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NITROGEN STORAGE AND CYCLING IN OLD- AND SECOND-GROWTH NORTHERN HARDWOOD FORESTS

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Abstract. Ecosystem retention of N is mediated by interactions among plant, soil, and microbial processes. These are likely to change with forest ecosystem development as living plant biomass accumulation slows and detrital biomass increases. We investigated linkages among N storage, N cycling processes, and N leaching losses in a study of replicate mid-successional (80-yr-old) and late-successional (uneven-aged old-growth) northern hardwood forests in the western upper peninsula of Michigan, USA. Our study tested hypotheses that detrital biomass and microbial immobilization of N function as larger N sinks and correspond to greater N retention in old-growth compared to maturing second-growth forests. Aboveground living and detrital biomass pools were greater in old-growth compared to second-growth forest, a difference due largely to coarse woody debris (CWD). The total amount of N in detrital pools was significantly greater in old-growth than second-growth forests. We also found more rapid rates of microbial N immobilization in old-growth forests than in second-growth forests. In situ net N mineralization ranged widely among individual stands and did not differ between old- and second-growth forests. Nitrogen (organic + inorganic) leaching did not differ significantly between old-growth and second-growth forests, and was substantially lower than wet deposition inputs. Nitrate leaching losses were significantly related to soil NO_3^- pools, litterfall N flux, and fine root biomass across both old- and second-growth forest stands. We conclude that CWD and microbial N uptake and turnover are greater N sinks in old-growth than in second-growth forests. This apparent N sink was not the primary factor influencing N leaching loss, however. Patterns of N dynamics among individual forest ecosystems indicate that N losses correspond to net rates of N mineralization and to litterfall N flux (indicators of plant N cycling), which are independent of forest age, biomass pools, and gross N transformations at the successional stages that we compared.

Key words: coarse woody debris; dissolved organic nitrogen; microbial nitrogen uptake; nitrogen cycling; nitrogen immobilization; nitrogen leaching; nitrogen storage; old-growth forests.

INTRODUCTION

Nitrogen (N) retention varies widely among forest ecosystems (Hedin et al. 1995), and explanation of the patterns and mechanisms of N retention are central to our general understanding of N limitation and of ecosystem function in N cycles. Losses of N that limit its ability to accumulate in an ecosystem depend upon rates of N transformations and upon quantities of mobile NO_3^- that are available in soil solution (Vitousek et al. 1998). Ecosystem theory predicts that these losses change with successional status, or time since large-scale disturbance, of forest ecosystems. For example, Vitousek and Reiners (1975) proposed that successional patterns of biomass accumulation regulate the loss of limiting nutrients, such as N, from terrestrial ecosystems (the nutrient retention hypothesis).

Experimental tests of the nutrient retention hypothesis have had different outcomes depending on forest age, suggesting that factors other than plant N demand and sequestration limit N losses in older forests. For example, low N losses have been observed early in succession (Vitousek 1977, Martin 1979, Pardo et al. 1995) when forest biomass accumulates rapidly. Available N pools were probably kept at a minimum by high plant demand in these young forests. However, N losses from old-growth forests (Martin 1979, Sollins et al. 1980), or those in which overstory biomass accumulation has ceased (C. T. Driscoll, *unpublished data*; Likens et al. 1994), have been lower than predicted by the nutrient retention hypothesis. Nitrogen retention apparently continues in some forest ecosystems after plant biomass accumulation slows.

As forests mature, N retention may be influenced to a greater extent by heterotrophic processes that regulate internal N supply and its storage in detritus. Several factors suggest changes in heterotroph processing of N as forests age. Organic matter substrates are likely to change in both quantity and quality with forest age.

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For example, total quantities of detrital biomass increase as forests age (Sollins et al. 1980, Harmon et al. 1986, McCarthy and Bailey 1994, Goodburn and Lorimer 1998, Hale et al. 1999). The chemical nature and decomposition of litter also differ in older forests (Gorham et al. 1979, Vitousek et al. 1988, Gower et al. 1996). It is not certain how microbial N cycling processes respond to these changes, but there is evidence of greater microbial N immobilization in old-growth compared to second-growth forests (Davidson et al. 1992). This immobilization can minimize NO_3^- production (Davidson et al. 1992) and thus might limit leaching losses. Furthermore, several studies indicate that microbial uptake and turnover can sequester N in forest soils (Vitousek and Matson 1985, He et al. 1988, Schimel and Firestone 1989*a*, Couteaux and Sallih 1994, Hart and Stark 1997, Fenn et al. 1998, Zogg et al. 2000). It is therefore plausible that a greater heterotrophic sink for N exists in old-growth forests.

To better understand the mechanisms by which microbial N cycling influences N losses, we examined relationships among N storage, microbial and plant N cycling processes, and leaching loss in second-growth (80-yr-old) and uneven-aged old-growth northern hardwood forests located in Michigan's upper peninsula. We expected the available NO_3^- pool to be the greatest potential source for N loss in these forests. Dissolved organic N was also considered as an avenue for N loss, although this is thought to be most important in forests that receive little to no N deposition (Hedin et al. 1995). In order to focus on the comparisons of detrital pools and microbial processes, we chose second-growth forests that were relatively mature so that N sequestration in aggrading overstory biomass would not be high. We expected detrital pools to influence microbial N cycling to a greater extent in old- than second-growth forests, decreasing available N pools and, ultimately, N losses. We tested these ideas as a series of related hypotheses, that in old-growth compared to second-growth forests: (1) detrital biomass is greater, (2) gross N transformations are greater, with proportionately higher microbial N immobilization, (3) net N mineralization and nitrification are limited by microbial immobilization to a greater extent, and (4) N leaching is lower because of lower available NO_3^- pools.

Methods

Study sites

We studied sugar maple (*Acer saccharum* Marsh.)-dominated northern hardwood forests in the southwestern upper peninsula of Michigan (Fig. 1). Other common tree species include yellow birch (*Betula alleghaniensis* Britton), basswood (*Tilia americana* L.), eastern hemlock (*Tsuga canadensis* (L.) Carr.), and ironwood (*Ostrya virginiana* (Mill.) K. Koch). Study sites were located in Albert's (1994) Sub-Subsection IX.3.2 (Winegar Moraine) on landforms that are irregular lobes of a terminal moraine with varying slope. Soils were fine sandy loam haplorthods or fragiorthods from the Gogebic Series. To minimize variation in the physical environment, sampling was limited to ecosystems mapped as Ecological Landtype Phase (ELTP) 38B or 38C within the Sub-Subsection (Albert 1994). Both ELTP's are *Acer–Tsuga–Dryopteris* associations on sandy loam soil, but they differ in slope (ELTP 38B 1–6%; ELTP 38C 6–18%). Within this framework, we selected forest stands that were similar in terms of plant species composition, soil characteristics, landform, and climate, but differed in successional stage. These criteria, plus management history and accessibility for research, limited the number of forest stands available and did not enable us to choose study sites from a larger pool of available sites.

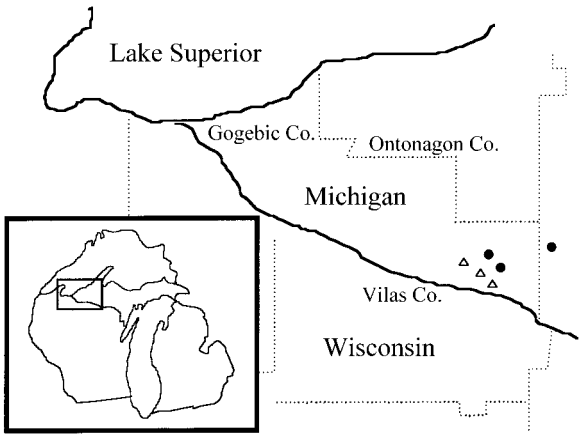

FIG. 1. Location of study sites in western Upper Michigan. Triangles represent old-growth forest ecosystems, and circles represent second-growth forest ecosystems.

