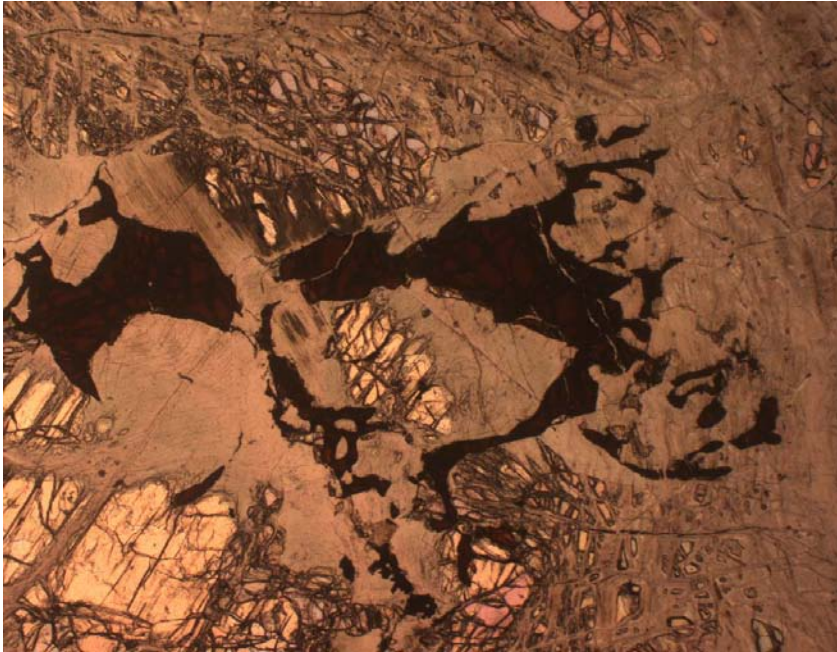
AT6. Approximate dissolution rate of olivine, p. 37.

AT7. Layout for calculating concentrations of dissolved Li and Si, p. 38.

**Figure F1.** Map of Mid-Atlantic Ridge (MAR), showing the 15°20' Fracture Zone and site locations and numbers from ODP Leg 209 (Kelemen, Kikawa, Miller, et al., 2004). The image identifies the location of the samples used in the peridotite experiment, collected during Leg 209. Depths are indicated by colors: red, orange, and yellow = 1500–3000 mbsl; green = 3000–4000 mbsl; blue = 4000–5000 mbsl; pink and purple = 5000–6000 mbsl; white = area with no data.



**Figure F2.** Plane-polarized light image taken on board *JOIDES Resolution* (Sample 209-1274A-8R-2, 101–103 cm) (Kelemen, Kikawa, Miller, et al., 2004). Field of view is 5 mm across. From ODP Leg 209 thin section 200.





**Figure F3.** Plane-polarized light image taken on board *JOIDES Resolution* (Sample 209-1274A-15R-1, 102–105 cm) (Kelemen, Kikawa, Miller, et al., 2004). Field of view is 2.75 mm. From ODP Leg 209 thin section 208.



Figure F4. Amount of dissolved Li in culture media through time. Solid diamonds = cultures inoculated with peridotite from Hole 1274A at 42 mbsf (with 20% fresh olivine), open circles = cultures from Hole 1274A at 76 mbsf (with 0% fresh olivine). Actual values are in Table AT4, p. 35.

