Fate of ^{15}N-Labelled Atrazine in a Wetland Mesocosm

An Undergraduate Thesis in Bioresource Research
Oregon State University

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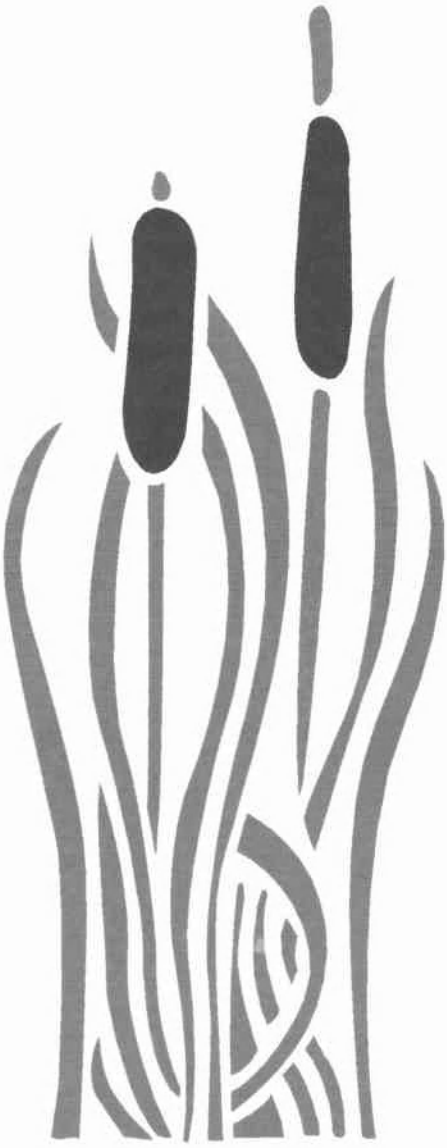

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Introduction

Atrazine is one of the most widely used herbicides in the world. In 1993, about 32,000 to 34,000 t of atrazine was applied in the United States (Mersie et al 1998). Unfortunately, 0.1-3.0% of atrazine has been found to move off-site after application (Jones et al 1982). As such, atrazine is frequently detected in surface water. A recent study of Willamette Basin surface water reported atrazine in 99% of the samples analyzed (Anderson et al 1996). In the Midwest, atrazine has been found in domestic wells (Paterson et al 1992). The EPA has set drinking water maximum contaminant level (MCL) for atrazine at 0.003 mg/L. The wide use of atrazine and its frequent detection in ground and surface water suggests treatment methods which minimize off-site movement need evaluation.

Constructed wetlands have proven useful for removing nutrients and can reduce biological oxygen demand (Kadlec et al 1996). Constructed wetlands also show promise at reducing concentrations of pesticides caused by agricultural run-off (Alvord et al 1996). However, the overall fate and site of degradation of pesticides in wetlands is not well understood. A better understanding of how wetlands work to sequester and degrade pesticides could be used to improve constructed wetland design.

Studies involving wetland mesocosms have shown that the water concentration of atrazine decreases after application (Lee et al 1995, Runes et al, McKinlay et al 1999). Biodegradation occurs slowly in an aqueous environment (Grover and Cessna 1991). The most significant pathway resulting in the decline of atrazine water concentration is the partitioning of atrazine into the soil. Alvord et al (1995) measured a K_{oc} value of 476 for a wetland soil. A study by Jones et al (1982) of the partitioning of atrazine between water and a wetland sediment found only 38% of the original ^{14}C activity in the water after 21 days, the rest was presumably in the soil. Of the residues extractable from soil, 75% were atrazine, 15% were hydroxyatrazine, and 10% were dealkylated atrazine.

(Figure 1) On subsequent sampling dates, the percent of extractable residue that was hydroxyatrazine increased. On day 45, 56% of extractable residue was hydroxyatrazine while dealkylated atrazine remained at 10%. Degradation to hydroxyatrazine is the most significant degradation pathway. (Ma et al 1996). In a study of submerged soil, only 0.48% of ring labelled ^{14}C-atrazine was recovered as $^{14}CO_2$ after 20 days suggesting that mineralization of atrazine is not significant (Goswami et al 1971).

Wetland vegetation may also play a role in atrazine degradation. Lee et al (1995) found that wetland hydrophyte community structure was the primary ecological determinant of atrazine degradation rate in a wetland mesocosm. Atrazine water concentration declined most rapidly in mesocosms possessing the emergent plants *Typha latifolia and T. augustifolia.* Plants are capable of taking up atrazine. Rice et al (1997) found 11.7±1.06% of ^{14}C from ^{14}C-atrazine in extracts from the aquatic plant *Elodea canadensis* after 16 days. Roeth et al (1971) found 23% of ^{14}C from ^{14}C-atrazine in corn seedlings after 4 weeks. Burken and Schnoor (1997) report that poplar trees took up 27.8% of soil applied atrazine in 13 days. However, plant uptake of atrazine has not been measured for wetland plants at the mesocosm scale.

If wetlands are to be used to treat atrazine in surface water, it is important to know how atrazine is distributed between the soil, water, and plant compartments. This information is basic to understanding how wetlands work to degrade atrazine and how they could be improved for performing this function. Previous mesocosm scale experiments have only measured the decline in water concentration of atrazine (Lee et al 1995, Runes et al, McKinlay et al 1999). The objective of the research reported here was to measure the movement of atrazine from water into soil and plants at the mesocosm scale.

