CONTROLS ON N RETENTION AND EXPORTS IN A FORESTED WATERSHED

K. NADELHOFFER[1], M. DOWNS[1], B. FRY[2], A. MAGILL[3] and J. ABER[3]

[1] *The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543,* [2] *Biology Department, Florida International University, University Park, Miami, FL 33199,* [3] *Complex Systems Research Center, University of New Hampshire, Durham, NH 03824*

Abstract. We conducted a ^{15}N-tracer study in a fertilized, forested catchment at the Bear Brook Watersheds in Maine (BBWM), USA, in order to characterize N cycling processes, identify sinks for ammonium-N additions, and determine the contribution of the experimental ammonium additions to nitrate exports from the treated catchment. Distributions of ^{15}N in plant tissues, soils, precipitation and streamwater collected before adding tracers showed that nitrate-N (the dominant form of inorganic N deposition at the site) inputs under ambient conditions were depleted in ^{15}N relative to plants and that soil was enriched in ^{15}N relative to plants. The ^{15}N content of streamwater nitrate was within the range of ^{15}N contents in natural plant tissues, suggesting that nitrate deposited from the atmosphere is reduced and assimilated into soil and plant N pools before being leached as nitrate from the catchment. Variations in ^{15}N natural abundances also suggested that most N uptake by trees is from the forest floor and that nitrification occurs in soils at this catchment under ambient conditions. Changes in ^{15}N contents of plant tissues, soils and streamwater after adding a ^{15}N tracer to the ammonium sulfate fertilizer applied to the treated catchment showed that soils were the dominant sink for the labeled ammonium. Surface soils (Oea horizon plus any underlying mineral soil to 5cm depth) assimilated 19 to 31 percent of the 42 kg ha^{-1} of ^{15}N-labelled ammonium-N during the tracer study. Aboveground biomass assimilated 8 to 17 percent of the labeled ammonium-N additions. Of the three forest types on the catchment, the soil:biomass assimilation ratio of labeled-N was highest in the spruce forest, intermediate in the beech-dominated hardwood forest and lowest in the mixed hardwood-spruce forest. Although ammonium sulfate additions led to increases in streamwater nitrate, only 2 of the 13 kg ha^{-1} of nitrate-N exported from the catchment during the 2 years of tracer additions was derived from the 42 kg ha^{-1} of labeled ammonium-N additions.

1. Introduction

Increased inputs of reactive N (ammonium and nitrate) to ecosystems from the atmosphere can lead to increases in plant growth over the short term. At longer time scales, however, elevated N deposition can lead to increased net N mineralization and nitrification in soils, base cation (Ca^{2+}, Mg^{2+}, K^{+}) depletion, increased soil acidification (greater H^{+} and Al^{3+} activity), nutrient imbalances in plant tissues, reductions in plant growth, changes in species composition, and increased nitrate exports to groundwater or streams (Nihlgård, 1985; Aerts and Berendse, 1988; Van Breemen and Van Dijk, 1988; Schulze, 1989). Ecosystems undergoing changes in these properties and processes as a result of chronically elevated N inputs can be viewed as approaching 'N saturation' (*sensu* Agren and Bosatta, 1988; Aber *et al.*, 1989; Gundersen, 1991), a condition in which the amount of inorganic N available to plants and microbes in an ecosystem exceeds the total N assimilation capacity of biological processes.

Environmental Monitoring and Assessment **55:** 187–210, 1999.

Forest ecosystems, particularly those on base-poor Spodosols and Inceptisols, are susceptible to N saturation. For example, Kahl *et al.* (1993) reported that nitrate (NO3-) concentrations in streamwater draining a ~10 ha forested catchment in eastern Maine (the West Bear Brook catchment) increased continuously during the first 3 years of fertilization at 25 kg N ha-1 yr-1 (as ammonium sulfate). Although N accumulated within the West Bear Brook catchment throughout the period of ammonium sulfate application, the rate of N accumulation and the N input:N loss ratio decreased during the first 6 years of treatment (Kahl *et al.*, this volume).

The rapid inducement of incipient N saturation in the West Bear Brook catchment following the onset of relatively moderate experimental N additions (Kahl *et al.*, 1993) was surprising given that forests in the catchment were presumed to be N limited and that background N deposition at the site was low (inorganic N wet deposition < 4.5 kg N ha^{-1} yr^{-1} as reported in Kahl *et al.*, 1993). We report here results of a catchment-scale ^{15}N tracer experiment conducted on a chronically fertilized catchment at the Bear Brook Watersheds in Maine (Norton *et al.*, this volume) that allowed us 1) to partition the fluxes of fertilizer N inputs into plant and soil compartments in the 3 forest ecosystem types within the catchment and 2) to identify the importance of fertilizer N versus native soil N as sources of nitrate exports from the treated catchment to streamwater.

2. Methods

2.1 SITE

The East and West Bear Brook catchments are located in eastern Maine (44°52' N, 68°06' W) at 300 to 450 m asl on the southeastern slope of Lead Mountain, ~60 km inland from the Gulf of Maine. Soils are Spodosols developed on 0 to 5 m of till. The upper third of each catchment is dominated by nearly pure stands of red spruce (*Picea rubens*, Sarg.) with small amounts of balsam fir (*Abies balsamea*, L.) and the lower third of each consists of northern hardwoods dominated by American beech (*Fagus grandifolia*, Ehrh.) and maples (*Acer rubrum*, L. and A. *saccharum*, Marsh. with some *A. pensylvanicum*, L.). Yellow birch (*Betula alleghaniensis*, Britton) is an important co-dominant species in the hardwood forests. The middle elevations consist of mixed hardwood-spruce stands. The hardwood and mixed hardwood-spruce stands are dominated by 40 to 60-year-old trees whereas the spruce stands in the upper elevations are somewhat older and appear not to have been harvested during this century. Both catchments are drained by first-order streams. More detailed descriptions of the site are in Norton *et al.* (this volume).

2.2. TREATMENTS

Additions of ammonium sulfate fertilizer to the West Bear Brook catchment (10.2 ha) began in November 1989 and have continued through to the present (1996). Pelletized fertilizer was applied at 25.2 kg N ha^{-1} yr^{-1} (or 1800 equivalents ha^{-1} yr^{-1}) as 6 bimonthly applications per year of 4.2 kg N ha^{-1} (300 equivalents ha^{-1}). An adjacent, non-fertilized catchment (10.9 ha) drained by East Bear Brook served as the reference. During the second and third treatment years, the ^{15}N abundance of the fertilizer N was increased from approximately 0.3663 atom % ^{15}N to 0.4366 atom % ^{15}N, or in natural abundance units[1] from 0 ‰ to 192 ‰ $\delta^{15}N$. The isotopically labeled fertilizer was prepared at the Tennessee Valley Authority Laboratory (Muscle Shoals, AL) by dissolving commercial grade ammonium sulfate fertilizer pellets ($\delta^{15}N$ = –0.7 ‰) in water, mixing $(^{15}NH_4)_2SO_4$ (98+ atom %^{15}N) into the fertilizer solution, evaporating the labeled solution, and repelletizing the precipitated, labeled fertilizer. Applications of ^{15}N-labelled fertilizer began on 18 April 1991 (first application at the start of the second treatment year) and were applied bimonthly using a helicopter through 10 December 1992 (the end of the third growing season of treatment), except for one application (March 1992) when the catchment was snow-covered and non-labeled fertilizer was applied. Thus, all labeled applications were made during snow-free periods between April 1991 and December 1992. Cumulative applications of labeled fertilizer were 21 kg N ha^{-1} yr^{-1} in 1991 and 42 kg N ha^{-1} yr^{-1} in 1992.

2.3. TREE BIOMASS AND PRODUCTION

Estimates of tree species size class distributions within each of the 3 forest cover types were derived from a timber survey conducted in March 1992. Aboveground biomass of individual trees was estimated using allometric regressions of the form:

$$\ln(B) = a + b \ln(D) \quad [1]$$

where D is diameter breast height (cm), B is total aboveground biomass (g dry mass of boles, branches, twigs plus foliage), and *a* and *b* are species specific parameters from Young *et al.* (1980). Tree biomass and stem size class distributions were combined to provide areal estimates of wood biomass for dominant species.

Wood increment in stands of the treated catchment was estimated using 3-year radial increments of tree cores collected in October 1992 from within 25 m of each of 6 sampling points. The sampling points were selected using a 50 m × 50 m survey grid and a stratified random procedure with 2 points being located in each of the 3 forest

---

[1] We follow the convention of expressing ^{15}N compositions of natural materials and of materials slightly enriched with ^{15}N using $\delta^{15}N$ notation in which units are expressed as per mil deviations from the atmospheric standard of 0.3663 atm % ^{15}N and are calculated as follows: $\delta^{15}N = [(\text{atm } \%\ ^{15}N \text{ sample}/0.3663)-1] \times 1000$.

cover types. These 6 points were also used for soil and tree tissue sampling. Radial increments were measured using single cores collected from 6 to 8 trees per dominant species and from 2 to 6 co-dominant species within each forest type. Tree cores were collected at ~1.5 m above the base of each stem using a 1.9-cm diameter keyhole saw to allow sufficient material for elemental (CN) and isotopic analyses. Estimates of wood accumulation in each forest type during the 3 years of fertilization were calculated by applying the mean percent biomass increase of each species to areal estimates of species aboveground biomass within each forest type. Although the allometric equations predicted total aboveground biomass (including foliage), we assumed that all biomass accumulated in trees during the 3-year measurement period was woody material (stems, branches and twigs).

