Nitrogen Saturation in Temperate Forest Ecosystems

Hypotheses revisited

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Nitrogen emissions to the atmosphere due to human activity remain elevated in industrialized regions of the world and are accelerating in many developing regions (Galloway 1995). Although the deposition of sulfur has been reduced over much of the United States and Europe by aggressive environmental protection policies, current nitrogen deposition reduction targets in the US are modest. Nitrogen deposition remains relatively constant in the northeastern United States and is increasing in the Southeast and the West (Fenn et al. in press).

The US acid deposition effects program in the 1980s (the National Atmospheric Precipitation Assessment Program, or NAPAP) funded research mostly on the effects of sulfur deposition rather than on those associated with nitrogen. Since the completion of this program and the passage of the 1990 Clean Air Act Amendments, the US regulatory community has not supported substantial additional research on acidic deposition. Consequently, the potential for nitrogen deposition to contribute to soil and surface water acidification remains unresolved. Unlike the European Community, which has pursued an active and well-coordinated international program on nitrogen deposition effects (the NITREX program; Wright and van Breeman 1995, Wright and Rasmussen 1998) and on critical loads for nitrogen (Nilsson and Grennfelt 1988, Henricksen et al. 1992, Warfvinge and Sverdrup 1992, Wright and Rasmussen 1998), research on this topic in the United States remains scattered and piecemeal. Policy and regulatory activity have also, until very recently, been virtually nonexistent.

> Recent research raises questions on the processes of N retention in soils, and how much protection these processes offer to forest and stream ecosystems

Despite this lack of support and direction, the US scientific community has continued to express concern over the long-term effects of nitrogen deposition on forests, grasslands, streams, and estuaries (e.g., Aber et al. 1995, Wedin and Tilman 1996, Asner et al. 1997, Vitousek et al. 1997, Fenn et al. in press). These concerns cover the deposition not only of oxides of nitrogen (NO_x and especially nitrate, NO_3^-), which originate mainly from fossil fuel combustion, but also of ammonium (NH_4^+), which originates from the production and use of fertilizers in agriculture. In parts of Europe and much of Asia, deposition of ammonium exceeds that of nitrate, sometimes by severalfold (Galloway 1995).

In 1989, we published a review in *BioScience* of the known effects of nitrogen deposition on temperate forest ecosystems in which we set forth a series of hypotheses about the long-term consequences of continuously elevated nitrogen inputs (Aber et al. 1989). We stressed the potential for nitrogen additions to lead to nitrate and aluminum mobility in soils, causing soil and stream acidification, nutrient:nitrogen imbalances in trees, and forest decline. The integrated set of hypotheses (Figure 1) suggested that responses to nitrogen deposition would not be linear and would therefore not be captured in simple dose-response functions. Rather, we expected them to be highly nonlinear, with critical thresh-

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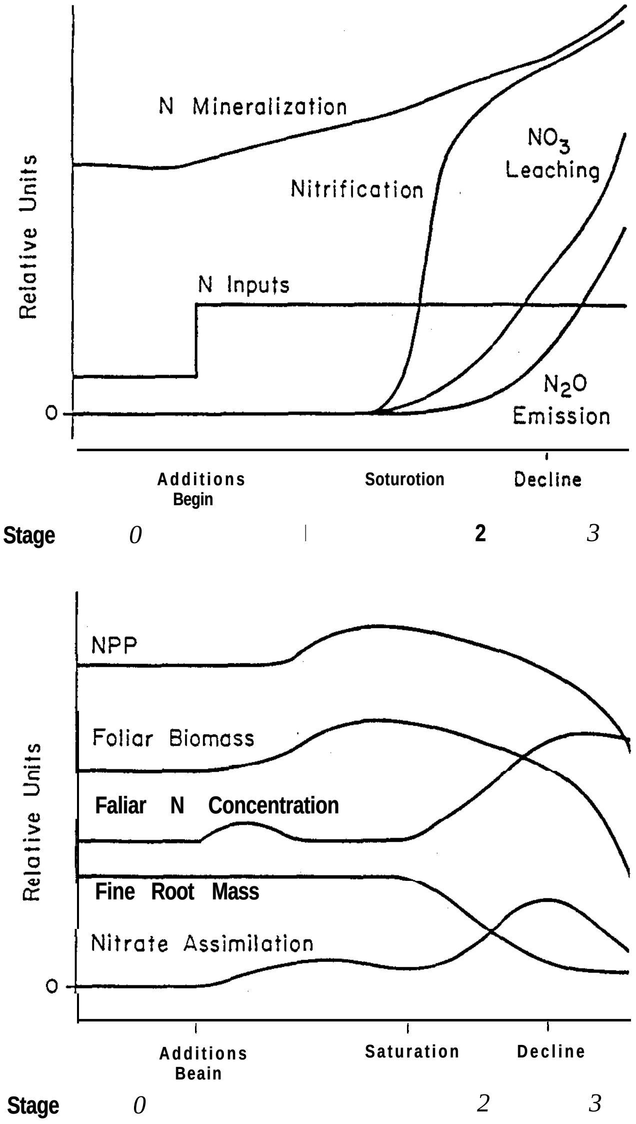


Figure 1. Initial set of hypotheses describing the integrated response of nitrogen-limited temperate forests to chronic nitrogen additions. The two graphs show predicted responses for soils (top) and plants (bottom). The stages of response are drawn from Smith (1974) and Bormann (1982), as summarized in Aber et al. (1989). Stage 0 is pretreatment and assumes strong nitrogen limitations on growth. Stage 1 is characterized by high nitrogen retention and a fertilizer effect of added nitrogen on tree growth. Stage 2 includes the induction of nitrification and some nitrate leaching, although growth is still high. In Stage 3, tree growth declines, and nitrification and nitrate loss continue to increase, as the fraction of mineralized ammonium that nitrifies increases. Figure redrawn from Aber et al. (1989).

old points. Central to most responses was the induction of, or increase in, net nitrification.

We used these hypotheses to frame a series of field experiments, similar to the NITREX studies in Europe, and one extensive survey in forest ecosystems across New York and New England. These recent studies allow a more complete summary of the process that has come to be called nitrogen saturation. Although this term has been defined in many ways (Agren and Bossata 1988, Aber et al. 1989, Stoddard 1994), all definitions include increases in nitrogen availability over time that result in the alleviation of nitrogen limitations on rates of biological function and increases in nitrate mobility in soils.

In this article, we review the theory behind the initial 1989 hypotheses. We then combine results derived from testing these hypotheses with results from other experiments to present our current understanding of nitrogen saturation. Finally, we examine the evidence for three different general mechanisms by which added nitrogen may be retained in forest ecosystems and offer a novel hypothesis as to how this retention may occur.

Theory behind the original hypotheses

Our initial review of the literature (see Aber et al. 1989 for references) pinpointed the production and mobility of nitrate as key processes controlling ecosystem responses to nitrogen deposition. Predicted changes in stream and sail chemistry, as well as plant responses, all followed from nitrate dynamics. From previously published studies, we derived the following general understanding of processes affecting nitrogen cycling and nitrate production.

Under background or low nitrogen deposition conditions, plant growth-in temperate forests is-most frequently limited by nitrogen availability, while the metabolism of free-living soil microbes is most generally limited by carbon (or energy). These essential constraints on the biogeochemistry of forested ecosystems are self-perpetuating in that plants incorporate available nitrogen released by microbial decomposition into carbon compounds that are energetically and structurally difficult to decompose. The partial decomposition of fresh plant tissues shed as litter results in both a further concentration of nitrogen in the resulting organic matter (humus) and a further degradation in the decomposability of this residue. The rate-limiting step in the nitrogen cycle of temperate forest ecosystems is thus the humus decay process, and the largest pool of nitrogen in these systems is in soil organic matter. In addition, published studies suggested that in acid forest soils, nitrification is controlled mainly by competition between plants and nitrifying microbes such that nitrification tends to occur only where the supply of ammonium is high relative to plant demand.