Traditional approaches to measure environmental compartmentalization possess undesirable features. One approach to measure atrazine partitioning into the soil, water, and plant compartments is to measure atrazine with gas chromatography (GC) or high

performance liquid chromatography (HPLC). However, this approach requires extraction and extensive sample cleanup. Even more effort is required if the same measurements are to be made for atrazine metabolites. Furthermore, some metabolites, such as hydroxyatrazine, have a low extraction efficiency making accurate measurement difficult (Ma et al 1996). Another approach is to use ^{14}C-labelled atrazine. Oxidation and liquid scintillation techniques can be used to measure ^{14}C in the plant, soil, or water compartments. This approach is undesirable due to the cost of ^{14}C-atrazine, handling requirements for radiolabeled compounds, and disposal costs.

This research employs atrazine labelled with the stable isotope ^{15}N. This isotope is a naturally occurring, non-radioactive isotope of nitrogen that is frequently used in studies of the nitrogen cycle (Knowles 1993). Since ^{15}N exists in relatively low concentrations (only 0.3663% of atmospheric N is ^{15}N), highly enriched ^{15}N compounds can be used to determine nitrogen fate by measuring the $^{15}N/^{14}N$ ratio in environmental compartments such as plants. This ratio, measured as $\delta^{15}N$, can be measured with great sensitivity using an elemental analyzer isotope ratio mass spectrometer (EA-IRMS). Since atrazine contains nitrogen, a small amount of highly enriched ^{15}N-labelled atrazine will be used to determine the fate of atrazine by its effect on the $\delta^{15}N$ of soil and plants. A search of the literature leads the author to believe that this will be the first time ^{15}N has been used to study atrazine fate at the mesocosm scale.

Since some atrazine will be transformed once it has been added to the mesocosm, changes in isotopic ratio of samples cannot be contributed solely to labelled atrazine. Degradation products will be partially responsible for the change in isotopic ratio. However, the position of the labelled nitrogen is such that the isotope will remain with the triazine ring through the initial degradation reactions. Primary degradation products of atrazine are hydroxyatrazine, desethylatrazine (DEA), and desisopropylatrazine (DIA), all of which contain the labelled nitrogen. (Figure 1) Hence, use of the ^{15}N label will

provide information not only for the compartmentalization of atrazine, but also for its primary degradation products.

For the first several weeks, enrichment of the plants will be caused primarily by ^{15}N-atrazine and only less significantly by degradation products. Hydroxyatrazine is often the primary initial degradation product of atrazine (Ma et al 1996). However, it can bind tightly to soil and may be less available for plant uptake than atrazine (Ma et al 1996). Degradation to DEA and DIA is less significant (Jones et al 1982). Hence, ^{15}N-atrazine will be most available for plant uptake.

Materials and Methods

Wetland Mesocosms

Six wetland mesocosms were constructed in 96.5 x 61.0 x 53.0 cm tubs in a greenhouse. Soil from a constructed wetland located at a container nursery near Gaston, Oregon was added to the mesocosms. The soil was 14 cm deep. The soil texture was 10.4% sand, 69.5% silt, and 20.1 % clay. Soil density was 660 g/dm^3.

Twenty cattails (*Typha latifolia)* were planted in each mesocosm. Plants were 18 weeks old and there were 14.5± 2.9 cattails per mesocosm that were at least 0.75 m tall when the atrazine was applied to the mesocosms.

The water in the mesocosms was maintained at 12.7 cm above the soil surface. Water was added twice weekly to maintain this depth. Once weekly the mesocosms received 3 liters of Miracle Gro for Tomatoes (18%N, 18%P, 21%K) at a dilution of 15 mL fertilizer per 12 L water.

Temperature in the greenhouse was maintained between 29.4 degrees C during daytime and 14.4 degrees C at night. Lighting consisted of two 500W metal halide lamps. Lamps were on from 6am to 9pm daily.

Mesocosm Treatments

Three mesocosms received 67.07 mg of ^{15}N-Atrazine (2-Chloro-4-ethyamino-^{15}N-6-isopropylamino-1,3,5-triazine, >99 atom%, >99% chemical purity) purchased from ISOTEC Inc. (Miamisburg, OH) in 20 ml acetone. Two mesocosms received 66.81 mg of unlabelled atrazine (99% chemical purity) purchased from Chem Service (West Chester, PA) in 20 ml acetone. One mesocosm received 20 ml of acetone with no atrazine. The atrazine addition produced an approximate initial water concentration of 0.9 ppm.

Mesocosm Sampling

Samples collected included surface soil (0-3 cm), soil cores (11-14 cm), cattail foliage, and cattail roots. Water was removed from the surface soil by vacuum using a Buchner funnel with a Whatman #41 filter paper. Soil core samples contained less water and did not receive vacuum drying. All soils were subsequently dried for seven days at 45 degree C. Soils were then ground in a mortar for isotopic analysis.

Cattail foliage from two plants per mesocosm was collected from the innermost four leaves which had achieved a height of at least 1.5 m, sampling 23-30.5 cm from the tip of the leaf. The foliage was dried for seven days at 45 degree C. Foliage samples were then ground in mortar with liquid nitrogen in preparation for isotopic analysis.

Isotopic Analysis

Samples were analyzed for ^{15}N and %N on a Finnigan Delta-Plus isotope ratio mass spectrometer linked to a Carlo Erba NC2500 elemental analyzer (Finnigan MAT GmbH, Bremen, Germany), and located at the U.S. Environmental Protection Agency Western Ecology Division, Corvallis, Oregon. The internal standard for isotopic and

concentration measurements was pine needles (NIST 1575). Stable isotope abundances are reported as

$$N = (R_{sample} / R_{standard} - 1) \times 1000,$$

where $R = {}^{15}N/{}^{14}N$ of either the sample or the reference standard (atmospheric N_2). The standard deviation of isotopic measurements for the ${}^{15}N$ standard was ±0.2. Samples with more of the heavy isotope are referred to as heavier, or enriched; samples with more of the light isotope are lighter, or depleted.

