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Research Paper

Inorganic Nitrogen Reduction and Stability under Simulated Hydrothermal Conditions

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Abstract

Availability of reduced nitrogen is considered a prerequisite for the genesis of life from prebiotic precursors. Most atmospheric and oceanic models for the Hadean Earth predict a mildly oxidizing environment that is conducive to the formation and stability of only oxidized forms of nitrogen. A possible environment where reduction of oxidized nitrogen to ammonium has been speculated to occur is aqueous hydrothermal systems. We examined a suite of transition metal oxides and sulfides for their ability to reduce nitrate and nitrite, as well as oxidize ammonia, under hot (300°C) high-pressure (50–500 MPa) aqueous conditions. In general, iron sulfides exhibited the most rapid and complete conversion noted, followed by nickel and copper sulfides to a much lower degree. Of the oxides examined, only magnetite exhibited any ability to reduce NO_3^- or NO_2^-. Ammonium was stable or exhibited small losses ($<20\%$) in contact with all the mineral phases and conditions tested. The results support the idea that hydrothermal systems could have provided significant amounts of reduced nitrogen to their immediate environments. The enhanced availability of reduced nitrogen in hydrothermal systems also has important implications for prebiotic metabolic pathways where nitrogen availability is critical to the production of amino acids and other nitrogenous compounds. Key Words: Nitrogen—Hydrothermal—Sulfides—Prebiotic—Ammonia. Astrobiology 8, 1113–1126.

1. Introduction

THE AVAILABILITY OF REDUCED NITROGEN, primarily ammonium, on early Earth has been a subject of investigation ever since published studies indicated a relatively oxidizing Hadean Era atmospheric composition (Kasting, 1987, 1990; Kasting *et al.*, 1989; Pavlov and Kasting, 2002). Prior to these studies, the assumption had been that early Earth's atmosphere was very reduced, with abundant ammonium available for prebiotic reactions. An atmosphere composed primarily of CO_2 and N_2, however, is much less hospitable to prebiotic atmospheric reactions that use electrical discharge (the Miller-Urey pathway) to produce amino acids and other important biological precursors (Schlesinger and Miller, 1983a, 1983b; Miller and Schlesinger, 1984). Thus, the search began for a mechanism by which N_2 could be converted to reduced N forms. Dinitrogen gas (N_2) is extremely stable and has to be converted to other forms either by lightning discharge (Navarro-Gonzalez *et al.*, 1998, 2001a, 2001b; Mvondo *et al.*, 2001; Nna-Mvondo *et al.*, 2005), shock (Nakazawa *et al.*, 2005) or mineral catalyst–mediated reduction (Brandes *et al.*, 1998; Schoonen and Yong, 2001; Dorr *et al.*, 2003; Schoonen *et al.*, 2004). Lightning or corona discharges in a CO_2-N_2 environment produce, at low efficiency, oxidized nitrogen species such as NO and NO_2 (Navarro-Gonzalez *et al.*, 1998; Nna-Mvondo *et al.*, 2005), which must be reduced via some other mechanism. Three such mechanisms have been proposed: reduction of nitrogen oxides by ferrous iron (Buresh and Moraghan, 1976; Summers and Chang, 1993; Summers and Lerner, 1998), photochemical reduction on titanium oxide catalysts (Hendersonsellers and Schwartz, 1980), or minerals within hydrothermal systems (Brandes *et al.*, 1998;

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Schoonen and Yong, 2001; Dorr *et al.*, 2003; Summers, 2005). In each case, NO and NO_2 must react with OH radicals to form NO_2^- or NO_3^-, which are then rained out into the prebiotic ocean. Under hydrothermal conditions, nitrate will react with iron sulfides to form ammonium (Blochl *et al.*, 1992; Brandes *et al.*, 1998). However, the stability of ammonium and the stability or reduction of nitrogen oxides as a function of different conditions and in the presence of different minerals have remained little studied. Given the continued interest in nitrogen chemistry on Mars (Klingler *et al.*, 1989; Mancinelli, 1996; Denlinger, 2005; Segura and Navarro-Gonzalez, 2005; Capone *et al.*, 2006; Krasnopolsky, 2006) and elsewhere, there is a need for studies that can constrain possible prebiotic nitrogen reactions in different environments.

Modern-day hydrothermal systems have varying levels of ammonium in the waters discharged from vents (Vondamm, 1990; Lilley *et al.*, 1993; Sansone and Resing, 1995). In most cases, the source of this reduced nitrogen is assumed to be ammonium released from organic matter decomposition in overlying sediment deposits, which is carried into the vent systems as part of the overall circulation patterns at each site. These high levels of ammonia could be generated by decomposition of organic matter within previously buried sediments (Lilley *et al.*, 1993). However, another possibility exists. Deep marine waters that surround mid-ocean ridge vent systems contain 30–40 micromoles/liter nitrate (Gruber and Sarmiento, 1997). The fate of fixed (*i.e.*, not N_2) nitrogen injected with source waters into vent systems has remained unclear; Brandes *et al.* (1998) suggested that fixed nitrogen would be quantitatively reduced to ammonium, while Summers (1999) suggested that ammonium as well as nitrite would be converted to N_2 and thus lost.

To investigate the possible reactions between nitrogen compounds and minerals under high temperature-pressure conditions, we undertook a series of experiments in which transition metal sulfides and oxides were allowed to react with solutions that contained nitrate, nitrite, or ammonium at a variety of temperatures with the intent of simulating conditions found in marine hydrothermal systems. The initial series, presented here, concentrated on the system N_2-H_2O-inorganic nitrogen-transition metal sulfide, with no added salts for pH or redox control. Addition of buffer salts, use of different dissolved and headspace gas compositions, and application of competing redox-sensitive compounds will be addressed in future articles.

2. Methods

Aqueous solutions of ammonium chloride, sodium nitrite, or sodium nitrate were prepared from reagent quality NH_4Cl, $NaNO_2$, or $NaNO_3$ (Sigma) dissolved in distilled, deionized water. Aqueous solution concentrations were standardized at 50 mmol/L. Initial experiments in which 50 μmol/L solution strengths were used had very high blank:sample ratios and required the use of chemiluminescent techniques to analyze the small amounts of N in each experiment. Mineral phases used were either high-quality natural samples obtained from the National Museum of Natural History or reagent-grade metal oxide (Sigma) (Table 1). Mineral samples were ground under liquid nitrogen to powder, and fines were removed by sieving. Following initial sample preparation, 15 mg aliquots of solution and 5–10 mg mineral were placed in 10 mm (length) by 2 mm (diameter) precleaned gold (99.95% purity) tubes. The gold tubing was cleaned by sequential washes in boiling 10% HNO_3 and 10% HCl solutions, followed by distilled water washes to neutrality and annealing at 900°C for 1 hour. Complete removal of HNO_3 was tested prior to annealing by measuring the final wash solution for NO_3^- content. One tube end was sealed by arc welding prior to sample introduction. During aqueous sample introduction and afterward, the gold tubes remained in a glove bag and were purged with N_2 for >30 minutes. The open tube ends were then crimped and sealed under N_2, followed by immersion in liquid N_2 and subsequent arc welding of the crimped tube head. Crimping of the tube was done to minimize the headspace volume within each tube. Maximum headspace volume was estimated at 25 μl per tube, or about 1 μmol of N_2. Final solution weights were determined by difference (before aqueous solution introduction and after crimp sealing). Sample tubes were stored at 4°C prior to incubation at temperature and pressure.

