APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1985, p. 1119–1123
0099-2240/85/051119-05$02.00/0

Vol. 49, No. 5

Nitrogen Dynamics in Stream Wood Samples Incubated with [^{14}C]Lignocellulose and Potassium [^{15}N]Nitrate†

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Received 26 December 1984/Accepted 14 February 1985

Surface wood samples obtained from a Douglas fir log (*Pseudotsuga menziesii*) in a Pacific Northwest stream were incubated in vitro with [^{14}C]lignocellulose in a defined mineral salts medium supplemented with 10 mg of N liter^{-1} of ^{15}N-labeled NO_3^- (50 atom % ^{15}N). Evolution of $^{14}CO_2$, distribution and isotopic dilution of ^{15}N, filtrate N concentrations, and the rates of denitrification, N_2 fixation, and respiration were measured at 6, 12, and 18 days of incubation. The organic N content of the lignocellulose-wood sample mixture had increased from 132 μg of N to a maximum of 231 μg of N per treatment after 6 days of incubation. Rates of [^{14}C]lignocellulose decomposition were greatest during the first 6 days and then began to decline over the remaining 12 days. Total CO_2 evolution was also highest at day 6 and declined steadily over the remaining duration of the incubation. Filtrate NH_4^+-N increased from background levels to a final value of 57 μg of N per treatment. Filtrate NO_3^- N completely disappeared by day 6, and organic N showed a slight decline between days 12 and 18. The majority of the ^{15}N that could be recovered appeared in the particulate organic fraction by day 6 (41 μg of N), and the filtrate NH_4^+ N fraction contained 11 μg of ^{15}N by day 18. The ^{15}N enrichment values of the filtrate NH_4^+ and the inorganic N associated with the particulate fraction had increased to approximately 20 atom % ^{15}N by 18 days of incubation, whereas the particulate organic fraction reached its highest enrichment by day 6. Measurements of N_2 fixation and denitrification indicated an insignificant gain or loss of N from the experimental system by these processes. The data show that woody debris in stream ecosystems might function as a rapid and efficient sink for exogenous N, resulting in stimulation of wood decomposition and subsequent activation of other N cycling processes.

Stream ecosystems in the Cascade Mountains of the Pacific Northwest are known to possess large accumulations of woody debris in the channels, particularly in mature forests dominated by Douglas fir (*Pseudotsuga menziesii*) (22). Although earlier studies have shown that wood decomposition in streams is a slow process, it may provide significant amounts of mineralized C and N to the biological community given the large accumulations of wood present (22, 23). Little is known about the physicochemical and nutritional factors that affect these decomposition processes in streams.

We previously demonstrated that measurements of decomposition with ^{14}C-labeled natural lignocellulose prepared from Douglas fir are a sensitive tool for investigating the dynamics of lignocellulose decomposition by the stream wood microflora (2). The results of our studies suggest that microbial communities on logs are restricted to the outer surfaces and respond to mineral nutrient supplements of N and P with increased rates of [^{14}C]lignocellulose mineralization. Previously it was shown that additions of NO_3^- in concentrations as low as 10.0 mg of N liter^{-1} can result in increased rates of [^{14}C]lignocellulose decay and concurrent accumulation of NH_4^+ in the culture filtrate (1). Preliminary data also demonstrated that the NO_3^- additions lead to a twofold increase in the organic N fraction of the [^{14}C]lignocellulose-wood inoculum mixtures (1). The kinetics of NO_3^- utilization and the subsequent transformations of N are fundamental aspects of wood decomposition given the low initial N content of the substrate and the low inorganic N concentrations in stream water (23, 24).

