

# Imprint of denitrifying bacteria on the global terrestrial biosphere

Benjamin Z. Houlton<sup>1</sup> and Edith Bai

Department of Land, Air, and Water Resources, University of California, Davis, CA 95616

Communicated by William H. Schlesinger, Cary Institute of Ecosystem Studies, Millbrook, NY, October 21, 2009 (received for review June 6, 2009)

**Loss of nitrogen (N) from land limits the uptake and storage of atmospheric CO<sub>2</sub> by the biosphere, influencing Earth's climate system and myriads of the global ecological functions and services on which humans rely. Nitrogen can be lost in both dissolved and gaseous phases; however, the partitioning of these vectors remains controversial. Particularly uncertain is whether the bacterial conversion of plant available N to gaseous forms (denitrification) plays a major role in structuring global N supplies in the nonagrarian centers of Earth. Here, we use the isotope composition of N (<sup>15</sup>N/<sup>14</sup>N) to constrain the transfer of this nutrient from the land to the water and atmosphere. We report that the integrated <sup>15</sup>N/<sup>14</sup>N of the natural terrestrial biosphere is elevated with respect to that of atmospheric N inputs. This cannot be explained by preferential loss of <sup>14</sup>N to waterways; rather, it reflects a history of low <sup>15</sup>N/<sup>14</sup>N gaseous N emissions to the atmosphere owing to denitrifying bacteria in the soil. Parameterizing a simple model with global N isotope data, we estimate that soil denitrification (including N<sub>2</sub>) accounts for ≈1/3 of the total N lost from the unmanaged terrestrial biosphere. Applying this fraction to estimates of N inputs, N<sub>2</sub>O and NO<sub>x</sub> fluxes, we calculate that ≈28 Tg of N are lost annually via N<sub>2</sub> efflux from the natural soil. These results place isotopic constraints on the widely held belief that denitrifying bacteria account for a significant fraction of the missing N in the global N cycle.**

denitrification | isotope | nitrogen | ecosystem | microbe

**W**e present a simple nitrogen (N) isotope model to investigate the partitioning of gaseous ( $f_{\text{gas}}$ ) vs. leaching ( $f_{\text{leaching}}$ ) pathways of N removal from the nonagricultural (i.e., unmanaged) terrestrial biosphere. N's isotopes, <sup>14</sup>N and <sup>15</sup>N, are stable but display a high degree of variation in biogenic materials owing to isotope fractionation, particularly kinetic fractionation, whereby organisms' enzymes more rapidly transfer the light isotope from substrates to products. <sup>15</sup>N is rare (≈0.37%), and small deviations in its concentration relative to <sup>14</sup>N have been widely used in the examination of N cycling on land and in the sea (1). In the case of the N isotope composition of the terrestrial biosphere, we consider a steady-state approximation to examine the partitioning of global N losses:

$$f_{\text{gas}} = \frac{\delta^{15}\text{N}_{\text{TB}} - \delta^{15}\text{N}_{\text{I}} + \varepsilon_{\text{L}}}{\varepsilon_{\text{L}} - \varepsilon_{\text{G}}} \quad [1]$$

in which  $\delta^{15}\text{N} = ((^{15}\text{N}/^{14}\text{N}_{\text{sample}}/^{15}\text{N}/^{14}\text{N}_{\text{std}} - 1) \times 1,000)$  of the terrestrial biosphere (TB) or atmospheric inputs (I);  $\varepsilon$  is the isotope effect associated with leaching (L) or gaseous (G) N removal; and  $1 = f_{\text{gas}} + f_{\text{leaching}}$ . We define  $\varepsilon$  as the ratio of the rate constants of the heavy and light isotopes (i.e.,  $\varepsilon = (^{15}\text{k}/^{14}\text{k} - 1) \times 1,000$ ) at steady state, whereby instantaneous isotope effects are equivalent to equilibrium ones. Here, we constrain global N losses by: (i) estimating the  $\delta^{15}\text{N}$  of natural plants and soils worldwide; (ii) compiling data on the  $\delta^{15}\text{N}$  of N inputs, and potential fractionations via leaching vs. gaseous N losses; and (iii) parameterizing Eq. 1 to quantify the N lost via microbial denitrification.