We studied three uneven-aged, old-growth forest stands (O-1, O-2, and O-3) in the Watersmeet District on the Ottawa National Forest (Fig. 1). USDA Forest Service records indicated that they had never been harvested or otherwise managed. They initially were part of a large privately owned reserve that became the Sylvania Wilderness Area. Old-growth stands had a wide variety of tree ages, but each was dominated by large-diameter individuals ranging from 200 to 300 yr old (Crow et al. 2001). The three additional stands (S-1, S-2, and S-3) were even-aged, second-growth forests located in the Watersmeet and Iron River Districts on the Ottawa National Forest, near and around the perimeter of the Sylvania Wilderness Area (Fig. 1). These stands originated after commercial clearcutting between 1918 and 1920, and they have not been managed since their initial harvest. They were dominated by trees 60–80 yr old with an occasional residual tree 120–200 yr old (Crow et al. 2001).

We randomly located eight points along a 100-m transect in each stand. From each point, we designated a sampling plot at a random distance (0 to 100 m)

perpendicular to the transect. These eight sampling plots, or random subsets of them, were used for all of our measurements with the exception of basal area and overstory biomass. Basal area and overstory biomass were quantified in separate, randomly located 833-m^2 plots in each stand. Replication of plots within a stand enabled us to make comparisons among individual stands, whereas replication of stands in an ecosystem type (i.e., second vs. old growth) enabled us to compare forests of different age. All measurements were made in 1995 unless otherwise noted.

Organic matter, total N, pH, and soil texture were measured on subsamples of soil cores (six per stand) collected from the surface of the Oe horizon to a depth of 15 cm. Oe plus Oa horizons varied from 0 to 4 cm depth, and accounted for ≤15% of the mass of the entire core. Organic matter was measured by loss on ignition at 450°C. Total N was measured using Kjeldahl digestion in H_2SO_4 with a cupric sulfate catalyst, followed by analysis for NH_4^+ using an Alpkem rapid flow analyzer (RFA 300, Alpkem, Clackamas, Oregon, USA). Soil pH was measured with a glass electrode in a 1:1 soil:water paste. Percentages sand, silt, and clay were quantified by the hydrometer method (Sheldrick and Wang 1993).

Biomass and nitrogen pools

Overstory biomass was estimated in three randomly located 833-m^2 plots in each second- and old-growth stand. Tree height and diameter at breast height (dbh) were measured for all stems >1.5 cm dbh. Species-specific allometric equations for trees in the Lakes States region were used to estimate total aboveground biomass (Host et al. 1989). Seven percent of stems (49% of basal area) exceeded upper diameter limits of the allometric equations (50 cm) in old-growth plots, leading to some uncertainty in our biomass estimates for those trees. Total aboveground biomass of each tree was partitioned into bole, bark, branch, twig, and leaf using the equations of Crow (1978). We used N concentations reported for each component by Pastor and Bockheim (1984) and estimated tree N content as the product of mass and N concentration.

We used a line intercept method to quantify coarse (>5 cm diameter) and fine (2–5 cm diameter) woody debris (Van Wagner 1968). We measured the diameter of all woody debris >2 cm in diameter that intersected three 120-m transects in each second- and old-growth stand. The 120-m transects were broken into equilateral triangles to avoid bias associated with nonrandom orientation of woody debris (Van Wagner 1968). Starting points and angle of orientation for the triangles were chosen randomly within each stand. All debris sampled was assigned a decay class from I (least decomposed) to V (most highly decomposed), using the classifications of Sollins (1982) and Tyrrell and Crow (1994).

Wood density and N concentrations were quantified for one cross-section taken from each piece of woody debris on one side of a triangle in each stand. Average densities, determined for cross sections of each decay class, were used to convert coarse woody debris (CWD) and fine woody debris (FWD) volumes into masses. Entire cross-section samples were ground (425-μm mesh) and subsamples were digested and analyzed for N concentration. Average N concentrations for each decay class were used to estimate woody debris N from mass.

Forest floor mass was estimated from Oe and Oa horizon material collected in 0.09 m^2 frames in six of our eight sampling plots in each stand. The Oi horizon, which we defined as the current year's fresh litter, varies seasonally and so was not included these forest floor samples. Leaf litterfall was collected in the same six plots in 0.20-m^2 baskets per stand in September and October following leaf-fall. Forest floor and litter samples were ground in a mill (425-μm mesh) and total N content was determined using the Kjeldahl procedure.

Fine root biomass (<2 mm diameter) was measured in 5 cm diameter cores collected to a depth of 15 cm from the Oe horizon surface. Six cores per stand were collected in the last week of June 1995 and in the first week of August 1996; samples were refrigerated for up to 3 wk until roots could be separated from soil by hand.

Gross nitrogen transformations

We used a short-term ^{15}N pool dilution technique to quantify rates of gross N transformations in July 1995. At three sampling locations in each stand, we collected six 5 cm diameter cores to a 15 cm depth from the surface of the Oe horizon. Cores were removed from the soil and ^{15}N was injected in solution using a spinal tap needle modified to have six side injection ports at its end; three cores received $^{15}NH_4Cl$ and three received $K^{15}NO_3$. Isotope was added in six 2-mL injections throughout the length of each core; both solutions were 0.1 μg N/mL (99% ^{15}N enrichment). These injections caused an increase from 37% to 42% in gravimetric soil water content.

From each set of six cores, one $^{15}NH_4^+$-labeled core and one $^{15}NO_3^-$-labeled core were homogenized 30 min following injection, and ~25-g fresh-mass subsamples were immediately extracted by shaking in 100 mL 2-mol/L KCl. From these extractions, we determined initial soil inorganic N concentrations and percentage ^{15}N enrichment. A second subsample from each core was used to measure gravimetric soil water content (24 h at 100°C). Additional subsamples were used to quantify microbial N using the chloroform fumigation–extraction procedure (K_n = 0.54; Brookes et al. [1985], Davidson et al. [1989]).

Remaining cores were incubated in the field for 48 h. Following incubation, two $^{15}NH_4^+$-labeled cores from each sample location were removed from the soil and composited in the field. A homogenized subsample from each set of composited cores was extracted in 2-

mol/L KCl for final soil inorganic N concentration and ^{15}N enrichment analyses. Gravimetric water content was measured on additional subsamples.

Soil extracts were allowed to settle for 24 h after shaking, filtered through 0.45-μm Nucleopore filter membranes, and stored at 4°C prior to analysis. Ammonium and NO_3^- concentrations in soil extracts were analyzed within 1 wk using the Alpkem RFA. Net N mineralization was calculated as the difference in NH_4^+ and NO_3^- concentrations between final and initial extracts. A diffusion procedure similar to that of Brooks et al. (1989) was used to collect $^{15}NH_4^+$ and $^{15}NO_3^-$; ^{15}N enrichment was determined using a Europa Scientific Integra CN mass spectrometer (Europa Scientific, Vandalia, Ohio, USA). Gross N mineralization, gross nitrification, and N consumption were calculated using the equations of Kirkham and Bartholomew (1954). Ammonium immobilization was estimated as NH_4^+ consumption minus gross nitrification (Hart et al. 1994*b*). Nitrate immobilization was assumed to be equal to NO_3^- consumption m. We also estimated total N immobilization (NH_4 plus NO_3^-) as gross N mineralization minus net N mineralization, measured in the same cores. In either approach, immobilization may be overestimated if addition of N stimulates its uptake.