A combination of measurements of stable isotopes and radioisotopes was used to determine the sources of the filtrate NH_4^+ and the increase in organic N, along with their relation to [^{14}C]lignocellulose decay. Stream wood samples and [^{14}C]lignocellulose were incubated in a mineral salts medium with the addition of $K^{15}NO_3$. Measurements of $^{14}CO_2$ evolution, isotope ratio determinations, chemical analysis of culture filtrates, and gas chromatographic studies were performed on replicate samples to follow the N dynamics and lignocellulose decomposition in laboratory incubations over an 18-day period.

MATERIALS AND METHODS

Sample collection and preparation. Surface wood scrapings were obtained in April 1984 from a stream-wetted section of a Douglas fir log that had been wind-felled 7 years previously into Mack Creek, a third-order stream located at an elevation of 830 m in the H. J. Andrews Experimental Ecological Reserve, Cascade Mountain Range, Oregon. Wood samples were placed in sterile Whirl-Pak bags, stored on ice, and returned to the laboratory for processing. The scrapings were homogenized in distilled water for 8 min at a setting of 30 in a VirTis model 45 homogenizer (VirTis Co., Inc., Gardiner, N.Y.). Subsamples (1.0 ml) of the homogenate containing approximately 11 mg (dry weight) of homogenized wood were used as microbial inocula for the series of decomposition studies described below.

[^{14}C]lignocellulose decomposition experiments. Douglas fir [^{14}C]lignocellulose was prepared by incubating freshly cut

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branches with [^{14}C]phenylalanine or [^{14}C]glucose to preferentially label the lignin or cellulose fractions, respectively. The methods used to prepare and thoroughly characterize the radiolabeled lignocellulose are those of Crawford (6) and are described in detail in a companion paper (1). The incubations were conducted over 18 days, with $^{14}CO_2$ accumulation measured at 6-day intervals.

^{15}N studies. A series of experimental incubations were prepared, using a defined mineral salts medium in which the supplemental N (10.0 mg of N liter^{-1}) contained $K^{15}NO_3$ (50 atom % abundance; Amersham Corp., Arlington Heights, Ill.). The culture filtrate and particulate organic matter of 10 replicate bottles were analyzed for the distribution of ^{15}N after 6, 12, and 18 days of incubation (7). The entire contents of each serum bottle were filtered through 0.45-μm filters (Millipore Corp., Bedford, Mass.), and the particulates remaining on the filter were rinsed with 5 ml of distilled water. A KCl extraction step was included in preliminary experiments and was shown not to increase recovery of NH_4^+-N in the filtrate. A portion of the filtrate was reserved for NO_3^-, NO_2^-, and NH_4^+ analysis on a Technicon Autoanalyzer II (Technicon Instrument Corp., Cherrytown, N.Y.). The remainder was stored at 4°C for no longer than 24 h before distillation. The NH_4^+-N in 5 ml of filtrate was recovered by steam distillation for 7 min in the presence of NaOH in an all-glass microdistillation apparatus with Teflon stopcocks. Distillate was collected in 20 ml of distilled water (pH 5.5) from which a 7-ml subsample was removed for determination of NH_4^+-N, and the remainder was acidified with 2 drops of 0.05 M H_2SO_4. Distillation of known amounts of NH_4^+-N demonstrated that the pH 5.5 distilled water completely trapped the low amounts of N encountered in our experiments. Acidified samples were spiked with 50 μg of N as unenriched $(NH_4)_2SO_4$ (0.3683 atom % ^{15}N) and evaporated to dryness. An unenriched sample of $(NH_4)_2SO_4$ was distilled after each ^{15}N sample to eliminate the chance of cross-contamination of ^{15}N between distillates.

The particulate material remaining on the Millipore filter was dried at 50°C and digested for 10 h in 2 ml of Kjeldahl catalyst digestion solution. The digest was diluted with 11 ml of distilled water, and 5 ml was used for distillation and recovery of NH_4^+-N as described above. NO_3^- plus NO_2^- N in the digest was recovered as NH_4^+-N by the addition of 0.2 g of Devarda alloy and distillation for an additional 7 min.

