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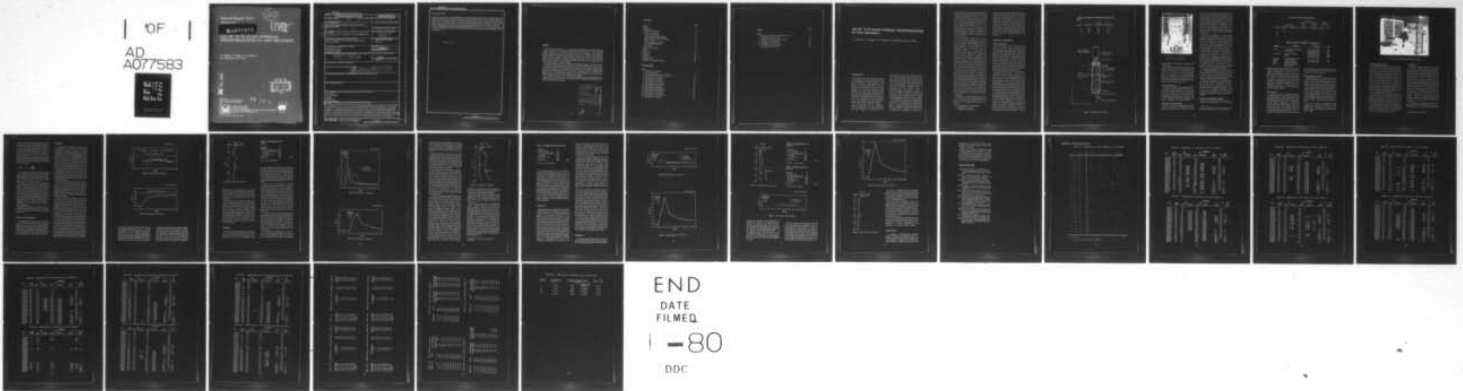
USE OF 15N TO STUDY NITROGEN TRANSFORMATIONS IN LAND TREATMENT. (U)

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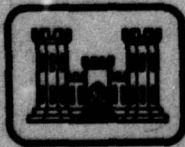
T.F. Jenkins, S.T. Quarry, I.K. Iskandar,
A.P. Edwards and H.E. Hare

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The objective of this study was to compare different strategies of using ¹⁵ N as a tracer to describe the fate of wastewater N in land application of wastewater. Four soil columns were packed with Windsor sandy loam soil and covered with forage grass. The columns were treated with 7.5 cm of either tapwater or wastewater according to four experimental strategies. The strategies varied the treatment given the soil prior to application of the ¹⁵ N label, the schedule and amounts of the applied ¹⁵ N label, and the type of water used for subsequent column leaching. Soil solution at depth and leachate were analyzed weekly for concentration and ¹⁵ N content of nitrate and ammonium. Plant samples were obtained periodically throughout the experiment and, together with soil samples collected at the end of the experiment,		

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analyzed for total nitrogen content and $^{15}\text{N}/^{14}\text{N}$ ratios. The results indicate that nitrification occurred rapidly in the column preconditioned with wastewater, and the resulting labeled nitrogen moved in a narrow band through the soil profile. In the other columns, nitrification was delayed, resulting in the formation of a lower total amount of labeled nitrate as well as a more diffuse band. A strategy consisting of a single application of the ^{15}N label in wastewater with a high enrichment, preceded and followed by wastewater applications, permitted the monitoring of nitrification, movement of NO_3^- in the soil and plant uptake subsequent to application of the label. The other strategies were found to be useful for obtaining specific types of information relative to the operation of land treatment systems and other soil N pathways.

NO₃(-)

PREFACE

This report was prepared by T.F. Jenkins, Research Chemist, S.T. Quarry, Physical Science Technician, Dr. I.K. Iskandar, Research Soil Chemist, and H.E. Hare, Physical Science Technician, of the Earth Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory, and Dr. A.P. Edwards, Senior Research Fellow, University of New Hampshire. The work discussed in this report was conducted under Corps of Engineers Civil Works Project CWIS 31314, as part of the Corps of Engineers Land Treatment of Wastewater Program. This report was technically reviewed by J.H. Cragin and D.C. Leggett of CRREL.

The authors would like to thank Christine McDade, Bethany Blake and James Coleman for the fine technical assistance in preparation of the soil columns and in sample collection, Patricia Schumacher for supervision of the water chemistry analyses—particularly the forms of nitrogen in soil solution samples, Ellen Foley for her help in assembling the information for the preparation of the manuscript, and Eleanor Huke for drafting of the figures used in the report. Daniel Leggett is also acknowledged for useful discussions which assisted in choosing various experimental strategies.

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USE OF ^{15}N TO STUDY NITROGEN TRANSFORMATIONS IN LAND TREATMENT

T.F. Jenkins, S.T. Quarry, I.K. Iskandar, A.P. Edwards and H.E. Hare

INTRODUCTION

Within the past decade, widespread interest has developed in the use of land application to treat and dispose of municipal sewage. A great deal of research has been conducted on the methods of applying wastewater to the land without adversely affecting the environment. Research on slow rate irrigation systems (Iskandar et al. 1976, Sopper and Kardos 1973) has consistently indicated that the leaching of nitrate nitrogen through the soil profile is a problem which limits the wastewater loading rate. Since land requirements largely determine system costs, methods to increase loading rates and minimize nitrate leaching are needed.

The predominant form of nitrogen in municipal wastewater is usually ammonium. While ammonium does not leach through the soil under ordinary circumstances, it is

transformed to nitrate by nitrifying bacteria in the soil. Once formed, nitrate has little interaction with soil particles and will readily leach downward in the soil profile with percolating water. The rate at which nitrification takes place during land treatment and its dependency on various environmental conditions such as temperature, pH, and moisture content is unknown (Leggett and Iskandar in press). In order to develop strategies to minimize the leaching of nitrate, an accurate understanding of the rate of this transformation and its quantitative dependency on these factors is needed.

One method of studying the transformations of nitrogen in soil systems is the use of the stable isotope ^{15}N . This isotope is present in the atmosphere at a natural abundance of 0.366% (Junk and Svec 1958) and is chemically equivalent to the more abundant isotope ^{14}N . Since ^{15}N is nonradioactive, it requires no licensing or

special handling and has been used extensively in agronomic research (Bremner 1965). Specialized analytical equipment, however, is needed to determine the extent of incorporation of the label in soil, plant material and water samples. The availability of this type of equipment and the cost of the labeled material have limited its use in land treatment studies. Thus few, if any, detailed studies on the slow rate mode of land treatment (crop irrigation) have utilized the ^{15}N labeling method. A capability at CRREL was recently developed, however, to utilize the ^{15}N tracer method for just such a purpose (Jenkins and Quarry 1978).

When any labeled material, such as ^{15}N , is used in small-scale simulations of natural systems, the experimental design should be as close as possible to real conditions. This is to allow extrapolation from the behavior of the labeled material in the simulation to that of unlabeled material in the real system. In agronomic studies, the ^{15}N is generally incorporated into the fertilizer which is applied as usual. No further application of nitrogen is involved, even if irrigation is required. In land treatment, however, nitrogen is added to the site with each application of wastewater. Since labeling experiments in slow rate land treatment studies have not often been conducted, the best way of applying the label has not been determined and several alternatives are possible. The ^{15}N label could be incorporated with each application during the entire study, or it could be applied only once followed by unlabeled applications. The label could be incorporated in the first wastewater application to study the initial buildup of nitrifying organisms or the soil could be conditioned with several wastewater applications prior to the addition of the label and the steady-state conditions investigated. Following the application of the label, the columns could be leached with additional wastewater to maintain the steady-state conditions throughout the study or with tapwater to minimize dilution of the label and simplify calculations.

In order to choose among these alternatives, we conducted a small-scale preliminary study, which is described in this report. Several combinations of the following alternatives were tested:

1. Pre-application soil conditioning,
2. ^{15}N application strategy,
3. Post-application leaching.

The results from this study will be used to design a more elaborate lysimeter study. The ultimate purpose of this type of research is to gain a quantitative understanding of the fate of wastewater nitrogen in the soil. The results from these studies will be used to obtain rate coefficients for a mathematical representation of the nitrogen cycle in the soil for slow rate land treatment systems. Another objective of this particular study was to test all procedures for sample acquisition, processing and analysis prior to the onset of the more elaborate lysimeter experiments.

MATERIALS AND METHODS

Column construction

Four soil columns were constructed from 11.1-cm i.d. Lucite tubing cut into 122-cm lengths. Holes (6.4 mm diam) were drilled every 15 cm along the length of the tubes in four rows 90° apart. The inner walls were coated with Ottawa sand (20-30 mesh). This was done by 1) pouring a small amount of methylene chloride (CH_2Cl_2) along the sides, causing the surface to soften, 2) pouring in sand, and 3) shaking vigorously to coat the entire inner surface. The excess sand was poured off and the remaining methylene chloride was allowed to evaporate. The coating process minimizes channeling of water along the soil/column interface.

One end of each column was then closed with a plexiglass cap in which a small hole was drilled; then a nylon fitting was placed in the hole and connected to Tygon tubing for collection of leachate. A layer of Fiberglas cloth was placed inside the tube at its base, covered with approximately 3 cm of sand and gravel, and finally another layer of Fiberglas cloth was placed on top of the gravel. The columns were then packed with Windsor sandy loam soil to a total soil profile depth of 107 cm, recreating as closely as possible the physical characteristics found in the undisturbed soil. The dry bulk density of each horizon is given in Table 1. Ceramic porous tensiometer tubes (0.64 cm diam) were placed in the soil through drilled holes at 15- and 45-cm depths to allow collection of soil solution samples. The remaining holes were plugged with glass wool to allow air to diffuse into the columns horizontally at depth, but to prevent outflow of soil solution. Opaque duct tape was later wrapped around the columns to prevent

Table 1. Dry bulk density of Windsor sandy loam soil (g/cm³).

