Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems

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Abstract. By synthesizing more than twenty years of research at the Fernow Experimental Forest, we have documented 7 symptoms of nitrogen saturation in two adjacent watersheds. The symptoms include: 1) high relative rates of net nitrification, 2) long-term increases in stream-water concentrations of nitrate and base cations, 3) relatively high nitrate concentrations in solution losses, 4) little seasonal variability in stream-water nitrate concentrations, 5) a high discharge of nitrate from a young aggrading forest, 6) a rapid increase in nitrate loss following fertilization of a young aggrading forest, and 7) low retention of inorganic nitrogen when compared with other forested sites. These data support current conceptual models of nitrogen saturation and provide a strong, and perhaps the best, example of nitrogen saturation in the United States.

Introduction

Ecologists have come to realize that there is more to acid rain than acidity. Whereas early studies often focused on the effects of hydronium ions (Likens et al. 1976 & 1979; Driscoll & Likens 1982; Schindler et al. 1985), we are now increasingly concerned about the detrimental effects of the other components of acid rain – sulfur and nitrogen. In particular, research during the last decade has investigated the effects of chronic nitrogen deposition (Nihlgård 1985; van Breeman & van Dijk 1988; Tietema et al. 1993; Aber et al. 1993; Christ et al. 1995) and has led to the concept of nitrogen saturation (År upper & Bosatta 1988; Aber et al. 1989).

Nitrogen saturation has been defined as the availability of inorganic nitrogen in excess of biotic demand (Aber et al. 1989; Aber 1992) and is best viewed as a continuum of changes that reduces the capacity of an ecosystem to retain nitrogen. Concern over nitrogen saturation arises because, once the capacity of an ecosystem to retain nitrogen is exceeded, the excess nitrogen may stress the biosphere by degrading water quality, altering the composition
of the atmosphere, and by contributing to forest decline (Aber et al. 1989). Furthermore, the current concern over nitrogen saturation is not likely to diminish in the near future. Economically important human activities have substantially elevated nitrogen deposition above background levels (Galloway et al. 1984) and these levels are predicted to increase by 25% in more-developed countries by the year 2020 (Galloway et al. 1994 & 1995).

Many measurements have been proposed as indicator symptoms of nitrogen saturation. These include high relative rates of net nitrification, elevated concentrations of nitrate in stream water, little seasonal variability in streamwater nitrate concentrations, a rapid increase in nitrogen discharge following fertilization, a close balance between the inputs and outputs of inorganic nitrogen, high rates of nitrous oxide production, and reduced rates of methane consumption (Aber et al. 1989; Aber 1992; Stoddard 1994). Several of these symptoms have been well documented for forests in Europe that receive exceptionally high rates of nitrogen deposition (van Breeman et al 1982; Nihlgård 1985; Schultze 1989; Haugs et al. 1989). However, fewer symptoms have been reported for forests in the United States, despite the fact that elevated nitrogen deposition has occurred for several decades. In the United States, elevated nitrogen deposition is concentrated in eastern portion of the country with some of the highest depositions occurring in Ohio, Pennsylvania, New York, western Maryland, and northern West Virginia.

The purpose of this paper is to document a strong, and perhaps the best, example of nitrogen saturation in the United States by synthesizing existing data from more than twenty years of research at the Fernow Experimental Forest near Parsons, West Virginia (39°3'15" N, 79°41'15" W).

Methods

Study sites

The study sites consist of two adjacent watersheds – Watersheds 3 and 4. Watershed 3 is 34.3 ha in size with an average slope of 27% and a southerly aspect. Trees greater than 12.7 cm in diameter at breast height (dbh) were cut in 1958–1959 and again in 1963. Between July 1969 and May 1970, Watershed 3 was clear cut to 2.5 cm dbh except for a 2.99-ha riparian buffer strip. The buffer strip was clear cut in November of 1972. After each cut, the vegetation regenerated naturally. The dominant tree species growing on this watershed are Prunus serotina, Acer rubrum, Betula lenta, and Fagus grandifolia. Aerial applications of fertilizer on Watershed 3 were begun in 1989. Since then, granular ammonium sulfate has been applied three times each year: 7.1 kg N ha\(^{-1}\) in March, 7.1 kg N ha\(^{-1}\) in November, and 21.3
kg N ha\(^{-1}\) in July. The total amount of nitrogen added each year (35.5 kg N ha\(^{-1}\)) is approximately twice the amount of nitrogen received as throughfall in a nearby undisturbed forest.

