

# Declines in soil-water nitrate in nitrogen-saturated watersheds

Pamela J. Edwards and Karl W.J. Williard

**Abstract:** Two forested watersheds (WS3 and WS9) in the central Appalachians were artificially acidified with ammonium sulfate fertilizer. WS9 was treated for 8 years, whereas WS3 has been treated for approximately 15 years. Soil leachate was collected from a depth of 46 cm (B horizon) in WS9 and below the A, B, and C horizons in WS3. Nitrate concentrations from WS3 increased for approximately 10–12 years (depending upon horizon) and then did not increase from 2000 through 2003 despite continued fertilizer treatments. Nitrate concentrations in WS9 soil water increased for the first 3 years of fertilization, and then declined for another 2 years. After that time, the concentrations remained relatively constant at approximately 15  $\mu\text{equiv}\cdot\text{L}^{-1}$ ; this period of low nitrate concentrations included 2.3 years of fertilization followed by 8.2 years of no fertilization. Stream-water nitrate concentrations from both watersheds indicate they were in stage 2 of nitrogen saturation; however, the soil-water nitrate behavior observed within the setting of continued elevated nitrogen inputs is at odds with responses predicted in current nitrogen saturation theory. We believe that the cessation of nitrate increases in at least the B and C horizons were due primarily to abiotic retention, with recalcitrant forms of dissolved organic carbon providing the carbon needed to induce retention. These results show that nitrogen cycling in forest soil ecosystems is more complex than current nitrogen saturation theory suggests.

**Résumé :** Deux bassins versants boisés (WS3 et WS9) dans les Appalaches centrales ont été artificiellement acidifiés par une fertilisation au sulfate d'ammonium. Le bassin WS9 a été traité pendant huit ans tandis que le bassin WS3 a été traité pendant approximativement 15 ans. Le lessivat du sol a été collecté à une profondeur de 46 cm (horizon B) dans le bassin WS9 et sous les horizons A, B et C dans le bassin WS3. La concentration de nitrates a augmenté dans le bassin WS3 pendant approximativement 10–12 ans (dépendamment de l'horizon) et n'a pas augmenté par la suite de 2000 à 2003 malgré la poursuite de la fertilisation. La concentration de nitrates dans l'eau du sol du bassin WS9 a augmenté pendant les trois premières années de fertilisation et par la suite a diminué pendant deux autres années. Ensuite, la concentration est demeurée relativement constante à environ 15  $\mu\text{equiv}\cdot\text{L}^{-1}$ ; cette période de faible concentration de nitrates incluait 2,3 ans de fertilisation suivie de 8,2 ans sans fertilisation. La concentration de nitrates dans l'eau des cours d'eau des deux bassins versants indique qu'ils étaient au stade 2 de saturation en azote. Cependant, le comportement des nitrates dans l'eau du sol observé en situation d'apport élevé continu d'azote ne correspond pas aux réponses que prédit la théorie actuelle de la saturation en azote. Les auteurs croient que l'arrêt de l'augmentation des nitrates, au moins dans les horizons B et C, était dû principalement à la rétention abiotique par des formes récalcitrantes de carbone organique dissout qui fournissent le carbone nécessaire pour induire la rétention. Ces résultats montrent que le recyclage de l'azote dans les écosystèmes des sols forestiers est plus complexe que le suggère la théorie actuelle de la saturation en azote.

[Traduit par la Rédaction]

## Introduction

Historically, northern temperate forest ecosystems were considered nitrogen (N) limited. However, in the past two decades, researchers have noted significant increases in N exports from forested watersheds (Driscoll et al. 1989; Johnson and Lindberg 1992; Stoddard 1994). The theory of N saturation was developed to explain this phenomenon of forest ecosystems' declining abilities to retain N (Agren and Bosatta 1988; Aber et al. 1989). Among other things, this hypothesis had as its basis that nitrate ( $\text{NO}_3^-$ ) leaching through soils increased as N became more available. Over

time, the N saturation theory has been revised as more research data have become available. The new hypothesis continues to have the premise of increased leaching with increased  $\text{NO}_3^-$  availability as its foundation, but greater emphasis is given to the importance of N retention, because a large percentage of N typically does not leach out of a watershed even when N deposition rates are high. As a result, N retention is considered to be an important pathway by which the deleterious effects of high N deposition are offset, at least in the short term. In current N saturation theory, N retention is most important during the first stages of N saturation; leaching increases with time as N inputs continue in-

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**P.J. Edwards.**<sup>1</sup> USDA Forest Service, Northeastern Research Station, P.O. Box 404, Parsons, WV 26287, USA.

**K.W.J. Williard.** Department of Forestry, Southern Illinois University, Mailcode 4411, Carbondale, IL 62901, USA.

<sup>1</sup>Corresponding author (e-mail: [pjedwards@fs.fed.us](mailto:pjedwards@fs.fed.us)).

creasing, and leaching does not decline at least until N inputs decrease (Aber et al. 1998).

Recently, N saturation theory has been challenged by researchers who have discovered long-term stream  $\text{NO}_3^-$  declines in New England forested watersheds (Goodale et al. 2003). Various hypotheses have been put forth to explain these findings including interannual climate variation (Goodale et al. 2003), increased immobilization of N during postdisturbance ecosystem recovery (Huntington 2005), and increased microbial immobilization and denitrification in soils and sediments owing to increased dissolved organic carbon levels (Goodale et al. 2005).

Two separate whole-watershed artificial acidification studies on and near the Fernow Experimental Forest showed  $\text{NO}_3^-$  concentration responses in soil leachate (Edwards et al. 2002a) and baseflow (Edwards et al. 2002b) were consistent with Aber and his colleagues' (1998) revised hypothesis through approximately 10 years of repeated treatments with ammonium sulfate ( $\text{NH}_4\text{SO}_4$ ) fertilizer. In this paper, we examine whether soil-water  $\text{NO}_3^-$  dynamics over the subsequent 5 years continued to support current N saturation theory.

## Materials and methods

The two treatment watersheds, WS3 and WS9, and the treatments themselves are described in detail in Edwards et al. (2002a, 2002b), so only brief descriptions are given here. WS3 (34.4 ha) is on the Fernow Experimental Forest, Tucker County, West Virginia. It currently supports an approximately 30-year-old stand of hardwood trees that regenerated naturally after clear-cutting. WS9 (11.6 ha) is approximately 10 km west of WS3 in the Monongahela National Forest. WS9 supports 20-year-old planted Japanese larch (*Larix leptolepis* Seib. & Zucc.) throughout most of the watershed and 80-year-old native hardwoods in the 1.42 ha bufferstrip (~20 m wide on both sides of the stream).

WS3 and WS9 were fertilized with  $\text{NH}_4\text{SO}_4$  three times per year (March, July, and November) at an annual rate of  $35.5 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$  and  $40.6 \text{ kg S}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ , which are approximately double the ambient N and sulfur inputs estimated from throughfall (Helvey and Kunkle 1986). Ambient N inputs during the period of study are listed in Table 1. Treatments on WS9 began in 1987 and on WS3 in 1989.