Column	Horizon		
	A(0-15 cm)	B(15-30 cm)	C(30-107 cm)
I	1.58	1.58	1.52
II	1.43	1.71	1.53
III	1.48	1.74	1.48
IV	1.41	1.71	1.53

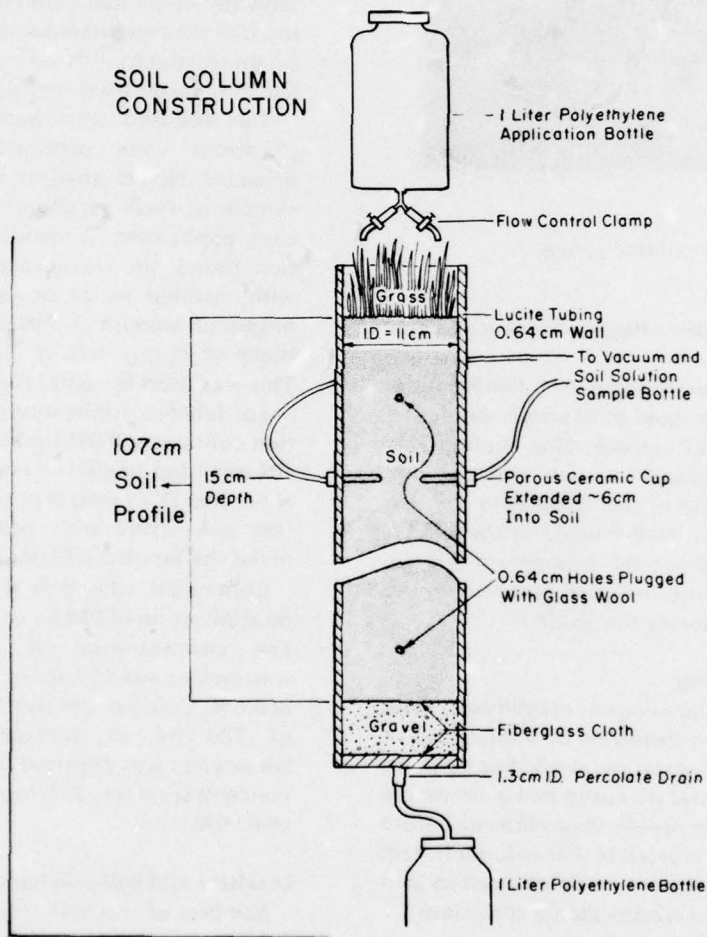


Figure 1. Diagram of soil columns.

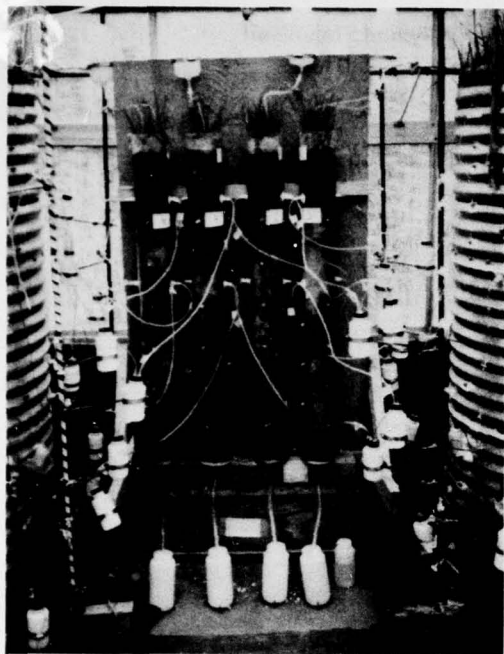


Figure 2. Soil column set-up.

algal growth and other effects of sunlight on the column walls.

Four sections of grass sod were taken from the CRREL lawn and trimmed to fit snugly inside the columns on the soil surface. One section was placed on the surface of each soil column. Figure 1 is a diagram of the completed soil columns. The columns were housed in the CRREL greenhouse throughout the experiment (Fig. 2). Greenhouse temperatures were generally in the range of 20-25°C during the study.

Column conditioning

The four soil columns were conditioned prior to the start of the experiment by application of 780 ml (8.05 cm) of water per week. For columns I, III and IV, tapwater was used and a conditioning period of three weeks was allowed before the application of labeled N. For column II, tapwater was applied for one week followed by four weeks of unlabeled wastewater applications.

Application of labeled nitrogen

After the columns were preconditioned, 780 ml (8.05 cm) of wastewater labeled with ^{15}N was applied to each column. For columns II, III and

IV, the ^{15}N was applied as ammonium at an atom %* of 6.6 and a concentration adjusted to approximately 25 mg/l $\text{NH}_4\text{-N}$. Application of the label was made on 25 October 1977 to columns III and IV and on 14 November to column II. Column I received wastewater containing ^{15}N labeled ammonium adjusted to 25 mg/l-N on 25 October but the ^{15}N atom % was only 0.99%. Column I received wastewater labeled with ^{15}N in this way once per week for the remainder of the experiment, for a total of 24 individual weekly applications.

Following the application of the labeled nitrogen, columns II and III received 780 ml of wastewater adjusted to 25 mg/l $\text{NH}_4\text{-N}$ once per week for the remainder of the experiment. Column IV was treated with the same volume of tapwater on an identical schedule. Table 2 summarizes the experimental strategies with respect to pretreatment, ^{15}N enrichment, and type of leaching water used for each of the columns.

The adjusted wastewaters and labeled applications were prepared as follows. Colorimetric $\text{NH}_4\text{-N}$ analysis was performed on a sample of fresh secondary wastewater prior to each application. Depending on the concentration found, the wastewater was either diluted with distilled water or enriched with an appropriate amount of NH_4Cl to obtain a 4-liter batch of 25 mg/l $\text{NH}_4\text{-N}$ "adjusted" wastewater. This was used for unlabeled applications.

For labeled applications, a 1-liter stock solution containing 0.0955 g NH_4Cl with 99 atom % ^{15}N was used to give a concentration of 26.3 mg N per liter (1.75 meq N per liter). This stock solution was mixed with adjusted wastewater to make the labeled solutions for application.

Columns II, III and IV received a single labeled application of 780 ml of adjusted wastewater. The concentration of ammonium in this wastewater was 25.1 mg-N/l with an atom % ^{15}N of 6.6%. Column I received a weekly application of 780 ml of adjusted wastewater. This wastewater was adjusted so that the ammonium concentration was 25.0 mg/l with an atom % ^{15}N of 0.99%.

Leachate and soil solution sampling

Samples of leachate from the base of the soil columns and soil solution at 15- and 45-cm

* Number of atoms of the mass 15 isotope of nitrogen compared with total number of atoms of nitrogen present.

Table 2. Experimental strategies used.

Column	Pretreatment		Enrichment (¹⁵ N)		Column Leaching	
			High-6.6%	Low-0.99%	Tap-	Waste-
	Tapwater	Wastewater	(Single Application)	(Multiple Applications)	water	water
I	*		*	*		*
II		*	*			*
III	*		*			*
IV	*		*		*	

Table 3. Summary of methods for water analysis.

Parameter	Method	Analysis of EPA std samples	% Error
Nitrate-N	Automated cadmium-reduction, diazo-coupling	Low range (0-1.0 ppm)	8.8%
		mid range (1-10 ppm)	5.8%
		High range (10-25 ppm)	3.9%
Ammonium-N	Automated colorimetric, phenate	Low range (0-1.0 ppm)	8.7%
		Mid range (1-10 ppm)	3.6%
		High range (10-25 ppm)	2.4%
Nitrite-N	Automated diazo coupling	---	
Total Phosphorus	Continuous digestion, automated molybdate	Low range (0-2.0 ppm)	5.0%
		High range (2-10 ppm)	1.3%
Chloride	Automated thiocyanate	---	
pH	Manual, Orion Research digital pH meter	---	

depths were collected once per week. The soil solution samples were obtained with porous ceramic tensiometers by application of a uniform 4-psi (27.6 kPa) suction for a period of 12-16 hours. In practice, the device controlling the vacuum occasionally malfunctioned, resulting in a suction of up to 15 psi (103.4 kPa). Leachate was obtained by gravity flow from the bottom of the columns and collected in 1-liter polyethylene bottles. All samples were immediately analyzed for pH and refrigerated at 4°C until further analysis.

Water analysis

A sample of the adjusted secondary wastewater applied to the columns was collected and analyzed for nitrate, ammonium, total phosphorus, pH and chloride. The samples were occasionally analyzed for nitrite. The leachate and soil solution samples were analyzed for nitrate, ammonium, total Kjeldahl nitrogen and occasionally nitrite. The methods used for analysis, reported in detail elsewhere (Iskandar et al. 1976), relied, for the most part, on use of Technicon II autoanalyzers. Table 3 summarizes

the methods used for each type of analysis and the results of analysis of EPA standard samples obtained during the experiment.

Plant sampling and analysis

Vegetation samples were obtained from each column twice before application of the labeled nitrogen and five times following application. In each case, the grass was clipped at approximately 6 cm above the surface. The fresh weight of each sample was obtained, the material was dried at 55°C and the dry weight measured.

The dried material was pretreated with KMnO₄ and iron powder for reduction of nitrate prior to digestion with H₂SO₄. The digest was steam distilled directly in the digestion flask according to Olsen (1929) and the distillate analyzed for NH₄-N colorimetrically (Nesslerization).

The atom % ¹⁵N in the digested, distilled samples was obtained by oxidation with hypobromite to nitrogen gas, and analysis by isotope ratio mass spectroscopy (Jenkins and Quarry 1978).

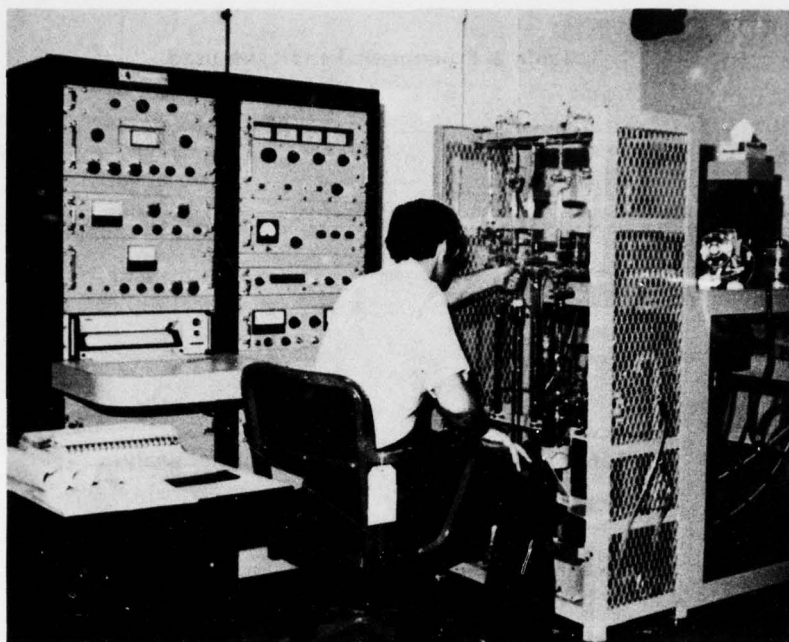


Figure 3. Nuclide 6-60 Ratio Mass Spectrometer.

Soil sampling and analysis

At the completion of the study, the soil was removed from each of the four columns and sectioned into 11 soil segments by depth, plus a vegetative mat sample. The vegetative mat sample was analyzed in an identical manner to the plant material described earlier. Each soil segment was analyzed for the concentration and atom % ^{15}N of mineral and total nitrogen. For mineral nitrogen determinations, a 5-g subsample of soil was mixed with 0.2 g MgO, 0.2 g Devarda's alloy, and 25 ml of a 1 N (1 normal) solution of potassium chloride and then steam distilled (Keeney and Bremner 1966). The ammonia produced was distilled and collected in a receiver acidified with 0.1 N H_2SO_4 .