High-pressure experiments were conducted with use of an internally heated gas pressure device (Yoder, 1950) located at the Geophysical Laboratory. Variations in temperature and pressure were controlled to better than ±1°C and ±2 MPa precision, respectively. Samples were pressurized and then ramp heated (15 minutes) to incubation temperature. At experiment's end, the samples were quenched to <100°C in ~20 seconds, depressurized, and removed from the apparatus. Most experiments were conducted at 50 MPa pressure and 300°C temperature. Typically, sets of 6 capsules—3 experimental variations with duplicates—were run during each experiment. Samples were then frozen and air-shipped to the University of Texas Marine Science Institute for analysis. Sample tubes were weighed, cleaned sequentially in dilute HCl and methanol, and then dried. Any tubes that exhibited more than a 0.1 mg weight change during the incubation and shipping processes were discarded. Afterward, the capsules were frozen in liquid N_2 and then quickly opened to prevent the possibility of rapid degassing. Sample tubes were placed in precleaned 40 ml Teflon-capped vials, and their contents were extracted in 30 ml of distilled, deionized water by vortexing sample contents for 30 seconds and overnight soaking at 4°C. Duplicate 10 ml aliquots of sample were removed for analysis for dissolved NO_2^-, NO_3^-, and NH_4^+ by way of a flow injection analysis nutrient analyzer. Nitrate, nitrite, and ammonia were measured colorometrically on a Lachat Quikchem 800 ion analyzer with computer-controlled sample selection and peak processing via the manufacturer's recommended chemistries (Quikchem method 31-107-04-1-A for nitrate and nitrite, Quikchem method 31-107-06-5-A for ammonium). Typical uncertainties in yields for replicate samples were $\pm5\%$, primarily due to errors in sample extraction and dilution, though in a few cases errors were larger and reached 15%. Detection limits for the methods used are 0.2 μmol/L N for NO_3^-, NO_2^- and NH_4^+.

The elemental compositions of mineral phases both before and after experiments were determined by a JEOL 8900 electron microprobe with 5 wavelength-dispersive spectrometers for quantitative analyses and an energy-dispersive spectrometer for semi-quantitative point analyses of elemental abundances. Standards used included synthetic NiS (mil-

TABLE 1. COMPOSITION OF MINERALS BEFORE AND AFTER REACTIONS

		Atoms per formula unit[a]				*Composition (wt %)*[b]		
Mineral	*Source*	*Fe*	*Ni*	*Cu*	*Zn*	*Before*	*After*	*Nitrogen content wt %*
Magnetite (Fe_3O_4)[c]	Natural Fe_3O_4	3.00	0	0	0	Fe = 72.6 ± 0.2%	Fe = 70.8 ± 0.5%	0.001
Bunsenite (NiO)[d]	NMNH	0	1.00	0	0	Ni = 74.2 ± 0.2%	Ni = 72.0 ± 0.2%	N/D
Cuprite (Cu_2O)	Reagent grade	0	0	2.00	0	Cu = 85 ± 0.5%	Cu = 83 ± 0.8%	N/D
Pyrrhotite ($Fe_{1-x}S$)[e]	NMNH R7568-6	0.908	0	0	0	Fe = 61.3% Fe/S = 0.909 ± 0.006	Fe = 60.6% Fe/S = 0.882 ± 0.004	0.003
Millerie (NiS)[f]	NMNH 128240	0.023	0.977	0	0	Ni = 62.2 ± 0.3% Fe = 1.4 ± 0.3%	Ni = 62.3 ± 0.4% Fe = 1.4 ± 0.3%	0.003
Chalcocite (Cu_2S)[g]	NMNH 63341	0	0	1.999	0.001	Cu = 79.6 ± 0.5% Fe = 0.1%	Cu = 80.4 ± 0.5% Fe = 0.1%	0.002
Chalcopyrite ($CuFe_2$)[h]	NMNH B3139	1.016	0	0.984	0	N/D	N/D	0.002

Experimental conditions: 300°C, 24 hours, 55 MPa.
NMNH, National Museum of Natural History.
[a] A value of 0 indicates < 0.001 atoms per formula unit.
[b] As determind by microprobe (see Methods).
[c] Fumerolic crystals from Valley of 10,000 Smokes, Alaska. Collected by E. G. Zeis in 1919. (E. G. Zeis, National Geographic Society, Continuing Technical Papers, Katmai Series, 1 #3)
[d] Synthetic NiO.
[e] Potosi Mine, Santa Eulalia, Chihuahua, Mexico.
[f] Otter Shoot, Kambalda, Western Australia.
[g] Massive sulfide from Butte, MT. Contains 15% bornite (Cu_5FeS_4).
[h] Victoria Mine, Littfeld, Germany, Contains ~2% pyrite (FeS_2).

lerite), Fe_3O_4 (magnetite) and Cu_2O (cuprite), as well as natural ZnS with 3% Fe. Internal standards were cross calibrated to NIST standards. Mineral samples and standards were embedded in SPI Epoxy Resin/Hardener #1300/1302 and subsequently ground to expose grains for analysis. The N content of each mineral phase was measured with a Carlo Erba NC1500 elemental analyzer. Typical uncertainties in N content for 60 mg samples were 0.5 weight percent.

3. Results

3.1. Nitrate

All the transition metal sulfides surveyed were, to varying extents, capable of reducing NO_3^- to NH_4^+ (Fig. 1, Table 2). Pyrrhotite exhibited the most rapid reactions and the greatest conversion efficiency. Complete conversion was observed within 90 minutes, which was higher than that seen in previous studies on pyrite and other iron sulfides (Blochl *et al.*, 1992; Brandes *et al.*, 1998). Reaction rates, however, should be considered only potential rates, as the experimental systems began far out of equilibrium, and the distilled water solution promoted dissolution of the solid phases. Recoveries in excess of 100% (115%, Table 2) were observed in chalcopyrite, which may have been due to possible hydrolysis of intercalated organics in the mineral matrix. Although blanks containing distilled water plus a mineral phase were run, the presence of a potential oxidant such as nitrate made it difficult to rule out completely an enhanced organic-matter breakdown rate (and thus release of ammonium) in the presence of nitrate and not distilled water. However, measurements of the nitrogen content of the minerals used indicated that there was sufficient nitrogen present in the minerals to account for up to a 10% increase in blank (Table 2). This process was most significant at high pressures (see Section 3.3 below). However, repeat runs with Ar atmospheres instead of N_2 atmospheres within the capsules failed to indicate that any N_2 reduction took place.

The iron-copper sulfide (chalcopyrite) was also effective, which converted 50% of the nitrate to ammonium and exhibited a further increase to 70% over 24 hours. In contrast to the iron sulfides, the nickel sulfide millerite was only able to achieve a 25% conversion efficiency, and all the conversion occurred within the first 90 minutes. Copper sulfide (chalcocite) alone was only able to convert a small amount (<5%) of the nitrate to ammonium. Every sulfide tested removed nitrate from solution very efficiently, and only trace amounts (<1%) remained even within the first 90 minutes of experiments.

The transition metal oxides exhibited markedly different reaction rates. Of the 3 oxides tested, only Fe_3O_4 (magnetite) exhibited any significant ability to reduce nitrate (Table 3, Fig. 1). This ability is seen in the production of NH_3, to about a 20% yield, but more significantly in the loss of nitrate, which was completely removed within 90 minutes at 300°C and 55 MPa (Fig. 2). Nearly all nitrate remained after 24 hours in contact with NiO and Cu_2O, though the latter exhibited a small decrease in nitrate recoveries and a small amount of ammonium (equivalent to a 5% conversion, Table 3).