On WS3, zero-tension pan lysimeters (DeWalle et al. 1988) were installed at the base of the A, B, and C horizons in 15 plots across the watershed (Fig. 1) to capture gravity draining soil water. Average depth to the bottom of the A, B, and C horizon lysimeters, respectively, is 13, 79, and 119 cm. On WS9, zero-tension pan lysimeters were installed at approximately 46 cm below the soil surface, which approximates the lowest rooting depth in the catchment, in six  $30.5 \text{ m} \times 30.5 \text{ m}$  plots across the watershed (Fig. 2). There was a paired control watershed for WS3 and six untreated control plots within WS9 from which soil water samples were collected as part of this study. The results for these controls are not described in this paper because their  $\text{NO}_3^-$  concentrations remained unchanged during the period of fertilization on both watersheds (see Edwards et al. 2006).

Initially it was planned that lysimeter samples would be collected monthly; however, most samples actually were collected only during dormant seasons or during very wet peri-

**Table 1.** Annual ambient nitrogen inputs ( $\text{kg}\cdot\text{ha}^{-1}$ ) by watershed.

Year	WS3	WS9
1986	17.65	27.28
1987	15.06	17.92
1988	9.02	15.05
1989	11.64	13.38
1990	10.04	11.75
1991	9.50	9.40
1992	9.64	12.07
1993	11.00	11.71
1994	10.69	14.43
1995	9.59	11.17
1996	12.41	15.77
1997	10.57	14.07
1998	10.00	13.49
1999	7.26	9.28
2000	8.30	10.26
2001	9.23	11.02
2002	8.59	9.62

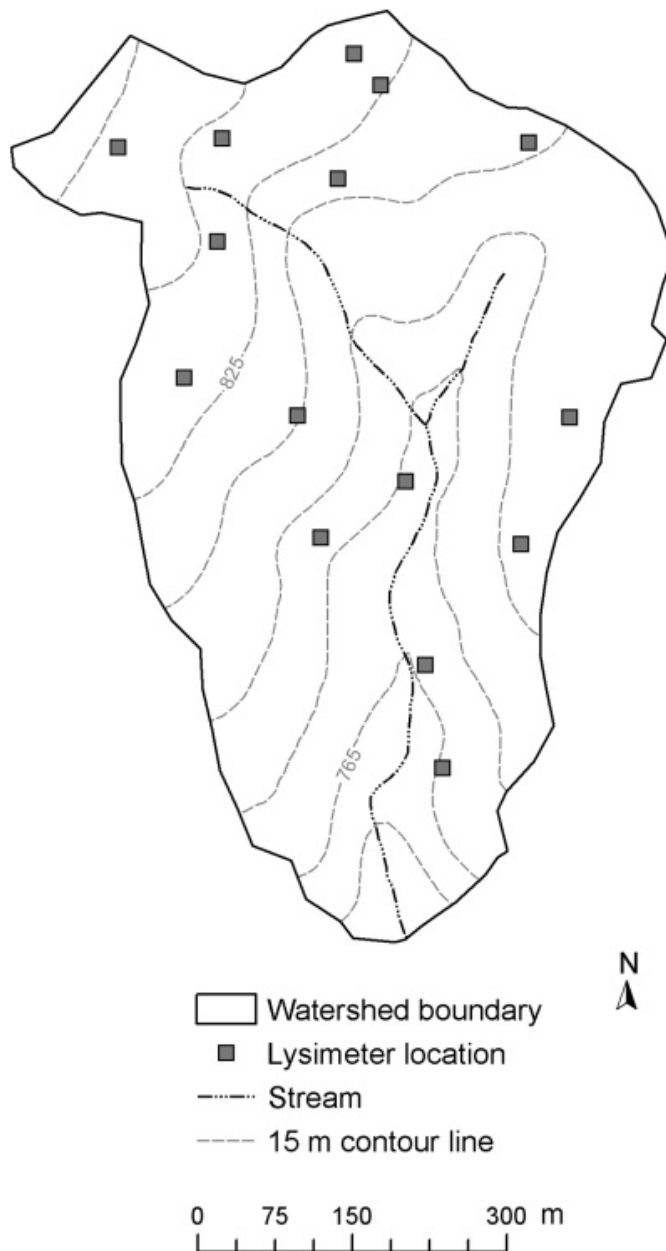
ods of growing seasons, as these were the times in which soils were moist enough to yield sufficient water for chemical analyses. After extended dry periods, if no samples or only minor amounts of water were present, the bottles were replaced with clean ones prior to the onset of the dormant season. On average, samples were collected 5 times per year from WS3 from 1989 to 2003, and 10 times per year from WS9 from 1986 to 2002. No lysimeter samples were collected from WS3 in 1994 and from WS9 in 1995 owing to budget limitations.

Soil water samples were analyzed at the USDA Forest Service, Northeastern Research Station's Timber and Watershed Laboratory in Parsons, West Virginia. Each lysimeter sample was analyzed for  $\text{NO}_3^-$  using ion chromatography and for  $\text{NH}_4$  using a Wescan  $\text{NH}_4$  analyzer. Samples were handled and processed using EPA-approved protocols (Edwards and Wood 1993).

To account for chemical differences that could be attributed to concentration or dilution effects because of differences in soil water volumes, volume-weighted mean concentrations by horizon were determined for WS3 and WS9 for each sampling period. Only lysimeters that had sufficient volume for chemical analyses were included in the calculation of the volume-weighted means for that horizon (WS3) or the 46 cm depth (WS9). The volume-weighted mean  $\text{NO}_3^-$  concentrations were graphed against time, and a locally weighted regression line (Cleveland and Devlin 1988) was fitted through the data using Locfit software (Loader 1998) to aid in showing trends (Hipel and McLeod 1994). Locally weighted regression does not provide regression coefficients, but it does display data trends very effectively (especially nonmonotonic trends) without the assumptions or limitations of predefined equation forms (i.e., linear, quadratic, cubic, etc.) that accompany least squares regression. Locally weighted regression also is appropriate for irregularly spaced nonparametric time-series data, which describe many of the data in this study.

To avoid oversmoothing the trend lines, various combinations of fitting degrees and smoothing values were applied to

**Fig. 1.** Locations of the lysimeters in WS3 (longitude 79° 41'10"N, latitude 39°03'15"W).



each  $\text{NO}_3^-$  vs. time data set. Residuals were then determined for each data point and were plotted against time. A locally weighted regression line developed using the same fitting degrees and smoothing values as for the original data was overlaid on the plotted data. The degree of fit and smoothing values were considered appropriate when the latter locally weighted regression line was essentially flat using the lowest possible smoothing value (Cleveland 1994).