From this general pattern of carbon and nitrogen limitations on plant and microbial function, we hypothesized that the addition of inorganic nitrogen to temperate forests in mineral form would result, at least initially, in increased nitrogen uptake and growth by plants, and that there would be little change in the immobilization of nitrogen by microbes due to preexisting energetic limitations (Figure 2a). Rather, most of the nitrogen added to these systems would be taken up by the nitrogen-limited organisms-the plants-and relatively little by soil microbes. The process of nitrogen saturation, by which forest ecosystems show decreased retention efficiency of added nitrogen, should then be controlled mainly by plant uptake, rather than by soil microbial uptake, with the secondary process of microbial nitrification increasing as nitrogen availability begins to saturate plant demand. This logic led directly to the initial overarching hypothesis (Figure 1) that plant processes would change first in response to nitrogen additions,

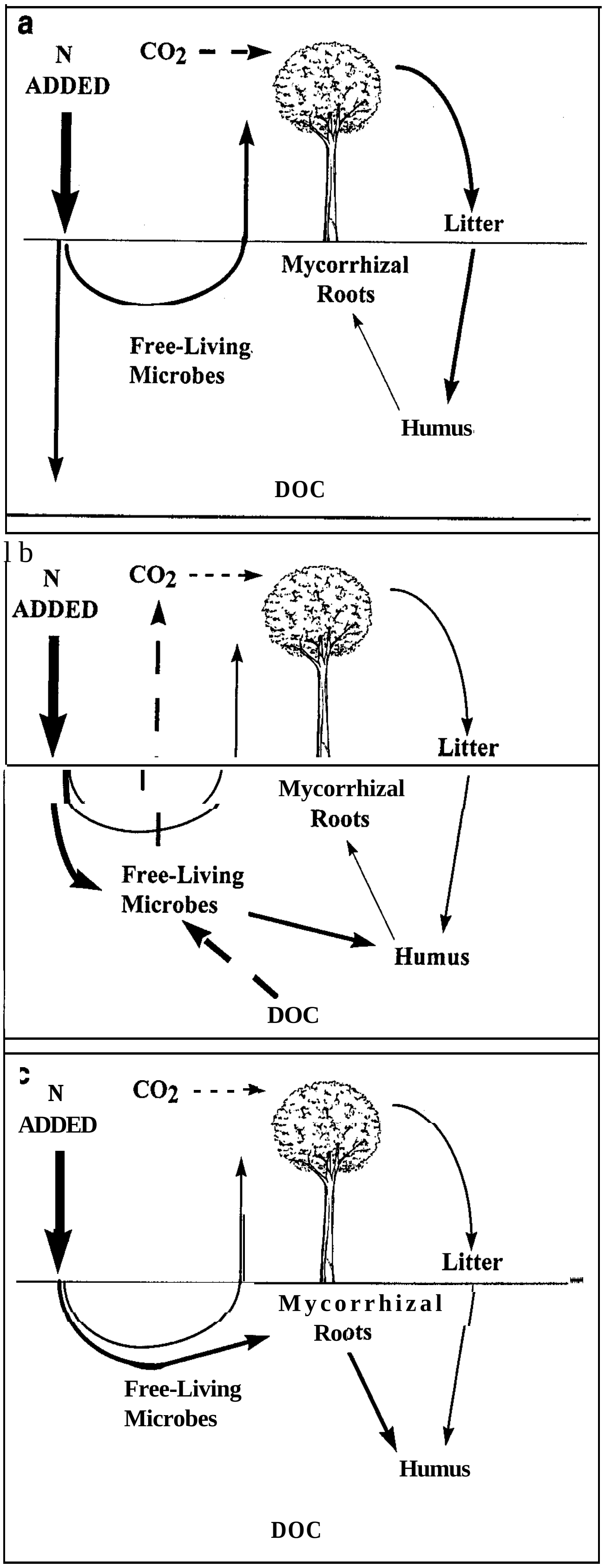


Figure 2. Evolution of hypotheses regarding the main pathway of nitrogen assimilation and retention in forest ecosystems exposed to chronically elevated nitrogen inputs: The width of the arrows expresses the relative amount of added nitrogen following each pathway. **(a)** The initial hypothesis was that plant uptake would be the main sink, resulting in increased photosynthesis and tree growth, with recycling of nitrogen through litter and humus to the available pool (Aber et al. 1989). This mechanism should saturate quickly, resulting in early increases in nitrate leaching. **(b)** The very large rates of nitrogen retention in soils led us to question the assumption that free-living soil microbes were carbon or energy limited. We hypothesized that pools of available carbon in soils were driving increased microbial growth in competition with plant uptake (Aber 1992). A requirement of this hypothesis was large increases in soil respiration of CO,. (c) Field measurements suggest significant immobilization of nitrogen in soils without increased CO, production. Mycorrhizal assimilation using plant carbon derived directly from photosynthate is a process that most closely matches the limitations on carbon and nitrogen cycling imposed by those measurements. DOC, dissolved organic carbon.

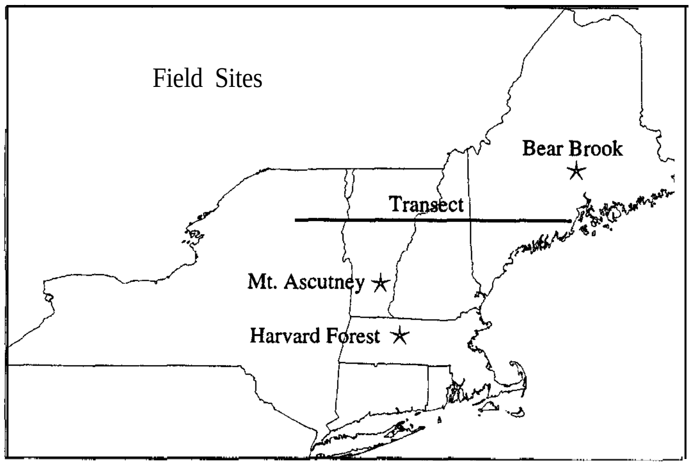
with changes in soil microbial processes such as nitrogen mineralization and nitrification changing secondarily in response to alterations in litter input and quality, and to the saturation of plant demand for inorganic nitrogen. Do our experiments and those of others support or refute this central hypothesis?

Experiments

To test the hypotheses contained in Figure 1, we established experimental manipulations in four site/stand combinations in New England, and also examined C:N and other element:nitrogen ratios in foliage and forest floor materials from a series of 161 spruce-fir forests along an nitrogen deposition gradient across the New York/New England region (Figure 3). Intensive site-level experiments can be questioned as to their generality or applicability over a larger region, whereas regional surveys can be questioned due to the simultaneous change in several environmental parameters (e.g., climate, geology, soils) along any spatial transect. Thus, a combination of space-for-time substitution in the regional survey, and controlled experiments at the intensive sites, provides a more robust set of conclusions.

