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Decadal responses in soil N dynamics at the Bear Brook Watershed in Maine, USA

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Abstract

Atmospheric nitrogen deposition to forested ecosystems is a concern because of both geochemical and biological consequences for ecosystem integrity. High levels of prolonged N deposition can lead to "N saturation" of the ecosystem. The Bear Brook Watershed in Maine is a long-term, paired forested watershed experiment with over a decade of experimental N additions ($\sim$34 kg ha^{-1} per year = ambient + treatment) to investigate the biogeochemical consequences of N saturation. Both in situ and laboratory studies of N mineralization and nitrification were carried out to evaluate the changes in N cycling brought about by the long-term N additions. Consistent with hypotheses set forth in the literature (sensu [BioScience 39 (1989) 378]), the treated watershed had higher rates of N cycling compared to the reference watershed. In addition, we report important differences in N cycling rates as a function of forest cover type and soil horizon. Higher rates of net N mineralization occurred in hardwood O horizons compared to softwoods, but the opposite was true in the mineral soils suggesting an important link between litter type and N mineralization that varies with depth in the pedon. Nitrification showed the greatest response to N treatments, with the majority of mineralized N subsequently oxidized to nitrate in the mineral soils. By comparing the data herein with that previously reported for the Bear Brook experiment, it appears that the ecosystem response to N treatment continues to evolve on a decadal time scale and inherent differences in forest cover types and their underlying soils alter the fate of depositional N.

Keywords: Nitrogen cycling; Nitrogen mineralization; Nitrification; N saturation

1. Introduction

Over the past century atmospheric N deposition has increased across both Europe (Gunderson, 1995; Dise and Wright, 1995) and the northeastern US (Galloway et al., 1995; Aber et al., 1993), and this is largely due to human activities including fossil fuel combustion and changes in agricultural practices. Although N is considered to be limiting to growth in most forest ecosystems throughout the world (Schlesinger, 1997), it can also be considered a major pollutant (Torseth and Semb, 1997). Nitrogen in excess of biological demand can lead to soil acidification, which in turn can cause root damage, thereby reducing the ability of plants to take up nutrients (Henriksen and Hessen, 1997). Other consequences of elevated N deposition include: acid fog damage and spruce decline (McNulty et al., 1996), soil aluminum (Al) mobilization (Lawrence and David, 1997), mercury (Hg) accumulation in fish (Driscoll et al., 1994), increased

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NO_3-N leaching resulting in acidification of streams, eutrophication of estuaries and coastal waters (Murdoch and Stoddard, 1992; Henriksen and Hessen, 1997), and NO_3-N contamination of drinking water (Spalding and Exner, 1993).

Most forest soils not influenced by high N deposition are N-limited, and contain small amounts of exchangeable inorganic N as compared to the pool of organic N in the forest floor. The inorganic N pool is usually dominated by NH_4-N, which is made available via mineralization from the organic pool and is then quickly utilized by plants, microbial communities or adsorbed to cation exchange sites. This can result in little NH_4-N being leached or available for nitrification. However, once assimilatory needs have been met, additional NH_4-N may be utilized by bacterial nitrifier communities that oxidize NH_4-N to NO_3-N thereby fixing atmospheric CO_2 with the energy gained. This NO_3-N can then be leached from the soil owing to its low retention by most soils. Under conditions of low oxygen availability, denitrification can convert much of the NO_3-N to N_2O or N_2 (Aber, 1993; McNulty et al., 1996; Mohn et al., 2000).

Nitrification and N mineralization rates are one among many microbial processes that have been studied to assess the effects of site factors (Christ et al., 2002) as well as ecosystem disturbances such as fertilization (Gilliam et al., 1996; McNulty and Aber, 1993), harvesting (Fenn et al., 1998; Frazer et al., 1990), historical land use and related disturbances (Goodale and Aber, 2001; Goodale et al., 2000) and climate (Mitchell et al., 1996a; Pastor and Post, 1988; Rustad et al., 2000; Peterjohn et al., 1994). Nitrogen mineralization has also been used to evaluate forest succession (Gower and Son, 1992) and the effects on forest types (Cole and Rapp, 1981; Campbell et al., 2000; Peterjohn et al., 1999) on ecosystem processes. A number of researchers have found O horizon soils under hardwoods have significantly higher rates of N mineralization compared to O horizon soils under softwoods (Campbell et al., 2000; Aber et al., 1993; Finzi et al., 1998; Ferrari, 1993). This has been attributed to differences in soil moisture, pH, temperature, carbon to nitrogen (C/N) ratios and biotic mechanisms related to competition between plants and microbes (Campbell et al., 2000; Aber et al., 1993). Campbell et al. (2000) and Aber et al. (1993) have hypothesized that the lower N cycling rates observed in softwoods could indicate that softwoods might be more sensitive to deleterious effects of increased N deposition than hardwoods.

Long-term elevated N deposition can result in N saturation at a site where N availability is chronically in excess of microbial and plant demand (Aber et al., 1989; Ågren and Bosatta, 1988). Nitrogen saturation is manifested in an ecosystem by initial increases in net N mineralization (NNM) rates (thought to be a fertilization effect) followed by sharp declines. These declines in N mineralization parallel increases in net nitrification (NN) rates and are often followed by declining tree growth as N saturation conditions develop (Aber et al., 1998).

Since many of Europe's ecosystems have progressed further on the N saturation continuum than in North America, researchers there have provided valuable insights into the progression of N dynamics during the evolution of N saturation. The EXperimental MANipulation of Forest Ecosystems project in Europe (EXMAN) (Rasmussen et al., 1990) and the NITRogen Saturation EXperiments in Europe (NITREX) (Dise and Wright, 1992) addressed the effects of increased N deposition on biogeochemical cycling in European coniferous forests. In both Europe and North America the most commonly used indicator of the N status of a site is stream NO_3-N export (e.g. Aber et al., 1993; Adams et al., 1997; Andersson et al., 2002). However, stream chemistry is a function of internal watershed processes such as N mineralization and nitrification in soils. The objectives of this research were to determine the effects of increased N deposition on NNM and NN at the Bear Brook Watershed in Maine (BBWM) after more than a decade of experimental whole-watershed N enrichment.

