ACS AuthorChoice

Environmental Science & Technology

Article

pubs.acs.org/est

Terms of Use

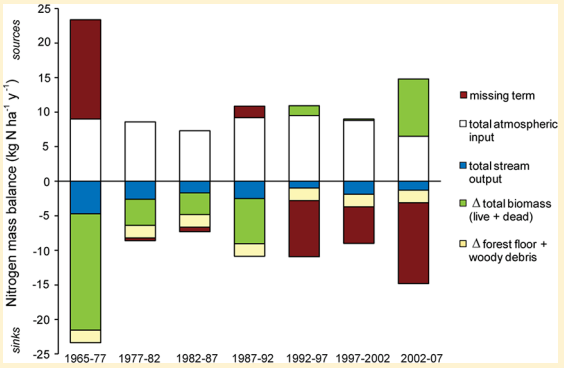
# From Missing Source to Missing Sink: Long-Term Changes in the Nitrogen Budget of a Northern Hardwood Forest

Ruth D. Yanai,[†] Matthew A. Vadeboncoeur,[‡] Steven P. Hamburg,[§] Mary A. Arthur,[∥] Colin B. Fuss,[⊥] Peter M. Groffman,[@] Thomas G. Siccama,[#] and Charles T. Driscoll*[,⊥]

[†]College of Environmental Science and Forestry, State University of New York, Syracuse, New York 13210, United States
[‡]Earth Systems Research Center, University of New Hampshire, Durham, New Hampshire 03824, United States
[§]Environmental Defense Fund, Boston, Massachusetts 02108, United States
[∥]Department of Forestry, University of Kentucky, Lexington, Kentucky 40546, United States
[⊥]Department of Civil and Environmental Engineering, Syracuse University, Syracuse, New York 13244, United States
[@]Cary Institute of Ecosystem Studies, Millbrook, New York 12545, United States
[#]School of Forestry and Environmental Studies, Yale University, New Haven, Connecticut 06511, United States

Supporting Information

**ABSTRACT:** Biogeochemical monitoring for 45 years at the Hubbard Brook Experimental Forest in New Hampshire has revealed multiple surprises, seeming contradictions, and unresolved questions in the long-term record of ecosystem nitrogen dynamics. From 1965 to 1977, more N was accumulating in living biomass than was deposited from the atmosphere; the "missing" N source was attributed to biological fixation. Since 1992, biomass accumulation has been negligible or even negative, and streamwater export of dissolved inorganic N has decreased from ~4 to ~1 kg of N $ha^{-1}$ $year^{-1}$, despite chronically elevated atmospheric N deposition (~7 kg of N $ha^{-1}$ $year^{-1}$) and predictions of N saturation. Here we show that the ecosystem has shifted to a net N sink, either storing or denitrifying ~8 kg of N $ha^{-1}$ $year^{-1}$. Repeated sampling over 25 years shows that the forest floor is not detectably accumulating N, but the C:N ratio is increasing. Mineral soil N has decreased nonsignificantly in recent decades, but the variability of these measurements prevents detection of a change of <700 kg of N $ha^{-1}$. Whether the excess N is accumulating in the ecosystem or lost through denitrification will be difficult to determine, but the distinction has important implications for the local ecosystem and global climate.




## INTRODUCTION

The northeastern United States has been experiencing elevated atmospheric N deposition for decades.[1−4] In the late 20th Century, chronically elevated N deposition was recognized as a potential threat to terrestrial and aquatic ecosystems.[1,2,5−7] This chronic N fertilization could be expected to increase forest growth and carbon sequestration (e.g., ref 8), although the magnitude of this impact is under considerable debate.[9−12] Excessive N inputs were predicted to eventually lead to a condition of N saturation,[2] in which forest ecosystems no longer sequester added N, with consequent losses of dissolved N to aquatic ecosystems and decreases in forest productivity.

Chronically elevated N deposition is theorized to induce a series of changes in ecosystem processes, including increases in foliar and soil N, decreases in soil C:N ratio, increases in the net production of nitrate in soil, and increased leaching of N, largely nitrate, in streamwater.[2,13,14] Detecting these changes requires long-term observations of a wide range of ecosystem characteristics, including vegetation and soil stocks and precipitation and streamwater fluxes. Biogeochemical data from the Hubbard Brook Experimental Forest in New Hampshire provide a unique opportunity to characterize and quantify long-term changes in ecosystem N cycling under chronically elevated N deposition, challenging some long-held assumptions and predictions.

## MATERIALS AND METHODS

**Site Description: Hubbard Brook Watershed 6.** Watershed 6 is the designated biogeochemical reference at the Hubbard Brook Experimental Forest (HBEF). This 13 ha area has not been cut since a moderate salvage logging following the 1938 hurricane, prior to which the forest had been cut over for red spruce (*Picea rubens* Sarg.) in the 1890s and for hardwoods

Received: June 10, 2013
Revised: September 12, 2013
Accepted: September 19, 2013
Published: September 19, 2013



and spruce between 1909 and 1917.[15] The elevation of this watershed ranges from 550 to 790 m. The dominant tree species are sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.). Above 750 m elevation, dominance shifts to paper birch (*Betula papyrifera* Marsh.), balsam fir (*Abies balsamea* L.), and red spruce. Soils are primarily coarse-loamy, mixed, frigid, Typic Haplorthods. Precipitation, streamwater, and vegetation have been monitored since the 1960s. In this paper, we report forest floor data sampled every 5 years from 1977 to 2002. Samples collected in 1976 and 1977 were combined to form a single sample set comparable in size to the later collections.

**Other White Mountain Sites.** Forest floors were sampled from 1979 to 2003 in stands of northern hardwoods in the central White Mountains of New Hampshire.[16] In this study, we report forest floor data from the six mature stands [>55 years of age at the time of the most recent sampling (Table 2)]. Elevations of these sites ranged from 320 to 580 m. The tree species with the greatest basal area were American beech, yellow birch, paper birch, sugar maple, red maple (*Acer rubrum* L.), and white ash (*Fraxinus americana* L.). Soils were predominantly coarse-loamy, mixed, frigid, Typic Haplorthods.

**Forest Floor Collection and Processing.** Methods of forest floor collection differed slightly between Hubbard Brook and the other White Mountain sites. At Hubbard Brook Watershed 6, sampling points were randomly selected within three elevation bands, with no more than one sample per 25 m × 25 m plot. The number of samples collected varied by sampling date, ranging from 57 to 100.[17] Samples were collected using a 15 cm × 15 cm template, which defined the vertical faces of the forest floor block. The surrounding soil was removed until the mineral soil was exposed, leaving a pedestal with the forest floor on top. The block was then removed and inverted and the mineral soil removed from the bottom of the sample.[17]

In the 1987 and later collections, the Oa was separated from the Oie before further processing; at the earlier sampling dates reported here, the forest floor was processed as a single sample. When the Oa was collected separately from the rest of the forest floor, the Oa was air-dried. Twigs and roots larger than 7 mm in diameter were removed, and the rest of the sample was oven-dried at 80 °C to constant mass, weighed, milled to pass a 2 mm screen, and archived. Samples were analyzed for C and N within a few years of collection, with a few samples analyzed up to 29 years postcollection (information about the Hubbard Brook sample archive is available at http://www.hubbardbrook.org). Note that the Oa horizons collected by this method likely include some A-horizon material, based on the organic matter content.[17]

In the six other White Mountain sites (Table 2), forest floor samples were collected in 1979 and 1980 and in 1994 and 1995; three sites were sampled again in 2003. Ten 10 cm × 10 cm forest floor samples were collected along each of five or six transects using the pin block method.[17] A template was placed on the ground, and steel pins were pushed into the soil to define the column to be sampled. The column was excavated and trimmed to the dimensions defined by the pins, and the mineral soil was removed from the bottom of the block. Sampling points on rocks or stumps counted as zeros. Blocks were composited by line for analysis of mass and nutrient contents. These samples were air-dried, sieved, weighed, subsampled for moisture content, and then stored for up to 15 years before being further analyzed.