Manipulated sites included two stands (red pine plantation and native mixed hardwoods) at the Harvard Forest in Petersham, Massachusetts, where a chronic nitrogen amendment experiment is one of the core activities of the National Science Foundation's Long Term Ecological Research (LTER) program (Aber et al. 1993, Magill et al. 1997). Started in 1988 and still continuing, this experiment involves additions of concentrated dissolved ammonium nitrate in six monthly doses from May to October of each year. Total nitrogen additions are 0, 5, and 15 g·m^{-2}·yr^{-1}. A similar plot-level experiment was conducted in a second-growth northern hardwood stand as part of the Bear Brook Watershed manipulation program in Maine, which is funded by the Environmental Protection Agency (Kahl et al. 1993, Rustad et al. 1993, Magill et al. 1996). This experiment, which occurred from ***1988*** to 1991, included nitrogen additions of 0, 2.8, and ***5.6*** g·m^{-2}·yr^{-1} as nitric acid. The fourth site, Mt. Ascutney in Vermont, was selected as a mature, healthy spruce-fir system to complement the regional spruce-fir survey described below. Nitrogen additions, which ranged from 0 to 3.1 g·m^{-2}·yr^{-1} (McNulty and Aber 1993, McNulty et al. 1996),

Figure 3. Location of study sites. Harvard Forest, Bear Brook, and Mt. Ascutney are sites of intensive manipulation experiments. The transect sites are a set of 161 spruce-fir forests used for an extensive survey of changes in forest floor and foliar chemistry along a nitrogen deposition gradient.



included a combination of ammonium and nitrate added as a concentrated solution three times per year, due to the shorter growing season. These additions are continuing. The 161 spruce-fir stands in the regional survey (Figure 3) were located in 11 areas from New York to Maine (McNulty et al. 1990, 1991) that receive from 0.4 to 1.3 $g \cdot m^{-2} \cdot yr^{-1}$ in estimated current nitrogen deposition (Ollinger et al. 1993).

At each of the intensive manipulation sites, continuous measurements were made of key components of the nitrogen cycle, including net annual nitrogen mineralization and nitrification, foliar nitrogen concentration and Mg:N and Ca:Al ratios, and either wood plus foliar net primary productivity (NPP) or basal area increment (net increase in the total cross-sectional stem area of all trees on a plot). Nitrogen leaching losses were measured at the Harvard Forest and Bear Brook Watershed sites with tension lysimeters placed below the rooting zone. Because tension lysimeters could not be buried in the shallow, stony soil at Mt. Ascutney, resin bags were used to assess relative rates of cation and anion movement below the rooting zone. Foliar nitrogen, magnesium, calcium, and aluminum concentrations were measured on samples collected in midsummer from all sites. At the regional survey sites, only foliar and forest-floor samples were collected, and these were analyzed for nitrogen and element:nitrogen ratios, as well as for potential net nitrogen mineralization and nitrification (as measured by a one-time, laboratory incubation of forest-floor samples).

Ecosystem responses and intersite comparisons

Results from the experiments described above (e.g., McNulty et al. 1991, 1996, Aber et al. 1995, Magill et al. 1996, 1997) can be combined with those from similar studies in Europe and elsewhere in the United States (e.g., Christ et al. 1995, Gilliam et al. 1996) to generate a more complete picture of the nitrogen saturation process. In particular, the European NITREX program (Wright and Rasmussen 1998) carried out a series of nitrogen additions to nitrogen-poor sites (by fertilizer additions) and nitrogen removals from nitrogen-rich sites (by the construction of under-canopy roofs). There have also been experimental additions of nitrogen at the watershed scale in the United States (Adams et al. 1993, Kahl et al. 1993) and observational studies of nitrogen cycling in nitrogen-saturated systems (Van Miegroet and Cole 1985, Johnson et al. 1991).

Nitrogen mineralization, nitrification, and nitrate loss. Our original hypothesis predicted that chronic nitrogen additions would result in long-term increases in nitrogen mineralization as added nitrogen became incorporated into soil organic matter, reducing C:N ratios and increasing nitrogen release during decomposition. All sites and studies did show an initial increase in net nitrogen mineralization, as did the stands in the midpoint of the spruce-fir transect relative to the lowest nitrogen deposition stands (Figure 4a). Maximum measured rates of net nitrogen mineralization ranged from 1.2 to 2.4 times control (or low nitrogen deposition) values. However, longer-term responses in all but one stand, the Harvard Forest hardwoods, show actual decreases in net nitrogen mineralization from early peak rates, dropping near or below control values (Figure 4a).

Reports of reduced rates of nitrogen mineralization under nitrogen-enriched conditions can be found in both recent and older studies (e.g., Baath et al. 1981, Soderstrom et al. 1983, Fog 1988). In the NITREX studies in Europe, Tietema (1998), using ^{15}N pool dilution, found increases in both gross mineralization and gross immobilization rates across a nitrogen deposition gradient, but net nitrogen mineralization peaked at intermediate nitrogen deposition. Gundersen et al. (1998) reported increases in field-measured net nitrogen mineralization rates with nitrogen addition only at nitrogen-limited sites, whereas those with high initial rates of nitrogen cycling showed decreases in net nitrogen mineralization with further nitrogen additions.

Two extant hypotheses could explain this decline in nitrogen mineralization rates. The first is that the addition of nitrogen randomizes chemical bond structures in soil organic matter, reducing efficiencies of extracellular catabolic enzymes and decreasing decay rates (e.g., Berg 1986). Thus, nitrogen applications result in higher nitrogen concentration in soil organic matter, which reduces decomposability and net nitrogen mineralization despite a narrower C:N ratio. The second hypothesis states that the production of humus-degrading enzymes by soil microbes (especially fungi) is suppressed in the presence of elevated concentrations of mineral nitrogen, thereby reducing nitrogen mineralization (Keyser et al. 1978, Fog 1988, Tien and Myer 1990). The relative importance of these two mechanisms under field conditions cannot be evaluated with available data, although the inhibition of enzyme production may be especially relevant where experimental manipulations result in very high rates of nitrogen addition.

We also predicted that chronic nitrogen additions would increase net nitrification, or induce it where previously absent, and that nitrate mobility and leaching losses would increase. Again, with one exception, this prediction was borne out. Both the Mt. Ascutney and regional transect data yielded a linear and highly significant relationship between forest-floor nitrogen concentration and the fraction of mineralized nitrogen nitrified (McNulty et al. 1996). This relationship is nearly identical to that derived from the NITREX data sets (Tietema and Beier 1995). The only experimental stand that showed neither a decrease in net nitrogen mineralization nor an increase in net nitrification was the Harvard Forest mixed hardwood site, the most nitrogen limited site as discussed below.

However, although increases in nitrate cycling and loss were detected in most stands, these increases were small relative to the additions, showing that nitrogen retention efficiency was relatively high in all sites. The NITREX study sites also showed rapid reductions in nitrate losses in stands to which nitrogen inputs were experimentally reduced, but small to no increases in nitrate losses with nitrogen additions (Bredemeier et al. 1998), suggesting efficient retention of added nitrogen in nitrogen-limited systems. In a broader survey of nitrate leaching in response to nitrogen deposition in over 100 stands in Europe, Dise and Wright (1995) showed that nitrate leaching losses average only approximately 30% of inorganic nitrogen deposition. Similar results have been compiled for North American sites by Peterjohn et al. (1996; see also Christ et al. 1995), although individual sites may approach input:output ratios of 1.0 (e.g., Johnson et al. 1991) or may greatly exceed this ratio in cases in which nitrogen fixation by root symbionts is a major pathway for nitrogen addition (Van Miegroet and Cole *1985).*

