Aspects of Carbon and Sulfur Transformations in MOFEP Surface Soils

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Abstract.—Carbon and sulfur transformations were studied in surface soils from plots in MOFEP sites from August 1993 to May 1996 and in plots in watersheds of MOFEP sites 1, 3, and 4 from May 1995 to May 1996. Element pools measured included total carbon, total sulfur, sulfate, and organic sulfur. Transformations quantified included lignocellulose mineralization and organic sulfur production. Most parameters measured were similar compared by plots and sites, with large differences observed when compared by date. This baseline data, compared with post-treatment data, may help determine mechanisms involved in soil carbon and sulfur transformations, and their relation to other soil nutrients, such as potassium and magnesium.

The elements carbon and sulfur are essential to forested ecosystems. As part of the extensive energy transformation system associated with food webs, carbon literally makes up the backbone of the forest. Carbon also interacts with other critical elements in their complex cycles through the ecosystem. Surface soils of forests play a major role in the cycling of both carbon and sulfur, providing decomposing microorganisms responsible for the transformations necessary to keep these elements from becoming sequestered within the soil. Forest primary producers provide the energy that keeps all of these transformations going. The form of carbon primary producers contribute to the forest floor in the greatest concentrations is lignocellulose. Soil microorganisms play critical roles degrading this relatively recalcitrant molecule, and help to recycle the carbon, releasing it to the atmosphere as carbon dioxide (CO_2) (Atlas and Bartha 1993, Stolp 1988). Certain bacterial and fungal species possess cellulases that are capable of splitting the β 1,4 linkages of cellulose (Crawford *et al.* 1977, Stolp 1988). Other bacteria and fungi are capable of producing oxidizing agents that lead to the depolymerization of lignin (Tien and Kirk 1983). Thus, the decomposition of lignocellulose in forest soils is dependent on the presence of bacteria and fungi possessing these degradative abilities.

Sulfur plays important roles in ecosystems both as an essential nutrient and as a reactant. Studies of sulfur cycling in Eastern U.S. forests have indicated that, as a nutrient, sulfur should generally not be limiting (Johnson *et al.* 1982, Likens *et al.* 1977, Shriner and Henderson 1978). However, sulfur interacts with a number of other nutrient elements, including nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), and potassium (K), in some cases influencing their mobility directly (Rechcigl and Sparks 1985, Watwood *et al.* 1993, Wiklander 1978), or indirectly (Homann and Harrison 1992, Mitchell *et al.* 1989). The major pools of sulfur in forest soils include sulfate (soluble or adsorbed) and organic sulfur (C-bonded or ester sulfate; Schindler *et al.* 1986). Studies of forest soils in the U.S., Canada, and Europe indicate that organic sulfur makes up the largest proportion of the soils' total sulfur constituents (Johnson *et al.* 1986, Mitchell and Zhang 1992, Van Loon *et al.* 1987, Zucker and Zech 1985).

Studies of sulfur cycling in forests may involve consideration of the many sources and sinks of sulfur in that habitat (fig. 1). Sulfur is supplied to the forest ecosystem via either weathering or precipitation in the form of sulfate (Mitchell and Lindberg 1992). Concern over the increased input of sulfate to forest soils, as a result of acidic precipitation, has resulted in numerous studies of this problem. These studies have led to a better understanding of the physico-chemical interactions that occur when sulfate is added to a forest soil (Foster 1985, Mitchell and Lindberg 1992, Rechcigl and Sparks 1985,

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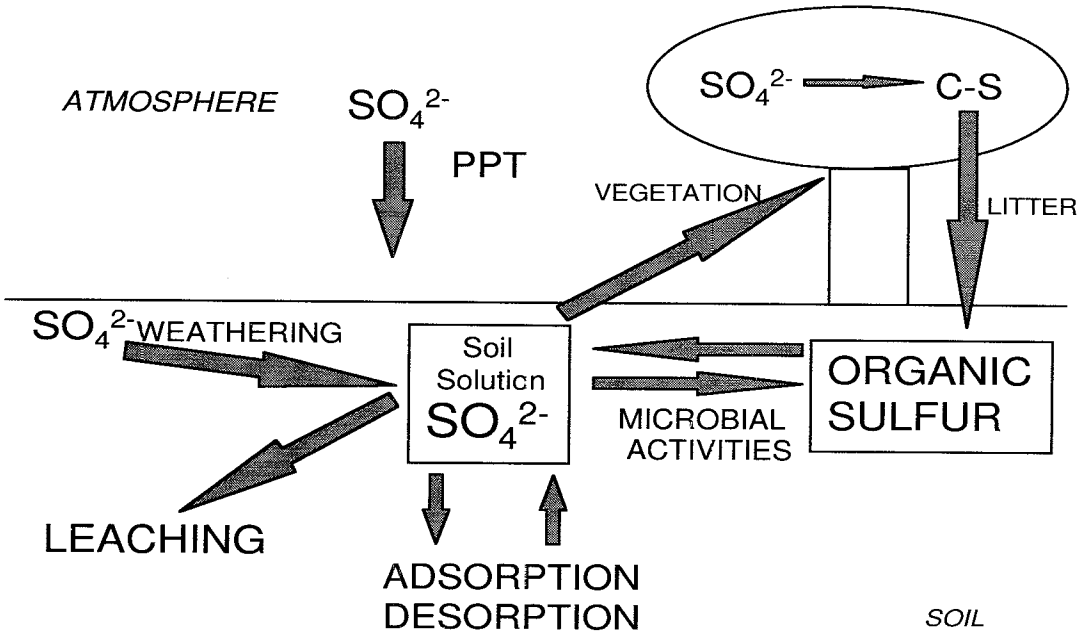
Forest Sulfur Cycling



Modified from Mitchell, David, and Harrison 1992

Figure 1.—*Forest sulfur cycling.*

Ulrich *et al.* 1980, Wiklander 1978). Within forest soils the added sulfate can be adsorbed via abiotic mechanisms to positively charged ion exchange sites, where it may then be taken up by soil microorganisms or plants and converted into a variety of organic sulfur compounds. These organic sulfur compounds of either plant or microbial origin may then be mineralized by soil microorganisms, with the sulfur released back to soil solution as sulfate (Strickland *et al.* 1986). In some mineral soils, however, organic sulfur has been found to be somewhat recalcitrant, resulting in lower potential for microbial mineralization (McLaren *et al.* 1985), and hence may accumulate in the soil. Organic sulfur compounds apparently play a critical role in the retention of nutrient cations within forest soils by possibly serving as cation exchange sites. Watwood *et al.* (1993) demonstrated that mineralization of the organic sulfur fraction in A-horizon forest soils correlates with the loss of nutrient cations (i.e., Ca^{+2}, Mg^{+2}, and K^{+}). Thus, disturbances that may contribute to organic sulfur loss from forest soils may be important to the availability of nutrient cations within the forest ecosystem.

One anthropogenic disturbance of forest ecosystems is the harvesting of timber. Studies of whole-tree harvesting at the Hubbard Brook Experimental Forest in New Hampshire indicated that the greatest short-term (ca. 2 years post-harvest) effect on sulfur cycling in these spodsols was a significant increase in the adsorbed sulfate pool (Mitchell *et al.* 1989). As far as organic sulfur pools were concerned, the only change observed as a result of the harvest was a reduction in the concentration of soil solution organic sulfur as the solution passed from the Oa to the Bs2 horizons, with no significant changes in solution organic sulfur observed for lower mineral horizons. Mitchell *et al.* (1989) made no mention of the potential for cation leaching from the surficial soil horizons as a result of the loss of organic sulfur from this soil.

A preliminary study of the effect that clear-cutting has on sulfur transformations in A-horizon soils of Deer Run State Forest, near Ellington, MO, indicated that significant changes in soil organic sulfur and exchangeable K^{+} and Mg^{2+} were observed for sites that had

been clearcut 2 to 3 or 8 to 10 years previously (Spratt 1997). Studies in other forested sites have indicated that soil bacterial activities are at first stimulated by timber harvest, followed approximately 2 years post harvest by significant reductions in these activities (Lundgren 1982, Pietikäinen and Fritze 1995). The pulse of labile organic materials available to soil microorganisms from decaying debris and root material appears to be instrumental in the pattern of bacterial activity observed following harvest. Once depleted, the concentrations of labile organic materials apparently fall below that necessary to support the populations of microorganisms present in the undisturbed soils. Hence, the marked decline in microbial activities approximately 2 years post harvest.

The results of the preliminary study in Deer Run A-horizon soils led to the development of a large-scale project involving the study of surface soil carbon and sulfur transformations as part of the Missouri Ozark Forest Ecosystem Project (MOFEP) (Brookshire *et al.* 1997). This report summarizes the findings of nearly 3 years of pre-treatment data generated in this component of MOFEP.

OBJECTIVES

The major objectives of this large-scale study are:

1. To determine the short-term and long-term effects of even-aged, uneven-aged, and non-manipulative forest management practices on soil carbon and sulfur constituents in MOFEP soils.
2. To assess any changes in soil microbial lignocellulose or sulfur processing due to even-aged, uneven-aged, and non-manipulative forest management practices in MOFEP soils.
3. To determine relationships that may exist between soil lignocellulose mineralization and organic sulfur production.
4. To determine relationships that may exist between soil microbial sulfur transformations and nutrient cations (e.g., K^+ or Mg^{2+}) as a result of the experimental treatments.

MATERIALS AND METHODS

Sample Sites and Collection

Sample site selection for this study was complicated by the need to keep total sample numbers as low as possible to allow completion of all analytical procedures necessary for that date. At the same time enough samples had to be collected to enable detection of changes in the measured parameters over the noise inherent in the system. It was also desirable to sample all nine MOFEP sites, and, preferably, different locations within the landscape. Sites used in this study were carefully chosen to reflect the above concerns. Beginning in August 1993 and continuing through May 1996, samples were collected from each of the nine MOFEP sites (see figure 1 in Brookshire *et al.* 1997). For each MOFEP site, three plots were randomly placed as described below, with three replicate samples collected from each plot. Soil collection sites were established as a subset of the permanent MOFEP plots selected (see table 1 for details of the locations of these soil collection sites within the sampled plots). All MOFEP plots sampled were located midslope, with south and west aspect (see table 1 for a summary of the plots sampled). Control plots were chosen at random from plots having similar aspect and slope within the site. To ensure that the harvest treatment designated for the site (e.g., even-aged or uneven-aged management) occurred on the experimental plots to be sampled the first year of treatment, the Missouri Department of Conservation (MDC) provided maps of the first timber sales and helped in the selection of sample plots for this study. For sites receiving even-aged harvest, plots were chosen at random from a pool of south and west aspect, midslope plots to be experimentally treated the first year. Because the effects of uneven-aged cutting are much less predictable, the exact location of uneven-aged harvests are unknown in advance of treatment. Plots with the greatest likelihood of being treated were chosen for sample collection (i.e., plots with basal area ≥ the site mean). Soil samples were collected from these MOFEP sites on the following sample dates [field A-horizon soil temperatures indicated in parentheses]: August 17 & 18, 1993 (32°C), December 3 & 4, 1993 (8°C), March 7 & 8, 1994 (7°C), June 1 & 2, 1994 (24°C), September 22 & 23, 1994 (18°C), December 15 & 16, 1994 (9°C), March 9 & 10, 1995 (5°C), and May 23-25, 1995 (20°C), September 21 & 22, 1995 (17°C), March 9 & 10, 1996 (3°C), and May 2 & 3, 1996 (18°C).

Beginning in May 1995, samples were also collected from three paired watersheds located in MOFEP sites 1, 3, and 4. The paired watersheds represented both south and west aspect

Table 1.—*MOFEP plots sampled in the soil carbon and sulfur transformation study.*

Site	Plot sampled [1]	Soil type[2]	ELT[3]
1	21	ultisol	17
1	31	ultisol	17
1	40	alfisol	17*
2	14	alfisol	17*
2	42	alfisol	17*
2	45	alfisol	17*
3	14	alfisol	17*
3	15	ultisol	17
3	37	ultisol	17
4	16	alfisol	17*
4	21	alfisol	17*
4	39	alfisol	17*
5	55	alfisol	17*
5	#1, located in stand 14[4]	alfisol	17*
5	#2, located in stand 14[4]	alfisol	17*
6	18	ultisol	19*
6	34	alfisol	17*
6	58	ultisol	17
7	3	alfisol	17*
7	9	alfisol	17*
7	65	ultisol	17
8	3	ultisol	17
8	16	alfisol	17*
8	70	ultisol	17*
9	26	ultisol	17
9	65	ultisol	17
9	67	alfisol	17*

[1] Soil samples are collected from positions within the plots indicated by small blue flags inserted into the ground. The location of the sampling positions within the plots is determined as follows (all measured from the plot's center post): Sample A - 45°, 70 feet; Sample B - 135°, 70 feet; Sample C - 225°, 70 feet. .

[2] As determined by Dennis Meinert in his study of MOFEP soils.

[3] Ecological landtype (ELT), or landscape classification, as estimated by Dennis Meinert after his soil survey of MOFEP plots. Note: * indicates that this ELT classification might change.

[4] Points #1 and #2 are not MOFEP plots. They are located in stand #14 along the side of a ridge on ELT 17. The first of these points is located about three chains, 338° from the center post of site 5, plot 55. The second point is about two chains from the first, also at 338°. The centers of both points are marked with green/black flagging, and the samples A, B, and C are found in the same relationship to the center as at all other plots, and are also marked by blue flags.

and north and east aspect habitat. Two sample collection plots were located in each of the watersheds, with one site located in a convex area high on the slope, and the other site located in a concave area on the slope near the bottom of the watershed. There were 12 plots total in the watershed habitats. Three replicate samples were collected from each of the sample collection sites. These three replicates were not pooled. Soil samples were collected from these watershed sites on the following dates [field A-horizon soil temperatures indicated in parentheses]: May 23 & 24, 1995 (20 °C), September 21, 1995 (17 °C), March 9, 1996 (3 °C), and May 2, 1996 (18 °C).

Also beginning in May 1995, surface water grab samples were collected from streams located near MOFEP plots and from the Current River at Owls Bend. A water sample was collected from a stream located between sites 2 and 3 near Bankers Cave. Three streams in Peck Ranch were sampled; one running between sites 7 and 8, Rodgers Creek (roughly midway between sites 7 and 9), and Mill Creek, at the edge of site 9. These water samples were filtered through 0.45-µm cellulose acetate filters at the site and placed on ice until they could be frozen (within 6 hours). The frozen samples were transported to the University of Tennessee at Chattanooga (UTC) where they were analyzed for SO_4^{2-} and NO_3^- using ion chromatography.

For the locations of MOFEP plots sampled, the watershed plots, and the surface water sample collection sites, please refer to figures 1 through 5 in Brookshire *et al.* (1997).

For the MOFEP plots sampled, A-horizon soil samples were collected by removing the overlying litter layer, and cutting into the A horizon with a sharp spatula. Care was taken to remove only the organic-rich A horizon, and not any of the B horizon (the A-horizon soils are generally much darker than the B-horizon soils). The soil samples were placed in sterile Whirl-pac® bags, and stored in a cooler for the return trip to a laboratory at Southeast Missouri State University (SMSU) in Cape Girardeau (samples taken from August 1993 through June 1994), or to a laboratory at the UTC (samples taken from September 1994 through May 1996).

