A biomarker based on the stable isotopes of nickel

Vyllinniskii Cameron[a,b,1], Derek Vance[b], Corey Archer[b], and Christopher H. House[a]

[a]Department of Geosciences and Penn State Astrobiology Research Center, The Pennsylvania State University, University Park, PA 16802; and [b]Bristol Isotope Group, Department of Earth Sciences, University of Bristol, Bristol BS8 1RJ, United Kingdom

Edited by Donald E. Canfield, University of Southern Denmark, Odense M., Denmark, and approved May 20, 2009 (received for review January 22, 2009)

The new stable isotope systems of transition metals are increasingly used to understand and quantify the impact of primitive microbial metabolisms on the modern and ancient Earth. To date, little effort has been expended on nickel (Ni) isotopes but there are good reasons to believe that this system may be more straightforward, and useful in this respect, than some others. Here, we present Ni stable isotope data for abiotic terrestrial samples and pure cultures of methanogens. The dataset for rocks reveals little isotopic variability and provides a lithologic baseline for terrestrial Ni isotope studies. In contrast, methanogens assimilate the light isotopes, yielding residual media with a complementary heavy isotopic enrichment. Methanogenesis may have evolved during or before the Archean, when methane could have been key to Earth's early systems. Our data suggest significant potential in Ni stable isotopes for identifying and quantifying methanogenesis on the early planet. Additionally, Ni stable isotope fractionation may well prove to be the fundamental unambiguous trace metal biomarker for methanogens.

biological fractionation | early life | methanogens | trace metal

Life has had a profound impact on the surface of the Earth, with perhaps the most obvious expression being the advent of oxygenic photosynthesis leading to the evolution of an oxidised atmosphere and oceans (1, 2). However, the microorganisms and metabolisms mediating oxygenic photosynthesis probably evolved relatively late in the Archean (3–5) and other metabolisms, such as methanogenesis, likely dominated both the early history of the biosphere itself (6–8) and the surface chemistry of the planet (9, 10). For example, atmospheric methane has been suggested as the dominant greenhouse gas before 2.3 Ga, before the evolution of oxic conditions (10). This inference is based on models of solar luminosity, atmospheric photochemistry and Archean microbial ecology, but little is known about the actual timing of the evolution of methanogens or methanogenesis as a metabolism. Phylogenetic studies indicate methanogens are deeply rooted in the tree of life (6, 8, 11), but this approach relies on biological evidence to constrain the timing of evolutionary events.

In the investigation of the history of life on Earth, isotopic and chemical biomarkers serve both to calibrate time in phylogenetic trees and to document the impact of the biosphere on the physical world. An array of tools has traditionally been harnessed, including organic molecules (12) and the isotopic composition of key bioactive elements whose surface geochemical cycles are dominated by the biosphere (13). The transition metals are a recent addition to the latter group, with Fe undergoing the most intensive study (14, 15). Fe has advantages and disadvantages in this regard, as the many studies conducted on its isotope system have shown. The ubiquitous role of Fe in the biosphere makes its isotopes a key system to study, though other isotope systems such as Se (16) and Cu (17) may also be promising. A substantial body of work (for example, see refs. 15 and 18–21) has documented the various issues characterizing the interpretation of isotopic fractionations of virtually every isotope system that has been developed. For example, transitions between oxidation states can impart fractionations that may be attributable to any number of abiological processes and biological vital effects. Pinpointing isotopic fractionations directly ascribed to the latter has not been shown to be a particularly easy task although this may be more true for some trace metal isotope systems than others.

Of key importance to our study is nickel's role as a bioessential trace metal. Microorganisms use trace metals typically as micronutrients, notably for vital roles in enzymes, and the amounts needed are analogous to the metal concentration in seawater (22). At least 3 of the 7 known Ni enzymes are found in methanogens (23) and only one, urease, is used by higher-order organisms in nitrogen metabolism (22). From a functional and evolutionary perspective, the absolute requirement for Ni by methanogens and particularly by one very special enzyme, methyl-coenzyme M reductase (MCR), exemplifies what could be termed a class-specific metal dependency. Briefly, methanogenesis is the process by which methanogens generate energy via a stepwise reduction of single-carbon substrates; concomitantly, methane is produced as a waste product. The terminal process of methane formation involves MCR whose active site is a unique Ni cofactor, F_{430}, which is found only in methanogens and whose only known function is the catalytic activity within MCR (23–25). An analogue of MCR that catalyzes reverse methanogenesis, the anaerobic oxidation of methane (AOM), has been proposed (26), but its identity, structure and function remain unknown. Another intriguing Ni enzyme is the bifunctional CODH/ACS cluster; microorganisms possessing these enzymes use the Wood-Ljungdahl pathway for growth and are thought to be evolutionarily primitive. Several lines of support for this concept are described in refs. 27 and 28.

