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Indicators of nitrate export from forested watersheds of the mid-Appalachians, United States of America

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Abstract. Soil net nitrogen mineralization and nitrification rates were studied on nine undisturbed, forested watersheds in an effort to explain large variations in nitrate export in streamflow within the mid-Appalachian region. Rates of soil net nitrogen mineralization and net nitrification were measured in the upper 10 cm of mineral soil over a 5-week summer incubation period (June-July) using nine buried bags in each of the three major soil types on each watershed. Watersheds with high, medium, and low nitrate export rates exhibited high, medium, and low mean net nitrogen mineralization and net nitrification rates, respectively. Exchangeable calcium (an index to site fertility), C/N ratios, and soil moisture content together explained 63% of the variation in soil nitrogen mineralization rates, and exchangeable calcium and soil moisture content explained 61% of the variation in soil nitrification rates using multiple regression analysis. The variation in watershed nitrate export was best explained by total nitrogen in the upper 10 cm of mineral soil (explained 46%) and the percentage of mineralization due to nitrification (explained 42%). Estimated rates of wet and dry atmospheric deposition of nitrogen were not significantly correlated with watershed nitrate export. Results from this study demonstrate that soil nitrogen pools and dynamics are the most critical factors controlling nitrate export from forested watersheds in the mid-Appalachians. Long-term changes in site fertility, C/N ratios, and soil moisture, which largely control microbial nitrogen cycling, should have a significant effect on long-term trends in nitrate leaching.

1. Introduction

Historically, northern temperate forests were considered to be nitrogen-limited systems. All of the available nitrogen was thought to be incorporated into vegetation, soil microbial biomass, or soil organic matter allowing little opportunity for nitrogen export. Recently, this assumption has been challenged as substantial exports of nitrogen from forested watersheds have been discovered over extended periods of time [*Aber et al.*, 1989; *Driscoll et al.*, 1989; *Johnson and Lindberg*, 1992; *Murdoch and Stoddard*, 1992; *Stoddard*, 1994].

Wet and dry atmospheric nitrogen deposition, rates of soil net nitrogen mineralization and net nitrification, soil C/N ratios, and foliar nitrogen have been measured in order to explain the presence of high-nitrogen export from forested watersheds [*Foster et al.*, 1989; *Strader et al.*, 1989; *Friedland et al.*, 1991; *McNulty et al.*,1991; *Aber*, 1992; *Johnson and Lindberg*, 1992; *Morecroft et al.*, 1992; *Kahl et al.*, 1993; *Stevens et al.*, 1994; *Likens and Bormann*, 1995]. In the Integrated Forest Study, the most comprehensive North American study in terms of number of watersheds included (17 watersheds), *Johnson and Lindberg* [1992] found that relative rates of soil nitrogen mineralization explained 3 times more variation in nitrogen export from forested watersheds than atmospheric deposition or nutrient uptake by trees.

The primary objective of this study was to determine if the relationship between soil nitrogen mineralization and watershed nitrate export was apparent within the mid-Appalachian region of North America, where marked differences in nitrate export from forested watersheds are known to exist [*DeWalle and Pionke*, 1996]. High-nitrate export rates have been reported at the Fernow Experimental Forest Watershed 4 in West Virginia (5.12 kg ha^{-1} yr^{-1}) and Whiskey Hollow Run (4.30 kg ha^{-1} yr^{-1}) and Peapatch Ridge Run (4.70 kg ha^{-1} yr^{-1}) watersheds in northwestern Maryland, while low rates of nitrate export (0.041 - 0.21 kg ha^{-1} yr^{-1}) were found in north central Virginia and central Pennsylvania [*DeWalle and Pionke*, 1996]. Another objective of the study was to determine if wet and dry atmospheric deposition rates exhibited any relationship with reported nitrate export from the study watersheds. The study also sought to determine which of the possible controlling factors of soil nitrogen mineralization, C/N ratios of the soil, soil temperature, and soil moisture [*Stanford et al.*, 1973; *Stanford and Epstein*, 1974; *Pastor et al.*, 1984; *Hill and Shackleton*, 1989; *Johnson*, 1992; *Tietema et al.*, 1992; *Yin*, 1992; *Zak et al.*, 1993; *Stottlemyer et al.*, 1995], were the most important in these ecosystems. Exchangeable calcium, an index to site fertility, also was measured to determine if it had any control over soil mineralization. It is changes in the

