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Fate and distribution of sulfur-35 in yellow poplar and red maple trees

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Summary. Two deciduous tree species (yellow poplar and red maple) on Walker Branch Watershed (WBW), near Oak Ridge, Tennessee, were radiolabeled with ^{35}S (87 day half-life) to study internal cycling, storage, and biogenic emission of sulfur (S). One tree of each species was girdled before radiolabeling to prevent phloem translocation to the roots, and the aboveground biomass was harvested prior to autumn leaf fall. Aboveground biomass, leaf fall, throughfall, and stemflow were sampled over a 13 to 24 week period. Sulfur-35 concentrations in tree leaves reached nearly asymptotic levels within 1 to 2 weeks after radiolabeling. Foliar leaching of ^{35}S and leaf fall represented relatively unimportant return pathways to the forest soil. The final distribution of ^{35}S in the nongirdled trees indicated little aboveground storage of S in biomass and appreciable (>60%) capacity to cycle S either to the belowground system by means of translocation or to the atmosphere by means of biogenic S emissions. Losses of volatile ^{35}S were estimated from the amount of isotope missing (~33%) in final inventories of the girdled trees. Estimated ^{35}S emission rates from the girdled trees were $\sim 10^{-6}$ to $\sim 10^{-7}$ μCi cm^{-2} leaf d^{-1}, and corresponded to an estimated gaseous S emission of approximately 0.1 to 1 μg S cm^{-2} leaf d^{-1}. Translocation to roots was a significant sink for ^{35}S in the red maple tree (40% of the injected amount). Research on forest biogeochemical S cycles should further explore biogenic S emissions from trees as a potential process of S flux from forest ecosystems.

Key words: Tree physiology – Nutrient cycling – Biogenic emission – Sulfur – Radioecology

The purpose of this research was to study the internal cycling, storage, and biogenic emission of S, as traced by ^{35}S, in two commonly occurring deciduous tree species on Walker Branch Watershed (WBW), in east Tennessee, USA. The sulfur cycle on WBW is dominated by geochemical processes (Johnson et al. 1982, 1986); uptake by forest trees does not appear to be of sufficient magnitude to account for an apparent net accumulation of S by the watershed based on input-output calculations (Johnson 1984). Nevertheless, little is known about the way that forest trees deal with S in environments (like WBW) impacted by atmospheric sulfate deposition.

Walker Branch Watershed is located within 20 km of three coal-fired power plants (Shriner and Henderson 1978). Approximately half of the annual sulfate input to the WBW forest is in the form of precipitation, and the other half is dry deposition (mostly vapors) (Lindberg et al. 1986). Johnson et al. (1982) showed that the atmospheric input of S to the watershed far exceeded the calculated total annual S requirement of the forest. Shriner and Henderson (1978) concluded that there was little internal recycling or storage of S by vegetation on the watershed. However, internal recycling, storage, and gaseous emissions reflect metabolic processes that can be used by trees to deal with excess S (ie., S inputs beyond nutritional requirements) in environments impacted by sulfate pollution. Plant survival in a sulfur-rich environment is not usually achieved by avoiding S uptake (Rennenberg 1984).

In this work, S behavior in trees was studied with radioactive ^{35}S as a tracer for stable sulfur. In trees radiolabeled with ^{35}S, the initial total activity injected (I) must balance the sum of aboveground leaching losses (L), leaf fall (LF), amounts present in aboveground biomass at harvest (W), root storage and turnover (R), and biogenic emissions of volatile S compounds (V), or:

$$I = L + LF + W + R + V. \quad (1)$$

Leaching losses, leaf fall, and aboveground inventories can be measured directly in a tracer experiment, but root storage and turnover of ^{35}S is very difficult, if not impossible, to measure directly. However, the role of roots as a sink for ^{35}S can be eliminated by girdling. In a girdled tree the downward translocation of S is eliminated and upper limits to the emission of volatile ^{35}S from the tree can be estimated as the difference between the amount injected and the remaining measured amounts, or:

$$V = I - (W + LF + L). \quad (2)$$

The estimated biogenic emission of ^{35}S from girdled trees (equation 2) is an upper limit for two reasons: 1) any underestimation of ^{35}S in aboveground biomass, leaf fall, or leachates through sampling errors will cause an overestimation of volatile S losses, and 2) as discussed later, girdling is a disturbance to the tree that may stimulate volatile S emissions from the foliage.

Given an upper limit on volatile S emissions, a lower estimate on the amount of ^{35}S sequestered by tree roots can be obtained from a rearrangement of the activity balance equation, or:

$$R = I - (L + LF + W + V).$$

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Materials and methods

Field methods

The study trees (2 red maples, *Acer rubrum* L., and 2 yellow poplars, *Liriodendron tulipifera* L.) were between 15 and 19 cm diameter at breast height and 12 to 15 m tall. Trees were located at two sites, separated by a distance of approximately 500 m, on ridgetops at Walker Branch Watershed, Oak Ridge National Laboratory (ORNL). The soil was a Fullerton cherty silt loam (pH 4.2–4.4). A precipitation chemistry collection site (part of the National Atmospheric Deposition Program/National Trends Network) was located within 350 m of the study trees.

