CHAPTER 12

Exploring the Process of Nitrogen Saturation

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Introduction and Historical Context

Plant growth in temperate zone forests is generally limited by the availability of nitrogen. Human activities may have played a major role in establishing this truism, and may play as large a role in its eventual contradiction.

Plants require more nitrogen (N) than any other element drawn from the soil. Nitrogen is generally absent from rocks and so is not released during weathering, the physical and chemical disintegration of minerals that provides calcium, magnesium, and many other essential elements. While N_2 gas constitutes 78 percent of the atmosphere, this form of nitrogen is not available to plants, so plants may be nitrogen limited while awash in an atmosphere rich in this element. Atmospheric N_2 must be converted to a reactive form either by microbial processes or by physical events in the atmosphere (lightning, for example) before it can be used for plant growth. Background inputs of nitrogen to native ecosystems are generally very low (1 to 2 kilograms nitrogen per hectare per year [$kg\ N \cdot ha^{-1} \cdot yr^{-1}$]) and can be more than overbalanced by losses through fire or by leaching of nitrate after disturbance, or by the continuous, slow loss of nitrogen in dissolved organic compounds to streams. This combination of a tenuous nitrogen balance, combined with relatively high rates of inputs of other elements to soils by weathering of geological substrates, causes nitrogen to be the most frequently limiting nutrient under natural conditions in recently glaciated areas such as New England.

Human use of the lands that now constitute the Harvard Forest has altered the nitrogen status of the landscape significantly. Early clearing and farming practices were extractive, with little in the way of fertilization or return of manure to pastures or plowlands. By the nineteenth century, pastures were enclosed and animals housed in barns, with manure returned to the fields. Nitrogen in grasses from extensive hayfields

and pastures was concentrated into manures that were then spread over the adjacent but smaller areas of plowed cropland. Consequently, adjoining parcels of land were differentially depleted or enriched in nitrogen and other elements depending on their specific land-use history. Farm abandonment then resulted in the establishment of a patchwork of forest types across a landscape in which nitrogen cycling differed markedly as a consequence of this history, as well as inherent site factors (see Chapter 9).

One effect of this land-use history is that species-site relationships in the modern landscape can be very different from those seen in natural areas. For example, pine plantations established at the Harvard Forest soon after its founding were sited preferentially on the remaining open areas. These were mainly plowed lands that had become pastures near the end of the agricultural period and tended to be among the most productive and enriched farmlands. So while most natural pine forests occur on nutrient-poor and acid soils with low fertility, these plantations occupy some of the richest sites in the landscape. In contrast, old woodlots, dominated by hardwoods that had sprouted after repeated cutting and/or burning, are most likely depleted in nutrients relative to their presettlement conditions.

Regional urbanization and industrialization over the past century have altered the nitrogen balance at the Harvard Forest once again. Dramatic increases in the generation of the waste products of combustion regionally and farther upwind in the Great Lakes–Ohio River area have led to increased deposition of nitric and sulfuric acids, the major components of acid rain. Although sulfur emissions have been reduced over the past two decades through legislation, nitrogen emissions, which result more from automobile exhausts than industrial processes, remain high. Concern now focuses on the potential for "nitrogen saturation" or the availability of nitrogen in excess of biotic demand due to atmospheric deposition.

Background for the Chronic Nitrogen Experiment

The chronic nitrogen addition experiment was designed to test the long-term consequences of increased nitrogen deposition in a region in which nitrogen is historically and naturally a limiting nutrient. Understanding the experiment and the process of nitrogen saturation requires some basic background on the rates and forms in which nitrogen cycles through forest ecosystems.

The nitrogen cycle of a forest connects four pools of very unequal size (Figure 12.1). Over 98 percent of all nitrogen generally resides in organic forms in plants and soils. Nitrogen in these large pools turns over very slowly; any one atom of nitrogen may reside in these pools for
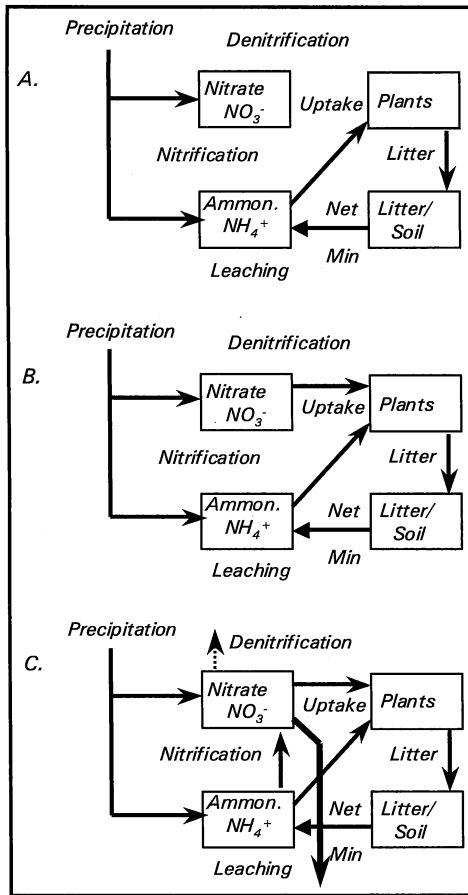
Figure 12.1. A simplified view of changes in the nitrogen (N) cycle in forest ecosystems in response to increased N deposition. A: Under N-limited conditions, N cycles mainly as ammonium due to competition for this form of N between plants (with mycorrhizal root symbionts) and free-living microbes. Net Min, net mineralization. B: Long-term N additions increase N availability, stimulating plant growth and partially alleviating N limitations. C: As deposited N enters the N cycle, further increases in availability occur, stimulating nitrification, nitrate cycling, and, eventually, leaching losses to groundwater.

decades to centuries. The remaining nitrogen is present in mineral form as ammonium and nitrate in soils with residence times measured in hours to weeks. Plants shed litter (dead plant parts such as leaves and roots) into the soil organic matter pool on an ongoing basis and contribute large woody debris like branches or whole stems after disturbance or tree death. Microbial decomposition of this dead organic matter releases ammonium, which can then be either reused by the

microbes or taken up by plants. Under conditions of severe nitrogen limitation (Figure 12.1A), plants and their associated root symbionts compete effectively for ammonium against the free-living microbes that use ammonium as an energy source (nitrifiers) and produce nitrate (nitrification). Thus, in ecosystems where nitrogen is very limited, nitrogen cycles primarily as ammonium and there is no appreciable loss of mineral nitrogen from the system. Nitrogen can, however, be lost from ecosystems in dissolved organic form (the arrow showing leaching loss directly from litter/soil). Measured rates for this flux in most systems are low and do not appear to change with nitrogen status, so this flux can be important in the long-term nitrogen balance of forests in which nitrogen inputs are low and inorganic forms of nitrogen are retained.