Here, we present the first Ni isotope data for abiotic terrestrial samples, for pure cultures of laboratory-grown methanogens and for cultures of one heterotrophic Archaea. Nickel is a first row transition metal. Although it can occupy a range of oxidation states from 0 to 4+, the 2+ state is essentially the only natural oxidation state. This makes for a key difference from the Fe system. To date, Ni isotope studies have focused almost exclusively on extraterrestrial materials and the search for radiogenic ^{60}Ni, the decay product of extinct ^{60}Fe (29–33). Within analytical uncertainties, these studies have demonstrated a fairly homogenous Ni isotopic composition for the early solar system, although 2 studies report moderate stable isotope variability for specific meteoritic fractions (30, 32). In the modern oceans, Ni distributions are dominated by scavenging and the element has an oceanic residence time of ≈10,000 years (34).

Results

Our new Ni stable isotope data, along with details of the experimental and analytical methods, are presented in the *SI Text* and summarized in Fig. 1. The first observation is that key abiotic terrestrial samples, representing the mantle and crust, show very little variation in Ni isotopic composition (Fig. 1*A*:

Author contributions: V.C. and C.H.H. designed research; V.C. performed research; V.C., D.V., and C.A. contributed new reagents/analytic tools; V.C. and D.V. analyzed data; and V.C. and D.V. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. E-mail: glxvc@bristol.ac.uk.

This article contains supporting information online at www.pnas.org/cgi/content/full/0900726106/DCSupplemental.

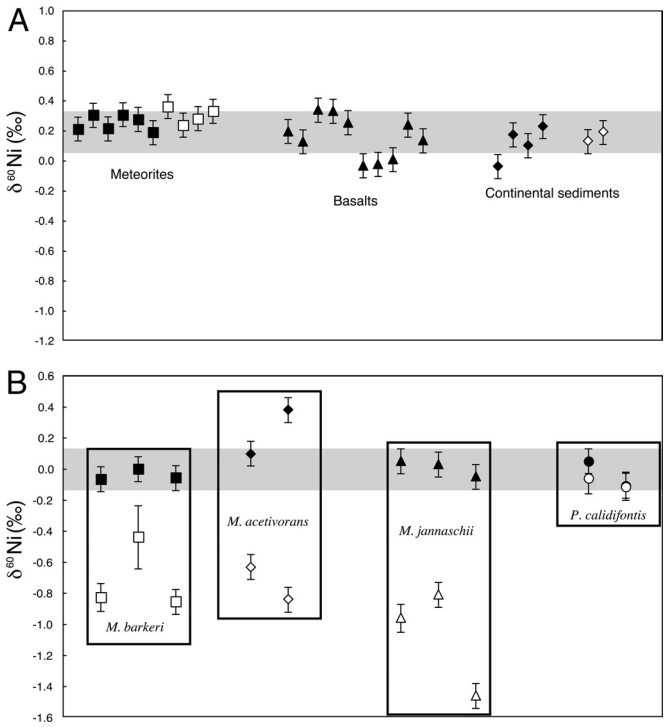

Fig. 1. Nickel stable isotope data for meteorites, terrestrial basalts and sediments (*A*) and cultures of Archaea (*B*) plotted on the same vertical scale. For the meteorite data in *A*, filled symbols are for stones and open symbols for irons. For the continental sediments filled symbols are for loess, and open symbols are for 2 samples of River Nile sediments. The average of all of the data in *A* is 0.2‰ and the gray band denotes 1 standard deviation either side of this average. The first 3 microorganisms in B are methanogens; the fourth on the right (*P. calidifontis*) is a heterotrophic archaea. Only data for experiments where reservoir effects are insignificant or small are plotted. Filled symbols represent residual media and open symbols Ni extracted from cultured cells. All data are plotted relative to a starting medium of 0‰ (center of the gray band), and the width of the gray band denotes 1 standard deviation of the abiotic data in *A* either side of the value for the medium.

average δ^{60}Ni of 0.15 ± 0.24‰, 2σ). Second, within the group of analyzed meteorites (Fig. 1*A*), there is even less deviation from the average value of δ^{60}Ni = 0.27 ± 0.06‰. The subtle isotopic variation within these 2 datasets is not our target here. Our main purpose in presenting these data are to demonstrate that analogues for inorganic or abiogenic processes occurring under diverse redox and temperature gradients show relatively insignificant Ni isotopic variations. This conclusion is broadly consistent with a previously reported larger meteoritic dataset (32) documenting average δ^{60}Ni in a range of meteorite types of 0.08–0.25‰. The dataset is as yet small but does provide a lithologic baseline within whose framework possible biological fractionations can be interpreted.

