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Nitrogen Cycling and Nitrogen Saturation in Temperate Forest Ecosystems

John D. Aber

The last decade has seen a dramatic shift in the focus of nitrogen cycling research in forest ecosystems. Concerns over nitrogen deficiencies and effects of removal in harvest have given way to concerns over excess nitrogen availability and the potential for forest decline and surface water pollution. Driving this paradigm shift is the increase in atmospheric deposition of nitrogen to forests due to industrial and agricultural activity. At the core of the new paradigm is the concept of 'nitrogen saturation' of forest ecosystems. The purpose of this review is to synthesize recent advances in research relating to nitrogen deposition effects on temperate zone forest ecosystems, and the further effects of nitrogen saturation on environmental quality.

The concept of nitrogen saturation was apparently unknown before 1981, when Ingestad *et al.*[1] presented a very simple model of N nutrition based on the nutrient flux density and N productivity concepts. This model was used to predict when N additions would be sufficient to remove all N limitations on forest growth under typical conditions in Sweden[2]. Simultaneously, van Breemen *et al.*[3] reported extreme soil acidification and increased aluminium mobility due to very high levels of ammonium (NH_4^+) deposition in the Netherlands. Nihlgard[4] brought together data from both soils and plant research suggesting that excess N availability was a serious concern for both forest decline and soil and stream-water acidification. His 'ammonium hypothesis' marks the beginning of the focused discussion of the potential environmental dangers of excessive N deposition.

Several definitions of N saturation have been proposed[5,6]. Some are based on the absence of a growth response from the vegetation[7], and some on either the initiation of nitrate leaching[8], or on an equivalence between N losses and N inputs (no further N retention[9]). All of these definitions describe stages of declining ability of an ecosystem to retain added N. Processes that contribute to N retention include plant uptake and accumulation, microbial uptake and incorporation into soil organic matter, and physical–chemical processes such as cation exchange and abiotic incorporation of mineral N into soil organic matter. We need not think of N saturation as occurring at a specific point in time, but as a set of changes in critical ecosystem processes which represent the integrated response of a system to increased N availability[6,8].

Implications of N saturation

Nitrogen saturation has at least three serious environmental impacts: (1) on soil chemistry and water quality, (2) on forest composition and productivity, and (3) on fluxes of radiatively active (or 'greenhouse') gases.

Negative impacts on water quality derive from the induction of nitrification in acid soils. Net nitrification (the conversion of NH_4^+ to NO_3^-) is generally very low to nonexistent in acid forest soils in the absence of elevated N deposition or N-fixing species[10]. However, nitrification can occur at low pH in the presence of elevated NH_4^+ concentrations, and is now common in heavily affected European and North American sites[11–13]. Nitrification appears to increase with increasing cumulative N deposition and storage in soils[14–16].

As an anion, nitrate is very mobile in temperate zone soils and tends to leach rapidly in the absence of plant uptake. By charge balance, this results in cation removal as well, with the nutrient cations tending to be drawn down first, followed by an increase in the concentration and mobility of hydrogen ions and inorganic aluminium[12,17,18]. Increases in nitrate concentration in surface waters have been reported for Scandinavia (Refs 19, 20 and see Ref. 21), and correlate with increased deposition in central Europe[22]. Increasing nitrate concentrations in

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stream water have recently been reported for the Catskill and Adirondack regions in New York state, USA[23,24]. Episodic increases in aluminium in stream water during snow melt, which are particularly critical for fish populations, correlate strongly with increased nitrate concentrations during this period[25,26].

Increased N deposition to N-limited forests will result initially in increased forest growth[27,28]. However, excessive inputs can result in severe imbalances in element concentrations in foliage (particularly N:Mg ratios[29]), causing chlorosis (yellowing) of foliage, premature needle drop, and declining tree vigor and even mortality[30]. Imbalances can be accentuated by soil leaching, which reduces Mg concentrations in soils and increases Al, which in turn can reduce the uptake of Mg^{2+} and other cations by roots[18,29]. Excess N content in foliage has also been implicated in forest decline through loss of frost-hardiness and winter desiccation[31,32].