## Results and Discussion.

Across natural terrestrial environments, <sup>15</sup>N/<sup>14</sup>N of bulk soils (integrated to 50-cm depth) and foliage vary systematically with climate (2–4); hence, large-scale (i.e., regions, biomes) terrestrial <sup>15</sup>N/<sup>14</sup>N ratios can be estimated from precipitation and temperature fields (3). Although this model can introduce errors at smaller scales, it reasonably captures the shift in plant and soil  $\delta^{15}\text{N}$  across biomes, pointing to its robustness at the global scale (Fig. S1 and Tables S1 and S2). Taking advantage of this model (3) and eliminating those areas of land that are managed by humans (*Methods* and *SI Text*), we estimate that the area-integrated  $\delta^{15}\text{N}$  of natural soil and vegetation leaves are equal to 5.4‰ (±2.2) and 0.43‰ (±2.5), respectively (Fig. 1A). Plants are known to have a lower <sup>15</sup>N/<sup>14</sup>N than soils owing to various mechanisms (5, 6), consistent with the offset in Fig. 1. Although plants are not expected to alter the <sup>15</sup>N/<sup>14</sup>N of bulk soils under quasiequilibrium conditions (7), we account for any such N recycling effects by combining components of the biosphere into a single vector. Because roots, shoots, and leaves are similar in their N isotope compositions (8) (Table S3), the <sup>15</sup>N/<sup>14</sup>N of leaves also reflects that of total vegetation. Using estimates (9) of the N stored in vegetation and soils worldwide, we show that the mass- and area-weighted  $\delta^{15}\text{N}$  of the unmanaged terrestrial biosphere is equal to 5.3‰ (Fig. 1A).

The <sup>15</sup>N/<sup>14</sup>N of the natural terrestrial biosphere is elevated compared with external N inputs (Fig. 1A). Biological N<sub>2</sub> fixation, the biochemical conversion of N<sub>2</sub> to ammonia, imparts a minor isotope effect; its  $\delta^{15}\text{N}$  ranges from 0 to –2‰ (10). The other major N input to natural systems occurs via atmospheric deposition, originating from natural and from fossil fuel sources, producing mainly NO<sub>x</sub> that rains down as nitrate on land (11). We show in Fig. 1B that the  $\delta^{15}\text{N}$  of bulk nitrate deposition observed for various latitudes (Table S4), altitudes and biomes (from deserts to tropical rainforests;  $n = 187$ ) approach a median of –1.3‰, with most values clustered between –3 and 1. Although ammonium and dissolved organic N compounds can also contribute deposition, their <sup>15</sup>N/<sup>14</sup>N ratios either overlap with or are somewhat <sup>15</sup>N-depleted with respect to that of nitrate in bulk precipitation (7, 12, 13). We therefore infer that there must be a pathway of N loss that preferentially removes <sup>14</sup>N from the land, acting to elevate the terrestrial biosphere's <sup>15</sup>N/<sup>14</sup>N over the long term.

Two mechanisms could underlie terrestrial <sup>15</sup>N enrichment. First, leaching of N to waterways could remove low <sup>15</sup>N/<sup>14</sup>N compounds from soil ( $\varepsilon_{\text{L}}$ ) (Eq. 1), thereby elevating the <sup>15</sup>N/<sup>14</sup>N of remaining ecosystem pools (1). In particular, nitrifying bacteria can produce low <sup>15</sup>N/<sup>14</sup>N nitrate ( $\varepsilon \approx -15$  to  $-35$ ‰) (1); if <sup>15</sup>N-depleted nitrate were to escape ecosystems via streams, it would increase the <sup>15</sup>N/<sup>14</sup>N