Mann–Kendall tests (Mann 1945) were used to determine whether the volume-weighted mean soil water concentrations increased or decreased significantly over time ( $p \leq 0.05$ ). These tests were performed on the concentrations and not the trend lines, since locally weighted regression does not provide estimates of regression coefficients to test for

significance. Because Mann–Kendall tests can determine the significance of only monotonic data trends, where the trend lines changed direction (indicating a change in the direction of the concentrations) a separate Mann–Kendall test statistic was calculated for the data on both sides of that change as recommended by Hipel and McLeod (1994). Sen slope estimates (Sen 1968) were calculated to identify the direction (i.e., positive or negative) of the data trends, since these were not always obvious for cases in which the data were quite variable or the change was small.

## Results and discussion

### Watershed 3

N inputs from the fertilizer were as  $\text{NH}_4$ , but the vast majority of applied  $\text{NH}_4$  on WS3 is nitrified rapidly (Gilliam et al. 1996) so that soil-water  $\text{NH}_4$  concentrations were very small and typically zero throughout most of the study in all soil horizons (Fig. 3). Furthermore, soils throughout WS3 also are generally well drained, so denitrification owing to saturated and (or) anoxic conditions is not believed to be common, nor is it supported by  $\text{NH}_4$  concentrations (Fig. 3).

$\text{NO}_3^-$  concentrations in soil water of all three horizons increased significantly for 11–12 years (Table 2, Fig. 4). After that, the trend lines and data show a decrease in  $\text{NO}_3^-$  (Fig. 4), though the Mann–Kendall tests indicate a flattening of the response, with neither increasing nor decreasing concentrations from 2000 to 2003 (Table 2).

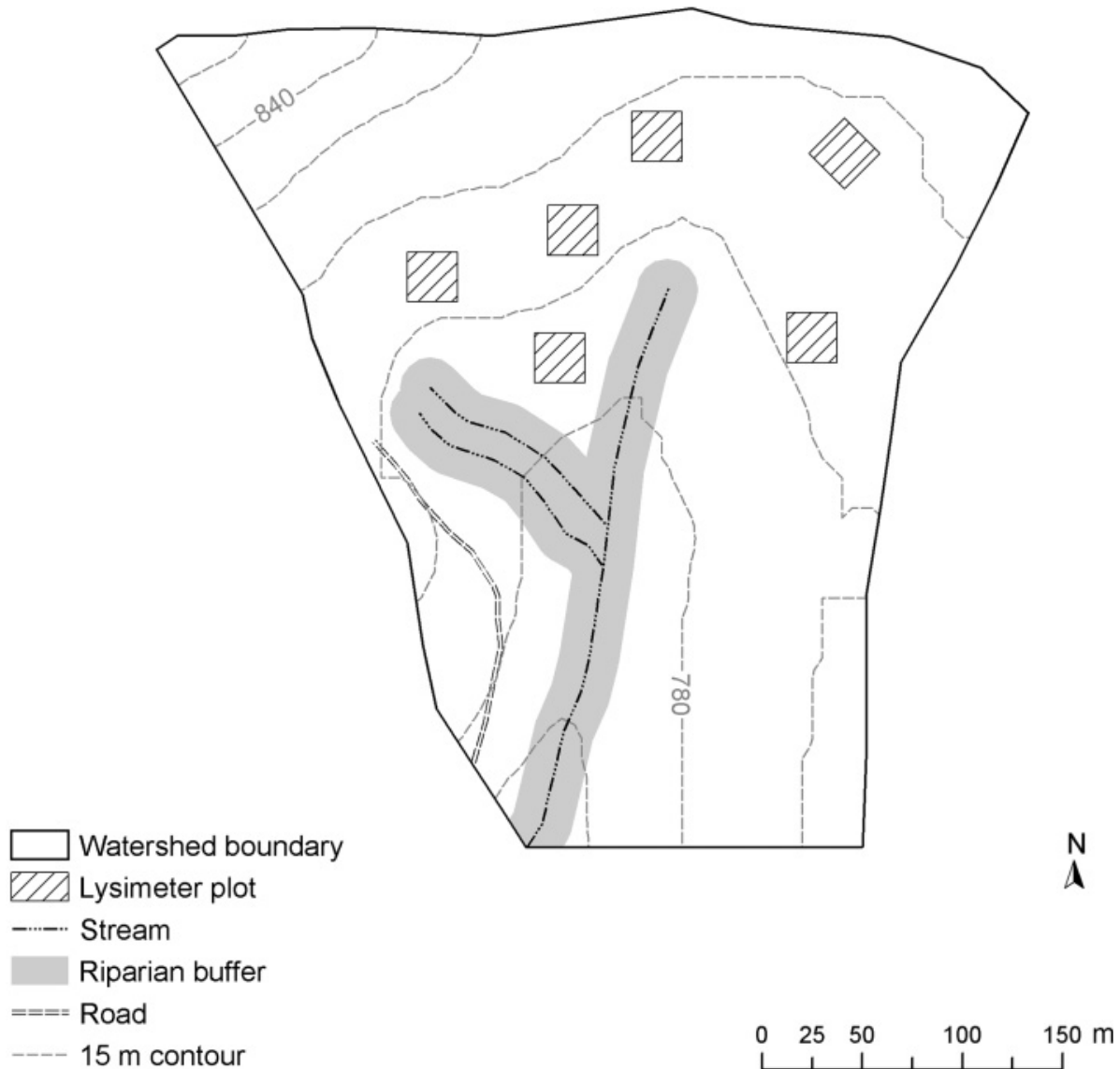
The discontinuation of rising  $\text{NO}_3^-$  concentrations was unexpected, particularly given that it occurred after more than a decade of  $\text{NH}_4\text{SO}_4$  applications and applications were ongoing even during 2000–2003. This behavior suggests that some type of  $\text{NO}_3^-$  retention or conversion to another chemical species must have occurred.

There are a number of documented ways that  $\text{NO}_3^-$  could be retained in WS3, but none of them as observed or currently hypothesized seem likely, except perhaps in the A horizon. An onset of adsorption of  $\text{NO}_3^-$  is improbable because sulfate adsorption was occurring due to the continued inputs of sulfate from the fertilizer (Edwards et al. 2002a), and the elevated levels of sulfate availability in WS3 soils would have induced sulfate adsorption preferentially rather than  $\text{NO}_3^-$  adsorption on available anion adsorption sites.

The trees on WS3 were approximately 25 years old in 1995. They grew vigorously, the canopy was fully closed, and the root systems already were well developed and exploiting the soil volume since coppicing was a dominant source of regeneration in this stand. Thus, an onset of biotic  $\text{NO}_3^-$  assimilation by vegetation or microorganisms would have been an unlikely cause of the retention at this time in the stand's age. If the watershed soils were N deficient, N retention by microorganism or vegetative assimilation would have occurred and been evidenced when the fertilizer applications were begun or soon thereafter (Aber et al. 1998).

Hansen et al. (1996, 2001) presented an abiotic  $\text{NO}_3^-$  retention method that could be important in subsoils in which organic matter and microorganisms are in limited supply. In this process, layered iron (II) and iron (III) hydroxides (green rusts) reduce  $\text{NO}_3^-$  to form ammonia and magnetite at a rate similar to microbial denitrification. However, green rusts are most prevalent in nonacidic soils, and the reduction of  $\text{NO}_3^-$

Fig. 2. Locations of the lysimeters in WS9 (longitude 79°48'49"N, latitude 39°09'15"W).