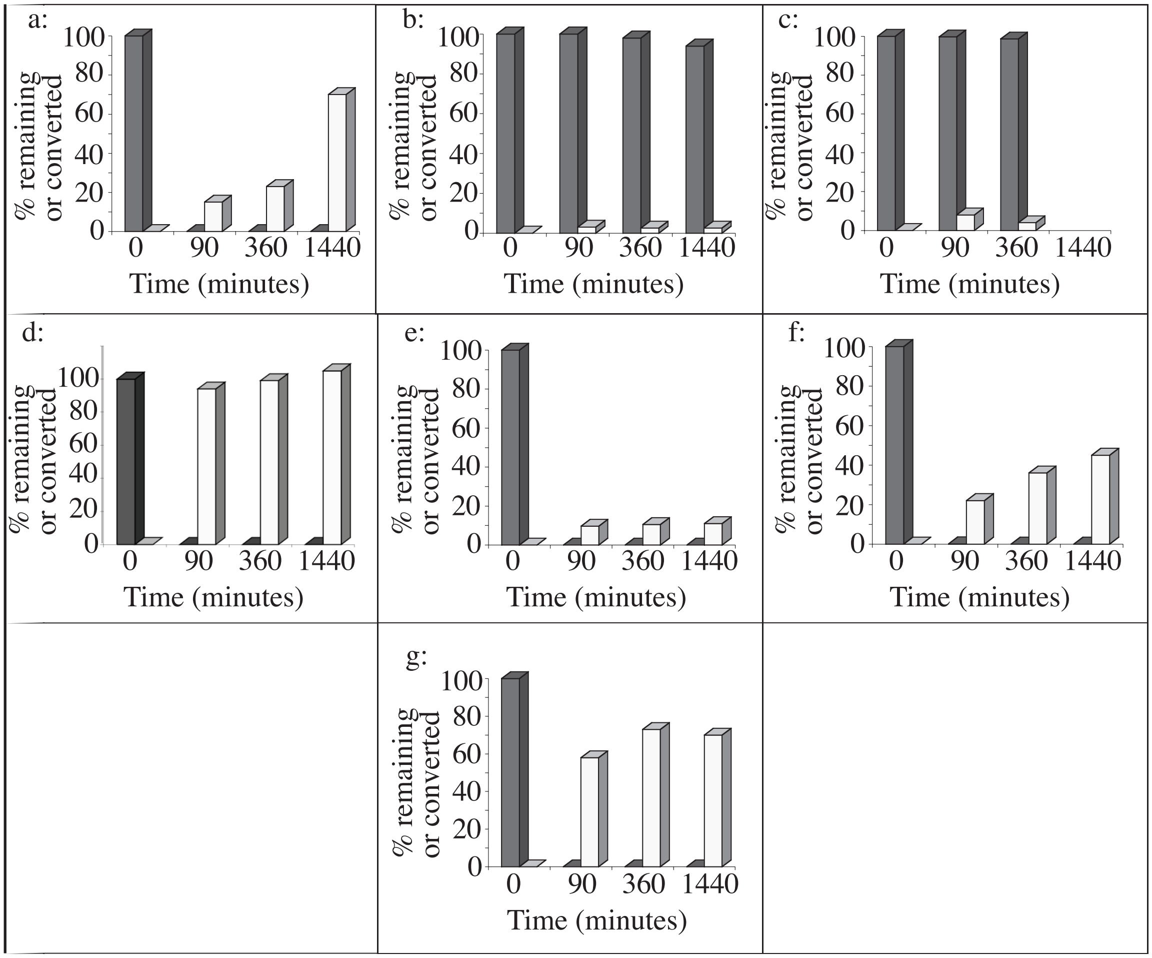

FIG. 1. Conversion of nitrate in contact with transition metal sulfides and oxides at 300°C. Dark bars represent the percentage of nitrate remaining, light bars the percentage of starting nitrate converted to ammonia. Minerals: a, magnetite; b, bunsenite; c, cuprite; d, pyrrhotite; e, millerite; f, chalcocite; g, chalcopyrite.

3.2. Effects of temperature on nitrate reduction

Sulfide-mediated nitrate reduction to ammonium reactions at 300°C primarily were completed by 90 minutes (Fig. 2). Removal of nitrate and nitrite from solution was very rapid, but conversion to ammonium varied among mineral catalysts. Therefore, the effect of decreasing reaction temperatures from 300° to 200°C for 90-minute reactions was investigated. For pyrrhotite, the yield at 200°C was about 10% greater than that which occurred at 300°C (Table 3). For chalcocite it was about 15% greater at the lower temperature, but for millerite the change was negligible. All these differences fall at, or near, the level of uncertainty for these measurements; thus, there does not appear to be a strong temperature effect with these sulfides. In the case of millerite at 200°C, however, there remained considerable (68%) unreacted nitrate. Thus, it is likely that the reaction rate for millerite reduction of nitrate to intermediate, metastable species or ammonium was slower than for the iron or iron-copper sulfides.

3.3. Effect of pressure on nitrate reduction

The effect of raising pressure tenfold (from ~50 MPa to 300–500 MPa) on nitrate reduction varied according to the mineral system (Fig. 3). All high-pressure experiments were run for 6 hours at 300°C, and they were compared to similar 6-hour experiments performed at ~50 MPa. Recoveries in the pyrrhotite-nitrate system remained constant from 98% at 53.9 MPa to 97% at 300 MPa. However, chalcopyrite exhibited increased yields, close to 100% NH_3 recovery, at high pressure. The most significant change was observed in the millerite-nitrate system, where conversion efficiencies increased from 22% at 54 MPa to 90% at 544 MPa.

In the transition metal oxide systems, each metal oxide exhibited a different trend (Fig. 3). Nitrate stability in the magnetite system increased markedly, from no nitrate recovered at 54 MPa to 90% recovery at 520 MPa. However, the bunsenite-nitrate system showed an opposite effect, with lower nitrate recoveries and higher conversion efficiencies to ammonium at higher pressure. This trend was also shown by the cuprite-nitrate system, where conversion efficiencies increased from 4% to 25% at 520 MPa. Thus, the overall oxide conversion patterns seen at 54 MPa, where magnetite>bunsenite>cuprite in effectiveness, were reversed at 520 MPa.

3.4. Comparisons with nitrite

Although the observations made for the nitrate-mineral systems in the previous paragraphs also held true for the ni-

Table 2. Recoveries of Nitrogen Oxides (Nitrate or Nitrite, as a Function of Starting Material) and Ammonium in High-Pressure, High-Temperature Experiments with Transition Metal Sulfides

