# Posttreatment Effects of Forest Fertilization on the Predominant Benthic Community of a Headwater Stream in Eastern Kentucky

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#### ABSTRACT

A posttreatment analysis was conducted on the benthic invertebrate community of a small headwater stream in eastern Kentucky that had been affected by a forest fertilization project in 1975. During the biological sampling period (1978–1981), significantly higher concentrations of nitrate-nitrogen were present in the affected stream when compared with two unaffected streams. Standing-crop biomass of invertebrates (wet weight), volumetric displacement, and the number of individuals were not significantly different between the treated stream and one untreated stream, suggesting that the fauna was not using the nitrate-nitrogen subsidy to increase biomass. However, the number of taxa and species diversity (Sequential Comparison Index) were significantly higher in the stream located within the unfertilized watershed.

The most abundant invertebrates in both streams were the Diptera, Ephemeroptera, and Plecoptera. Most species were dipterans. The observed differences between the streams could indicate that the affected stream is in the process of recovery. This possibility seems unlikely considering the length of time since fertilization, the abundant sources of recolonizers, and the absence of any disturbance since fertilization. The benthic fauna is, therefore, considered presently unaffected by the previous forest fertilization.

# INTRODUCTION

Forests are fertilized primarily during the establishment of new forests (1). However, fertilization of established forests is being conducted with increasing frequency (2) and is known to increase forest biomass and nutrient uptake (1). Nitrogen has been identified as a limiting factor in many forests (3), and nitrate loss through leaching is generally very small or confined to the non-growing season (4). Nitrogen has been suggested also as a possible limiting factor in lotic systems (5) because the energy base for stream ecosystems is detrital decomposition and algal production (6, 7). Increased levels of nitrogen have been shown to increase algal growth (8, 9) and nitrification (5) but not detrital decomposition (5, 10). Benthic macroinvertebrate density in leaf packs in a second-order stream in Tennessee did not increase upon fertilization with ammonium hydroxide (5). Even so, it is conceivable that benthic macroinvertebrate community structure may be affected by forest fertilization; however, information concerning the community's response to this practice is lacking. An opportunity arose in 1978 to investigate the

posttreatment effects of ammonium nitrate fertilization upon the benthic community structure of a first-order stream in eastern Kentucky as part of a more extensive project investigating the composition, structure, and biomass of the benthic macroinvertebrate community within a relatively undisturbed third-order stream system (11).

In 1975 a forest fertilization project was conducted in the watershed (41 ha) of Field Branch (located 24 km southeast of Jackson in Breathitt County, Kentucky). The stream is located within the University of Kentucky's Robinson Forest, a 6,075 ha forest research experiment station. The forest has remained relatively undisturbed since 1920 when the last major harvest of the area took place. The physiography, topography, climate, soils (12, 13), and vegetation (14) are characteristic of the Cumberland Plateau.

The purpose of the Field Branch fertilization project was to measure and evaluate the effects of fertilization on the quality and quantity of streamflow in a forested Appalachian watershed (2). Since nitrogen fertilization does increase forest biomass, it was postulated that increased evapotranspiration would result in a concomitant reduction in streamflow. No such decrease was found relative to the similar and untreated stream, Falling Rock (94 ha watershed), located approximately 1.6 km east of Field Branch; however, nitrate-nitrogen levels remained significantly higher in the treated stream a full two years after treatment (2).

Because significantly higher nitrate-nitrogen levels had existed within Field Branch before our investigation of the benthic macroinvertebrate community, 3 distinct questions were posed. First, were the elevated nitratenitrogen levels persistent through the biological sampling period? Second, did the benthic communities of the treated differ significantly from the untreated streams in the area? Third, if differences were found, could they be correlated with the elevated nitrate-nitrogen levels resulting from the forest fertilization?

# Methods

## Nitrate-Nitrogen

In late April 1975 ammonium nitrate fertilizer was aerially applied to the watershed of Field Branch at a rate of 504 kg/ha. No effort was made to avoid direct application to the stream. Weekly water samples of streamflow had been collected and analyzed by university personnel since 1971 and continued throughout the biological sampling period for various streams throughout Robinson Forest. The analysis of nitrate-nitrogen was a modified nitrate reductase procedure (15) using *Escherichia coli* as the reducing agent (2).