2. Materials and methods

2.1. Site description

The BBWM is located in eastern Maine at 44°52′N latitude and 68°06′W longitude, approximately 60 km from the coast of Maine (Fig. 1), situated in the upper 210 m of the southeast slope of Lead Mountain. BBWM is a paired watershed experiment that began in 1987. The study was established to evaluate

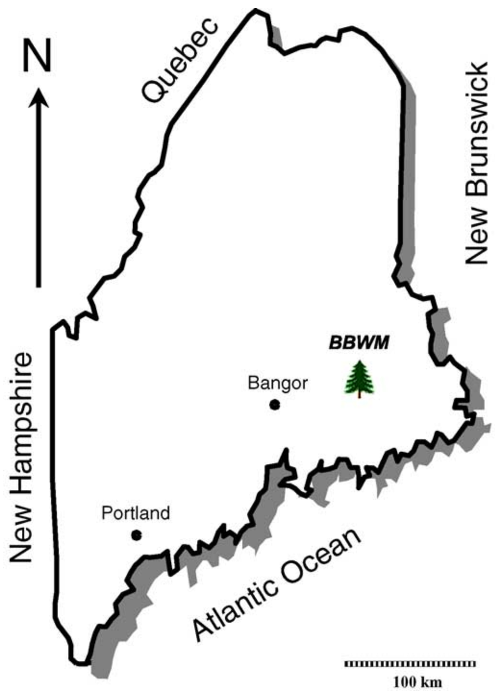

Fig. 1. The geographic location of the BBWM.

whole-ecosystem response to elevated N and S deposition in a low alkalinity forested stream watershed in northern New England (Norton et al., 1999), utilizing the paired watershed approach (Likens et al., 1977). Both watersheds are topographically similar (Wang and Fernandez, 1999) and had similar patterns of output fluxes for elements prior to manipulation (Norton et al., 1999). The East Bear watershed is 11.0 ha while West Bear is 10.3 ha. A first order stream drains each watershed with an average slope from the top of the watershed to the weirs of 31% (Norton et al., 1999).

The vegetation at BBWM includes both hardwoods and softwoods, with hardwoods and mixed woods dominating the lower ~60% of the watersheds. Hardwoods include American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), with minor yellow birch (*Betula alleghaniensis* Britt.) and white birch (*Betula papirifera*). The hardwood forest is successional following logging prior to 1945 (Wang and Fernandez, 1999). The upper areas of the watersheds are nearly pure softwood stands 80–120 years old including red spruce (*Picea rubens* Sarg.), balsam fir (*Abies balsamea* L.) and hemlock (*Tsuga canadensis* L. Carr). Softwood, mixed wood, and hardwoods cover approximately 25, 40, and 35% of the total watershed areas, respectively (Wang and Fernandez, 1999).

The soils are acidic, have low base saturation, cation exchange capacity, and sulfate adsorption capacity (Norton et al., 1999). Bedrock geology consists of metamorphosed quartzites and calc-silicate gneiss. Further details of the study site can be found in Norton et al. (1999) and Fernandez and Adams (2000).

Nitrogen additions to the West Bear watershed were initiated in 1989 and consisted of bimonthly additions of dry $(NH_4)_2SO_4$, typically with two applications to the snowpack, two during the growing season, one in the spring and one in the fall. The West Bear watershed receives 25.2 kg N ha^{-1} per year of N treatments resulting in estimated total N inputs (wet + estimated dry + treatment) of 33.6 kg N ha^{-1} per year. The reference East Bear watershed receives 8.4 kg N ha^{-1} per year of ambient wet plus estimated dry deposition (Norton et al., 1999).

2.2. Experimental design

Within each watershed, four 10 m × 10 m plots were established with two of the four plots in each watershed in hardwoods and two in softwoods. Plots were chosen to have comparable slopes, dominant tree species, and proximity to streams between watersheds. Four replicate soil samples were collected from each 10 m × 10 m plot on five different dates (19 September 2000, 11 and 12 June 2001, 2 July 2001, 6 and 7 August 2001 and 17 September 2001). In situ incubations varied for logistical reasons by sampling period and were 35, 28, 21, 14 and 14 days, respectively. Soil sampling depth increments included the O horizon and the uppermost 15 cm of the B horizon using a 15 cm × 15 cm frame for sampling, excluding the E horizon when present. At the laboratory, samples were sieved and divided into three subsamples. Subsamples were numbered and used for (1) "time zero" or the initial NO_3-N and NH_4-N extractions prior to incubations, (2) the 14-day laboratory incubations, or (3) in situ NNM for 14–35 days according to Eno (1960) by placing them in labeled polyethylene bags and re-burying them.

Soils were transported on ice and stored at 4 °C prior to extraction. Soil extractions were completed within 24 h of collection. Extracts were frozen until they could be analyzed for NH_4-N and NO_3-N. Analyses were conducted by comparable methods at both the Institute of Ecosystem Studies (IES) in Millbrook, NY and the University of Maine's Analytical Laboratory. A subset of samples were analyzed at both laboratories to assure data quality with excellent agreement between facilities.

2.3. NNM and NN

NNM and NN were assessed using both a 14-day laboratory incubation (Hart et al., 1994) and an in situ method (Eno, 1960). Methods were chosen to estimate NNM and NN rates in the field (in situ method) and the substrate potential for NNM and NN under the controlled temperature of a laboratory incubation (14-day laboratory incubation method). While laboratory incubations do not measure actual rates of N processes under field conditions, they are widely used as an index of N dynamics in forest soils for practical reasons. NNM is defined as the difference between the sum of NO_3-N and NH_4-N at the initiation (e.g. time $= 0$) of incubation and at some final point in time, while NN is the difference for NO_3-N alone. Field moist O horizon soils were sieved through a 6 mm mesh sieve and mineral soils were sieved through a 2 mm mesh sieve. A 15 ± 0.05 g subsample of field moist soil was placed in a polycarbonate cup and incubated in the dark at ~22 °C for 14 days. Another ~5 g subsample of field moist soil was used to determine oven-dry moisture content (O horizon soils were dried at 65 °C and mineral soils were dried at 105 °C). At the initiation of the experiment, time zero subsamples were extracted immediately with 100 ml of 2 M KCl to determine initial NO_3-N and NH_4-N concentrations. After 14 days of laboratory incubation or 14–35 days of in situ incubation, soils were extracted as above. Soil pH was determined in deionized water according to Hendershot et al. (1993) for samples collected in September 2000. Soil pH from this research was highly correlated with soil pH measured during a quantitative soil study in 1998 at BBWM (Fernandez et al., 2003) when evaluated at the plot level. Concentrations of NO_3-N and NH_4-N were determined on an OI® Analytic Dual-Channel Automated Ion Analyzer at the University of Maine's Analytical Laboratory and on a Perstop® Flow Solutions 3000 Injection Analyzer at IES.