One tree of each species was girdled at a height of approximately 50 cm above the ground by carefully removing the bark, phloem, and cambium several weeks prior to isotope labeling. Each tree received between 182 and 243 μCi of ^{35}S (as sodium sulfate) on June 25 or 26, 1986. The amount of stable S, as sulfate, added to each tree in the labeling solution was <20 μg. The calculated amount of ^{35}S in each solution was confirmed by liquid scintillation beta counting. A leak-proof collar (the injection well) was constructed around each bole, above the girdle (when present), with aluminum foil and electrical sealant. Tests with the sealant showed that it did not sorb ^{35}S. Ten to 12 cuts, about 1 cm into the xylem, were made under distilled water at the base of the injection well around each bole with a 0.6 cm wide chisel. Sulfur-35 was then added to distilled water in the injection well, and the ^{35}S was drawn into the tree by acropetal transport.

Injection wells were covered with plastic sheeting for several days, then removed from each tree and soaked for one week in a known volume of dilute hydrogen peroxide. The soak solution was assayed to determine the amount of ^{35}S not assimilated by the tree. Plastic sheeting remained over the injection sites throughout the study to prevent contamination of other samples.

The ground area (plot size) beneath the crown of each tree was determined by projecting the drip line of each tree onto the ground. Each plot was covered with polypropylene netting (2 cm mesh) to separate fresh leaf fall from existing litter. Leaf fall collections were made whenever leaf fall was present. Leaf fall was weighed, mixed in a large plastic bag, and subsampled. The recovery of leaf fall from the ground beneath the girdled trees was estimated at $>95\%$.

Stemflow was collected by building a leakproof collar around the bole (similar to the injection well) about 25 cm above the injection well. The stemwell drained into a calibrated 58 L plastic carboy. Following rainfall, the volume of stemflow in each carboy was measured, mixed, and a 1 L sample was removed for analysis. Background samples of stemflow were obtained from an unlabeled maple tree fitted with a stemwell. Sulfur-35 return in stemflow was calculated from concentrations (μCi L^{-1}) and stemflow amounts (L $plot^{-1}$).

Throughfall was randomly sampled on a 1×1 m grid beneath each tree using four high density polyethylene buckets (18 L capacity), each with a surface area opening of 638 cm^2. The buckets were covered with 3 mm nylon mesh to exclude falling leaves and debris. The position of each bucket was changed when throughfall was collected. Following rainfall, the volume of throughfall in the four buckets was combined, measured, mixed, and a 1 L sample was removed for analysis. The cumulative return of ^{35}S in throughfall was calculated from concentrations (μCi L^{-1}), throughfall amounts (L m^{-2}), and plot area (m^2 $plot^{-1}$).

The average amount of rainfall each week was determined from continuous rainfall recorders located at two clearings less than 400 m from the trees. Precipitation measurements were confirmed using rain gauges located at each tree. The average volume of rainfall measured at each clearing was highly correlated ($r>0.98$) with both rainfall and throughfall volumes at the individual trees.

Samples of leaves, twigs, bole wood, stump wood, and leaf fall were collected from the trees at approximately four week intervals. Leaf and twig samples were obtained at 4 to 8 m above the ground on opposite sides of each tree with a pole pruner. Leaves were separated from twigs and samples from different sides of the tree were pooled. Bole wood samples were obtained (~ 30 cm above the stemwell) by drilling three holes (0.6 cm diam) equidistant around the bole to a depth of ~ 5 cm and collecting the wood shavings in a plastic bag. Stump wood samples were collected from the base of each tree in a similar manner.

In order to obtain a complete accounting of aboveground amounts of ^{35}S, the girdled maple and girdled poplar tree were harvested prior to autumn leaf fall (13 and 15 weeks, respectively, after radiolabeling). The nongirdled trees were harvested after autumn leaf fall (23 and 24 weeks after radiolabeling). At harvest, each tree was cut at the stump and divided into five sections on the basis of tree height (Fig. 1). The foliage, if any, was completely removed from each section, weighed, and subsampled for analysis after mixing the leaves in a large plastic bag. Each of the five sections was separated into bole, branches (>1.5 cm diam), twigs (<1.5 cm diam), and all parts were weighed.

Twig, branch, and bole samples were taken for analysis from each section of the tree. There were two subsections of bole below the stemwell (Fig. 1). Twigs were cut into small pieces (<10 cm long) and randomly sampled. Each branch was drilled with a 0.6 cm diameter bit and the wood shavings were composited. The bole above the stemwell was sampled by drilling holes on different sides of the tree to the center of the bole at 1 m intervals along each section.

The bole section below the stemwell was subdivided by making a cut directly below the injection well (Fig. 1). The two subsections were weighed and wood shavings were removed with a drill. The bole section bearing the injection site was intensively sampled by drilling from 16 to 28 holes at regularly spaced intervals around the perimeter of the bole to a depth of 6.5 cm. The remaining section was sampled with approximately half as many holes. In the case of the girdled trees, the bole section below the stemwell was later resampled to provide an independent estimate of the ^{35}S activity associated with this portion of bole. During the resampling, the bole below the stemwell was subdivided further by making a cut above and below the injection well which yielded three subsections for sampling (Fig. 1).