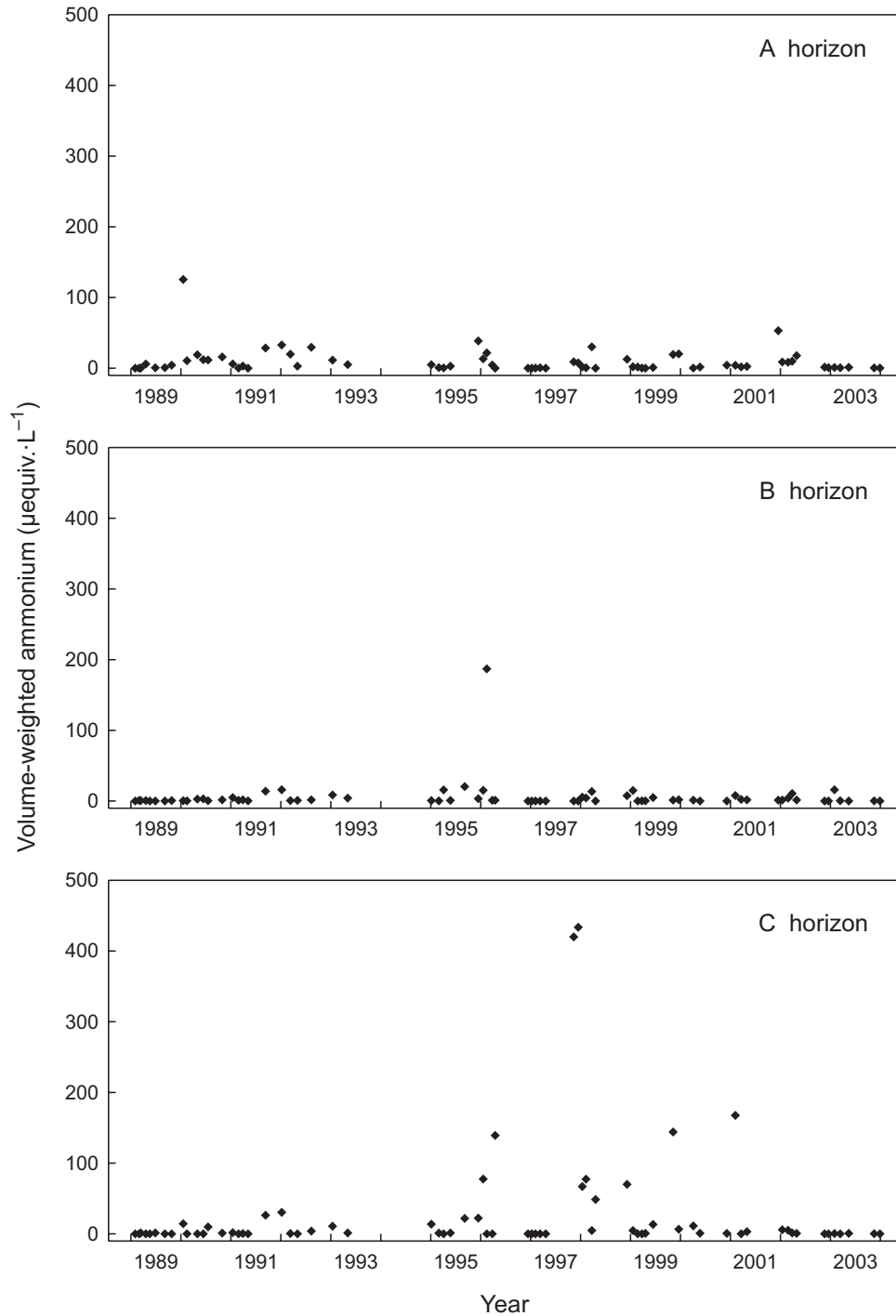
by green rusts requires anoxic conditions (Hansen et al. 1996), neither of which describes the conditions on WS3.

N retention through biotic immobilization by mycorrhizae (Aber et al. 1998) and abiotic immobilization of  $\text{NH}_4$  and nitrite ( $\text{NO}_2^-$ ) in soil organic matter (Berntson and Aber 2000; Dail et al. 2001; Fitzhugh et al. 2003) are mechanisms that have been presented more recently to account for N retention. However, the situations in which these mechanisms operate are at odds with those present on WS3. Mycorrhizae associations on fine roots would be concentrated in upper, rather than lower, soil horizons; as a result, effects of mycorrhizal immobilization on  $\text{NO}_3^-$  concentrations should have been demonstrated first in the A horizon instead of occurring first in the B and C horizons (Fig. 4). It is possible that  $\text{NO}_2^-$  formed during oxidation could have become fixed abiotically into soil organic matter if the  $\text{NH}_4$  inputs and supplies in the soil (due to the fertilizer) exceeded biotic sinks (Fitzhugh et al. 2003). In this situation, neither  $\text{NH}_4$  nor  $\text{NO}_3^-$  levels in leachate would have increased (Berntson and Aber 2000), which is consistent with what was observed

in the soil leachate. But again, it is more likely that such abiotic immobilization of  $\text{NH}_4$  and  $\text{NO}_2^-$  would have been reflected first and to a greater degree in the A horizon, especially since soil organic matter levels decreased substantially with depth, particularly below 10 cm (Adams and Kochenderfer 1999).

Abiotic retention by iron and possibly manganese catalysts also has been suggested as a retention mechanism of N (Davidson et al. 2003). In this hypothesis, soil organic matter reduces Fe(III) and Mn(IV) oxides to Fe(II) and Mn(II). In turn, both reduced metals reduce  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , and  $\text{NO}_2^-$  then reacts with phenolic compounds in soil solution to form dissolved organic N compounds that can adsorb onto soil surfaces (Currie et al. 1996; Kaiser et al. 1996). This mechanism also requires soil organic matter to react with  $\text{NO}_2^-$  before it reoxidizes to  $\text{NO}_3^-$ , so again one would have expected it to occur first in the A horizon rather than deeper soils.