Canopy foliar mass was estimated using litterfall data from 4 litter traps (0.112 m^2 each) located in each of 4 permanent sampling plots (900 m^2 each) in the treated catchment. One litter sampling plot was located in the spruce, one in the hardwood-spruce, and two in the hardwood forest. We estimated hardwood foliar mass as 1.1 times 1991 litterfall, assuming that 10 percent of foliar mass was either leached, metabolized, retranslocated or consumed by herbivores. Foliar mass of red spruce was estimated to be 7.7 times 1991 litterfall, assuming a 7-year needle retention (S. McNulty, *pers. comm.*) and 10 percent mass loss as in hardwood leaves.

2.4. TREE TISSUE AND SOIL SAMPLING

Elemental and ^{15}N analyses were conducted on tree core wood sampled from at least 6 dominant or co-dominant trees located within 25 m of each of the 6 sampling points in the treated catchment in October 1990 prior to adding ^{15}N-labelled fertilizer. The trees sampled for elemental and ^{15}N analyses were a subset of the trees sampled for radial increment. The same trees were resampled in October 1991 and October 1992 after the first and second years of ^{15}N-labelled fertilizer application and after the second and third years of treatment (non-labeled plus labeled fertilizer additions). The 3 most recent annual rings were separated from bark and from older rings prior to ^{15}N analysis.

Green foliage was sampled in the treated catchment in mid-August in 1991 and 1992 from the same trees from which bolewood samples were collected. Because foliage was not sampled in the treated catchment before ^{15}N was applied, foliage samples collected in 1991 from trees in matching forest cover types in the reference (East Bear) catchment served as baselines for estimating ^{15}N enrichment of foliage in the treated catchment. Foliage was collected from exposed leaves using a shotgun. Foliar samples were composited for individual trees and, for red spruce, by age class (current-year, 1-year-old, and >1-year-old). In all, at least 12 red spruce, 8 beech, 8 maple, and 4 birch trees were sampled annually for tissue chemistry from the treated catchment.

Soils were sampled using two schemes. In both schemes, soils were sieved free of fine and woody roots and mineral fragments >2 mm diameter. The first method involved collecting 3 10 × 10 cm forest floors (O_{ea}) yearly from within 10 m of each of the 6 grid points on the treated catchment (n = 6 per forest type per year). Mineral soil cores (6.5-cm diameter, 10-cm deep) were collected from beneath each of these forest floor samples. Information from these samples was used to estimate dry masses and soil N pool sizes (to a 10 cm mineral soil depth) in each ecosystem type. Although ^{15}N and CN analyses were conducted on these samples, results of these analyses were not used to estimate retention of labeled N. This was because natural variations in ^{15}N abundances in forest floor and mineral soil samples collected in this manner were too large to detect changes in ^{15}N abundances following the labeled fertilizer additions. Therefore, a second sampling method involving repeated sampling from small quadrats was used to estimate shifts in forest floor and soil ^{15}N abundances. In this method, permanent 20 cm × 20 cm quadrats (6 per forest cover type) were resampled annually both before and after the start of ^{15}N addition. Samples were collected from just below the fresh litter (O_i horizon) to 5 cm below the O_{ea} surface using a 2.2-cm diameter soil corer. Three replicate cores were taken from each quadrat before and after the first year of ^{15}N additions. After the second year of ^{15}N additions, we collected only 1 core per quadrat (within-quadrat variations in ^{15}N abundance were small). Variances in ^{15}N abundance were considerably smaller at this scale than at the 1 to 10 m scale used in the first sampling method. Paired comparisons of δ^{15}N values between years within quadrats were used to test for changes in ^{15}N abundances of the top 5 cm of forest floor plus mineral soil in each forest type during the course of ^{15}N additions to the treated catchment.

2.5. STREAMWATER AND PRECIPITATION SAMPLING

Flow-weighted streamwater samples were collected at the gauging weirs located at the base of each watershed (See Norton *et al.*, this volume) during 7 to 65-day base flow intervals and during high-flow (daily) events. We measured ^{15}N abundances of dissolved inorganic N in 25 solution samples from West Bear Brook (treated) and in 9 samples from East Bear Brook (reference) collected between 1 April 1991 and 30 June 1993. Bulk precipitation samples analyzed for ^{15}N abundance (2 to 6-week composites, n = 16) were collected at the East Bear Brook weather station between August 1990 and November 1992. Solution samples were stored frozen until the ^{15}N abundances in dissolved ammonium and nitrate were analyzed (Downs *et al.*, this volume).

2.6. ^{15}N MASS BALANCES

We estimated the assimilation of the ^{15}N-labelled ammonium additions into soils and aboveground biomass components of all three forest types using 1) estimates of N pool sizes, 2) changes in vegetation and soil ^{15}N abundances following labeled fertilizer additions, and 3) the following mass balance equation (Nadelhoffer and Fry, 1994):

$$m_{lab} = m_f \times (\delta^{15}N_f - \delta^{15}N_i)/(\delta^{15}N_{lab} - \delta^{15}N_i) \qquad [2]$$

where,

m_{lab} = mass of ^{15}N-labeled compound incorporated into the N pool;

m_f = final mass of the ecosystem N pool;

$\delta^{15}N_f$ = final ^{15}N abundance of the ecosystem N pool;

$\delta^{15}N_i$ = initial ^{15}N abundance in the ecosystem N pool;

and

$\delta^{15}N_{lab}$ = ^{15}N abundance of the labeled N additions.

The contributions of ^{15}N-labelled fertilizer to inorganic N exports to streamwater from the fertilized catchment were also estimated by mass balance (below). The value of $\delta^{15}N_{lab}$ (labeled fertilizer) in this study was 192 ‰. Initial or reference ^{15}N abundances ($\delta^{15}N_i$) varied among ecosystem pools (Figure 1), but δ^{15}N values for all measured ecosystem components prior to labelling of the catchment were at least 175 ‰ lower than the labeled fertilizer.

2.6.1. *Vegetation*

Estimates of ^{15}N fertilizer assimilation into aboveground biomass within forest types during the 2 years of labeled N additions were calculated using N pool size estimates for each tree species (Table I) as follows. For tree tissues, mean $\delta^{15}N_f$ and $\delta^{15}N_i$ values for each species within a forest type were used in Equation [2]. Assimilation of ^{15}N fertilizer into spruce foliage was calculated as the sum of assimilation into current-year, 1-year-old, and >1-year-old needles by August 1992. Assimilation of labeled N into foliage of deciduous species was calculated as the sum of assimilation as of August 1991 and as of August 1992 in order to account for N assimilated into foliage that was shed after the first year. Total labeled N assimilation into total foliar mass was calculated as the sum of labeled N assimilated into foliage of each species within a forest type.

Assimilation of ^{15}N-labelled fertilizer into wood was calculated for individual species within each forest type using the pool size of N in wood produced from 1990 through 1992 and mean δ^{15}N values of 3-year radial increments collected in November 1990 (before labelling) and November 1992 (after labelling). Pool sizes of N in 1990-1992 wood for each species within each forest type was estimated using mean three-year growth rates (above) and N concentrations (Table I).