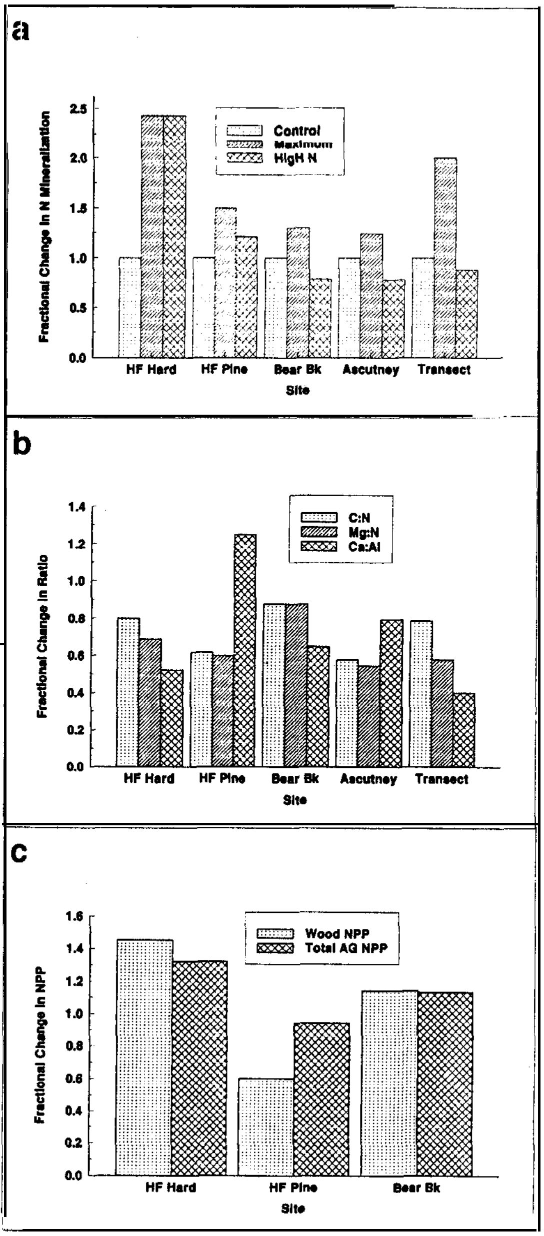


Figure 4. Summary of changes over time in several ecosystem processes in response to long-term, chronic nitrogen additions. (a) Fractional change in nitrogen mineralization ("Maximum" is the highest value obtained in the high nitrogen deposition stands before declining to the "High nitrogen" value at the end of the experiment or transect). (b) Element ratios in foliage in the highest nitrogen deposition treatments expressed as a fraction of control values (e.g., a value of 0.8 under C:N means that high nitrogen treatment foliar C:N ratios are 80% of control values). (c) Change in wood and total aboveground NPP in the highest nitrogen deposition treatment expressed as a fraction of control-site values. Data from Aber et al. (1995).

Foliar nitrogen. As we hypothesized, all sites showed increases in foliar nitrogen concentration, with the largest proportional changes occurring in the Harvard Forest pine stand and at the Mt. Ascutney site. These increases resulted in decreased Mg:N ratios in all sites (Figure 4b). Interestingly, foliar Ca:Al ratios also decreased in all but one site. The most probable explanation for these decreases is that increased nitrate mobility in soils increases cation leaching losses and soil acidification. These results are also consistent with those from a watershed-level experiment in North America (Gilliam et al. 1996) and from the NITREX experiments (Dise and Wright 1995, Gundersen et al, 1998). Because forest decline has been linked to Ca:Al imbalances (e.g., Shortle and Smith 1988), the finding that this condition can be induced by experimental increases in nitrogen availability alone may provide a unifying principle in forest-decline research,

Increased foliar nitrogen concentration can also affect ecosystem carbon balance through increased rates of net photosynthesis (Field and Mooney 1986, Reich et al. 1995). This increase can increase aboveground biomass production (the fertilizer effect) and may also provide additional carbon (and energy) to fuel belowground processes.

Net primary production. Although we had hypothesized, based on research in Europe (e.g., Schulze 1989), that nitrogen saturation could lead to forest decline, we did not expect to see any response of this type within the lifetime of the intensive experiments. However, both of the experimentally manipulated evergreen stands (Harvard Forest pine and Mt. Ascutney) and the regional survey have shown either declining tree growth (Figure 4c) or increased tree mortality (McNulty et al. 1996). This evidence of decline occurred despite the increases in foliar nitrogen, which might be expected to increase net photosynthesis (Reich et al. 1995). Increased mortality at Mt. Ascutney is significantly correlated with both nitrogen addition rate and foliar Ca:Al ratio (McNulty et al. 1996) and occurred within the first 6 years of fertilization. Declining pine growth rates at the Harvard Forest have not yet led to increased mortality, but surveys of mortality along

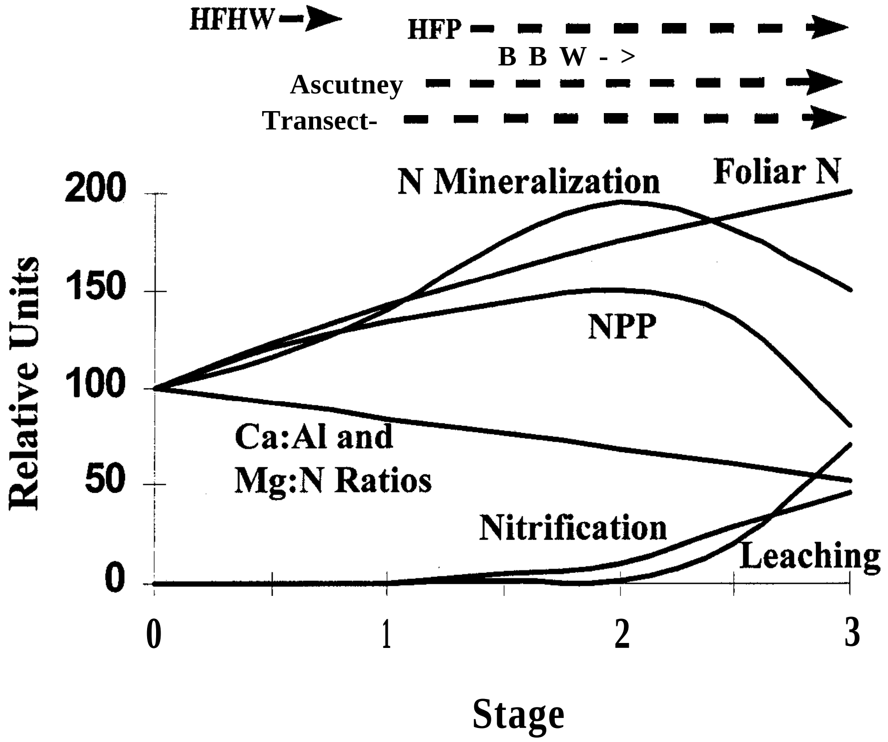


Figure 5. Revised set of hypotheses on the response of temperate forest ecosystems to long-term, chronic nitrogen additions. Changes from initial hypotheses (Figure 1) include the reduction in nitrogen mineralization in stage 3 and the addition of foliar Ca:Al and Mg:N ratios. Abbreviations at the top of the figure denote relative degree of nitrogen saturation in each of the study sites and the transect stands prior to increased deposition. The position of the arrowheads summarize how far toward saturation each site moved, as summarized by the data presented in this article. We hypothesize that previous land-use history determines the initial position and that deciduous stands move more slowly toward saturation than evergreen stands. HFP, Harvard Forest pine stand; HFHW, Harvard Forest hardwood; BBW, Bear Brook Watershed.

the spruce-fir transect (Rock et al. *1986*, McNulty et al. 1991) show consistent trends of increasing mortality with increasing nitrogen deposition. These findings suggest that the significant decline in high-elevation spruce-fir forests in the last three decades in western New England and eastern New York (Siccama et al. 1982, Hornbeck and Smith 1985) may have been due in part to increased nitrogen deposition. In addition, Boxman et al. (1998) report increased tree growth in two NITREX sites with high nitrogen cycling following the reduction of nitrogen inputs through undercanopy roof construction.