Mineral	*Starting material*	*Pressure (MPa)*	*Temperature (°C)*	*Time (min)*	*% N oxides*	*% ammonium*
Pyrrhotite	Nitrate	0.1	25	0	99.8	0.0
Pyrrhotite	Nitrate	0.1	25	0	99.7	0.0
Pyrrhotite	Nitrate	55.6	300	90	0.0	94.5
Pyrrhotite	Nitrate	55.6	300	90	0.0	94.1
Pyrrhotite	Nitrate	53.9	300	360	0.0	97.2
Pyrrhotite	Nitrate	53.9	300	360	0.0	99.0
Pyrrhotite	Nitrate	53.7	300	360	0.0	99.0
Pyrrhotite	Nitrate	53.7	300	1440	0.0	103
Pyrrhotite	Nitrate	53.7	300	1440	0.0	106
Pyrrhotite	Nitrate	51.9	200	90	0.0	100
Pyrrhotite	Nitrate	51.9	200	90	0.0	103
Pyrrhotite	Nitrate	50.9	250	90	0.0	90.9
Pyrrhotite	Nitrate	50.9	250	90	0.0	94.5
Pyrrhotite	Nitrate	300	300	360	0.2	95.8
Pyrrhotite	Nitrate	300	300	360	0.2	93.2
Pyrrhotite	Nitrate:Ar	300	300	360	0.1	101
Pyrrhotite	Nitrate:Ar	300	300	360	0.0	95.7
Pyrrhotite	Nitrite	0.1	25	0	100.1	0.0
Pyrrhotite	Nitrite	0.1	25	0	100.0	0.0
Pyrrhotite	Nitrite	55.6	300	90	0.0	46.6
Pyrrhotite	Nitrite	55.6	300	90	0.0	46.3
Pyrrhotite	Nitrite	55.3	300	360	0.0	55.0
Pyrrhotite	Nitrite	55.3	300	360	0.0	56.3
Pyrrhotite	Nitrite	56.5	300	1440	0.0	83.3
Pyrrhotite	Nitrite	56.5	300	1440	0.0	78.2
Pyrrhotite	Ammonium	0.1	25	0	0.0	100
Pyrrhotite	Ammonium	0.1	25	0	0.0	100
Pyrrhotite	Ammonium	55.6	300	90	0.0	100
Pyrrhotite	Ammonium	55.6	300	90	0.0	100
Pyrrhotite	Ammonium	55.3	300	360	0.0	99.8
Pyrrhotite	Ammonium	55.3	300	360	0.0	100
Pyrrhotite	Ammonium	56.5	300	1440	0.0	99.4
Pyrrhotite	Ammonium	56.5	300	1440	0.0	100
Millerite	Nitrate	0.1	25	0	100.1	100
Millerite	Nitrate	0.1	25	0	100.0	100
Millerite	Nitrate	51.9	300	90	0.0	27.1
Millerite	Nitrate	51.9	300	90	0.0	24.1
Millerite	Nitrate	56.3	300	360	0.0	21.3
Millerite	Nitrate	56.3	300	360	0.0	23.6
Millerite	Nitrate	52.0	300	1440	0.0	24.1
Millerite	Nitrate	52.0	300	1440	0.0	24.8
Millerite	Nitrate	51.9	200	90	66.3	30.3
Millerite	Nitrate	51.9	200	90	68.7	27.9
Millerite	Nitrate	50.9	250	90	0.0	34.8
Millerite	Nitrate	50.9	250	90	0.0	29.7
Millerite	Nitrate	542.2	300	360	0.0	95.8
Millerite	Nitrate	542.2	300	360	0.0	100
Millerite	Nitrite	0.1	25	0	100.0	0.0
Millerite	Nitrite	0.1	25	0	100.0	0.0
Millerite	Nitrite	51.9	300	90	0.0	9.5
Millerite	Nitrite	51.9	300	90	0.0	10.2
Millerite	Nitrite	56.3	300	360	0.0	10.4
Millerite	Nitrite	56.3	300	360	0.0	10.7
Millerite	Nitrite	52.0	300	1440	0.0	11.0
Millerite	Nitrite	52.0	300	1440	0.0	11.7
Millerite	Ammonium	0.1	25	0	0.0	100
Millerite	Ammonium	0.1	25	0	0.0	100
Millerite	Ammonium	51.9	300	90	0.0	91.9
Millerite	Ammonium	51.9	300	90	0.0	93.1
Millerite	Ammonium	56.3	300	360	0.0	79.4
Millerite	Ammonium	56.3	300	360	0.0	84.8

(*continued*)

Table 2. Recoveries of Nitrogen Oxides (Nitrate or Nitrite, as a Function of Starting Material) and Ammonium in High-Pressure, High-Temperature Experiments with Transition Metal Sulfides (Cont'd)

Mineral	*Starting material*	*Pressure (MPa)*	*Temperature (°C)*	*Time (min)*	*% N oxides*	*% ammonium*
Millerite	Ammonium	52.0	300	1440	0.0	91.8
Millerite	Ammonium	52.0	300	1440	0.0	94.5
Chalcocite	Nitrate	0.1	25	0	100.0	0.0
Chalcocite	Nitrate	0.1	25	0	100.2	0.0
Chalcocite	Nitrate	51.5	300	90	0.1	23.5
Chalcocite	Nitrate	51.5	300	90	0.1	23.0
Chalcocite	Nitrate	55.9	300	360	0.0	36.5
Chalcocite	Nitrate	55.9	300	360	0.0	34.7
Chalcocite	Nitrate	53.2	300	1440	0.0	45.3
Chalcocite	Nitrate	53.2	300	1440	0.0	46.3
Chalcocite	Nitrite	0.1	25	0	100.0	0.0
Chalcocite	Nitrite	0.1	25	0	100.0	0.0
Chalcocite	Nitrite	51.5	300	90	0.1	3.1
Chalcocite	Nitrite	51.5	300	90	0.1	3.0
Chalcocite	Nitrite	55.9	300	360	0.0	4.6
Chalcocite	Nitrite	55.9	300	360	0.0	5.1
Chalcocite	Nitrite	53.2	300	1440	0.0	8.2
Chalcocite	Nitrite	53.2	300	1440	0.0	7.8
Chalcocite	Ammonium	0.1	25	0	0.0	100
Chalcocite	Ammonium	0.1	25	0	0.0	100
Chalcocite	Ammonium	51.5	300	90	0.1	91.5
Chalcocite	Ammonium	51.5	300	90	0.1	85.9
Chalcocite	Ammonium	55.9	300	360	0.0	82.8
Chalcocite	Ammonium	55.9	300	360	0.0	82.9
Chalcocite	Ammonium	53.2	300	1440	0.0	80.2
Chalcocite	Ammonium	53.2	300	1440	0.0	80.0
Chalcopyrite	Nitrate	0.1	25	0	100.0	0.0
Chalcopyrite	Nitrate	0.1	25	0	100.0	0.0
Chalcopyrite	Nitrate	55.6	300	90	0.0	58.0
Chalcopyrite	Nitrate	55.6	300	90	0.0	57.9
Chalcopyrite	Nitrate	55.3	300	360	0.0	73.8
Chalcopyrite	Nitrate	55.3	300	360	0.0	73.1
Chalcopyrite	Nitrate	53.7	300	1440	0.0	69.4
Chalcopyrite	Nitrate	53.7	300	1440	0.0	70.1
Chalcopyrite	Nitrate	51.9	200	90	0.0	77.4
Chalcopyrite	Nitrate	51.9	200	90	0.0	79.7
Chalcopyrite	Nitrate	50.9	250	90	0.0	56.4
Chalcopyrite	Nitrate	50.9	250	90	0.0	58.6
Chalcopyrite	Nitrate	542.2	300	360	0.0	98.3
Chalcopyrite	Nitrate	542.2	300	360	0.0	95.1
Chalcopyrite	Nitrite	0.1	25	0	100.0	0.0
Chalcopyrite	Nitrite	0.1	25	0	100.0	0.0
Chalcopyrite	Nitrite	55.6	300	90	0.0	15.8
Chalcopyrite	Nitrite	55.6	300	90	0.0	13.8
Chalcopyrite	Nitrite	55.3	300	360	0.0	22.8
Chalcopyrite	Nitrite	55.3	300	360	0.0	22.8
Chalcopyrite	Nitrite	56.5	300	1440	0.0	26.2
Chalcopyrite	Nitrite	56.5	300	1440	0.0	31.4
Chalcopyrite	Ammonium	0.1	25	0	0.0	100
Chalcopyrite	Ammonium	0.1	25	0	0.0	100
Chalcopyrite	Ammonium	55.6	300	90	0.0	99.0
Chalcopyrite	Ammonium	55.6	300	90	0.0	98.5
Chalcopyrite	Ammonium	55.3	300	360	0.0	109
Chalcopyrite	Ammonium	55.3	300	360	0.0	108
Chalcopyrite	Ammonium	53.7	300	1440	0.0	110
Chalcopyrite	Ammonium	53.7	300	1440	0.0	112

All reactions took place under an N_2 atmosphere unless otherwise indicated.