Clearly, the N retention mechanisms that developed over time on WS3 are not understood. However, the observed retention in the deeper soil layers may be due to abiotic reac-

**Fig. 3.** Volume-weighted mean concentrations of ammonium from the A, B, and C horizon lysimeters in WS3.

tions with recalcitrant carbon compounds, which can be relatively common in deeper soil horizons compared with labile carbon (Qualls et al. 2002; Davidson et al. 2003). We hypothesize that dissolved organic carbon (DOC) leached from litter, microbial biomass, humus, and root exudates, and not incorporated soil organic matter per se, provided the primary sources of carbon involved with N retention in these deep soil layers. Root exudate derived DOC may have been particularly important in the lower soil. It is commonly as-

sumed that the vast majority of roots are located in the upper 10–20 cm of soil; however, a recent study in northern hardwood forests has shown that more than half of all root turnover was below 20 cm (Hendrick and Pregitzer 1996). They also found that early growing season fine root growth was even more prevalent at depth, with 50%–80% of the root production occurring below 50 cm. Whatever its source, as DOC leaches, it becomes more recalcitrant and can accumulate over long periods of time by sorption onto clay minerals



**Table 2.** Mann–Kendall and Sen slope results for soil-water nitrate from WS3, by horizon.

Horizon	Period	Mann–Kendall probability	Sen slope
A	1	0.000	17.50
	2	0.149	–33.34
B	1	0.000	28.47
	2	0.537	–21.76
C	1	0.000	31.94
	2	0.115	18.73

**Note:** Period 1: 1989 through 31 March 2000; period 2: 1 April 2000 through 2003.

(Magill and Aber 2000). In turn, large supplies of DOC would be available for extensive Fe(III) and Mn(IV) oxide reduction.

It is possible that abiotic transformations of  $\text{NO}_3^-$  to dissolved organic N (DON) occurred with little net retention. However, DON can exist in stable cyclic structures in soils (Davidson et al. 2003) and can also be adsorbed onto soil surfaces and held in soil aggregates (Moore et al. 1992; Strickland et al. 1992; Kaiser et al. 1996). As annual exports of DON comprise only a small portion of the DON produced in forested watersheds, DON retention is an important N controlling process (Davidson et al. 2003). Consequently, whereas we did not measure DON concentrations and they may have increased in soil water at the expense of  $\text{NO}_3^-$ , abiotic N retention probably was still very important on WS3.

Retention of  $\text{NO}_3^-$  in the A horizon may have been due to biotic immobilization by mycorrhizae (Aber et al. 1998), abiotic immobilization of  $\text{NH}_4$  and  $\text{NO}_2^-$  in soil organic matter (Berntson and Aber 2000; Dail et al. 2001; Fitzhugh et al. 2003), and (or) abiotic retention by iron and possibly manganese catalysts (Davidson et al. 2003) because organic matter was present at much higher levels above 10 cm than below 10 cm (Adams and Kochenderfer 1999). Abiotic transformation to DON also may have occurred in the A horizon. However, the reason for later retention of  $\text{NO}_3^-$  in the A horizon compared with the B and C horizons is not known.

### Watershed 9

$\text{NO}_3^-$  concentrations in soil leachate on WS9 increased for about 4 years and then decreased (Fig. 5, Table 3) in a manner similar to that in WS3 (Fig. 4), indicating that the phenomenon on WS3 was not an isolated anomaly. Edwards et al. (1999) originally reported this decline in  $\text{NO}_3^-$  concentrations for WS9 soil leachate but attributed it to vegetative uptake by the Japanese larch planted on the site, because WS9 had limited soil nutrient (including N) pools owing to its past farming history (DeWalle et al. 1995). Edwards et al. (1999) suggested that the roots did not fully utilize the N applied to the site because the tree roots had not yet become established sufficiently during the first years of the study to fully occupy the soil and exploit available nutrients. However, there are several pieces of evidence and reasoning that indicate this original interpretation was incorrect: the similar response in WS3, the fact that Japanese larch do not require or even thrive in high N-available environments (VanGoor 1953), no coincidental increases in  $\text{NH}_4$  concentrations (Fig. 6), the lack of other more gradual changes in the slope

of the trend line that would be expected as N uptake increased with root establishment, and that biotic assimilation, as currently understood, cannot be induced simply by adding N if the site was not initially N deficient. All of these pieces of evidence indicate that the  $\text{NO}_3^-$  decline on WS9 was due to something other than biotic immobilization. Abiotic immobilization in WS9 by the same scenario described for the B and C horizons in WS3 (i.e., recalcitrant DOC) seems to be a plausible explanation for the  $\text{NO}_3^-$  decreases since the WS9 lysimeters are in the B horizon.