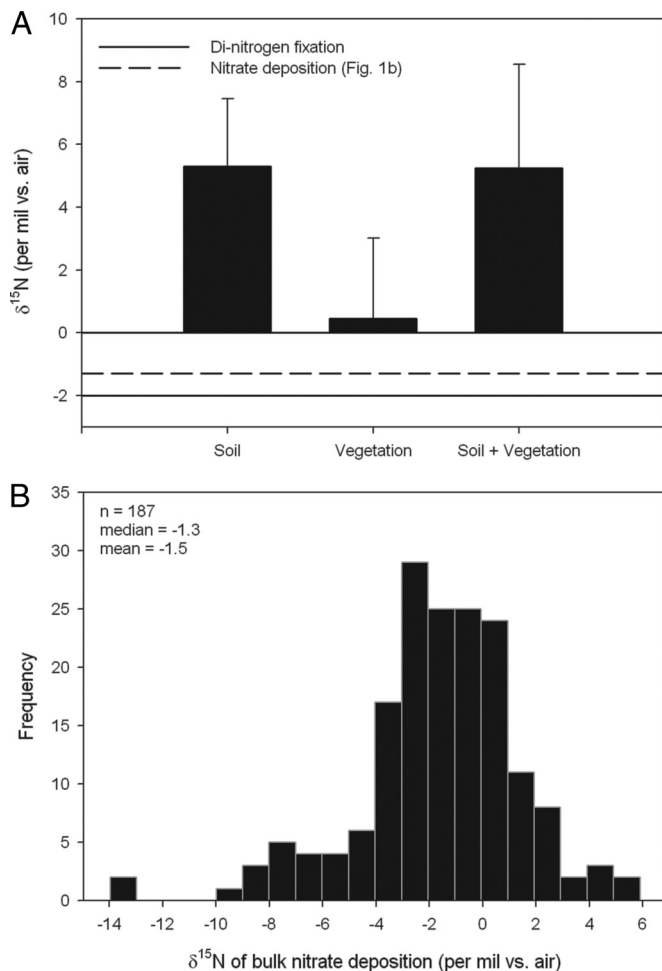
Author contributions: B.Z.H. designed research; B.Z.H. and E.B. performed research; B.Z.H. and E.B. analyzed data; and B.Z.H. and E.B. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

To whom correspondence should be addressed at: Department of Land, Air, and Water Resources, University of California, One Shields Avenue, Davis, CA 95616. Email: bzhoulton@ucdavis.edu.

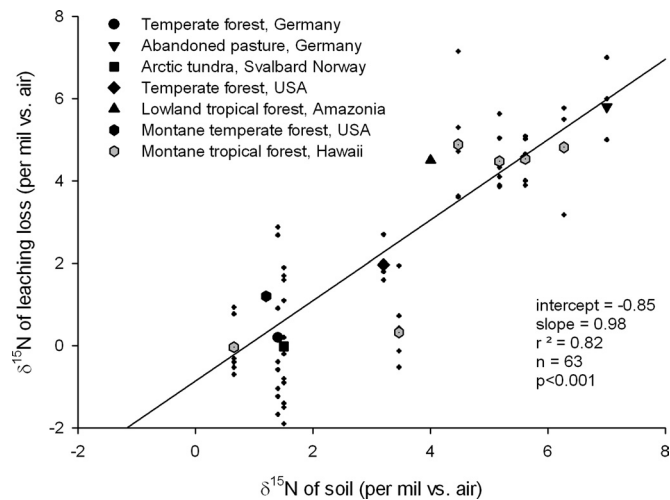
This article contains supporting information online at [www.pnas.org/cgi/content/full/091211106/DCSupplemental](http://www.pnas.org/cgi/content/full/091211106/DCSupplemental).



**Fig. 1.**  $^{15}\text{N}/^{14}\text{N}$  of soil, vegetation and inputs to the terrestrial biosphere. (A)  $\delta^{15}\text{N}$  of soil and vegetation were estimated from multiple regression models (3) and integrated at the global scale in the absence of human-managed environments (See *Methods* and *SI Text*). The  $\delta^{15}\text{N}$  of the terrestrial biosphere was estimated as follows:  $(\delta^{15}\text{N}_{\text{soil}} \times N_{\text{soil}} + \delta^{15}\text{N}_{\text{veg}} \times N_{\text{veg}}) / (N_{\text{soil}} + N_{\text{veg}})$ , where  $N$  is total  $\text{N}$  (i.e.,  $^{14}\text{N} + ^{15}\text{N}$ ). The solid lines delineate the  $\delta^{15}\text{N}$  range for biological dinitrogen fixation (10); the dashed line is the median of bulk nitrate deposition from *B*. Errors for soil and vegetation reflect the SE of the regression models, which were propagated to the terrestrial biosphere (i.e.,  $(\text{SE}_{\text{soil}}^2 + \text{SE}_{\text{veg}}^2)^{1/2}$ ). (B)  $^{15}\text{N}/^{14}\text{N}$  of bulk nitrate deposition from different locations around the globe, including sites spanning temperate to tropical biomes, and low to high altitudes (see *SI Text*).

