Acid rain mitigation experiment shifts a forested watershed from a net sink to a net source of nitrogen

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Decades of acid rain have acidified forest soils and freshwaters throughout montane forests of the northeastern United States; the resulting loss of soil base cations is hypothesized to be responsible for limiting rates of forest growth throughout the region. In 1999, an experiment was conducted that reversed the long-term trend of soil base cation depletion and tested the hypothesis that calcium limits forest growth in acidified soils. Researchers added 1,189 kg Ca^{2+} ha^{-1} as the pelletized mineral wollastonite ($CaSiO_3$) to a 12-ha forested watershed within the Hubbard Brook Experimental Forest in the White Mountains of New Hampshire. Significant increases in the pH and acid-neutralizing capacity of soils and streamwater resulted, and the predicted increase in forest growth occurred. An unanticipated consequence of this acidification mitigation experiment began to emerge a decade later, with marked increases in dissolved inorganic nitrogen (DIN) exports in streamwater from the treated watershed. By 2013, 30-times greater DIN was exported from this base-treated watershed than from adjacent reference watersheds, and DIN exports resulting from this experiment match or exceed earlier reports of inorganic N losses after severe ice-storm damage within the study watershed. The discovery that $CaSiO_3$ enrichment can convert a watershed from a sink to a source of N suggests that numerous potential mechanisms drive watershed N dynamics and provides new insights into the influence of acid deposition mitigation strategies for both carbon cycling and watershed N export.

calcium | acid deposition | nitrate

Decades of acid deposition throughout North America and Europe have resulted in acidified soils and freshwaters in regions with inherently low rates of base cation supply. Acidification of soils and freshwaters impairs the health of sensitive trees and aquatic species, leading to declines in aquatic biodiversity and forest growth (e.g., refs. 1–3). Although clean air regulations have reduced levels of acid deposition below their historic highs, the marked depletion of base cations of affected ecosystems remains a legacy of acid deposition (4–6). In 1999, researchers at the Hubbard Brook Experimental Forest (HBEF) attempted to reverse this trend by adding 1,189 kg Ca^{2+} ha^{-1} as the pulverized mineral wollastonite ($CaSiO_3$) to an entire watershed ecosystem ("Watershed 1" or W1) (7). The goal of this whole-ecosystem experiment was to replace soil calcium that had been lost as a result of elevated leaching since the onset of acid deposition. Researchers predicted that this experiment (adding $CaSiO_3$) would increase the soil base saturation, pH, and acid neutralizing capacity (ANC) of soil solution and streamwater, resulting in a sustained increase in forest growth. The experiment also offered the opportunity to test the specific prediction that the addition of soil calcium would lead to enhanced growth of *Acer saccharum* (sugar maple), a canopy dominant with a high calcium requirement that has been declining in relative abundance throughout northeastern forests (1, 8).

Thus far, the experimental results have confirmed all of these initial predictions. The wollastonite addition led to changes in the soils of W1, including increased concentrations of Ca^{2+} and H_4SiO_4 and significant declines in H^+ and dissolved inorganic Al concentrations (9). Soil solution and streamwater pH and ANC increased significantly after the treatment and have remained above reference conditions (9, 10). Mass balances indicate that soils have been a net sink for the Ca and Si added to the watershed (9, 11), and that only 5% of the added wollastonite was lost via stream export within the first 12 y after treatment (12). Tree biomass, leaf area index, and total evapotranspiration increased after the whole-watershed $CaSiO_3$ enrichment (13–15). The total leaf area index, foliar Ca, and seed production of existing sugar maple trees responded particularly strongly to the addition of $CaSiO_3$ (15, 16).

Observations of enhanced forest growth in the $CaSiO_3$-treated watershed led to predictions of a "tightening N cycle" (in the sense of ref. 17), whereby trees accumulate N in biomass and prevent its export from the watershed. This prediction arises from three classic tenets of watershed science: Ecosystems with higher primary production tend to retain more limiting nutrients (18, 19); forest biomass should accumulate N when N enrichment increases, until plant demand is saturated (20–22); and undisturbed and unpolluted ecosystems strongly retain inorganic nitrogen (23, 24).