Sampling in the watershed plots included collection of litter, A-horizon soils, and B-horizon soils. The litter was removed from the forest floor in an area of ca. 100 cm^2 and placed in a sample bag. The A-horizon soil was then carefully cut with a sharp spatula and removed to a sample bag. Finally, B-horizon soils were collected down to a total depth of ca. 15 cm using a small trowel, carefully avoiding contamination of the B-horizon soil with litter or A-horizon soil, and placed in a sample bag.

White oak (*Quercus alba*) distribution on the MOFEP plots sampled in this study was determined using data provided by MOFEP administrators (Brookshire *et al.* 1997). Details of the methodology used to survey the woody vegetation on MOFEP plots may be found in Kabrick *et al.* (1997). All white oak > 4 cm d.b.h. on plots sampled in this study were summed to yield the data presented in table 2.

Table 2.—*Number of white oaks > 1.5 in. d.b.h. on MOFEP plots sampled for the soil carbon and sulfur transformation study.*

Site	Mean white oak	+/- 1SE	Plots	Range white oak
	- - Number per plot - -		*Number*	*Number per plot*
1	51.7	12.7	3	31-82
2	46	16.3	3	12-81
3	46	7.6	3	33-64
4	58	11.1	3	31-75
5	42.3	0.3	3	42-43
6	20.3	5.6	3	12-34
7	18	3.1	3	11-24
8	13	7.0	3	3-30
9	30	10.7	3	14-56
All 27 plots	36.1	4.3	27	3-82

Once at the laboratory, the soils and other samples were stored at 5°C and, within 3 days of collection, processed according to the chart in figure 2. From August 1993 until June 1994, each of the 81 individual replicate samples collected from the MOFEP plots and all samples collected from the watershed sample collection sites were processed separately. Beginning in September 1994, samples collected from the MOFEP plots were pooled using equal weight aliquots of the three replicate soil samples from each plot before continuing with sample processing. Unwanted root material, rocks, and any other recognizable litter were removed by passing the soils through a 2-mm polyethylene sieve. The sieved samples were then subdivided into four fractions: one for measurement of extractable sulfate; a second for percent moisture determination, total sulfur measurement, and determination of exchangeable bases (e.g., Mg^+ and K^+); a third to measure ^{35}S-sulfate incorporation; and a fourth to measure ^{14}C-lignocellulose mineralization. The exchangeable sulfate samples were placed in sealed vials and frozen at -20°C until further processing (see below); the samples for percent moisture were weighed and then dried at 60°C until a constant weight was obtained to determine the weight of moisture lost. After the percent moisture was determined, the dried soils were used to determine the soil total sulfur content and extractable base content (see below). Note: all data are presented on a gram dry weight basis to negate changes due to different moisture content throughout the 3 years of sampling.

For the watershed samples, A-horizon soils were treated exactly as the MOFEP plot samples, although replicates were not mixed. Watershed litter and B-horizon soils were dried at 60 °C for ca. 1 week. The litter was then chopped up in a Waring Blender and ground in a mortar and pestle, dried again, and then used for elemental analysis (see below). Dried B-horizon soils were also used for elemental analysis.

Production of ^{14}C-Labeled Lignocellulose

Published techniques to specifically label the lignin or cellulose portion of woody plant tissue were followed (Benner *et al.* 1984, Benner *et al.* 1985, Crawford and Crawford 1976, Crawford *et al.* 1977, Hackett *et al.* 1977). White oak was chosen as the species to be radiolabeled, based on its distribution throughout the MOFEP sites. Cuttings were collected from MOFEP site 8 (well away from any of the plots—the nearest plot

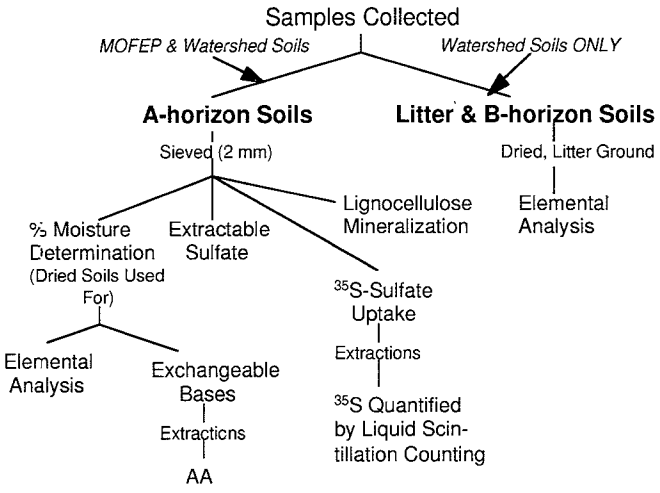


Figure 2.—*Sample processing for carbon and sulfur cycling studies of forest soils.*

was #70) in late July 1993. These cuttings were immediately immersed in water and transported to the Biology Department greenhouse at SMSU, where they were placed on a misting bench. Shortly thereafter, the cuttings were cut into smaller pieces approximately 30 cm in length, ensuring that the leaves were not damaged. The stems of these plants were immersed in water as soon after cutting as possible. About 100 smaller cuttings total were used. Under a hood, the cut ends of the cuttings were carefully cleaned with sterile distilled H_2O, and placed in small beakers containing 10 ml of the ^{14}C-precursor to either lignin or cellulose mixed in distilled H_2O. Uniformly labeled ^{14}C-phenylalanine (New England Nuclear, 50 µCi total for 50 small cuttings) was used as the precursor of lignin (Crawford *et al.* 1977). Uniformly labeled ^{14}C-glucose (ICN, 50 µCi total for the remaining 50 small cuttings) was used as the precursor of cellulose (Crawford *et al.* 1977). The cuttings were kept under constant illumination while the 10 ml of precursor was taken up by the plants, requiring between 2 and 3 hours. At that time, and for the remaining time in the 72-hour incorporation incubation, sterile distilled H_2O was added to the beakers to keep the plants from drying out. The plants were kept under constant illumination throughout the 72-hour period to ensure maximal photosynthetic activity.

After the incorporation process, all of the lignin-labeled and cellulose-labeled plants were pooled into "^{14}C-lignin" and "^{14}C-cellulose" groups and maintained thusly for the remainder of processing. The plants (both leaves and twigs) were cut into pieces no larger than 1 cm in length and

dried at 55 °C for 72 hours. Once dry, the plant material was placed in a Waring blender and ground until it would pass through a #30 sieve (600 μm particles will pass). All work was conducted within a fume hood.

To ensure that no unincorporated ^{14}C-phenylalanine or ^{14}C-glucose remained in the plant materials, a procedure to produce extractive-free lignocellulose was followed (Benner *et al.* 1984, Benner *et al.* 1985). Using a Soxhlet extraction unit, the material was first washed with distilled water for approximately 5 hours. The plant material was then extracted with a 95-percent-ethanol:benzene mixture (1:2 vol:vol) for approximately 24 hours (until the extracted fluid ran clear). Next, the plant material was extracted with 95 percent-ethanol for approximately 24 hours (again until the extracted fluid ran clear). Finally, the plant material was washed with distilled water overnight. The extractive-free plant material was carefully removed from the extraction thimble, placed in a beaker, and dried at 60°C for 48 hours. The total amounts of labeled plant material recovered were: 31.4 g "^{14}C-lignin" and 30.8 g "^{14}C-cellulose." The specific activity (DPM/g dry material) of both the radiolabeled lignin and cellulose material was determined by combusting variable weights of plant material in a Schoniger combustion flask (A.H. Thomas, Swedesboro, NJ), in which 25 ml of a 0.1N NaOH solution was placed. Aliquots of the NaOH were removed and quantified using liquid scintillation counting (see below). The plant material was (and still is) stored desiccated in a -80°C freezer. Over the first 3 years of this project, approximately one-third of the radiolabeled lignocellulosic material was used.

^{14}C-Lignocellulose Mineralization Experiments

Mineralization of white oak ^{14}C-lignin and ^{14}C-cellulose was determined using a modification of previously published techniques (Benner *et al.* 1985, Crawford *et al.* 1977). Microcosms were constructed using 200-ml screw-capped bottles (see figure 3 for a diagram of the microcosm). In place of the screw caps, butyl rubber stoppers were inserted. Suspended below the stoppers was a test tube (3-ml capacity) into which a short length of small diameter tygone tubing was placed. The tygone tubing was connected to a large gage syringe needle, which was inserted through the stopper. On the outside of the stopper, the needle's luer-lock

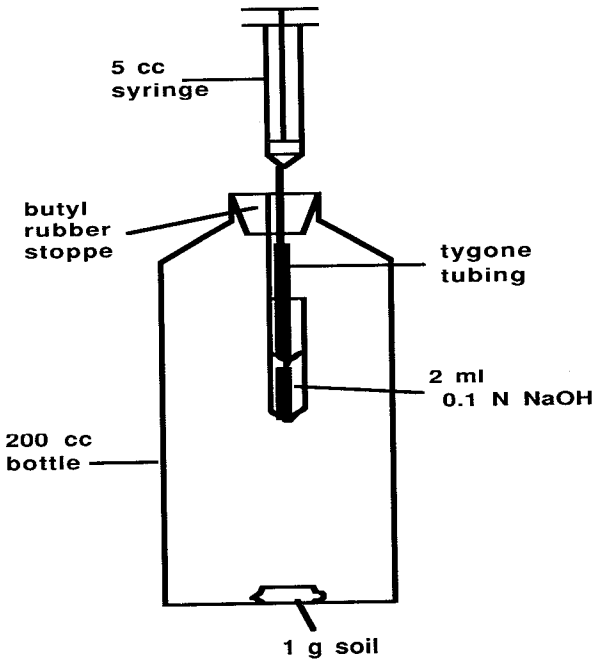


Figure 3.—*Diagram of a microcosm used in the lignocellulose mineralization studies. The volume of the bottle was 200 ml.*

was sealed using a 5-cc plastic syringe. The syringe was used to place exactly 2.0 ml of 0.1N NaOH into the test tube. This NaOH served as the $^{14}CO_2$ trap during the incubation. At each time point during a time-course incubation, the NaOH in the test tube was completely removed by drawing it up into the syringe. Fresh NaOH was immediately added back into the test tube via a second (clean) syringe. This second syringe was left locked in place until the next sampling, effectively sealing the microcosm and minimizing any loss of $^{14}CO_2$. During each incubation, potential loss of $^{14}CO_2$ from the microcosms was monitored via several NaOH traps placed within the incubator. Only on one occasion (August 1993, the first time this procedure was performed) was there any indication of minor leaks.

To initiate the experiments, approximately 1 g of the sieved soil (maintained at field moisture) was placed into a microcosm bottle for the lignin study, and one additional gram was placed into a second microcosm bottle for the cellulose study. Next, approximately 10 mg of the dried extractive-free ^{14}C-labeled lignin or cellulose plant material was added to the microcosms. The soil and plant material were shaken to ensure a homogeneous mixture. "Time zero" in the time course experiments was indicated as the time 0.5-ml distilled H_2O was added to the

soil in each microcosm. This amount of water was found to minimize soil drying, while not saturating the soil. The microcosms were then placed in a dark incubator maintained at field temperature for the duration of the incubations. From this point on, the microcosms remained sealed, except for the time involved to collect samples. This procedure is designed to produce aerobic conditions throughout the experiment (Benner *et al.* 1985). Samplings were made every 3 to 5 days for 3 to 4 weeks. Of the 2 ml of NaOH removed from the microcosm, 1 ml was placed in a scintillation vial for liquid scintillation counting. Maximal rates of lignin or cellulose mineralization were determined by calculating the maximal change in DPM (backgrounds subtracted) recovered for different times in the time course of the incubation. This helped avoid factoring potential lag periods into the rate of lignocellulose mineralization.

^{35}S-Sulfate Incorporation Experiments

Incorporation of ^{35}S-sulfate into different soil sulfur pools was monitored using a modification of the technique of Watwood and Fitzgerald (1988). Two slightly different techniques were used over the sampling period. In the first technique (used in August and December 1993), sieved soil was added to Ace Glass filter sticks (with a fritted glass porosity of 25 to 50 μm), and in the second technique (used for the remainder of sampling dates), sieved soil was added directly into 12-ml conical centrifuge tubes. The reason for the change in techniques was the high rate of breakage of the filter sticks (in December 1993, nearly 15 percent of the samples were lost due to breakage) and the resultant loss of the samples. Approximately 1 g of sieved soil was used in each technique. ^{35}S-sulfate, as $Na_2{}^{35}SO_4$, was added (0.2 ml, ca. 1 μCi containing a total of 8 pmols sulfate) to the top of the soil samples to initiate the incubations. The soils were incubated at field temperature, under aerobic conditions, for 48 hours in the dark. The soils were then placed in a -80°C freezer to arrest any further transformations of the ^{35}S-sulfate, until further processing occurred (within 2 weeks of the completion of the incubation).

^{35}S-Organic Sulfur Mineralization Experiments

For samples collected from the watershed plots in March and May 1996, rates of organic sulfur mineralization were determined using a modification of the technique of Strickland *et al.* (1986). Radiolabeled organic sulfur was prepared using mixed A-horizon soils collected from each of the nine MOFEP sites. Twenty grams total of mixed soil was subdivided into six - 50-cc centrifuge tubes, and a total of 160 μCi of $Na_2{}^{35}SO_4$ was evenly distributed among all of the tubes. The soil was incubated at 20°C for approximately 2 weeks, at which point it was frozen at -20°C. The $^{35}SO_4$ remaining in the soils was removed by washing the soil first with dH_2O, followed by a salt mixture (see below), and again with dH_2O. The soil washes were accomplished by adding the dH_2O or salt mixture to each centrifuge tube (3.0 ml dH_2O, 2.0 ml salt mixture), mixing on a vortex mixer, and centrifuging (2,000 x g, 10 minutes). The washes were repeated four times for the initial dH_2O wash, two times for the salt wash, and then five times for the final dH_2O wash. The soils were then removed from the centrifuge tubes and dried at 60°C for 72 hours. The specific activity of the soils was determined by combusting aliquots of the dried soil in a Schoniger combustion flask, following the technique of Spratt and Morgan (1990). An aliquot of the dH_2O present in the combustion flask was removed after combustion, and the ^{35}S present was quantified by liquid scintillation counting. The specific activity of the soil generated was 1.25 μCi/g. This radiolabeled soil is stored desiccated at -80°C.

Organic sulfur mineralization experiments were set up by adding approximately 1 g of sieved A-horizon soil from each of the watershed plots to conical centrifuge tubes (12 cc), followed by the addition of approximately 10 mg of the dried ^{35}S-organic sulfur-labeled soil. The centrifuge tubes were shaken thoroughly to mix the soils, and 0.3-ml dH_2O was added to initiate the incubation. Separate sets of soils were set up to generate time courses of organic sulfur mineralization. One set of soils, designated t_0, was placed in a freezer at -80°C immediately after addition of the 0.3 ml dH_2O. The remaining sets of soils were incubated at the field temperature of A-horizon soil on the date of collection for various times up to 2 weeks. At the appropriate time in the time course, the incubations were halted by freezing at -80°C. The ^{35}S-sulfate liberated from the mineralized ^{35}S-organic sulfur was recovered using extractions of the soils with dH_2O and a mixture of salts (see below for the details of these extractions). ^{35}S present in the extracts was quantified by liquid scintillation counting.

