

Follow the Nitrogen

Douglas G. Capone, Radu Popa, Beverly Flood, Kenneth H. Nealson

“Follow the water” is the mantra used by NASA in the search for life in the universe. It is hard to imagine life without a fluid, or to imagine that the fluid could be anything other than water. From our Earth-centric point of view, it is equally difficult to imagine life without carbon and nitrogen. These two elements, by virtue of their ability to form double bonds, allow the formation of a multitude of unsaturated and aromatic compounds that impart both structural strength and energetic versatility to earthly life. We propose here that the lack of a substantial geological reservoir of nitrogen (1, 2) makes it an ideal candidate in the search for life on Mars and other planetary bodies: “follow the nitrogen.”

As shown in the figure, the carbon and nitrogen cycles involve similar conversions of various redox states (from completely oxidized to completely reduced). These transformations are catalyzed at the organismal level and lead to the movement of carbon and nitrogen among the biosphere, atmosphere, geosphere, and ocean; to this extent, the two cycles seem quite similar. However, when the scale is global rather than organismal, carbon and nitrogen offer remarkable contrasts with regard to both their abundance and distribution (see the figure).

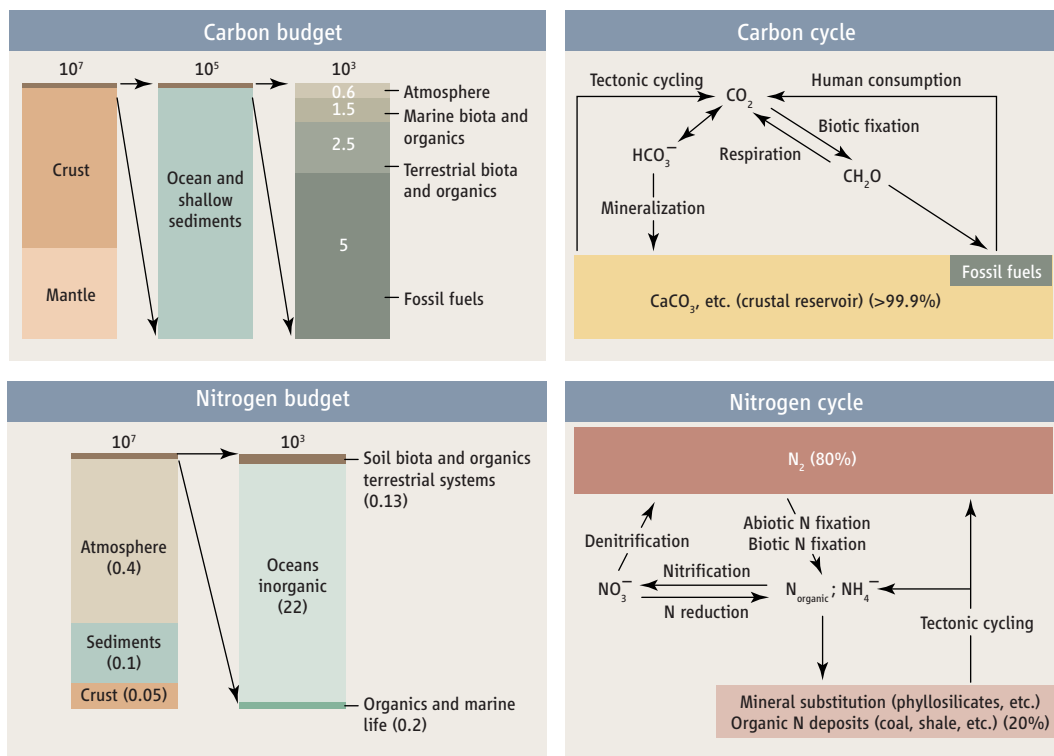
Carbon resides primarily in the lithosphere (mantle-derived rocks, crust, and sediments), with less than 0.05% being cycled through the biosphere, hydrosphere, and atmosphere. In contrast, nitrogen resides primarily in the atmosphere as dinitrogen (N_2) and trace amounts of N_2O (an important atmospheric biosignature) (2). With the exception of a few phyllosilicates that contain ammonia substituted for potassium, nitrogen has no substantial geological reservoirs. Because of this unusual distribution, the amount of nitrogen found in the biosphere (in living species and

deposited as shales) is actually a few percent of the total fixed nitrogen.