Watershed 4 consists of 38.7 ha with an average slope of 20% and a east-southeasterly aspect. The vegetation on this watershed was heavily cut in 1905. The only disturbance since that time has been the removal of dead American chestnut trees \((\text{Castanea dentata})\) in the 1940s. The dominant tree species growing on this watershed are \(Acer saccharum\), \(Acer rubrum\), \(Fagus grandifolia\), and \(Quercus rubra\).

The dominant soil type in both watersheds is Calvin channery silt loam (loamy-skeletal, mixed, mesic Typic Dystrochrept) that is derived from acidic sandstone and shale of the Hampshire formation. The average pH\(_{\text{KCl}}\) in the upper soils of both watersheds is about 3.7 (Gilliam et al. 1996). The soils are shallow and the depth to bedrock is about 1 m.

Precipitation, streamflow, air temperature and relative humidity have been measured routinely since 1951 (Adams et al. 1994) and the climate experienced by both watersheds is similar. Mean monthly air temperatures range from about −2 °C in January to about 20 °C in July. The average annual precipitation and streamflow for Watershed 3 are 148 and 66.6 cm, respectively. Annual precipitation and streamflow for Watershed 4 averages 146 and 64.0 cm. Whereas precipitation is uniformly distributed throughout the year, streamflows exhibit strong seasonal changes with the greatest flows occurring between November and April.

Watersheds at the Fernow Experimental Forest receive relatively high inputs of inorganic nitrogen in rainfall. Between 1982 and 1993, we estimate that these watersheds received an average of 6.7 kg N ha\(^{-1}\) yr\(^{-1}\) in wetfall. This amount is greater than 93% of the observations reported during the same period for 197 sites in the contiguous United States and Canada (National Atmospheric Deposition Program 1993).

**Precipitation and streamflow**

The amount of precipitation is measured weekly using standard rain gauges (20 cm in diameter) located in clearings on both watersheds. A weight-recording rain gauge also measures rainfall on Watershed 4. In July of 1978, a site was established for monitoring precipitation chemistry. This site is located approximately 3 km north of the Fernow Experimental Forest and is part of the National Atmospheric Deposition Program (NADP site WV18). Wetfall samples at this site are collected with an Aerochem-Metrics Model 301 wet/dry collector. Nitrogen inputs were estimated by multiplying the amount of precipitation at each site by the concentration of inorganic N measured in rainfall at NADP site WV18.
Streamflow from both watersheds is measured using 120° sharp-crested V-notch weirs and FW-1 water-level recorders. Grab samples of stream water for chemical analysis have been collected at weekly or biweekly intervals since 1969. Samples are collected at permanently marked locations upstream of the weirs on each watershed. Details of the quality assurance and quality control protocols are given by Edwards & Wood (1993).

**Soil leachate**

To collect soil leachate, 15 soil pits were dug on each watershed in 1988. In each pit, zero-tension lysimeters were positioned at the base of the A and B horizons. Whenever possible, lysimeters were also placed at the base of the C horizon. After a 6-month acclimation period, leachate samples were collected at monthly intervals or whenever the sample volume was sufficient for chemical analysis.

**Chemical analysis of water samples**

All precipitation, stream-water, and leachate samples were analyzed for a variety of chemical parameters using EPA approved protocols at the USDA Forest Service’s Timber and Watershed Laboratory in Parsons, WV (Edwards & Wood 1993). This paper will focus on the measurements of inorganic nitrogen, but we also report values for electrical conductivity, calcium, and magnesium.

**Net mineralization and net nitrification**

The buried-bag technique was used to make monthly measurements of net mineralization and nitrification rates in seven circular plots located in each watershed (Gilliam et al. 1996). Five samples of mineral soil were taken to a depth of 5 cm in each plot. These samples were thoroughly mixed and divided to form two composite samples. One composite sample was placed in a polyethylene bag, buried 5 cm beneath the mineral soil surface, and allowed to incubate for approximately 30 days. The remaining sample was returned to the laboratory for chemical analysis of extractable ammonium and nitrate. In the laboratory, subsamples of both the initial and incubated soils were extracted with 1 N KCl and distilled water. The KCl extracts were analyzed for ammonium and the distilled water extracts were analyzed for nitrate using an Orion 720A pH/ISE meter in conjunction with ammonium- and nitrate-sensitive electrodes. Changes in the amount of extractable inorganic nitrogen during the incubation period were used to calculate net mineralization and net nitrification rates.
Results and discussion

In synthesizing the results of over twenty years of research at the Fernow Experimental Forest seven symptoms of nitrogen saturation are evident.