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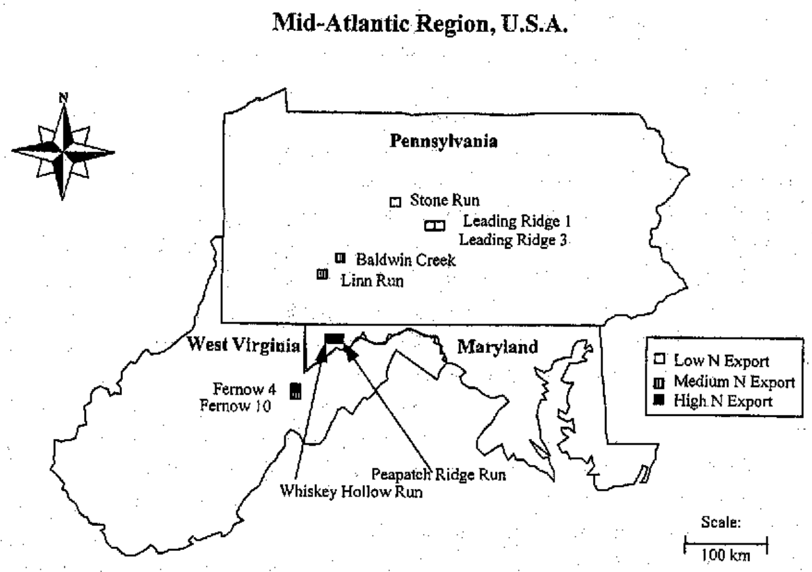

Figure 1. Watershed locations and nitrate-nitrogen export categories.

controlling factors of soil mineralization which could have the most impact on long-term trends in nitrogen export from forested watersheds.

2. Methods

2.1 Study Sites

The nine study watersheds (Figure 1) were placed into high-, medium-, and low-nitrate export categories based upon published data (Table 1). All study watersheds have mature forest stands (60-90 years old), except for Leading Ridge 3 which was cut 19 years prior to sampling. The watersheds contain primarily deciduous stands with oaks and maples predominating. All nine watersheds have residual stony soils, derived from shale and sandstone parent materials. The soils are classified as Typic Fragiaquults, Typic Dystrochrepts, Typic Hapludults, and Aquic Fragiudults and are either fine loamy or loamy skeletal in texture. Inputs of atmospheric wet and dry nitrogen deposition for the nine watersheds were estimated from published data (Table 1). Dry deposition inputs included only fluxes of NO_3 and HNO_3, resulting in an underestimate of the actual dry deposition of nitrogen.

2.2. Field Measurements

Three sampling transects were located on each watershed in the three most prevalent soil types to ensure representative sampling of the entire watershed. The sampling transects were stratified by watershed elevation (high, medium, and low). Net nitrogen mineralization and nitrification rates were determined by using the buried polyethylene bag technique in the top 10 cm of mineral soil [*Eno,* 1960; *Hart et al.,* 1994]. The buried bag technique [*Eno,* 1960] is a widely used and cost effective method of obtaining in situ indices of soil mineralization [*Matson and Vitousek,* 1981; *Nadelhoffer et al.,* 1983; *Pastor et al.,* 1984; *Hart and Firestone,* 1989; *Johnson and Lindberg,* 1992; *Aber et al.,* 1993; *Gilliam and Adams,* 1996]. The buried bag method is superior to laboratory incubations because of its sensitivity to on-site temperature and moisture regimes [*Binkley and Hart,* 1989].

The incubation period was 5 weeks long, from mid-June to late July. At each sampling transect, nine 10-cm-diameter x 10-cm-deep mineral soil cores were taken and incubated in zipper-lock polyethylene bags buried beneath the organic soil horizon. Nine cores were taken adjacent to the nine buried bags and composited by threes to yield three samples for the determination of preincubation levels of ammonium and nitrate in the soil. After the 5-week incubation period, the buried bags were recovered and composited by threes for analysis. Nine percent of the 243 bags used for in situ incubations were not analyzed because of bag disturbance and tearing caused by animals.