Recovery of ^{35}S in Soil Sulfur Fractions

The fate of ^{35}S-sulfate added to the soils was determined by sequential extraction of the soils to quantify the radiolabel present in the water soluble and adsorbed sulfate pools, and the organic sulfur fraction (Watwood and Fitzgerald 1988). For August and December 1993 samples, the water soluble fraction was determined by three successive washes through the soils in filter sticks (200 µl of dH_2O each), with centrifugation (2,000 x g, 10 minutes) between each wash. The filtrate recovered in the bottom of the centrifuge tubes was pooled in a scintillation vial. The ^{35}S-sulfate present in this vial represented the radiolabel that remained soluble during the incubation period. The soils collected on all other sample dates were also washed successively (200 µl dH_2O), but, five rinses were used, and the soil/rinse water was thoroughly mixed before centrifugation. After centrifugation, the supernatant was carefully collected using a pipet without removing any of the soil.

Sequential extraction with salts was used to determine the amount of ^{35}S-sulfate adsorbed onto soil surfaces during the incubation. For the August and December 1993 samples, following the water washes, the soil in the filter stick was washed six times with solutions of salt (2-200 µl washes each of 1M Na_2SO_4, 1M NaH_2PO_4, and 1M NH_4Cl). Between each wash, the filter sticks with soil were centrifuged (2,000 x g, 10 minutes), and the filtrate was transferred to a labeled scintillation vial. Soil samples collected on all other sample dates were washed with each of the salts one more time than were the filter stick soils, with mixing before centrifugation, and supernatant collection via pipet (as above with the water rinses).

Determination of the radiolabel incorporated into the organic sulfur fraction of the soil was made using a strong acid/high temperature hydrolysis followed by a strong base extraction. For the acid extraction on samples collected in August and December 1993, 300 µl of 6N HCl was added to each filter stick, and the filter sticks were placed in an autoclave (121°C, 15 PSI) for 20 hours. After cooling, the soils were centrifuged to collect the HCl and then washed (2-300 µl dH_2O washes). These washes were added to a scintillation vial. The strong base extraction involved the addition of 300 µl of 2N NaOH, followed by a 12-hour extraction period at room temperature. After this period, the soils were centrifuged to collect the NaOH and finally washed (2-300 µl dH_2O washes). These washes were also added to a scintillation vial. The ^{35}S present in these fractions were determined using liquid scintillation counting. Soil samples collected on all other sample dates were treated to the same hydrolytic reactions as their filter stick counterparts; the only difference was the rinsing, which used one additional dH_2O rinse, and mixing between centrifugations.

Liquid Scintillation Counting

Quantification of the ^{14}C and ^{35}S used in all of the above experiments was made using a Beckman LS 5000 TA liquid scintillation counter from August 1993 until June 1994. The ^{14}C samples processed from September 1994 until May 1995 were also quantified on the Beckman LS 5000 TA scintillation counter. Beginning in September 1994, the ^{35}S samples were quantified using a Wallac 1409 liquid scintillation counter. Finally, ^{14}C samples were also quantified on the Wallac instrument for the March and May 1996 sample dates. Care was taken to ensure comparability of the samples quantified on different scintillation counters. A biodegradable scintillation cocktail was used (Packard - Ultima Gold XR) for both radionuclides on all dates. Quenching of the samples was accounted for using external quench monitoring techniques (Beckman's "H" number, and Wallac quench correction). For the ^{35}S-extraction samples, specific quench curves were prepared for each scintillation counter using soils with no added ^{35}S, but extracted exactly as the radiolabeled soils. This was necessary because of the dark colors obtained from the soils, due to extracted organic acids, which caused significant color quench.

Determination of Sulfur Pools

Soil total sulfur and the pools of water soluble and adsorbed sulfate were determined for all A-horizon soils sampled. The only analyses performed on litter and B-horizon soils from watershed plots were total sulfur and total carbon (see below). From August 1993 to September 1994, total sulfur was determined by combustion of an aliquot (ca. 30 mg) of soil (initially dried for the percent moisture determination) in a Schoniger flask, followed by quantification of the sulfate adsorbed into dH_2O in the

flask, using a Shimadzu HIC-6A ion chromatograph (Spratt and Morgan 1990). Beginning in January 1995, a Leco CNS 2000 elemental analyzer was available for use on this project. Total carbon and sulfur were quantified in the CNS 2000 by combusting an aliquot (ca. 200 mg) of the dried soils. Sulfamethazine was used to standardize the instrument, and an NIS-traceable soil standard was used for drift correction. To validate the Schoniger flask combustion technique for the analysis of total sulfur, soil samples collected over the period August 1993 to September 1994 were also analyzed on the CNS 2000.

The water soluble sulfate pool in these soils was determined using the soil fraction frozen after sieving. Approximately 0.4 g of soil was transferred to a filter funnel fitted with a 0.45-µm filter, 1 ml of dH_2O was added, and the mixture was shaken for 15 minutes. The filtrate was collected and used to determine the soluble sulfate pool. The soil was rinsed (two 1-ml dH_2O washes), and the final volume extracted from the soil totaled approximately 3 ml. The adsorbed sulfate pool was determined for the soil remaining on the filter in the funnel. One ml of 20 mM Na_2HPO_4 was added to the funnel; the soil was resuspended and then shaken for 1 minute. The phosphate solution was then filtered and collected in a vial. This process was repeated two times, and the total 3 ml of phosphate solution was pooled and used to determine adsorbed sulfate. Both the water soluble and adsorbed sulfate concentrations were quantified using ion chromatography (Watwood and Fitzgerald 1988). Organic sulfur present in the soil was calculated by difference (Organic Sulfur = Total Sulfur - (Water Soluble Sulfate + Adsorbed Sulfate)).

Exchangeable Bases

The exchangeable bases K^+ and Mg^{+2} were determined for all samples using an ammonium acetate extraction procedure (Simard 1993). Five grams of dried soil was placed in a centrifuge tube along with 5 ml of 1N NH_4OAc, pH 7.0. The tube was thoroughly mixed using a vortex mixer, and centrifuged for 10 minutes (2,000 x g). The supernatant was collected and the mixing/centrifugation procedure was repeated twice; 15 ml was the final volume of supernatant collected. This supernatant was analyzed for K^+ and Mg^{+2} using a Perkin-Elmer 1100B atomic adsorption spectrophotometer for August 1993 to June 1994 samples. For samples from September 1994 to May 1996, exchangeable bases were quantified using a Varian Spectr AA10 atomic adsorption spectrophotometer. Atomic adsorption standards were prepared in 1N NH_4OAc, pH 7.0 to reduce the possibility of errors due to matrix effects.

Statistical Methods

Trends in the data were determined by a multivariate repeated-measures analysis of variance (alpha=0.10) using SYSTAT® 5.03 (SPSS, Inc.). Relationships among variables were examined using a Pearson correlation analysis.

RESULTS

MOFEP Plots

For 2 of the 3 years of pre-treatment study on MOFEP plots presented here, seasonal trends were evident for both carbon and sulfur pools in A-horizon soils. The data set for the third year contains only three seasons and was not included in these analyses. The range in A-horizon soil total carbon over all plots and sample dates was from 6 to 32 µmol C/g dry. Overall, the largest differences in total carbon were observed in seasonal comparisons. Samples collected in the late summer/early fall, compared with samples collected in the early spring (fig. 4, $p<0.01$, appendix 1), were noticeably different. Consideration of total carbon in

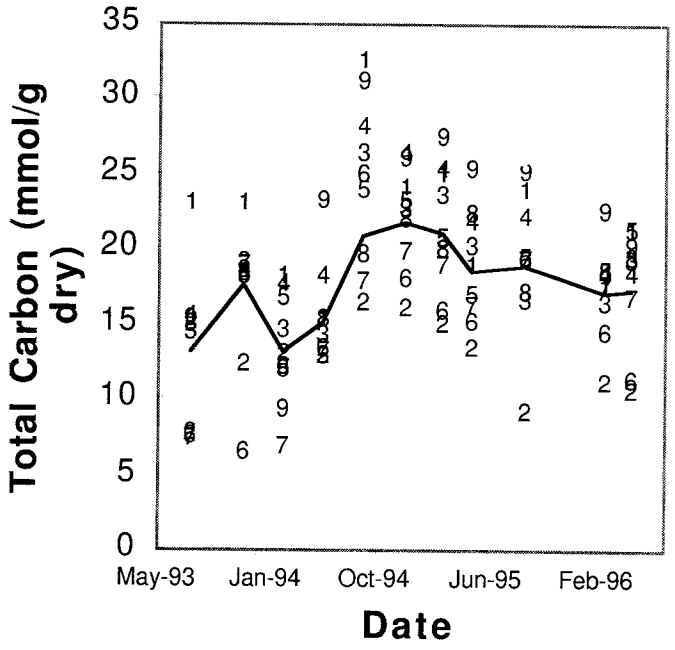


Figure 4.—*MOFEP plots, A-horizon soils - total carbon, August 1993 to May 1996, numbers 1 through 9 represent mean values of 3 plots per site, line represents mean of all 27 plots.*

A-horizon soils by block or treatment indicated no substantial differences.

Total sulfur in MOFEP plot A-horizon soils also exhibited marked yearly trends over the 2 years analyzed ($p<0.01$, appendix 2). Seasonally, the greatest concentrations of total sulfur were observed in late summer/early fall, and the lowest concentrations were observed in late spring (fig. 5). Total sulfur concentrations in A-horizon soils observed for all plots over all sample dates were approximately 10 to 65 µmol/g dry. No substantial differences were observed for A-horizon soil total sulfur when compared by treatment or block.

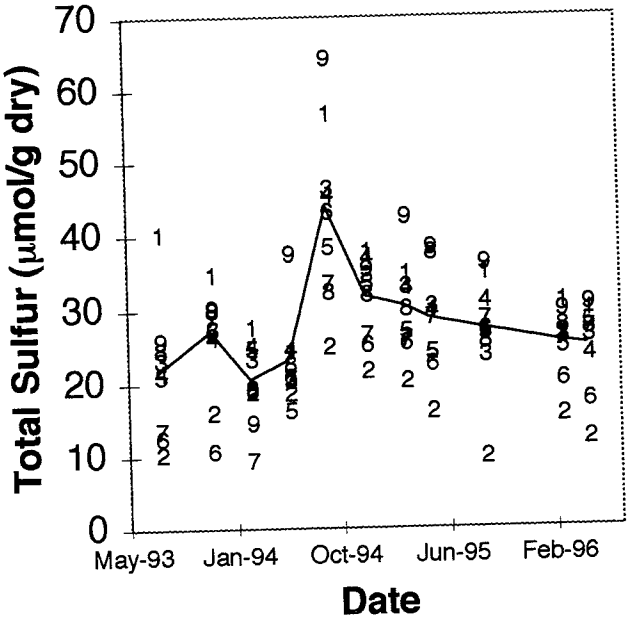


Figure. 5.—*MOFEP plots, A-horizon soils - total sulfur, August 1993 to May 1996; numbers 1 through 9 represent mean values of three plots per site; line represents mean of all 27 plots.*

Organic sulfur in MOFEP plot A-horizon soils was also found to change year to year ($p<0.01$, appendix 3). Seasonally, the highest concentrations of organic sulfur were found in late summer/early fall, and the lowest concentrations were measured in the late spring (fig. 6). Organic sulfur concentrations for all plots over all sample dates ranged from 9 to 64 µmol/g dry. Comparisons of organic sulfur data by treatment or block yielded no noticeable differences. Organic sulfur production rates in A-horizon soils also exhibited large differences from date to date over the pre-treatment period (fig. 7).

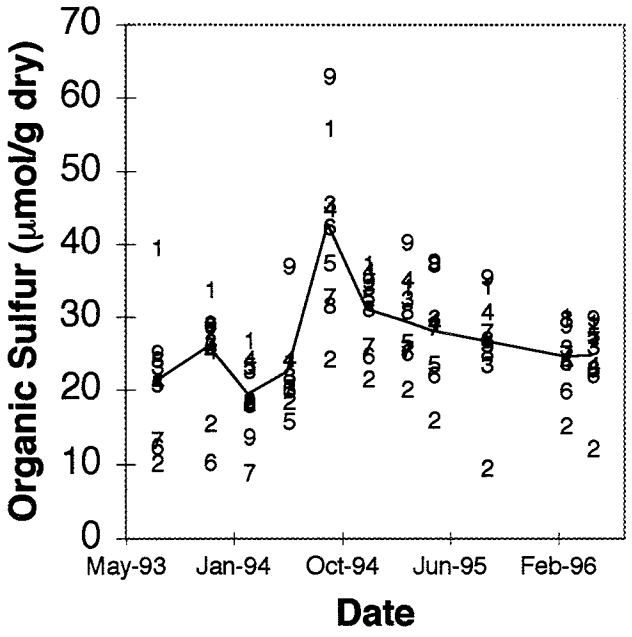


Figure 6.—*MOFEP plots, A-horizon soils - organic sulfur, August 1993 to May 1996; numbers 1 through 9 represent mean values of three plots per site; line represents mean of all 27 plots.*

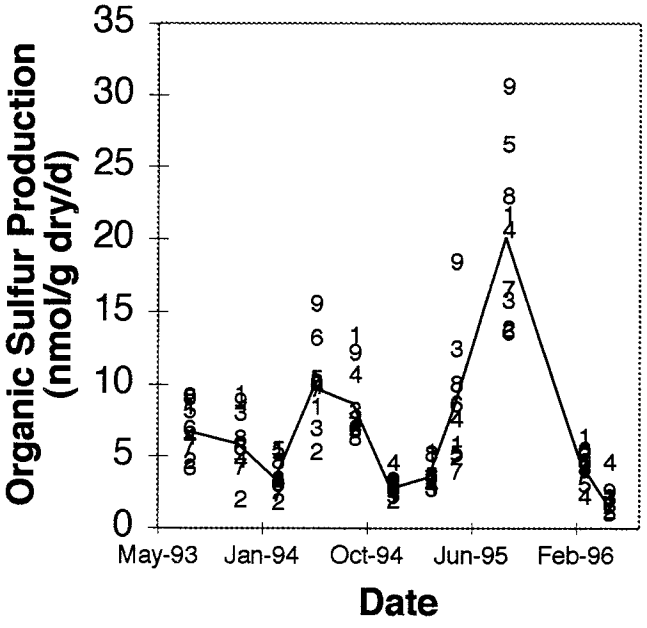


Figure 7.—*MOFEP plots, A-horizon soils - organic sulfur production, August 1993 to May 1996; numbers 1 through 9 represent mean values of three plots per site; line represents mean of all 27 plots.*

Organic sulfur production rates for A-horizon soils over all dates and plots ranged from 1 to 39 nmol/g dry/d. Over the 2 years considered, organic sulfur production exhibited some seasonality (p=0.114, appendix 4), but no other differences in the data were evident.

The presence of white oak on MOFEP plots used for the soil carbon and sulfur transformation study was determined by summing all white oaks > 1.5 in. in diameter (table 2). Some differences (p=0.113, appendix 5) in the numbers of white oak present on the plots sampled were detected when compared by block. No differences were observed in numbers of white oaks on the plots in comparisons by treatment. Plots in blocks 1 and 2 had similar mean numbers of white oak trees present (47.8 and 40.2, respectively), while plots in block 3, on average, had many fewer white oaks (20.3).