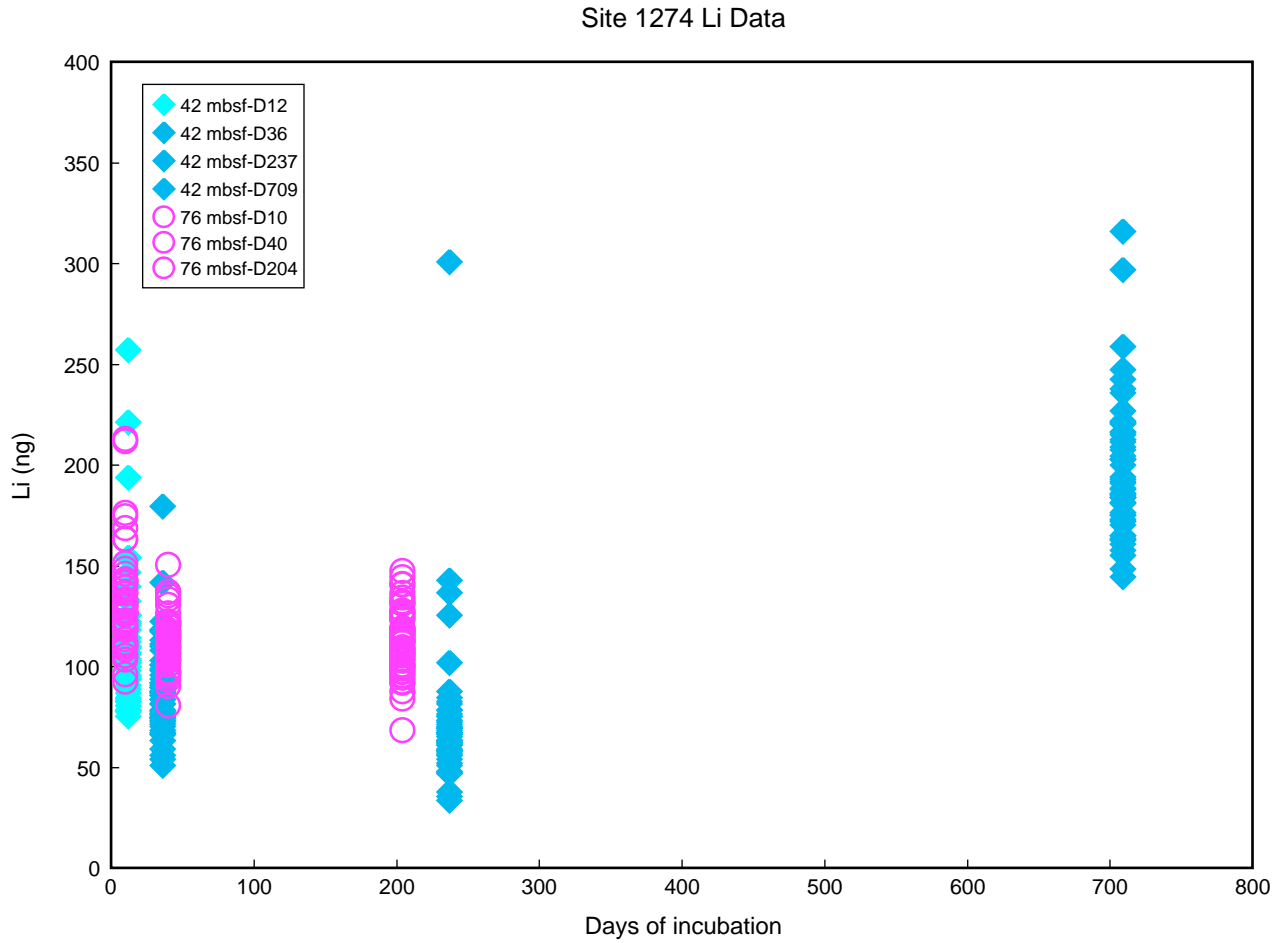
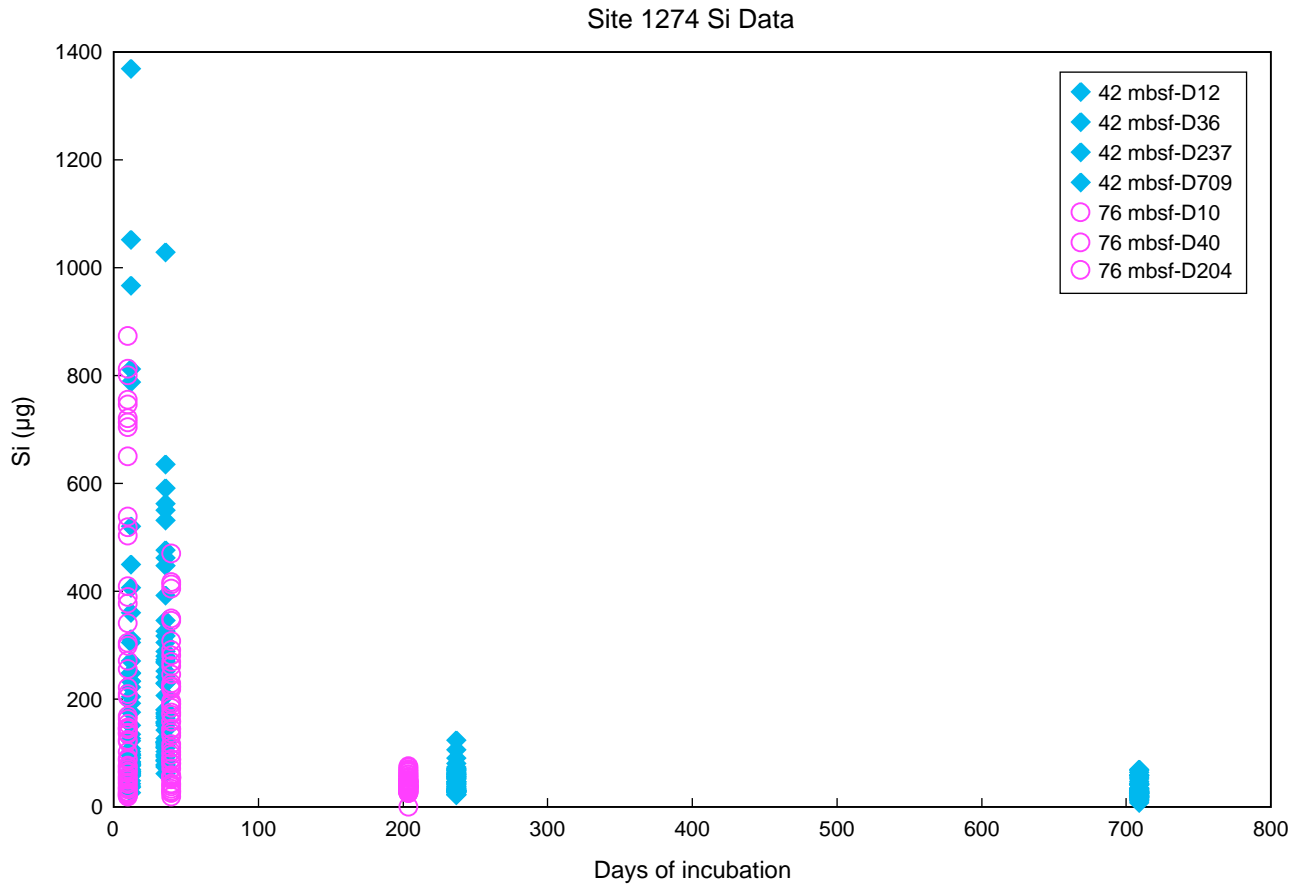
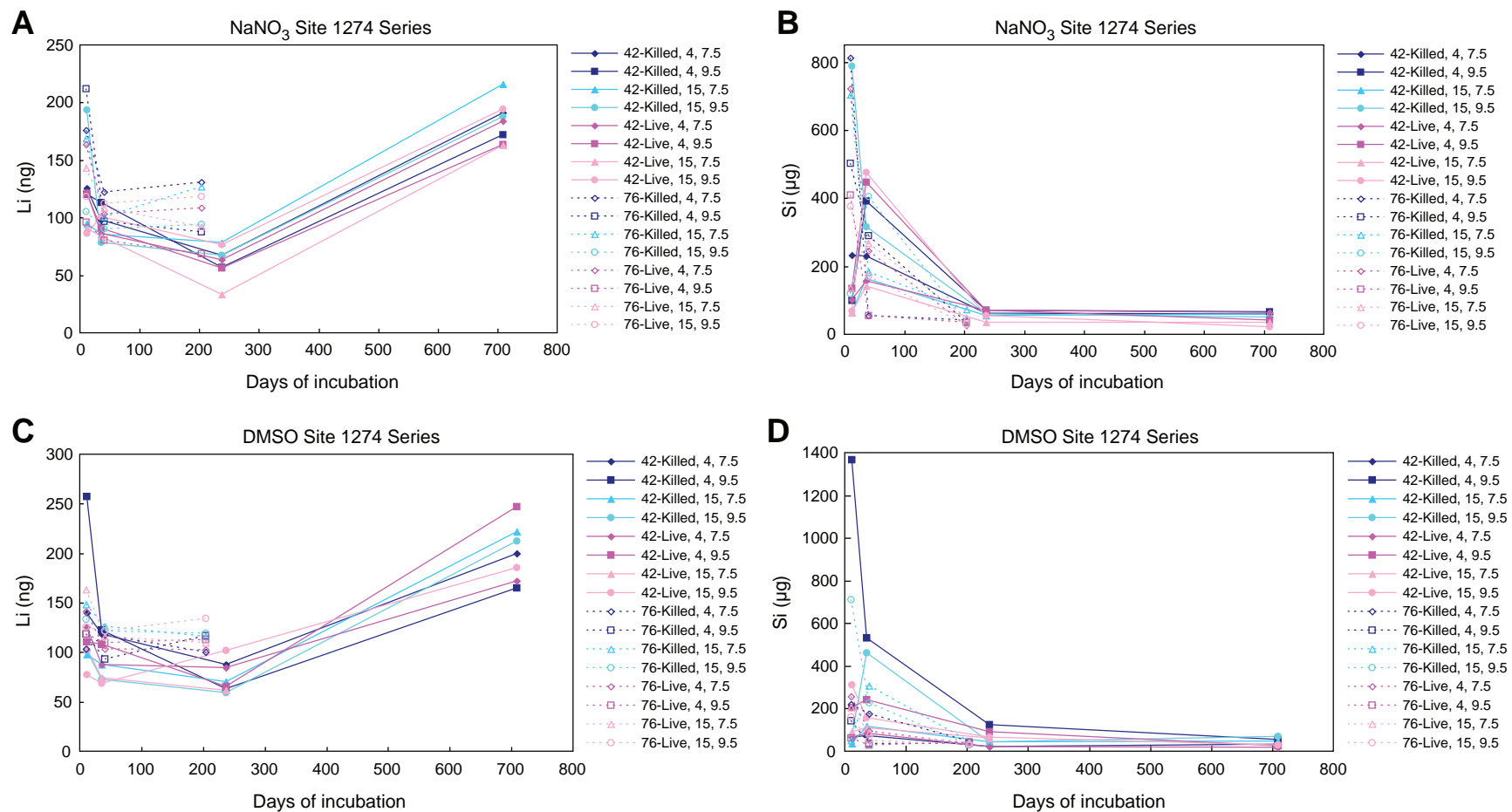