Laboratory methods

Samples of leaves, wood, and leaf fall were weighed, frozen, freeze-dried, and reweighed to determine the fresh weight to dry weight (DW) conversion factor. Twigs were cut into small (<0.5 cm) pieces prior to freeze drying. Leaf or leaf fall samples were crushed into small fragments by manipu-

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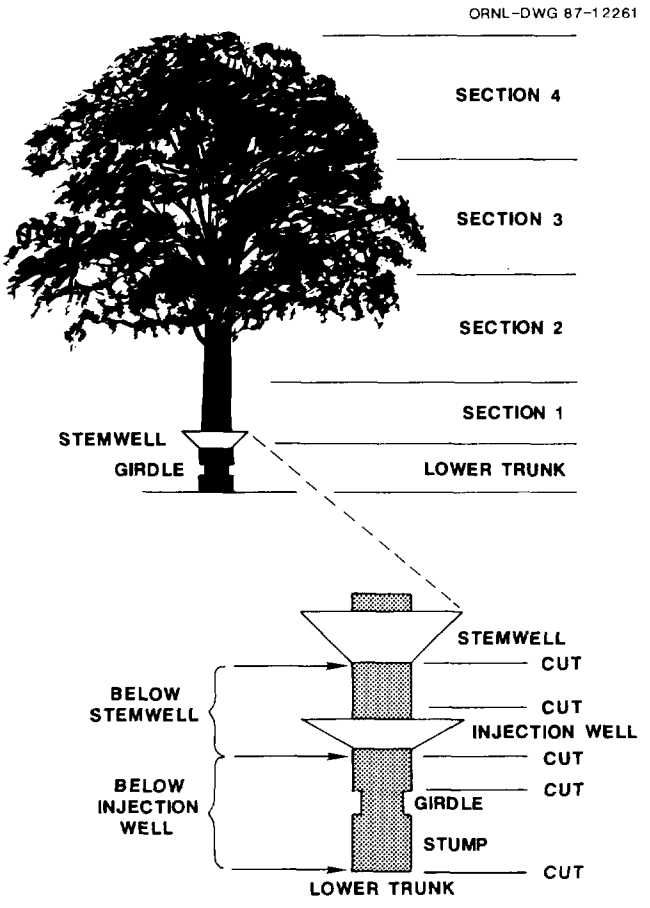

Fig. 1. Schematic representation of a study tree showing the sections produced during harvest of aboveground biomass and detail of subsections for sampling from the lower trunk (bole below the stemwell)

lating the dry samples in plastic bags. Samples of wood shavings taken from branches and the bole did not require pulverization after drying. However, wood samples taken below the stemwell (in the vicinity of the injection well) were ground in a blender to produce as homogeneous a sample as possible for analysis. Blending was necessary because of the possibility of higher levels of activity present on or in the bark relative to the wood beneath the bark.

Samples were prepared for ^{35}S analysis using methods outlined by the Association of Official Analytical Chemists (Williams 1984). One gram of dry sample was weighed into a large crucible and saturated with 7.5 mL of 3.7 M magnesium nitrate (AR grade) solution. The crucibles were heated on a hot plate until no further reaction occurred and were then ashed in a furnace (450° C maximum). The ash was dissolved in 1 M HCl (known weight) and a 2 g and 4 g subsample of the acid solution was transferred to individual plastic scintillation vials. Counting results from the subsamples, corrected to the total sample, were averaged to give a final ^{35}S concentration.

Throughfall and stemflow samples were prepared for analysis on the day of collection or were refrigerated until preparation was possible. Samples were filtered through a Whatman #541 filter paper to remove debris. A 10 to 15 mL sample was immediately taken for stable sulfate analysis (ion chromatography by the ORNL Analytical Chemistry Division). Ten mL of 30% hydrogen peroxide was added to each filtered throughfall and stemflow sample, ranging in volume from 400 to 600 mL, after neutralization (pH 7) with ammonium hydroxide. Samples were then evaporated to approximately 75 mL on a hot plate and transferred to 150 mL tall form beakers with two 5 mL washes of 1 N HCl. One mL of 30% hydrogen peroxide was added to each sample which was taken to complete dryness with gentle heat. Dry residues were taken up in 2 mL of 1 N HCl and transferred to plastic scintillation vials. Sulfur-35 activity in the sample was adjusted for sample volume to obtain activity per mL or activity per L.

Samples for ^{35}S analysis were analyzed by liquid scintillation counting. All samples were counted until the count rate stabilized at a standard deviation of 2% or the counting time reached 30 minutes. All ^{35}S data presented in this paper were corrected for radioactive decay (87 d half-life) to the date of tree labeling.

Total stable S concentrations in foliage samples were determined using methods described by Bushman et al. (1983). Briefly, leaf samples were ground in a blender and 50 mg portions were burned in a Schoniger-type oxygen flask. After combustion, the flasks were allowed to stand for one hour during which the gases produced on combustion were absorbed by 10 mL of deionized water containing 2 drops of 30% hydrogen peroxide. Absorbing solutions were analyzed by ion chromatography (ORNL Analytical Chemistry Division).

Leaf area: dry weight ratios were determined for maple and poplar leaves before the beginning of the experiment (late May) and before leaf fall (late August). Leaf areas ($N=20$) were read directly with a Li-Cor LI 3100 Area Meter. Leaves were dried (72 h at 100° C) and weighed. An average leaf area: dry weight ratio was calculated for each species.