Nitrogen is “fixed” from N_2 through both physical (such as nitrogen oxide formation by lightning) and biological processes. Minerals containing fixed nitrogen, such as nitrate and ammonium salts, tend to dissolve in the pres-

On Mars, the jovian satellites, and other planetary bodies, nitrogen species may provide important clues to the presence of life.

the progressive loss of N_2 from the atmosphere and the accumulation of nitrates in the oceans. On a tectonically active planet such as Earth, some nitrogen is returned on geological time scales via volcanic emissions, but given the absence of nitrogen-rich minerals, this effect will be relatively small (4). Thus, if biological



Comparative carbon and nitrogen cycles on Earth. (Left) Budgets of carbon and nitrogen in the major reservoirs on Earth. Values are taken from many different sources, as summarized in (1, 2). (Right) Major changes in redox chemistry catalyzed by biota. The reactions due to anaerobic ammonium oxidation (11) are not included in the diagram. Budget values are in petagrams (10^{15} g).

ence of even small amounts of liquid water. Thus, except for extremely dry sites such as the Atacama desert, nitrogen moves from the continents to the oceans as soluble nitrates in water flow (1, 2) and returns to the atmosphere as N_2 via denitrification, the return pathway that keeps the nitrogen cycle going.

Nitrogen fixation—the transformation of N_2 to a reduced, biologically useful form—is often touted as the great evolutionary invention that allowed life to prosper on Earth (3). Although there is some truth to this with regard to organic productivity, it may well be that an important point with regard to the development of life on Earth has been missed. Nitrates are not readily converted to N_2 by abiotic processes, so that in an abiological oxidizing environment, any conversion of N_2 to soluble nitrates should result in

pathways such as denitrification (including anaerobic ammonium oxidation) (5) did not occur, the terrestrial nitrogen cycle (and terrestrial life as we know it) could not be sustained. Even on Earth, over the course of billions of years, abiotic processes such as lightning would substantially deplete the atmosphere of N_2 , leaving an ocean of soluble nitrogen and a land mass substantially depleted in this element (6, 7). Denitrification is the driving force for the return of nitrogen to the land and its equitable redistribution via nitrogen fixation. In our view, biological nitrogen fixation was needed only when the productivity of Earth exceeded the ability of existing fixed reservoirs and abiotic nitrogen fixation to supply it (8); in contrast, we see denitrification as essential for the maintenance of the nitrogen cycle itself.

D. G. Capone and B. Flood are in the Department of Biological Sciences, and K. H. Nealson is in the Department of Earth Sciences, University of Southern California, Los Angeles, CA 90089, USA. R. Popa is in the Department of Biology, Portland State University, Portland, OR 97207, USA. E-mail: knealson@usc.edu

On Mars, things are very different. N_2 constitutes only about 2.7% of a very thin atmosphere (9). Isotopic measurements of the martian atmosphere suggest that the nitrogen has been lost from the planet over geological time (6, 10), and to date, no ammonia has been identified. If nitrogen-containing life ever existed on Mars, then we would expect evidence of this to exist in the form of sedimentary deposits containing substantial amounts of nitrogen (for example, fixed nitrogen in organics, such as alkyl porphyrin derivatives, and its stable isotopic signatures in shales and kerogen) (11). Given the lack of tectonic processing and the absence of continuing life that might recycle the evidence of the past, there might be relatively accessible and unaltered evidence for this life, perhaps visible even today in outflow channels. Alternatively, life may have never evolved, leaving perhaps only abiotic nitrate deposits, the understanding of which might reveal the workings of past planetary chemistry (6, 7, 12). For any of these scenarios, “follow the nitrogen” is a mantra that makes sense both for understanding the past planetary chemistry and for searching for life, be it extant or extinct, on Mars.

The presence of nitrogen in any form should be a signal to planetary scientists to take notice. It may well be that the form and amount of nitrogen could constitute a roadmap for understanding whether chemical or biological processes were involved in its deposition. At least on a body that has had a

separation of continental and oceanic components, the existence of nitrogen on continents is not easy to explain without special life-supplied chemistry.

Does this logic extend to considerations of the jovian satellites Europa, Ganymede, and Callisto? We think so. These moons may have liquid oceans hidden under layers of ice, presenting a very different scenario with regard to their nitrogen cycles; it is easy to imagine a nitrogen cycle that does not involve N_2 , perhaps instead moving from organic nitrogen to nitrate and back, or perhaps even a nonredox cycle involving organic and inorganic forms of reduced nitrogen. Saturn’s moon Titan, already known to be replete with nitrogen, offers other problems and opportunities (9). Life at such low temperatures may be a special case with regard to nitrogen.

Is N_2 in planetary atmospheres a useful signal for life? On those bodies where N_2 should otherwise be lost by diffusional escape from the atmosphere, the presence of fixed nitrogen would be an exciting finding. Recent mass spectrometric analyses of the atmosphere of Enceladus, a moon of Saturn, revealed a peak at 28 mass units, which could be either N_2 or CO; no definitive assignment could be made (13–15). Thus, the reports are not particularly encouraging with regard to life; as the authors say, it is worthy of more study (13–15).