Calculations

Surface soil measurements were fit to a Boltzman sigmoidal funtion. A linear regression was performed for the soil core measurements. Absolute enrichment was calculated for each sampling date as the curve-fit value minus the control value. Soil enrichment was assumed to decline with depth in the soil profile as the function

$$y = ae^{-kx}$$

where y = enrichment and x = soil depth. For surface soil measurements x = 1.5 cm. For soil core measurements x = 12.5 cm. From the soil-enrichment model, the total amount of additional ${}^{15}N$ in the soil was calculated. (Figure 2)

For each sampling date, absolute enrichment of the treated cattails was calculated as the mean $\delta^{15}N$ value minus the mean of the $\delta^{15}N$ values of the pre-treatment samples. The measured enrichment of the shoots 23-30.5 cm from the leaf tip was assumed to be the same for the entire shoot mass. Enrichment of the roots was assumed to be 25% of the mean shoot enrichment. This value is from Roeth et al (1971). The ratio of ${}^{14}C$ concentration from ${}^{14}C$-atrazine of roots/leaves in corn seedlings was 25% (Roeth et al 1971).

Results and Discussion

The average percent nitrogen was 0.16±0.04, 0.12±0.03, 3.4±0.4 for the surface soil, soil cores, and cattails, respectively. In order, the mean shoot mass and root mass for the treatment mesocosms was 581 g and 704 g.

Results for the soil are given in Figures 3 and 4. The surface soil (0-3 cm) was enriched by 18 $^{0}/_{00}$ (thousandths) by day 21. The soil cores were enriched by approximately 1.5 $^{0}/_{00}$ by day 21. This enrichment of the soil is reciprocal to the decline in water concentration that has been observed. Runes et al found that the water concentration of atrazine had declined by 74% after 20 days in a wetland mesocosm system employing the same plant species and soil as used in this research. McKinlay et al (1999) found that the concentration of atrazine declined by 63% after 18 days upon the second addition of atrazine.

Results for the enrichment of cattails are given in Figure 5. The cattails steadily increased in enrichment until day 28. The decline in enrichment after day 28 may have resulted from the sampling design. The protocol called for sampling the innermost four cattail leaves that were at least 1.5 m tall. For the later sampling dates, leaves meeting these criteria may have been newer growth that was not present earlier in the experiment when atrazine water concentration was probably higher. Thus, these leaves would have a lower enrichment. In retrospect, sampling the same leaf over time would have been a better strategy. In addition, some research suggests that atrazine accumulates towards the base of plant leaves. In oats, progressively more atrazine accumulates towards the base of the blade (Ashton et al 1973). If this is true of cattails, then data reported here may underestimate atrazine uptake.

It is possible to calculate the quantity of ^{15}N label in each environmental compartment once the enrichment of the compartment is known and the size of the nitrogen pool in that compartment has been estimated. Such a mass balance model was

generated. (Figure 6) Since atrazine concentration in water was not measured, data from Runes et al was used to complete the mass balance. It was assumed that, proportionally, the same amount of atrazine was remaining in the water as was measured by Runes et al with GC-MSD. Use of this data is reasonable because the mesocosms used were very similar. Both studies used soil from the same source. Mesocosms were approximately equal in size and were planted with *T. latifolia.* Initial water concentrations of atrazine were comparable. At day 21, the enrichment of the soil and plants would require 47% and 8% of the applied ^{15}N-atrazine to be present in each respective compartment. On this day, Runes et al found 30% of the applied atrazine present in the water. Plant uptake of atrazine was not insignificant. The amount of atrazine that partitions into cattails could potentially increase as the density of the cattail population increased. Indeed, if cattails do accumulate atrazine towards the base of the plant, then uptake could be substantially underestimated. More measurements are needed to determine whether cattails are evenly enriched.

After a decline during the first two days after application, the amount of total ^{15}N that is accounted for by the mass balance model remains between 80-90%. A greatly inaccurate mass balance model would not likely possess this consistency in the accounting of the label. However, this mass balance model was meant to provide only a rough estimate of atrazine distribution. The 10-20% deficit could result from inaccurate estimates of the atrazine in each compartment. Soil enrichment may differ from the exponential decline assumed in the soil-enrichment model. This would lead to inaccuracy in the total atrazine estimated in the soil. As well, the enrichment of the plants may have been underestimated. Samples were taken near the tips of leaves while it is not certain that atrazine is distributed evenly throughout the plant. Atrazine is evenly distributed in the water compartment and hence the total amount of dissolved atrazine can be well determined by techniques such as GC-MSD. However, use of data from the slightly different mesocosms used by Runes et al is also a potential source of error.

Future experiments could improve the accuracy of similar mass balance models by measuring soil enrichment at a smaller interval and thereby improving the accuracy of the soil-enrichment model. Similarly, measurements could be made to determine if atrazine is indeed distributed homogeneously in cattail shoots.

Use of the stable isotope ^{15}N to trace a herbicide has proved to possess several advantages over existing techniques. Preparing samples for isotopic analysis is simpler than preparing samples for liquid scintillation counting, GCMS, or HPLC. No extraction from soil or plant matter, or sample clean-up is necessary. Samples need only be dried and ground. The sample preparation is the same for soil, plant, or even insect samples. It is even feasible to measure the $\delta^{15}N$ of water samples. However, since EA-IRMS requires solid samples, it would be necessary to first concentrate solutes on a solid substrate.