At each sampling transect, two additional cores were bagged and transported back to The Pennsylvania State University for soil moisture content measurements and C/N determinations of the top 10 cm of mineral soil. Exchangeable calcium also was determined on one of these additional cores, yielding one calcium concentration measurement per transect. A maximum-minimum thermometer was buried 2 cm deep at each transect to measure the maximum and minimum temperatures during the 5-week incubation period. The dominant overstory, woody understory, and ground cover species were noted at each sampling area.

2.3. Laboratory Measurements

Ammonium and nitrate analyses were performed at the Soil and Plant Analysis Laboratory, College of Agricultural Sciences, Pennsylvania State University. Samples were analyzed quickly with a maximum storage time of 3 days at 4°C. Nitrate was extracted from dry sieved soil with an ammonium sulfate and boric acid solution and analyzed with a specific ion electrode [*Griffin,* 1991]. Ammonium also was extracted from the dry sieved soil with 0.5 N potassium chloride solution and analyzed with a Technicon autoanalyzer [*Keeny and Nelson,* 1982]. Results were expressed as milligrams of NO_3-N and NH_4-N per kilogram of dry soil. Soil samples were analyzed for total C and N by combustion using a Carlo Erba elemental analyzer at the U.S. Forest Service Timber and Watershed Laboratory in Parsons, West Virginia [*Baccanti et al.,* 1993]. Soil moisture content was determined on a dry-weight basis in the laboratory [*Gardner,* 1965]. Exchangeable calcium was measured using the Mehlich 3

Table 1. Annual Mean Nitrate-N Watershed Export, Atmospheric Wet Deposition (1989-1994), and Atmospheric Dry Deposition (1990-1992) for the Nine Study Watersheds

Watershed	Area, km^2	Latitude	Longitude	Watershed Aspect	NO_3-N Export, kg ha^{-1} yr^{-1}		Wet Deposition NO_3-N + NH_4-N, kg ha^{-1} yr^{-1}	Wet Deposition Monitoring Station	Dry Dep.[g] NO_3-N, kg ha^{-1} yr^{-1}	Dry Deposition Monitoring Station
					Years	Mean				
					Low-Export Watersheds					
Leading Ridge 1	1.23	40°40'25"N	77°56'15"W	SE	1974-1987	0.04[a]	7.19[f]	Leading Ridge	3.17	University Park
Leading Ridge 3	1.04	40°39'45"N	77°57'15"W	SE	1974-1987	0.08[a]	7.19[f]	Leading Ridge	3.17	University Park
Stone Run	11.60	41°05'52"N	78°26'48"W	SE	1988	0.21[b]	7.61[f]	S.B. Elliot State Park	3.17	University Park
					Medium-Export Watersheds					
Fernow 10	0.15	39°03'15"N	79°40'45"W	S	1985-1994	1.20[c]	7.88[c]	Fernow 4	2.30	Parsons, W.Va.
Linn Run	10.00	40°08'40"N	79°12'37"W	NW	1988	1.97[b]	8.10[f]	Laurel Hill	3.00	Laurel Hill
Baldwin Creek	5.40	40°21'05"N	79°03'04"W	NW	1988	2.12[b]	8.10[f]	Laurel Hill	3.00	Laurel Hill
					High-Export Watersheds					
Whiskey Hollow	3.40	39°34'45"N	79°10'50"W	ENE	1990-91	4.30[d]	6.86[c]	Parsons, W.Va.	2.30	Parsons, W.Va.
Peapatch Ridge	1.80	39°33'45"N	79°08'35"W	SW	1989-91	4.70[d]	6.86[c]	Parsons, W.Va.	2.30	Parsons, W.Va.
Fernow 4	0.39	39°03'15"N	79°41'20"W	ESE	1984-94	5.12[e]	7.88[c]	Fernow 4	2.30	Parsons, W.Va.