2.4. Estimates of ecosystem N

Biomass N was estimated as the sum of above- and belowground biomass N content using allometric equations from the literature and stem data from Eckhoff (2000). Total soil N was calculated from a quantitative pedon soil study conducted in 1998 at BBWM (Fernandez, unpublished data). Total N in the mineral soils was defined in this study as all soil below the O horizons, excluding the E horizon, but including all B and C horizons to a 1 m depth. Total pedon N values were the sum of the O horizon and all mineral soils. Extractable N data from the 1998 soil studies were only available for the O horizons and the upper 5 cm depth increment of mineral soils. Annual in situ NNM was taken as growing season NNM and calculated for 1 May 2001 to 31 October 2001. Since soils were collected in June–September 2001, June and September 2001 were used to extrapolate estimates for both May and October.

2.5. Statistical design

The statistical design was a split–split plot among treatments, forest types and time. In this design, factor A was the East and West Bear watersheds, factor B was the hardwood and softwood forest types, and factor C was time. Analyses were performed separately on O horizons and mineral soils given the different characteristics of each horizon. Data required rank transformations and were subsequently analyzed by ANOVA on the statistical analysis system (SAS Institute, 1999–2000) with an alpha level of 0.05.

3. Results and discussion

3.1. NNM and NN—watershed effects

Table 1 shows O and mineral soil means for the main effects of watershed and forest type from this study. O horizon potential NNM was significantly higher in the treated West Bear watershed compared to the reference East Bear watershed, however there were no significant differences between mineral soils

Table 1
NNM and NN (mg N kg^{-1} soil per day) for the main effects of both watersheds and forest types (standard errors are given in parentheses)

	Soil horizon	NNM (mg N kg^{-1} soil per day)		NN (mg N kg^{-1} soil per day)	
		In situ	14-Day lab incubation	In situ	14-Day lab incubation
Watershed					
East Bear	O horizon	3.00 (0.46)	4.82 (0.53)*	0.07 (0.03)*	0.14 (0.05)*
	Mineral	0.24 (0.09)	0.91 (0.09)	0.13 (0.02)*	0.21 (0.04)*
West Bear	O horizon	4.25 (0.59)	10.30 (1.07)	0.83 (0.15)	2.68 (0.37)
	Mineral	0.21 (0.07)	1.01 (0.19)	0.19 (0.07)	0.71 (0.18)
Forest type					
Softwood	O horizon	2.17 (0.25)*	4.76 (0.47)*	0.19 (0.04)	0.55 (0.11)
	Mineral	0.32 (0.09)	1.28 (0.09)*	0.20 (0.02)	0.61 (0.07)
Hardwood	O horizon	5.07 (0.66)	10.36 (1.09)	0.72 (0.16)	2.27 (0.40)
	Mineral	0.13 (0.07)	0.65 (0.18)	0.12 (0.07)	0.31 (0.17)

* Significance at the 0.05 level for contrasts within either watershed or forest type within horizons.

using the laboratory or in situ NNM method. The detection of N mineralization differences in the O horizon attributed to fertilization is new; Wang and Fernandez (1999) reported potential NNM after 4 years of treatment at BBWM and found no significant differences between the watersheds. However, they pointed out that their watershed-level comparisons did not take into account the potential effects of forest type and thus the impetus for those aspects of the current study. Significantly higher potential NNM in West Bear O horizons after 12 years of treatment could reflect the evolution of N accumulation in the treatment watershed or may have been detectable earlier had forest type been taken into account. Regardless of method used, NNM (Table 1) trends were for consistently higher rates in the O horizons from West Bear, but relatively minor differences between watersheds for underlying mineral soils. One interpretation of the differences between the in situ and laboratory results for O horizon NNM is that under the more ideal laboratory conditions, incipient changes in N dynamics were more easily detected, but these changes were not large enough to detect in the field where ambient conditions generate greater variability in the data.

Increased N mineralization rates in northeastern forests soils in response to experimental N enrichment have been reported previously for Harvard Forest, Massachusetts (Magill et al., 1997), Mt. Ascutney, Vermont (Aber et al., 1995), and the Fernow Experimental Forest, West Virginia (Gilliam et al., 2001). An increase in N mineralization rates at the beginning of N additions is attributed to a fertilization effect (Aber et al., 1998). Aber et al. (1998) hypothesized that during the latter stages of N saturation, however, N mineralization rates would decrease due to one of the two reasons: (1) increased N deposition results in the randomization of chemical bond structures in soil organic matter containing N, thereby reducing extracellular catabolic enzyme efficiencies resulting in decreased decomposition rates, or (2) the production of humus-degrading microbes is suppressed in the presence of elevated N in available forms. Using stream NO_3-N concentrations as an indicator, stream chemistry for the treated West Bear watershed indicated that the treated watershed was at stage 2 of N saturation (Aber et al., 1995; Fernandez and Adams, 2000). Stage 2 of the N saturation continuum would also be expected to have higher rates of N mineralization as shown here for West Bear compared to East Bear.

Rates of both potential and in situ NN were significantly higher in West Bear compared to East Bear in both the O horizon and mineral soils (Table 1). In terms of treatment effects, the O horizons had much higher NN when compared to mineral soils. This finding was consistent with that of Wang and Fernandez (1999) who also found significantly higher potential NN rates in the forest floor of West Bear after only 4 years of treatment. This also reinforces the notion that West Bear reflects stage 2 of N saturation (Aber et al., 1998) where both N mineralization and nitrification have increased. Future measurements of these processes will be necessary to determine if N mineralization is increasing or decreasing over time, reflecting the ecosystems progress between stages 2 and 3 of the N saturation continuum.

Inorganic N in acidic forest soils not influenced by high N inputs is mostly found as NH_4-N rather than NO_3-N. This is due in part to nitrification being limited in forest soils by poor organic matter quality leading to low N turnover, high acidity, low soil retention of any NO_3-N produced, and rapid biological immobilization of NO_3-N. In O horizon soils, using either the in situ or laboratory incubation, we confirmed that >90% of mineralized N was recovered as NH_4-N with little conversion to NO_3-N (Table 2). This is consistent with N mineralization reported for other sites in the northeastern US (McNulty et al., 1996; Aber and Mellilo, 1991). Potential and in situ NN appeared to most responsive to treatments in this study (Table 1), but significant differences in NN did not generally translate into significant differences in N mineralization because in most instances mineralized N was almost entirely attributable to NH_4-N (Table 2). This was especially true in O horizons, but mineral soils showed relatively high potential nitrification rates, e.g. NO_3-N contributing >20% to total N mineralized at the cessation of incubation.