In situ net nitrogen transformations

Net N mineralization and nitrification were measured in situ using a buried bag method (Eno 1960). These measurements were initiated on 15 June 1995 and continued through 15 October 1995. At six sampling locations in each forest stand, four soil cores (5-cm diameter) were collected from the surface of the Oe horizon to a depth of 15 cm. Two soil cores were mixed together and extracted in 2-mol/L KCl, and analyzed for NO_3^- and NH_4^+ concentrations The remaining two cores were enclosed in plastic bags and returned to their original positions in the soil. Following 1 mo of field incubation, these cores were collected from the field, mixed together, and analyzed for NH_4^+ and NO_3^-. Net N mineralization was estimated as the difference between the incubated and initial inorganic N for each composited sample; net nitrification was estimated as the difference in NO_3^-.

Nitrogen loss

Nitrogen leaching is probably the major pathway for N loss in our study sites, given the relatively low denitrification in the well-drained soils of the sugar-maple-dominated northern hardwood forests in the Lake States region (Merrill and Zak 1992, Holmes and Zak 1999). We estimated the leaching loss of organic and inorganic N in second- and old-growth stands with porous-cup tension lysimeters placed at a depth of 1 m. Lysimeters were installed in June 1995, left at a tension of 30 kPa, and evacuated monthly from July 1995 to October 1997. Although soil solution was collected monthly, analyses for inorganic and organic N were not initiated until April 1996, to allow regrowth of roots and minimize disturbance effects. Beginning April 1996, soil solution from each lysimeter was filtered (0.45 μm) within 12 h of field collection and stored at 4°C prior to analyses for NO_3^- and NH_4^+. Nitrate always was the dominant form of inorganic N (>98%) in soil solution; therefore, soil solution NH_4^+ concentrations and leaching are not presented. Total dissolved N was quantified using a persulfate oxidation procedure (D'Elia et al. 1977), followed by NO_3^- analysis on an Alpkem RFA 300. Dissolved organic N was estimated as the difference between total dissolved N and NO_3^-.

We used the BROOK water-balance model (Federer and Lash 1978, Vorosmarty et al. 1998) to estimate the volume of water leaching below the rooting zone of these second- and old-growth forests. Precipitation data for the model were obtained from the nearby (45 km) Trout Lake National Atmospheric Deposition Program (NADP) station (National Atmospheric Deposition Program (NRSP-3)/National Trends Network [1999]). Maximum and minimum daily temperatures were obtained from our study sites. We estimated monthly N leaching as the product of soil solution N concentration and the volume of water moving below the rooting zone.

We also used data from the Trout Lake National Atmospheric Deposition Program (NADP) station (National Atmospheric Deposition Program (NRSP-3)/National Trends Network [1999]) to estimate N deposition in second- and old-growth stands. Nitrogen contained in wet deposition was available for this site (NO_3^--N plus NH_4^+-N), but we were unable to obtain estimates of dry N deposition. Consequently, the exclusion of dry deposition provides a conservative estimate of atmospheric N input to our sites.

Statistical analyses

We used a mixed-model ANOVA to analyze differences in biomass pools, N transformations, and N leaching between second- and old-growth stands. The model had forest stands nested as random effects within forest age (fixed effects). In this model, we tested for significant forest age effects using the mean square of the stands × forest age interaction in the denominator of the *F* ratio. Significance of stand effects was tested using the mean square error in the denominator of the *F* ratio (Sokal and Rohlf 1981). This nested design allowed us to make comparisons among individual forest stands as well as between replicate old- and second-growth forest stands (Sokal and Rohlf 1981). For those measurements conducted over time, we used a repeated-measures ANOVA with time as the within-subjects factor. Relationships among variables were explored using correlation analysis. SAS (SAS Institute, Cary, North Carolina, USA) was used for all analyses, and significance for all analyses was accepted at $\alpha = 0.05$.

TABLE 1. Overstory and soil characteristics (15 cm depth from Oe surface) in old- and second-growth northern hardwood forest ecosystems in western Upper Michigan, USA.

Ecosystem	Basal area (m^2/ha)	Stem density (number/ha)	Soil organic matter (g/kg)	Soil N (g/kg)	Soil pH	Soil texture (percentage compostion)		
						Sand	Silt	Clay
Old Growth								
O-1	29 (2.0)	1110 (204)	59 (5)	1.8 (0.1)	4.5 (0.1)	57 (2)	36 (2)	6 (1)
O-2	36 (3.8)	970 (130)	50 (7)	1.7 (0.2)	4.9 (0.2)	69 (2)	28 (2)	3 (1)
O-3	33 (3.3)	1300 (205)	52 (5)	1.6 (0.1)	4.8 (0.2)	64 (4)	32 (3)	4 (1)
Mean	33 (2.0)	1130 (96)	54 (3)	1.6 (0.1)	4.7 (0.1)	63 (3)	32 (2)	4 (1)
Second Growth								
S-1	28 (1.8)	750 (160)	34 (3)	1.3 (0.1)	4.6 (0.2)	81 (1)	15 (1)	4 (1)
S-2	30 (1.2)	1120 (124)	68 (8)	2.0 (0.2)	4.6 (0.1)	62 (3)	32 (3)	6 (1)
S-3	34 (1.1)	1040 (97)	64 (6)	1.8 (0.1)	5.2 (0.2)	68 (2)	27 (2)	5 (1)
Mean	31 (1.8)	970 (112)	55 (10)	1.7 (0.2)	4.8 (0.2)	70 (6)	25 (5)	5 (1)

Notes: Standard errors of the mean are in parentheses. For each stand, $n = 3$ for basal area and stem density, and $n = 6$ for soil characteristics. For old- and second-growth forest means, $n = 3$ stands.

RESULTS

Stand characteristics

Stand basal area and stem density varied little between the old-growth and second-growth forests (Table 1), whereas stem size-class distribution clearly differed between forests of different age. Most of basal area in second-growth stands occurred in intermediate size classes (20–40 cm dbh), whereas basal area in old-growth stands was dominated by large stems (>50 cm dbh; Fig. 2). Soil organic matter, total N, pH, and soil texture varied little among forest stands (Table 1) and were similar in second- and old-growth ecosystems.

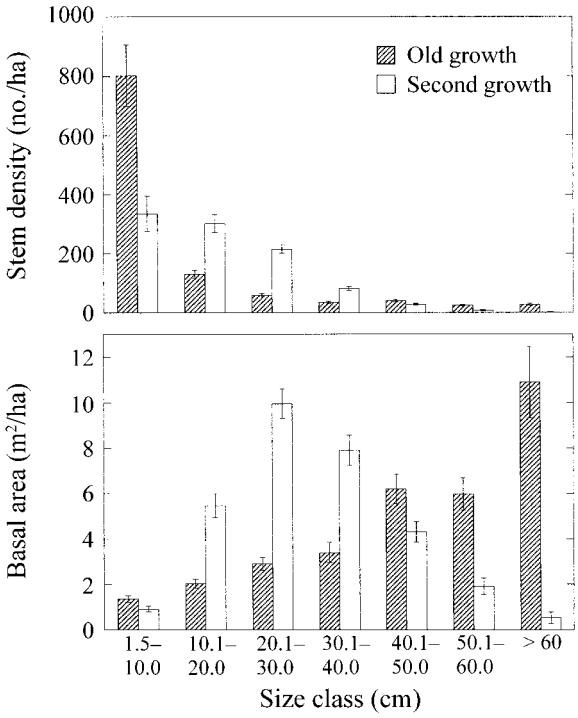

FIG. 2. Size class distributions (10-cm classes) of (top panel) stem numbers and (bottom panel) basal areas for all living woody stems >1.5 cm diameter in old- and second-growth northern hardwood forests. Data are means ± 1 standard error of the mean (SE).

