Complex response of the forest nitrogen cycle to climate change

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Climate exerts a powerful influence on biological processes, but the effects of climate change on ecosystem nutrient flux and cycling are poorly resolved. Although rare, long-term records offer a unique opportunity to disentangle effects of climate from other anthropogenic influences. Here, we examine the longest and most complete record of watershed nutrient and climate dynamics available worldwide, which was collected at the Hubbard Brook Experimental Forest in the northeastern United States. We used empirical analyses and model calculations to distinguish between effects of climate change and past perturbations on the forest nitrogen (N) cycle. We find that climate alone cannot explain the occurrence of a dramatic >90% drop in watershed nitrate export over the past 46 y, despite longer growing seasons and higher soil temperatures. The strongest climate influence was an increase in soil temperature accompanied by a shift in paths of soil water flow within the watershed, but this effect explained, at best, only ∼40% of the nitrate decline. In contrast, at least 50–60% of the observed change in the N export could be explained by the long-lasting effect of forest cutting in the early 1900s on the N cycle of the soil and vegetation pools. Our analysis shows that historic events can obscure the influence of modern day stresses on the N cycle, even when analyses have the advantage of being informed by 0.5-century-long datasets. These findings raise fundamental questions about interpretations of long-term trends as a baseline for understanding how climate change influences complex ecosystems.

forest ecosystems | long-term monitoring | streamwater chemistry | precipitation chemistry | nutrient cycles

Our understanding of how climate change impacts complex ecological systems depends on our conception of a baseline against which change can be judged and knowledge of how this baseline has been shaped by historical conditions. At the Hubbard Brook Experimental Forest (HBEF) in New Hampshire, for example, we know that current concentrations of nitrate in watershed streams are the lowest in 46 y of measurement and that ecosystem nitrate losses have decreased by >90% over this time (Fig. 1*A*). If we were to take the early high nitrate period (1969–1976) as the historical reference, we would estimate that nitrate export has dropped by a total of ∼125 kg nitrogen (N) ha^{-1} during the 30 y of the decline (1977–2007) (Fig. 1*A*). Such a large drop in N export is ecologically relevant and constitutes a dramatic shift in the ecosystem N cycle: from a leaky cycle that retained only ∼30% of external inputs in the high stream water nitrate period to a highly retentive cycle that currently captures ∼90% of atmospheric inputs (*Methods*).

We adopt a watershed mass balance approach (1, 2) to examine the factor(s) responsible for this dramatic change in the forest N cycle (Fig. 2). Because climate is an overriding and powerful driver of biological process, we pay particular attention to whether the observed changes in ecosystem N dynamics were linked to climate change over the past five decades. Previous examinations of this trend have discounted effects of changing N deposition and forest maturation, and they have suggested that there may exist a previously unrecognized ecosystem sink for N (3, 4), possibly associated with the soil (5, 6). We evaluate these hypotheses and other competing hypotheses using an analysis that integrates several unique long-term records of biological, physical, and biogeochemical factors within the HBEF. From a mass balance perspective, we show in Fig. 2*A* how the decline in nitrate export (vector 1) could be caused by one or several mechanisms: (*i*) decreased N deposition (vector 2), (*ii*) increased export of dissolved organic N compounds (DON; vector 3), (*iii*) increased net accumulation of N in vegetation (sum of vectors 4 and 5), (*iv*) increased gaseous N loss by denitrification (vector 6), or (*v*) net increase in N stored in the soil pool (balance of vectors 1–6).

Results and Discussion

We first examine atmospheric N deposition (vector 2 in Fig. 2*A*), which has strong potential to influence trends in surface water nitrate (1, 7, 8). Neither the 46-y record of bulk deposition at HBEF (Fig. 1*B*) nor shorter records from nearby locations (*SI Results and Discussion*) can explain the observed drop in watershed nitrate export. Bulk N deposition at HBEF is today similar to values in the 1960s, and there has been no systematic decline large enough to explain the ∼125 kg N ha^{-1} reduction in nitrate export (Fig. 1 *A* vs. *B*). DON contributes <20% to bulk deposition and has declined only marginally since first measured routinely in 1995 (Fig. 1*B*). In addition, dry deposition contributes negligibly to atmospheric N inputs in this densely forested region [0.37 ± 0.02 (volume weighted average ± SE) kg N ha^{-1} y^{-1} or <6% of total N deposition] and does not show any clear trend since first measured routinely in 1989. We conclude that the long-term decline in nitrate export from HBEF watersheds cannot be explained by any coincident change in atmospheric deposition (Fig. 2*B*).

A second possibility is that watershed DON export (vector 3 in Fig. 2*A*) has increased since the 1970s to the extent of quantitatively offsetting the observed reduction in nitrate export. Our analyses show no increase in DON or evidence of such a shift in N forms since DON was first measured routinely in 1994 (Fig. 1*A*). Moreover, present day export (1 ± 0.1 kg N ha^{-1} y^{-1}) is too low to balance N inputs (6.8 ± 0.2 kg N ha^{-1} y^{-1}) or offset the historic nitrate decline. For example, a sustained linear increase of DON export from zero in the 1969–1976 reference period to 1 kg N ha^{-1} y^{-1} by 1995 (when it was first measured) (*SI Results and Discussion*) followed by export of 1 kg N ha^{-1} y^{-1} during 1995–2007 could explain <22 kg N ha^{-1} or <18% of the ∼125 kg ha^{-1} nitrate decline. We conclude that increased DON export cannot resolve the long-term nitrate decline in HBEF watersheds (Fig. 2*B*).

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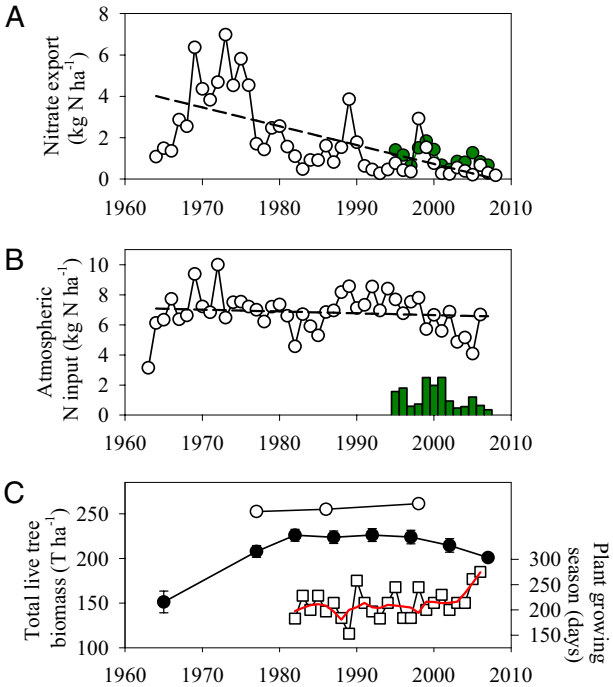

Fig. 1. Historic N loads and forest dynamics at HBEF. (*A*) Annual hydrological export of nitrate (1964–2008; n = 45) and DON (since 1995; n = 13) from watershed 6 (W6; white and green circles, respectively). The linear trend of nitrate across years is shown with a dashed line (r^2 = 0.38, slope ± SE = −0.08 ± 0.02 kg N ha^{-1} y^{-1}, P < 0.001). Nitrate export declined from an average (± SE) of 5.13 ± 0.39 kg N ha^{-1} y^{-1} during the 1969–1976 high nitrate reference period to 0.42 ± 0.07 kg N ha^{-1} y^{-1} in 2000–2007. We quantified the nitrate decline (missing nitrate in the text) between 1977 and 2007 by subtracting the annual nitrate export observed each year from the average value of the high nitrate reference period (5.13 kg N ha^{-1} y^{-1}; 1969–1976); this decline in export was equivalent to 125 ± 12 kg N ha^{-1} over 30 y. Similar quantities result from alternative calculations based on the trend in nitrate export determined from either linear or curvilinear fits to the long-term record (1969–2007). DON export showed no linear trend over time (P > 0.05). (*B*) Annual bulk deposition of inorganic N (nitrate + ammonium) in W6 (1964–2008; n = 45) did not display any significant linear trend over time (dashed line; P > 0.05). There was no significant linear trend in DON deposition since 1995 (green bars; n = 13, P > 0.05). (*C*) Total live tree biomass [≥10 cm diameter at breast height (DBH)] in W6 (1965–2007; n = 8) and the Bird Area (1981–2001; n = 3; black and white circles, respectively; error bars are as in ref. 14; error bars in the Bird Area are smaller than symbols). Length of the growing season from 1982 to 2006 is shown by squares (n = 25); locally weighted scatterplot smoothing is in red. Although there was no significant linear trend before 2002 (P > 0.05), the long-term trend became significantly positive after 2002 (r^2 = 0.89, slope = 19.7 ± 4 d y^{-1}, P < 0.02).