of remaining terrestrial matter. Alternatively, by discriminating against  $^{15}\text{N}$ , gaseous  $\text{N}$  losses ( $\epsilon_G$ ) (Eq. 1) could impact the terrestrial  $^{15}\text{N}/^{14}\text{N}$  system in manner similar to that of leaching (7).

However, nitrate leaching does not seem to significantly discriminate against ecosystem  $^{15}\text{N}$  (Fig. 2). Across major biomes and latitudes, including arctic tundra, temperate and boreal forest, and tropical lowland and montane forest (Table S5), there is good agreement between the  $\delta^{15}\text{N}$  of nitrate in small drainage streams and the  $\delta^{15}\text{N}$  of soil particulate matter (Fig. 2). Together the slope (0.98) and intercept ( $-0.85$ ) of this significant ( $r^2 = 0.82$ ;  $n = 63$ ;  $P < 0.001$ ) linear relationship point to  $\epsilon_L < -1\text{‰}$  that is systematically conserved across these diverse biomes. Missing from Fig. 2 are arid sites with high average soil  $\delta^{15}\text{N}$  ( $\approx 4\text{--}15\text{‰}$ ) (14, 15). Although leaching is limited in such environments (i.e., evaporation  $>$  precipitation), observations of high nitrate- $\delta^{15}\text{N}$  in biologically active soils ( $8\text{--}15\text{‰}$ ) (14) and in water that percolates from soils to ground water ( $10\text{--}13\text{‰}$ ) (16) suggest a similarly small leaching effect in natural arid lands. Finally, although other forms



**Fig. 2.**  $^{15}\text{N}/^{14}\text{N}$  of  $\text{N}$  leaching losses to small streams across the terrestrial biosphere. These data were compiled from the primary literature (see *SI Text*). The small black symbols are individual observations. The large black symbols reflect site means for the  $\delta^{15}\text{N}$  of stream nitrate; the gray symbols reflect the means for the  $\delta^{15}\text{N}$  of total dissolved  $\text{N}$  in small streams. The regression line is drawn through the site means.

of dissolved  $\text{N}$  might also influence soil  $\delta^{15}\text{N}$ , neither is there evidence for major  $^{15}\text{N}$ -discriminations against soil  $\text{N}$  isotopes by total  $\text{N}$  leaching losses (gray circles) (Fig. 2). Thus the available evidence points to a small leaching term; based on the intercept in Fig. 2, it seems that leaching could at most raise the  $\delta^{15}\text{N}$  of the terrestrial biosphere by  $\approx 1\text{‰}$  above  $\text{N}$  inputs (see Fig. 1A)

The only remaining mechanism involves isotope fractionation via gaseous pathways, particularly bacterial ones. Ammonia volatilization is not likely to affect the integrated  $^{15}\text{N}/^{14}\text{N}$  of the terrestrial system; the short atmospheric lifespan of ammonia means that this  $\text{N}$  form mainly recycles within and among terrestrial regions (17). Regional-scale anomalies in  $\delta^{15}\text{N}$ , which contain important information beyond the global mean, may be influenced by processes such as volatilization; but our integrated estimate would be minimally affected by this process. Although emission and transport of ammonia from the terrestrial biosphere to the ocean has increased in response to anthropogenic activities (18), particularly  $\text{N}$  fertilizer applications, ammonia emissions from natural soils are relatively small (i.e.,  $2.4\text{--}10\text{ Tg N/yr}$ ) (19, 20). By contrast, bacterial denitrifiers produce substantial quantities of long-lived gaseous compounds ( $\text{N}_2$ ,  $\text{N}_2\text{O}$ ) which mix evenly in the atmosphere before returning to land. Denitrification can also produce  $\text{NO}_x$ , especially in dry environments (21), some of which rapidly washes out of the atmosphere as deposition (17, 22). Nevertheless, this term is small compared with the combined losses of  $\text{NO}_x$ ,  $\text{N}_2\text{O}$ , and (especially)  $\text{N}_2$  (22).