Biomass and nitrogen pools

Living plus detrital biomass was significantly greater in old-growth (350 ± 6 Mg/ha) compared to second-growth forests (290 ± 11 Mg/ha; Table 2). Live overstory biomass of 262 Mg/ha in old-growth forest and 230 Mg/ha in second-growth forest did not differ significantly (Table 2), despite obvious differences in overstory structure (Fig. 2). Biomass in standing dead trees, forest floor, litterfall, and fine roots (0–15 cm depth) also did not differ significantly between old- and second-growth forests (Table 2). Fine woody debris (FWD) was significantly greater in second-growth forest but was a very small biomass component (1–2 Mg/ha) in both old- and second-growth forests. Coarse woody debris was the only biomass pool that differed substantially and significantly between second- and old-growth forest; it was 4× greater in the old- compared to the second-growth forests (Fig. 3, Table 2).

Although mean litterfall and forest floor mass were similar between the old-growth and second-growth forest, there were significant differences among individual stands (Table 2). Litterfall mass varied between 2.5 Mg/ha and 4.2 Mg/ha among individual stands. Forest floor mass varied >10× (4.2–48.5 Mg/ha). Among the six stands, we observed a significant, linear relationship between litterfall mass and forest floor mass ($r = 0.96$, $P = 0.004$).

Nitrogen pools in living and detrital biomass averaged 1200 ± 142 kg N/ha in old-growth and 950 ± 169 kg N/ha in second-growth forests (Fig. 4). The difference in N content between old- and second-growth forests resulted largely from the greater quantities of detrital N found in old-growth forests. Nitrogen content of detrital biomass (sum of standing dead trees,

TABLE 2. Living and detrital biomass in old- and second-growth northern hardwood forest stands in western Upper Michigan.

Category	Biomass (Mg/ha)		*F* values	
	Old growth	Second growth	Age (df = 1)	Stand{Age} (df = 4)
Overstory trees	262 (16.2)	230 (6.6)	3.32	0.51
Standing dead trees	28 (10.3)	17 (3.8)	1.00	1.87
Coarse woody debris	26 (4.2)	6 (1.2)	20.10*	0.50
Find woody debris	1 (0.1)	2 (0.3)	18.74*	0.55
Forest floor	26 (9.9)	24 (13.0)	0.01	11.64**
Annual litterfall	3.3 (0.44)	3.5 (0.35)	1.05	4.67**
Fine roots	3.6 (0.54)	2.3 (0.28)	1.68	11.68**
Living + Detrital†	350 (6)	280 (11)		

Notes: F values and significance levels are results of ANOVA testing effects of forest age (old growth vs. second growth). Stands are nested as random effects within forest age. Standard errors of the mean are in parentheses, $n = 3$ stands.

* $P < 0.05$; ** $P < 0.01$.

† Sum of measured biomass components in second- and old-growth stands. Living + detrital pool does not include biomass contained in soil organic matter, coarse roots, or fine roots deeper than 15 cm from forest floor surface.

CWD, FWD, forest floor (Oe and Oa horizons), and litterfall) was significantly greater in old-growth (560 kg N/ha) than second-growth (400 kg N/ha) forests, because of five times greater N in CWD in old growth.

Gross nitrogen transformations

Gross N mineralization was almost two times greater in old-growth than in second-growth forests (Table 3). Variation among stands was high and this difference was only marginally significant given our sampling design (Table 3; $P = 0.06$). Gross nitrification did not differ between old- and second-growth forests (Table 3), but it accounted for a higher proportion of gross mineralization in second-growth (41% of gross N mineralization) compared to old-growth (12%).

Total microbial immobilization ($NH_4^+ + NO_3^-$) was significantly greater in old- than second-growth forests, whether estimated either as the sum of NH_4^+ and NO_3^- consumption minus gross nitrification or as the difference between gross and net N mineralization (Table 3). Moreover, rates of microbial immobilization of N exceeded rates of gross N mineralization in old-growth forests, whereas N immobilization was similar or less than gross N mineralization second-growth forests (Table 3). Ammonium was immobilized to a much greater extent than NO_3^- in both old- and second-growth forests (Table 3). Microbial immobilization of NO_3^- was

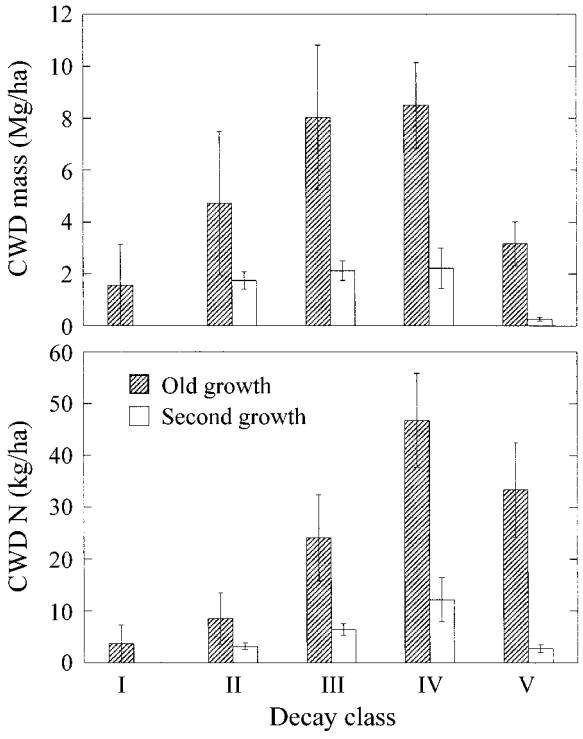

FIG. 3. Coarse woody debris mass and nitrogen in old- and second-growth northern hardwood forest ecosystems in western Upper Michigan. Decay class progresses from I (least decayed) to V (most highly decayed). Data are means ± 1 SE; $n = 3$ stands.

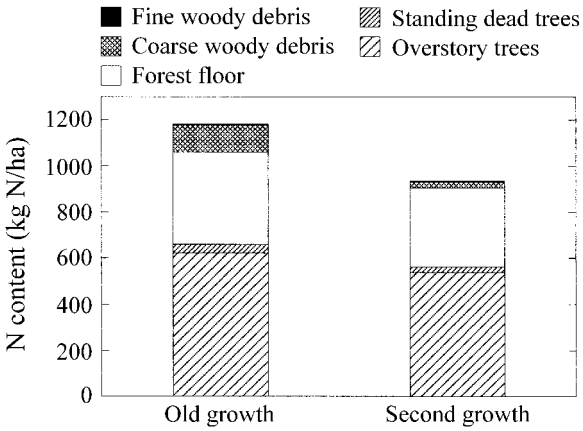

FIG. 4. Nitrogen (kg/ha) contained in living and detrital biomass in old- and second-growth northern hardwood forests. Values are means for three replicate forest stands.

TABLE 3. Nitrogen fluxes and pools in old- and second-growth northern hardwood forest soils (from surface of Oe to 15-cm depth), July 1995.

Measure	Old growth	Second growth	Age (df = 1)	Stand{Age} (df = 4)
Gross N mineralization	3.7 (0.57)	2.1 (0.31)	6.57	1.00
Gross nitrification	0.4 (0.21)	0.9 (0.11)	2.42	2.47
Microbial immobilization				
NH_4^+	4.2 (0.81)	1.6 (0.45)	5.29	1.06
NO_3^-	0.2 (0.9)	0.2 (0.10)	3.35	3.09
Total N†	4.4 (0.93)	1.8 (0.98)	ND	ND
Total N‡	4.4 (1.31)	0.5 (1.13)	9.92*	1.54
Microbial N	89 (11.2)	102 (8.5)	1.84	2.31
Residence time	25	59		

Notes: Data show gross N transformation rates (kg N·ha^{-1}·d^{-1}), microbial biomass N (kg N/ha), and N residence time (in days). The last two columns show results (F values and significance levels) of ANOVA testing effects of forest age (old-growth vs. second-growth). Stands are nested as random effects within forest age. Standard errors of the means are in parentheses; n = 3 stands; ND = not determined.