**Forest Floor Analysis.** To determine the C and N content of forest floors in Hubbard Brook Watershed 6, archived samples dating from 1976 to 2002 were subsampled, 5% of them in triplicate to assess methodological precision. Samples were analyzed for C and N on a CE Instruments model NC2100 elemental analyzer. Replicate analyses indicate an analytic precision of approximately ±5% of each value. Reference samples were analyzed every 10th sample to assess accuracy; the root-mean-square (rms) error was 3% for N and 2% for C.

Samples from the 1979 and 1980 collections and the 1994 and 1995 collections from the other White Mountain sites were analyzed together to minimize the risk of systematic differences in chemical analysis using a LECO CN 2000 instrument (LECO Corp., St. Joseph, MN). Samples from 2003 were analyzed at a later date also on a LECO CN 2000 instrument.

**Statistical Analysis of Forest Floor Repeat Measures.** We combined the data from 1976 and 1977 to provide a sample size comparable to the sample sizes of data from later dates. We report mean C and N content per unit area of forest floor at Hubbard Brook Watershed 6 and 95% confidence intervals [±1.96 standard errors (see the Supporting Information)]. The C:N ratio for each sampling date was calculated as the ratio of mean C to mean N, not the mean of the ratios for each sampled point, consistent with the watershed-scale budget. Trends in N content, C content, and C:N ratio were analyzed using linear regression for the watershed means. All observations were used; omitting outliers changed the means but did not markedly affect the trends.

For the six additional sites in the White Mountain National Forest, we found the change over time for each stand using linear regression and described all six using the mean slope of the regression and the 95% confidence interval on the slope (Table 2). Similarly, for the C:N ratio, we used linear regression to find the slope and reported the mean slope and 95% confidence interval.

**Mineral Soil N Calculations.** There are two long-term mineral soil N data sets at and near Hubbard Brook. The first is from Watershed 5, adjacent to Watershed 6, and consists of spatially paired 0.5 $m^2$ quantitative pits excavated at 59 distinct sites across the watershed, with the first set excavated prior to whole-tree harvesting in 1983 and the second excavated 15 years later. We compared mean mineral soil N content after removing outliers [samples more than three standard deviations (SDs) from the mean in mineral soil C or N content]. The variance of the difference in mean N content was calculated by summing the variances of the two data sets ($n$ = 57 in 1983; $n$ = 58 in 1998).

In three second-growth postagricultural stands (35−90 years of age) 10 km south of Hubbard Brook, soils were sampled in the same manner as at Watershed 5 in three pits per stand in 1980[18] and 2005. Mean soil N stock changes were calculated using stand as the experimental unit; the reported confidence interval reflects the variance among stands in the difference in mean N content between the two sampled years.

Changes were reported as nonsignificant at an $\alpha$ level of 0.10.

**N Budget Calculations.** The N budget for Watershed 6 (Table 1 and Figure 3) was assembled using long-term data collected at the HBEF. Annual hydrologic fluxes were based on a June 1 water year using hydrological data provided by the U.S. Forest Service and chemical data provided by Gene Likens[19,20]

(http://www.hubbardbrook.org). Stream fluxes of dissolved inorganic N (DIN) were calculated using daily discharge and weekly concentrations, using the average concentration of the preceding and following measurements for the intervening days. Bulk precipitation fluxes of DIN were based on weekly collections. Changes over time in analytical methods were detailed by Buso et al.;[21] when changes were made, instruments were run in parallel to ensure consistency in the long-term record.

Stream-dissolved organic N (DON) was calculated from analysis of total dissolved nitrogen minus DIN on samples collected monthly[22] (http://www.hubbardbrook.org). Stream DON fluxes prior to 1992 when measurements began were assigned the average annual flux from 1992 to 2007.[23] Similarly, precipitation DON fluxes prior to 1997 were assigned the average annual flux from 1997 to 2007.

Dry N deposition includes gaseous ammonia, nitric acid vapor, and particulate nitrate and ammonium. We have estimates of the atmospheric concentrations of the latter three N species at Hubbard Brook through the U.S. Environmental Protection Agency CASTNET program (http://epa.gov/castnet), which uses a modeled deposition velocity.[24] The ratios of dry to bulk deposition of oxidized, reduced, and total N have not changed significantly from 1990 to present (see the Supporting Information). To estimate dry N deposition, we used the linear relationship between dry and wet inorganic N deposition based on the CASTNET record (see the Supporting Information).

Fluxes of N in soil solution draining the Oa and Bs soil horizons were calculated by assuming vertical flow and partitioning water loss by evapotranspiration by the horizontal distribution of fine root biomass[22] for the years 1992−2008. The uncertainty [95% confidence interval (CI)] was estimated from interannual variability.

Total biomass was estimated using the Watershed 6 biomass calculator.[25] This is an improvement over the method used to estimate biomass accumulation in the original N budget, which was based on tree ring analysis.[26] Standing dead trees were not measured in 1977; to estimate their biomass, we interpolated between 1965 and 1982. Woody debris accumulation[27] was estimated using data from the same regional sites where the forest floor data were taken (Table 2).

In the case of denitrification, we converted the missing N sink of 8.4 kg of N $ha^{-1}$ $year^{-1}$ (1992−2007, Table 1) to a greenhouse gas equivalent based on a $N_2O$:N ratio of 15%[28] and the IPCC GWP (100 years) of 298. For the case of an N sink in the soil, we estimated the C sink associated with an N sink in the soil by assuming a C:N ratio of 20.[29]

## RESULTS AND DISCUSSION

**Missing Nitrogen Source: 1965−1977.** In the early years of ecosystem monitoring at Hubbard Brook, from 1965 to 1977, the living vegetation was an active sink for N, largely because of the accumulation of biomass in the second-growth northern hardwood forest. In fact, the rate of N accumulation in living biomass (9 kg of N $ha^{-1}$ $year^{-1}$, estimated from tree cores) was greater than could be explained by atmospheric N inputs. The forest floor was also thought to be accumulating N at the rate of 8 kg of N $ha^{-1}$ $year^{-1}$.[30] Thus, the original Hubbard Brook N budget presented by Bormann et al.[26] reported a missing source of 14 kg of N $ha^{-1}$ $year^{-1}$, which was attributed to biological N fixation. Subsequent measured N fixation, however, was found to be 1 order of magnitude smaller than this budget discrepancy.[31]

An alternative explanation for the missing N source is a net flux of N from the mineral soil. This mechanism is difficult to evaluate because a change of this magnitude in the mineral soil N stock would be nearly impossible to measure directly. Organic N stocks in the mineral soil are very large and heterogeneous (5900 kg of N $ha^{-1}$; SD of 2700[32]), representing more than 70% of the total ecosystem N stock (Figure 1).