For statistical analyses, two control (unfertilized) streams were included: the immediately adjacent Little Millseat (79 ha watershed) and Falling Rock (94 ha watershed), approximately 1.6 km southeast of Field Branch and Little Millseat watersheds. The General Linear Model (GLM) (16) procedure was used to perform analyses of variance on ten years of weekly nitrate-nitrogen concentrations among the 3 streams, among seasons, and for season-stream interaction, beginning 1 May 1972 through 30 April 1982. Pretreatment time includes years 1, 2, and 3; posttreatment includes years 4–10.

### Benthic Macroinvertebrate Community

Three Surber samples  $(0.1 \text{ m}^2)$  were taken from similar riffles in the treated stream and the adjacent untreated Little Millseat during all seasons on 11 dates from May 1978 to August 1981 (N = 66). Both streams are primarily riffles. The material collected was fixed in the field in 10% formalin; handpicked and sorted in the laboratory using a number 35 U.S.A. standard testing seive (500  $\mu$  mesh size) to retain organisms; and subsequently stored in 80% ethyl alcohol. The Sequential Comparison Index (SCI) was used to measure diversity (17). Organisms were counted and assigned to Order for the statistical analysis. Standing crop wet weight (gm/m<sup>2</sup>) was obtained for each sample. Wet weights were obtained so that specimens could be retained for further analvsis. Volumetric displacement (ml/m<sup>2</sup>) was determined by measuring the volume of water displaced by the sample when placed in a known volume of water. Volumetric displacement should correlate directly with wet weight and, thus, serve as a check for accuracy.

The occasional salamanders, fishes, snails, clams, crayfishes, and terrestrial insects sporadically captured were excluded from the samples due to the fact that a Surber net is not an effective or reliable method to capture those groups. The Statistical Analysis System (SAS) t tests (16) were employed to compare the SCI values, wet weights, volumetric displacements, and numbers of taxa and individuals between the two streams.

#### RESULTS

#### Nitrate-Nitrogen

The nitrate-nitrogen concentrations in Little Millseat (non-fertilized) and Field Branch (fertilized) are presented in Figure 1. The concentrations in Falling Rock follow closely those in Little Millseat. Following treatment very high concentrations of nitrate-nitrogen were immediately detected in Field Branch (640 mg/liter), and levels potentially toxic to humans and other animals persisted in the stream for several days (2). Mean weekly pretreatment concentrations of nitrate-nitrogen for the 3 streams ranged from 0.0-0.91 mg/liter with mean values of 0.13-0.14 mg/liter. Pretreatment nitrate-nitrogen concentrations were significantly different among seasons (P < 0.0318) but not among streams. There was no significant season-stream interaction (P > 0.05, Fig. 1). After treatment (1975), differences among



FIG. 1. Instream nitrate-nitrogen levels for the fertilized and non-fertilized streams from 1 May 1972 through 30 April 1982. F = fall, W = winter, S = spring, S = summer. Data modified from Coltharp et al. (1978).

the streams became a significant source of variation (P < 0.0001) which continued to 1980. Differences among seasons generally remained significant after treatment. Significant seasonstream interaction effects also were noted after treatment. Therefore, seasonal and stream effects could not be separated. To ascertain the source of variation among streams, nitrate-nitrogen levels were contrasted by years for Stream 1 (Falling Rock, untreated) versus 2 (Little Millseat, untreated); stream 1 versus 3 (Field Branch, treated); stream 2 versus 3, and streams 1 and 2 versus 3. During pretreatment vears 1972-1974, differences between all pairs of streams were not significant. After treatment the contrast of stream 1 vs. 2 (both untreated) for nitrate-nitrogen was generally nonsignificant while the remaining contrasts were highly significant after treatment (P = 0.0001). Furthermore, the differences between nitratenitrogen levels in the stream persisted through year 9, a span of 6 years after treatment which includes all biological sampling dates. To determine how season-stream interaction might have affected the contrasts, the data were further analyzed by seasons within years. The results indicated that posttreatment nitrate-nitrogen levels among streams remain significantly different (P < 0.04) over all seasons for contrasts 1 vs. 3, 2 vs. 3, and 1 + 2 vs. 3 through year 9.

# Benthic Macroinvertebrate Community

Mean (N = 3) standing crop net weight of benthic invertebrates ranged from 1.14-12.57  $g/m^2$  in the fertilized stream and from 2.35-10.50 g/m<sup>2</sup> in the unfertilized stream for all sample dates. As expected, volumetric displacement revealed a direct relationship to wet weight with mean values ranging from 0.91-20.65 ml/m<sup>2</sup> in the fertilized stream and 2.68- $11.85 \text{ ml/m}^2$  in the unfertilized one (Little Millseat). Both measurements varied in a similar seasonal fashion, with lowest values found during the winter and the highest between June and July. Variability between streams was not significant for either wet weight (P =(0.7188) or volumetric displacement (P =0.5729).