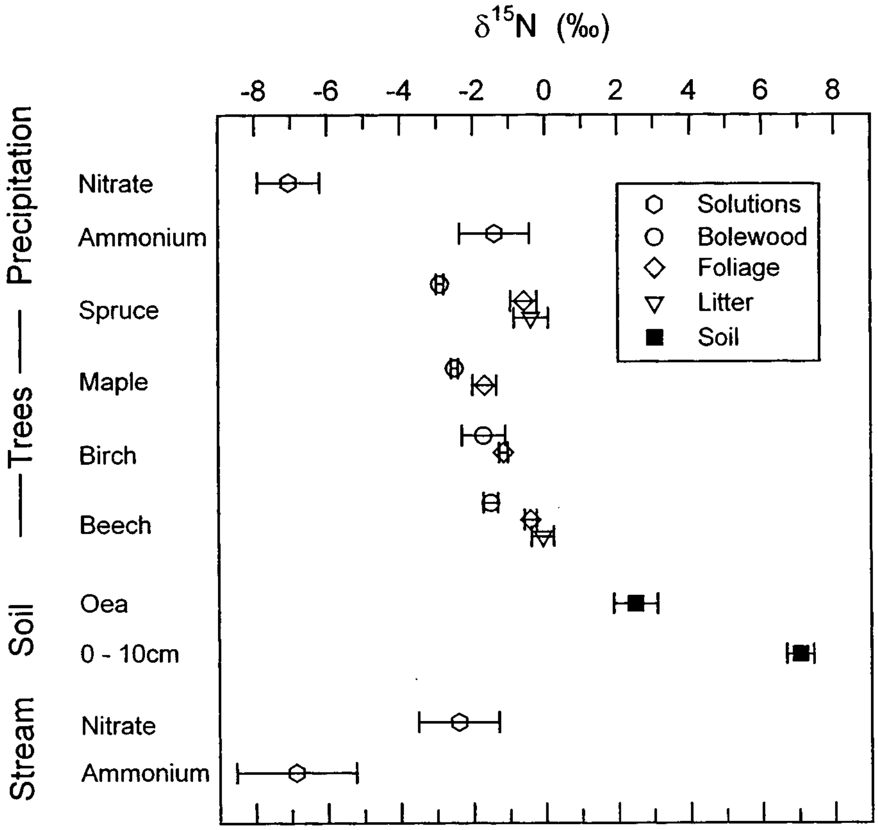

Fig. 1. Natural ^{15}N abundances (means ± 1 sem) in non-labeled forests at the Bear Brook Watersheds in Maine (BBWM) in Maine, USA. Precipitation values are from ammonium (n = 15) and nitrate (n = 16) composites collected from September1990 through October. Streamwater values are from East Bear Brook samples collected from April 1991 and April 1993 (n = 8 for ammonium, n = 9 for nitrate). Wood values are from spruce (n = 14), beech (n = 12), maples (n = 8) and birch (n = 4) sampled from trees on the West Bear catchment prior to ^{15}N additions. Litter (< 1 year old) values are from composite samples of red spruce (n = 4) and beech (n = 7) litter collected from sample points in both catchments prior to ^{15}N additions. Forest floor (O_{ea} horizon) and mineral soil (0-10 cm depth) values are means of 3 samples collected from each of 6 grid points on the West Bear catchment (n = 18) before ^{15}N additions. Foliage values are means of 3 trees per species sampled in 1991 from the East Bear (non-labeled) catchment.

2.6.2. *Soil*

Estimates of labeled N assimilation into forest floor and soil pools (top 5 cm of O_{ea} + mineral soil, if any) used quadrat-specific N masses and $\delta^{15}N$ values ($\delta^{15}N_f$) derived from samples taken in 1992, quadrat-specific $\delta^{15}N$ values from our 1990 sampling ($\delta^{15}N_i$), and Equation [2] to estimate retention within each 20 cm x 20 cm quadrat. Labeled N retention in the top 5 cm of forest floor + soil was estimated as the mean retention of the 6 quadrats sampled within each forest type. This method of calculation was possible because measures of soil mass, percent N, and ^{15}N abundance were available for each sample.

TABLE I

Total carbon and nitrogen pool sizes and element concentrations in aboveground biomass and surface soil of the three forest types in the West Bear Brook catchment. Values in parentheses are standard errors of means ($n = 4$ to 8 for foliar mass, $n = 4$ to 12 for foliage and wood element concentrations, $n = 6$ for forest floor and soil masses and element concentrations).

Forest Ecosystem Type	Total C (kg/ha)		C (% of total mass)		N (% of total mass)		C:N		Total N (kg ha^{-1})	
SPRUCE										
Foliage: Beech	8	(7)	50.4	(0.25)	2.97	(0.254)	17.2	(1.64)	0	
Maple	40	(12)	49.9	(0.38)	2.22	(0.045)	22.5	(0.28)	2	
Birch	17	(5)	50.0	(0.30)	3.02	(0.133)	16.7	(0.71)	1	
Spruce, current-year	858	(201)	51.8	(0.24)	1.23	(0.048)	42.4	(1.47)	20	
Spruce, 1 year-old	872		52.6	(0.29)	1.15	(0.060)	46.3	(2.04)	19	
Spruce, >1 year-old	4,335		52.3	(0.29)	1.01	(0.028)	52.1	(1.29)	84	
Total Foliage	6,130								126	
Wood: Beech	0								0	
Maple	4,024		44.4	(0.95)	0.14	(0.010)	318.2	(15.94)	13	
Birch	4,444		45.1	(0.30)	0.15	(0.009)	296.9	(17.32)	15	
Spruce	46,085		46.5	(0.28)	0.12	(0.010)	390.1	(33.14)	122	
Total Wood	54,553								150	
Aboveground Biomass	60,683								276	
Soil: Forest Floor (Oea)	26,183	(4,062)	33.2	(3.84)	1.56	(0.173)	21.3	(0.52)	1144	(98)
Mineral (0-10cm)	48,751	(10,947)	11.6	(1.60)	0.56	(0.086)	21.0	(0.43)	2105	(334)
Sum of Oea and 0-10cm soil	74,934								3,249	
Sum of Measured Pools	135,617								3,525	
HARDWOOD- SPRUCE										
Foliage: Beech	141	(83)	49.4	(0.19)	2.41	(0.117)	20.8	(1.03)	7	
Maple	174	(4)	49.8	(0.69)	2.40	(0.119)	20.9	(0.77)	8	
Birch	33	(22)	49.3	(0.06)	2.57	(0.084)	19.3	(0.67)	2	
Spruce, current-year	535	(69)	51.3	(0.19)	1.18	(0.089)	44.8	(3.11)	12	
Spruce, 1 year-old	539		51.7	(0.36)	1.06	(0.052)	49.5	(2.25)	11	
Spruce, >1 year-old	2,720		52.1	(0.21)	1.07	(0.043)	49.3	(1.86)	56	
Total Foliage	4,143								96	

Table I (continued)

Forest Ecosystem Type	Total C (kg/ha)	C (% of total mass)	N (% of total mass)	C:N	Total N (kg ha^{-1})
Wood: Beech	22,047	46.2 (0.25)	0.16 (0.006)	297.4 (12.53)	75
Maple	17,371	46.1 (0.19)	0.14 (0.02)	384.6 (58.60)	52
Birch	5,664	46.6 (0.14)	0.12 (0.006)	390.5 (19.79)	15
Spruce	16,747	47.2 (0.20)	0.10 (0.009)	500.9 (41.48)	35
Total Wood	61,830				176
Aboveground Biomass	65,972				272
Soil: Forest Floor (Oea)	11,414 (2,216)	28.3 (4.45)	1.31 (0.173)	21.5 (0.90)	483 (78)
Mineral (0-10cm)	42,328 (2,473)	5.6 (0.79)	0.31 (0.040)	17.9 (0.55)	2295 (217)
Sum of Oea and 0-10cm soil	55,742				2,779
Sum of Measured Pools	119,714				3,050
HARDWOOD					
Foliage: Beech	385 (48)	49.3 (0.27)	2.16 (0.153)	23.5 (1.79)	17
Maple	308 (97)	49.0 (0.40)	1.97 (0.118)	25.2 (1.58)	12
Birch	24 (13)	48.2 (0.48)	2.49 (0.124)	19.4 (0.82)	1
Spruce, current-year	2 (1)	51.4 (0.24)	1.08 (0.055)	48.3 (2.32)	<1
Spruce, 1 year-old	2	51.8 (0.38)	1.00 (0.055)	52.8 (3.24)	<1
Spruce, >1 year-old	8	51.9 (0.45)	1.00 (0.057)	53.2 (3.63)	<1
Total Foliage	728				31
Wood: Beech	31,527	46.4 (0.17)	0.17 (0.011)	291.2 (21.68)	112
Maple	14,196	46.7 (0.18)	0.12 (0.008)	411.0 (31.44)	35
Birch	9,320	46.6 (0.28)	0.10 (0.009)	457.2 (35.47)	21
Spruce	2,520	47.3 (0.23)	0.10 (0.005)	494.1 (20.67)	5
Total Wood	57,563				173
Aboveground Biomass	58,291				204
Soil: Forest Floor (Oea)	19,469 (1,003)	35.5 (3.60)	1.56 (0.120)	22.9 (1.78)	871 (103)
Mineral (0-10cm)	39,656 (5,923)	6.0 (0.71)	0.33 (0.051)	18.8 (1.09)	1974 (191)
Sum of Oea and 0-10cm soil	59,125				2,845
Sum of Measured Pools	117,416				3,048

2.6.3. *Streamwater*

Streamwater chemistry and streamflow data were provided by our collaborators on the Bear Brook Watershed project (See Kahl *et al.* and Norton *et al.*, this volume.). These data, together with our measurements of ^{15}N abundances in dissolved nitrate and ammonium in samples collected from April 1991 through June 1993, were used to estimate cumulative DIN exports from the treated catchment and the contributions of ^{15}N-labelled fertilizer to DIN exports as follows. Using hourly flow data and weekly (or more frequent at high flows) DIN concentrations, the masses of nitrate- and ammonium-N exported were calculated for the time period from which each streamwater ^{15}N sample was composited. The measured ^{15}N abundances of nitrate and ammonium were applied to the mass of nitrate or ammonium to estimate the mass of ^{15}N exported as DIN during that period. These masses were accumulated over the year to make conservative estimates of ^{15}N exported as both DIN forms. We were unable to make direct measurements of ammonium or nitrate ^{15}N abundances for about half of the April 1991 to June 1993 interval, due to low flow and sample storage problems. For these periods, we estimated ^{15}N abundances to be the same as those of the prior measured period.