These assumptions have been developed through comparative studies that contrast N dynamics across forests at various successional stages (19, 23, 24) or through vegetation removal experiments (e.g., ref. 25). Experimental manipulations that lead to increased plant growth (as in this $CaSiO_3$ enrichment experiment) are typically too small in extent to allow watershed-scale nutrient retention studies (e.g., free air CO_2 enrichment experiments) (26). We used watershed mass balance comparisons between the experimental watershed and from adjacent, unmanipulated reference watersheds. We wanted to assess whether $CaSiO_3$ fertilization has

Significance

Acid rain has stripped forests of soil calcium, with consequences for forest health and downstream ecosystems. In 1999, researchers initiated a whole-watershed experiment, with the goal of replacing all the calcium lost. This experiment increased the pH and acid-neutralizing capacity of soils and streamwater, and forest growth increased. In 2010, nitrogen export from the treated watershed began to increase, and by 2013, annual inorganic N losses from the experimental watershed were 30-times higher than from the adjacent reference watershed, a proportional increase only seen in whole-watershed clear-cutting experiments. The discovery that calcium enrichment can convert a watershed from a sink to a source of N suggests unforeseen consequences of acid rain mitigation and provides new insights into watershed dynamics.

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further enhanced the already strong capacity for N retention in these watersheds (5) or, alternatively, whether soil organic matter (SOM) turnover in response to increasing alkalinity may reduce watershed N retention. Thus, the whole-ecosystem calcium-enrichment experiment, and the resulting enhancement in forest growth, offers a test of our understanding of how plant growth, carbon turnover, and terrestrial N cycling influence watershed-ecosystem nutrient exports.

Results and Discussion

Within the decade after the $CaSiO_3$ addition, a large and unanticipated increase in inorganic nitrogen export from the treated watershed was observed (Fig. 1*A*). Indeed, by 2012, exports of NO_3^- from the treated watershed were 30 times higher than from the adjacent reference watershed. This relative increase in watershed N export is comparable to the effects of a previous entire-watershed, whole-tree harvest experiment (W5) (Fig. 1*A*) (27).

This marked increase in N export after wollastonite addition represents a distinct and extended excursion from the 35 or more year trend of declining N losses from these and other northeastern forested watersheds. Since 1976, dissolved inorganic N (DIN) concentrations in streamwater have declined markedly from peak annual concentrations of ~0.8 mg NO_3-N L^{-1} to peak concentrations of less than 0.2 mg NO_3-N L^{-1} since 1991 (Fig. 1*B*). There is no evidence that other forms of dissolved N export have changed through time (28); NH_4 concentrations remain near detection limits, whereas dissolved organic nitrogen (DON) is typically 0.1 mg N L^{-1} (29). Over the course of four decades, nongrowing season N concentrations have declined even more rapidly than during the growing season (Fig. 1*B*, *Inset*) (29). In contrast to these declining trends, peak snowmelt N concentrations for W1 now exceed historic highs (Fig. 1*B*). Despite declines in atmospheric N deposition regionally (30), the $CaSiO_3$-treated watershed has become a net source of inorganic N, with streamwater N exports now exceeding N inputs in precipitation (Figs. 2 and 3) (5).

This result does not fit with commonly used explanations for high nitrogen exports from watershed ecosystems. High nitrogen inputs are theoretically required to sustain high nitrogen exports (the N saturation hypothesis) (20, 31), yet the N deposition to the experimental watershed has continued to decline over the course of the last two decades (5). Similarly, unfertilized watersheds are not expected to release DIN in the absence of physical disturbances that damage vegetation or alter soil profiles (5, 23, 24). Finally, other studies of watersheds with high N export have linked this phenomenon to low C:N of soils and dominant trees (32, 33), or to changes in the residence time or routing of water through soil (34), neither of which has been observed here. Indeed, the experimental $CaSiO_3$ enrichment, if anything, led to short-term reductions in stream flows via increased forest evapotranspiration (14).