The process of nitrogen saturation involves a number of integrated changes in biogeochemical cycling that are induced as increasing inputs of nitrogen from human sources gradually decrease nitrogen limitation in the system. Deposition of nitrogen from the atmosphere adds both nitrate and ammonium (Figure 12.1B). Plants and microbes can take up this added nitrogen, partially relieving nitrogen limitations. The increased availability of nitrogen stimulates increased plant growth and production of organic matter, resulting in larger inputs of litter, an acceleration of nitrogen cycling, and increased production of ammonium. As nitrogen inputs from the atmosphere accumulate, nitrogen limitations on microbes and plants are reduced, free-living nitrifiers in the soil have increasing access to ammonium, and the production of nitrate begins or increases (Figure 12.1C). Although both plants and microbes can use nitrate, production can eventually outstrip uptake potential, and nitrate will begin to leach to streams. Nitrate (NO_3^-) is a negatively charged ion. When it leaches from the system, it carries along positively charged ions such as calcium, potassium, and magnesium (preferentially), followed by aluminum and hydrogen, leading to acidification of soils and streams, increased aluminum concentrations in soil and stream water, and nutrient imbalances in soils and plants. All of these responses can stress forests, leading to declining growth rates and increased mortality.

At the initiation of the chronic nitrogen addition experiment, we published a set of hypotheses on the expected integrated response of nitrogen-limited forests to chronic nitrogen additions (Figure 12.2). A key feature of this set of hypotheses was that responses would be nonlinear; that different components and processes of the ecosystem would change at different rates and in different directions. A second key feature was that the initiation of nitrification would mark a critical step in the overall response. A third key prediction, and one we considered very unlikely to be realized, was that excess nitrogen availability would lead to reductions in forest growth, a phenomenon often termed *forest decline.*
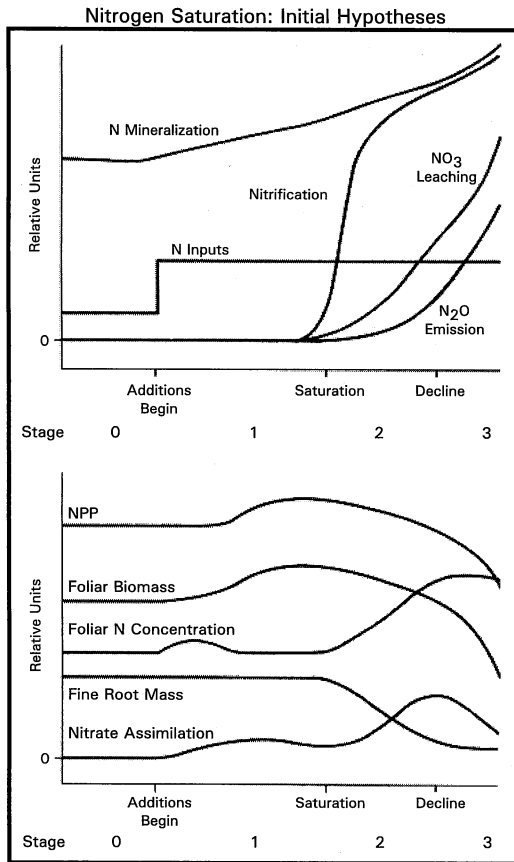
Figure 12.2. Initial set of hypotheses regarding the sequential responses of N-limited forests to chronic N additions. Key characteristics of this set of hypotheses are the nonlinear responses of several processes and the central role of nitrification. NPP, net primary production. Reprinted from Aber, Nadelhoffer et al. 1989.

Experimental Design

We selected a red pine plantation and an adjacent mixed hard-wood (red oak, red maple, black birch) forest on the Prospect Hill tract for this study. The land now supporting the red pine stand was cleared and plowed during the eighteenth and nineteenth centuries and was in pasture at the time of acquisition by the Harvard Forest. This open field was planted to red pine in the 1920s. A distinct plow layer is visible in the mineral soil, and only a thin forest floor (organic soil horizon) has developed since the stand was planted. In contrast, the mixed hard-

wood stand was never plowed, as evidenced by the natural appearance of the soil horizons and high stone content of the upper soil. This stand, however, was harvested and used for pasture until around 1850, when it was abandoned and allowed to reforest naturally. The resulting forest was logged again in the 1930s, and at least part of the stand was burned by an intense surface fire in 1957. The stand is very slow growing and has a thick, densely rooted forest floor, which suggests strong competition for nutrients.

Our first surprise in this experiment was the difference in nitrogen cycling rates at these two sites at the beginning of the experiment. Planted on enriched plowland, the red pine stand had an annual rate of nitrogen mineralization (supply of N through decomposition) of 92 kg $N \cdot ha^{-1} \cdot yr^{-1}$, much higher than normal for needle-leaved evergreen stands. In contrast, the heavily used but never-plowed hardwood stand was cycling only 84 kg $N \cdot ha^{-1} \cdot yr^{-1}$, at the low end of the range for temperate deciduous forests. Nitrification was 21 kg $N \cdot ha^{-1} \cdot yr^{-1}$ in the pines and undetectable in the hardwoods. The imprint of two centuries of land use is still evident in these two stands nearly 100 years after the end of agricultural management.