Interactions between N saturation and trace gas fluxes are just beginning to emerge. Tietema *et al.*[33] report losses of 20 kg N ha^{-1} yr^{-1} as nitrous oxide (N_2O) from a heavily affected Dutch forest with a fluctuating water table. This compares with near-zero flux rates from N-limited pine and mixed deciduous forests in the northeast US[34]. Steudler *et al.*[35] report significant decreases in methane (CH_4) consumption following N additions to two N-limited forests, apparently due to the inability of the dominant enzyme systems to distinguish between CH_4 and NH_4^+. Both increased N_2O production and decreased CH_4 consumption would tend to increase atmospheric concentrations of these important greenhouse gases.

Mechanisms of N retention in forest ecosystems

It is certain that N deposition has increased dramatically over eastern North America and much of Europe[22,36,37]. Yet there has been a clear lag between increases in N deposition and increased losses of N from forests, either as nitrate[22,26] (Fig. 1) or in gaseous forms[34]. This implies that most temperate forest ecosystems have a significant capacity to assimilate and retain added N[17,38]. Defining the kinetics and capacity of the processes responsible for retention of added N is the major challenge in determining 'critical loads' of N deposition for forest ecosystems.

A simplified diagram of the N cycle within forest ecosystems (Fig. 2) emphasizes the critical nature of the pool sizes and transformations of ammonium and nitrate. Nitrogen retention requires effective conversion of mineral N to organic form, and to an organic form that will reside within the system for an extended period.

Plants, heterotrophic microbes and nitrifiers all compete for the available pool of ammonium. In addition, ammonium is held relatively strongly on cation exchange sites, and can also be chemically immobilized directly into soil organic matter[39,40], so that biotic sinks must also compete with chemical sinks[41,42]. The relative strength of these alternate sinks for N – along with the gross N mineralization rate – determines the degree of N limitation on biological function, and the residual concentration of inorganic N in the soil solution and in leachate.

There have been very few studies that directly measure the relative strength of alternate sinks for N within intact forest ecosystems. In an excellent review, Johnson[42] identifies three levels of N availability, and discusses the relative competitiveness of plants and microbes at each level. (1) The first level is a 'control' situation where N deposition is low. Under these conditions, plants actually 'mine' soil N, acquiring an amount greater than atmospheric N inputs, and thus competing effectively with decomposers for mineral N. (2) In chronic, low-level additions typical of N deposition studies, the main response is the incorporation of N into soil organic matter, or the stimulation of nitrification; this suggests that the microbial community competes very effectively for any additional N entering the system (retention in soil organic matter can be as high as 95%[38], even with additions of up to 150 kg N ha^{-1} yr^{-1}). (3) In large, pulse N additions typical of forest fertilization experiments, a much larger proportion of the added N is taken up by trees (up to 40%[43]), with an accompanying increase in growth; this suggests that high or discontinuous rates of addition swamp the potential for microbial N immobilization and facilitate increased uptake by plants. Abiological incorporation of N into soils should also be favored by large, pulse additions.

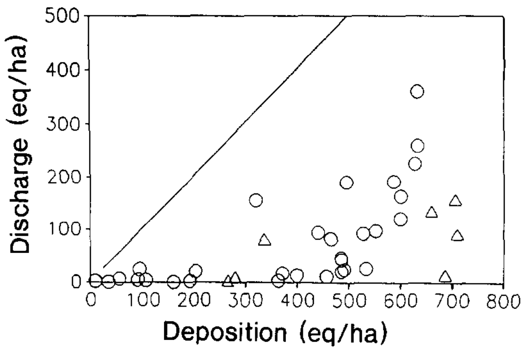

Fig. 1. Relationship between measured wet deposition N inputs and stream-water outputs for several sites in North America (circles are US sites, triangles are Canadian sites). The line represents discharge equal to deposition. *From Ref. 26.*

To the extent that immobilization of added N is microbial and not abiotic, these results suggest that there is an unmet demand for N within the microbial community in most forest soils. The need for reduced carbon compounds to drive