An increasing contribution of NO_3-N to NNM shown in WB is consistent with hypotheses regarding the progression of the stages of N saturation (Aber et al., 1998), where enhanced NH_4-N availability increases N cycling rates and ultimately the NH_4-N available to support nitrification. Fenn et al. (1998) hypothesized that elevated N additions may increase nitrification rates simply by increasing the substrate NH_4-N. Magill et al. (1997) similarly reported an increased percentage of NNM attributable to nitrification in N enriched plots at the Harvard Forest, Massachusetts. They showed that NN, as a percentage of NNM, increased from 17% at the beginning of N additions in 1988 to 51% in 1993, with most of this increase occurring in the mineral soil of a monoculture red pine stand. Controls on nitrification are numerous and not simply NH_4-N concentration as it would not fully explain why NO_3-N was >90% of NNM in the untreated East Bear hardwood mineral soils, where rates of N turnover were also exceedingly low (Table 1).

Gilliam et al. (2001) reported that environmental factors such as soil temperature and soil moisture

Table 2
Percentage of NNM comprised of NH_4-N and NO_3-N averaged over the five collection periods

Watershed	Forest type	Horizon	Method	N mineralization dominated by NH_4-N (%)	N mineralization dominated by NO_3-N (%)
East Bear	Hardwood	O horizon	14-Day	98	2
			In situ	98	2
		Mineral	14-Day	78	22
			In situ	0	100
East Bear	Softwood	O horizon	14-Day	98	2
			In situ	98	2
		Mineral	14-Day	77	23
			In situ	60	40
West Bear	Hardwood	O horizon	14-Day	75	25
			In situ	79	21
		Mineral	14-Day	21	79
			In situ	7	93
West Bear	Softwood	O horizon	14-Day	88	12
			In situ	84	16
		Mineral	14-Day	28	72
			In situ	16	84

strongly influenced NN rates in the untreated forested watersheds at the Fernow Experimental Forest (FEF) in West Virginia. However, they found that the strength of the correlation between these environmental factors and NN rates decreased in their experimental watershed between 4 and 6 years after the beginning of treatments with $(NH_4)_2SO_4$. They suggested that N additions might alter microbial communities in ways that make them more sensitive to N than other environmental factors. Koopmans et al. (1995) found similar results in coniferous forests in The Netherlands where environmental factors were not as strongly correlated with NN rates in high N deposition plots compared to the ambient and low N deposition plots. It could be that soil eutrophication brought about by N additions selects for a nitrifier community that is less responsive to the soil microclimate, however little is known about the shifts in soil microbial populations undergoing this sort of nutrient manipulation. It must be noted that for our study, no significant correlations between soil temperature or soil moisture and potential or in situ NNM were detected. The apparent difference between this study and that of Gilliam et al. (2001) may be due to climatic or other edaphic features that differ between the sites; some notable differences include the fact that the Fernow Experimental Forest has a mean annual temperature 5 °C higher and receives on average 15 cm per year more precipitation than BBWM resulting in warmer soil temperatures and possibly higher moisture contents in the soil (Fernandez and Adams, 2000).

3.2. NNM and NN—forest type effects

Table 1 shows the potential and in situ NNM by major forest type at the BBWM. Both potential and in situ methods resulted in significantly higher rates of NNM in hardwood compared to softwood O horizons. In contrast, the underlying mineral soil NNM assays suggested lower potential NNM in hardwood compared to softwood stands. NN data showed no significant differences between forest types although trends paralleled the NNM patterns when comparing O and mineral soil horizons.

Higher NN in O horizons compared to mineral soils for hardwoods, but in mineral soils compared to O horizons for softwoods, is a contrast that has been reported in other studies in the northeastern US (Aber et al., 1993; Fernandez et al., 2000; Campbell et al., 2000; Finzi et al., 1998). Campbell et al. (2000) attributed higher N cycling rates in hardwood O horizons compared to softwoods to differences in soil moisture, pH, and biotic controls reflecting competition between plants and microbes. They found lower rates of N mineralization in the mineral soils for hardwoods although there were no significant differences between forest types in mineral soils.

Fernandez et al. (2000) looked at potential NNM, potential NN and potential net ammonification at 20 hardwood stands and 9 softwood stands across the 4 major climatic regions of Maine, including the reference watershed at BBWM. They found significantly higher potential NNM and potential NN in O horizons from hardwood stands as compared to those under softwoods. They also found similar O horizon N concentrations in both forest types; however, total C concentrations were higher under softwoods with the net result being higher C/N ratios. Higher C/N ratios are often correlated with lower NNM rates in forest soils (Blair et al., 1990; Fernandez and Adams, 2000; Vitousek et al., 1982). Quantitative soil excavations and analyses at BBWM (Fernandez, unpublished data) found softwoods had significantly higher C/N ratios than hardwoods in both organic and mineral horizons (Table 3) which could explain why hardwoods had significantly higher NNM rates in the O horizons compared with softwoods.

Results from the European NITREX project showed C/N ratios could be a predictor of NO_3-N leaching (Gunderson et al., 1998; Dise et al., 1998). Gunderson et al. (1998) found that conifer stands in temperate forest ecosystems in Europe having a C/N ratio below 25 leached NO_3-N or had elevated surface water NO_3-N concentrations. They suggested a C/N ratio continuum where the potential for NO_3-N leaching is low with O horizon C/N ratios above 30, moderate for sites with C/N ratios between 25 and 30, and high for those with C/N ratios below 25. Therefore soil moisture, pH, biotic competition, and C/N are determinates of NNM and NN. Table 3 shows the softwood stands at BBWM have O horizon C/N ratios of 29 and 26 for East and West Bear watersheds, respectively, indicating that the softwood stands at BBWM may be leaching moderate amounts of NO_3-N using the C/N continuum concept of Gunderson et al. (1998). During 1997 the West Bear watershed discharged ~5 kg N ha^{-1} while East Bear

Table 3
Soil pH, total N, total C and total C:N ratio by horizon and depth from the quantitative soil excavations in 1998 (standard errors are given in parentheses)