Lignocellulose was mineralized in the microcosms used for these analyses following a characteristic time course. Rates of $^{14}CO_2$ released from the soils were not linear, but followed a more logistic-type function (fig. 8-A). For soils cellulose degradation, in August 1993, there was little lag, with rapid exponential mineralization. Emission of $^{14}CO_2$ from the soil then stabilized, with a total of 51 percent of the added labeled plant material mineralized over the 5-week incubation. The lignin moiety of the radiolabeled plant material produced a similar time course of mineralization; however, there was a notable lag period before the onset of exponential $^{14}CO_2$ release (fig. 8-B). Comparison of cellulose and lignin mineralization indicates that the cellulose moiety is much more labile, being mineralized approximately twice as fast as the lignin moiety (1.8 to 2.5 times faster, as calculated for all soils tested from August 1993 to June 1994).

Maximum rates of white oak cellulose mineralization, calculated from the exponential portion of time course experiments, exhibited seasonal differences across the pre-treatment period. The overall range of cellulose mineralization calculated for all plots and dates was from 0.02 to 1.18 mgC/g dry/d (fig. 9-A). Substantial differences (p<0.01, appendix 6) in rates of cellulose mineralization for A-horizon soils were detected in comparisons of the data by season. Rates of cellulose mineralization were lowest in the late fall and winter sampling periods, and highest in the spring and summer sample dates. No block or treatment differences were observed in comparisons of cellulose mineralization rates for all sample dates.

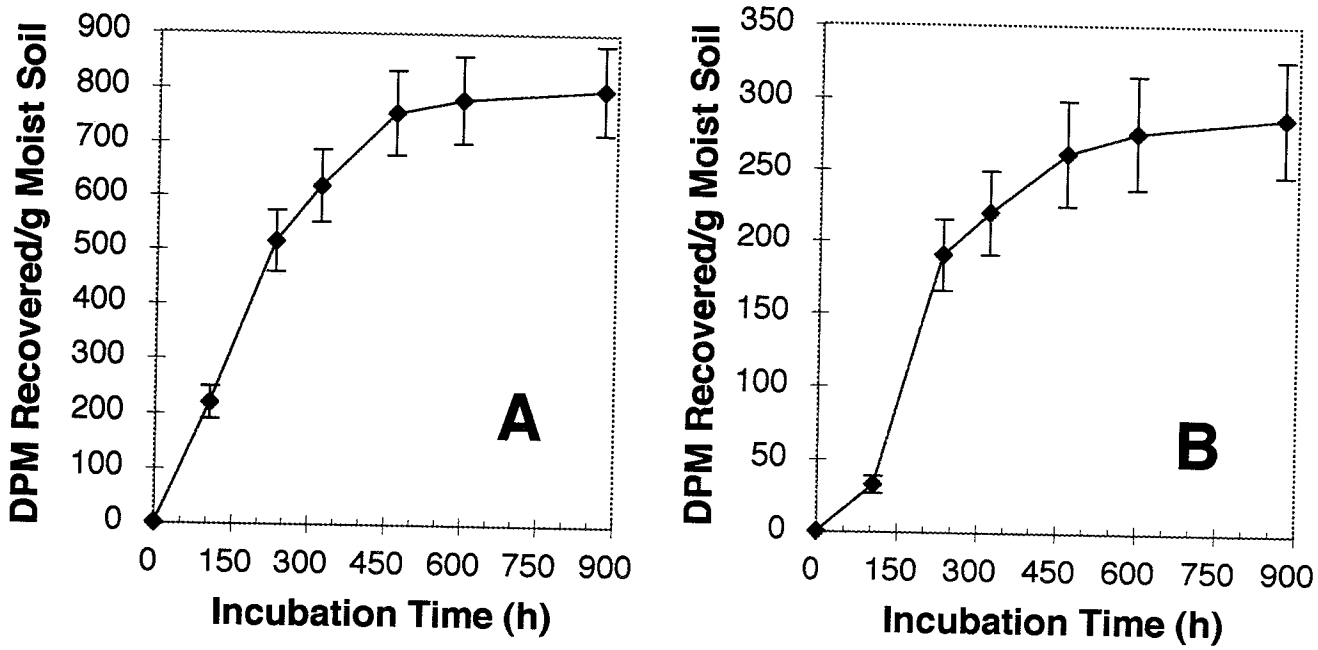


Figure 8.—*MOFEP plots, A-horizon soils - lignocellulose mineralization time course, August 1993, 32°C; mean values for all plots +/- 1 SE, n=9; note differences in vertical scale; A) cellulose mineralization, B) lignin mineralization.*

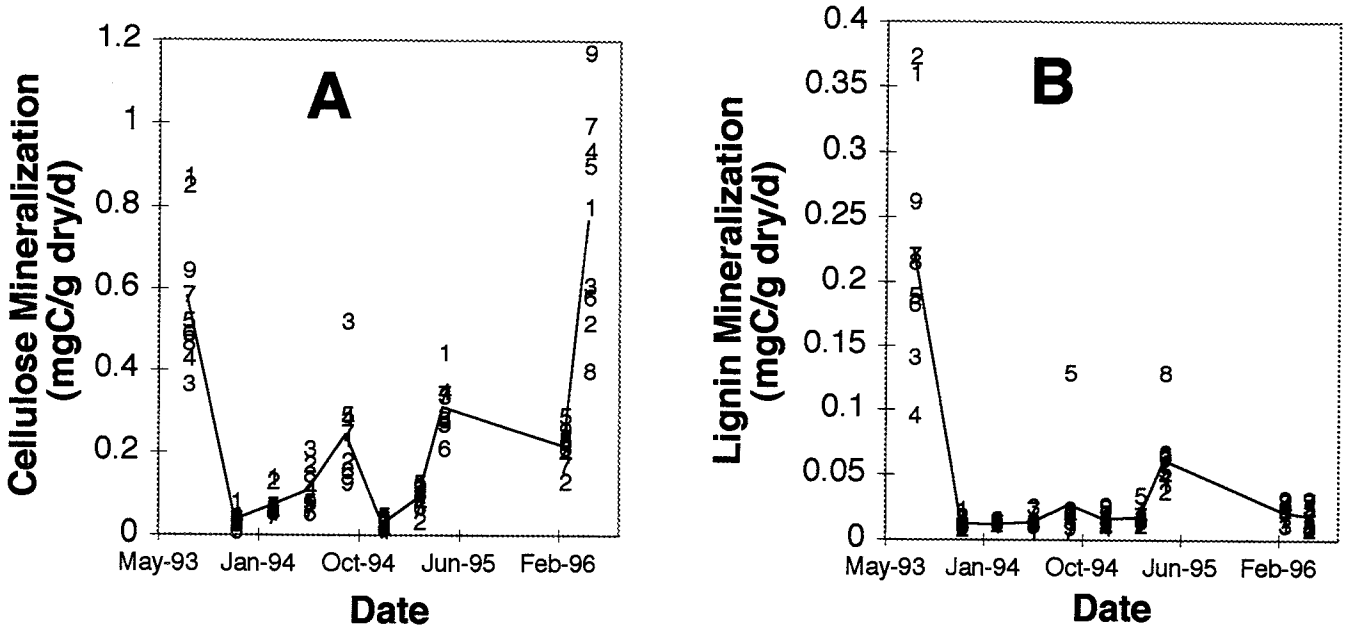


Figure 9.—*MOFEP plots, A-horizon soils - white oak lignocellulose mineralization, August 1993 to May 1996; numbers 1 through 9 represent mean values of three plots per site, line represents mean of all 27 plots; note differences in vertical scale; A) cellulose mineralization, B) lignin mineralization.*

White oak lignin was mineralized at rates that were much lower than the rates of cellulose mineralization for all plots on all dates (compare figs. 9-A and 9-B). Lignin mineralization also exhibited noticeable seasonal differences ($p<0.05$, appendix 7) over the dates sampled. The rates of lignin mineralization for all plots over all dates ranged from 0.01 to 0.37 mgC/g dry/d (fig 9-B). Lowest rates of lignin mineralization for A-horizon soils occurred in late fall or winter. Comparisons of A-horizon soil lignin mineralization by block and future treatment indicated no noticeable differences for the dates sampled.

Comparisons of rates of white oak cellulose or lignin mineralization with the numbers of white oak > 1.5 in. diameter present on the plots studied were made using a Pearson correlation test. No significant correlation between the number of white oak trees present on the plots and the rates of white oak cellulose or lignin mineralization was detected ($r=0.012$ and 0.018, respectively, $n=27$).

Exchangeable K^+ in MOFEP A-horizon soils exhibited noticeable seasonal differences ($p=0.05$, appendix 8) over the period sampled. On an annual basis, the highest concentrations of K^+ were detected in late fall, and the lowest concentrations were measured in late summer (fig. 10-A). For all plots and dates, A-horizon soil K^+ concentrations ranged from 7 to 35 μmol/g dry. There were no differences in the concentration of exchangeable K^+ for A-horizon soils compared by either block or future treatment.

Exchangeable Mg^{+2} in MOFEP A-horizon soils, like exchangeable K^+, also exhibited large seasonal differences ($p<0.01$, appendix 9). A-horizon soils collected in late fall had the greatest concentrations of Mg^{+2}, while late summer samples had lowest concentrations of Mg^{+2} (fig. 10-B). Variation for A-horizon soil Mg^{+2} across all plots and dates ranged from 12 to 76 μmol/g dry. Some differences in A-horizon soil exchangeable Mg^{+2} were detected in comparisons of data from the dates sampled by block ($p=0.062$), while no differences were observed in comparisons by treatment.

A-horizon soil moisture exhibited seasonal variation (table 3). The greatest soil moisture was measured on late fall or winter sample dates; soils were the driest in the late summer.

Stream water SO_4^{2-} for streams in the vicinity of MOFEP plots also exhibited seasonal trends. The lowest concentrations of surface water SO_4^{2-} were measured in September 1995, SO_4^{2-} concentrations varied only little over the other

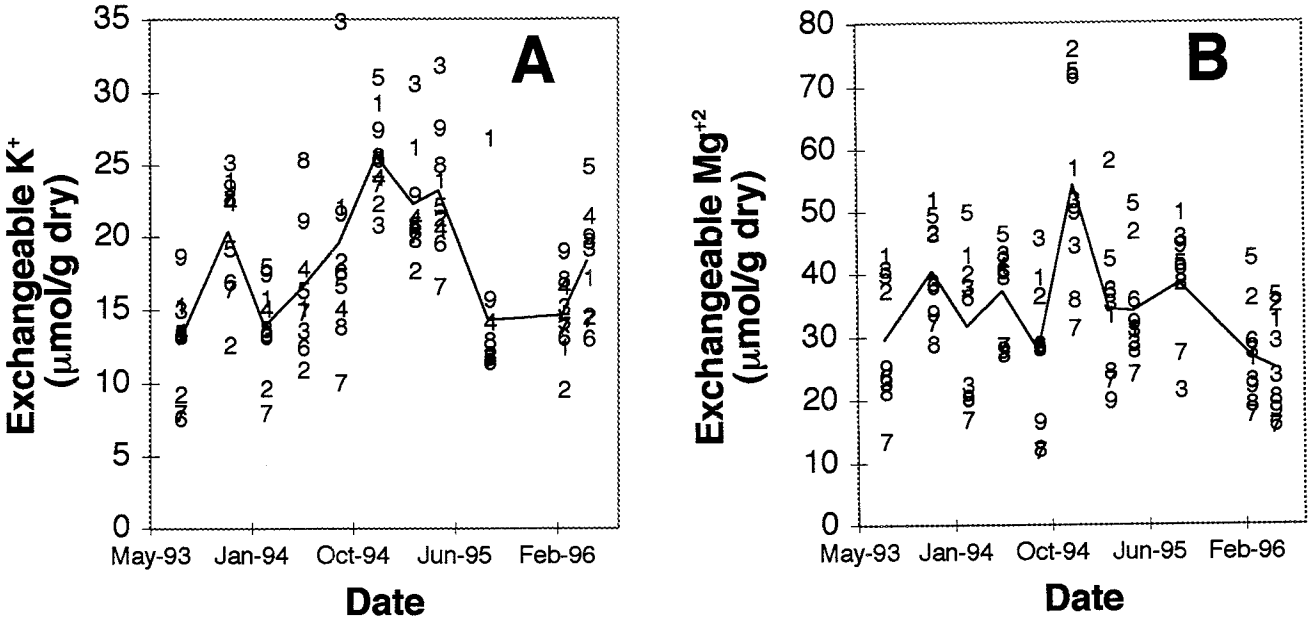


Figure 10.—*MOFEP plots, A-horizon soils - exchangeable potassium and magnesium, August 1993 to May 1996; numbers 1 through 9 represent mean values of three plots per site, line represents mean of all 27 plots; A) potassium, B) magnesium.*

sample dates (fig. 11). Sulfate concentrations from all collection sites over all dates ranged from 12 to 57 µM.

Watershed Plots

The watershed plots, located in MOFEP sites 1, 3, and 4, represent a subsample of the larger carbon and sulfur study of MOFEP, including sample plots with both south and west aspect and north and east aspect, as well as plots positioned both high and low on the slopes. If we consider data from two dates (March and May 1996), total carbon in watershed plots was greatest in forest floor litter on the winter sampling date (mean values across south and west and east and west aspects, and both landscape positions were approximately 40 µmol/g dry), and somewhat lower in the spring (mean values ranging from 37 to 39 µmol/g dry, figs. 12-A and 12-B, $p<0.01$, appendix 10). Total carbon in litter from watershed plots exhibited no noticeable differences when compared by aspect or slope position. In March 1996, A-horizon soil total carbon ranged from 18 to 24 µmol/g dry; the largest difference between the total carbon in litter and A-horizon soil was found for samples from south and west aspect sites, high in the landscape. Total carbon in B-horizon soils exhibited noticeable differences ($p=0.086$, appendix 11) when compared by season. Sample site aspect and slope location resulted in little difference in the B-horizon total carbon. From March to May 1996, the largest change in total carbon for litter, A-, and B-horizon soils was measured for A-horizon soils collected from south and west aspect plots, high in the landscape (figs. 12-A and 12-B). The change in total carbon for these soils from March to May was an increase of nearly 30 percent (from 18 to 23 µmol/g dry).

A closer look at total carbon in A-horizon soils from south and west aspect watershed plots high in the landscape indicated these soils followed the same basic pattern for total carbon observed in A-horizon soils from the MOFEP plots (see fig. 4). The highest concentrations of total carbon in these watershed A-horizon soils (up to 30 µmol/g dry) were measured in the early fall; the lowest concentrations were observed in the winter (as low as 20 µmol/g dry, fig. 13). The only possible difference in A-horizon soil total carbon for watershed plots ($p=0.145$, appendix 12), occurred when the data were compared by season. Comparison of the data by aspect or slope position indicated minimal differences in the A-horizon soil total carbon.