Fine roots. Because it is difficult to measure fine-root biomass, fewer data are available on responses of fine roots to nitrogen deposition. At both Harvard Forest stands, no changes in fine-root biomass were measured through year 4 of nitrogen additions, although root nitrogen concentration nearly doubled (Magi11 et al. 1997). In the NITREX studies, by contrast, fine-root biomass increased significantly following nitrogen exclusion from the site with the highest nitrogen availability (Boxman et al. 1998, Gundersen et al. 1998).

Hypotheses revisited

Information gained through nearly a decade of intensive research can be synthesized into a set of summary relationships that define the response of temperate-zone forests to accumulated nitrogen deposition (Figure 5). The principal differences between Figure 5 and our initial hypotheses are the downturn in nitrogen mineralization as saturation is approached and the addition of the foliar Mg:N and Ca:Al trends. This description of nitrogen saturation as a progressive "syndrome" of concurrent responses to long-term, chronic nitrogen deposition is in agreement with the use of a generalized indicator of "nitrogen status" derived from principle components analysis for the NITREX sites by Gundersen et al. (1998).

It is apparent that not all of the control or pretreatment stands fall on the same part of the x-axis in Figure 5. Measured values for net nitrogen mineralization, nitrification, nitrate leaching, foliar nitrogen concentration, and NPP all suggest that the Harvard Forest hardwood control stand is extremely nitrogen limited and that it has not moved very far toward nitrogen saturation. In contrast, the Bear Brooks hardwood control stand shows some nitrification and nitrate leaching, suggesting that this stand started at a different point along the x-axis. Initial differences between the Harvard Forest pine stand and the spruce-fir stands can also be expressed as different initial positions along this x-axis, with those locations being determined by measured rates of nitrogen cycling and loss.

Nor do all stands move toward saturation at the same rate. The arrows in Figure 5 suggest how far each site has moved toward saturation and possible decline during the treatment period. The deciduous stands did not move as rapidly toward saturation as did the evergreen stands. The Harvard Forest hardwood stand, which was the most nitrogen limited initially, received nitrogen in the amount of nearly 90 g/m’ over the first 6 years without the initiation of significant nitrification or nitrate leaching. In contrast, total nitrogen inputs of less than 15 g/m^2 were sufficient to induce significant decreases in foliar Mg:N and Ca:Al ratios and increases in tree mortality in spruce-fir forests on Mt. Ascutney. Despite the presence of some nitrification and nitrogen leaching in control plots, the Bear Brook watershed deciduous forest also exhibited only moderate increases in nitrate loss (Magi11 et al. 1996), whereas those in the red pine stand at the Harvard Forest increased dramatically. Across all experiments, growth declined or mortality increased in evergreen stands but not in deciduous stands.

Do these findings suggest that greater cumulative inputs of nitrogen are required to induce nitrogen saturation in deciduous forests? Do they further suggest that nitrogen saturation does not pose a problem for forest health in deciduous forests, or only that higher nitrogen retention rates will allow a delay in the onset of decline? Only the continuation of these long-term experiments will provide answers to these questions.

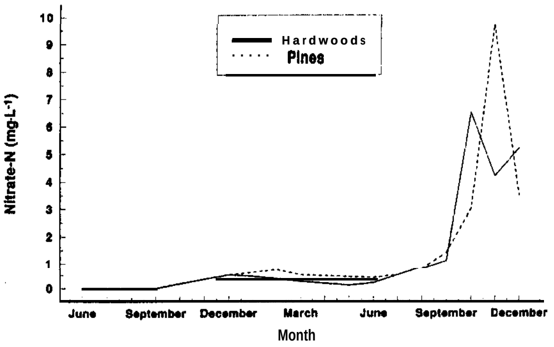


Figure 6. One-year change in nitrate concentration in soil solution collected below the rooting zone in trenched plots within a red pine plantation and a native hardwood stand at the Harvard Forest. In contrast to stands showing long-term retention of added nitrogen, stands with disrupted canopy–root connections begin to leach nitrogen within 1 year (from Aber et al. 1983).

Does land-use history determine ecosystem response?

Why do stands appear to start at different points within the nitrogen-saturation continuum? Before beginning our experiments, we thought that the Bear Brook Watershed hardwood stand in Maine would have a higher nitrogen retention capacity than the Harvard Forest hardwoods because of higher cumulative nitrogen deposition at the Harvard Forest site. The opposite is true. We now hypothesize (Aber and Driscolll997, Foster et al. 1997) that prior land-use history, reaching back even 100–200 years, can play a significant role in preconditioning forest response to nitrogen deposition. Essentially, the greater the previous extraction of nitrogen from a site by agricultural conversion, fires, or harvesting, the greater the nitrogen limitation on net photosynthesis and forest growth and the larger the amount of nitrogen deposition required to move toward saturation. Previous land-use history appears to be more important than either current or total accumulated nitrogen deposition in determining current nitrate leaching losses across the landscape in the northeastern United States. The same hypothesis can be applied to European sites, where forest-floor C:N ratio, reflective of both past land use and current nitrogen status, is significantly correlated with nitrogen leaching losses (Tietema and Beier 1995, Gundersen et al. 1998). In high-elevation sites in the southeastern United States, both the nitrogen deposition rate (which increases with elevation) and previous land use have been shown to affect nitrate concentrations in streams (Silsbee and Larson 1982, 1983, Nodvin et al. 1995).

This view implies that under undisturbed, mature, or steady-state conditions, all forests should be near the middle of stage 2 (Figure 5), where nitrogen losses are sufficient to balance nitrogen inputs in deposition (see discussion below). Stands to the left of this stage in Figure 5 represent disturbed conditions, in which the removal of nitrogen from the site has resulted in a slower nitrogen cycle. For example, the Harvard Forest hardwood site was part of a working farm for over a century, and although it was never plowed, it was adjacent to a plowed field and was undoubtedly harvested many times. There was also a ground fire through this stand in the 19.50s. In contrast, the Bear Brook stand is in a remote area of eastern Maine, and only one commercial harvest can be documented. It is thus closer to the “natural,” or stage 2, condition.

A paradox: Nitrogen retention in carbon-limited soils

From our initial literature review, we had hypothesized that adding nitrogen in the amount of 15 $g \cdot m^{-2} \cdot yr^{-1}$ to the Harvard Forest sites would move those systems rapidly to nitrogen saturation due to limitations on the increase in photosynthesis that could be achieved and would be needed to assimilate additional nitrogen (Figure 2a). This assumption was based in part on the results from an older trenching experiment conducted in similar stand types at the Harvard Forest, in which 1 x 2 m soil monoliths were isolated from aboveground plant processes by installation of a 1 m deep trench surrounding the plot. This experiment resulted in highly elevated nitrate concentrations in soil solutions within a year of treatment (Figure 6; Aber et al. 1983). Other stands examined in a comparative study showed nearly immediate nitrate losses (Vitousek et al. 1979). Trenching effectively cuts off plant uptake of mineral nitrogen. In a system that cycles nitrogen in the amount of 7.5–8.0 $g \cdot m^{-2} \cdot yr^{-1}$ (Magi11 et al. 1997), trenching would be expected to result in fairly large nitrogen losses from the system in a short period of time. We thought the 1 year delay was quite a long response time, and we expected the delay before the initiation of nitrate losses from the nitrogen amended plots to be similar, that is, 1-2 years.