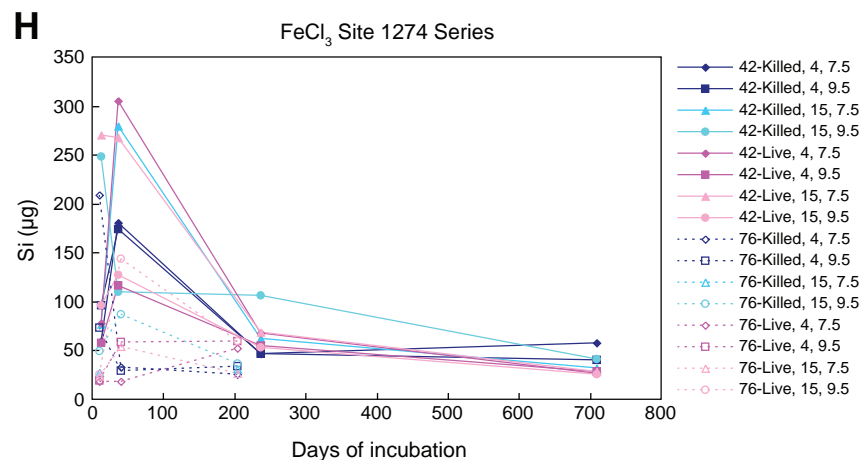
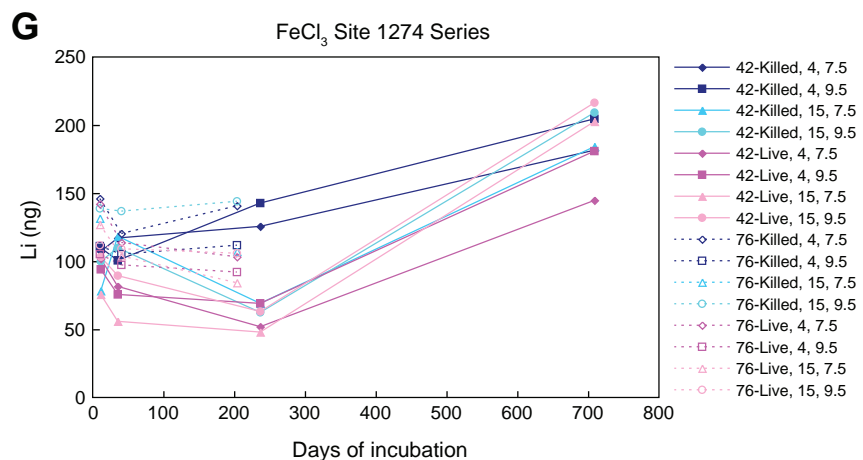
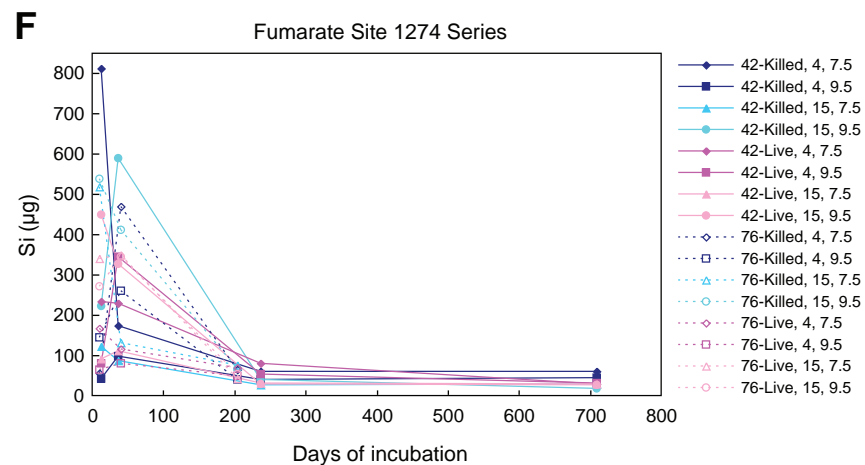
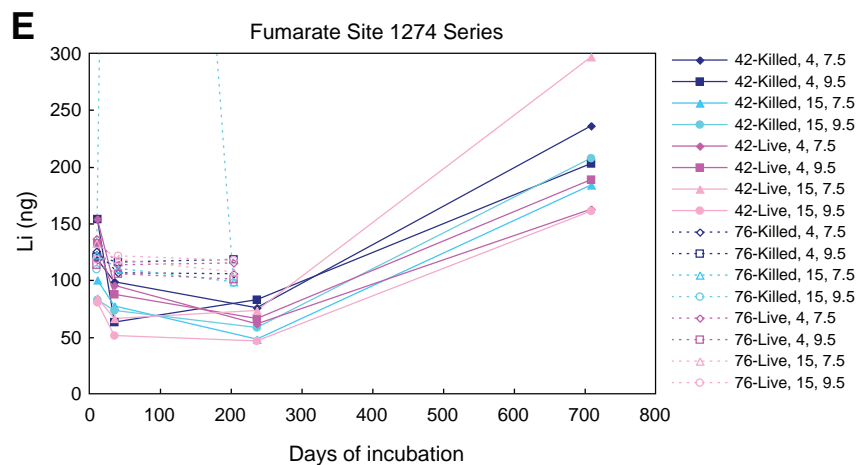
Figure F5. Amount of dissolved Si in culture media through time. Solid diamonds = cultures inoculated with peridotite from Hole 1274A at 42 mbsf (with 20% fresh olivine), open circles = cultures from Hole 1274A at 76 mbsf (with 0% fresh olivine). Actual values are in Table AT5, p. 36.