2.7. ELEMENTAL AND N ISOTOPE ANALYSES

All samples subjected to C, N or ^{15}N analysis were ground to a fine powder and dried (50°C) prior to analysis. Total C and N contents of plant tissues and soils were measured using either a Perkin-Elmer 240C or a 2400 elemental analyzer with acetanilide as a reference standard. The ^{15}N abundances of finely ground plant tissue and soil samples were determined by combustion to N_2 gas, cryogenic purification, and analysis using a trapping box and a Finnegan MAT Delta S isotope ratio mass spectrometer (Fry *et al.*, 1992). Analytical precision using this method is typically better than 0.2 ‰ $\delta^{15}N$. Nitrate and ammonium in streamwater and precipitation samples were extracted, concentrated, and diffused onto filter papers as described by Downs *et al.* (this volume). The N-impregnated filter papers were then ground and subjected to ^{15}N analysis in the same manner as plant and soil samples.

3. Results

3.1. ECOSYSTEM N POOLS

Aboveground tree biomass (wood plus foliage) in the three ecosystem types of the West Bear Brook catchment ranged from ~130 Mg ha^{-1} spruce forest (3.3 ha) in the upper elevations to ~140 Mg ha^{-1} in the hardwood-spruce forest (3.6 ha) in the middle elevations (Table I). Tree biomass in the hardwood forest (3.3 ha) in the lower third of the catchment was intermediate at ~135 Mg ha^{-1}. Foliage comprised ~9, 6, and 1 percent of tree biomass, respectively, in the spruce, hardwood-spruce and hardwood forests. Wood ranged from 44 to 47 percent C and from 0.10 to 0.17 percent N across species and forest types. Percent N was lower in spruce (wood = 0.10 to 0.12 %N) than in either beech or maple (wood = 0.12 to 0.17 %N). Carbon concentrations were higher in foliage (50 ± 2 %C) than in wood (46 ± 2 %C). Hardwood leaves ranged from ~2 to 3 percent N whereas red spruce needles ranged from 1.0 to 1.2 percent N with higher concentrations in younger needles. Forest floor (Oe + Oa horizons) C content ranged from 11.4 Mg ha^{-1} in the hardwood-spruce forest to 26.2 Mg ha^{-1} in the spruce forest. Forest floors ranged from 28 to 36 percent C and from 1.3 to 1.6 percent N. Mineral soils had lower C and N concentrations than did forest floors but total C and N contents were higher due to higher bulk densities.

Total N contents in measured pools ranged from ~3.1 to 3.5 Mg ha^{-1} in the 3 forest types on the treated catchment (Table I). Nitrogen concentrations in deeper soils (>10 cm below the forest floor) and roots were not measured. Inclusion of these pools, particularly deep soils, would increase ecosystem N estimates considerably. Of the pools measured, >90 percent of the N content in all 3 forest types was contained in the forest floor and underlying 10 cm of mineral soil and <10 percent of ecosystem N was in aboveground biomass. Aboveground biomass accounted for about 200 to 275 kg N ha^{-1} across forest types. Smaller wood biomass and lower foliar and wood N concentrations in spruce than in hardwoods were compensated for by larger foliar mass in the spruce and hardwood-spruce forests. As a result, aboveground biomass N was highest in the spruce forest and lowest in the hardwood forest.

3.2. ^{15}N NATURAL ABUNDANCES

Natural abundances of ^{15}N in soils, aboveground biomass, and solutions (precipitation and streamwater) at the Bear Brook catchments (Figure 1) provide references for determining the movements of ^{15}N-labelled fertilizer into ecosystem components of forests in the treated catchment and also provide insights into N cycling processes in non-labeled forests. Prior to ^{15}N additions, plant N pools were depleted in ^{15}N relative to soil pools with $\delta^{15}N$ values of plant tissues ranging between approximately –3 and 0 ‰ and increasing from ~0 ‰ in fresh litter to +2.5 ‰ in the forest floor (O_{ea} horizon) and to +7 ‰ in the mineral soil (0-10 cm).

Precipitation at the catchments was depleted in ^{15}N relative to N in both soils and plants (Figure 1). The mean $\delta^{15}N$ value of nitrate in precipitation (–7.1 ‰) was 4 to 7 ‰ less than plant N whereas ammonium in precipitation (–1.4 ‰) occupied the middle of the plant $\delta^{15}N$ range. Because the wet deposition rate of nitrate-N was ~2 times that of ammonium-N (Kahl *et al.*, 1993), the weighted mean $\delta^{15}N$ of inorganic N in precipitation during 1991 and 1992 was approximately –5 ‰. Outputs of inorganic N in streamwaters of the non- and pre-treated catchments were dominated by nitrate which averaged –2.4 ‰ $\delta^{15}N$ in East Bear Brook from April 1991 through May 1993. The mean $\delta^{15}N$ value for streamwater ammonium was much lower (–7.3 ‰) but ammonium export was extremely low with almost all inorganic N being exported as nitrate (Kahl *et al.*, 1993; this volume). Assuming that >90 percent of all inorganic N loss from non-fertilized catchments was nitrate-N, the weighted $\delta^{15}N$ value of streamwater inorganic N was approximately –2.8 ‰.

3.3. ^{15}N TRACERS

3.3.1. Vegetation and Soil Pools

The $\delta^{15}N$ values of recently formed bolewood in the 4 dominant tree genera increased after each year of ^{15}N-labelled fertilizer addition (Figure 2). Values for maple, spruce and beech wood increased by 5.4 to 6.4 ‰ between October 1990 and October 1992 after 9 applications of ^{15}N labeled fertilizer (37.8 kg N ha^{-1}). Increases were greatest in birch (11.4 ‰). However, the percent of birch in aboveground biomass was small in all three forest types (Table I). Foliar ^{15}N abundances also increased during both years of ^{15}N application (Figure 3). By August 1992, after 8 applications of ^{15}N-labelled fertilizer (33.6 kg N ha^{-1}), beech and maple foliar $\delta^{15}N$ values had increased by ~6.5 ‰ and birch foliage by 8.8 ‰. Changes in ^{15}N abundances of red spruce needles during ^{15}N fertilizer application were greater in current-year than in older needles. By August 1992, the mean $\delta^{15}N$ value of current-year needles had increased by 4.9 ‰ over the reference value –0.4 ‰, while 1-year-old needles means had increased by 3.6 ‰ (from –0.5 ‰) and the oldest needles (2 to 6 years old) had increased by 3.1 ‰ (from –0.9 ‰).

Although we detected increases in ^{15}N abundances of the O_{ea} horizons following addition of ^{15}N-labelled fertilizer to the West Bear catchment, we did not detect increases in the underlying 10 cm of mineral soil (data not shown). Also, repeated sampling of microplots to 5 cm below the surface of the forest floor increased our ability to detect differences during the course of treatment (See Methods). Therefore, we report here the results of repeated samples (forest floor plus any mineral soil present within 5 cm of the O_{ea} surface) taken from permanent microplots in the West Bear catchment. We detected increases in $\delta^{15}N$ values in surface soils of all 3 forest types in the treated catchment during the course of ^{15}N additions (Figure 4). Increases in $\delta^{15}N$ values during the 2 years of treatment ranged from 1.6 ‰ in the hardwoods to ~3 ‰ in both the hardwood-spruce and spruce forests. Soil $\delta^{15}N$ increased significantly (paired, one-tailed *t*-test, $P < .05$, $n=6$) in all three forest types after the first year of ^{15}N additions and did not change significantly thereafter.

3.3.2. Streamwater

Nitrate $\delta^{15}N$ values in streamwater draining the reference catchment averaged –2.4 ‰ and ranged from –7.9 to +0.1 ‰ (Figure 5) from April 1991 through June 1993, while nitrate values in the treated catchment averaged 16.9 ‰ and ranged from –3.8 to +53.7 ‰ (Figure 6). Values for treated catchment samples composited over 7 to 65 day baseflow intervals (mean $\delta^{15}N$ = +17.6 ‰, n = 17) did not differ significantly from values for single day, high flow events (mean $\delta^{15}N$ = +15.3 ‰, n = 8). During the

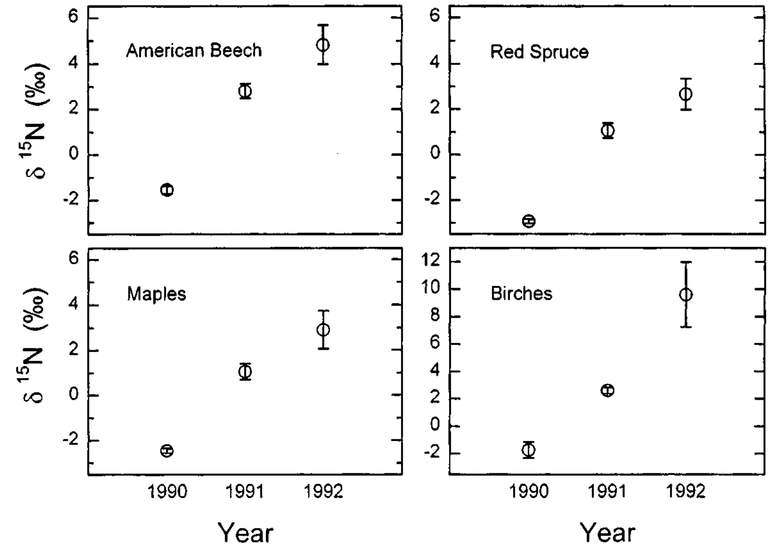

Fig. 2. Bolewood ^{15}N abundances in trees on the West Bear catchment before, and after 1 and 2 years of ^{15}N fertilizer additions. Symbols are means (across forest types, ±1 sem) of wood formed in four tree genera during the 3 years preceding each sampling event. Increases in $\delta^{15}N$ values between years were significant (Student's *t*-test, $p < 0.05$) within each genus.