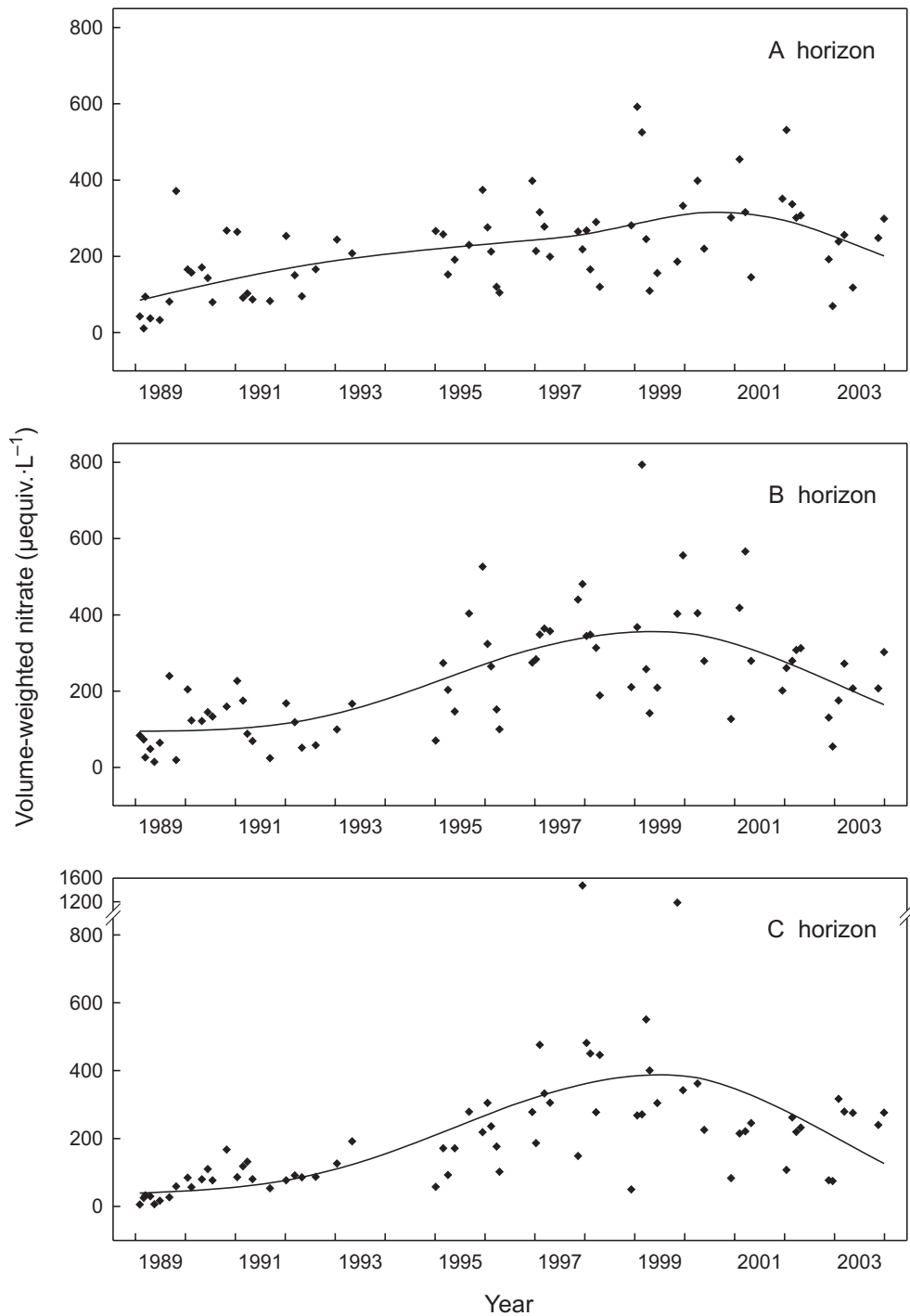
### Application to N saturation theory

Stoddard (1994) described N saturation in a series of stages based on seasonal stream  $\text{NO}_3^-$  concentrations. In stage 0, stream  $\text{NO}_3^-$ -N concentrations are near zero year round. In stage 1,  $\text{NO}_3^-$ -N becomes elevated (0.5 to 0.8 mg  $\text{NO}_3^-$ -N·L<sup>-1</sup>) in the dormant season because of a lack of microbial and vegetative uptake. Stage 2 is characterized by elevated stream  $\text{NO}_3^-$ -N concentrations year round. A reference watershed (WS4) adjacent to WS3 has been cited as the best example of a stage 2 N-saturated watershed in the northeastern United States, exhibiting elevated year-round  $\text{NO}_3^-$  concentrations (Peterjohn et al. 1996; Fenn et al. 1998; Williard et al. 2003). At the beginning of this study, weekly stream water samples collected at the mouth of WS3 showed the  $\text{NO}_3^-$  concentrations from WS3 to be approximately equal to those from WS4. Now WS3 concentrations are about 3 times that level (Fig. 7), so based on stream water concentrations, WS3 too was and is in stage 2 N saturation.  $\text{NO}_3^-$  concentrations from streamflow collected at the mouth of WS9 were lower at the beginning of treatment (Fig. 7) than on WS3 or WS4, but the most recent years of data show that  $\text{NO}_3^-$  concentrations in stream water on WS9 are now approximately equal to that on WS4 and equal to that in the mid 1980s on WS3. The degree of seasonality for WS9  $\text{NO}_3^-$  concentrations is somewhat greater than WS3 and WS4, but as the WS9 data are plotted on a different vertical scale, seasonality is slightly exaggerated for WS9 in Fig. 7. Thus, stream water concentrations on WS9 also indicate that it is now behaving in accordance with stage 2 N saturation.

Whereas WS3, WS4, and WS9 all can be defined as being in stage 2 N saturation, the leveling off of  $\text{NO}_3^-$  concentrations in soil water (Fig. 4) and stream water (Fig. 7) in WS3 coupled with the soil-water  $\text{NO}_3^-$  decreases in WS9 (Fig. 5) while N was being applied, illustrate that N cycling processes and mobility controls are much more complex than current N saturation theory suggests. Our results present a situation in which N retention (or at least conversion to DON) at depth in acidic, moderately well-drained forest soils can occur in the presence of, or perhaps be induced by, high levels of N deposition. Both WS3 and WS4 experienced  $\text{NO}_3^-$  concentration reductions in stream water only after they reached values of at least 100  $\mu\text{equiv}\cdot\text{L}^{-1}$  (Fig. 7). Stream water concentrations on WS9 have not yet reached 100  $\mu\text{equiv}\cdot\text{L}^{-1}$  (Fig. 7) and they have not yet experienced a decline, even though fertilization ended in 1994. This response is in direct contrast to N saturation theory that suggests retention will be confined to early periods of deposition, and increasing N deposition will result in increasing  $\text{NO}_3^-$  leaching losses (Aber et al. 1998).

On a budget basis (Fig. 8), both WS3 and WS9 showed

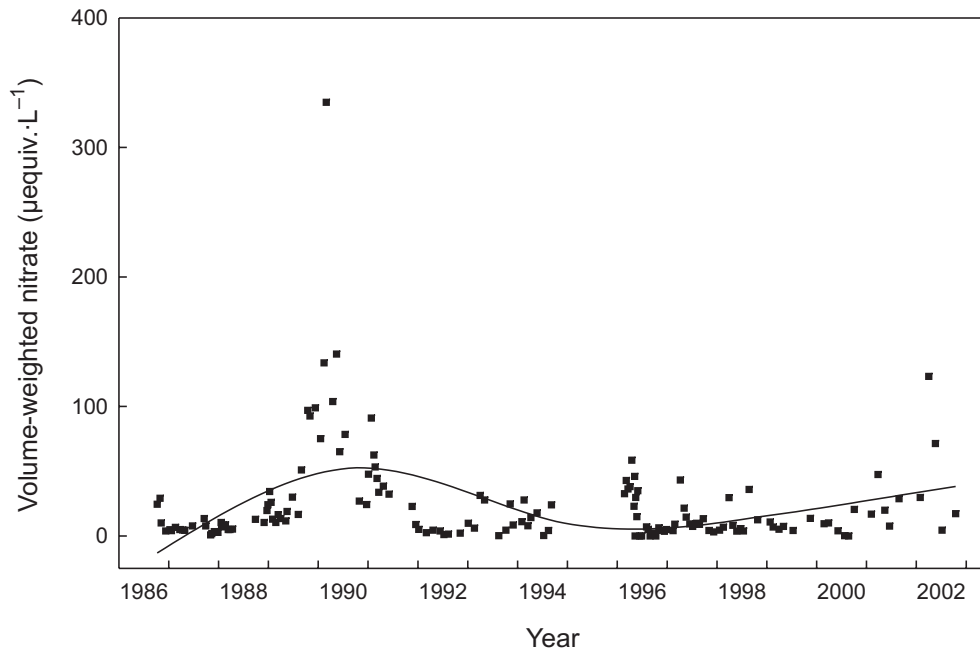
**Fig. 4.** Volume-weighted mean concentrations of nitrate from the A, B, and C horizon lysimeters in WS3 with locally weighted regression trend lines overlaid.