Quality assurance measures included the following: 1) analysis of background samples to correct for naturally occurring radioactivity in vegetation, throughfall, and stemflow, 2) repeated counting of selected samples to check the decay rate of ^{35}S, 3) trial analyses of vegetation and stemflow with known additions of ^{35}S, 4) replicate analysis of throughfall, stemflow, and vegetation samples, 5) comparison of ^{35}S concentrations in samples ashed by the magnesium nitrate method with the Schoniger flask method, and 6) comparison of subsamples drawn from larger samples.

Results

Quality assurance

Repeated counting of 12 samples (including stemflow, throughfall, and vegetation samples) over a period of 100 to 150 days showed that the sample radioactivity decayed with a mean half-life of 84 (S.D. = 10) days. Therefore, the radioactive decay in these samples was consistent with that expected for ^{35}S and not potassium-40 (half-life = 1.2×10^9 years), the principal naturally occurring radioisotope expected in low-level samples.

Analysis of six leaf samples with known additions of ^{35}S (as sodium sulfate) showed that between 94 and 102% of the ^{35}S was recovered using our methods (mean = 97%; S.D. = 3.8%). Recovery of ^{35}S from known additions to four samples of stemflow was also high (mean = 101%;

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Table 1. Properties of the four study trees injected with ^{35}S on Walker Branch Watershed, Tennessee, in June 1986

Property	Units	Red maple		Yellow poplar	
		Non-girdled	Girdled	Non-girdled	Girdled
Time from labeling to harvest	weeks	23	13	24	15
Total foliage[a]	kg DW	6.5	7.0	6.2	6.8
Branch and twig[b]	kg DW	22.5	9.9	15.0	18.5
Bole[b]	kg DW	69.6	64.5	66.3	65.1
Total above-ground	kg DW	98.6	81.4	87.5	90.4
Cumulative leaf fall	kg DW	4.7	0.8	4.5	3.9
Plot area[c]	m^2	23	12	18	22
Total leaf sulfur	mg g^{-1} DW	1.15	0.90	2.85	1.73

[a] Estimated from whole-tree harvests and collections of total leaf fall
[b] Determined from whole-tree harvest
[c] Approximate area defined by drip line of the tree

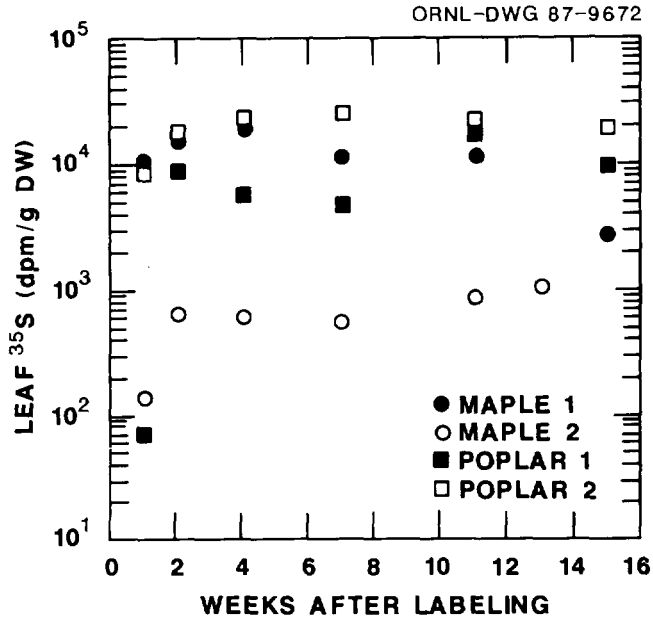

Fig. 2. Concentrations of ^{35}S in tree leaves from red maple and yellow poplar trees at different times after radiolabeling (tree number 1 is not girdled and tree number 2 is girdled); 2.22 disintegration per minute (dpm) = 1 pCi

S.D. = 4.2%). These analyses demonstrated that sulfate-^{35}S was not lost during sample preparation.

Seventeen samples of throughfall and stemflow (ranging in concentration from 61 to 3864 pCi ^{35}S L^{-1}) from the radiolabeled trees were analyzed twice. A paired *t*-test showed that the mean difference between sets (25 pCi L^{-1}) was not statistically significant (at the $P = 0.05$ level). Sulfur-35 determinations in approximately 90% of the replicate samples were within 10% of results from the first ^{35}S analysis.

Twenty vegetation samples (including bole, leaf, twig, and leaf fall) were analyzed twice using the magnesium nitrate ashing procedure. Samples ranged in concentration from 347 to 41 775 pCi ^{35}S g^{-1} DW. A paired *t*-test showed that the mean difference between sets was not statistically significant. Sulfur-35 determinations in approximately 85% of the replicate samples were within 15% of results from the first ^{35}S analysis.

Sulfur-35 determinations by the magnesium nitrate ashing method were not different from those determined by the Schoniger flask method. Ten vegetation samples (including leaves, leaf fall, and wood) were analyzed for ^{35}S using the magnesium nitrate ashing method and the Schoniger flask method. The mean difference between the two sets of analyses was 3% (S.D. = 8.7), which was not a statistically significant difference (at the $P = 0.05$ level).

Two subsamples were drawn from each of eight bags of leaf fall, ranging in size from 400 to 1300 g DW, to determine how successfully a single representative sample could be drawn from a larger sample of leaves after mixing. Mean ^{35}S concentrations in the two sets of subsamples differed by 7% and a paired *t*-test showed that this difference was not statistically significant (at the $P = 0.05$ level). It was decided that after thorough mixing, the ^{35}S concentration in a single subsample of leaves (approximately 50 g DW) was representative of the larger sample.