The observed $CaSiO_3$-induced N export is most likely the result of enhanced N mineralization within watershed soils in excess of increased plant N demand. However, measurements of both soil N mineralization and nitrification made in the first 5 y after the $CaSiO_3$ treatment found lower rates of both processes in the treatment watershed compared with the reference (35). More recent extensive soil surveys, however, have documented a 40% decline in forest floor organic matter mass in soils in the $CaSiO_3$-treated watershed (11). The resulting estimate of N mineralization during the last 10 y (~420 kg N ha^{-1}) (11) is very large relative to estimates of biomass N sequestration (~20 kg N ha^{-1}), so the net effect of enhanced plant growth and SOM turnover has been to increase the inorganic N available for stream export. Together,

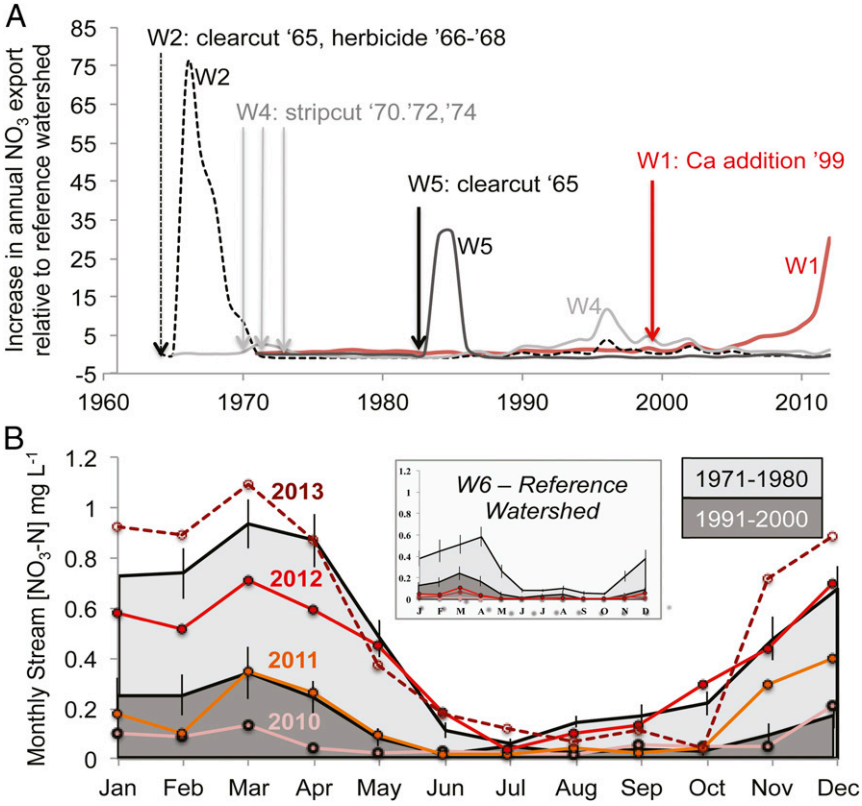

Fig. 1. (*A*) Watershed nitrate exports relative to values observed for the long-term reference watershed (W6) are shown for W2 (clear-cut in 1965), W4 (strip cut over the course of 4 y between 1970 and 1974), W5 (whole-tree harvest in 1983), and W1, the watershed experimentally enriched with $CaSiO_3$ in 1999. (*B*) Monthly average streamwater concentrations of NO_3-N for both the $CaSiO_3$-enriched and reference watersheds (*Inset*) are shown for two historic decades (gray shading) and for the last 4 y.

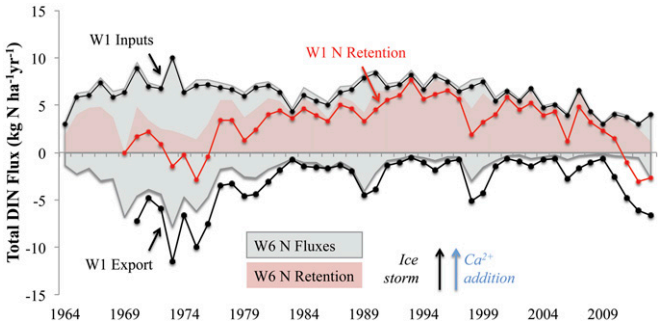

Fig. 2. Inputs of DIN in precipitation are compared with streamwater export of DIN from W1 (treated) and W6 (reference). (*Lower*) These numbers are used to calculate the percentage DIN retained by each watershed relative to annual inputs. Note that flux calculations extend only to 2011 (because of delays in final water budget calculations). On the basis of concentration data (Fig. 1), it is clear that W1 has become an even greater source of DIN in 2012–2013.