Proximally, whether originating from denitrification or nitrifier-denitrification, bacterial pathways of gaseous  $\text{N}$  removal would be expected to elevate the  $^{15}\text{N}/^{14}\text{N}$  of nitrate relative to any upstream isotope effects (23, 24). Indeed, the average isotope effect of denitrification on nitrate is substantial in both pure culture ( $-20 \pm 1.0\text{‰}$ ) and in natural soil communities ( $-16 \pm 1.6\text{‰}$ ) (Table S6). The slightly lower ( $P < 0.1$ ; two-tailed  $t$  test) value for soil might be due to heterogeneities in nitrate consumption, which reduces expression of the isotope effect relative to homogenous (culture) conditions (7, 25). Perhaps more important, the isotope effect of denitrification seems to control entire ecosystems: the near  $\approx 1:1$  relationship in Fig. 2 can only be explained by fractionating losses via denitrification under steady state conditions (7). The similarities between nitrate  $\delta^{15}\text{N}$  and soil  $\delta^{15}\text{N}$  indicates that the rather high isotope



scheme of VUB and VITO, derived from a full year cycle (1998–1999) of 10 daily composites of SPOT-VEGETATION ([www.geosuccess.net/Geosuccess](http://www.geosuccess.net/Geosuccess)). Areas classified as croplands, urban and built-up, and cropland and natural vegetation mosaics were considered as human-managed.

Data on the  $\delta^{15}\text{N}$  of N leaching losses to small streams and atmospheric N inputs, and the isotope effect of denitrification in pure culture and in native

soil communities were collated from the primary literature. Details of our literature syntheses can be found in the *SI Text*.

**ACKNOWLEDGMENTS.** We thank Bryant A. Browne for influential discussions, and Audrey Niboyet for comments. This work was supported by the Andrew W. Mellon Foundation.