We next examined a mechanism that is highly sensitive to climate change and can exert strong influence on the forest N cycle: accelerated plant growth (vectors 4 and 5 in Fig. 2*A*). More favorable climate, increased atmospheric CO_2, and longer growing seasons can, in theory, trigger growth and increase N stored in plant biomass (9, 10). We used satellite observations of normalized difference vegetation index (NDVI) to quantify any secular trend in the HBEF growing season (11) (*SI Results and Discussion*). Our analysis revealed an increase in growing season length by several weeks (Fig. 1*C*) since satellite imagery first became available in 1982. Closer inspection shows that the trend was caused by increasingly benign autumn conditions since 2002, a period during which nitrate export did not change (Fig. 1 *A* vs. *C*). There was no consistent trend in growing season length before 2002. Although limited to conditions after 1982, this analysis fails to establish any clear link between changes in the plant growing season and nitrate export at HBEF.

We also evaluated the possibility of a climate–ecosystem link by examining direct measures of soil temperatures, which in contrast to NDVI, are available for the long-term record at HBEF. We examined temperatures in deeper soil layers, because these temperatures are less influenced by day to day weather deviations (*Methods* and *SI Results and Discussion*). We found that temperatures have consistently increased since 1961 at 30-cm soil depth, with the steepest change occurring from December to March (Fig. 3*A* and *SI Results and Discussion*). Air temperatures and records of lake ice cover also indicate a warming trend over the same period in the Hubbard Brook Valley (12). These findings show that HBEF forests have experienced substantial warming in late fall to early spring over the past five decades, which in turn, may have caused increased plant growth.

The existence of regular censuses of above- and belowground live biomass since 1965 allows us to examine directly whether the observed warming trend has caused increased plant growth and storage of plant N in HBEF forests—either through temperature alone or the combined effect of temperature plus increasing CO_2. Although tree biomass increased between 1965 and 1982 as expected for an aggrading forest (13), net biomass accumulation declined to approximately zero after 1982 and decreased by ∼10% between 1997 and 2007 (Fig. 1*C*) (14, 15) (*SI Results and Discussion*). Such deceleration of growth is not only inconsistent with conditions of improved climate and increased CO_2 but also with the observed decline in nitrate export (3). Growth deceleration acts to reduce plant N demand, which in turn, should cause increased rather than decreased export of N at the watershed scale (16–18). That we observe a large drop in nitrate as biomass accumulation decelerates indicates that factors other than plant biomass govern the long-term N dynamics of this forest. This rather counterintuitive result raises fundamental questions about the role of climate and plant growth in shaping the forest N cycle over decades. The results do not, however, support the idea that increased plant growth (vectors 4 and 5 in Fig. 2) can resolve the observed nitrate decline.

We next evaluated whether increased microbial denitrification (vector 6 in Fig. 2*A*) could explain the drop in nitrate (NO_3^--N) export. This mechanism might seem unlikely on theoretical grounds, because denitrification generally is high in nitrate-rich (i.e., early HBEF record) but low in nitrate-poor conditions (late HBEF record) (19, 20). We used a stable isotope approach that takes advantage of the substantial isotopic discrimination of $\delta^{15}N$-NO_3^- that occurs during microbial denitrification (21). We expected a negative correlation between $\delta^{15}N$-NO_3^- and nitrate concentration if denitrification was the primary determinant of the stream water nitrate trend. We analyzed archived samples of stream water and bulk deposition since 1990 and fresh samples from 2008 (*Methods* and *SI Results and Discussion*). The $\delta^{15}N$-NO_3^- signature in archived bulk precipitation [0.47 ± 0.78‰ (average ± SE), n = 8] did not differ from the precipitation in 2008 samples (−1.07 ± 0.44‰, n = 6; Wilcoxon rank sum test, z = −1.48, P > 0.05) and showed no trend with nitrate concentration (Fig. 4*C*). In contrast, $\delta^{15}N$-NO_3^- in archived stream water (4.1 ± 0.29‰, n = 11) was strongly but positively correlated with stream water nitrate concentration (Fig. 4*C*) (Spearman's ρ = 0.74, P < 0.01). This positive correlation does not support the idea that denitrification was the primary determinant of stream water nitrate. Rather, the pattern supports the theoretical expectation that denitrification is high when nitrate also is high. We conclude that stream nitrate does not bear the identifying signature of denitrification acting as the major determinant of the nitrate trend (vector 6 in Fig. 2).

We next considered two mechanisms related to increased N storage in soils (SOIL in Fig. 2*A*): (*i*) enhanced N immobilization caused by changing paths of soil water flow and (*ii*) increased N

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accumulation caused by historic disturbance events. Both mechanisms are difficult to detect by direct soil sampling, because the change in N needed to resolve the observed nitrate decline (~125 kg N ha^{-1}) is <2% of total soil N—an amount too small to detect given uncertainties and errors inherent in quantifying the soil N pool (22).

We considered whether climate-induced changes in watershed hydrology might have increased the immobilization of nitrate by soils. Several lines of evidence imply the existence of climate-mediated effects of hydrology on nitrate in the HBEF record, especially during spring snowmelt, which is a period of peak export of both nitrate and water (68% and 54% of annual total, respectively) (1). First, stream nitrate declined most strongly during December to April when soil warming was most rapid, with the steepest drop in March and April when snowmelt volumes are greatest (1) (*SI Results and Discussion*). Second, we found substantial long-term changes in the dynamics of snow cover and snowmelt. Although annual rain and snowfall amounts have increased slightly (5), the period of snow cover has shortened by ~6 d $decade^{-1}$, whereas maximum snow depth has declined by ~6 cm $decade^{-1}$ between 1956 and 2006 (*SI Results and Discussion*). Moreover, the snowfall volume that melted in intermittent warming events (rather than as a single spring melt pulse) has more than doubled from <20% in the 1950s to >40% today (Fig. 3*B*) (*Methods* and *SI Results and Discussion*). The frequency of short-term soil freezing events has increased in response to these intermittent warming periods (5). These results point to dramatic changes in watershed hydrology at HBEF, with potentially strong links to warming soils and periods of changing nitrate export.

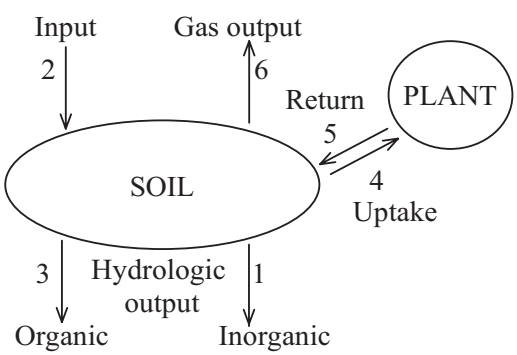
We next evaluated whether these observed hydrological changes might trigger differences in soil nitrate immobilization. Microbial immobilization and root uptake of N depend on the contact time between dissolved nitrate and the soil complex, such that nitrate can escape to watershed streams when water flows on top of frozen soil or in macropores during large snow melt events (23). It is, therefore, plausible that the observed long-term decline of large snowmelt events would have caused increased contact time between nitrate and soils, thus increasing microbial and plant immobilization and decreasing nitrate export. In addition, warmer early spring conditions could also act to enhance microbial/plant immobilization.