Watershed	Forest type		pH_w	Total N ($mg\ kg^{-1}$)	Total C ($mg\ kg^{-1}$)	Total C:N	Fine earth ($kg\ ha^{-1}$)
East Bear	Hardwoods	O horizon	4.01 (0.06)*	1.56 (0.06)	34.3 (1.49)*	22 (0.99)*	82652 (9957)*
		5 cm	4.28 (0.04)*	0.49 (0.06)	8.83 (0.84)	19.41 (0.81)*	146059 (20177)
		5–25 cm	4.66 (0.03)*	0.32 (0.03)	6.14 (0.52)	20.24 (0.56)*	804855 (99647)
East Bear	Softwoods	O horizon	3.54 (0.04)*,**	1.41 (0.03)	40.64 (0.81)*,**	29.20 (0.94)*,**	159393 (27270)*
		5 cm	4.07 (0.05)*	0.39 (0.04)	9.28 (0.82)	24.39 (0.62)*,**	144733 (12750)
		5–25 cm	4.36 (0.06)*	0.32 (0.03)	7.69 (0.70)	24.08 (0.41)*,**	680275 (60916)
West Bear	Hardwoods	O horizon	3.97 (0.06)*	1.44 (0.05)	33.63 (1.45)	23.31 (0.54)	86111 (19293)*
		5 cm	4.18 (0.05)	0.43 (0.03)	8.59 (0.64)	19.92 (0.37)*	120254 (11001)
		5–25 cm	4.59 (0.05)*	0.33 (0.03)	6.65 (0.57)	20.60 (0.39)*	704367 (81687)
West Bear	Softwoods	O horizon	3.69 (0.05)*,**	1.49 (0.05)	37.54 (1.26)**	25.68 (1.25)**	139519 (11661)*
		5 cm	4.09 (0.04)	0.46 (0.05)	9.81 (0.95)	22.22 (0.67)*	143093 (12571)
		5–25 cm	4.43 (0.04)*	0.30 (0.02)	6.47 (0.38)	22.28 (0.68)*,**	768772 (50000)

* Significance between vegetation types within a watershed at the 0.05 level.
** Significance between watersheds within a vegetation type at the 0.05 level.

discharged ~0.1 kg N ha^{-1} (Kahl et al., 1999) consistent with the differences in the C/N ratios between watersheds. Dise et al. (1998) also examined the hypothesis that C/N ratios of the O horizons could be used to estimate the level of NO_3-N leaching from an ecosystem, but went further and examined a range of N deposition conditions to evaluate how N deposition affects both C/N ratios and NO_3-N leaching. Dise et al. (1998) found that at low levels of N deposition (<9 kg N ha^{-1} per year), NO_3-N leaching was minimal regardless of the O horizon C/N ratio. At intermediate (9–18 kg N ha^{-1} per year), high (18–30 kg N ha^{-1} per year), and very high (>30 kg N ha^{-1} per year) inputs of N deposition, NO_3-N leaching increased with increasing N deposition and decreasing C/N ratios. BBWM receives an estimated ambient total N deposition of 8.4 kg N ha^{-1} per year, with the treated West Bear watershed receiving 33.6 kg N ha^{-1} per year as both ambient atmospheric deposition plus treatment (Kahl et al., 1999). According to Dise et al. (1998) the very high N deposition (>30 kg N ha^{-1} per year) to West Bear should induce higher NO_3-N leaching compared to East Bear. This is consistent with higher stream NO_3-N export in West Bear compared with East Bear (Kahl et al., 1999). Ultimately, other parameters need to be taken into account when predicting NO_3-N leaching besides C/N ratios and amounts of N deposition such as plant demand, site and land-use history (Goodale and Aber, 2001; Ollinger et al., 2002; Gunderson et al., 1998; Dise and Wright, 1995; Dise et al., 1998).

Researchers in the US also have examined the relationship between C/N ratios and N mineralization, nitrification and NO_3 leaching, typically reporting negative correlations between O horizon soil C/N ratios and N mineralization and nitrification rates (Goodale and Aber, 2001; McNulty et al., 1991, 1996; Ollinger et al., 2002). The strongest correlations are usually between soil C/N ratios and nitrification rates. Lovett and Rueth (1999), Ollinger et al. (2002) and McNulty et al. (1996) all reinforced the premise that a threshold forest floor C/N ratio of 20–25 exists in both softwoods and hardwoods where nitrification sharply increases at or below this range.

The typical negative correlation between C/N ratio and NNM and NO_3-N leaching is a logical mechanism to explain why the O horizons in hardwoods have significantly higher NNM rates compared to softwoods, e.g. hardwoods have a lower C/N. It does not explain why the opposite trend exists in the softwood mineral soils. We hypothesize one of the two possibilities for the softwood mineral soil results. The first hypothesis is that O horizons in hardwoods mineralize N at a faster rate than softwoods because of higher tissue N concentrations (Nadelhoffer et al., 1995) and more rapid rates of litter decomposition

(Finzi et al., 1998). This leaves only the more recalcitrant humic materials to illuviate into the mineral soils below. In contrast, softwood litter is slower to decompose in the O horizons because of its higher lignin:N and C/N ratios (Ferrari, 1993), resulting in less O horizon mineralization and humification with more mineralizable substrate illuviating into the mineral soils. Thus, more labile C is available in softwood mineral soils to respond to N enrichment. While this mechanism remains plausible, Parker et al. (2002) did not find clear evidence for higher labile C in softwood compared to hardwood B horizon soils at BBWM. The second hypothesis is that softwood and hardwood mineral soils mineralize N at similar rates, but softwood mineral soils are more sensitive to increased N deposition. This would cause a more rapid increase in NNM to result in the softwoods with N enrichment compared to hardwoods, a characteristic also reported by others (Campbell et al., 2000; Aber et al., 1995). Campbell et al. (2000) hypothesized that softwood species may be more sensitive to N deposition if softwood sites have lower rates of N assimilation into foliage and bolewood compared to hardwood sites (Nadelhoffer et al., 1995), resulting in a greater effect on soil processes.

No significant differences were observed for potential or in situ NNM and NN for the interaction between watersheds and forest type (Fig. 2). Similar numerical patterns were observed in these data as in the main effects results (Table 1): (1) O horizons had higher NNM and NN rates than mineral soils; (2) potential NNM and NN rates were higher than in situ NNM and NN rates; (3) hardwood soils, particularly in West Bear, had higher nitrification rates compared to softwoods. West Bear hardwood O horizon potential and in situ NNM means were twice those of East Bear (Fig. 2) after 12 years of continuous treatment. It is possible that there is indeed no effect on N dynamics from the treatments. However, the lack of significant differences in potential NNM between watersheds by dominant stand type in both this study and Wang and Fernandez (1999) could also be influenced by: (a) high variability, (b) rapid immobilization of added N by soil microbes and plant roots, (c) colloidal adsorption of NH_4-N, (d) denitrification, or (e) nitrification and subsequent NO_3-N leaching. In response to these possibilities, the high variability in this study was