In stark contrast to the abiotic materials, Ni assimilation during methanogenic growth produced substantial fractionation of Ni isotopes (Fig. 1*B*), resulting in a separation between isotopically light cells and, where the mass balance is known, relatively heavy residual media. Methanogens were primarily investigated as they arguably have the greatest requirement for Ni. Cultures consisted of 2 mesophiles (*Methanosarcina barkeri*, *Methanosarcina acetivorans*) and a hyperthermophile (*Methanococcus jannaschii*). The source of Ni in the starting media for all methanogens was very similar and the analyses of Ni separated from these media yielded a δ^{60}Ni from + 0.42 ± 0.10 to + 0.60 ± 0.08‰. These results are analytically indistinguishable from Ni analyzed in the trace metal solution added to all growth media (0.47 ± 0.08‰, Table S1). In contrast, methanogen cells are universally isotopically light in Ni relative to the starting media and Ni was fractionated irrespective of phylogenetic differences. Many of the experiments resulted in no significant Ni depletions in the medium (i.e., no reservoir effect) so that the measured cell δ^{60}Ni yields a direct estimate of the fractionation of Ni isotopes into methanogen cells. As shown in Fig. 1*B*, cells of *M. barkeri* harvested from 2 separate experiments yielded values that are different from the starting medium ($\Delta\delta^{60}Ni_{cells\text{-}starting\ medium}$) to an identical degree (−0.83 ± 0.09 and −0.86 ± 0.09‰), whereas a third experiment (experiment 2) had a smaller fractionation but larger uncertainty (−0.44 ± 0.20). Two of 5 *M. acetivorans* experiments yielded $\Delta\delta^{60}Ni_{cells\text{-}starting\ medium}$ of −0.63 and −0.84. Two of the five *M. jannaschii* experiments yielded analytically identical values of −0.96 ± 0.09 and −0.81 ± 0.06, whereas the third gave a large fractionation of −1.46 ± 0.08.

In a few experiments (e.g., *M. acetivorans* experiment 2) substantial proportions of the total starting Ni in the system is contained within the cells, and as a result the residual media are, as expected from mass balance, significantly heavier than the starting Ni. The results, along with the data for all experiments where the total Ni mass balance is also available, are presented in Fig. 2. The Ni uptake experiment (Fig. 3) conducted with *M. acetivorans* was specifically designed to monitor the isotopic evolution of the residual medium as cells grow, removing and sequestering more Ni from the system. Cells were harvested only once at the end of this experiment and during the exponential phase of growth. For this terminal stage of the experiment, the amount of Ni taken up into cells (1.2 μg) represents ≈25% of total originally available Ni. The data for the final cells, and all but one of the analyses of the evolving residual media, fit reasonably well to a closed-system Rayleigh model with an alpha $[(^{60}Ni/^{58}Ni)_{cells}/(^{60}Ni/^{58}Ni)_{medium}]$ = ≈0.9990–0.9993 [i.e., cellular Ni −1.0 to −0.7‰ relative to medium, where ‰ = (1000 × ln alpha)]. Indeed, all methanogen data with the notable exception of the one anomalous cell sample from an *M. barkeri* experiment, are consistent with this small range in alpha values describing Ni isotopic fractionation between cells and medium.

In addition to the methanogens, isotope measurements were also made of cultures of the heterotrophic archaeal hyperthermophile, *P. calidifontis* (Fig. 1*B*). In contrast to the methanogens, very little fractionation is observed relative to the starting medium. Nickel is an important bioessential trace metal even to nonmethanogens but not to the same extent. *P. calidifontis* typically grows in an organic medium. Experiments were conducted under *normal* growth conditions, without the addition of trace metals (experiment 4; Table S1); in this case, the cells obtain bioessential trace metals from the organic substrates used to make up the growth medium. In separate experiments (experiments 1 and 2), the organic substrates were first treated with a metal-binding resin (Chelex; Bio-Rad) before the medium was made with the addition of a known quantity of trace metals (via the trace metal solution). Within analytical uncertainties, the results suggest that Ni isotopes were not fractionated by *P. calidifontis*. However, the extremely small dataset for nonmethanogens represented by *P. calidifontis* needs to be expanded by analysis of other microorganisms. At this time, the general conclusion which can be drawn from the results is that Ni isotopic fractionations potentially produced by microorganisms in general, will not be as significant as the fractionation produced by methanogens.

Conclusion

The combined results of our study suggest significant potential for Ni stable isotopes as a biomarker and as a biogeochemical tracer for understanding interactions between the geosphere and biosphere. Nickel's inimitable and specific niche in life may have important implications for understanding biogeochemical processes not only in modern environments but, perhaps critically, those occurring back through time to the very early stages of life on Earth. Particularly, the extent of the fractionation produced by methanogens may result in an additional role for Ni isotopes