The unfertilized Little Millseat had significantly higher (P = 0.0015) SCI values than the treated Field Branch (ranges = 24–54 and 17– 52, respectively). In both streams, the highest (29 Apr 1979 and 11 May 1981) and lowest (25 May 1978 and 24 Feb 1979) SCI values occurred on the same sampling dates (Fig. 2).

The number of taxa/0.1 m<sup>2</sup> in the unfertilized stream (range = 28–51) was significantly higher (P = 0.0001) than the number in the affected stream (range = 18--55, Fig. 3). Most taxa were classified into 5 orders of insects (Coleoptera, Diptera, Ephemeroptera, Plecoptera, and Trichoptera). The category referred to as "others" includes oligochaetes, isopods, amphipods, and odonates (Fig. 4). In 4 of the 6 groups (Coleoptera P = 0.0005, Diptera P =0.0032, Ephemeroptera P = 0.0467, and others P = 0.0184) there were significantly fewer taxa (3.27, 4.1, 1.4, and 1.1 fewer, respectively) in the stream affected by fertilization.

The number of individuals was not significantly different (P = 0.08) between the fertilized stream (range = 48–489 individuals/0.1 m<sup>2</sup>) and the unaffected stream (range = 88– 538) (Fig. 5). In general, there were no significant differences between the numbers of individuals in the predominant groups of each stream (Fig. 6) with the exception of Coleoptera, in which there were significantly (P =



FIG. 2. Mean sequential comparison index values (N = 3) of the benthic macroinvertebrate community in the fertilized and non-fertilized streams from May 1978 through August 1981.

0.0019) fewer (52.8 fewer) in the fertilized stream when compared with the unfertilized stream. Field Branch (fertilized) had 5.8 Coleoptera/0.1 m<sup>2</sup>, but Little Millseat had 48.0/ 0.1 m<sup>2</sup> (Table 1). The lower numbers of Coleoptera were primarily due to the much lower numbers (per 0.1 m<sup>2</sup>) of Optioservus ovalis (2 versus 96), Oulimnius nitidulus (119 vs. 1,439), Stenelmis n. sp. (22 vs. 137), Helichus basalis (8 vs. 72), and Ectopria sp. (27 vs. 137) in Field Branch. The two obvious peaks (Fig. 6) in the Coleoptera found in Little Millseat were due to the large number of O. nitidulus.

In each stream, the Ephemeroptera, Diptera, and Plecoptera were the most abundant orders each having over 64 individuals/ $0.1 \text{ m}^2$ (20% of the total number). Dipterans were the most speciose group (14 taxa/ $0.1 \text{ m}^2$  in Field Branch, 18 taxa/ $0.1 \text{ m}^2$  in Little Millseat).



FIG. 3. Mean number of benthic macroinvertebrate taxa (N = 3) in the fertilized and non-fertilized streams from May 1978 through August 1981.

# DISCUSSION

Pretreatment concentrations of nitrate-nitrogen were similar among the 3 streams as well as to those of comparable streams in West Virginia (18) and Tennessee (5). All possible pair-wise contrasts revealed that the nitratenitrogen concentrations remained significantly higher for a full 6 years (1975–1981) after treatment in Field Branch within the fertilized watershed when compared with 2 unaffected streams.

The concentration of nitrogen in natural surface waters, including areas of intense agriculture, ranges from 0 to 100 ppm (19). When nitrogen is present at unnaturally or experimentally elevated levels primary producers and eutrophication increase (9, 19). However, as stated previously, detrital decomposition (5, 10) and benthic macroinvertebrate density (5) have

TABLE 1. Averages, ranges, and a ranking for the numbers of taxa and individuals in the predominant insect orders in Field Branch (fertilized) and Little Millseat (non-fertilized). All values represent the average of three Surber collections taken on eleven dates (N = 33).