Thus, the most striking and unexpected result from this set of studies was the very high nitrogen retention efficiency in all treatments over long periods of time (Table 1). Even the highest nitrogen addition plot in the pine stand at the Harvard Forest, in which nitrogen leaching losses rose dramatically after the first 2 years, still maintained an overall retention efficiency of 85% over the initial 6 years of treatment. The hardwood stand at the Harvard Forest retained nearly 90 g/m^2 of nitrogen in the first 6 years with no loss of nitrate and extremely low soil-extractable nitrate. This long-term net nitrogen accumulation is a major factor reducing the negative impacts of nitrogen deposition on forests and surface waters (e.g., nitrate and aluminum mobility, cation depletion, soil and water acidification, and forest decline; Schulze 1989, Herrick

Table 1. Mean annual inorganic nitrogen retention efficiency for several experimental plots at the Harvard Forest and Bear Brook Watershed.”

Location	Time period	Stand	Treatment	Nitrogen inputs (g/m^2)[b]	Nitrogen outputs (g/m^2)[b]	Retention efficiency[c]
Harvard forest	1988-1993	Pine	Control	4.8	0.2	.96
		Pine	Low nitrogen	32.4	0.6	.98
		Pine	High nitrogen	87.4	2.7	.85
		Hardwood	Control	4.8	0.1	.98
		Hardwood	Low nitrogen	32.4	0.6	.98
		Hardwood	High nitrogen	87.4	0.5	.99
Bear Brook	1988-1991	Hardwood	Control	2.0	0.1	.95
		Hardwood	Low nitrogen	11.9	0.3	.97
		Hardwood	High nitrogen	24.3	1.8	.93

“Data from Magill et al. (1996, 1997).

[b]Nitrogen inputs include both background deposition (0.8 $g \cdot m^{-2} \cdot yr^{-1}$) and fertilizer additions.

‘Retention efficiency calculated as (1– (nitrogen outputs/nitrogen inputs)).

and Friedland et al. 1991, Richter et al. 1992, Kahl et al. 1993, McNulty and Aber 1993, Boxman et al. 1995, Emmett et al. 1995).

The difference between the effects of trenching and nitrogen additions on nitrate losses suggests that severing the connection between plants and the root and soil systems, despite the potential creation of a sizable pool of decomposition substrate for nitrogen immobilization by microbes due to root death, causes a much larger and more immediate decrease in nitrogen retention efficiency than even very large additions of mineral nitrogen. This hypothesis in turn suggests that the presence of functional roots in the soil matrix may have an effect on nitrogen cycling and retention beyond the process of nitrogen uptake for plant use and that a functional root system, with associated mycorrhizal symbionts, may be more important than the free-living microbial community or soil detrital pools in determining nitrogen retention.

The high nitrogen retention rates reported here are not unusual. They are generally consistent with a large survey of European data (Dise and Wright 1995), in which average retention of dissolved inorganic nitrogen (DIN) was approximately 70%, and did not appear to decline significantly or consistently even at very high nitrogen deposition rates. Retention was somewhat higher in systems in which ammonium inputs dominated over nitrate. A smaller set of US data shows similar efficiencies (Peterjohn et al. 1996). Although a few sites have shown increases in DIN loss over long time periods (Murdoch and Stoddard 1991, 1993, Peterjohn et al. 1996) similar to those in the Harvard Forest pine stand (Magi!! et al. 1997), DIN losses do not generally approach input levels in the absence of forest decline or disturbance (but see Johnson et al. 1991).

DIN exports are not the only nitrogen losses from forested systems. Dissolved organic nitrogen (DON) can be the dominant form of nitrogen exported from systems in which DIN losses are low (e.g., McDowell and Asbury 1994, Hedinet al. 1995). At moderate rates of DIN deposition, inclusion of DON losses in stand nitrogen balances can reduce calculated nitrogen retention efficiencies significantly (Table 2). Because DON exports appear to cover a much narrower range than DIN losses and do not appear to increase significantly with high nitrogen addition rates (Table 2; Currie et al. 1996), total nitrogen retention efficiencies, including DON, may actually increase rather than decrease at higher levels of nitrogen addition (Table 2). Other pathways for ecosystem nitrogen loss, such as denitrification and ammonia volatilization, appear to be too small to be important in most sites with high nitrogen inputs (but see Tietema and Verstraten 1992).

What mechanism(s) might account for this very high rate of nitrogen retention? Studies on altered C:N ratios in forest-floor organic matter under coniferous forests (McNulty et al. 1991, McNulty and Aber 1993, Tietema and Beier 1995), on long-term distribution of added ^{15}N tracers (Buchman et al. 1995, Nadelhoffer et al. 1995, Koopmans et al. in press), on ecosystem-level nitrogen budgets (Aber et al. 1993, Magi!! et al. 1997), and on soil nitrogen partitioning (e.g., Matschonat and Matzner 1995) indicate that two-thirds or more of added nitrogen resides in soil organic matter. Moreover, most of this nitrogen did not pass through aboveground plant tissues (see also Johnson 1992). These results may at first appear to contradict our hypothesis that plant processes should both respond first to nitrogen deposition and control progress toward nitrogen saturation. However, only the final, long-term sink for nitrogen is in soils. We do not know the mechanisms (plant, microbial, or soil-chemical) through which this stabilization occurred. Strong correlations between nitrate mobility and foliar nitrogen concentration in several studies (Tietema and Beier 1995, Magi!! et al. 1997) suggest that plant nitrogen status and nitrogen processing might still be a critical part of the nitrogen saturation process.

The importance of soil organic matter as the final sink for added nitrogen caused us first to rethink our initial assumption that soil microbes are energy and carbon limited (Aber 1992). What if the soils at the Harvard Forest were so nitrogen depleted that microbes were limited by availability of nitrogen? If so, then there would have to be a pool of unused but bioavailable carbon in the soils that soil microbes used to immobilize the added nitrogen. The nitrogen additions should then result in a pulse of CO, from soils as this available carbon pool was tapped. We then hypothesized that dissolved organic carbon (DOC) might be this available soil pool, and should therefore be converted to CO, and de-

Table 2. Nitrogen retention efficiency in different Harvard Forest stands under different nitrogen treatments, with and without the inclusion of dissolved organic nitrogen (DON).[a]

	Pine			Hardwood		
Parameter	Control	Low nitrogen	High nitrogen	Control	Low nitrogen	High nitrogen
DIN deposition	0.8[b]	0.8	0.8	0.8	0.8	0.8
DON deposition	.06	.06	.06	.06	.06	.06
Nitrogen additions	0	5.0	15.0	0	5.0	15.0
DIN leaching	0.03	0.1	2.1	0.02	0.1	0.1
DON leaching	.54	.44	.36	.32	.46	.35
Without DON	0.8					
Total inputs	0.03	5.8	15.8	0.8	5.8	15.8
Total outputs		0.1	2.1	0.02	0.1	0.1
Nitrogen retention efficiency (%)	96	98	87	97	98	99
With DON						
Total inputs	.86	5.86	15.86	0.86	5.86	15.86
Total outputs	.57	0.54	2.46	0.34	0.56	0.36
Nitrogen retention efficiency (%)	34	91	85	60	90	98

[a] Data are for the period October 1992–September 1993 and come from the chronic nitrogen addition experiment at the Harvard Forest (Currie et al. 1996, Magill et al. 1997). All values are given in g·m^{-2}·yr^{-1}. Measured nitrogen trace gas fluxes were found to be near zero.

pleted by the microbial response to nitrogen additions (Figure 2b).

None of these occurred. Differences in soil CO$_2$ efflux across treatments were undetectable, although a simple calculation of the CO$_2$ production that should result from immobilization of added nitrogen through microbial biomass production suggested that fluxes should be very large and easily detected (Micks 1994). Similar results have been found in other studies (Roberge 1976, Foster et al. 1980, Flanagan and Van Cleve 1983, Soderstrom et al. 1983, Mattson 1995). In addition, DOC flux below the forest floor was not altered significantly, and DON fluxes increased only slightly through a shift in the DOC:DON ratio (Currie et al. 1996). There were also no significant reductions in the bioavailability of DOC in leachate from the forest floor (Yano et al. in press).