A subsample of the ammonium solution produced was analyzed colorimetrically, while the remainder was saved for ^{15}N analysis. The total nitrogen determinations were obtained by digesting the soil by the reduced iron method as described previously in the plant analysis section. This procedure results in the inclusion of nitrate and nitrite-N as well as ammonium and organic nitrogen in the total value. The digests were steam distilled and the resulting ammonium solutions analyzed as above.

^{15}N Analysis

All the water samples were analyzed for atom % ^{15}N with a Nuclide 6-60 Ratio Mass Spectrometer (Fig. 3) as described in detail elsewhere (Jenkins and Quarry 1978). In this procedure, the water samples are mixed with MgO in a distillation flask, steam distilled and the condensate collected in 0.5 ml of 0.1 N H_2SO_4 . The ammonia in this condensate represents the ammonia in the original sample plus any extraneous N from the reagents and glassware. Devarda's alloy (0.2 g) is then added to the distillation flask and the sample is steam distilled again. The Devarda's alloy reduces nitrate in the sample to ammonia gas which is distilled and collected as ammonium in the acidified condensate. The ammonium represents the nitrate found in the original sample. If a sufficient quantity of nitrogen is present in both the ammonium and nitrate forms (>100 ug), both are subjected to ^{15}N analysis.

The ^{15}N analysis is obtained by placing 1 ml of the acidified ammonium solution in one arm of a Rittenburg tube*, 1 ml of sodium hypobromite

*As modified by Dr. A.P. Edwards.

solution in the other arm and attaching the tube to the vacuum system of the mass spectrometer. The tube is then evacuated and the contents of the two arms mixed, generating nitrogen gas. This gas is then allowed to enter the mass spectrometer. The mass spectrometer is tuned to focus the peak of mass 28 ($^{14}\text{N}_2$) on the major isotope collector and the peak of mass 29 ($^{14}\text{N}^{15}\text{N}$) on the minor isotope collector. The ratio (R) of mass 28 to 29 is then obtained from the balance panel and the atom% ^{15}N calculated from the equation:

$$\text{Atom\% } ^{15}\text{N} = \frac{100}{2R + 1}$$

The ammonium solutions resulting from steam distillation of the plant material digests were analyzed in an identical manner. For soils, the ammonium solutions resulting from distillation of mineral nitrogen and total nitrogen digests were also treated as above.

Since there is no universally accepted standard for ^{15}N analysis, all values were determined relative to a particular bottle of Fisher Certified Primary Standard $(\text{NH}_4)_2\text{SO}_4$ which on analysis was found to have an identical atom % ^{15}N as atmospheric nitrogen (0.366%). The difference in the ^{15}N atom % of this standard was compared to that for Mallinckrodt AR $(\text{NH}_4)_2\text{SO}_4$ over the course of the study. After 50 determinations, the mean difference in atom % ^{15}N was found to be 0.0010% with a standard deviation of $\pm 0.0006\%$ or 0.16% of the ratio $^{15}\text{N}/^{14}\text{N}$ at natural abundance.

Secondary wastewater was analyzed for 10 different days and was found to have a ^{15}N atom % of 0.371 ± 0.002 relative to the Fisher standard (0.366% ^{15}N).

RESULTS AND DISCUSSION

A complete tabulation of the results obtained from this experiment is presented in Appendix Tables A1-A21. This includes the quantity and composition of the water applied to the columns (Table A1), soil solution samples (Tables A2-A13), soil samples taken at the completion of the study (Tables A18-A21), and vegetative yield and composition over time (Tables A14-A17).

COLUMN I

The atom % ^{15}N in the nitrate from soil solution samples from column I over the study is presented in Figure 4. Column I received wastewater in which the ammonium was labeled with 0.99 atom % ^{15}N weekly throughout the experiment by addition of labeled NH_4Cl . Since this column was not conditioned before the onset of the experiment, the nitrate concentrations were too low for ^{15}N analysis in the early weeks of the experiment for both the soil solution at 15 and 45 cm and the leachate. Once concentrations were sufficient for analysis, the atom % ^{15}N tended to increase slowly and then level off between 0.6 and 0.7 atom %. This value is considerably lower than the 0.99 atom % applied in the wastewater. This difference is to be expected and is due to dilution of the label with nitrate nitrogen produced by mineralization of soil organic matter.

Analysis of the plant material taken from column I over the study (Fig. 5) indicates a gradual rise in the ^{15}N content through the first 10 weeks and then a leveling off at about 0.7 atom % through the remainder of the study. This value is slightly higher than the ^{15}N content of the nitrate in the soil solution samples, possibly due to the ability of the plant to take up both ammonium and nitrate nitrogen.

Analysis of the total nitrogen in the surface soil at the end of the experiment for column I (Fig. 6) indicates an increase in the atom % ^{15}N from natural abundance of about 0.366% to 0.385%. This is due to the incorporation of the labeled nitrogen into soil organic matter by microbial activity and plant detritus. The atom % ^{15}N in the plant material is a measure of the proportion of nitrogen taken from the wastewater relative to that from mineralization of soil organic matter. Since the wastewater had a ^{15}N atom % of 0.99% while the initial surface soil had a ^{15}N concentration of 0.366 atom %, a value of about 0.7% in plant material indicated that about 53% originated from wastewater-N. Thus only a little over half of the nitrogen taken up by the vegetation was from the nitrogen applied in the wastewater, while the rest came from nitrogen that originated from soil organic matter. The same is true of the nitrate found in soil solution and leachate samples. Thus applying the label with each application, as was done

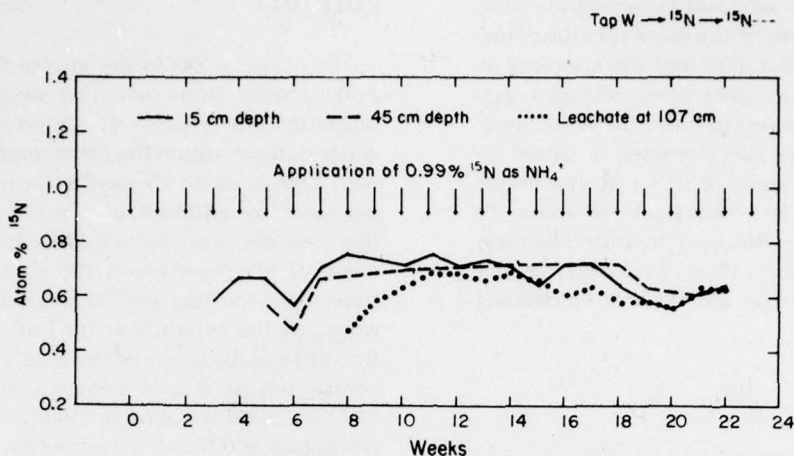


Figure 4. Soil solution ^{15}N , column I.

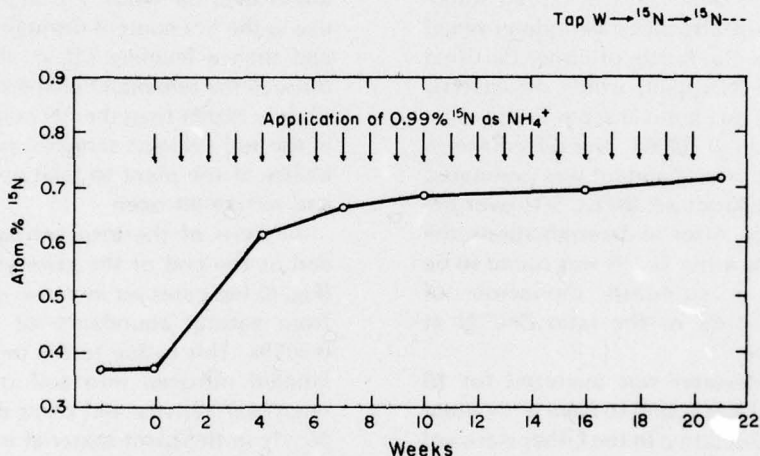


Figure 5. Plant material ^{15}N , column I.

in column I, allows an estimate of the source of the nitrogen absorbed by plants or leached through the soil. This strategy is not useful for studying the rate of nitrogen transformation since it is not possible to discriminate between the nitrogen coming from different applications. The lack of preconditioning before the onset of the study caused a lack of a sufficient quantity

of nitrogen in the soil solution samples to obtain ^{15}N data for the first several weeks of the experiment. Isotope dilution techniques could have been used on some of these samples but would have resulted in somewhat lower analytical precision. This lack of preconditioning presumably introduced a lag period in nitrification since the time required for movement of

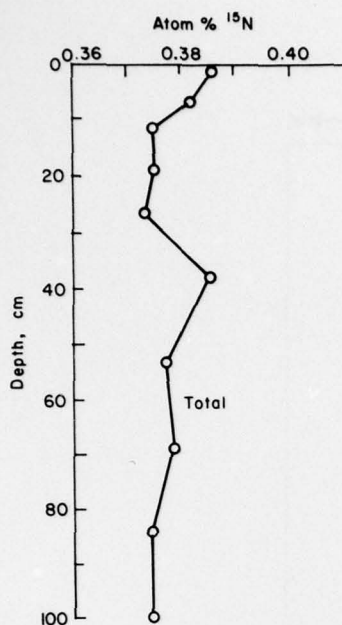


Figure 6. Soil profile ¹⁵N, column I.

labeled nitrate to the leachate was several weeks longer than in column II where preconditioning was used. This is due to the time required to build up the population of nitrifying organisms to the level where significant nitrification occurs.

Table 4 presents the amount of ¹⁵N applied to column I and the amount of ¹⁵N recovered in soil solution, leachate, plant material and soils. Only about 67% of the amount of ¹⁵N was recovered. The recovered ¹⁵N was about equally divided between soil, plant and water samples. Following the completion of this study, column I was leached with several applications of water to obtain hydraulic characteristics. The leachate solutions obtained were not analyzed for ¹⁵N and part of the low recovery is attributable to this oversight. Other possible sources of ¹⁵N loss will be discussed later.

COLUMN II

Column II was preconditioned with applications of adjusted wastewater for four weeks. Wastewater in which the ammonium was enriched to 6.6 atom % ¹⁵N with labeled NH₄Cl

Table 4. ¹⁵N budget (mg) for soil column I.

¹⁵ N Applied*	2.98	
Recovered		
¹⁵ N in water samples as NO ₃	0.53	
¹⁵ N in water samples as NH ₄	0.01	
¹⁵ N in plants	0.49	
¹⁵ N in vegetative mat	0.22	
¹⁵ N in soil	0.76	
Recovery of ¹⁵ N	2.01	67.4%

*In excess of natural abundance.

was then applied. Soil solution samples taken the following day at the 15-cm depth indicated that part of this labeled ammonium had already been transformed to nitrate. The lack of any significant lag time in nitrification is undoubtedly due to the preconditioning of the column, resulting in the buildup of a viable population of nitrifying organisms before the application of the ¹⁵N label.

