

## Nutrient Loss Accelerated by Clear-Cutting of a Forest Ecosystem

**Abstract.** *The forest of a small watershed-ecosystem was cut in order to determine the effects of removal of vegetation on nutrient cycles. Relative to undisturbed ecosystems, the cut ecosystem exhibited accelerated loss of nutrients: nitrogen lost during the first year after cutting was equivalent to the amount annually turned over in an undisturbed system, and losses of cations were 3 to 20 times greater than from comparable undisturbed systems. Possible causes of the pattern of nutrient loss from the cut ecosystem are discussed.*

One-third of the land surface of the United States supports forest, and much of it is occasionally harvested. Yet, apart from nutrient losses calculated on the basis of extracted timber products, we have little quantitative data on the effects of harvesting on either the nutrient status of forest ecosystems or the chemistry of stream water draining from them—which is closely related to the increasingly important problem of eutrophication of stream and river water (1). This paucity of information partly reflects the difficulties of measuring characteristics of massive forest ecosystems, and the fact that nutrient cycles are closely tied to hard-to-measure hydrologic parameters (2).

The input and output of chemicals can be measured and nutrient budgets constructed for forest ecosystems by use of the small-watershed approach (2). For several years we have measured these parameters on six small undisturbed watersheds in the Hubbard Brook Experimental Forest in central New Hampshire (3); here the bedrock is practically impermeable (4), and all liquid water leaves the watersheds by way of first- or second-order streams; the runoff pattern is typical of northern regions having deep snow packs (5). Additional information on topography, geology, climate, and biology is given by Likens *et al.* (4).

Chemical relations for these undis-

turbed forest ecosystems (watersheds) are being established by weekly measurements of dissolved cations and anions entering the ecosystem in all forms of precipitation and leaving the system in stream water. These data, combined with measurements of precipitation and stream flow, enable computation of the input and output of these various elements, as well as annual budgets (2). These results have been reported (4, 6, 7).

In 1965 the forest of one watershed (W-2) was clear-cut in an experiment designed to: (i) determine the effect of clear-cutting on stream flow, (ii) examine some of the fundamental chemical relations of the ecosystem, and (iii) evaluate the effects of forest manipulation on nutrient relations and on eutrophication of stream water. This is a preliminary report of chemical effects observed during the subsequent year.

The experiment began during the winter of 1965–66 when the beech-maple-birch forest (15.6 hectares) was leveled by the U.S. Forest Service. All trees, saplings, and shrubs were cut, dropped in place, and limbed so that no slash was more than 1.5 m above ground. No products were removed from the forest, and great care was taken to minimize erosion of the surface. On 23 June 1966, regrowth of vegetation was inhibited by application of the herbicide Bromacil ( $C_9H_{13}BrN_2O_2$ ) at 28 kg/hectare; approximately 80 percent of the mixture applied was Bromacil; 20 percent was largely inert carrier (8).

Samples of stream water were collected and analyzed weekly, as they had been for 2 years before the cutting; the loss of ions was calculated in terms of kilograms per hectare. Similar measurements on adjacent undisturbed watersheds provided comparative information.

The cutting had a pronounced effect on runoff, which began to increase in May 1966; the cumulative runoff value for 1966 exceeded the expected value by 40 percent. The greatest difference occurred during June through September, when runoff values were 418 percent greater than expected. The difference is directly attributable to the removal of transpiring surface and probably reflects wetter conditions within the soil profile.

The striking loss of nitrate nitrogen in stream water (Fig. 1) suggests that alteration of normal patterns of nitrogen flow played a major role in loss of nutrients from the cutover ecosystem. This loss is best understood by consideration of nitrogen patterns in the undisturbed ecosystem. Runoff data from such systems (7) (Fig. 1) indicate a strong and reproducible seasonal cycle of concentration of nitrate in stream water. High concentrations are associated with the winter period from November through April, while low concentrations persist from April through November.

The decline of nitrate concentrations during May and the low concentrations throughout the summer correlate with heavy nutrient demands by the vegetation and increased heterotrophic activity associated with warming of the soil. The winter concentration pattern of  $NO_3^-$  may be explained in strictly physical terms, since the input of nitrate in precipitation from November through May largely accounts for nitrate lost in stream water during this period. Evaporation from the snow pack may account for some increase in concentration of  $NO_3^-$  in the stream water in the spring. Also, since yearly input of nitrate in precipitation exceeds losses in stream water (Table 1), concentrations in stream water provide little conclusive evidence of the occurrence of nitrification in these undisturbed acid soils.