[a] *Lynch and Corbett* [1991].

[b] *Dow* [1992].

[c] P. Edwards, U.S. Forest Service, Parsons, W.V. - written communication, 1995.

[d] R. Morgen and K. Eshleman, Appalachian Environmental Laboratory, Frostburg, MD - written communication, 1994.

[e] *Adams et al.* [1993].

[f] *Lynch et al.* [1989-1994].

[g] *Environmental Science and Engineering, Inc.* [1995].

method [*Wolf and Beegle,* 1991]. The maximum-minimum thermometers were calibrated to 1°C using an Ertco certified thermometer.

2.4. Statistical Methods

A log transformation on the raw data plus a constant (5) was performed to stabilize the error variance and make the error terms homogeneous. All subsequent statistical procedures were performed on log-transformed data. A nested analysis of variance was performed on export categories, watersheds within export categories, and sampling locations within watersheds and export categories. Differences among export categories and watersheds within export categories were tested using the least significant difference (LSD) mean separation procedure [*SAS Institute Inc.,* 1985] which was applied to each dependent variable: net mineralization (Net_{min}), net nitrification (Net_{nit}), net nitrification/net mineralization expressed as a percentage (Nit/Min), percentage of C in the soil (C), percentage of N in the soil (N), C/N ratio (C/N), exchangeable calcium (Ca), maximum temperature (T_{max}), minimum temperature (T_{min}), maximum-minimum temperature (T_{range}), and percentage of soil moisture (M_s) (n=72-81).

Correlation coefficients were calculated between the $\log_{10}$ transformed dependent variables, C, N, C/N, Ca, M_s, T_{max}, T_{min}, and T_{range} to find which variables were correlated with Net_{min} and Net_{nit}. Models predicting Net_{min} and Net_{nit} from C, N, C/N, Ca, M_s, T_{max}, T_{min}, and T_{range} were built using a maximum r-square improvement (stepwise multiple regression) technique [*SAS Institute Inc.,* 1985]. Variables included in the multiple regression models also were run against Net_{min} and Net_{nit} in single parameter regression models. Correlation tests between the mean watershed values for the dependent variables (including wet and dry nitrogen deposition) and mean watershed nitrate-N export were also run. A linear regression model was developed for each dependent variable which had a significant correlation (α = 0.05) with watershed nitrate export.

3. Results and Discussion

3.1. Net Nitrogen Mineralization and Net Nitrification Rates

The mean net N mineralization and net nitrification rates for the three export categories were significantly different from one another using an LSD mean separation procedure (α=0.05). The high-, medium-, and low-export categories exhibited high, medium, and low mean mineralization and nitrification rates, respectively (Table 2). Net N mineralization rates (0.17 - 7.42 mg kg^{-1} week^{-1}) and nitrification rates (0.17 - 7.42 mg kg^{-1} week^{-1}) in the top 10 cm of mineral forest soil of the nine watersheds were within the range found by other researchers [*Mladenoff,* 1987, *Persson and Wiren,* 1995]. Results for Fernow watershed 4 also were similar to the monthly buried bag results obtained by *Gilliam and Adams* [1996] on Fernow 4.

Baldwin Creek and Peapatch Ridge Run were exceptions to the observed relationship between N mineralization and nitrification rates and nitrate export (Figure 2). Baldwin Creek and Peapatch Ridge Run exhibited relatively low net N mineralization and nitrification rates even though export data placed them in the medium- and high-export categories,

Table 2. Mean Values of Net Nitrogen Production, Carbon and Nitrogen Content, Moisture, Exchangeable Calcium, and Temperature of the Upper 10 cm of Mineral Soil in the Nine Study Watersheds