Four large (30-by-30-meter) plots were established in each stand. Single large plots were used rather than smaller replicate plots to reduce edge effects and to include full-sized trees in each plot, allowing us to examine feedbacks between plants and soils. Each plot was assigned one treatment (control, low N, high N, and N+S [sulfur]). The low nitrogen treatment is 50 kg $N \cdot ha^{-1} \cdot yr^{-1}$ and the high nitrogen treatment is 150 kg $N \cdot ha^{-1} \cdot yr^{-1}$, added as a concentrated solution of NH_4NO_3 in six equal monthly doses applied to the soil surface with a backpack sprayer between May and September. The N+S treatment included the low nitrogen addition plus 74 kg $S \cdot ha^{-1} \cdot yr^{-1}$ as SO_4^{-2} and was intended to mimic the mid-1980s ratio (albeit at higher dosages) of nitrogen to sulfur in deposition over New England. In general, results from the N+S plots were indistinguishable from those from the low-nitrogen plot and will not be discussed further here.

One of the major goals of the chronic nitrogen experiment was to determine the fate of nitrogen added to the two stands through time: did it end up in the soil, the plants, or the microbes, or was it leached from the system? Although net budgets (measurements of changes in storage within different parts of the forest over time) provide partial insights to this important question, we also chose to add distinctive isotopes of nitrogen that can be traced through the measured pools to provide more precise measurements of nitrogen dynamics. In 1991 and 1992 nitrogen fertilizer additions in the low-nitrogen plots contained slightly elevated (natural abundance levels) of ^{15}N. At the same time, we added very small amounts of highly enriched ^{15}N to the control plots. This procedure allowed us to compare measurements between these two stands

without adding sufficient nitrogen to induce a fertilizer response in the control plots. We added ^{15}N as NH_4NO_3 but split the labeling so that $^{15}NH_4NO_3$ was added to one half of each plot and $NH_4^{15}NO_3$ was added to the other half.

Process Measurements

We began by measuring processes that were relevant to the hypotheses in Figure 12.2. Nitrogen mineralization (the conversion of organic nitrogen to mineral nitrogen) and nitrification (the conversion of ammonium to nitrate) were measured by on-site soil incubation. We also measured nitrogen leaching losses into deeper soil layers using tension cup lysimeters, which collect soil water below the rooting zone. Concentrations of ammonium and nitrate in lysimeter samples were multiplied by monthly hydrologic fluxes derived from the PnET-II model (see Chapter 17). Net fluxes of important gases (nitrous oxide [N_2O], methane [CH_4], and CO_2) between the soil and the atmosphere were measured using chambers positioned on the soil surface. In experiment 1, below, the soda-lime trap technique was used to measure CO_2 efflux. Aboveground net primary production (ANPP, the production of new plant biomass) was calculated as the sum of litterfall and stem growth. Nitrogen concentration in tree foliage was measured on samples collected by shooting leaves from the canopies of trees with a shotgun in midsummer, and concentrations in fine roots have been measured intermittently.

Summary of Initial Results

In general, the two forests responded to the ongoing chronic additions of nitrogen as hypothesized, but on very different timescales. Nitrification increased quickly in both pine plots and eventually in the hardwood high-nitrogen plot as well. Nitrate leaching into lower soil horizons increased almost immediately in the pine high-nitrogen plot and was occasionally elevated in the low-nitrogen plot. In contrast, significant leaching in the hardwood stand did not occur in the high-nitrogen plot until the eighth year of treatment (Figure 12.3). In parallel with trends in nitrate loss, the concentrations of nitrogen in leaves and fine roots have increased dramatically in the pine stand, whereas increases in the hardwood stand have been less pronounced (Table 12.1). All of these results suggest that both stands are progressing toward nitrogen saturation but that the hardwood stand was more nitrogen limited initially and has responded more slowly to nitrogen additions than the red pine stand.

There were also surprises in the results. Nitrous oxide efflux from the soil, which we hypothesized would increase with nitrogen addi-
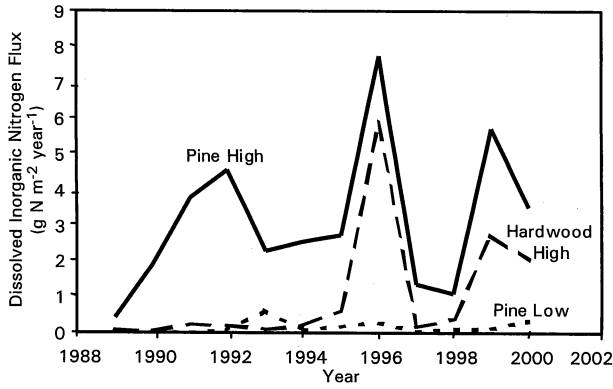
Figure 12.3. Pattern of nitrate loss from the chronic N plots at the Harvard Forest. Lines for high and low N additions in the pines and high additions in the hardwood stand are shown. Values for other stands are combined and are not different from zero.

tions (Figure 12.2), remained nearly undetectable. In contrast, there was an immediate and major decrease in the rate of methane consumption in both the pine and hardwood nitrogen-amended stands. Methane consumption in the high-nitrogen plots was approximately half that of the control stands in year two and has continued to decrease over time (Figure 12.4). The potential for microbes to switch from methane to ammonium as substrates for metabolism depending on relative abundances of the two molecules appears to underlie this result. Methane is a potent greenhouse gas, approximately twenty times as effective as CO_2 in trap-

Table 12.1. Differences in Nitrogen Concentrations in Foliage and Fine Roots in the Chronic Nitrogen Experiment

Stand	Treatment		
	Control (%)	Low N (%)	High N (%)
Pine			
Foliage	1.0	1.5	1.9
Fine roots			
Forest floor	1.6	2.2	2.0
Mineral horizon	1.1	1.8	1.6
Hardwood			
Foliage (oak)	2.4	2.6	3.1
Fine roots			
Forest floor	1.2	1.5	1.6
Mineral horizon	0.8	0.9	1.2