Results from the cut watershed demonstrate nitrogen relations of such an

Table 1. Partial nitrogen budgets for watersheds 6 (13.2 hectares) and 2 (15.6 hectares); all data are in kilograms of elemental nitrogen per hectare. Gains by biological fixation and losses by volatilization are not included. Watershed 2 was cut in the winter of 1965–1966.

Year	Input in precipitation		Output in stream water		Net gains (+) losses (–)	
	$NH_4-N$	$NO_3-N$	$NH_4-N$	$NO_3-N$	$NH_4-N$	$NO_3-N$
<i>Watershed 6 (undisturbed)</i>						
1965	2.1	2.8	0.6	1.0	+1.5	+1.8
1966	2.0	4.5	0.5	1.5	+1.5	+3.0
<i>Watershed 2 (cutover)</i>						
1965	2.1	2.8	0.4	1.3	+1.7	+1.5
1966	2.0	4.5	1.2	58.1	+0.8	–53.6

ecosystem and indirectly those of the undisturbed ecosystem. Comparison of nitrate concentrations in stream water from watersheds W-6 (undisturbed) and W-2 (cutover) indicates a similar pattern of concentrations throughout 1964 and 1965, prior to cutting, and through May of 1966 (Fig. 1). Beginning on 7 June 1966, 16 days before application of the herbicide, nitrate concentrations in W-2 show a precipitous rise, while the undisturbed ecosystem shows the normal late-spring decline. Allison (9) has documented similar losses of nitrate from uncropped fields or fields carrying poorly established crops. The increase in nitrate concentrations is a clear indication of the occurrence of nitrification in the cutover ecosystem. Since an  $\text{NH}_4^+$  substrate is required, the occurrence of nitrification also indicates that soil C:N ratios were favorable for the production of  $\text{NH}_4^+$ , in excess of heterotrophic needs, sometime before 7 June.

Some of these conclusions must hold for the undisturbed ecosystem; that is to say, sometime prior to 7 June C:N ratios were favorable for the flow of ammonium either to higher plants or to the nitrification process. The low levels of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the drainage water of the undisturbed ecosystem (W-6) may attest to the efficiency of the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ , and to the efficiency of the vegetation in utilizing  $\text{NO}_3^-$ . However, Nye and Greenland (10) state that growing, acidifying vegetation represses nitrification; thus the vegetation may draw directly on the  $\text{NH}_4^+$  pool, and little nitrate may be produced within the undisturbed ecosystem. In this case, one must assume that cutting drastically altered conditions controlling the nitrification process.

The action of the herbicide in the cutover watershed seems to be one of reinforcing the already well-established trend, of loss of  $\text{NO}_3^-$ , induced by cutting alone. This action is probably effected through herbicidal destruction of the remaining vegetation—herbaceous plants and root sprouts. In the event of rapid transformation of all nitrogen in the Bromacil, this source could at best contribute only 5 percent of the nitrogen lost as nitrate.

During 1966 the cutover area showed a net loss of N of 52.8 kg/hectare, compared to a net gain of 4.5 kg/hectare for the undisturbed system (Table 1). If one assumes that the cutover system would have normally

gained 4.5 kg/hectare, the adjusted net loss from the cutover system is about 57 kg/hectare. The annual turnover of nitrogen in undisturbed systems is approximately 60 kg/hectare on the basis of an equilibrium system in which annual leaf fall is about 3200 kg/hectare (11) and annual losses of roots are about 800 kg/hectare. Consequently an amount of elemental nitrogen, equivalent to the annual turnover, was lost during the first year following cutting.

Nitrogen losses from W-2 do not take into account volatilization, which accounted for about 12 percent of the total losses from 106 uncropped soils (9). Moreover, denitrification, an an-

aerobic process, requires a nitrate substrate generated aerobically (12); consequently, for substantial denitrification to occur in fields, aerobic and anaerobic conditions must exist in close proximity. The large increases in subsurface flow of water from the cutover watershed suggests that such conditions may have been more common throughout the watershed.