Watershed	Net N Mineralization, mg kg^{-1} week^{-1}	Net Nitrification, mg kg^{-1} week^{-1}	Nitrification / Mineralization, %	C in Soil, %	N in Soil, %	C/N Ratio	Exchangeable Ca, meq 100 g^{-1}	Soil Moisture, % Water Content	Maximum Soil Temperature, °C	Minimum Soil Temperature, °C
Leading Ridge 1	1.14	0.35	36.40	3.026	0.174	16.562	0.37	32.37	24.3	14.1
Leading Ridge 3	0.96	0.17	26.64	2.575	0.152	17.004	0.37	19.76	24.0	15.0
Stone Run	0.75	0.34	45.70	2.473	0.169	14.179	0.30	29.43	24.6	11.9
Low export	0.95 a	0.29 a	36.25 a	2.691 a	0.165 a	15.915 ab	0.35 a	27.19 a	24.3 a	13.67 a
Fernow 10	4.64	4.32	93.00	4.496	0.302	14.812	0.77	34.77	22.6	13.9
Linn Run	2.76	2.00	73.35	9.698	0.508	18.968	0.37	47.67	22.6	13.0
Baldwin Creek	0.17	0.23	117.08	6.655	0.303	21.702	0.30	28.10	20.1	14.3
Medium export	2.52 b	2.18 b	94.48 b	6.950 c	0.371 b	18.494 a	0.48 a	36.85 b	21.77 b	13.73 ab
Whiskey Hollow	6.26	4.44	70.01	4.024	0.306	13.292	1.20	29.30	22.0	13.0
Peapatch Ridge	1.03	0.53	40.93	4.054	0.266	15.291	0.63	28.85	22.8	12.8
Fernow 4	7.42	7.42	108.33	5.897	0.432	13.220	0.77	47.76	24.8	13.1
High export	4.90 c	4.13 c	73.09 b	4.658 b	0.335 b	13.934 b	0.87 b	35.3 b	23.2 ab	12.97 ac

Export category means with different letters are significantly different at α=0.05 using the least significant difference mean separation procedure.

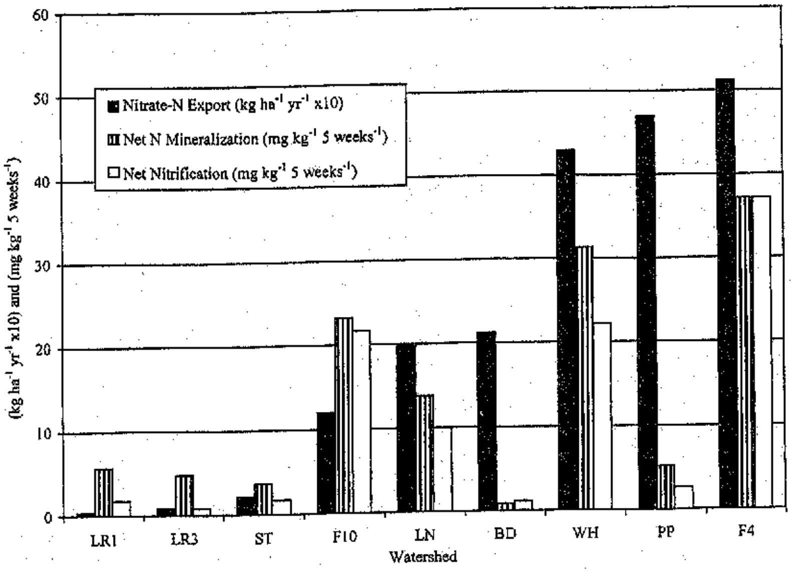

Figure 2. Mean nitrate-nitrogen export, net nitrogen mineralization, and net nitrification rates for the nine study watersheds. LR1, Leading Ridge 1; LR3, Leading Ridge 3; ST, Stone Run; F10, Fernow 10; F4, Fernow 4; LN, Linn Run; BD, Baldwin Creek; WH, Whiskey Hollow Run; and PP, Peapatch Ridge Run.

respectively. These low net mineralization and nitrification rates can be explained, in part, by the low soil moisture contents on Baldwin Creek and Peapatch Ridge Run which were lower than for all other medium- and high-export watersheds (Table 2). Lower soil moisture contents affect mineralization and nitrification rates by depressing microbial activity. Normal soil moisture regimes could well be higher than the measured soil moisture contents, resulting in greater mineralization and nitrification rates which would fit the observed nitrate exports.