Table 2. Concentrations (pCi g^{-1} DW) of ^{35}S in boles and stumps of nongirdled and girdled red maple and yellow poplar trees at various times after radiolabeling

Treatment	Month	Red maple		Yellow poplar	
		Bole	Stump	Bole	Stump
Nongirdled	July	545	252	338	40
	August	327	318	291	295
	September	329	443	219	222
	October	257	275	250	571
	November	290	361	291	535
Girdled	July	2094	0	827	0
	August	1478	0	596	37
	September	1014	106	632	14
	October	–	–	608	8

Properties of the study trees

The four trees were similar with respect to foliar biomass and total aboveground biomass (Table 1). Foliar biomass was estimated from measured cumulative leaf fall plus the amount of foliage at harvest. Estimates of foliar biomass, when determined from leaf fall collections, were corrected for an average 28% weight loss prior to leaf abscission (Grizzard et al. 1976).

Beta radiation levels on each tree bole in the vicinity of the injection well were monitored 4 weeks after radiolabeling with an end window Geiger-Mueller survey meter. Surveys showed that there was some seepage of ^{35}S into the tree bark immediately above and below the injection well site on each maple tree. Poplars showed negligible amounts of surface beta radioactivity associated with the bole in the vicinity of the injection well. Sulfur-35 uptake

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through the injection well was more efficient in the poplar trees than in the maples. Beta radiation measurements around the tree boles indicated that ^{35}S uptake was least efficient from the injection well on the girdled maple.

Because of a dry summer in 1986, poplar trees in the Oak Ridge area began to drop their leaves in July. The monthy departure from normal rainfall in July, August, and September was −2.3 cm, −6.0 cm, and +2.3 cm, respectively (NOAA 1986). Approximately 88% of the total leaf fall from the girdled poplar tree occurred between 6 and 9 weeks after radiolabeling with ^{35}S, corresponding to an estimated 75% loss of foliage biomass in July and August. By comparison, 88% and 67% of the total leaf fall from the nongirdled maple and poplar, respectively, occurred during October and November, weeks 18 through 20 after radiolabeling.

Time history of ^{35}S concentrations in trees

Irrespective of the treatment, foliar ^{35}S concentrations reached nearly asymptotic levels within 1 to 2 weeks after radiolabeling (Fig. 2). Concentrations in leaves were $\sim 10^4$ disintegrations per minute ^{35}S g^{-1} in three of the trees and were $< 10^3$ disintegrations per minute ^{35}S g^{-1} in the girdled maple because of poor absorption of ^{35}S from the injection well (2.22 disintegrations per minute = 1 pCi). Except for the girdled maple, ^{35}S concentrated in tree leaves; levels measured in wood from the bole or stump (Table 2) were less than 10% of those found in foliage.

Girdling prevented significant basipetal translocation of ^{35}S in both the maple and poplar tree (Table 2), although a small amount of isotope reached the roots of the girdled maple tree just prior to whole-tree harvest. Concentrations in the bole wood of both maples decreased by ~50% between July and September, while levels in yellow poplar boles declined by 14 to 26% over the same period. Sulfur-35 concentrations in wood from the nongirdled maple bole and stump were similar after July. The ^{35}S concentration in the nongirdled poplar stump exhibited more than a tenfold increase from July to November and was eventually almost double the ^{35}S concentration found in bole wood (Table 2).

Cycling of ^{35}S in throughfall, stemflow, and leaf fall

There was less leaching of ^{35}S from the girdled trees (Table 3) because they were harvested prior to autumn leaf fall. Leaching losses of ^{35}S from the nongirdled trees increased markedly just prior to and during autumn leaf fall (Garten et al., in press). Stemflow accounted for <10% and throughfall for >90% of the ^{35}S reaching the forest floor by means of rainfall leaching of the tree canopy. The total amount of ^{35}S leached from the nongirdled trees was <20% of the ^{35}S reaching the forest floor by autumn leaf fall (Table 3). Leaf fall was more important to S cycling than leaching of ^{35}S in throughfall and stemflow.

The measured μCi amounts lost from the nongirdled trees through leaf fall (Table 3) were comparable to or greater than estimated μCi amounts calculated as the product of concentration data and foliage biomass estimates from allometric relationships (Ker 1984; Sollins et al. 1973). Although the exact percent recovery of leaf fall from the nongirdled trees is unknown, the measured returns in Table 3 do not appear to substantially underestimate ^{35}S losses from each tree by leaf fall.

Table 3. Losses of ^{35}S from the study trees in stemflow, throughfall, and leaf fall

Item	Nongirdled maple	Girdled maple	Nongirdled poplar	Girdled poplar
Cumulative ^{35}S flux (μCi plot^{-1})				
Stemflow	0.06	0.01	0.30	0.06
Throughfall	1.21	0.11	5.53	0.76
Total leached	1.27	0.12	5.83	0.82
Leaf fall (μCi plot^{-1})				
July	0.02	0	0.72	3.03
August	0.10	0.02	0.43	39.87
September	0.07	0.29	0.84	0.32
October	14.49	–	6.97	0.84
November	0.46	–	22.81	–
Total	15.14	0.31	31.8	44.05

Harvest of aboveground tree biomass

With the exception of the girdled maple, the highest ^{35}S concentrations were usually found in the upper portions of each tree. For example, the ^{35}S concentration in bole wood from section 4 (Fig. 1) was approximately 2.5 times greater than that found in section 1 or 2. Sulfur-35 concentrations in stems and branches from section 4 were 2.0 to 8.2 times greater than those found in section 2. Sulfur-35 concentrations in the girdled poplar were 1.8 times greater in leaves from section 4 as compared with section 2 or 3. Sulfur-35 concentrations in leaves from the girdled maple did not exhibit a gradient (although branches did).