A high level of nitrate ion in the soil solution implies a corresponding concentration of cations and ready leaching (10); precisely this situation prevailed in W-2. Simultaneously with the rise of nitrate, concentrations of  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Na}^+$ , and  $\text{K}^+$  rose ultimately severalfold. These increases, in

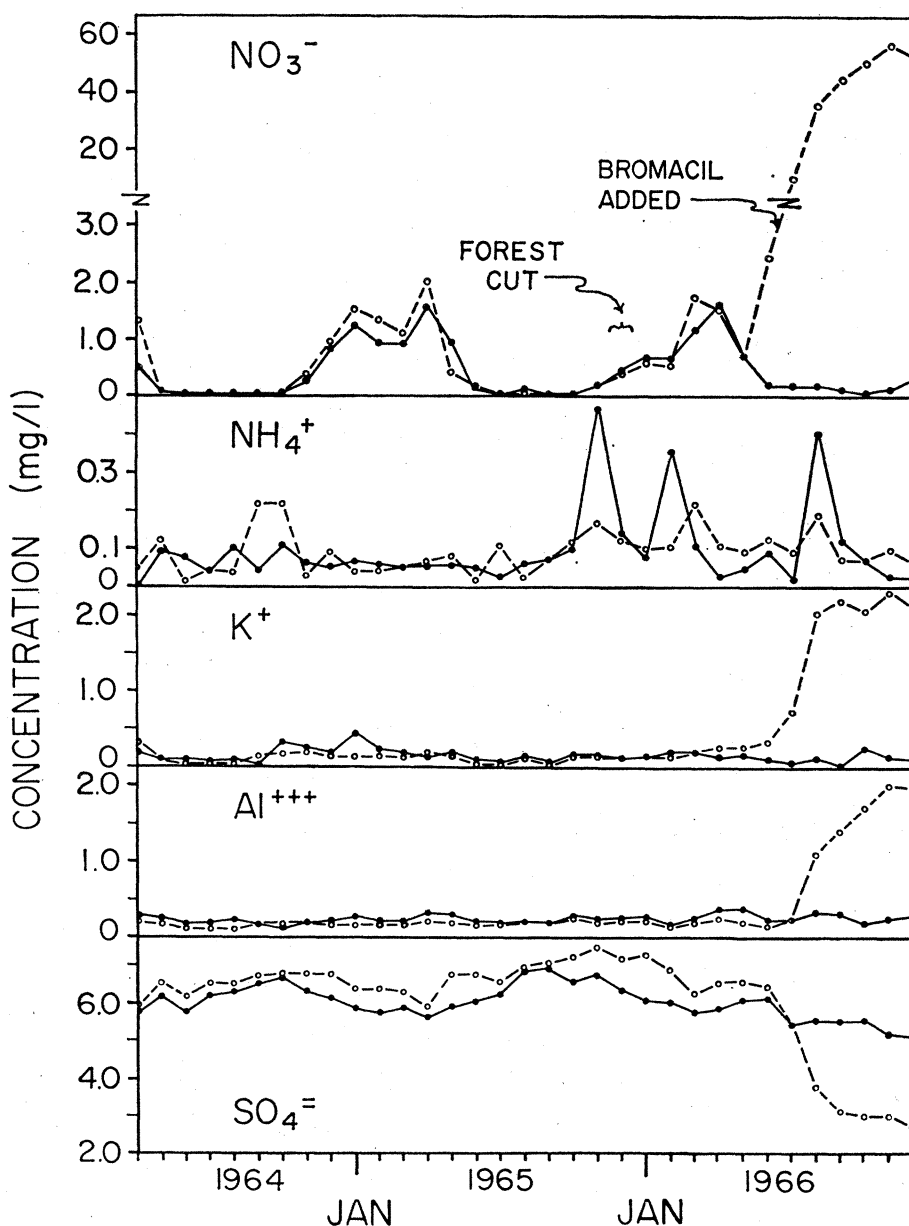


Fig. 1. Average monthly concentrations of selected cations and anions in stream water draining from forest ecosystems undisturbed (solid lines) and clear-cut during the winter of 1965-66 (dashed lines).

combination with the increase in drainage water, led to net losses 9, 8, 3, and 20 times greater, respectively, than similar losses from five undisturbed ecosystems between June 1966 and June 1967. Concentrations of  $Al^{+++}$  rose about 1 month later than the initial rise in nitrate, while sulfate showed a sharp drop in concentration, coincident with the rise in nitrate (Fig. 1).