* $P < 0.05$.

† Calculated as NH_4^+ consumption − gross nitrification + NO_3^- consumption.

‡ Calculated as gross N mineralization − net N mineralization.

similar between old- and second-growth forest and was a greater proportion of total N immobilization in second-growth (11%) than old-growth (4%) forests. The microbial N pool was similar between old- and second-growth forests (Table 3).

Rates of soil N transformations can be used to estimate the residence or turnover time of N in soil pools (Davidson et al. 1992). In both old- and second-growth forests, the daily input of NH_4^+ from gross N mineralization was similar to the average extractable soil NH_4^+ pool; gross nitrification (NO_3^- input) also was similar to the extractable soil NO_3^- pool. Mean residence times (MRT) for both NH_4^+ and NO_3^- thus were ~1 d and did not differ between old- and second-growth forests. In contrast, N residence time in the microbial pool (microbial N/N immobilization) was two times longer in second-growth (59 d) compared to old-growth forest (25 d; Table 3).

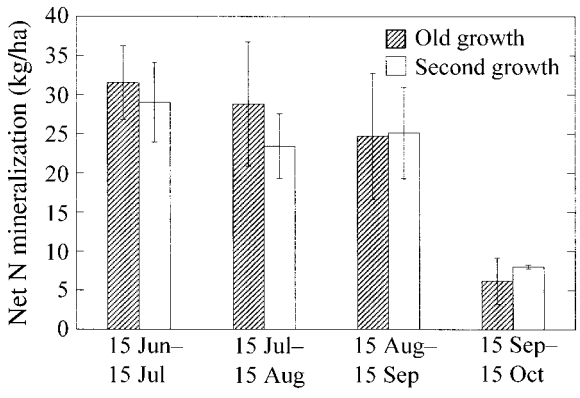

FIG. 5. Net nitrogen mineralization in old-growth and second-growth forest ecosystems using monthly in situ buried-bag incubations throughout the snow-free season, 1995. Data are means ± 1 SE; n = 3 stands.

In situ net nitrogen transformations

Monthly in situ net N mineralization and nitrification varied up to fourfold among stands and also differed over time, with lower rates in the fall compared to the summer months (Fig. 5). Expressed on an areal basis and summed over the snow-free season, mean net N mineralization and nitrification were similar in old- and second-growth forest (Table 4). However, annual net N mineralization varied from 44 to 129 kg N/ha among stands, independent of stand age. Net nitrification followed the same pattern and accounted for a high proportion of net N mineralized in forests of both ages (Table 4).

Despite the similarity between net N mineralization and nitrification, patterns of extractable soil NH_4^+ and NO_3^- (0–15 cm depth) differed from each other. Extractable NH_4^+ varied from 1.8 to 6.2 kg N/ha and did not differ between old- and second-growth soils or among individual stands. Extractable NO_3^- concentrations were far lower than NH_4^+ (0.1–2.5 kg N/ha), and differed significantly among individual stands. Across stands, average growing season soil NO_3^- was significantly correlated with litterfall N flux ($r = 0.80$, $P < 0.05$) and forest floor N ($r = 0.90$, $P < 0.01$).

Nitrogen leaching

Nitrate in soil solution at 1-m depth differed significantly among stands, varying from 0.02 to 6.0 mg N/L, but did not differ between second- and old-growth forest (Table 5). Dissolved organic N (DON) concentrations were less variable (0.02–1.2 mg N/L), differing neither among stands nor between old- and second-growth forests (Table 5). As a result, soil solution N was not consistently dominated by one form of N. Nitrate varied among stands from 15% to 80% of total soil solution N.

TABLE 4. Total net N mineralization and nitrification (kg N/ha) measured in monthly in situ forest soil incubations (from surface of Oe to 15 cm depth) from 15 June to 15 October 1995.

Measure	Old growth	Second growth	Age (df = 1)	Stand{Age} df = 4
Net N mineralization	93 (25.5)	91 (17.9)	0.03	17.82**
Net nitrification	70 (23.5)	79 (14.0)	0.01	13.97**
Net nitrification as a percentage of net N mineralization	75 (2.8)	87 (3.0)	9.09**	3.17*

Notes: The last two columns show results (F values and significance levels) of ANOVA testing effects of forest age (old growth vs. second growth). Stands are nested as random effects within forest age. Standard errors of the means are in parentheses; $n = 3$ stands.
* $P < 0.05$; ** $P < 0.01$.

No seasonal trends were evident in soil solution N concentrations. However, water flux estimates differed substantially between growing season (June–October) and nongrowing season (April, May, November) months. We therefore found a pronounced seasonal pattern of N leaching, with the largest quantities during snowmelt (April and May), and after leaf fall (November) (Figs. 6 and 7).

Leaching loss of inorganic plus organic N averaged 1.8 kg N·ha^{-1}·yr^{-1} in old-growth and 2.8 kg N·ha^{-1}·yr^{-1} in second-growth forests and over both years (Table 6). Consistent with soil solution N concentrations, leaching did not differ between forest ages. These average estimates of N leaching were less than wet deposition of N to the nearby (45 km) Trout Lake NADP monitoring site (Table 6). Significant differences in leaching losses existed among forest stands, which ranged from 0.6 to 5.2 kg N/ha in 1996 and from 0.7 to 5.6 kg N/ha in 1997. Wet deposition of N exceeded leaching from all individual forest stands except S-2, which leached 5.6 kg N/ha in 1997.

These comparisons of leaching loss to wet deposition depend upon the accuracy of our water flux estimates. Although we cannot verify these estimates or even estimate variability in water flux through our soil profiles, we did estimate potential error in the model by varying soil and canopy parameters. Our manipulation of canopy (height and LAI) or soil (type, depth, and organic matter content) parameters produced at most 20% variation in water flux, which should far exceed the relatively small variation in these parameters that occurs within our stands or between old- and second-growth forests. This maximum potential error corresponds to N leaching of ±0.4 kg N·ha^{-1}·yr^{-1} in old-growth and ±0.6 kg N·ha^{-1}·yr^{-1} in second-growth forests. With the

TABLE 5. Nitrogen concentrations (mg/L) in soil solution sampled monthly in old- and second-growth northern hardwood forests.

	1996		1997	
Month	Old growth	Second growth	Old growth	Second growth
Nitrate				
Apr	0.8 (0.35)	1.5 (0.76)	0.9 (0.44)	1.1 (0.58)
May	0.8 (0.33)	1.6 (1.06)	0.9 (0.43)	1.6 (1.01)
Jun	1.0 (0.33)	1.2 (0.68)	0.7 (0.35)	2.5 (1.60)
Jul	0.7 (0.28)	1.1 (0.62)	0.9 (0.42)	2.2 (1.40)
Aug	0.5 (0.21)	1.0 (0.53)	0.9 (0.48)	1.7 (0.95)
Sep	0.4 (0.21)	1.0 (0.53)	0.9 (0.40)	1.4 (0.85)
Oct	0.8 (0.48)	1.5 (0.46)	0.2 (0.16)	1.2 (0.57)
Nov	0.9 (0.52)	1.6 (0.60)	ND	ND
Dissolved organic N				
Apr	0.3 (0.01)	0.2 (0.04)	0.6 (0.09)	0.4 (0.08)
May	0.3 (0.11)	0.5 (0.08)	0.5 (0.04)	0.5 (0.10)
Jun	0.2 (0.09)	0.1 (0.10)	0.3 (0.10)	0.3 (0.05)
Jul	0.4 (0.06)	0.2 (0.07)	0.3 (0.05)	0.4 (0.14)
Aug	0.5 (0.04)	0.3 (0.07)	0.5 (0.15)	0.4 (0.06)
Sep	0.8 (0.21)	0.9 (0.19)	0.1 (0.02)	0.2 (0.05)
Oct	0.7 (0.16)	0.5 (0.15)	0.4 (0.09)	0.4 (0.07)
Nov	0.3 (0.06)	0.4 (0.01)	ND	ND

Notes: Standard errors of the means are in parentheses; $n = 3$ stands. ND indicates not determined because lysimeters collected no soil solution.