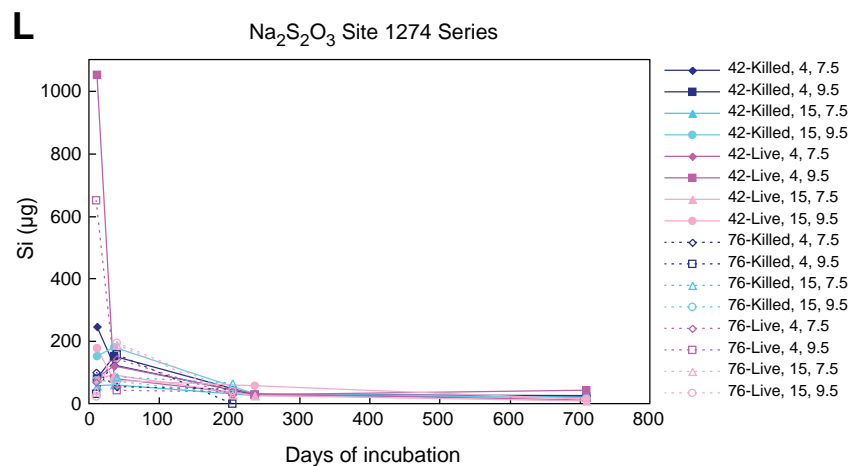
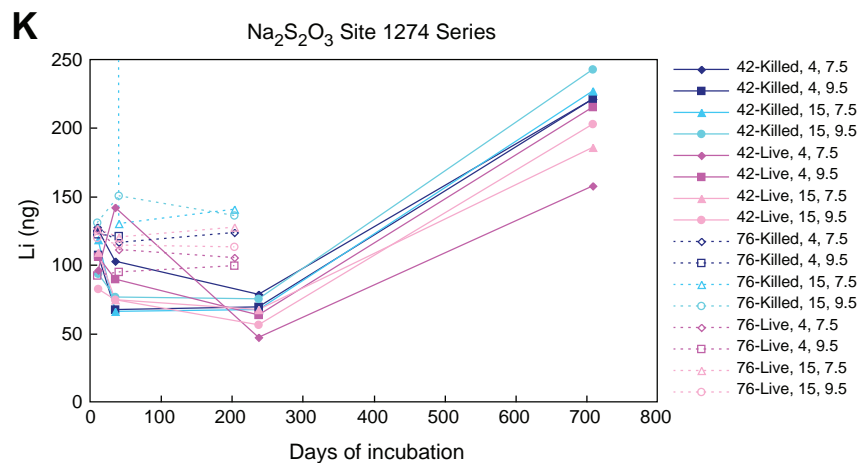
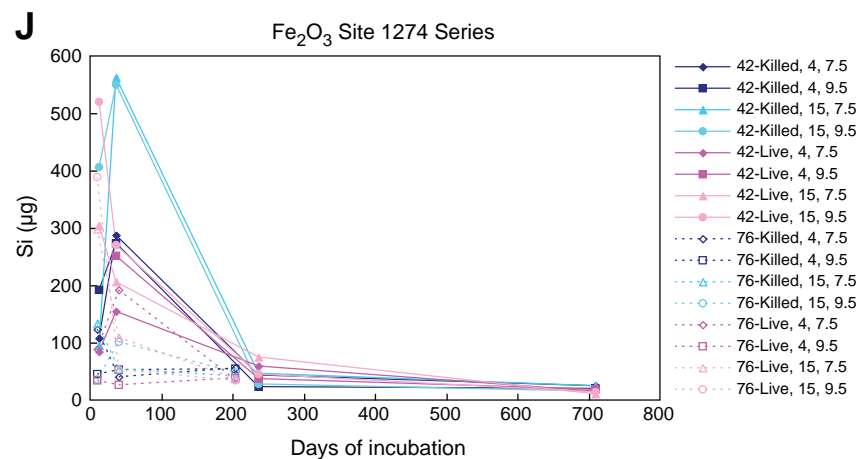
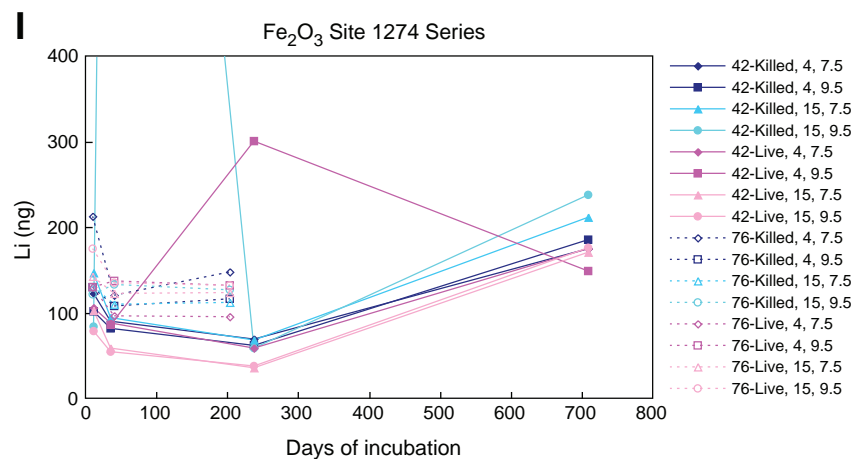
**Figure F6.** A. Changes in the concentrations of Li through time for each individual culture tube with the NaNO<sub>3</sub> enrichment media. B. Changes in the concentrations of Si through time for each individual culture tube with the NaNO<sub>3</sub> enrichment media. C. Changes in the concentration of Li through time for each individual culture tube with the dimethyl sulfoxide (DMSO) enrichment media. D. Changes in the concentration of Si through time for each individual culture tube with the DMSO enrichment media. The 42 or 76 refers to which inoculum the culture was started with. Killed = cultures that were treated with mercuric chloride and we used as controls, live = inoculated cultures. The 4 or 15 refers to the incubation temperature, and the 7.5 or 9.5 indicates the initial pH of the enrichment media. (Continued on next three pages.)



**Figure F6 (continued).** E. Changes in the concentration of Li through time for each individual culture tube with the fumarate enrichment media. F. Changes in the concentration of Si through time for each individual culture tube with the fumarate enrichment media. G. Changes in the concentration of Li through time for each individual culture tube with the FeCl<sub>3</sub> enrichment media. H. Changes in the concentration of Si through time for each individual culture tube with the FeCl<sub>3</sub> enrichment media.



**Figure F6 (continued).** I. Changes in the concentration of Li through time for each individual culture tube with the  $\text{Fe}_2\text{O}_3$  enrichment media. J. Changes in the concentration of Si through time for each individual culture tube with the  $\text{Fe}_2\text{O}_3$  enrichment media. K. Changes in the concentration of Li through time for each individual culture tube with the  $\text{Na}_2\text{S}_2\text{O}_3$  enrichment media. L. Changes in the concentration of Si through time for each individual culture tube with the  $\text{Na}_2\text{S}_2\text{O}_3$  enrichment media.



**Figure F6 (continued).** M. Changes in the concentration of Li through time for each individual culture tube with the MnO<sub>2</sub> enrichment media. N. Changes in the concentration of Si through time for each individual culture tube with the MnO<sub>2</sub> enrichment media.

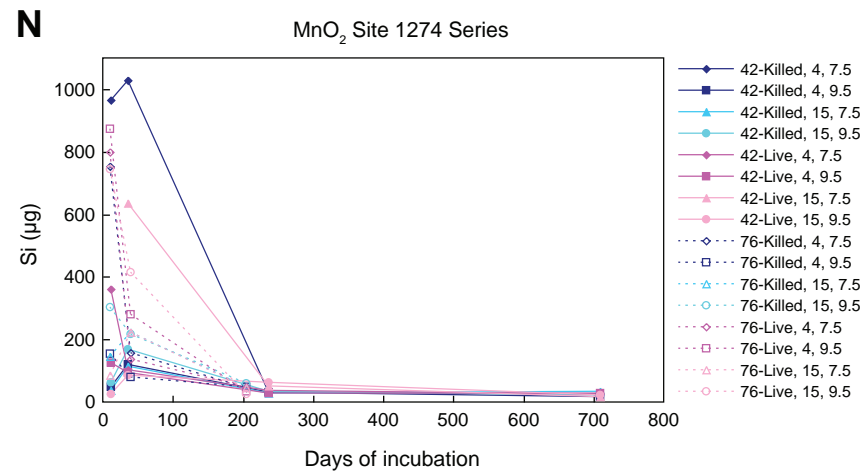
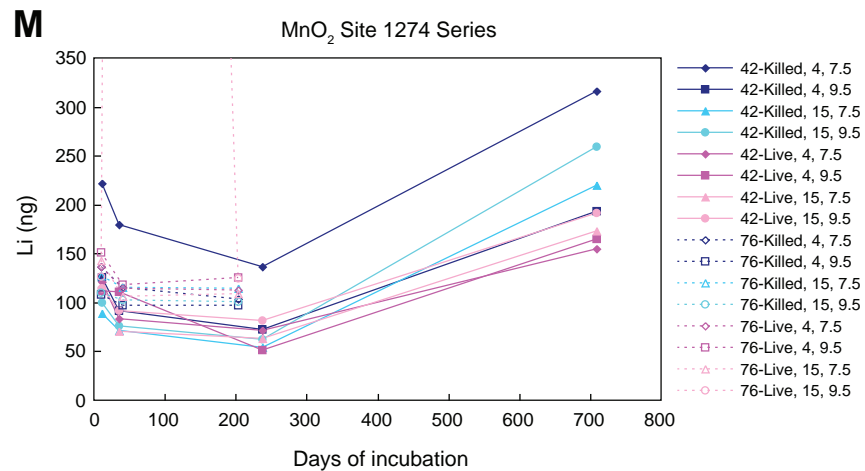