Lignocellulose mineralization in A-horizon soils of watersheds also followed the general trends

Table 3.—*Mean site A-horizon soil percent moisture on sample dates (+/- 1 SE, n=3)*

Date	Site 1	2	3	4	5	6	7	8	9
Aug 1993	41.8 (6.6)	23.7 (1.2)	18.5 (3.1)	23.6 (0.7)	17.5 (2.2)	17.9 (1.5)	21.4 (4.4)	26.7 (1.4)	26.7 (4.6)
Dec 1993	64.3 (2.8)	47.1 (2.8)	61.6 (3.4)	57.5 (1.9)	60.0 (1.7)	52.1 (4.0)	51.2 (1.6)	57.5 (2.5)	54.2 (4.3)
Mar 1994	53.1 (4.8)	45.5 (2.5)	49.4 (3.2)	51.5 (0.7)	58.3 (1.2)	49.2 (3.4)	44.0 (2.5)	52.9 (1.0)	49.3 (2.2)
Jun 1994	38.4 (2.4)	36.0 (3.0)	36.6 (3.3)	40.9 (2.1)	38.5 (2..0)	35.6 (1.3)	30.0 (3.0)	39.7 (4.6)	39.9 (3.6)
Sep 1994	31.0 (3.6)	29.1 (2.2)	45.6 (4.1)	38.0 (1.4)	49.4 (3.2)	39.2 (5.1)	31.6 (4.8)	38.3 (2.5)	43.9 (1.3)
Dec 1994	56.9 (2.2)	43.0 (2.4)	48.2 (2.2)	53.4 (2.1)	50.4 (2.4)	44.2 (2.9)	54.6 (6.8)	59.4 (1.0)	60.6 (1.4)
Mar 1995	63.1 (2.7)	43.2 (1.8)	54.9 (4.1)	51.6 (4.8)	54.7 (5.5)	48.6 (4.5)	50.0 (2.1)	52.6 (2.1)	55.8 (2.2)
May 1995	53.5 (2.1)	45.3 (4.6)	52.5 (2.1)	50.9 (2.5)	44.2 (1.0)	46.8 (3.3)	51.0 (4.7)	58.0 (2.1)	63.1 (0.5)
Sep 1995	53.2 (5.6)	35.9 (2.4)	41.7 (3.4)	46.8 (1.7)	46.9 (1.6)	42.9 (3.1)	41.0 (4.6)	48.1 (1.2)	51.0 (1.2)
Mar 1996	46.2 (5.0)	39.8 (3.0)	48.3 (2.2)	50.0 (1.3)	49.1 (1.1)	45.5 (2.4)	44.5 (5.2)	47.5 (1.0)	54.5 (1.1)
May 1997	57.0 (6.7)	42.5 (2.5)	53.5 (2.7)	57.9 (1.2)	57.3 (2.3)	39.3 (3.7)	49.5 (3.4)	53.3 (3.0)	56.1 (3.1)

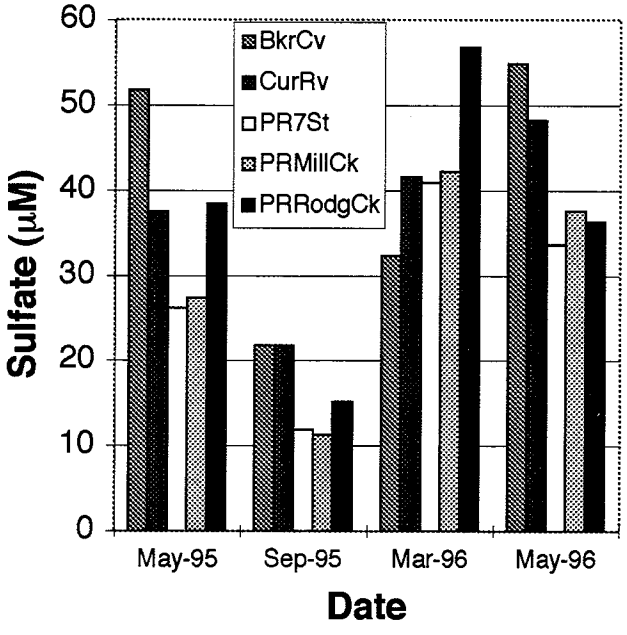


Figure 11.—*MOFEP streams - surface water sulfate, May 1995 to May 1996; BkrCv=Bankers Cave, CurRv=Current River, PR7St=Peck Ranch stream (site 7), PRMillCk=Peck Ranch Mill Creek, PR RodgCk=Peck Ranch Rodgers Creek.*

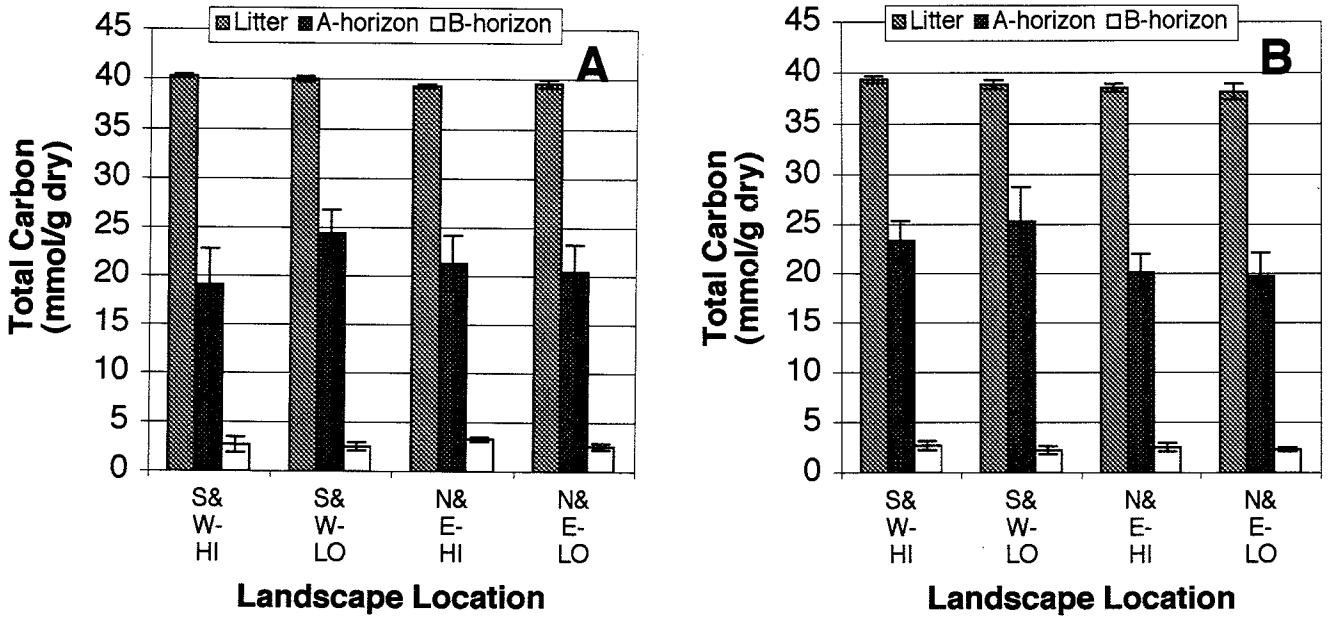


Figure 12.—*Watershed plots, litter, A- and B-horizon soils - total carbon, March and May 1996; S&W=southwest aspect, N&E=northeast aspect, HI=near top of slope, LO=near bottom of slope; mean values +/- 1 SE, n=9; A) March 1996, B) May 1996.*

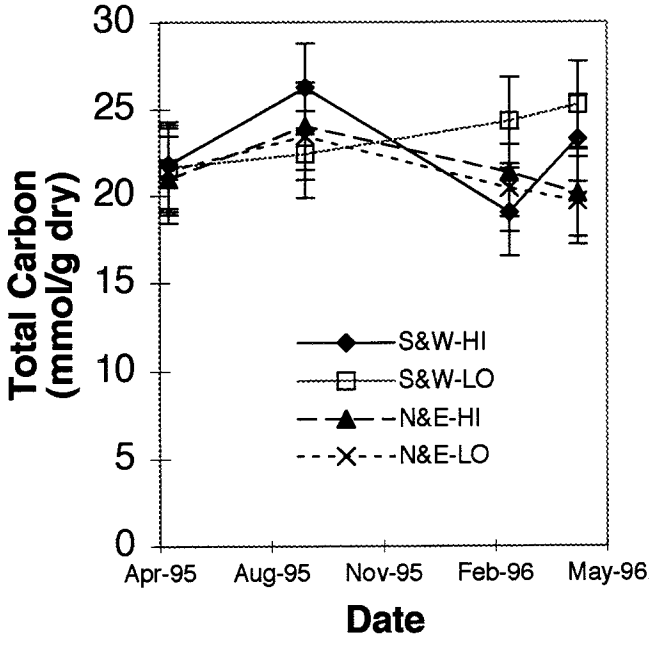


Figure 13.—*Watershed plots, A-horizon soils - total carbon, May 1995 to May 1996; S&W= southwest aspect, N&E=northeast aspect, HI=near top of slope, LO=near bottom of slope; mean values +/- 1 SE, n=9.*

observed for A-horizon soil from MOFEP plots over the period May 1995 to May 1996 (see fig. 9-A). Noticeable differences in cellulose mineralization were observed for A-horizon soils comparing May 1995 and March 1996; May 1996 had the highest rates (fig. 14-A, p=0.024, appendix 13). The rates of cellulose mineralization in May 1995 and March 1996 ranged from 0.2 to 0.3 mgC/g dry/d for A-horizon soils from all watershed plots. No notable differences in rates of cellulose mineralization were detected in comparisons of site aspect or slope location.

White oak lignin mineralization in A-horizon soils from watershed plots, as observed for MOFEP plots (see figs. 9-A and 9-B), was much lower than cellulose mineralization for all dates and sample locations. The differences in rates of lignin and cellulose mineralization for watershed A-horizon soils ranged from 2.5- to 12.5-fold, with lignin mineralization always lower than cellulose mineralization (fig. 14-B). For all dates and plots, rates of lignin mineralization ranged from 0.02 to 0.08 mgC/g dry/d. Rates of lignin mineralization in A-horizon soils were marginally greater in May 95 than March 96 (p=0.159, appendix 14). No differences in lignin mineralization for watershed plots were observed when compared by site aspect or slope location.

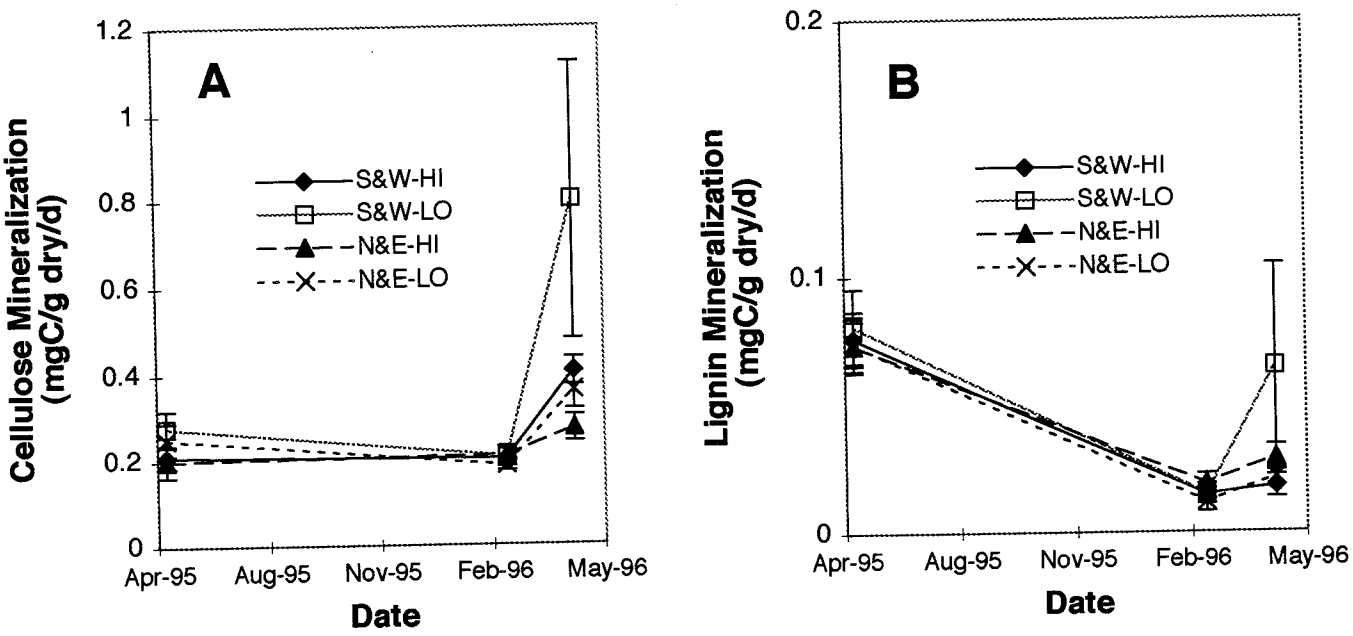


Figure 14.—*Watershed plots, A-horizon soils - white oak lignocellulose mineralization, May 1995 to May 1996; S&W=southwest aspect, N&E=northeast aspect, HI=near top of slope, LO=near bottom of slope; mean values +/- 1 SE, n=9; note differences in vertical scale; A) cellulose mineralization, B) lignin mineralization.*

Total sulfur in the watershed plots was usually greater in the A-horizon soils than in either litter or B-horizon soils, ranging from approximately 31 to 47 μmol/g dry for both aspects studied and landscape positions on both dates analyzed (figs. 15-A and 15-B). In all cases, the total sulfur concentrations in B-horizon soils were much lower than in either litter or A-horizon soil, ranging from 2 to 5 μmol/g dry for all samples on the two dates. Substantial differences ($p<0.01$, appendix 15) were detected in comparisons of B-horizon soil total sulfur on the different dates sampled. Litter had total sulfur concentrations ranging from 27 to 42 μmol/g dry for all samples on both dates. Comparison of litter total sulfur by sample collection date indicated notable differences in this data ($p<0.01$, appendix 16). A-horizon soils from south and west aspect sites had the highest concentration of total sulfur measured for litter, or A- or B-horizon soils, on both sample dates. Soils from both south and west aspect, and north and east aspect sites had essentially the same concentrations of total sulfur in March 1996. In May 1996, north and east aspect A-horizon soils had very slightly increased concentrations of total sulfur compared with March 1996 soils, while south and west aspect A-horizon soils had noticeable increases ($p=0.022$, appendix 17) in total sulfur compared with March 1996 soils (increases of from 21 to 35 percent). B-horizon soils also exhibited substantial changes in total sulfur from March to May 1996 ($p<0.01$, appendix 15), losing approximately 50 percent of the March concentration by May (loss of approximately 2 μmol/g dry).

Extending the study of A-horizon soil total sulfur in watershed plots to 1 year indicated that south and west aspect plots tend to have somewhat higher concentrations of total sulfur than do north and east aspect plots on all sampling dates (fig. 16, $p=0.069$, appendix 17). Comparison of the A-horizon soil total sulfur data for watershed plots with the 3-year database of total sulfur from MOFEP plots (see fig. 5) indicates that the same trend (highest concentrations of total sulfur found in early fall, lowest concentrations in May for MOFEP plots) was not evident for the watershed plots over the year sampled, although the differences measured were significant ($p=0.022$). The year sampled, however, did not include a late fall sample collection.