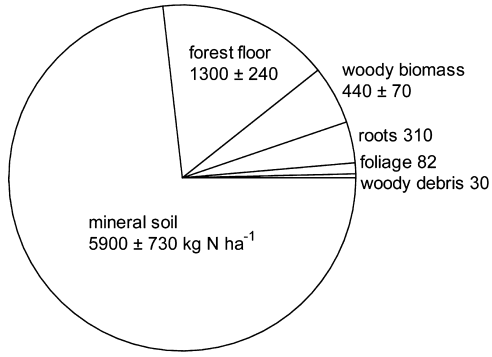



**Figure 1.** Ecosystem nitrogen stocks measured at Hubbard Brook Watershed 5 (adjacent to Watershed 6) in 1982 and 1983, in kilograms of N per hectare. Data are from Arthur et al.[36] (woody biomass), Fahey et al.[66] (roots), Huntington et al.[32] (forest floor and mineral soil), and Siccama and Denny[25] (foliage). Woody debris data are for mature stands throughout the region.[27]

Huntington et al.[32] estimated that the minimum detectable change in the mineral soil would be 730 kg of N $ha^{-1}$, using 60 pits each 0.5 $m^2$ in area. Thus, ∼50 years would be required to detect a change in soil N storage of 14 kg of N $ha^{-1}$ $year^{-1}$.

**Living Biomass Is No Longer a Nitrogen Sink.** From 1965 to 1977, forest growth was vigorous, and biomass accumulation of N was 17 kg of N $ha^{-1}$ $year^{-1}$. From 1977 to 1992, the net accumulation of live and standing dead biomass slowed, averaging 3.7 kg of N $ha^{-1}$ $year^{-1}$. Since 1992, mortality has exceeded growth with a net loss averaging 4.5 kg of N $ha^{-1}$ $year^{-1}$ (Table 1). The biomass of the reference watershed is based on a complete census of more than 12000 trees every 5 years since 1977, a sample of tree heights, and allometric equations developed at this site relating tree diameter and height to biomass.[33] While the uncertainty caused by the allometric relationships can be readily quantified (0.5−1.0 kg of N $ha^{-1}$ $year^{-1}$),[34] changes in the N concentrations of tissues are not well-known. The N content of vegetation has been calculated using concentrations measured in trees sampled in 1966 and 1967.[35] In 1983, the aboveground vegetation on three 0.25 ha plots, measured by whole-tree removal and chipping,[36] had a total N content only 8% greater than that calculated using the 1965−1967 N concentrations and allometric equations, which suggests that any change in N content in the vegetation resulting from a changing concentration in tissues between 1965 and 1985 has been small (1.6 kg of N $ha^{-1}$ $year^{-1}$). Foliar N concentrations have been monitored since 1992,[37] and the 1965 values[35] are within the range of current interannual variation. Roots, branches, and wood represent larger N stocks but have not been systematically monitored over time.

**Atmospheric Inputs and Streamwater Outputs: The Missing Sink.** Hubbard Brook Experimental Forest has a continuous record of bulk precipitation and stream chemistry dating from the early 1960s.[38,39] Bulk deposition of nitrate plus

**Table 1. Mass Balance of N (kilograms of N per hectare per year) in Watershed 6 of the Hubbard Brook Experimental Forest over Various Time Intervals[a]**

| | 1956–1976 | 1965–1977 | 1977–1982 | 1982–1987 | 1987–1992 | 1992–1997 | 1997–2002 | 2002–2007 | 1977–1992 mean | 1992–2007 mean |
|---|---|---|---|---|---|---|---|---|---|---|
| inputs and outputs (kg of N $ha^{-1}$ $year^{-1}$) | | | | | | | | | | |
| precipitation, DIN | 6.5 | 7.3 | 7.0 | 5.8 | 7.5 | 7.8 | 6.9 | 5.3 | 6.8 | 6.7 |
| precipitation, DON | not included | *1.1* | *1.1* | *1.1* | *1.1* | *1.1* | 1.4 | 0.8 | 1.1 | 1.1 |
| dry deposition | not included | *0.6* | *0.5* | *0.4* | *0.6* | *0.6* | *0.5* | *0.4* | 0.5 | 0.5 |
| streamflow, DIN | −4.0 | −4.1 | −2.0 | −1.1 | −1.9 | −0.6 | −1.2 | −0.5 | −1.7 | −0.8 |
| streamflow, DON | not included | *−0.6* | *−0.6* | *−0.6* | *−0.6* | −0.4 | −0.7 | −0.8 | −0.6 | −0.6 |
| input−output imbalance | 2.5 | 4.3 | 6.0 | 5.6 | 6.7 | 8.5 | 6.9 | 5.2 | 6.1 | 6.9 |
| internal stock changes | | | | | | | | | | |
| living biomass | −9.0 | −16.9 | −3.4 | −2.3 | −5.4 | 1.2 | 2.4 | 10.0 | −3.7 | 4.5 |
| standing dead biomass | not included | not included | −0.4 | −0.8 | −1.1 | 0.3 | −2.2 | −1.7 | −0.8 | −1.2 |
| woody debris (fine + coarse) | not included | *−0.1* | *−0.1* | *−0.1* | *−0.1* | *−0.1* | *−0.1* | *−0.1* | −0.1 | −0.1 |
| forest floor | −7.7 | *−1.7* | −1.7 | −1.7 | −1.7 | −1.7 | −1.7 | *−1.7* | −1.7 | −1.7 |
| internal stock changes | −16.7 | −18.7 | −5.6 | −4.9 | −8.4 | −0.4 | −1.6 | 6.5 | −6.3 | 1.5 |
| missing source or sink | −14.2 | −14.4 | 0.4 | 0.7 | −1.7 | 8.1 | 5.3 | 11.7 | −0.2 | 8.4 |

[a]Positive values are releases of N from the ecosystem; negative values are inputs of N into the ecosystem or increases in ecosystem stocks. The first column shows the 1956−1977 budget published in ref 26, followed by our estimate for a similar time period, including additional data. Fluxes and stock changes shown in italics are estimated rather than measured. The 1997−2002 pentad includes high N export in streams following the 1998 ice storm.

ammonium (DIN) averaged ~7 kg of N $ha^{-1}$ $year^{-1}$ from 1965 to 2007 (Table 1), which was similar to other observations in the northeastern United States.[4,5,23] As expected from the relatively high atmospheric deposition, streamwater export of DIN increased from the 1960s to the mid-1970s, approaching the rate of precipitation input. Surprisingly, streamwater DIN fluxes decreased thereafter, averaging 0.8 kg of N $ha^{-1}$ $year^{-1}$ from 1992 to 2007. This decrease in stream nitrate preceded the recent decline in atmospheric nitrate deposition due to controls on nitrogen oxide emissions from power plants that came into effect in 2003[40] (Table 1). Similar decreases in streamwater $NO_3^-$ concentrations between the 1970s and 1990s have been observed in other mature forests throughout the region.[41−43] These ecosystems appear now to be retaining ~6 kg of N $ha^{-1}$ $year^{-1}$, or >90% of incoming DIN. Including dry deposition (~0.4 kg of N $ha^{-1}$ $year^{-1}$) and DON in the budget, we found atmospheric inputs exceeded streamwater export by 5−9 kg of N $ha^{-1}$ $year^{-1}$ since 1992 (Table 1). This trend challenges the theory of impending N saturation, which predicts increasing streamwater losses of N.[2,44]

**The Forest Floor as Putative Sink.** In the original N budget for Watershed 6 at Hubbard Brook,[26] the forest floor was considered to be a strong N sink (7.7 kg of N $ha^{-1}$ $year^{-1}$), based on a chronosequence study by Covington.[30] However, the hypothesis that the forest floor loses and then regains mass and nutrient content after harvesting has not been substantiated experimentally or theoretically.[45] We tested the hypothesis that the N concentration and content of the forest floor have increased in recent decades. At Hubbard Brook Watershed 6, the forest floor has been sampled every 5 years from 1977 to 2002, allowing a 25 year analysis of changes in N content, reported here for the first time.