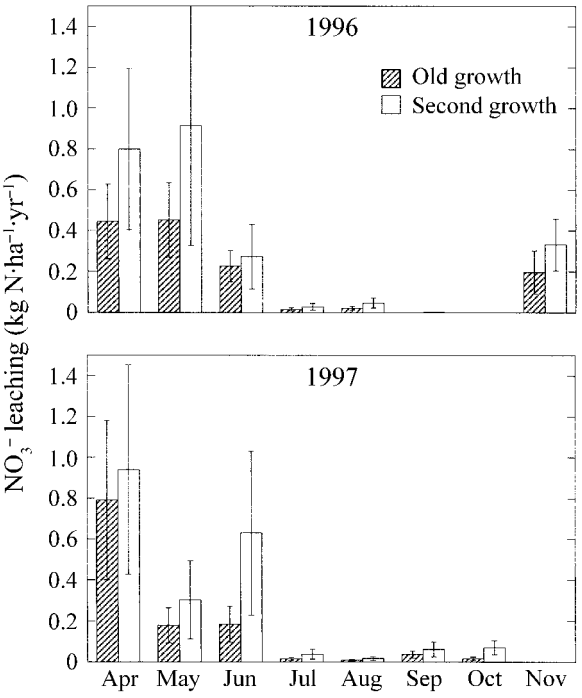

FIG. 6. Nitrate leaching (kg N/ha) at 1-m depth in old-growth and second-growth forest stands in 1996 and 1997. Data are means ± 1 SE; $n = 3$ stands.

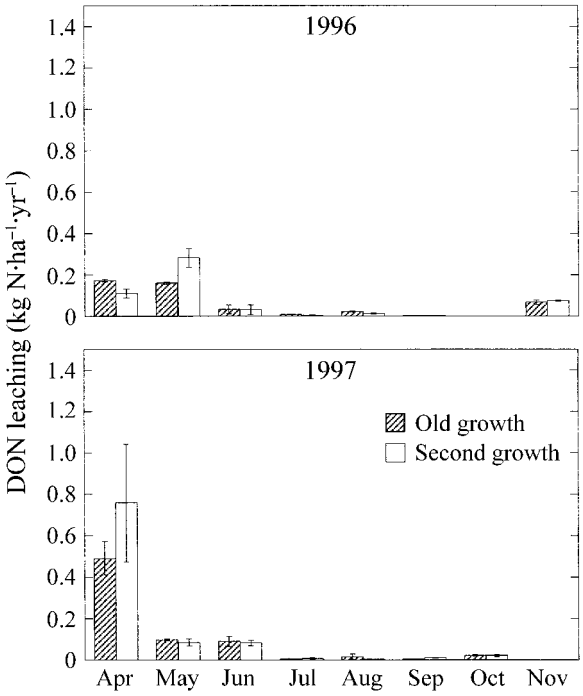

FIG. 7. Dissolved organic nitrogen (DON; kg N/ha) at 1-m depth in old-growth and second-growth forest stands in 1996 and 1997. Data are means ± 1 SE; $n = 3$ stands.

exception of stand S-2, these potential errors are not large enough to suggest net N loss from these forest stands.

The pattern of N loss from individual stands paralleled that of several ecosystem pools and N cycling variables. Nitrate leaching loss from each stand was significantly and positively related to average growing season extractable soil NO_3^- (in surface 15 cm; $r = 0.96$; $P = 0.004$; Fig. 8A), litterfall N flux ($r = 0.91$; $P = 0.01$; Fig. 8B), and forest floor N ($r = 0.83$; $P = 0.04$). Nitrate leaching showed a significant negative relationship with fine root biomass ($r = -0.88$, $P = 0.02$; Fig. 8C). Nitrate leaching was not significantly related to net N mineralization, primarily because of the very high NO_3^- leaching from stand S-2 (Fig. 8D). Excluding this point from the analysis resulted in a significant positive relationship between net N mineralization and NO_3^- loss ($r = 0.99$; $P = 0.0002$), growing season extractable soil NO_3^- ($r = 0.93$; $P = 0.02$), and litterfall N flux ($r = 0.88$, $P = 0.05$). Nitrate leaching was not related to N pools or transformations that differed between old- and second-growth forests. For instance, no relationships were evident between NO_3^- leaching and gross N immobilization rates (Fig. 8E) or N in CWD (Fig. 8F).

DISCUSSION

We investigated the linkages among N storage, N cycling processes, and N leaching losses in this comparison of N retention between uneven-aged old-growth and even-aged second-growth northern hardwood forests in western Upper Michigan. Our exploration of relationships among different aspects of ecosystem N cycling provides insights into patterns of N cycling that differ between successional stages and contribute to ecosystem N storage vs. those that are not age dependent, but rather covary strongly across sites and are predictive of N leaching losses. We did not find differences in net N retention between old- and second growth forest ecosystems, and we found only partial support for our general hypothesis that the accumulation of detrital biomass in old-growth northern hardwood forests creates a heterotrophic N sink that leads to lower N leaching losses than those from maturing second-growth hardwoods. While our findings did suggest that rapid rates of microbial N turnover and the incorporation of N into woody detritus create a heterotrophic sink for N late in forest ecosystem succession, this potential N sink did not correspond with patterns of net N retention.

Patterns of N storage and microbial N immobilization also did not correspond with other indicators of internal N cycling in our study sites. Net N mineralization and litterfall N did not differ between old- and second-growth forests, contrary to our hypothesis that microbial N demand would limit the availability of inorganic N to a greater extent in old-growth forest. Although our results did not support the idea that slower net N mineralization and nitrification rates limit NO_3^- leaching losses to a greater extent from old-growth than from second-growth forests, they did suggest that processes leading to available NO_3^- pools regulated NO_3^- leaching losses among the forest stands that we studied. The primary control of N leaching from these individual forest stands appears to be related to stand-specific rates of net N cycling that are not dependent on forest age.

TABLE 6. Nitrogen leaching losses (kg N·ha^{-1}·yr^{-1}) and retention in old- and second-growth northern hardwood forests.

Measure	Old growth	Second growth
1996		
Total dissolved N	1.6 (0.55)	2.8 (1.29)
NO_3^--N	1.4 (0.54)	2.4 (1.28)
Input − Leaching	3.9	2.7
N input retained (%)	71	51
1997		
Total dissolved N	2.0 (0.77)	2.8 (1.42)
NO_3^--N	1.2 (0.59)	2.1 (1.20)
Input − Leaching	1.5	0.7
N input retained (%)	43	20

Notes: Total dissolved N is the sum of NO_3^--N and dissolved organic N. Retention was estimated as the difference between losses and annual wet deposition inputs of 5.5 kg N·ha^{-1}·yr^{-1} in 1996 and 3.5 kg N·ha^{-1}·yr^{-1} in 1997 (data courtesy of National Atmospheric Deposition Program (NRSP-3)/National Trends Network [1999], Trout Lake field station). Standard errors of the means are in parentheses, $n = 3$ stands.