Use of ^{15}N-labelled atrazine allows experiments of a larger scale to be more readily conducted. In fact, field scale experiments are feasible. Of the studies involving ^{14}C-atrazine that are relevant to this research, all have been conducted with systems no more than 2 liters in size (Goswami et al 1971, Jones et al 1982, Mersie et al 1996, Mersie et al 1998). None of these systems contained plants. Use of larger systems will likely provide results more representative of the fate of atrazine in the environment. The small systems that have been used may not possess microbial populations as diverse as natural systems. There was so little soil used in these studies that the soil structure was likely very homogenous. Mersie et al (1996) used only 1 g of soil. Channels along plant roots or macropores due to insect life probably did not exist. In contrast, the mesocosms used in this experiment contained approximately 59 kg dry weight soil, 75 liters water, approximately 20 mature cattails and a community of microfauna.

Other experiments have been conducted to trace atrazine in wetland mesocosms (Lee et al 1995, McKinlay et al 1999, Runes et al). These studies did not employ ^{14}C-atrazine. Using ^{14}C-atrazine on a large scale would involve large disposal costs from the

disposal of the contaminated material. As such, these experiments measured non-labelled atrazine using GCMS or HPLC. These studies only measured the decline in the water concentration of atrazine. No soil or plant measurements were made. This is likely because of the laborious nature of extracting atrazine from soil or plant matter. By employing ^{15}N-labelled atrazine, soil and plant measurements can be easily made for mesocosm scale experiments. These measurements facilitate the completion of mass balance calculations.

Another advantage of the use of ^{15}N as a tracer is the sensitivity of measurements that are made with IRMS. In fact, the lower the %N of the environmental compartment being sampled, the larger the change in the $\delta^{15}N$ value caused by a given amount of added ^{15}N. For example, a 2 $^{0}/_{00}$ change in plant matter that is 3.4%N would be caused by a 3.6 ppbw concentration of ^{15}N-atrazine. But a 2 $^{0}/_{00}$ change in a soil core that is 0.12%N would be caused by a 0.127 ppbw concentration of ^{15}N-atrazine. (Both examples assumed the samples were originally 0 $^{0}/_{00}$) In addition, the sensitivity at which atrazine can be detected can be increased by labelling additional nitrogen atoms. For each additional nitrogen atom that is labelled after the first, the detection limit for the labelled atrazine is decreased by a factor 1/X where (X = 2, 3, 4, 5....). A 2 $^{0}/_{00}$ change in a soil core that is 0.12%N could be caused by a 0.042 ppbw concentration of atrazine labelled at the three triazine nitrogens.

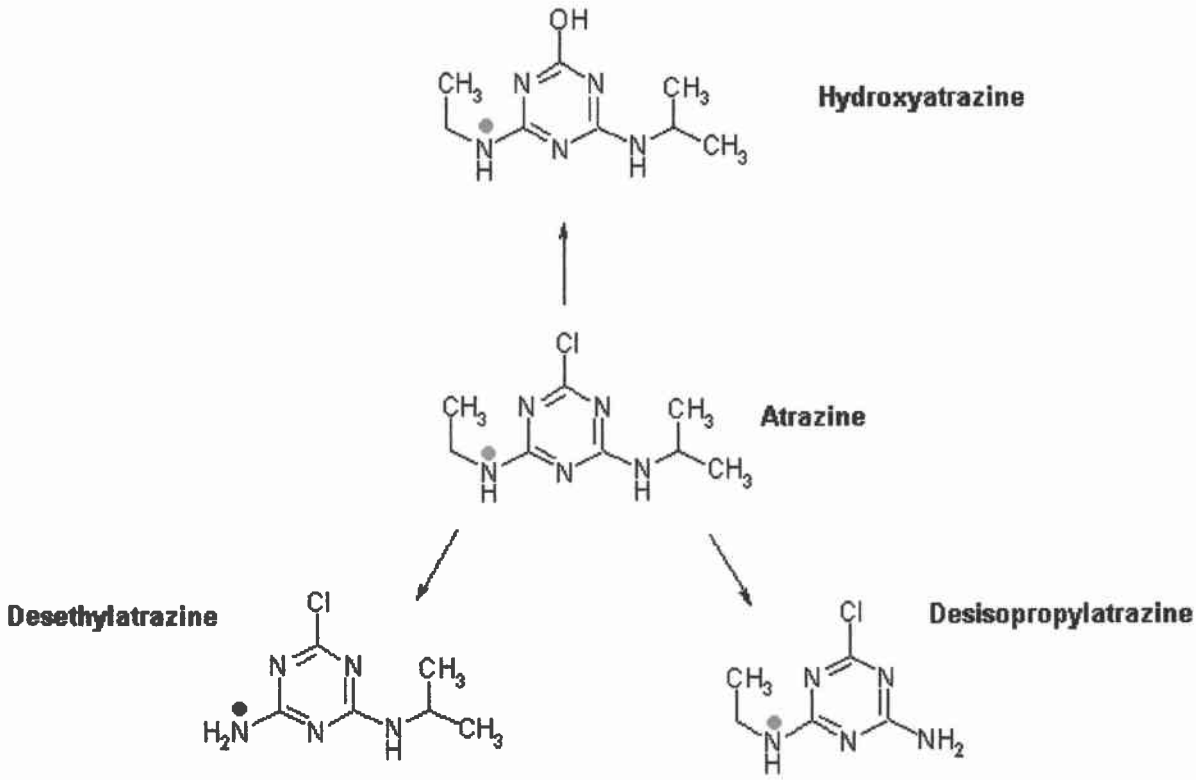
Conclusions

This experiment suggests that compartmentalization into soil is the major dissipation pathway for dissolved atrazine. Plant uptake, as calculated from enrichment, was not large. However, uptake was estimated from foliage samples 23-30.5 cm from the leaf tip. If plants accumulated atrazine towards their base, then their uptake may have been underestimated. Further, increased plant density may increase total plant uptake. It