Organic sulfur concentrations in A-horizon soils of watersheds from south and west aspect plots high in the landscape followed the same basic seasonal pattern observed for A-horizon soils in MOFEP plots of the same aspect (see fig. 6). The organic sulfur concentrations in A-horizon

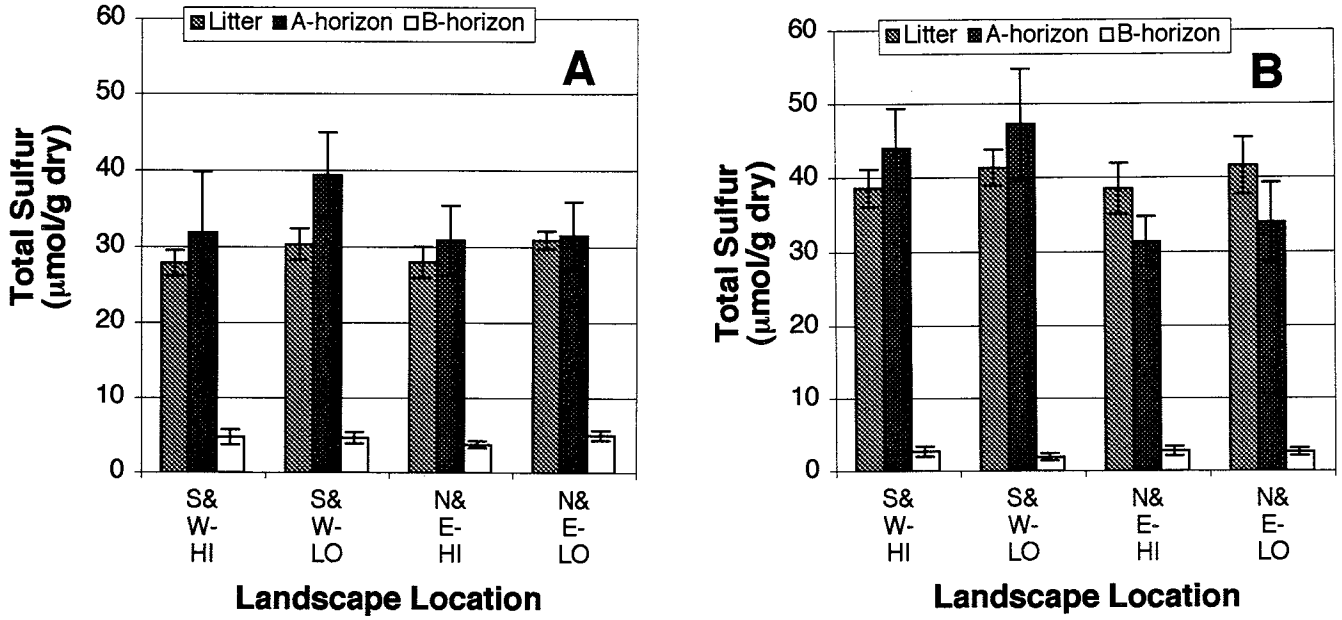


Figure 15.—*Watershed plots, litter, A- and B-horizon soils - total sulfur, March and May 1996; S&W=southwest aspect, N&E=northeast aspect, HI=near top of slope, LO=near bottom of slope; mean values +/- 1 SE, n=9; A) March 1996, B) May 1996.*

soils from south and west aspect watershed plots were greatest in the early fall and declined steadily through the next spring (fig. 17, p=0.019, appendix 18). A-horizon soils from south and west aspect plots located low in the landscape had a slight increase in organic sulfur over the sample period (from 33 up to 38 µmol/g dry). Soils from both slope locations in

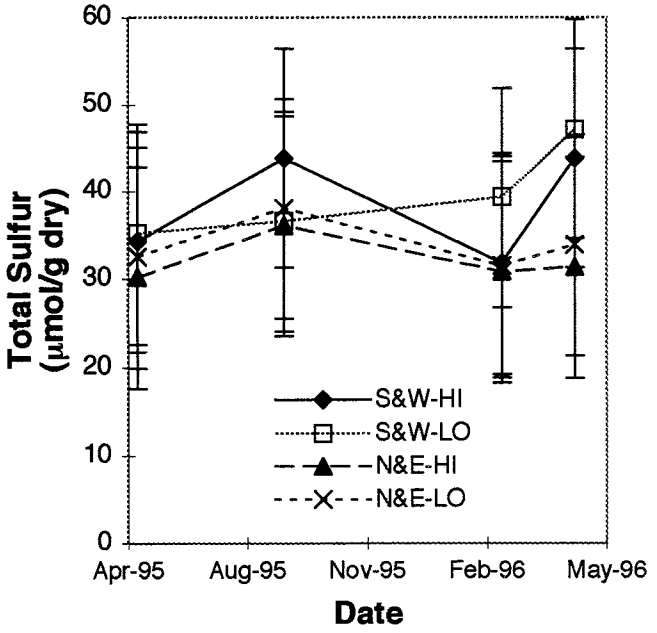


Figure 16.—*Watershed plots, A-horizon soils - total sulfur, May 1995 to May 1996; S&W=southwest aspect, N&E=northeast aspect, HI=near top of slope, LO=near bottom of slope; mean values +/- 1 SE, n=9.*

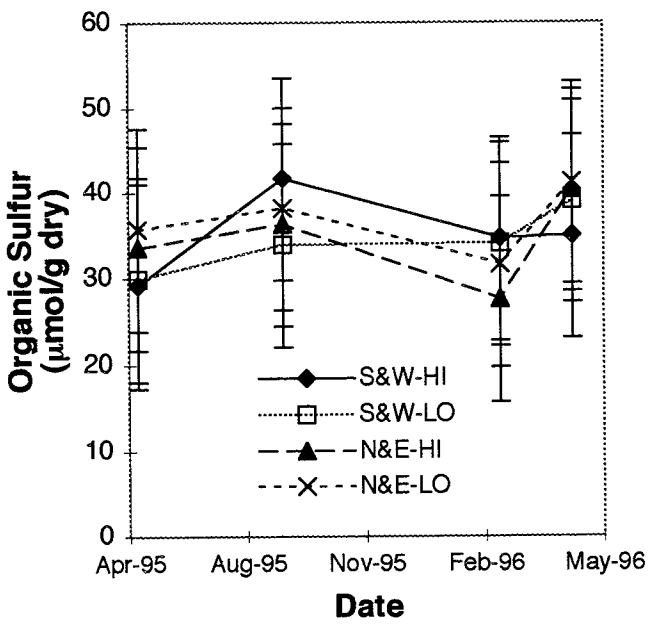


Figure 17.—*Watershed plots, A-horizon soils - organic sulfur, May 1995 to May 1996; S&W= southwest aspect, N&E=northeast aspect, HI=near top of slope, LO=near bottom of slope; mean values +/- 1 SE, n=9.*

north and east aspect sites had declining concentrations of organic sulfur in A-horizon soils from early fall through late winter, but then increased about 34 percent (from 30 to 41 µmol/g dry) in the late spring.

A-horizon soils from watershed plots supported the production of organic sulfur consistently over the sample dates, with notable differences (p=0.020, appendix 19) observed based on comparisons by date (fig. 18). For all watershed plots, organic sulfur production rates ranged from 3 to 55 nmol/g dry/d. The highest rates of organic sulfur production were measured in late fall, with rates declining during the winter, basically supporting the seasonal trend observed for organic sulfur production in MOFEP plots (fig. 7). The rate of organic sulfur production measured for samples collected in May 1996 was much lower than that measured for May 1995 samples.

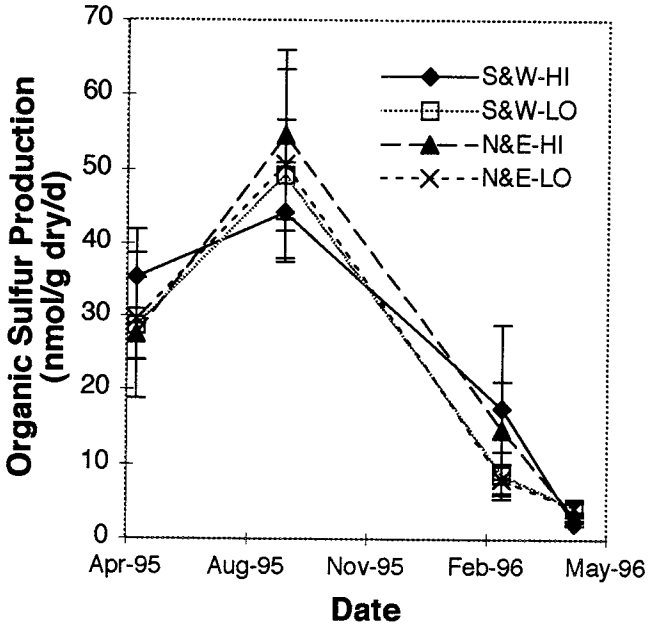


Figure 18.—*Watershed plots, A-horizon soils - organic sulfur production, May 1995 to May 1996; S&W=southwest aspect, N&E=northeast aspect, HI=near top of slope, LO=near bottom of slope; mean values +/- 1 SE, n=9.*

Mineralization of organic sulfur for A-horizon soils from watershed plots was measured in March and May 1996. In March 1996, the rate of organic sulfur mineralization for A-horizon soils from all watershed plots ranged from approximately 150 to 300 nmol/g dry/d (fig. 19). In May 1996, the rate of organic sulfur

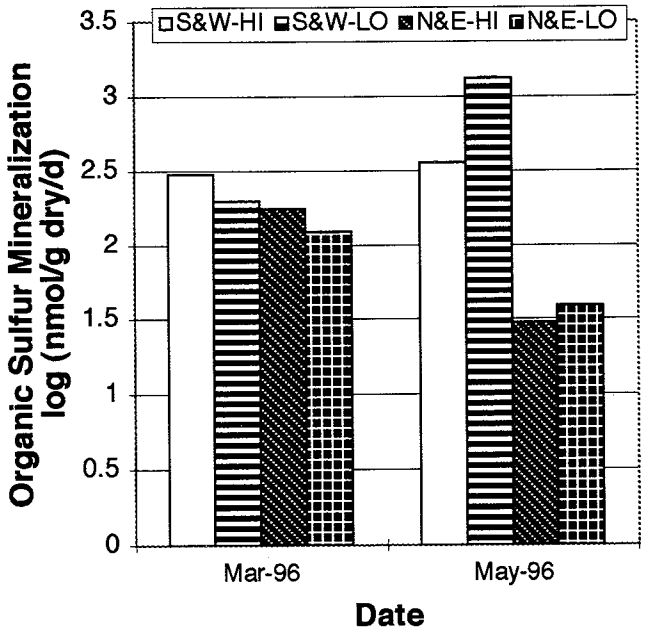


Figure 19.—*Watershed plots, A-horizon soils - organic sulfur mineralization, March and May 1996; S&W=southwest aspect, N&E=northeast aspect, HI=near top of slope, LO=near bottom of slope; mean values +/- 1 SE, n=9.*

mineralization increased for south and west aspect plots low on the landscape, compared with the March 1996 data (increases of greater than fourfold, up to approximately 1,300 nmol/g dry/d, p=0.104, appendix 20).

Exchangeable K^+ in A-horizon soils from watershed plots followed the same seasonal pattern as observed for MOFEP plots (see figs. 10-A and 10-B). Highest concentrations were observed on spring sample dates (fig. 20-A), while concentrations were lowest from late fall through winter. For A-horizon soils from south and west aspect plots the change in K^+ from March to May 1996 was approximately 34 percent, increasing from 22 to 30 µmol/g dry. Potassium in A-horizon soils of north and east aspect plots also increased between March and May 1996, but only by about 13 percent (from 22 to 25 µmol/g dry). Comparison of exchangeable K^+ in A-horizon soils indicated some differences (p=0.137, appendix 21) from date to date.

A-horizon soil exchangeable Mg^{+2} concentrations in watershed plots were not appreciably different when compared by date, aspect, or slope location (fig. 20-B, p>0.2, appendix 22). Exchangeable Mg^{+2} was higher in A-horizon soils from north and east aspect sites than in

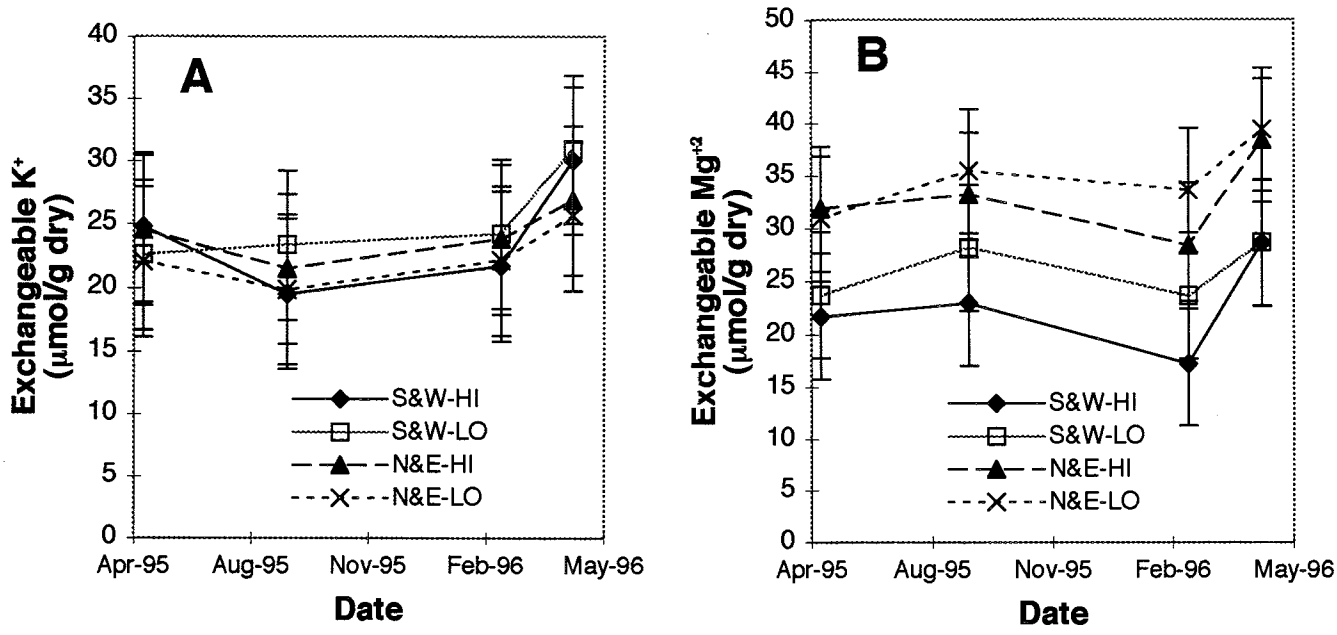


Figure 20.—*Watershed plots, A-horizon soils - exchangeable potassium and magnesium, May 1995 to May 1996; S&W=southwest aspect, N&E=northeast aspect, HI=near top of slope, LO=near bottom of slope; mean values +/- 1 SE, n=9; A) potassium, B) magnesium.*

soils from south and west aspect sites. Comparison of the watershed exchangeable Mg^{+2} data with that from the MOFEP plots (see fig. 10-B) indicates some differences in the two datasets. For example, between March and May 1996, Mg^{+2} A-horizon soils declined somewhat for MOFEP plots, but increased for watershed plots.