Table T1. Leg 209 Samples used for peridotite culture experiments.

Hole, core, section, piece	Rock type	Latitude	Longitude	Depth (mbsf)	Estimated fresh olivine (%)
209-					
1268D-2R-1, 7	Serpentinized harzburgite	14°50.76'N	45°4.64'W	14.38	0
1270D-4R-1, 14	Serpentinized harzburgite	14°43.27'N	44°53.08'W	24.52	
1271B-7R-1, 6	Altered dunite	15°2.19'N	44°56.91'W	36.52	
1272A-13R-1, 17	Altered harzburgite	15°5.67'N	44°58.30'W	61.91	
1274A-8R-2, 16	Altered harzburgite	15°38.87'N	46°40.58'W	42.36	20
1274A-15R-2, 7	Highly altered harzburgite	15°38.87'N	46°40.58'W	76.01	0
1274A-27R-1, 6	Altered harzburgite	15°38.87'N	46°40.58'W	146.79	15
1275D-10R-2, 3	Altered troctolite	15°44.44'N	46°54.22'W	47.79	0-3

Note: Highlighted rows are samples analyzed in this paper.



Table T2. Electron donors used in the culture experiments.

Compound	Redox pair	$E_0'$
$\text{Na}_2\text{S}_2\text{O}_3$	$\text{S}_2\text{O}_3^{2-}/\text{HS}^- + \text{HSO}_3^-$	-0.40
Dimethyl fumarate	Fumarate <sup>2-</sup> /Succinate <sup>2-</sup>	+0.033
Dimethyl sulfoxide	DMSO/DMS	+0.16
$\text{NaNO}_3$	$\text{NO}_3^-/\text{NO}_2^-$	+0.43
$\text{FeCl}_3$	$\text{Fe}^{3+}/\text{Fe}^{2+}$	+0.77
$\text{Fe}_2\text{O}_3$	$\text{Fe}^{3+}/\text{Fe}^{2+}$	+0.77
$\text{MnO}_2$	$\text{Mn}^{4+}/\text{Mn}^{2+}$	+0.798

Notes: From Madigan et al. (2000). DMSO = dimethyl sulfoxide,  
DMS = dimethyl sulfide.

Table T3. Microsphere tracer results.

Hole, core, section	Filtered wash (mL)	Exterior concentration (ms/mL)	Weight of rock (g)	Interior concentration (ms/mL)
209-				
1268D-2R-1	1	$6 \times 10^4$	—	0
1271B-7R-1	38	$9 \times 10^5$	0.5	0
1272A-13R-1	34	$1 \times 10^3$	0.4	0
1274A-8R-2	25	300	0.6	0
1274A-15R-2	10	100	0.2	0
1274A-27R-1	38	0	0.3	0
1275A-10R-2	10	$3 \times 10^3$	0.3	0

Note: ms = microspheres.

**Table T4.** Average amount of dissolved Si (in  $\mu\text{g}$ ) for Hole 1274A at 42 mbsf and 76 mbsf.

Enrichment media	Number of days	NaNO <sub>3</sub>	DMSO	Fumarate	FeCl <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	MnO <sub>2</sub>
Hole 1274A (42 mbsf)								
Inoculated	12	100	150	200	100	300	350	200
Inoculated	36	300	150	250	200	200	100	250
Inoculated	237	50	50	50	50	50	50	50
Inoculated	709	50	0	50	50	0	0	0
Control	12	300	400	300	100	200	150	300
Control	36	300	300	200	200	400	150	350
Control	237	50	50	50	50	50	50	50
Control	709	50	50	50	50	0	0	50
Hole 1274A (76 mbsf)								
Inoculated	10	400	200	200	0	200	200	600
Inoculated	40	200	50	200	50	100	150	300
Inoculated	204	50	50	50	50	50	50	50
Control	10	500	300	300	100	100	50	350
Control	40	200	200	300	50	50	100	200
Control	204	50	50	50	50	50	50	50

Notes: Averages are calculated from values in Table [AT5](#), p. 36. Number of days indicates the number of days that have elapsed since the culture was set up to when the media was sampled. DMSO = dimethyl sulfoxide.

**Table T5.** Average amount of dissolved Li (in ng) for Hole 1274A at 42 mbsf and 76 mbsf.

Enrichment media	Number of days	NaNO <sub>3</sub>	DMSO	Fumarate	FeCl <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	MnO <sub>2</sub>
Hole 1274A (42 mbsf)								
Inoculated	12	100	100	100	100	100	100	100
Inoculated	36	100	100	100	100	50	100	100
Inoculated	237	50	100	50	50	100	50	50
Inoculated	709	200	200	200	200	150	200	200
Control	12	100	150	100	100	100	100	100
Control	36	100	100	100	100	100	100	100
Control	237	50	100	50	100	50	100	100
Control	709	200	200	200	200	200	200	250
Hole 1274A (76 mbsf)								
Inoculated	10	150	150	100	100	150	100	150
Inoculated	40	100	100	100	100	100	100	100
Inoculated	204	100	100	100	100	100	100	100
Control	10	150	150	100	150	150	150	100
Control	40	100	100	500*	100	100	150	100
Control	204	100	100	100	100	100	100	100

Notes: Averages were calculated from values in Table AT4, p. 35; these are the averages of both temperatures (4°C and 15°C) and pHs (7.5 and 9.5) (*N* = 4). The standard deviation for the averages = 20%. Number of days indicates the number of days that have elapsed since the culture was set up to the date when the media was sampled. DMSO = dimethyl sulfoxide. \* = an unusually high measurement is responsible for the elevated average.

**Table T6.** Estimated concentration of dissolved Li (ppb) and Si (ppm) at the sampling times in Hole 1274A (42 mbsf) sample set.