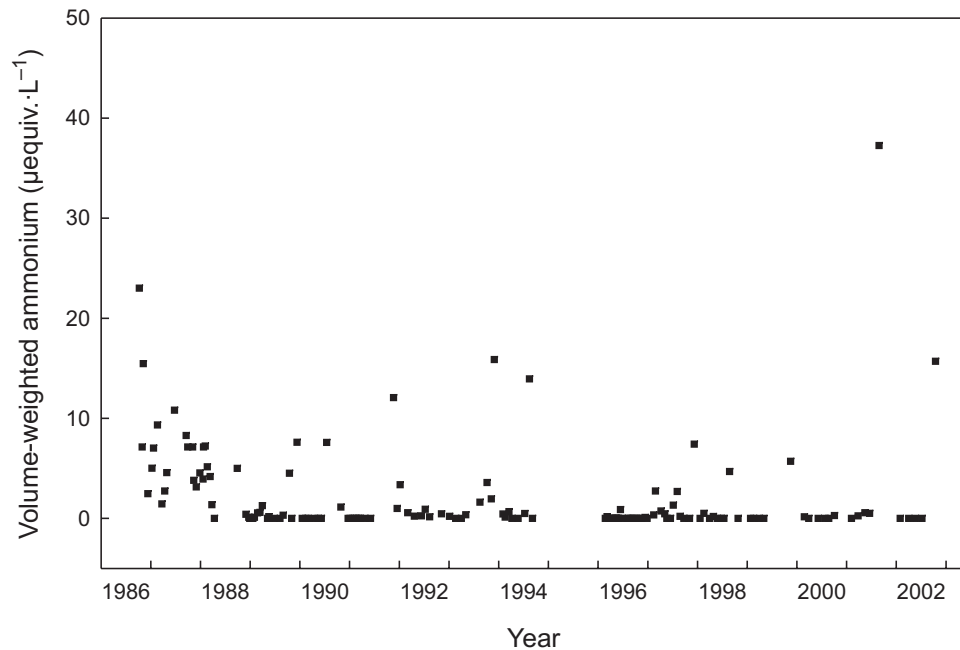
net retention of N throughout the study period. In WS3, the degree of net retention during the fertilization period decreased ( $p = 0.011$ ) through 1996 (Fig. 8), as expected under N saturation theory. In 1997, net retention increased slightly and then remained fairly constant ( $p = 0.953$ ) for the next 5 years (Fig. 8), in contrast to N saturation theory. This general pattern of retention agrees with the soil water results. In WS9, the degree of net N retention decreased slightly during both the fertilization (1987–1994;  $p = 0.084$ ) and the post-fertilization time periods (1995–2002;  $p = 0.084$ ) (Fig. 8),

meeting expectations of N saturation theory. These results are in contrast with the decreasing soil-water  $\text{NO}_3^-$  concentrations in WS9 (Fig. 5). However, watershed N budgets were based on stream water versus upland soil water responses, and we have demonstrated elsewhere (Edwards et al. 2006) that N responses in soil water and stream water behaved very differently in WS9. This inconsistent response between the uplands and stream system on WS9 may be attributable to its contrasting upland and riparian area characteristics. The 20 m wide buffer strip that was retained

**Fig. 5.** Volume-weighted mean concentrations of nitrate from the 46 cm deep lysimeters in WS9 with a locally weighted regression trend line overlaid.



**Fig. 6.** Volume-weighted mean ammonium concentrations from lysimeters in WS9.



**Table 3.** Mann–Kendall and Sen slope results for soil-water nitrate from 46 cm deep lysimeters in WS9.

Period	Mann–Kendall probability	Sen slope
1986 – Oct. 1990	0.000	12.44
Nov. 1990 – 1994	0.005	–7.01
1995–2002	0.524	0.35

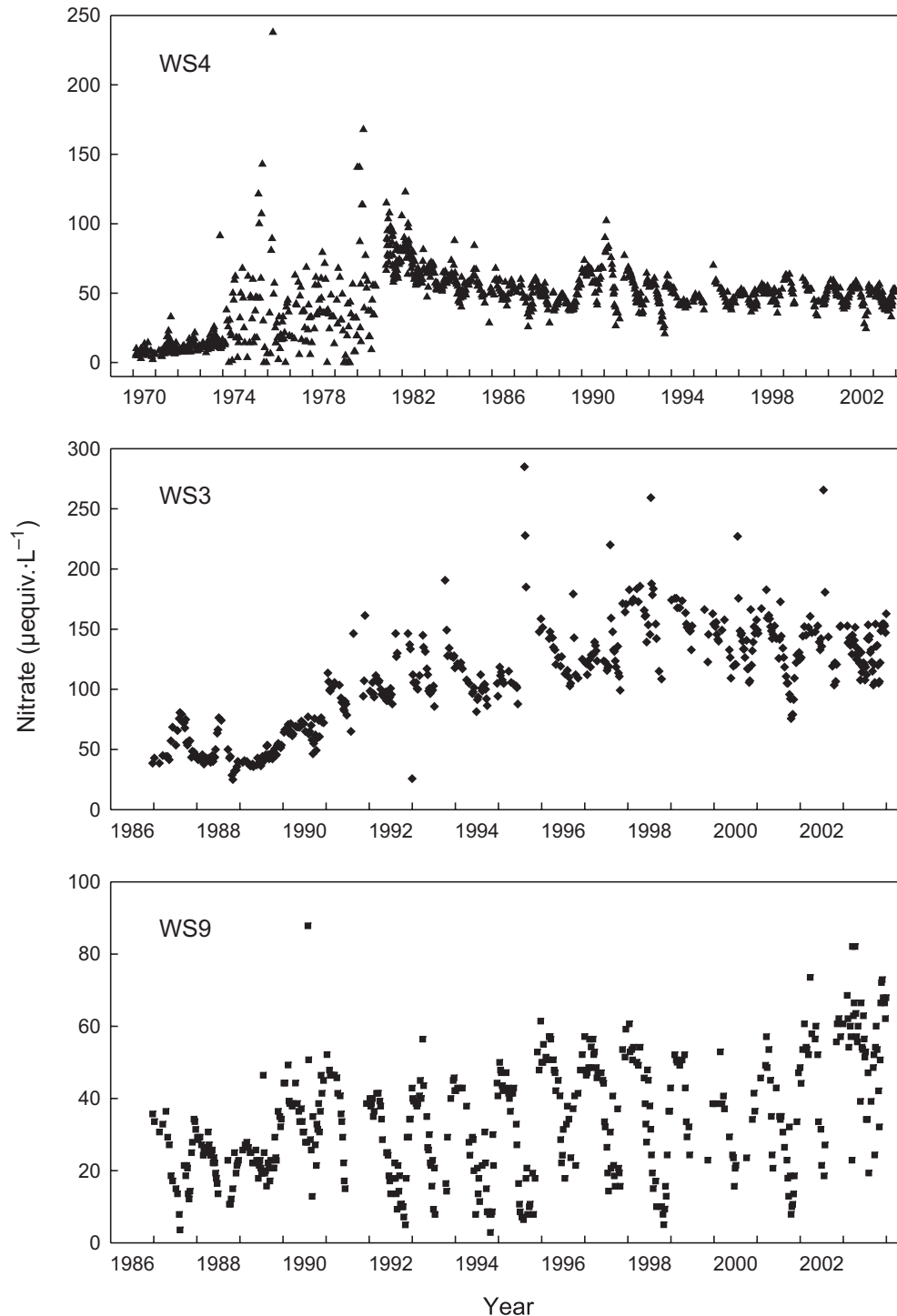
around each side of the entire stream length (including the ephemeral reaches) (Fig. 2) is composed of 80-year-old mixed hardwoods; the uplands support 20-year-old planted

larch. We do not know the mechanisms in the riparian area that are controlling stream-water chemistry, but if DOC is involved, the lower concentrations associated with hardwood leachate (Cronan and Aiken 1985; DeWalle et al. 1985) could reduce streamside  $\text{NO}_3^-$  retention via abiotic mechanisms and (or) denitrification, allowing greater  $\text{NO}_3^-$  release to stream water.