To test this hypothesis directly, we monitored watershed discharge, dissolved nitrate, and natural abundance isotopes of oxygen in nitrate ($\delta^{18}O\text{-}NO_3^-$) in bulk deposition, accumulated snow, and stream water during the 2008 snowmelt period (*Methods* and *SI Results and Discussion*). Stream nitrate and $\delta^{18}O\text{-}NO_3^-$ values were both low during base flow, but they increased substantially during large snowmelt events (Fig. 4 *A* and *B*). An isotopic mixing model indicated that >95% of stream nitrate originated from soil nitrifying bacteria during base flow but that up to 40% derived directly from snowmelt during large melt events (Fig. 4*B*). We conclude that large melt events can short circuit water flow paths and allow nitrate to bypass the soil immobilization trap. This finding raises the question of whether the HBEF nitrate decline could be explained by changes in snowmelt dynamics: from few and large melt events early to frequent and small events later in the long-term record.

We can place an upper limit to this hydrologic mechanism by assuming that snowmelt bypassed soils during the high nitrate period of 1969–1976 but was fully intercepted and immobilized by microbes and fine roots since 1977. This calculation indicates a maximum soil sink of 54 kg N ha^{-1}, which can explain up to 43% of the long-term decline in N export (*Methods* and *SI Results and Discussion*). As discussed earlier, however, there is no evidence that such an induced root sink for N has translated into faster plant growth or higher aboveground biomass (Fig. 1*C*). We conclude that changes in hydrological flow paths and soil temperature could influence nitrate retention by soils but that the effect is not large enough to cause a detectable increase in plant growth or explain the entire decline in nitrate export (Fig. 2*B*).

Finally, we explored whether historical vegetation disturbances could explain the observed decline in N export through changes in the soil N pool (SOIL in Fig. 2). The long-term influence of such disturbances on the N cycle is often elusive; long-term records are scarce, and it is difficult to disentangle past and present perturbations in real world datasets. Model calculations can help resolve such complex historical interactions, and we used a modeling approach to examine two influences: (*i*) major events of tree mortality (>20% of total live biomass) caused by harvests in 1906 and 1917, the 1938 hurricane, and the 1998 ice storm at HBEF (24–26),

A



B

Hypothesis	Results	Contribution to the missing N: 125 kg N ha^{-1} over 30 y
Decreased N deposition (vector 2)	Rejected	
Increased DON losses (vector 3)	Unlikely	
Longer growing season	Rejected	
Plant accumulation (vector 4 - vector 5)	Rejected	
Increased denitrification (vector 6)	Unlikely	
Changes in soil water flowpaths/temperature (SOIL)	Plausible	up to 43%
Sugar maple decline (SOIL)	Plausible	up to 18%
Historical disturbances (SOIL)	Plausible	up to 60%*

* Using published estimates of tree mortality and harvest removal (24). Using other reasonable tree mortality scenarios the contribution could be even greater (*SI Results and Discussion*).

Fig. 2. Schematic representation of N fluxes and storage pools for a typical temperate hardwood forest and synthesis of competing hypotheses related to the long-term declining trend in nitrate export. (*A*) Conceptual model of fluxes and storage pools of N in the northeastern US hardwood forests. Atmospheric deposition (vector 2) is the major input flux of nitrogen to the forest. Denitrification (vector 6) and leaching (vector 1 and 3) are the major output fluxes of nitrogen from the ecosystem. Nitrogen is taken up and stored by vegetation (vector 4), returning eventually to the soil pool as litterfall or root exudates (vector 5); also, it may be cycled by microbes and stored in the soil. (*B*) Synthesis of the competing hypotheses considered here and their potential contribution to declining nitrate export observed in W6 at the HBEF.

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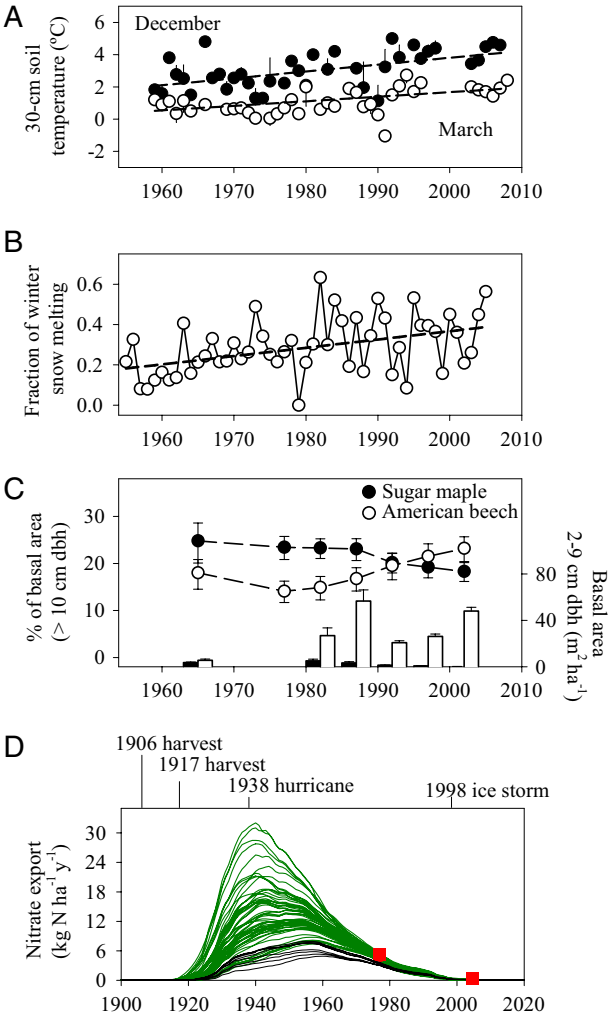

Fig. 3. Historic soil temperature and hydrological data, changes in forest species composition, and simulated long-term trend of nitrate export with the LM3V model. (*A*) Mean (error bars are ± SE) soil temperature at 30-cm depth in December and March (1959–2007). The linear fit (dashed lines) was significant in both cases ($P < 0.001$; December: $r^2 = 0.28$, slope = 0.046 ± 0.011 °C y^{-1}, $n = 39$; March: $r^2 = 0.27$, slope = 0.028 ± 0.007 °C y^{-1}, $n = 38$). There was no significant trend in late spring and summer ($P > 0.05$ for April to September). (*B*) Fraction of intermittent warming events that caused winter snowmelt (1955–2007). The dashed line is the linear fit ($r^2 = 0.18$, slope = 0.004 ± 0.001 y^{-1}, $P < 0.01$, $n = 51$ y). (*C*) Relative contribution to the total basal area (≥10 cm DBH; circles, $n = 7$) and basal area for the saplings (2–9 cm DBH; bars, $n = 6$) of sugar maple and American beech in the mid- and low-elevation areas in the W6 forest (1965–2002). Error bars are 95% confidence intervals of the mean are based on plot-scale variation. (*D*) Nitrate export at HBEF simulated with the LM3V model. The lines represent a subset of 500 simulations, where different intensities of tree mortality have been prescribed (*SI Results and Discussion*). Red squares are volume weighted average nitrate export for the high N export period (1969–1976) and the low N export period (2000–2007) measured at HBEF. Green lines represent simulations that reproduce peak nitrate export as observed during the high N period. The range in tree mortality for this subset of runs was 0–55% and 45–95% for the 1906 and 1917 events, respectively (*SI Results and Discussion*) Black lines denote simulations with tree mortality from the literature (24) (20% ± 10% and 60% ± 10% for the 1906 and 1917 events, respectively). In all cases, tree mortality was set to 20% and 30% for the 1938 hurricane and the 1998 ice storm (25, 26).

and (*ii*) shifts in tree species caused by the onset of sugar maple (*Acer saccharum*) decline (27).