First estimates of ^{35}S amounts associated with the bole section between the stemwell and the girdle were 64.3 and 24.4 μCi in the girdled maple and girdled poplar, respectively. Resampling of these bole sections following further subdivision into sections above, below, and at the injection site yielded estimates of 68.4 and 19.1 μCi. The difference between estimates was less than 3% of the ^{35}S injected into each tree.

The final distribution of ^{35}S was different in each tree (Fig. 3). In all cases, the amount of ^{35}S not absorbed from the injection well was small (<3% of the isotope added). Foliar leaching (<3%) and ^{35}S in twigs and branches (<10%) also made up a small amounts in the final inventory. The percentage of the ^{35}S label found in foliage and leaf fall varied greatly (between 2 and 31%) as did the percentage distribution in the bole above and below the stemwell (Fig. 3). In the girdled maple most of the ^{35}S was associated with the bole, particularly the section bearing the injection well (lower trunk). Approximately 63 to 69% of the isotope added to nongirdled trees and 31 to 34% of that added to girdled trees could not be accounted for in the final inventory (Fig. 3).

Calculated volatilization and translocation to roots

Table 4 presents a summary of the ^{35}S activity balance (summation of the μCi amounts in the aboveground portions of each tree at harvest, plus amounts in leaching and leaf fall). The unaccounted for portion of ^{35}S, which was the difference between the injected amount and measured amounts, was ~56 and ~67 μCi in the girdled maple and

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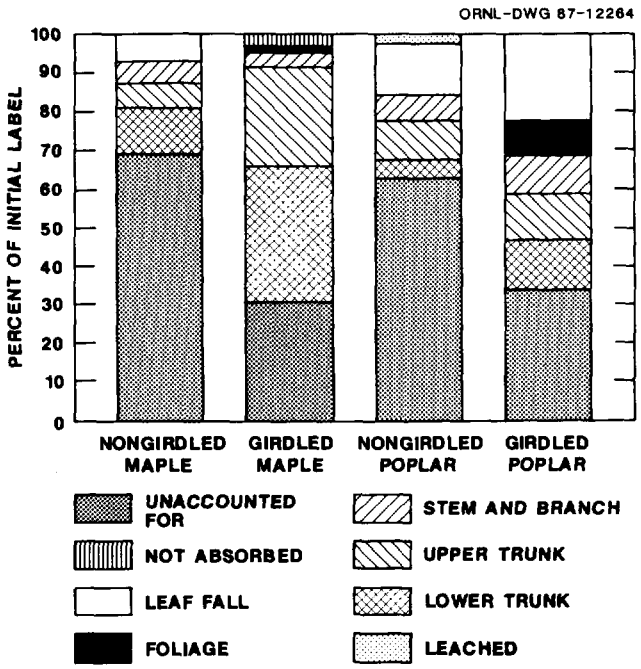

Fig. 3. Percentage distribution of ^{35}S in red maple and yellow poplar trees after harvest and aboveground inventory

Table 4. Activity balance for ^{35}S in the four study trees (all amounts are in microcuries)

Item	Non-girdled maple	Girdled maple	Non-girdled poplar	Girdled poplar
^{35}S added to injection well	243	182	243	200
Not absorbed[a]	0.3	5.3	0.1	0.1
S-35 absorbed (I)	242.7	176.7	242.9	199.9
Leached prior to harvest (L)	1.3	0.1	5.8	0.8
Cumulative leaf fall (LF)	15.1	0.3	31.8	44.1
Total in aboveground biomass at harvest (W)	58.3	120.4	52.8	87.7
Volatile S emission $V = I - (L + LF + W)$	71[b]	55.9	105[b]	67.3
Root storage and losses $R = I - (L + LF + W + V)$	97	[c]	48	[c]

[a] Amount remaining with the injection well
[b] Estimate based on ^{35}S loss rate from girdled trees (see Table 5)
[c] Root losses were considered negligible in girdled trees

girdled poplar, respectively. Because the isotope not accounted for in the girdled trees theoretically represented losses from biogenic emissions, a ^{35}S loss rate per leaf area was calculated based on the duration of the experiment and the total one-sided leaf area for each girdled tree (Table 5). The average one-sided surface area: dry weight ratio for maple and poplar leaves was 227 and 171 $cm^2\ g^{-1}$, respectively.

Sulfur-35 loss rates from the girdled trees were $\sim 10^{-6}$ to 10^{-7} $\mu Ci\ cm^{-2}$ leaf d^{-1}. Assuming no differential loss of stable S and ^{35}S from the leaves, the ^{35}S flux corresponded to an estimated foliar S emission ranging from 0.15 to 1.0 μg S cm^{-2} leaf d^{-1} (Table 5). The measured ratio of total S to ^{35}S in leaves averaged 1.8×10^5 to 2.7×10^6. Within each tree, the total S: ^{35}S ratio varied by less than a factor of two during the summer (Garten et al., in press). Extraction data indicated that the percentage of sulfate-^{35}S was similar to the percentage of total leaf sulfate, therefore the calculation of stable S emission used the measured ratio of total S to ^{35}S in the whole leaf rather than that in the sulfate pool.