is notable that plants may contribute to atrazine dissipation in other ways than direct uptake. The rhizosphere may provide a hospitable environment for atrazine-degrading microbes. Dissolved atrazine may be pulled into the soil through the absorption of water by the roots of transpiring plants. This research builds on other work suggesting that constructed wetlands could serve as low-maintenance, low-cost tools for reducing pesticides and other pollutants in surface water.

There is great potential for future experiments with ^{15}N-labelled pesticides. Mesocosm and field-scale experiments are possible. Soil, plant or insect samples can be easily measured. Any pesticide containing nitrogen may be traced. Sensitivity can be increased by labelling multiple nitrogen positions within a pesticide.

Figure 1: Primary Atrazine Degradation Products



• = Labelled Nitrogen

Figure 2

Model for the enrichment of soil. Soil enrichment was assumed to decline exponentially. The top 6 cm were assumed to be 0.16%N. The bottom 8 cm were assumed to be 0.12%N.

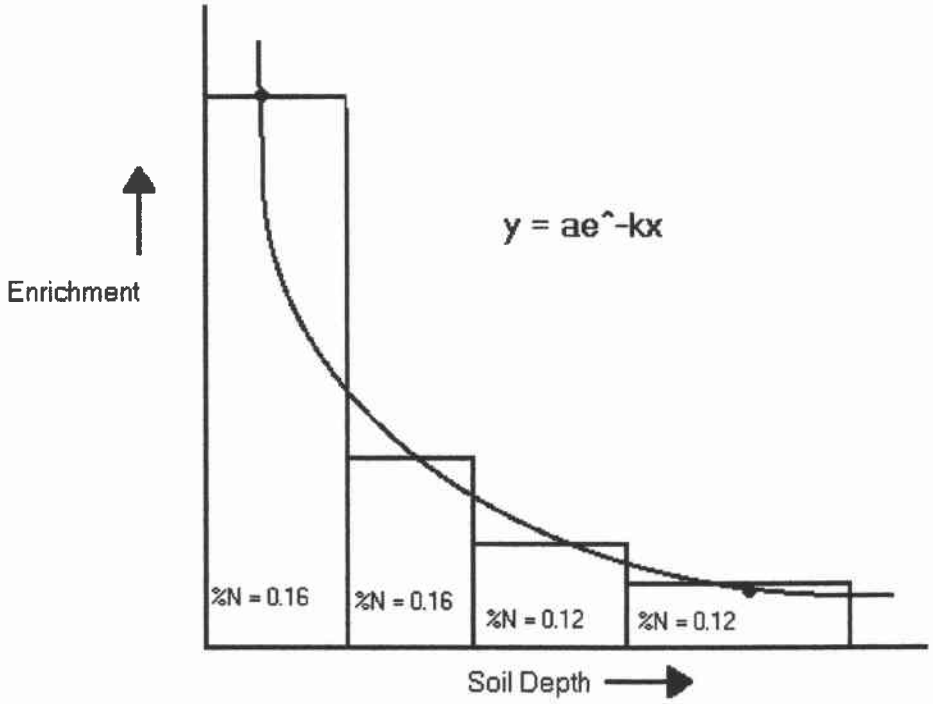


Figure 3. $\delta^{15}N$ of surface soil (0-3 cm) from a wetland mesocosm after addition of ^{15}N-labelled atrazine to surface water. Addition occurred after sampling on day 7. Dashed line represents a Boltzman Sigmoidal fit to the data from three treated mesocosms.

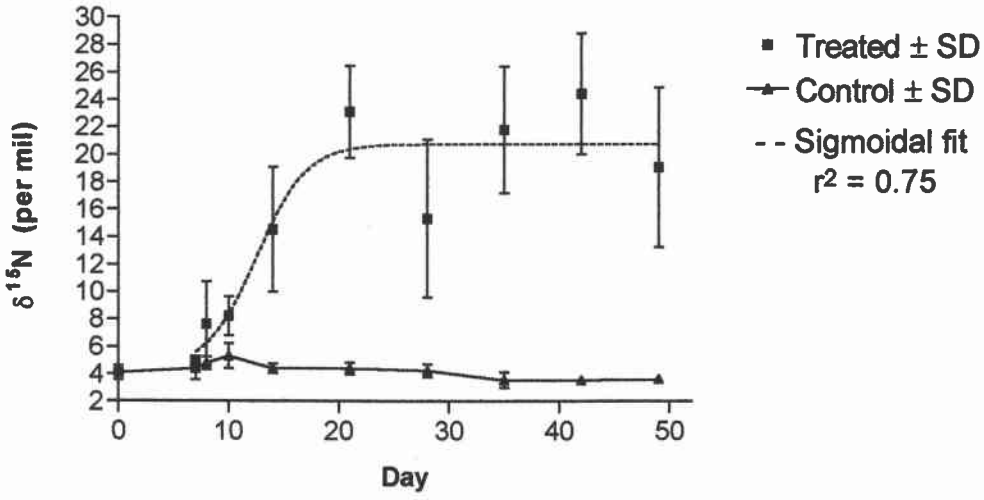


Figure 4. $\delta^{15}N$ of soil cores (11-14 cm) from three wetland mesocosms after addition of ^{15}N-labelled atrazine to surface water. Addition occurred after sampling on day 7.