Sodium hypobromite was added to the reconstituted samples to release N_2, and the isotopic ratio was determined in a Nuclide RMS 3-60 mass spectrometer by Isotope Services, Inc., Los Alamos, N.M. Two to four subsamples of each sample were analyzed, and the mean isotopic ratio was determined.

Gas chromatography. Additional replicates of the N-enriched incubation treatments were removed at 6, 12, and 18 days for determination of respiration, denitrification, and N_2 fixation rates. Respiration measurements on six replicate samples were made on each date by injecting 0.2-ml samples of headspace gas into a Hewlett-Packard 5700A gas chromatograph fitted with a 1.5-m Porapak Q column and a thermal conductivity detector. Gas samples were removed from each bottle at 0, 2, 4, and 6 h. Results were standardized against injections of various amounts of a standard gas mixture (1.06 ml of CO_2 liter^{-1} of N_2). Six replicate samples were used for determination of denitrification by the acetylene block technique (14). Calcium carbide-generated acetylene was injected to provide a final concentration of 10% (vol/vol) in air. An additional six replicates containing no acetylene were used to assay for acetylene-independent N_2O production.

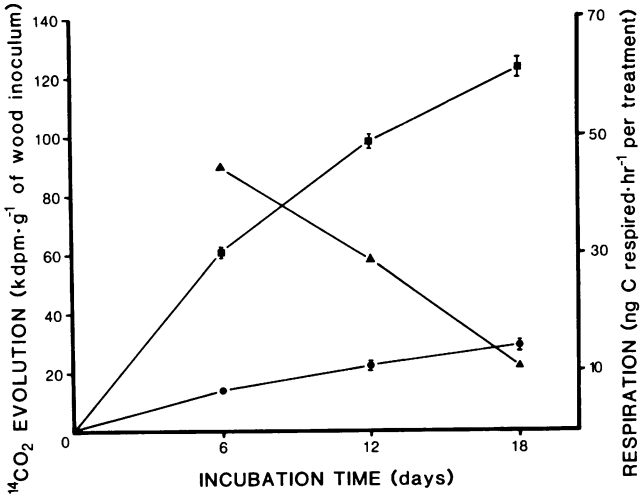

FIG. 1. [^{14}C]lignocellulose decomposition and respiration rates over time in samples incubated in mineral salts solution with supplemental KNO_3 (10 mg of N liter^{-1}). Symbols: ■, [^{14}C]cellulose; ●, [^{14}C]lignin; ▲, respiration rate. Each point is the mean of five or six replicates. Bars, Standard errors of the means.

Samples of headspace gas (0.2 ml) were injected at 0-, 2-, 4-, and 6-h intervals into a Hewlett-Packard 5840A gas chromatograph fitted with a 4-m Porapak Q column and a ^{63}Ni electron capture detector. The sample bottles containing 10% (vol/vol) acetylene were also assayed for acetylene reduction (N_2 fixation) over the same time intervals by injecting 0.2-ml gas samples into a Hewlett-Packard 5830A gas chromatograph containing a 1.5-m Porapak R column and a flame ionization detector. The denitrification and acetylene reduction results were standardized by injections of various amounts of an N_2O-in-N_2 mixture (4 μl of N_2O liter^{-1} of N_2) and an ethylene-in-N_2 mixture (2 μl of C_2H_4 liter^{-1} of N_2) respectively. Results were corrected for background levels of N_2O and ethylene contained in the acetylene and the CO_2 in air.

RESULTS

Previous data from our laboratory have shown that high concentrations of NO_3^- (318.0 mg of N liter^{-1}) stimulate the decomposition of both the lignin and cellulose fractions of [^{14}C]lignocellulose. Results presented in Fig. 1 show that a lower concentration of NO_3^- (10.0 mg of N liter^{-1}) can also support relatively high rates of $^{14}CO_2$ evolution from both fractions, with the greatest rates observed between days 0 and 6 of the incubation. The subsequent rates of lignocellulose decomposition began to diminish over the remaining 12 days of the incubation period. This pattern coincided with a decrease in the rates of microbial respiration measured by gas chromatography on samples which had been incubated for 6, 12, and 18 days, respectively (Fig. 1).