The theoretical volatile ^{35}S emission from each nongirdled tree (Table 4) was estimated based on one-sided leaf surface area, the time between injection and the beginning of autumn leaf fall (119 days), and estimated ^{35}S volatilization rates of 4×10^{-7} and 8×10^{-7} $\mu Ci\ cm^{-2}$ leaf d^{-1} for the maple and poplar trees, respectively (Table 5). The calculated volatile ^{35}S emission from the nongirdled trees was based on a rate estimated from the girdled trees, rather than amount, so that the difference in harvest times did not bias the final ^{35}S inventories.

Translocation of ^{35}S to roots in the nongirdled trees was estimated by difference between injected amounts and all other measured or estimated amounts (Table 4). Translocation to roots was a significant sink for ^{35}S in the nongirdled maple (40% of the injected amount). It was estimated that twice as much ^{35}S was translocated to roots of the nongirdled maple as compared with the nongirdled poplar.

Discussion

Calculations show that the soluble S pool available to forest tree roots in the soil (A and B horizons) on WBW is 5 times greater than the total amount of S in forest trees and 15 times greater than the forest's annual root uptake of S (Johnson et al. 1982). The magnitude of soil reserves and dry deposition of S (Lindberg et al. 1986) on WBW raises questions about what mechanisms (ie. foliar leaching, volatilization, and translocation) trees use to eliminate S in excess of their nutritional requirements.

The final distribution of ^{35}S for the nongirdled maple and the nongirdled yellow poplar tree (Table 4) indicated little aboveground storage of the isotope in tree biomass ($<25\%$) and appreciable ($>60\%$) elimination from aboveground biomass by translocation and volatile S emissions. Plant roots do not cope with excess sulfate in soil by avoidance of its uptake and because sulfate, or its associated metabolic products, appear to be freely mobile in higher plants (Rennenberg 1984), translocation and biogenic S emissions could be important pathways for trees to eliminate excess S taken up from the environment.

The relative importance of ^{35}S pathways to the forest soil was translocation > leaf fall > leaching. A more than ten-fold increase in ^{35}S concentrations in the stump of the nongirdled poplar from July to November indicated that phloem translocation of S to roots was a gradual process. By comparison, a considerable amount of the ^{35}S in the nongirdled red maple tree was translocated to roots soon after radiolabeling (rapid transport of ^{35}S to the roots may be artificial because the isotope had to cross the phloem during absorption). Root sloughing represents a large organic matter flux and a potentially large nutrient flux to forest soil (Harris et al. 1973). Fine root turnover could

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Table 5. Calculated loss of volatile ^{35}S and stable S flux from the leaves of red maple and yellow poplar trees

Item	Nongirdled maple	Girdled maple	Nongirdled poplar	Girdled poplar[a]
^{35}S unaccounted for (μCi)		56		67
Duration of experiment (d)	119	91	119	105
One-sided leaf area (cm^2)	1.5×10^6	1.6×10^6	1.1×10^6	7.9×10^5
^{35}S loss rate (μCi cm^{-2} d^{-1})	[b]	4×10^{-7}	[b]	8×10^{-7}
Volatile ^{35}S lost (μCi)	71		105	
Ratio stable S: ^{35}S (μg μCi^{-1})		2.7×10^6		1.8×10^5
Stable sulfur flux from leaf (μg S cm^{-2} leaf d^{-1})		1.0		0.15

[a] One-sided leaf area was calculated from an average of weekly foliar biomass estimates over 15 weeks to compensate for early leaf drop
[b] ^{35}S loss rate in the nongirdled tree was set equal to that in the girdled tree

be one mechanism by which trees temporarily eliminate excess S and increase organic sulfur in the soil.

Available data (Table 4) show that only a small percentage of the tree's S content could be lost by foliar leaching and autumn leaf fall. Therefore, gaseous S and soil S that enters the tree in excess of nutritional demand must be eventually stored, translocated, or volatilized back to the atmosphere.

Volatile S emissions by trees might be an effective strategy for eliminating excess S taken up from the environment. Hydrogen sulfide emission is commonly observed when plants are exposed to S in excess of their needs (Rennenberg 1984). Hydrogen sulfide is the only reduced sulfur gas emitted from SO_2 fumigated pine needles (Hallgren and Fredriksson 1982), but other reduced sulfur gases (carbonyl sulfide, dimethyl sulfide, carbon disulfide) have been detected in measurements of volatile S emissions from oak and pine leaves (Westberg 1984; Lovelock et al. 1972). If most of the estimated volatile S emission from Table 5 was H_2S, then the estimated S flux from yellow poplar and red maple leaves (0.4 and 2.6 nmol cm^{-2} leaf h^{-1}, respectively) would be in reasonable agreement with published rates (typically 1 to 2 nmol cm^{-2} leaf h^{-1}) of H_2S emission from plant leaves in response to S exposure (Taylor and Tingey 1983; Rennenberg 1984).