Note: Forest floor measurements include roots from the Oa and Oe horizons; mineral horizon measurements include roots from 0 to 10 centimeters. All root and foliar samples were collected in 1999. Foliar measurements from 1999.
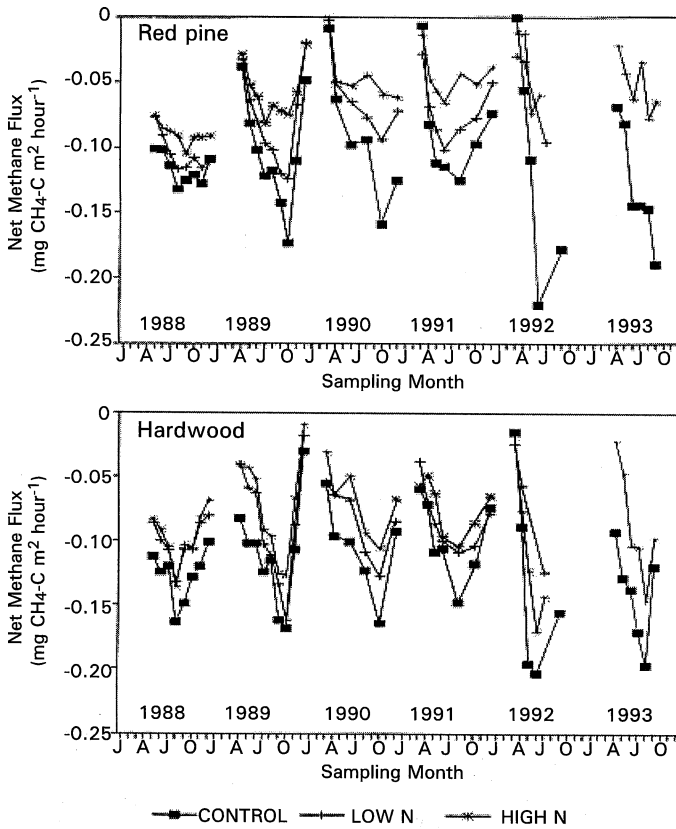
Figure 12.4. Changes in net methane flux in the red pine (top) and hardwood (bottom) stands in response to the first six years of chronic N additions. Modified from Castro, Steudler et al. 1995, by permission of the American Geophysical Union (copyright 1995, American Geophysical Union).

ping long-wave radiation. Increased nitrogen deposition over large parts of North America and Europe could actually be contributing to the measured rapid increase in atmospheric concentrations of methane.

We were surprised as well by a measurable decline in productivity in the red pine stands receiving nitrogen additions (Figure 12.5). While we had hypothesized that such an outcome was a feasible long-term concern on the basis of the literature on forest decline in both North America and Europe (Figure 12.2), we did not expect this response to occur this quickly. The decline in growth occurs despite an increase in foliar nitrogen concentration and is accompanied by a reduced foliar retention time and severe reduction in total needle mass (Figure 12.6).

It could be argued that the red pine stand is old and nearly mature
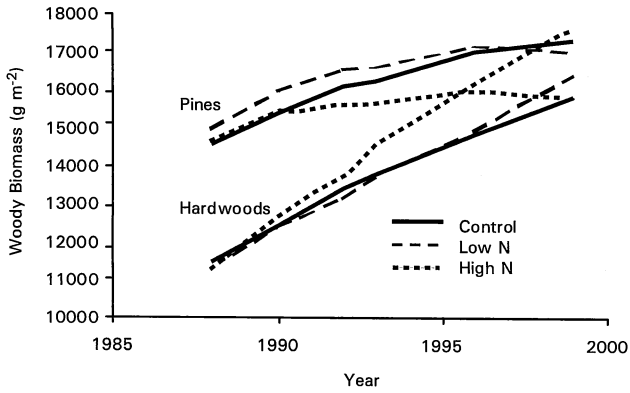
Figure 12.5. Comparison of changes in total woody biomass over time in the red pine and hardwood plots as a consequence of chronic N additions. Total accumulation in the high-N pine plot is significantly lower than in the control and low-N plots, while in the hardwood stand accumulation is highest in the high-N plot.
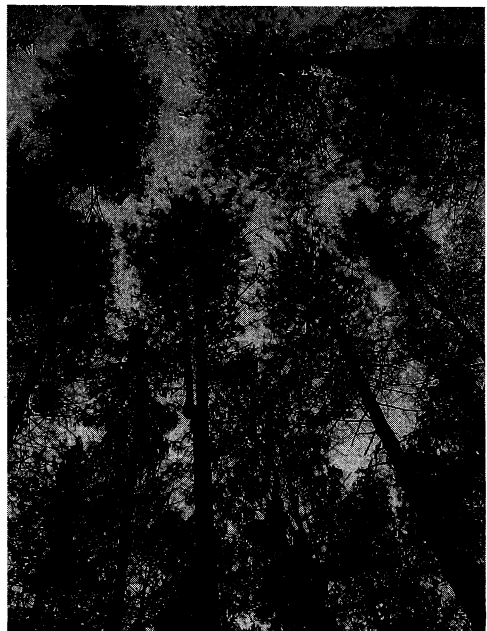
Figure 12.6. Foliar biomass has been greatly reduced in the pine stand receiving high-N additions (left) compared with the control (right). Photographs by D. R. Foster.

and therefore is being pushed to overmaturity by excess fertilization. However, similar results in a spruce-fir stand on Mount Ascutney, Vermont, and across a long transect of spruce-fir stands from New York to Maine, combined with results from Europe, suggest that high levels of nitrogen deposition may indeed endanger forest health in needle-leaved evergreen stands. The decline in tree growth that we have recorded is accompanied by decreasing ratios of magnesium to nitrogen and/or calcium to aluminum in foliage in the pine stand, at Mount Ascutney, and along the regional nitrogen deposition transect. The ratios of these elements are used as indicators of forest health, and our results suggest that nutritional imbalances involving elements other than nitrogen may be induced by excess nitrogen deposition. Measurements of photosynthesis and stress response at both the Harvard Forest and Mount Ascutney sites provide process-level explanations for declining wood production.