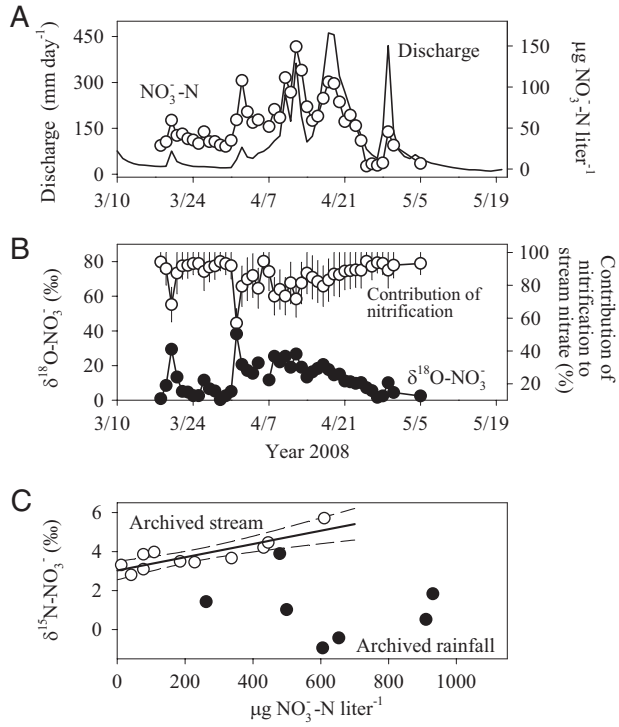

Fig. 4. Stream discharge and stream water nitrate concentration during the winter and spring of 2008, and isotopic natural abundance of nitrate for actual and archived water samples. (*A*) Discharge ($n = 79$) and stream nitrate concentration ($n = 46$) from March 18, 2008 to May 5, 2008 in W6. (*B*) The $\delta^{18}O$-NO_3^- (‰) in stream water (black circles; $n = 45$) and the relative contribution of internal sources (nitrification) to stream nitrate (white circles) during the winter and spring of 2008 in W6. We assumed $\delta\delta^{18}O$-NO_3^- in stream water results from two end members: nitrate in atmospheric deposition (84.2 ± 1.8‰; our measures) vs. nitrate produced by microbial nitrification in soils. Bars represent the range in the relative contribution of nitrification after sensitivity analyses for microbially produced nitrate ranging from −10‰ to +15‰ (29). (*C*) Relationship between stream nitrate concentrations and the $\delta^{15}N$-NO_3^- (‰) signal for archived bulk deposition (black circles; $n = 8$) and stream (white circles; $n = 11$) samples for the period of 1990–2007. The linear fit was significant only for the archived stream water samples (black line; $r^2 = 0.72$; $P < 0.001$). Dashed lines show the ±95% confidence interval.

We applied a dynamic terrestrial vegetation model (28) designed to explicitly resolve C–N interactions and feedbacks, including N limitation of plant productivity and N dependence on organic matter decomposition (*SI Results and Discussion*). The model simulates the effects of historical disturbances and tree mortality on soil N dynamics and nitrate leaching. We considered scenarios of tree mortality caused by the major historic events reported at HBEF as outlined in *SI Results and Discussion*. Across all these scenarios, our model showed a distinct postdisturbance pattern of a pulse increase in nitrate losses followed by a sustained decline from high to low nitrate export over the past five decades (Fig. 3*D* and *SI Results and Discussion*). In all scenarios, the nitrate decline was caused by net accumulation of soil N over decades, because the forest progressively recovered from abrupt losses of internal ecosystem N pools (28) (*SI Results and Discussion*).

We explored the sensitivity of our result to different assumptions about the severity of individual disturbances on vegetation N pools (*SI Results and Discussion*). A wide range of values recreated the overall pattern observed in the HBEF long-term record: high nitrate export in the 1970s followed by a strong decline over the next 30 y (Fig. 3*D*, green lines). Although historical values of tree mortality

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are poorly known, our use of published estimates for the HBEF (24–26) generated a nitrate decline of 48–60% of the missing N (Fig. 3*D*, black lines and *SI Results and Discussion*). These results indicate that watershed nitrate export is highly sensitive to historical legacies of forest disturbance and their influence on soil N pools. In the case of HBEF, the observed decline in nitrate export is broadly consistent with the series of disturbances that these forests have experienced since the 1906 harvest (Fig. 2*B*).

We also evaluated whether sugar maple decline could influence nitrate export from HBEF watersheds (*Methods* and *SI Results and Discussion*). Sugar maple litter promotes nitrate production in soils, which in turn, could promote watershed nitrate export. Historical HBEF census records show a 26% decline in basal area of adult sugar maples and a 12-fold loss of sapling basal area since 1977 (Fig. 3*C*). We estimated the effect of this decline using known decomposition and nitrification rates for sugar maple and American beech (*Betula allegheniensis*), the two most common hardwood species at HBEF (*Methods* and *SI Results and Discussion*). These calculations indicate that decline in sugar maples would cause only small changes in the soil N pool (3 kg N ha^{-1} 25 y^{-1}) (*SI Results and Discussion*). Even our extreme case scenario, in which we assumed that all nitrogen mineralized by sugar maple is exported as nitrate, implied a decrease in nitrate export of only 19 kg N ha^{-1} 25 y^{-1}. We conclude that sugar maple decline can explain only a minor fraction (<16%) of the observed decline in N export at HBEF (Fig. 2*B*).

Our analysis of the unique long-term record of N flux and cycling at HBEF offers several unexpected findings, which we summarize in the form of competing mechanisms in Fig. 2*B*. Most startling is perhaps the lack of any evidence for direct effects of climate change on net vegetation growth and plant N demand, despite substantial increases in air and soil temperatures and growing season length. We could identify only one potential climate effect: a shift in snowmelt hydrology induced by the progressive warming of soils with limited influence on nitrate export. In contrast, our analyses indicated that historical disturbances of vegetation could have very large and lasting influences on soil N pools and in turn, patterns of nitrate export over many decades.

Our results raise the critical issue of what constitutes the baseline against which climate change and other anthropogenic impacts should be evaluated in the HBEF record. Our finding of long-term influences of forest disturbances, for instance, suggests that the 1969–1976 high nitrate period may be a transient response to earlier disturbances rather than a baseline that characterizes the historic condition of the N cycle at HBEF. We conclude that historic disturbances can obscure the effects of climate change on the forest N cycle, even when analyses are advantaged by the availability of half-century long datasets. Resolution of how present day impacts interact with historical trajectories of ecosystem function represents one of the greatest challenges in understanding natural ecosystems today.

Methods

Historical Data Analysis. We analyzed historical hydrometeorological and chemistry data and forest inventories from the HBEF for the biogeochemical reference watershed [watershed 6 (W6); 43°56′ N, 71°45′ W]. For annual budgets, we considered the first of June as the start of the water year (1). The hydrological retention of nitrate for each water year was estimated as the difference between bulk inorganic N deposition (N_{input}) and stream water nitrate load (N_{export}) in relative terms ($100 \cdot [1 - N_{export}/N_{input}]$). We analyzed weekly 30-cm-depth soil temperature data recorded by the US Forest Service from 1961 to 2003. We also analyzed $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ in archived samples of stream water and bulk precipitation from 1990 to 2007. We used weekly measures of snow depth recorded by the US Forest Service at the W6 snow course from 1956 to 2005 to quantify the fraction of winter precipitation melted in individual intermittent events. We estimate monthly inputs of N as snow using historic records of monthly volume weighted average inorganic N concentration in bulk deposition at HBEF rain gauge 6. Data sources and calculation details are in *SI Methods*.

Spring Sample Collection. Stream samples were collected in W6 before, during, and after the 2008 spring pulse of snowmelt discharge (n = 50). Bulk deposition (n = 5) and snow samples (n = 3) were collected at approximately monthly intervals. Samples were filtered through prewashed GF/F filters before laboratory analysis. All samples were analyzed for NO_3^-, $\delta^{15}N\text{-}NO_3^-$, and $\delta^{18}O\text{-}NO_3^-$ (details for chemical analysis are in *SI Methods*).

Forest Floor N Model. We first calculated whether N retention caused by the slower decomposition rate of American beech litter could increase HBEF soil N pools such that it could explain the long-term nitrate decline. In subsequent scenarios, we tested whether reduction in sugar maple abundance could cause lower nitrification and thus, reduced losses of nitrate from the forest floor pool (model structure and parameters used are in *SI Methods*).

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Supporting Information

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SI Methods

Hubbard Brook Experimental Forest Historical Datasets and Statistical Analysis. We analyzed hydrometeorological and historical chemistry data as well as forest inventories from the Hubbard Brook Experimental Forest (HBEF) for watershed 6 (W6)—the biogeochemical reference watershed at HBEF (43°56′ N, 71°45′ W). These data are available at http://www.hubbardbrook.org/ (unless otherwise specified in the text).