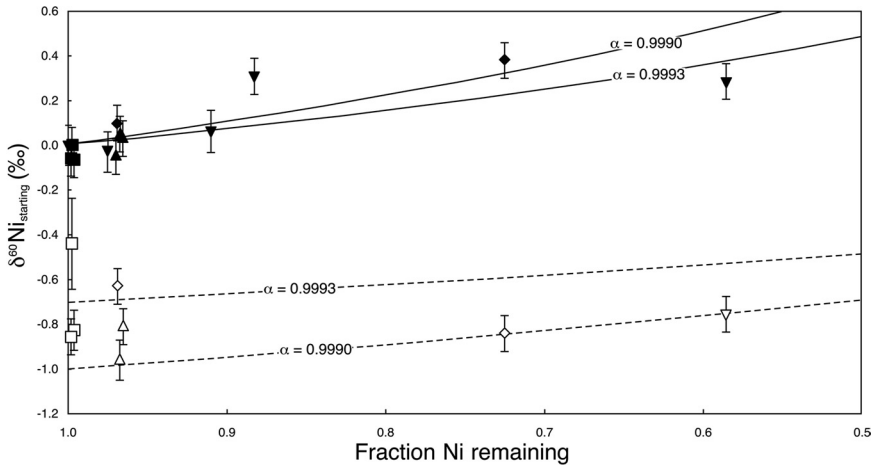

Fig. 2. Nickel stable isotope data for all methanogen cultures for which the total Ni mass balance is also available (but omitting one extreme value for *M. jannaschii* cells, which plots at −1.4‰; see Fig. 1*B*), plotted as a function of the fraction of total starting Ni remaining in the medium at the end of the experiment. All Ni isotope data for residual media (filled symbols) and cells (open symbols) are plotted relative to the starting medium for the respective experiments. Symbols for different species as in Fig. 1 except that the nonmethanogen is not plotted and the inverted triangles represent data for the *M. acetivorans* Ni uptake experiment. Also plotted are model closed system Rayleigh fractionation curves for residual media (solid lines) and accumulated cells (dashed lines) assuming fractionation factors for Ni uptake into cells of 0.9990 and 0.9993.

as a class-specific indicator for these microorganisms, and possibly for other microbial groups as well. Furthermore, the extent of Ni isotopic fractionation produced by specific groups of methanogens (contrast *M. acetivorans* and *M. jannaschii*) may be potentially exploited as a signature of metabolic processes. Our dataset is small but, as a unique bioessential metal, we foresee the possibility that the biological cycling of Ni may have the potential of being an important contributor of Ni isotopic variations in the rock record. The pursuit of this goal will require the analysis of a variety of rocks such as BIFs, organic-rich materials (shales, kerogen) and modern sediments before such claims can be made with certainty.

In addition to influencing Earth's modern environment, particularly the carbon cycle (35), methanogens may have had a profound and significant impact on the early Earth, before the evolution of oxygen. Carbon isotope evidence suggests life arose before ≈3.8 Ga (36, 37) and the presence of several prokaryotic groups in the Archean including sulfate reducers (38), sulfur-disproportionaters (39), iron reducers (15, 40), and methanogens (8, 41) have been proposed. Carbon isotopic evidence also indicates that a significant global methane cycle developed by the late Archean, indicating a growing ecological role for methanogens during this eon (42). The nature and timing of the origin of methanogenesis and the development of the 2.7 Ga global methanogen ecosystem may be recorded in sedimentary trace metals deposited by Archean microbial cells. Our data suggest that such sediments may record isotopic fractionations in Ni, which relate specifically and intimately to ancient microbial metabolisms. The biological fractionation of Ni potentially provides a powerful new tool for elucidating the nature and impact of early life on this and other planets.

Materials and Methods

Abiotic Samples. Powdered basalt and river sediment samples were available in-house from the Department of Earth Sciences, University of Bristol. Loess samples were provided by K. Pye (Kenneth Pye Associates, UK). Concentrated stock samples of previously digested meteorites were supplied by T. Elliot (Department of Earth Sciences, University of Bristol). Stock solutions of all terrestrial materials (data in Table S2) were prepared through a series of concentrated acid digestions (described in *SI Text*).

Biological Samples. Cell cultures (see Table S3 for summary) were obtained from established and maintained cultured stocks in the laboratory of C. House (Department of Geosciences, Pennsylvania State University, University Park, PA). The microorganisms used in this work are all from the domain Archaea and include several types of methanogens (*M. acetivorans*, *M. barkeri*, *M. jannaschii*) and a facultatively aerobic heterotrophic hyperthermophile (*Pyrobaculum calidifontis*), which was originally furnished to the House laboratory by Professor Tadayuki Imanaka (Department of Synthetic Chemistry and Biological Chemistry, Kyoto University, Japan) (43). *M. acetivorans* was provided by J. G. Ferry (Department of Biochemistry, Pennsylvania State University). Cell growth experiments and collection of all biological samples were carried out in the geomicrobiological laboratory of E. Hornibrook (Department of Earth Sciences, University of Bristol), before digestion and chemical processing. Conditions for the cultivation of all microorganisms, media preparation, sample collection and digestions are described in *SI Text*.