A mass balance of N in these NO_3^--supplemented systems showed a large increase (112 μg of N per treatment) in the Kjeldahl N fraction over the first 6 days of incubation (Fig. 2). The initial NO_3^- addition (200 μg of N per treatment) decreased below the limits of detection by day 6 of incubation (Fig. 2). Since filtrate NH_4^+ and NO_2^--N concentrations were very low (~3 μg of N per treatment) over the same time period, the data suggest that the majority of the NO_3^- was assimilated into organic fractions and not simply

reduced to NH_4^+ and immediately excreted into the medium. Measurements of denitrification and acetylene reduction showed that no appreciable losses or gains of N occurred through either of these pathways over the course of the experiment (Table 1). Filtrate NH_4^+-N increased to 17 and 57 μg of N per treatment by days 12 and 18, respectively. In fact, this latter value is equivalent to 20% of the total combined N found in the 18-day sample and coincides with a decrease in the organic N fraction of the particulates. The increase in filtrate NH_4^+-N occurred during the time when NO_3^- was undetectable in the medium. The addition of Devarda alloy to the Kjeldahl samples after distillation of the NH_4^+ resulted in the detection of additional NH_4^+ (10 to 15 μg of N per treatment), which we infer originated from NO_3^- sequestered by the woody material and not reduced by microbiological activity.

Results of the ^{15}N analyses of the samples show that the majority of ^{15}N that was recovered was associated with the particulate fraction (Fig. 3). The ^{15}N incorporated into the organic fraction between days 0 and 6 amounted to 44 μg of ^{15}N per treatment. When this value is corrected for the 50 atom % ^{15}N of the NO_3^- amendment, 81% of the N increase in the particulate fraction originated from the NO_3^- supplement. The amount of ^{15}N found in the filtrate NH_4^+-N fraction was small, but significantly above the natural abundance of ^{15}N, over the first 12 days of incubation. There was a large increase in the filtrate $^{15}NH_4^+$ content between days 12 and 18, with 11 μg of ^{15}N eventually recovered in the filtrate NH_4^+-N fraction at day 18. It can be seen that 53 μg of the original 100 μg of ^{15}N per treatment was accounted for in the filtrate and particulates at the end of the incubation period.

The data presented in Fig. 4 provide some insight into the complexity of the NO_3^- processing within the system. The initial large increase in ^{15}N enrichment of the organic N fraction and in the inorganic N fraction associated with the particulate matter coincided with increases in absolute amounts of ^{15}N shown in Fig. 3. The filtrate NH_4^+-N fraction was enriched to 4.5 atom % ^{15}N within 1 h of the addition of the $K^{15}NO_3$ to the [^{14}C]lignocellulose-wood inoculum mixture. The atom percent ^{15}N values declined between that time and day 6 of the incubation and then increased to a final value of 20 atom % ^{15}N. By 18 days of incubation, the enrichment of all three N fractions was similar. The data suggest that, although much of the NO_3^- was rapidly sequestered and metabolized into organic N, further turnover of this fraction, involving mineralization and nitrification, became significant during the later stages of the incubation and coincided with the decrease in respiration (Fig. 1).

TABLE 1. Denitrification and acetylene reduction rate in [^{14}C]lignocellulose-wood sample incubations over time[a]

Incubation time (days)	Production of N_2O (μg of N day^{-1} $treatment^{-1}$)[b]		Acetylene reduction
	With acetylene	Without acetylene	
6	0.74 (0.23)	0.17 (0.06)	ND[c]
12	0.03 (0.02)	0.02 (0.01)	ND
18	ND	0.01 (0.01)	ND

[a] Each value is the mean of six replicates, and standard errors are in parentheses.
[b] Samples were incubated with and without 10% (vol/vol) acetylene to determine acetylene-dependent and -independent N_2O generation.
[c] ND, Not detectable.