Another explanation for the lack of fit to the generalized relationship is that the mineralization and nitrification rates at the sampling sites on Baldwin Creek and Peapatch Ridge were not representative of the two watersheds. There could have been significant areas with higher soil mineralization and nitrification that were not sampled. Unmeasured atmospheric nitrogen deposition could also help explain how low nitrification rates could support such high nitrate export. Baldwin Creek may receive greater amounts of atmospheric nitrogen deposition than measured by the Laurel Hill monitoring station (Table 1), because of a coal-fired electric generation plant located only 3 km north of the watershed. Differences in nitrogen fixation by free-living soil microorganisms between Baldwin Creek and Peapatch Ridge Run and the other watersheds could also exist. Vast differences in nitrogen fixation, which can be a significant contributor to nitrogen pools [*Dawson*, 1993; *Likens and Bormann*, 1995], can occur among forested watersheds [*Swank and Crossley*, 1988].

Interestingly, Fernow watershed 10 is a medium-exporting watershed and Fernow watershed 4 is a high-exporting watershed, even though the two watershed outlets are only 580 m apart. Fernow 10 had lower mineralization and nitrification rates than Fernow 4 which could help cause the difference in export. These lower mineralization and nitrification rates could be explained by Fernow 10's lower soil moisture content and higher C/N ratio than Fernow 4 (Table 2). Soil moisture contents and C/N ratios are discussed in detail later in the text.

3.2. Ratio of Net Nitrification to Net Mineralization

Ratios of net nitrification/net mineralization explained 42% of the variation in nitrate exports from the nine watersheds based on linear regression between the two $\log_{10}$-transformed variables (Table 3). *Van Miegroet et al.* [1992] reported that soil mineralization rates were the single most important factor in explaining the variation in nitrate leaching (explained 44%) from the 17 Integrated Forest Study sites. Even though the ratio of net nitrification/net mineralization is not equal to net mineralization rates, it demonstrates that microbial cycling of nitrogen is a critical determinant of nitrate export.

Mean net nitrification/net mineralization ratios for the medium- and high-export categories, 100% and 74%, respectively, were significantly greater than for the low-export category (31%) using an LSD mean separation procedure at $\alpha = 0.05$ (Table 2). According to *Aber*'s [1992] hypothesis, lower net nitrification/mineralization ratios on the low-export watersheds indicate soils on these basins are more nitrogen-limited. Either the ammonifying bacteria are not supplying the nitrifying bacteria with sufficient ammonium, or the immobilizing bacteria are actively consuming the nitrate produced by nitrifying bacteria.

In this study, Fernow 4 soil exhibited a 100% nitrification/mineralization ratio which agrees with the calculated nitrification/mineralization ratio of 90% for Fernow 4 soil [*Gilliam and Adams*, 1996]. A high nitrification/mineralization ratio implies that there is an ample supply of ammonium available to nitrifying bacteria and that the nitrifying bacteria are producing far more nitrate than bacteria can immobilize.

Baldwin Creek had a nitrification/mineralization percentage over 100%, meaning net nitrification was greater than net mineralization. Net losses of ammonium from volatilization, substantial microbial immobilization, and/or ammonium fixation during the incubation period could explain the lower mineralization values. Sampling and soil analysis errors also

Table 3. Regression Equations of Nitrate Export, Net Mineralization, and Net Nitrification

Parameter	Equation	R^2_{adj}	Probability > F
Nitrate export	$\log_{10} NO_3$ export^a = $58.51 \log_{10}$ %N - 42.38	0.46	0.03
	$\log_{10} NO_3$ export = $2.46 \log_{10}$ Nitr/Min (%) - 4.44	0.42	0.04
Net mineralization	$\log_{10} Min^b = -1.18 \log_{10} C/N + 0.39 \log_{10} [Ca]^c + 0.98 \log_{10} SM^d + 0.97$	0.63	0.0001
	$\log_{10} Min = 0.59 \log_{10} [Ca] + 0.83$	0.46	0.0001
	$\log_{10} Min = 0.58 \log_{10} SM - 4.63$	0.20	0.0001
	$\log_{10} Min = -1.90 \log_{10} C/N + 3.65$	0.17	0.0001
Net nitrification	$\log_{10} Nitr^e = 0.54 \log_{10} [Ca] + 1.13 \log_{10} SM - 1.01$	0.61	0.0001
	$\log_{10} Nitr = 0.61 \log_{10} [Ca] + 0.72$	0.45	0.0001
	$\log_{10} Nitr = 1.32 \log_{10} SM - 0.99$	0.26	0.0001

[a] Nitrate export is given in kg ha^{-1} yr^{-1}.