Column Chemistry. Nickel separation and purification was achieved through a 2-column ion exchange procedure, modified from Quitte and Oberli (44). Microbial growth media are rarely simple solutions. Additionally, a number of the media used in this study are analogous to seawater and thus contain significant quantities of Na, Mg, and other constituents, which must be removed to eliminate matrix effects and isobaric interferences. We tested several chemical procedures for this task (31, 45–49) but, although all can successfully separate Ni from simple solutions, they were unsuccessful when applied to our complex media. Our technique has been adapted for a relatively small sample volume. After sample digestion, the dried residue is taken up and dissolved in 1 M HCl (2 mL). Ammonium citrate (0.4 mL, 1 M) is added and the pH adjusted to 8–9 with 5 M NH_4OH. Samples are loaded on to columns (Teflon, made in house) prefilled with Ni resin (Eichrom Technologies). Before sample loading, the resin is washed with 18 MΩ water (2 mL) and 0.2 M ammonium citrate (1.5 mL), the latter also being used for resin conditioning (2 mL). The Ni-DMG complex forms and is retained, on the resin. Matrix elements are washed off with 0.2 M ammonium citrate (6 mL) and Ni is collected in 3 M HNO_3 (3 mL). After addition of 15.3 M HNO_3 (2 mL), the Ni solutions are dried down overnight. The residue is dissolved in 15.3 M HNO_3 (2 mL) and the solution digested overnight at 170 °C in closed beakers. This process, which separates the Ni-DMG complex and oxidizes DMG, is repeated twice more.

Fe and Zn are the major atomic interferences on Ni (^{58}Fe on ^{58}Ni and ^{64}Zn on ^{64}Ni, although the latter is not relevant here and will not be discussed further) and, although these elements are removed in the first column step, we use a second column filled with the macroporous anion resin, AG MP-1 M (Bio-Rad) to ensure their complete removal. Previous use of this resin to separate Fe and Zn is described elsewhere (50, 51). The dried, digested samples from the first column are treated with 10.7 M HCl (0.3 mL) followed by 7 M HCl + 0.01% H_2O_2 (0.3 mL); this last step is repeated twice. The residue is dissolved (0.75 mL), loaded and eluted from the second column in 7 M HCl + H_2O_2. At acid concentrations >2 M HCl, Fe and Zn are retained on the resin while Ni passes through. ^{56}Fe was routinely checked before the full Ni isotopic analysis and the possible interference on ^{58}Ni was never significant. The purified Ni

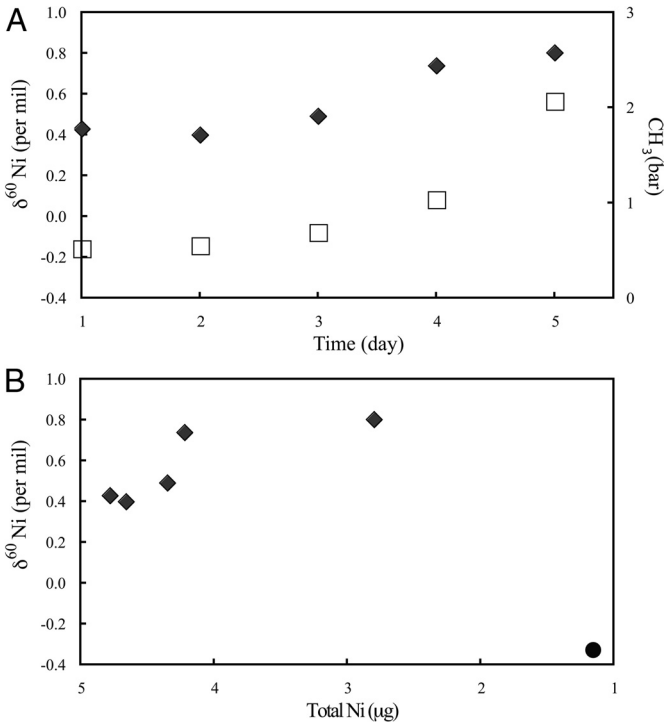

Fig. 3. Results of the Ni uptake experiment conducted with the methanogen *M. acetivorans*. (*A*) Individual bottles of growth were sacrificed for each residual medium sample collected for isotope analysis (filled diamonds) and headspace gas measurement (open squares) made over the 5-day period. δ^{60}Ni values and the amount of CH_4 evolved over the growth period, as a function of headspace pressure, are plotted on the y-axes vs. time on the *x* axis. The CH_4 value on day 1 (≈0.5 bar) is the amount added to the bottles at the beginning of the experiment. As would be predicted, a positive trend is observed for cell growth, with increasing amounts of CH_4 being produced as growth proceeds. Correlating with the production of CH_4 is the increasingly heavier isotopic values in the same residual media samples. (*B*) Total Ni amounts measured in the residual media are plotted on the bottom axis against δ^{60}Ni values on the left-hand axis. Cells (filled circle) were harvested for isotopic analysis only on day 5 of the experiment. A correlating effect is seen with the isotope values and Ni amounts; i.e., the residual medium becomes increasingly heavier and depleted as the cells grow with preferential removal of the lighter Ni isotope.

fractions are dried down, treated with 15.3 M HNO_3 and finally dissolved in 2% HNO_3 for analysis.