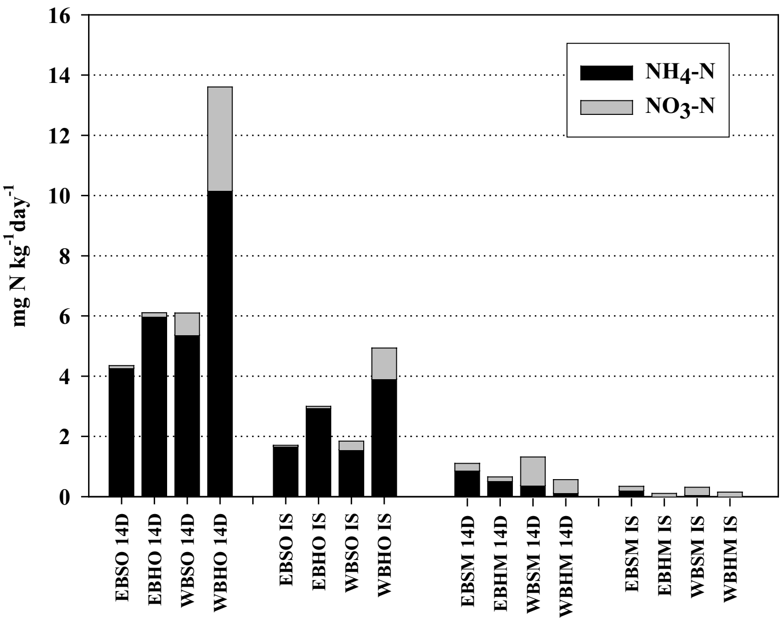

Fig. 2. NNM by watershed, forest type and soil horizon in both the laboratory and in situ incubated samples. Note: WB—the West Bear watershed; EB—the East Bear watershed; S—softwood forest type; H—hardwood forest type; O—O horizons; M—mineral soils; 14D—14-day laboratory incubations; IS—in situ incubations.

evident by the large standard deviations of the mean (120% of the mean for the 14-day laboratory incubations and 160% of the mean for the in situ incubations). Immobilization by soil microbes and plant roots was not in the scope of this study; however, there was evidence for this immobilization mechanism in both soil biota based on previous work at BBWM (White et al., 1999; Nadelhoffer et al., 1999). Ammonium fixation in clays is likely insignificant in these sandy loam Spodosols, but given the high CEC of the O horizons at BBWM as well as the greater exchangeable NH_4-N in the West Bear watershed, it is probable that there was adsorptive retention of NH_4-N. Unfortunately, there is no denitrification data from BBWM at this time although research is ongoing to address this question. Ample evidence exists of both increased nitrification and NO_3-N leaching in the treated watershed streams at BBWM (Kahl et al., 1999).

3.3. NNM and NN—temporal patterns

Fig. 3 shows the results of the in situ NNM measurements over the course of this study, with lower values evident at the end of the 2001 growing season. These trends over time appear to be attributable to declining moisture over the study period (Fig. 4). Both temperature and moisture regulate microbial activity in soils and subsequently N mineralization and nitrification rates (Arnold et al., 1999; Sarathchandra et al., 1989). Although warmer soils can lead to increased N mineralization rates (Fenn et al., 1998), warmer soils can also lead to decreases in soil moisture that may have negative effects on microbial populations (Van Gestel et al., 1993). Arnold et al. (1999) reported on microbial biomass as influenced by experimental soil warming treatments and resultant moisture regimes in a spruce–fir forest in Maine. They suggested that a moisture threshold might exist in O horizons between 20 and 120% soil moisture content above which soil temperature exerted strong controls on microbial activity, and below which moisture availability became the dominant limiting factor. Results from this study support the hypothesis that a moisture threshold exists in the O horizons of these forest soils, with Fig. 3 indicating that this moisture threshold might be between approximately 75–130% moisture content. Since in situ NNM rates declined after the July 2001 collection coincidental with an O horizon soil moisture decline to approximately 75%, we hypothesize that moisture was the primary driver for reduced in situ NNM rates. This does not exclude the possibility of substrate availability, substrate quality, plant demand or other factors contributing to these results. It should be noted that this trend was not

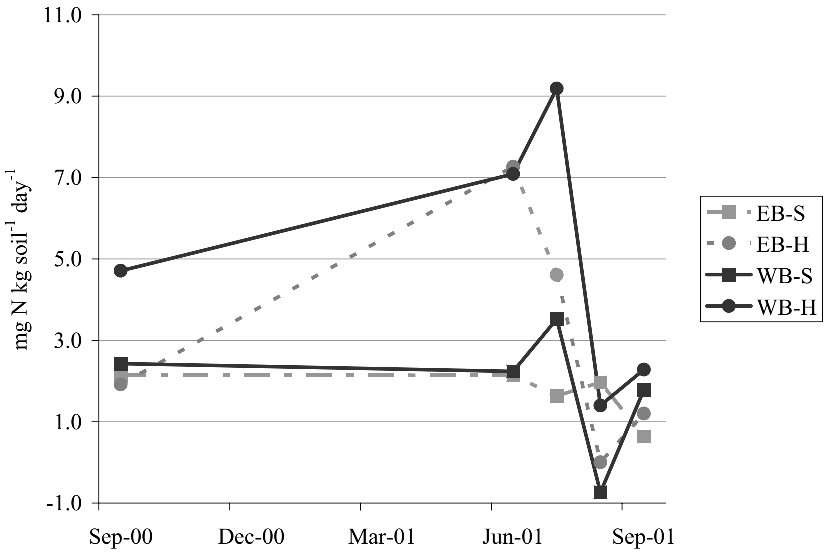

Fig. 3. In situ NNM compartmental rates over time. EB-S: East Bear watershed, softwood forest type; EB-H: East Bear watershed, hardwood forest type; WB-S: West Bear watershed, softwood forest type; WB-H: West Bear watershed, hardwood forest type.

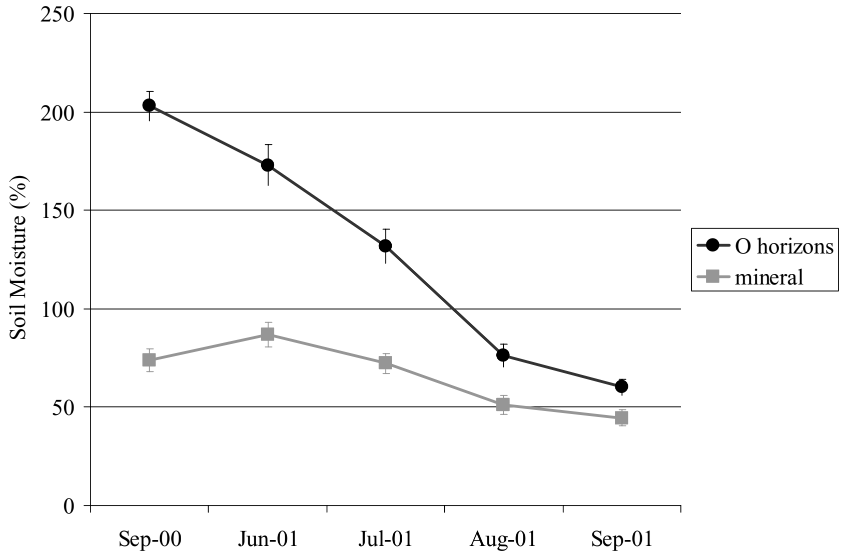

Fig. 4. O horizon and mineral soil mean percent initial moisture over time (standard errors are given in parentheses).

demonstrated in the mineral soils where relative moisture declines were less marked over the duration of the study.