Symptom #1 – High relative rates of net nitrification

A pivotal change that occurs during the process of nitrogen saturation is the increase in the relative importance of net nitrification. Net nitrification is often low in acid forest soils but can be stimulated by increased nitrogen additions. Under these conditions nitrification has been attributed to either heterotrophic fungi, add-sensitive autotrophs living in neutral microsites, or acid-tolerant chemolithotrophs (Stroo et al. 1986; De Boer et al. 1991 & 1992). It has been predicted that as elevated nitrogen deposition continues, rates of net nitrification will increase and approach those of net mineralization (Aber et al. 1989).

The soils in Watersheds 3 and 4 are very acidic (pH_{KCI} ≈ 3.7; Gilliam et al. 1996) and, since they are derived from acidic bedrock, they have undoubtedly always been characterized by a low pH. Thus, in the absence of elevated nitrogen deposition, we would expect low relative rates of net nitrification. Estimates of the annual rates of net mineralization and nitrification in these watersheds (Gilliam et al. 1996) indicate that net nitrification (ca. 70 kg N ha^{-1} yr^{-1}) accounts for >95% of net mineralization (ca. 72 kg N ha^{-1} yr^{-1}; Figure 1). The currently high relative rates of net nitrification are consistent with the occurrence of nitrogen saturation, but they do not prove it conclusively. Unfortunately, no measurements of net mineralization or nitrification were made prior to 1993. As a result, we cannot determine whether the relative rates of net nitrification have increased over time.

The measured rates of net mineralization and nitrification at our study sites are within the range (30–200 kg N ha^{-1} yr^{-1} for net mineralization; 2.5–98 kg N ha^{-1} yr^{-1} for net nitrification) reported for other temperate forests (Nadelhoffer et al. 1992; Pastor et al. 1984) and may actually be higher than most since they exclude the contribution of mineralization and nitrification occurring in the organic horizon. For example, in a fertilized (150 kg N ha^{-1} yr^{-1}) deciduous forest in Massachusetts, net rates of mineralization and nitrification in the mineral soil averaged ca. 29.6 and 0.8 kg N ha^{-1} yr^{-1}, respectively (Aber et al. 1993). Surprisingly, the net rates of mineralization and nitrification in soils at the Fernow Experimental Forest do not appear to increase in response to fertilization (Gilliam et al. 1996). The reason for this is not immediately apparent and deserves further study.
Symptom #2 – Long-term increases in stream-water nitrate and cation concentrations

Once significant amounts of net nitrification occur, continued nitrogen deposition should result in increased stream-water losses of nitrate and associated cations (Aber et al. 1989, Aber 1992). Enhanced nitrate leaching is undesirable because it may contribute to the eutrophication of receiving waters, pose a risk to human health, and accelerate soil acidification by increasing the loss of base cations. Few sites have records of stream-water chemistry that are long enough to document the changes that may result from nitrogen saturation. However, twenty years of streamwater chemistry data from Watershed 4 at the Fernow Experimental Forest (Figure 2; Adams et al. 1994; Edwards & Helvey 1991) show significant increases in electrical conductivity (16.4 to 24.9 uS cm⁻¹) and the concentrations of nitrate (0.095 to 0.975 mg N L⁻¹),
calcium (1.14 to 1.74 mg L\(^{-1}\)), and magnesium (0.490 to 0.823 mg L\(^{-1}\)). Similar changes in the composition of precipitation did not occur (Adams et al. 1994). This indicates that the long-term changes observed in stream-water chemistry are not merely a reflection of altered nutrient inputs; rather they represent an inherent modification of the ecosystem itself.

**Symptom \#3 – High nitrate concentrations in leachate and stream water**

With the continued deposition of elevated amounts of nitrogen, not only should the concentrations of nitrate in stream water and soil leachate increase through time (symptom \#2), but they should achieve concentrations that are substantially greater than those measured in forests receiving lower amounts of nitrogen deposition (Aber 1992; Hedin et al. 1995). The average annual concentration of nitrate in stream water leaving Watershed 4 achieved its highest level in 1981 (1.13 mg N L\(^{-1}\)) and in 1990 it was 0.975 mgN L\(^{-1}\).
(Figure 2). In the same watershed from 1989–1991, the concentration of nitrate in water leaching through the A horizon ranged from ~0.564–2.94 mg N L⁻¹ (Edwards et al. 1992). In a separate one-year study, De Walle et al. (1988) sampled soil leachate in the upper portion of Watershed 4 and found the mean biweekly concentration of nitrate in water leaching through the B horizon to be ~4.29 mg N L⁻¹. These values are much higher than those reported for solution losses from unpolluted, old-growth temperate forests (0.0001–0.0042 mg N L⁻¹) and they are similar to, or greater than, values reported for temperate forests in areas subject to strong anthropogenic atmospheric nitrogen deposition (Vitousek 1977; Driscoll et al. 1989; Kahl et al. 1993; Likens & Bormann 1995; Hedin et al. 1995).