Analysis of the soil solution at 15 cm during the following two weeks indicated a sharp increase in the nitrate-¹⁵N to over 2 atom %, followed by a drop to just over 1 atom % (Fig. 7). In the following weeks, the nitrate-¹⁵N at the 15-cm depth continued to decline toward natural abundance levels. These results seem to indicate a rapid nitrification process in which all the labeled ammonium that was to nitrify did so in the first week. The remaining ¹⁵N which did not nitrify was either assimilated into the soil nitrogen pool or taken up by the grass. This resulted in very little formation of labeled nitrate following this initial week and thus a sharp decline in the nitrate-¹⁵N at 15 cm after this period.

A movement of this peak of labeled nitrate through the soil column was found by analysis of the soil solution at 45 cm and the column percolate at 107 cm (Fig. 7). The highest ¹⁵N values appeared at the 45- and 107-cm levels at 2-3 and 6 weeks, respectively. Assuming a 1-week period of nitrate formation from ammonium, this indicates a retention time of about 5 weeks for nitrate in the 107-cm profile at the 8-cm/wk application rate.

The ammonium concentrations in soil solution at the 15-cm depth (Table A3) were quite low in the beginning of the experiment, rising to over 10 mg/l during the study, and declined

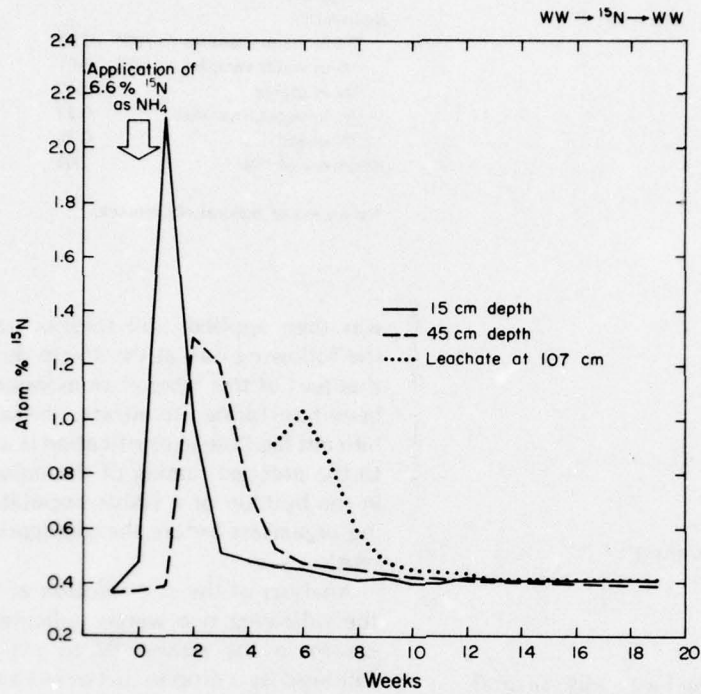


Figure 7. Soil solution ¹⁵N, column II.

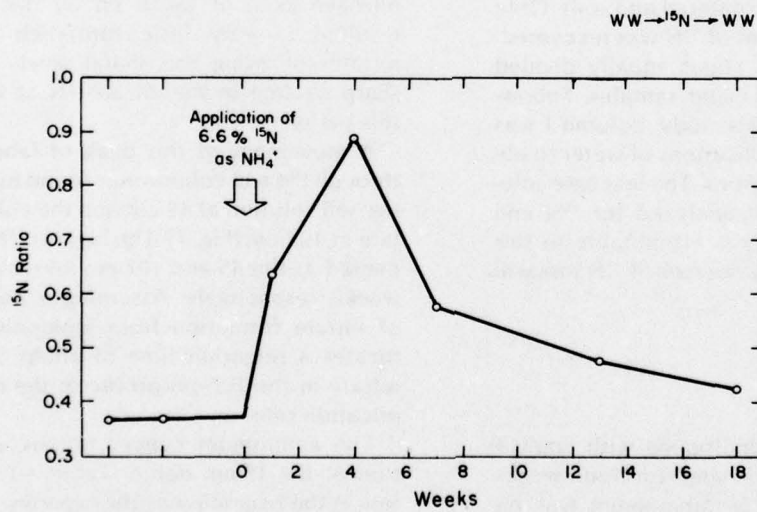


Figure 8. Plant material ¹⁵N, column II.

again to low levels toward the end. ^{15}N analysis of the ammonium demonstrated a much slower rate of movement of the labeled ammonium than found for nitrate, undoubtedly a result of interaction with cation exchange sites on the soil.

High concentrations of NH_4 (over 10 mg/l) were observed at 45 cm near the end of the experiment, but no values greater than 1 mg/l were found at the 107-cm level. This movement of ammonium in the soil columns was unexpected and may be due to the high suction applied in the soil solution samplers or to the method of drip application which did not evenly apply the water over the entire soil surface.

Plant uptake of the ^{15}N labeled nitrogen is shown in Figure 8. Analysis of the plant before the label was applied revealed ^{15}N content to be near natural abundance (0.366 atom %). Analysis of plant material a week after application of the label showed rapid uptake of the labeled nitrogen into the foliar portion of the plant. By the fourth week after application, the ^{15}N declined rapidly after this point due to dilution of the labeled application with further applications of wastewater N, continued mineralization of soil organic matter and the movement of labeled nitrate below the rooting zone of the plant.

^{15}N analysis of the soil at depth following the experiment (Fig. 9) indicated little change in the ^{15}N content of the total soil nitrogen, with the highest values only slightly elevated above natural abundance levels. Analysis of the inorganic portion of the soil nitrogen showed somewhat higher values. The profile of ^{15}N values for inorganic nitrogen shows a broad peak centered around 30 cm in depth and a second peak at the bottom of the column. There are several possible explanations for these elevated values near 30 cm. Labeled ammonium could be moving slowly through the column, an explanation consistent with results from soil solution samples at 15- and 45-cm depths showing relatively high concentrations of ammonium (Appendix Tables A3 and A7) and the low cation exchange capacity of this soil (about 7 meq/100 g). Another explanation is that root material and microbial biomass, having ^{15}N incorporated, could be mineralizing at depth. Alternately it is possible that some nitrate could be held on anion exchange sites on hydrous iron and aluminum oxides in the soil. This would also have shown up in the soil mineral nitrogen

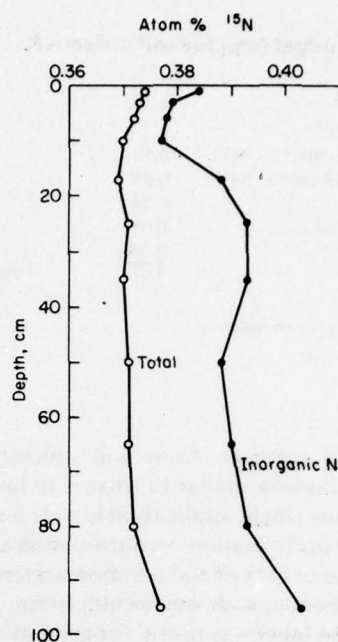


Figure 9. Soil profile ^{15}N , column II.

analyses. The ^{15}N peak for the soil at the base of the column is probably due to some residual ^{15}N labeled nitrate not completely drained.

After the completion of the experiment the dead plant material on the soil surface was also analyzed for total nitrogen (Appendix Table A15). This nitrogen was found to contain 0.441 atom % ^{15}N .

Table 5 presents the amount of ^{15}N applied to column II and the amount of ^{15}N found in the various categories. Only about 70% of the ^{15}N applied to column II could be recovered. Of this, the largest portion was found to be in the soil and plant material. The leachate accounted for only about 12% of the ^{15}N applied. The fate of the 30% ^{15}N not recovered is unknown. Some possibilities are loss of ^{15}N to the atmosphere as ammonia by volatilization or as nitrogen gas through denitrification of nitrate. The latter is more likely, as shown by the results from columns III and IV presented below. Labeled ammonium could also be lost by fixation within clay particles.* A small portion of the labeled nitrogen could also have been incorporated into some organic nitrogen form not amenable to Kjeldahl digestion.

*This possibility is currently under investigation by A.P. Edwards.

Table 5. ¹⁵N budget (mg) for soil column II.

¹⁵ N Applied*	1.30	
Total ¹⁵ N recovered*		
¹⁵ N in water samples as NO ₃ ⁻	0.16	
¹⁵ N in water samples as NH ₄ ⁺	0.01	
¹⁵ N in plants	0.28	
¹⁵ N in vegetative mat	0.08	
¹⁵ N in soil	<u>0.38</u>	
Recovery of ¹⁵ N	0.91	70.0%

*In excess of natural abundance.

The labeled nitrogen from soil column II behaved in a fashion similar to what is to be expected from any single application in a slow rate system where no limitation on nitrification such as temperature or lack of soil aeration is present. The preconditioning with wastewater before application of the label was useful for generating a viable population of nitrifying organisms and establishing nitrate concentrations high enough for ¹⁵N analysis. The application of a single pulse of labeled wastewater enabled the movement of the label through the system to be studied and the fate of the nitrogen applied under a specific set of conditions to be documented. This approach is also well suited for obtaining data on rates of nitrification and translocation of nitrate in land treatment.

COLUMN III

Adjusted wastewater with ammonium labeled with 6.6 atom % ¹⁵N was applied to column III. Prior to this application, only tapwater had been applied to the column. The results of ¹⁵N analysis of the nitrate in soil solution at 15 and 45 cm depth and percolate are given in Figure 10. Because of the lack of wastewater additions before the label was applied, nitrate concentrations in soil solution at 15 cm were only about 1 mg/l for the first two weeks of the study and under 10 mg/l for the first 4 weeks. Ammonium concentrations remained at or below 2 mg/l during this same period. Thus the ammonium in this column was being effectively removed from solution but nitrification was inhibited, probably due to an inadequate population of nitrifying organisms. A small peak in the nitrate ¹⁵N concentration corresponding to this analysis was

only 7.4 mg/l (Table A4). Very little of the ¹⁵N label was found during the entire study in soil solution samples below the 15-cm depth. Toward the end of the study, relatively large concentrations of nitrate (up to 95 mg/l) were found in soil solution but this material contained very little of the ¹⁵N label.

Since the labeled nitrogen did not move through the soil profile in significant quantity, it must have been tied up in the upper soil more efficiently than that found for column II. Plant analysis over the study (Fig. 11) does show substantially higher ¹⁵N incorporation than found for column II. This is undoubtedly due to the larger time period the labeled nitrogen was present in the root zone since little labeled nitrate or ammonium was found below the 15-cm depth. Soil analysis at the end of the experiment (Fig. 12) also shows a higher incorporation of the label into the soil nitrogen pool, as is particularly evident in the inorganic analysis. This incorporation is limited to the surface soil, demonstrating why little labeled material was found moving through the profile in soil solution.