TABLE 3. RECOVERIES OF NITROGEN OXIDES (NITRATE OR NITRITE, AS A FUNCTION OF STARTING MATERIAL) AND AMMONIUM IN HIGH-PRESSURE, HIGH-TEMPERATURE EXPERIMENTS WITH TRANSITION METAL OXIDES

Mineral	*Starting material*	*Pressure (MPa)*	*Temperature (°C)*	*Time (min)*	*% N oxides*	*% ammonium*
Magnetite	Nitrate	0.1	0	0	99.0	0.0
Magnetite	Nitrate	0.1	0	0	101.0	0.0
Magnetite	Nitrate	55.0	300	90	0.0	12.3
Magnetite	Nitrate	55.0	300	90	0.0	17.5
Magnetite	Nitrate	53.9	300	360	0.0	19.6
Magnetite	Nitrate	53.9	300	360	0.0	27.8
Magnetite	Nitrate	56.1	300	1440	0.0	21.8
Magnetite	Nitrate	56.1	300	1440	0.0	24.1
Magnetite	Nitrate	520.1	300	360	86.4	12.5
Magnetite	Nitrate	520.1	300	360	90.6	9.6
Magnetite	Nitrite	0.1	0	0	100.0	0.0
Magnetite	Nitrite	0.1	0	0	98.0	0.0
Magnetite	Nitrite	55.0	300	90	2.3	6.6
Magnetite	Nitrite	55.0	300	90	2.2	3.7
Magnetite	Nitrite	53.9	300	360	1.2	8.0
Magnetite	Nitrite	53.9	300	360	1.5	13.9
Magnetite	Nitrite	56.1	300	1440	0.2	20.1
Magnetite	Nitrite	56.1	300	1440	0.1	22.1
Magnetite	Ammonium	0.1	0	0	100.0	0.0
Magnetite	Ammonium	0.1	0	0	97.0	0.0
Magnetite	Ammonium	55.0	300	90	0.2	46.2
Magnetite	Ammonium	53.9	300	360	0.2	46.0
Magnetite	Ammonium	53.9	300	360	0.6	62.8
Magnetite	Ammonium	56.1	300	1440	0.3	89.7
Magnetite	Ammonium	56.1	300	1440	0.3	74.6
Bunsenite	Nitrate	0.1	0	0	100.0	0.0
Bunsenite	Nitrate	0.1	0	0	100.0	0.0
Bunsenite	Nitrate	55.2	300	90	101.6	5.9
Bunsenite	Nitrate	55.2	300	90	100.0	3.4
Bunsenite	Nitrate	53.9	300	360	98.0	3.0
Bunsenite	Nitrate	53.9	300	360	99.0	6.6
Bunsenite	Nitrate	53.7	300	1440	94.2	1.9
Bunsenite	Nitrate	53.7	300	1440	95.0	3.0
Bunsenite	Nitrate	520.1	300	360	89.7	13.2
Bunsenite	Nitrate	520.1	300	360	90.1	16.1
Bunsenite	Nitrite	0.1	0	0	100.0	0.0
Bunsenite	Nitrite	0.1	0	0	100.0	0.0
Bunsenite	Nitrite	55.0	300	90	2.3	6.6
Bunsenite	Nitrite	55.0	300	90	2.1	3.7
Bunsenite	Nitrite	53.9	300	360	1.2	8.0
Bunsenite	Nitrite	53.9	300	360	1.5	13.9
Bunsenite	Nitrite	56.1	300	1440	0.2	20.1
Bunsenite	Nitrite	56.1	300	1440	0.1	8.1
Bunsenite	Ammonium	0.1	0	0	0.0	97.0
Bunsenite	Ammonium	0.1	0	0	0.0	99.0
Bunsenite	Ammonium	53.7	300	90	0.0	91.6
Bunsenite	Ammonium	53.7	300	90	0.0	94.0
Bunsenite	Ammonium	56.6	300	360	0.7	76.9
Bunsenite	Ammonium	56.6	300	360	0.1	78.5
Bunsenite	Ammonium	56.1	300	1440	0.4	77.4
Bunsenite	Ammonium	56.1	300	1440	0.7	74.2
Cuprite	Nitrate	0.1	0	0	100.0	0.0
Cuprite	Nitrate	0.1	0	0	100.0	0.0
Cuprite	Nitrate	55.2	300	90	99.2	7.3
Cuprite	Nitrate	55.2	300	90	99.7	9.4
Cuprite	Nitrate	56.6	300	360	98.7	3.4
Cuprite	Nitrate	56.6	300	360	98.8	4.6
Cuprite	Nitrate	520.1	300	360	81.8	23.6
Cuprite	Nitrate	520.1	300	360	79.4	26.8
Cuprite	Nitrite	0.1	0	0	99.0	0.0
Cuprite	Nitrite	0.1	0	0	101.0	0.0

(*continued*)

TABLE 3. RECOVERIES OF NITROGEN OXIDES (NITRATE OR NITRITE, AS A FUNCTION OF STARTING MATERIAL) AND AMMONIUM IN HIGH-PRESSURE, HIGH-TEMPERATURE EXPERIMENTS WITH TRANSITION METAL OXIDES (CONT'D)

Mineral	*Starting material*	*Pressure (MPa)*	*Temperature (°C)*	*Time (min)*	*% N oxides*	*% ammonium*
Cuprite	Nitrite	55.2	300	90	99.3	0.0
Cuprite	Nitrite	55.2	300	90	98.0	1.0
Cuprite	Nitrite	56.6	300	360	98.1	0.3
Cuprite	Nitrite	56.6	300	360	97.8	1.0
Cuprite	Ammonium	0.1	0	0	0.0	100
Cuprite	Ammonium	0.1	0	0	0.0	99.7
Cuprite	Ammonium	55.2	300	90	0.0	85.4
Cuprite	Ammonium	55.2	300	90	0.0	85.9
Cuprite	Ammonium	56.6	300	360	0.8	81.0
Cuprite	Ammonium	56.6	300	360	0.3	85.8

trite-mineral systems, we observed several differences. In general, conversion efficiencies were lower for the nitrite-mineral systems (Fig. 2). Of the transition metal sulfides, pyrrhotite was again the most effective in converting nitrite to ammonium, to 46% in 90 minutes and increasing to 80% over 24 hours. Millerite converted 10% of the nitrite to ammonium within 90 minutes, but the reaction did not appear to continue, while chalcopyrite converted 15% within 90 minutes and rose to 30% over 24 hours. Copper sulfide exhibited about the same low efficiency for nitrite conversion as nitrate. In a similar manner as nitrate, nitrite was removed from these systems rapidly with no traces left after 90 minutes.

Nitrite reactivity in the oxide mineral systems showed surprising variability. Although magnetite showed efficacy in converting nitrite to ammonium (20%), bunsenite exhibited