Perhaps the most surprising result is the very high potential for nitrogen retention in both forest ecosystems and the fact that most of the nitrogen retained was incorporated rapidly into soils without passing through plant biomass. This was shown by both mass budget calculations (direct measurement of changes in pool size and flux) and by the fraction of added ^{15}N recovered in each pool (Table 12.2). This result has been confirmed in a number of other studies in both the United States and Europe. We had anticipated that plants, being nitrogen limited, would be the strongest sink for added nitrogen and that this capacity would soon be exceeded by the high rates of nitrogen addition. Instead, total retention was much higher than we expected, and the most significant retention processes apparently occur in the soil. Efficient, long-term retention of added nitrogen is a critical result, as all of the negative

Table 12.2. Comparison of Two Methods for Estimating the Distribution of Added Nitrogen in Low-Nitrogen Plots

Sinks (%)	Pine Low		Hardwood Low	
	Budget	^{15}N	Budget	^{15}N
Foliage	12.7	9.6	5.3	5.9
Wood	2.9	1.4	12.7	4.4
Roots	9.6	8.7	11.6	13.7
Total plant	25.2	19.7	29.6	24.0
Soil (0–20 cm)	—	49	—	71.9
Soil (0–60 cm)	74.8	(80.3)	70.4	(76.0)
Total	100	68.7	100	95.9
		(100)		(100)

Note: Values are percentage of total nitrogen retained in each pool. The "Budget" column uses data collected as described in Magill et al. 2000. The "^{15}N" data represent percentage recovery by compartment after two years of natural abundance-level ^{15}N additions to control and low-nitrogen plots (Nadelhoffer, Downs, and Fry, 1999). The (est. 0–60 cm) values assume that the unrecovered ^{15}N resides in the soil compartment in all cases but could not be fully detected because of low concentrations and sampling limited to 20 centimeters' depth.

effects of nitrogen saturation are linked to elevated rates of nitrification and nitrate leaching that occur when nitrogen is no longer limiting (Figure 12.1). Consequently, if forests have a greater-than-expected potential to retain and store nitrogen before nitrate leaching begins, then the damage to forests and waters predicted to occur in the wake of nitrogen saturation is reduced, or at least delayed.

## Mechanisms for Nitrogen Retention: A Series of Miniexperiments

Overview

We need to understand how added nitrogen is incorporated into soils and what the capacity of this mechanism is (how long will this protective function continue?). After the first three years of the experiment, it was clear that the soil was the biggest nitrogen sink. At that time (about 1991), new experiments tracing the short-term (twenty-four-hour) dynamics of ^{15}N in soils suggested that gross rates of uptake and release of nitrogen by microbes were many times faster than previously thought. Also, new insights into the possible role of dissolved organic carbon (DOC) in forest soils were emerging. Combining these perspectives, we hypothesized that DOC was an important substrate and energy source for microbes as they incorporated (and immobilized) the additional nitrogen into microbial biomass. This was something of a radical proposal in that basically all previous evidence indicated that soil microbes are carbon (or energy)–limited rather than nitrogen-limited.

We devised three experiments to test this new hypothesis that nitrogen incorporation was due to microbial immobilization using DOC as an additional energy source. The first looked for changes in the efflux of CO_2 from microbial respiration in response to nitrogen additions; the second examined the effect of nitrogen additions on concentrations of DOC and associated nitrogen (DON) in soil water; and the third measured nitrate dynamics using ^{15}N tracer-pool dilution techniques. Because our nitrogen additions are made directly to the soil surface, we also examined the results of a long-term leaf litter decomposition experiment for evidence of increased biological immobilization.

Experiment 1: Soil CO_2 Flux versus Nitrogen Incorporation

If soil microbes were limited by nitrogen, we hypothesized that the addition of mineral nitrogen would result in both the disappearance of that nitrogen and an increase in the flux of CO_2 from the soil as the microbes increased their metabolic activity and respiration in order to produce more biomass. To test this, we measured CO_2 efflux from soils to the atmosphere and the rate of disappearance of the added nitrogen in both pine and hardwood plots.
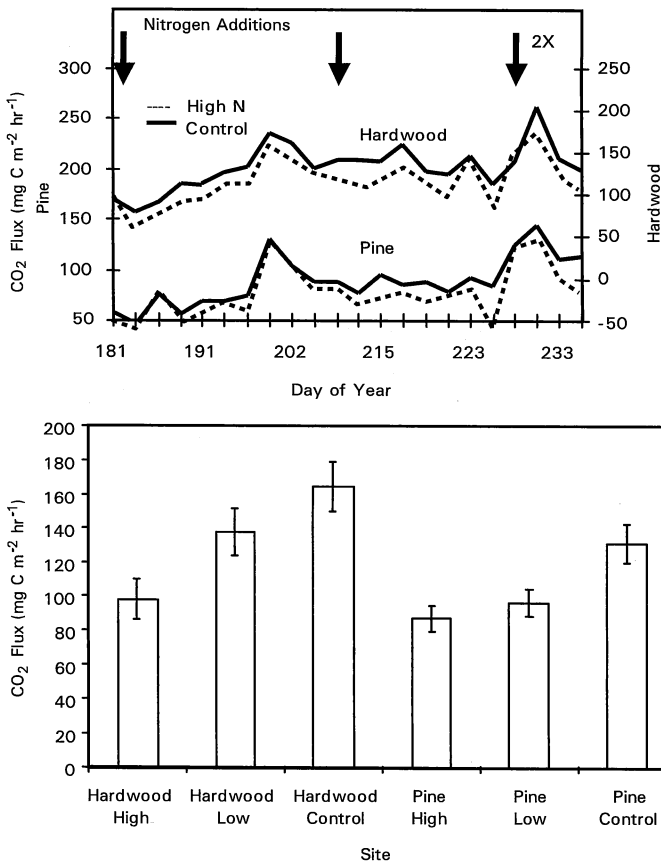
Figure 12.7. Comparison of measured rates of CO_2 efflux from the control and N-treated stands. Top: Changes immediately after N additions at doses equivalent to chronic N additions. 2X indicates timing of a double-high N dose. These data show no significant increase in CO_2 efflux after N additions (Micks 1994, unpublished thesis). Bottom: Mean soil CO_2 flux rates by treatment, June to August 2001, showing a strong reduction with the addition of nitrogen. Modified from C. Arabia and E. Davidson (unpublished).