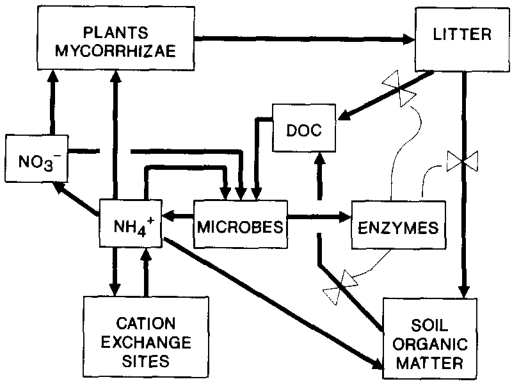

Fig. 2. A generalized N cycle in forest ecosystems. Plants and associated mycorrhizae compete with free-living heterotrophic microbes and nitrifiers for the pool of available ammonium. Net production of either nitrate or ammonium will be the balance between gross production and gross immobilization. Biotic sinks for ammonium compete with chemical processes such as cation exchange and chemical incorporation of inorganic N into soil organic matter. Biotic immobilization by free-living microbes requires the availability of labile carbon substrates (microbially available DOC) produced by decomposition of plant litter, including root/mycorrhizal material. Heterotrophic microbes produce extracellular enzymes that cleave long-chain, plant-derived polymers (such as cellulose and lignin) into smaller molecules, which can be taken up and metabolized. These also play a role in the conversion of litter to soil organic matter.

Table 1. Characteristics of N-limited and N-saturated forest ecosystems

Characteristic	N-limited	N-saturated
Form of N cycled (net, as plant uptake)	100% NH_4^+	25–50% NO_3^-, 50–75% NH_4^+
Soil DOC concentration	High	Low
Ratio of gross NO_3^- immobilization to gross nitrification	Near 100%	Near 0%
Ratio of gross NH_4^+ immobilization to gross mineralization	High (90–95%)	Low (50%?)
Fraction of soil fungi that are mycorrhizal	High	Low
Nitrate loss during snow melt	Low	High
Nitrate loss at base flow	Zero	High
Foliar lignin concentration	High	Low
Foliar N concentration	Low	High
Foliar free amino acid (e.g. arginine) concentration	Zero	High
Soil C:N ratio	High	Low
N_2O production	Zero	High
CH_4 production	High	Low (zero?)

microbial growth and N immobilization implies that there is also an excess of available carbon in soils that can be tapped when N additions occur. To date, studies of dissolved organic carbon (DOC) in forest soils have been few and have dealt mainly with concentrations, budgets and effects on soil and stream-water chemistry[44,45]. Further research on the production and utilization of microbially available carbon within the soil–litter system may improve our understanding of N immobilization considerably, and may provide a sensitive indicator of the degree of N saturation.

The fact that pulse additions can swamp microbial demand and increase plant uptake suggests that the kinetics of microbial response to N additions is another important characteristic. The timing of the additions of N and the state of the microbial community, including soil reserves of available carbon, may be critical to the pulse acidification processes that occur in certain systems.

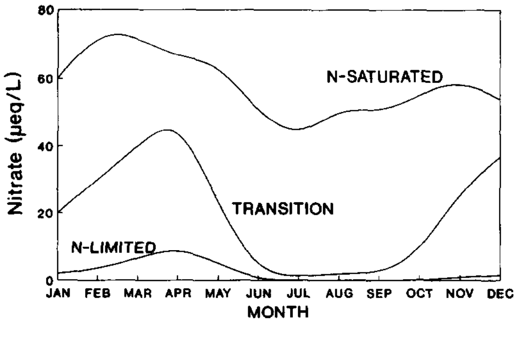

Fig. 3. Generalized changes in seasonal trends in nitrate concentration in stream water accompanying the transition from N-limited to N-saturated forest ecosystems[22,50].

Total long-term storage capacity for added N is apparently much larger in soils than in plants. For example, data from coniferous forests in the northeastern US suggest that soil N storage has increased by nearly 1000 kg ha^{-1} as a result of elevated N deposition[14]. This is equivalent to 30–40 years accumulated N deposition at current rates, or nearly all of the cumulative anthropogenic N loading. Estimated retention within plants is one to two orders of magnitude lower. This difference is due to the larger total amount of organic matter in soils versus plants, and a much larger shift in C:N ratio in soils than in plant biomass components.