The results obtained for recovery of the ¹⁵N label for column III are presented in Table 6. About 92% of the applied ¹⁵N was accounted for in various categories, the largest portion being in plant and soil material. The proportion of the label found in water samples was about 9%, somewhat lower than that found for column II. This was probably due to the delay in nitrification found for this column, due to the lack of preconditioning with wastewater. The delay in nitrification allowed more time for incorporation of the ¹⁵N label into plant material and so there was less competition for NH₄-N by soil microorganisms. This was demonstrated by the greater amount of ¹⁵N taken up in plant material in column III than in column II (Tables 5 and 6).

The experimental strategy used in column III does not seem to be particularly useful for land treatment simulations unless one is particularly interested in the first few weeks of operation of a new system. If so, the treatment with tapwater before application probably will simulate rainfall events and a preapplication condition.

COLUMN IV

The experimental strategy used for column IV involved pretreatment with tapwater, application of adjusted wastewater with ammonium

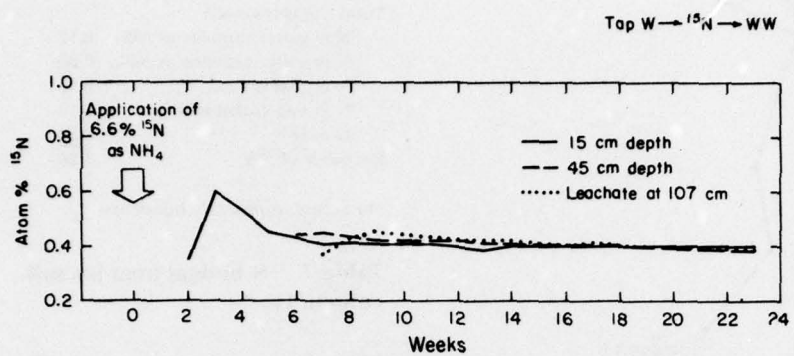


Figure 10. Soil solution ¹⁵N, column III.

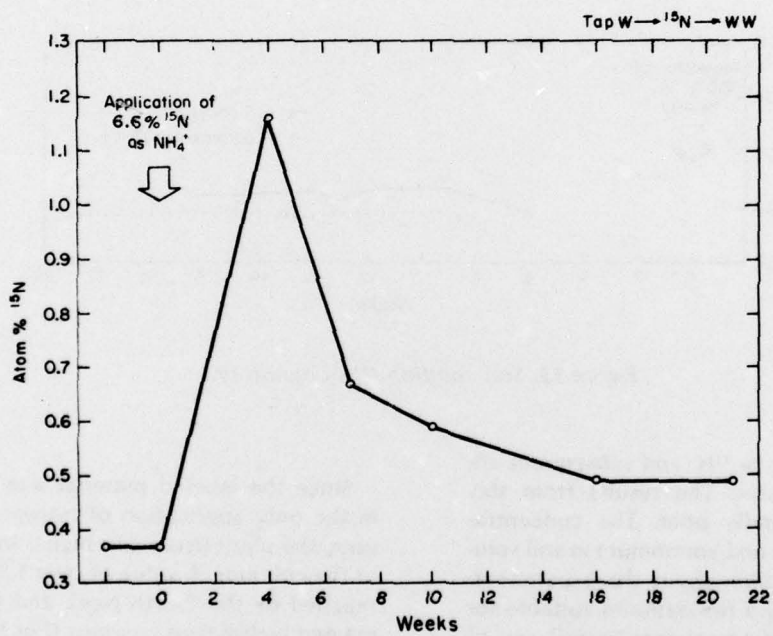


Figure 11. Plant material ¹⁵N, column III.

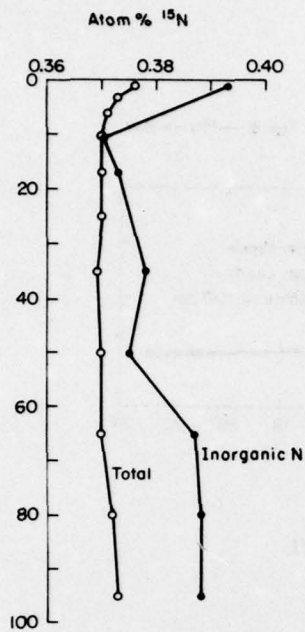


Figure 12. Soil profile ^{15}N , column III.

Table 6. ^{15}N budget (mg) for soil column III.

^{15}N Applied*	1.30	
Total ^{15}N recovered*		
^{15}N in water samples as NO_3^-	0.12	
^{15}N in water samples as NH_4^+	0.00	
^{15}N in plants	0.51	
^{15}N in vegetative mat	0.19	
^{15}N in soil	<u>0.38</u>	
Recovery of ^{15}N	1.20	92.3%

*In excess of natural abundance

Table 7. ^{15}N budget (mg) for soil column IV.

^{15}N Applied*	1.30	
Total ^{15}N recovery*		
^{15}N in water samples as NO_3^-	0.00	
^{15}N in water samples as NH_4^+	0.00	
^{15}N in plants	0.49	
^{15}N in vegetative mat	0.24	
^{15}N in soil	<u>0.52</u>	
Recovery of ^{15}N	1.25	96.2%

*In excess of natural abundance.

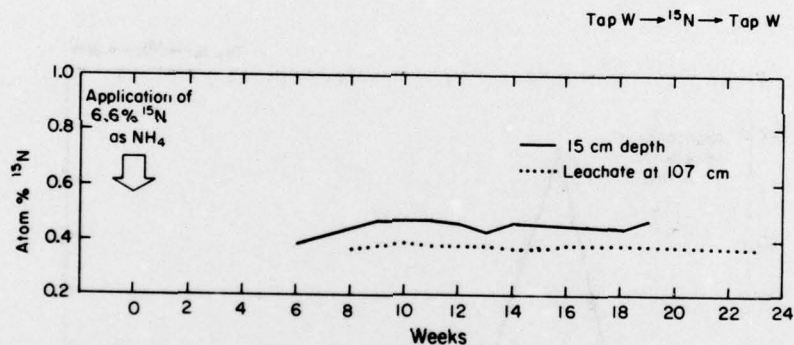


Figure 13. Soil solution ^{15}N , column IV.

containing 6.6 atom % ^{15}N , and subsequent applications of tapwater. The results from this strategy were generally poor. The concentrations of both nitrate and ammonium in soil solution remained low throughout the experiment. This resulted in only a few samples suitable for ^{15}N analysis, but did reveal that little if any of the labeled nitrogen moved into the profile (Fig.13). No ^{15}N values above natural abundance levels were found in the percolate throughout the study.

Since the labeled material was incorporated in the only application of nitrogen to this column, the plant tissue was higher in ^{15}N than any of the columns. A value of over 1.3 atom % was reached by the fourth week and the values remained higher than columns II or III throughout the study (Fig. 14). Soil analysis (Fig. 15) revealed a much higher incorporation of ^{15}N in the surface soil than in any of the other columns and little movement of labeled material below 5 cm.

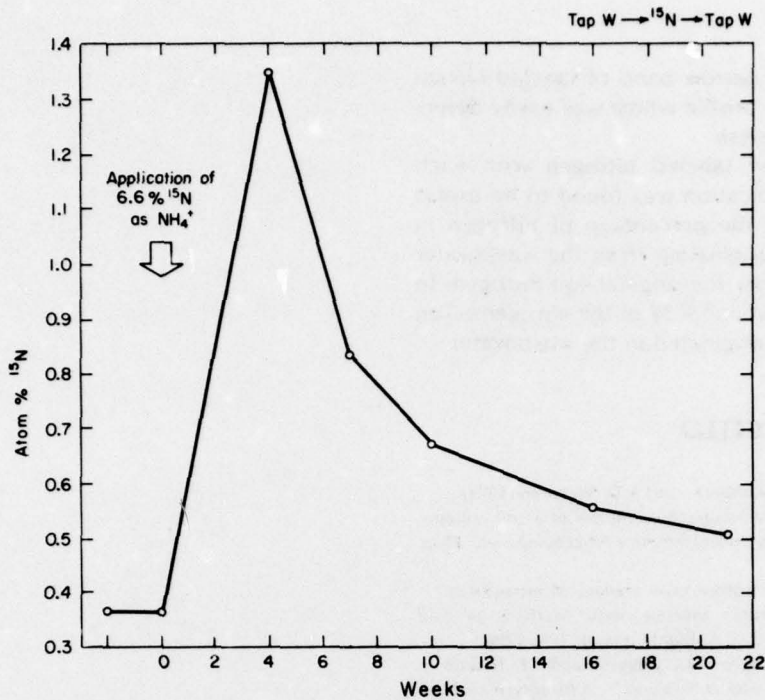


Figure 14. Plant material ¹⁵N, column IV.

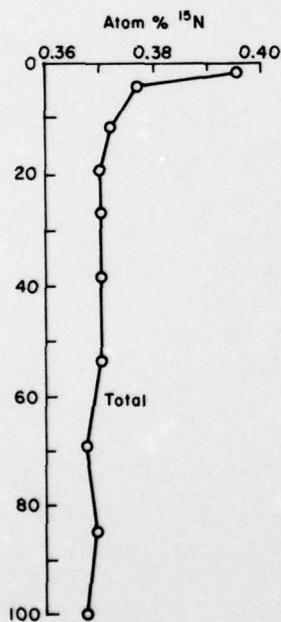


Figure 15. Soil profile ¹⁵N, column IV.

The lack of conditioning with wastewater before or after the label was applied resulted in the lack of any significant nitrification occurring in this column throughout the study. Nitrate-N concentrations were below 3 mg/l in soil solution at 15 cm until the last week.

The recovery of the ¹⁵N label for column IV is presented in Table 7. Over 96% of the ¹⁵N applied was found. Very little of this was observed in water samples, however, due in large measure to the lack of significant nitrification in column IV during the entire study. The mass of ¹⁵N taken by the plants was similar to that in column III but much more was found in the soil than for either columns II or III.

Since this is such a drastic deviation from conditions in the nutrient-rich environment of land application of wastewater, plant uptake or soil nitrogen mechanisms probably are not representative of land treatment conditions.

CONCLUSIONS

To simulate an on-going land treatment system, treatment with wastewater prior to application of the label was essential. Applications of the labeled N at high enrichment and with a single wastewater application resulted in the

movement of a narrow band of labeled nitrate through the soil profile which was easily detectable by ^{15}N analysis.

Application of labeled nitrogen with each wastewater application was found to be useful for determining the percentage of nitrogen in plant material originating from the wastewater vs that taken from the original soil nitrogen. In this study, only about 50% of the nitrogen taken up by the grass originated in the wastewater.