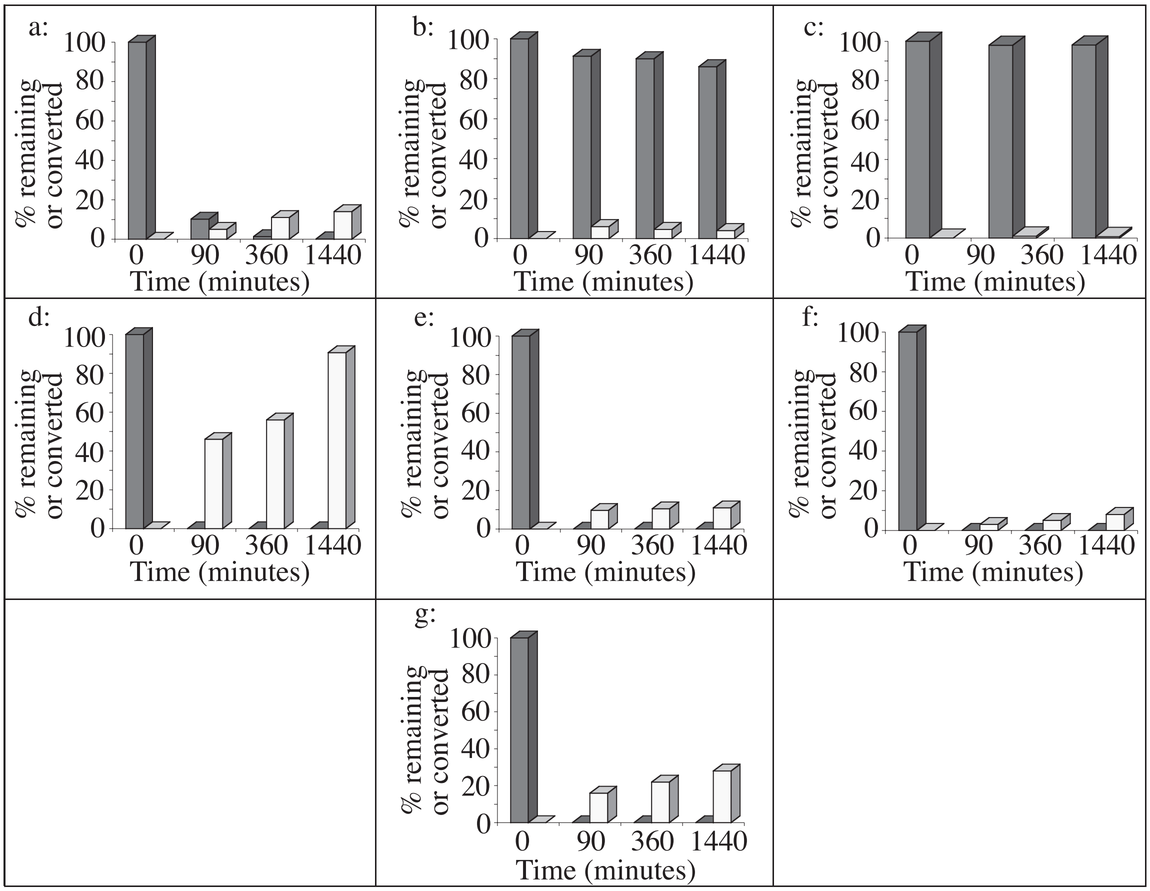

FIG. 2. Conversion of nitrite in contact with transition metal sulfides and oxides at 300°C. Dark bars represent the percentage of nitrite remaining, light bars the percentage of starting nitrate converted to ammonia. Minerals: a, magnetite; b, bunsenite; c, cuprite; d, pyrrhotite; e, millerite; f, chalcocite; g, chalcopyrite.

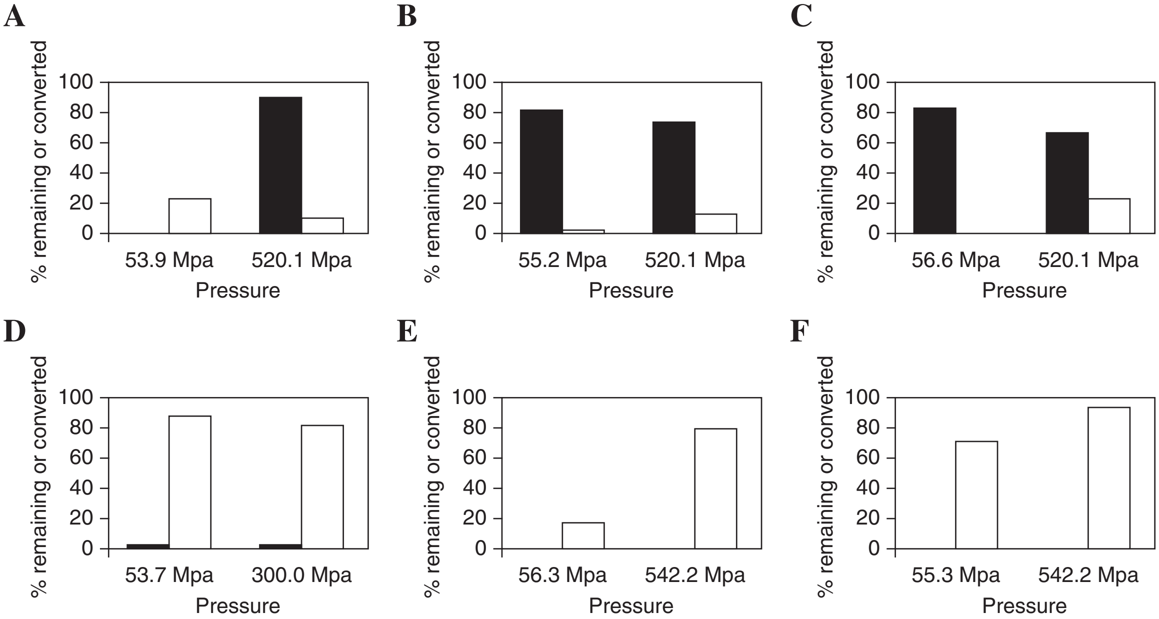

FIG. 3. Influence of pressure upon nitrate conversion and stability in contact with transition metal sulfides and oxides at 300°C. Dark bars represent the percentage of nitrate remaining after 6 hours, light bars the percentage of nitrate converted to ammonia. Minerals: a, magnetite; b, bunsenite; c, cuprite; d, pyrrhotite; e, millerite; f, chalcopyrite.

an equal ability to convert some nitrite to ammonium (15–20%). This result was unexpected, given the behavior of the nitrate system. Cuprite was less reactive with nitrite; over 80% of the nitrogen oxide was recovered after 6 hours. In comparison, both magnetite and bunsenite removed nearly all nitrite from solution within 90 minutes.

3.5. Ammonium stability

Ammonium proved stable in contact with all transition metal sulfides examined (Fig. 4, Table 2). The largest losses were in the millerite and chalcocite systems, with 10% and 15% losses, respectively. Recovery of ammonium averaged 100% after 24 hours in the pyrrhotite-ammonium system. Chalcopyrite-ammonium experiments had higher-than-predicted yields, increasing to 111% at 24 hours, which may have been due to hydrolysis of intercalated organic matter from the mineral phase. The amount of nitrogen present within the minerals, if converted to ammonium, would have been capable of raising apparent yields by 8–10%. In either case, ammonium appears to be stable in the presence of these sulfides.

The transition metal oxide systems exhibited a slight to moderate reactivity toward ammonium (Fig. 4, Table 3). Although only trace amounts of nitrogen oxides were found in the experiments (entirely nitrate), recoveries of ammonium were 90% or less in all three systems studied. Magnetite removed ~50% of ammonium within 90 minutes, though recoveries increased over 24 hours to >70%. Bunsenite converted 25% of ammonium to other forms, presumably gaseous, as only minor (<1%) amounts of nitrate were measured. Recoveries steadily dropped over time, which suggests that more extensive oxidation of ammonium is possible (Fig. 4). Cuprite converted 15% of ammonium to other forms, again with only minor amounts of nitrate formed.

4. Discussion

The stability or reduction/transformation of inorganic compounds under high temperature/high pressure conditions has been rarely studied. Blochl *et al.* (1992) examined the reduction of nitrate with iron sulfide and found that it could be reduced under conditions of 100°C and ambient pressure. They reported ammonium yields of about 41% when H_2S was included in a pyrrhotite-nitrate system but less than 15% when H_2S was omitted. The final concentrations of nitrate in their systems were not reported. Brandes *et al.* (1998) examined a variety of possible nitrogen reduction reactions under hydrothermal conditions and noted the efficacy of pyrrhotite in reducing nitrate even without H_2S added. Summers (2005) also noted the ability of FeS to reduce nitrate partially and nitrite completely under room-temperature conditions. The ability of pyrrhotite to reduce nitrate under elevated temperature (>150°C), high-pressure conditions as reported here is thus not surprising. The reputed requirement of H_2S for nitrate reduction in FeS systems (Blochl *et al.*, 1992) may have been met by partial dissolution of the mineral sulfide:

$$FeS + 2H_2O \rightarrow Fe^{2+} + H_2S + 2OH^- \qquad [1]$$

This reaction may be enhanced at high pressure, and we observed significant dissolution of pyrrhotite and millerite (as well as the bornite minor phase in the chalcocite samples) in our experiments. Often only 1 or 2 grains of solid phase remained in the capsules after incubation. If released, Fe^{2+} is effective as a reductant for nitrite (Summers and Chang, 1993; Summers and Lerner, 1998) and presumably for nitrate as well. However, dissolved ferrous iron may not be the only or primary oxidant in these systems. Every mineral studied showed significant signs of oxidation, either by a decreased metal:S ratio or by a decreased metal content