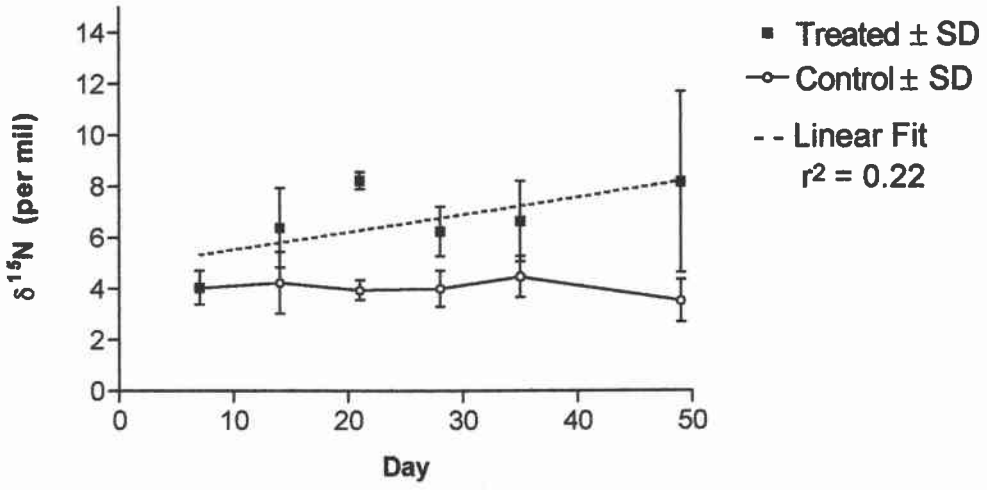


Figure 5. $\delta^{15}N$ of cattail foliage after addition of ^{15}N-labelled atrazine to the surface water of three wetland mesocosms. Addition occurred after sampling on day 7.

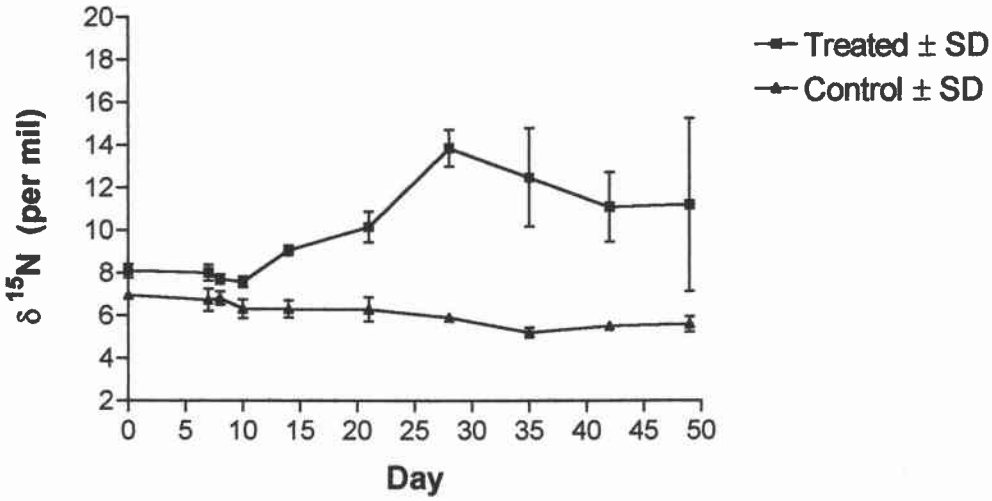
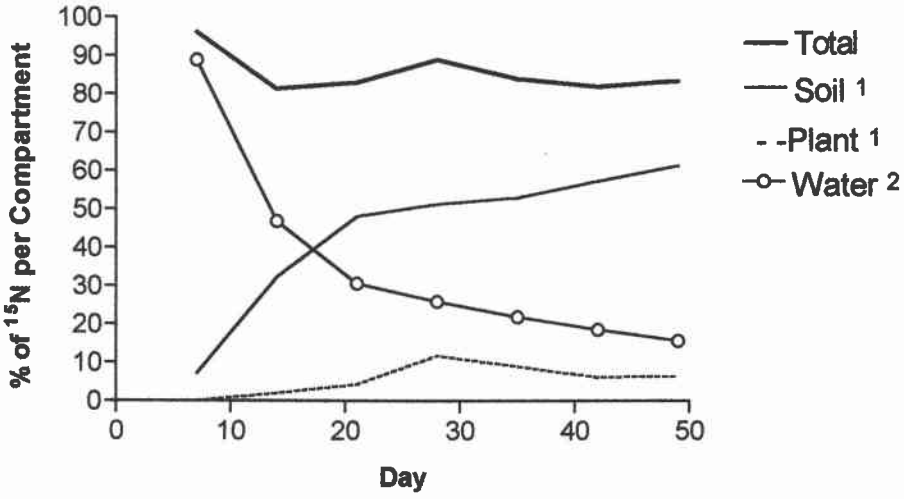


Figure 6. Percent of ^{15}N per compartment as calculated from enrichment. Labelled atrazine was added on day 7. Water measurements from Runes et al have been added to facilitate mass balance.