The yield for the column chemistry, as obtained from the double spike-derived Ni amount before the separation procedure and the Ni signal size relative to a standard after the separation procedure, is close to 100%—this approach does not give precise yields because signal size depends on tuning of the mass spectrometer and even minor quantities of residual matrix. Because samples are spiked before chemical separation, quantitative yields are not required. We also note that the yield is only 100% if care is taken to break down the Ni-DMG complex (as above) after the first column. If this is not complete, Ni is lost on the second column. Total procedural blanks are ≈2 ng and are ≈5% of the smallest sample. No corrections were done to the isotopic data. In an extreme case of a Ni blank isotopic composition 1.5‰ different from the sample, the size of the blank would suggest that the maximum magnitude of the correction would be 0.075‰, or the same as the reproducibility of std-spike mixtures.

Mass Spectrometry. Of the 3 methods available for correcting instrumental mass discrimination, the double spike approach has been shown to be a robust and rigorous technique when applied to isotope systems that have 4 or more isotopes (52–55). In this regard, the 5 stable isotopes of Ni make it an ideal candidate for this approach. The advantages of using the double-spike method are well known and include elimination of issues concerning differences in the behavior of samples and dopant or standards, used respectively in the doping and standard-bracketing methods, and removing potential fractionation problems induced due to incomplete yield from the column chemistry (53, 54).

The quality of isotopic analyses depends critically on the exact composition of the double spike and the optimum composition was first established using simulations of mass spectrometer runs for various combinations of double spike isotopes. The optimum spike was found to be a mixture of ^{61}Ni and ^{62}Ni. These isotopes were obtained from Oak Ridge National Laboratories (^{61}Ni, batch 127890; ^{62}Ni, batch 233026). Each isotope was weighed and digested separately with concentrated HNO_3 in sealed PFA beakers. The solutions were then transferred to individual 125-mL FEP bottles and diluted with 18 MΩ water to 2% HNO_3. These solutions were then mixed, resulting in a ^{61}Ni-^{62}Ni double spike with subequal proportions of the 2 isotopes. The isotopic composition of the spike was obtained via isotopic analysis of a series of standard-spike mixtures, with std/spike ratios ranging from 2 to 0.1. Ni standard, NIST SRM 986, was used to determine the isotopic composition and all isotopic data are thus reported relative to a value of 0‰ for the isotopic composition of this standard. The CRM 1,000 ppm single-element Ni standard (Peak Performance) was used to calibrate the total Ni concentration of the spike. Our double spike reduction procedure has been described for Zn (53), and the procedure used here is identical to that with ^{60}Ni/^{58}Ni, ^{61}Ni/^{58}Ni and ^{62}Ni/^{58}Ni as the 3 isotope ratios used in the double spike calculation. As well as the composition of the double spike, the sample/spike ratio of the mixture measured is also critical for obtaining precise and accurate isotopic analyses. At extreme sample/spike ratios error magnification during the reduction procedure increases, and accuracy is also compromised. The behavior of the spike in terms of these issues was characterized using spike/standard mixtures and is illustrated in Fig. S1. All samples measured here had sample/spike mixtures with ratios between 0.1 and 1. Nonexponential mass discrimination was dealt with in exactly the same way as described by Bermin et al. (53) for Zn.

All analyses were carried out at the University of Bristol using a ThermoFinnigan Neptune multicollector (MC) ICPMS coupled to an Aridus desolvating nebuliser system (CETAC) fitted with a PFA nebuliser and spray chamber (CPI). Nickel cones were used in this work and, as discussed in ref. 44, contributed very little instrumental blank to our measurements. Sweep gas was typically ≈4.25 L min^{-1} Ar + 10 mL min^{-1} N_2, with the latter being critical to the reduction of a background at mass 58, presumably ^{40}Ar^{18}O. All isotopes were measured simultaneously in static mode using a multiple Faraday collector array, but ^{64}Ni is not used in the calculation. Data collection consisted of 15 4-s integrations of the 2% HNO_3 blank followed by measurement of standards or samples for 30 4-s integrations. Blank contributions were subtracted from the standards and samples and generally accounted for <0.02% (for ^{58}Ni) of the ion beams. Typically 150 to 300 ng of Ni was used for each measurement with an associated internal error on the ^{60}Ni/^{58}Ni ratio of 0.001–0.002% at the 2σ level. The amount of Ni measured in some of the biological samples (see Table S1) fell outside this range, with associated internal errors of up to 0.0035% at the 2σ level.

All Ni data are reported relative to NIST SRM 986, in the standard delta notation:

$$\delta^{60}\mathrm{Ni} = \left[\frac{^{60}\mathrm{Ni}/^{58}\mathrm{Ni}_{\,\mathrm{sample}}}{^{60}\mathrm{Ni}/^{58}\mathrm{Ni}_{\,\mathrm{SRM986}}}\right] \times 1000.$$

All reported uncertainities are 2σ.