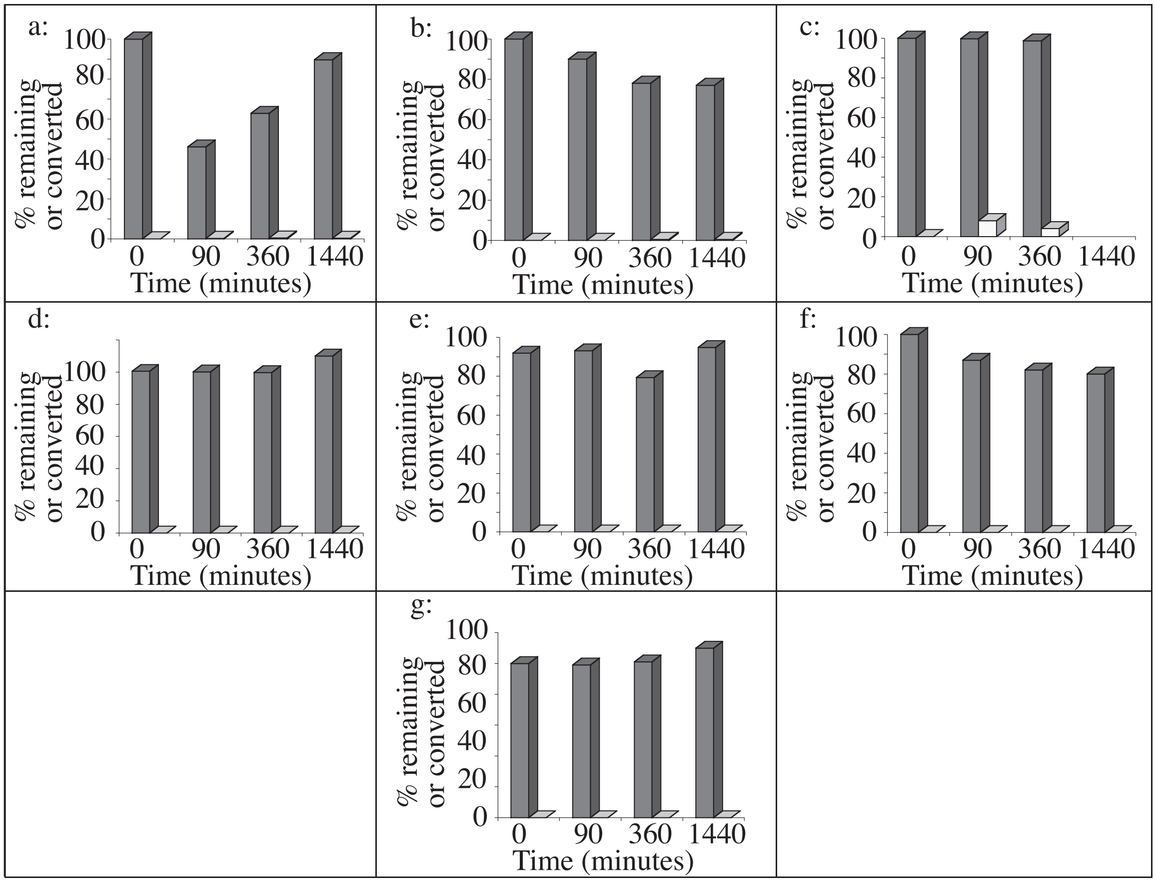

FIG. 4. Stability of ammonia in contact with transition metal sulfides and oxides at 300°C. Dark bars represent the percentage of ammonia remaining, light bars the percentage of starting ammonia converted to nitrate + nitrite. Minerals: a, magnetite; b, bunsenite; c, cuprite; d, pyrrhotite; e, millerite; f, chalcocite; g, chalcopyrite.

(Table 1). In addition, if release of Fe^{2+} were the only important factor, then the nickel sulfide millerite and the copper sulfide chalcocite should have shown negligible conversion efficiencies. In fact, both mineral phases were capable of reducing a significant (>25%) percentage of nitrate. Both the observed increases in mineral oxidation and the apparent lack of correlation between iron content and nitrification ability indicate that all the mineral sulfide phases are capable of nitrate and, to a lesser extent, nitrite reduction. A generalized reaction can be written:

$$8MeS + NO_3^- + 2H_2O \rightarrow 4MeS_2 + 4MeO + N\,H_3 + OH^- \quad [2]$$

or, if no oxidized solid phase was formed,

$$8MeS + NO_3^- + 4H_2O \rightarrow 4MeS_2 + 4Me^{2\,+} + NH_3 + 5OH^- + O_2 \quad [3]$$

where Me represents a transition metal. Similarly, the oxide minerals were also observed to become more oxidized during our experimental runs, (*e.g.*, formation of hematite from magnetite). Our oxide phases were a contained mixture of metal oxidation states; thus, some reducing power was available in these systems, though to a lesser extent than for the sulfide systems. The composition of the nickel and copper oxides also became more oxygen rich during our experiments. With this information, a generalized reaction between metal oxides and nitrate can be written:

$$4MeO + 2H_2O + NO_3^- \rightarrow NH_3 + 4MeO_2 + OH^- \quad [4]$$

or

$$4Me_2O + 2H_2O + NO_3^- \rightarrow NH_3 + 8MeO + OH^- \quad [5]$$

However, it is clear that under the reaction condition studied, only iron exhibited any efficacy in nitrate reduction (Fig. 1). In our experiments, the final metal phases (both sulfide and oxide) observed were a mixture of more oxidized phases and some remaining reduced phase. For example, the final composition of the copper-oxide solid phase became slightly more metal poor and thus oxygen rich, though the shift was only a few percent (Table 1). This small change indicated that there remained additional reducing ability within our experiments; thus, the incomplete reduction observed in most experiments was not due to exhaustion of the mineral's reducing capacity.

Pressure played a complex role in nitrate and N_2 reduction. In some systems (chalcopyrite), a tenfold increase in pressure appeared to increase yields slightly. Most of this increase was likely due to an increased level of release of organic N contained in our mineral catalysts, perhaps through increased mineral dissolution. Repeated experiments with Ar instead of N_2 in the pyrrhotite-nitrate-water system showed no difference in reaction yields. However, in others (bunsenite, cuprite, millerite) it enhanced nitrate reduction to a greater extent than expected from blank enhancement alone, while in 2 cases (magnetite, chalcopyrite) it decreased nitrate reduction. Additionally, in the magnetite system, nitrate stability (as opposed to conversion to ammonium) was significantly enhanced at high pressure. It is possible that nitrate is stable (or, at least, reaction rates are extremely slow) at a high enough pressure in the presence of magnetite at 300°C. Although nitrate-bearing fluids subducted into hydrothermal systems likely encounter hot reducing conditions prior to reaching depths that correspond to 544 MPa (~4000–5000 m below sea level) on Earth and thus are likely be reduced prior to this point, these results underscore the complexity of nitrate-mineral interactions and indicate that further research is warranted.