Atmospheric N deposition and stream water nitrate data. We calculated monthly bulk inorganic N (NO_3^--N and NH_4^+-N) deposition at W6 since 1963 by multiplying monthly precipitation by volume weighted average (VWA) monthly concentration for bulk precipitation. We estimated the relative contribution of dry atmospheric N inputs to bulk N precipitation using wet and dry N deposition data recorded by the Clean Air Status and Trends Network (http://www.epa.gov/CASTNET/) for locations nearby the HBEF area since 1989 (WST109 station). Atmospheric input of dissolved organic nitrogen was measured since 1995 (1). We calculated VWA monthly stream water nitrate loads at W6 since 1963 by multiplying monthly stream discharge by VWA monthly nitrate concentration recorded at the W6 stream gauging station.

Stream flow and precipitation measurements as well as accuracy of chemical analysis have varied little since the beginning of the HBEF records (2, 3). However, quantification of N fluxes is subjected to uncertainties inherent to both water flow measurement and analytical precision. These uncertainties are additive, and they result in a 5–15% variation in estimates of annual N fluxes for both stream water and precipitation (2, 4, 5).

Global Inventory Modeling and Mapping Studies satellite data. We used the satellite-derived Global Inventory Modeling and Mapping Studies (GIMMS) dataset to assess changes on the length of the growing season over time. The GIMMS dataset is a normalized difference vegetation index (NDVI) available each 15 d since 1982. These data are obtained from the Advanced Very High Resolution Radiometer (AVHRR) onboard the National Oceanic and Atmospheric Administration satellite (http://www.landcover.org/data/gimms). We analyzed GIMMS NDVI values from a 10 × 10 km^2 cell centered on the HBEF area (43°57′ N, 71°45′ W) using $NDVI_{max}/2$ as a proxy of the start and the end of the plant growing season. The first day of the year (DOY), when $NDVI > NDVI_{max}/2$ (DOY_0), and the first DOY > 180, when $NDVI < NDVI_{max}/2$ (DOY_f), were considered the beginning and the end of the plant growing season, respectively. We calculated the length of the plant growing season as $DOY_f - DOY_0$ (6) for the period of 1982–2006.

Soil temperature data. We analyzed weekly soil temperature records provided by the US Forest Service. Data were recorded with Colman fiberglass sensors at different depths (8, 15, and 30 cm) located at 300 m southwest of the stream gauge for watershed 4 from 1961 to 1998 (2). We also analyzed weekly soil temperature data recorded since 2003 by means of encapsulated thermistors installed at the same location and at similar depths provided by the US Soil Climate and Analysis Network (SCAN) (http://www.wcc.nrcs.usda.gov/scan/).

For each soil depth, we estimated monthly average ± SD soil temperature, and we performed statistical tests to ensure no bias between the two different datasets. The existing period for SCAN soil temperature data (2003–2008) was compared against the initial and the final 6-y period recorded with Colman sensors (1961–1966 and 1993–1998, respectively). Monthly soil temperatures recorded during 1993–1998 (last 6 y of Colman data) and 2003–2008 (SCAN data) were not significantly different (Table S1). In all cases, soil temperature during these two periods was higher than during 1961–1966, albeit differences were not always significant (Table S1). Based on this intercomparison analysis, we concluded that there was no evidence of bias between records.

Forest inventory data. The HBEF forest inventory is one of the most complete US inventories to date (7–10). Starting in 1965 and occurring every 5 y after 1977, the inventory includes information about the health status and above- and belowground biomass for all trees ≥10 cm dbh in the HBEF watersheds. A subsample of trees ≥2 and <10 cm dbh is also measured each time.

We analyzed the censuses from 1965 to 2002 at W6 to quantify changes in total live tree biomass, and changes on the basal area of healthy trees [diameter at breast height (DBH) ≥10 cm] and saplings (2 cm ≤ dbh < 10 cm) of the two most common hardwood species in the HBEF area: sugar maple (*Acer saccharum*) and American beech (*Betula allegheniensis*). The 2007 total live tree biomass was as reported in the work by Lindenmayer and Likens (7).

Snow deposition and snowpack data. We used weekly snow depth data recorded since 1956 by the US Forest Service at the W6 snow course to assess the maximum snow depth (M) reached every winter season. We quantified the total amount of precipitation stored in the snowpack during every season (S) by summing up only the increases in snow depth recorded every week at the snow course. For a given year, we quantified the fraction of snow precipitation melted in intermittent warming events (f; rather than exiting the catchment in the spring snowmelt pulse; i.e., $1 - f$) with $1 - M/S$, and therefore, f approaches zero when almost no intermittent winter melt events occur.

We used weekly snow depth records at W6 to assess the beginning and the end of the snowpack period for every winter season. We estimated precipitation input of inorganic N during the snowpack period by multiplying VWA monthly concentration of bulk inorganic N deposition at W6 by monthly precipitation. Then, we placed an upper limit of the potential effect of increased soil microbial immobilization during snowmelt on reducing nitrate export at the HBEF by assuming that inorganic N in snowmelt bypassed soils during the high nitrate period of 1969–1976 but was fully intercepted and immobilized by soils since 1977.

Statistical analysis. We used linear regression to analyze annual long-term trends for climatic, hydrological, and chemical variables. We calculated the slope ± SE and the 95% confidence interval of the linear regression, and we tested each linear model with ANOVA. We also analyzed long-term trends by month for soil temperature and stream water nitrate (concentration and flux). Correlation between pairs of variables was determined by means of the Spearman's ρ coefficient. Differences between groups of variables were determined by means of the Wilcoxon rank sum test. Nonparametric analyses were chosen, because data were not assumed to be normally distributed (11).

Water Sample Collection During the 2008 Spring, Archived Samples, and Chemical Analysis. ***Spring sample collection.*** We collected daily stream water samples from W6 before, during, and after the 2008 spring pulse of snowmelt discharge (from March 18, 2008 to May 5, 2008; $n = 50$). Samples were kept at ambient temperature (on average <4 °C) and immediately frozen on arrival at the laboratory. Samples were thawed and filtered through prewashed GF/F filters before laboratory analysis. Bulk deposition and snow samples were collected on a monthly basis from March to May ($n = 5$ and $n = 3$, respectively). These samples were immediately filtered through prewashed GF/F filters and kept frozen until analyzed. The first

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10-mL water sample was discarded each time, and only one GF/F filter per sample was used.

Archived samples. Stream water and bulk deposition samples from the different weirs and meteorological stations at HBEF have been stored at the US Department of Agriculture, Forest Service headquarters from as early as 1963. These archived samples had been kept at room temperature after adding reagent-grade chloroform for preservation (3) (1 mL L^{-1}). We had access to archived samples from 1982 to 2007 collected at the weir of W3 (the hydrological reference watershed at HBEF) and bulk deposition samples from the rain gauge station 1 (43°57′ N, 71°44′ W). The HBEF historical data indicate that stream nitrate loads at W3 and W6 had a similar trend over time (12), and we were confident that any pattern exhibited by stream water samples at W3 would resemble the pattern at W6. We selected a subset of 75 archived stream water samples to investigate whether the isotopic signal of nitrogen in nitrate ($\delta^{15}N\text{-}NO_3^-$) changed with stream water nitrate concentration. We also gleaned 25 archived bulk deposition samples to assess whether changes in the $\delta^{15}N\text{-}NO_3^-$ in stream water archived samples were caused by changes in the isotopic signature of atmospheric N input. Based on our preliminary results, we chose only archived samples from December to April (the dormant period) when warming was most rapid and drop in nitrate export was more remarkable over time.

Chemical analyses. All water samples were analyzed for nitrate with a Dionex ionic chromatograph, and the $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ isotopic signatures were analyzed using the denitrifier method (13, 14). To minimize the influence of any storage effects on the isotopic signature of nitrate, we considered only archived samples for which there was no evidence that nitrate levels had changed significantly since they were first measured. Our criterion was fulfilled only in 19 of 100 samples and comprised 11 stream water samples and 8 bulk deposition samples from winter and early spring (January to April) for the period of 1990–2007.