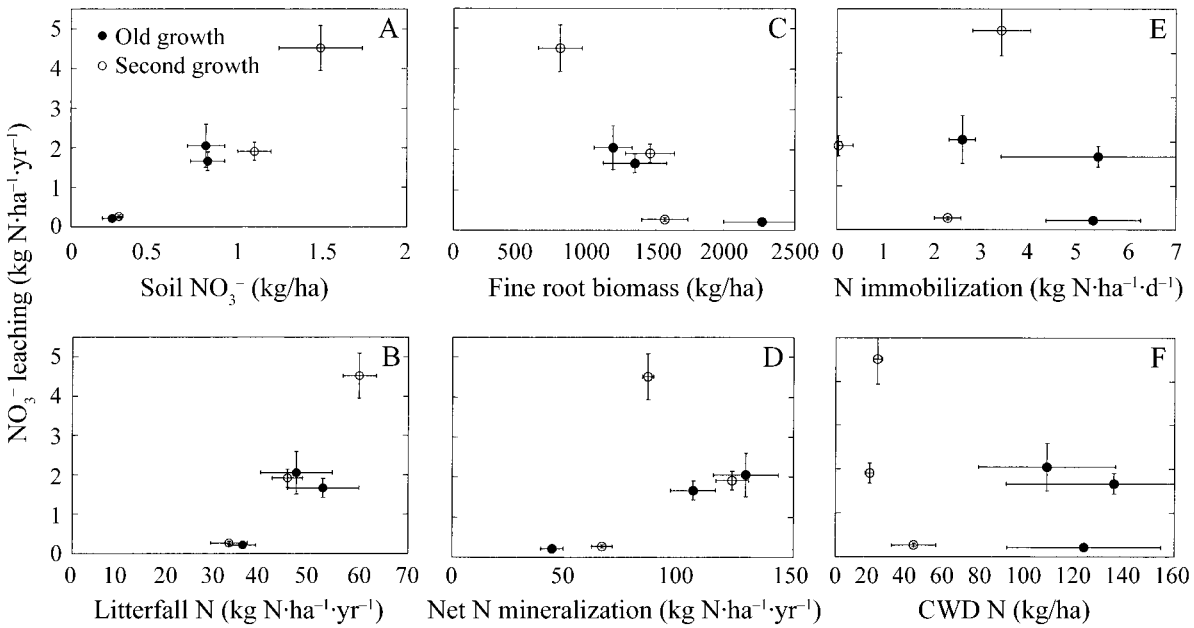

FIG. 8. Relationships between mean NO_3^--N losses in 1996 and 1997 and (A) extractable soil NO_3^- pools (mean for growing season 1995), (B) litterfall N flux (1995), (C) fine root biomass (average, midsummer 1995 and 1996), (D) net N mineralization (1995), (E) gross N immobilization (July 1995), and (F) N contained in coarse woody debris, for three old-growth and three second-growth northern hardwood forests. Error bars are standard errors of within-stand means; $n = 8$ replicates for NO_3^--N leaching; 6 replicates for soil NO_3^-, fine root biomass, leaf litterfall, and net N mineralization; and 3 replicates for gross N immobilization and N in coarse woody debris.

Biomass and nitrogen pools

Despite differences in successional development that were evident from overstory structure, second growth had accumulated an average of 88% of the overstory biomass found in old-growth forests, and neither overstory biomass nor N differed significantly between old- and second-growth forests. Our observations contrast with some predictions suggesting that overstory biomass can accumulate for well over 100 yr after harvest of northern hardwood forests (Botkin et al. 1972, Bormann and Likens 1979), but they concur with more recent evidence that overstory biomass in northern hardwood forests can attain maximum values in as little as 80 yr (Likens et al. 1994). Although we did not quantify coarse structural roots, results of Whittaker et al. (1974) indicate that coarse roots are ~20% of overstory biomass in northern hardwood forests. Based on this average estimate, total biomass in overstory trees averaged 310 Mg/ha in old-growth and 280 Mg/ha in second-growth forests. However, Whittaker et al. (1974) also found that the contribution of coarse roots to total biomass increased with tree size. We may have therefore underestimated coarse root biomass in our old-growth forests, compared to second growth. Our estimates of plant biomass also lack the understory component. This is likely to be small (3% of total in second growth [Crow et al. 1991]), but would nevertheless further increase the difference between old- and second-growth biomass and N content. Crow et al. (2001) found four times the stem density of woody understory plants in the old-growth compared to the second-growth stands that we studied.

We were particularly interested in the biomass of detrital components because of their potential to influence microbial activity and ecosystem N storage. We found more detrital biomass in old-growth forests, primarily due to greater mass of coarse woody debris. These results are consistent with the idea that nonliving biomass becomes a greater proportion of total biomass as forests mature (Gorham et al. 1979, Vitousek et al. 1988). Furthermore, the N content of detrital biomass is generally greater than N in living biomass, because N is immobilized in detritus (Schimel and Firestone 1989*b*, Hart et al. 1993) and conserved during wood decomposition (Harmon et al. 1986). Accordingly, we found a greater biomass N capital in old-growth forests, primarily due to N in standing dead trees and CWD. Together these pools totaled 160 kg N/ha in old-growth forest and 50 kg N/ha in second-growth forest. Forest floor mass or N did not differ between old- and second-growth, concurring with Covington's (1981) model of forest floor recovery following forest harvest.

Sollins et al. (1980) concluded that tree mortality in old-growth Douglas-fir represented an organic matter input to soil equal in quantity to aboveground litterfall, and our data indicate that CWD inputs are of similar importance in old-growth northern hardwood forests. We used the relationship $I = C_e k$; where I is CWD

inputs, C_e is CWD mass at equilibrium, and k is the first-order decay constant (Jenny et al. 1949, Sollins et al. 1980), to estimate annual CWD production required to sustain the current pool size in old-growth forest. We assumed that CWD was close to equilibrium and was no longer accumulating in old-growth forest. Based on the distribution of CWD among decay classes in our study (Fig. 3), this assumption is consistent with the model of Tyrrell and Crow (1994). Decay constants for mass loss in northern hardwood boles vary from 0.080 yr^{-1} for *Populus tremuloides* in Minnesota (Alban and Pastor 1993) to 0.096 yr^{-1} for *Fagus grandifolia, Betula alleghaniensis,* and *Acer saccharum* in New Hampshire (Arthur et al. 1993). Using these decay constants, annual inputs of 2.1–2.5 Mg/ha are required to maintain current CWD mass (26.0 Mg/ha) in old-growth forest. This estimate, equal to 60–70% of leaf litterfall, represents an important organic matter input in the old-growth forests.

Such an estimate is not possible for second-growth forests, because CWD is relatively low and will likely increase fourfold as these forests attain old-growth status. Nevertheless, it is apparent that our second-growth forests have accumulated CWD at a slow rate. Our observation of 6.0 Mg/ha CWD in second growth was far lower than values from 50- to 100-yr-old northern hardwoods in the northeastern United States (16.8 Mg/ha [Tritton 1980], 32.1–54.4 Mg/ha [Gore and Patterson 1986]), but was identical to other quantities documented in second-growth Lake States northern hardwoods (Goodburn and Lorimer 1998). Furthermore, the low biomass of standing dead trees in second growth (60% of that in old growth) suggests that inputs to the second-growth CWD pool will remain low for some period of time. Our second-growth forest ecosystems thus appear to be at a transition point, during which living overstory biomass accumulation is slow but mortality and detrital biomass accumulation are still minimal.

Gross nitrogen transformations

Substantially greater gross N immobilization and turnover of N through microbial biomass in old-growth forests suggest that microbial N demand is a more important component of N cycling processes compared to second-growth forests. We made this comparison only once during the year (mid growing season); although we would not expect the relative pattern between old- and second-growth forests to change dramatically, further investigation of seasonality would be informative for more in-depth comparison of microbial N cycling between old- and second-growth forests. Nevertheless, our results concur with comparisons between old- and second-growth conifer forests (Davidson et al. 1992). High microbial N demand also has been suggested by an increase in microbial N following N additions in old-growth conifer forests (Hart and Stark 1997). Davidson et al. (1992) and Hart et al. (1994*a*) proposed that labile C availability regulated NO_3^- immobilization in conifer-dominated old-growth forests. The possibility that the same is true for NH_4^+ and NO_3^- immobilization warrants further study in these northern hardwood forests. It is also possible that microbial activity and N immobilization respond to the greater annual inputs of C in woody detritus in the old-growth forests.