- Hogberg P (1997) Tansley review No 95 - N-15 natural abundance in soil-plant systems. *New Phytol* 137:179–203.
- Handley LL, et al. (1999) The N-15 natural abundance ( $\delta^{15}\text{N}$ ) of ecosystem samples reflects measures of water availability. *Aust J Plant Physiol* 26:185–199.
- Amundson R, et al. (2003) Global patterns of the isotopic composition of soil and plant nitrogen. *Glob Biogeochem Cycles* 17:1031.
- Craine JM, et al. (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol* 183:980–992.
- Houlton BZ, Sigman DM, Schuur EA, Hedin LO (2007) A climate-driven switch in plant nitrogen acquisition within tropical forest communities. *Proc Natl Acad Sci USA* 104:8902–8906.
- Hobbie EA, Macko SA, Shugart HH (1999) Interpretation of nitrogen isotope signatures using the NIFTE model. *Oecologia* 120:405–415.
- Houlton BZ, Sigman DM, Hedin LO (2006) Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. *Proc Natl Acad Sci USA* 103:8745–8750.
- Pardo LH, et al. (2006) Regional assessment of N saturation using foliar and root  $\delta^{15}\text{N}$ . *Biogeochemistry* 80:143–171.
- Schlesinger WH (1997) *Biogeochemistry: An Analysis of Global Change* (Academic, San Diego).
- Shearer G, Kohl DH (1986) N-2-fixation in field settings—Estimations based on natural N-15 abundance. *Aust J Plant Physiol* 13:699–756.
- Holland EA, et al. (1997) Variations in the predicted spatial distribution of atmospheric nitrogen deposition and their impact on carbon uptake by terrestrial ecosystems. *J Geophys Res* 102:15849–15866.
- Cornell S, Rendell A, Jickells T (1995) Atmospheric inputs of dissolved organic nitrogen to the oceans. *Nature* 376:243–246.
- Heaton THE, Spiro B, Madeline S, Robertson C (1997) Potential canopy influences on the isotopic composition of nitrogen and sulphur in atmospheric deposition. *Oecologia* 109:600–607.
- Densmore JN, Bohlke JK (2000) Use of nitrogen isotopes to determine sources of nitrate contamination in two desert basins in California. *Interdisciplinary Perspectives Drinking Water Risk Assessment Management* 260:63–73.
- Shearer G, et al. (1983) Estimates of N-2-fixation from variation in the natural abundance of N-15 in Sonoran Desert ecosystems. *Oecologia* 56:365–373.
- McMahon PB, Bolke JK (2006) Regional patterns in the isotopic composition of natural and anthropogenic nitrate in groundwater, high plains, USA. *Environ Sci Technol* 40:2965–2970.
- Galloway JN, et al. (2004) Nitrogen cycles: Past, present, and future. *Biogeochemistry* 70:153–226.
- Duce RA, et al. (2008) Impacts of atmospheric anthropogenic nitrogen on the open ocean. *Science* 320:893–897.
- Bouwman AF, et al. (1997) A global high-resolution emission inventory for ammonia. *Glob Biogeochem Cycles* 11:561–587.
- Schlesinger WH, Hartley AE (1992) A global budget for atmospheric  $\text{NH}_3$ . *Biogeochemistry* 15:191–211.
- Hartley AE, Schlesinger WH (2000) Environmental controls on nitric oxide emission from northern Chihuahuan desert soils. *Biogeochemistry* 50:279–300.
- Gruber N, Galloway JN (2008) An Earth-system perspective of the global nitrogen cycle. *Nature* 451:293–296.
- Brandes JA, Devol AH (2002) A global marine-fixed nitrogen isotopic budget: Implications for Holocene nitrogen cycling. *Glob Biogeochem Cycles* 16:1120.
- Perez T, et al. (2000) Isotopic variability of  $\text{N}_2\text{O}$  emissions from tropical forest soils. *Glob Biogeochem Cycles* 14:525–535.
- Brandes JA, Devol AH (1997) Isotopic fractionation of oxygen and nitrogen in coastal marine sediments. *Geochim Cosmochim Acta* 61:1793–1801.
- Deutsch C, Sigman DM, Thunell RC, Meckler AN, Haug GH (2004) Isotopic constraints on glacial/interglacial changes in the oceanic nitrogen budget. *Glob Biogeochem Cycles* 18:GB4012.
- Prentice IC, Heimann M, Sitch S (2000) The carbon balance of the terrestrial biosphere: Ecosystem models and atmospheric observations. *Ecol Appl* 10:1553–1573.
- Hungate B, Dukes J, Shaw M, Luo Y, Field C (2003) Nitrogen and climate change. *Science* 302:1512–1513.
- Schlesinger WH (2009) On the fate of anthropogenic nitrogen. *Proc Natl Acad Sci USA* 106:203–208.
- Van Drecht G, Bouwman AF, Knoop JM, Beusen AHW, Meinardi CR (2003) Global modeling of the fate of nitrogen from point and nonpoint sources in soils, groundwater, and surface water. *Glob Biogeochem Cycles* 17:1115.
- Seitzinger S, et al. (2006) Denitrification across landscapes and waterscapes: A synthesis. *Ecol Appl* 16:2064–2090.
- Delwiche CC (1970) Nitrogen cycle. *Sci Am* 223:136–146.
- Galloway JN, Schlesinger WH, Levy IH, Michaels A, Schnoor JL (1995) Nitrogen fixation: Anthropogenic enhancement-environmental response. *Glob Biogeochem Cycles* 9:235–252.
- Cleveland CC, et al. (1999) Global patterns of terrestrial nitrogen ( $\text{N}_2$ ) fixation in natural ecosystems. *Glob Biogeochem Cycles* 13:623–645.
- Bai E, Houlton BZ (2009) Coupled isotopic and process-based modeling of gaseous nitrogen losses from tropical rainforests. *Global Biogeochem Cycles* 23.
- Bowden WB (1986) Gaseous nitrogen emissions from undisturbed terrestrial ecosystems—An assessment of their impacts on local and global nitrogen budgets. *Biogeochemistry* 2:249–279.
- Potter CS, Matson PA, Vitousek PM, Davidson EA (1996) Process modeling of controls on nitrogen trace gas emissions from soils worldwide. *J Geophys Res* 101:1361–1379.