1. Estimated % of mass from ^{15}N enrichment. (mean, n = 3)
2. Estimated % of mass derived from GC-MSD data in a separate experiment conducted by Runes et al. (mean, n = 3).

Table 1: Data Values for Figures 3 - 5

Figure 3 Data

Day	Treated Soil			Control Soil		
	Cosm 1	Cosm 3	Cosm 5	Cosm 2	Cosm 4	Cosm 6
0	4.62	3.98	3.66	4.31	3.99	3.96
7	4.64	5.33	4.5	5.35	3.92	3.91
8	11.11	6.69	5.17	5.29	4.35	4.72
10	9.62	8.29	6.78	6.31	4.56	5.05
14	16.7	9.31	17.62	4.79	4.08	4.33
21	27	21.33	21.02	4.89	4.09	4.21
28	21	15.59	9.51	4.53	3.72	4.51
35	26.1	16.89	22.48	4.15	3.45	3.06
42	23.22	29.36	20.77	3.71	3.51	3.44
49	21.87	22.99	12.42	3.85	3.34	3.67

Figure 4 Data

Day	Treated Soil			Control Soil		
	Cosm 1	Cosm 3	Cosm 5	Cosm 2	Cosm 4	Cosm 6
7	4.61	4.61	4	4.63	4.19	3.32
14	5.85	8.14	5.19	5.39	2.98	4.36
21	8.59	8.18	7.93	4.35	3.57	3.95
28	5.29	7.21	6.27	3.39	4.79	3.87
35	8.46	5.68	5.82	4	5.42	4.04
49	6.42	5.85	12.22	4.49	3.09	3.03

Figure 5 Data

Treated Cattails			Control Cattails		
Cosm 1	Cosm 3	Cosm 5	Cosm 2	Cosm 4	Cosm 6
7.62	8.67	8	6.74	7.27	6.8
7.79	8.71	7.5	5.77	6.83	7.59
7.39	8.17	7.54	6.24	7.32	6.87
7.43	8.04	7.23	5.53	6.36	7.03
9.06	9.45	8.64	6.25	5.64	7
8.73	11.13	10.58	5.46	7.37	6.01
13.47	15.51	12.61	5.7	6.18	5.79
16.43	12.6	8.41	4.82	5.59	5.2
14.26	8.8	10.18	5.42	5.46	5.61
19.22	6.13	8.2	4.95	6.2	5.63

Appendix: Pilot Study

Pilot Study

Introduction

Synthesized ^{15}N-atrazine is expensive. The purpose of this pilot study was to test the experimental design for an experiment using ^{15}N-atrazine. Inexpensive ^{15}N-ammonium sulfate served as the surrogate for ^{15}N-atrazine. Specifically, this experiment aimed to:

- Determine if the estimated quantity of ^{15}N was sufficient to cause measurable changes in environmental compartments, particularly in plants.
- Determine if intermittent fertilizations necessary for healthy plants would cause noise in the isotopic ratio of environmental samples, particularly plants.
- Determine if the plant sampling strategy was unbiased and provided interpretable results.
- Determine the best handling and preparation technique for enriched samples.
- Determine an atrazine dosage that was not lethal to cattails.

Materials and Methods

Wetland Mesocosm

One mesocosm was constructed as described in the ^{15}N-atrazine experiment. The mesocosm was fertilized as described in the ^{15}N-atrazine experiment. The water level was brought to 12.7 cm above the soil surface once per week. Plants were grown for 9 weeks before the initial sampling. The mesocosm contained 10 plants taller than 2.5 ft at the time of spiking. Greenhouse conditions were as for the ^{15}N-atrazine experiment except that only natural light was used.

Mesocosm Treatment

The mesocosm received 412.0 mg of Ammonium-$^{15}N_2$ Sulfate (5 atom%) purchased from ISOTEC Inc. (Miamisburg, OH) dissolved in 200mL deionized water. The mesocosm also

received 85.80 mg of Atrazine (99% chemical purity, Chem Service) dissolved in 20mL of acetone.

Mesocosm Sampling

Samples collected included surface soil (0-3cm), cattail foliage, and green algae. Water was removed from the surface soil using a Buchner funnel with a Whatman #41 filter paper over a vacuum. The first 15.2 cm from the tip of the leaf were collected form three cattail plants and pooled at each sampling date. All samples were then dried for seven days at 45 degree C. Samples were then ground in a mortar for isotopic analysis. Isotopic analysis was completed as for the ^{15}N-atrazine experiment.

Results and Discussion

Results are given in Figures A1, A2, and A3. Three days after spiking, the soil, cattails, and algae were 10, 120, and 860 per mil enriched respectively. No known natural process could lead to changes is isotopic ratio that are this large in only three days. This indicates that plants and algae could immediately access the ^{15}N. Clearly, 412 mg ^{15}N-ammonium sulfate (5 atom%) was sufficient to cause measurable changes in all of the environmental compartments sampled.

Periodic unlabelled fertilizations caused no noticeable noise in the measurements. Nor would such noise be expected to be noticeable with such large changes in isotopic ratio that resulted from the addition of the labelled ammonium sulfate. This fertilization regime was used in a later experiment.

Changes in the isotopic ratio of the cattails were so large that it was conjectured that this may have resulted from sampling the tips of the leaves. If newly acquired nitrogen accumulates at leaf tips, then the enrichment of tips would not be representative of the enrichment of plant matter as a whole. As such, foliage further from the leaf tip was collected in future experiments.