Possible mechanisms for nitrogen incorporation

Multiple sets of observations from our field experiments and those of others allow us to provide criteria that any process must meet to be considered an important mechanism in the retention of added nitrogen in the Harvard Forest sites. Such a process must:

- Incorporate nitrogen into soil organic matter without first incorporating it into aboveground plant biomass;
- Neither alter concentrations of DOC or the bioavailability of DOC in soil solution nor increase CO$_2$ efflux from soils (therefore, it must not draw on previously untapped pools of labile carbon in soils);
- Use only additional carbon made available through increased rates of net photosynthesis resulting from higher foliar nitrogen concentrations;
- Require the continued integrity of the plant-root-soil system.

Three classes of soil processes might account for the measured conversions of mineral nitrogen to soil organic nitrogen. In order from most to least conventional, these are:

- Immobilization by free-living microbes through biomass production;
- Abiotic incorporation into existing soil organic matter;
- Conversion to organic nitrogen forms by mycorrhizae, without biomass production.

We have calculated the amount of carbon required to immobilize the nitrogen added in the high nitrogen treatments at the Harvard Forest and the increase in soil CO$_2$ efflux that would result for each of these mechanisms (Table 3). These estimated fluxes can be compared with the estimated increase in net photosynthesis predicted to occur through increased foliar nitrogen concentration (reduced by carbon going to increased wood NPP; Table 4), and with measured changes in soil CO$_2$ efflux (i.e., undetectable; Micks 1994). The first comparison places an upper limit on the amount of additional carbon that might be available through detritus production or root exudation. The second comparison bounds the increase in microbial respiration that could result from an increase in biomass production.

Microbial immobilization. Microbial immobilization is frequently mentioned as the primary process for incorporation of mineral nitrogen into soil organic matter. Short-term ^{15}N pool dilution experiments have demonstrated that gross nitrogen mineralization and immobilization rates measured in soils may be many times higher than net rates, such that large quantities of nitrogen could be added to soil organic matter by this mechanism (Davidson et al. 1992, Hart et al. 1994). Stark and Hart (1997) proposed that gross fluxes of nitrate are also high in forest soils, even when no evidence for net nitrification, and no extractable nitrate, can be found. These authors hypothesized that rapid microbial immobilization could be the primary mechanism involved in the retention of added DIN. In contrast, Tietema (1998) found no evidence for gross nitrate immobilization in several forest soils of differing nitrogen availability.

If microbial immobilization is the primary process incorporating mineral nitrogen into soil organic mat-

Table 3. Calculated demand for carbon and production of CO, for the three hypothesized pathways for incorporation of the nitrogen (15 $g \cdot m^{-2} \cdot yr^{-1}$) added to the chronic nitrogen stands at the Harvard Forest.

Pathway	Nitrogen incorporation	X	C:N ratio of product’	X	Carbon-use efficiency	=	Total carbon requirement[b]	Total carbon respired’
Nitrogen incorporation through production by free-living microbes	15 $g \cdot m^{-2} \cdot yr^{-1}$		8		3 g C required per g C produced		360 $g \cdot m^{-2} \cdot yr^{-1}$	240 $g \cdot m^{-2} \cdot yr^{-1}$
Abiotic incorporation	15 $g \cdot m^{-2} \cdot yr^{-1}$		Not known		0 g C required per g C produced		0 $g \cdot m^{-2} \cdot yr^{-1}$	0 $g \cdot m^{-2} \cdot yr^{-1}$
Nitrogen incorporation by mycorrhizal assimilation	15 $g \cdot m^{-2} \cdot yr^{-1}$		6.25		1 g C required per 0.8 g C produced		117.2 $g \cdot m^{-2} \cdot yr^{-1}$	23.4 $g \cdot m^{-2} \cdot yr^{-1}$

[a] C:N ratio of product in the case of microbial production is the assumed C:N ratio of microbial biomass. For mycorrhizal assimilation, the C:N ratio of product is the C:N ratio in proteins-the assumed product-not biomass.

[b] Total carbon requirement is obtained by multiplying nitrogen incorporation by C:N ratio of product by carbon-use efficiency.

‘Respired carbon is the fraction of total carbon demand that is not incorporated into product (e.g., two-thirds of 360 $g \cdot m^{-2} \cdot yr^{-1}$ for microbial growth; 20% of 117.2 $g \cdot m^{-2} \cdot yr^{-1}$ for mycorrhizal assimilation).

ter, then what is the source of the labile carbon required to drive this process? Nitrogen immobilization through biomass production by free-living soil microbes carries high carbon requirements resulting from the C:N ratio in biomass produced and the relatively low efficiency of carbon utilization, which results from the need to degrade soil organic compounds to obtain carbon and energy. Microbial carbon-use efficiency is both very difficult to measure and varies widely between and within studies (e.g., Schimel 1988). Soil fungi (e.g., mycorrhizae) may be more efficient users of carbon than free-living prokaryotes (Zak et al. 1996), but this efficiency is offset by the relatively high C:N ratio of fungi as compared with bacteria (Tietema 1998). Low carbon-use efficiency has the additional effect of driving relatively large CO, fluxes from soils. If soil microbial activity under field conditions is energy or carbon limited, not nitrogen limited (e.g., Foster et al. 1980, Baath et al. 1981, Flanagan and Van Cleve 1983), then additions of DIN should not stimulate increased metabolic rates, nor should soils contain an untapped pool of labile carbon available to drive increased immobilization of added DIN. Thus, even if background rates of DIN cycling are high in untreated stands, an additional source of carbon would be required to immobilize the additional DIN received.

Our calculations of both carbon required and carbon respired for immobilization through microbial biomass production (Table 3) exceed estimated fluxes. Multiplying 15 g nitrogen added by a C:N ratio in microbial biomass of 8.0, and dividing by an assumed carbon-use efficiency of 33%, yields a total net increase in carbon demand of 360 $g \cdot m^{-2} \cdot yr^{-1}$. This calculated value greatly exceeds the estimated increases in net photosynthesis (less the increased carbon allocation to wood; Table 4). The 33% carbon-use efficiency means that two-thirds of the carbon used is respired, so that soil CO, efflux should increase by 240 $g \cdot m^{-2} \cdot yr^{-1}$. An increase of this magnitude at the Harvard Forest would have been detectable with the methods used, but no increase was measured there or in several other locations. Assuming that most soil microbial biomass is fungal rather than bacterial and applying Tietema's (1998) C:N ratios (25:1) and carbon-use efficiencies (60%) results in an even higher total carbon demand of 626 $g \cdot m^{-2} \cdot yr^{-1}$, with respiration about the same (2.50 $g \cdot m^{-2} \cdot yr^{-1}$).

These calculations indicate that there is neither enough of an increase in carbon fixation, nor enough of an increase in soil CO, efflux, to suggest that microbial immobilization through biomass production is the primary mechanism involved in the immobilization of added nitrogen at the Harvard Forest. Moreover, this process would also be highly dependent on existing soil detrital pools, and not affected by continuing carbon inputs from plants, and so is not in agreement with the comparison of trenched plot and nitrogen amendment results.