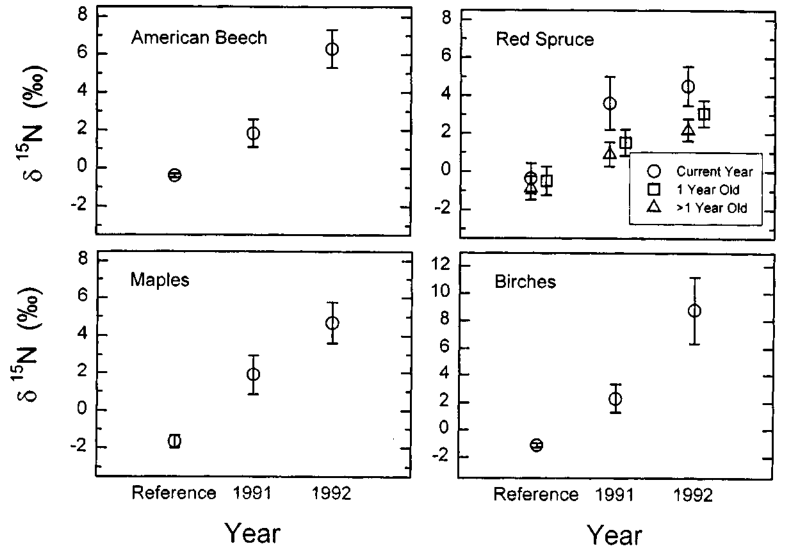

Fig. 3. Foliar ^{15}N abundances in foliage collected from the East Bear (non-labeled reference) catchment in August 1991 and from the West Bear catchment during mid-August of the first (1991) and second (1992) years of ^{15}N fertilizer application. Symbols are means (±1 sem) of foliage sampled across forest types. Foliar $\delta^{15}N$ values were significantly greater (Student's *t*-test, $p < 0.05$) in foliage collected during Year 1 of labelling (1991) on the treated catchment than in foliage collected from the reference catchment during the same year for all species. Values increased significantly between Years 1 and 2 of labelling in beech, maple and birch, but not in red spruce foliage.

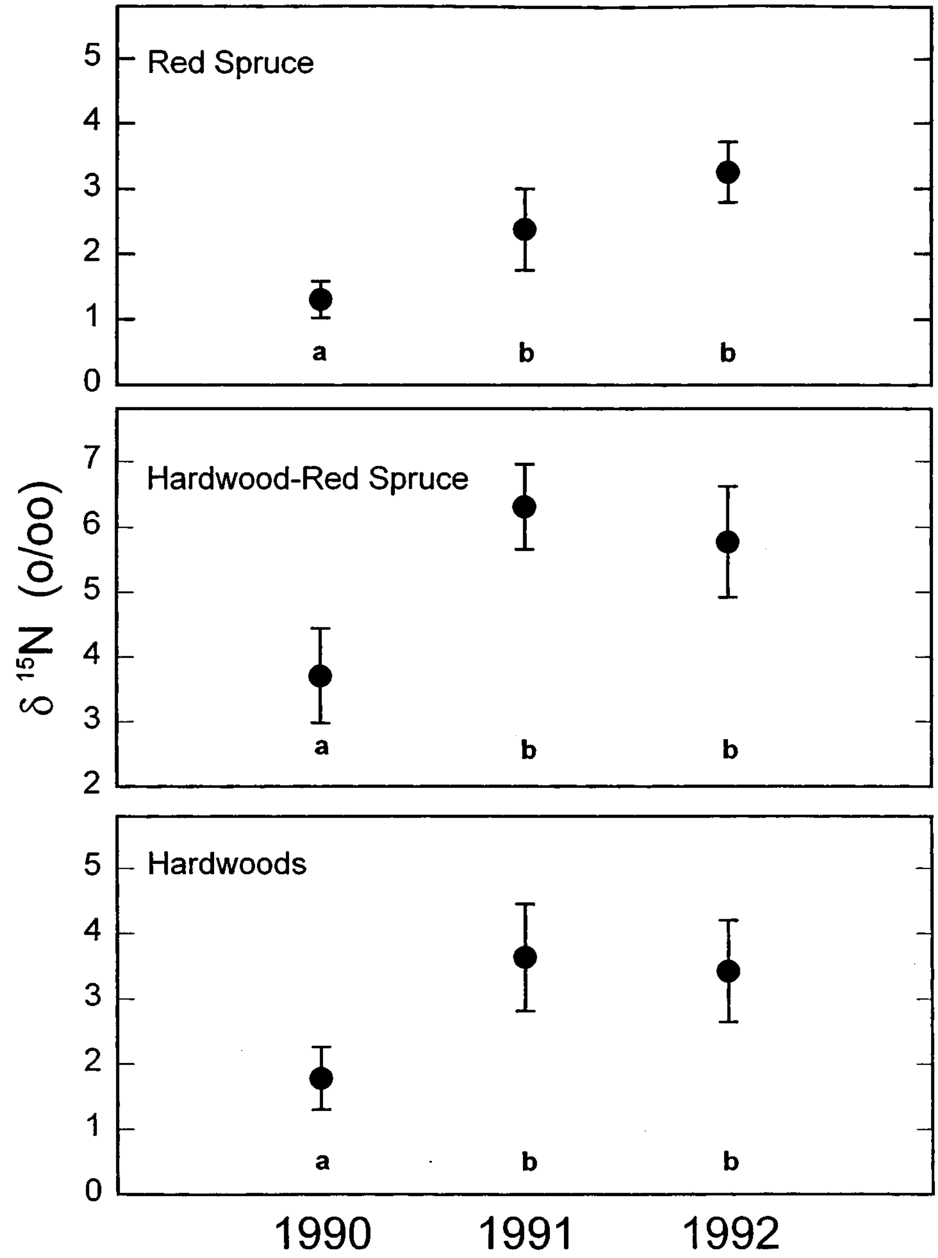

Fig. 4. Soil ^{15}N abundances before and after 1 and 2 seasons of ^{15}N fertilizer application. Sample cores (2.2 cm diam.) were taken each year from the top 5 cm of soil (forest floor including mineral soil, if present within 5 cm) in 6 permanent quadrats (400 cm^2 each) located in each of the three forest types in the catchment. Samples were collected in November of each year after additions of 0 (1990), 16.8 (1991) and 37.8 (1992) kg N ha^{-1} yr^{-1} of labeled fertilizer to the catchment. Different lower case letters within forest types show significant differences in mean $\delta^{15}N$ values between years (paired *t*-test, one tailed, $P < .05$).

same period, reference streamwater ammonium $\delta^{15}N$ values averaged −7.3 ‰ and ranged from −13.8 to −2.8 ‰ (Figure 5), while ammonium values in the treated catchment stream averaged +7.0 ‰ and ranged from −10.6 to +110.8 ‰ (Figure 6).

Nitrate $\delta^{15}N$ values were greater than ammonium values in the treated catchment on all but three sampling dates (Figure 6). These dates were all in either late March or early April during periods of high flow. Between April 1991 and June 1993, volume weighted averages in West Bear Brook streamwater were 34 μmol l^{-1} for nitrate and 3 μmol l^{-1} for ammonium, while in East Bear Brook averages were 13 μmol l^{-1} nitrate and 2 μmol l^{-1} ammonium. Therefore, although the streamwater ammonium ^{15}N abundances were occasionally much higher than nitrate ^{15}N abundances, the amount of fertilizer-derived N exported from the treated catchment as ammonium was much less than the amount exported as nitrate. Nitrate $\delta^{15}N$ values in West Bear Brook, although almost always greater than those in East Bear Brook, tended to be highest during the spring and fall when baseflow was high and were lowest during summer when baseflow was low (See Norton *et al.*, this volume). The highest nitrate $\delta^{15}N$ values in West Bear Brook occurred in April-May 1993, 4 to 5 months after the last application of ^{15}N fertilizer.

3.4. FATE OF ^{15}N ADDITIONS: MASS BALANCES

Of the total 42 kg ha^{-1} of ^{15}N-labelled fertilizer added to the West Bear Brook catchment between April 1991 and December 1992, we account for between 14 and 17 kg N ha^{-1} in vegetation plus surface soil (mainly forest floor) in the three forest ecosystem types (Table II). The weighted (by forest area) mean retention of ^{15}N-labelled fertilizer in aboveground vegetation and 0-5 cm soil was 15.4 kg ha^{-1} , or ~37% of the total mass of ^{15}N-labelled NH_4-N additions.