No evidence from this experiment gave reason to suspect that contamination resulted from the failure to isolate and contain samples. However, the high signature of algae samples may have been caused by the lack of sufficient preparation. Algae samples were not washed prior to drying. Hence, ^{15}N dissolved in the water surrounding the algae samples may not have evaporated and thereby increased the signature of the algae.

The 85.80 mg of atrazine used in this experiment noticeably affected plant health. Leaves yellowed and several smaller plants died. Plants were clearly less robust than in untreated, but otherwise identical, mesocosms that were being prepared for future experiments. This amount of atrazine was considered to be the maximum amount that could be used in future experiments without causing serious harm to plants.

Figure A1. $\delta^{15}N$ of surface soil (0-3 cm) from a wetland mesocosm after addition of ^{15}N-labelled ammonium sulfate to surface water. Addition occurred after sampling on day 6.

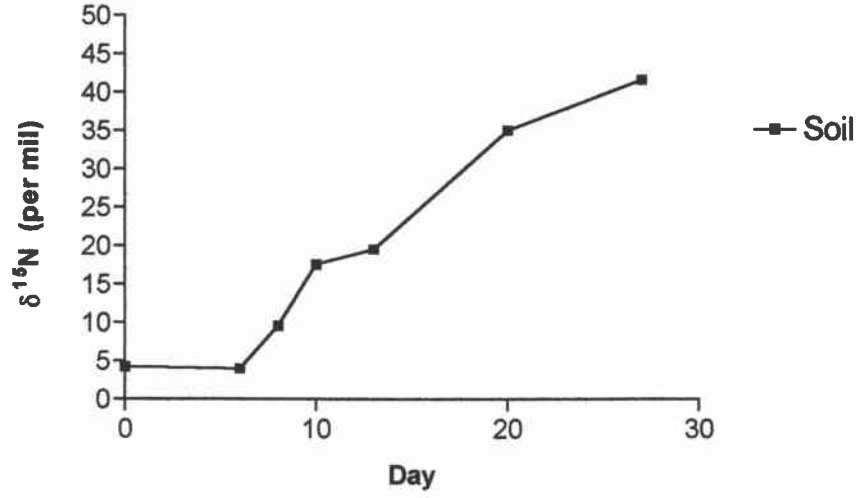


Figure A2. $\delta^{15}N$ of cattail foliage from a wetland mesocosm after addition of ^{15}N-lablelled ammonium sulfate to surface water. Addition occurred after sampling on day 6.

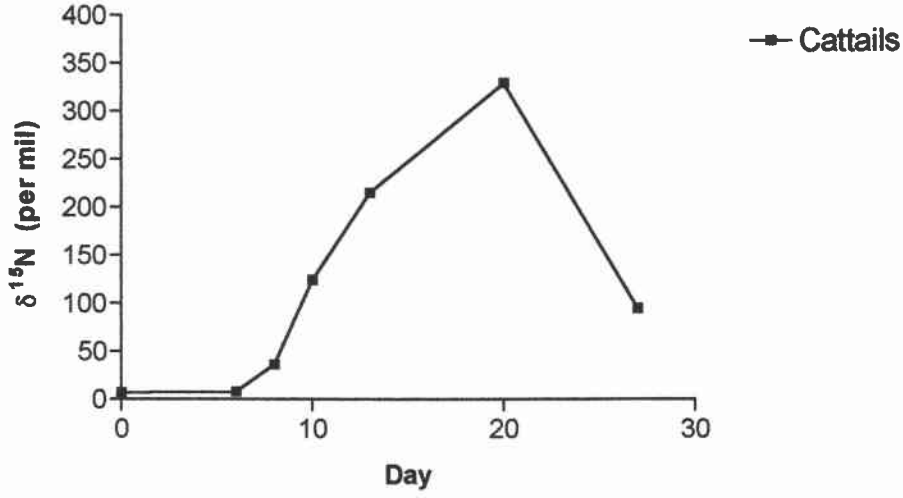
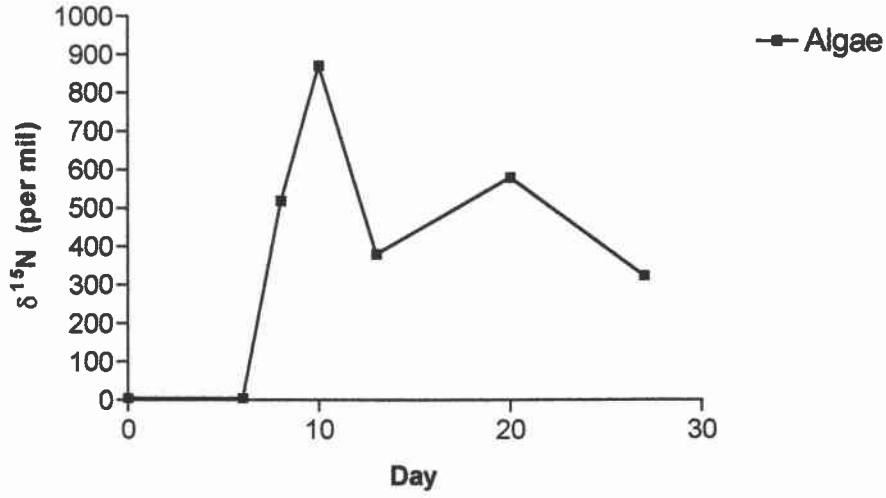


Figure A3. $\delta^{15}N$ of algae from a wetland mesocosm after addition of ^{15}N-labelled ammonium sulfate to surface water. Addition occurred after sampling on day 6.



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