[b] Net mineralization rate is given in mg kg^{-1} $week^{-1}$.

[c] Calcium concentration is given in meq 100 g^{-1}.

[d] Soil moisture content is given in percent dry weight.

[e] Net nitrification rate is given in mg kg^{-1} $week^{-1}$.

could have been magnified for Baldwin Creek's low mineralization rates (0.17 mg kg^{-1} $week^{-1}$).

3.3. Exchangeable Calcium

Exchangeable calcium served as an index to site fertility in this study. It had the most control over net mineralization ($r^2 = 0.46$) and net nitrification rates ($r^2 = 0.45$) of any of the measured variables (Table 3). Mean exchangeable calcium on the high-export watersheds was significantly greater than on the medium- and low-export watersheds (Table 2), indicating that site fertility is related to nitrate leaching. These results are supported by *DeWalle et al.* [1988], who demonstrated that nitrate in soil leachate was significantly greater from a fertile watershed (Fernow 4), in terms of calcium concentrations, than from a nonfertile watershed. Calcium is an essential nutrient for microorganisms, and it has been found to limit the activity of some nitrogen-fixing bacteria species [*Alexander*, 1977] which could substantially decrease nitrogen available to nitrifiers.

3.4. Soil Moisture Content

Soil moisture was another important factor for predicting net mineralization and net nitrification rates during the 5-week period. Soil moisture explained 20% of the variation in net mineralization rates and 26% of the variation in net nitrification rates (Table 3). Mean soil moisture on the medium- and high-export watersheds were significantly greater (α=0.05) than on the low-export watersheds (Table 2), which is expected given the positive correlation between soil moisture and net N mineralization. Previous researchers also found a positive correlation between soil moisture and in situ measurements of net mineralization [*Hill and Shackleton*, 1989; *Tietema et al.*, 1992; *Stottlemyer et al.*, 1995]. *Stanford and Epstein* [1974] and *Stottlemyer et al.* [1995] found a positive correlation between soil moisture and N mineralization from laboratory incubations for unsaturated soil moisture conditions (<25-35% dry weight).

3.5. Soil Temperature

Mean separation procedures on minimum temperatures, maximum temperatures, and temperature ranges did not yield any consistent significant differences among the three export categories (Table 2). This may be due to the fact that sampling was performed during June and July when soil temperatures were warmed similarly across the sampling region. Watershed aspect did appear to affect soil temperatures, as the three north facing watersheds (Linn Run, Baldwin Creek, and Whiskey Hollow Run) (Table 1) all had as low or lower maximum soil temperatures than the other watersheds.

3.6. Soil C, N, and C/N Ratios

The percentage of N in the upper 10 cm of mineral soil explained the most variation (46%) in watershed nitrate export of the $\log_{10}$-transformed variables tested (Table 3), even though soil N pools are normally thought to control N mineralization and nitrification rates more directly than nitrate export. C/N ratios did not vary as expected among watershed export classes. An LSD mean separation procedure (Table 2) revealed that the only significant differences in mean C/N were between the medium- and high-watershed-nitrate-export categories. The high-export category had the lowest C/N ratio. C/N ratios of watershed soils were correlated negatively with net mineralization rates, explaining 17% of the variation in net mineralization rates (Table 3). *Pastor et al.* [1984] also found a high negative correlation between the C/N ratio of litter and N mineralization rates.