Soil was a greater sink for labeled fertilizer than was aboveground plant biomass in all three forest types, ranging from 7.6 kg N ha^{-1} in hardwood-spruce to 13.3 kg N ha^{-1} in spruce (Table II). Aboveground vegetation retained only 3.4 kg ha^{-1} of labeled N additions in the spruce forest and more than twice as much (7.1 kg N ha^{-1}) in the hardwood-spruce forest. In the spruce forest, foliage assimilated 2.4 of the 3.4 kg ha^{-1} labeled N additions that were retained in aboveground biomass. In contrast, foliage in the hardwood forest assimilated only 1.0 of 4.8 kg ha^{-1} of the labeled N additions retained in aboveground biomass. The remaining 3.8 kg ha^{-1} of labeled N additions retained in aboveground biomass was retained in newly formed (1990-1992) wood. The relatively large canopy N mass in the spruce forest was a greater sink for N additions than was the smaller N mass in the deciduous forest canopy. Conversely, higher percentages of labeled N were retained in wood of deciduous species than of spruce (Table II).

Nitrogen (mainly nitrate) exports via streamwater from the West Bear catchment have increased steadily relative to the reference catchment since the start of ammonium-N additions in November 1989 (Kahl *et al.* 1993; this volume). However,

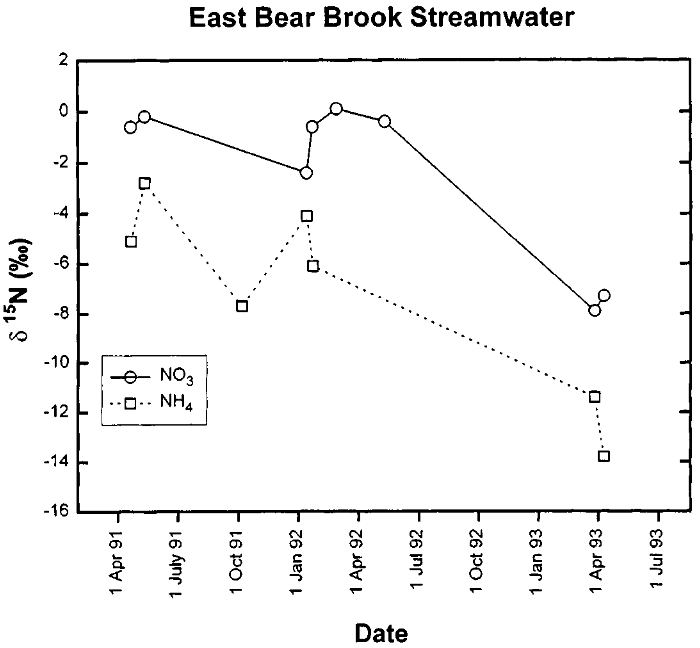

Fig. 5. Ammonium and nitrate $\delta^{15}N$ values in streamwater draining the East Bear Brook (reference) catchment from April 1991 through July 1993.

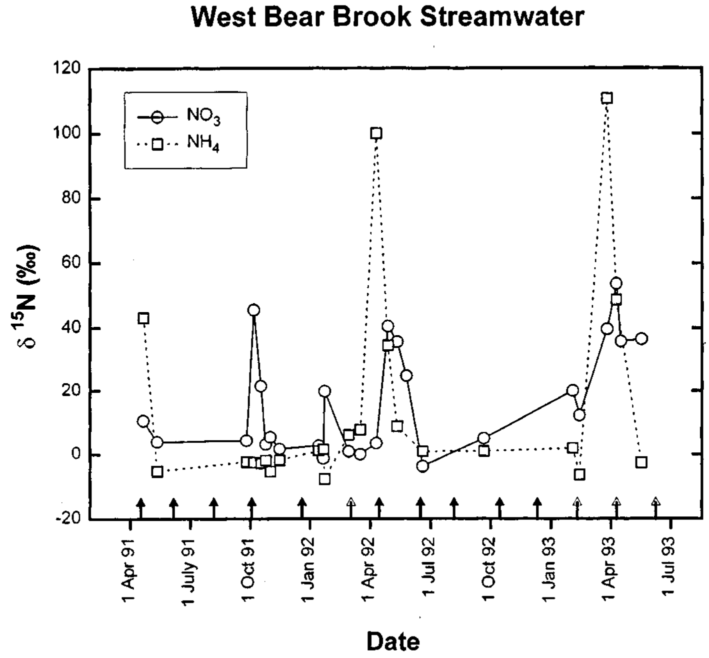

Fig. 6. Ammonium and nitrate $\delta^{15}N$ values in streamwater draining the West Bear (fertilized and ^{15}N–labelled) catchment from April 1991 through July 1993. Filled arrows show dates of ^{15}N-labelled fertilizer ($\delta^{15}N = 192$ ‰) additions. Open arrows show dates of non-labeled fertilizer ($\delta^{15}N = 0$ ‰) additions. Non-labeled fertilizer was also added to the West Bear catchment for one year prior to ^{15}N applications (See methods.).

TABLE II

Retention of ^{15}N-labelled fertilizer additions in forests of the West Bear Brook catchment. Labelled N retention is calculated by mass balance using N pool size and δ^{15}N values before and after labelling. See text for details.

Forest Ecosystem Type (and area)	N Pool (kg/ha)	Pre-treatment δ^{15}N (‰)	1991 δ^{15}N (‰)	1992 δ^{15}N (‰)	Labelled N retention (kg/ha)
SPRUCE (3.3 ha)					
Foliage: Beech	<1	-0.40	0.40	8.90	<0.01
Maple	2	-1.67	-1.20	9.55	0.1
Birch	1	-1.15	3.15	2.40	0.0
Spruce, current-year	20	-0.37	2.05	5.02	0.6
Spruce, 1 year-old	19	-0.50	1.10	2.87	0.3
Spruce, >1 year-old	84	-0.87	0.40	2.18	1.3
Total Foliage	126				2.4
Wood†: Beech	nd				
Maple	4	-1.40		10.45	0.2
Birch	10	-2.40		-0.30	0.1
Spruce	27	-2.85		1.87	0.6
Total Wood	41				1.0
Above-ground Biomass	167				3.4
Soil: top 5 cm (Oea)	851	1.30		3.25	13.3
Sum of Measured Pools	1018				16.7
HARDWOOD- SPRUCE (3.6 ha)					
Foliage: Beech	7	-0.40	3.95	7.18	0.4
Maple	8	-1.67	3.03	6.60	0.6
Birch	2	-1.15	1.30	7.90	0.1
Spruce, current-year	12	-0.37	7.20	6.15	0.4
Spruce, 1 year-old	11	-0.50	3.05	5.35	0.3
Spruce, >1 year-old	56	-0.87	2.15	3.58	1.3
Total Foliage	96				3.1
Wood†: Beech	67	-1.48		5.33	2.3
Maple	16	-2.50		2.96	0.4
Birch	20	-2.50		5.40	0.8
Spruce	13	-2.95		3.33	0.4
Total Wood	115				4.0
Above ground Biomass	211				7.1
Soil: top 5 cm (Oea + some mineral)	1047	3.71		5.72	7.6
Sum of Measured Pools	1258				14.7

HARDWOOD (3.3ha)					
Foliage: Beech	17	-0.40	0.40	4.13	0.5
Maple	12	-1.67	-1.20	3.90	0.4
Birch	1	-1.15	3.15	8.10	0.1
Spruce, current-year	<1	-0.37	2.05	2.13	<0.01
Spruce, 1 year-old	<1	-0.50	1.10	1.05	<0.01
Spruce, >1 year-old	<1	-0.87	0.40	0.80	<0.01
Total Foliage	31				1.0
Wood[†]: Beech	47	-1.69		5.37	1.7
Maple	23	-2.40		4.35	0.8
Birch	17	-1.70		12.10	1.2
Spruce	1	-3.08		3.18	0.0
Total Wood	89				3.8
Above-ground Biomass	119				4.8
Soil: top 5 cm (Oea + some mineral)	738	1.78		3.42	9.8
Sum of Measured Pools	857				14.6

[†] Trees were cored at the end of the 1992 growing season (final season of ^{15}N additions) and ^{15}N contents of 1990-1992 growth rings were used in mass balance calculations.

only a small proportion of the nitrate leaving this treated catchment originated as fertilizer (Table III). For example, ~6 percent of the nitrate-N exported to streamwater from the start of ^{15}N labelling in April 1991 through 1 June 1992 originated from the labeled ammonium-N additions. Approximately 24 percent of the ammonium exported to streamwater during this period originated as fertilizer. Because nitrate losses dominated, however, only 7 percent of total inorganic N losses through 1 June 1992 could be attributed to labeled fertilizer additions. By June 1993, the cumulative fractions of streamwater N derived from fertilizer additions increased to 45 percent for ammonium and to 14 percent for nitrate losses. The weighted average contribution of labeled N to total DIN loss during the 26 month period was 15 percent of the 12.5 kg N ha^{-1} exported to streamwater. Therefore, although DIN losses from April 1991 through 1 June 1992 were equivalent to 30 percent of the 42 kg ha^{-1} of labeled ammonium-N additions (and to 22 percent of 58 kg N ha^{-1} of labeled plus non-labeled fertilizer), only ~5 percent of the labeled N additions was exported as DIN in streamwater during the period of measurement.