ACKNOWLEDGMENTS. We thank K. Muehlenbachs and C. Hawkesworth for helpful comments and suggestions in the preparation of this manuscript; Z. Zhang for assisting with the preparation of initial microbial cultures; J. Godwin for methane measurements; and S. Cobb. This work was supported by a National Aeronautics and Space Administration Astrobiology Institute Research Scholarship and a Worldwide Universities Network Research Grant, Pennsylvania State University (to V.C.).

1. Canfield DE (2005) The early history of atmospheric oxygen: Homage to Robert M. Garrels. *Annu Rev Earth Planet Sci* 33:1–36.
2. Holland HD (1984) in *The Chemical Evolution of the Atmosphere and Oceans* (Princeton Univ Press, Princeton), pp 277–428.
3. Eigenbrode JL, Freeman KH (2006) Late Archean rise of aerobic microbial ecosystems. *Proc Natl Acad Sci USA* 103:15759–15764.
4. Brocks JJ, Buick R, Summons RE, Logan GA (2003) A reconstruction of Archean biological diversity based on molecular fossils from the 2.78 to 2.45 billion-year-old Mount Bruce Supergroup, Hamersley Basin, Western Australia. *Geochim Cosmochim Acta* 67:4321–4335.
5. Summons RE, Jahnke LL, Hope JM, Logan GA (1999) 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature* 400:554–557.