The study revealed that total CO_2 flux might actually be lower in the nitrogen-amended stands and that respiration in both control and amended stands changed in response to moisture and temperature, but that there was no detectable increase in CO_2 efflux after nitrogen additions (Figure 12.7, top). The added nitrogen disappeared from the soil over a period of one to two weeks, and a very simple model of microbial dynamics suggested that CO_2 fluxes during this period would have increased severalfold if microbial biomass production were the primary process by which mineral nitrogen was converted to organic nitrogen.

More recent and more detailed measurements of soil respiration have indeed shown that CO_2 flux from soils has decreased in the high-nitrogen plots (Figure 12.7, bottom). There are several possible explanations for this, but none of them would support the hypothesis that nitrogen limitations were suppressing microbial metabolism in these stands before nitrogen additions began.

Experiment 2: Effects of Nitrogen Additions on DOC and DON Flux

A second line of evidence that could support the hypothesis of microbial incorporation driven by metabolism of DOC would be a reduction in measurable soil DOC. We measured the leaching losses of DOC from the forest floor to the mineral soil as an indicator of total DOC availability. The mean values for this DOC flux were somewhat higher, rather than lower, in the nitrogen-amended stands, but the differences were not statistically significant. There was, however, a significant increase in DON flux and a narrowing of the DOC:DON ratio in the water passing from the forest floor into the mineral soil. The size of the increase in DON flux (5 to 6 kg $N \cdot ha^{-1} \cdot yr^{-1}$ in the high-nitrogen stands) is too small to account for a large part of the added nitrogen, but it may suggest a more important immobilization mechanism, as discussed below.

Experiment 3: Pool Dilution Studies and Abiotic ^{15}N Incorporation

We employed ^{15}N pool dilution techniques to examine rates of microbial uptake and release of nitrate. This technique involves injecting very small amounts of NO_3 that is highly enriched with ^{15}N into soils and then determining the form in which this nitrogen occurs at different time intervals up to twenty-four hours after addition. For example, if a small amount of enriched nitrate is added to a soil, then at time zero a given fraction of the extractable nitrate pool consists of ^{15}N. The gross rate of nitrogen uptake or immobilization of that nitrate is equivalent to the rate at which the extractable ^{15}N disappears through time. The rate of nitrate production is determined by the rate at which the remaining $^{15}NO_3$ pool is diluted by new $^{14}NO_3$. Different sampling times (often fifteen to thirty minutes and twenty-four hours) are used to identify and separate the potential roles of "fast" and "slow" immobilization processes. Rapid disappearance of ^{15}N (that is, a fast process) is often interpreted as being due to chemical or abiotic reactions, while disappearance between this first sample and twenty-four hours is generally attributed to microbial uptake.

We applied this technique using $^{15}NO_3$ in the pine and hardwood control and high-nitrogen plots, using several sampling periods and a
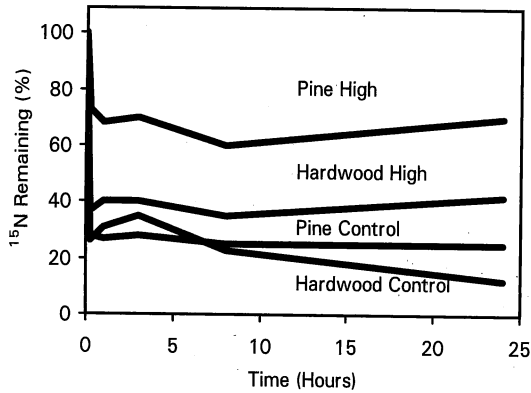
Figure 12.8. Gross nitrate immobilization in the control and high-N plots for the pine and hardwood stands as measured by ^{15}N pool dilution. Modified from Berntson and Aber 2000, 153, with permission from Elsevier Science (copyright 2000).

unique statistical method to determine more accurately the dynamics of the fast and slow processes. Surprisingly, our results suggest that the fast process is the only significant mechanism for nitrate incorporation in all but the hardwood control stand (Figure 12.8). In the pine stand, there was no significant slow immobilization, but fast immobilization was greater in the control plot than in the high-nitrogen plot. In the hardwoods, fast immobilization was similar between control and high-nitrogen plots, but the control plot showed additional slow nitrate immobilization while the high-nitrogen plot did not.

It is notable that total (fast plus slow) nitrate immobilization in each of the four plots measured was highly correlated with the concentration of nitrate in soil water below the rooting zone. This suggests that the processes measured by our modified pool dilution method could be important in nitrogen retention. The results also clearly indicate that fast immobilization is an important process in nitrogen retention. Much more work needs to be done here to determine whether "fast" and "slow" equate to abiotic and biotic reactions. If they do, then much of the efficient retention of nitrate in the high-nitrogen plots could be dominated by abiotic chemical reactions in the soil about which we know very little indeed.

Experiment 4: Carbon and Nitrogen Dynamics of Nitrogen-Amended Leaf Litter

To look at the role of the litter layer in nitrogen retention, we conducted two experiments on the mass loss and the nitrogen dynamics

of litter. The first was a laboratory experiment in which NO_3 and NH_4 were added to freshly collected leaf litter in amounts equivalent to the field nitrogen applications. As an innovation, we also examined DOC and DON fluxes from this material in order to provide complete carbon and nitrogen balances and to link to the field DOC and DON measurements described above. The second was a long-term field study of leaf litter decay under the different nitrogen-addition treatments.

In the lab study, the addition of inorganic nitrogen produced small increases in rates of leaf litter decay and substantial increases in nitrogen immobilization. What was surprising was the large fraction of mass loss (5 to 35 percent) that occurred through the leaching of DOC, rather than as CO_2 fluxes from microbial respiration. This DOC flux has been largely ignored in decomposition studies and suggests that serious errors in the calculation of ecosystem carbon balances can occur when litter mass "disappearance" is equated with microbial respiration. These results also emphasize the importance of knowing the fate of DOC leached from litter. Is it then respired within the forest floor (in which case it would be equivalent to respiration from the litter itself), or is it eventually stabilized through chemical reactions?