**Symptom #4 – Little seasonal variability in stream-water nitrate concentrations**

As a system becomes increasingly saturated with respect to nitrogen, the reduced biotic demand for inorganic nitrogen should be reflected in the seasonal changes of nitrate concentrations in stream water (Hauhs et al. 1989; Driscoll et al. 1989; Aber 1992; Stoddard 1994). When plant and microbial growth is nitrogen limited, we expect extremely low concentrations of nitrate in stream water throughout the year. As nitrogen limitations diminish, a distinct seasonal pattern in nitrate concentrations should emerge – low concentrations during the growing season and high concentrations outside the growing season. Finally, when plant and microbial growth are no longer limited by nitrogen availability, we expect high concentrations and little seasonal variability in stream-water nitrate concentrations. The eleven-year mean monthly nitrate concentrations for stream water leaving Watershed 4 between 1984 and 1994 are consistent with our expectations of a system nearing saturation (Figure 3) – high concentrations (~0.8 mg N L⁻¹) that show little seasonal variability (coefficient of variation ~20%). Furthermore, the twenty-four-year record of mean monthly nitrate concentrations demonstrates that the variability in stream-water chemistry has changed over time (Figures 4 & 5). From this record, three periods are evident: an initial period (1971–1973) of low concentrations and low variability; a period (1974–1983) of high peak concentrations and high variability, and, most recently, a period of high concentrations and low variability (1984–1994). This sequence of changes in the variability of stream-water chemistry is in broad agreement with our expectations, but the lack of a distinct seasonal pattern is not. More specifically, there is no consistent difference between nitrate concentrations during the growing and non-growing seasons (Figure 4). The lack of a distinct seasonal pattern in nitrate concentrations deserves further study and suggests that several factors
Figure 3. A comparison between the expected seasonal patterns in stream-water nitrate concentrations for various stages of nitrogen saturation (from Aber 1992) and the average monthly concentration of nitrate in stream water leaving Watershed 4 for the years 1984–1994 (Adams et al. 1994).

may be important in regulating stream-water concentrations of nitrate in this watershed.

Symptom #5 – A high discharge of nitrate from a young aggrading forest

Stream-water concentrations and losses of limiting nutrients are often low in young aggrading forests. However, during the course of ecosystem succession, nutrient losses are expected to increase as a result of declining net ecosystem productivity until nutrient inputs balance nutrient outputs (Vitousek & Reiners 1975; Vitousek 1977). As a result of this temporal pattern, it may be difficult to distinguish successional effects from those of nitrogen saturation in mature ecosystems receiving high nitrogen inputs. Thus, an unusually high discharge of nitrate from a young forest contradicts successional expectations and may be a strong indication of nitrogen saturation. Watershed 3 at the Fernow Experimental Forest was last cut about 23 years ago and is a vigorously growing forest (M.B. Adams, unpublished data). However, in the 3 years prior to its fertilization, the discharge of nitrate from Watershed
3 nearly equaled the loss of nitrate from an older and undisturbed forest – Watershed 4 (Figure 6). From this data, it appears that the nutrient dynamics of a regenerating forest can be fundamentally altered by elevated rates of nitrogen deposition.

Symptom #6 – A rapid increase in nitrate loss after fertilization of a young aggrading forest

In forests experiencing severe nutrient limitations, the addition of nutrients in low amounts should stimulate growth and be efficiently retained within the ecosystem. For example, after 3 years of fertilization with 50 or 150 kg N ha\(^{-1}\) yr\(^{-1}\), Aber et al. (1993) found that essentially all nitrogen inputs were retained by both a pine and hardwood forest. In contrast, once a forest is near saturation, even the ability of an aggrading forest to utilize nutrient additions can be exceeded. For example, an immediate increase in stream-
water nitrate concentrations was observed when an approximately 50 year-old hardwood forest in Maine was fertilized with 25 kg N ha\(^{-1}\) yr\(^{-1}\) (Kahl et al. 1993). A similar result was observed for an even younger forest at the Fernow Experimental Forest. Prior to fertilization, nitrate loss from Watershed 3 was about 15% lower than the amount of nitrate lost from an adjacent and more mature forest – Watershed 4 (see Symptom #5). One year after nitrogen additions had begun, nitrate losses from Watershed 3 were about 20% greater than losses from unfertilized Watershed 4. And two years after nitrogen additions had started, nitrate losses were about 70% greater than those from Watershed 4 (Figure 6). Corresponding to these rapid changes in stream-water concentrations were equally rapid increases in the concentration of nitrate leaching through the A horizon of Watershed 3. Eight months after the beginning of nitrogen additions, the nitrate concentrations in soil leachate samples from Watershed 3 were consistently higher than those from Watershed 4 (Edwards et al. 1992). Leachate concentrations of nitrate in both watersheds exhibited cyclical changes and ranged from about 0.56–2.94 mg N L\(^{-1}\) in Watershed 4 and from about 1.24–4.43 mg N L\(^{-1}\) in Watershed 3.