Interpretation of the fate and distribution of ^{35}S in the study trees depends on the accuracy of the final microcurie amount inventories and the assumption that the girdling did not significantly alter aboveground S metabolism. Based on resampling and quality assurance data, it appears that measured amounts of ^{35}S (Table 4) were not significantly underestimated.

Prior experiments have shown that nutrient reserves in the stumps and roots of yellow poplar trees are large enough to maintain a functional root system for more than two years after girdling (Edwards and Ross-Todd 1979). Girdling causes a gradual depletion of food reserves below the girdle and an increase in catabolic activity above the girdle (probably resulting from accumulation of photosynthate). Since there is no indication of increased fine root death for up to six months after girdling (Edwards and Ross-Todd 1979), the girdling treatment was regarded as a minimal disturbance in the current experiment.

Total S (Table 1) and sulfate concentrations (Garten et al., in press) in leaves from the girdled trees were less than or equal to those measured in the normal trees, indicating no accumulation of sulfur in the leaves of girdled trees. However, girdling may cause a disruption of S metabolism that is not apparent from monitoring of total foliar S or sulfate. An accumulation of foliar glutathione or L-cysteine as a result of disruption to phloem transport in the girdled trees could stimulate hydrogen sulfide emissions from the leaves (Rennenberg 1984) and exaggerate the relative importance of biogenic emissions.

Volatile S emissions from trees and the translocation of S to tree roots are not well studied processes in forest sulfur cycles. Although the forest on WBW has little capacity for storage of excess S in aboveground biomass, there appears to be appreciable capacity to cycle S either to the belowground system by means of translocation or to the atmosphere by means of biogenic S emissions from tree leaves. Both processes may provide forest trees with a mechanism for eliminating S in excess of nutritional needs. Geochemical processes have been proven important to the biogeochemical cycling of sulfur in forests (Johnson 1984; Johnson et al. 1986), but the potential importance of biological processes could be underestimated.

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References

Bushman LM, Dick RP, Tabatabai MA (1983) Determination of total sulfur and chlorine in plant materials by ion chromatography. Soil Sci Soc Am J 47:1167–1170

Edwards NT, Ross-Todd BM (1979) The effects of stem girdling on biogeochemical cycles within a mixed deciduous forest in eastern Tennessee. Oecologia (Berlin) 40:247–257

Garten Jr CT, Bondietti EA, Lomax RD (1988) Contribution of foliar leaching and dry deposition to sulfate in net throughfall below deciduous trees. Atmos Environ (in press)

Grizzard T, Henderson GS, Clebsch EEC, Reichle DE (1976) Seasonal nutrient dynamics of foliage and litterfall on Walker

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Branch Watershed, a Deciduous Forest Ecosystem, ORNL/TM-5254. Oak Ridge National Laboratory, Oak Ridge, Tennessee

Hallgren JE, Fredriksson SA (1982) Emission of hydrogen sulfide from sulfur dioxide-fumigated pine trees. Plant Physiol 70:456–459

Harris WF, Goldstein RA, Henderson GS (1973) Analysis of forest biomass pools, annual primary production and turnover of biomass for a mixed deciduous forest watershed. In: Young HE (ed) IUFRO Biomass Studies. Mensuration, Growth, and Yield. University of Maine Press, Orono, Maine, pp 41–64

Johnson DW (1984) Sulfur cycling in forests. Biogeochemistry 1:29–43

Johnson DW, Henderson GS, Huff DD, Lindberg SE, Richter DD, Shriner DS, Todd DE, Turner J (1982) Cycling of organic and inorganic sulphur in a chestnut oak forest. Oecologia (Berlin) 54:141–148

Johnson DW, Richter DD, Miegroet HV, Cole DW, Kelly JM (1986) Sulfur cycling in five forest ecosystems. Water Air Soil Pollut 30:965–979

Ker MF (1984) Biomass equations for seven major maritime tree species. Information Report M-X-148. Canadian Forestry Service, Department of the Environment, Ottawa

Lindberg SE, Lovett GM, Richter DD, Johnson DW (1986) Atmospheric deposition and canopy interactions of major ions in a forest. Science 231:141–145

Lovelock JE, Maggs RJ, Rasmussen RA (1972) Atmospheric dimethyl sulfide and the natural sulfur cycle. Nature (London) 237:452–453

NOAA (1986) Climatological Data: Tennessee 1986. National Oceanic and Atmospheric Administration, National Climatic Data Center, Asheville, North Carolina

Rennenberg H (1984) The fate of excess sulfur in higher plants. Ann Rev Plant Physiol 35:121–153

Shriner DS, Henderson GS (1978) Sulfur distribution and cycling in a deciduous forest watershed. J Environ Qual 7:392–397

Sollins P, Reichle DE, Olson JS (1973) Organic matter budget and model for a southern Appalachian *Liriodendron* forest. PhD Dissertation, University of Tennessee, Knoxville, Tennessee

Taylor Jr GE, Tingey DT (1983) Sulfur dioxide flux into leaves of *Geranium carolinianum* L. Plant Physiol 72:237–244

Westberg H (1984) Estimation of biogenic sulfur emissions from the continental U.S. In: Aneja VP (ed) Environmental Impact of Natural Emissions. Air Pollution Control Association, Pittsburgh, Pa, pp 41–53

Williams S (ed) (1984) Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Inc., Arlington, Va

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