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APPENDIX A: TABULATION OF RESULTS

Table A1. Composition of water applied to soil columns

Date	Type	Applied To Column	Volume (ml)	NH ₄ ⁺ (mg/l)		NO ₃ ⁻ (mg/l)		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)	P(T) (mg/l)
				(mg/l)	Atom% ¹⁵ N	(mg/l)	Atom% ¹⁵ N				
10/18/77	W*	II	780								
	T**	I, III, IV	780	0.0					6.7		
10/21/77	T	I, II, III, IV	500	0.2		0.0			6.7		
10/25/77	W	II	780								
	W-12 ¹⁵ N	I	780								
	W-72 ¹⁵ N	III, IV	780								
11/1/77	W	II, III	780	27.9		7.2			7.7		
	W-12 ¹⁵ N	I	780								
	T	IV	780	0.2		0.0			6.9		
11/8/77	W	II, III	780	21.8		12.5			7.8		
	W-12 ¹⁵ N	I	780								
	T	IV	780	0.0		0.2			7.1		
11/14/77	W	III	780	22.8		0.1			8.1		
	W-12 ¹⁵ N	I	780								
	W-6.62 ¹⁵ N	II	780								
	T	IV	780	0.0		0.0			7.0		
11/21/77	W	II, III	780							5.4	
	W-12 ¹⁵ N	I	780							0.2	
	T	IV	780							4.6	
11/28/77	W	II, III	780								0.1
	W-12 ¹⁵ N	I	780								
	T	IV	780								
12/3/77	W	II, III	780								
	W-1 ¹⁵ N	I	780								
	T	IV	780								
12/12/77	W	II, III	780	23.7		1.0		69.8		0.1	
	W-1 ¹⁵ N	I	780								
	T	IV	780	0.0		0.0		8.9	7.8	0.0	
12/19/77	W	II, III	780				0.373				
	W-12 ¹⁵ N	I	780								
	T	IV	780								
12/23/78	W	I	780	27.1	0.374	0.1		40.6		0.0	3.8
	W-12 ¹⁵ N	II, III, IV	780								
1/3/78	W	II, III	780								
	W-12 ¹⁵ N	I	780								
	T	IV	780								
1/9/78	W	II, III	780								
	W-12 ¹⁵ N	I	780								
	T	IV	780								
1/16/78	W	II, III	780	23.5	0.367	0.6			7.8		
	W-12 ¹⁵ N	I	780								
	T	IV	780								
1/23/78	W	II, III	780	31.6	0.368	0.0			7.7		
	W-12 ¹⁵ N	I	780								
	T	IV	780								
12/30/78	W	II, III	780								
	W-12 ¹⁵ N	I	780								
	T	IV	780								
2/6/78	W	II, III	780								
	W-12 ¹⁵ N	I	780								
	T	IV	780								
2/13/78	W	II, III	780	11.2		0.1					3.0
	W-12 ¹⁵ N	I	780								
	T	IV	780								
2/21/78	W	II, III	780	25.2		0.7		31.9		0.0	
	W-12 ¹⁵ N	I	780								
	T	IV	780								
2/28/78	W	II, III	780								
	W-12 ¹⁵ N	I	780								
	T	IV	780								
3/7/78	W	II, III	780						7.4		
	W-12 ¹⁵ N	I	780								
	T	IV	780								
3/13/78	W	II, III	780							8.1	
	W-12 ¹⁵ N	I	780								
	T	IV	780								
3/20/78	W	II, III	780							8.1	
	W-12 ¹⁵ N	I	780								
	T	IV	780								
3/27/78	W	II, III	780							7.9	
	W-17 ¹⁵ N	I	780								
	T	IV	780								

* W - wastewater

** T - tap water

- W-12¹⁵N - wastewater containing 0.99 atom % ¹⁵N in the NH₄⁺ form

-- W-72¹⁵N - wastewater containing 6.62 atom % ¹⁵N in the NH₄⁺ form

Table A2. Composition of soil water at 15 cm, Column I

Date	Volume (ml)	NH ₄ ⁺		PARAMETER		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		(mg/l)	atom% ¹⁵ N	(mg/l)	NO ₃ ⁻ atom% ¹⁵ N			
10/26/77	57	2.6		2.6			8.4	
11/2/77	57	1.3		0.4			8.4	
11/9/77	57	0.6		5.2			8.5	
11/15/77	60	0.5		4.8	0.578		7.7	
11/22/77	50	5.2		9.4	0.661		7.7	
11/29/77	75	2.7	0.371	17.6	0.660	38.2	7.8	0.0
12/6/77	75	1.0	0.384	11.8	0.565	16.0	6.7	
12/13/77	57	3.6	0.377	17.4	0.704	45.5	7.6	0.0
12/20/77	57	5.4	0.397	25.9	0.751	76.2	7.5	0.1
12/28/77	60	7.5	0.395	28.9		72.5	7.8	0.3
1/4/78	57	7.2	0.398	17.9	0.711	47.8	7.9	0.0
1/10/78	30	5.7	0.416	17.4	0.745		8.0	
1/17/78	80	7.4		16.5	0.710		7.8	
1/24/78	80	8.9	0.447	20.3	0.726		8.3	
1/31/78	65	10.4	0.464	19.8			8.2	
2/7/78	50	12.7	0.473	17.6	0.658	88.0	7.9	0.0
2/14/78	20	13.3	0.474	16.6	0.754		8.0	
2/22/78	70	17.0		38.4	0.712	78.8	7.9	0.3
3/1/78	80	13.4	0.492	68.0	0.643	71.6	7.8	
3/8/78	30	6.6	0.487	110.	0.578	62.7	7.9	0.3
3/14/78	30	3.5		111.	0.598	67.3	7.5	0.1
3/21/78	50	0.8		71.4	0.612	55.1	7.3	0.1
3/28/78	15	0.0		53.6		40.8		0.1
4/4/78		0.1		59.1	0.642	45.6		0.0

Table A3. Composition of soil water at 15 cm, Column II

Date	Volume (ml)	NH ₄ ⁺		PARAMETER		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		(mg/l)	atom% ¹⁵ N	(mg/l)	NO ₃ ⁻ atom% ¹⁵ N			
10/26/77	38	2.5		2.4			7.8	
11/2/77	38	1.8		4.2			7.9	
11/9/77	38	2.9	0.373	9.2	0.367		7.4	
11/15/77	50	3.6	0.371	15.7	0.482		8.0	
11/22/77	40	12.2	0.397	13.1	2.087		7.9	
11/29/77	50	5.3	0.397	15.0	1.041	37.1	7.8	0.0
12/6/77	38	8.1	0.400	23.7	0.511	33.8	7.8	
12/13/77	38	8.7	0.415	23.5	0.479	48.9	7.7	0.0
12/20/77	38	10.9	0.428	19.0	0.455	74.6	8.3	0.0
12/28/77	40	14.7	0.443	26.0	0.448	65.0	8.0	0.1
1/4/78	38	11.4	0.442	21.5	0.431	47.2	8.0	0.0
1/10/78	20	8.7	0.446	18.0	0.409		7.9	
1/17/78	40	12.3	0.433	21.6	0.418		7.9	
1/24/78	30	12.4	0.439	17.7	0.404		8.2	
1/31/78	35	15.1	0.434	20.6	0.404		8.2	
2/7/78	35	16.8	0.429	25.1	0.415	114.	7.9	0.1
2/14/78	15	17.9		47.5	0.411		7.8	
2/22/78	45	14.8		65.1	0.407	104.	7.6	0.4
3/1/78	30	6.7		85.5	0.403	75.1	7.7	
3/8/78	20	7.2		90.8	0.400	71.1	8.0	0.1
3/14/78	20	2.2		90.2	0.397	63.4	7.5	0.2
3/21/78	20	0.1		91.3	0.394	60.2	7.5	0.0
3/28/78	12	0.1		27.4	0.391	38.1		0.0
4/4/78	27	1.4		38.3		47.3		0.1

Table A4. Composition of soil water at 15 cm, Column III

Date	Volume (ml)	NH ₄ ⁺		PARAMETER NO ₃ ⁻		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		(mg/l)	atom% ¹⁵ N	(mg/l)	atom% ¹⁵ N			
10/26/77	57	1.0		0.5				
11/2/77	57	1.0		1.2		8.6		
11/9/77	57	2.2		5.9	0.388	8.2		
11/15/77	40	2.0		7.4	0.604	7.4		
11/22/77	40	4.3		12.2	0.533	8.0		
11/29/77	60	3.4	0.388	21.8	0.454	43.6	7.6	0.0
12/6/77	57	2.6	0.371	19.3	0.431	35.7	7.1	
12/13/77	57	1.9	0.378	27.8	0.411	58.3	7.5	0.2
12/20/77	57	1.2	0.366	23.4	0.409	70.5	7.7	0.2
12/28/77	70	1.3		35.6	0.413	63.8	7.6	0.1
1/4/78	57	0.4		32.4	0.408	60.8	7.7	0.1
1/10/78	30	0.6		24.7	0.407		7.9	
1/17/78	75	0.4	0.433	23.1	0.401		7.7	
1/24/78	45	0.9	0.366	33.9	0.391		8.5	
1/31/78	100	1.4	0.374	23.2	0.398		8.0	
2/7/78	55	2.8		21.5	0.398	111.	7.8	0.1
2/14/78	35	1.8		29.5	0.398		7.6	
2/22/78	115	4.5		41.1	0.398	92.9	7.4	0.1
3/1/78	70	4.7		48.6	0.398	92.2	7.7	
3/8/78	45	4.4		64.2	0.398	82.5	7.8	0.1
3/14/78	40	2.0		74.7	0.398	80.2	7.4	0.1
3/21/78	75	0.4		75.1	0.398	69.4	7.1	0.0
3/28/78	38	0.2		73.7	0.398	42.2		0.0
4/4/78	50	0.2		83.1	0.399	54.4		0.1

Table A5. Composition of soil water at 15 cm, Column IV

Date	Volume (ml)	NH ₄ ⁺		PARAMETER NO ₃ ⁻		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)	
		(mg/l)	atom% ¹⁵ N	(mg/l)	atom% ¹⁵ N				
10/26/77	72	1.5		0.0			8.3		
11/2/77	72	1.2		0.3			8.4		
11/9/77	72	2.0		2.0			8.1		
11/15/77	100	1.3		0.5			7.7		
11/22/77	60	1.8		0.6			7.9	0.0	
11/29/77	90	1.9	0.399	0.6		2.5	12.2	7.9	0.1
12/6/77	72	2.0	0.372	2.2	0.392		22.2	7.7	
12/13/77	72	1.8	0.366	0.3			17.0	7.7	0.0
12/20/77	72	2.1	0.366	0.5		3.7	21.8	7.7	0.0
12/28/77	90	3.3	0.375	0.9	0.469	3.5	31.2	7.9	0.0
1/4/78	72	2.6	0.376	1.3		3.5	23.1	7.9	0.0
1/10/78	55	2.4	0.378	1.9	0.468		7.8		
1/17/78	95	3.4		2.4	0.463	3.4	7.8		
1/24/78	50	3.3	0.376	1.1	0.425		8.2		
1/31/78	60	3.0	0.382	1.5	0.455	4.1	8.2		
2/7/78	50	3.1		1.1		4.3	24.5	7.8	0.0
2/14/78	20	3.6		1.5			8.0		
2/22/78	60	4.9		1.5			19.6	7.8	0.0
3/1/78	40	5.5		0.9	0.441		21.5	7.9	
3/8/78	20	6.2	0.377	1.1	0.465		23.0	8.1	0.0
3/14/78	20	5.2		1.4			22.4	7.7	0.0
3/21/78	40	6.0		1.9			15.9	7.8	0.0
3/28/78	20	4.1							0.0
4/4/78		4.8		13.0			13.0		0.0