Nitrogen immobilization into litter in this lab experiment did increase with nitrogen additions and accounted for a significant fraction of total added nitrogen. Using measured litterfall rates, and extending the results of the lab experiment to the point of humus production, the nitrogen sink associated with increased nitrogen immobilization in litter is estimated at 11 and 17 kg $N \cdot ha^{-1} \cdot yr^{-1}$ in the hardwood and pine high-nitrogen plots, respectively, or 7 to 11 percent of the total addition. Whether or not this immobilization occurs through microbial activity cannot be determined from these results.

The long-term field study confirms an increase in nitrogen retention in litter during decay. In this case, rates of decomposition in the nitrogen-amended plots were reduced in the later stages of the process (Figure 12.9), particularly through reduced rates of lignin decomposition. Nitrogen immobilization into this litter continued in the high-nitrogen plots, even though mass loss was reduced. At the end of the experiment, the concentration of nitrogen in litter was similar across treatments, but the total mass of litter remaining was higher in the high-nitrogen plots, so total nitrogen retained was also higher. Calculations of the total additional amount of nitrogen in the soil organic matter produced at the end of the entire decay sequence also suggest a net retention by this process of around 15 kg $N \cdot ha^{-1} \cdot yr^{-1}$, similar to the estimates obtained in the lab study. Results from both litter studies are consistent with those obtained from the ^{15}N additions in the field, in which 11 percent of the total ^{15}N added to the low-nitrogen plots was recovered in the litter layer.
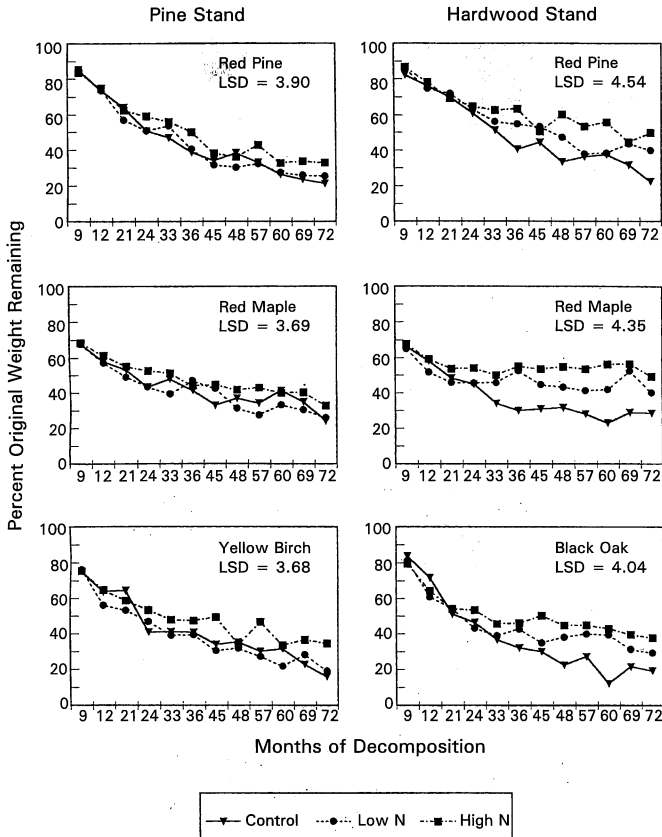
Figure 12.9. Effects of long-term chronic N additions on decay rates of different foliar litters in the field. N concentrations in older litter material did not differ between treatments, but these differences in total mass remaining in later stages of decay leads to increased total N content and higher estimated N incorporation and retention. Modified from Magill and Aber 1998, 304: fig. 1, with kind permission from Kluwer Academic Publishers (copyright Kluwer Academic Publishers 1998).

Summarizing the Experiments

CONSTRAINING THE IMMOBILIZATION PROCESSES

All four of these experiments contradict the hypothesis from which they were generated. Soil CO_2 efflux did not increase, DOC concentrations did not decrease, most of the immobilization appeared to occur very rapidly, and nitrogen concentrations in older litter increased in the absence of mass loss (or apparent microbial processing). All of these suggest that immobilization is not occurring through microbial uptake and biomass production as traditionally viewed. What other processes

might drive this rapid movement of mineral nitrogen into soil organic matter?

In a 1998 review paper, we described two processes that fit within the constraints imposed by the results of the four experiments: abiotic immobilization and mycorrhizal assimilation without biomass production. Abiotic immobilization results from chemical reactions between mineral forms of nitrogen and organic compounds in soils and so requires no energy and generates no CO_2. Mycorrhizal assimilation into proteins would use carbon provided by the host plant. The efficiency of conversion of this carbon to proteins is much higher than the conversion of litter carbon to microbial biomass because of the cost of producing the enzyme systems required to decay organic matter. Because of this and the slightly lower ratio of carbon to nitrogen in proteins versus microbial biomass, less carbon is required and much less CO_2 is generated. The question remains as to what the fate of nitrogen assimilated by mycorrhizae might be. Is there other evidence to support either of these processes?

ABIOTIC INCORPORATION OF INORGANIC NITROGEN
INTO SOIL ORGANIC MATTER

It has been known for several decades that significant rates of direct chemical incorporation of NH^4 into organic materials can occur under the right conditions. Before the invention of chemical fertilizers, nitrogen-rich soil amendments were produced by combining peat moss with ammonium under conditions of high pH, temperature, and pressure. Several studies over the past two to three years have shown that chemical incorporation of both nitrate and ammonium can also occur under ambient conditions in lab incubations. One study estimates that abiotic immobilization accounts for 6 to 90 percent of total immobilization, with an average of about 40 percent across a very wide range of ecosystems. Two others showed that most nitrate immobilization at the Harvard Forest occurred in the first few minutes of ^{15}N additions, a result generally associated with chemical rather than biological immobilization, and that sterilization of soils did not alter this pattern. It is particularly surprising that these fast reactions occur for both nitrate and ammonium. There are no known or generally accepted mechanisms for abiotic incorporation of nitrate.