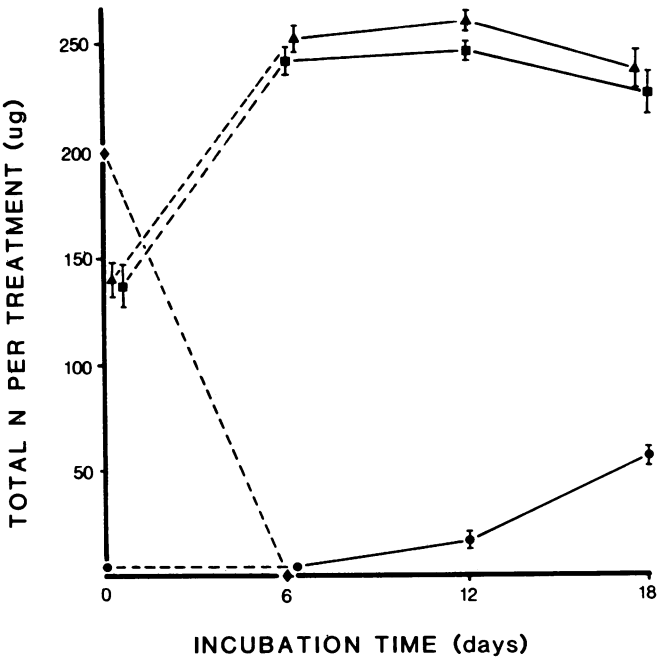

FIG. 2. Distribution of N in [^{14}C]lignocellulose-wood sample incubations over time. Symbols: ■, organic N; ▲, organic N plus inorganic N associated with particulates; ●, filtrate NH_4^+; ◆, filtrate NO_3^--N. Each point is the mean of 10 replicates. Bars, Standard errors of the means.

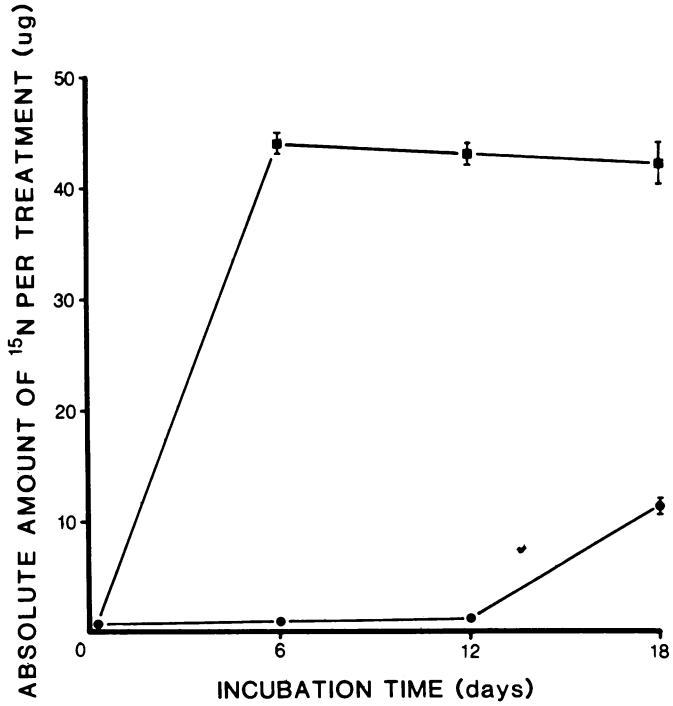

FIG. 3. Distribution of ^{15}N in [^{14}C]lignocellulose-wood sample incubations over time. Symbols: ■, organic N; ●, filtrate NH_4^+-N. Each point is the mean of 10 replicates. Bars, Standard errors of the means.