the observed increases in aboveground productivity in W1, coupled with the even larger increase in SOM degradation, suggests $CaSiO_3$ enrichment is promoting new biomass accrual at the cost of older, stored SOM. Similar conclusions have been drawn from several free air CO_2 enrichment experiments in which increases in forest biomass under elevated CO_2 do not lead to the expected increases in C sequestration, as they are coupled with enhanced heterotrophic respiration (26) and N mineralization (36). In this case, $CaSiO_3$ enrichment may simply be enabling enhanced microbial processing of dead organic matter either by reducing soil acidity (soil pH has increased from ∼3.5 to 4.5 in W1) (11) or through Si mitigation of aluminum toxicity (37).

Important questions remain about what mechanisms or feedbacks produced the long lag between the watershed $CaSiO_3$ treatment and the enhanced watershed N export response and the fate of the majority of N mobilized from the forest floor. Stream export can account for only ∼5.6 additional kg N ha yr^{-1} in recent years. This level of elevated N loss could continue for a decade and would still represent only ∼12% of the 420 kg N ha^{-1} that has been lost from surface soil SOM pools as a result of $CaSiO_3$ enrichment (11). This excess N may be exported from the watershed via enhanced gaseous losses of N_2 and N_2O. Recent work has suggested that denitrification may be a much more important N loss pathway in HBEF forests than previously thought (ref. 38; but see ref. 39). Although there has been no research investigating the response of denitrification rates to the $CaSiO_3$ addition, both higher soil NO_3^- concentrations and higher soil pH should promote higher rates of denitrification (40). An alternative hypothesis is that N has been transported vertically to deep soils below the 15-cm limit of most soil-sampling methods.

Perhaps the most intriguing implications from this experiment are the opportunity these data provide to reconsider the long-term consequences of acid deposition for forest ecosystems. Nitrate exports from forested watersheds throughout the region have been declining during the last several decades (5, 41). Our results suggest that large amounts of organic N are being stored within the forest floor and that these standing stocks are highly susceptible to mineralization as a result of base cation enrichment. The mechanisms responsible for "unlocking" this stored N are thus far not clear. Enhanced mineralization may result directly from microbial responses to the increases in soil Ca, Si, and alkalinity and the decreases in soil Al. It is also possible that enhanced mineralization results indirectly from increases in the quality and quantity of root and leaf litter inputs associated with the shifts in soil chemistry (15, 16, 42). Regardless, results from the W1 $CaSiO_3$ enrichment pose interesting questions about whether acid deposition has left a legacy of enhanced soil carbon and nutrient sequestration, and if so, how these "acid protected" organic matter standing stocks will change in response to the recovery of historic pH conditions throughout the northeastern United States. The long lag between the $CaSiO_3$ addition and the phenomenon of high N export may be the result of slow vertical redistribution of the $CaSiO_3$ minerals (11) or the gradual acclimation of vegetation to experimentally altered soil chemistry.