Time (d):	Hole 1274A (42 mbsf) sample set			
	12	36	237	709
pH 7.5, temp 25°C				
Ol dissolved (g)	0.0825	0.2474	1.6290	4.8731
Li released (ng) calculated	150	500	3000	10,000
Li released (ng) measured	100 ± 30	100 ± 30	50 ± 20	200 ± 40
Si released (µg) calculated	15,000	50,000	300000	1,000,000
Si released (µg) measured	200 ± 200	200 ± 200	50 ± 20	30 ± 15
pH 9.5, temp 25°C				
Ol dissolved (g)	0.0165	0.0495	0.3258	0.9746
Li released (ng) calculated	30	100	500	2,000
Li released (ng) measured	100 ± 40	100 ± 20	100 ± 50	200 ± 30
Si released (µg) calculated	3,000	10,000	50000	20,0000
Si released (µg) measured	250 ± 330	250 ± 160	50 ± 25	30 ± 15

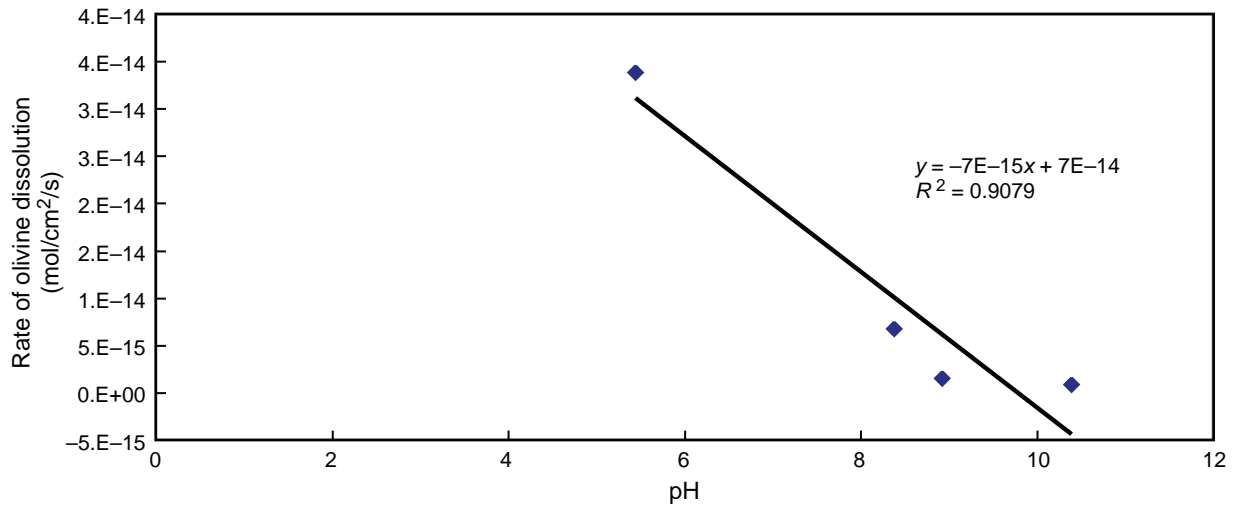
Notes: Based on a Li concentration of 2 ppm in the olivine. The measured averages are the combined average of the inoculated and control cultures, the two temperatures, and the seven amendments ( $N = 28$ ). Error =  $1\sigma$ .

**Table T7.** Estimated concentration of dissolved Li (ppb) and Si (ppm) at the sampling times in Hole 1274A (76 mbsf) sample set.

Time (d):	Hole 1274A (76 mbsf) sample set		
	10	40	204
pH 7.5, temp 25°C			
Ol dissolved (g)	0.0687	0.2749	1.4021
Li released (ng) calculated	150	500	3,000
Li released (ng) measured	150 ± 20	100 ± 10	100 ± 15
Si released (µg) calculated	10,000	50,000	300,000
Si released (µg) measured	250 ± 250	150 ± 100	50 ± 15
pH 9.5, temp 25°C			
Ol dissolved (g)	0.0137	0.0550	0.2804
Li released (ng) calculated	30	100	500
Li released (ng) measured	100 ± 20	100 ± 15	100 ± 30
Si released (µg) calculated	2,000	10,000	50,000
Si released (µg) measured	250 ± 250	150 ± 150	50 ± 15

Notes: Based upon a Li concentration of 2 ppm in the olivine. The measured averages are the combined average of the inoculated and control cultures, the two temperatures, and the seven amendments ( $N = 28$ ). Error =  $1\sigma$ .

Figure AF1. Pokrovsky and Schott (2000b) rates, T = 25°C.



**Table AT1.** Artificial seawater recipe for 1 L of sterile H<sub>2</sub>O.

Salt	Concentration (g/L)
NaCl	24.32
MgCl <sub>2</sub>	5.14
CaCl <sub>2</sub>	1.14
KCl	0.69
NaHCO <sub>3</sub>	0.2
KBr	0.1
H <sub>3</sub> BO <sub>3</sub>	0.027
SrCl <sub>2</sub>	0.026
NH <sub>4</sub> Cl	0.0064
NaF	0.003
NaSiO <sub>3</sub>	0.002
FePO <sub>4</sub>	0.001

Note: Based on ZoBell (1946).



Table AT2. Electron donors and acceptors added to 1 L artificial seawater solutions to make culture media.

Electron donor/acceptor	Concentration (g/L)
$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$	0.472
Dimethyl fumarate	0.275
Dimethyl sulfoxide	0.149
$\text{NaNO}_3$	0.054
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	1.029
$\text{Fe}_2\text{O}_3$	0.304
$\text{MnO}_2$	0.165

**Table AT3.** Composition of Riddle Nickel Mountain peridotite used in the enrichment cultures and specific surface area (SSA) measurements.

Element	Oxide (wt%)
SiO <sub>2</sub>	42.81
Al <sub>2</sub> O <sub>3</sub>	ND
FeO	9.5
CaO	0
MgO	45.12
NiO	0.26
Cr <sub>2</sub> O <sub>3</sub>	0.79
LOI	0.57
Li, ppm	1.5*
Sample	SSA (m <sup>2</sup> /g)
#1	0.7494 ± 0.0065
#2	0.7735 ± 0.0078

Notes: Composition of peridotite is from Pecora and Hobbs (1942).

ND = not determined. LOI = loss on ignition. \* = assumed based on average Li in peridotites (Kent and Rossman, 2002).