These results indicate that this ecosystem has limited capacity to retain nutrients when the bulk of the vegetation is removed. The accelerated rate of loss of nutrients is related to the cessation of uptake of nutrients by plants and to the larger quantities of drainage water passing through the system. Accelerated losses may also relate to increased mineralization resulting from changes in the physical environment, such as change in temperature or increase in available substrate.

However, the effect of the vegetation on the process of nitrification cannot be overlooked. In the cutover ecosystem the increased loss of cations correlates with the increased loss of nitrate; consequently, if the intact vegetation inhibits the process of nitrification (13) and if removal of the vegetation promotes nitrification, release from inhibition may account for major losses of nutrients from the cutover ecosystem.

These results suggest several conclusions important for environmental management:

1) Clear-cutting tends to deplete the nutrients of a forest ecosystem by (i) reducing transpiration and so increasing the amount of water passing through the system; (ii) simultaneously reducing root surfaces able to remove nutrients from the leaching waters; (iii) removal of nutrients in forest products; (iv) adding to the organic substrate available for immediate mineralization; and (v), in some instances, producing a microclimate more favorable to rapid mineralization. These effects may be important to other types of forest harvesting, depending on the proportion of the forest cut and removed. Loss of nutrients may be greatly accelerated in cutover forests where the soil microbiology leads to an increase of dissolved nitrate in leaching waters (10).

2) Management of forest ecosystems can significantly contribute to eutrophication of stream water. Nitrate concentrations in the small stream from the cutover ecosystem have exceeded

established pollution levels (10 parts per million) (14) for more than 1 year, and algal blooms have appeared during the summer.

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## Antigen Binding to Cells: Determination by Enzymic Fluorogenic Group Hydrolysis

**Abstract.** A sensitive method for detecting cells containing antibody to  $\beta$ -galactosidase has been devised. The enzyme attached to the cells containing antibody can hydrolyze a fluorogenic substrate and yield fluorescent products which are measured microphotofluorometrically. This method of detecting a few molecules of antibody is applicable to other enzyme antigen systems.

A method for measuring cellular antibody in very small amounts is necessary when only a few molecules of antibody may be present in a cell, such as very early in the antibody response, during states of immunologic unresponsiveness, or possibly at receptor sites on the membranes of cells bearing immunologic memory (1). Our method was designed to detect only a few molecules of cellular antibody.

Using the substrate fluorescein-di- $\beta$ -galactopyranoside (FD $\beta$ G), Rotman *et al.* measured the  $\beta$ -galactopyranoside activity of single molecules (2), individual bacterial cells (3), and ribosomes (4). This substrate is nonfluorescent and upon hydrolysis of its glycosidic bonds yields fluorescent products (fluorescein-mono- $\beta$ -galactoside or fluorescein, depending on whether one or both glycosidic bonds are cleaved, respectively). Antibody to  $\beta$ -galactosidase does not impair the activity of the enzyme (5), and so the enzyme-antigen complex can hydrolyze the substrate also.

For the assay, the spleens of im-

munized or normal A/Jax mice were removed and macerated; the fragments were screened through a 60-gauge and then a 250-gauge stainless steel gauze in Hanks's balanced salt solution, with 50  $\mu$ g of streptomycin, 60 units of penicillin, and 20 units of heparin per milliliter. The resultant single-cell suspension was washed once in the same solution and centrifuged. The cell pellet was treated with 95 percent ethanol for 10 minutes at room temperature, washed again, and suspended in medium to yield a 20-percent suspension of cells.

A portion of the suspension was removed for measurement of "intrinsic"  $\beta$ -galactosidase, including the activity of endogenous mouse-spleen  $\beta$ -galactosidase and activity of any possible residual antigen from the immunization. The rest of the cells were incubated with 15  $\mu$ g of  $\beta$ -galactosidase per milliliter of cell suspension for 1 hour at 37°C and then overnight at 4°C (6). The cells were then washed four times with 40 ml of medium to remove the free enzyme, and suspended