Abiotic incorporation of DIN into soil organic matter. Significant rates of direct chemical incorporation of ammonium into soil organic matter have been measured by mass balance using sterilized soil samples (Nommik 1970, Axelsson and Berg 1988, Schimel and Firestone 1989, Sen and Chalk 1995). Measured rates of direct chemical incorporation of NH,-N per kg of carbon range from 0.0036 mg/day (Nommik 1970) to 112 mg/day (Sen and Chalk 1995). It is difficult to interpret these results in terms of field nitrogen retention rates because the methods used (e.g., incubation times, ammonium concentrations, extraction procedures, and soil sterilization techniques) vary between studies. In particular, all methods of soil sterilization (i.e., gaseous fumigation, solutions of heavy metal salts, gamma irradiation, and autoclaving) may influence the incorporation of ammonium by alteration of the substrate. However, Foster et al. (1985a, 1985b) have shown that significant immobilization can occur through reactions between ammonia and organic matter in forest soils treated with urea, which creates both high pH and high ammonia concentrations-conditions that foster this reaction.

Abiotic incorporation of DIN into soils has also been estimated by the rapid (15-30 min) disappearance of ^{15}N tracer in pool dilution experiments (Davidson et al. 1991). This rapid incorporation was not strongly influenced by autoclaving of soil cores. Both the speed of immobilization and the insensitivity to autoclaving suggest that a biotic processes were involved.

The incorporation of mineral nitrogen into soil organic matter through chemical fixation reactions has been verified by ^{15}N NMR spectrometry. Laboratory studies have shown that ammonium (Thorn and Mikita 1992) and other nitrogen compounds, such as glycine (Benzing-Purdie et al. 1983), undergo reactions with soil organic matter that result mainly in heterocyclic forms of nitrogen (e.g., indoles and pyrroles) and some amides (Thorn and Mikita 1992). Biotic nitrogen incorporation in soils transforms nitrogen primarily into amides (Almendros et al. 1991, Knicker et al. 1993, Clinton et al. 1995).

However, ^{15}N NMR spectra from soil organic matter and compost residues in which biotic processes were not excluded suggest that direct chemical fixation explains 15% or less of the total nitrogen retention. Natural abundance spectra of soil organic matter (Knicker et al. 1993), spectra from labeled organic matter amendments (Almendros et al. 1991), and spectra from labeled mineral nitrogen amendments (Clinton et al. 1995) show that approximately 85 % of the nitrogen retained occurs in the amide form.

Abiotic nitrogen incorporation requires only the existence of suitable organic substrates and environmental conditions. It requires no additional carbon inputs and generates no additional CO_2 efflux (Table 3), so it is compatible with the carbon balance restrictions already discussed. However, estimates of nitrogen immobilization rates derived from both rapid short-term loss of ^{15}N tracers and from ^{15}N NMR spectrometry are too small to account for the magnitude of nitrogen retention observed in our experimental sites in northeastern North America and throughout the NITREX sites in Europe.

Table 4. Estimated net increase in carbon available to drive nitrogen immobilization.[a]

Control	Pine (low nitrogen)	Pine (high nitrogen)	Deciduous (low nitrogen)	Deciduous (high nitrogen)
0	99	249[b]	49	75

[a] Net increase in carbon availability is calculated as the estimated increase in total net photosynthesis due to increased foliar nitrogen concentration (from a model of canopy photosynthesis validated at the Harvard Forest, PnET-Day [Aber et al. 1996]) minus carbon allocated to increased aboveground production. Values in $g \cdot m^{-2} \cdot yr^{-1}$.

[b] This number may be too high. Actual growth rates of trees have declined in this stand, as have both Mg:N and Ca:Al ratios in foliage, suggesting that net photosynthesis may be declining.

Mycorrhizal assimilation. A third, and less frequently discussed process for conversion of DIN to soil organic nitrogen is through mycorrhizal assimilation and exudation. Mycorrhizae are ubiquitous in forest soils (Fahey and Hughes 1994) and can constitute a large fraction of the total fungal biomass, which, in turn, can be a large fraction of the total microbial biomass (Killham 1994). This fraction may be especially large under nitrogen-limiting conditions and may decline with nitrogen saturation (Tietema 1998). 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 (Rygiewicz et al. 1984, Yanai 1994). Still, there is some uncertainty about which partner, the plant or the fungus, primarily controls this interaction (Rygiewicz and Andersen 1994).

If mycorrhizae were to assimilate mineral nitrogen, convert the nitrogen into extracellular enzymes, and exude these enzymes back into the soil, where they could be stabilized by well-known condensation reactions between proteins and humic compounds, the estimated carbon requirements and CO_2 production would be much lower than for free-living microbial immobilization (Table 3) and well within the range of estimated increases in net carbon availability (Table 4). Mycorrhizal nitrogen assimilation reduces the estimated carbon cost of nitrogen incorporation by using photosynthate carbon directly rather than requiring that the carbon be first converted to biomass by plants and then shed as litter and decomposed by soil microbes. The respirational efficiency of conversion of photosynthate to structural polymers is estimated to be approximately 80% (growth respiration equals 25% of total carbon required for growth; Penning de Vries etal. 1974,SprugelandBenecke 1991), in contrast to the 33% efficiency we have used for free-living microbial immobilization. Thus, carbon requirements are one-third as large and CO_2 effluxes one-tenth as large for the hypothesized mycorrhizal pathway (Table 3), in comparison with microbial biomass production.

Because of the direct reliance of mycorrhizae on continued allocation of photosynthate from crowns to roots, this mechanism would be the most sensitive of the three to disruption of the plant-root-soil linkage. If rapid uptake by mycorrhizae is responsiblefor the high rates of gross nitrogen immobilization in soils measured by ^{15}N pool dilution, then the question of the carbon source to drive this process may also be answered—it is plant photosynthate and not detrital or soil organic carbon.

A new hypothesis

Several sets of studies described in this article have established boundary conditions on the mechanism by which the majority of nitrogen added to forests in nitrogen addition experiments becomes incorporated into soil organic matter. Of the three general classes of processes discussed in this article, only the third—mycorrhizal assimilation and exudation, using photosynthate from the host plant as the carbon source-fits within the limits set by field observations. We hypothesize (Figure 2c) that mycorrhizal assimilation and exudation is the dominant process involved in immobilization of added nitrogen. Tietema (1998) has suggested that during nitrogen saturation, soil microbial communities move from being fungal, and probably mycorrhizal, dominated to being bacterial dominated. This loss of mycorrhizal function could be a key process leading to increased nitrification and nitrate mobility.

Unfortunately, no methods are currently available to determine the assimilation-exudation balances of mycorrhizae. Still, the hypothesis can be tested inferentially. We predict that severing the connections between plants and mycorrhizal roots, through a simple set of trenched plot experiments, will lead to immediate reductions in the rate of gross nitrogen immobilization (as measured by ^{15}N pool dilution) and to increased nitrate mobility. If our hypothesis is correct, gross immobilization of ^{15}N would decrease within days to weeks of trenching as the carbon source fueling immobilization, plant photosynthate, is excluded. If our hypothesis is incorrect, then the pulse of detrital material created by root death as a result of trenching should lead, also in days to weeks, to increased gross nitrogen immobilization.

At the beginning of this article, we set up a dichotomy between plant and microbial control over the retention of nitrogen added to forest ecosystems and the timing of nitrogen saturation. Our new hypothesis suggests that this dichotomy was an oversimplification of the problem, and recognizes the fundamental importance of mycorrhizal symbioses in mediating the carbon and nitrogen cycles of temperate forest ecosystems. Although the processes involved are microbial, and would be measured as such by ^{15}N pool dilution methods, gross rates of nitrogen cycling in soils are driven by, and tied directly to, current rates of plant photosynthesis. This view reconciles the high rates of gross nitrogen immobilization reported for many systems with the strongly carbon and energy limited nature of the soil ecosystem. One of the key implications of this hypothesis is that increased nitrate mobility requires "saturation" of plant nitrogen demand. Consequently, accurately determining plant nitrogen status-for example, by foliar analysis-could provide the best indicator of ecosystem nitrogen status and degree of nitrogen saturation.

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