3.4. Soil N content and watershed N budgets

The previous discussion about soil N dynamics from this study was based on data expressed on a concentration basis. Almost all the findings are the same whether the data are expressed as concentrations or on a mass of N per unit surface area basis. Exceptions included: (1) in situ NNM was significantly higher in West Bear compared with East Bear in the O horizons, and (2) using data expressed on an aerial basis potential NNM was no longer significantly higher in hardwood O horizons compared to softwood O horizons, or softwood mineral soils compared to hardwood mineral soils. These differences were a matter of statistical significance, and trends remained the same regardless of the manner in which the data were expressed. Differences in statistical significance between the two ways of expressing our data were due to slight differences in soil mass between watersheds. Table 4 shows mass per unit area data for the interaction between watershed and forest type for both in situ NNM and NN. West Bear in situ NNM was significantly higher than East Bear for both softwoods and hardwoods. West Bear O horizon in situ NNM was

Table 4
In situ NNM and nitrification (kg N ha^{-1} per day) for watersheds by forest type (standard errors are given in parentheses)

	West Bear (kg N ha^{-1} per day)		East Bear (kg N ha^{-1} per day)	
	Softwoods	Hardwoods	Softwoods	Hardwoods
NNM				
O horizon	0.476 (0.064)*	0.464 (0.079)*	0.100 (0.029)	0.386 (0.075)
Mineral	0.076 (0.012)	0.071 (0.069)	0.139 (0.059)	0.065 (0.021)
NN				
O horizon	0.066 (0.015)	0.103 (0.020)	0.004 (0.002)	0.009 (0.004)
Mineral	0.071 (0.008)	0.063 (0.067)	0.055 (0.011)	0.063 (0.015)

* Significance between watersheds by forest type at the 0.05 level.

~5× East Bear for softwoods but only ~1.2× for hardwoods. Using the in situ NNM method, we detected a treatment response that suggested a greater apparent increase in softwood O horizon soil NNM as compared to those under hardwoods. This suggests that softwood soils may be more responsive than hardwood soils to changes in N inputs after 12 years of treatment. This is in contrast to results from earlier in the BBWM program where, after 3 years of treatment, it appeared that hardwood O horizons were more responsive than softwoods (Wang and Fernandez, 1999).

Annual NNM and NN were estimated using data extrapolated from 1 May to 31 October and assuming no N turnover during the dormant season. These data were then used to construct a simple N budget for BBWM to place our N cycling data within a whole-ecosystem context (Figs. 5 and 6). As we did not perform N cycling rate estimates in the lower mineral soil depths, we report only the total N data for the lower soil depth increment. Estimated aboveground biomass N was small compared to total soil N, and represented only ~6–7% of total soil N in both watersheds, while estimated annual NNM was ~1% of total soil N in West Bear and ~0.6% in East Bear. Although the majority of total soil N was found in the mineral soils, higher rates of NNM occurred in the O horizons in both the treated and reference watersheds. It is thought that a lower rate of N turnover in the mineral soils reflects soil organic matter that is older and progressively more recalcitrant with depth (Federer, 1983; Persson and Wirén, 1995). The O horizons also contained more extractable N than the mineral soils, a logical byproduct of higher rates of N cycling. Extractable inorganic N (2 M KCl) was 0.2–0.6% of total N in both watersheds for the O horizons and upper mineral soil. The most notable difference between watersheds was that total NNM from the O and upper mineral soil of West Bear was approximately 1.7× than that in East Bear, due largely to the higher cycling rates in West Bear O horizons. Although O horizons had much higher NNM than mineral soils, mineral soils had higher NN compared to O horizons. NN in the treated West Bear mineral soil was much higher than East Bear and comprised nearly all the N mineralized in these soils for the data in this study. Other researchers have also found similar increases in NN in the mineral soils compared to the O horizons at N treated sites (Andersson et al., 2002; Magill et al., 1997). Although we did not have NNM and NN data

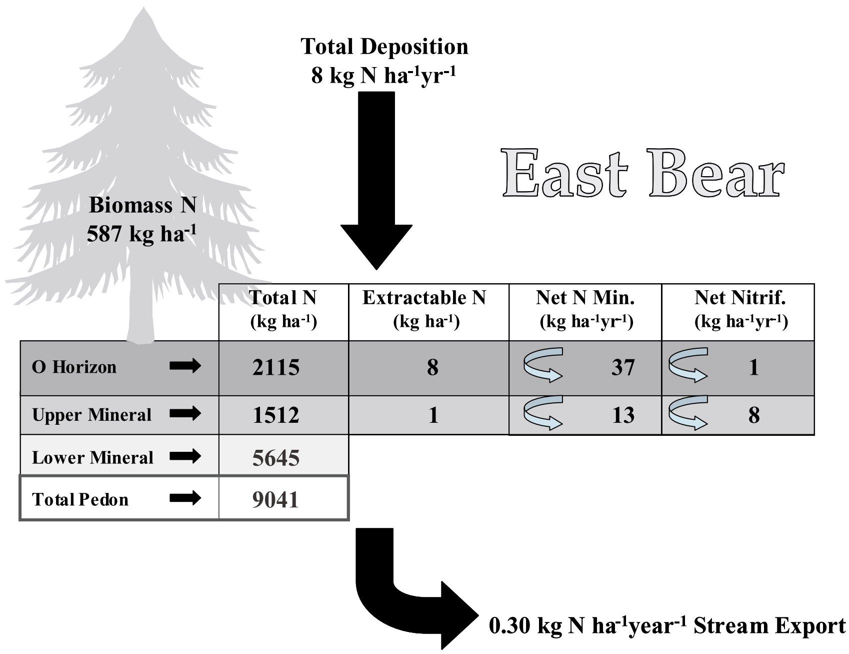

Fig. 5. Estimates of N budget components in 2001 for East Bear at BBWM.