If nitrogen was a strongly limiting nutrient, then the regenerating forest growing on Watershed 3 should have a high capacity to retain supplemental
Figure 6. Annual discharge of nitrate in stream water leaving Watersheds 3 and 4 at the Fernow Experimental Forest (from Adams et al. 1993). The value for Watershed 3 in 1991 is corrected for an error in the original article (M.B. Adams, pers. comm.). Watersheds 3 and 4 were last cut in 1972 and 1905 respectively. Aerial fertilization with 35 kg N ha\(^{-1}\) yr\(^{-1}\) in 3 applications of ammonium sulfate was begun on Watershed 3 in 1989. Watershed 4 has not been fertilized.

additions of nitrogen. The fact that a rapid increase in nitrate loss followed the onset of fertilization strongly indicates that, prior to fertilization, the forest growing on this watershed was near saturation.

Symptom #7 – Low retention of inorganic nitrogen when compared with other forested sites

Once nitrogen availability exceeds biotic demand, the ability of an ecosystem to retain nitrogen should diminish until inputs of nitrogen balance outputs. When assessing the ability of an ecosystem to retain nitrogen, both organic
and inorganic forms of nitrogen should be measured. In practice, however, most investigators measure only inorganic nitrogen (nitrate and ammonium) because nitrogen in this form is readily assimilated by plants and microbes. The balance between the input and output of inorganic nitrogen has been compared for a large number of forested watersheds in the United States, Canada, and Europe (Driscoll et al. 1989; Correll & Weller in press). In general, forests receiving <4 kg N ha\(^{-1}\) yr\(^{-1}\) of inorganic nitrogen lose very little inorganic nitrogen in streamflow. However, forests receiving >4 kg N ha\(^{-1}\) yr\(^{-1}\) of inorganic nitrogen exhibit variable stream-water losses.

In a comparison of 37 forested watersheds, the greatest stream-water losses of inorganic nitrogen occurred in Watershed 4 at the Fernow Experimental Forest (Figure 7). In the 13 years from 1978 to 1990, the average loss of inorganic nitrogen in streamflow from Watershed 4 was 6.1 kg N ha\(^{-1}\) yr\(^{-1}\) and the average input from rainfall was estimated to be 8 kg N ha\(^{-1}\) yr\(^{-1}\) (Adams et al. 1994). Thus, stream-water losses of inorganic nitrogen from

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**Figure 7.** A comparison between the throughput of inorganic N (ammonium + nitrate) for 36 watersheds in the United States and Canada (from Driscoll et al. 1989) and Watershed 4 at the Fernow Experimental Forest. Data for Watershed 4 are from Adams et al. (1994) and represent the average input and output for the 13 years between 1978 and 1990. The solid line is the one-to-one line.
Watershed 4 typically represent about 76% of rainfall inputs and indicate that the demand for available nitrogen in this watershed is relatively low.

Conclusions

By synthesizing data from more than 20 years of research at the Fernow Experimental Forest, seven symptoms of nitrogen saturation were documented for two adjacent watersheds. Each of these symptoms is consistent with the predictions of Aber et al. (1989) and supports their conceptual model of nitrogen saturation. Given the variety and extent of the symptoms measured in Watersheds 3 and 4, the data presented here may be the clearest example of nitrogen saturation in the United States.

From the data synthesized, two observations deserve special attention in the future. The first observation is that a young aggrading forest can exhibit symptoms of nitrogen saturation. These symptoms indicate that the nitrogen requirement of the regrowing forest is being met and that a nutrient other than nitrogen may be limiting growth. The second important observation is the variable response of forests to elevated amounts of nitrogen deposition. By better understanding the mechanisms responsible for this variability, we could begin to identify forests that are resistant to nitrogen saturation, and formulate management practices that may avoid the undesirable effects of this process in more sensitive forests.

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