**Table AT4.** Amount of dissolved Li (in ng) in media through time for two culture sets.

Sample- Tube #	Media	pH	Temp (°C)	Sample set: Time: Incubation (days): I/C	Hole 1274A (42 mbsf)				Hole 1274A (76 mbsf)		
					T0	T1	T2	T3	T0	T1	T2
					Li	Li	Li	Li	Li	Li	Li
1274-6	DMSO	7.5	4	Control	140	118	88	200	103	117	100
1274-34	DMSO	9.5	4	Control	257	122	63	165	119	93	117
1274-8	DMSO	7.5	15	Control	98	88	70	222	148	126	116
1274-36	DMSO	9.5	15	Control	102	72	59	213	133	122	119
1274-5	DMSO	7.5	4	Inoculated	125	88	85	172	141	104	103
1274-33	DMSO	9.5	4	Inoculated	110	108	65	247	118	109	113
1274-7	DMSO	7.5	15	Inoculated	104	74	61		163	116	109
1274-35	DMSO	9.5	15	Inoculated	78	68	102	185	126	121	134
1274-22	Fe <sub>2</sub> O <sub>3</sub>	7.5	4	Control	122	90	69	175	213	121	147
1274-50	Fe <sub>2</sub> O <sub>3</sub>	9.5	4	Control	102	82	62	185	130	108	117
1274-24	Fe <sub>2</sub> O <sub>3</sub>	7.5	15	Control	147	94	68	212	131	110	112
1274-52	Fe <sub>2</sub> O <sub>3</sub>	9.5	15	Control	84	1725	58	238	121	133	127
1274-21	Fe <sub>2</sub> O <sub>3</sub>	7.5	4	Inoculated	106	88	58	175	130	96	95
1274-49	Fe <sub>2</sub> O <sub>3</sub>	9.5	4	Inoculated		86	301	148	130	137	132
1274-23	Fe <sub>2</sub> O <sub>3</sub>	7.5	15	Inoculated	103	59	36	170	142	122	123
1274-51	Fe <sub>2</sub> O <sub>3</sub>	9.5	15	Inoculated	79	54	38	176	175	135	131
1274-10	FeCl <sub>3</sub>	7.5	4	Control	107	117	125	182	146	120	141
1274-38	FeCl <sub>3</sub>	9.5	4	Control	110	101	143	204	111	105	112
1274-12	FeCl <sub>3</sub>	7.5	15	Control	79	118	69	184	131		107
1274-40	FeCl <sub>3</sub>	9.5	15	Control	100	110	62	209	139	137	144
1274-9	FeCl <sub>3</sub>	7.5	4	Inoculated	102	82	52	145	142	114	104
1274-37	FeCl <sub>3</sub>	9.5	4	Inoculated	94	75	69	181	105	97	92
1274-11	FeCl <sub>3</sub>	7.5	15	Inoculated	75	56	48	203	127	104	84
1274-39	FeCl <sub>3</sub>	9.5	15	Inoculated	103	90	63	216	112	109	106
1274-14	Fumarate	7.5	4	Control	118	99	75	236	126	107	106
1274-42	Fumarate	9.5	4	Control	154	63	83	203	121	116	119
1274-16	Fumarate	7.5	15	Control	100	78	48	184	123	110	99
1274-44	Fumarate	9.5	15	Control	83	73	58	208	110	1497	98
1274-13	Fumarate	7.5	4	Inoculated	154	96	61	163	137	114	115
1274-41	Fumarate	9.5	4	Inoculated	133	88	66	189	113	106	101
1274-15	Fumarate	7.5	15	Inoculated	85	66	73	297	133	118	107
1274-43	Fumarate	9.5	15	Inoculated	81	51	47	161	118	122	118
1274-18	MnO <sub>2</sub>	7.5	4	Control	221	180	137	316	137	115	104
1274-46	MnO <sub>2</sub>	9.5	4	Control	125	92	72	193	108	97	97
1274-20	MnO <sub>2</sub>	7.5	15	Control	89	71	54	220	127	116	114
1274-48	MnO <sub>2</sub>	9.5	15	Control	100	77	62	259	110	103	101
1274-17	MnO <sub>2</sub>	7.5	4	Inoculated	121	84	71	155	136	114	113
1274-45	MnO <sub>2</sub>	9.5	4	Inoculated	112	111	51	165	151	118	126
1274-19	MnO <sub>2</sub>	7.5	15	Inoculated		70	63	173	143	106	109
1274-47	MnO <sub>2</sub>	9.5	15	Inoculated	114	92	82	192	127		125
1274-26	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	7.5	4	Control	125	103	79	221	128	117	124
1274-54	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	9.5	4	Control	107	67	69	221	123	121	0
1274-28	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	7.5	15	Control	118	66	67	227		131	141
1274-56	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	9.5	15	Control	94	77	75	243	131	150	136
1274-25	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	7.5	4	Inoculated	96	142	47	158	126	112	106
1274-53	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	9.5	4	Inoculated	106	90	63	215	93	95	100
1274-27	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	7.5	15	Inoculated	109	74	67	186	126	120	128
1274-55	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	9.5	15	Inoculated	83	74	56	203	120	114	113
1274-2	NaNO <sub>3</sub>	7.5	4	Control	125	98	67	191	176	122	131
1274-30	NaNO <sub>3</sub>	9.5	4	Control	120	113	57	172	212	97	88
1274-4	NaNO <sub>3</sub>	7.5	15	Control	95	86	79	216	169	101	127
1274-32	NaNO <sub>3</sub>	9.5	15	Control	194	79	67	188	105	90	95
1274-1	NaNO <sub>3</sub>	7.5	4	Inoculated	122	87	63	184	163	103	109
1274-29	NaNO <sub>3</sub>	9.5	4	Inoculated	121	91	56	164	96	81	68
1274-3	NaNO <sub>3</sub>	7.5	15	Inoculated	91	84	34	163	143	109	93
1274-31	NaNO <sub>3</sub>	9.5	15	Inoculated	87	101	77	194	118	112	119

Notes: Blank spaces are time points that were not measured. Each value is the average of two measurements within the ICP-MS run. The total counts were converted into concentrations using the instrument generated standard curve created on each day of analysis. To calculate the total dissolved Li in each culture, the ICP-MS measured concentration was multiplied by 102 to calculate the concentration of Li in the 50 µL of media diluted by 5 mL of nitric acid and the Be internal standard (added to each sample for the ICP-MS analysis). Then the concentration was multiplied by 20 to determine the total Li within the media (20 mL) and not the Li within 1 mL of media. Finally, the concentration was converted to an amount of dissolved Li in ng. DMSO = dimethyl sulfoxide.

Table AT5. Amount of dissolved Si (in µg) in media through time for two culture sets.