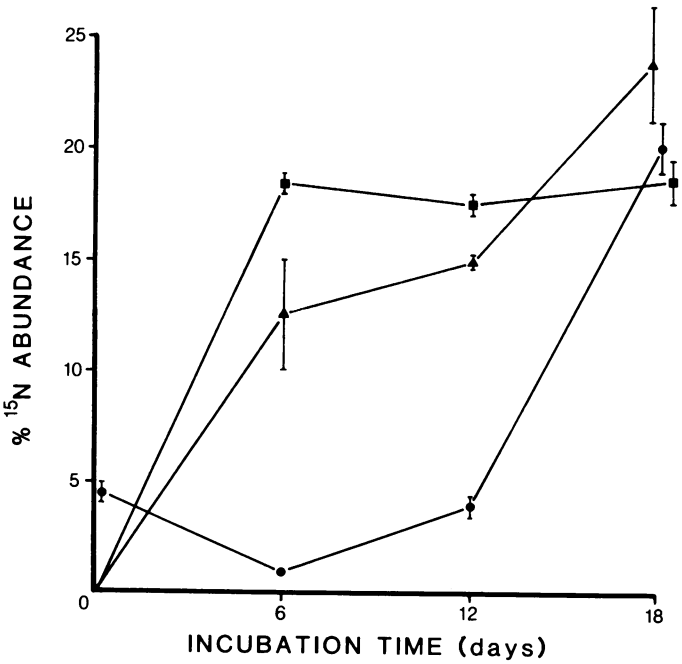

FIG. 4. Percent abundance of ^{15}N in [^{14}C]lignocellulose-wood sample incubations over time. Symbols: ■, organic N; ▲, inorganic N associated with particulates; ●, filtrate NH_4^+-N. Each point is the mean of 10 replicates. Bars, Standard errors of the means.

DISCUSSION

The experimental results presented here represent the first use of a combination of stable isotopes and radioisotopes to examine the interaction of supplemental combined N with lignocellulose decomposition in stream wood samples. The data from the ^{15}N analyses demonstrate that the addition of NO_3^--N to laboratory incubations of stream wood samples and [^{14}C]lignocellulose can lead to N accretion in the organic fraction (Fig. 2 and 3). Even though the organic substrate in this experiment consists mainly of lignocellulose, which is decomposed relatively slowly, NO_3^- from the surrounding medium may be immobilized rapidly. Absolute increases in N content of organic detritus have been reported, from both laboratory decomposition experiments and weight-loss studies conducted in situ (3, 10, 12, 13, 15, 19, 23). The mechanisms for this N increase have not been verified, but have been suggested to include N_2 fixation (23) and microbial uptake of combined N from the surrounding environment (J. M. Melillo, R. J. Naiman, J. D. Aber, and A. F. Linkins, Bull. Mar. Sci., in press; D. L. Rice and R. B. Hanson, Bull. Mar. Sci., in press). A third possible mechanism not often considered includes abiotic surface charge phenomena such as anion and cation exchange. Whereas the latter two pathways can only be speculated upon at this point, the acetylene reduction assays performed on replicates of our experimental treatments suggest that N_2 fixation was not a substantial source of combined N in this instance.

The most intense biological activity in the experimental treatments occurred during the first 6 days of incubation. The greatest rates of [^{14}C]lignocellulose degradation and respiration (as measured by CO_2 production) and all of the N uptake occurred during this period (Fig. 1 and 2). Although surprising from the perspective of lignocellulose recalcitrance to microbial decomposition, the rapid response to nitrogen supplementation agrees with our previous observations of the effect of N on the enhancement of lignocellulose decay (2). Given the low concentrations of N in large woody debris and in the stream waters of the Pacific Northwest, external N supplementation would be expected to stimulate microbial activity. The subsequent decline in activity observed in our studies and those of other investigators suggest that available N is depleted or that C limitation eventually becomes the controlling factor (11, 15, 20). Further study with flowthrough systems with continual additions of N are needed, however, to eliminate the nutrient depletion in our closed systems.