An understanding of mechanisms of competition between plants and microbes is critical for the accurate prediction of the timing of N saturation, and is as yet poorly developed. Recent applications of ^{15}N pool-dilution methods to native ecosystem soils have revealed that the internal gross cycling of ammonium and nitrate in N-limited systems is much faster, perhaps as much as 10–20 times faster, than net cycling rates measured by longer-term soil incubations or as plant uptake[46,47]. This finding is critical in that it helps to explain the rapid disappearance of inorganic N added to soils[38]. Results from N-saturated forests suggest that this ratio of gross to net mineralization becomes much smaller in N-saturated systems (perhaps approaching 2:1; Ref. 48). For longer-term N retention, however, immobilized N must be converted to more stable forms of soil organic matter. Very little is known about the rates or mechanisms of this conversion.

Large amounts of labile carbon are required to drive the gross immobilization process – more than is generally provided through plant litter production. This suggests that other sources of plant-derived reduced carbon, perhaps either root exudation or rapid turnover of mycorrhizal hyphae, are important to the N immobilization process.

Integrated effect of N deposition on N retention mechanisms

In an earlier paper[8] we described a generalized set of integrated responses of N-limited forest ecosystems to chronic N additions. More recent research summarized here makes it now possible to add several additional qualitative, but process-based, distinctions between N-limited and N-saturated forest ecosystems. These are presented here as two end-points (Table 1), with the understanding that movement from one end-point to the other will be continuous.

In strongly N-limited systems, the majority of net N cycling (through plant uptake) is as ammonium. This is because gross nitrification is reduced by the combined competitive demands of plant uptake and gross immobilization by decomposers, and because gross immobilization of nitrate is nearly equivalent to gross nitrification (near-zero net nitrification). High rates of gross immobilization are driven by relatively large pools of labile DOC produced by the decomposition of N-poor plant litter and/or by root exudation and turnover of mycorrhizal hyphae. The decomposition of this material produces low C:N soil organic matter. Plant roots are heavily mycorrhizal and mycorrhizal fungi dominate the soil fungal community[49]. All of this results in low to zero rates of nitrate leaching and N_2O emissions, and high consumption of CH_4.

Chronic N additions lead to increased uptake by both plants/mycorrhizae and free-living microbes. If gross N immobilization is as much as 20 times net plant uptake, most of the added N will cycle first through the microbial pool and effective availability to plants will increase only slightly[42]. As chronic N additions continue, there should be both a decrease in the production of available DOC (as N concentrations in plant litter increase and internal plant carbon pools decline through higher biomass production) and increased utilization of existing pools of DOC (N limitation on carbon metabolism reduced). As combined plant and microbial demand for inorganic N is increasingly met by N additions and gross mineralization, gross nitrate immobilization should be reduced (inhibited by increased NH_4^+ concentrations due to the high energy requirement for nitrate reduction[40,48]), and gross nitrate production should increase (induced by increasing NH_4^+ concentrations). This will result in increased net nitrification, increased nitrate accumulation in soils, and increased nitrate leaching. The ratio of gross to net mineralization and nitrification should narrow. Increased availability of ammonium and nitrate in soils will lead to higher rates of N_2O loss and reduce CH_4 consumption.

Increased plant uptake of N will result in higher N concentrations in foliage, and perhaps reduced lignin concentration[14], reflecting reduced excess carbon availability. The accumulation of arginine in foliage may be a sensitive indicator of the initiation of luxury consumption of N (uptake in excess of physiological requirements)[17]. Higher foliar N will result in plant litter with a narrower C:N ratio, the decomposition of which will produce soil organic matter with a narrower C:N ratio as well.

Movement toward saturation will also affect the seasonal patterns of N availability and loss. Stoddard[50] has outlined a sequence of changes in stream nitrate concentrations (Fig. 3) that would accompany progressive stages of N saturation, using three stages described by Aber *et al.*[8] A stage 0 or N-limited system would show near-zero nitrate concentrations at all times, with the exception of snow melt where overland flow minimizes contact between soils and water. In stage 1, where N limitations are being alleviated, nitrate losses occur consistently outside the growing season and during storm events within the growing season, but are near zero at base flow. In stage 2, where saturation has occurred, nitrate concentrations are elevated at all times.