As noted above, there are some planetary bodies with adequate gravity and/or very low temperatures that might be expected to main-

tain their nitrogen gases without the help of the biota. Thus, our recommended approach might be to search for the nitrogen; characterize and quantify it; if its abundance and chemistry cannot be explained by abiotic processes, do not leave until it is explained; and when it comes to sample return—bring back anything that is enriched in nitrogen!

References and Notes

1. J. Galloway *et al.*, *Biogeochemistry* **70**, 153 (2004).
2. J. N. Galloway, in *Treatise on Geochemistry*, H. D. Holland, K. K. Turekian, Eds. (Elsevier, Amsterdam, 2004), vol. 8, pp. 557–583.
3. V. Smil, *Enriching the Earth* (MIT Press, Cambridge, MA, 2001).
4. J. F. Kasting, D. P. Whitmire, R. T. Reynolds, *Icarus* **101**, 108 (1993).
5. A. A. van de Graaf *et al.*, *Appl. Environ. Microbiol.* **61**, 1246 (1995).
6. Y. L. Yung, M. B. McElroy, *Science* **203**, 1002 (1979).
7. R. L. Mancinelli, A. Banin, *Int. J. Astrobiol.* **2**, 217 (2003).
8. J. A. Brandes *et al.*, *Nature* **395**, 365 (1998).
9. Y. L. Yung, W. B. DeMore, *Photochemistry of Planetary Atmospheres* (Oxford Univ. Press, Oxford, 1999).
10. M. B. McElroy, T. Y. Kong, Y. L. Yung, *J. Geophys. Res.* **82**, 4379 (1977).
11. M. I. Chicarelli *et al.*, *Geochim. Cosmochim. Acta* **57**, 1307 (1993).
12. R. Plumb, R. Tantayanon, M. Libby, W. W. Xu, *Nature* **338**, 633 (1989).
13. J. S. Kargel, *Science* **311**, 1389 (2006).
14. J. H. Waite Jr. *et al.*, *Science* **311**, 1419 (2006).
15. C. C. Porco *et al.*, *Science* **311**, 1393 (2006).
16. We thank NASA, the U.S. Department of Energy, and NSF for financial support. We also thank the USC students of Geobiology and Astrobiology (BISC 483, spring 2004) for some of the discussion leading to this Perspective.

10.1126/science.1111863

IMMUNOLOGY

B Cells Spread and Gather

Margaret M. Harnett

B lymphocytes play an important role in the immune response to infection, secreting neutralizing antibodies to combat invading pathogens. They are activated to clonally proliferate and differentiate into such antibody-secreting cells after binding of their cell surface antigen receptors to specific foreign antigen that gets trapped in lymphoid tissue. This interaction with antigen also activates B cell receptor signaling, resulting in the internalization, processing, and presentation of antigen to T lymphocytes in the context of cell surface proteins of the major histocompatibility complex class II family. Recognition of such processed antigen by the T cell receptor

induces the formation of a stable association, or synapse, between the two cell types (1), resulting in the transmission of signals required for regulating the B cell response.

The B cell receptor has often been portrayed as simply binding and internalizing soluble antigen, with the degree of receptor activation reflecting the affinity of interaction with the antigen. What has not been clear is how B cells deal with antigens that are not soluble but rather are integral to, or tethered to, cellular membranes. How do B cells recognize, discriminate by affinity, and acquire membrane-associated antigens? Indeed, given experimental findings that antigens associated with membranes are particularly effective at activating B cells and promoting consequential B cell-driven T cell activation in vitro, it is thought that such B cell receptor recognition and affinity discrimination of membrane-associated anti-

Immune cells that produce effective antibodies against foreign proteins are selected by a synapse-like interaction with antigen-presenting cells that promotes the survival of the cells with high-affinity binding.

gens is likely to be of paramount importance in vivo. For example, in the germinal centers of lymphoid tissues, B cells undergo a maturation process that selects for those cells that produce antibodies with high affinity for antigen and for those that will recognize the same antigen in the future (memory B cells). Here, follicular dendritic cells trap, retain, and present antigen to B cells in the form of immune complexes (antigen associated with antibodies) that bind to receptors at the follicular dendritic cell surface (2).

On page 738 of this issue (3), Batista and colleagues explain how, in the early stages of the selection process, B cells dynamically spread and contract over cell membranes that bear antigen (either anchored in the membrane or tethered to the membrane as an immune complex) (see the figure). This allows the B cell receptor to differentially sense antigens of widely varying affinities by the amount of anti-

The author is in the Division of Immunology, Infection and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow G12 8TA, UK. E-mail: M.Harnett@bio.gla.ac.uk