Insect order	Average number of taxa		Average number of individuals	
	Field Branch	Little Millseat	Field Branch	Little Millseat
Ephemeroptera	5.4 (2.3-7.0)	6.8 (4.0-11.0)	64.9 (9.7-160.7)	68.0 (14.7-145.0)
Plecoptera	5.6(2.3-9.0)	5.0(2.3 - 9.0)	97.4 (3.3-242.7)	67.1 (22.7-145.0)
Coleoptera	2.2(0.3-4.0)	5.6(2.5-9.0)	5.8(0.3-19.3)	48.0 (5.0-125.7)
Diptera	14.1 (7.3-25.0)	18.3 (11.0-25.0)	81.5 (18.0-204.7)	78.7 (29.7-196.3)
Trichoptera	4.2(0.7-7.3)	4.6(1.7-6.7)	12.5 (0.7-26.3)	17.4(1.7-32.0)
Others	2.7 (1.0-5.3)	4.0(1.7-6.7)	12.1 (1.0-51.7)	15.8 (3.7-85.0)
Total	34.2	44.3	274.2	295.0



FIG. 4. Mean number of taxa (N = 3) in each order and others for the fertilized (triangle, dashed line) and the non-fertilized (open circle, solid line) streams May 1978 through August 1981.

been shown to be unaffected by such nitrogen subsidies.

In this study there were no significant differences in the standing crop biomass wet weights and volumetric displacements of the benthic communities between control and enriched streams. Standing-crop biomasses were similar to those found by Kraft and Mundahl (20) in benthic invertebrate riffle communities in a Michigan river. Furthermore, the significantly higher levels of nitrate-nitrogen in the affected stream are considered low in comparison with streams, known to support a varied biota (21), in heavily agriculturalized areas of Kentucky where nitrate-nitrogen levels as high as 14.0 mg/liter have been reported.

Cairns and Dickson (17) found that in polluted or disturbed situations the SCI value is less than 2. In their least disturbed sites, index values ranged from 20–24. The SCI values for the two streams reported herein greatly exceeded those of Cairns and Dickson's (17) least disturbed sites indicating these streams are highly diverse.

There was a lower number of taxa and species diversity (SCI) in the fertilized stream. The number of taxa in 4 groups (Coleoptera, Diptera, Ephemeroptera, and others) accounted for the difference. The Coleoptera also had a significantly higher number of individuals in the unaffected stream while other groups showed no significant differences.

The trophic dynamics of headwater streams are maintained chiefly by detritus, specifically allochthonous leaf material (7, 22, 23, 25) and that, quantitatively, the detrital pathway of energy flow is much more significant than the living pathway (24). If the benthic community is dependent primarily on detritus, and detrital decomposition is unaffected by experimentally elevated nitrogen concentrations, then increasing nitrogen in a stream system should not increase the availability of detrital material to the benthic community. In this study, benthic biomass was not significantly different between the control stream and the stream with experimentally elevated nitrate-nitrogen levels, supporting this hypothesis.

In general, faunal similarities between the 2 streams were more striking than the differences. The total number of individuals and standing-crop biomass as well as volumetric displacement were not significantly different



FIG. 5. Mean number of benthic macroinvertebrate individuals (N = 3) in the fertilized and non-fertilized streams from May 1978 through August 1981.

suggesting that the community in Field Branch was not using the nitrate-nitrogen subsidy to increase biomass during the sampling period.

If nitrogen fertilization had an effect on the benthos it would most likely have been either a toxic one or one of enhancement. In the absence of pretreatment biological samples, the possibility remains that Field Branch was a system still undergoing recovery at the time of sampling. However, considering the length of time allowed for recovery (6 years), the absence of any other human-caused disturbance since fertilization, and the abundant sources of recolonizers within Robinson Forest, it would seem that recovery would have preceded the biological sampling (26, 27, 28, 29, 30, 31).

The evidence suggests that the treatment did not produce lasting effects upon the benthic community of Field Branch. The present community is not aberrant and, thus, is considered unaffected by the forest fertilization. However, considering the intimate relationship between a stream and its watershed (32) forestry management should advocate the accurate and judicious application of fertilizer. Care should be taken to avoid the direct application of nitrogen fertilizers into stream courses in order to decrease the possibility of toxic instream concentrations.

### ACKNOWLEDGMENTS

This work was funded in part by the Department of Forestry and the School of Biological Sciences at the University of Kentucky.



FIG. 6. Mean number of individuals (N = 3) in each order and others for the fertilized (triangle, dashed line) and non-fertilized (open circle, solid line) streams May 1978 through August 1981.

We thank the staff of Robinson Forest for their technical assistance. The late Dr. Robert Kuehne provided critical advice throughout this project. Mr. Melvin L. Warren, Jr. graciously reviewed the manuscript. Drs. Harley P. Brown and Guenter A. Schuster provided the identifications of the beetles and caddisflies, respectively.

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