SI Results and Discussion

Local Effect of Global Climate Warming at the HBEF and Possible Links to Nitrate Decline. Temperature is a key factor controlling catchment water budgets and biological processes, and not surprisingly, climate warming is inducing significant changes in hydrological processes and biological activity in the northeastern United State and Europe (15–19). At the HBEF, for instance, historical records revealed local changes in climate and snow hydrology. First, there was a long-term increase in air temperature since 1955, most strongly during winter (20). Concordantly, growing degree days (the accumulation of °C > 4 °C in air temperature since the first of January) have increased by the end of March and April (March: $r^2 = 0.23$, slope = 0.53 ± 0.14 °C y^{-1}; April: $r^2 = 0.12$, slope = 0.93 ± 0.35 °C y^{-1}; in the two cases, $n = 52$ and $P < 0.02$). Annual mean stream water temperature has also increased since 1980 (0.038 °C y^{-1}) (21). Second, we found a long-term trend increase in monthly mean soil temperature (0.024–0.048 °C y^{-1}) since the 1960s, particularly during the dormant season (from December to March) (Table S2) when the soil at HBEF was covered by snow. Monthly mean soil temperatures, however, showed no trend in late spring and summer (from April to September; for all months, $P > 0.05$). Finally, the number of days when ice covers the Mirror Lake (located within the Hubbard Brook Valley) has declined at a rate of 0.5 d y^{-1} since 1968 (18). Previous examinations of the HBEF snow pack records indicated significant declines in snow depth and snow cover duration (20). We also analyzed such records in detail; we found that the period of snow cover has shortened by 6 ± 2 d decade^{-1} ($r^2 = 0.14$, $P < 0.01$, $n = 51$), and maximum snow depth has declined by 6 ± 2 cm decade^{-1} ($r^2 = 0.16$, $P < 0.01$, $n = 51$) since 1956 at W6. These findings indicate that the HBEF has experienced a substantial warming over the past five decades and more notably, that climate conditions from late fall to early spring are becoming more favorable for biological activity.

Historical records also raised the possibility of a significant link between climate and nitrate trends in the HBEF. First, stream nitrate concentration has declined most strongly during the December to April Period when concentrations are the highest (Fig. S1), coinciding with the period of most rapid soil warming. Analysis of the long-term trend of nitrate export by month also revealed the strongest decline in export during April (at a rate of 0.14 ± 0.03 kg N ha^{-1} y^{-1}, $r^2 = 0.38$, $P < 0.001$, $n = 45$); declines were significant but small in other months. We found a moderate negative correlation between soil temperature and stream water nitrate in winter, especially in December and March when not only stream water concentrations but also nitrate fluxes were related to soil temperature (Table S3). That stream water nitrate declines with increases in soil temperature suggests that more favorable climatic conditions over time could be inducing increased immobilization and/or removal of nitrate by biota during the nonvegetative period, which could be, at least, partially responsible for the decline in nitrate loss observed at HBEF in the last decades.

Effect of Changes in Species Composition on Soil N Immobilization and Leaching: A Modeling Approach. There is an increasing body of knowledge showing the interaction between forest species composition and soil nutrient cycling. Litter from sugar maple, for instance, tends to decompose faster than litter from other hardwood species, bringing about higher nitrification rates and lower C and C:N ratios in the forest floor (22–26). Forest disturbances, whether natural or human-induced, promoting species replacement in hardwood forests could, therefore, have a dramatic impact on the N cycling at the scale of the entire ecosystem.

In the HBEF, sugar maple is being replaced by American beech—a species with tissues that are more recalcitrant to decomposition (22). Forest censuses at HBEF revealed that the contribution of sugar maple to the total basal area of W6 has decreased by 26%, whereas American beech's contribution has increased by 29% since they were first measured in 1965. We investigated the possibility that such changes in species composition would modify N immobilization in the soil pool and N leaching over time by applying a simple mechanistic model (Fig. S2) based on first-order kinetics (27). We considered specific decomposition rates for sugar maple and beech leaf litter, the two most common hardwood species at the HBEF (together contributing to 40% of the total basal area of W6). To model solely the effect of changes in species composition, we considered a constant input of leaf litter to the forest floor pool over time. For a given year, the fraction of leaf litter for each tree species was equivalent to the fraction by which each of these species contributed to the total basal area of healthy trees in the low and mid-elevations (545–783 m above sea level) of the W6, where hardwood forest predominates (http://www.hubbardbrook.org/data). We approximated the forest floor dynamics with a single pool linear model for each species (Eq. **S1**):

$$\frac{dL_i}{dt} = f(t) - k_i L_i. \qquad \textbf{[S1]}$$

Where L is the amount of litter in the forest floor, k is the decomposition rate for each tree species i, and $f(t)$ is the input of leaf litter to the forest floor at each time step that is linearly interpolated between times of the census. The total amount of leaf litter entering the system was considered to be constant over time and equal to 3,117 kg of dry weight (DW) ha^{-1} y^{-1}, which is the average for the whole period from 1965 to 2002 (8) according to the Landscape Biomass Tool of the HBEF (http://www.hubbardbrook.org/w6_tour/biomass-stop/biomassw6.htm). We ran the model using the decomposition rates (k_i) reported for the HBEF (0.25 and 0.08 for sugar maple and American beech, respectively) (22). We assumed the steady state solution for litter inputs in year 1965 as initial condition for L_i.

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In a first scenario, we assessed whether changes in the litter/soil organic matter stock caused by species shift were responsible for the decline in nitrate loss (that is, if slower decomposing litter of American beech has led to retention of nitrogen). We estimated the relevant nitrogen content in the litter/soil organic matter ($N_{L,i}$) complex with (Eq. **S2**)

$$N_{L,i}(t) = \frac{\rho L_i(t)}{r_i}, \qquad \textbf{[S2]}$$

where ρ denotes the amount of carbon per unit DW (0.4 kg kg^{-1}) and r_i is the C:N ratio of the specific litter type (23.7 and 22.0 for sugar maple and American beech, respectively; http://www.hubbardbrook.org/data). The C:N ratios were kept constant over time, because available data (1992–2003) do not show any consistent temporal pattern in C:N of leaf litter for these two tree species (http://www.hubbardbrook.org/data).

Our calculations support the common expectation that a higher proportion of American beech litter in the forest floor promotes N accumulation in the litter soil complex (Fig. S3*A*). According to our results, however, a shift from sugar maple to American beech, such as reported for HBEF, would imply an increase in soil N immobilization of only ∼3 kg N ha^{-1} in a 25-y period, an amount that does not explain the drop in nitrate export (Fig. S3*A*).

In subsequent scenarios, we tested whether the tendency to reduced sugar maple abundance can account for the decline of nitrate exports caused by reduced nitrification and N losses in the forest floor pool. At each time step, the amount of mineralized litter for each species (M_i) based on Eqs. **S1** and **S2** was given by (Eq. **S3**)

$$M_i(t) = k_i \frac{\rho L_i(t)}{r_i} = k_i N_{L,i}(t), \qquad \textbf{[S3]}$$

and we partitioned the mineralization flux into ammonium and nitrate using different mineralization to nitrification (M:N) ratios. In scenario 2, we considered empirical M:N ratios as a proxy of the NH_4^+-N:NO_3^--N ratios in the forest floor. For each tree species, we estimated a mean M:N ratio based on values reported in empirical studies from HBEF and locations nearby (24, 25) (1.7:1 and 3.4:1 for sugar maple and beech, respectively). As a simplification, we considered that all NO_3^--N is leached from the system and that all NH_4^+-N is retained. In scenario 3, we assessed how large the effect of a shift from high- to low-quality litter could be on the leaching of available N from the forest floor pool by assuming an M:N ratio of 1:1 (all available N is nitrified and consequently leached) for high-quality litter (sugar maple) and 1:0 (there is no nitrification) for low-quality litter (American beech). We acknowledge that such phenomenological nitrification rates are not expected to occur in nature, but they illustrate the upper limit of the impact that a species replacement of the nature and the magnitude recorded at the HBEF may have on the leaching of available N from the forest floor.