Forest floor N content averaged 1800 kg of N $ha^{-1}$ in Watershed 6 and did not change significantly between 1977 and 2002 (linear regression $p$ = 0.84; Figure 2a; see Figure SI.1 of

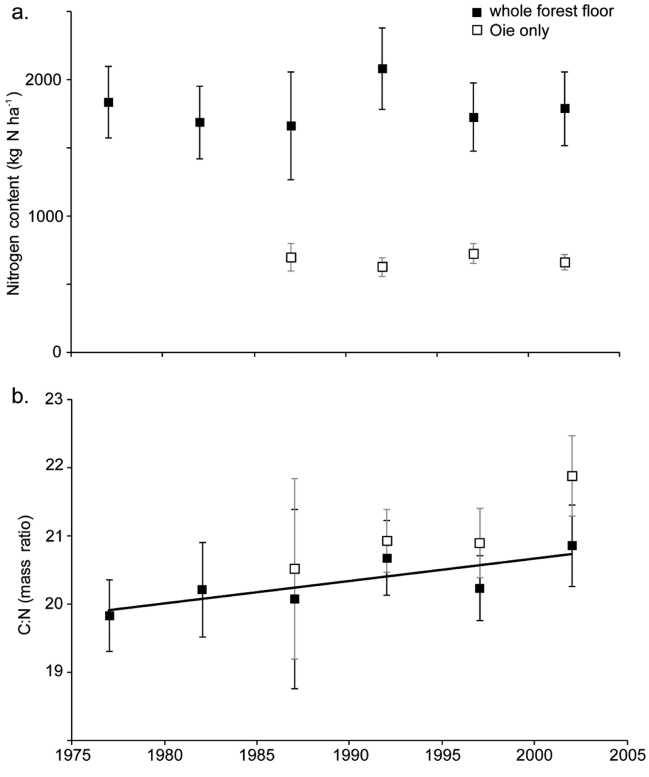



**Figure 2.** (a) Nitrogen content of the forest floor as a whole and the Oie horizon alone (when sampled separately), at Hubbard Brook Watershed 6. Error bars show 95% CIs for the mean. Total forest floor regression: slope of 1.7 ± 19.2 kg of N $ha^{-1}$ $year^{-1}$ (95% CI for the slope); $p$ = 0.84; $R^2$ = 0.01. Oie only: slope of −0.2 ± 19.6 kg of N $ha^{-1}$ $year^{-1}$; $p$ = 0.96; $R^2$ = 0.001. (b) C:N ratio on the forest floor. Total forest floor: slope of 0.03 ± 0.03; $p$ = 0.05; $R^2$ = 0.65. Oie: slope of 0.081 ± 0.118; $p$ = 0.10; $R^2$ = 0.81.

**Table 2. Forest Floor C and N Data[a]**

| | | | | Oie horizon | | | | entire forest floor | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| site | year | age | *n* | C (kg ha$^{-1}$) | CI | N (kg ha$^{-1}$) | CI | C (kg ha$^{-1}$) | CI | N (kg ha$^{-1}$) | CI |
| HB Watershed 6 | 1976–1977 | 67 | 117 | – | – | – | – | 36359 | 5059 | 1834 | 260 |
| HB Watershed 6 | 1982 | 72 | 68 | – | – | – | – | 34106 | 4950 | 1687 | 268 |
| HB Watershed 6 | 1987 | 77 | 70 | 14282 | 2025 | 696 | 99 | 33339 | 7412 | 1661 | 392 |
| HB Watershed 6 | 1992 | 82 | 80 | 13136 | 1408 | 628 | 69 | 43008 | 6428 | 2080 | 297 |
| HB Watershed 6 | 1997 | 87 | 87 | 15108 | 1441 | 723 | 71 | 34854 | 4941 | 1723 | 251 |
| HB Watershed 6 | 2002 | 92 | 100 | 14498 | 1386 | 661 | 58 | 37303 | 5958 | 1788 | 272 |
| H3 | 1979 | 105 | 60 | 14927 | 2185 | 597 | 73 | 51549 | 6237 | 1929 | 196 |
| H3 | 1994 | 120 | 50 | 16259 | 2876 | 639 | 98 | 44492 | 7654 | 1699 | 213 |
| M3 | 1980 | 70 | 50 | – | – | – | – | 43231 | 9125 | 1751 | 479 |
| M3 | 1995 | 85 | 50 | – | – | – | – | 67599 | 15576 | 2849 | 577 |
| H4 | 1979 | 44 | 60 | 21164 | 8724 | 912 | 323 | 28135 | 9519 | 1228 | 339 |
| H4 | 1994 | 59 | 50 | 8069 | 1527 | 363 | 66 | 20312 | 6082 | 913 | 283 |
| H4 | 2003 | 68 | 50 | 12474 | 2456 | 488 | 72 | 20972 | 1607 | 830 | 43 |
| H1 | 1979 | 40 | 60 | 7630 | 1278 | 311 | 51 | 33450 | 7208 | 1459 | 322 |
| H1 | 1994 | 55 | 50 | 10240 | 1417 | 455 | 68 | 35040 | 6560 | 1558 | 311 |
| H1 | 2003 | 64 | 50 | 11696 | 2359 | 468 | 98 | 39871 | 4326 | 1841 | 123 |
| T30 | 1980 | 32 | 50 | – | – | – | – | 43196 | 14601 | 1946 | 600 |
| T30 | 1995 | 47 | 50 | – | – | – | – | 22864 | 5583 | 1121 | 285 |
| T30 | 2003 | 55 | 50 | – | – | – | – | 37131 | 12367 | 1762 | 427 |
| M4 | 1980 | 30 | 50 | – | – | – | – | 34485 | 8938 | 1433 | 303 |
| M4 | 1995 | 45 | 50 | – | – | – | – | 42745 | 12920 | 1862 | 610 |

[a]For HBEF Watershed 6 and for six mature White Mountain forest stands.

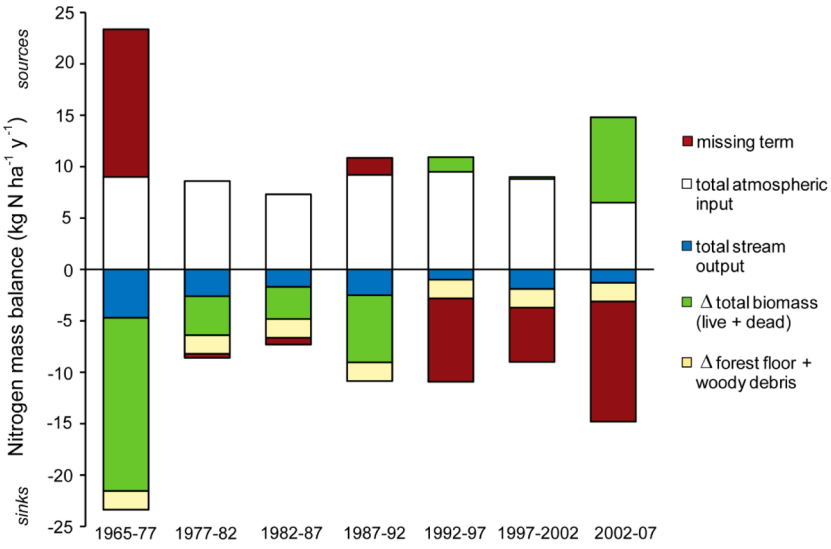



**Figure 3.** Ecosystem-scale mass balance of N at Hubbard Brook Watershed 6, a northern hardwood forest that was aggrading biomass until approximately 1982, for a 37 year period during which the vegetation and forest floor were measured seven and six times, respectively.

the Supporting Information). No sampling date had N content statistically distinguishable from that of any other date. On average, the N content increased 2 kg of N ha$^{-1}$ year$^{-1}$; however, the 95% CI on the regression slope ranged from −21 to 24 kg of N ha$^{-1}$ year$^{-1}$, and the $R^2$ of the regression was 0.01.