In the uplands, soil-water  $\text{NO}_3^-$  concentrations stopped increasing when they reached about  $400 \mu\text{equiv}\cdot\text{L}^{-1}$  on WS3 (Fig. 4) and slightly less than  $200 \mu\text{equiv}\cdot\text{L}^{-1}$  (though one mean value was  $\sim 350 \mu\text{equiv}\cdot\text{L}^{-1}$ ) on WS9 (Fig. 5). Larch is



**Fig. 7.** Weekly stream-water nitrate concentrations from WS4 (top), WS3 (middle), and WS9 (bottom). Note the time scale on the WS4 graph is longer than those for WS3 and WS9. Nitrate concentrations determined prior to 1981 on WS4 were determined by the Hach method (Hach Chemical Co. 1977). Concentrations in 1981 and later were determined by ion chromatography.



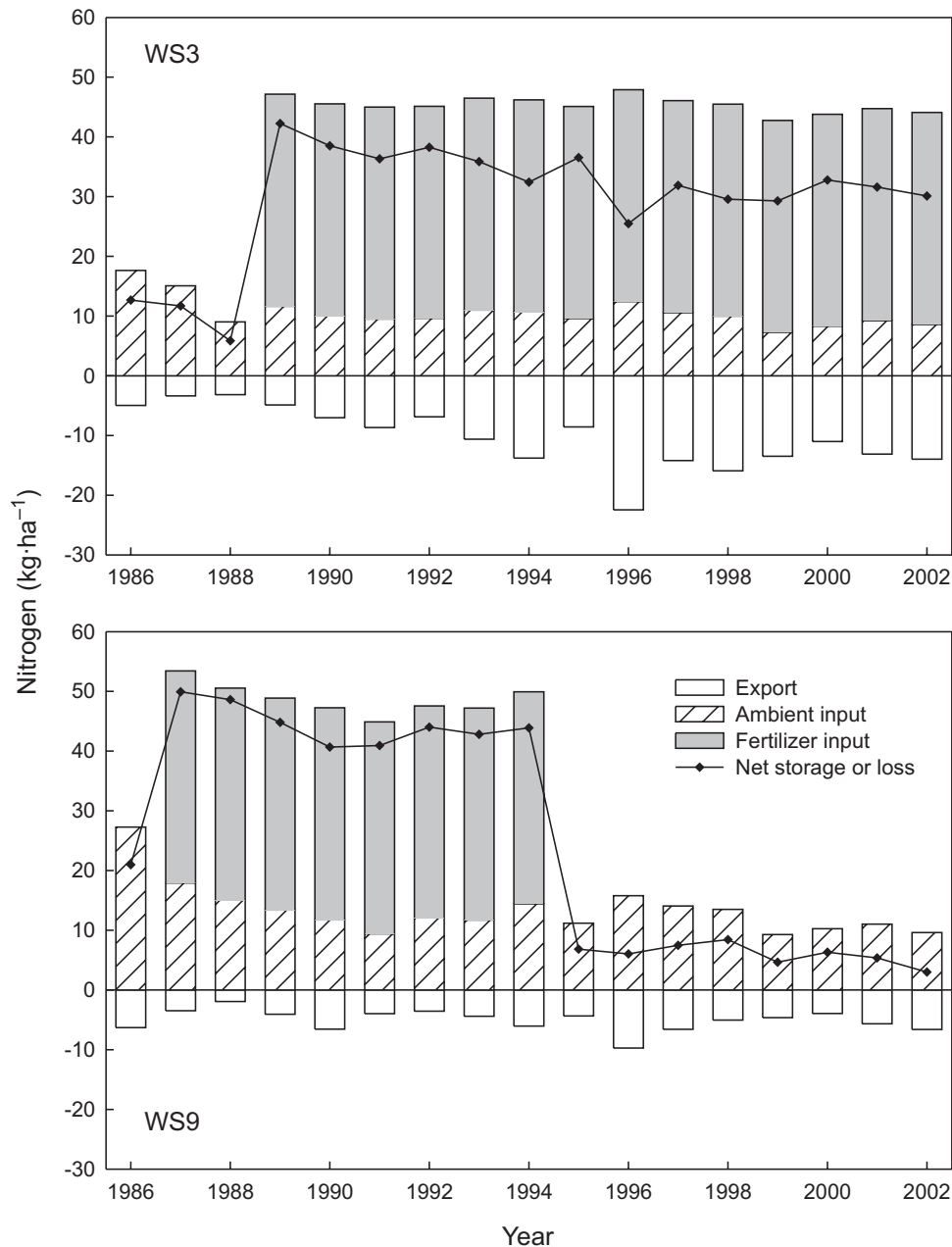
known to be a species that does not need nor thrive in high N conditions (VanGoor 1953), and DOC concentrations can be much greater under conifers than hardwoods (Cronan and Aiken 1985; DeWalle et al. 1985). Thus, greater DOC in soil water on WS9 could explain why retention of  $\text{NO}_3^-$  in the uplands occurred at lower  $\text{NO}_3^-$  concentrations on WS9 than WS3. On WS9, the last application of  $\text{NH}_4\text{SO}_4$  was made in 1994 and there was no evidence of increased re-

mobilization in the lysimeters through the end of data collection (Fig. 5). In terms analogous to sulfate desorption theory (Reuss and Johnson 1986),  $\text{NO}_3^-$  retention on WS9 would be characterized as completely irreversible.

## Conclusion

Two Appalachian watersheds that were fertilized with

**Fig. 8.** Nitrogen inputs and outputs for WS3 (top) and WS9 (bottom). Ambient inputs are wet + dry values; 1986–1988 dry values were not available so they were estimated from (wet + dry)/bulk ratios for 1989–2003.



$\text{NH}_4\text{SO}_4$  fertilizer over approximately 8 and 15 years to induce soil acidification are currently in stage 2 N saturation based on stream-water  $\text{NO}_3^-$  concentrations. Soil leachate indicates that after initially increasing in concentration for a number of years,  $\text{NO}_3^-$  experienced some type of retention. Current explanations of  $\text{NO}_3^-$  retention center on abiotic assimilation, which we believe is occurring in these watersheds. However, it is doubtful that N retention in the lower soil horizons is occurring by the specific mechanisms currently presented in the literature, as those mechanisms typically depend upon high levels of incorporated soil organic matter and reduction of metals, particularly iron and manganese. Because high levels of incorporated soil organic matter are not present in the lower soil horizons, we instead hy-

pothesize that recalcitrant DOC leached and stored by sorption on clay minerals in deeper soil provides an alternative source of carbon that then promotes abiotic retention of  $\text{NO}_3^-$  through reduction of metals. That  $\text{NO}_3^-$  retention can occur or be induced even as N is added to soil illustrates that N cycling processes and mobility controls are much more complex than current N saturation theory suggests.

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