We have proposed the concept of the "DOM conveyor" as a possible mechanism for the rapid, abiotic immobilization of mineral nitrogen. This derives from the observation that increases in DON are a measurable if short-term response to the addition of mineral nitrogen to soil solutions. It is generally assumed that pools of DOC or dissolved organic matter (DOM) in the soil solution represent an equilibrium between very rapid rates of sorption and desorption from/to the solid soil phase. If there are chemical reactions by which mineral nitrogen can be incor-
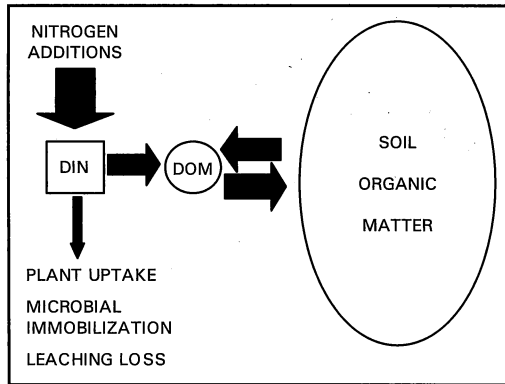
Figure 12.10. Diagrammatic presentation of a hypothesized mechanism for the incorporation of mineral N into soil organic matter. Pools of dissolved organic matter (DOM) are small but exchange rapidly with the solid phase of soil organic matter through sorption/desorption reactions. If chemical reactions occur that incorporate dissolved inorganic nitrogen (DIN) into DOM, then resorption of this DOM into the solid phase would provide a "conveyor belt" effect, loading added N into soil organic matter.

porated into DOM before resorption, then a rapid "conveyor belt" effect would be established by which relatively large quantities of nitrogen could be transported into soil organic matter (Figure 12.10), although the enrichment of the DON pool would be small at any one time. To test this, we need to establish both that DOC is exchanged rapidly with soil organics (a pool dilution–like experiment would do this), and that reactions between DOM and mineral nitrogen occur.

MYCORRHIZAL ASSIMILATION

If we listed components of forests in order by their importance in ecosystem processes divided by how much we know about them, there can be no doubt that mycorrhizae would be right up at the top. Mycorrhizae, the symbiotic combination of plant roots with soil fungi, are ubiquitous in forest soils and can compose a large fraction of the total fungal biomass and total microbial biomass. Mycorrhizae are longer-lived than free-living microbes, act as an extension of root systems, and are generally thought of as a mechanism by which plants increase the efficiency of nutrient uptake, but many uncertainties about processes and controls in this interaction remain.

We do know that the degree of mycorrhizal infection of roots and the species composition of the fungi involved change with nitrogen status, but we know almost nothing about the physiological processes of mycorrhizae in the symbiotic state in the field. This is especially true when compared against our knowledge of other critical processes such as pho-

tosynthesis. There are very few methods for the measurement of, for example, the simultaneous uptake of nitrogen and release of CO_2 by mycorrhizae in the field. This is one of the most important areas for future work in forest ecosystem studies. Without this knowledge, we can say only that mycorrhizae might assimilate mineral nitrogen with a low respirational cost, or they might not. Speculations on the evolutionary adaptability of such a process are premature.

Although direct studies of carbon and nitrogen processing by mycorrhizae in the field have not been undertaken, innovative isotopic approaches are beginning to provide some insights into changes in the role of mycorrhizae over nitrogen availability gradients. For example, studies on the fractionation of ^{15}N among soil organic matter, extractable nitrogen, roots, and foliage show that fractionation varies with nitrogen status. A modeling analysis suggests that increased fractional retention of nitrogen by mycorrhizae is the process most consistent with measured results. Reduced retention by mycorrhizae at high nitrogen availability may then contribute to nitrate leaching.

Synthesis and Conclusions

Results from the chronic nitrogen experiment, especially when considered along with those from similar experiments conducted elsewhere, have resolved several qualitative hypotheses about the process of nitrogen saturation. A generalized pattern of forest response to the increased availability of nitrogen has emerged that includes the following: (1) increasing rates of nitrification and nitrate leaching; (2) increasing foliar nitrogen concentration and decreasing nitrogen:element ratios; (3) initially increasing and then possibly decreasing rates of soil respiration and nitrogen mineralization; and (4) initially increasing and then perhaps decreasing rates of total net primary production (Figure 12.1). Growth declines in needle-leaved evergreens both in experimental trials and at the high end of ambient nitrogen deposition gradients here and in other places around the world suggest that reduced forest growth and yield in these types of forests in response to nitrogen deposition is a real possibility.

We have also documented a remarkable capacity for uptake and retention of nitrogen in soils and demonstrated that understanding the mechanisms responsible for this retention is the key to developing predictions of the timing of nitrogen saturation. By working on sites where the history of past land-use and land-cover changes are well-known, we have also recognized the critical role that the legacies of this history play in determining nitrogen status of forests and conditioning ecosystem response to nitrogen additions. Species composition also plays a role here. Broad-leaved deciduous forests appear to have a greater capacity to retain added nitrogen and to delay the onset of nitrogen saturation.

Why this is true remains unclear, although different physiological capacities to fix carbon, take up nitrogen, and produce biomass with higher nitrogen concentrations are logical candidates.

To put quantitative substance on the qualitative skeleton of our understanding of nitrogen saturation is more challenging. Negative effects of nitrogen saturation all relate to production and leaching of nitrate, so the key policy question relating to nitrogen saturation is the timing, magnitude, and spatial extent of excess nitrogen availability and the associated increases in nitrate leaching, cation loss, and soil and water acidification. Previous research has developed quantitative relationships regarding nitrogen availability and net primary production and also between litter quality and decomposition rates. The largest remaining unknown involves the greatest sink for added nitrogen in forest ecosystems, the organic and mineral soils pools. Consequently, although nitrogen deposition and saturation appears to pose a potential threat to forest ecosystems, we cannot yet judge the significance of that threat because of a lack of understanding of nitrogen cycling, both biotic and abiotic, belowground.