4. Discussion

The fertilizer additions of 25 kg N ha^{-1} yr^{-1} to the West Bear Brook catchment were less than 1 percent of the 3,000 to 3,500 kg N ha^{-1} contained in aboveground biomass and surface soils in the catchment forests. However, the rate of N addition was large relative to rates of N cycling between plants and soils within the forests. We estimate, for example, that N uptake into aboveground biomass ranged from 24 kg ha^{-1} yr^{-1} in

TABLE III

Cumulative N losses from the West Bear Brook Catchment from 1 April 1991 through 1 June 1993. Units are kg N ha^{-1} exported via streamwater from a catchment fertilized with 25.2 kg N ha^{-1} yr^{-1} since November 1989. Dissolved inorganic N (DIN) is the sum of ammonium-N and nitrate-N. Estimates of N losses originating from the 42 kg ha^{-1} of ^{15}N-labelled ammonium-N added to the catchment from April 1991 through December 1992 (equal bi-monthly additions) were derived using ^{15}N abundances in streamwater ammonium and nitrate and mass balance.

Cumulative N Losses	June 1991			June 1992			June 1993		
starting 1 April 1991	NH_4-N	NO_3-N	DIN	NH_4-N	NO_3-N	DIN	NH_4-N	NO_3-N	DIN
Total N	.02	0.88	0.90	0.20	5.50	5.70	0.59	11.94	12.54
N mass from labelled fertilizer	trace	0.04	0.04	0.05	0.33	0.38	0.26	1.66	1.92
Percent N from labelled fertilizer		4%	4%	24%	6%	7%	45%	14%	15%

TABLE IV

Estimated annual N uptake into aboveground vegetation from soils in forests of the West Bear Brook catchment.

		Annual N uptake			Percent soil N
Forest type	Table soil N[†] Oea + 10cm (kg ha^{-1})	Foliage[‡] (kg ha^{-1} yr^{-1})	Wood[§] (kg ha^{-1} yr^{-1})	Total aboveground (kg ha^{-1} yr^{-1})	to aboveground biomass (yr^{-1})
Spruce	3200	10	14	24	0.8%
Hardwood-Spruce	2800	12	28	40	1.4%
Hardwood	2800	13	30	43	1.5%

[†] From Table I.

[‡] Estimated as leaf litterfall times %N in litter summed for dominant tree species.

[§] Estimated as 1/3 of 3-year N uptake; from Table I.

the spruce forest to 43 kg ha^{-1} yr^{-1} in the hardwood forest type (Table IV). If annual N uptake by belowground biomass (mostly fine roots) is assumed to be two times uptake by foliage (e.g., Nadelhoffer *et al.*, 1985), then N uptake into plant biomass ranges from ~55 kg ha^{-1} yr^{-1} in the spruce forest to ~70 kg ha^{-1} yr^{-1} in the hardwood-spruce forest. This range compares well with the mean annual net N mineralization rates 85 kg N ha^{-1} yr^{-1} for hardwood-spruce plots adjacent to the East Bear Brook catchments (Magill *et al.*, 1996). Therefore, although annual fertilizer N inputs are small relative to total N stocks in these forests, they are equivalent to between 1/3 and 1/2 of estimated annual N exchanges between vegetation and soils in the catchment forests. Given that the cumulative amount of N addition was large relative to actively cycling N pools, it is not surprising that some N was exported even if forest growth within the catchment is N limited.

The pattern of variation in ^{15}N natural abundance in the Bear Brook forests (Figure 1) is characteristic of the general pattern reported for forests elsewhere (e.g. Nadelhoffer and Fry, 1988; Gebauer and Schulze, 1991; Garten, 1993; Garten and Van Miegroet, 1994; Buchmann *et al.*, 1995) with plant tissues having relatively low ^{15}N abundances and soils having higher ^{15}N abundances. This pattern likely results from the preferential mineralization of ^{14}N during decomposition. This isotopic discrimination gradually enriches soil organic matter in ^{15}N as fresh litter progresses to well-decomposed humus (Nadelhoffer and Fry, 1994). The mineralized N in soil, consequently, is depleted in ^{15}N which leads to lower plant tissue $\delta^{15}N$ values.

The variations in ^{15}N abundances among plant tissues, soils, and inorganic N inputs and outputs at the Bear Brook site (Figure 1) provide insights into N cycling dynamics. First, plant tissue $\delta^{15}N$ values are intermediate between values in soil and precipitation, suggesting that plants take up N from both sources. However, precipitation adds <5 kg ha^{-1} yr^{-1} and annual N uptake by plants and net N mineralization in soil range between 55 and 85 kg N ha^{-1} yr^{-1}. Thus, most N taken up by trees is likely derived from soil pools. Soil pools from which plant-available N is derived, therefore, are likely depleted in ^{15}N relative to bulk soil due to isotopic fractionation during mineralization and nitrification (Shearer *et al.*, 1974). Plant N could also be depleted in ^{15}N as a result of greater rates of N mineralization and plant N uptake occurring in upper soil horizons than in deeper, more ^{15}N-enriched horizons. This is consistent with results of *in situ* measures by Magill *et al.* (1996) conducted in plots adjacent to the East Bear catchment. They reported that net N mineralization was about 2 times greater in the forest floor than in the underlying 10 cm of mineral soil.

Differences in natural ^{15}N abundances in precipitation and streamwater N also suggest that background inorganic N inputs undergo biochemical transformations within forests and have long residence times in the catchment. For example, the mean $\delta^{15}N$ value of nitrate in precipitation was approximately –7 ‰ while the value for streamwater nitrate from the non-fertilized catchment was approximately –2 ‰. Moreover, the mean $\delta^{15}N$ value for streamwater nitrate in the reference catchment was within the –3 to 0 ‰ range of values for plant tissues in the forests, suggesting that exported nitrate was derived from the same pools as plant-assimilated nitrate. Ammonium in reference streamwater was highly depleted in ^{15}N relative to ammonium in precipitation and to both plant tissue and soil N. However, because ammonium concentrations in streamwater from the reference and pre-fertilized catchments were extremely low and often undetectable, we do not speculate as to the cause of the low $\delta^{15}N$ values.

The relative abundance of ^{15}N in the total catchment N pool is probably decreasing slowly, both because inorganic N inputs in wet deposition exceed stream outputs by ~1.5 kg ha^{-1} yr^{-1} in the reference watershed (Kahl *et al.*, 1993) and because the $\delta^{15}N$ value of measured N inputs (–5 ‰) exceeds that of outputs (–3 ‰). Any changes in the N isotope composition of total ecosystem pools in catchment forests would occur slowly, because N fluxes into and out of the forests via streams are small (< 5 kg ha^{-1}

yr^{-1}) relative to total N pools (> 3,000 kg N ha^{-1}). However, sustained changes in N fluxes either into or out of smaller, actively cycling N pools such as in plant biomass (<300 kg N ha^{-1}) could result in detectable changes in biomass ^{15}N abundances at decadal time scales (Gebauer and Schulze 1991; Högberg and Johannisson 1993).

The narrow ranges of ^{15}N abundances in plant tissues (Figure 1), small pool sizes of N in aboveground plant tissues (Table I), and large changes in foliage and wood δ^{15}N values after ^{15}N fertilizer additions (Figures 2 and 3) resulted in relatively reliable estimates of assimilation of ^{15}N-labelled ammonium into aboveground biomass. Although tree biomass served as a sink for fertilizer additions, of the 42 kg N ha^{-1} of ^{15}N-labelled ammonium added to each forest type, aboveground biomass assimilated only ~8 percent in the spruce forest, 11 percent in the hardwood forest, and 17 percent of N additions in the hardwood-spruce forest. Assimilation of the labeled N additions into belowground biomass (roots) was not measured. However, it is unlikely that assimilation into belowground biomass exceeded assimilation into aboveground biomass. Assuming that N assimilation into roots did not exceed assimilation into aboveground tissues, less than one-third of the labeled ammonium additions was assimilated into growing forest biomass in any of the three forest types.

Foliage was a comparatively strong sink for N additions to the spruce forest, assimilating 2.5 times more ^{15}N-labelled fertilizer N than wood (Table II). Although older needles (>1 year old) accounted for over half of the labeled N additions retained in the spruce forest canopy, current-year needles were a stronger sink on a concentration basis. Approximately 3 percent of the N in current-year needles was derived from the ^{15}N-labelled additions whereas only 1.6 to 1.7 percent of the N in older needles was derived from the labeled additions. In contrast, the hardwood forest assimilated ~4 times more labeled N fertilizer into wood than into foliage. This was partially due to the fact that total N accumulation from 1990 to 1992 by hardwood forest wood was ~2 times greater than by spruce wood while canopy N content was ~4 times greater in the spruce than in the hardwood forest (Table II). However, the percentages of labeled N additions accounted for in wood (1990-1992 growth) were ~4.3 percent of the total N accumulated in the hardwood forest wood and ~2.4 percent of total N accumulated in the spruce forest wood, suggesting that biomass accumulation was more limited by N availability in hardwoods than in spruce.