Table A6. Composition of soil water at 45 cm, Column I

Date	Volume (ml)	NH ₄ ⁺		PARAMETER NO ₃ ⁻		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		(mg/l)	atom% ¹⁵ N	(mg/l)	atom% ¹⁵ N			
10/26/77	20	0.7		0.2			7.9	
11/2/77	20	0.2		0.1			8.1	
11/9/77	20	0.3		0.8			7.7	
11/15/77	25	1.0		1.3			7.5	
11/22/77	7	0.5						
11/29/77	25	0.7		7.4	0.569	35.3	7.6	0.0
12/6/77	50	0.2		4.3	0.480	14.1	6.8	
12/13/77	20	1.1	0.366	18.6	0.656	37.9	7.5	
12/20/77	20	1.2		14.8	0.670	50.0	7.5	0.1
12/28/77	25	1.8		15.3	0.697	70.1	7.9	0.3
1/4/78	20	1.6	0.389	23.0	0.693	70.0	7.9	0.5
1/10/78	10	0.6		22.2			7.9	
1/17/78	35	0.9	0.387	14.2	0.697		7.9	
1/24/78	15	0.9		12.4	0.709		8.1	
1/31/78	20	1.2		13.5	0.711		8.2	
2/7/78	10	0.6		18.6		85.0	7.4	0.1
2/14/78	5	1.1		12.8	0.708			
2/22/78	25	2.8		22.6	0.717	123.	8.0	0.1
3/1/78	30	3.3	0.399	26.0	0.714	101.	7.9	
3/8/78	30	4.1	0.373		0.629		8.0	0.0
3/14/78	30	6.2		47.2	0.655	70.1	7.8	0.0
3/21/78	65	8.9	0.416	54.2	0.595	55.0	7.6	0.0
3/28/78	27	13.0		81.6		60.5		0.0
4/4/78		10.3	0.440	51.7	0.633	54.6		0.0

Table A7. Composition of soil water at 45 cm, Column II

Date	Volume (ml)	NH ₄ ⁺		PARAMETER NO ₃ ⁻		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		(mg/l)	atom% ¹⁵ N	(mg/l)	atom% ¹⁵ N			
10/26/77	50	0.4		0.4			7.9	
11/2/77	50	0.4		1.6			7.9	
11/9/77	50	0.5		3.6	0.366		7.4	
11/15/77	80	0.5		4.8	0.373		7.7	
11/22/77	40	0.5		8.8	0.389		7.8	0.0
11/29/77	30	0.9		10.0	1.303	38.2	7.8	0.0
12/6/77	50	1.3	0.374	20.4	1.195	37.5	7.7	
12/13/77	50	1.1	0.386	16.9	0.753	37.4	7.6	0.0
12/20/77	50	1.5	0.366	17.2	0.526	41.8	7.8	0.0
12/28/77	60	2.3	0.386	14.0	0.465	67.1	7.9	0.0
1/4/78	50	2.1	0.391	20.0	0.456	72.0	7.9	3.0
1/10/78	40	1.8	0.386	19.8	0.447		8.0	
1/17/78	85	2.0	0.383	14.9	0.433		7.8	
1/24/78	30	2.0	0.400	16.5	0.418		8.2	
1/31/78	45	2.7	0.382	14.4	0.409		8.2	
2/7/78	30	2.9	0.380	12.2	0.405	86.6	8.0	0.0
2/14/78	10	4.4		15.4	0.404		8.1	
2/22/78	30	8.3		35.5	0.404	75.2	8.0	0.1
3/1/78	25	9.6		48.2	0.404	90.6	7.7	
3/8/78	25	9.7		47.3	0.404	68.8	8.1	0.4
3/14/78	30	10.6		49.7	0.404	65.5	7.5	0.4
3/21/78	55	11.8		75.7	0.404	47.4	7.6	0.8
3/28/78	22	7.9		95.2	0.404	51.1		1.2
4/4/78	40	2.1	0.405	79.8	0.403	53.0		1.4

Table A8. Composition of soil water at 45 cm, Column III

Date	Volume (ml)	NH ₄ ⁺		PARAMETER NO ₃ ⁻		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		(mg/l)	atom% ¹⁵ N	(mg/l)	atom% ¹⁵ N			
10/26/77	66	0.3		0.2			7.6	
11/2/77	66	0.2		0.6			8.3	
11/9/77	66	0.2		1.7			7.8	
11/15/77	80	0.5		3.4			7.4	
11/22/77	40	0.8		2.3			7.8	
11/29/77	80	0.6		10.5		42.0	7.6	0.0
12/6/77	66	0.8	0.366	29.7	0.436	34.8	7.4	
12/13/77	66	0.8	0.366	20.8	0.451	40.5	7.4	0.0
12/20/77	66	1.5	0.375	28.4	0.429	57.4	7.5	0.0
12/28/77	100	2.2	0.388	19.5	0.424	67.4	7.7	0.0
1/4/78	66	2.2	0.372	28.8	0.424	88.9	7.6	0.0
1/10/78	45	2.0	0.381	28.4	0.422		7.8	
1/17/78	100	2.3	0.392	23.1	0.416		7.6	
1/24/78	45	2.3	0.372	23.9	0.413		7.9	
1/31/78	85	2.9	0.386	29.2	0.413		7.8	
2/7/78	70	3.2		25.4	0.409	88.4	7.5	0.1
2/14/78	25	2.0		17.0	0.406		7.8	
2/22/78	110	2.6		22.2	0.404	94.9	7.5	0.3
3/1/78	70	1.6		23.3	0.402	110.	7.5	
3/8/78	50	0.8		23.3	0.400	72.7	7.7	0.2
3/14/78	45	0.2		31.1	0.398	73.8	7.4	0.2
3/21/78	80	0.1		62.8	0.396	58.9	7.3	0.4
3/28/78	30	0.0		83.1	0.395	52.4		0.6
4/4/78	50	0.0		76.5	0.394	52.6		0.8

Table A9. Composition of soil water at 45 cm, Column IV

Date	Volume (ml)	NH ₄ ⁺		PARAMETER NO ₃ ⁻		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		(mg/l)	atom% ¹⁵ N	(mg/l)	atom% ¹⁵ N			
10/26/77	0	0.5		0.4			7.9	
11/2/77	0	0.3		0.4			8.8	
11/9/77	0	1.0		1.1			8.6	
11/15/77								
11/22/77	7	1.0						
11/29/77								
12/6/77								
12/13/77	0	2.0		3.4			8.1	
12/20/77	0	1.4		1.0		25.3	8.2	0.0
12/28/77								
1/4/78								
1/10/78								
1/17/78								
1/24/78								
1/31/78								
2/7/78								
2/14/78								
2/22/78	5	0.0		0.4				
3/1/78	125	1.2		1.2		18.8	7.7	
3/8/78	75	1.2		1.0		17.0	7.8	0.0
3/14/78	60	1.1		1.1		16.5	7.7	0.0
3/21/78	70	1.3		1.4		22.7	7.6	0.1
3/28/78	47	1.1						0.0
4/4/78		1.4		2.6		17.2		0.0

Table A10. Composition of soil water at percolate, Column I

Date	Volume (ml)	NH ₄ ⁺		PARAMETER NO ₃ ⁻		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		(mg/l)	atom% ¹⁵ N	(mg/l)	atom% ¹⁵ N			
10/19/77		0.0		0.0			7.8	
10/22/77		0.2		0.0			7.7	
10/26/77	565	0.0		0.0			7.9	
10/27/77		0.0		0.0			7.9	
11/2/77	408	0.0		0.0			7.8	
11/4/77							7.9	
11/9/77	436	0.0		0.1			7.8	
11/11/77							8.0	
11/15/77	465	0.0		0.0			7.7	
11/17/77							7.9	
11/22/77	485	0.0		0.0		10.9	7.8	0.1
11/23/77		0.0		0.0		9.6	8.0	0.0
11/29/77	478	0.0		0.1		10.8	7.8	0.0
12/6/77	465	0.0		0.0		19.6	7.8	
12/13/77	425	0.0		0.0		28.8	7.9	0.0
12/20/77	532	0.1		1.3	0.481	39.0	7.8	0.0
12/28/77	420	0.0		3.6	0.581	47.9	7.8	0.0
1/4/78	500	0.0		9.3	0.627	57.2	7.8	0.1
1/10/78	606	0.1		11.5	0.678		8.0	
1/17/78	480	0.1		14.4	0.683		8.1	
1/24/78	571	0.3		21.0	0.658		8.1	
1/31/78	581	0.2		20.9	0.680		8.2	
2/7/78	485	0.3		18.0	0.648	73.7	8.0	0.0
2/14/78	465	0.1		15.0	0.601		7.9	
2/22/78	460	0.1		14.9	0.629	87.9	8.0	0.1
3/1/78	440	0.1		16.8	0.583	111.	8.0	
3/8/78	475	0.1		17.8	0.580	118.	8.1	0.0
3/14/78	445	0.1		17.4	0.561	129.	8.0	0.0
3/21/78	370	0.2		25.1	0.630	143.	7.9	0.0
3/28/78	480	0.1		31.1		126.		0.0
4/4/78		0.3	0.425	38.2	0.629	95.9		0.0

Table A11. Composition of soil water at percolate, Column II

Date	Volume (ml)	NH ₄ ⁺		PARAMETER NO ₃ ⁻		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		(mg/l)	atom% ¹⁵ N	(mg/l)	atom% ¹⁵ N			
10/19/77		0.4		0.0			7.7	
10/22/77		0.8		0.0			7.8	
10/26/77	392	0.2		0.0			7.9	
10/27/77		0.1		0.0			8.2	
11/2/77	336	0.3		0.0			8.0	
11/4/77							8.2	
11/9/77	282	0.3		0.2			8.0	
11/11/77							8.2	
11/15/77	395	0.3		0.0			8.0	
11/17/77							8.1	
11/22/77	377	0.2		0.0		36.0	7.9	0.2
11/23/77		0.0		0.0		30.1	8.2	0.0
11/29/77	420	0.3		0.0		26.3	7.9	0.0
12/6/77	420	0.2		0.0		29.9	7.9	
12/13/77	345	0.1		0.0		37.8	8.0	0.0
12/20/77	475	0.1	0.366	0.3	0.914	44.5	8.3	0.0
12/28/77	505	0.0		0.8	1.032	49.7	8.0	0.0
1/4/78	500	0.3		3.2	0.824	50.3	8.0	0.1
1/10/78	538	0.5	0.456	7.7	0.590		7.9	
1/17/78	480	0.3	0.449	9.0	0.479		8.1	
1/24/78	493	0.6	0.458	14.4	0.448		8.1	
1/31/78	563	0.3		15.9	0.437	75.8.	8.1	
2/7/78	440	0.5		13.4	0.425	75.8	7.8	0.0
2/14/78	535	0.5		15.6	0.411		8.1	
2/22/78	600	0.7		19.1	0.409	83.3	7.9	0.2
3/1/78	410	0.6		14.2	0.411	118.	7.9	
3/8/78	485	0.6		15.7	0.405	127.	8.0	0.0
3/14/78	525	0.5		31.3	0.409	135.	7.9	0.0
3/21/78	475	0.2		52.2	0.400	130.	7.9	0.0
3/28/78	490	0.1		58.9	0.400	103.		0.1
4/4/78	525	0.1		74.9	0.406	91.0		0.1