The increase in filtrate NH_4^+ enriched in ^{15}N toward the latter stages of incubation suggests that immobilized N is mineralized (Fig. 2). If the utilizable N and C sources become depleted, as indicated by the decline in [^{14}C]lignocellulose decomposition and overall respiration, the subsequent use of microbial C and release of immobilized N could increase. This possibility is supported by the observation of a decrease in the organic N fraction which coincided with the increase in filtrate NH_4^+-N between days 12 and 18. The possibility of nitrification processes occurring is supported by the increase in the ^{15}N abundance in the NO_3^--N associated with the particulate organic fraction.

Even though several different pathways of N processing can be inferred by the results of this experiment, a discrepancy exists in that 15% of the total N that was present at the beginning of the incubation period was unaccounted for at day 18. An apparent N loss such as this is typically explained by denitrification activity, yet our measurements of denitrification rates in replicate samples at days 6, 12, and 18 by the acetylene block technique suggest that an insignificant amount of N was lost as N_2 or as N_2O (Table 1). Although it is conceivable that the bulk of denitrification could have occurred between days 0 and 6 of the incubation, before the filtrate NO_3^--N was depleted, earlier denitrification studies in our laboratory on [^{14}C]lignocellulose-wood inoculum incubations under conditions conducive for denitrification (high NO_3^--N concentrations, anaerobic incubations) also showed insignificant losses of N (unpublished data). The percentage of ^{15}N unaccounted for at the end of this study (47%) was greater than the percentage of total N missing (15%). This observation implies that the missing N might be sequestered either in its original form of NO_3^- by the lignocellulose and wood substrate or in an organic form that was not recovered quantitatively by the procedures used in this study.

The appearance of ^{15}N enrichment in the filtrate NH_4^+-N fraction after only 1 h of incubation could have resulted from dissimilatory reduction of NO_3^- to NH_4^+. This process has been reported in soils, aquatic sediments, and water, although usually under oxygen-limited conditions (4, 5, 9, 16, 18). Even then, the process is usually responsible for only a small percentage of the total N flux, as was the case here (8, 17, 21). The results do indicate, however, that a microbial population capable of this pathway exists on decomposing wood substrates in stream environments.

The rapidity and efficiency of NO_3^- uptake exhibited in our laboratory experiments have important implications for nutrient cycling and wood decay in aquatic ecosystems. Stream water concentrations of N are low in Pacific Northwest streams and are mainly in the form of NO_3^- (24). Concentrations of NO_3^- increase during the high stream flows in the fall and winter and might result from soil solution inputs to the stream channel (24). Instead of these occasional pulses of NO_3^- being lost to the stream commu-

nity, the presence of extremely large accumulations of woody debris, along with their resident surface microflora, could serve as a rapid sink for this N. Whether the mechanism of N uptake is biotic or abiotic, the increase in N could result in C release from lignocellulose and the eventual slow release of NH_4^+-N through mineralization for use by aquatic biota.

ACKNOWLEDGMENTS

We thank D. Myrold for reviewing the manuscript, K. Fairchild and D. Coffey-Flexner for excellent technical assistance, R. Y. Morita for use of the gas chromatographs, and The Central Chemistry Analytical Laboratory of the U.S. Forest Science Laboratory, Corvallis, Ore., for use of the Technicon Autoanalyzer. We also acknowledge Isotope Services, Inc., Los Alamos, N.M., for analysis of the ^{15}N samples and C. Pelroy for typing the manuscript.

This research was supported by National Science Foundation grants DEB80-04642, DEB80-12162, DEB80-22634, and DEB81-12455 and the Oregon Agricultural Experiment Station and is Riparian Contribution no. 21.

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