The forest floor was collected in multiple layers at most sampling dates, usually the Oie (the litter and fermented layers) and Oa (humic layers).[17] The boundary between the Oa and the mineral soil is difficult to determine in the field, and forest floors tend to be sampled to greater depths when soils are wetter, as in 1992, because mineral particles are harder to distinguish when wet.[17] It is not surprising, therefore, that interannual variation in N content was lower in the Oie horizons than in the forest floor as a whole (Figure 2a). The change in the N content of the Oie alone was an insignificant decrease of −0.2 ± 19.6 kg of N ha$^{-1}$ year$^{-1}$ [$p$ = 0.96; $R^2$ = 0.001 (Figure 2a)] of a total of 680 kg of N ha$^{-1}$.

Similarly, the C content of the forest floor of Watershed 6 showed no significant trend. Over time, the C content averaged 37 Mg of C ha$^{-1}$, with a minimum of 33 Mg of C ha$^{-1}$ in 1987 and a maximum of 43 Mg of C ha$^{-1}$ in 1992. The trend was an insignificant increase of 0.10 ± 0.50 Mg of C ha$^{-1}$ year$^{-1}$. The C content of the Oie alone averaged 14 Mg of C ha$^{-1}$ with an insignificant gain of 0.05 ± 0.35 Mg of C ha$^{-1}$ year$^{-1}$. Surprisingly, the C:N ratio in the forest floor increased slightly over the 25 year period, from 19.8 in 1977 to 20.9 in 2002 (Figure 2b). The increase in the C:N ratio was 0.03 ± 0.03 g of N (g of C)$^{-1}$ year$^{-1}$ [linear regression $p$ = 0.05; $R^2$ = 0.65


dx.doi.org/10.1021/es4025723 | Environ. Sci. Technol. 2013, 47, 11440–11448

(Figure 2b)]. This trend runs counter to expectation if the forest floor is a net sink for N and counter to the theory about the long-term trajectory of the forest floor under N saturation.[2,44] For the 15 years of separate Oie measurements, the change in the C:N ratio was also positive [0.08 ± 0.12 g of N (g of C)$^{-1}$ year$^{-1}$; $R^2$ = 0.81; $p$ = 0.10 (1987−2002)]. The causes of the long-term increase in forest floor C:N ratio are not clear but might be related to increases in atmospheric $CO_2$[46,47] or decreases in decomposition rates associated with N addition,[48] which may result from inhibition of lignin-degrading microbial enzymes.[49,50]

In six additional mid- to old-age northern hardwood stands in the White Mountain region, forest floors were sampled in 1979 and 1980 and in 1994 and 1995; three of these stands were resampled in 2003, providing a 24 year record comparable to that at Hubbard Brook (Table 2). These sites ranged from 55 to 128 years of age in 2003. As at Hubbard Brook, the trend in forest floor N was not significant ($p$ = 0.24), with an average decrease of 5 ± 20 kg of N ha$^{-1}$ year$^{-1}$ (Figure 2a; see Figure SI.1 if the Supporting Information). The C:N ratio of the forest floor did not change significantly ($p$ = 0.21; the average change over all six sites was −0.03 ± 0.05). Overall, we found no evidence that the forest floor is currently a substantial sink for N at Hubbard Brook (HB) or in the region.

**Where Has All the Nitrogen Gone?** We constructed a time series mass balance for N at Hubbard Brook Watershed 6. We chose a 5 year time step to correspond to the sampling interval for vegetation and forest floor. Summing the N fluxes for ecosystem inputs and outputs and changes in living and dead organic matter stocks gives the residual flux required to balance the N budget for each time interval.

The N balance shifted from a missing source in the 1965−1977 period to a missing sink later in the record (Table 1 and Figure 3). Our 1965−1977 budget is similar to that of Bormann et al.[26] but includes more N accumulation in biomass and less in the forest floor, based on improved information. After 1977, when the rate of biomass accumulation slowed and streamflow losses declined, the budget was approximately balanced until the early 1990s. From 1992 to 2007, further declines in biomass and in streamwater export resulted in a missing sink of ∼8 kg of N ha$^{-1}$ year$^{-1}$.

There are several possible explanations for the residual flux required to balance the Hubbard Brook N budget. Gaseous N fluxes in or out of the ecosystem would appear in this term. While some measurements of $N_2O$ flux were made during the 1980s (e.g., ref 51), sustained monitoring of $N_2O$ and measurements of fluxes of $N_2$ began relatively recently at Hubbard Brook.[52] Alternatively, a budget discrepancy could be hidden in the net error of all the other measured and estimated stocks and fluxes,[53] such as the uncertainty in the assumed lack of change in the mineral soil.

Changes in the mineral soil have not been monitored in Watershed 6, because such measurements are inherently destructive, and the high spatial variation in soil mass gives large uncertainties in repeated sampling.[32] At Hubbard Brook Watershed 5, adjacent to Watershed 6, four sets of 0.5 m$^2$ quantitative pits were excavated at 59 distinct sites across the watershed over a 15 year period. Between 1983 (just prior to whole-tree harvesting) and 1998, there was a weakly significant decline in mineral soil N of 54 ± 53 kg of N ha$^{-1}$ year$^{-1}$ ($p$ = 0.05). Repeated measurements over 25 years in three second-growth stands (35−90 years of age) and two nearby mowed fields on abandoned agricultural land 10 km away give similar results: nonsignificant declines of 15 ± 22 kg of N ha$^{-1}$ year$^{-1}$ in O + Ap (disturbed) horizons and 33 ± 41 kg of N ha$^{-1}$ year$^{-1}$ in the soil profile.[18] Because these uncertainties are larger than the "missing" fluxes, other evidence must be brought to bear to explain the change over time in the N budget.

Measurements of N in soil solution in Watershed 6 between 1992 and 2008 can shed some light on the mass balance of N in soil stores. The flux of total N (DIN plus DON) from the forest floor to the mineral soil exceeds the total N flux leaving the Bs horizon by 8.5 ± 2.1 kg of N ha$^{-1}$ year$^{-1}$, which is consistent with the current missing N sink. It is not clear, however, whether the sink is due to the accumulation of N in the mineral soil or to gaseous losses from this horizon.