Table A12. Composition of soil water at percolate, Column III

Date	Volume (ml)	PARAMETER		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		NH ₄ ⁺ (mg/l)	atom% ¹⁵ N			
10/19/77		0.1			7.7	
10/22/77		0.2			7.8	
10/26/77	650	0.0			7.9	
10/27/77		0.2			8.1	
11/2/77	460	0.0			8.0	
11/4/77					8.1	
11/9/77	416	0.0		0.2	7.9	
11/11/78					8.1	
11/15/77	515	0.0		0.0	7.8	
11/17/77					8.0	
11/22/77	490	0.0		0.0	34.0	7.9
11/23/77		0.0		0.0	34.3	8.2
11/29/77	465	0.0		0.4	40.9	7.9
12/6/77	405	0.0		1.0	41.0	7.9
12/13/77	295	0.0		1.1	46.0	7.9
12/20/77	395	0.5		2.0	51.8	8.3
12/28/77	450	0.0		6.5	74.9	8.0
1/4/78	450	0.0		13.8	76.1	8.1
1/10/78	575	0.0		19.0		7.9
1/17/78	430	0.0		20.4		8.2
1/24/78	465	0.0		22.9		8.3
1/31/78	415	2.1	0.366	29.0		8.5
2/7/78	420	0.2		27.9	84.2	8.1
2/14/78	450	0.1		26.8		8.5
2/22/78	370	0.0		26.6	89.7	8.0
3/1/78	330	0.0		29.2		0.2
3/8/78	380	0.1		31.7	113.	7.7
3/14/78	460	0.1		33.7	122.	8.0
3/21/78	420	0.0		36.2	128	7.9
3/28/78	430	0.0		35.6	127.	7.9
4/4/78	470	0.0		40.4	114.	8.0
					103	0.1

Table A13. Composition of soil water at percolate, Column IV

Date	Volume (ml)	PARAMETER		Cl ⁻ (mg/l)	pH	NO ₂ ⁻ (mg/l)
		NH ₄ ⁺ (mg/l)	atom% ¹⁵ N			
10/19/77		0.2			7.8	
10/22/77		0.4			7.8	
10/26/77	574	0.1			7.9	
10/27/77		0.4			8.2	
11/2/77	485	0.0			8.0	
11/4/77					8.2	
11/9/77	523	0.0		0.1	7.8	
11/11/77					8.5	
11/15/77	475	0.0		0.0	7.8	
11/17/77					8.4	
11/22/77	395	0.0		0.0	24.5	8.0
11/23/77		0.0		0.0	21.8	8.6
11/29/77	475	0.0		0.0	25.0	7.9
12/6/77	450					
12/13/77	430	0.0		0.3	30.2	8.2
12/20/77	455	0.2		0.9	33.4	8.3
12/28/77	380	0.0		1.6	37.9	8.1
1/4/78	440	0.0		3.3	43.1	8.2
1/10/78	531	0.0		4.5		8.1
1/17/78	380	0.0		2.6		8.6
1/24/78	436	0.0		3.5		8.6
1/31/78	511	0.0		4.2		8.6
2/7/78	290	0.0		6.0	45.7	8.3
2/14/78	500	0.0		8.3		0.0
2/22/78	450	0.0		11.0	46.4	8.0
3/1/78	220	0.0		8.6	48.7	8.0
3/8/78	395	0.0		7.5	49.1	8.2
3/14/78	270	0.0		7.6	49.1	8.0
3/22/78	390	0.1		7.5	46.4	8.0
3/28/78	280	0.0		7.4	48.0	8.0
4/4/78		0.4		9.1	43.1	0.0

Table A14. Composition of plant material, Column I Table A15. Composition of plant material, Column II

Date Sampled	Dry Weight (g)	% N	Atom% ¹⁵ N	Date Sampled	Dry Weight (g)	% N	Atom% ¹⁵ N
13 Oct 1977	0.78	2.88	0.368	13 Oct 1977	0.44	2.81	0.368
25 Oct 1977	--	2.50	0.372	25 Oct 1977	--	3.54	0.370
22 Nov 1977	0.68	4.50	0.614	22 Nov 1977	0.69	4.75	0.638
13 Dec 1977	0.35	4.96	0.663	13 Dec 1977	0.30	4.63	0.890
3 Jan 1978	0.22	5.44	0.688	3 Jan 1978	0.18	4.18	0.578
13 Feb 1978	0.25	4.52	0.695	13 Feb 1978	0.11	4.20	0.474
23 Mar 1978	0.37	3.91	0.719	23 Mar 1978	0.15	4.29	0.422
12 May 1978	0.31	3.19	0.626	12 May 1978	0.62	2.84	0.400
12 May 1978 (Dead)	3.92	2.17	0.575	12 May 1978 (Dead)	4.24	2.04	0.441

Table A16. Composition of plant material, Column III Table A17. Composition of plant material, Column IV

Date Sampled	Dry Weight (g)	% N	Atom% ¹⁵ N	Date Sampled	Dry Weight (g)	% N	Atom% ¹⁵ N
13 Oct 1977	0.70	2.84	0.368	13 Oct 1977	1.01	2.63	0.367
25 Oct 1977	--	3.44	0.367	25 Oct 1977	--	2.85	0.365
22 Nov 1977	0.65	3.90	1.156	22 Nov 1977	0.55	3.33	1.357
13 Dec 1977	0.37	6.27	0.671	13 Dec 1977	0.34	3.41	0.834
3 Jan 1978	0.24	4.11	0.589	3 Jan 1978	0.24	3.41	0.672
13 Feb 1978	0.28	4.21	0.491	13 Feb 1978	0.24	3.64	0.559
23 Mar 1978	0.53	3.06	0.488	23 Mar 1978	0.30	3.34	0.506
12 May 1978	0.95	2.92	0.406	12 May 1978	--	2.86	0.457
12 May 1978 (Dead)	3.50	2.33	0.534	12 May 1978 (Dead)	3.64	2.05	0.567

Table A18. Soil analysis, Column I

Depth (cm)	Wt(g)	Total Nitrogen		Mineral Nitrogen	
		N(ug/g)	Atom% ¹⁵ N	N(ug/g)	Atom% ¹⁵ N
0- 2.5	177.9	1504	0.386	18.35	0.384
2.5- 5.1	350.6	1584	--	17.85	0.379
5.1- 7.6	370.1	1457	0.382	16.15	0.378
7.6-15.2	938.6	1501	0.375	18.15	0.377
15.2-22.9	1095.6	615	0.375	34.35	0.388
22.9-30.5	1275.7	700	0.373	14.00	0.393
30.5-45.7	2474.6	139	0.385	15.85	0.393
45.7-61.0	2184.7	205	0.377	22.00	0.388
61.0-76.2	2163.0	129	0.378	14.35	0.390
76.2-91.4	2304.4	171	0.374	19.65	0.393
91.4-107	2303.7	193	0.374	65.00	0.402

Table A19. Soil analysis, Column II

Depth (cm)	Wt(g)	Total Nitrogen		Mineral Nitrogen	
		N(ug/g)	Atom% ¹⁵ N	N(ug/g)	Atom% ¹⁵ N
0- 2.5	306.2	1602	0.375	18.35	0.384
2.5- 5.1	326.1	1537	0.373	17.85	0.379
5.1- 7.6	340.8	1720	0.372	16.15	0.378
7.6-15.2	1136.4	1168	0.370	18.15	0.377
15.2-22.9	1186.5	457	0.372	34.35	0.388
22.9-30.5	1159.7	553	0.371	14.00	0.393
30.5-45.7	2410.8	165	0.370	15.85	0.393
45.7-61.0	2292.8	245	0.371	22.00	0.388
61.0-76.2	1962.3	155	0.371	14.35	0.390
76.2-91.4	2185.8	143	0.372	19.65	0.393
91.4-107	2610.7	163	0.377	65.00	0.402

Table A20. Soil analysis, Column III

Depth (cm)	Wt(g)	Total Nitrogen		Mineral Nitrogen	
		N(ug/g)	Atom% ¹⁵ N	N(ug/g)	Atom% ¹⁵ N
0- 2.5	220.5	1758	0.376	22.65	
2.5- 5.1	350.2	1700	0.373	6.60	
5.1- 7.6	354.6	1400	0.372	11.95	
7.6-15.2	1010.3	1665	0.370	10.80	
15.2-22.9	1137.8	675	0.370	14.60	
22.9-30.5	1124.7	658	0.370	12.89	
30.5-45.7	2434.5	200	0.369	7.41	
45.7-61.0	2212.9	255	0.370	18.17	
61.0-76.2	1978.1	190	0.370	13.95	0.387
76.2-91.4	2176.7	175	0.372	23.56	0.388
91.4-107	2220.5	238	0.373	50.00	0.388

Table A21. Soil analysis, Column IV

Depth (cm)	Wt(g)	Total Nitrogen	
		N(ug/g)	Atom% ¹⁵ N
0- 2.5	279.9	1842	0.395
2.5- 5.1	343.8	1702	0.377
5.1- 7.6	333.0	1587	--
7.6-15.2	1044.3	1562	0.372
15.2-22.9	1219.6	752	0.370
22.9-30.5	1157.5	619	0.370
30.5-45.7	2411.5	245	0.370
45.7-61.0	2347.9	232	0.369
61.0-76.2	2034.7	112	0.368
76.2-91.4	2204.7	195	0.369
91.4-107	2334.4	149	0.367

Table A22. Composition of vegetative mat, Columns I-IV.

<u>Column</u>	<u>Dry Weight</u> (g)	<u>Nitrogen Concentration</u> ($\mu\text{g/g}$)		<u>Atom % ^{15}N</u>
			<u>Excess ^{15}N</u>	
I	51.56	8300	0.215394	0.413
II	47.48	8702	0.084069	0.385
III	77.42	8900	0.191852	0.392
IV	85.28	7800	0.235074	0.399