Conversion of nitrite was lower than nitrate in all experimental systems. On one hand, this is expected, as nitrite is more reactive than nitrate. However, it can be considered somewhat surprising, as the reduction of nitrate would logically require the production of nitrite as an intermediate step. Biological nitrate reduction proceeds through several steps:

$$NO_3{}^- \rightarrow NO_2{}^- \rightarrow NO \rightarrow NH_4\,OH \rightarrow NH_3 \qquad [6]$$

Thus, it could be assumed that nitrite would show higher yields than the nitrate systems. It is possible that the mineral-mediated reduction of nitrate bypasses the first 2 steps, perhaps proceeding with an initial hydrogenation step as proposed by Blochl *et al.* (1992). Alternatively, nitrite may be destroyed prior to reduction by a competing reaction to a gaseous species that we did not measure. One possibility is reaction with ammonia to yield N_2 (Rubin *et al.,* 1987). In this case, the higher yields in nitrate reduction may be explained if any nitrite formed on mineral surfaces or in solution is immediately reduced further prior to contact with ammonium. If nitrate or nitrite is reduced to N_2O in the presence of Fe^{2+}, it will be reduced to ammonia (Moraghan and Buresh, 1977); so the early stages of reduction are apparently the most critical in determining final yields. In spite of possible conversion to N_2, we observed 50% yields of ammonia after 90 minutes with the pyrrhotite-nitrite system; so even though a significant amount of nitrite may be lost prior to reduction, a significant fraction is reduced in the iron sulfide system.

Ammonia stability within hydrothermal systems has remained unstudied. Summers and Chang (1993) and Summers and Lerner (1998) assumed, based upon possible chemical reactions between nitrite and ammonium to produce N_2 (Rubin *et al.,* 1987), complete destruction of any ammonium and nitrite carried into vent systems. Results of the mineral-ammonia experiments presented here suggest little or no destruction under our conditions. In the case of magnetite, the eventual yield is close to 100%, though there are some early losses. Ammonia in this case may be partially oxidized by Fe^{3+} before final reduction by Fe^{2+} or other reducing agent (Fe^{2+} is also present in magnetite). Although the systems were unbuffered, the dissolution of sulfides would presumably raise the pH above that of neutrality. In this case, the sulfide surfaces are likely to be negatively charged, and inorganic N tilted toward ammonia. Thus, little surface adsorption would be expected.

It is thought that much of the high-temperature circulation in mid-ocean ridge systems originates within 10 km of the ridge crests (Lowell and Rona, 1985; Lowell, 1991; Sleep, 1991; Lowell *et al.,* 1995). These geologically recent environments generally have very little sediment coverage; thus, the pool of organic matter (presumably dissolved) and ammonium (presumably released from organic matter diagenesis under suboxic conditions) that enter into hydrothermal systems through the recharge zone is limited. Although many hydrothermal systems exhibit relatively low ammonium concentrations ($<10\ \mu M$) in vent waters (Vondamm, 1990), there are exceptions to this (Lilley *et al.,* 1993), and the origins of this reduced nitrogen are still unclear. The results presented here suggest a potential for a high degree of conversion of this nitrate to ammonia; however, there have to be loss mechanisms to account for the discrepancy between inputs of fixed dissolved nitrogen that are about 3–4 times greater than nitrogen effluxes. Certainly, some of the ammonium that leaves these systems will be oxidized or utilized by bacteria (Mevel and Prieur, 1998) and, thus, not a component of the efflux. Some of the nitrate that enters these systems may be denitrified or otherwise consumed and lost. Some of the ammonium may react with crustal minerals to form a variety of stable minerals (Hallam and Eugster, 1976). Finally, some conversions may be difficult to examine directly or will be complicated by biological activity. Hydrothermal vent systems contain a variety of environments where hot (>300°C) sulfide-rich waters are in close contact with nitrate-rich, cold oceanic bottom waters. Examples of these environments are the trapped high-temperature pools under flanges found at a number of Juan de Fuca sites (Delaney *et al.,* 1992; Kerr and Turner, 1996; Kerr, 1997). Any nitrate entrained into these pools will be rapidly converted to ammonium, especially if iron sulfides or reduced iron are present. There are also a whole host of "warm" off-axis hydrothermal systems (Mottl *et al.,* 1998; Kelley *et al.,* 2001, 2005; Dias and Barriga 2006; Kristall *et al.,* 2006) that are just now beginning to be explored and understood. Such environments may have provided milder conditions for N reduction or perhaps improved survivability for labile organic compounds

Another issue is that of the prebiotic and protobiotic nitrogen cycle. Given our current understanding of the Hadean Earth's atmosphere, the predominant form of nitrogen in that environment was probably N_2 (Kasting, 1982; Navarro-Gonzalez *et al.,* 2001a). Conversion of this N_2 into nitrogen oxides by lightning may have been a major source of reactive nitrogen (Mancinelli and McKay, 1988; Navarro-Gonzalez *et al.,* 1998, 2001a, 2001b). Nearly all studies of prebiotic chemistry, however, have presumed the availability of ammonia (if not CN), rather than oxidized nitrogen, as a necessary starting ingredient. The extensive nitrate and nitrite reduction and concomitant ammonia stability under hydrothermal conditions presented here suggest that minerals within Earth could have played an important role in the prebiotic nitrogen cycle. Modern Earth's oceanic nitrogen cycle is dominated by biologically catalyzed nitrogen fixation, balanced by denitrification in marine sediments and suboxic waters (Codispoti *et al.,* 2001; Brandes and Devol, 2002). Prebiotic Earth's nitrogen cycle was likely dominated by N_2 oxidation by lightning, which was augmented with nitrogen reduction within Earth (Brandes *et al.,* 1998; Schoonen and Yong, 2001; Dorr *et al.,* 2003) and perhaps organic nitrogen from meteorites (Tajika and Matsui, 1993). If hydrothermal vents were not a major sink for reduced or oxidized nitrogen, as suggested here, then the primary loss terms would have been photolytic oxidation of ammonia to N_2 in the upper atmosphere (Kasting, 1982). If indeed hydrothermal vents converted most nitrogen oxides to ammonia and passed what ammonia that entered the systems through without major loss, then hydrothermal systems would have contained the highest ammonium concentrations found on prebiotic Earth. The critical environments for the synthesis of early life from prebiotic precursors (whether on Earth or elsewhere) remain a subject of speculation. However, the rates or production of nitrogenous compounds may have been highest in hydrothermal systems, where a combination of high concentrations of starting materials (NH_3, CO, CO_2), reducing conditions, and a wide variety of mineral catalysts would have led to a conducive environment for synthesis. It is presently thought that biologically mediated nitrogen fixation did not arise until atmospheric CO_2 levels fell enough to preclude major formation of NO_x compounds (Navarro-Gonzalez *et al.,* 2001b), about 2 billion years ago. Between the presumed rise of organisms 3.5–3.8 billion years ago and the rise of N_2 fixation, the ecology of early Earth would have been potentially limited by fixed nitrogen (phosphorous being available under suboxic conditions and CO_2 being non-limiting). Hydrothermal vents, even if nitrogen oxides were taken up by early photosynthesizers, may have operated in a similar manner to modern systems, where reduced nitrogen from a variety of sources is concentrated in vent waters. Thus, vents may have functioned as "hot spots" of activity simply because a major limiting nutrient was more easily available at these locations than elsewhere. This could have been even more important on other planets such as Mars, where nitrogen is less common than on Earth (Klingler *et al.,* 1989; Capone *et al.,* 2006).

5. Conclusions

The availability of reduced nitrogen to early Earth played several possible critical functions. The presence of ammonium makes the production of amino acids and other critical organic molecules possible without the presence of intrinsically labile cyano compounds (Cole *et al.,* 1994; Hafenbradl *et al.,* 1995). If the primary sources of fixed (*i.e.,* not N_2) forms of nitrogen to prebiotic and earliest biotic Earth were nitrogen oxides from atmospheric lightning discharges, then the problem becomes: how are these compounds reduced in the absence of evolved biological nitrogen reduction in organisms? It is equally important to understand the possible destructive pathways for fixed nitrogen. Given the ubiquitous presence of hydrothermal vent systems on early Earth (Chyba, 1993), the question has been posed whether these systems were sinks or sources for fixed nitrogen. The results presented here suggest that hydrothermal systems, especially those rich in iron minerals, will reduce oxidized nitrogen forms and pass ammonia through them without major losses. Although the conversion patterns are complex and dependent upon temperature and pressure, the overall picture is of a facile ability of transition metal sulfides and even some oxides to reduce nitrate to ammonia.

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