**Is Gaseous Loss the Missing Sink?** Gaseous losses of $N_2$ and $N_2O$ via nitrification and denitrification represent a potentially important pathway for the loss of N from the ecosystem.[28] These fluxes are inherently difficult to measure, especially at the spatial and temporal scales of an ecosystem *in situ* budget. New intact soil core methods allow for direct *in situ* measurements of both $N_2O$ and $N_2$, which is especially difficult to measure because of its high background concentration.[54,55] Annual flux estimates of $N_2O$ ranged from 0.27 to 1.4 kg of N ha$^{-1}$ year$^{-1}$ between 1997 and 2004.[52,56] These estimates are similar to those for other sites in the northeastern United States[57,58] but are lower than fluxes in highly N-saturated European forests.[59,60]

The partitioning of gaseous N losses between $N_2$ and $N_2O$ (a potent greenhouse gas) is important for assessing the environmental impact of denitrification. Balancing the N budget from 1992 to 2007 (Table 1) without invoking any unmeasured changes in ecosystem stocks (such as accumulation in the mineral soil) would require gaseous N losses of ∼5−12 kg of N ha$^{-1}$ year$^{-1}$. We can estimate total gaseous N losses at Hubbard Brook by considering our measured annual $N_2O$ fluxes of ∼1.0 kg of N ha$^{-1}$ year$^{-1}$,[56] and assuming that $N_2O$ accounts for 8−17% of total gaseous N losses from the soil. This is a reasonable assumption as a global review suggests that $N_2O$ accounts for 3−100% of the total gaseous N flux from forest soils, with a median of 25%.[28] Recent data collected at Hubbard Brook suggest an $N_2O$ contribution at the low end of this range.[54]

If gaseous loss via nitrification and denitrification accounts for a large fraction of the "missing sink", our budget (Table 1 and Figure 3) suggests that it may have increased markedly since the early 1990s. Measurements of $N_2O$ flux on Watershed 6 in the early 1980s were 1 order of magnitude lower (0.17 ng cm$^{-2}$ h$^{-1}$)[51] than our recent observations (1.05−3.22 ng cm$^{-2}$ h$^{-1}$).[52] Both air[61] and soil[39] temperatures have increased at Hubbard Brook, and nitrate availability has likely also increased,[62] consistent with reduced demand for N by vegetation. The frequency of anoxic soil conditions necessary for denitrification may also be increasing: soil moisture[63] and the number of days when precipitation is >25 mm,[61] those that are most likely to create anaerobic conditions, have increased significantly over the long-term records at Hubbard Brook. In addition, Groffman et al.[52] showed that soil freezing induced by reduced snow cover increased nitrification rates. In-stream denitrification may also have increased at Hubbard Brook because coarse woody debris can provide "hot spots" of denitrification,[64] and woody debris may have increased in streams since harvest removals ceased in the early 1900s. These aspects of forest change and climate change might contribute to accelerated $N_2O$ loss, creating an important feedback to the

climate system in forests receiving chronically elevated N deposition.

**Consequences of a Changing Nitrogen Cycle.** Predicting the long-term consequences of anthropogenic N deposition on forest health, water quality, and feedbacks on global climate requires understanding the controls of ecosystem sinks for N. If forests are retaining N, whether in living biomass, in dead biomass such as woody debris, in the forest floor, or in any form in the mineral soil, these stocks might be expected to saturate in the future. Our ignorance of the fate of anthropogenic N makes it difficult to predict how much more N the ecosystem can retain and consequently whether and when export of N to surface water and groundwater will begin to increase. The missing N sink may include gaseous N losses rather than N retention in the ecosystem, which could explain the lack of symptoms of N saturation. If denitrification or other gaseous N losses have increased in response to N deposition, these potentially represent an important anthropogenic source of greenhouse gases (~0.7 Mg of $CO_2$ $ha^{-1}$ $year^{-1}$). Alternatively, the long-term storage of N in the soil would require a net flux of approximately 0.6 Mg of $CO_2$ $ha^{-1}$ $year^{-1}$ to soil organic matter.[29] Dissolved inorganic N inputs and outputs continue to decline because of controls on power plant emissions since 2003;[65] how the ecosystem mass balance of N will change as a result of this sustained decrease in inputs, given the legacy of cumulative past N inputs, remains to be seen.

## ■ ASSOCIATED CONTENT

### Supporting Information

Long-term patterns in regional forest floor nitrogen and carbon to nitrogen ratios and long-term patterns in dry to wet nitrogen deposition from the U.S. Environmental Protection Agency CASTNET site at Hubbard Brook. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*Department of Civil and Environmental Engineering, Syracuse University, 151 Link Hall, Syracuse, NY 13244. E-mail: ctdrisco@syr.edu. Phone: (315) 443-3434.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Susan Woontner, Elizabeth Hane, and Jeffrey Beem-Miller for their efforts in assembling the long-term forest floor data sets for Watershed 6. Concentrations of DIN in precipitation and streamflow were provided by Gene E. Likens with financial support from the National Science Foundation and the Andrew W. Mellon Foundation. Concentrations of DON in precipitation were provided by John Campbell. Steve Stehman provided statistical consultation. This work is a contribution of the Hubbard Brook Ecosystem Study. Hubbard Brook is part of the Long-Term Ecological Research (LTER) network, which is supported by the National Science Foundation. The Hubbard Brook Experimental Forest is operated and maintained by the U.S. Department of Agriculture Forest Service, Northern Forest Research Station, Newtown Square, PA.

## ■ REFERENCES

(1) Galloway, J. N.; Aber, J. D.; Erisman, J. W.; Seitzinger, S. P.; Howarth, R. W.; Cowling, E. B.; Cosby, B. J. The nitrogen cascade. *BioScience* **2003**, *53* (4), 341−356.

(2) Aber, J. D.; Nadelhoffer, K. J.; Steudler, P.; Melillo, J. M. Nitrogen saturation in northern forest ecosystems. *BioScience* **1989**, *39* (6), 378−386.

(3) Bowen, J. L.; Valiela, I. Historical changes in atmospheric nitrogen deposition to Cape Cod, Massachusetts, USA. *Atmos. Environ.* **2001**, *35*, 1039−1051.

(4) Galloway, J. N.; Dentener, F. J.; Capone, D. G.; Boyer, E. W.; Howarth, R. W.; Seitzinger, S. P.; Asner, G. P.; Cleveland, C. C.; Green, P. A.; Holland, E. A.; Karl, D. M.; Michaels, A. F.; Porter, J. H.; Townsend, A. R.; Vorosmarty, C. J. Nitrogen cycles: Past, present, and future. *Biogeochemistry* **2004**, *70* (2), 153−226.

(5) Driscoll, C. T.; Whitall, D.; Aber, J.; Boyer, E.; Castro, M.; Cronan, C.; Goodale, C. L.; Groffman, P.; Hopkinson, C.; Lambert, K.; Lawrence, G.; Ollinger, S. Nitrogen pollution in the northeastern United States: Sources, effects, and management options. *BioScience* **2003**, *53* (4), 357−374.

(6) Likens, G. E.; Driscoll, C. T.; Buso, D. C. Long-term effects of acid rain: Response and recovery of a forest ecosystem. *Science* **1996**, *272* (5259), 244−246.

(7) Pardo, L. H.; Fenn, M.; Goodale, C. L.; Geiser, L. H.; Driscoll, C. T.; Allen, E.; Baron, J.; Bobbink, R.; Bowman, W. D.; Clark, C.; Emmett, B.; Gilliam, F. S.; Greaver, T.; Hall, S. J.; Lilleskov, E. A.; Liu, L.; Lynch, J.; Nadelhoffer, K.; Perakis, S.; Robin-Abbott, M. J.; Stoddard, J.; Weathers, K.; Dennis, R. L. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecol. Appl.* **2011**, *21* (8), 3049−3082.

(8) Townsend, A. R.; Braswell, B. H.; Holland, E. A.; Penner, J. E. Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. *Ecol. Appl.* **1996**, *6* (3), 806−814.

(9) Magnani, F.; Mencuccini, M.; Borghetti, M.; Berbigier, P.; Berninger, F.; Delzon, S.; Grelle, A.; Hari, P.; Jarvis, P. G.; Kolari, P.; Kowalski, A. S.; Lankreijer, H.; Law, B. E.; Lindroth, A.; Loustau, D.; Manca, G.; Moncrieff, J. B.; Rayment, M.; Tedeschi, V.; Valentini, R.; Grace, J. The human footprint in the carbon cycle of temperate and boreal forests. *Nature* **2007**, *447* (7146), 848−850.