DISCUSSION

The pre-treatment portion of this study has served a vital role in helping to establish baseline data that will be used to determine if changes in the parameters measured after treatment might be due to the treatment. Natural variation in forest ecosystems is great; however, if any trends in data sets can be determined prior to an experimental treatment, then a higher level of certainty of the treatment effect should be obtained. In the pre-treatment carbon and sulfur transformation data presented here, the data have been compared by season, replicate grouping of sites (block), site aspect, and slope location (high or low). In many cases very noticeable differences ($p<0.01$) in the pre-treatment data exist when compared by season. This finding reflects the variability that might be expected of biological processes over different seasons. In many cases differences detected in the parameters measured in this report may be due to changes in soil moisture content over the year. Moisture levels can have pronounced effects on the activity of microorganisms (Atlas and Bartha 1993), and if the parameter being tested is the result (either direct or indirect) of some microbial activity, then it should be expected to differ by soil moisture content. Whatever the difference detected in the pre-treatment dataset, having a baseline of the parameter of interest, and knowing something of the natural variation over several seasons occurring in that parameter should help in comparing data collected after the experimental treatment.

In looking at the data sets presented in this report, we found that soil total carbon varied from the litter layer down through the A and B horizons. This would be expected, because the primary source of carbon to the surface soils would be litter fall. As soil microorganisms decompose this litter, labile components of the litter will be released as CO_2, leaving behind more refractile compounds to become part of the soil humus (Atlas and Bartha 1993). For MOFEP soils, the major accumulation of organic carbon appears to be found in the A-horizon soils, at least in a comparison of A-horizon soils with B-horizon soils from no deeper than approximately 15 cm. The A-horizon soil total carbon was also found to differ by sample date.

These variations may be due to differences in rates of microbial activities in these soils, which in turn may be due to physical parameters such as soil moisture and temperature. Finally, activities of decomposers are critical to the larger ecosystem, since they are required to recycle essential nutrients used by primary producers. Therefore, indications of substantial changes in surface soil microbial activities may foreshadow future nutrient limitations to the producers.

The means used here to monitor rates of soil microbial metabolism is the rate of white oak lignocellulose mineralization in A-horizon soils. One of the major sources of carbon to microorganisms found on the forest floor is the lignocellulose of trees present in these Ozark forests. The choice of white oak lignocellulose for studies of A-horizon soil microbial activity was made after consultation with forest ecologists working on MOFEP. White oak was determined to be the dominant tree species of MOFEP south and west aspect plots. Due to equipment availability and experience, the ^{14}C-lignocellulose mineralization assay was chosen to monitor soil microbial activity. Time courses of $^{14}CO_2$ emission from soils amended with ^{14}C-lignocellulose in the microcosms used here were very similar to those obtained for other studies of cellulose and lignin mineralization by microorganisms found in soils (Benner *et al.* 1984, Benner *et al.* 1985, Crawford and Crawford 1976, Crawford *et al.* 1977, Hackett *et al.* 1977), indicating that similar microbial processes occur in different forest soils.

Is white oak lignocellulose mineralization an adequate measure of A-horizon soil microbial metabolic activity? It's possible that differences in the structures of lignin, and possibly cellulose, known to exist from plant species to species (Atlas and Bartha 1993), might predispose the decomposing microorganisms in the soil to lignocellulose from a particular species. Hence, the rates of lignocellulose mineralization determined using radiolabeled white oak lignocellulose might be expected to correlate with the presence or absence of this species in the plots studied if the decomposers preferred one species lignocellulose over another. To address this question, rates of cellulose or lignin mineralization were compared with the number of white oaks >1.5 in. diameter found on the plots studied. No correlations were detected between white oak number and either cellulose or lignin mineralization rates. This finding suggests that A-horizon soil microorganisms, at least on the MOFEP plots sampled, do not discriminate between lignocellulose sources based on the species from which the lignocellulose comes. Therefore, in this study, the rate of lignocellulose mineralization is used to represent microbial metabolic activity.

Lignocellulose from one of the dominant tree species on the MOFEP plots may also be a good indicator of future changes to these plots following experimental treatment. Because leaf litter will be greatly reduced in plots where trees are harvested, provision of the principal carbon source to the soil will be greatly altered. The reduced contribution of organic matter due to lower inputs of leaf litter to A-horizon soils of clearcut sites may also affect the microorganisms in these soils by removing potential carbon and energy sources. Pietikäinen and Fritze (1995) observed an approximate 25 percent decrease in soil total microbial carbon from clearcut forests in Finland 2 years after harvest. Clearcutting has been found to increase soil bacterial biomass for the first 2 years after harvest, followed by a decrease in soil bacterial biomass in subsequent years as labile carbon sources from the decaying woody-debris and roots are depleted (Lundgren 1982). Although not specifically measured in a study of sulfur transformations in Deer Run State Forest (Spratt 1997), there is suggestive evidence that lower inputs of labile carbon from litter or decaying woody-debris or roots may have resulted in reduced microbial growth in these soils, as measured 2 to 3 or 8 to 10 years after harvest. Hence, rates of white oak lignocellulose mineralization presented here may offer a good baseline against which estimation of any changes in soil microbial processes after harvest may be made.

Total sulfur in MOFEP soils was generally higher than that determined for non-leached U.S. soils (Jordan and Reisenauer 1957, Stevenson 1986). The grand mean of A-horizon soil total sulfur, calculated for all MOFEP plots on all dates, was 27.6 μmol/g dry, which is considerably greater than the average for non-leached U.S. soils (16.9 μmol/g dry). At the concentrations observed in the MOFEP plots, sulfur should not be limiting to vegetation in the ecosystem (Shriner and Henderson 1978). In a previous study of sulfur transformations in A-horizon soils of Deer Run State Forest (one of

the State Forests included in MOFEP), on plots not part of MOFEP, clearcutting led to a significant reduction (54 percent, $p<0.01$) in the total sulfur of these soils when compared with control soils. The lack of substantial differences in A-horizon soil total sulfur, when compared by MOFEP block or treatment, should provide a good baseline to observe any changes in soil total sulfur of the magnitude observed in the Deer Run soils.

One potential concern in comparing forest soil total sulfur analyzed in different laboratories has to do with the method used to quantify the sulfur. Dry combustion techniques, similar to those used in this study, require dried soils and have been found to underestimate total sulfur content of some soils (Amaral *et al.* 1989). The greatest loss of sulfur on drying, however, appears to occur for aquic or udic soils. Other researchers have not observed substantial loss of total sulfur when analyzing dried and moist forest soils (David *et al.* 1982, Wieder *et al.* 1985). Since MOFEP soils are mostly xeric, there is the possibility that samples collected during the wetter sampling periods may actually have slightly higher total sulfur values than are reported here.

Sulfur in MOFEP A-horizon soils was dominated by organic sulfur. Organic sulfur made up from 90 to 99 percent of the A-horizon soil total sulfur over all dates and sites sampled. This finding is in keeping with findings from diverse sites around the world (Mitchell and Zhang 1992), indicating that organic sulfur is the predominant form of sulfur in most forest soils. Organic sulfur of plant origin was not directly measured, but it may be inferred that the very large seasonal increases in this compound in the fall must be due to litter drop or some form of root release.

^{35}S-sulfate added to MOFEP A-horizon soils was principally incorporated into the organic sulfur fraction in short-term incubations, similar to other soils amended with this isotope (Fitzgerald *et al.* 1983, McLaren *et al.* 1985, Schindler *et al.* 1986, Strickland and Fitzgerald 1984, Strickland *et al.* 1986). Microbially produced organic sulfur also represents a major portion of the organic sulfur found in MOFEP A-horizon soils. It is possible that the rates of organic sulfur production presented here for MOFEP A-horizon soils may be somewhat underestimated. The methodology used here to quantify organic sulfur utilizes an extraction of soluble and adsorbed sulfate before the organic sulfur fraction is quantified. If appreciable quantities of soluble organic sulfur (e.g., sulfur-containing amino acids) are present in MOFEP soils, then the methodology used here would not detect this soluble organic sulfur. However, soluble organic sulfur compounds have not made up a substantial fraction of other forest soil total organic sulfur (Strickland and Fitzgerald 1984, Strickland *et al.* 1986).

Microbial production of organic sulfur measured for MOFEP soils was found to correlate with rates of lignocellulose mineralization in those soils, suggesting that microorganisms play a role in the formation of this compound in the soil. Abundant evidence is available supporting microbial involvement in the production of organic sulfur in forest soils (David *et al.* 1982, Fitzgerald *et al.* 1983, Schindler *et al.* 1986, Spratt 1997, Strick *et al.* 1982, Swank *et al.* 1984, Watwood *et al.* 1993).

Rates of organic sulfur mineralization in A-horizon soils for two sampling dates were much higher than rates of microbial organic sulfur production over the same period. This suggests that maintenance of organic sulfur in MOFEP A-horizon soils at the levels found in these pretreatment soils over many years requires the annual contribution of organic sulfur that comes from litter fall. The implications that reductions in litter fall, as a result of timber harvest, may negatively affect A-horizon soil organic sulfur are great, at least in the short term (<10 years). In a previous study of sulfur transformations in A-horizon soils from Deer Run State Forest (Spratt 1997), substantial differences ($p<0.01$) in total sulfur (again, mostly organic sulfur) were found for soils clearcut either 2 to 3 or 8 to 10 years prior to sampling. Mitchell *et al.* (1989) came to a different conclusion in their study of whole-tree harvesting, where no significant change in A-horizon total sulfur was found 2 years after whole-tree harvesting in the Hubbard Brook Experimental Forest in New Hampshire. Although no mention was made in the Hubbard Brook study of any changes in litter layers after harvest, the clearcut Missouri sites in Deer Run State Forest had much thinner litter layers than control sites. In addition, A-horizon soils of clearcut sites in Deer Run State Forest were all much thinner than soils of control sites. This finding suggests that erosion of the A-horizon soils down the steep slopes may have been greater for the clearcut sites than the control

sites, leading to loss of the A-horizon soils observed in the Missouri study. This loss, coupled with reduced litter layers, may have resulted in lower concentrations of sulfur, especially organic sulfur, in clearcut A-horizon soils. There is good evidence from other forested ecosystems indicating that A-horizon soils generally contain much higher total and organic sulfur fractions than the lower mineral horizons (Schindler *et al.* 1986). By comparing post-treatment data on soil organic sulfur in MOFEP plots with the baseline data on A-horizon soil organic sulfur presented here, potential mechanisms of organic sulfur loss observed in Deer Run State Forest soils after clearcutting (Spratt 1997) may be elucidated.

Another important aspect of A-horizon soil organic sulfur to nutrient availability in the ecosystem is the role these compounds play in the retention of exchangeable bases. Other researchers have noted the relationship between sulfate adsorption (the result of a physico-chemical process) in the B and lower soil horizons and ecosystem-wide retention of cations (e.g., Johnson *et al.* 1980, 1982). Little emphasis has been placed on A-horizon soils and the role they play in cation retention. A study by Watwood *et al.* (1993) suggested that ecosystem leaching of Ca^{+2}, Mg^{+2}, and K^{+} was positively correlated with the loss of soil organic sulfur from the A horizons of a wide range of soils. Loss of nutrient cations from forest ecosystems might have a negative effect on production in those ecosystems.

Soils sampled in this study were classified as either alfisols or ultisols. Both of these soil types tend to be highly weathered, and have very distinct demarcations between A and B horizons (Hausenbuiller 1978). One characteristic of these soils that helps differentiate them is their level of exchangeable bases. Alfisols have higher exchangeable base concentrations than ultisols. Another characteristic of alfisols and ultisols is their limited K-supplying power. In these soils, K that is available to primary producers comes primarily from exchangeable and soluble forms of the mineral. As a result of the limited K-supplying power of the soils of the MOFEP plots, the predominant source of this base to the forest ecosystem must be atmospheric deposition, a noted source of K to eastern U.S. forests (Ragsdale *et al.* 1992). As the vegetation utilizes base cations, deciduous trees tend to accumulate exchangeable bases in surface soils (Johnson 1992). Because the soils sampled in this study were well drained, any changes that might lead to loss of ion exchange sites in the soils for exchangeable bases in the surface soils might lead to a deficit in these nutrients. A-horizon soil K^{+} and Mg^{+2} were selected for study here because they represent vital nutrients to the forest ecosystem, and they have been shown to correlate with organic sulfur concentrations in A-horizon soils (Spratt 1997, Watwood *et al.* 1993). Spratt (1997) has provided evidence that in A-horizon soils from Deer Run State Forest plots that were clearcut 2 to 3 or 8 to 10 years prior to sampling, both exchangeable K^{+} and Mg^{+2} were substantially reduced compared with controls (K^{+} by 40 percent, and Mg^{+2} by 40 to 70 percent). These reductions in exchangeable bases were correlated with loss of organic sulfur from the A-horizon soils as a result of clearcutting.

Is there a minimal limit to the level of organic matter, including organic sulfur, that will retain adequate levels of K^{+} and Mg^{+2} from precipitation to help keep the Missouri Ozark forest ecosystem adequately supplied with these nutrients? The need for further study of relationships between forest disturbance and soil microbial processes, related to nutrient status of the ecosystem, should be evident. Comparison of post-treatment surface soil organic sulfur and nutrient cation data with the baseline data presented here may help answer this question.

Post-treatment Goals

The pre-treatment goals of this project will continue to be the focus of ongoing research. These goals concentrate on identification of potential long-term changes in soil sulfur transformations and lignocellulose mineralization as a result of the experimental treatments, and any relationship they might have with ecosystem nutrient status. During winter and spring 1997, samples were collected from the watershed plots as soon after harvest as possible. These data will help indicate any short-term (on the order of months) changes in sulfur transformations or lignocellulose mineralization that may occur as a result of the harvest. From studies of sulfur transformations conducted in Deer Run State Forest A-horizon soils, we already know that very large changes in sulfur transformations in A-horizon soils from clearcut sites, compared with control sites, have occurred previously (Spratt 1997).

As a result of the study in Deer Run State Forest, this project will concentrate on several things after harvest in the MOFEP plots. First, the status of microbial organic sulfur production and the pools of organic sulfur in A-horizon soils will be carefully monitored after harvest. The pilot study indicated substantial changes in these aspects of soil sulfur cycling. Future research will attempt to determine the relative importance of microbial vs. plant derived organic sulfur to the soil sulfur pool. Because litter drop from clearcut managed sites should be noticeably less than from control plots, the role microorganisms play in the production of soil organic sulfur may gain importance. Monitoring soil organic sulfur mineralization will also be of great importance after harvest. If the balance between organic sulfur production (both microbial and plant) and mineralization is shifted towards mineralization, then the potential for nutrient loss (e.g., K^+ and Mg^{+2}) similar to that observed in the pilot study may exist.

Lignocellulose mineralization is expected to increase in the short-term following harvest (Lundgren 1982, Pietikäinen and Fritze 1995), but later diminish along with litter fall. As with the sulfur study, short-term changes in lignocellulose mineralization should be evident during the 1997 study of watershed plots. Information from the lignocellulose mineralization study will be helpful as an indicator of microbial activity in these soils, and to some degree will be related to carbon cycling in these soils. Any correlations between lignocellulose mineralization and sulfur transformations in these soils after harvest will be noted.