Our tracer results suggest that soil (primarily forest floor) was a greater sink for added N than was plant biomass in all three forest ecosystem types on the treated catchment, particularly in the spruce forest. We are less confident in our estimates of ^{15}N-labelled fertilizer retention in soils than in aboveground biomass, however, because mass balance-based estimates in soils (with large N stocks) are highly sensitive to small shifts in δ^{15}N values following tracer additions. For example, although natural variations in mineral soil ^{15}N abundances did not allow for detecting 1 ‰ shifts in the δ^{15}N values, a 1 ‰ increase in a 2,000 kg ha^{-1} mineral soil (0-10 cm depth) N pool would account for ~11 kg ha^{-1} of N fertilizer with a δ^{15}N value of 192 ‰. Therefore, we strongly recommend that the δ^{15}N value of fertilizers used in future large-scale tracer studies exceed 1000 ‰. Although our estimates of ^{15}N labeled fertilizer retention in soils were less reliable than our estimates of retention in plant biomass, we suspect that most of the labeled fertilizer was in fact retained in soils and decomposing litter. This is because vegetation (including roots, above) probably accounted for less than one-third of the ^{15}N-labelled additions (above) and because only ~5 percent of the labeled N added to the catchment was exported via streamwater through the first 6 months following the final ^{15}N fertilizer application (Table III).

Finally, our tracer results suggest that most of the nitrate exported from the chronically fertilized catchment at the Bear Brook site was derived mainly from sources within the catchment rather than directly from ammonium additions. Although cumulative inorganic N losses from the West Bear catchment between 1 June 1991 (just after the start of ^{15}N additions) and 1 June 1993 were 12.5 kg N ha^{-1}, only ~2 kg N ha^{-1} was derived from the 42 kg ha^{-1} of labeled ammonium-N additions made between April 1991 and December 1992 (Table III). A much greater percentage of ammonium than nitrate in streamwater draining the treated catchment during this period was derived from fertilizer. Ammonium, however, comprised only ~5 percent of inorganic N losses from the treated catchment and the cumulative exports of fertilizer-derived ammonium via streamwater represented less than 1 percent of the labeled fertilizer added to the catchment. Taken together, these data suggest that elevated nitrate exports from ammonium-fertilized catchment resulted primarily from increased nitrification in soils, decreased nitrate assimilation by the biota, or both factors together.

5. Conclusion

Variations in ^{15}N natural abundances in plant tissues, soils, precipitation and streamwater at the BBWM site provided insights into forest N cycling processes prior to labelling of the experimental catchment. Labelling the ammonium sulfate added to the treated catchment with $^{15}NH_4^+$ (fertilizer $\delta^{15}N$ = 192 ‰) during years 2 and 3 of fertilization allowed us to compare the relative importance of soils and vegetation as sinks for N additions in the three forest types on the catchment.

Natural ^{15}N abundances (before tracer additions) showed:

- Patterns of ^{15}N abundances were typical of those reported from other forests with $\delta^{15}N$ values of plant and litter materials ranging from -3 to 0 ‰ and with soil values increasing from 2 (±1) ‰ in the O_{ea} (humus) horizon to about 7 ‰ in the underlying mineral soil.
- Precipitation N was depleted in ^{15}N relative to biomass and soil N while the ^{15}N abundances in streamwater nitrate and tree tissues were similar, thereby suggesting that background N inputs to the catchments are cycled through biomass, litter and soils before being remineralized, nitrified and exported.

The ^{15}N tracer additions to the fertilized catchment showed:

- Total assimilation of ^{15}N-labelled N additions into measured pools was similar in all three forest types and ranged from 15 to 17 kg ha^{-1}, or ~35 to 40 percent of labeled N inputs.
- Aboveground biomass assimilated only 3 to 7 kg ha^{-1} (or 8 to 17 percent) of 42 kg ha^{-1} of labeled fertilizer N applied during 2 years of fertilizer addition.
- The proportion of labeled N inputs retained in aboveground biomass was lowest in the spruce forest, highest in the hardwood-spruce forest, and intermediate in the hardwood forest.
- Hardwood species assimilated greater proportions of the labeled N additions into woody tissues while spruce trees assimilated more labeled N into foliage.
- Surface soils (O_{ea} horizon plus any mineral soil within 5 cm of the surface of the O_{ea}) assimilated 8 to 13 kg ha^{-1} (19 to 31 percent) of labeled fertilizer added.
- Non-sampled pools (mostly in soils) probably served to retain large fractions of N inputs.
- Only 2 of the 13 kg NO_3-N ha^{-1} exported in streamwater from the fertilized catchment was derived from the labeled 42 kg NH_4-N ha^{-1} additions.
- Most nitrate exported from the treated catchment was derived from N pools with residence times >2 years.
- Ammonium sulfate additions either stimulated nitrification, inhibited biological nitrate assimilation, or affected both processes simultaneously.

Acknowledgments

We gratefully acknowledge the support and cooperation of our collaborators from the University of Maine including Drs. Stephen Norton, J. Stephen Kahl, Ivan Fernandez, and Lindsey Rustad. We are indebted to Dr. Roland Hauk and Mr. George Jones of the Tennessee Valley Authority (Muscle Shoals, AL) for advice and for preparing the ^{15}N-labelled fertilizer. We also thank Jon Scofield for sending solution samples from the field on a regular basis; Daniel Sperduto, Todd Drummey, Katherine Michmerhuizen, and Jennifer Ellis for field and laboratory assistance; Kris Tholke for conducting ^{15}N analyses; and Dr. William Currie for valuable comments on an earlier draft. Carbon and nitrogen analyses were performed using equipment donated by the Perkin-Elmer Corporation to the Marine Biological Laboratory. Nadelhoffer thanks the U. S.-Norway Fulbright Program and the Norwegian Institute for Water Research who provided resources during manuscript preparation. Financial support was provided by the U. S. Environmental Protection Agency (EPA CR-818633-01-0) and the National Science Foundation (NSF-BSR 9009190 and NSF-DEB 9408794). The research has not been subjected to the review of either agency and therefore does not necessarily reflect the views of either agency, and no official endorsement should be inferred.

References

Aber, J. D., Nadelhoffer, K. J., Steudler, P. A., Melillo, J. M.: 1989, *BioScience* **39**, 378-386.

Aerts, R. and Berendse, F.: 1988, *Vegetatio* **76**, 63-69.

Agren, G. I. and Bosatta, E.: 1988, *Environ. Pollut.* **54**, 185-197.

Buchmann, N., Schulze, E.-D., Gebauer, G.: 1995, *Oecologia* **102**, 361-370.

Downs, M. R., Michener, R. H., Fry, B., Nadelhoffer, K. J., this volume.

Fry, B., Brand, W., Mersch, F. J., Tholke, K., Garritt, R.: 1992, *Anal. Chem.* **64**, 288-291.

Garten, C. T., Jr.: 1993, *Ecology* **74**, 2098-2113.

Garten, C. T., Jr. and Van Miegroet, H.: 1994, *Can. J. For. Res.* **24**, 1636-1645.

Gebauer, G. and Schulze, E.-D.: 1991, *Oecologia* **87**, 198-207.

Gundersen, P.: 1991, *In*: R. Hantschel and F. Beese (Editors), *Effects of Forest Management on the Nitrogen Cycle with Respect to Changing Environmental Conditions*, Report of a European workshop held at GSF München, May 9-13, 1990. GSF-Report 43/91, 183-186.

Högberg, P. and Johannisson, C.: 1993, *Plant and Soil* **157**, 147-150.

Kahl, J. S., Norton, S. A., Fernandez, I. J., Nadelhoffer, K. J., Driscoll, C. T., Aber, J. D.: 1993, *Environ. Sci. Technol.* **23**, 565-568.

Kahl *et al.*, this volume.

McNulty, S., *pers. comm.*

Magill, A. H., Downs, M. R., Nadelhoffer, K. J., Hallett, R. A., Aber, J. D.: 1996, *For. Ecol. Manage.*, in press.

Nadelhoffer, K. J., Aber, J. D., Melillo, J. M.: 1985, *Ecology* **66**, 1377-1390.

Nadelhoffer, K. J. and Fry, B.: 1988, *Soil Sci. Soc. Am. J.* **52**(5), 1633-1640.

Nadelhoffer, K. J. and Fry, B.: 1994, *In*: K. Lajtha and R. Michener (Editors), *Stable Isotopes in Ecology*, Blackwell Scientific Publications, Oxford, UK., 22-44.

Nihlgård, B.: 1985, *Ambio* **14**, 2-8.

Norton *et al.*, this volume.

Schulze, E.-D.: 1989, *Science* **244**, 776-783.

Shearer, G., Duffy, J., Kohl, D. H., Commoner, B.: 1974, *Soil Sci. Soc. Am. Proc.* **38**, 315-322.

Van Breemen, N. and Van Dijk, H. F. G.: 1988, *Environ. Pollut.* **54**, 249-274.

Young, H. E., Ribes, J. H., Wainwright, K.: 1980, *Weight tables for tree and shrub species in Maine*, Life Sciences and Agricultural Experiment Station, University of Maine at Orono. Miscellaneous Report No. 230, 84 pp.