(10) Magnani, F.; Mencuccini, M.; Borghetti, M.; Berninger, F.; Delzon, S.; Grelle, A.; Hari, P.; Jarvis, P. G.; Kolari, P.; Kowalski, A. S.; Lankreijer, H.; Law, B. E.; Lindroth, A.; Loustau, D.; Manca, G.; Moncrieff, J. B.; Tedeschi, V.; Valentini, R.; Grace, J. Ecologically implausible carbon response? Reply. *Nature* **2008**, *451* (7180), E3−E4.

(11) Nadelhoffer, K. J.; Emmett, B. A.; Gundersen, P.; Kjonaas, O. J.; Koopmans, C. J.; Schleppi, P.; Tietema, A.; Wright, R. F. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature* **1999**, *398* (6723), 145−148.

(12) Janssens, I. A.; Luyssaerts, S. Carbon cycle: Nitrogen's carbon bonus. *Nat. Geosci.* **2009**, *2*, 318−319.

(13) Aber, J. D.; Magill, A.; McNulty, S. G.; Boone, R. D.; Nadelhoffer, K. J.; Downs, M.; Hallett, R. Forest biogeochemistry and primary production altered by nitrogen saturation. *Water, Air, Soil Pollut.* **1995**, *85* (3), 6.

(14) Aber, J.; McDowell, W.; Nadelhoffer, K.; Magill, A. Nitrogen saturation in temperate forest ecosystems: Hypotheses revisited. *BioScience* **1998**, *48* (11), 14.

(15) Likens, G. E.; Bormann, F. H. *Biogeochemistry of a Forested Ecosystem*, 2nd ed.; Springer-Verlag: New York, 1995; pp 159.

(16) Federer, C. A. Organic matter and nitrogen content of the forest floor in even-aged northern hardwoods. *Can. J. For. Res.* **1984**, *14* (6), 763−767.

(17) Yanai, R. D.; Siccama, T. G.; Arthur, M. A.; Federer, C. A.; Friedland, A. J. Accumulation and depletion of base cations in forest floors in the northeastern US. *Ecology* **1999**, *80*, 2774−2787.

(18) Hamburg, S. P. Effects of forest growth on soil nitrogen and organic matter pools following release from subsistence agriculture. In *Forest Soils & Treatment Impacts*; Stone, E. L., Ed.; Department of

Forestry, Wildlife and Fisheries, The University of Tennessee: Knoxville, TN, 1984; pp 145−158.
(19) Likens, G. E. Chemistry of Bulk Precipitation at HBEF WS-6. Hubbard Brook Ecosystem Study, 2012 (http://www.hubbardbrook.org/data/dataset.php?id=20).
(20) Likens, G. E. Chemistry of Streamwater at HBEF WS-6. Hubbard Brook Ecosystem Study, 2012 (http://www.hubbardbrook.org/data/dataset.php?id=8).
(21) Buso, D. C.; Likens, G. E.; Eaton, J. S. Chemistry of precipitation, streamwater, and lakewater from the Hubbard Brook Ecosystem Study: A record of sampling protocols and analytical procedures. General Technical Report NE-275; U.S. Department of Agriculture, Forest Service: Newtown Square, PA, 2000; pp 52.
(22) Dittman, J. A.; Driscoll, C. T.; Groffman, P. M.; Fahey, T. J. Dynamics of nitrogen and dissolved organic carbon at the Hubbard Brook Experimental Forest. *Ecology* **2007**, *88* (5), 1153−1166.
(23) Campbell, J. L.; Hornbeck, J. W.; Mitchell, M. J.; Adams, M. B.; Castro, M. S.; Driscoll, C. T.; Kahl, J. S.; Kochenderfer, J. N.; Likens, G. E.; Lynch, J. A.; Murdoch, P. S.; Nelson, S. J.; Shanley, J. B. Input-output budgets of inorganic nitrogen for 24 forest watersheds in the Northeastern United States: A review. *Water, Air, Soil Pollut.* **2004**, *151* (1−4), 24.
(24) Lovett, G. M.; Bowser, J. J.; Edgerton, E. S. Atmospheric deposition to watersheds in complex terrain. *Hydrol. Processes* **1997**, *11* (7), 645−654.
(25) Siccama, T. G.; Denny, E. Interactive Hubbard Brook Landscape Biomass Program. Watershed 6 at Hubbard Brook, 2004 (http://www.hubbardbrook.org/w6_tour/biomass-stop/biomassw6.htm).
(26) Bormann, F. H.; Likens, G. E.; Melillo, J. M. Nitrogen budget for an aggrading northern hardwood forest ecosystem. *Science* **1977**, *196* (4293), 981−983.
(27) Acker, M. Base cation concentration and content in litterfall and woody debris across a northern hardwood forest chronosequence. Ph.D. Dissertation, University of Kentucky, Lexington, KY, 2006.
(28) Schlesinger, W. H. On the fate of anthropogenic nitrogen. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106* (1), 203−208.
(29) de Vries, W.; Solberg, S.; Dobbertin, M.; Sterba, H.; Laubhann, D.; van Oijen, M.; Evans, C.; Gundersen, P.; Kros, J.; Wamelink, G. W. W.; Reinds, G. J.; Sutton, M. A. The impact of nitrogen deposition on carbon sequestration by European forests and heathlands. *For. Ecol. Manage.* **2009**, *258* (8), 1814−1823.
(30) Covington, W. W. Changes in forest floor organic-matter and nutrient content following clear cutting in northern hardwoods. *Ecology* **1981**, *62* (1), 41−48.
(31) Roskoski, J. P. Nitrogen fixation in hardwood forests of the northeastern United States. *Plant Soil* **1980**, *54*, 33−44.
(32) Huntington, T. G.; Ryan, D. F.; Hamburg, S. P. Estimating soil nitrogen and carbon pools in a northern hardwood forest ecosystem. *Soil Sci. Soc. Am. J.* **1988**, *52* (4), 1162−1167.
(33) Whittaker, R. H.; Bormann, F. H.; Likens, G. E.; Siccama, T. G. The Hubbard Brook Ecosystem Study: Forest biomass and production. *Ecol. Monogr.* **1974**, *44*, 233−254.
(34) Yanai, R. D.; Battles, J. J.; Richardson, A. D.; Blodgett, C. A.; Wood, D. M.; Rastetter, E. B. Estimating uncertainty in ecosystem budget calculations. *Ecosystems* **2010**, *13* (2), 239−248.
(35) Likens, G. E.; Bormann, F. H. Chemical analyses of plant tissues from the Hubbard Brook Ecosystem in New Hampshire. Bulletin 79; Yale University School of Forestry: New Haven, CT, 1970; pp 25.
(36) Arthur, M. A.; Hamburg, S. P.; Siccama, T. G. The accuracy of allometric estimates of aboveground living biomass and nutrient contents of a northern hardwood forest. *Can. J. For. Res.* **2001**, *31*, 11−17.
(37) Fahey, T. J. Watershed 6 Temporal Canopy Leaf Chemistry. Hubbard Brook Ecosystem Study, 2012 (http://www.hubbardbrook.org/data/dataset.php?id=44).
(38) Likens, G. E.; Bormann, F. H.; Pierce, R. H.; Eaton, J. S.; Johnson, N. M. *Biogeochemistry of a Forested Ecosystem*; Springer-Verlag: New York, 1977; p 146.
(39) Bernal, S.; Hedin, L. O.; Likens, G. E.; Gerber, S.; Buso, D. C. Complex response of the forest nitrogen cycle to climate change. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109* (9), 3406−3411.
(40) Driscoll, C. T.; Cowling, E. B.; Grennfelt, P.; Galloway, J.; Dennis, R. Integrated assessment of ecosystem effects of atmospheric deposition: Lessons available to be learned. *EM Magazine* **2010**, No. November, 6−13.
(41) Houlton, B. Z.; Driscoll, C. T.; Fahey, T. J.; Likens, G. E.; Groffman, P. M.; Bernhardt, E. S.; Buso, D. C. Nitrogen dynamics in ice storm damaged forest ecosystems: Implications for nitrogen limitation theory. *Ecosystems* **2003**, *6*, 431−443.
(42) Goodale, C. L.; Aber, J. D.; Vitousek, P. M. An unexpected nitrate decline in New Hampshire streams. *Ecosystems* **2003**, *6* (1), 75−86.
(43) Martin, C.; Driscoll, C.; Fahey, T. Changes in streamwater chemistry after 20 years from forested watersheds in New Hampshire, USA. *Can. J. For. Res.* **2000**, *30* (8), 1206−1213.
(44) Agren, G. I.; Bosatta, E. Nitrogen saturation of terrestrial ecosystems. *Environ. Pollut.* **1988**, *54* (3−4), 185−197.
(45) Yanai, R. D.; Currie, W. S.; Goodale, C. L. Soil carbon dynamics after forest harvest: An ecosystem paradigm reconsidered. *Ecosystems* **2003**, *6* (3), 197−212.
(46) Ollinger, S. V.; Goodale, C. L.; Hayhoe, K.; Jenkins, J. P. Potential effects of climate change and rising $CO_2$ on ecosystem processes in northeastern U.S. forests. *Mitig. Adapt. Strategies Gl. Chang.* **2008**, *13*, 467−485.
(47) Ollinger, S. V.; Aber, J. D.; Reich, P. B.; Freuder, R. J. Interactive effects of nitrogen deposition, tropospheric ozone, elevated $CO_2$ and land use history on the carbon dynamics of northern hardwood forests. *Glob. Chang. Biol.* **2002**, *8* (6), 545−562.
(48) Janssens, I. A.; Dieleman, W.; Luyssaert, S.; Subke, J. A.; Reichstein, M.; Ceulemans, R.; Ciais, P.; Dolman, A. J.; Grace, J.; Matteucci, G.; Papale, D.; Piao, S. L.; Schulze, E. D.; Tang, J.; Law, B. E. Reduction of forest soil respiration in response to nitrogen deposition. *Nat. Geosci.* **2010**, *3* (5), 315−322.
(49) Carreiro, M. M.; Sinsabaugh, R. L.; Repert, D. A.; Parkhurst, D. F. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* **2000**, *81* (9), 2359−2365.
(50) Weand, M. P.; Arthur, M. A.; Lovett, G. M.; McCulley, R. L.; Weathers, K. C. Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. *Soil Biol. Biochem.* **2010**, *42* (12), 2161−2173.
(51) Keller, M.; Goreau, T. J.; Wofsy, S. C.; Kaplan, W. A.; Mcelroy, M. B. Production of nitrous oxide and consumption of methane by forest soils. *Geophys. Res. Lett.* **1983**, *10* (12), 1156−1159.
(52) Groffman, P. M.; Hardy, J. P.; Driscoll, C. T.; Fahey, T. J. Snow depth, soil freezing, and fluxes of carbon dioxide, nitrous oxide and methane in a northern hardwood forest. *Glob. Chang. Biol.* **2006**, *12* (9), 1−13.
(53) Yanai, R. D.; Levine, C. R.; Green, M. B.; Campbell, J. L. Quantifying uncertainty in forest nutrient budgets. *J. For.* **2012**, *110* (8), 448−456.
(54) Kulkarni, M. V.; Burgin, A. J.; Groffman, P. M.; Yavitt, J. B. Direct flux and $^{15}N$ tracer methods for measuring denitrification in forest soils. *Biogeochemistry* **2013**, http://download.springer.com/static/pdf/720/art%253A10.1007%252Fs10533-013-9876-7.pdf?auth66=1380905000_69398f2c64f812e079c41a131a15ddb8&ext=.pdf.
(55) Wang, R.; Willibald, G.; Feng, Q.; Zheng, X.; Liao, T.; Brüggemann, N.; Butterbach-Bahl, K. Measurement of $N_2$, $N_2O$, NO, and $CO_2$ emissions from soil with the gas-flow-soil-core technique. *Environ. Sci. Technol.* **2011**, *45*, 6066−6072.
(56) Groffman, P. M.; Hardy, J. P.; Fisk, M. C.; Fahey, T.; Driscoll, C. T. Climate variation and soil carbon and nitrogen cycling processes in a northern hardwood forest. *Ecosystems* **2009**, *12* (6), 927−943.
(57) Peterjohn, W. T.; McGervey, R. J.; Sexstone, A. J.; Christ, M. J.; Foster, C. J.; Adams, M. B. Nitrous oxide production in two forested watersheds exhibiting symptoms of nitrogen saturation. *Can. J. For. Res.* **1998**, *28* (11), 1723−1732.