Microbial N uptake and turnover has been proposed as an important pathway for N sequestration in forest floor or soil organic matter (Couteaux and Sallih 1994, Magill et al. 1997, Seely and Lajtha 1997). Experimental tracer studies have shown N uptake by soil microbial biomass, followed by incorporation in soil organic matter to varying degrees (Hart et al. 1993, Stark and Hart 1997, Zogg et al. 2000, Perakis and Hedin 2001). Although the total quantity of N cycled to organic matter varies among forests, it is likely to be a substantial longterm sink, as organic matter can slowly accumulate in forest soils long after overstory biomass reaches a maximum (Schlesinger 1977). Mechanisms that regulate microbial N uptake and turnover deserve further attention for understanding changes in the function of soil microorganisms late in forest succession, especially the movement of N from microbial biomass into stable forms of soil organic matter with long turnover times.

In addition to possibly sequestering N in organic forms, microbial immobilization of N can limit N losses by limiting nitrification and thus quantities of NO_3^- in the available soil pool. We found that more rapid rates of NH_4^+ immobilization in old-growth forests did correspond to lower gross nitrification (12% of gross mineralization in old-growth forests, compared to 41% of gross mineralization in second-growth). However, this pattern did not carry through to affect net nitrification rates, available NO_3^- pools, or NO_3^- leaching. Instead, we found low rates of NO_3^- immobilization and similarly high rates of net nitrification in forests of both ages. These results differ from the pathway of microbial transformations that determined net nitrification in conifer forests, where high NO_3^- immobilization limited net nitrification in old growth relative to second growth (Davidson et al. 1992).

In situ net nitrogen transformations

Despite greater microbial N demand in old growth, we did not find differences in net N mineralization between old- and second-growth forests. Successional patterns of net N mineralization differ among ecosystems, such that generalization is difficult (Ryan et al. 1997). Some studies suggest that net N mineralization declines as forest ecosystems mature (Vitousek et al. 1989, Frazer et al. 1990, Binkley et al. 1995), and higher N immobilization is one mechanism that can lead to lower net N mineralization in older forests (Davidson et al. 1992). In our study, more rapid N immobilization in old-growth than second-growth forests

did not correspond to uniformly lower net N mineralization, because gross N mineralization and immobilization varied proportionately among all stands. This produced similar net N mineralization rates between second- and old-growth forests in our short-term pool-dilution incubations, and it is possible that the same was true for monthly net N mineralization.

Nitrogen mineralization and cycling are known to vary widely among Lake States forests that differ in soil type and overstory composition (Nadelhoffer et al. 1983, Pastor et al. 1984, Zak et al. 1989, Reich et al. 1997). We were surprised to find an equally wide range of net N mineralization rates among our study sites (40–130 kg $N \cdot ha^{-1} \cdot yr^{-1}$) despite efforts to minimize within-ecosystem variation. Soil organic matter, soil texture, and soil pH were similar among stands, and soil water content did not differ among stands (*data not shown*). Overstory species composition varied only slightly, with sugar maple accounting for 61–89% of total litterfall. One difference among forest stands was the absence of a forest floor horizon in one old-growth and one second-growth stand, which may be related to locally abundant earthworm populations (M. C. Fisk, *personal observation*). Although our study was not designed to investigate such an influence, these two stands obviously differed in some manner that corresponds to a more conservative pattern of N cycling, as net N mineralization, extractable soil NO_3^- pools, and litterfall N flux all were lower in these second-growth (S-3) and old-growth (O-3) stands.

Nitrogen leaching

Atmospheric N deposition was greater than N leaching from the forests that we studied, suggesting that these forests retain substantial quantities of N. Our estimates of retention (Table 6) do not include dry deposition or biological N fixation as inputs; they are therefore conservative. No good estimates of biological N-fixation exist for our study sites, but general estimates for hardwood forests average ~1.5 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (Cleveland et al. 1999). Furthermore, tension lysimeters can sample water that is more tightly held to soil rather than water that moves through soil in bulk flow (Barbee and Brown 1986, Joslin et al. 1987). Although there is no simple consensus on the accuracy of N concentrations sampled in porous-cup tension lysimeters (Litaor 1988), it is possible that these samplers overestimate soil solution N concentrations (Hendershot and Courchesne 1991). Any of these factors suggest that we have overestimated N leaching losses and underestimated N retention.

Nitrogen retention in these old-growth forests is not consistent with predictions of the nutrient retention hypothesis that N loss from old-growth forests will equal N input (Vitousek and Reiners 1975). Our data do not allow us to test whether biomass accumulation has ceased in these forests, an underlying assumption of the nutrient retention hypothesis. Nevertheless, our study contributes to a growing body of literature that points to the need to better understand soil N sinks, and it suggests that the nutrient retention hypothesis could be augmented with a heterotrophic N sink whose importance increases late in secondary succession.

The seasonal patterns of N leaching that we found further emphasize heterotroph regulation of patterns of N loss. Plant uptake of N minimizes the availability of NO_3^- for leaching during the growing season and thus limits total quantities of N leaching. However, microbial processes regulate NO_3^- pools available for leaching loss outside of the growing season. In this study, 82% of N leaching in old-growth and 88% of N leaching in second-growth forests occurred in the spring before leafout and in the autumn after leaf fall. Microbial processes that mediate available NO_3^- pools during these times are therefore critical for determining patterns of N leaching losses.

We found no differences in N leaching between old- and second-growth forests; high variability suggests that inherent differences in net N cycling among stands were greater than any differences that might be related to forest age. Most noteworthy were differences among stands in the dominant form of N in soil solution. Nitrate was the major component of N leaching in two old-growth and two second-growth stands, whereas DON composed the majority of N lost from the remaining two stands (S-3 and O-3; *data not shown*). Hedin et al. (1995) noted that N leaching was dominated by DON in old-growth Chilean forests that receive small amounts of atmospheric N deposition, and Vitousek et al. (1998) suggested that the relative contribution of DON to N leaching declines as atmospheric N deposition increases. Because our study sites received a moderate amount of N deposition (5 kg $N \cdot ha^{-1} \cdot yr^{-1}$, 4–10% of annual net N mineralization), we expected NO_3^- to be the dominant form of N leached from soil. Large differences in the contribution of DON to N leaching within the same N deposition regime concur with patterns of litterfall N and soil NO_3^- to suggest that fundamental differences in N cycling exist among our forest stands.

Conclusions: relationships among N cycling processes and N retention

Two patterns emerged in this study of N dynamics in northern hardwood forests. First, detrital N storage and microbial N cycling were greater in old-growth than second-growth forests. Second, we found that N leaching losses corresponded to indicators of plant N cycling (net N mineralization and litterfall N flux) that were independent of forest age, biomass pools, or gross N transformations at the successional stages that we compared. Ecosystem N storage thus was distinct from and did not necessarily covary with net N retention. We conclude that detrital N storage and microbial N immobilization probably influence N leaching from these forests, but that they do not appear to be the

primary factors limiting N losses in the forests that we studied. Instead, overall N cycling rates, indicated by net N transformations and litterfall N flux, were more closely related to the wide variation in N leaching that we found among northern hardwood forests in Upper Michigan. The underlying factors that regulate spatial variation in N cycling among stands need to be better understood to fully evaluate short- and long-term controls of N retention in these northern hardwood forest ecosystems.

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