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APPENDIXES

Appendix 1.—*ANOVA table, total carbon in MOFEP A-horizon soils (August 1993 to May 1996).*

Source	DF	MS	F	P
Between site effects				
Block	2	1.040	0.007	0.993
Treatment	2	60.612	0.400	0.694
Error A (Block*Treatment)	4	151.410		
	F	**Num DF**	**Den DF**	**P**
Within sitet effects				
Year	92.68[1]	1	4	0.001
Year*Treatment	0.446[1]	2	4	0.668
Season	16.361[2]	3	2	0.058
Season*Treatment	0.814[2]	6	6	0.596
Year*Season	6.003[2]	3	2	0.146
Year*Season*Treatment	0.243[2]	6	6	0.945

[1] Systat® outputs analysis for year, with only 1 DF, in the univariate ANOVA table. F-value from univariate tables.
[2] Pillai's Trace F.

Appendix 2.—*ANOVA table, total sulfur in MOFEP A-horizon soils (August 93 to May 96)*

Source	DF	MS	F	P
Between site effects				
Block	2	51.006	0.130	0.882
Treatment	2	291.512	0.741	0.532
Error A (Block*Treatment)	4	393.380		
	F	**Num DF**	**Den DF**	**P**
Within site effects				
Year	61.640[1]	1	4	0.001
Year*Treatment	0.755[1]	2	4	0.527
Season	4.961[2]	3	2	0.172
Season*Treatment	0.826[2]	6	6	0.589
Year*Season	26.518[2]	3	2	0.037
Year*Season*Treatment	0.297[2]	6	6	0.917

[1] Systat® outputs analysis for year, with only 1 DF, in the univariate ANOVA table. F-value from univariate tables.
[2] Pillai's Trace F.

Appendix 3.—*ANOVA table, organic sulfur in MOFEP A-horizon soils (August 1993 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Block	2	46.328	0.120	0.890
Treatment	2	260.543	0.675	0.559
Error A (Block*Treatment)	4	386.223		
	F	**Num DF**	**Den DF**	**P**
Within site effects				
Year	66.632[1]	1	4	0.001
Year*Treatment	0.640[1]	2	4	0.574
Season	3.778[2]	3	2	0.216
Season*Treatment	0.945[2]	6	6	0.527
Year*Season	13.832[2]	3	2	0.068
Year*Season*Treatment	0.338[2]	6	6	0.894

[1] Systat® outputs analysis for year, with only 1 DF, in the univariate ANOVA table. F-value from univariate tables.
[2] Pillai's Trace F.

Appendix 4.—*ANOVA table, organic sulfur production in MOFEP A-horizon soils (August 1993 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Block	2	9.745	0.582	0.600
Treatment	2	28.013	1.672	0.297
Error A (Block*Treatment)	4	16.755		
	F	**Num DF**	**Den DF**	**P**
Within site effects				
Year	2.085[1]	1	4	0.222
Year*Treatment	0.807[1]	2	4	0.508
Season	7.917[2]	3	2	0.114
Season*Treamentt	0.363[2]	6	6	0.879
Year*Season	35.086[2]	3	2	0.020
Year*Season*Treatment	2.235[2]	6	6	0.175

[1] Systat® outputs analysis for year, with only 1 DF, in the univariate ANOVA table. F-value from univariate tables.
[2] Pillai's Trace F.

Appendix 5.—*ANOVA table, white oak enumeration (>1.5 in. diam.), MOFEP plots*

Source	DF	MS	F	P
Block	2	606.827	3.959	0.113
Treatment	2	138.531	0.904	0.474
Error A (Block*Treatment)	4	153.272		

Appendix 6.—*ANOVA table, white oak cellulose mineralization, MOFEP A-horizon soils (August 1993 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Block	2	0.029	6.254	0.059
Treatment	2	0.000	0.041	0.960
Error A (Block*Treatment)	4	0.005		
	F	**Num DF**	**Den DF**	**P**
Within site effects				
Year	1.335[1]	1	4	0.312
Year*Treatment	0.494[1]	2	4	0.646
Season	502.776[2]	3	2	0.002
Season*Treatment	1.187[2]	6	6	0.420
Year*Season	9.219[2]	3	2	0.099
Year*Season*Treatment	0.912[2]	6	6	0.543

[1] Systat® outputs analysis for year, with only 1 DF, in the univariate ANOVA table. F-value from univariate tables.
[2] Pillai's Trace F.

Appendix 7.—*ANOVA table, white oak lignin mineralization, MOFEP A-horizon soils (August 1993 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Block	2	0.001	0.697	0.550
Treatment	2	0.000	0.298	0.757
Error A (Block*Treatment)	4	0.001		
	F	**Num DF**	**Den DF**	**P**
Within site effects				
Year	19.009[1]	1	4	0.012
Year*Treatment	0.734[1]	2	4	0.535
Season	34.300[2]	3	2	0.028
Season*Treatment	0.687[2]	6	6	0.670
Year*Season	46.316[2]	3	2	0.021
Year*Season*Treatment	0.944[2]	6	6	0.527

[1] Systat® outputs analysis for year, with only 1 DF, in the univariate ANOVA table. F-value from univariate tables.
[2] Pillai's Trace F.

Appendix 8.—*ANOVA table, exchangeable potassium, MOFEP A-horizon soils (August 93 to May 96)*

Source	DF	MS	F	P
Between site effects				
Block	2	15.583	0.315	0.747
Treatment	2	187.124	3.777	0.120
Error A (Block*Treatment)	4	49.549		
	F	**Num DF**	**Den DF**	**P**
Within site effects				
Year	60.624[1]	1	4	0.001
Year*Treatment	0.446[1]	2	4	0.668
Season	19.211[2]	3	2	0.050
Season*Treatment	0.716[2]	6	6	0.652
Year*Season	0.732[2]	3	2	0.621
Year*Season*Treatment	0.881[2]	6	6	0.559

[1] Systat® outputs analysis for year, with only 1 DF, in the univariate ANOVA table. F-value from univariate tables.
[2] Pillai's Trace F.

Appendix 9.—*ANOVA table, exchangeable magnesium, MOFEP A-horizon soils (August 1993 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Block	2	2023.293	6.009	0.062
Treatment	2	3.111	0.009	0.991
Error A (Block*Treatment)	4	336.724		
	F	**Num DF**	**Den DF**	**P**
Within site effects				
Year	1.045[1]	1	4	0.365
Year*Treatment	0.275[1]	2	4	0.773
Season	345.518[2]	3	2	0.003
Season*Treamentt	1.019[2]	6	6	0.491
Year*Season	7.065[2]	3	2	0.127
Year*Season*Treatment	2.083[2]	6	6	0.197

[1] Systat® outputs analysis for year, with only 1 DF, in the univariate ANOVA table. F-value from univariate tables.
[2] Pillai's Trace F.

Appendix 10.—*ANOVA table, total carbon in litter of watershed plots (March and May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	0.111	0.667	0.562
ELT	1	3.342	3.094	0.221
Error A (Rep*ELT)	2	1.080	6.476	0.056
Slope	1	0.306	1.837	0.247
ELT*Slope	1	0.139	0.831	0.414
Error B (Rep*Slope+Rep*Slope*ELT))	4	0.167		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	413.279	1	4	0.000
Season*ELT	0.036	1	4	0.858
Season*Slope	15.958	1	4	0.016
Season*ELT*Slope	3.748	1	4	0.125

[1] F-value from univariate tables.

Appendix 11.—*ANOVA table, total carbon in B-horizon soils of watershed plots (March and May 96)*

Source	DF	MS	F	P
Between site effects				
Rep	2	1.695	6.824	0.051
ELT	1	0.119	0.293	0.642
Error A (Rep*ELT)	2	0.405	1.632	0.303
Slope	1	0.857	3.448	0.137
ELT*Slope	1	0.036	0.146	0.721
Error B (Rep*Slope+Rep*Slope*ELT))	4	0.248		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	5.138	1	4	0.086
Season*ELT	1.680	1	4	0.265
Season*Slope	0.116	1	4	0.750
Season*ELT*Slope	1.840	1	4	0.246

[1] F-value from univariate tables.

Appendix 12.—*ANOVA table, total carbon in A-horizon soils of watershed plots (May 1995 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	48.538	1.602	0.308
ELT	1	30.608	0.304	0.637
Error A (Rep*ELT)	2	100.595	3.320	0.141
Slope	1	0.473	0.016	0.907
ELT*Slope	1	3.700	0.122	0.744
Error B (Rep*Slope+Rep*Slope*ELT))	4	30.300		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	6.068	3	2	0.145
Season*ELT	3.438	3	2	0.233
Season*Slope	2.633	3	2	0.287
Season*ELT*Slope	2.741	3	2	0.279

[1] F-value from univariate tables.

Appendix 13.—*ANOVA table, white oak cellulose mineralization in A-horizon soils of watershed plots (May 1995 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	0.058	1.398	0.346
ELT	1	0.128	3.879	0.188
Error A (Rep*ELT)	2	0.033	0.792	0.513
Slope	1	0.081	1.948	0.235
ELT*Slope	1	0.042	1.015	0.371
Error B (Rep*Slope+Rep*Slope*ELT))	4	0.042		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	12.618	1	4	0.024
Season*ELT	3.726	1	4	0.126
Season*Slope	3.014	1	4	0.158
Season*ELT*Slope	0.937	1	4	0.388

[1] F-value from univariate tables.

Appendix 14.—*ANOVA table, white oak lignin mineralization in A-horizon soils of watershed plots (May 1995 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	0.607 e-03	0.794	0.512
ELT	1	0.364 e-03	0.556	0.534
Error A (Rep*ELT)	2	0.654 e-03	0.855	0.491
Slope	1	0.378 e-03	0.494	0.521
ELT*Slope	1	0.001	1.910	0.239
Error B (Rep*Slope+Rep*Slope*ELT))	4	0.765 e-03		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	2.979	1	4	0.159
Season*ELT	0.629	1	4	0.472
Season*Slope	1.238	1	4	0.328
Season*ELT*Slope	1.256	1	4	0.325

[1] F-value from univariate tables.

Appendix 15.—*ANOVA table, total sulfur in B-horizon soils of watershed plots (March and May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	3.541	11.182	0.023
ELT	1	0.004	0.011	0.926
Error A (Rep*ELT)	2	0.350	1.105	0.415
Slope	1	0.022	0.070	0.805
ELT*Slope	1	1.363	4.303	0.107
Error B (Rep*Slope+Rep*Slope*ELT))	4	0.317		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	54.672	1	4	0.002
Season*ELT	1.532	1	4	0.284
Season*Slope	3.272	1	4	0.145
Season*ELT*Slope	0.444	1	4	0.542

[1] F-value from univariate tables.

Appendix 16.—*ANOVA table, total sulfur in litter of watershed plots (March and May 96)*

Source	DF	MS	F	P
Between site effects				
Rep	2	77.449	5.211	0.077
ELT	1	0.304	0.009	0.933
Error A (Rep*ELT)	2	32.575	2.192	0.228
Slope	1	46.124	3.104	0.153
ELT*Slope	1	0.218	0.015	0.909
Error B (Rep*Slope+Rep*Slope*ELT))	4	14.862		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	39.599	1	4	0.003
Season*ELT	0.008	1	4	0.931
Season*Slope	0.004	1	4	0.954
Season*ELT*Slope	0.000	1	4	0.992

[1] F-value from univariate tables.

Appendix 17.—*ANOVA table, total sulfur in A-horizon soils of watershed plots (May 1995 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	166.023	1.099	0.416
ELT	1	449.342	0.868	0.450
Error A (Rep*ELT)	2	517.272	3.425	0.136
Slope	1	22.135	0.147	0.721
ELT*Slope	1	3.163	0.021	0.892
Error B (Rep*Slope+Rep*Slope*ELT))	4	151.028		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	44.283	3	2	0.022
Season*ELT	12.281	3	2	0.076
Season*Slope	13.628	3	2	0.069
Season*ELT*Slope	17.424	3	2	0.055

[1] F-value from univariate tables.

Appendix 18.—*ANOVA table, organic sulfur in A-horizon soils of watershed plots (May 1995 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	140.957	0.990	0.448
ELT	1	507.906	1.039	0.415
Error A (Rep*ELT)	2	488.813	3.432	0.136
Slope	1	22.469	0.158	0.712
ELT*Slope	1	0.424	0.003	0.959
Error B (Rep*Slope+Rep*Slope*ELT))	4	142.440		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	51.247	3	2	0.019
Season*ELT	9.943	3	2	0.093
Season*Slope	3.189	3	2	0.248
Season*ELT*Slope	14.791	3	2	0.064

[1] F-value from univariate tables.

Appendix 19.—*ANOVA table, organic sulfur production in A-horizon soils of watershed plots (May 1995 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	802.524	3.238	0.146
ELT	1	5.197	0.024	0.891
Error A (Rep*ELT)	2	215.256	0.869	0.486
Slope	1	25.568	0.103	0.764
ELT*Slope	1	49.916	0.201	0.677
Error B (Rep*Slope+Rep*Slope*ELT))	4	247.842		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	50.038	3	2	0.020
Season*ELT	18.122	3	2	0.053
Season*Slope	1.132	3	2	0.501
Season*ELT*Slope	1.527	3	2	0.419

[1] F-value from univariate tables.

Appendix 20.—*ANOVA table, organic sulfur mineralization in A-horizon soils of watershed plots (March and May 1996, note: data were log transformed before analysis)*

Source	DF	MS	F	P
Between site effects				
Rep	2	0.019	2.029	0.246
ELT	1	0.091	7.000	0.118
Error A (Rep*ELT)	2	0.013	1.417	0.343
Slope	1	0.008	0.838	0.412
ELT*Slope	1	0.005	0.564	0.494
Error B (Rep*Slope+Rep*Slope*ELT))	4	0.009		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	4.396	1	4	0.104
Season*ELT	1.358	1	4	0.309
Season*Slope	0.116	1	4	0.751
Season*ELT*Slope	1.789	1	4	0.252

[1] F-value from univariate tables.

Appendix 21.—*ANOVA table, exchangeable potassium in A-horizon soils of watershed plots (May 1995 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	78.118	1.104	0.415
ELT	1	21.653	0.485	0.558
Error A (Rep*ELT)	2	44.639	0.631	0.578
Slope	1	0.710	0.010	0.925
ELT*Slope	1	27.946	0.395	0.564
Error B (Rep*Slope+Rep*Slope*ELT))	4	70.757		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	6.467	3	2	0.137
Season*ELT	0.418	3	2	0.761
Season*Slope	0.854	3	2	0.579
Season*ELT*Slope	0.435	3	2	0.752

[1] F-value from univariate tables.

Appendix 22.—*ANOVA table, exchangeable magnesium in A-horizon soils of watershed plots (May 1995 to May 1996)*

Source	DF	MS	F	P
Between site effects				
Rep	2	6.722	0.094	0.912
ELT	1	1101.826	3.522	0.201
Error A (Rep*ELT)	2	312.877	4.392	0.098
Slope	1	84.748	1.190	0.337
ELT*Slope	1	6.962	0.098	0.770
Error B (Rep*Slope+Rep*Slope*ELT))	4	71.233		

	F[1]	Num DF	Den DF	P
Within site effects				
Season	3.556	3	2	0.227
Season*ELT	1.740	3	2	0.385
Season*Slope	6.401	3	2	0.138
Season*ELT*Slope	0.715	3	2	0.628

[1] F-value from univariate tables.