(58) Venterea, R. T.; Groffman, P. M.; Verchot, L. V.; Magill, A. H.; Aber, J. D. Gross nitrogen process rates in temperate forest soils exhibiting symptoms of nitrogen saturation. *For. Ecol. Manage.* **2004**, *196* (1), 14.
(59) Brumme, R.; Borken, W.; Finke, S. Hierarchical control on nitrous oxide emission in forest ecosystems. *Global Biogeochem. Cycles* **1999**, *13* (4), 1137−1148.
(60) Butterbach-Bahl, K.; Breuer, L.; Gasche, R.; Willibald, G.; Papen, H. Exchange of trace gases between soils and the atmosphere in Scots pine forest ecosystems of the northeastern German lowlands 1. Fluxes of $N_2O$, $NO/NO_2$ and $CH_4$ at forest sites with different N-deposition. *For. Ecol. Manage.* **2002**, *167* (1−3), 123−134.
(61) Hamburg, S. P.; Vadeboncoeur, M. A.; Richardson, A. D.; Bailey, A. S. Climate change at the ecosystem scale: A 50-year record in New Hampshire. *Clim. Change* **2013**, *116* (3−4), 457−477.
(62) Duran, J.; Morse, J. L.; Groffman, P. M. Comparison of *in situ* methods to measure N mineralization rates in forest soils. *Soil Biol. Biochem.* **2012**, *46*, 145−147.
(63) Groffman, P. M.; Rustad, L. E.; Templer, P. H.; Campbell, J. L.; Christenson, L. M.; Lany, N. K.; Socci, A. M.; Vadeboncoeur, M. A.; Schaberg, P. G.; Wilson, G. F.; Driscoll, C. T.; Fahey, T. J.; Fisk, M. C.; Goodale, C. L.; Green, M. B.; Hamburg, S. P.; Johnson, C. E.; Mitchell, M. J.; Morse, J. L.; Pardo, L. H.; Rodenhouse, N. L. Long-term integrated studies show complex and surprising effects of climate change in the northern hardwood forest. *BioScience* **2012**, *62* (12), 1056−1066.
(64) Bernhardt, E. S.; Likens, G. E.; Buso, D. C.; Driscoll, C. T. In stream uptake dampens effects of major forest disturbance on watershed nitrogen export. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (18), 5.
(65) Likens, G. E.; Buso, D. C. Dilution and the elusive baseline. *Environ. Sci. Technol.* **2012**, *46* (8), 4382−4387.
(66) Fahey, T. J.; Hughes, J. W.; Pu, M.; Arthur, M. A. Root decomposition and nutrient flux following whole-tree harvest of northern hardwood forest. *For. Sci. (Bethesda, Md)* **1988**, *34* (3), 744−768.