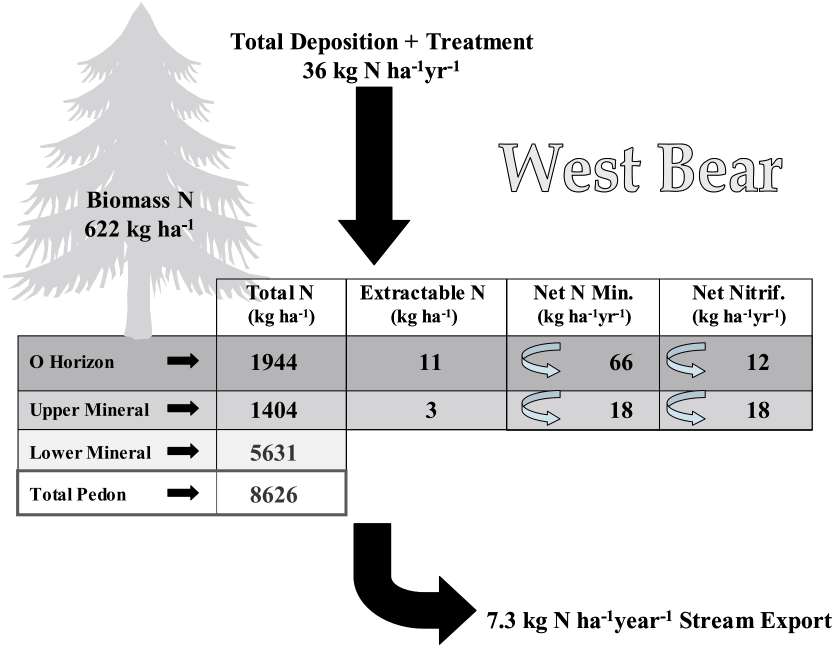

Fig. 6. Estimates of N budget components in 2001 for West Bear at BBWM.

for the lower mineral soils for these ecosystem N estimates, we expect the majority of the N turnover to be attributable to upper soil increments as has been reported elsewhere (Federer, 1983; Persson and Wirén, 1995). Nevertheless, these estimates of NNM and NN did not encompass the entire pedon and are for perspective only. It is also worthy to note these estimates assume no N cycling beyond the growing season of northern New England, and are therefore likely to be an underestimate of N cycling.

The data reported here appear consistent with similar forest soil N data reported in the literature. Literature values for total soil N range from 1034 and 2275 kg N ha^{-1} for a low elevation spruce–fir forest in Howland, Maine (Fernandez et al., 1993), to 1932 and 877 kg N ha^{-1} in the O horizon and mineral soil, respectively, for the Adirondack Region of New York (Mitchell et al., 2001). Federer (1983) reported the total N in a softwood stand in Maine was 1802 and 3224 kg N ha^{-1}, and NNM was 15 and 27 kg N ha^{-1} per year, for O horizons and mineral soils, respectively. Cole and Rapp (1981) analyzed data from 14 sites included in the International Biological Program (IBP) from around the world and found temperate coniferous forest soils averaged 6821 kg N ha^{-1} and temperate deciduous forest soils averaged 5177 kg N ha^{-1}. Devito et al. (1999) reported NNM values for O horizons in Canadian soils ranged from 114 kg N ha^{-1} per year for deciduous forest types to 140 kg N ha^{-1} per year for mixed conifer forest types. They also reported N mineralization rates of 52 and 46 kg N ha^{-1} per year for deciduous and mixed forest types, respectively, in the upper 10 cm of the mineral soils, which is ~4 times than that at BBWM. In their measurements of annual NNM, they included the winter months for which they reported high rates of NNM (49–92% of annual NNM). It will be important in future research to better define dormant season forest soil N dynamics across a range of climatic regimes for northern forest types.

Input–output estimates showed that ~20% of the total N inputs were exported annually in West Bear stream after 12 years of treatments, while this figure was ~4% in the reference East Bear stream. Therefore ~80% of total input N was still retained in West Bear, despite the long-term N amendments to this watershed. Other investigators in both Europe and US have reported high retention of inorganic N even after experimental N additions in forested ecosystems. Bergholm and Majdi (2001) reported 93% retention of

N inputs in a Norway spruce stand in Sweden treated with $(NH_4)_2SO_4$, and 96% retention for their reference watershed. They suggested that the spruce stand had a relatively high capacity to accumulate N due to high aboveground production. Mitchell et al. (1996b) reported that an untreated watershed in the Adirondack State Park of New York retained 74% of wet inputs of N. Similarly, Magill et al. (1997) observed that 85–99% of varying N additions (50 and 150 kg N ha^{-1} per year of NH_4NO_3) were retained at the Harvard Forest in Massachusetts. Despite NO_3-N leaching in the softwood site at Harvard Forest occurring fairly early in the study, these soils were still retaining greater than 90% of very high N amendments. It must be noted that none of these studies measured gaseous loss, ostensibly by way of denitrification, which may modify these estimates of N retention. Even so, it is likely that under moderate N treatments these forest soils have both a large potential for further accumulation of N, mostly in soil N pools, and a high susceptibility for accelerated N mineralization, nitrification, and NO_3-N export in soil solutions and streams.

4. Conclusions

Our results indicate that after 12 years of whole-watershed experimental N enrichment, the West Bear watershed demonstrated higher rates of NNM and NN consistent with the evolution of N saturation. While the majority of the soil N pool is in the mineral soil, higher rates of N mineralization as determined by NNM assays are in the O horizon. It is the O horizon that shows the greatest response to enhanced N deposition. Watershed retention of N input was still nearly 80% in the treated watershed and over 95% in the reference watershed, indicating a significant potential in both watersheds for continued N accumulation. Perhaps of greater importance are the changes in N dynamics within the treated watershed that are developing over time. These results indicate that the rapid changes in N dynamics evident early in the BBWM experiment (Wang and Fernandez, 1999) continue but are evolving. With chronically elevated N inputs, soils under the softwood forest types have recently begun to respond to treatments, as indicated by changes in N cycling rates, whereas hardwood soils were first to respond to treatments earlier in the experiment. We note that there are important differences in the response attributable to surface O and subsoil mineral horizons for different forest types. We believe that the type and availability of carbon under these two forest cover types may be driving the differences we see in soil horizons. Nitrification is becoming increasingly important in the overall NNM of the treated soils as might be expected. The findings from this whole-watershed N enrichment experiment are generally consistent with current concepts of N saturation and N cycling in forested watersheds. Yet they also indicate the need to better understand the interactions between C and N that relate to forest type and impinge upon deeper, mineral soil layers as N saturation evolves on decadal time scales.

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