According to our model, a shift in tree species composition, such as reported at HBEF, would imply a drop of nitrate leaching from the forest floor of 5 kg N ha^{-1} 25 y^{-1} (scenario 2) to 19 kg N ha^{-1} 25 y^{-1} (scenario 3) (Fig. S3*B*). Our results suggest that changes in N immobilization and N leaching from the forest floor promoted by the shift in species composition reported at HBEF could contribute only moderately, from 4% to <16%, to decreased nitrate losses from W6.

Modeling the Long-Term Effect of Historical Perturbations on Forest N Dynamics. Although past disturbances can promote significant disruptions on ecosystem functioning at different temporal scales, the long-term impact of natural perturbations and land use history on N cycling remains elusive in many cases (28–30). In HBEF, harvesting was a common practice until the beginning of the 20th century. The W6 forest was harvested two times (in 1906 and 1917), and some tree mortality resulted from the 1938 hurricane and the 1998 ice storm in the W6 area (31–33). Although historical values of tree mortality are poorly known, estimates published in the literature (31) suggest that harvesting was light in 1906 (∼20%) and substantial in 1917 (∼60%). We explored, using the Princeton Geophysical Fluid Dynamic Laboratory LM3V land model, a dynamic vegetation model with prognostic C–N cycles (34), the effect of past events of tree mortality on soil N dynamics and nitrate leaching over time at HBEF. In LM3V, carbon and nitrogen in vegetation are allocated to five pools: leaves, roots, storage, sapwood, and wood. Photosynthesis and nitrogen intake are adjusted to maintain C:N ratios in the different pool, whereas the storage pool is used to (*i*) store retranslocated carbon before leaf senescence and (*ii*) buffer nitrogen in plants against seasonal asynchrony of productivity and nitrogen supply. A plant functional type-specific fraction of N is retranslocated before leaf senescence (34, 35). Litterfall is partitioned according to quality in a fast and slow litter pool, and decomposition of the slow litter pool is incomplete and results in formation of soil organic matter, with immobilization of N. Nitrogen exports include both organic and inorganic forms. Soil organic matter and plants have precedence for inorganic N forms over exports. The model is forced with a recurring climate, where the climate variables are recycled over a period of 16 y. The climate forcing (temperature, solar radiation, wind, surface pressure, and relative humidity) is based on 3-h reanalysis data (36) integrating observations over the years 1951–1966. LM3V was spun up from bare ground until equilibrium (negligible drifts in carbon and nitrogen pools) before entering the transient simulation with the disturbances.

We subjected the model to probable scenarios with a Monte Carlo approach considering different harvest intensities for the 1906 and 1917 events for which the severity of perturbation was more uncertain. We ran 500 scenarios with tree mortality ranging from 5% to 95% and vegetation removal after harvesting ranging from 50% to 90% for each of the two harvest events. Parameters were varied in 5% intervals, and Monte Carlo draws were based on a uniform distribution. In all scenarios, the mortality of trees because of the 1938 hurricane and the 1998 ice storm was 20% and 30% of the total live biomass, respectively (32, 33).

The model captured the general pattern of increasing nitrate losses that gradually returned to predisturbance conditions (Fig. S4*A*). This pattern was explained mainly by the disequilibrium between litter inputs and soil pools: a first phase of high N immobilization occurred because of harvest residuals or windthrow material and vegetation uptake; subsequent losses were caused by a decrease in the soil organic matter pool with litter inputs below the predisturbance value. A final phase was characterized by soil organic matter buildup as vegetation pools and litter input returned to the long-term steady state (Fig. S4 *B* and *C*). Model simulations suggest that it takes several centuries for the vegetation and soil pools to go back to predisturbance levels, highlighting the long legacy of perturbations on forest ecosystems.

Increasing the severity of individual disturbances resulted in higher peaks of nitrate export that occurred earlier in time. The simulated long-term trend in nitrate export was sensitive to the severity of individual disturbances. Several combinations of parameters could recreate a peak of nitrate export as high as the observed empirically in the 1970s during the high N period at the HBEF (∼5 kg N ha^{-1} y^{-1}) (Fig. S4*A*, green lines). However, some levels of disturbance were more likely to happen than others according to the model (Fig. S5). Best matches between model and data were obtained when tree mortality was low (<10%) for the 1906 event and moderate to high (65–85%) for the 1917 event (Fig. S5). For this subset of runs, the simulated drop in nitrate export calculated in the same way as with the empirical data ranged from 86.8 to 117 kg N ha^{-1} over the period of 1977–2007 (60–90% of the N missing). In a second scenario, we used values

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of tree mortality and harvest removal as estimated in the literature (31–33) (Fig. S4, black lines). In this case, the simulated drop in nitrate export was 60.4–73.5 kg N ha^{-1} for the period 1977–2007, explaining 48.3–58.8% of the observed missing N (125 kg N ha^{-1}).

Our simulations show that the impact of past disturbances can influence N dynamics in the soil pool and concomitantly, nitrate export for many decades. Moreover, the results indicate that historical perturbations can account, at least partially, for the general pattern exhibited by nitrate losses in the HBEF, and they pose the question of how effects of present day impacts, such as climate warming, can be resolved on forest ecosystems still under the influence of past perturbations.

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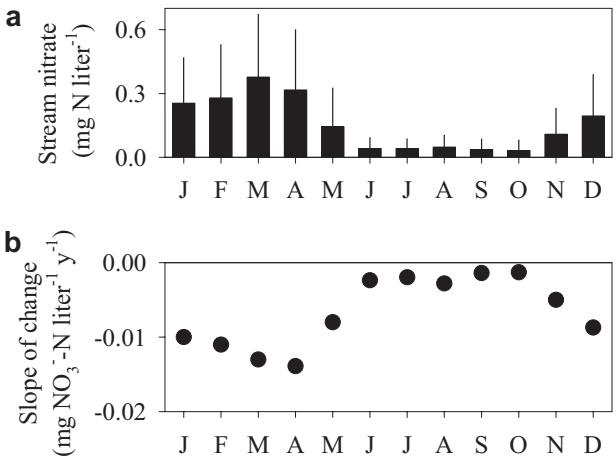

Fig. S1. Monthly VWA stream water nitrate concentration in W6 at the HBEF. (*A*) Monthly VWA stream water nitrate concentrations from 1963 to 2007 (whiskers are ±SD). (*B*) Slope of the linear fit of the long-term trend in stream water nitrate concentration by month (in all cases, $P < 0.05$). The more negative the slope, the more pronounced the nitrate decline over time.

DOVER 002413

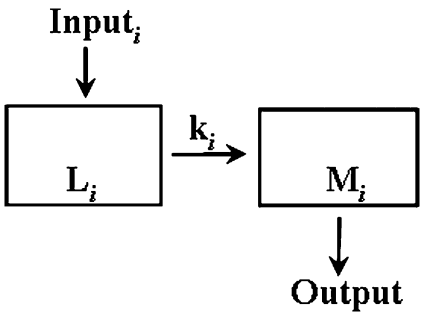

Fig. S2. Schematic representation of the applied forest floor N model. At each time step, there is an input of leaf litter to the forest floor compartment. After mineralization, nitrate is leached from the pool. *i*, respective tree species; k, mineralization rate; L, organic forest floor pool; M, available inorganic nitrogen in the forest floor pool.

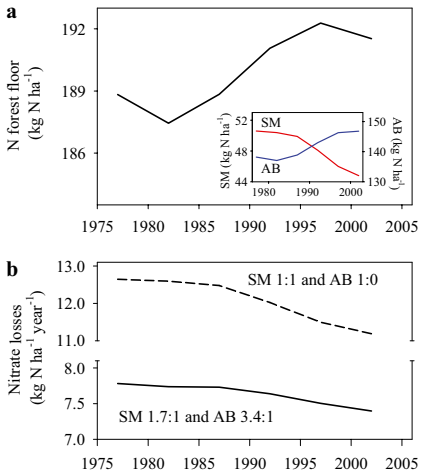

Fig. S3. Simulation of the N content in and nitrate leaching from the forest floor at the HBEF for the 1977–2002 period obtained with the forest floor N model. (*A*) Nitrogen immobilized in the forest floor over time (scenario 1). *Inset* shows the contribution from sugar maple (red line) and American beech (blue line) to the total N content. (*B*) Nitrate leached from the forest floor over time. The solid line shows simulated nitrate losses when approaching mineralized nitrate with empirical M:N rates (scenario 2). The dashed line shows nitrate losses when applying phenomenological M:N ratios (1:1 for sugar maple and 1:0 for American beech; scenario 3).