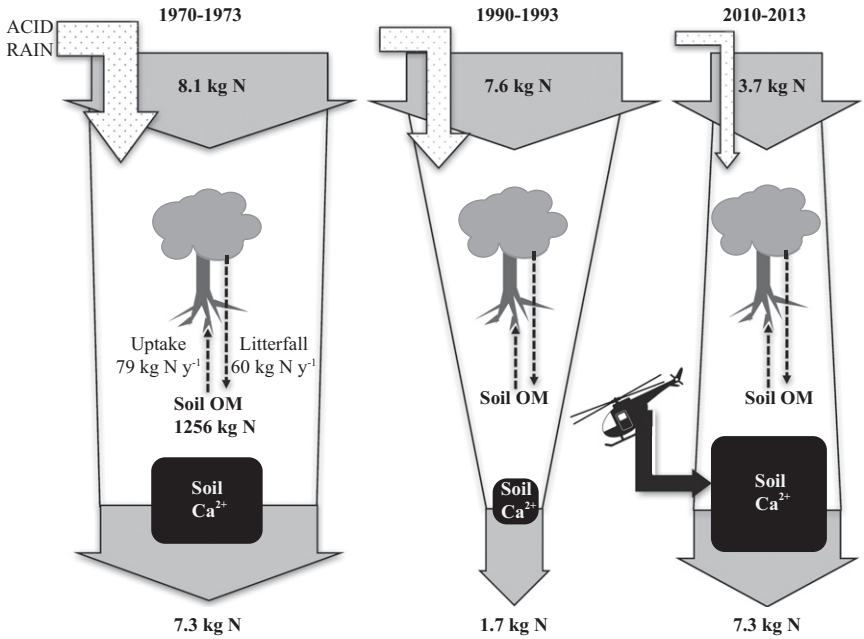

Fig. 3. Annual nitrogen mass balances for the experimental watershed (W1) are contrasted between three distinct, 3-y periods within the long-term record. The first 3 complete water years available for W1 (1970–1973), the most recent 3 y of complete data records (2010–2013), and an intermediate 3-y period (1990–1993). To provide context for these rates, we have also included estimated internal N stocks (k·ha^{-1}) in biomass and SOM and the annual rate at which N is transferred between these pools as reported for the reference watershed in the early years of the HBEF long-term record (5). More recent biomass N standing stock estimates have been developed for W1 (15).

If enhanced SOM mineralization is the source of excess N, there are sufficient supplies to sustain enhanced watershed N export for more than a century. Ecosystem theory, drawn largely from vegetation removal experiments, provides little guidance to predict the shape, magnitude, and duration of this long-term N loss pathway. The conceptual framework built from vegetation removal experiments must be expanded to adequately explain how a vegetation growth-enhancing experiment can create a persistent source of excess N export with the potential to exceed the losses associated with prior forest clear-cutting experiments. Significant advances in the use of element ratios and isotopic tracers allow modern watershed studies to expand beyond mass balance approaches and provide new opportunities for unraveling these complex biogeochemical dynamics. Ultimately, these approaches will be necessary to build new conceptual and empirical models that can explain how soil acidity and alkalinity affect the sequestration and mineralization of SOM. These findings also illustrate the importance of long-term research, as the unanticipated, high-magnitude effects of an experimental manipulation on ecosystem element cycling took ∼10 y to emerge (43).

Materials and Methods

The watersheds examined are located in the HBEF in the White Mountain National Forest, New Hampshire. Several south-facing watersheds have been gaged, and the export of solutes has been studied since 1963. The forests of the watersheds are dominated by the hardwood species sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula alleghaniensis*). In addition, red spruce (*Picea rubens*), balsam fir (*Abies balsamea*), and white birch (*Betula papyrifera*) are common on the ridges of these watersheds. For additional details on these ecosystems, see ref. 5. The procedures used to collect, store, and analyze rain and streamwater samples have been detailed by Buso et al. (44). Here we describe the procedures briefly. Since 1963, streamwater samples have been collected at least weekly from all six south-facing watersheds at HBEF (Table S1). Samples are collected ∼10 m upstream of the stream gauges in acid-washed plastic bottles. Historically, samples were not filtered because of the extremely low particulate content of streamwater and the limited effects of storage on solute concentrations (see ref. 39). In 1999, researchers at the HBEF added 1,189 kg/ha^{-1} Ca^{2+} as the finely ground mineral wollastonite ($CaSiO_3$) to an 11.8-ha watershed 1 (W1) in an attempt to restore the soil Ca^{2+} depleted by acid deposition. Procedures for determining annual N deposition and N flux have followed the same approach over the long-term record. A detailed description of these methods and the data handling and processing approaches has been published previously (5, 39).

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