6. Bapteste E, Brochier C, Boucher Y (2005) Higher-level classification of the Archaea: Evolution of methanogenesis and methanogens. *Archaea* 1:353–363.
7. Tice MM, Lowe DR (2006) The origin of carbonaceous matter in pre-3.0 Ga greenstone terrains: A review and new evidence from the 3.42 Ga Buck Reef Chert. *Earth Sci Rev* 76:259–300.
8. Battistuzzi FU, Feijao A, Hedges SB (2004) A genomic timescale of prokaryote evolution: Insights into the origin of methanogenesis, phototrophy, and the colonization of land. *Bmc Evol Biol* 4:44.
9. Catling DC, Zahnle KJ, McKay C (2001) Biogenic methane, hydrogen escape, and the irreversible oxidation of early Earth. *Science* 293:839–843.
10. Kasting JF (2005) Methane and climate during the Precambrian era. *Precambrian Res* 137:119–129.
11. Xue H, Tong K-L, Marck C, Grosjean H, Wong JT-F (2003) Transfer RNA paralogs: Evidence for genetic code-amino acid biosynthesis coevolution and an archaeal root of life. *Gene* 310:59–66.
12. Brocks JJ, Logan GA, Buick R, Summons RE (1999) Archean molecular fossils and the early rise of eukaryotes. *Science* 285:1033–1036.
13. Horita J (2005) Some perspectives on isotope biosignatures for early life. *Chem Geol* 218:171–186.
14. Beard BL, et al. (1999) Iron isotope biosignatures. *Science* 285:1889–1892.
15. Johnson CM, Beard BL, Roden EE (2008) The iron isotope fingerprints of redox and biogeochemical cycling in modern and ancient Earth. *Annu Rev Earth Planet Sci* 36:457–493.
16. Herbel MJ, Johnson TM, Oremland RS, Bullen TD (2000) Fractionation of selenium isotopes during bacterial respiratory reduction of selenium oxyanions. *Geochim Cosmochim Acta* 64:3701–3709.
17. Zhu XK, et al. (2002) Mass fractionation processes of transition metal isotopes. *Earth Planet Sci Lett* 200:47–62.
18. Anbar AD, Roe JE, Barling J, Nealson KH (2000) Nonbiological fractionation of iron isotopes. *Science* 288:126–128.
19. Marechal C, Albarede F (2002) Ion-exchange fractionation of copper and zinc isotopes. *Geochim Cosmochim Acta* 66:1499–1509.
20. Jang JH, Mathur R, Lierman LJ, Ruebush S, Brantley SL (2008) An iron isotope signature related to electron transfer between aqueous ferrous iron and goethite. *Chem Geol* 250:40–48.
21. Chapman JB, Weiss DJ, Shan Y, Lemburger M (2009) Iron isotope fractionation during leaching of granite and basalt by hydrochloric and oxalic acids. *Geochim Cosmochim Acta* 73:1312–1324.
22. Fraústo da Silva JJR, Williams RJP (2001) in *The Biological Chemistry of the Elements: The Inorganic Chemistry of Life* (Oxford Univ Press, Oxford), pp 436–449.
23. Watt RK, Ludden PW (1999) Nickel-binding proteins. *Cell Mole Life Sci* 56:604–625.
24. Ermler U (2005) On the mechanism of methyl-coenzyme M reductase. *Dalton Trans* 21:3451–3458.
25. Thauer RK (1998) Biochemistry of methanogenesis: A tribute to Marjory Stephenson. *Microbiology* 144:2377–2406.
26. Kruger M, et al. (2003) A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. *Nature* 426:878–881.
27. Huber C, Wachtershauser G (1997) Activated acetic acid by carbon fixation on (Fe,Ni)S under primordial conditions. *Science* 276:245–247.
28. Lindahl PA, Chang B (2001) The evolution of acetyl-CoA synthase. *Orig Life Evol Bios* 31:403–434.
29. Birck JL, Lugmair GW (1988) Nickel and chromium isotopes in Allende inclusions. *Earth Planet Sci Lett* 90:131–143.
30. Cook DL, Clayton RN, Wadhwa M, Janney PE, Davis AM (2008) Nickel isotopic anomalies in troilite from iron meteorites. *Geophys Res Lett* 35:L01203.
31. Morand P, Allegre CJ (1983) Nickel isotopic studies in meteorites. *Earth Planet Sci Lett* 63:167–176.
32. Moynier F, Blichert-Toft J, Telouk P, Luck JM, Albarede F (2007) Comparative stable isotope geochemistry of Ni, Cu, Zn, and Fe in chondrites and iron meteorites. *Geochim Cosmochim Acta* 71:4365–4379.
33. Shimamura T, Lugmair GW (1983) Ni isotopic compositions in Allende and other meteorites. *Earth Planet Sci Lett* 63:177–188.
34. Sclater FR, Boyle E, Edmond JM (1976) Marine geochemistry of nickel. *Earth Planet Sci Lett* 31:119–128.
35. Ragsdale SW (2007) Nickel and the carbon cycle. *J Inorg Biochem* 101:1657–1666.
36. Mojzsis SJ, et al. (1996) Evidence for life on Earth before 3,800 million years ago. *Nature* 384:55–59.
37. Schidlowski M (2001) Carbon isotopes as biogeochemical recorders of life over 3.8 Ga of Earth history: Evolution of a concept. *Precambrian Res* 106:117–134.
38. House CH, Runnegar B, Fitz-Gibbon ST (2003) Geobiological analysis using whole genome-based tree building applied to the Bacteria, Archaea, and Eukarya. *Geobiology* 1:15–26.
39. Philippot P, et al. (2007) Early Archaean microorganisms preferred elemental sulfur, not sulfate. *Science* 317:1534–1537.
40. Archer C, Vance D (2006) Coupled Fe and S isotope evidence for Archean microbial Fe(III) and sulfate reduction. *Geology* 34:153–156.
41. Ueno Y, Yamada K, Yoshida N, Maruyama S, Isozaki Y (2006) Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature* 440:516–519.
42. Hayes JM (1994) in *Early Life on Earth*, ed Bengtson S (Columbia Univ Press, New York), pp 220–236.
43. Amo T, Paje M-L-F, Inagaki A, Ezaki S, Atomi H (2002) Pyrobaculum calidifontis sp. nov., a novel hyperthermophilic archaeon that grows in atmospheric air. *Archaea* 1:113–121.
44. Quitte G, Oberli F (2006) Quantitative extraction and high precision isotope measurements of nickel by MC-ICPMS. *J Anal At Spectrom* 21:1249–1255.
45. Wu JF, Boyle EA (1997) Low blank preconcentration technique for the determination of lead, copper, and cadmium in small-volume seawater samples by isotope dilution ICPMS. *Anal Chem* 69:2464–2470.
46. Victor AH (1986) Separation of nickel from other elements by cation-exchange chromatography in dimethylglyoxime/hydrochloric acid acetone media. *Anal Chim Acta* 183:155–161.
47. Strelow FWE, Weinert CHS, Eloff C (1972) Distribution coefficients and anion-exchange behavior of elements in oxalic acid hydrochloric acid mixtures. *Anal Chem* 44:2352–2356.
48. Korkisch J, Ahluwali SS (1967) Cation-exchange behaviour of several elements in hydrochloric acid—organic solvent media. *Talanta* 14:155–170.
49. Wu JF, Boyle EA (1998) Determination of iron in seawater by high-resolution isotope dilution inductively coupled plasma mass spectrometry after Mg(OH)(2) coprecipitation. *Anal Chim Acta* 367:183–191.
50. Marechal CN, Telouk P, Albarede F (1999) Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry. *Chem Geol* 156:251–273.
51. Archer C, Vance D (2004) Mass discrimination correction in multiple-collector plasma source mass spectrometry: An example using Cu and Zn isotopes. *J Anal At Spectrom* 19:656–665.
52. Dodson MH (1963) A theoretical study of use of internal standards for precise isotopic analysis by the surface ionization technique: Part 1—general first-order algebraic solutions. *J Sci Instrum* 40:289–295.
53. Bermin J, Vance D, Archer C, Statham PJ (2006) The determination of the isotopic composition of Cu and Zn in seawater. *Chem Geol* 226:280–297.
54. Bermin JH (2006) Determination of the isotopic composition of Cu and Zn in seawater. PhD thesis (University of London, London).
55. Johnson CM, Beard BL (1999) Correction of instrumentally produced mass fractionation during isotopic analysis of Fe by thermal ionization mass spectrometry. *Int J Mass Spectrom* 193:87–99.