DOVER 002414

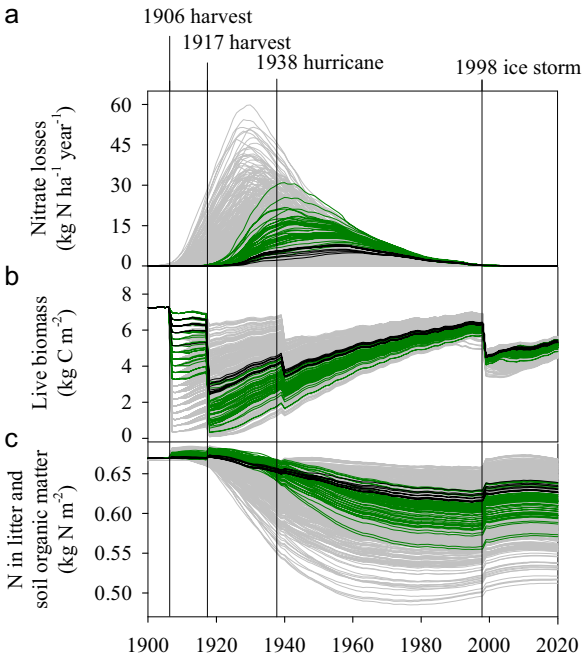

Fig. S4. Simulations of the long-term effect of historical forest perturbations on nitrate losses and the soil and vegetation pools in the HBEF obtained with the LM3V model (n = 500). The tree mortality caused by the 1938 hurricane and the 1998 ice storm was set to 20% and 30%, respectively (1, 2). The disturbance severity prescribed for the 1906 and 1917 harvest events was different in each run: tree mortality ranged from 5% to 95%, and biomass removal after harvesting ranged from 50% to 90%. Green lines represent simulations that reproduce a peak in nitrate export as observed at the HBEF in the 1970s (5.1 $\pm$ 0.4 kg N ha^{-1} y^{-1}; n = 57). Black lines denote simulations with tree mortality and harvest removal as estimated in the literature (3) (20% $\pm$ 10% and 60% $\pm$ 10% for the 1906 and 1917 harvest events, respectively; n = 9). (*A*) Stream water nitrate losses. Simulated values were smoothed using a 16-y window running average. (*B*) Carbon content in the aboveground live biomass. (*C*) Nitrogen content in the forest floor and the soil organic pool.

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3. Bormann FH, Likens GE (1979) Catastrophic disturbance and the steady state in northern hardwood forest. *Am Sci* 67:660–669.

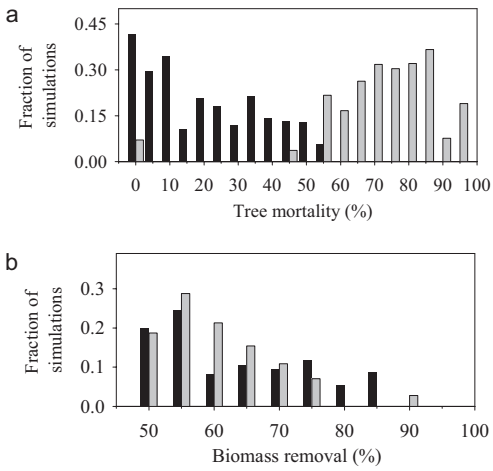

Fig. S5. Fraction of simulations that result in an export of nitrate for the 1970s similar to the export observed at the HBEF for different levels of disturbance. (*A*) Percentage of biomass killed during harvest. (*B*) Percentage of biomass removal after harvesting. Black and gray bars show the fraction of simulations for the 1906 and 1917 harvest events, respectively.

DOVER 002415

Table S1. Comparison of historic monthly average soil temperature during different periods at the HBEF

	Colman period		
Depth (cm)	1961–1966	1993–1998	SCAN period (2003–2008)
December			
8	1.6 ± 1.33*	2.65 ± 0.27*	2.28 ± 0.59*
15	2.04 ± 1.19*	3.6 ± 0.24†	3.06 ± 0.57*†
30	2.68 ± 1.23*	4.15 ± 0.36*†	4.2 ± 0.61†
January			
8	0.51 ± 0.36*	0.98 ± 0.85*†	1.08 ± 0.38†
15	1.03 ± 0.47*	1.95 ± 0.68†	1.72 ± 0.33†
30	1.42 ± 0.46*	2.27 ± 0.89*†	2.6 ± 0.35†
February			
8	0.33 ± 0.42*	0.48 ± 0.89*	0.78 ± 0.44*
15	0.66 ± 0.44*	1.61 ± 0.41†	1.32 ± 0.44†
30	1.10 ± 0.47*	1.63 ± 0.82*†	2.1 ± 0.39†
March			
8	0.31 ± 0.29*	1.06 ± 0.51†	0.8 ± 0.33†
15	0.71 ± 0.46*	1.12 ± 1.19*	1.22 ± 0.15*
30	0.86 ± 0.36*	1.53 ± 1.33†	1.88 ± 0.33†
April			
8	2.48 ± 1.82*	3.79 ± 1.73*†	4.36 ± 0.66†
15	1.9 ± 1.31*	3.18 ± 1.3*†	4.26 ± 0.61†
30	1.88 ± 0.33*	3.68 ± 1.29†	3.88 ± 0.33†

Monthly soil temperature (mean ± SD) at 8-, 15-, and 30-cm depths from December to April for the 1961–1966 and 1993–1998 periods (using Colman fiberglass sensors) and the 2003–2008 period (using encapsulated thermistors) at HBEF. For each depth, different asterisks and daggers indicate significant differences between groups ($P < 0.05$).

Table S2. Long-term trend of monthly average soil temperature at the HBEF

	Depth (cm)		
Month	8	15	30
December	+0.17* (0.34)	+0.26† (0.41)	+0.29† (0.48)
January	+0.14* (0.24)	+0.22‡ (0.35)	+0.33‡ (0.41)
February	ns	+0.23‡ (0.26)	+0.28‡ (0.34)
March	ns	+0.21‡ (0.27)	+0.32† (0.36)
April	ns	ns	ns

Goodness of the linear fit (r^2) of the long-term trend in monthly average soil temperature (8-, 15-, and 30-cm depths) from December to April for the 1961–2007 period at HBEF. The plus sign indicates increasing soil temperatures across years. The rate of increase is shown in parenthesis (°C decade^{-1}). ns, not significant.

*$P < 0.05$.

†$P < 0.001$.

‡$P < 0.01$.

DOVER 002416

Table S3. Relationship between soil temperature and stream nitrate concentration and fluxes

	Depth (cm)					
	8		15		30	
Month	NO_3-N mg L^{-1}	NO_3-N kg ha^{-1}	NO_3-N mg L^{-1}	NO_3-N kg ha^{-1}	NO_3-N mg L^{-1}	NO_3-N kg ha^{-1}
December	−0.73*	−0.63*	−0.68*	−0.62*	−0.66*	−0.61*
January	−0.41†	ns	−0.49‡	ns	−0.49‡	ns
February	ns	ns	−0.63*	−0.44†	−0.43†	ns
March	−0.50‡	−0.43†	−0.53‡	−0.42†	−0.61*	−0.49‡
April	ns	ns	ns	ns	ns	ns

Correlation coefficient (Spearman's ρ) between soil temperature (8-, 15-, and 30-cm depths) and stream nitrate concentrations (mg N L^{-1}) and between soil temperature (8-, 15-, and 30-cm depths) and stream nitrate fluxes (kg N ha^{-1}) from December to April for the 1961–2007 period at HBEF. ns, not significant.

*$P < 0.001$.

†$P < 0.05$.

‡$P < 0.01$.

DOVER 002417