Prediction of time to N saturation

Predicting the timing of N saturation in relation to rates of N deposition is crucial for the establishment of critical loads of N to sensitive systems. However, although conceptual and numerical models have been constructed that outline processes and indicators of the approach to saturation (Table 1)[6,8,51], quantitative predictions remain elusive. This is due directly to a lack of quantitative understanding of the state of soils in most systems relative to maximum retention capacity, and a similar lack in the understanding of competition between plants/mycorrhizae and microbes for inorganic N. Considerable additional research on the effects of different levels of chronic N additions will be required to fill these gaps in our understanding. The demands of a signed protocol[52] on emissions of NO_x lend urgency to this research.

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Echinoderm Phylogeny: Morphology and Molecules Approach Accord

Andrew B. Smith

Phylogenetic relationships of echinoderms at various taxonomic levels have come under intense focus recently from both a morphological and a molecular standpoint. Initial conflict between molecular and morphological results is gradually being resolved to produce a consensus that places echinoderm phylogeny on a new robust footing.

Echinoderms are both abundant and morphologically diverse in today's oceans. Their complex calcite skeleton confers two advantages for biologists. Firstly, it has given them a reasonably good fossil record stretching back some 560 million years; secondly, there is little difficulty in integrating living and fossil taxa, since the skeleton provides the main suite of characters on which the living groups are classified.

Recently there has been renewed interest in echinoderm phylogeny, aimed at establishing relationships both within and amongst the five classes: Crinoidea, Asteroidea, Ophiuroidea, Echinoidea and Holothuroidea. This began in the early 1980s when cladistic methodology was first used to investigate echinoderm relationships, but in the past few years molecular data have become increasingly important. Three principal techniques have been used to obtain molecular data for constructing phylogenetic trees: DNA–DNA hybridization, analysis of gene order within the mitochondrial genome, and sequencing of ribosomal RNA genes (Box 1).

During the initial stages, when molecular data first became widely available, the results from morphological and molecular phylogenies were disappointingly at variance. But as this field has developed, better and better accord has been achieved, as morphological criteria are re-examined and methods of constructing and rooting molecular trees are improved. The reciprocal illumination gained by comparing morphological and molecular phylogenies is helping to highlight problem areas where future work needs to be concentrated.

When molecular and morphological phylogenies conflict

Echinoids have by far the best fossil record of any echinoderm class and consequently have been investigated more thoroughly than any other. Broad phylogenetic relationships are reasonably well established based on morphology[1,2]; recent work has concentrated on better resolution within specific groups, such as holasteroids[3], clypeasteroids[4] and cidaroids[5]. Although echinoids separated from other echinoderm classes in the Ordovician, it is clear that all living species in this class form a monophyletic group which originated around the end of the Palaeozoic, some 260 million years ago. There is a good fossil record of the group, documenting the step-by-step divergence of today's major groups during the Mesozoic and Tertiary. Palaeontologists and morphologists therefore felt that echinoid phylogenetic relationships were on a relatively secure footing.

Yet when molecular phylogenies began to appear, some results were markedly at variance with the expectations. It is these areas that have attracted much attention and have led to reappraisal of one or other data set.

DNA–DNA *hybridization and echinoid phylogenies*

DNA–DNA hybridization is a technique pioneered by Britten and Kohne[6] and is highly suitable for studying taxa that have separated within the past 50 million years or so. When the technique was first developed and applied to echinoids, molecular biologists naturally chose to work with those species that were the easiest to obtain, namely, coastal echinoids belonging to the group Camarodonta. But unfortunately, this group has one of the poorest fossil records, mainly because its members live mainly in rocky shore environments where the chances of preservation are poorest[2,7]. Consequently, phylogenetic relationships had been neglected and divergence times for this group, estimated from the fossil record, varied enormously. Prompted by the increasing availability of DNA–DNA hybridization data for camarodonts, their morphology and their fossil record were reviewed, with the earliest records of clades being used to construct and calibrate an evolutionary tree[8,9]. In this case morphological and molecular data were not in conflict and the development of molecular information prompted morphological re-evaluation.

Work on the phylogeny of sand-dollars (Scutellina, Clypeasteroida) produced less concord-

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