Sample- Tube #	Media	pH	Temp (°C)	Sample set: Time: Incubation (days): I/C	Hole 1274A (42 mbsf)				Hole 1274A (76 mbsf)		
					T0	T1	T2	T3	T0	T1	T2
					12	36	237	709	10	40	204
					Si	Si	Si	Si	Si	Si	Si
1274-6	DMSO	7.5	4	Control	65	74	22	35	221	174	46
1274-34	DMSO	9.5	4	Control	1369	531	124	54	144	38	38
1274-8	DMSO	7.5	15	Control	38	119	42	48	53	308	46
1274-36	DMSO	9.5	15	Control	68	462	45	69	713	228	31
1274-5	DMSO	7.5	4	Inoculated	65	85	24	19	255	96	36
1274-33	DMSO	9.5	4	Inoculated	205	241	90	26	63	28	43
1274-7	DMSO	7.5	15	Inoculated	90	112	61		204	88	41
1274-35	DMSO	9.5	15	Inoculated	312	159	67	29	162	51	33
1274-22	Fe <sub>2</sub> O <sub>3</sub>	7.5	4	Control	108	288	43	25	124	41	56
1274-50	Fe <sub>2</sub> O <sub>3</sub>	9.5	4	Control	193	273	23	20	46	53	55
1274-24	Fe <sub>2</sub> O <sub>3</sub>	7.5	15	Control	96	562	47	26	135	55	41
1274-52	Fe <sub>2</sub> O <sub>3</sub>	9.5	15	Control	407	550	29	16	36	102	48
1274-21	Fe <sub>2</sub> O <sub>3</sub>	7.5	4	Inoculated	84	155	60	16	89	192	35
1274-49	Fe <sub>2</sub> O <sub>3</sub>	9.5	4	Inoculated		251	38	18	34	26	39
1274-23	Fe <sub>2</sub> O <sub>3</sub>	7.5	15	Inoculated	305	206	74	11	298	109	37
1274-51	Fe <sub>2</sub> O <sub>3</sub>	9.5	15	Inoculated	521	270	46	14	389	53	35
1274-10	FeCl <sub>3</sub>	7.5	4	Control	59	181	47	57	209	33	25
1274-38	FeCl <sub>3</sub>	9.5	4	Control	96	174	47	40	74	29	34
1274-12	FeCl <sub>3</sub>	7.5	15	Control	77	279	63	32	28		31
1274-40	FeCl <sub>3</sub>	9.5	15	Control	248	110	106	41	50	87	37
1274-9	FeCl <sub>3</sub>	7.5	4	Inoculated	78	305	68	27	18	19	53
1274-37	FeCl <sub>3</sub>	9.5	4	Inoculated	58	117	55	28	19	59	60
1274-11	FeCl <sub>3</sub>	7.5	15	Inoculated	271	268	69	29	24	54	27
1274-39	FeCl <sub>3</sub>	9.5	15	Inoculated	96	127	53	26	26	144	60
1274-14	Fumarate	7.5	4	Control	812	173	59	59	56	470	64
1274-42	Fumarate	9.5	4	Control	43	97	41	44	145	261	39
1274-16	Fumarate	7.5	15	Control	123	86	27	29	518	132	75
1274-44	Fumarate	9.5	15	Control	222	591	39	18	538	413	67
1274-13	Fumarate	7.5	4	Inoculated	233	228	81	28	168	116	69
1274-41	Fumarate	9.5	4	Inoculated	80	346	53	32	65	79	50
1274-15	Fumarate	7.5	15	Inoculated	92	110	32	30	340	349	43
1274-43	Fumarate	9.5	15	Inoculated	450	327	29	27	271	344	53
1274-18	MnO <sub>2</sub>	7.5	4	Control	967	1028	31	26	754	159	45
1274-46	MnO <sub>2</sub>	9.5	4	Control	48	119	32	18	153	81	49
1274-20	MnO <sub>2</sub>	7.5	15	Control	37	115	29	36	147	222	47
1274-48	MnO <sub>2</sub>	9.5	15	Control	62	168	34	22	304	218	60
1274-17	MnO <sub>2</sub>	7.5	4	Inoculated	361	103	37	25	800	138	48
1274-45	MnO <sub>2</sub>	9.5	4	Inoculated	127	96	28	29	873	280	33
1274-19	MnO <sub>2</sub>	7.5	15	Inoculated		636	51	18	86	224	46
1274-47	MnO <sub>2</sub>	9.5	15	Inoculated	27	87	63	24	746	417	26
1274-26	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	7.5	4	Control	248	122	30	26	101	54	45
1274-54	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	9.5	4	Control	79	152	30	23	32	158	0
1274-28	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	7.5	15	Control	58	62	26	20		87	66
1274-56	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	9.5	15	Control	152	180	31	21	76	71	31
1274-25	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	7.5	4	Inoculated	82	120	31	12	70	144	28
1274-53	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	9.5	4	Inoculated	1051	79	29	44	649	43	39
1274-27	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	7.5	15	Inoculated	79	93	26	7	42	190	28
1274-55	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	9.5	15	Inoculated	176	76	56	14	22	196	39
1274-2	NaNO <sub>3</sub>	7.5	4	Control	233	230	63	59	812	56	42
1274-30	NaNO <sub>3</sub>	9.5	4	Control	99	392	71	67	502	290	36
1274-4	NaNO <sub>3</sub>	7.5	15	Control	65	164	56	59	704	183	73
1274-32	NaNO <sub>3</sub>	9.5	15	Control	787	317	60	52	120	405	38
1274-1	NaNO <sub>3</sub>	7.5	4	Inoculated	103	156	72	64	721	246	27
1274-29	NaNO <sub>3</sub>	9.5	4	Inoculated	135	447	70	42	409	55	36
1274-3	NaNO <sub>3</sub>	7.5	15	Inoculated	63	142	34	34	137	269	48
1274-31	NaNO <sub>3</sub>	9.5	15	Inoculated	69	476	55	22	377	170	29

Notes: Blank spaces are time points that were not measured. Each value is the average of two measurements within the ICP-MS run. The total counts were converted into concentrations using the instrument generated standard curve created on each day of analysis. Then to calculate the total dissolved Si in each culture tube the ICP-MS measured concentration was multiplied by 102 to find the concentration of Si in the 50 µL of media diluted by 5 mL of nitric acid and the Be internal standard (added to each sample for the ICP-MS analysis). Then the concentration was multiplied by 20 to determine the total Si within the media (20 mL) and not the Si within 1 mL of media. The concentration of dissolved Si was converted into an amount of dissolved Si (µg). DMSO = dimethyl sulfoxide.

**Table AT6.** Approximate dissolution rate of olivine at 25°C and pH 7.5 and 9.5.

pH	Rate (mol/cm <sup>2</sup> /s)
7.5	$1.75 \times 10^{-14}$
9.5	$3.50 \times 10^{-15}$

**Table AT7.** Step-by-step layout for calculating concentrations of dissolved Li (ppb) and Si (ppm) in Hole 1274A at 42 mbsf.

BET SSA (cm <sup>2</sup> /g)	7,494			
SSA in tube (cm <sup>2</sup> )	29,976			
Nickel Mountain olivine is Fo <sub>83</sub> ; molecular weight = 151.648				
g of Si in 1 mol Fo <sub>83</sub> =	0.185			
g of Li in 1 mol Fo <sub>83</sub> =	2.00 × 10 <sup>-6</sup>			
Rate of dissolution	mol/cm <sup>2</sup> /s			
pH 7.5	1.75 × 10 <sup>-14</sup>			
pH 9.5	3.5 × 10 <sup>-15</sup>			
Time (days)	12	36	237	709
Time (s)	1,036,800	3,110,400	20,476,800	61,257,600
mol olivine dissolved = rate (mol/cm <sup>2</sup> /s) × SSA in tube (cm <sup>2</sup> ) × time (s)				
g Fo <sub>83</sub> dissolved =	mol olivine dissolved × molecular weight of Fo <sub>83</sub>			
g Li released =	g Fo <sub>83</sub> dissolved × g of Li in 1 g Fo <sub>83</sub>			
g Si released =	g Fo <sub>83</sub> dissolved × g of Si in 1 g Fo <sub>83</sub>			
@ pH 7.5	12 days	36 days	237 days	709 days
mol Fo <sub>83</sub> dissolved =	0.0005	0.0016	0.0107	0.0321
g Fo <sub>83</sub> dissolved =	0.0825	0.2474	1.6290	4.8731
g Li released =	1.65 × 10 <sup>-7</sup>	4.95 × 10 <sup>-7</sup>	3.26 × 10 <sup>-6</sup>	9.75 × 10 <sup>-6</sup>
g Si released =	0.015	0.046	0.301	0.902
@ pH 9.5				
mol Fo <sub>83</sub> dissolved =	0.0001	0.0003	0.0021	0.0064
g Fo <sub>83</sub> dissolved =	0.0165	0.0495	0.3258	0.9746
g Li released =	3.30 × 10 <sup>-8</sup>	9.90 × 10 <sup>-8</sup>	6.52 × 10 <sup>-7</sup>	1.95 × 10 <sup>-6</sup>
g Si released =	0.003	0.009	0.060	0.180

Note: SSA = specific surface area.