Riha et al. [1986] suggested that high nitrogen exports should be associated with low C/N ratios based on the premises that heterotrophic bacteria are the most successful short-term competitors for N [*Johnson and Edwards*, 1979; *Schimel and Firestone*, 1989], and heterotrophic demand for N is determined by available C. Thus substrates with less available C create lower heterotrophic bacterial demand for ammonium, and more ammonium is available to be nitrified [*Riha et al.*, 1986]. In turn, more nitrate can be exported in streamflow. At higher C/N ratios, heterotrophic demand for N is greater, and less N is available to nitrifying bacteria. Subsequently, less nitrate is available for leaching and export.

Alexander [1977] reported that at C/N ratios >30 net immobilization of nitrate occurs, while at C/N ratios <20 net mineralization occurs. At C/N ratios of 20-30, net immobilization or net mineralization can occur, depending on the amount of N available to nitrifiers. In this study, except for Baldwin Creek, all of the average C/N ratios were below 20. This finding supports the hypothesis that net mineralization generally occurs at C/N ratios <20, since all the watersheds with a C/N ratio below 20 had net N mineralization rates greater than 3 mg kg^{-1} 5 weeks^{-1}. Net mineralization <1 mg kg^{-1} 5 weeks^{-1} on Baldwin Creek can be explained in part by its higher C/N ratio (C/N = 21.702). Baldwin Creek had a percentage of C mean of 6.655%, second highest only to Linn Run (Table 2), which could create a higher heterotrophic demand for the available N. The soil nitrogen on Baldwin Creek (0.303%) was lower than on Fernow 4 (0.432%) and Linn Run (0.508%), which both had greater mineralization rates. Peapatch Ridge Run also had relatively low soil nitrogen (0.266%) which may help explain its low mineralization rates.

Differences in N mineralization and nitrification rates between Fernow 4 and 10 can be attributed in part to the lower C/N ratio (13.22) in Fernow 4 soils. *Gilliam and Adams* [1996] measured a C/N ratio of 19.9 in the upper 5 cm of mineral soil on Fernow 4. A higher C/N ratio would be expected in the upper 5 cm than in the 10 cm of mineral soil, since the percentage of C generally decreases with soil depth [*Craul*, 1964]. In the Integrated Forest Study [*Johnson and Lindberg*, 1992], mineral soil was sampled to a depth of 10 cm, and 15 out of the 21 sites sampled across North America had C/N ratios <20, agreeing with the relatively low C/N ratios found in our study.

3.7. Nitrogen Deposition

In the current study, total nitrogen deposition (wet and dry nitrate-N and wet ammonium-N) (Table 1) received by the nine watersheds was not significantly correlated with nitrate-N export from the watersheds. The nine watersheds received similar levels of nitrogen deposition, from 9.16 to 11.1 kg ha^{-1} yr^{-1}. The atmospheric deposition estimates should be interpreted with caution, since only two of the nine nitrogen deposition values were obtained from monitoring stations located within the respective watershed boundaries.

Other researchers have found that nitrogen deposition is not an important factor in explaining variations in nitrate export. In the Integrated Forest Study [*Van Miegroet et al.*, 1992], nitrogen input from atmospheric deposition was the least important regulating factor, explaining only 11% of the variation in nitrate leaching from the 17 forested watersheds. *Foster et al.* [1989] also found that nitrogen levels in the soil were more important than atmospheric nitrogen deposition in determining solution chemistry in the Turkey Lakes Watershed, Ontario.

4. Conclusions

High nitrate export from nine watersheds in the Appalachians of Pennsylvania, Maryland, and West Virginia appears to be explained, in part, by high soil N pools (46% variation explained) and high ratios of net nitrification/net N mineralization rates (42% variation explained) in the upper 10 cm of mineral soil. High rates of N mineralization and nitrification are associated with high exchangeable calcium concentrations, low soil C/N ratios, and high soil moisture. Available data suggest that atmospheric wet plus dry nitrogen deposition rates are not causing the variations in nitrate exports observed in these forested streams. The study results illustrate that soil microbial conversions and utilization of nitrogen is, indeed, the major determinant of nitrate leaching across the existing broad range of nitrate export from mid-Appalachian forested watersheds. Gradual changes in factors that affect microbial nitrogen dynamics, such as soil C and N pools, exchangeable calcium, and soil moisture, should have significant effects on the long-term status of nitrate leaching from forested ecosystems.

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